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RESEARCH ON CHINA'S TOURISM CARBON EMISSION EFFICIENCY AND ITS INFLUENCING FACTORS

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ABSTRACT

The paper uses super-efficiency DEA to measure the carbon emission efficiency of China's 30 provinces in 2010-2017. The research shows that carbon emission efficiency in Beijing, Tianjin, Liaoning, Shanghai, Jiangsu, Zhejiang, Shandong, Henan, Guangdong and Chongqing. Both are above 0.6; tourism carbon emission efficiency in Hebei, Shanxi, Inner Mongolia, Jilin, Heilongjiang, Anhui, Fujian, Jiangxi, Hubei, Hunan, Guangxi, Hainan, Sichuan, Guizhou, Yunnan and Shaanxi is between 0.3 and 0.6, which is moderate. Efficiency range; low-efficiency provinces below 0.3 have Gansu, Qinghai, Ningxia, Xinjiang and other regions. At the same time, Tibot model is used to verify the influencing factors affecting the carbon emission efficiency of China's tourism industry. It is concluded that the service industry development level, urbanization level, location conditions, opening level and socio-economic development level are negatively correlated with China's tourism carbon emission efficiency. However, there is no significant relationship between tourism resource endowment and carbon emission efficiency. On this basis, suggestions for improving tourism carbon emission efficiency are proposed.

KEYWORDS:
China, tourism, carbon emission, efficiency

INTRODUCTION

In recent years, global climate change has become the biggest environmental problem facing human society, and CO2 produced by human activities. Emissions are an important cause of climate change. At present, China has become the world's largest CO2 emitter, and the task of energy conservation and emission reduction is extremely arduous. As a pillar industry of China's national economy, tourism has seen its CO2 emissions increase significantly with the rapid growth of tourism scale. China National Tourism Administration has successively issued the "Opinions on Tourism to Address Climate Change", "Guiding Opinions on Further Promoting Energy Saving and Emission Reduction in Tourism Industry" and other documents to guide the development of low carbonization in tourism. At the same time, according to the report "Climate Change and Tourism: Responding to Global Challenges" issued by the World Tourism Organization in 2008, it is believed that tourism has at least 5% responsibility for global greenhouse gas emissions, and if no measures are taken, in the future In the next 30 years, tourism carbon emissions will increase by 1.5 times. Therefore, the carbon emission problem of tourism has become one of the important issues to be studied and solved in the development of low-carbon economy. As an important indicator to measure the relationship between tourism economic development and tourism carbon emissions, tourism carbon emission efficiency is based on the development efficiency of tourism under the constraints of carbon emissions, which refers to the input of production factors in the tourism production process. The extent of CO2 emissions at the output level is minimized. Carbon emission efficiency is essentially the efficiency of production technology that incorporates carbon emissions. Improving tourism carbon emission efficiency is a key path for tourism carbon emission reduction. Therefore, scientific measurement and analysis of China's tourism carbon emission efficiency and its key influencing factors are low for promoting tourism. Carbon transformation has important theoretical guidance and practical reference significance.

LITERATURE REVIEW

Research on tourism carbon emission efficiency. Wang Kun et al. (2015) used the SBM model to measure the carbon emission efficiency of tourism in 30 provinces and autonomous regions of China, and studied the spatial pattern of tourism carbon emission efficiency and the spatial and temporal heterogeneity of its influencing factors by means of ESDA and GWR methods [1]. Han Yuanjun et al. (2015) measured tourism carbon emissions in Beijing, Shandong, Zhejiang, Hubei, and Hainan using the tourism consumption stripping coefficient, and combined the carbon emission indicators to evaluate
the tourism industry efficiency of the five provinces and regions using the DEA model [2]. Wang Kai et al. (2017) used the coefficient of variation and spatial autocorrelation analysis method to study the regional differences and pattern evolution of China's tourism carbon productivity [3]. Based on the input-output analysis and IPCC's CO2 conversion method, Zhang Jianping (2016) estimated the regional tourism energy consumption and CO2 emissions in 17 cities in Hubei Province, and used the SBM-Undesirable model to low-carbon for regional tourism. Efficiency and its total factor productivity have been measured and analyzed. The conclusions of the study show that: the efficiency assessment shows that if the undesired output is not considered, the tourism development efficiency may be underestimated; the low carbon efficiency of tourism in Hubei Province is generally at a low level, and the low carbon efficiency level of tourism in different regions. There is a big difference, and the potential for the use of production factors in the regional tourism economic system remains to be discovered. During the analysis period, the low-carbon efficiency of regional tourism in Hubei Province is generally on the rise, and the technological progress promoted by scale factors is regional low-carbon tourism. The key driving force for the economic efficiency to climb, and the change in pure technical efficiency is not conducive to productivity [4]. Li Peng and Li Tianying. (2017) selected the CO2 emissions of tourists and tourism activities as indicators of eco-efficiency, and constructed a measurement model for the eco-efficiency of tourism route products. The calculation and analysis of eco-efficiency was carried out in Yunnan as an example [5]. Liu Changsheng and Jian Yufeng. (2012) Based on the input-output analysis, this paper constructs the data envelopment method (DEA) and stochastic frontier function method (SFA) for the evaluation of low-carbon tourism service efficiency. Taking the environmental protection traffic in Zhangjiajie scenic spot as an example, the efficiency of low-carbon tourism service is provided. The evaluation shows that the environmentally-friendly transportation low-carbon tourism service provides low efficiency and relatively severe seasonal volatility, but it shows an increasing pattern of change; total tourism population, human capital investment, and fixed-asset investment provide efficiency for low-carbon tourism services. Positive impact, while the number of workers, fuel consumption has a negative impact on it [6].

Research on the factors affecting tourism carbon emissions. Some researchers have further explored the factors affecting the carbon dioxide emissions of tourism. The index decomposition method is the most widely used. For example, Robaina-Alves M et al. (2016) used the exponential decomposition method to study the factors affecting the tourism carbon dioxide emissions in Portugal, and pointed out that the scale of tourism is The key to its rising carbon dioxide emissions, energy structure, carbon intensity and energy intensity also have an important impact [7]; Wang Kai et al. (2016) use the exponential decomposition method to measure and analyze the key factors affecting China's tourism carbon emissions[8]. A few researchers have used input-output analysis and data envelopment analysis to analyze the influencing factors of tourism-related CO2 emissions [9-11]. Tao Yugu et al. (2014) used the LMDI method to find that the main factors affecting tourism carbon emissions are energy intensity, consumption structure, tourism income and tourist visits [12]; Wang Jia and Yang Jun (2014) adopted the improved IPAT model for tourism carbon emission intensity and tourism. Quantitative analysis of the factors affecting the proportion of CO2 emissions, per capita tourism income, and tourist reception times, found that tourism carbon emission intensity is a key factor in inhibiting CO2 emissions [13].

In summary, it can be seen that tourism development efficiency and environmental factors under the constraints of tourism development have achieved remarkable results, but tourism CO2 emissions as an important part of the tourism industry's environmental impact; into the tourism production process Related literature on the efficiency of tourism carbon emissions is relatively rare. In view of this, this paper takes 30 provinces in mainland China (the missing data in Tibet) as an example, and introduces the ultra-efficient DEA model to measure the carbon emission efficiency of tourism in China's provinces and cities from 2010 to 2017. Based on this, the Tobit model is used to test carbon emissions. And to analyze the influencing factors of tourism carbon emissions, in order to provide reference and enlightenment for the development of low carbon tourism in China.

**EMPIRICAL ANALYSIS**

**Method selection.** Data Envelopment Analysis (DEA) is a method for evaluating the relative effectiveness of decision units (DMUs) with multiple inputs and multiple outputs using DEA models based on known data to obtain corresponding production fronts. DEA was originally proposed by Charnes, Cooper and Rhodes (1978) and is the first DEA model - the CCR model. Post-Banker, Charnes and Cooper (1984) changed the assumption that the scale return is unchanged in the CCR model, and the assumption of the change in scale returns is the BCC model. Up to now, the most representative DEA models have CCR, BCC, FG and ST models. Among them, the FG model assumes that the scale returns are diminished, and the ST model assumes that the scale returns are increasing. In DEA, the relative efficiency of firms is distributed within the (0,1)
interval, and the efficiency of firms at the efficiency front is 1. DEA can calculate distribution efficiency and technical efficiency, which in turn can be decomposed into scale efficiency and pure technical inefficiency. Each model has both input-oriented and output-oriented forms. The model can be set to variable scale return (CRS) and variable scale return (VRS). The output-oriented DEA model is set to give a certain amount of input factors and obtain the largest output value. Conversely, an input-oriented DEA model refers to minimizing input costs at a given output level. The DEA model has the following advantages.

First, the DEA method can be used to evaluate the production (operating) performance of multi-input, multi-output decision-making units. The DEA method does not need to specify the production function form of input and output, so it is possible to evaluate the efficiency of decision-making units (DMUs) with more complex production relationships.

Second, it has the characteristic of unit invariant, that is, the result of DMU measured by DEA is not affected by the unit selected by input-output data. As long as the units for inputting and outputting data are unified, any unit that inputs and outputs data will not affect the efficiency results. It can process both proportional and non-proportional data, that is, both input and output data can use both proportional and non-proportional data, as long as the data is the main indicator that reflects the input or output side of the decision unit.

Third, the weight of the model in DEA is generated by mathematical planning based on data. It is not necessary to set the weight of input and output beforehand, so it is not affected by subjective factors. The method of setting weights beforehand, such as the expert evaluation method, is susceptible to subjective factors.

Fourth, DEA can perform comparative analysis, sensitivity analysis, and efficiency analysis of target and actual values. You can further understand the use of resources in the decision-making unit and can be used as a reference for managers’ business decisions.

The DEA model refers to the “unit” or “department” to be evaluated as the DMU, and each $U_{DMj}$ $(j = 1, 2, 3, \ldots, n)$ has r term inputs. $x_i = \{x_{ij1}, x_{ij2}, \ldots, x_{ijr}\}$ and s term output $y_i = \{y_{ij1}, y_{ij2}, \ldots, y_{ijr}\}$, where $x_{mj}$ represents the mth type of input of the jth $U_{DMj}$. The quantity, $y_{ij}$ denotes the input quantity of the jth $U_{DMj}$, $x_{mj} > 0$. $y_{ij} > 0$, $m = 1, 2, 3, \ldots, r$, $i = 1, 2, \ldots, s^+$. The construction model is as follows:

$$\text{min} \theta_i :$$

$$\begin{align*}
\sum_{j=1}^{r} \lambda_j x_{ij} + s^- &= \theta_i x_i, \quad i = 1, 2, \ldots, n, \\
\sum_{j=1}^{r} \lambda_j x_{ij} - s^- &= y_i, \quad i = 1, 2, \ldots, n, \\
\lambda_j &\geq 0, \quad j = 1, 2, \ldots, m, \\
s^+ &\geq 0, \quad s^- \geq 0.
\end{align*}$$

Where $\theta_i$ is the effective value of $U_{DMj}$ and the closer the effective value is to 1, the more effective the input of this DMU is. The validity judgment method is: if $\theta_i = 1$, $U_{DMj}$ is called valid or weak effective for DEA. When $S^+ = S^- = 0$, $U_{DMj}$ is called DEA is valid; if $\theta_i < 1$, then $U_{DMj}$ is invalid for weak DEA. The input data slack variable is $s^-$, and $S^-$ represents an input surplus, that is, an unused resource. If $S^- \neq 0$ indicates that the output is unchanged, the input can also reduce $s^-$; the output slack variable For $S^+$, $S^+$ means that there is insufficient output, and $S^+ \neq 0$ means that if the input is constant, the output can also increase $S^+$. Therefore, if a DMU is not valid, DEA can be effectively adjusted by not writing input and output indicators. Assuming a fixed output level, the input variable is adjusted to $X_i = \theta_i X_i - s^-$; if Assuming a fixed input level, the output variable is adjusted to $Y_i = Y_i + S^+$.

For the traditional DEA model, in the process of analyzing the Carbon emission efficiency, multiple DEAs may be effective. At this time, their comprehensive technical efficiency index is $\theta = 1$, which makes it impossible to further evaluate the effective of DEA. Therefore, the use of a super-efficiency model allows for a more in-depth production efficiency ranking of all DEA effective decision making units. When calculating the super-efficiency value of the DEA effective decision unit K, the principle is to exclude the DMUK from the model, and replace the input and output of the DMUK by the input-output linear combination of other decision units. The result of the solution is the decision unit K’s super-efficiency value, due to the backward movement of its production frontier, the measured effective unit efficiency value is often greater than the traditional model’s efficiency value 1, and the corresponding super-efficiency values of different DEA effective decision-making units are different. Make DEA effective decision-making unit has the characteristics of ecological efficiency comparability. Anderson and Peterson (1993) established an investment-oriented hyper-efficiency DEA model to compensate for this deficiency, and can make effective decision-making units with efficiency values greater than one. The super-efficient DEA (SE-DEA) model is as follows.

$$\begin{align*}
\min [\theta - \varepsilon(S_{i=1}^{m} s_i^- + \sum_{r=1}^{s^+} s_r^+)] \\
\sum_{j=1}^{r} \lambda_j x_{ij} - s^- &\leq \theta X_0 \\
\sum_{j=1}^{r} \lambda_j y_{ij} - s^+ &= Y_0 \\
\lambda_j &\geq 0, \quad j = 1, 2, \ldots, n; \quad s^+ \geq 0; \quad s^- \geq 0.
\end{align*}$$

Among them, $\lambda$ is the weight variable of DMU, $\theta$ is the parameter to be determined, slack variable $s^+, s^-$, X is the input quantity, and Y is the output quantity. The solution to the model is denoted by $\theta^*$. If $\theta^* < 1$, it indicates that there is a virtual decision unit whose output is not lower than the output of the
first $j_0$ decision unit, and the input ratio is the input of the $j_0$ decision units. Below, this shows that $j_0$ is non-DEA valid. If $\theta^* = 1$ and the slack variables are all 0, then the $j_0$ decision unit is DEA valid; and $\theta^* < 1$ but the slack variable is not 0, the $j_0$ decision unit is valid for weak DEA. This paper takes 30 provinces in mainland China (the missing data in Tibet) as an example, and introduces the ultra-efficient DEA model to measure the carbon emission efficiency of tourism in China’s provinces and cities from 2005 to 2017.

**Variable selection.** Tourism carbon emission efficiency comprehensively reflects the coordination between tourism economic growth, tourism energy consumption and CO2 emissions. Therefore, the above three aspects should also be reflected in the selection of input and output indicators. In the selection of input indicators, capital, labor and land are the most basic production factors in the economic sense. The capital and labor factors in the tourism production process are measured by the amount of fixed assets investment in tourism and the number of employed persons in the tourism industry. Land is an important place for carrying out tourism activities and material carriers, but capital investment in tourism production is developed through tourism projects, infrastructure construction, tourism environment creation, etc. can indirectly measure the level of tourism land input, so this article does not include the land elements of tourism production. In addition, the energy consumption in the tourism production process is also an important input indicator for measuring the carbon emission efficiency of tourism, but the Energy Statistics Yearbook does not make detailed statistics on the energy consumption of the tourism industry or related services. The tourism industry mainly involves the tertiary industry sectors such as accommodation and catering, transportation, warehousing and postal services, wholesale and retail trade and other services. Only part of the energy consumption of these sectors is the energy consumption of the tourism industry. It is stripped out in proportion. Using tourism consumption stripping coefficient to calculate the energy consumption of tourism, the formula is:

$$m_i = M_i \times u_i; \quad M_i = s_i \times \beta_i; \quad u_i = \frac{q_i}{v_i}; \quad q_i$$

$$= p_i \times U_i; \quad p_i = \frac{v_i}{w_i}$$

Where $m_i$ is the energy consumption of the tourism industry in the $i$ industry; $M_i$ is the energy consumption of the $i$ industry; $u_i$ is the coefficient of tourism consumption divestiture; $s_i$ is the various consumption of various industries in the China Energy Statistics Yearbook regional energy balance sheet Energy quantity; $\beta_i$ is the standard coal reference coefficient of various energy sources; $q_i$ is the value added of $i$ industry tourism; $v_i$ is the added value of industry $i$; $p_i$ is the value added rate of industry $i$; $U_i$ is the income of $i$ industry in tourism; For the industry’s total output value.

Output indicators include expected output and non-expected output. Expected output usually reflects the economic results of tourism industry and can be represented by total tourism income indicators. Non-expected output indicators are expressed by tourism CO2 emissions. The CO2 emissions of tourism have been measured from different scales and perspectives, but there is no uniform measurement method.

According to the easy accessibility and operability of the data, this paper measure the tourism energy consumption converted into standard coal in each relevant industry, and then multiply it by the standard coal CO2 emission. And then get the tourism CO2 Emissions.

The above input-output variable data is derived from the corresponding year’s China Tourism Statistical Yearbook and its copy, the Tourism Sample Survey Data, the China Transportation Yearbook and the annual statistical reports of tourism in various provinces and regions have been compiled and calculated.

**Calculation result.** This paper takes the tourism carbon emission efficiency of 30 provinces, autonomous regions and municipalities directly under the Central Government (because the data of Tibet is incomplete, so it does not enter the analysis) as the analysis object, and input the panel data of 2010-2017 into EMS1.3 software for super-efficiency DEA analysis. The measurement results of China’s tourism carbon emission efficiency are shown in Table 1 below.

It can be seen from the above table that according to the average value of tourism carbon emission efficiency in 2010-2017, China’s provinces are divided into three levels: high, medium and low: 1 high-efficiency provinces (efficiency average is above [0.6]): Beijing, Tianjin, Liaoning, Shanghai, Jiangsu, Zhejiang, Shandong, Henan, Guangdong, and Chongqing; 2 medium-efficiency provinces (efficiency average [0.3, 0.6]): Hebei, Shanxi, Inner Mongolia, Jilin, Heilongjiang, Anhui, Fujian, Jiangxi, Hubei, Hunan, Guangxi, Hainan, Sichuan, Guizhou, Yunnan, Shaanxi; 3 low-efficiency provinces (efficiency average [0.0, 0.3]): Gansu, Qinghai, Ningxia, Xinjiang; It can be seen that there are obvious inter-provincial differences in the mean carbon emission efficiency of tourism in China’s provinces. The provinces with higher carbon emission efficiency in tourism are mainly distributed in the eastern part of China where the economy is more developed, and the provinces with lower carbon emission efficiency are concentrated in the western region. It can be seen that although the carbon emission efficiency level of tourism in most provinces and regions has improved, most of the provinces and regions are still in an inefficient state. It is still a long way to go to further reduce CO2 emissions from tourism and improve the efficiency of carbon
emissions in tourism. In order to improve the carbon emission efficiency of China's tourism industry, we first need to know what are the main influencing factors affecting carbon emission efficiency. This paper uses Tobit model to further study its main influencing factors, and hopes to provide reference for the low carbon development of China's tourism industry.

AN EMPIRICAL ANALYSIS OF THE IMPACT OF CHINA'S TOURISM CARBON EMISSION EFFICIENCY

The above measures the carbon emission efficiency of 30 provinces in China. We all know that the improvement of tourism carbon emission efficiency is not a simple task, and the factors affecting China's tourism carbon emission efficiency may be relatively complicated. The factors of tourism carbon emission efficiency are analyzed by Tobit regression model to analyze the influencing factors of China's tourism carbon emission efficiency.

Tobit model. The Tobit regression model is a generalization of the Probit model, and the founder of the Tobit model is Tobin. In 1958, Tobin studied related issues such as the upper or lower limit or the extreme value of the explanatory variables in the model, and the research of such problems attracted the attention of many scholars. Since then, in order to commemorate Tobin's outstanding contribution to the study of this type of model problem, the model that limits the value of such dependent variables is called the Tobit model. This model is actually divided into two different equations, one is a discrete data model that can reflect the selection problem, and the other is a continuous variable model with interpreted explanatory variables. And the second model is often more concerned by people.

Since China's tourism carbon emission efficiency deserves a minimum value of 0, this indicates that the data is truncated. If we use the traditional current regression method to perform regression analysis on the model, we may get a negative value for the fitted value, so this chapter uses explanatory variables. The Tobit model with limited values analyzes the influencing factors of China's ecology. In
order to avoid the situation where the parameter estimation is biased but inconsistent, the subject uses the Tobit model for regression analysis. The model is constructed as follows:

\[ y_{it} = \begin{cases} a_{it} + \beta^t x_{it} + \epsilon_{it} & y_{it} \geq 0 \\ 0 & y_{it} < 0 \end{cases} \]

Among them, the interpreted variable \( y_{it} \) is the ecological efficiency of the \( t \)-th year of the \( i \)-th region. \( X_{it} \) is the explanatory variable, \( \beta^t \) is the unknown parameter, \( \epsilon_{it} \sim N(0, \sigma^2) \) This model is the intercepted regression model of the panel data, the explanatory variable \( x_{it} \) takes the actual observation value, and the interpreted variable \( y_{it} \) is in a restricted manner. Value: When \( y_{it} \geq 0 \) takes the actual observation value; when \( y_{it} < 0 \), the observation is truncated to 0. \( a_{it} \) is the fixed effect of the \( t \)-year of region \( i \), which is an unknown constant.

**Variable Influence Factors Selection in Tobit Model.** Starting from the dimensions of social and economic development, the level of social and economic development determines the level of service industry. The speed, depth, and plays an important role in the service industry. Therefore, the level of service industry development is quite different. The level of economic efficiency, and then obtain an indicator that can relatively comprehensively reflect the level of foreign economic opening.

(3) **Level of opening up (OP).** The level of openness reflects the degree of exchange of people; finances, materials and information between provinces and cities, and this exchange mechanism may have a “spill effect”, which will have a certain impact on the scale and structure of the tourism industry, which may affect Tourism carbon emission efficiency. This paper chooses to use principal component analysis to weight the two indicators of foreign investment dependence and foreign trade dependence, and then obtain an indicator that can relatively comprehensively reflect the level of foreign economic opening.

(4) **Tourism resource endowment (TE).** The tourism resource endowment of a region determines the abundance of tourism resources in the region, which shows to some extent the attractiveness of tourism resources in the region, which may have an important impact on the scale of tourism economy and its carbon emissions. This paper selects the total number of national key cultural relics protection units and national-level scenic spots from the two dimensions of human landscape and natural landscape to indicate the endowment of tourism resources.

(5) **Location conditions (DC).** Accessibility is a basic condition for the development of the tourism industry. It is also an important part of the tourism carbon emissions. Good location conditions will not only enhance the local tourism attractiveness, but also change the local tourism consumption structure, which will affect the tourism industry carbon. Emission efficiency. This article selects the tourism revenue of each province / the province’s GDP and national tourism income / National GDP.

(6) **Urbanization level (UN).** Urban tourism is an indispensable part of modern tourism and an important place for tourism to cause carbon emissions. Urban tourism includes six elements of “food, shelter, travel, travel, purchase, entertainment” and is closer to the tourist attractions away from the city. During the urban tourism process, tourists stay longer and have more tourism activities (including tourism). High energy consumption and high emission activities such as transportation, tourism, catering and tourism accommodation have a greater impact on tourism carbon emissions. This paper chooses to measure the urbanization level by the proportion of urban population in the provinces to the total population.

The above data comes from the China Statistical Yearbook, China Tourism Statistics Yearbook, China Economic and Social Development Statistics Database, and provincial tourism statistics bulletin.

**Tobit model regression analysis.** Based on the existing literature, this paper considers the availability of data, the level of social and economic...
development, the level of service industry development (SD), the level of openness (OP), tourism resource endowment (TE), location conditions (DC) and the level of urbanization (UN) as an explanatory variable, the tourism carbon emission efficiency (recorded as CEE) as the explanatory variable, the sampling interval is 2010-2017, the following regression equation is established. The regression results are shown in Table 2 below:

\[
CEE = \alpha + \beta_1 \text{DE} + \beta_2 \text{SD} + \beta_3 \text{OP} + \beta_4 \text{TE} + \beta_5 \text{DC} + \beta_6 \text{UN} + \mu
\]

**TABLE 2**

<table>
<thead>
<tr>
<th>coefficient</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>DE</td>
<td>-1.336***</td>
</tr>
<tr>
<td>SD</td>
<td>-0.189***</td>
</tr>
<tr>
<td>OP</td>
<td>-0.026**</td>
</tr>
<tr>
<td>TE</td>
<td>0.2255</td>
</tr>
<tr>
<td>DC</td>
<td>-0.836***</td>
</tr>
<tr>
<td>NN</td>
<td>-0.018***</td>
</tr>
</tbody>
</table>

Note: * *, ** *, *** indicate significant at the 10%, 5%, and 1% levels, respectively.

As can be seen from the above table, the level of social and economic development (DE) is significantly negative at the 1% level, indicating that the development and progress of the social economy has led to a rise in the carbon intensity of tourism, which has led to a decline in carbon emission efficiency. With the development of social economy and the improvement of residents' income level, the structure of tourism services and product consumption in various regions has also changed. Various “high energy consumption and high emission” tourism services and products have emerged, compared with previous tourism. The development model will have higher energy intensity and emission intensity and lower carbon emission efficiency.

The level of service industry development (SD) has a negative impact on tourism carbon emissions efficiency and is significant at the 1% level. That is to say, the prosperity and development of the service industry can not only enhance the attractiveness of local tourism resources, but also enrich the local tourism service and product mix, and boost the consumption intensity of “high energy consumption and high emission” products and services on the supply side. In turn, the carbon intensity of tourism is boosted, thereby reducing carbon emission efficiency.

The level of openness (OP) has a significant negative impact on the carbon intensity of tourism, and the level of openness will also affect the carbon emission efficiency of tourism. In the case that foreign investment has not been completely “loose”, the scope of external capital inflows in the tourism industry is limited, and more tends to be “high energy consumption and high emission” service industries such as hotels, shopping malls and entertainment. The larger the scale, the higher the grade. High, the greater the energy consumption, each unit is a high source of urban emissions, which will undoubtedly boost the carbon intensity of tourism and reduce the efficiency of carbon emissions.

The estimated coefficient of tourism resource endowment (TE) is positive, but not significant, indicating that tourism resource endowment does not have a significant impact on tourism carbon emission efficiency. The tourism resource endowment can have an important impact on the scale of the local tourism economy, and the expansion of the tourism economy will increase the total carbon emissions of the tourism industry, but the total output of the tourism industry will also increase, so this does not mean tourism. The efficiency of carbon emissions will change accordingly.

Location conditions (DC) have a significant negative impact on tourism carbon intensity. Good geographical location and accessibility contribute to the development of tourism economy, but also boost the carbon intensity of tourism. Compared with other consumer categories, tourism transportation has the highest energy consumption and carbon emission intensity. Therefore, the advantages and disadvantages of location conditions will not only affect the attractiveness of tourism resources, but also stimulate the scale of tourists, which will also promote the local tourism service and product consumption structure. For higher emissions, reduce carbon emissions efficiency.

The impact of the level of urbanization (UN) on the carbon intensity of tourism is significantly negative, which is in line with the above expectations. The urbanization process will boost the carbon intensity of tourism and reduce the efficiency of carbon emissions.

**CONCLUSION**

This paper uses the ultra-efficient DEA to measure the tourism carbon emission efficiency of 30 provinces in China. It is concluded that the carbon emission efficiency of Beijing, Tianjin, Liaoning, Shanghai, Jiangsu, Zhejiang, Shandong, Henan, Guangdong and Chongqing is above 0.6. The tourism carbon emission efficiency of Hebei, Shanxi, Inner Mongolia, Jilin, Heilongjiang, Anhui, Fujian, Jiangxi, Hubei, Hunan, Guangxi, Hainan, Sichuan, Guizhou, Yunnan and Shaanxi is between 0.3 and 0.6, which is a medium efficiency range; The provinces with efficiency below 0.3 are in Gansu, Qinghai, Ningxia and Xinjiang. At the same time, Tibet model is used to verify the influencing factors affecting the carbon emission efficiency of China’s tourism industry. It is concluded that the service industry development level, urbanization level, location conditions, opening level and socio-economic development
level are negatively correlated with China’s tourism carbon emission efficiency. There is no significant relationship between tourism resource endowment and carbon emission efficiency.

**Suggestion. Improve energy efficiency.** To optimize the energy consumption structure of tourism, in addition to focusing on the development of clean energy, we should further improve the efficiency of the use of major energy sources. Energy efficiency is a comprehensive indicator reflecting the level of energy consumption and utilization. Low utilization rate will result in great waste of energy and further exacerbate the situation of energy shortage. The energy consumption per unit of traffic volume in the province, the energy consumption per unit of hotel rooms, and the energy consumption per catering enterprise all contribute to the increase of carbon footprint. It can be seen that the current tourism energy consumption is too large and the utilization efficiency is low. This paper believes that there are two main ways to improve energy efficiency: one is to upgrade the operation technology, for example, to transform the power generation equipment and transmission technology of the power plant to improve the power generation efficiency, and to carry out the cycle transformation of the water supply and drainage system inside the hotel to improve water conservation. Efficiency, renovate the door, window, wall, roof, building orientation of the guest room to improve heating efficiency, reduce building energy consumption, etc.; one is the repeated use of waste, such as the aforementioned methanol fuel, which can use inferior coal, coke oven exhaust and natural gas production, to maximize the utilization of primary energy, while minimizing the consumption of non-renewable resources.

**Improve the infrastructure and low carbonization construction.** China's tourism industry started late, infrastructure construction could not keep up with the reality, and it had a negative impact on tourism energy consumption and environmental changes. With the development of the economic level and the improvement of national understanding, the current requirements for the construction of tourism infrastructure have also put forward higher requirements. While ensuring the convenience and comfort of the tourism process, we must minimize the pollution to the environment. Therefore, effective actions must be taken to reduce the building energy consumption, water and electricity consumption, and other aspects of infrastructure and low carbonization.

**Give play to the leading role of tourism enterprises and implement a green and low-carbon business model.** Tourism enterprises are the main implementers and operators of low-carbon tourism. Tourism enterprises should first change from the concept, from the traditional extensive tourism development model to the innovative low-carbon tourism development model, and between tourism enterprises. Low carbon cooperation. The government can introduce the business philosophy and development model of low-carbon tourism into the entire tourism industry, and guide and encourage tourism enterprises to implement a green and low-carbon business model. The development of low-carbon tourism is bound to play a leading role in the development of low-carbon tourism. The development of low-carbon tourism is under the leadership of tourism enterprises. On the one hand, it expands the scale of tourism development, on the other hand, it vigorously promotes and introduces low-carbon technologies in order to achieve the goal of low-carbon development of tourism.

**Scientific management of tourism waste.** The impact of tourism waste on the environment is increasing, and the per capita waste output of tourists is significantly increased, mainly because the number of tourists has increased year by year and the average length of stay of tourists has also increased. Since the generation of tourism waste is inevitable and can only be alleviated, the reduction of tourism waste should be started from two aspects: 1 reduce the amount of tourism waste generated. Promote environmental protection through media and logos, and encourage tourists and tour operators to reduce the use of disposables, save water, reduce the amount of discarded solid waste and waste water; thus reduce the generation of tourism waste from the source classification of tourism waste. The waste generated by tourists and tour operators is uniformly recycled, classified and treated, and the recyclable waste is separated and reused [14]. The waste can not be used for harmless treatment, which not only reduces the cost of tourism waste treatment, but also reduces tourism waste disposal carbon emissions.

**Optimize the internal structure of tourism carbon emissions while promoting the use of low carbon tools.** Tourism transportation carbon emissions are the most important part of tourism carbon emissions. Therefore, the use of low-carbon travel methods to gradually replace high-carbon emissions travel modes is one of the ways to effectively reduce tourism carbon emissions, such as promoting green tourism routes, advocating Train travel instead of flying [15]. At the same time, the reform of scenic spot transportation, using green new energy vehicles to replace cars for sightseeing, adhere to the development path of low carbon tourism. Not only that, but also can reduce tourism carbon emissions from other tourism departments, for hotels, promoting low-carbon hotels, on the one hand to save the cost of wine cellar suppliers, while not reducing the tourist experience.
REFERENCES


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BACTERIAL PROFILES OF THE MUD FORMATIONS OBSERVED FROM A REMOTELY OPERATED VEHICLE (ROV) IN THE DEEP OF THE CANAKKALE STRAIT (DARDANELLES), TURKEY

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ABSTRACT

The Çanakkale Strait, as a part of the Turkish Strait System (TTS), is an important water route in the world which connects the Mediterranean Sea to the Sea of Marmara and hence to the Black Sea via the Istanbul Strait. Due to its peculiar hydrodynamicic characteristics, the area offers unique opportunities for researching profiles of bacteria under different, poorly described conditions. The samples of the mud formations, observed by "Remotely-Operated Vehicles" (ROV) with a diameter of roughly 120m and a height of 1.5-2m at a depth of 24m on the seabed of the Çanakkale Strait, were investigated regarding bacterial composition, metabolic characteristics of heterotrophic bacteria and environmental variables. Gram-negative fermenting and non-fermenting bacteria were the most common group in terms of species numbers, compared to Gram-positive cocci and non-spore-forming and spore-forming bacilli, both also found in the sample of mud formations and surface sediment around them. In the study, four species, *Micrococcus lylae*, *Lysinibacillus fusiformis*, *Bordetella trematum* and *Roseomonas gilardii* were recorded for the first time in the Turkish Seas. The results of the study contribute to an increasing knowledge on bacterial diversity and bacterial interactions regarding metabolically-talented bacteria and the environmental conditions of different texture on the floor of the seabed.

KEYWORDS:
Dardanelles, bacterial community, heterotrophic bacteria, mud hills, sediment.

INTRODUCTION

The discovery of the deep biosphere has shown that a major part of the microbial biosphere might be present in surface sediments [1-3]. Bacteria have a critical role in the decomposition of organic matter and recycling of nutrients in marine environments. Major biogeochemical processes in marine environments are related to the activities of heterotrophic microbes [4-6]. Since microbial communities play an important role in biogeochemical cycles, knowledge of bacterial diversity and the community structure of surface sediments is crucial for understanding marine ecosystem functioning.

When community profiles of bacteria have been linked to variable environmental factors, it is clear that influences of various and variable environmental and hydrographic conditions, shape peculiar sediment profiles of each micro-geographical marine area. In view of this, marine areas, which have unique hydrographical peculiarities, such as the Çanakkale Strait in Turkey, offer interesting opportunities for bacteriological studies.

In addition to common methods in detecting heterotrophic bacterial diversity, are culture-independent studies, where many studies report that cultured strains of marine bacteria can represent significant fractions of the bacterial biomass. Based on DNA-DNA hybridization of the genomic DNAs of isolates obtained by the traditional medium, against community DNA, it has been suggested that readily-cultivable bacteria are abundant in the marine water column [7-10]. Bacteria that are active in situ can be identified using molecular biological methods. However, these methods cannot reveal the whole spectrum of physiological capabilities that are essential to understanding the ecology of a single bacterial species. Therefore, for investigation of microbial adaptations to environmental conditions, pure cultures remain crucial [11].

In this study, mud formations, observed from a remotely-operated vehicle (ROV) and the surface sediment around them, were sampled from the bottom of the Çanakkale Strait in Turkey. The community profiles of mud-associated bacteria and the surface sediments around mud hills, regarding variable environmental parameters were investigated for the first time, with an aim to understanding the variances of bacterial community profiles and enzymatic reactions between mud formation and...
the surface sediment of nearby mud hills. The metabolic reactions of mud-associated bacteria were compared with the bacteria isolated from the surface sediments of nearby mud knolls. These samples, investigated for the first time, were to describe bacterial community profiles, metabolic peculiarities of mud-associated bacteria and potentials of the isolates for possible use in the industrial application.

**MATERIALS AND METHODS**

**Study area and sampling.** The terms of "mud hill/mud knoll" were used to describe the mud formations with a diameter of roughly 120 m and a height of 1.5-2 m at a depth of 24 meters on the seafloor of the Çanakkale Strait, Turkey. The mud formations were observed with Remotely Operated Vehicles (ROV) at 22 meters depth of the Çanakkale Strait, Turkey in spring 2014. The samples were collected both from the mud knolls and the normal surface sediments nearby the mud formations. The sampling area was shown in Figure 1.

View of the mud hills on the seafloor of the Çanakkale Strait, Turkey April 2014 was shown in Figure 2.

The sampling coordinates: **EC7 East: 496089.00 North 4487098.00, EN2 East: 495631.00 North 4488047.00, EC6 East: 495998.00 North 495998.00, ES2 East: 495496.00 North 4487982.00**
Bacteriological analyses. The samples were collected, serial dilutions of $10^{-5}$ were prepared in 9-mL amounts of sterile seawater (artificial seawater, Sigma) and inoculated (0.2 mL) in duplicate on Marine Agar (Difco), and the plates were incubated for 5 days at $22 \pm 0.1 \, ^\circ\text{C}$ (15). At the end of the incubation, colonies were counted and picked colonies were restreaked several times to obtain pure cultures.

Metabolic profiling of the isolates. The VITEK 2 Compact 30 (bioMérieux, France) automated micro identification system was used for detecting biochemical responses of the bacterial isolates against various substrates. The pure isolates were Gram-stained and then identified using GN (Gram-negative fermenting and nonfermenting bacilli), GP (Gram-positive cocci and nonspore-forming bacilli), and BCL (Gram-positive spore-forming bacilli) cards in the automated micro identification system VITEK 2 Compact 30 (bioMerieux, France). The identification cards are based on biochemical tests (46 tests for BCL, 43 tests for GP, 47 tests for GN) measuring carbon source utilization, enzymatic activities, inhibition, and resistance. Calculations are performed on raw data and compared to thresholds to determine reactions for each test. On the VITEK 2 Compact, test reaction results appear as “(−)” or “(+)”. Reactions that appear in parentheses were evaluated as an indicator of weak reactions that are too close to the test threshold [12].

Hydrographic Parameters. Temperature, salinity and density values were measured in situ using the CTD (RBR Concerto) at the sampling areas.

FlowQuest (LinkQuest) acoustic current profiler was used to measure current speed (mm/sec, operation frequency 1000 kHz) of the sampling location. Directions of currents were measured using fixed RDCP (Recording Doppler Current Profiler 600).
RESULTS

The recorded values of variable environmental parameters; temperature, salinity and density of the sampling areas were summarized in Figure 3. The recorded Flow Quest acoustic current profilers of the sampling location were shown in Figure 4. The recorded directions of currents in the sampling location were shown in Figure 5.

Heterotrophic aerobic bacteria count (HPC) /total colony forming unit (cfu/g) were shown in Table 1.

Heterotrophic plate count was found higher in the samples of the mud hills than the surface sediment around them.

Cultivable aerobic heterotrophic bacteria species isolated from the mud formations and the surface sediments in the deep of the Çanakkale Strait, Turkey were shown on the Table 2.

Total numbers of the identified isolates were found higher in the samples of the mud knolls than the surface sediment around them.

Gram-negative fermenting and non-fermenting bacteria were the most common group in terms of species number in comparison to Gram-positive cocci and non-spore-forming and spore forming bacilli in the samples both mud formations and the surface sediment around them.

The presence of four bacteria species; Micrococcus lylae, Lysinibacillus fusiformis, Bordetella trematum and Roseomonas gilardii, belonging to four different families from the mud knolls and surface sediments were reported for the first time in the Turkish Seas.

All of the Gram-negative isolates displayed positive reactions at various rates against tested substrates. However, the frequency of the positive reactions of the strains isolated from the mud samples was higher than the strains isolated from the surface sediments.

More than 50% of Gram-negative isolates that exhibit a positive reaction against substrates tested was summarized in Figure 6. Biochemical characteristics of the strains identified by using GN card in VITEK 2 Compact 30 were shown in Table 3. Biochemical characteristics of the strains identified by using BCL card in VITEK 2 Compact 30 were shown in Table 4. Biochemical characteristics of the strains identified by using GP card in VITEK 2 Compact 30 were shown in Table 5.

<table>
<thead>
<tr>
<th>Sampling site</th>
<th>Heterotrophic plate count (cfu/g)</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mud knoll 1</td>
<td>31x10^{11}</td>
<td>29 x10^{11}</td>
</tr>
<tr>
<td>Mud knoll 2</td>
<td>27x10^{11}</td>
<td></td>
</tr>
<tr>
<td>Surface sediment1</td>
<td>18x10^{10}</td>
<td></td>
</tr>
<tr>
<td>Surface sediment 2</td>
<td>15x10^{10}</td>
<td>16x10^{10}</td>
</tr>
</tbody>
</table>
TABLE 2
Bacteria species isolated from the mud formations and surface sediments on seafloor of the Çanakkale Strait, Turkey

<table>
<thead>
<tr>
<th>Phylum/Class</th>
<th>Family</th>
<th>Species</th>
<th>Mud</th>
<th>Sedim</th>
<th>The areas that species isolated previously isolated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Actinobacteria/Actinomycetales</td>
<td>Actinobacteria/Actinomycetales</td>
<td>Micrococcus luteus Lehmann and Neumann, 1896</td>
<td>+</td>
<td>+</td>
<td>Aegean Sea [21], Istanbul Strait [27], Gülük Bay, Aegean Sea [28], Coastal areas of the Lebanon and Syria [29]</td>
</tr>
<tr>
<td>Bacillaceae</td>
<td>M. lylae</td>
<td>+</td>
<td>+</td>
<td>Ballast waters [22], Gülük Bay, Aegean Sea [28]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Kocuria kristinae</td>
<td>+</td>
<td>-</td>
<td>Istanbul Strait [27], Gülük Bay, Aegean Sea [28]</td>
<td></td>
</tr>
<tr>
<td>Firmicutes/Bacilli</td>
<td>Bacillaceae</td>
<td>Bacillus cereus Frankland and Frankland 1887</td>
<td>+</td>
<td>+</td>
<td>Istanbul Strait [27], Gülük Bay, Aegean Sea [28], Gökköda Island, Aegean Sea [30]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>This study</td>
</tr>
<tr>
<td></td>
<td>Bacillus mycoides Flügge 1886</td>
<td>+</td>
<td>-</td>
<td>Istanbul Strait [27], Gökköda Island, Aegean Sea [30]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bacillus pumilus Meyer and Gottheil 1901</td>
<td>+</td>
<td>+</td>
<td>Istanbul Strait [27], Gülük Bay, Aegean Sea [28], Gökköda Island, Aegean Sea [30]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>B. thuringiensis Berliner 1915</td>
<td>+</td>
<td>-</td>
<td>Istanbul Strait [27], Gökköda Island, Aegean Sea [30]</td>
<td></td>
</tr>
<tr>
<td>Proteobacteria/Alcaligenaceae</td>
<td>Proteobacteria/Alcaligenaceae</td>
<td>Sphingomonas paucimobilis (Holmes et al. 1977), Yabuuchi et al. 1990</td>
<td>+</td>
<td>+</td>
<td>Ballast waters [22], Istanbul Strait [27], Gülük Bay, Aegean Sea [28], Aegean Sea [30]</td>
</tr>
<tr>
<td></td>
<td>Ochrobactrum anthropi Holmes et al., 1988</td>
<td>+</td>
<td>+</td>
<td>Ballast waters [22]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Achromobacter denitrificans Roger and Tan (1983) Coenye et al., 2003</td>
<td>+</td>
<td>+</td>
<td>Ballast waters [22]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A. xylosidans Roger and Tan (1983) Coenye et al., 2003</td>
<td>+</td>
<td>+</td>
<td>Ballast waters [22]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bordetella trematum</td>
<td>+</td>
<td>-</td>
<td>This study</td>
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<tr>
<td></td>
<td>Capriavidus pauculus</td>
<td>+</td>
<td>+</td>
<td>Gülük Bay [28], Coastal areas of the Lebanon and Syria [29]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Burkholderia mallei</td>
<td>-</td>
<td>+</td>
<td>Gülük Bay [28], Aegean Sea [30]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Chromobacterium violaceum Bergonzini 1880</td>
<td>+</td>
<td>-</td>
<td>Gülük Bay [28], Coastal areas of the Lebanon and Syria [29]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Delftia acidovorans (den Dooen de Jong 1926) Wen et al. 1999</td>
<td>+</td>
<td>+</td>
<td>Gülük Bay [28], Coastal areas of the Lebanon and Syria [29]</td>
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<tr>
<td></td>
<td>Stenotrophomonas maltophilia Palleroni and Bradbury 1993</td>
<td>+</td>
<td>+</td>
<td>The Sea of Marmara [21], ballast waters [22], Istanbul Strait [27]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Klebsiella oxytoca</td>
<td>+</td>
<td>+</td>
<td>This study</td>
<td></td>
</tr>
<tr>
<td></td>
<td>K. pneumoniae ssp pneumoniae</td>
<td>-</td>
<td>+</td>
<td>The Sea of Marmara [21], ballast waters [22], Istanbul Strait [27]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Enterobacter cloacae</td>
<td>+</td>
<td>+</td>
<td>The Sea of Marmara [21], ballast waters [22], Istanbul Strait [27]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>E. coli</td>
<td>+</td>
<td>+</td>
<td>The Sea of Marmara [21], ballast waters [22], Istanbul Strait [27]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Roseomonas gilardii</td>
<td>+</td>
<td>-</td>
<td>The Sea of Marmara [21], ballast waters [22], Istanbul Strait [27]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pseudomonas luteola Kodama et al., 1985 Holmes et al., 1987</td>
<td>+</td>
<td>+</td>
<td>The Sea of Marmara [21], ballast waters [22], Istanbul Strait [27]</td>
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<td></td>
<td>Xanthomonadaceae</td>
<td>Stenotrophomonas maltophilia Palleroni and Bradbury 1993</td>
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<td>+</td>
<td>The Sea of Marmara [21], ballast waters [22], Istanbul Strait [27]</td>
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<tr>
<td></td>
<td>Enterobacteriaceae</td>
<td>Klebsiella oxytoca</td>
<td>+</td>
<td>+</td>
<td>The Sea of Marmara [21], ballast waters [22], Istanbul Strait [27]</td>
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<tr>
<td></td>
<td></td>
<td>K. pneumoniae ssp pneumoniae</td>
<td>-</td>
<td>+</td>
<td>The Sea of Marmara [21], ballast waters [22], Istanbul Strait [27]</td>
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<td></td>
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<td>Enterobacter cloacae</td>
<td>+</td>
<td>+</td>
<td>The Sea of Marmara [21], ballast waters [22], Istanbul Strait [27]</td>
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<tr>
<td></td>
<td></td>
<td>E. coli</td>
<td>+</td>
<td>+</td>
<td>The Sea of Marmara [21], ballast waters [22], Istanbul Strait [27]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>This study</td>
</tr>
</tbody>
</table>

First records counts: 4
Total isolates: 145
Species: 22
The positive reaction percentage of the Gram-negative isolates against tested substrates.

<table>
<thead>
<tr>
<th>Test Substrates</th>
<th>Representation</th>
<th>Positive Reaction %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mud</td>
</tr>
<tr>
<td>ILATk</td>
<td>L-LACTATE alkalinisation</td>
<td>88.9</td>
</tr>
<tr>
<td>SUCT</td>
<td>SUCCINATE alkalinisation</td>
<td>87.3</td>
</tr>
<tr>
<td>ProA</td>
<td>L-Proline ARYLAMIDASE</td>
<td>63.5</td>
</tr>
<tr>
<td>TyrA</td>
<td>Tyrosine ARYLAMIDASE</td>
<td>63.5</td>
</tr>
<tr>
<td>ELLM</td>
<td>ELLMAN</td>
<td>61.9</td>
</tr>
<tr>
<td>PyrA</td>
<td>Pyrrolidonyl-ARYLAMIDASE</td>
<td>58.7</td>
</tr>
<tr>
<td>GGT</td>
<td>GAMMA-GLUTAMYL-TRANSFERASE</td>
<td>58.7</td>
</tr>
<tr>
<td>PHOS</td>
<td>PHOSPHATASE</td>
<td>57.1</td>
</tr>
<tr>
<td>CIT</td>
<td>CITRATE (SODIUM)</td>
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</tr>
<tr>
<td>URE</td>
<td>UREASE</td>
<td>52.4</td>
</tr>
<tr>
<td>CMT</td>
<td>COUMARATE</td>
<td>47.6</td>
</tr>
<tr>
<td>APPA</td>
<td>Ala-Phe-Pro ARYLAMIDASE</td>
<td>46.0</td>
</tr>
<tr>
<td>BGLU</td>
<td>BETA-GLUCOSIDASE</td>
<td>46.0</td>
</tr>
<tr>
<td>LIP</td>
<td>LIPASE</td>
<td>36.5</td>
</tr>
<tr>
<td>GGAA</td>
<td>Glu-Gyl-Arg-ARYLAMIDASE</td>
<td>34.9</td>
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<tr>
<td>AGLTtp</td>
<td>Glutamyl Arylamidase pNA</td>
<td>31.7</td>
</tr>
<tr>
<td>dGLU</td>
<td>D-GLUCOSE</td>
<td>31.7</td>
</tr>
<tr>
<td>AGLU</td>
<td>ALPHA-GLUCOSIDASE</td>
<td>28.6</td>
</tr>
<tr>
<td>dMAL</td>
<td>D-MALTOSE</td>
<td>27.0</td>
</tr>
<tr>
<td>O129R</td>
<td>O/129 RESISTANCE</td>
<td>27.0</td>
</tr>
<tr>
<td>BNAg</td>
<td>BETA-ACETYL-GLUCOSAMINIDASE</td>
<td>23.8</td>
</tr>
<tr>
<td>IMLa</td>
<td>L-MALATE assimilation</td>
<td>15.9</td>
</tr>
<tr>
<td>dTRE</td>
<td>D-TRHALOSE</td>
<td>14.3</td>
</tr>
<tr>
<td>SAC</td>
<td>SACCHAROSE/SUCROSE</td>
<td>12.7</td>
</tr>
<tr>
<td>MNT</td>
<td>MALONATE</td>
<td>12.7</td>
</tr>
<tr>
<td>Dcel</td>
<td>D-CELLOBIOSE</td>
<td>7.9</td>
</tr>
<tr>
<td>dMNe</td>
<td>D-MANNNOSE</td>
<td>7.9</td>
</tr>
<tr>
<td>dMAN</td>
<td>D-MANNITOL</td>
<td>6.3</td>
</tr>
<tr>
<td>BXYL</td>
<td>BETA-XYLOSIDASE</td>
<td>6.3</td>
</tr>
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<td>BGAL</td>
<td>BETA-GALACTOSIDASE</td>
<td>4.8</td>
</tr>
<tr>
<td>PLE</td>
<td>PALATINASE</td>
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</tr>
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<td>HisSa</td>
<td>L-HISTIDINE assimilation</td>
<td>4.8</td>
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<tr>
<td>GlyA</td>
<td>Glycerine ARYLAMIDASE</td>
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</tr>
<tr>
<td>BAlap</td>
<td>ETA-Alanine arylamidase pNA</td>
<td>1.6</td>
</tr>
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<td>dTAG</td>
<td>D-TAGATOSE</td>
<td>1.6</td>
</tr>
<tr>
<td>AGAL</td>
<td>ALPHA-GALACTOSIDASE</td>
<td>1.6</td>
</tr>
<tr>
<td>ILAta</td>
<td>L-LACTATE assimilation</td>
<td>1.6</td>
</tr>
<tr>
<td>ADO</td>
<td>ADONITOL</td>
<td>0</td>
</tr>
<tr>
<td>LARL</td>
<td>L-ARABITOL</td>
<td>0</td>
</tr>
<tr>
<td>H2S</td>
<td>H2S PRODUCTION</td>
<td>0</td>
</tr>
<tr>
<td>OFF</td>
<td>FERMENTATION/GLUCOSE</td>
<td>0</td>
</tr>
<tr>
<td>dSOR</td>
<td>D-SORBITOL</td>
<td>0</td>
</tr>
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<td>5-KETO-D-GUUCONATE</td>
<td>0</td>
</tr>
<tr>
<td>NAGA</td>
<td>Beta-N-NCETYL-GALACTOSAMINIDASE</td>
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</tr>
<tr>
<td>ODC</td>
<td>ORNITHINE DECARBOXYLASE</td>
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<tr>
<td>LDC</td>
<td>LYSINE DECARBOXYLASE</td>
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</tr>
<tr>
<td>BGar</td>
<td>BETA-GLUCORONIDASE</td>
<td>0</td>
</tr>
</tbody>
</table>

FIGURE 6
The positive reactions percentages against tested substrates of the Gram-negative bacteria isolated from the mud hills
(The percentage of the strains displayed positive values more than 50% were shown).
TABLE 4
The positive reaction percentage of the bacilli isolates against tested substrates.

<table>
<thead>
<tr>
<th>Test Substrates</th>
<th>Representation</th>
<th>Positive Reaction %</th>
</tr>
</thead>
<tbody>
<tr>
<td>LeuA</td>
<td>Leucine-ARYLAMIDASE</td>
<td>100 20</td>
</tr>
<tr>
<td>APPA</td>
<td>Ala-Phe-Pro ARYLAMIDASE</td>
<td>100 20</td>
</tr>
<tr>
<td>ESC</td>
<td>Esculin hydrolysis</td>
<td>100 20</td>
</tr>
<tr>
<td>PyrA</td>
<td>L-Pyrrolidonyl-ARYLAMIDASE</td>
<td>87.5 10</td>
</tr>
<tr>
<td>AlaA</td>
<td>Alanine ARYLAMIDASE</td>
<td>87.5 10</td>
</tr>
<tr>
<td>PheA</td>
<td>Phenylalanine ARYLAMIDASE</td>
<td>75 10</td>
</tr>
<tr>
<td>PVATE</td>
<td>PYRUVATE</td>
<td>75 10</td>
</tr>
<tr>
<td>dTRE</td>
<td>D-TREHALOSE</td>
<td>75 20</td>
</tr>
<tr>
<td>dGLU</td>
<td>D-GLUCOSE</td>
<td>75 20</td>
</tr>
<tr>
<td>dRIB</td>
<td>D-RIBOSE</td>
<td>75 10</td>
</tr>
<tr>
<td>NaCl 6.5%</td>
<td>GROWTH IN 6.5% NaCl</td>
<td>75 20</td>
</tr>
<tr>
<td>POLYB_R</td>
<td>POLYMIXIN_B RESISTANCE</td>
<td>75 10</td>
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<td>MTE</td>
<td>MALTOTRIOSE</td>
<td>62.5 10</td>
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<tr>
<td>NAG</td>
<td>N-ACETYL-D-GLUCOSAMINE</td>
<td>62.5 10</td>
</tr>
<tr>
<td>TTZ</td>
<td>TETRAZOLIUM RED</td>
<td>62.5 10</td>
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<td>dMNE</td>
<td>D-MANNOSE</td>
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<td>Tyrosine ARYLAMIDASE</td>
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<td>BETA-N-ACETYL GLUCOSAMINIDASE</td>
<td>37.5 5</td>
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<td>PHOSPHORYL CHOLINE</td>
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<tr>
<td>old</td>
<td>OLEANDOMYCIN RESISTANCE</td>
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<td>L-Proline ARYLAMIDASE</td>
<td>25 10</td>
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<tr>
<td>MdG</td>
<td>METHYL-A-D-GLUCOPYRANOSIDE acidification</td>
<td>25 50</td>
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<td>ELLM</td>
<td>ELMAN</td>
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<td>BXYL</td>
<td>BETA-XYLOSIDASE</td>
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</tr>
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<td>AspA</td>
<td>L-Aspartate ARYLAMIDASE</td>
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<td>BETA-GALACTOSIDASE</td>
<td>12.5 0</td>
</tr>
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<td>ALPHA-GALACTOSIDASE</td>
<td>12.5 0</td>
</tr>
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<td>ALPHA-MANNOSIDASE</td>
<td>12.5 0</td>
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<tr>
<td>GlyA</td>
<td>Glycine ARYLAMIDASE</td>
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</tr>
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<td>dMAN</td>
<td>D-MANNITOL</td>
<td>12.5 0</td>
</tr>
<tr>
<td>BGLU</td>
<td>BETA-GLUCOSIDASE</td>
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<td>dTAG</td>
<td>D-TAGATOSE</td>
<td>12.5 0</td>
</tr>
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<td>L-Lysine-ARYLAMIDASE</td>
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<td>CDEX</td>
<td>CYCLODEXTRIN</td>
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<td>D-GALACTOSE</td>
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<td>GLYCOCEN</td>
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<tr>
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<tr>
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DISCUSSION

In the present study, "unusual mud formations", observed via a "Remotely Operated Vehicle" (ROV) at the bottom of the Çanakkale Strait, Turkey were investigated regarding their bacterial community profile and metabolic response of the isolated bacteria with regard to enzyme expression capability against tested substrates. The mud formations and normal surface sediment around them were compared in order to understand bacterial differences regarding analyses of the counts of cultivable aerobic heterotrophic bacteria, bacterial enzyme expression capacity, diversity and composition.

The Çanakkale strait, as a study area for observing the mud formations, has a counter-current system, formed as a result of the less saline waters (17 psu) of the Black Sea (upper currents) and the concentrated saline waters (38 psu) of the Mediterranean Sea (undercurrents) (Fig. 2). Additionally, this current system was described as an important constituent in chemical oceanographic structure, ecological states and the productivity of the Sea of Marmara and the Turkish Strait System.

The Çanakkale and Istanbul Strait is prone to biological and chemical pollution due to the environmental pollution from the Sea of Marmara [13]. [14], using the accumulation rate of labile organic C in the sediments, showed that changes in ecosystem functioning can increase the efficiency of heterotrophic prokaryotes in transforming organic detritus pools into biomass. However, there is no available data on opposite currents, system-related particle distribution, and abundance of particle-associated...
TABLE 5

The positive reaction percentage of the Gram-positive isolates against tested substrates.

<table>
<thead>
<tr>
<th>Test Substrates</th>
<th>Representation</th>
<th>Positive Reaction %</th>
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<td></td>
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and free-living bacteria at the bottom of the Çanakkale Strait. It is known, however, that particle-associated bacteria levels are higher than free-living bacteria in marine environments. High levels of heterotrophic bacteria detected in mud formations, rather than the surface sediments around the mud knolls, showed that these formations are appropriate environments in which to induce bacterial heterotrophic activity and growth.

Additionally, the initial detection of high bacteria counts and enzymatic dynamics in mud hills may be related to the tendency of bacteria to attach themselves to suspended particles at the bottom of the sea. It is known that when pollutants arrive at natural water environments, their most common accumulation site is within sediments. High epibacteria levels detected in the mud knoll samples, allow us to suggest that these formations may occur as a result of an accumulation of organic and inorganic substances via water movements such as undercurrents (Figure 3, 4), at certain points at the bottom of the sea.

It is known that decomposition of organic substance occurs by reactions of the bacteria to specific adaptations. For investigation of microbial adaptations to environmental conditions and to understand the ecology of a single bacterial species, description of physiological capabilities of pure cultures remain crucial [11].

In the present study, bacterial isolates were compared between the samples taken from the mud formations and natural sediment samples, with an aim to describing the metabolic response types of bacteria regarding two different habitats. A total of 242 cultivable bacterial isolates were characterized by an automated micro identification system, based on biochemical tests measuring carbon source utilization, enzymatic activities and inhibition. Metabolic response rates, against tested substrates, regarding positive reaction frequency of the isolated bacteria from the mud hills, were higher than surface sediment samples.
While the community profiles of the cultivated bacteria were markedly different (as a result of the abundance rates of the bacterial species), 72% of the bacterial species was similar in both the sediment and the mud formation samples. As an example, the Sphingomonas paucimobilis, aerobic Gram-negative bacillus, both belonging to the family Sphingomonadaceae and Alpha Proteobacteria class, were found to be the most common species in the samples of mud formations. Sphingomonas has been found in aquatic environments, both freshwater and seawater, and also in terrestrial habitats [15].

S. paucimobilis was reported to be the most abundant surface-associated bacteria in the sponges taken from the northern part of the Aegean Sea in Turkey [16]. It was also reported that the Sphingomonas species was predominant in all biofilms [17, 18] and has a high production capacity for extracellular polymeric substances and strong adhesion properties [19]. In addition, due to their metabolic diversity, they have been considered a potential microbial agent for biological remediation studies. S. paucimobilis was also reported as able to degrade lignin-related biphenyl chemical compounds [20].

Independent from this study, we also tested oil hydrocarbon degradation capacity of S. paucimobilis isolated from mud formations. At the end of a three week incubation period of individual bacteria with oil hydrocarbon in experiments, the degradation rate, regarding the GC-MS analyses, were recorded to be over 80% (data not shown). In this study, some bacterial species isolated from the mud formation, as in S. paucimobilis, offered some signs for possible use as a candidate species for further study.

Due to the fact that bacterial communities are all different and dynamic and relate to various environmental factors, each marine environment offers unique opportunities for understanding bacterial roles in marine ecosystem functioning. However, it is still unknown how a bacterial community responds to its environmental changes regarding pollution factors at the bottom of the seas. For instance, in this study, members of the phylum Firmicutes and Proteobacteria, including nitrogen-fixing bacteria and various pathogenic bacteria, were recorded to be the dominant group in the mud-hill samples.

The most common group in terms of species number, in both mud formations and surface sediment around them, were Gram-negative pathogenic bacteria. Detection of high pathogenic bacteria consisting of Gram-negative fermenting and non-fermenting bacteria belonging to the Enterobacteriaceae family, and multi-drug resistant isolates (data not shown), allowed us to conclude that the bottom of the Çanakkale Strait indicates some evidence of human-source pollution. For instance, isolation of the multi-drug resistant Stenotrophomonas malto-

CONCLUSION

In this study, the bacterial analysis was appropriate for detecting compositional differences in bacterial communities inhabiting extraordinary formations such as mud knolls and normal surface sediments, from samples taken simultaneously at the bottom of the Çanakkale Strait. Variations in bacterial community composition between the two different samples were recorded, to correspond with differences in habitat characteristics. Our data suggest that detected differences between natural surface sediment and mud formations, regarding composition and metabolic response of the bacteria, may relate to environmental conditions where pollution exists at the bottom of the sea.

Extracellular enzymes, produced by sediment bacteria, play an important role in accumulated and buried organic matter decomposition, nutrient recycling, and earth element transformation and mobilization [26]. As such, our hypothesis is that the interactions between hydrographical processes and variable environmental conditions, accumulation of organic-inorganic substances, including heterotrophic activity may induce a "sludging" tendency and produce unusual occurrences such as the mud knolls at the bottom of the sea.

The comparative analysis of bacterial communities in the samples provided, increase our knowledge about bacterial diversity and composition, in poorly described conditions at the bottom of the Çanakkale Strait. This data will allow us to go forward with studies in which the effects of environmental pollution on bacterial communities and their functions on the seabed of the Çanakkale Strait, will be evaluated. In addition, this study offers us a knowledge with which to compare exoenzyme activities of cultivable bacteria, and for a better understanding of their biochemical roles and the biomaterials to be used as a source for further biotechnological studies.
ACKNOWLEDGEMENTS

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REFERENCES


Adsorption and Separation of CO₂ and CH₄ on Activated Carbon Modified by Acetic Acid

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ABSTRACT

Compared with the modification of activated carbons by alkalis for gas adsorption, fewer studies of that by acids have been reported, especially by organic acids. The acid modified activated carbons are usually utilized to treat wastewater, whereas the application in the separation of CO₂/CH₄ has less been studied. In this study, acetic acid was used to modify activated carbon. N₂ adsorption/desorption isotherms, FT-IR and SEM were adopted to describe the properties of the samples. The adsorption of CO₂ and CH₄, selectivity for binary gas mixtures and thermodynamics were analyzed. The adsorption capacity of CO₂ and CH₄ calculated by the Sips model is 12.87 mmol/g and 7.02 mmol/g on 15H-AC, increasing about 29.48% and 15.84%, respectively. The selectivity of CO₂/CH₄ gas mixtures at 298 K and 1 MPa is reached up to 4.70 for a binary mixture of 50/50 vol.% and 4.60 for that of 40/60 vol.%.

INTRODUCTION

Renewable energy sources such as biogas and landfill gas, which are mainly composed of methane and carbon dioxide, have attracted more attentions as alternative fuels [1]. Separating methane from carbon dioxide to achieve fuel grade quality brings much more energy, economic and environmental benefits [2].

Pressure swing adsorption (PSA) is considered as a promising and economical technology, in which a proper and efficient kind of adsorbent is the one of the most important factors. Among the numerous adsorbents, activated carbons (ACs), have many advantages due to its well-developed pore structure, large specific surface area, high adsorption capacity and inexpensive raw material cost [3], which are applied widely in industrial and technological processes.

Improvements of the adsorption properties and separation effects of ACs can be achieved by various methods [4,5]. Most studies focus on the treatment of ACs with basic maters. It has been recognized that introdution of basic functionalities into the carbon surface can be favorable for their adsorption of acidic gases such as CO₂ [6]. Przepliński et al [7] performed a series studies on the treatment of commercial ACs with ammonia, finding that the optimal CO₂ adsorption capacity was 0.076 g CO₂/g adsorbent. Plaza et al [8] modified the ACs with different amine compounds through a wet impregnation method, with the results that the micropore volume of the samples reduced and the adsorbed amount of CO₂ at room temperature decreased. AC samples treated with K₂CO₃ at 700°C by Mestre et al [1] had narrower micropores and an excellent CO₂/CH₄ selectivity of 4-7. Shafeeyan et al [9] adopted ammonia to treat granular ACs with its CO₂ adsorption capacity increasing. Tan et al [10] impregnated the coconut shell AC with alkaline NaOH and found that the highest adsorbed amount of CO₂ reached to 27.1 mg/g at 35°C.

Additionally, researches on modification of ACs with acids have also been reported. Sun [11] modified ACs with H₃PO₄, finding that the saturation adsorption amounts of CO₂ and CH₄ were only 1.5 mmol/g and 0.1 mmol/g, respectively. Li [12] used nitric acid, hydrochloric acid and sulfuric acid to treat the coal ACs, with the consequence that the adsorption capacity of CO₂ and CH₄ increased but the selectivity effect decreased. Gęsikiewicz-Puchalska et al [13] adopted nitric acid and hydrochloric acid to modify three kinds of commercial ACs, achieving a significant CO₂ adsorption capacity improvement of up to 36% than the raw materials.

Modification of ACs with basic compounds can increase the CO₂ adsorption capacity and improve the selectivity of CO₂ from the gas mixtures, but the regeneration is a bit energy-intensive due to the strong interaction between the carbon surface and the molecules [14]. Besides, compared with the modification of ACs by alkalis in the area of gas separation, fewer studies on treatments of ACs by acids have been reported, especially by organic acids.
acids. Moreover, the organic acid modified ACs are usually utilized in the treatment of wastewater [14], but the application in the separation of CO$_2$ and CH$_4$ have rarely been studied.

In this study, acetic acid was chosen as modifying agent to treat the commercial coconut shell ACs for improving the adsorption capacity and selectivity. The modified AC was compared with the raw sample. The pure component adsorption isotherms of CO$_2$ and CH$_4$ were measured and the selectivities of CO$_2$/CH$_4$ mixtures were predicted by the Ideal Adsorbed Solution Theory (IAST). The sample obtained in this study was hoped to be helpful in the industrially separation of CO$_2$/CH$_4$ mixtures of landfill gas or biogas.

**MATERIALS AND METHODS**

**Materials.** The commercial activated carbon made from cocoanut shell was chosen as a starting material. The sample was sieved to 40-60 mesh and impregnated by an acetic acid solution (15 vol.%) for 24 h at 298 K with horizontal vibrating of 140 r/min. The mixture was filtrated to separate out the modified AC and washed by deionized water. Then the sample was dried at 383 K for 12 h. The raw and modified samples were referred to as R-AC and 15H-AC, respectively.

**Characterization of the activated carbon samples.** N$_2$ adsorption/desorption isotherms at 77 K using a Micromeritics ASAP 2000M analyzer were obtained to understand the textural characterization of the samples. Prior to the measurements, the samples were degassed at 473 K under vacuum for 4 h. The Brunauer-Emmett-Teller (BET) specific surface area ($S_{BET}$) was calculated through adsorption data in the relative pressure (P/P$_0$) range of 0.05-0.2. The total pore volume ($V_{total}$) was computed according to the adsorption data at relative pressure of 0.99. The micropore volume ($V_{micro}$) and mesopore volume ($V_{meso}$) were estimated using t-plot method and BJH method, respectively. The macropore volume was calculated through $V_{total}$ minus by $V_{micro}$ and $V_{meso}$. And pore size distribution was analyzed by non-local density functional theory (DFT).

The surface chemical properties of the unmodified and modified ACs were observed by FTIR spectrometer (Nicolet iS5). Before the measurements, the samples were ground and sieved by 200 mesh. Small quantity of the sample powder was mixed with KBr, compressed to discs, placed in the FTIR instrument and scanned through wave-numbers range from 4000 cm$^{-1}$ to 400 cm$^{-1}$.

The surface morphology, which helps to observe the variation of the solid surface, was examined by scanning electron microscopy (Nova Nano 400) operating at an accelerating voltage of 15 kV. Before scanning process, the samples were dried and coated with gold under vacuum to enhance the electron conductivity.

**Measurement of adsorption isotherms.** The pure component adsorption isotherms of CO$_2$ and CH$_4$ on the raw and modified activated carbons were measured by an Intelligent Gravimetric analysis (IGA-100B, UK) at the pressure of up to 1 MPa and at 298 K, 308 K and 318 K. To avoid the influence of water and other gases adsorbed on the adsorbents, the samples were pretreated in a vacuum at 473 K for 4 h before the measurements.

**Theory and method. Adsorption models for single gas component.** Adsorption models are very important to predict the behavior of the equilibrium adsorption over a wide range of temperature and pressure [15]. A variety of adsorption models have been used to describe the adsorption isotherms of a single gas component [16,17], such as Langmuir, Freundlich and Henry model [18]. Among these models, Langmuir model has been utilized widely due to its simplicity. Therefore, Langmuir model and its two extended models Sips and Toth models were employed in this study.

Langmuir model is based on the theory that the adsorbate layer adsorbed on the solid surface belongs to monolayer pattern [19]. The equation of this model is presented by Eq.1.

$$ q = \frac{q_m b_1 p}{1 + b_1 p} \quad \text{Eq.1} $$

The Sips model can describe the adsorption isotherms of pure gases and predict the behaviors of heterogeneous adsorption systems [20]. The model is expressed as Eq.2.

$$ q = \frac{q_m (b_3 p)^\gamma}{1 + (b_3 p)^\gamma} \quad \text{Eq.2} $$

On the other hand, the Toth model, which is based on the Langmuir model and derived from the potential theory [17], is given by Eq.3.

$$ q = \frac{q_m b_2 p}{(1 + (b_2 p)^\gamma)^\gamma} \quad \text{Eq.3} $$

In Eq.1-Eq.3, $q$ and $q_m$ are the adsorbed amount at the pressure $p$ and the maximum adsorption capacity, respectively; $b_1$ represents the adsorption equilibrium constant ($b_2$ or the affinity parameter ($b_3$ and $b_4$); and $n$ ($n_s$ and $n_I$) is the parameter indicating the heterogeneity of the adsorption system. The heterogeneity is related with the adsorbent structure and energy properties or the adsorbate [15]. The value of $n$ is often above the unity and the larger it is, the more heterogeneous the system is.

To compare and evaluate the fitting results of the above models to the experimental data, two error functions, the nonlinear regression coefficient ($R^2$) and the average relative error (ARE) were em-
ployed. The average relative error, which can describe the deviation between the experimental data and the fitted data [20], is given by Eq.4.

\[
ARE = \frac{100}{n} \sum_{i=1}^{n} \left| \frac{q_{exp} - q_{cal}}{q_{exp}} \right|
\]

where \(q_{exp}\) is the experimental data and \(q_{cal}\) represents the values calculated by the models.

**Adsorption models for binary gas components.** The adsorption behaviors of binary gas mixtures can be predicted with Ideal Adsorption Solution Theory (IAST) according to the parameters obtained by the pure gas isotherms. The theory assumes that the adsorbed phase and the gas phase comply with gas phase [21]. And the equilibrium between the adsorbed phase and the gas phase comply with Raoult’s law, which can be expressed as Eq.5.

\[
P_{\text{total}} y_i = x_i p_i^0 (\pi)
\]

where \(p_{\text{total}}\) is the total pressure of the gas phase; \(x_i\) and \(y_i\) are the molar fraction of component in the adsorbed phase and the gas phase, respectively; \(p_i^0(\pi)\) is the equilibrium gas phase pressure of component \(i\) corresponding to the solution pressure \(\pi\) and to the solution temperature. For a pure gas, \(p_i^0(\pi)\) can be obtained by Gibbs equation given by Eq.6.

\[
\frac{\pi_i^0 A}{RT} = \int_0^{\pi_i^0} q_i(p) dp
\]

where \(\pi_i^0\) is the spreading pressure of component \(i\) in the gas phase; \(A\) is the surface area of the adsorbent; \(R\) is the gas constant; \(T\) is the absolute temperature; and \(q_i(p)\) represents the pure gas adsorption equilibrium equation of component \(i\).

Because the spreading pressure \(\pi_i^0\) of every component in the gas phase is identical, then Eq.6 can be expressed as Eq.7.

\[
\int_0^{\pi_i^0} \frac{q_i(p)}{p} dp = \int_0^{\pi_i^0} \frac{q_j(p)}{p} dp
\]

Additionally, Lewis relations given as Eq.8, and other relational expressions given as Eq.9, are also needed to compute the adsorbed amounts of the binary gas mixtures.

\[
\frac{1}{q_{\text{total}}} = \frac{x_i}{q_i(p_i^0)} + \frac{x_j}{q_j(p_j^0)}
\]

\[
q_i = q_{\text{total}}x_i, \quad q_j = q_{\text{total}}y_j
\]

\[
x_i + x_j = 1, \quad y_i + y_j = 1
\]

According to \(q_i^0(p)\), it can be known that the IAST model are on the basis of pure gas adsorption isotherms. Therefore, the accuracy of the single adsorption model used in the IAST model is of great importance to the prediction precision. In this study, the optimal model among the three single adsorption isotherms was employed in IAST model to predict the binary adsorption equilibrium.

**Selectivity.** The performance of the adsorbents for separating two different gases can be described by selectivity, which is a vital indicator to estimate the adsorbents. The IAST is well-known to predict the adsorptive selectivity of the gas mixtures in many porous adsorbent materials [22]. The expression is presented by Eq.10.

\[
S = \frac{x_i}{y_i} / \frac{x_j}{y_j}
\]

where \(x_i, y_i\) are the equilibrium mole fractions of component \(i\) in the adsorbed and gas phase, respectively.

**RESULTS AND DISCUSSION**

**Characterization. Textural properties.** \(N_2\) adsorption/desorption curves of the raw and modified ACs are illustrated in Figure 1. According to the classification of International Union of Pure and Applied Chemistry (IUPAC), all the curves are combination of type I and type IV which are characteristic for micropore and mesopore, respectively [23]. The adsorbed amounts increase sharply at low relative pressure, which indicates that the materials contain a large amount of micropores. However, the slopes of the curves decrease gradually at high relative pressure and the adsorption curves differ with the desorption ones. The findings show that capillary condensation happens in the samples, which is related with mesopores. The hysteresis hoops at the relative pressure above 0.4 belong to type H4, indicating the existence of narrow slit pores. Similar phenomena have been reported in the other literatures [13, 20].

**FIGURE 1**

\(N_2\) adsorption/desorption curves of R-AC and 15H-AC
The pore structure distribution of all samples is shown in Figure 2. It can be observed that the samples exhibit a concentrated pore size distribution mainly on the range of 1.6-1.9 nm and 3.97 nm, manifesting the existence of the micropores and mesopores. These results are consistent with the N₂ adsorption/desorption isotherms. Most of the micropores of R-AC are distributed in 1.61-2.02 nm, whereas that of 15H-AC is concentrated in 1.61-1.93 nm. It is obvious that the pore size becomes narrower after the modification by acetic acid. The reason is mainly that the blocked pores were opened and some new micropores were formed after the treatment. The pore structure parameters of the samples are listed in Table 1.

From Table 1, it can be seen that the specific surface area of the ACs slightly increases after the modification. For instance, the specific surface area and the total pore volume of the modified sample 15H-AC is 874.62 m²/g and 0.439 cm³/g, which is larger than that of the raw sample. The micropore volume also increases, but its percentage becomes smaller which is about 90.6% in R-AC while only 88.36% in 15H-AC. The mesopore volume varies little and both the macropore volume and its percentage become larger after the modification. The possible reason of the phenomenon is that acetic acid opens some blocked pores and produces new micropores, enlarging the specific surface area, total pore volume and the micropore volume. Meanwhile, the corrosion of the acid results in the translation of mesopores to macropores, increasing the macropore volume. Similar results were obtained in other researches [13].

**Surface chemical properties.** The FT-IR spectra of the raw and modified activated carbons are demonstrated in Figure 3. The main characteristic absorption peaks of the samples are almost the same. The broad and strong peaks at 3443 cm⁻¹ are attributed to O-H groups stretching vibrations in alcohol, phenol and carboxylic acid [23,24] and water absorbed on the surface. The peak at 3443 cm⁻¹ of the modified sample strengthens, suggesting the increasing number of O-H groups. The bands located at 2925 cm⁻¹ are related to the asymmetric stretching vibration of –CH₃ groups [25]. The peaks at 1634 cm⁻¹ can be assigned to C=C bond stretching vibrations in aromatic rings of carbon structure [26]. The weak sharp peaks at around 1384 cm⁻¹ and the broad peaks at around 1087 cm⁻¹ are associated with the C-O bonds stretching of phenol, alcohol and carboxylic acid [24]. It can be concluded that the samples have similar chemical properties that the skeleton structure are made up of multiple aromatic rings with oxygen-containing functional groups such as methyl, hydroxyl and carboxyl.

**Surface morphology properties.** As shown in Figure 4, the external surface of the raw sample R-AC is relatively smooth and shows several shallow holes. However, the surface of the modified sample 15H-AC is out of flatness and exhibits more scorings and deep pores which are attributed to the corrosion of acetic acid. These scorings and pores are helpful for increasing the surface specific area and pore volume. The well-developed porosity and large surface specific area are conducive to the diffusion of gases within the adsorbents and the adsorption performance of the adsorbents.

**TABLE 1**

<table>
<thead>
<tr>
<th>Sample</th>
<th>S_{BET} (m²/g)</th>
<th>V_{tota} (cm³/g)</th>
<th>V_{micro} (cm³/g)</th>
<th>V_{meso} (cm³/g)</th>
<th>V_{macro} (cm³/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>R-AC</td>
<td>787.07</td>
<td>0.425</td>
<td>0.385 (90.61%)</td>
<td>0.018 (4.24%)</td>
<td>0.022 (5.15%)</td>
</tr>
<tr>
<td>15H-AC</td>
<td>874.62</td>
<td>0.439</td>
<td>0.388 (88.36%)</td>
<td>0.021 (4.78%)</td>
<td>0.030 (6.86%)</td>
</tr>
</tbody>
</table>
Adsorption isotherms of single component. The adsorption isotherms of pure CO\textsubscript{2} and CH\textsubscript{4} measured at the temperature of 298 K, 308 K and 318 K and the pressure up to 1 MPa on R-AC and 15H-AC are graphically represented in Figure 5.

As evident in Figure 5, the adsorbed amounts of CO\textsubscript{2} and CH\textsubscript{4} on the samples increase with pressure. The growth rate of adsorbed amount reduces gradually with pressure and finally approaches the equilibrium. The CO\textsubscript{2} and CH\textsubscript{4} adsorption capacity of the modified AC rises. As reported in the previous literatures, the specific surface area and pore volume are related to the adsorption capacity of the adsorbents [26]. Thus, the growth of the adsorbed amount could be ascribed to the enhanced effect of porosity and surface area after the modification [27].

In addition, the adsorbed amount of CO\textsubscript{2} is larger than that of CH\textsubscript{4} when at the same condition, which is due to the difference of the physical properties of the two gases. The molecules of CO\textsubscript{2} could enter into the smaller micropores because of the smaller gas kinetic diameter, which is 0.34 nm for CO\textsubscript{2} while 0.38 nm for CH\textsubscript{4} [26]. For CH\textsubscript{4} molecules which are the critical gases, the phenomenon of micropore filling happens merely in the micropores. However, for CO\textsubscript{2} molecules, both micropore filling and capillary condensation could occur in the micropores, mesopores and macropores [28]. The results demonstrate that the adsorption of CO\textsubscript{2} and CH\textsubscript{4} is relevant to not only the pore structure but also its distribution [24]. The calculated adsorption parameters of CO\textsubscript{2} and CH\textsubscript{4} on the samples, which were predicted by Langmuir, Sips and Toth models respectively, are summarized in Table 2-4.

![FIGURE 5](image)

**FIGURE 5**
Adsorption isotherms of CO\textsubscript{2} and CH\textsubscript{4} on (a) R-AC and (b) 15H-AC at 298 K, 308 K and 318 K.

### TABLE 2

Adsorption parameters of CO\textsubscript{2} and CH\textsubscript{4} on the samples fitted by Langmuir model

<table>
<thead>
<tr>
<th>Gas</th>
<th>Temperature (K)</th>
<th>R-AC</th>
<th>15H-AC</th>
<th>ARE (%)</th>
<th>R\textsuperscript{2}</th>
<th>ARE (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO\textsubscript{2}</td>
<td>298</td>
<td>7.58</td>
<td>4.603</td>
<td>0.9939</td>
<td>7.764</td>
<td>8.19</td>
</tr>
<tr>
<td></td>
<td>308</td>
<td>7.39</td>
<td>4.254</td>
<td>0.9964</td>
<td>6.227</td>
<td>8.19</td>
</tr>
<tr>
<td></td>
<td>318</td>
<td>7.12</td>
<td>3.928</td>
<td>0.9975</td>
<td>4.844</td>
<td>8.06</td>
</tr>
<tr>
<td>CH\textsubscript{4}</td>
<td>298</td>
<td>5.09</td>
<td>2.370</td>
<td>0.9986</td>
<td>6.657</td>
<td>5.15</td>
</tr>
<tr>
<td></td>
<td>308</td>
<td>4.95</td>
<td>2.136</td>
<td>0.9993</td>
<td>3.384</td>
<td>5.05</td>
</tr>
<tr>
<td></td>
<td>318</td>
<td>4.79</td>
<td>1.957</td>
<td>0.9994</td>
<td>4.533</td>
<td>4.90</td>
</tr>
</tbody>
</table>
As can be seen in Table 2-4, the parameters obtained by one model are different from that by another. The saturation adsorbed amounts calculated by the three models are all positive related to the concentration of acetic acid used to modify the activated carbons. Meanwhile, the adsorption constants \(b\) in the Langmuir model, which are relevant to the interaction between the adsorbates and the adsorbents, have similar rules of the values of \(CO_2\) larger than that of \(CH_4\), indicating that the interactions between \(CO_2\) molecules and the samples are stronger. The reason may be that the larger quadrupole moment of \(CO_2\) than \(CH_4\) (no quadrupole moment) strengthens the adsorbate-adsorbent interaction [29]. The value of adsorption constant \(b\) also increases after the modification, suggesting that the interaction between the gas molecules and the adsorbents is enhanced after the modification. The parameter \(m\) in Sips and Toth models represents the system heterogeneity [30]. Thus, the values of \(m\) of the Sips model (\(n>1\)) and the Toth model (\(n<1\)) show the high degree of heterogeneous adsorption [20].

Based on the small values of \(R^2\) and \(ARE\), the excellent agreement between the fittings of the models and the experimental data confirm that the three models can be adopted to accurately represent the adsorption equilibrium of the two gases. Among these models, the Sips model fitted the adsorption curves best and then followed by the Toth and Langmuir model successively. Thus, the optimal fitting lines of Sips model are given in Figure 5. As shown in Table 3, though the adsorbed amounts of both \(CO_2\) and \(CH_4\) increase after the modification, the growth of \(CO_2\) is much larger than that of \(CH_4\) when at the same condition. The saturation adsorption amount of \(CO_2\) on 15H-AC is 2.93 mmol/g larger than that on R-AC, increasing by 29.48%.

However, that of \(CH_4\) on 15-AC is only 0.96 mmol/g larger than that on R-AC, increasing by 15.84%. This is probably because that some new micro pores and macropores, which are beneficial to the adsorption of \(CO_2\) rather than \(CH_4\), were formed in the activated carbons during the modification with acetic acid. Besides the increase of oxygen-containing functional groups like hydroxyl and carboxyl in the surface results in the increase of \(CO_2\) adsorbed amount according to Figure 3.

Besides, it can be seen from Figure 5 that temperature strongly influences the adsorption capacity at the same pressure for both the two samples. The adsorbed amounts of the two adsorbates decrease with the increasing temperature, which is due to the exothermic nature of the adsorption processes of \(CO_2\) and \(CH_4\). The adsorption parameters of the samples at three temperatures are calculated by Sips model. It comes out that for R-AC the saturation adsorption capacity of \(CO_2\) are 9.95 mmol/g, 9.06 mmol/g and 5.96 mmol/g and that of \(CH_4\) are 6.06 mmol/g, 5.58 mmol/g and 5.05 mmol/g at 298 K, 308 K and 318 K, respectively; whereas for 15H-AC the values of \(CO_2\) are 12.87 mmol/g, 11.27 mmol/g and 10.08 mmol/g and that of \(CH_4\) are 7.02 mmol/g, 6.69 mmol/g and 6.02 mmol/g, respectively. Álvarez-Gutiérrez et al [15] prepared two kinds of activated carbons CS-H2O and CS-CO2, and the adsorption capacity of \(CO_2\) and \(CH_4\) were 10.88 mmol/g and 6.25 mmol/g for CS-H2O and 9.90 mmol/g and 5.96 mmol/g for CS-CO2, respectively, which were measured at 303 K and obtained by Sips model. In this study, the adsorption capacity of R-AC is less than that mentioned in the literature, but that of 15H-AC is greater. The comparisons of the adsorption capacity of \(CO_2\) and \(CH_4\) on R-AC and 15H-AC with other ACs reported in the literatures are listed in Table 5.
Comparison of the adsorption capacity of gases on R-AC and 15H-AC with other activated carbons obtained from the literatures at the pressure of 0.1 MPa

<table>
<thead>
<tr>
<th>Adsorbent</th>
<th>T(K)</th>
<th>Adsorption capacity (mmol/g)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>15H-AC</td>
<td>298</td>
<td>2.74</td>
<td>1.23</td>
</tr>
<tr>
<td>308</td>
<td>2.41</td>
<td>1.11</td>
<td></td>
</tr>
<tr>
<td>318</td>
<td>2.13</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>MAC (microwave AC)</td>
<td>298</td>
<td>1.69</td>
<td>0.81</td>
</tr>
<tr>
<td>308</td>
<td>1.71</td>
<td>-</td>
<td>20</td>
</tr>
<tr>
<td>OXA-GAC (ammonia-modified AC)</td>
<td>333</td>
<td>1.12</td>
<td>-</td>
</tr>
<tr>
<td>345</td>
<td>5.05</td>
<td>-</td>
<td>22</td>
</tr>
<tr>
<td>AC-KOH-N</td>
<td>303</td>
<td>1.88-2.98</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>308</td>
<td>0.85</td>
<td>23</td>
</tr>
<tr>
<td>H.Po-AC</td>
<td>313</td>
<td>0.65</td>
<td>31</td>
</tr>
<tr>
<td>JX101</td>
<td>321</td>
<td>1.22</td>
<td>32</td>
</tr>
<tr>
<td>ACD18-053 (AC disc)</td>
<td>298K</td>
<td>3.51</td>
<td>1.22</td>
</tr>
</tbody>
</table>

Table 5 shows that the adsorbed amount of CO₂ on the modified sample used in this study is larger than that of most samples in the literatures, but less than that of AC-KOH-N which possessed basic group. However, the adsorbed amount of CH₄ is not much different with that of the other carbonaceous adsorbents. Therefore, in terms of adsorption capacity, the adsorbent obtained in this study can be well utilized in adsorption of CO₂ and in the separation of CO₂/CH₄ gas mixture.

Prediction of binary adsorption equilibrium from single component data. Based on Table 2-4, the Sips model is the optimal to describe the single component adsorption isotherms. So Sips-IAST method was used to predict the adsorption isotherms of binary gases and to analyze the adsorption process of each component. Two conditions of CO₂/CH₄ binary gas mixtures were considered, namely 50/50 vol.% which was usually adopted in most studies and 40/60 vol.% which was characteristic of landfill gas.

As can be seen in Figure 6, for both two conditions of CO₂/CH₄ mixtures, the adsorption of CO₂ is predominated and the adsorbed amount of CO₂ takes up most of the total adsorbed amount. For an equivalent binary gas mixture of CO₂ and CH₄ in the gas phase, the total adsorbed amount on R-AC is 5.37 mmol/g and the amount of CO₂ and CH₄ is 4.41 mmol/g and 0.96 mmol/g when at the pressure of 1 MPa; whereas those parameters on 15H-AC are up to 5.90 mol/g, 4.86 mol/g and 1.044 mol/g, respectively. The increment of the adsorbed amount of CO₂ on 15H-AC is 0.45 mmol/g compared with that on R-AC, whereas that of CH₄ is only 0.08 mmol/g. Under the condition that the ratio of CO₂/CH₄ gas mixture is 40/60 vol.%, the total adsorbed amount, the amount of CO₂ and CH₄ on R-AC is 5.32 mmol/g, 3.99 mmol/g and 1.33 mmol/g at 1 MPa, and those on 15H-AC are 5.84 mmol/g, 4.40 mmol/g and 1.44 mmol/g, respectively. The adsorbed amount of CO₂ on 15H-AC is 0.41 mmol/g larger than that on R-AC and that of CH₄ 0.11 mmol/g larger. Although the adsorption capacity of the two gases increases after the modification, the increased degree of CO₂ is more significant than that of CH₄.

![FIGURE 6](https://via.placeholder.com/150)

Absorbed amounts of CO₂/CH₄ gas mixtures on R-AC and 15H-AC under the pressure range of 0-1 MPa at 298 K with different gas ratio of (a) CO₂:CH₄=50:50 and (b) CO₂:CH₄=40:60

Figure 7 depicts the adsorption behaviors of CO₂/CH₄ mixtures in various ratios on R-AC and 15H-AC at 298 K and 0.1 MPa. With the increase of the percentage of CH₄ in the mixtures, the total adsorbed amounts on both two samples decrease gradually. When the percentage of CH₄ is below a certain value, which is 0.8 in this study, the adsorption of CO₂ is dominant. This finding is similar
with that reported before [16]. At the same ratio of CH4 in the mixtures, the total adsorbed amount increases after the modification. Besides, the increase of CO2 adsorbed amount is more obvious but that of CH4 changes slightly, which is in agreement with the adsorption isotherms obtained in Section 3.2.1. Thus, the modification of acetic acid is more favorable to the adsorption of CO2. Additionally, for a mixture of 40% CO2 and 60% CH4, which represents the landfill gas, the value is around 4.60 for 15H-AC and 4.52 for R-AC at 298 K and 1 MPa. It can be concluded that both the two kinds of ACs can separate CO2/CH4 mixture well, but the sample modified by acetic acid shows better performance. These values are significantly higher than those obtained in the literatures before. Álvarez-Gutiérrez et al [15] reported the optimal selectivities of about 4.3 on CS-H2O ACs and 4.4 on CS-CO2 ACs for a 50% CO2 and 50% CH4 mixture at 303 K and 1 MPa. And a brief survey of the selectivity of CO2/CH4 gas mixture on the other carbon adsorbents mentioned in the previous is summarized in Table 6.

Selectivity of CO2/CH4 Figure 8 shows that the selectivities for both R-AC and 15H-AC first reduce at low pressure and then increase with the pressure. These behaviors may be accounted for the difference of the adsorbed amount growth rate for the two gases with increasing pressure. Under the same condition, the selectivity for 15H-AC is apparently higher than that for R-AC. Two main reasons can be used to explain this phenomenon. One is that the smaller dynamic diameter, higher quadrupole moment and larger polarizability of CO2 molecule make the stronger interaction with the adsorbents; another is that the decreasing pore diameter, larger pore volume after the modification by acetic acid are conducive to the adsorption of CO2 rather than CH4, benefiting the separation of the two gases.

The maximum selectivity for separating the binary CO2/CH4 (50/50 vol.%) is reached at 298 K and 1 MPa: 4.70 for 15H-AC and 4.60 for R-AC. It can be found clearly that the adsorbent used in this work has a great CO2/CH4 selectivity among the carbonaceous adsorbents mentioned in Table 6, except the monolith activated carbon whose selectivity up to 6.5. Although the selectivity of the modified sample increases slightly, taking the adsorption capacity into consideration simultaneously, the AC modified by acetic acid could be a promising carbonaceous material for the separation of CO2/CH4 mixture.

### TABLE 6

<table>
<thead>
<tr>
<th>adsorbent</th>
<th>Temperature(K)</th>
<th>Pressure (MPa)</th>
<th>(y_{\text{CH4}})</th>
<th>Selectivity</th>
<th>refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>15H-AC</td>
<td>298</td>
<td>0.2</td>
<td>0.5</td>
<td>4.24</td>
<td>This study</td>
</tr>
<tr>
<td>Pitch-based activated carbon beads</td>
<td>303</td>
<td>0.1</td>
<td>0.5</td>
<td>3.6</td>
<td>16</td>
</tr>
<tr>
<td>Activated carbon prepared from pine cone</td>
<td>298</td>
<td>0.1</td>
<td>0.5-0.6</td>
<td>1.2</td>
<td>29</td>
</tr>
<tr>
<td>Norit R1 Extra</td>
<td>298</td>
<td>0.2</td>
<td>0.5</td>
<td>1.8</td>
<td>33</td>
</tr>
<tr>
<td>BPL activated carbon</td>
<td>298</td>
<td>0.2</td>
<td>0.5</td>
<td>1.9</td>
<td>34</td>
</tr>
<tr>
<td>Massorb activated carbon</td>
<td>298</td>
<td>0.2</td>
<td>0.5</td>
<td>2.3</td>
<td>35</td>
</tr>
<tr>
<td>activated carbon</td>
<td>298</td>
<td>0.1</td>
<td>0.7</td>
<td>2</td>
<td>36</td>
</tr>
<tr>
<td>Norit R1 Extra</td>
<td>298</td>
<td>0.1</td>
<td>0.57</td>
<td>1.4</td>
<td>35</td>
</tr>
<tr>
<td>monolith activated carbon</td>
<td>303</td>
<td>0.1</td>
<td>-</td>
<td>6.5</td>
<td>36</td>
</tr>
</tbody>
</table>

**FIGURE 7**

Completive absorption behaviors of CO2/CH4 gas mixtures on R-AC and 15H-AC at 298 K and 0.1 Mpa

**FIGURE 8**

Sips-IAST selectivities of CO2/CH4 for R-AC and 15H-AC at 298 K up to 1 MPa with different ratio of 50:50 (solid points) and 40:60 (hollow points)
CONCLUSIONS

In order to improve the properties of ACs in the separation of CO2/CH4 mixture, acetic acid was used to modify the commercial coconut shell activated carbons. It was found that the specific surface area, total pore volume, micropore volume increase after the modification. The saturation adsorption capacity and the micropore diameter decreases slightly after the modification. The selectivity of CO2 and CH4 on ACs modified by 15% acetic acid solution is 12.87 mmol/g and 7.02 mmol/g respectively, with the values increasing about 29.48% and 15.84% in contrast with that on the raw sample and larger than that on the other porous carbon samples reported in the literatures. The Sips-IAST model was utilized to predict the adsorption of the binary gas mixtures and to evaluate the separation effect of the adsorbents. The selectivity of CO2/CH4 gas mixtures at 298 K and 1 MPa is reached up to 4.70 for a binary mixture of 50/50 vol.% which was frequently used in most studies and 4.60 for a binary mixture of 40/60 vol.% which could be considered as typical landfill gas. The adsorbent used in this work exhibit a greater selectivity than the other carbonaceous materials. In conclusion, the method of modification with acetic acid is feasible to improve the adsorption capacity and the separation effect of the activated carbons. And the adsorbent obtained in this study is promising in the separation of CO2/CH4 gas mixtures industrially.

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REFERENCES


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EFFECT OF STRONG STRUCTURE ON RCPTU TEST RESULTS FOR MARINE CLAYS DEPOSITED IN NINGBO CITY, CHINA

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ABSTRACT

The structure of soft soil has major effects on its properties, and this structure varies from place to place. This study investigates the effect of structure on resistivity piezocone penetration (RCPTU) test results for soft marine clay in the Ningbo area, China through analysis of the values obtained for soil parameters such as electrical resistivity, sensitivity, undrained shear strength and coefficient of consolidation. The results show that the penetration index is high at Ningbo, namely, qa = (1.0–3.0) MPa, and the average value of the friction ratio, Rf is about 0.85%; highly accurate soil classification can be achieved by RCPTU testing for this strong-structured soft marine clay; the electrical resistivity of soft clay is a good reflection of its properties and microstructure; in strongly structured soil, the deeper the soil is beneath the surface, the shorter the CPTU pore pressure dissipation time becomes and the larger the consolidation coefficient of the soil becomes and the larger the consolidation coefficient of the soil becomes; shear dilatancy occurs at the initial stage of pore pressure dissipation; damage done to the soil structure by the process of penetration causes the permeability of the soil around the probe to decrease rapidly and index distortion to occur. These research results provide a reference for the design of foundation treatments for structured soft soil and have important significance for engineering applications.

KEYWORDS:
Marine clay, structure, resistivity piezocone test (RCPTU), dissipation of pore pressure, China

INTRODUCTION

The structure of soil refers to the character of pore and arrangement and interaction between soil particles. Natural sedimentary soil always has structural characteristics, and its structure has a significant impact on its engineering properties. Loose sediments, both terrestrial and marine, were formed as the Ningbo area of China transitioned from submarine to land, and strongly structured marine clay is widely deposited in Ningbo city, China. Rapid industrialization and urbanization in this area have resulted in an ever-increasing number of construction projects such as highways or railways. The high moisture content, high compressibility, high sensitivity, and high viscosity of the marine clay means that it is necessary to pretreat the soil foundation [1, 2] as, otherwise, these poor engineering properties can result in hazards such as excessive settlement, landslides, and building collapse [3-5]. Therefore, a systematic site investigation for characterization of subsurface soil and determination of geotechnical parameters is of important and necessary. Recently, in-situ testing, the cone penetration test (CPT) has been used to determine the geotechnical engineering properties of soils and characterize soil stratigraphy. A new in-situ technique, the piezocone penetration test (CPPTU), is a commonly used and very convenient method that allows for rapid, continuous soil profiling and has good performance for determining the in-situ characteristics of clay deposits [6-8]. The CPTU can provide near-continuous measurements of tip resistance (qt), sleeve friction (βs), and pore water pressure (uw) induced during penetration, making it a powerful in-situ testing technique for determining the properties of soft clay deposits. Since uw (and possibly qt) and electrical resistivity (ρ) are among the parameters that can be reliably obtained from in-situ testing, it seems logical to attempt to combine them (in RCPTU) to characterize and classify soft marine clays [9-11].

The term ‘soil structure’ is often used to describe the fabric of natural sedimentary soils, especially clays [12], and the effects of bonding on the soil’s mechanical properties; it thus distinguishes natural clay from remolded clay. In recent years, numerous experimental or theoretical studies have been attempted to evaluate the effects of clay structure, such as preconsolidation stress, compression characteristics, shear characteristics, coefficient of consolidation and pore pressure characteristics and so on [5, 13-18]. However, most of these experimentally results are based on laboratory tests. As in-situ testing is widely used for site investigation, the effect of the structure of clay on the results of in-situ testing is of great importance for engineering practice. Shen (1998) used in-situ test data to study several aspects of the engineering properties of structured soil: the generation and dissipation of pore pressure, lateral displacement, and settlement disturbance...
[19], and Cruz and Mayne (2006) used CPTU to evaluate the geotechnical design parameters for the soft structural lacustrine deposits of Mexico City Valley [20]. These reported results are not sufficient to develop a full understanding of the in-situ structural characteristics of soils, and further study is necessary. Additionally, the results show that there are site-specific structural differences between soils, indicating that the systematic study of the structure of soft marine clay in a specific area is of great interest.

The main objective of this paper is to study the effect of strong soil structure on RCPTU test results for Ningbo Clay area. For this purpose, a series of in situ RCPTU tests are carried out at the sites of a subway construction project in the Ningbo area. The soil parameters of resistivity, undrained shear strength, sensitivity and coefficient of consolidation are analyzed, and the differences between the values obtained for these parameters and those resulting from laboratory tests and other in-situ tests are investigated. It is believed that such investigations can facilitate a better understanding of the effect of strong soil structure on RCPTU test results.

FIELD TESTING

Resistivity Piezocone Penetration Tests (RCPTU). The development of in-situ testing techniques has resulted in the development of the advanced RCPTU method and its application in practice [21]. The RCPTU is a rapid, reliable, and economical in-situ method that can obtain both the basic engineering properties of the soil and the stratigraphy of the subsurface [22]. Moreover, the RCPTU provides nearly continuous resistivity soundings and can characterize the structure of marine clays.

SITE DESCRIPTION

Figure 1 shows a map of Zhejiang province with the approximate location of the testing sites used for this study marked. The sites are in Ningbo City, which is located in the eastern coastal area of Zhejiang Province, China. The geologic formations at the testing sites include sediments associated with the Yangtze River Delta and coastal plain. Mineral composition and the proportion of clay directly affect the structure of soils in this area. The clay minerals present in the Ningbo area are mainly illite and a small amount of montmorillonite and kaolinite that exhibit structured characteristics. The Ningbo clay typically has a spongy structure and bedding. The depositional process of soil also has a major influence on its structure. If flocculation structures are formed during deposition, the soil will show a very strong structure; otherwise, it presents a weak structure. In addition, at Ningbo the clay structure has been strengthened due to pore changes and soil particle cementation under the action of external load. Weathering and changes to the surface temperature lead to changes in the connections between soil particles and in the overall strength of the clay. In generally, the thickness of the marine clays varies from 20m to 30m with a hard crust layer on the surface of 1m to 1.5m. Table 1 summaries information regarding the distribution of soil layers and their main physical indexes at the site surveyed.
The RCPTU tests were carried out by using the multi-functional, digital, vehicle-mounted RCPTU system, which is in accordance with ASTM D 5778 [23] and [24]. The specifications of the resistivity probe are as follows: its diameter is 35.7 mm, apex angle is 60°, projected area is 10 cm², friction sleeve area is 150 cm², and the pore pressure filter element is located in the \( r_e \) position [25]. Near-continuous RCPTU profiling was conducted by pushing the cone into the soil at the standard penetration rate of 20 mm/s. During the penetration process, the readings for \( q_c \), \( f_s \), \( u_e \), and \( \rho \) were measured and recorded synchronously. A schematic diagram of the RCPTU probe is shown in Figure 2. The key components of RCPTU equipment are four copper electrodes and an internal circuit system that uses insulating plastic between the electrodes to form an O-type ring seal system. A voltage difference is applied to the two outer electrodes, and the resistivity of the soil is determined with the aid of the two inner electrodes. A calculation program based on Ohm's law is compiled to compute the electrical resistivity of soils along the line of penetration. Field data were collected in real time with an E4FCS computer system, and data processing was carried out with CONEPLLOT and CLEANUP software. The measured total cone tip stress \( q_c \) is often transformed to cone resistance corrected for unequal end area effects \( q_c = q_c + \alpha \times u_e \), \( \alpha \) is area ratio of the cone. The groundwater table varied from 3.0 m to 3.5 m below ground level and was recorded immediately after the RCPTU tests. In-situ vane shear tests (FVT) and flat dilatometer tests (DMT) were also carried out.

**Sampling and Laboratory tests.** In order to ensure the quality of the samples collected and to meet the needs of engineering design, samples were collected by using a 76 mm-diameter stationary piston sampler at 1.0 m intervals from the surface to the penetration depth. When the sampler was withdrawn from the borehole, the soil sample at the end of the tube was excavated, and waxing sealing was applied at both ends. Laboratory tests were carried out on the basic geotechnical properties of the soil samples from the investigated sites. A summary of the soil properties at the site surveyed is given in Table 1.

![Schematic diagram of RCPTU probe](image_url)

**FIGURE 2**
Schematic diagram of RCPTU probe

**TABLE 1**
Summary of the soil engineering properties index

<table>
<thead>
<tr>
<th>Number</th>
<th>Soil layer</th>
<th>Depth(m)</th>
<th>( \gamma ) (kN/m²)</th>
<th>( w ) (%)</th>
<th>( e )</th>
<th>( w_l ) (%)</th>
<th>( L_c )</th>
<th>( E_S ) (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-2</td>
<td>Clay</td>
<td>1.3~1.6</td>
<td>19.2</td>
<td>30.7</td>
<td>0.852</td>
<td>40.4</td>
<td>0.46</td>
<td>4.20</td>
</tr>
<tr>
<td>1-3b</td>
<td>Mucky clay</td>
<td>8.0~9.2</td>
<td>17.2</td>
<td>48.8</td>
<td>1.356</td>
<td>39.4</td>
<td>1.56</td>
<td>2.48</td>
</tr>
<tr>
<td>2-2b</td>
<td>Mucky clay</td>
<td>3.5~3.6</td>
<td>17.1</td>
<td>51.3</td>
<td>1.447</td>
<td>45.4</td>
<td>1.25</td>
<td>2.15</td>
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<tr>
<td>2-2c</td>
<td>Silty clay</td>
<td>1.7~2.1</td>
<td>16.9</td>
<td>49.2</td>
<td>1.420</td>
<td>36.5</td>
<td>1.76</td>
<td>2.52</td>
</tr>
<tr>
<td>4-2a</td>
<td>Clay</td>
<td>3.0~3.4</td>
<td>17.9</td>
<td>41.0</td>
<td>1.167</td>
<td>41.6</td>
<td>0.91</td>
<td>3.61</td>
</tr>
<tr>
<td>5-1a</td>
<td>Clay</td>
<td>2.0~2.5</td>
<td>19.8</td>
<td>27.7</td>
<td>0.766</td>
<td>38.4</td>
<td>0.36</td>
<td>6.67</td>
</tr>
<tr>
<td>5-3b</td>
<td>Silty sand</td>
<td>9.0~12.0</td>
<td>19.4</td>
<td>27.5</td>
<td>0.775</td>
<td>—</td>
<td>—</td>
<td>9.48</td>
</tr>
</tbody>
</table>

*Note: \( \gamma \)=unit weight; \( w \)=water content; \( e \)=void ratio; \( w_c \)=liquid limit; \( L_c \)=liquid index; \( E_s \)=compression modulus.
**Typical Test Results.** Figure 3 depicts a typical RCPTU profile obtained at the Ningbo sites and the associated profiles for $q_t$, $f_t$, $u_2$, friction ratio $R_f (\equiv f_t/q_t \times 100\%)$, pore pressure ratio $B_t (\equiv (u_2-u_0)/(q_t-\sigma_{vo}))$, and soil electrical resistivity $\rho$ with penetration depth. It can be observed from the figure that the ground surface is covered by a weathered crust to a depth ($h$) of approximately 0.3 m, in which $q_t$ is about 0.7 MPa and displays a clear decrease with increasing depth. Two types of soft clay layers are present below the surface crust: (1) mucky clay, with a depth of 19 m and (2) clay, with a depth of 3 m. In the mucky clay layers, positive pore water pressure is generated during penetration and dramatically increases with depth, while $q_t$ increases very slightly over the depth range 0.5-19 m. The penetration indexes in clay and silty clay layers are higher than in the mucky clay, with $q_t$ values of about 1.0-3.0 MPa, an average $R_f$ value of about 0.85% and $u_2$ values of about 400–800 kPa. The morphological trends of the $q_t$–$h$, $f_t$–$h$, and $R_f$–$h$ curves are smooth and, whereas their range of variation in mucky clay is small, there is a large variation between clay and silt, indicating that the mucky clay is more uniform than the other layers. It is noteworthy that the curves for $u_2$–$h$ and $q_t$–$h$ increase linearly with depth, indicating that the changes in pore water pressure and soil electrical resistivity are more sensitive than that of cone tip resistance. The trend of pore water pressure with depth can be used to identify soil layering. Where the pore pressure ratio $B_t<0$, it can be inferred that the soil layer contains sand with good drainage conditions and permeability. In the test results, $B_t$ has both positive and negative values and the corresponding absolute value is not large, indicating that the soil layer is between drained and undrained and contains silt; the soil layer is clay when $B_t>0$. The engineering characteristics of the soil layer can be preliminarily evaluated by the value of $B_t$, and the development of the sedimentary characteristic of soil bedding could also be inferred.

**RESULTS**

**Soil Classification.** The data points for the Ningbo sites can be projected in a soil classification map using the CPTU-based normalized soil behavior type (SBTn) method proposed by Roberston [26], as shown in Figure 4. Extensive engineering experience [27-29] has shown that the combination of the $q_t$, $f_t$ and $u_2$ parameters is a valuable means of soil classification. The rate of pore-pressure dissipation during a pause in the penetration process can also be measured. The CPTU can also provide equilibrium pore pressure after 100% dissipation ($u_0$), which is useful to define the in-situ piezometric profile at that time. Due to the fact that the value of the excess pore water pressure is closely related to the soil type, the change in clay structure can be characterized by the measurement of $u_2$ with RCPTU, and more obvious changes in the values of the testing parameters of strong-structured clay can be seen than with CPT. For instance, the boundary between a sand layer and a clay layer can be identified accurately on the basis of positive and negative values of $B_t$. The soil layer between 22 m and 24 m is identified as a mechanically stratified sandy clay or silt based on CPT. However, it can be observed from the $u_2$ curve that the pore pressure dramatically decreases with depth, indicating that the drainage conditions have improved. The values of $B_t$ change from positive to negative with depth, and the absolute value of $B_t$ is small. The results further indicate that the soil can be classified as silty clay; this is in agreement with the results obtained from drilling reports. The results indicate that it is difficult to discriminate silt or silty clay only by means of traditional CPT, and that the CPTU tests allow more accurate and reliable soil classification than CPT, especially in strong-structured marine clay.

**Electrical Resistivity of Soil.** The electrical resistivity ($\rho$) of soil is the basic parameter used to represent its electrical conductivity, which is highly
correlated to other soil parameters such as porosity, saturation, particle shape and size, gradation, and grain orientation [30]. Therefore, it is considered that it may be possible to infer the structure of a soil on the basis of $\rho$. Previous studies have shown that $\rho$ has a close relationship with the salt content, clay content, plasticity index, moisture content, sensitivity, compression modulus and shear strength of soil [12,31-32]. Figure 3 shows clear changes in the $\rho$ value in the layer from the ground surface to 0.5-0.6 m, which indicates changes to the soil structure. The $\rho$ value also changes dramatically in the depth range 24-25 m because the structures of silty clay and silty sand are quite different. The results also illustrate the electrical resistivity can be used as a reference for soil identification.

The microstructure of clay can be characterized using the electrical resistivity test by introducing the structure factor and establishing a corresponding structural model [30]. Figure 5 presents a schematic diagram of changes to the electrical conduction mechanism with differences in the water content of clays. The process can be divided into three parts: when there is little pore water, the main means of electrical conduction is between particles and there is basically no conductivity between the particles and the pore gas. When a small amount of water is added to the soil, a water film is formed on the surface of the soil particles, and the conductive performance is enhanced; the bound water is the main medium of electrical conduction. When soil water enters continually, the thickness of the water film increases and the pore air inside the soil is partially replaced by the water; the free water is the main medium of electrical conduction.

---

**FIGURE 4**
CPTU data in SBTn chart (Robertson, 1990) [26]
The sensitivity \( S_t \) of clay is defined as the ratio of the undrained shear strength of an undisturbed sample to that of a remolded sample. \( S_t \) is an indicator of structural strength owing to the influence of structure on the undrained strength of soil. The value of \( S_t \) can be estimated from CPTU tests using the friction ratio \( f_t \) method proposed by Schmertmann (1979). The formula is as follows [33]:

\[
S_t = \frac{N_t}{R_t}
\]  

(1)

where \( N_t \) is an empirical coefficient what typically varies from 5 to 10 (Rad and Lunne [34] suggest using the average value, 7.5, and this is adopted in the present study) and \( R_t \) is the friction ratio \( (R_t=f_t/q_t\times100\%) \). FVT tests were also performed at depths that corresponded to the depths of the piezocone penetration tests. There is widespread use of FVT tests to determine the \( S_t \) of natural soft clays [35]; the specific calculation process can be seen in the relevant reference. The change in \( S_t \) with depth is shown in Figure 6. It can be observed that the \( S_t \) values obtained from FVT tests are about 4-5 for mucky soil and about 3-5 for clay, while those obtained with CPTU tests are about 4-13 and 2-8, respectively. The range in \( S_t \) values is large between 2m and 3m, which indicates that \( f_t \) changes dramatically with depth. Sleeve friction \( (f) \) can be considered to reflect remolded strength, and its values may become very small after soil failure, leading to a low \( f_t \) value and thus a large calculated \( S_t \) value as per Equation (1).

The values of \( S_t \) from CPTU tests in the range of 20m to 24m are small than those from FVT tests due to structure effect. The results show that structure has a great influence on sensitivity, and the change in sensitivity reflects the structural characteristics of the soil to a certain extent.

**Undrained Shear Strength.** Undrained shear strength \( (S_u) \) is one of the most important mechanical properties of cohesive soil and plays an important role in the analysis of the soil’s strength and stability. The \( S_u \) values determined from the CPTU data correspond to those measured in an anisotropically consolidated undrained triaxial test sheared in compression-UAUC [24]. The following relationship was employed to calculate the value of \( S_u \):

\[
S_u = \frac{q_t - \sigma_{vo}}{N_t} \times 100
\]

(2)

where \( N_t \) is the cone coefficient, typically \( N_t \) varies from 10 to 18 , and \( \sigma_{vo} \) is total overburden stress. Figure 7 depicts the change in \( S_u \) values from CPTU tests, FVT tests and laboratory tests with the depths. This shows that FVT testing gives \( S_u \) values ranging approximately 14-8 kPa for mucky soil and 20-35 kPa for clay and that CPTU gives values of about 12-5 kPa and 25-0 kPa, respectively. The range in \( S_u \) values obtained from CPTU is larger than that for values from FVT tests. The laboratory test gives \( S_u \) values of about 5.5-10 kPa for mucky soil and
about 10-14 kPa for clay, while the $S_u$ values obtained with FVT tests are about 3-7 kPa for natural soil and 4-8 kPa for remolded soil, respectively. Consequently, the range in the remolded value, $S_u$, obtained with FVT tests are larger than in laboratory tests. This observation illustrates that CPTU testing is more sensitive and accurate for detecting changes in the $S_u$ value of structured clays.

![FIGURE 7 The $S_u$ versus depth](image)

The Coefficient of Lateral Stress. The magnitude of the coefficient of lateral stress, $K_0$ is very important in geotechnical engineering design (such as for the assessment of the bearing capacity of piles). $K_0$ values determined by the CPTU data using the method proposed by Kulhawy and Mayne [36].

Table 2 gives the calculated $K_0$ values from CPTU, laboratory and flat dilatometer (DMT) tests. The $K_0$ values obtained with CPTU are larger than those from laboratory tests and DMT tests, and the differences gradually reduce with depth. It is worth mentioning that the $K_0$ values obtained with CPTU near the ground surface are high, which is a result of the large measured values of $q_i$ in the weathered crust layer. On the whole, the $K_0$ values from CPTU are closer to those from the laboratory test than those from the DMT test, and the $K_0$ values from the DMT test are more dependent on the regional empirical formula, but the overall bias is small. Due to the marine clay in the Ningbo area being strong-structured clay, the change law of the $K_0$ values is not obvious. Undisturbed and remolded clays with different structural characteristics exhibit different mechanical properties. The cementation of the undisturbed soil increases the structural strength of the clay and enhances the lateral deformation of the specimen.

Consolidation Characteristics. In order to better understand the pore pressures dissipation data, the pore water pressures are normalized:

$$U = \frac{U_s - U_0}{u_s - u_0}$$

where $u_s$, $u$, and $u_0$ are a measurement under pore water pressure, the initial pore water pressure and the static pore water pressure, respectively and the units are kPa. The horizontal coefficient of consolidation $(c_h)$ is calculated using the method proposed by Teh and Housley [37], which takes into account the influence of the soil stiffness index $I_s$. The equation can be expressed as:

$$c_h = \frac{I_s \cdot r^2 \cdot \sqrt{h}}{t_{so}}$$

where $t_{so}$ is the time factor that corresponds to the $t_{so}$, 0.245; $R$ is probe radius, which equals 17.85 mm; $I_s$ is stiffness index, which is equal to the ratio of $G_0$ and $S_u$, where $G_0$ is the shear modulus (MPa); $t_{so}$ corresponds to the time of the off dissipation of excess pore pressures up to 50% ($s$).

Figure 8(a) presents the typical eight pore pressure dissipation curves with logarithm of time. Generally speaking, a curve of pore pressure dissipation should show a gradual, monotonic downward trend, whereby the $u_2$ reading is at a maximum during penetration and, when penetration stops, the measured pore water pressure gradually reduces and eventually reaches hydrostatic conditions ($u_0$). It can be observed from Figure 8 that the pore pressure dissipation curves at $h=20$ m, 22 m and 24 m are different from the others; in these, the pore water pressure increases with time until it reaches a peak value, after which it decreases with time. The reason for this phenomenon may be that the structured clay exhibits dilatancy at a low confining pressure. The compressive deformation caused by the effect of probe penetration will cause the structure of the soil to be partly or completely lost. Stress will be released, and the unloading action will put the soil in a negative state. When penetration stops, and the dissipation test begins, the deformation of the structure must be coordinated, and the rest of the soil will be squeezed, which will increase the pore water pressure in this part of the soil. In addition, it can be seen from the depths of pore pressure dissipation that the characteristics of Ningbo marine soft soil are deep.

Figure 8(b) depicts typical curves of normalized excess pore water pressure versus the logarithm of time at the Ningbo test sites. The normalized excess pore-water pressure ($\Delta u/\Delta u_0$) versus the logarithm of time can be used to check for uniformity and homogeneity in clay deposits, where $\Delta u = (u_2 - u_0)$ is measured where there is pore water pressure after penetration stops. It can be observed that these readings are fall into three distinct groupings: (1) the muddy clay at depths of 0 to 4 m; (2) a middle clay layer from depths of 4 to 22 m; and (3) lower silty sand at depths of 23 to 26 m. The degree of consolidation can be obtained by the expression, $U = 100 \cdot (1 - \Delta u/\Delta u_0)$. It should be noticed that the normalized ($\Delta u/\Delta u_0$) dissipation curves exceed 1, which indicated that a slight dilatory response was measured. The slight dilatory behavior appears during CPTU dissipations due to the structure of the marine clays.
TABLE 2

Comparison of the K0

<table>
<thead>
<tr>
<th>Number</th>
<th>Soil layer</th>
<th>Thickness /m</th>
<th>CPTU- K0</th>
<th>Laboratory- K0</th>
<th>DMT- K0</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1)2</td>
<td>Clay</td>
<td>1.3-1.6</td>
<td>0.85</td>
<td>0.52</td>
<td>0.53</td>
</tr>
<tr>
<td>(1)3b</td>
<td>Mucky clay</td>
<td>8.0-9.2</td>
<td>0.63</td>
<td>0.62</td>
<td>0.64</td>
</tr>
<tr>
<td>(2)2b</td>
<td>Mucky clay</td>
<td>3.5-3.6</td>
<td>0.64</td>
<td>0.62</td>
<td>0.55</td>
</tr>
<tr>
<td>(2)2c</td>
<td>Silty clay</td>
<td>1.7-2.1</td>
<td>0.62</td>
<td>0.59</td>
<td>0.49</td>
</tr>
<tr>
<td>(4)2a</td>
<td>Clay</td>
<td>3.0-3.4</td>
<td>0.52</td>
<td>0.50</td>
<td>0.51</td>
</tr>
<tr>
<td>(5)1a</td>
<td>Clay</td>
<td>2.0-2.5</td>
<td>0.44</td>
<td>0.43</td>
<td>0.34</td>
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<tr>
<td>(5)-3b</td>
<td>Silty sand</td>
<td>9.0-12.0</td>
<td>0.34</td>
<td>0.33</td>
<td>0.36</td>
</tr>
</tbody>
</table>

**FIGURE 8**

Pore pressure dissipation curves: (a) excess pore water pressure, (b) normalized excess pore water

**Coefficient of Consolidation.** $c_h$ values estimated based on CPTU data together with estimated values of $c_h$ from the laboratory tests are listed in Table 3. It can be observed that the values of $c_h$ obtained from the CPTU dissipation tests are five times larger than those from the laboratory consolidation tests: the multiple is 1.5 to 8 in mucky clay layers, 1.6 in silty clay and about 14.5 in the clay layer at 20 m. Laboratory tests measure $c_h$ vertically, and its accuracy is influenced by the structure and stress history of soil, while the values of $c_h$ estimated by CPTU dissipation are more accurate and reliable. It is well known that the $c_h$ is the time required for the consolidation of the soil during testing; the larger the $c_h$ value, the faster consolidation will be and the higher the pore water discharge rate. Generally, the pore pressure dissipation time increases with depth but drops substantially at depths of 20 m and 22 m. It can be observed that estimated $c_h$ values from CPTU dissipations decrease with the depth, but there are some local fluctuations, such as $c_h = 7.72 \times 10^{-3}$ cm$^2$/s at a depth of 18 m and $c_h = 44.06 \times 10^{-3}$ cm$^2$/s at a depth of 20 m. The reason for this may be that the structural strength of clay soil increases with depth, indicating that the law of drainage consolidation of clay is affected by its structural characteristics.
TABLE 3
Comparison of consolidation coefficient $C_h$

<table>
<thead>
<tr>
<th>Number</th>
<th>Soil layer</th>
<th>Thickness/m</th>
<th>Measured depth/m</th>
<th>$t_{90}$/s</th>
<th>$C_h$/10^5cm²/s</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CPTU- $C_h$</td>
</tr>
<tr>
<td>1/2</td>
<td>Clay</td>
<td>1.3-1.6</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>1/3b</td>
<td>Mucky clay</td>
<td>8.0-9.2</td>
<td>4.0</td>
<td>216</td>
<td>41.20</td>
</tr>
<tr>
<td>2/2b</td>
<td>Mucky clay</td>
<td>3.5-3.6</td>
<td>15.0</td>
<td>1060</td>
<td>8.40</td>
</tr>
<tr>
<td>2/2c</td>
<td>Silty clay</td>
<td>1.7-2.1</td>
<td>18.0</td>
<td>1153</td>
<td>8.04</td>
</tr>
<tr>
<td>4/2a</td>
<td>Clay</td>
<td>3.0-3.4</td>
<td>20.0</td>
<td>202</td>
<td>44.06</td>
</tr>
<tr>
<td>5/1a</td>
<td>Clay</td>
<td>2.0-2.5</td>
<td>22.0</td>
<td>251</td>
<td>35.45</td>
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<tr>
<td>5/3b</td>
<td>Silty sand</td>
<td>9.0-12.0</td>
<td>24.1</td>
<td>2128</td>
<td>4.18</td>
</tr>
</tbody>
</table>

DISCUSSION

Shear failure occurs when the probe penetrates evenly into the soil under the driving force of the vehicle. This is because the soil structure within a certain range around the probe will be squeezed, producing a remolding effect. According to the Saint Venant principle, the degree of disturbance will be greater with greater proximity to the probe. The damage to the soil structure caused by rod penetration is made more obvious by the high sensitivity and strong structure of the Ningbo clay. The soil around the probe becomes dense and the permeability of the soil decreases rapidly due to the compression effect when the structure is damaged, which makes test indexes inaccurate and distorted. Therefore, in-situ testing can reflect soil deformation under certain stress and boundary conditions.

CONCLUSIONS

In this paper, the RCPTU tests were performed to study the structure of the marine soft clay. The main conclusions are as follows:

1. The penetration index of Ningbo marine clay is high, with an average value of $q_c = 1.0-3.0$ MPa, and its $R_t$ is about 0.85%. The electrical resistivity of soil is a good indicator of the soil’s microstructure and can be used as a reference for identifying different soil layers. Compared with CPT tests, RCPTU tests can more accurately classify soil layers, especially for strong-structured marine clay.

2. The values of $S_t$ and $S_r$ from the CPTU tests are larger and more variable than those from a vane shear test. The values of $S_t$ and remolded $S_r$ varied substantially due to the strong structure of the soil. The values of $S_r$ from the CPTU tests are close to those from laboratory tests, further indicating that CPTU is a reliable and accurate in-situ method.

3. The cementation of natural soil particles enhances the structural strength of clay, reinforcing the resistance of the soil sample to lateral deformation. The influence of soil structure on the change law of $K_0$ values is not obvious.

4. The greater the burial depth of the soil, the shorter the dissipation time and the larger the coefficient of consolidation, due to the influence of the soil’s strong structure. A dilatory response was measured in the early stage of pore pressure dissipation. The coefficients of consolidation of Ningbo marine clay from CPTU tests are 1.5-14.5 times those from laboratory tests, indicating that the influence of structure on the consolidation of the soil is very significant.

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REFERENCES


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NEW APPROACH TO TEST RUST DISEASES USING SOWING TIME FOR WHEAT RESISTANCE UNDER NATURAL CONDITIONS

Hasan Ay*

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ABSTRACT

Main objective is to assess rust diseases severity of different wheat varieties under different sowing time to find best sowing time with highest rusts levels under natural conditions. High level of diseases existence is important to escape diseases inoculation and its costs during breeding studies.

Experiment was conducted during 2008-2009, 2009-2010 and 2010-2011 wheat growing seasons for 3 years in Adana in Turkey. In this study, 10 bread and 2 durum wheat varieties were tested for yellow rust, leaf rust and stem rust diseases. Rust diseases severities were assessed with 5 different sowing time under natural rust disease inoculations.

There were strong relationships between sowing time and stem rust disease in 3 years. When sowing time delayed from November to April, stem rust disease severity significantly increased due to increased stem rust spores in April sown wheat materials. Instead, yellow rust disease severity was not significantly affected from sowing time. This result may be related to low yellow rust infection coefficients of wheat varieties. Leaf rust showed different responses to sowing times in 3 years, so different sowing time method may probably not function properly for this disease.

As a sole result of this research, April sowing will be the best adopted sowing time to obtain high stem rust diseases existence in wheat in Mediterranean climate conditions to test breeding materials for stem rust under natural conditions.

KEYWORDS:
Wheat, sowing time, rust diseases, disease assessment

INTRODUCTION

Wheat crop ranks first in production area in the world due to ease of cultivation, variety richness, appropriateness for animal nutrition and industrial purposes and high adaptation ability to ecological conditions [1]. The rust diseases caused by fungi are among the most important biotic factors in wheat which drastically decrease grain yield and quality [2]. Therefore, wheat varieties should be improved to resistance to rust diseases like other biotic and abiotic factors [1].

Races of yellow rust (*Puccinia striiformis*) cause major losses at low temperature and high humidity conditions where wheat crops are severely affected in the middle and west Anatolian plateau of Turkey. Additionally, similar epidemics were occurred in past in costal lines and south west of Turkey at suitable climate conditions.

Wheat leaf rust (*Puccinia recondita*) occurs wherever wheat is grown and it is the most common of all cereal rust. Therefore, the leaf rust causes more damage than other wheat rusts at the cereal crops [3, 4]. Minimum leaf rust severity was recorded on early wheat sown 30th November where maximum disease severity was observed on wheat sown on 30th of November in Pakistan in 2009 [5]. Stem rust or stem rust caused by *Puccinia graminis* is one of the earliest known diseases of wheat. The stem rust severely affects wheat crops when plant vegetation period is long and production zone locates in high altitudes but also can causes diseases in coastal zones such as Cukurova region of Turkey. Stem rust can be more detrimental than any other cereals diseases which can cause epidemics and destroy whole crop in a less than a month [2].

Main objective is to assess rust diseases severity of different wheat varieties under different sowing time to find best sowing time with highest rusts levels under natural conditions.

MATERIALS AND METHODS

The research was conducted under natural conditions during 2008-2009, 2009-2010 and 2010-2011 growing seasons for 3 years in Cukurova Region of Turkey where Mediterranean climate is existing. The experimental design was a randomized complete block design with two replications. In the study, 10 bread wheat varieties (Adana-99, Ceyhan-99, Seri-82, Karatopak, Osmaniym, Yüreğir-89, Quaiu, Chonto, Munal) and two durum wheat varieties (Amanos-97 and Fuatbey-2000) were tested with 5 different sowing times (December, January,
February, March and April) in Adana, Turkey. Research field was fertilized with pure 60 kg ha⁻¹ P₂O₅ applied pre-sowing and 150 kg ha⁻¹ pure nitrogen split in twice, which first was applied at sowing time and second was at tillering times. The plant material was individually sown above mentioned times, if seedlings and plants needed water they were appropriately irrigated.

Severity rust diseases was recorded regularly according to the modified Cobb’s scale under the natural inoculations [6]. Infection coefficients were used described by [7] and [8]. Disease intensity and host reaction were multiplied. The coefficient of infection (CI) was calculated by multiplying disease severity (DS) and constant values of infection type (IT). The constant values for infection types were used based on; immune: 0, resistant (R): 0.1, moderately resistant (MR): 0.4, moderately susceptible (MS): 0.8 and susceptible (S): 1, [7, 8] and 9]. The CI value is very important when many host varieties to compare for resistance where the CI value low denotes low IT [9]. Meanwhile, monthly rainfalls (Figure 1), relative humidity (Figure 2) and temperature (Figure 3) were regularly monitored to understand any relationships between the sowing time, disease severity and environmental factors.

![Rainfall (mm) for Adana-Turkey](image1)
**FIGURE 1**
Average monthly rainfalls for three years in Adana, Turkey

![Relative Humidive for Adana-Turkey](image2)
**FIGURE 2**
Averages of relative humidity in Adana, Turkey

![Temperature (celcius) for Adana-Turkey](image3)
**FIGURE 3**
Averages of temperature in Adana, Turkey
RESULTS

Different sowing time and rust disease severity interactions were studied using 10 bread and 2 durum wheat cultivars under natural inoculations for 3 years at Çukurova region in Turkey.

Relationship of sowing time and stem rust diseases. Generally, wheat-sowing times are November and December months for Çukurova region. Wheat plantings have not been infected with stem rust because optimum growing temperature is 25 °C for stem rust. This temperature reaches in the middle of the May in Çukurova Region. When wheat varieties sow in November and December, they achieve physiologically matured in Çukurova Region. They escaped to stem rust due to physiologically matured and seems be resistant, despite most of wheat varieties are susceptible. When wheat materials were sown in February, March and April months, they were infected heavily with the stem rust and they had revealed more sensitivity to the fungal pathogen within delayed sowing time (Figure 4, 5 and 6).

Influence of stem rust on wheat varieties in different sowing time in 2008-2009, 2009-2010 and 2010-2011 years given Figure 4, 5 and 6.

![Effect of stem rust on bread and durum wheat varieties in 2008-10 years](image1)

**FIGURE 4**
Influence of stem rust on wheat varieties in different sowing time in growing seasons 2008-09

![Effect of stem rust on bread and durum wheat varieties in 2009-10 years](image2)

**FIGURE 5**
Influence of stem rust on wheat varieties in different sowing time in growing seasons 2009-10
Total coefficients of stem rust infections were increased each delayed month and the highest susceptibility were obtained in April sowing plantings during the 3-year field trials (Figure 7,8 and 9). This situation was distinctive for stem rust which caused severe diseases on all tested wheat varieties except Osmaniye bread wheat variety (Figure 4,5 and 6). Similarly; rust development occurs optimum at 26 °C but its development could be reduced significantly under 15 °C and above 40 °C temperatures. High level of yield losses are occurred by appropriate climate conditions with elevated air temperature leading rust diseases in later stages of wheat development [12]. The results clearly indicate that, if breeding wheat materials would be tested with the stem rust pathogen under natural inoculation, the materials should be sown in April at Çukurova conditions. In this study, the Osmaniye cultivar is the most resistant wheat variety against the stem rust disease under natural inoculations at all sowing times in Çukurova region.

Total coefficient of stem rust infection on sowing time interactions in 2008-2009, 2009-2010 and 2010-2011 years given Figure 7, 8 and 9.

Relationship of sowing time and stripe (yellow) rust diseases. Under natural inoculations, 10 bread and 2 durum wheat cultivars were assessed using 5 different sowing times with yellow rust diseases from 2008 to 2011 growing seasons. There is no strong relationship were found between wheat varieties coefficients of infection to stripe rust and different sowing times (Figure 10, 11 and 12). However, February sown wheat varieties seem to be more susceptible in 2 years field trials. Results are not reliable because coefficients of infection to yellow rust disease are low and only a few cultivars are infected with the pathogen (Figure 13,14 and 15). Therefore, the results could not provide reliable assessment method to test wheat materials in delayed sowing time contrary to [10] due to different location. Adjustment of sowing date is also an important and effective way of decreasing and eliminating early infection. Delaying sowing date can greatly reduce disease incidence in autumn-sown seedlings [10]. Delay of sowing date of wheat is an important and effective way of avoiding early infections in autumn-sown wheat [11].

Effect of yellow rust on wheat varieties in different sowing time in 2009, 2010 and 2011 years was given Figure 10, 11 and 12 respectively.

Total coefficient of yellow rust infection on sowing time interactions in 2008-09, 2009-10 and 2010-11 years given Figure 13, 14 and 15.

Relationship of sowing time and leaf rust diseases. 12 bread wheat materials are tested in 5 different sowing times with leaf rust pathogens for 3 years. In each year, the leaf rust diseases occurred on 12 wheat cultivars where were sown in different months had not shown specific disease severities (Figure 16, 17 and 18). There are no interactions obtained between plant materials sown in 5 different times and disease coefficients of leaf rust infections in three years results (Figure 19, 20 and 21). Thus, the different sowing times could not use for testing to identify resistance breeding materials against leaf rust pathogen under natural inoculations.

Influence of leaf rust on wheat varieties in different sowing time in 2008-09, 2009-10 and 2010-11 years given Figure 16, 17 and 18 respectively.

Total coefficient of leaf rust infection on sowing time interactions in 2008-09, 2009-10 and 2010-11 years given Figure 19, 20 and 21.
FIGURE 7
Total coefficient of stem rust infection on sowing time interactions in 2008-09

FIGURE 8
Total coefficient of stem rust infection on sowing time interactions in 2009-10

FIGURE 9
Total coefficient of stem rust infection on sowing time interactions in 2010-11

FIGURE 10
Effect of yellow rust on bread and durum wheat varieties in 2008-09
Effect of yellow rust on bread and durum wheat varieties in 2010

FIGURE 11

Effect of yellow rust on bread and durum wheat varieties in 2009-10

FIGURE 12

Interaction of total coefficient of yellow rust of wheat varieties on sowing time in 2011

FIGURE 13

Interaction of total coefficient of yellow rust of wheat varieties on sowing time in 2008-09

FIGURE 14

Interaction of total coefficient of yellow rust of wheat varieties on sowing time in 2009-10
Interaction of total coefficient of yellow rust of wheat varieties on sowing time in 2010-11

FIGURE 15

Interaction of total coefficient of yellow rust of wheat varieties on sowing time in 2008-09

Effect of leaf rust on bread and durum wheat varieties in 2008-09

FIGURE 16

Effect of leaf rust on bread and durum wheat varieties in 2009-10

FIGURE 17
FIGURE 18
Effect of leaf rust on bread and durum wheat varieties in 2010-11

FIGURE 19
Interaction of total coefficient of leaf rust of wheat varieties on sowing time in 2008-09

FIGURE 20
Interaction of total coefficient of leaf rust of wheat varieties on sowing time in 2009-10
Leaf rust caused by *Puccinia triticina* is a common disease on wheat in the coastal regions of Turkey. The brown rust mainly affects irrigated crop fields in Middle Anatolia, Aegean, Marmara and Black Sea where minimum 50% crop yield reduced due to the fungal disease [14, 13]. The leaf rust does not cause sudden epidemics and great crop losses unlikely stripe and stem rusts. Nevertheless, the leaf rust continuously makes diseases every year with various degrees on wheat fields considering significant level crop lost for long periods in total [15]. The damage occurred by leaf rust on leaves producing pustules and limiting photosynthesis areas. Hence, crop loss, 1000 seeds weight, hectoliter weight is reduced leading less protein concentration and quality in wheat grains [3, 15].

**DISCUSSION**

Normally, delayed sowing is not advice for farmers; however, if breeding material would test instantly against rust diseases delayed sowing is a suitable way to understand resistant wheat cultivar(s). Delayed sowing in April could allow for identifying resistant cultivars to stem rust disease under natural conditions at Çukurova region, Turkey. Because resistant wheat cultivars that were sown in November or December have already reached maturity stages where stem rust pathogen is very active. This is a possibility to make mistakes during selecting resistant plant materials in natural conditions. Under these circumstances, optimum-sowing time for testing against stem rust disease could be April month. Delayed sowings require supplementary irrigation should be applied for all breeding materials.

In warm climates like Çukurova region, wheat is planted in late fall and harvested in early summer. Stem rust grows above 25 °C temperatures where these conditions reaches after May 10th. Meanwhile, wheat plants reach physiological maturity in the same time and stem rust could not affect all the plant materials due escaping disease. They seem to be resistance to the stem rust.

Delayed sowing times encourage intensities of stem rust rather than normal sowing times. Moreover, this testing method cheaper for stem rust to the other common way

There is not significant relationship obtained between wheat materials and yellow rust disease incidence, February sowing time sown that, wheat varieties had elevated disease severity on wheat varieties in different 2 years. But, the results could not be reliable because of infection coefficients are low and few wheat varieties are infected with yellow rust.

Also, there is no relationship between sowing time and leaf rust diseases under natural inoculations. Wheat varieties coefficients of infection changed year by year. Therefore, delayed sowing time is not a suitable way to assess plant material against leaf rust.

Different sowing times and testing wheat materials for resistance against stem rust could be new approach that will be able to provide quick, reliable and sensitive technique under natural inoculation at Çukurova region, Turkey.

**ACKNOWLEDGEMENTS**

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## REFERENCES


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MATERIALS AND METHODS

Plant material was collected from a total of 183 walnut trees from 13 sites. Sampling the plant materials collected from Eastern Anatolia and different locations such as; Erzincan (Üzümli, Kemah, Kemaliye, Çatal Armut Köyü, Merkez-Bahçe Bitkileri Araştırma Merkezi), Erzurum (Narman-Tortum, Tortum-Pazaryolu (Yedi Gölér); Uzundere, Oltu, İspir, Pazaryolu-Yesil vadi Aktaş, Maden Köy), Artvin (Yusufeli), and Istanbul (Sultan Ahmet Camisi). The height of walnut trees were about 20-30 m and their diameter about 0.2-1 m. The area covered with snow from 15 December-30 April. There are daily and partly rainfall between May-June and then the rainfall start from September to early December.

The trees were not sprayed with pesticide and fungicides. At least ten leaves from each tree collected and transformed into the laboratory. From infected areas of leaves and shoots cut to 5x5 mm pieces and with surface sterilization with 70% ethanol for one minute and cultivated on PDA (LAB) medium and incubated at 25 °C and darkness condition. The fungal isolates investigated under the limited studies on the microbial flora of walnut [2]. Some fungal pathogen reported on walnut from different countries [3]. Also, there are studies about the microbial biodiversity of European walnuts [2]. Besides that, there is a different report about microbial pathogens of walnut from Turkey [4]. The fungal pathogens such as; Microstroma juglandis [4]; Ascochyta juglandis [5]; Ophiognomonia leptostyla [5]; Pestalotiopsis guenii [6]; Phyllosticta juglandis [5]; Phytophthora chamissydouspora, Fusarium moniliforme, F. solani, Alternaria alternata [7]; some bacterial diseases such as; Xanthomonas campestris pv. juglandis [8] and Xanthomonas arboricola pv. Juglandis [7] have been reported on walnut trees from Turkey. This study was conducted to investigate the microbial biodiversity on leaves, shoots, and fruits on walnut trees in Anatolia region of Turkey to determine the diversity of fungal and bacterial species.
TABLE 1
The Fungal isolates from walnut

<table>
<thead>
<tr>
<th>Fungus Species</th>
<th>Leaves</th>
<th>Fruit</th>
<th>Shoots</th>
<th>Number of Observed Isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alternaria alternata</td>
<td>*</td>
<td>*</td>
<td></td>
<td>50</td>
</tr>
<tr>
<td>Alternaria solani</td>
<td>*</td>
<td>*</td>
<td></td>
<td>39</td>
</tr>
<tr>
<td>Aspergillus falkus</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>3</td>
</tr>
<tr>
<td>Aspergillus niger</td>
<td>*</td>
<td>*</td>
<td></td>
<td>5</td>
</tr>
<tr>
<td>Aspegillus ochraceus</td>
<td>*</td>
<td></td>
<td></td>
<td>9</td>
</tr>
<tr>
<td>Aspergillus parasiticus</td>
<td>*</td>
<td></td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Cladosporium cladosporioides</td>
<td>*</td>
<td>*</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Chaetomium globosum</td>
<td>*</td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Drechslera sp.</td>
<td>*</td>
<td>*</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Fusarium solani</td>
<td>*</td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Fusarium incarnatum</td>
<td>*</td>
<td>*</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Melanospora zamiae</td>
<td>*</td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Microstroma juglandis</td>
<td>*</td>
<td></td>
<td></td>
<td>7</td>
</tr>
<tr>
<td>Penicillium chrysogenum</td>
<td>*</td>
<td></td>
<td></td>
<td>6</td>
</tr>
<tr>
<td>Penicillium citrinum</td>
<td>*</td>
<td></td>
<td></td>
<td>12</td>
</tr>
<tr>
<td>Penicillium expansum</td>
<td>*</td>
<td></td>
<td></td>
<td>5</td>
</tr>
<tr>
<td>Penicillium italicum</td>
<td>*</td>
<td></td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>Phoma glomerata</td>
<td></td>
<td>*</td>
<td>*</td>
<td>10</td>
</tr>
<tr>
<td>Rhizopus stolonifer</td>
<td>*</td>
<td>*</td>
<td></td>
<td>13</td>
</tr>
<tr>
<td>Mucor hiemalis</td>
<td>*</td>
<td>*</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Sclerotinia sclerotiorum</td>
<td>*</td>
<td></td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>Septofusidum sp.</td>
<td>*</td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Stempyllium sp.</td>
<td>*</td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Trichothecium roseum</td>
<td>*</td>
<td>*</td>
<td></td>
<td>4</td>
</tr>
<tr>
<td>Ophiognomana leptostyla</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>111</td>
</tr>
</tbody>
</table>

Total Fungal Isolates 292

TABLE 2
The bacterial isolates from walnut

<table>
<thead>
<tr>
<th>Bacteria species</th>
<th>Leaves</th>
<th>Number of isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacillus subtilis</td>
<td>*</td>
<td>51</td>
</tr>
<tr>
<td>Erwinia carotovora</td>
<td>*</td>
<td>12</td>
</tr>
<tr>
<td>Xanthomonas arboricola pv. juglandis</td>
<td>*</td>
<td>33</td>
</tr>
<tr>
<td>Pseudomonas sp.</td>
<td>*</td>
<td>18</td>
</tr>
</tbody>
</table>

Total Bacteria Isolates 104

light microscope (BH2) with sterile water, lactophenol, and acid fusion slide microscopic preparation. To identification the fungal isolates at least 50 spores measured and different identification key and fungal description have been used [8-11]. Also, the bacterial isolate identified with the GC-MS apparatus library in Phytobacteriological Laboratory in Department of Plant Protection, Faculty of Agriculture, at Ataturk University.

RESULTS AND DISCUSSION

During the investigation of walnut leaves and shoots, different fungal and bacterial species isolated in mediums and microscopical sampling. According to the identification keys, the fungal species classified in 17 genera and 23 species and total 292 fungal isolates collected. Also, four bacterial species from 104 bacterial isolates were identified by GC-MS apparatus library in Phytobacteriological Laboratory, Department of Plant Protection, at Ataturk University.

Although the studied area has cold climate and covered with snow for nine months in a year, the results of this survey showed us there is significant fungal biodiversity on walnut shoots and leaves in Eastern Anatolia. The leaves of walnut trees in this area start to emerge in mid-April. The leaf spots on walnut observed in last of May and early June and the last of September and early of December, the walnut leaves fall down the trees. In short period of growing season, different fungal propagation could infect and colonization on the walnut leaves. Among the fungal species, the asexual stage of Ophiognomana leptostyla was made most abundant leaf spots on the leaves, shoots, and fruits of trees. The infected trees with walnut anthracnose casual agents showed
a high percentage of infection. Almost all the walnut leaves were infected with this pathogen and the leaf spots were covered until to 10-50% of each leaf. There were acervulus and conidia of fungi in most of the leaf spots and because of this disease, there were limited numbers (20-50) of the nut on each old tree. Another most abundant fungal species on the leaves were *Alternaria alternata*, *A. solani*, *Aspergillus ochraceus*, *Rhizopus stolonifer*, *Penicillium chrysogenum* and *Microstroma juglandis*. Except for three fungi (*Fusarium solani*, *Chaetomium globosum*, and *Phoma glomerata*), all other fungal species were isolated from walnut leaves, too (Table 1). Besides that, four bacterial isolates have been identified from walnut leaves such as; *Bacillus subtilis*, *Erwinia carotovora*, *Xanthomonas arboricola pv. juglandis* and *Pseudomonas* sp. (Table 2). All the bacterial isolates were isolated from walnut leaves.

In previous studies on walnut in Turkey, some fungal (*Alternaria alternate*, *Ascochyta juglandis*, *Fusarium moniliforme*, *F. solani*, *Microstroma juglandis*, *Ophiognomonia leptostyla*, *Pestalotiopsis guepinii*, *Phyllosticta juglandis*, *Phytophthora chlamydospora*) and bacteria (*Xanthomonas campestris pv. juglandis* and *Xanthomonas arboricola pv. juglandis*) species have been reported [5-8, 12]. According to the result, there are significant fungal and bacterial diversity on the walnut in Eastern Anatolia, Turkey and some of the have potential to be a disease problem in walnut production.

**REFERENCES**


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STUDY OF DECOLORIZATION KINETICS OF REACTIVE RED B-2BF IN FENTON OXIDATION PROCESS

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ABSTRACT

Online spectrophotometric technique was adopted to monitor the degradation of simulated Reactive Red B-2BF (RR B-2BF) solution with Fenton oxidation process. The effects of initial FeSO₄ dosage, initial H₂O₂ dosage, pH value, and initial dye concentration on chroma removal have been discussed. The kinetic mechanics of the decolorization of RR B-2BF, the decolorization rate (R) and the constant (kₚ) were investigated. The results show that the color removal rate of RR B-2BF is 93.47% under the optimum dosage of H₂O₂ 0.96 mM, pH 4.0, FeSO₄ (Fe²⁺) dosage 0.09 mM, and the initial dye concentration 16 mg/L after 300 s. The chroma removal process can be divided into two stages. The intrinsic reaction rate constant of •OH with RR B-2BF in aqueous solution is 2.09 ×10⁶ M⁻¹s⁻¹ in Fenton oxidation process. The molecule structure of RR B-2BF is decomposed and not mineralized thoroughly in Fenton oxidation process (300 s). Online spectrophotometric technique is an accurate, fast, and feasible technique to monitor color removal rate of RR B-2BF with Fenton process.

KEYWORDS:
Fenton oxidation process, Reactive Red B-2BF, kinetics, wastewater, online spectrophotometry, decolorization

INTRODUCTION

In recent years, reactive dyes have been widely used in the textile industry and generate serious pollution problems to human’s living environment. The reactive azo dye wastewater discharged from various chemical industries contains many organic components [1], which are resistant to water, light and oxidizing agents. Therefore, it is very difficult to degrade reactive dye wastewater once released into aquatic systems. The low concentrations reactive dyes in effluent can also produce visible chroma (different colors) and undesirable feeling [2, 3] in living environment. Moreover, the reactive dye wastewater maybe has an adverse effect on the exposed organisms and high risk to the ecosystems [4]. With the environment preservation becoming one of major social concern, the discharge standard for the textile dye wastewater have gradually become more stringent, more effective processes are required to deal with the toxic pollutants [5]. Nevertheless, the conventional physical, chemical and biological methods commonly used to treat the reactive dye wastewater cannot completely remove organic compounds and are commonly non-destructive, inefficient, costly and resulted in the production of secondary waste products [2, 4, 6, 7, 8]. Therefore, it is very necessary to develop an effective technology to treat reactive azo dye wastewater.

The Fenton oxidation process is an effective homogeneous advance oxidation process (AOP) [9-17] to degrade reactive dyes. The Fenton process can produce highly oxidative hydroxyl radicals (•OH) from an acidic mixture of hydrogen peroxide (H₂O₂) and ferrous ions (Fe²⁺) [18] by Fe²⁺+H₂O₂ →Fe³⁺+OH+OH. Hydroxyl radicals are powerful non-selective oxidants with high oxidizing potential, which can react with dissolved contaminants and effectively remove chromaticity and reduce chemical oxygen demand. The hydroxyl radicals easily attack the unsaturated functional group of dye molecule and the chromophore is destroyed and decolorized [18]. In the present paper, online spectrophotometric monitoring method is selected because it is a potential technique to investigate instantaneous color concentration during dyes wastewater treatment [6, 11, 17]. This technique has many merits such as high effectiveness, real time and small sample volume, and so on. Online spectrophotometric system provides continuous and real-time measurement of the degradation process of the azo dye. It can continuously monitor to identify color variation, study the reaction kinetics, analyze the results in real time, and reveal the transient processes of Fenton reactions. In recent years, online spectrophotometric technique has been used to investigate many dyes color removal and degradation processes. It is a convenient monitoring method for fast color removal. It can reduce the experimental error and improve experimental efficiency.
In this study, the Reactive Red B-2BF (RR B-2BF) was chosen as a model pollutant. The RR B-2BF dye is an azo type with aromatic organic compounds. Fenton oxidation was applied to decompose simulated RR B-2BF solution. The feasibility analysis of Fenton oxidation process of degradation was monitored using the online spectrophotometry system. The effect of various experimental parameters including the ferrous ions catalyst (Fe²⁺) dosage, hydrogen peroxide (H₂O₂) dosage, initial dye concentration, and the pH value. Fenton oxidation kinetic performance was investigated based on experimental result and kinetic parameters were estimated.

**MATERIALS AND METHODS**

**Reagents.** The structure of RR B-2BF is listed in Figure 1. Reactive Red B-2BF (RR B-2BF) was purchased from Shijiazhuang Dyestuffs Company (China) and the RR B-2BF solution was prepared by dissolving a requisite quantity of dye in doubledistilled water. Ferrous sulfate (FeSO₄·7H₂O) was purchased from Sigma-Aldrich (>99.5% purity). Hydrogen peroxide (H₂O₂) was purchased from Fisher Scientific (30% purity; density 1.13 kg/L). Sulfuric acid (H₂SO₄) was supplied by Merck (AR). They were of reagent analytical grade.

![FIGURE 1](image1)

**FIGURE 1**

The structure of Reactive Red B-2BF (RR B-2BF)

**Apparatus set-up.** Online spectrophotometric system is shown in Figure 2. Reaction section (degradation device) includes a digital magnetic stirrer apparatus (Shanghai Instrument company, China), and a 250 mL beaker. Optical measuring unit contains UV-Vis spectrometer (UNICO 2802, Shanghai, China), cycle peristaltic pump and cuvette (1 mL). The velocity of wastewater in system was 22 mL/min. Recording part is a computer with the monitoring frequency of 12 min⁻¹ during the oxidation process.

**Experimental procedure.** Fenton oxidation process was performed in a beaker with 250 mL the vessel. 200 mL simulated wastewater which was made up with the concentration of RR B-2BF (8–32 mg/L) and ferrous ion (0.04–0.24 mM) was added into vessel. The designed pH value (2.2–4.4) was adjusted by adding H₂SO₄. With the help of peristaltic pump, the simulated dye wastewater was pumped into the cuvette of UV-Vis spectrophotometer. Absorbance at maximal absorption peak of dye was obtained by spectrophotometer. The certain concentration H₂O₂ (0.80–2.00 mM) was added into the wastewater, and the computer began to record experimental absorbance results.

**IC method.** Inorganic ions (Cl⁻, NO₃⁻, and SO₄²⁻) in aqueous solution were measured by Metrohm-881 Ion Chromatography (IC) with conductivity detector, tower type IC column (4×250 mm) and MagIC Net software. The eluent was a mixture of Na₂CO₃ (1.8 mmol/L) and NaHCO₃ (1.7 mmol/L) with the flow rate of 1.0 mL/min.

**Feasibility analysis of online spectrophotometric technique.** Online spectrophotometry method was applied to analyze in Fenton process. The UV-Vis spectra of RR B-2BF, H₂O₂, H₂SO₄, Fe²⁺, and Fe³⁺ are presented in Figure 3. Azo dye RR B-2BF has a maximum adsorption peak of 542 nm, which do not vary with addition of H₂SO₄, Fe²⁺, Fe³⁺ (Figure 4). Therefore, the 542 nm was selected as monitoring wavelength in the present paper. Instant recorded absorbance results were transferred to concentrations of dye on the basis of the criterion equation. The absorbance (A) of the 542 nm over concentration (C) of RR B-2BF relationship is \( A = 0.003 + 0.01487C \) (\( R = 0.99995 \)).

![FIGURE 2](image2)

**FIGURE 2**

Online spectrophotometric system

![FIGURE 3](image3)

**FIGURE 3**

Comparison of dye UV–Vis spectra between dye and dye (+H₂SO₄+ Fe²⁺+ Fe³⁺)
RESULTS AND DISCUSSION

Time-dependent degradation of RR B-2BF. The relationship of C/C₀ value of RR B-2BF dye over reaction time is illustrated in Figure 5. C₀ and C represent the initial concentration of dye and the instant concentration of dye with time, respectively. Fenton oxidation process of RR B-2BF solution consists of two stages. The decolorization is very fast at the first stage (less than 25 s). In the second stage, decolorization is considerably slow. This experimental result can be explained by two reasons: Firstly, the catalyst FeSO₄ and hydrogen peroxide in solution is consumed in Fenton oxidation. On the other hand, intermediate products or byproducts that produced in the reaction decreased apparent rate of dye degradation. Fenton oxidation process obviously abode by first-order kinetics (ln(C₀/C) = kₚt) in the first stage. The slope (kₚ) represents the first-order rate constants. In this paper, the decolorizing reaction rate constant (kₚ) and decolorization rate of dye (R = (C₀−C)/C₀×100%, where R denotes the color removal rate of dye at 300 s) were examined under different reaction conditions.

Effects of FeSO₄ dosage. A series of FeSO₄ concentrations (Fe²⁺) on the rate constant kₚ and the decolorization rate R of RR B-2BF are shown in Figure 6 and Table 1. The decolorization rate of RR B-2BF and the kₚ increase from 90.52% to 93.47%, and from 0.03039 to 0.04532 s⁻¹, respectively, with the FeSO₄ dosage from 0.04 to 0.09 mM. However, the decolorization rate decreases to
79.43% and the rate constant was 0.03573 s⁻¹ when FeSO₄ dosage rises to 0.24 mM. At the beginning of the Fenton oxidation process, reactions \( \text{Fe}^{2+} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{3+} + \text{OH}^− + \text{OH}^− \) and RR B-2BF + OH− → Product could happen and the reactive azo dye RR B-2BF begins to decolorize. But as \( \text{Fe}^{2+} \) dosage increases and reaches a certain value, the reaction \( \text{Fe}^{2+} + \text{OH}^− \rightarrow \text{Fe}^{3+} + \text{OH} \) [19] could happen, that ferrous ion \( \text{Fe}^{2+} \) competing for OH with dye molecules. This experimental phenomenon suggests that higher FeSO₄ dosage cannot improve decolorization rate of azo dye RR B-2BF. Therefore, 0.09 mM of initial FeSO₄ concentration can be regarded as an optimum dosage. The decomposition of \( \text{H}_2\text{O}_2 \) and production of \( \cdot \text{OH} \) can be accelerated by FeSO₄ catalyst.

![Graph](image)

**FIGURE 7**

Effect of initial \( \text{H}_2\text{O}_2 \) concentration

\((|\text{RR B-2BF}|=16 \text{ mg L}^{-1}, |\text{Fe}^{2+}|=0.09 \text{ mM}, \text{pH}=4.0)\)

**TABLE 1**

The \( k_{dp} \) and decolorization rate of RR B-2BF dye in different \( \text{Fe}^{2+} \) concentrations in the Fenton oxidation process

<table>
<thead>
<tr>
<th>( \text{Fe}^{2+} ) (mM)</th>
<th>Decolorization rate (%)</th>
<th>( k_{dp} ) (s⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.04</td>
<td>90.52</td>
<td>0.03039</td>
</tr>
<tr>
<td>0.09</td>
<td>93.47</td>
<td>0.04532</td>
</tr>
<tr>
<td>0.12</td>
<td>91.75</td>
<td>0.04175</td>
</tr>
<tr>
<td>0.16</td>
<td>86.80</td>
<td>0.040009</td>
</tr>
<tr>
<td>0.20</td>
<td>81.73</td>
<td>0.0379</td>
</tr>
<tr>
<td>0.24</td>
<td>79.43</td>
<td>0.03573</td>
</tr>
</tbody>
</table>

**TABLE 2**

The \( k_{dp} \) and decolorization rate of RR B-2BF dye in different \( \text{H}_2\text{O}_2 \) concentrations in the Fenton oxidation process

<table>
<thead>
<tr>
<th>( \text{H}_2\text{O}_2 ) (mM)</th>
<th>Decolorization rate (%)</th>
<th>( k_{dp} ) (s⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.80</td>
<td>92.96</td>
<td>0.03917</td>
</tr>
<tr>
<td>0.96</td>
<td>93.62</td>
<td>0.04387</td>
</tr>
<tr>
<td>1.20</td>
<td>92.90</td>
<td>0.04169</td>
</tr>
<tr>
<td>2.00</td>
<td>90.77</td>
<td>0.03775</td>
</tr>
</tbody>
</table>

**Effects of initial \( \text{H}_2\text{O}_2 \) dosage.** The powerful oxidative hydroxyl radical that generated by ferrous ion \( \text{Fe}^{2+} \) with \( \text{H}_2\text{O}_2 \) can degrade RR B-2BF in solution [20-22]. Figure 7 and Table 2 display the decolorizing kinetics constant \( k_{dp} \) and decolorization rate of RR B-2BF under various \( \text{H}_2\text{O}_2 \) dosages and a fixed initial \( \text{Fe}^{2+} \) concentration. The decolorization rate of RR B-2BF increases from 92.96% to 93.62% with the \( \text{H}_2\text{O}_2 \) concentration rising from 0.80 to 0.96 mM. The decolorization rate is 90.77% when \( \text{H}_2\text{O}_2 \) concentration is 2.00 mM. As seen from Figure 7, color removal rate has a slight decrease with the range of experimental \( \text{H}_2\text{O}_2 \) dosage. The range of \( k_{dp} \) is between 0.03917 s⁻¹ and 0.04387 s⁻¹ with \( \text{H}_2\text{O}_2 \) concentration from 0.80 to 0.96 mM. When \( \text{H}_2\text{O}_2 \) dosage is 0.96 mM, the \( k_{dp} \) is 0.04387 s⁻¹. The experimental result manifests that R and \( k_{dp} \) of reactive azo dye RR B-2BF increase with certain \( \text{H}_2\text{O}_2 \) dosage range (0.80-0.96 mM). This result can be explained by the enhancement in the quantum yield of formation of \( \cdot \text{OH} \) radical at the beginning process [6, 23] \( \text{Fe}^{2+} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{3+} + \text{OH} + \cdot \text{OH} \) and RR B-2BF + OH− → Product. When the \( \text{H}_2\text{O}_2 \) dosage is above the optimum value (0.96 mM), decolorization rate decreases due to hydroperoxy radical (as a scavenger of hydroxyl radical) which is generated by excess \( \text{H}_2\text{O}_2 \) \( (\text{H}_2\text{O}_2 + \cdot \text{OH} \rightarrow \text{H}_2\text{O} + \text{OH}^−) \) [23, 24]. Hydroperoxy radical promotes the radical chain reactions, but its oxidation potential is much lower than that of \( \cdot \text{OH} \). For this reason, hydroperoxy radical does not contribute to the oxidative destruction of RR B-2BF dye molecule in the aqueous solution [23]. It is important to control the initial \( \text{H}_2\text{O}_2 \) concentration. The amount of hydrogen peroxide should be enough for the degradation of reactive azo dye B-2BF, but a high concentration would be adverse to the degradation of azo dye and would increase the cost of the wastewater treatment [8]. Therefore, we selected 0.96 mM as an optimum \( \text{H}_2\text{O}_2 \) dosage for the most effective degradation of RR B-2BF during Fenton oxidation process.

![Graph](image)

**FIGURE 8**

Effect of pH value \((|\text{RR B-2BF}|=16 \text{ mg L}^{-1}, |\text{Fe}^{2+}|=0.09 \text{ mM}, |\text{H}_2\text{O}_2|=0.96 \text{ mM})\)
Effects of pH value. The pH of the solution plays an important role on dye degradation for Fenton process [6, 17, 25]. It is also an important operational variable in real wastewater treatment. The influences of pH value on decomposition of RR B-2BF by Fenton oxidation are illustrated in Figure 8 and Table 3. The results indicated that the degradation of azo dye RR B-2BF was largely influenced by the pH of the solution. With the pH value increasing from 2.2 to 4.0, the dye decolorization rate constant and k_{ap} increase from 72.15% to 93.00% and from 0.012 to 0.03073 s^{-1}, respectively. But the decolorization rate and k_{ap} drop to be 86.37% and 0.02891 s^{-1}, respectively, with the further increasing of pH 4.4. So, pH value 4.0 is considered the optimum value of degrading RR B-2BF in Fenton oxidation. The decolorization rate and reaction rate are limited at lower pH (<4.0) [25, 26]. The reason could be explained from three aspects. First, hydrogen peroxide can form an oxonium ion with a proton (H_{2}O_{2} + H^{+} → H_{3}O^{+}). The oxonium ion makes hydrogen peroxide to reduce the reactivity with ferrous ion [27]. Second, the formed complex compounds [Fe(H_{2}O_{6})_{4}]^{2+} and [Fe(H_{2}O_{6})_{5}]^{3+} also restrict reaction of ferrous ion with hydrogen peroxide [28].

<table>
<thead>
<tr>
<th>pH</th>
<th>Decolorization rate (%)</th>
<th>k_{ap} (s^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.2</td>
<td>72.15</td>
<td>0.012</td>
</tr>
<tr>
<td>3.1</td>
<td>89.55</td>
<td>0.02004</td>
</tr>
<tr>
<td>3.5</td>
<td>91.61</td>
<td>0.02462</td>
</tr>
<tr>
<td>4.0</td>
<td>93.00</td>
<td>0.03073</td>
</tr>
<tr>
<td>4.4</td>
<td>86.37</td>
<td>0.02891</td>
</tr>
</tbody>
</table>

In addition, -OH radical is consumed by the excessive hydrogen ion (the scavenging effect: -OH + H^{+} + e^{-} → H_{2}O) [25, 29]. In conclusion, when pH value of dye solution is below 4.0, the Fenton oxidation reaction (Fe^{2+}+H_{2}O_{2} → Fe^{3+}+OH+OH^{-} and Fe^{3+}+H_{2}O_{2} → Fe^{2+}+H^{+} + OOH) could be slowed down because of above reasons [8]. While pH value is higher than 4.0 in Fenton process, Fenton oxidizing ability decreases because of the decomposition of hydrogen peroxide and deactivation of the ferrous catalysts which formed ferric hydroxy complexes [8]. These results show that the Fenton oxidation has the best activity in a weakly acidic environment. Therefore, Fenton oxidation is sensitive to the pH value of the dye RR B-2BF aqueous solution. Similar experimental results were reported Table 4. We have compared various initial dye concentrations, H_{2}O_{2} dosages, pH value, Fe^{2+} dosages and removal efficiency with Fenton oxidation in previous papers.

FIGURE 9 Effect of initial dye concentration ([Fe^{2+}]_0=0.09 mM, [H_{2}O_{2}]_0=0.96 mM, pH=4.0)

Effects of initial RR B-2BF concentration. The effect of initial dye concentration on decolorization rate and the decolorizing reaction constant k_{ap} was listed in Figure 9 and Table 5. The decolorization rate of RR B-2BF and the k_{ap} decrease from 95.22% to 90.12% and from 0.08381 to 0.0478 s^{-1}, respectively, with the Fe^{2+} concentration increases from 8 mg/L to 32 mg/L. As seen from Figure 9, there is a slight change in the decolorization rates with the selected dye concentration range. It is observed that the lower the dye concentrations were, the higher the color removal and k_{ap} for degradation of RR B-2BF are. The rise of dye concentration leads to an increase in the number of dye molecules in aqueous solution but the amounts of hydroxyl radical concentration keeps the same (constant [H_{2}O_{2}]_0 and [Fe^{2+}]_0). So the decolorization and the decolorizing constant (k_{ap}) exhibit a decrease with increasing dye concentration [25].

<table>
<thead>
<tr>
<th>Dye type</th>
<th>Initial dye concentration</th>
<th>Optimum H_{2}O_{2} dosage</th>
<th>optimumpH</th>
<th>Optimum Fe^{2+} dosage</th>
<th>Removal efficiency</th>
<th>Sources</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid Red 66 and</td>
<td>2.94×10^{-5}M</td>
<td>5.00×10^{-5}M</td>
<td>3.5</td>
<td>2.50×10^{-5}M</td>
<td>99.8%</td>
<td>[6]</td>
</tr>
<tr>
<td>Direct Blue 71</td>
<td>50 mg/L</td>
<td>0.50mM</td>
<td>3.5</td>
<td>0.025mM</td>
<td>99.25%</td>
<td>[8]</td>
</tr>
<tr>
<td>Amido Black 10B</td>
<td>4×10^{-5}M</td>
<td>2.5×10^{-5}M</td>
<td>3.85</td>
<td>3.5×10^{-5}M</td>
<td>-100%</td>
<td>[11]</td>
</tr>
<tr>
<td>Reactive Red 6B</td>
<td>6.63×10^{-5}M</td>
<td>1.0×10^{-5}M</td>
<td>4.0</td>
<td>3.5×10^{-5}M</td>
<td>94.6%</td>
<td>[30]</td>
</tr>
<tr>
<td>Orange G</td>
<td>100 mg/L</td>
<td>125 mg/L</td>
<td>3.0</td>
<td>3 mg/mL</td>
<td>94%</td>
<td>[31]</td>
</tr>
<tr>
<td>Direct Blue 71</td>
<td>20 mg/L</td>
<td>0.15 mM</td>
<td>3.5</td>
<td>0.015 mM</td>
<td>92.7%</td>
<td>[32]</td>
</tr>
<tr>
<td>reactive Red B-2BF</td>
<td>16 mg/L</td>
<td>0.96 mM</td>
<td>4.0</td>
<td>0.09mM</td>
<td>93.47%</td>
<td>This study</td>
</tr>
</tbody>
</table>

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TABLE 5
The $K_{ap}$ and decolorization rate of RR B-2BF dye in different dye concentrations in the Fenton oxidation process

<table>
<thead>
<tr>
<th>Dye concentration (mg L$^{-1}$)</th>
<th>Decolorization rate (%)</th>
<th>$K_{ap}$ (10$^{-3}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>95.22</td>
<td>0.08381</td>
</tr>
<tr>
<td>16</td>
<td>93.60</td>
<td>0.07201</td>
</tr>
<tr>
<td>24</td>
<td>91.35</td>
<td>0.06051</td>
</tr>
<tr>
<td>32</td>
<td>90.12</td>
<td>0.0478</td>
</tr>
</tbody>
</table>

Kinetics study. The kinetics can be described as pseudo-first order with respect to the dye concentration. That is: $-dc/dt = K_{ap}$, $\ln C_0/C = K_{ap}t$.

The mechanism for degradation of RR B-2BF dye is expressed in Eqs. (1)-(9) [33]. The reactions rate of degradation of dye may be defined as:

$$Fe^{2+}+H_2O_2 \xrightarrow{k_1} Fe^{3+}+OH^+OH^-, \quad k_1= 76 \text{ M}^{-1}\text{s}^{-1}$$  \hspace{1cm} (1)

$$\text{RR B-2BF} + OH^- \xrightarrow{k_2} \text{Product}_{\text{oxid}}$$  \hspace{1cm} (2)

$$\text{Fe}^{2+}+OH^- \xrightarrow{k_3} \text{Fe}^{3+}+OH^-, \quad k_3=3.2 \times 10^8 \text{ M}^{-1}\text{s}^{-1}$$  \hspace{1cm} (3)

$$H_2O_2 + \text{OH}^- \xrightarrow{k_4} \cdot OH + H_2O_2, \quad k_4=4.5 \times 10^7 \text{ M}^{-1}\text{s}^{-1}$$  \hspace{1cm} (4)

$$\cdot OH + \text{OOH} \xrightarrow{k_5} H_2O+O_2, \quad k_5=6.6 \times 10^{11} \text{ M}^{-1}\text{s}^{-1}$$  \hspace{1cm} (5)

$$\text{Fe}^{3+}+H_2O_2 \xrightarrow{k_6} \text{Fe}^{2+}+H^++\text{OOH}, \quad k_6=0.02 \text{ M}^{-1}\text{s}^{-1}$$  \hspace{1cm} (6)

$$\text{Fe}^{3+}+\text{OOH} \xrightarrow{k_7} \text{Fe}^{2+}+H^++O_2, \quad k_7=3.1 \times 10^5 \text{ M}^{-1}\text{s}^{-1}$$  \hspace{1cm} (7)

$$\text{Fe}^{2+}+\text{OOH} \xrightarrow{k_8} \text{Fe}^{3+}+\text{HO}_2^-, \quad k_8=1.2 \times 10^6 \text{ M}^{-1}\text{s}^{-1}$$  \hspace{1cm} (8)

The reaction rate of dye can be defined as:

$$\frac{d[R][B-2BF]}{dt} = k_1 \cdot \text{OH} \cdot [\text{RR B-2BF}][9]$$  \hspace{1cm} (9)

According to the steady-state assumption, [-OH] can be obtained as follow:

$$\frac{d\cdot \text{OH}}{dt} = k_2 \cdot [\text{Fe}^{2+}][\text{H}_2\text{O}_2] \cdot k_3 \cdot \text{OH} \cdot [\text{RR B-2BF}] \cdot k_4 \cdot [\text{Fe}^{3+}] \cdot [\cdot \text{OH}] \cdot [\text{H}_2\text{O}_2] \cdot k_5 \cdot [\cdot \text{OH}] \cdot [\cdot \text{OOH}]=0$$  \hspace{1cm} (10)

$$\frac{d\cdot \text{OOH}}{dt} = k_2 \cdot [\text{Fe}^{3+}][\cdot \text{OH}] \cdot [\cdot \text{OH}] \cdot [\text{H}_2\text{O}_2] \cdot k_5 \cdot [\cdot \text{OH}] \cdot [\cdot \text{OOH}] \cdot k_6 \cdot [\cdot \text{Fe}^{2+}] \cdot [\cdot \text{Fe}^{3+}][\cdot \text{OOH}]$$  \hspace{1cm} (11)

On account of $k_6$ being negligible, compared to $k_7$, $k_8$, $k_9$, and $k_8$, so Eq.11 can be written as:

$$k_7 \cdot [\cdot \text{OH}] \cdot [\cdot \text{OH}] \cdot [\cdot \text{OH}] \cdot [\text{H}_2\text{O}_2] \cdot k_5 \cdot [\cdot \text{OH}] \cdot [\cdot \text{Fe}^{3+}] \cdot [\cdot \text{Fe}^{3+}][\cdot \text{OOH}]$$  \hspace{1cm} (12)

$k_7$ and $k_8$ were also negligible compared to $k_9$, so Eq.12 can be written as:

$$k_9 \cdot [\cdot \text{OH}] = k_9 \cdot [\text{H}_2\text{O}_2] \cdot [\cdot \text{OH}]$$  \hspace{1cm} (13)

According to Eqs. (10) and (13), we obtain:

$$\frac{d\cdot \text{OH}}{dt} = k_2 \cdot [\text{Fe}^{3+}][\cdot \text{OH}] \cdot [\cdot \text{OH}] \cdot [\text{H}_2\text{O}_2] \cdot k_4 \cdot [\cdot \text{OH}] \cdot [\cdot \text{Fe}^{3+}]$$  \hspace{1cm} (14)

$$\cdot \text{OH} = \frac{k_3 \cdot [\cdot \text{Fe}^{3+}][\cdot \text{H}_2\text{O}_2]}{k_1 \cdot [\cdot \text{Fe}^{3+}][\cdot \text{H}_2\text{O}_2] + k_2 \cdot [\cdot \text{Fe}^{3+}] + 2k_1 \cdot [\cdot \text{H}_2\text{O}_2]}$$  \hspace{1cm} (15)

Combined Eqs. (9) with (15), we obtained:

$$\frac{d[R][B-2BF]}{dt} = k_1 \cdot [\cdot \text{OH}] \cdot [\cdot \text{OH}] \cdot [\cdot \text{OH}] \cdot [\cdot \text{OH}] \cdot [\cdot \text{Fe}^{3+}] \cdot [\cdot \text{Fe}^{3+}] \cdot [\cdot \text{Fe}^{3+}] \cdot [\cdot \text{Fe}^{3+}]$$  \hspace{1cm} (16)

Thus, Eq. (16) deduces to:

$$\frac{d[R][B-2BF]}{dt} \cdot \text{OH} \cdot [\cdot \text{OH}] \cdot [\cdot \text{OH}] \cdot [\cdot \text{OH}] \cdot [\cdot \text{Fe}^{3+}] \cdot [\cdot \text{Fe}^{3+}] \cdot [\cdot \text{Fe}^{3+}]$$  \hspace{1cm} (17)

Fenton oxidation process follows first-order kinetics, it can be described:

$$\frac{d[R][B-2BF]}{dt} = k_{ap} \cdot [R][B-2BF]$$  \hspace{1cm} (18)

The $k_{ap}$ is apparent reaction rate constant, we combine Eqs. (17) with (18) and obtain:

$$\frac{[H_2O_2]}{k_{ap}} = \frac{k[R][B-2BF]}{B}$$  \hspace{1cm} (19)

The experimental results were displayed in Figure 10. The linear relationship between $[H_2O_2]/k_{ap}$ and $[RR B-2BF]_0$ ([RR B-2BF]_0 = 16 mg L$^{-1}$, $Fe^{3+}$] = 0.09 mM, $[H_2O_2]$ = 0.96 mM, pH = 4.0) Analysis of products after Fenton oxidation.
Aimed at analyzing structure of RR B-2BF after Fenton oxidation, UV-Vis and IC were used to investigate intermediates or byproducts. The reaction condition is described as below: the dosage of FeSO₄ is 0.09 mM; the dosage of H₂O₂ is 0.96 mM; RR B-2BF concentration is 16 mg/L; pH value is 4.0; temperature is 25°C; the reaction time is 300 s.

**UV-Vis spectrum.** Figure 11 listed the UV-Vis spectral changes of dyes in Fenton oxidation process. RR B-2BF dye has a maximum absorbability (λₘₐₓ = 542 nm) in visible spectral area and intensive absorption from 450 to 600 nm at 0 s. However, there is no absorbability in visible area but only in UV range after Fenton oxidation within 300 s. The chemical structure of RR B-2BF dye is destroyed by Fenton reagent, and decolorization of RR B-2BF wastewater can effectively be realized. However, strong absorbance in ultraviolet region indicates that some intermediates or byproducts may produce after Fenton oxidation process. Therefore, RR B-2BF dye is not mineralized thoroughly by Fenton reagent.

**FIGURE 11**

UV-Vis spectral of dyes during Fenton oxidation

**Analysis of IC.** Inorganic products were analyzed by Ion Chromatography. There are a number of SO₄²⁻ ions (10.6 mg/L) in solution. It illustrates that the chemical structure of RR B-2BF is damaged by Fenton oxidation. However, NO₃⁻ (3.76 mg/L) and Cl⁻ ions (2.66 mg/L) exist in oxidation process embodies that the oxidation reaction is not thorough in less than 300 s in aqueous solution. If the oxidation is fully completed, there are plenty of NO₃⁻ and Cl⁻ ions in solution. Therefore, RR B-2BF is not mineralized in 300 s during Fenton oxidation process.

**CONCLUSION**

Online monitoring spectrophotometric technology is greatly advantageous in recording detailed color removal information of RR B-2BF regarding the Fenton process. The color removal process can be divided into two stages: the first stage (25 s) is faster decolorization with high decolorization efficiency, while the second stage is fairly slower with low decolorization rate.

The decolorization rate (R) and its constant (kₒ) are investigated in Fenton process. The optimum initial dosages for H₂O₂ and FeSO₄ (Fe²⁺) are 0.96 mM and 0.09 mM under pH value of 4.0. The lower the dye concentration, the higher the decolorization rate and kₒ. According to the mechanism of Fenton oxidation process, a kinetic modeling has been obtained to acquire intrinsic reaction rates of ·OH and dyes. The intrinsic rate constant of RR B-2BF solution is 2.09×10⁶ M⁻¹ s⁻¹ in Fenton oxidation process.

Fenton oxidation process can rapidly decompose the dye RR B-2BF after 300 s, and decolorization of dye wastewater is effectively accomplished by the UV-Vis and IC analysis. However, mineralization is not completely performed in 300 s with Fenton oxidation process.

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**REFERENCES**


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EFFECT OF DIFFERENT CHEMICALS INJECTED SURGES ON CHEMICALS APPLICATION AND DISTRIBUTION UNIFORMITY THROUGH FURROW IRRIGATION IN CLAY SOIL UNDER DELTA REGION OF EGYPT

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ABSTRACT

Increasing water and chemicals efficiencies always has been one of the main concerns of experts and farmers. Using surge to inject fertilizers and chemicals through the irrigation system led to increase the efficiency of distribution of fertilizers and chemicals in the effective root zone of plants which means applying the correct amount of chemicals in the right time with right concentration subsequently reduce wastage rate, health protection for farmers and reduce the risk of environmental pollution of ground water. The developing of management for surge flow irrigation led to access a high application efficiency, water distribution uniformity, fertilizer application and fertilizer uniform in comparison to traditional furrow irrigation. In the context, the experiment was carried out at a private farm on Tanta city, Gharbeia Governorate, Egypt during season 2017. The aim of the present work was to developing and improving water and fertilizer application efficiency through surge flow technique under clay textured soil for corn crop in Delta Egypt. Treatments were 4, 5 and 6 surges with 0.56, 0.75 and 0.95 l sec⁻¹ flow rates of water and chemicals injected surge applied uniformly in a 140 meters long line. The results showed that: Water application efficiency. The treatment of 6 surges was recorded the highest water application efficiency at flow rate 0.95 l sec⁻¹ which was 79 % and the lowest value was 56% for continuous flow with flow rate 0.56 l sec⁻¹ treatment. Water distribution uniformity. The best value was obtained under surge flow with 5 surges which was 93 % and the lowest value was 56% for continuous flow with flow rate 0.56 l sec⁻¹ treatment. Water distribution uniformity. The best value was obtained under surge flow with 5 surges which was 93 % and the lowest value was 56% for continuous flow with flow rate 0.56 l sec⁻¹ treatment. Chemicals application efficiencies. The highest value was 91 % that obtained under 3rd and 4th injected surge with chemicals for 5 and 6 surges treatments respectively at 0.56 l sec⁻¹ flow rate. Chemicals distribution uniformity. The highest value was 70 % under 5 surges treatment through 3rd and 4th injected surge and 6 surges treatment during 4th injected surge using 0.75 l sec⁻¹ flow rate.

KEYWORDS:
Surge, furrow irrigation, chemicals, fertilizer, uniformity.

INTRODUCTION

The river Nile is the main source of water in Egypt. Which the share of Egypt in the flow of the river Nile is at least 55.5 billion m³/year. In the recent years the Government established large scale agricultural projects in order to compensate the population growth. surface irrigation considered the main irrigation system for old lands. These additional water demands require an increased efficiency in water use [1].

Surge flow irrigation is a surface irrigation method, consisting of furrows, which convey the water from a pipe or open channel to the field. In many cases the water is applied continuously, but for surge flow irrigation the flow is intermittent [2]. In surge flow irrigation the recommended number of surges is expected to front of water to the end of furrow ranges from 4 to 6 surges. This number of surges is expected to give the highest irrigation efficiency and consequently the highest distribution uniformity [3], [4] and [5]. Fertigation can be defined as the application of chemicals or soil amendments via an irrigation system by injecting the soluble into water flowing [3]. Fertilizer can be added by two methods by allow soluble flow into the irrigation water or by using injection pump [2]. Fertigation by pulses could reduce leaching and runoff losses in surface irrigation systems [6]. Applied fertilizer through irrigation system usually used by drip and sprinkler systems, while it is much less use with surface irrigation methods [7]. The aim of the present work was to developing and improving water and fertilizer distribution uniformity through surge flow technique under clay textured soil in Delta region.

MATERIALS AND METHODS

The field experiments were conducted at a private farm on Tanta city, Gharbeia Governorate, Egypt during season 2017. The experimental site
was ploughed by a nine mounted shares chisel plough, the average value of ploughing depth was 0.3 m, two passes and using self-rotary levelling laser to level the soil at slope 0.1 %. The furrow was designed to be V shape, 0.7 m spacing and 140 m length. Fertilizer requirement for Zea Maize were designed to be V shape, 0.7 m spacing and 140 m length. Fertilizer was added during seedbed preparation, 300 kg/fed ammonium nitrates 33.5%. 100 kg/fed Potassium sulfates 48% was divided into two doses and added with irrigation water which used as a fertilizer by fertigation solution and allow it to flow by gravity through some form of a constant head metering valve at some distance between furrows. Irrigation systems networks are consisting of the following components:

- Control head unit. Centrifugal pump, 6.5 hp (4.8 kW), gasoline engine was used for pumping water from main canal to irrigation system.

- Gated pipes. PVC gated pipes, 110 mm diameter were constructed at the head of the field experiment to converting the water through gates under control.

- Inflow rate. Three different inflow rates were used in the present study (0.56, 0.75 and 0.95 l sec⁻¹). Flow rate was calculated by calibration method, where a constant volume of water and the time required for receiving this volume of water.

- Number of surges. Three numbers of surges were used S1, S2, S3 and C (4, 5, 6 surges and continues flow respectively). Based on the guidelines of [3], [4] and [5] for surge flow irrigation the recommended number of surges for front of water to reach the end of furrow ranges from 4 to 6 surges. This number of surges is expected to give the highest irrigation efficiency and consequently the highest distribution uniformity.

d- Fertilizer injection method. Chemical injection tank 200 liters volume was connected 16 mm hose by- pass valves to isolate the tank output chemicals. Tow tanks were placed on the rise one and half meters above soil surface. Each tank was connected with valve 16 mm and calibrated in liters for fertilizer solution and allow it to flow by gravity through some form of a constant head metering valve at some convenient an open channel. Inject the soluble will depend on the discharge flow rate and on the concentration needed during the on-time of the irrigation time.

Experimental Factors. The experiment included on the following factors:

a- Irrigation systems. Traditional furrow irrigation system and surge flow technique were considered in the present study. Furrow length was 140 m and 0.7 m distance between furrows. Irrigation systems networks are consisting of the following components:

- Control head unit. Centrifugal pump, 6.5 hp (4.8 kW), gasoline engine was used for pumping water from main canal to irrigation system.

### TABLE 1

<table>
<thead>
<tr>
<th>Depth, cm</th>
<th>Particle size distribution, %</th>
<th>Texture</th>
<th>Bulk density, gm/cm³</th>
<th>W.P., %</th>
<th>Available water, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-15</td>
<td>22.8 30.4 46.8</td>
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<tr>
<td>15-30</td>
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<td>Clay</td>
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<tr>
<td>30-45</td>
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<td>Clay</td>
<td>1.22 37.1 17.3 19.2</td>
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<tr>
<td>45-60</td>
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<td>Clay</td>
<td>1.25 35.3 16.8 18.3</td>
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</tr>
<tr>
<td>Average</td>
<td>25.4 29.4 45.2</td>
<td>Clay</td>
<td>1.12 37.8 18.7 20.1</td>
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</table>

### TABLE 2

<table>
<thead>
<tr>
<th>Depth, cm</th>
<th>EC, ds/m</th>
<th>PH</th>
<th>Cations</th>
<th>Anions</th>
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<td>45-60</td>
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<td>0.75</td>
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<tr>
<td>Average</td>
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</tr>
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</table>

### TABLE 3

<table>
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<th>PH</th>
<th>Cations</th>
<th>Anions</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.47</td>
<td>8.1</td>
<td>4.00</td>
<td>2.6</td>
</tr>
</tbody>
</table>
Injected surges for chemicals were:
- 2nd and 3rd surges for 4 surges treatments,
- 2nd, 3rd and 4th surges for 5 surges treatments,
- 2nd, 3rd, 4th and 5th surges for 6 surges treatments.

Taking into consideration for all treatments, the first and the last surge of irrigation were not used for chemigation.

The previous factors affected the following:

**Soil moisture.** Soil moisture percentage was determined gravimetrically at three selected sites along the furrow. The soil samples were taken at three points along furrow and four depths at root zone (0-15, 15-30, 30-45 and 45-60 cm) before and 48 hours after each irrigation. The samples are usually collected from the field over some depths interval. The soil sample was weighted and placed in an oven maintained at 105°C. Usually, the soil samples are left in the oven for 24 hours and reweighed after drying. The results are expressed as a ratio of the mass of water lost to the mass of dry soil, to determine moisture content as percentage on dry mass basis as shown below:

\[
\% \text{Moisture} = \frac{M_w - M_d}{M_d} \times 100
\]

Where:
\( \% \text{Moisture} \) = Moisture content on dry mass basis, %
\( M_w \) = Mass of wet sample, g.
\( M_d \) = Mass of oven dry sample, g.

**Water application efficiency.** Water application efficiency is the ratio between water added to the root zone to the total water applied. It was calculated according to [8] as follows:

\[
E_a = \frac{D_{ad}}{D_{ap}} \times 100
\]

Where:
\( E_a \) = Water application efficiency (%),
\( D_{ad} \) = Depth of water added to the root zone (mm), and
\( D_{ap} \) = Depth of water applied to the furrow (mm).

**Water Distribution uniformity.** Distribution uniformity was calculated as [9] as follows:

\[
DU = \frac{D_{min}}{D_{sf}} \times 100
\]

Where:
\( DU \) = Distribution uniformity (%),
\( D_{min} \) = The minimum infiltrated depth (mm), and
\( D_{sf} \) = The mean infiltrated over the furrow length (mm).
**Fertilizer injection rate.** The fertilizer injection rate was calculated using, [10] equations as follow:

\[ Q = \frac{F \cdot A}{C \cdot T \cdot I} \]

Where:
- \( Q \) = Injection rate of completely soluble fertilizer into the irrigation system, l h\(^{-1}\)
- \( F \) = Fertilizer application rate per irrigation cycle, kg fed\(^{-1}\)
- \( A \) = Irrigation area in limited time, fed.
- \( C \) = Concentration of the actual nutrients in liquid fertilizer, kg l\(^{-1}\)
- \( T \) = Irrigation time, h
- \( I \) = Ratio between fertilizing and irrigation time.

**Salt Distribution Patterns.** Salt distribution and accumulation under different irrigation treatments is an important factor for evaluation of each irrigation systems. The accepted system produces a remarkable moisture distribution in the root zone and removes salts far from it. Electrical conductivity (EC) for each gravimetric soil samples has been measured using EC meter. The values of EC were used in constructing the pattern (1:5) of chemicals distribution for each treatment.

**Fertilizer distribution uniformity.** Statistical uniformity of fertilizer was evaluated by using the following equation [11]:

\[ U_s = 100 \left( 1 - \frac{S_q}{q'} \right) \]

Where:
- \( U_s \) = The statistical uniformity coefficient, %
- \( S_q \) = The sum of absolute deviation of each sample from the mean (g l\(^{-1}\)).
- \( q' \) = The mean of solution concentration, (g l\(^{-1}\)).

**Grain yield (ton fed\(^{-1}\)).** From one square meter at each treatment the grain yield were determined and multiplied by area of fedden.

**Fertilizer use efficiency (FUE).** Fertilizer use efficiency has been used to describe the relationship between corn crop production and the amount of fertilizer used. It was calculated according to [12] as follow:

\[ FUE \left( \frac{kg}{kg - N} \right) = \frac{\text{total grain yield}}{\text{total applied of N - fertilizer}} \]

**RESULTS AND DISCUSSION**

**Amount of applied water.** The amount of irrigation water which added to each treatment illustrated in Figure 2; the obtained results indicated that all treatments of surge flow received less amount of water than that continuous flow. The lowest values of water applied were obtained from using 6 surges treatment which were 2463.1, 2139.4 and 2003.6 m\(^3\) fed\(^{-1}\) for different inflow rate 0.56, 0.75 and 0.95 l sec\(^{-1}\) respectively. It is interesting to mention that the water savings were 21.3, 27.2 and 30.1 % respectively in comparison with surface irrigation (the control treatment). These results may be due to by using the surge flow water is applied intermittently causes the advance time in all surge treatments was longer than that in continuous flow. Consequently, a little of water is lost by deep percolation at beginning of the furrow and water can be advance through the furrow faster. Reducing of deep percolation at the upper of furrow and runoff at the end led to more uniform distribution of water along the furrow and less total water applied. These results agree with the results obtained by [13] and [14].

![Figure 2](image-url)  
**FIGURE 2**  
Amount of applied irrigation water under different number of surges, inflow rates and continuous flow irrigation treatments.
Water application efficiency. The highest application efficiency was recorded in case of 6 surges treatment and flow rate 0.95 l sec\(^{-1}\) which was 79\%. While the lowest application efficiency was recorded with continuous flow irrigation treatment which was 56\% under flow rate 0.56 l sec\(^{-1}\) as presented in Figure 3. This might be due to the surge irrigation improves the irrigation process and decreased applied water this might be attributed to the reduction of run-off and deep percolation losses under surge irrigation. Generally water application efficiency increases as the amount of water applied during each irrigation decreases. However, the very small irrigation may not fill the root zone adequately and may reduce crop yields, and in the long run increase the salt problems due to inadequate leaching. Similar results were obtained by [15].

Water distribution uniformity. The best values of distribution uniformity were obtained under surge flow with 5 surges which were 83, 93 and 86\%, while the lowest values were occurred under continuous irrigation which was 66, 69 and 73\% at the same inflow rate 0.56, 0.75 and 0.95 l sec\(^{-1}\) respectively as illustrated in Figure 4. These results attributed to that surge flow technique tends to reduce the water infiltrated down under effective depth of root zone and leads to a faster water advance along furrow, which causes in a suitable distribution uniformity of the water more along the line water. Similar results were obtained by many workers such as [17], [15] and [16].
**Fertilizer distribution uniformity.** The highest percent values of chemicals distribution uniformity were obtained was 63 % under 5 surges through 3rd and 58 % at 4th injected surge with chemicals under flow rate 0.95 l sec\(^{-1}\) and with 6 surges treatment during 3rd and 4th injected surge with chemicals under flow rate 0.95 l sec\(^{-1}\) which was 58 % as shown in Table 4 and illustrated in Figures 5 and 6.

We can noticed that the fertilizer concentrate with low values at the upper depth, while it is evident that the water added with surge flow technique plays an important role in concentrate chemicals and minerals in the root zone of plants. This probably due to the ratio between fertilizer concentration and amount of applied water where the application and distribution of water and fertilizer are efficient and uniform, with lowest runoff at the end of the furrow, and less deep percolation at the inlet flow of the furrow which means applying the chemicals with less wastage rate by deep percolation or loses by tailwater and reduce the risk of environmental pollution of ground water. The following step wise regression equations showed that the highest chemicals distribution uniformity was (R\(^2\) = 96 %):

\[
FDU = 37.59 Q + 4.27 N - 0.34 J.S
\]

Where:
- \(FDU\) = Fertilizer distribution uniformity, (%)
- \(Q\) = Inflow rate l sec\(^{-1}\),
- \(N\) = Number of surges,
- \(J.S\) = Injected surge.

Similar results were obtained by many workers such as [2] reported that fertilizer can be added by two methods by allow soluble flow into the irrigation water or by using injection pump.

**FIGURE 5**

A and B chemicals distribution uniformity under different number of surges, (2\(^{nd}\), 3\(^{rd}\)) injected surge with chemicals and inflow rates.
### TABLE 4
Fertilizer distribution uniformity %, Yield, kg/fed and Fertilizer use efficiency under different treatments.

<table>
<thead>
<tr>
<th>Discharge, l/s</th>
<th>Fertilizer injected surge</th>
<th>Fertilizer Distribution uniformity, %</th>
<th>Grain yield, kg/fed.</th>
<th>Fertilizer use efficiency, kg/kg-P</th>
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<td>43</td>
<td>3240</td>
<td>32.4</td>
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**Grain yield.** Data presented in Table 4 showed that the highest value grain yield was obtained by the surge flow treatment under 5 surges which was 3650 kg fed<sup>-1</sup> at discharge of 0.75 l sec<sup>-1</sup>. In generally, all surge flow irrigation system treatments recorded higher values of corn grain yield at different discharge rates compared with continuous furrow irrigation treatments. Mean values of the grain yields for continuous method were 3060, 3125 and 3190 kg fed<sup>-1</sup> for the discharge of 0.56, 0.75 and 0.95 l sec<sup>-1</sup> respectively. While it was 3650, 3730 and 3575 kg fed<sup>-1</sup> under 5 surges at 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> treatments respectively, at the same discharge rate. These results may have been attributed to encourage plants to grow in favorable conditions of soil aeration, moisture distribution uniformity and suitable concentrate chemicals and minerals in the root zone of plants along the furrow.

**Fertilizer use efficiency (FUE).** The fertilizer use by the crop is generally described in terms of fertilizer use efficiency in kg/kg-potassium. It can be defined as the crop fertilizer use efficiency which is the ratio of crop yield to the amount of fertilizer applied. Data presented in Table 4 showed that the treatment 5 surges give the best results for fertilizer use efficiencies 37.3 kg/kg-potassium compared with all other treatments.

From previous data by increasing discharge rate increase value of FUE at the same number of surges, we also find by increment number of surges from 4 to 5 surges the values of WUE increased. These results may be due to that surge flow irrigation treatments had higher water distribution uniformity, less water losses by deep percolation and less amount of water applied, this conditions leads to high grain yield and less nitrogen fertilizer lost to deep percolation therefore obtain high fertilizer use efficiency.

**CONCLUSIONS**

From these results, it can be concluded that: The different number of surges should be considered according to the length of irrigation furrow and water flow rate. Soil physical characteristics should also be undertaken. Meanwhile [4] reported that, the optimal surge flow regime has to be determined for each field situation according to cycle time, flow rate, advance phase, intake opportunity time and depth of application. In general, from the sum of the averages of the results of water and chemical use and distribution efficiencies under the same test condition we can see that; using pulsed irrigation 5 surges treatment with a flow rate of 0.95 l sec<sup>-1</sup>, gives a satisfactory result for the development of surface irrigation to raise the skills of the use and distribution of irrigation, fertilizers and chemicals.
It can be also concluded that, as for surge flow technique the fertilizer concentration after 48 hours after irrigation were taken lower values at the upper depth of all treatments, while it is evident that the water added with surge flow technique plays an important role in concentrate chemicals and minerals in the root zone of plants at 60 cm approximately and keep them from leaching with ground water to be lowest at the deeper depths. On the other hand, it can be concluded that, fertilizer can be added through irrigation systems and growers can used this methods and automated it in their farms.

ACKNOWLEDGEMENTS

Analysis of the mechanical and chemical properties for soil and water was procedure in Central Laboratory for Environmental Studies, Kafrelsheikh University.

REFERENCES

DETERMINATION OF OPTIMUM SOWING DATES OF SAFFLOWER (CARTHAMUS TINCTORIUS L.) IN DRY CONDITIONS

Hasan Koc*

Bahri Dagdas International Agricultural Research Institute, Konya, Turkey

ABSTRACT

This study was conducted in three years 2009-2010 and 2011 under the ecological conditions of Konya possessing all characteristics of dry conditions of the Central Anatolia Region and being responsible 10% of Turkey’s safflower sowing area. The effects of 15 February, 1 March, 15 March, 1 April, 15 April and 1 May sowing dates on safflower cultivars (Dinçer and Remzibey-05) were investigated. Field experiments were conducted in a randomized complete block design as a split plot with four replications at the Turkey Konya Bahri Dağdaş International Agricultural Research Institute farms. As the sowing date delaying, number of days to %50 emergence decreased but the emergence was negatively affected due to the decreasing rainfall. February and March months were the best sowing dates for yield and oil content. Effects of the sowing dates in the years on the yield were determined visually according to Biplot method.

KEYWORDS:
Safflower, sowing date, seed yield, oil yield, oil ratio, biplot analysis.

INTRODUCTION

Safflower is higher yield than other oil crops in semi-arid and infertile areas. Due to its high oil content it can be used in areas such as margarine, oil, varnish, polish, soap industry [1].

Safflower seeds have about 30-35% oil and it is about 50-60% for kernels. It is valuable for human nutrition due to 77% linoleic acid in its oil. Safflower oil cake have about 20-25% crude protein and 4-8% oil and so it is valuable for animal food. The “carthamin” substance in its flowers is used as dye in textile and food industry and it is utilized in medicine and industry for many properties [2, 3].

Safflower can be grown under different conditions because it is not selective for climate and soil. Considering the ecological factors, the safflower plant has the opportunity to enter the crop rotation with wheat in the Central, Eastern and Southeastern Anatolia Regions. Because of it is drought resistant and can be grown without watering, it is one of the plants to be recommended for fallow fields especially in semi-arid regions.

Considering the ecological requirements of other oilseed crops other than sunflower, increasing safflower cultivation that has the opportunity to more easily cultivated will make a significant contribution to the agriculture of oil plants [4].

Although all kinds of suitable cultivation techniques have been applied, it has been observed that many farmers received low yield due to poor plant growth and insufficient plant frequency in the field due to the fact that only the correct sowing date has not been selected. The yield of safflower plants varies depending on annual rainfall in the province of Konya like all growing areas.

In Konya, while the mean rainfall for many years (1996-2006) was 313 mm, it is 372 mm for the trial years (2009-2010-2011). In the semi-arid regions such as Konya, because of the rainfall amount and rainfall distribution according to growing stages are effecting factors to sunflower production, it is possible to benefit maximum from this rainfall with optimum planting.

In some studies [5, 6, 7, 8, 9], it is reported that the seed yield decreases as the sowing date is delayed in safflower, the effect of ecological conditions on Plant characteristics is important and the time is changed according to the regions. Therefore, it is important to determine the sowing date for high seed yield in safflower.

MATERIALS AND METHODS

In Konya conditions field experiments have been established in order to determine the optimum sowing date of safflower. The sowing dates was composed of 6 topics as 15 February, 1 March, 15 March, 1 April, 15 April and 1 May. Dinçer and Remzibey-05 safflower cultivars registered in Turkey were used in the study. This study was carried out at the Konya Bahri Dağdaş International Agricultural Research Institute fields in 2009, 2010 and 2011 years. Field experiments were conducted in a randomized complete block design as a split plot with four replications (sowing dates in main plots and cultivars in sub-plots).
### TABLE 1
The monthly total rainfall in the trial years (mm)*

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(Many years average)**</td>
<td>35 28 27</td>
<td>32</td>
<td>42</td>
<td>23</td>
<td>6</td>
<td>5</td>
<td>11</td>
<td>29</td>
<td>32</td>
<td>43</td>
<td>313</td>
<td></td>
</tr>
<tr>
<td>2009</td>
<td></td>
<td>60 45 24</td>
<td>45</td>
<td>55</td>
<td>3</td>
<td>12</td>
<td>0</td>
<td>21</td>
<td>13</td>
<td>56</td>
<td>74</td>
<td>354</td>
<td></td>
</tr>
<tr>
<td>2010</td>
<td></td>
<td>44 28 12</td>
<td>41</td>
<td>18</td>
<td>40</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>75</td>
<td>3</td>
<td>85</td>
<td>350</td>
<td></td>
</tr>
<tr>
<td>2011</td>
<td></td>
<td>46 52 35</td>
<td>64</td>
<td>64</td>
<td>62</td>
<td>4</td>
<td>3</td>
<td>6</td>
<td>1</td>
<td>45</td>
<td>9</td>
<td>24</td>
<td>413</td>
</tr>
</tbody>
</table>


### TABLE 2
Analysis of combined variance at different sowing dates

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>SY.</th>
<th>OR.</th>
<th>OY.</th>
<th>NDF</th>
<th>BB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Year</td>
<td>2</td>
<td>23187**</td>
<td>15**</td>
<td>1418**</td>
<td>1145**</td>
<td>20415*</td>
</tr>
<tr>
<td>Rep. [Year]</td>
<td>9</td>
<td>2537</td>
<td>0.6</td>
<td>302</td>
<td>5.2</td>
<td>88</td>
</tr>
<tr>
<td>Sowing Date</td>
<td>5</td>
<td>163824**</td>
<td>18.5**</td>
<td>12582**</td>
<td>6622**</td>
<td>3936**</td>
</tr>
<tr>
<td>Year*Sowing Date</td>
<td>10</td>
<td>3154</td>
<td>11.5**</td>
<td>260.5*</td>
<td>214**</td>
<td>805.7**</td>
</tr>
<tr>
<td>Error1</td>
<td>45</td>
<td>6823</td>
<td>1.81*</td>
<td>525.1</td>
<td>4.27**</td>
<td>40.48*</td>
</tr>
<tr>
<td>Year*Variety</td>
<td>2</td>
<td>4384</td>
<td>12.5**</td>
<td>172.8</td>
<td>6.7</td>
<td>7.0</td>
</tr>
<tr>
<td>Sowing Date *Variety</td>
<td>5</td>
<td>2777</td>
<td>3.7**</td>
<td>242</td>
<td>7</td>
<td>12</td>
</tr>
<tr>
<td>SD<em>V</em>Y</td>
<td>10</td>
<td>678</td>
<td>3.6**</td>
<td>68</td>
<td>3.2</td>
<td>7</td>
</tr>
<tr>
<td>Error2</td>
<td>54</td>
<td>1758</td>
<td>1.2</td>
<td>131</td>
<td>5.02</td>
<td>47</td>
</tr>
<tr>
<td>Total</td>
<td>143</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CV (%) | 22 | 3.8 | 21 | 2.4 | 8 |

SY: Seed Yield, OR: Oil ratio, OY: Oil Yield, NDF: Number of days of flowering, PH: Plant height.

MS: Mean Square, DF: Degrees of Freedom, CV: coefficient of variation, **P<0.01 significant, *P<0.05 significant

### TABLE 3
Number of days to emergence (day) at different sowing dates in Safflower

<table>
<thead>
<tr>
<th>Years</th>
<th>2009(DE)</th>
<th>2010(DE)</th>
<th>2011(DE)</th>
<th>Average (DE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 February</td>
<td>20</td>
<td>23</td>
<td>22</td>
<td>22</td>
</tr>
<tr>
<td>1 Marc</td>
<td>17</td>
<td>23</td>
<td>21</td>
<td>20</td>
</tr>
<tr>
<td>15 Marc</td>
<td>15</td>
<td>15</td>
<td>19</td>
<td>16</td>
</tr>
<tr>
<td>1 April</td>
<td>10</td>
<td>11</td>
<td>14</td>
<td>12</td>
</tr>
<tr>
<td>15 April</td>
<td>7</td>
<td>16</td>
<td>10</td>
<td>11</td>
</tr>
<tr>
<td>1 May</td>
<td>7</td>
<td>9</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>Average</td>
<td>12</td>
<td>16</td>
<td>16</td>
<td></td>
</tr>
</tbody>
</table>

DE: Day of emergence

### TABLE 4
Plant height values at different sowing dates in safflower (cm)

<table>
<thead>
<tr>
<th>Years</th>
<th>2009 Varieties</th>
<th>2010 Varieties</th>
<th>2011 Varieties</th>
<th>Average Varieties</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sowing Dates</td>
<td>Remzibeeyy</td>
<td>Dinçer</td>
<td>Remzibeeyy</td>
<td>Dinçer</td>
</tr>
<tr>
<td>15 February</td>
<td>106 ab</td>
<td>113 a</td>
<td>94 c-f</td>
<td>99 bc</td>
</tr>
<tr>
<td>1 Marc</td>
<td>97 b-e</td>
<td>106 ab</td>
<td>93 c-g</td>
<td>98 b-d</td>
</tr>
<tr>
<td>15 Marc</td>
<td>98 b-d</td>
<td>100 bc</td>
<td>86 f-1</td>
<td>89 c-g</td>
</tr>
<tr>
<td>1 April</td>
<td>87 c-h</td>
<td>94 c-f</td>
<td>74 jk</td>
<td>77 ij</td>
</tr>
<tr>
<td>15 April</td>
<td>78 h-j</td>
<td>84g-i</td>
<td>52 m-p</td>
<td>61 k-m</td>
</tr>
<tr>
<td>1 May</td>
<td>60 l-n</td>
<td>63 kl</td>
<td>43 pq</td>
<td>54 m-p</td>
</tr>
<tr>
<td>Average</td>
<td>87 b</td>
<td>94 a</td>
<td>74 d</td>
<td>79 c</td>
</tr>
</tbody>
</table>

Variety*Year LSD(%5) : 3.9  Variety*X*Sowing date LSD(%5) :5.5

Distance between inter-rows were 20 cm. Hoeing was done when the plants were 3-4 leaves, the intra-row spaces were set to 10 cm with thinning. Irrigation was done before sowing when the soil moisture was not suitable.

**Observations and measurements.** Number of days to %50 emergence (day): The duration from planting to %50 plant emergences in each plot were recorded as day.

Number of days to %50 flowering (day): The duration from planting to %50 plant flowering in each plot were recorded as day.
Plant height: The distance from the soil level to the top of the plant receptacle was measured as cm.

Seed yield (kg/ha): Plants were harvested in each plot, seeds were cleaned, weighed with 0.01 g precision weighing scale and seed yield was calculated as kg per hectare.

Oil content (%): Crude oil analysis was carried out by Bahri Dağdaş UTAE laboratory according to Soxhlet method.

Oil yield (kg/ha): The seed yields (kg/da) calculated from each plot were multiplied with the crude oil content of each plot.

The climate values of the research area. The total amount of precipitation in April, May, and June, the highest water consumption of safflower, was 103 mm in 2009, 99 mm in 2010 and 193 mm in 2011. The long-term average (1954-2011) was 98 mm, 2010 rainfall is in the long-term average, more rainfall in 2009 and 2011 than the long-term average [10].

RESULTS AND DISCUSSION

The variance analysis table of seed yield, oil ratio, oil yield, number of days to %50 flowering and plant height values at different sowing dates is given in Table 2.

Number of days to emergence (day). Average number of days to %50 emergence at different sowing date are given in Table 3. During the early sowing date, the average emergence duration was 20-22 days, but it decreased to 9-11 days on April 15 and May 1.

As the sowing date lags, the shortening of the days of emergence is related to the soil temperature [11]. As seen here, soil temperature was 4°C in February, 9°C in March, 13°C in April and 16°C in May [10].

Baydar and Turgut, [12] reported that the minimum soil temperature for safflower germination is 4.4°C and the optimum soil temperature is 15.6°C. The minimum temperature for germination of safflower in the experimental area was reached in the middle of February, and the optimum germination temperature was reached only in April and May [10]. April and May are suitable for optimum germination temperature, but there is a possibility of not being able to provide adequate soil moisture for the emergence due to the imbalance that may occur in the amount of rainfall distribution in these months.

Plant Height. Year, cultivar, sowing date and year x sowing date interactions effects on plant height were found to be important (Table 2).

As shown in Table 4, the mean plant height values at the first sowing date were 87 cm, while the last sowing date decreased to 51 cm at 1 May. Dinger cultivar has been taller than Remzibey.

Plants that have been planted at early planting dates are much longer than those planted in late vegetation because of the duration of benefiting from early spring rains. It can be said that the plant height gradually decreased as the planting date was delayed. In accordance with this research, (Baydar & Turgut, [12], Özkanay et al., [9], Kılıç & Küçükler [13], Şakir & Baştan, [15], Koç et al., [7], have stated that plant height shortens as the planting time lags.

Number of days to %50 flowering (day). Year, sowing date and year x sowing date interactions effects on number of days to %50 flowering were found to be important (Table 2).

As shown in Table 5, the average numbers of days to %50 flowering varies between 61 and 103 days. At the early planting time, the plant has a longer vegetation period, so the time from the beginning to the flowering is long. Safflower prefer cool and rainy weather conditions from the beginning to the flowering period for optimum growth. From flowering to maturity, hot and arid conditions are important for the normal growing and the completion of the vegetation period.

In late plantings, especially in May and June, due to the high temperature and rainfall insufficiency, safflower plants were caught early to the drought and so they passed early to the generative stage and stunted.

Seed Yield. Year and sowing date effects on seed yield were found to be important (Table 2).

As seen in Table 6, the effect of all sowing dates according to the 3-year average is statistically different. As the sowing dates lapsed, yields decreased significantly. Average seed yields were found to be 2840, 2570, 2160, 1850, 1307 and 640 kg/ha in the sowing dates of April 15, April 1, March 15, April 1, April 15 and May 1 respectively.

Our research has shown that sowing must be done in February and March in order to obtain a satisfactory yield in terms of safflower seed production. After this date, the yields decreased considerably. The reason for the higher seed yield at early planting dates can be due to the raining of a great part of the spring rains in March and April in the Central Anatolia Region. In late sowing dates, the plant enters to generative cycle early and the vegetation cycle is shortened due to the fact that a significant part of the plant growth period will encounter hot and relatively low rainfall cycles. As a result of the shortening of the vegetation cycle, both the vegetative and the roots parts of the plant grow less and the yield decreases.
TABLE 5
Number of days to %50 flowering (day) at different sowing dates in the safflower

<table>
<thead>
<tr>
<th>Years</th>
<th>Sowing dates</th>
<th>2009</th>
<th>2010</th>
<th>2011</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 February</td>
<td>114</td>
<td>95</td>
<td>101</td>
<td>103</td>
<td>a</td>
</tr>
<tr>
<td>1 Marc</td>
<td>106</td>
<td>95</td>
<td>90</td>
<td>97</td>
<td>b</td>
</tr>
<tr>
<td>15 Marc</td>
<td>92</td>
<td>90</td>
<td>80</td>
<td>87</td>
<td>c</td>
</tr>
<tr>
<td>1 April</td>
<td>84</td>
<td>70</td>
<td>72</td>
<td>75</td>
<td>d</td>
</tr>
<tr>
<td>15 April</td>
<td>70</td>
<td>64</td>
<td>67</td>
<td>67</td>
<td>e</td>
</tr>
<tr>
<td>1 May</td>
<td>60</td>
<td>60</td>
<td>64</td>
<td>61</td>
<td>f</td>
</tr>
<tr>
<td>Average</td>
<td>88 a</td>
<td>79 b</td>
<td>79 b</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Years LSD(%5) : 1        Sowing dates LSD(%5) : 1.3

TABLE 6
Seed yield values obtained at different sowing dates in safflower (kg / ha)

<table>
<thead>
<tr>
<th>Years</th>
<th>Sowing dates</th>
<th>2009</th>
<th>2010</th>
<th>2011</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 February</td>
<td>2740</td>
<td>2710</td>
<td>3080</td>
<td>2840</td>
<td>a</td>
</tr>
<tr>
<td>1 Marc</td>
<td>2330</td>
<td>2470</td>
<td>2920</td>
<td>2570</td>
<td>b</td>
</tr>
<tr>
<td>15 Marc</td>
<td>2070</td>
<td>2180</td>
<td>2220</td>
<td>2160</td>
<td>c</td>
</tr>
<tr>
<td>1 April</td>
<td>1890</td>
<td>1600</td>
<td>2060</td>
<td>1850</td>
<td>d</td>
</tr>
<tr>
<td>15 April</td>
<td>1320</td>
<td>720</td>
<td>1760</td>
<td>1207</td>
<td>e</td>
</tr>
<tr>
<td>1 May</td>
<td>670</td>
<td>510</td>
<td>730</td>
<td>640</td>
<td>f</td>
</tr>
<tr>
<td>Average</td>
<td>1890 b</td>
<td>1700 c</td>
<td>2130</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Years LSD (%5) : 168    Sowing dates LSD (%5) : 240

TABLE 7
The values of the oil content obtained in different sowing date of safflower (%)

<table>
<thead>
<tr>
<th>Years</th>
<th>Varieties</th>
<th>2009</th>
<th>2010</th>
<th>2011</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 Feb.</td>
<td>Remzibey</td>
<td>29.3 a</td>
<td>27.9 a-g</td>
<td>25.8 k-o</td>
<td>26.1 j-o</td>
</tr>
<tr>
<td>1 Marc</td>
<td>Remzibey</td>
<td>26.6 a-e</td>
<td>26.8 f-m</td>
<td>27.1 e-k</td>
<td>25.1 n-p</td>
</tr>
<tr>
<td>15 Marc</td>
<td>Remzibey</td>
<td>27.5 c-j</td>
<td>26.0 j-o</td>
<td>28.7 a-d</td>
<td>27.7 b-t</td>
</tr>
<tr>
<td>1 April</td>
<td>Remzibey</td>
<td>27.0 f-k</td>
<td>26.2 h-o</td>
<td>27.7 b-u</td>
<td>26.9 f-k</td>
</tr>
<tr>
<td>15 April</td>
<td>Remzibey</td>
<td>27.0 f-k</td>
<td>25.9 k-o</td>
<td>27.5 d-j</td>
<td>26.5 g-n</td>
</tr>
<tr>
<td>1 May</td>
<td>Remzibey</td>
<td>26.2 i-o</td>
<td>25.3 m-p</td>
<td>25.7 k-o</td>
<td>26.8 f-k</td>
</tr>
<tr>
<td>Average</td>
<td>Remzibey</td>
<td>27.6 a</td>
<td>26.3 c</td>
<td>27.1 ab</td>
<td>26.5 bc</td>
</tr>
</tbody>
</table>

Varieties *Years LSD(5): 0.6    Varieties * Sowing dates LSD(5): 0.8

TABLE 8
Oil yield values obtained at different sowing dates in safflower (kg / ha)

<table>
<thead>
<tr>
<th>Years</th>
<th>Sowing dates</th>
<th>2009</th>
<th>2010</th>
<th>2011</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 February</td>
<td>784</td>
<td>705</td>
<td>876</td>
<td>788</td>
<td>a</td>
</tr>
<tr>
<td>1 Marc</td>
<td>646</td>
<td>647</td>
<td>797</td>
<td>696</td>
<td>b</td>
</tr>
<tr>
<td>15 Marc</td>
<td>550</td>
<td>605</td>
<td>576</td>
<td>577</td>
<td>c</td>
</tr>
<tr>
<td>1 April</td>
<td>506</td>
<td>439</td>
<td>496</td>
<td>480</td>
<td>d</td>
</tr>
<tr>
<td>15 April</td>
<td>349</td>
<td>238</td>
<td>495</td>
<td>360</td>
<td>e</td>
</tr>
<tr>
<td>1 May</td>
<td>173</td>
<td>135</td>
<td>174</td>
<td>160</td>
<td>f</td>
</tr>
<tr>
<td>Average</td>
<td>501 b</td>
<td>461 b</td>
<td>569 a</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Years LSD(%5) : 45    Sowing Date LSD(%5) : 65

It has been determined that safflower planting in the Central Anatolia Region should be done in February and March namely at the earliest possible date of field entry. Seed yield decreases considerably in sowing after these dates.

As a result of the works done by Baydar & Turgut [12], Kızıl & Gül [6]: Koç et al. [7], Öztürk et al. [8], Kılıç & Küçüker [13], Keleş & Öztürk [14] it has been found similarly that the seed yield decreases as the planting time is delayed.

The seed yield effect of years and sowing dates can be visually understood better by biplot (Figure 1) analysis. In biplot analysis years and planting dates were divided into three groups. 2010 was in the first group, 2009 was in the second group and sowing dates were only in the third group with 2011.
It is understood that there is no similarity between the years when the experiment is evaluated within itself for 3 years. It is different in every 3 years. The reason for this is that the climate conditions are different every year as well as the effect of the place where the experiment is carried out. Climate factors, especially rainfall and temperature, have caused the years to be different from each other.

The highest seed yield was obtained in 2011 when compared with each other over the years. The seed yields of 2009 and 2010 were close to each other and the difference between these two years was not statistically significant. On biplot, the reason why 2009 and 2010 are in the different places is due to the fact that the reactions of the cultivars to different sowing dates in these two years are different. While the seed yield values of cultivars given in early sowing dates in 2009 and 2010 are close to each other, the seed yield values obtained from late sowing date in 2009 and early sowing date in 2010 caused the years to be different.

When the planting dates are evaluated on the Biplot graph, it is seen that two different planting groups are formed. Late sowing dates seems to be a group, early sowing dates is a different group in itself. Even the first two sowing dates (15 February and 1 March) seem to be closer to each other. This is why there is an important difference between the soil temperature. Early planting dates are related to high yield and late planting dates are related to low seed yield. As seen on Biplot, the highest seed yield in 2011 was obtained on October 15th. The fact that this is followed by the March 1 sowings confirms this interpretation. Biplot analysis method based on the visual examination and evaluation of relations has been widely used recently [16, 17, 18, 19, 20].

Oil content (%). The effect of all subjects and interactions on oil content was found important (Table 2).

As shown in Table 7, the highest oil content (28.0-28.3%) was obtained from 15 February and 1 March crops of Remzibey variety. The lowest oil content (24.4%) was obtained from May 1 planting of Dincer cultivar. With these results, it can be said that early sowing in terms of Remzibey variety generally gives more satisfactory results in terms of oil content. When both the Remzibey variety and the Dincer variety are evaluated among themselves, the 1 May crops, which are the last sowing date, do not seem appropriate in terms of oil content.

Similar to the results of this study, Basalma et al. [21] reported that the effect of sowing date and variety applications on crude oil ratio is important. In contrast Samanci et al. [22] reported that the effect of sowing date on oil content is insignificant.

Oil Yield (kg/ha). The effect of year, sowing date and year x sowing date interaction was found significant (Table 2).

As can be seen in Table 8, the oil yield is significantly reduced over the years as the sowing date lags. These values were found as 788, 696, 577, 480, 360 and 160 kg/ha in the plantations of February 15, March 1, March 15, April 1, April 15 and May 1 respectively.

All conditions affecting seed yield and oil content will affect the oil yield because this value is obtained by multiplying the seed yield and oil content. Just as in the seed yield, safflower must be planted in February or March for a sufficient oil yield.
In accordance with the results obtained in this study, it has been reported by Kızıl [5] and Başalma [21] that the oil yield decreases as the sowing date is delayed.

As a result, crude oil yield is an important consideration when working with oilseed plants.

CONCLUSION

In this study: effects of sowing dates on number of days to %50 emergence, number of days to %50 flowering, plant height, seed yield, oil content, oil yield of two safflower variety are determined. While the most suitable sowing date is determined as April and May in terms of emergence, this planting time is not recommended because the rainfall is an important limiting factor for germination and emergence in arid conditions. Plant height and number of days to %50 flowering are higher in early sowing, while they are decreasing as sowing date is delayed. In terms of seed yield and oil content, February and March were determined as the most suitable sowing date. Late planting significantly reduces the oil content.

As a result of the research; it has been determined that in the Central Anatolia Region of Turkey, safflower planting should be done in February and March of early spring in terms of seed yield, oil content and oil yield, which are important for oil seeds.

ACKNOWLEDGEMENTS

This study was supported by The General Directorate of Agricultural Research and Policies, Republic of Turkey. I am thankful to Rıza ÜLKER and Ramazan KELEŞ for their valuable contributions to the research.

REFERENCES


WATER QUALITY CHANGES AND GOLDFISH GROWTH (CARASSIUS AURATUS) IN MICROGREEN AQUAPONIC AND RECIRCULATING SYSTEMS

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ABSTRACT

Goldfish (Carassius auratus) growth was studied comparatively in microgreen aquaponic (APS) and sump filter systems (SFS), and the effects of these two different filter systems on water quality parameters were investigated. Arugula (Eruca vesicaria) was used in APS and produced as microgreens. NH3-N, NO2-N, NO3-N, PO4 and NH4 were measured at specific periods for 60 days. No statistical differences were found between both systems in terms of food conversion ratio (FCR), specific growth rate (SGR), survival and mean body weight of goldfish. In the beginning of the experiment, water pH level was around 8.5 while trend towards declining at the end of period. Nitrification process was successful in both systems, and declining pH towards from basic medium to approaching neutral levels made positive contribution to performance of nitrifying bacteria. Although there was a significant decrease in the level of NH4, the level of NO3-N increased continuously after the 30th day in APS. According to these results, arugula microgreens used mostly NH4 instead of NO3-N in higher pH level. Goldfish-arugula microgreen aquaponic system is similar with SFS in terms of keeping water quality in optimal conditions and any adverse effect on goldfish growth and welfare was not observed in both systems. Although in high pH, it was also seen that arugula seedlings can be produced efficiently as microgreens.

KEYWORDS:
Aquaponic, Recirculating, Goldfish, Arugula, Microgreen, Water Quality.

INTRODUCTION

Recirculating aquaculture systems have been used for almost fifty years. For instance, an eigthy liters home aquarium, which is a miniature recirculating system, would have to maintain at least five kilograms of fish [2]. Sump filter system, which is a filtration equipment of the aquarium, enable mechanical and biological filtration of effluents and also provide the environment in which the nitrification process takes place effectively. Nitrification is the biochemical conversion by nitrifying bacteria of NH3 to NO3 and is a critical component of aquaculture biofilters [3]. Recently, new production systems integrated with recirculating systems are also being developed to produce different products. Aquaponic systems, which are integrated of intensive fish culture and hydroponics [4, 5], consists of a fish tank, a biofilter and a grow bed [6]. The effluent is used as a by-product to produce plants as a second crop in aquaponics [7, 8]. In this system, nitrifying bacteria convert NH3 which is derived from excretion of fish and uneaten feed, to NO3 as a plant fertilizer [9]. Media-filled type is the simplest aquaponic system that does not require separate biofilters because it contains media in the grow bed for nitrification [6, 10].

In the present study, the growth of goldfish (Carassius auratus) was studied comparatively in microgreen aquaponic (APS) and sump filter systems (SFS as a recirculating system), and the effects of these two different filter systems on water parameters were investigated. Arugula (Eruca vesicaria) was used in APS and produced as microgreens. The effect of the plant on water quality in APS was compared with SFS and the productivity of filtration systems was examined.

MATERIALS AND METHODS

Material. This research was conducted in Aquaculture Application and Research Center of Munzur University (Tunceli - Turkey) between May-June 2016. The study area was a closed space and isolated from sunlight partially. The experiments were carried out with goldfish (Carassius auratus) and Arugula (Eruca vesicaria). Goldfish with a mean body weight of 1.76 ± 0.03 g were stocked...
into six glass aquariums (fish tank) at a rate of fifteen fish per 56 L water volume (n=90). Fish were fed with commercial granular feed (Sera Pond Granulat) containing 32.1% crude protein, 5% crude fat, 1.9% raw fiber and 6.8% ash. Arugula seeds which are organic and chemical-free, were purchased from a commercial store.

**Setup of aquaponic and sump filter systems (APS and SFS).** APS and SFS were conducted as triplicate in trials. Fish tanks had dimensions of 64 cm x 25 cm x 35 cm. The water depth is set to 25 cm and the water volume to about 56 L. Water temperature was adjusted around 22-24°C with heater (200 watt). Water pump (600 L h⁻¹, 10 Watt), air pump (5 Watt) and air stones were used for water circulation and oxygenation. The flow rate of a pump was 10 L min⁻¹ for APS and SFS fish tanks.

Media-filled type aquaponic system was used in this experiment. Water pumped from fish tank to grow bed by pump and discharged from grow bed to fish tank by bell siphon technique in APS. The siphoning time was around 17 s. The dimensions of grow bed reservoir was 70 cm x 15 cm x 15 cm with 15.7 L volume. Quartz sands (0.7-1.5 mm) were used as grow bed media with 10 L of those per reservoir and the media height was setup as 30 cm. Plants were lightened 11 hours from 7 am to 6 pm per day by using a timer and three LED grow lamps were placed 30 cm high from the plants. In SPS, biological sponge, activated carbon, ceramic, zeolite and bioballs were used for mechanical and biological filtrations.

**Activation of APS and SFS.** Water cycles were maintained day-long in APS and SFS. Both of systems were conducted for 2 days without fish after being installed, and then the goldfish were stocked into fish tanks. Fish were fed at least a week to obtain adaptation of those fish and development of nitrifying bacteria. Experiments were started after morphometric measurement of fish. Almost 2 L de-chlorinated water was added for each fish tank weekly due to evaporation.

**Feeding, growth and morphometric parameters of goldfish.** Fish were fed ad-libitum twice daily by hand on weekdays and fed by automated feeding machine approximately 3% of their own weight at the weekend. An electronic balance (precision 0.01 g) and a measuring scale (total length to 1 mm) were used to determine growth performance of fish within 60 days. Fish were anesthetized with 2-phenoxylethanol (600 μL L⁻¹) before sampling.

Specific growth rate (SGR) and feed conversion rate (FCR) were calculated as stated below.

\[ \text{SGR} \% = \left[ \ln (W_2) - \ln (W_1) / t \right] \times 100 \]

\[ \text{FCR} = \frac{\text{Consumed feed (g)}}{\text{Weight gain (g)}} \]

where \( W_2 \) is final weight, \( W_1 \) is initial weight and \( t \) is the period of trial (days).

**Seed planting and morphometric measurement of arugula.** Arugula seeds were germinated by being kept in wet cotton for three days. These seeds were then planted by sprinkling over aquaponic growing beds each with about two hundred seeds. Length of leaf and seedlings were measured using a measuring scale (1 mm) every 20 days of experiment.

**Water analysis.** Water temperature, pH and dissolved oxygen measurements were taken every two days by portable multi-parameter device (YSI), while NH₃-N, NO₂-N, NO₃-N and PO₄ were measured by portable colorimeter (HACH DR/890) every 10 days during experiment. NH₄ was analyzed by ICS-1000 Ion Chromatography (DIONEX) at zero, 30th and 60th days. Measurement were applied as triplicates.

**FIGURE 1**

Water dissolved oxygen (mg L⁻¹) and pH in both of systems during experiment.
TABLE 1

NH₃-N, NO₂⁻-N, NO₃⁻-N, PO₄ and NH₄ values for APS and SFS (mg L⁻¹) (mean ± SE)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Day 0</th>
<th>Day 10</th>
<th>Day 20</th>
<th>Day 30</th>
<th>Day 40</th>
<th>Day 50</th>
<th>Day 60</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH₃-N (mg L⁻¹) APS</td>
<td>0.001 ± 0.000</td>
<td>0.001 ± 0.000</td>
<td>0.007 ± 0.006</td>
<td>0.008 ± 0.017</td>
<td>0.007 ± 0.019</td>
<td>0.005 ± 0.007</td>
<td>0.001 ± 0.000</td>
</tr>
<tr>
<td>NH₃-N (mg L⁻¹) SFS</td>
<td>0.001 ± 0.000</td>
<td>0.000 ± 0.000</td>
<td>0.006 ± 0.003</td>
<td>0.006 ± 0.003</td>
<td>0.007 ± 0.003</td>
<td>0.006 ± 0.003</td>
<td>0.001 ± 0.000</td>
</tr>
<tr>
<td>NO₂⁻-N (mg L⁻¹) APS</td>
<td>0.007 ± 0.036</td>
<td>0.007 ± 0.003</td>
<td>0.017 ± 0.019</td>
<td>0.008 ± 0.019</td>
<td>0.008 ± 0.007</td>
<td>0.008 ± 0.001</td>
<td>0.007 ± 0.001</td>
</tr>
<tr>
<td>NO₂⁻-N (mg L⁻¹) SFS</td>
<td>0.003 ± 0.000</td>
<td>0.003 ± 0.000</td>
<td>0.017 ± 0.003</td>
<td>0.000 ± 0.000</td>
<td>0.001 ± 0.000</td>
<td>0.000 ± 0.000</td>
<td>0.001 ± 0.000</td>
</tr>
<tr>
<td>NO₃⁻-N (mg L⁻¹) APS</td>
<td>0.53 ± 8.73</td>
<td>9.83 ± 2.78</td>
<td>12.28 ± 6.48</td>
<td>8.02 ± 6.48</td>
<td>8.43 ± 6.48</td>
<td>12.8 ± 6.48</td>
<td>17.38 ± 6.48</td>
</tr>
<tr>
<td>NO₃⁻-N (mg L⁻¹) SFS</td>
<td>0.53 ± 6.23</td>
<td>9.83 ± 2.78</td>
<td>12.28 ± 6.48</td>
<td>8.02 ± 6.48</td>
<td>8.43 ± 6.48</td>
<td>12.8 ± 6.48</td>
<td>17.38 ± 6.48</td>
</tr>
<tr>
<td>PO₄ (mg L⁻¹) APS</td>
<td>0.31 ± 0.40</td>
<td>1.14 ± 1.85</td>
<td>2.60 ± 1.85</td>
<td>3.41 ± 1.85</td>
<td>3.57 ± 1.85</td>
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</tr>
<tr>
<td>PO₄ (mg L⁻¹) SFS</td>
<td>0.31 ± 0.75</td>
<td>1.31 ± 1.60</td>
<td>1.92 ± 1.60</td>
<td>2.50 ± 1.60</td>
<td>2.78 ± 1.60</td>
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<tr>
<td>NH₄ (mg L⁻¹) APS</td>
<td>0.0015 ± 0.000</td>
<td>0.0005 ± 0.000</td>
<td>0.0005 ± 0.000</td>
<td>0.0005 ± 0.000</td>
<td>0.0005 ± 0.000</td>
<td>0.0005 ± 0.000</td>
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</tr>
<tr>
<td>NH₄ (mg L⁻¹) SFS</td>
<td>0.0015 ± 0.000</td>
<td>0.0005 ± 0.000</td>
<td>0.0005 ± 0.000</td>
<td>0.0005 ± 0.000</td>
<td>0.0005 ± 0.000</td>
<td>0.0005 ± 0.000</td>
<td>0.0005 ± 0.000</td>
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</tbody>
</table>

*Within the same columns, values with asterix are significantly different (p<0.05).
*Within the same line, values with same superscripts are not significantly different (p>0.05).

RESULTS AND DISCUSSION

Physico-chemical parameters of water. Water temperature were recorded as 21.2°C – 24.7°C and 21.3°C – 24.5°C ranges for APS and SFS, respectively during experiment. pH and dissolved oxygen distributions along study were given in Figure 1.

NH₃-N, NO₂⁻-N, NO₃⁻-N, PO₄ and NH₄. These parameters were measured at specific periods for 60 days (Table 1).

Nitrification process (NH₃-N → NO₂⁻-N → NO₃⁻-N). Nitrification in APS and SFS were shown in Figures 2-3.
FIGURE 3  
Nitrification process (NH₃-N → NO₂-N → NO₃-N) in SFS.

FIGURE 4  
Growth of arugula leafs and seedlings for 60 days (mean ± SD)

TABLE 2  
Growth parameters and survival of APS and SFS (mean ± SE)

<table>
<thead>
<tr>
<th></th>
<th>APS</th>
<th>SFS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrification</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NH₃-N</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NO₂-N</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NO₃-N</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Leaf and seedling lengths of arugula were recorded every 20 days period and mean length of those were shown in Figure 4.

Growth parameters of goldfish and arugula. Growth parameters in APS and SFS were given in Table 2.

Leaf and seedling lengths of arugula were recorded every 20 days period and mean length of those were shown in Figure 4.

Higher feeding rate and protein content provide more nitrogen for plants. The quality of the feed has a major impact on fish and plant growth, and quality composition of plants. Poor quality feeds that have an impact on system functioning, are associated with more faecal and other wastes, and in the absence of highly efficient settling devices, these solids will tend to build up in the system [11, 12]. Goldfish was fed with high crude protein content (32.1%) feed and any problem on fish or systems was not observed in both trials. No statistical differences were found between both systems in terms of FCR, SGR, survival and mean body weight (p>0.05) (Table 2). The results are in ac-
In the beginning of the experiment, pH level was around 8.5 while trend towards declining at the end of period (Figure 1). Two main factors affect the water pH level in the recirculating aquaculture system; production of CO2 from fish and biological activity of biofilter. CO2 reacted with water and converted to carbonic acid that cause water more acidic. The nitrifying process produces acid (H+) and the pH level falls. All of these reactions give rise to low level of pH in water [15,16].

An acceptable nitrification rate can be achieved by water temperatures within 10 to 35°C (optimum around 30°C) and pH levels between 7 and 8 [16]. On the other hand, there are five most important factors for optimal nitrification according to [9]; high surface area media for bacteria to grow and colonize, pH (6-7), water temperature (17-34°C), dissolved oxygen (4-8 mg L-1) and cover from direct exposure to sunlight. Generally, water parameters were kept at optimum levels in our study. As lower pH level reduces the efficiency of biofiltration, the pH should be kept above 7 in order to reach a high rate of bacterial nitrifying in recirculating systems [16]. NO2 and NH4 were reduced and kept at certain levels in APS and SFS. Nitrification process has been successfully performed in both systems (Figures 2-3), and most probably declining pH towards from basic medium to approaching neutral levels (Figure 1) made positive contribution to performance of nitrifying bacteria. However, the nitrification process in SFS seems to work relatively better than APS. Bioballs provide high surface area for nitrifying bacteria and the filter materials in SFS such as ceramic, zeolite, activated carbon have also many functionalities due to their porous structures. Zeolites are known to have an affinity for NH4, other cations and a potential to remove NH4 from municipal, industrial wastewaters and aquacultural effluents [17]. These materials give SFS an advantage in keeping some parameters at a lower level than APS.

Plants and bacteria in aquaponic system have significant influence at nitrogen transformation and play an important role in the processing of nitrogenous wastes [18]. NO3-N level was recorded slightly higher in APS than SFS. (p<0.05) (Table 1). NO3 is the end-product of the nitrification process and high levels of NO3 (>100 mg L-1) have negative impacts on fish growth and FCR. Eliminating the NO3 accumulation can be achieved by exchanging a high amount of water or applying denitrification process which reducing NO3 to atmospheric nitrogen with denitrifying bacteria [16]. However, the use of NO3 and NH4 by plants depends on pH level of water. Previously reported that plants prefer NO3 in acidic environments, whereas NH4 is the favored nitrogen source in alkaline environments [19]. In the present study, the NH4 values was measured as 0.0054 mg L-1 in APS and 0.0019 mg L-1 in SFS on the 30th day (Table 1). At the end of the experiment (60th day) NH4 was recorded as 0.0033 mg L-1 and 0.0022 mg L-1 in APS and SFS, respectively. Although there was a significant decrease in the level of NH4 (p<0.05), NO3-N increased continuously after the 30th day of this study. According to these findings, arugula microgreens used mostly NH4 instead of NO3-N in high pH level.

Microgreens are young, tender, edible crops that are harvested as seedlings. Many seedlings will be ready for harvest in two weeks. Microgreens are harvested at the first true-leaf stage that seedlings will be approximately 3.5 to 5 cm tall [20]. In the present study, arugula seedlings reached an average length of 3.78 cm in 20 days (Figure 4). After this period, a decreasing in the rate of growth was observed. The possible reasons of this slowdown might be high hydric load, high pH, full-day cycle and lack of fertilizer addition. In general, aquaponic systems show a slightly lower growth rate than soil or hydroponic production in the first six weeks [9]. It has been reported that plant growth is low in aquaponic systems due to high pH level [15,21]. pH of 6.5-7 is recommended for aquaponic systems because high pH can lead to nutrient lock-out which is nutrient deficiency of iron, phosphorus and manganese [9,15,21].

The higher level of PO4 in APS than in SFS indicates that arugula seedlings were not able to use PO4 sufficiently (Table 1). Anoxic incubation of the filter material in the presence of an external carbon source results in considerable denitrification activity and phosphate uptake. Phosphate removal from the water in the system is mainly mediated by denitrifying organisms [22]. In addition to the carbon cycle, denitrifiers also are associated with sulfur and phosphorus cycles in recirculating systems. Orthophosphate uptake by some denitrifiers takes place in excess of their metabolic requirements and may result in a considerable reduction of orthophosphate from the culture water [23]. Bell siphon technique was used for water discharge in APS and water was siphoned automatically from the reservoir to fish aquarium at certain periodic intervals. As the water was drawn quickly from the reservoir during this flushing, less denitrification bacteria may be located in the APS than SFS. It can be said that the anaerobic conditions are more available in which denitrification process works better and consequently the PO4 is lower in SFS.

**CONCLUSION**

In conclusion, goldfish-arugula microgreen aquaponic system is similar with SFS in terms of keeping water quality in optimal conditions and any adverse effect on goldfish growth and survival was
not observed in both systems. Although in high pH, it was also seen that arugula seedlings can be produced efficiently as microgreens which have many advantages like higher added value, increased demand and short growing period for aquaponics. As an environmental friendly technique, microgreen aquaponic is a promising production method for aquaculture and agriculture.

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REFERENCES


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VARIABILITY OF HYDROMORPHIC SOILS IN THE FLOODPLAIN OF THE SAVA RIVER IN SERBIA

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ABSTRACT

The paper presents the results of a research of properties of hydromorphic soils in different forest communities in the Sava River floodplain in the area of Upper Srem. The soil cover of the investigated area consists of a number of soil units. On the basis of detailed field and laboratory examinations of the physical and chemical properties of the soil, five soil types have been studied and described, including the riparian black soil (humogley), wetland gley soil (eu-gley), pseudogley-gley, fluvial meadow soil (humofluvisol) and illimerized soil (luvisol).

KEYWORDS:
Hydromorphic soils, production potential, the Sava River floodplain, Upper Srem, Serbia

INTRODUCTION

The forests of the Flat Srem are divided into two large areas (Figure 1):

---the Upper Srem area, which includes all forests of Srem west of Sremska Mitrovica up to the border with the Republic of Croatia, and

---the Lower Srem area, which includes all forests of Srem east of Sremska Mitrovica up to the territory of the Municipality of Zemun.

The forests of Upper and Lower Srem differ in terms of their geographical position, age, quality and tree species. Oak (Quercus robur L.) occurs in the highest and driest positions, while moist habitats tend to be inhabited by field ash (Fraxinus angustifolia Vahl). At higher altitudes, these two species as edifiers are supplemented with hornbeam (Carpinus betulus L.) and field maple (Acer campestre L.), and in lower areas with white willow (Salix alba L.) and black poplar (Populus nigra L.).

The area of Upper Srem is located in the southwestern part of Vojvodina on the border with the Republic of Croatia and Bosnia and Herzegovina, where it occupies the left riverbank of the Sava River. The forest complexes of Upper Srem cover an area of 24,836.26 hectares of forest and forest land. This area is characterized by a flat terrain, with an altitude between 78 and 85 meters and an alternation of "sequences" and "beams", which leads to different soil humidification regimes. One of the characteristics of the investigated area is the embankment that extends along the entire length from the border with R. Croatia to Sremska Mitrovica and divides the forest into the "defended" and "undefended" parts. The "defended" part of the forest includes compartments that are separated from the Sava River by an embankment and they make up the largest part of the forest area of Upper Srem. The "undefended" part of the forest occupies a smaller share of the territory. Its compartments are located along the Sava River and they are subject to frequent flooding.

The entire Srem forest area, and therefore also the investigated area are characterized by a temperate continental climate with distinct seasonal changes. The average annual air temperature is 10.9 °C, while during the vegetation period it reaches 17.6 °C.

The average annual precipitation for the area of Upper Srem is 657 mm, while during the growing season the average amount of precipitation reaches 334.3 mm or 51%. The annual rainfall distribution is very favorable and highly suitable for forest production. Relative air humidity, which expresses the degree of air saturation by steam, is the highest in winter (December and January 89%), and the lowest in May (68%). Barometric pressures are fairly uniform in the investigated area, so that there are no strong winds. Winds blow mostly from east and west, and they are the strongest in winter and spring.

The parent rock of the investigated area is mainly the alluvial sediment of the Sava River. The average altitude of the alluvial plain is 83-84m. To the north the alluvial plain continues into a loess terrace, which in some parts extends to the very bank of the Sava River. The alluvial plain of the Sava is the widest along the border with Croatia (23.4 km), and the transitions between alluvial plains and the loess terraces are gradual and difficult to notice.

According to the results of previous research [1, 2, 3, 4, 5, 6, 7], 12 groups of ecological units were identified in the area of Flat Srem. A total of 36 forest types (ecological-production units of forests) were determined in these ecological units. The largest area is occupied by the group of ecological units of field ash and pedunculate oak (Fraxino-Querce-tum roboris) with 40.3% (Figure 2), followed by the...
group of pedunculate oak, hornbeam and field ash (Carpino-Fraxino-Quercetum roboris) with 17.7%, and the group of pedunculate oak, hornbeam and Turkey oak (Carpino-Quercetum robori-cerris) with 13.8%, the group of black poplar plantations with 12% (Figure 3) and the group of pedunculate oak and hornbeam (Carpino-Quercetum roboris) with 8% of the total forest covered area. The other groups of ecological units occupy less than 10% of the total forest covered area.

The paper presents the results of a soil study in the area of Upper Srem. The forest complexes in the Sava River floodplain in the territory of Upper Srem are among the most valuable forests in Serbia and are made up of flood-prone forests of pedunculate oak, ash and hornbeam and in a small percentage of Euroamerican poplar plantations. Frequent flooding and long-term water retention lead to a change in the soil moisture regime, as well as to the change of its properties.

The soils of the investigated area were subject to research by several researchers: [8, 9, 10, 11, 12, 13, 14, 15, 16].

According to the nature of humidification [17], soils in Upper Srem can be divided into two orders as the highest system units: the order of hydromorphic and automorphic soils. The largest part of the area is covered by the soil types from the order of hydromorphic soils, whose evolution is in addition to precipitation influenced by additional moistening of the profile with underground and flood water. Soils from the order of automorphic (terrestrial) soils, whose formation is exclusively under the impact of atmospheric precipitation, occurs on smaller areas.

The specific microrelief of the area of Flat Srem had a high impact on the formation of various soil formations. The microrelief differentiation in the form of beams, plateaus and sequences leads to differences in soil moisture regimes and the topographic and hydrological regimes.

The floods that occurred in this area during 2014 caused remaining of large quantities of water in the "defended" part of the forest for a long time after the Sava River withdrew. As a result of long water retention and possible changes in soil properties, the regeneration of a certain part of the forest is impossible.

Due to the increased drying of the main tree species of pedunculate oak (Quercus robur L.) and field ash (Fraxinus angustifolia Vahl), as well as the impossible regeneration of individual compartments with hybrid poplar (clones M-1, I-214, S 1-3, S-665) there is a need to gain new knowledge on the soils in this area.

In order to select the most favorable silvicultural measures in different forest communities in the Upper Srem area, it is necessary to expand knowledge about the properties and production potentials of these soils. A detailed study of morphological, physical and chemical properties, the definition of the soil system units [18], as well as their connection with the ecological and vegetation types of forests is an important basis for estimating the production potentials of these soils.
The research included the "defended" and "un-defended" parts of the Sava River floodplain. The investigated area contains hardwood stands dominated by pedunculate oak (*Quercus robur* L.) and plantations of soft broadleaves (clones M-1, I-214, S 1-3, S-665). The selection of soil study sites was carried out depending on the microrelief conditions.

A total of 21 profiles were opened in the research area, and the external and internal morphology was studied. In addition, a corresponding number of samples in the deteriorated condition were taken for the laboratory testing of standard physical and chemical properties of the soils.

Laboratory studies comprised a set of standard physical and chemical analyses: Hygroscopic moisture content was determined by drying soil samples in an oven at a temperature of 105°C for 6 to 8 hours. Particle size distribution was determined by treating the samples with sodium pyrophosphate. Soil particle sizing was carried out by combining the pipette method and the elutriation method using Atterberg sieves were used to determine the percentages of the following fractions: 2 to 0.2 mm, 0.2 to 0.06 mm, 0.06 to 0.02 mm, 0.02 -0.006 mm, 0.006 to 0.002 mm and less than 0.002 mm. Soil texture was determined using the texture triangle of The Soil Science Society of America.

Active acidity (pH in H₂O) was determined electrometrically by using pH meter apparatus. Exchangeable acidity (pH in a 0.01M CaCl₂) was determined electrometrically by using pH meter apparatus. Hydrolytic acidity was determined by the method of Kappe. The amount of adsorbed alkaline cations (S in cmol*kg⁻¹) was determined by the method of Kappe. The total cation-exchange capacity (T in cmol*kg⁻¹) was determined by calculation. The sum of acidic cations (T - S u cmol*kg⁻¹) was calculated from the hydrolytic acidity. Soil base saturation was calculated by Hissink (%). Total nitrogen in the soil was determined by the method of Kjeldahl (%). Carbon to nitrogen ratio (C: N) was determined by calculation. The availability of phosphorus and potassium (mg/100 g soil) was determined by the AL method.

Analytical procedures of the methods which were used to carry out the laboratory soil investigations are described in the *Soil testing manuals of JDPZ*. Field studies were carried out according to [19], using the methods described in the "Soil testing manual, Book IV". The mechanical composition of soil was determined after [20], using the methodological procedures described in the *Soil testing manual, Book V*, (1997), while the study of chemical...
soil properties applied the methodology described in the “Soil testing manual, Book I” [21]. The classification of the studied soil types was based on the “Classification of soils in Yugoslavia” [18].

RESULTS

The main aim of this research stems from the need to gain new knowledge on the ecological and production potentials of forest sites, especially those located in the Sava River floodplain, due to changes in the humidification regime and the emergence of new sites, with the aim of creating a quality basis for the selection of the best possible management system. It should be noted that changes to the hydrologic regime, in the River floodplain, have a major impact not only on soil genesis, but also the living world [22], and water quality [23].

The floods that affected this area in 2014 caused large quantities of water to remain in the defended part of the forest for a long time after the withdrawal of the Sava River into its riverbed. According to research [24], extreme hydrological events have a major impact on the changes in soil properties. As a result of long water retention and possible changes in soil properties, it is impossible to restore certain tree species. Getting familiar with the production potentials of the soil, through a research of its physical and chemical properties and the knowledge of vegetation-floristic conditions are the basis for defining the choice of tree species, tending and regeneration measures and management objectives in certain types of sites. The hydromorphological assessment [25] includes the assessment of the hydrological regime, river continuity and riverbed morphology, also the connection of surface waters to groundwater.

The morphological, chemical and physical properties of the soil cover in some forest communities in the investigated area have not been sufficiently investigated yet. On the basis of detailed soil studies, which included the study of the inner and outer soil morphology in the field and the laboratory testing of standard physical and chemical properties, a total of 5 soil types were distinguished in the investigated area according to the Soil classification [18] and [26]:
1. Riparian black soil or humogley [18] - Calcic Gleysol [26];
2. Wetland gley soil or eugley [18] – Gleysol [26];
3. Pseudogley-gley [18]– Planosol [26];
4. Fluvial meadow soil (humofluvisol) [18] – Fluvisoli [26];
5. Illimerized soil or luvisol [18] – Albeluvisol [26].

Riparian black soil or humogley with the A-AC-CGso profile structure. Riparian black soil or humogley [18] - Calcic Gleysol [26]: belongs to the order of hydromorphic soils, and the class of gley soils. The name is derived from the term "riparian area", which refers to the area in which the soil is formed. This type of soil is mainly formed in depressions on alluvial deposits, deluvial loess or loess with the A-AC-CGso profile structure.

The studied riparian black soils are characterized by a powerful humus-accumulating horizon (Figure 6), usually up to 70 cm, and in some cases over 100 cm. The groundwater level oscillates in the zone between 35-100 cm. The zone of reduction processes and long-term water retention is 80-100 cm from the surface of the soil. In comparison with wetland gley soils, riparian black soils have a shorter period of flooding.

The studied soils are characterized by a heavy mechanical composition, according to the texture composition - clay to clay loam. They are characterized by a large polyhedral to lumpy structure. There is abundant moisture in the profile that comes from both surface and groundwater. In a dry state, these soils are very hard with pronounced horizontal and vertical cracks. Riparian black soils are characterized by a larger share of coarse and fine sand compared to wetland gley soil (Table 1). The mechanical properties of riparian black soils are mostly poor due to poor drainage and unfavorable water-air properties.

Chemical properties (Table 2) are characterized by the presence, i.e. the absence of calcium carbonate. According to the criteria of soil classification [18] two subtypes can be distinguished: carbonate type and non-carbonate type. The studied riparian black soils are characterized by a neutral, low alkaline to moderate alkaline reaction. Alkalinity increases slightly with soil depth. The surface horizon is moderately to highly humic (the humus content ranges from 4-5%), while the content decreases with depth and the layers become poor in humus content (the humus content is lower than 2%). The total nitrogen content is in accordance with the humus content. So, the surface layers of the soil are rich in this macroelement.

The soils are poor in terms of the content of readily available phosphorus, while in terms of readily available potassium content they are mostly medium supplied. The production potential of these soils is high, and the forests of field ash and pedunculate oak (Fraxino-Quercetum roburis) occur naturally on these soils.

Wetland gley soil or eugley with the A-CGso-Gr and IP-G profile structure. Wetland gley soil or eugley [18] - Gleysol [26] is formed on the lowest parts of river terraces or in recessed relief forms with a high groundwater level. This type of soil is characterized by a small physiological depth (Figure 4). The humus horizon is 20-40 cm deep, dark brown, very compact, clayey, with a prismatic large lumpy structure with distinct cracks. The gley
horizon has clearly differentiated Gso and Gt subhorizons. The soil is clayey, heavy, compacted, damp, sticky and plastic. The color is gray-blue, with many orange-red accumulations in the zone of groundwater level fluctuation or in the Gso subhorizon.

According to the percentage share of granulometric fractions, this soil belongs to the texture class of silty clay soil or silty clay loam (Table 1). The content of the coarse sand fraction is rarely higher than 0.50%, while the clay fraction content reaches 80-90%. The air regime and microbiological activity are completely dependent on the granulometric composition and the underground water and flood water regimes.

The surface part of the profile is rich in humus and decomposition occurs in moist conditions, while the lower parts are humus poor profiles constantly saturated with water. The presence of water is manifested by the occurrence of the reduction process, and in the intermediate zone, where water oscillates, reduction processes occur interchangeably with oxidation.

Wetland gley soil in Flat Srem is carbonate throughout the entire solum, or the carbonates are washed from the surface layer to a depth that often coincides with the zone of permanent water stagnation [27]. Two forms can be distinguished in relation to the presence of carbonates: the carbonate form and non-carbonate form. The soil is saturated with base cations and the pH of the soil is high. The soil reaction in the studied wetland gley soils ranges from neutral to low and moderate alkaline soil reaction. In terms of the content of readily available phosphorus, the soils are poorly supplied, while in terms of readily available potassium they are mainly medium supplied.

<table>
<thead>
<tr>
<th>Profile</th>
<th>Horizon</th>
<th>Depth (cm)</th>
<th>Hygroscopic water (%)</th>
<th>Granulometric composition of the soil (%)</th>
<th>Textural class</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>2.0 – 0.2 mm</td>
<td>0.2 – 0.06 mm</td>
<td>0.06 – 0.02 mm</td>
</tr>
<tr>
<td>WETLAND GLEY SOIL (EUGLEY)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A 12/17</td>
<td>0-10</td>
<td>4.18</td>
<td>0.50</td>
<td>2.30</td>
<td>16.80</td>
</tr>
<tr>
<td>I 15/17</td>
<td>10-65</td>
<td>3.42</td>
<td>0.30</td>
<td>2.50</td>
<td>16.70</td>
</tr>
<tr>
<td>Ab</td>
<td>65-75/80</td>
<td>5.26</td>
<td>0.00</td>
<td>1.30</td>
<td>1.00</td>
</tr>
<tr>
<td>G</td>
<td>75/80-110</td>
<td>4.47</td>
<td>0.40</td>
<td>3.80</td>
<td>6.40</td>
</tr>
<tr>
<td>ILLIMERIZED SOIL ON LOESS ALLUVIUM (LUVISOL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A 15/17</td>
<td>0-2</td>
<td>2.83</td>
<td>2.50</td>
<td>11.90</td>
<td>30.00</td>
</tr>
<tr>
<td>Eg</td>
<td>2-20/25</td>
<td>1.70</td>
<td>1.60</td>
<td>10.10</td>
<td>24.40</td>
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<tr>
<td>Bt</td>
<td>20/25-75</td>
<td>3.57</td>
<td>0.10</td>
<td>12.80</td>
<td>16.30</td>
</tr>
<tr>
<td>BC</td>
<td>75/90</td>
<td>2.60</td>
<td>1.00</td>
<td>18.40</td>
<td>18.90</td>
</tr>
<tr>
<td>RIPARIAN GLEY SOIL (HUMogleY)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A 3/17</td>
<td>0-45</td>
<td>4.91</td>
<td>1.10</td>
<td>2.80</td>
<td>6.90</td>
</tr>
<tr>
<td>AC</td>
<td>45-70</td>
<td>4.85</td>
<td>1.10</td>
<td>4.70</td>
<td>8.70</td>
</tr>
<tr>
<td>CGSO</td>
<td>70-110</td>
<td>4.25</td>
<td>1.50</td>
<td>7.10</td>
<td>8.70</td>
</tr>
<tr>
<td>PSEUDOGLY-GLEY</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ag 4/17</td>
<td>0-10/13</td>
<td>5.72</td>
<td>0.10</td>
<td>0.30</td>
<td>12.50</td>
</tr>
<tr>
<td>A 4/17</td>
<td>10/13-45</td>
<td>6.15</td>
<td>0.20</td>
<td>0.30</td>
<td>3.80</td>
</tr>
<tr>
<td>CGOR</td>
<td>45-90</td>
<td>5.76</td>
<td>1.20</td>
<td>4.50</td>
<td>6.00</td>
</tr>
<tr>
<td>MEADOW GLEY SOIL (HUMOFLUVISOL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A 11/17</td>
<td>0-30</td>
<td>4.75</td>
<td>0.00</td>
<td>8.90</td>
<td>16.60</td>
</tr>
<tr>
<td>AC</td>
<td>30-45</td>
<td>3.60</td>
<td>0.30</td>
<td>14.00</td>
<td>17.70</td>
</tr>
<tr>
<td>C</td>
<td>45-80</td>
<td>2.54</td>
<td>0.30</td>
<td>28.90</td>
<td>23.30</td>
</tr>
</tbody>
</table>
Grey willow (Salix cinerea L.) occurs on the shallowest varieties of this soil, and with the increase in depth other species also occur, and primarily field ash (Fraxinus angustifolia Vahl).

**Pseudogley-gley with the Ag-A-CGor-Gr profile structure.** Pseudogley-gley [18] - Planosol [26]: This soil type occurs in closed depressions on two-layer substrates by large rivers. In this case, the lower part the two-layer substrate is composed of heavy and compact clay, and the upper part of porous materials such as loess or alluvial deposits.

On the excavated soil profile, the soil has a shallow A horizon (Figure 7), dark in color, clayey, compact, with a large lumpy structure with plenty of reddish spots and vertical crevices. CGor and Gr horizons are composed of a clayey alluvial material marbled with rusty and bluish stains.

According to the textual composition, these soils have the character of clay with an almost total absence of the coarse sand fraction. The chemical properties of pseudogley soils are generally unfavorable, as they are impoverished in terms of nutrients as well as humus. Active acidity ranges from moderate to slightly acidic in the surface layer and neutral to low alkaline in deeper layers (Table 2). These soils are mostly inhabited by monodominant forests of pendulate oak (Quercus robur caricetosum remota).

**Fluvial meadow soil (humofluvisol) with the A-AC-C-CG profile structure.** Fluvial meadow soil (humofluvisol) [18] - Fluvisol [26]: are soils formed in alluvial plains of river valleys formed in the conditions of the meadow process of pedogenesis. The parent rock of the investigated humofluvisol is loess and lime-rich deluvial loess. These soils have a well developed humus horizon (Figure 8), which is 0-45 cm thick. They are dark gray with a polyhedral lumpy structure. The lower horizons are mostly composed of clayey and gleic alluvial material.

According to the textural composition humofluvisols that are closer to rivers are sandy clay soils, while clay loams occur in the farthest parts and depressions (Table 1). In terms of chemical composition, these soils are humic, with a neutral to alkaline reaction and moderately rich in nutrients. These

---

### TABLE 2

<table>
<thead>
<tr>
<th>Profile</th>
<th>Horizon</th>
<th>Depth (cm)</th>
<th>pH H2O</th>
<th>Y1 ml. NaO H50 %</th>
<th>CaC</th>
<th>Xum</th>
<th>C</th>
<th>N</th>
<th>C/N</th>
<th>Ready available P2O5 % K2O mg/100g</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0-10</td>
<td>7.77</td>
<td>7.31</td>
<td>-</td>
<td>5.12</td>
<td>7.40</td>
<td>4.29</td>
<td>0.40</td>
<td>10.73</td>
<td>21.28 25.42</td>
</tr>
<tr>
<td>I</td>
<td>10-65</td>
<td>8.16</td>
<td>7.37</td>
<td>-</td>
<td>6.82</td>
<td>1.90</td>
<td>1.10</td>
<td>0.15</td>
<td>7.36</td>
<td>5.48  14.12</td>
</tr>
<tr>
<td>12/1 G</td>
<td>75/8</td>
<td>8.06</td>
<td>7.24</td>
<td>1.25</td>
<td>0.81</td>
<td>40.60</td>
<td>41.41</td>
<td>98.04</td>
<td>-</td>
<td>2.90  1.68 0.23 7.32 4.40 21.65</td>
</tr>
<tr>
<td>12/1 G</td>
<td>0-110</td>
<td>8.14</td>
<td>7.26</td>
<td>0.94</td>
<td>0.61</td>
<td>35.60</td>
<td>36.21</td>
<td>98.31</td>
<td>-</td>
<td>0.98  0.57 - - 8.60 14.54</td>
</tr>
</tbody>
</table>

**WETLAND GLEY SOIL (BUGLEY)**

**ILLIMIZED SOIL ON LOESS ALLUVIUM (LUVISOL)**

**RIPARIAN GLEY SOIL (HUMOGLY)**

**PSEUDOGLEY-GLEY**

**MEADOW GLEY SOIL (HUMOFLUVISOL)**
FIGURE 4
Wetland gley soil (eugley)

FIGURE 5
Luvisol on loess alluvium

FIGURE 6
Riparian black soil (humogley)

FIGURE 7
Pseudogley-gley

FIGURE 8
Meadow black soil (humofluvisol)
soils are mostly used for the growth of euroamerican poplar, although they can also be used for the growth of mixed hard broadleaf forests of pedunculate oak, ash and hornbeam (Carpino-Fraxino-Quercetum roboris).

Ilimerized soil (pseudo-gley variety) with the A-Eg-Bt-C profile structure. Pseudogley illimerized soil [18] - Albeluvisol [26]: is mainly lowland soil with a developed eluvial and illuvial horizons (Figure 5) where the processes of eluvial-illuvial migration by base cations and unmodified colloidal particles leaching are pronounced. The diagnostic horizon of these soils is the E horizon, from which the leaching usually occurs and the B horizon in which the migrating ingredients are collected.

The subtypes of illimerized soil are distinguished by the parent rock type [19]. The horizon generally has a small thickness of 2-3 cm. It is loose, brown with a gray shade, and with a silty-crumbly structure. The Eg horizon is yellow-grayish in color, compact and without a structure, with a thickness of 3-25 cm, with rusted stains, which engage in the horizon below in the form of small tongues. The argiluvic-Bt horizon is formed below the Eg horizon, with a capacity of 25-90 cm. It is brown with gray zones and rusty stains, clayey and with a heavy mechanical composition.

According to the textual composition, the humus-accumulative and Eg horizons belong to the textural class of silty clay. The Eg horizon is characterized by a high presence of the silt fraction, while in the illuvial horizon the share of colloidal clay fraction increases and it belongs to the class of silty clay in terms of texture.

The chemical properties of the illimerized soils are characterized by an acid reaction. However, pH varies within the profile as a result of cation migrations. The reaction of the humus accumulating horizon is moderately to low acidic.

The Eg the horizon is characterized by a very strong to strong acidity, while the illuvial horizon is characterized by a low acidic to moderate reaction.

DISCUSSION

The soils of Upper Srem were investigated by a large number of researchers [13, 17, 7, 10, 23, 15]. In accordance with the findings of previous research [17], this study shows that the soils of the investigated area are divided into two orders: the order of hydromorphic soils and the order of the automorphic soils. According to the opened soil profiles (Figure 4), the largest number of the investigated soils belong to the systematic soil unit wetland gley soil or eugley.

Considering that the wetland gley soil was formed on the lowest parts of the river terrace, by depositing the finest material of the alluvium, this soil is very clayey and belongs to the texture class of clay or silty clay. The percentage of the coarse sand fraction is in rare cases higher than 0.5%, while the content of the clay fraction sometimes reaches up to 90%, which causes the unfavorable water conditions in this soil. A reduction of forest production potentials is expected for the wetland gley soils due to the less favorable conditions for silviculture.

Two monodominant forest communities of field ash in Ravni Srem [28] have also been studied: the association of field ash and grey willow (Salicetum cinerea-Pratetum angustifoliae Jovanovic et Tomić 1979) and the association of field ash and remote sedge (Caricetum remotae-Pratetum angustifoliae Jovanovic et Tomić 1979). The community Salicetum cinerea-Pratetum angustifoliae Jovanovic et Tomić 1979, is the most moist variant of the forest of field ash in Upper Srem and it occurs on wetland gley soils, under unfavorable weather conditions with a long period of flooding.

In accordance with previous research [28], the edaphic conditions in the investigated area show that the most optimal results on the soils of wetland gley type can be expected by cultivating, first of all the monodominant forests of ash (Salicetum cinerea-Pratetum angustifoliae Jovanovic et Tomić 1979). In two compartments that are located on wetland gley soils and on which poplar clones are cultivated, the recommended management measure is replacement of the species and regeneration with field ash.

CONCLUSIONS

The soil surveys were carried out in pure and mixed forests of pedunculate oak, field ash and hornbeam in the riparian zone of the Sava River in the Upper Srem area in Serbia.

A total of 21 pedological profiles were opened for the purpose of the study of edaphic characteristics of the site. The physical and chemical properties of the horizons were subjected to laboratory analyses by horizons.

A total of 5 soil types were distinguished considering the principles of soil classification [18]. From the class of gley soils the distinguished types are riparian black soil (humogley), wetland gley soil (eugley) and pseudogley-gley. From the class of semigley soils, the distinguished type is meadow black soil (humofluvisol) and from the class of eluvial-illuvial soils the distinguished type is limerized soil (luvisol).

The majority of opened soil profiles belong to the systematic soil unit wetland gley soil (11 profiles).

The most common fraction in the wetland gley soil is the fraction of silt ans clay. The high content of colloidal clay can also be explained by the way of soil formation in a closed depression. The C/N ratio is 10.73 in the humus-accumulating horizon. The
content of carbonates is high and it fluctuates around 7.30, which causes a neutral to low alkaline reaction. Field ash (Fraxinus angustifolia Vahl) has been recognized as the most suitable species for regeneration on the most common systematic soil unit identified in this research.

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REFERENCES


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FILM MULCHED CO-APPLIED WITH COMPOST IMPROVES REVEGETATION IN SAND DUNES FIXED BY CEMENT CHECKERBOARD ALONG THE QINGHAI, TIBET RAILWAY

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ABSTRACT
The ever-increasing sand dunes are destroying alpine ecosystem and threatening the Qinghai–Tibet Railway safety. However, sand dunes revegetation gained little success. As a part of the ongoing efforts to exploit practical revegetation techniques, we designed a comparative experiment in the sand dune that over 4560 m above sea level. Four techniques efficiency on the growth conditions (cover, height, tiller and leaf number, total root length, average root diameter, root volume, and root tips) of two indigenous grasses, Elymus breviaristatum and E. sibiricus cv. duoye, and top 30 cm soil nutrients (available potassium, phosphorus, nitrogen, and organic matter) were monitored. Biological measures comprised adding agriculture/forestry–animal manure compost (compost) and sowing grasses, and engineering measures involved nonwoven fabric film (film) mulched in sand dune where established cement checkerboard barriers. Sowing, compost, and film (SCF); sowing, and compost (SC); sowing, and film (SF); and only sowing (CK) were designed in barrier zone. The results showed that SCF and SC improved edaphic and grasses growth conditions considerably, whereas SF and CK improved lightly. The amendment capacity rank was SCF > SC > SF > CK. The E. sibiricus cv. duoye grown were better than E. breviaristatum in fertile habitat while it in turned in poor soil. In conclusion, integrating engineering measures with biological measures is practical in alpine sand dunes revegetation. Plant growth and edaphic conditions are the strong indictors to monitor revegetation efficiency.

KEYWORDS:
Elymus sp., revegetation, compost, nonwoven fabric, mechanical sand barrier, root morphological characteristics

INTRODUCTION
Sand dunes inhabited extensive land areas worldwide, especially spread over Africa, Australia, and Asia [1]. It known as relatively coarse particles, big pore spaces, high rate of permeability or leaching, and low grain particles cohesion [2]. The ever-increasing sand dune land is destroying the balance of the alpine ecosystem in the Qinghai–Tibet Plateau, a harsh and extremely fragile ecological region, plays a vital ecological barrier role in China [3]. In recent decades, changes in the alpine environment, such as climate change [4], overgrazing [5], or rodent gnawing [6], have seriously expanded the sand dunes area, which threatened the safety of the Qinghai–Tibet Railway and hindered development of the Belt and Road Initiative [7, 8]. Aimed to control the worsening sand dunes situation along the railway, many strategies (e.g., engineering, chemical, and biological measures) have been studied and applied to minimise the associated losses [9]. Classical engineering measures, mainly including the construction of retaining walls, sand-resistance fences, and stone checkerboard barriers, have been widely used in sandy areas to control sand erosion [7, 10]. For example, stone checkerboard barriers, retaining walls, and sand-resistance fences have been built along the Honglianghe River, Beiluhe River, Tuotuohe River, Zajazangbu River, and Cuona Lake in the Qinghai–Tibet Plateau to protect the Qinghai–Tibet Railway from sand [11]. Disappointedly, most of the above engineering measures would cease to be effective due to little vegetation cover and sand unceasing accumulation [7, 10].

With the development of technology, chemical or biological sand-fixing materials that including inorganic material, organic material and inorganic–organic hybrid material gradually have been paid great attention [12]. These materials could improve the edaphic structure and nutrient [2, 13]. Using phosphoric acid mulching liquid could fix some shifting sand dunes, stop their drifting and change them to windbreakers [14]. The desert region of the...
Qinghai–Tibet Plateau possesses the characteristics of low and concentrated precipitation, great temperature differences, frequent strong windy weather, and extremely strong ultraviolet radiation. Hence, most of the current chemical or biological sand-fixing materials lack sufficient resistance to ultraviolet radiation and are difficult to stabilise active sand dunes [13]. Consequently, this major limitation severely hinders the use of most chemical sand-fixing materials in the desert region of the Qinghai–Tibet Plateau.

Essentially, the most efficient method of reducing erosion is plant communities cultivation in the desert region [15, 16]. Therefore, major attention has been paid to vegetation reconstruction, and specific plants with beneficial properties (e.g., drought endurance and cold resistance) have become popular species in revegetation engineering, such as Carex brunnescens was applied in sand-fixing in degraded alpine meadows in northwestern China [17, 18]. Generally, sand dune is mainly characterized by the soil nutrients and humus decline [19, 20], which limited the plants growth and production severely [21]. This is particularly true in the Qinghai–Tibet Plateau, which has an extreme climate and edaphic conditions; revegetation without soil conditioner generally achieves minor success. Thus, the demand for additional methods to control active sand dunes has been increasing.

This study explored the theoretical techniques that may promote revegetation in alpine sand dunes along with the Qinghai-Tibet Railway. Though there was mechanical sand barrier in sand dune zone, the edaphic condition is extremely barren and no vegetation in it. Aimed to amend the barren edaphic condition in the premise of sand barrier construction, organic soil conditioner and mulch film were selected, and different indigenous grasses were sown. We designed a technical contrast field experiment to establish artificial vegetation in the sand dunes. Revegetated plant communities were investigated, plant roots and soil (top 30 cm) was collected to compare amendments under different techniques. Therefore, we expected purposes that: (1) whether the compost conditioner co-applied with non-woven film is practical to promote revegetation in the sand dune that fixed by cement checkerboard sand barrier; and (2) whether there are adaption differences between E. breviaristatum and E. sibiricus cv. duoye.

MATERIALS AND METHODS

Study area. The experiment site (34°53'11.04"N, 92°55'42.47"E; 4563 m ASL) was located in the desertification prevention and control comprehensive demonstration zone that is adjacent to the Qinghai–Tibet Railway in Beiluhe River, which is in the Three-River Source Region. The study area has a typical alpine climate and a severe degree of desertification. The mean annual temperature in the area is approximately −3.8 °C. The mean annual precipitation is approximately 300 mm, which is concentrated during the growing season (May to October) [22]. Additionally, the mean annual potential evaporation in the area is up to 1782.9 mm, and the mean annual wind speed is approximately 3.9–4.1 m/s [23]. The frozen period lasts from September to April next year and the frozen soil depths range from 2 to 3 m approximately [24]. The main soil type belongs to the alpine meadow soil in the mature vegetation region. The plant community in the typical alpine swamp meadow mainly comprises cold-tolerant mesophytic perennial herbs, such as Kobresia tibetica and K. pyramidata. The alpine cold steppe and the alpine desert are also important ecological niches in the region. The plant community of the alpine cold steppe mainly consists of xeric herbs (e.g., Stipa purpurea and Littledalea racemosa), and the alpine desert ecosystem is dominated by xerophytes (i.e., drought-enduring plants), such as Leontopodium nanum or Saussurea arenaria [25]. The total vegetation cover varies among the different desertification gradients, ranging from 0 in sand dune zones to more than 90% in alpine meadows.

Experimental design. Cement checkerboard sand barrier, an important innovation in barrier technology that is portable and inexpensive, was laid out in active sand dune near the Qinghai–Tibet Railway in Beiluhe district. The checker was 1.5 × 1.5 m² in area, and the cement board thickness was approximately 5 cm. No original vegetation cover was found in the cement checkerboard barrier zone. Moreover, the sand dune habitat is highly flat and homogeneous.

The compost used in our study comprised agriculture and forestry waste, animal manure, and microorganisms. Agriculture and forestry waste were crushed and the animal manure and compost fermentation agent were added into it. The mixture moisture content was approximately 55%. With the operation of mechanical aeration, we conducted an artificial turning every 2–3 days in the former 8-day, and then every 3–4 days. The fermentation temperature increased firstly and then dropped to a stable status which was similar to surrounding environment. The total nitrogen is about 17.40g/kg, the total phosphorus is about 7.10g/kg, and the total organic carbon is about 47.69g/kg. A nonwoven fabric film (hereafter referred to as “film”) was also used in this study; it had a mass of approximately 30 g/m². Because of the advantages of drought endurance, low-temperature resistance, and increased plant quality and yield, this film is an excellent material for use in artificial vegetation reconstruction in fragile alpine regions with desertification [26, 27]. We chose the indigenous perennial...
Elymus bunch grasses *E. breviaristatum* (*Bre.* ) and *E. sibiricus* cv. *duoye* (*Sib.* ) as indicator plants. Comparative field experiments were performed in May 2016. Four techniques were designed in the active sand dune where established cement checkerboard barrier (hereafter referred to as ‘barrier’; Table 1, Fig. 1).

**TABLE 1**

Techniques for seeding *E. breviaristatum* and *E. sibiricus* cv. *duoye* in sand dune that stabilised by cement checkerboard barrier

<table>
<thead>
<tr>
<th>Plots</th>
<th>Sowing</th>
<th>Compost</th>
<th>Film</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCF</td>
<td>+</td>
<td>+(0.5cm thickness)</td>
<td>+</td>
</tr>
<tr>
<td>SC</td>
<td>+</td>
<td>+(0.5cm thickness)</td>
<td>-</td>
</tr>
<tr>
<td>SF</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CK</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Note: The “+”/“-” indicates that a particular management was performed/unperformed. All techniques were established in the cement checkerboard barrier zone.

**FIGURE 1**

Illustration and scene of the field experiment region

Data collection. We conducted the field investigation in September 2016. Three 1 m² quadrats were set in each plot, and the plants’ height, cover, and abundance were recorded. After the investigation, more than 10 plant individuals in each plot were collected, washed out in a nylon bag (80 mesh), and the roots scanned using the WinRHIZO root scanner software (Regent Instruments, Quebec, CA) and an Epson Perfection v700 scanner (Epson, Suwa, JP), which calculated the total length, surface area, volume, average diameter, and tips of plant roots. In addition, approximately 500 g of the top 30 cm of the local soil was sampled, with five replicates for each plot and the maximum and minimum values are removed when analysed the data. The available phosphorus (extracted by 0.5 mol/L NaHCO₃ and tested by SmartChem 200, France Alliance), available potassium (extracted by 1.0 mol/L CH₃COONH₄ and tested by flame photometer FP640), nitrate nitrogen (extracted by 2.0 mol/L KCl and tested by SmartChem 200, France Alliance), and soil organic carbon (estimated by oxidizing organic matter in samples with K₂Cr₂O₇ after addition of concentrate sulfuric acid, left for 30 min, and the excess of K₂Cr₂O₇ was titrated against ferrous ammonium sulfate) in the soil were measured [28].

Data analysis. Statistical analysis was performed using SPSS Statistics 16.0 (SPSS Inc., Chicago, IL, US). One-way analysis of variance (ANOVA) was used to determine the significant differences among treatments. (\( P < 0.05 \)). Figures were drawn using OriginPro 2016 (OriginLab Corporation, Northampton, MA, US) and Photoshop CS6 (Adobe Systems, San Jose, CA, US).

**RESULTS**

Soil amendment variances under different techniques. In this study, soil organic carbon increased greatly under compost amendment, particularly under SCF, which increased the top 30 cm soil organic carbon content to 4.51 mg/g compared CK (1.87 mg/g). Available potassium, available phosphorus, and nitrate nitrogen differed markedly between techniques with (SCF and SC) and without (SF and CK) compost amendment. The available nutrients increased considerably under compost amendment. Moreover, the available phosphorus under SCF was significantly more than that under SC, while the available nutrients under SF and CK showed little variance which indicated that the film could preserve the added nutrients (Table 2).

**TABLE 2**

Soil nutrient content under different technologies

<table>
<thead>
<tr>
<th>Plots</th>
<th>Available potassium (mg/kg)</th>
<th>Available phosphorus (mg/kg)</th>
<th>Nitrate nitrogen (mg/kg)</th>
<th>Organic carbon (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCF</td>
<td>146.67±18.56a</td>
<td>131.71±8.91a</td>
<td>13.05±0.31a</td>
<td>4.51±0.20a</td>
</tr>
<tr>
<td>SC</td>
<td>133.33±18.56a</td>
<td>107.36±1.61b</td>
<td>12.96±0.13a</td>
<td>3.54±1.01ac</td>
</tr>
<tr>
<td>SF</td>
<td>73.33±3.33b</td>
<td>79.36±8.03c</td>
<td>11.42±0.44b</td>
<td>2.56±0.58ab</td>
</tr>
<tr>
<td>CK</td>
<td>73.33±3.33b</td>
<td>86.61±2.02c</td>
<td>11.87±0.23b</td>
<td>1.87±0.53bc</td>
</tr>
</tbody>
</table>

Note: Means in columns, within each treatment, followed by different letter(s) are significantly different at \( P < 0.05 \) using least significant difference (LSD)
FIGURE 2
Root morphological characteristics variations of *E. sibiricus* cv. *duoye* and *E. breviaristatum* under different technologies.
The different lowercase (s) and capital letter (s) on the bar are significantly different of Bre.’s and Sib’s root morphological characteristics among SCF, SC, SF, and CK at $P < 0.05$ using least significant difference (LSD), respectively. The ‘*’ means the significant difference of root morphological characteristics between Bre. and Sib. in a certain technique using LSD.

Plant root morphological characteristic variances under different techniques. In the present study, both *E. sibiricus* cv. *duoye* and *E. breviaristatum* root morphological characteristics showed considerable variability among SCF, SC, SF, and CK (Fig. 2). The total root length, root surface area, average root diameter, total root volume, and root tip number increased greatly after the sandy soil was amended by compost in SCF and SC compared with the no compost conditioner in SF and CK. Additionally, SCF substantially promoted these root growth parameters compared with SC. Although root traits differed slightly between SF and CK, overall, the contribution of SF was greater. The total root length of *E. breviaristatum* and *E. sibiricus* cv. *duoye* under SCF increased by 59.75%, 143.48%, and 277.90% and by 115.81%, 381.63%, and 446.67%, respectively, compared with that under SC, SF, and CK. The tips of *E. breviaristatum* and *E. sibiricus* cv. *duoye* under SCF increased by 52.15%, 217.73%, and 186.43% and by 123.54%, 463.49%, and 573.56%, respectively, compared with that under SC, SF, and CK. The promotion effect of SC was weaker than that of SCF, whereas that of CK was the weakest.

Aboveground plant growth variances under different techniques. Compost amendment (SCF and SC) promoted artificial vegetation cover, individual plant height, and individual density. The individual tiller and leaf numbers varied greatly among CK, SF, SC, and SCF. For example, the vegetation cover of *E. breviaristatum* and *E. sibiricus* cv. *duoye* increased by 30.67% and 31.67%, respectively, under SCF compared with those under CK. SF contributed weakly to plant growth compared with CK. Additionally, the combined amendments of compost and film (SCF) showed the largest promotion on plants growth.

The two grasses growth conditions varied greatly under different techniques. *E. sibiricus* cv. *duoye* showed a better growth status than *E. breviaristatum* in SCF and SC. Although the *E. breviaristatum* individual density differed slightly in all plots, its density still showed a similar pattern (CK < SF < SC < SCF) with *E. sibiricus* cv. *duoye*. Overall, the combined amendments of film and compost (SCF) contributed considerably to the growth of two grasses in sand dune where established cement checkerboard barrier. The *E. sibiricus* cv. *duoye* grown better than *E. breviaristatum* when the compost was applied in sand dune whereas it in turned in SF and CK. (Table 3)
TABLE 3

Growth condition variations of *E. sibiricus cv. duoye* and *E. breviaristatum* under different techniques

<table>
<thead>
<tr>
<th>Techniques</th>
<th>Coverage / %</th>
<th>Height / cm</th>
<th>Density</th>
<th>Tiller number</th>
<th>Leaf number</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sib.</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SCF</td>
<td>31.67±4.91Aa</td>
<td>4.83±0.17Aa</td>
<td>119.33±17.48Aa</td>
<td>11.50±1.71Aa</td>
<td>26.50±4.37Aa</td>
</tr>
<tr>
<td>SC</td>
<td>27.67±2.91Aa</td>
<td>4.33±0.33Aa</td>
<td>105.00±9.24Aa</td>
<td>3.17±0.44Ab</td>
<td>8.83±1.30Ab</td>
</tr>
<tr>
<td>SF</td>
<td>2.00±0.58Ab</td>
<td>2.67±0.33Ab</td>
<td>32.33±5.78Ab</td>
<td>1.00±0.00Ac</td>
<td>3.75±0.25Ac</td>
</tr>
<tr>
<td>CK</td>
<td>1.00±0.00Ab</td>
<td>1.00±0.00Ac</td>
<td>17.33±1.45Ab</td>
<td>1.00±0.00Ac</td>
<td>2.25±0.31Ac</td>
</tr>
<tr>
<td><strong>Bre.</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SCF</td>
<td>30.67±4.33Aa</td>
<td>4.50±0.29Aa</td>
<td>98.33±7.69Aa</td>
<td>4.50±0.87Bb</td>
<td>17.25±2.95Aa</td>
</tr>
<tr>
<td>SC</td>
<td>21.33±2.40Ab</td>
<td>4.33±0.33Aa</td>
<td>67.00±10.44Aa</td>
<td>2.50±0.22Ab</td>
<td>7.83±1.40Ab</td>
</tr>
<tr>
<td>SF</td>
<td>5.00±0.58Bc</td>
<td>3.00±0.00Ab</td>
<td>70.33±15.98Aa</td>
<td>1.00±0.00Ac</td>
<td>2.88±0.23Bc</td>
</tr>
<tr>
<td>CK</td>
<td>4.33±0.67Bb</td>
<td>2.33±0.17Bb</td>
<td>68.67±9.53Bb</td>
<td>1.00±0.00Ac</td>
<td>2.88±0.23Ac</td>
</tr>
</tbody>
</table>

Note: Means in columns, within each treatment, followed by different letter (s) are significantly different at *P* < 0.05 using LSD. The different lowercase (s) are significantly different of *Bre.’s* and *Sib’s* growth characteristics among SCF, SC, SF, and CK at *P* < 0.05 using LSD, respectively. The different capital letter (s) means the significant difference of growth characteristics between *Bre.* and *Sib.* in a certain technique using LSD.

DISCUSSION

Principle of limiting factors put forward that the limiting factors are the heart of the matter in vegetation restoration. Artificial measures that based on the “Ecology suitability principles” and “Niche theory” amends poor soil to establish a suitable habitat for plant growth [29, 30], including change the edaphic conditions, light features, and thermal characteristic. Our study based on the “self-design versus design theory” that combine the engineering measures and vegetation reconstruction to manage the sand dune restoration [31]. Simply sowing under the sand barrier engineering measure (CK) yielded weak revegetation results. The added film amendment (SF) had a greater contribution than CK. Nonetheless, the compost amendment contributed considerably to underground and aboveground growth of both grasses. It implied that the contribution of compost to plant growth was better than that of the film. The plant root length, root surface area, and root volume play critical capacities of plants, particularly in arid areas [32]. Vigorous root growth has been found to be beneficial to plants [33], obtaining more water and nutrients and improving the productivity and quality of plants’ aerial parts [34]. In our study, organic amendments in sand dune showed a strong promotion to plant growth and soil quality in the sand dune, indicating that the proper utilisation of compost is a very efficient method to promote plant growth rates, productivity or soil fertility [29, 35]. It also has been proved that these changes could improve root abundance and fractal dimension in *Impatiens hawkeri* [36] and stimulated the root number, volume growth, and root vitality of cucumber plants [37]. In greenhouse experiments, pruning waste compost could be used as substrates of peat, which was in turn used in the cultivation of perennial ryegrass (*Lolium perenne*) and cypress (*Cupressus sempervirens*) [38] and in stimulating the growth rate of bean plants (*Phaseolus vulgaris*) in Southern Africa [39] in previous studies.

The edaphic condition (e.g., nitrogen, phosphorus, potassium, and organic matter contents) in sand barrier was promoted by organic amendments or non-woven film in our study. These amendatory edaphic conditions promoted plant growth in original poor sand dune soil, including the above- and underground growth of two grasses utilised in our study [29, 40, 41]. Agriculture or forestry waste and animal manure have been commonly proven to be rich in nutrients and organic matter, ameliorating poor soil capacity in desertification zones [42, 36]. Unstable organic matter can also be converted into stable humus by composting, so that the waste material gradually turns into biological resources [43, 44]. Moreover, the soil carbon pool, nitrogen, available potassium, and phosphorus contents, and physical characteristics (e.g., soil total porosity) can be improved by organic compost amendments, as revealed in previous studies [34, 45, 46].

Film-mulched amendment successfully achieved soil stabilisation and improvement, grasses germination and growth rate promotion, as illustrated by higher tiller and leaf numbers for *E. sibiricus cv. duoye* and *E. breviaristatum*. These achievements were more obvious when the sand dune was amended by organic amendments. The film is known for its uses in thermal insulation and water conservation. One proposed model has shown that the vertical movement of water within sand can be interrupted, resulting in suppressed water evaporation [47, 48, 49]. These advantages can prevent nutrients from being blown away by the wind and can improve the seed germination rate, protecting plants in the early bud stage and promoting continued plant growth. In the present study, the effective root area of nutrients and moisture absorption increased, and vigorous growth of *E. sibiricus cv. duoye* and *E. breviaristatum* was observed.

Substantial differences were observed in the response sensitivity of *E. sibiricus cv. duoye* and *E. breviaristatum* to different techniques. With compost amendment, the response of *E. sibiricus cv. duoye* was more sensitive than that of *E. breviaristatum*, as evaluated by grass growth parameters.
(height, abundance, tiller number, leaf number, root surface area, root diameter, root volume, root tips, and root length). Furthermore, this type of response sensitivity variance was much more compelling under the combined amendments of film and compost. Although *E. sibiricus* cv. *duoye* and *E. breviaristatum* are both ecological variants of *E. sibiricus*, *E. sibiricus* cv. *duoye* was cultivated by artificial breeding, whereas *E. breviaristatum* was derived from domesticated breeds [26]. Their productivity (e.g., seed production and herbage yield) and habitat adaptability (e.g., cold resistance and drought endurance) might be different in harsh environment. So the *E. breviaristatum* grown better in the poor edaphic habitat where was no compost, and the *E. sibiricus* cv. *duoye* preferred fertile soil in this study.

CONCLUSIONS

The major conclusions that can be drawn from this study are as follows:

1. Integrating non-woven film-mulched and organic compost-amended is practical in ameliorating the harsh edaphic conditions and accelerating grasses growth in sand dune where established sand barrier.

2. *E. sibiricus* cv. *duoye* and *E. breviaristatum* can be used to construct artificial vegetation in alpine sand dunes. The *E. sibiricus* cv. *duoye* grown better in fertile sand dune while the *E. breviaristatum*’s poor soil tolerance was stronger.

3. Plant growth and edaphic conditions are the strong indicators to evaluate efficiency of re-vegetation.

ACKNOWLEDGEMENTS

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REFERENCES


PHYSIOLOGICAL AND BIOCHEMICAL CHANGES OF POPULUS EUPHRATICA SEEDLINGS UNDER SALINE IRRIGATION STRESSES

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ABSTRACT

In the lower reaches of Tarim River, based on the habitat characteristics and salt-tolerant characteristics of P. euphratica, different concentrations of NaCl solution (0 mM; 50 mM; 150 mM; 300 mM) were used to irrigate on seedlings of P. euphratica. Na⁺, K⁺, Cl⁻ concentration in different parts of the P. euphratica seedlings were measured. Meanwhile, chlorophyll (Chl-a; Chl-b), Proline (Pro) content in leaves of the P. euphratica seedlings also were measured, and water potential of the leaves (ψp), stomatal conductance (gs), stem xylem hydraulic conductivity and sap flow flux (Fs) were monitored in situ. The aim was to explore biochemical and physiological effects of seedlings of P. euphratica under salinity stress. Experimental results showed that with the increasing concentration of NaCl solution for irrigation, P. euphratica seedlings under osmotic stress. In various parts of the seedlings Na⁺, Cl⁻ with K⁺ contents had significantly negative correlation relationships respectively (sig.<0.05). Na⁺ and Cl⁻ concentrations increased to inhibit the absorption and transport of K⁺, this effects mainly reflects in K⁺ concentration significantly reduced (sig.<0.05) in the leaves. Ions content ratios change showed that ion balance was disturbed, especially in the roots and leaves. This relationship was more noticeable in higher concentration NaCl solution treatment (150 mM and 300 mM). The chlorophyll content was increased under salt stress of NaCl solution at lower concentration (50 mM), but Chl-a / Chl-b value decreased, and no significant changes in other salt irrigation treatment. Pro in Populus seedlings accumulated more significantly at a higher level of salinity (150mM and 300mM). Leaf water potential monitoring results showed, NaCl solution irrigation decreased the predawn water potential with the extension of stress time, and decreased the leaf water potential with the increase of NaCl concentration. Under salt water irrigation, the peak time of stomatal conductance was advanced. Under severe salt stress 300 mM, hydraulic conductivity safety and efficacy of water transportation of stem xylem in P. euphratica seedlings significantly decreased. Stem sap flow flux decrease significant (p<0.05). Comprehensive analysis think that the physiological and biochemical parameters of P. euphratica seedlings can be used for determining the index of salt tolerance, Seedlings of P. euphratica can maintain normal growth when irrigation with the concentration of 50 mM NaCl solution, under stress when with 150 mM NaCl solution, salt water irrigation in 300 mM subjected to severe stress, equivalent drought stress.

KEYWORDS: P. euphratica seedling, salinity stress, growth factor, osmotic regulation, Salt tolerance

INTRODUCTION

Abiotic stresses, such as drought, salt, extreme temperatures, chemical toxicity and oxidative stress on agriculture and natural environment poses a serious threat [1-4]. Salt stress constitutes an increasing agricultural and environmental problem on global scale [5-8]. It is expected that until 2050, more than 50% of all arable land will experience salinization [9]. Salt stress on the influence of different plants in physiological and biochemical aspects usually include protein synthesis [10-11], plant hormone regulation [12], respiration [13], photosynthetic capacity, stomatal regulation [14], water relations [15], enzymatic antioxidant activity [16], and compound enzyme levels and inorganic nutrients [17]. In essence, salt stress and water deficit are closely related. The molecular responses of plants to water and salt stress are basically the same except for ionic components [18]. Salt dissolved in soil reduces soil water potential (i.e., reduced availability of soil water to plants) and root uptake of water by thermodynamic barriers [19]. Plants exposed to high salinity can suffer damage in minutes due to the high osmotic pressure of the soil.
solution [20-21]. Plant function and growth involves many interrelated biological and physical factors, due to its complexity, at present, the quantitative description of plant responses to salt depends on the understanding and analysis of response to the whole tree and field scale [22-23].

*P. euphratica* is the oldest and the most primitive arid riparian forest constructive species [24], is considered to be drought resistant, salt tolerant species [25]. It formed a natural tree climax community of arid inland river basin in process of natural vegetation succession. *P. euphratica* as a typical hydro-halophyte, is ideal for studying salt stress responses in woody plants [26]. Global 60% of *P. euphratica* in China, of which 89.1% of *P. euphratica* is concentrated in the Tarim Basin of Xinjiang [27]. For extremely drought the lower reaches of Tarim River, the superposition effect of natural and artificial factors makes the channel long-term zero flows. Groundwater due to the lack of surface water supply, the water level continued to decline [28]. At the same time due to the intense evaporation salt accumulation at ground surface, has seriously affected the growth and reproduction of desert riparian forest and ecosystem function. As a result, large area of natural *P. euphratica* degradation, poor growth, shoot blight, bald, unfruitful and even death. Low salinity is considered to be the key to germination and growth of *Populus euphratica* in early stage [29]. *P. euphratica* reproduction and regeneration modes have seeds, root turion and stump turion. Despite the lower reaches of Tarim River has carried out eco-water transfer project since 2000, the sustainable decline of groundwater level has been alleviated and the desert vegetation has been restored to a certain extent. However, under natural conditions, the existing habitat conditions, no matter what kind of the reproduction and regeneration modes were unable to realize the *P. euphratica* population update and expand. One of the reasons is that although the river channel water conveyance to curb the declining groundwater levels of desert riparian forest in the lower reaches of Tarim River, but it also limits the natural overflow, makes the surface soil lacks enough moisture. *P. euphratica* seeds are difficult to germinate naturally; Another reason is that the strong evaporation causes the salinity to accumulate on the surface, which seriously restricted the germination and settlement of *P. euphratica* seeds. As a result, the natural regeneration ability of *P. euphratica* is weak. A few of juvenile *P. euphratica* were observed along the channel [30]. Wang and Yang [31] studies have shown that The salt tolerance of *P. euphratica* is different at different growth stages, Soil salinity amounted to 0.3%, the low survival rate of *P. euphratica* seedlings and the growth began to decline; reach 0.7% of soil salinity cannot take root, salt the upper limit is 0.8%, but the forest, salt can be increased to 2%. The total salt content of soil in the area of natural vegetation is 0.497% ~ 29.96%[32], Therefore, in the process of restoration and reconstruction of vegetation in the lower reaches of the Tarim River, a moderate artificial regeneration is needed. However, based on the characteristics of severe water shortage, and the high degree of groundwater mineralization (4.37~11.11g/l) [26], using high salinity of groundwater irrigation is inevitable. Moreover, the use of saline irrigation has also become a growing concern due to increasing irrigation demands and competition among humans [33-36]. Saline irrigation is a common practice, especially in arid and semi-arid areas, although it may lead to decreased plant yields and progressive soil salinization [37]. In this paper, the physiological and biochemical reactions of *P. euphratica* seedlings under different concentrations of saline irrigation were monitored by artificial control experiment. The aim of this study is to explore the possibility of using high salinity groundwater to plant *Populus euphratica* under the desert environment in the lower reaches of the Tarim River, and to provide some theoretical basis and data support for reconstruction and regeneration of *Populus euphratica* forest in the lower reaches of Tarim River.

**MATERIALS AND METHODS**

**Plant materials and treatments.** The experiment was carried out at the ecological monitoring experimental station of the lower reaches of Tarim River (87.698 E,40.648 N), Xinjiang Institute of Ecology and Geography, Chinese Academy of Sciences. The climatic characteristics of the study area are shown in Fig. 1. *P. euphratica* seedlings, which have been cultivated for 2 years in the lower reaches of the Tarim River, have been used as experimental materials. The seedlings height, root length, crown width uniform, the average plant height was 51.9 ±3.4 cm, and the average basal stem diameter was 1 + 0.17cm. The experimental containers were 30 cm diameter, 50 cm high cylindrical PVC pipes. The bottoms were covered with plastic pallets. The culture medium was taken from natural *P. euphratica* forest soil, soil field moisture capacity was 36%, and the basic properties were shown in table 1. *P. euphratica* seedlings were transplanted in April 7, 2010. each basin planting a *P. euphratica* seedling, a total of 30 basins. In the early stage of the experiment, deionized water was used for irrigation, the irrigation amount of each pot was the same before the group control treatment. After the seedlings were planted for 3 months, Saline water irrigation control processing per-
formed.

Processing begins on July 18, 2010 and ends on August 2, 2010. The seedlings of *P. euphratica* were divided into 5 groups with 6 replicates in each group, 4 groups were treated with different concentrations (0 Mm, 50 mM, 150 mM, 300 mM) of NaCl solution by deionized water to salinity treatment of *P. euphratica* seedlings, and the soil water content was maintained at 80% - 90% of field moisture capacity. One group did not water during the experiment as a control. Soil water content measurements were used Moisture Meter type HH2 (Britain, Delta-T) for the determination.

**K⁺, Na⁺, Cl⁻ contents measurements.** Populus euphratica seedlings were treated with NaCl solution. After 20 days, 12 to 18 leaves and stems and roots samples were collected from the *P. euphratica* seedlings treated with each group. The samples were rinsed with deionized water, deactivation of enzymes at 105°C for 25 min, at 70 °C drying to constant weight, then the samples were ground and placed in a desiccator to be measurement. The contents of K⁺ and Na⁺ were determined by flame photometry. The content of Cl⁻ was determined by spectrophotometry with gelatin - ethanol - water solution as a protectant[38].

**Chlorophyll, Proline and Malondialdehyde content measurements.** Chlorophyll a (Chl a), Chlorophyll b (Chl b) contents were estimated by the Arnon [39] method. Leaf samples (0.25 g) homogenized in 5 ml of acetone (80%). Absorbance was recorded at 645 and 633 nm (Spectrophotometer CECIL Model 2000, Cambridge, UK). For proline (Pro) measurement, 0.5 g of plant material was boiled in 25 ml water for 2 h at 100°C in a dry heat bath. This hot water extract was cooled and filtered using Whatman no. 42 filter paper, followed by Proline (Pro) determination according to Bates et al [40]. For malondialdehyde (MDA) determination, a 0.5 g leaf sample was homogenized in 5 ml 0.1% trichloroacetic acid and centrifuged at 10000 g for 10 min. The amount of MDA in the supernatant was estimated by the thiobarbituric reaction according to Dhindsa and Matowe [41]. All measurements had three replicates.

**Water potential measurements.** Populus euphratica seedlings of predawn water potential (ψP) measured by C-52 thermocouple psychrometer chambers and HR-33Τ dew point microvolt-meter (Wescor Inc., Logan, UT, USA). Leaf discs corresponding to the third youngest leave were cut, placed inside the psychrometer chamber and allowed to reach temperature and water vapour equilibrium for 30 min before measurements were made by the dew point method [42].

**Stomatal conductance measurements.** During the experiment stomatal conductance of *P. euphratica* seedlings leave (Gs) was measured. Every two hours measurements of stomatal conductance were performed throughout the entire day using a Dynamic Porometer (AP4, Delta-T devices, Cambridge, UK). The measurements were performed under natural conditions and on leaves that were exposed to direct sunlight.

**Stem xylem hydraulic conductivity measurements.** The *P. euphratica* seedings stem xylem hydraulic conductivity were measured by xylem hydraulic conductivity and embolism measurement system (xylem embolism meter, Bronkhorst, Montigny-Les-Cormeilles, France). The measured parameters including initial specific conductivities (Ks0),maximum specific conductivities (Ks max),Percentage loss of hydraulic conductivity (PLC) [43].

![FIGURE 1](image)

**Climate characteristics of the research area**
**TABLE 1**

<table>
<thead>
<tr>
<th>Property</th>
<th>Soil Sample</th>
<th>Mean ±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organic matter /g·kg⁻¹</td>
<td>16.33±1.69</td>
<td></td>
</tr>
<tr>
<td>Total nitrogen /g·kg⁻¹</td>
<td>1.05±0.12</td>
<td></td>
</tr>
<tr>
<td>Total phosphor /g·kg⁻¹</td>
<td>0.99±0.11</td>
<td></td>
</tr>
<tr>
<td>Total Kalium /g·kg⁻¹</td>
<td>17.7±0.41</td>
<td></td>
</tr>
<tr>
<td>Available nitrogen /mg·kg⁻¹</td>
<td>71.68±29.72</td>
<td></td>
</tr>
<tr>
<td>Available phosphor /mg·kg⁻¹</td>
<td>86.38±18.96</td>
<td></td>
</tr>
<tr>
<td>Available kalium /mg·kg⁻¹</td>
<td>222.62±43.75</td>
<td></td>
</tr>
</tbody>
</table>

**Sap flow measurements.** The sap flow rate was continuously measured during the experiment using heat balance sensors with a constant heat supply (Dynagage SGA11-WS, Dynamax Inc., Houston, TX, USA)[11]. Three P. euphratica seedlings under each treatment were selected for repeated simultaneous monitoring. At the same time, meteorological factors such as air temperature, air relative humidity, wind speed and soil temperature were monitored by automatic weather station.

**RESULTS**

**Na⁺, K⁺, Cl⁻ contents in different parts of seedlings of P. euphratica.** Compared with 0 mM saline solution treatment, the K⁺ content in roots and stems of P. euphratica seedlings treated with 50 mM and 150 mM saline solution was not significantly changed, but it decreased significantly (P<0.05) under 300 mM NaCl solution treatment. In the leaves, the K⁺ content decreased with the increase of the concentration of the NaCl solution. The contents of Na⁺ and Cl⁻ in various parts of P. euphratica seedlings showed a tendency to increase with the increase of treatment concentration, which only in the stem, The change of Na⁺ content between 50 and 150 mM was not significant, The change of Cl⁻ content between 50 and 150 mM treatment and between 150 and 300 mM treatment also did not reach significant.

Na⁺ and Cl⁻ were significantly accumulated in different parts of the treatment concentration (P<0.05), this accumulation better apparent in roots and leaves. Stems Na⁺, Cl⁻ accumulation is relatively slow (Fig.2). Analysis of the ratio of each ion...
content, compared with the 0 mM NaCl solution treatment. With the increase of concentration, K+/Na+ and K+/Cl- ratio in different parts of Populus seedlings were significantly decreased. Na+, Cl- ion concentration reduces uptake and transport of K+ in P. euphratica seedlings. make each part of the P. euphratica seedlings of ion balance is affected, compared with 0 mM NaCl solution treatment, especially apparent significant on the leaves.

Leaf Chlorophyll, Proline contents of *P. euphratica* seedlings under different salt treatment. Chlorophyll to absorb light energy and transfer light energy in photosynthesis, its content is a reflection of the growing status of plants, some environmental factors such as drought, salinity, temperature, air pollution, element deficiency can affect the composition and content of chlorophyll, photosynthetic rate and effect of plant. Therefore, determination of chlorophyll a and chlorophyll b content on plant photosynthesis and stress physiology has important significance.

The full irrigation (CK) and no irrigation (WD) proline content in leaves of *p. euphratica* can be seen (Fig 3), proline content in seedlings of *p. euphratica* leaf was 214.3 μg/g in under full irrigation, significantly higher than that of no irrigation condition of 149.4 μg/g (p<0.01). Under water stress, plant by the synthesis of soluble sugar, proline, betaine, material, to reduce the cell osmotic potential and water potential, so as to prevent cell dehydration [44], decreased transpiration. As osmotic adjustment, to maintain osmotic balance protoplasm and the environment. And may be formed with some compounds of intracellular polymer, similar to the hydrophilic colloid to prevent moisture loss. *p. euphratica* seedlings under non-irrigated conditions showed a large number of Proline accumulation is the result of plants in arid environment osmotic regulation.
The proline content of *P. euphratica* seedlings under different salt treatments (Fig. 3) analysis, the proline content of *P. euphratica* seedlings increased with the increase of salt stress, with the same performance under water stress. Chlorophyll content of leaves decreased with the increase of salt stress intensity. To see changes in the salinity range from chlorophyll content, chlorophyll content appeared 2 obvious decline, indicated that the increase of plants to salinity have a process of adaptation.

**Water potential of *P. euphratica* seedlings under different salt stress.** Leaf water potential data show that during the whole experiment, under 0 Mm irrigation of *P. euphratica* seedling leaf predawn water potential change was not significant (sig>0.05). No significant difference of *P. euphratica* seedling leaves the previous 7.19 treatments under the predawn water potential; 5g/l saline irrigation of *P. euphratica* seedlings under 7.19 and 7.23 leaf predawn water potential did not change significantly, in 7.28 of water potential has slightly increased at day 8.2 and 8.7 have large decline, decline significant (sig<0.05). 10g/l, 20 g/l and leaves of drought treated seedlings of *P. euphratica* predawn water potential increased with the stress time showed a significant trend (sig<0.01), and salt stress decreased about the potential of greater magnitude. Seedlings of *P. euphratica* leaf water potential under drought stress under the largest decline.

**FIGURE 4**
Changes in leaf predawn water potential of *P. euphratica* seedlings during the experiment

**FIGURE 5**
Hydraulic conductivity (a) and curves of xylem vulnerability to cavitation (b) of roots and stems of *P. euphratica* seedlings under different concentration salt stresses (mean ±SE).

Ks0, initial hydraulic conductivity per unit volume. Ksmax, maximum hydraulic conductivity. PLC, percentage loss of hydraulic conductivity
Leaf stomatal conductance of *P. euphratica* seedlings under different salt stress. Stomatal resistance includes lower chamber and stomatal pore shape and volume, including the opening of pores, which stomatal aperture based. Stomatal resistance, transpiration slow; small stomatal resistance, transpiration fast. Conditions by full irrigation (CK) and no irrigation (WD) the diurnal variation of the *p. euphratica* seedling leaf stomatal conductance (Fig 5), you can see that under the sufficient irrigation of *p. euphratica* seedling leaf stomatal conductance at 8:00 tendency for 73.08 mmol/m²/s, then rapid increase, slow increase amplitude after 10:00, peaked at 12:00 tendency for 133.98 mmol/m²/ss, then fell rapidly, to slow down after 2:00, PM 40.18 mmol/m²/s tendency low value. Under the condition of no irrigation, stomata conductance of *p. euphratica* seedling at 8:00 tendency for 32.71 mmol/m²/s, maximum proposed 10:00 now, but only 41.22 mmol/m²/s tendency, rising amplitude is small, then slow down, low value of 11.22 mmol/m²/sat 20:00 tendency. Under the sufficient irrigation of *p. euphratica* seedling leaf stomatal conductance is far higher than that of the *p. euphratica* seedlings under drought stress, the former days, on average, stomatal conductance is 3 times of the latter.

Under different salt processing by *p. euphratica* seedlings stomata conductance live changes (Fig 4), you can see that in the control group (CK) under the condition of sufficient water, the *p. euphratica* leaf stomatal conductance is higher, with the intensification of the degree of salt stress, the stomatal conductance have varying degrees of decline, the greater the degree and the stronger the stress drop. By paired samples T test, the control group (CK) spend Euphrates poplar seedlings stomatal conductivity changes and 5 g/L because the salt treatment there is no significant difference between stomatal conductance (p = 0.285), and 10 g/L, 20 g/L and drought treatment of *p. euphratica* seedling stomatal conductance were extremely significant differences (p < 0.01); Euphrates poplar seedlings under different times each treatment stomatal conductance difference have different performance, in 8, 14, 16, 18 points, CK and 5 g/L processing Euphrates poplar seedlings stomatal conductance difference was not significant, 5 g/L ten o’clock and 10 g/L processing difference was not significant, in 18 and 20 points, 10 g/L and 20 g/L deal with no significant difference. At 12:00 when all treatments showed significant differences, evaporation noon relatively strong, the impact of salinity stress generated at this time is more prominent among the treatments showed significant differences in treatment under full irrigation, Populus stomatal conductance peak time in the 12 o’clock, and when subjected to salt stress treatment, stomatal conductance peak time in advance to 10:00 AM. Sophora japonica under salt stress and walnuts stomatal conductance peak hours in advance, the peak decreases consistent with the results, *P. euphratica* seedlings in not affected by salt stress, stomatal conductance is large, this can make full use of light energy for photosynthesis condition. After salt stress, *P. euphratica* under water stress, stomatal closure of part of the loss of water to minimize. The reaction of *P. euphratica* self-feedback regulation mechanism to reduce transpiration loss through the regulation of stomata, and avoid high temperature period. At the same time also showed that stomatal conductance was significantly inhibited under salt stress.

Xylem hydraulic of *P. euphratica* Seedlings under Different Salt Stress. Compared with the irrigation treatment of 0mM salt solution, under the treatment of 50mM salt solution, Populus euphratica seedling stem xylem specific conductivity and initial potential maximum specific conductivity had no significant change (P>0.05), But the difference between the maximum ratio and the initial ratio of the initial ratio (Kmax-Ks0) was reduced by 18.3% compared with that of 0mM salt solution (Fig 6). In 150 mm and 300 mm salt solution treatment of Populus euphratica seedling stem xylem maximum water rate and initial water flowing rate officer is 0mm treatment decreased (P < 0.05) decreased 56.75% and 52.35% respectively, difference of maximum ratio of conductivity and initial specific conductivity decreases the 28.3% and 63.3%. That along with the salt stress increase the transmission efficiency of *P. euphratica* seedling stem xylem water adjustable range becomes smaller. Different salt treatments of *P. euphratica* seedlings PLC curve display, under the condition without negative pressure, Populus euphratica PLC value increases with the increase of salt concentration, namely of Populus euphratica guide water loss rate increased and the difference was significant (P < 0.01). Hydraulic conductivity loss of 50% of the xylem pressure P50 respectively 3.05 MPa, 2.94 MPa, 1.98 MPa and 1.01 MPa, in 0 mm and 50 mm, there were no significant differences between treatment with salt stress, and in 150 mm and 300 mm handle P50 corresponding under negative pressure and 50 mm dealing with significant difference with 0 mm, explain stem xylem embolism vulnerability increased significantly, the resistance ability weakened hole.
FIGURE 6
Diurnal variation of stomatal conductance of *P. euphratica* Olive seedlings Under different salt stress level.

FIGURE 7
SAP flow of *Populus euphratica* seedlings under different salt treatments daily average value changes.

Sap flow of *P. euphratica* seedlings under different salt stress. Sap flow of *P. euphratica* seedlings under different salt continuous observations for trend analysis (Fig 4), no-salt treatment and with adequate irrigation, during the experiment changes linearly with the average daily transpiration of seedlings of *P. euphratica* and daily transpiration of the seedlings of *P. euphratica* under drought stress with drought stress shows the tendency of increase then decrease.

Compared to the control experiments of different salt treatments of *P. euphratica* seedlings liquid flow, it can be seen from Fig 7, during the experiment, the seedlings of *P. euphratica* solution 5 g/L saline irrigation flow daily average flux with time trend line for the two time curve ($R^2=0.0556$), but more slowly, linearly. With the 10 g / L and 20 g / L *P. euphratica* seedlings liquid L saline irrigation treatment in flux of daily mean value with the stress time curve showed two distinct, the trend line equation was: $y = 0.061x^2-23.82x+2387.8 \ R^2 = 0.4691$; $y = 0.076x^2-29.82x+2965.7 \ R^2 = 0.5841$. Were decreased with the increase of stress time trend, and it can be seen that the 20 g / L 10 g / L saline irrigation fluid flow decreased quickly. Research shows that, *Broussonetia papyrifera* seedlings under different salt stress, transpiration flux and stomatal conductance with the increase of soil salt content decreased [45]. Study on three species of mangrove also shows that the net photosynthetic rate, stomatal conductance and transpiration rate decreased with the increase of salinity.
Osmotic regulation of P. euphrates seedlings under different salt stress. Osmotic adjustment refers to plants under drought, salinity or temperature environment initiative accumulation of intracellular solutes, lower osmotic potential; thereby reducing the potential, continue to absorb water from the water potential decline in external medium, in order to maintain a normal physiological function. Osmotic adjustment occurred due to stress, plants should continue to absorb water from the lower water potential of the medium to maintain water balance; to maintain turgor potential basically unchanged to ensure normal operation of physiological and biochemical process. Its physiological function is to maintain the plant transpiration stomatal opening, stomatal limitation of photosynthesis to reduce. Under generally, salt or water stress plants osmoregulation there are usually two ways, one is the accumulation of inorganic ion absorption and Na+, Cl-, etc., the second is the synthesis and accumulation of small molecules such as proline (Pro).

Plant habitat salt over certain concentration can cause harm to the growth of plants. In the salinization environment, plant cell excessive intake of Na⁺ and Cl⁻ after the first damage cells of ion balance and enzyme activity of cells and membrane system institutions have a specific effect, which affects a series of metabolic reactions, such as photosynthesis, respiration, nucleic acid metabolism and hormone metabolism, etc., which seriously affected plant growth and development, makes the plant growth and stunted growth. Low water potential conditions seriously affect plant cell water deficit, transpiration decreases, thus affecting the absorption and transport of mineral nutrition, organic synthesis and transport. Due to the high concentration of salt reduces the soil water potential, so that the plant cannot absorb water, and even the body of water leakage, which manifests itself in physiological salt damage drought.

Salt damage to plants mainly for the toxic effects of salt ions, salt ions lead to osmotic stress and nutrient deficiency [46-48]. Plants in high permeability environments through the accumulation of inorganic ions and small molecules of organic substances reduce their potential, to absorb soil moisture and nutrients needed for growth while maintaining normal cell turgor [49]. Therefore, under salinity stress, plants of different organizations onion absorbents segmentation features are important aspects of plant salt-tolerance mechanism. K⁺, Na⁺ and Cl⁻ are under salt stress in plants for osmotic adjustment of major inorganic ions, but excessive accumulation of Na⁺ and Cl⁻ are harmful, salt is one of the main causes of stress in plants caused by growth [50], through selective restrictions roots, promoting Na⁺ efflux and compartmentalization, maintaining a high K⁺ / Na⁺ values and ion balance [51]. Under salt stress, K⁺, Na⁺ absorption and plasma membrane K⁺, Na⁺ exchange with salt tolerance in plants are closely related, in organ and whole plant level, transportation and distribution of K⁺ and Na⁺ also showed distinct characteristics.

Terrestrial plants most frequently subjected to environmental stress of drought, when water consumption is greater than the plant absorbs water, which makes the water deficit within the organization. Proline (Pro) is one of the components of plant proteins, and can exist in the free state widespread in plants. Under drought, salinity and other stress conditions, many plants Proline accumulation by osmotic adjustment of protoplasm maintain osmotic balance with the environment, the formation of a similar hydrocolloid polymer with other compounds to prevent moisture loss, thereby enhancing the plant’s drought tolerance and resistance. Bates [52] first observed stomatal closure could significantly stimulate the accumulation of proline. Then some people have noticed this phenomenon, and confirmed that the plant wilting of proline content increased significantly, and the drought resistance and stomatal closure process synchronization, and that the number of proline accumulation in certain stage, time depends on the regulation of stomatal for adaptability to drought, also points out at the same time of water stress, abscisic acid, cytokinin, temperature on proline accumulation may play an equally important role, but there are direct and indirect and the difference of order. Under salt stress, the plant cell membrane permeability increased, cell loss, make plants produce water deficit. Therefore, salt stress and drought stress is very similar, the proline accumulation will cause the proline in plant. The study of P.euphratica Leaf Proline concentration increased with the salt concentration increasing. From the analysis of proline increased the amplitude of 10G / L salt treatment only for Populus seedlings have slight stress; 20 g / L salt treatment on seedlings of P. euphratica constitute the stress is more serious.

The mechanism of plant resistance to salt research is a hotspot of research on plant physiological ecology, therefore many scholars to salt stress under the condition of plant morphological development, photosynthesis, carbon metabolism and changes of endogenous hormones carried on the thorough research. Considering the chlorophyll as the main plant leaf photosynthetic pigment, to the growth and development at the same time under the condition of the same plant in adversity leaf chlorophyll content changes can reflect the differences in photosynthetic performance, so some scholars
through the experiment proves that the plant chlorophyll content of leaves under salt stress decreases, caused the main reason of this change is caused by chlorophyll enzyme in the degradation of chlorophyll b.

Plant stomatal closure under salt treatment, through a balanced pressure maintaining high moisture condition has been proved [53]. With the increase of salt treatment, p. euphratica seedling stomatal conductance presents the obvious downward trend, and the more the greater the fall of the strong stress. And stomatal conductance peak time in advance from 12:00 to 10:00. Under normal circumstances, the sweet earth plant in soil salinity amounted to 0.2% to 0.25 %, the water will be difficult; when the salt content is higher than 0.4%, it is easy to extravasation dehydration plant, grow short, dark green leaves. Flow characteristics of p. euphratica seedlings under salt stress display, 50mM of salt treatment did not produce stress on it, so little during the experiment average daily flow flux. 150 mM and 300 mM salinity treatments for p. euphratica produce stress makes liquid circulation is reduced. Comprehensive analysis, p. euphratica has some salt tolerance, normal growth in the salt content of 0.5%, in 1% or higher salt content would be coercion.

In this study, p. euphratica seedlings under salt stress in the amount of proline accumulation and stomatal closure (reduced stomatal conductance), indicating that by salt stress, osmotic adjustment by itself, to maintain osmotic balance cells and the environment, to prevent plant loss of body water to accommodate moisture deficit. P. euphratica seedlings sap flow flux reduction is the result of osmotic adjustment under salt stress. Due to the ability of plants to adjust is reversible, osmotic adjustment of the plant has been established after rehydration can disappear, and then subjected to still establish the role of osmotic adjustment when stress. Salt stress can cause osmotic pressure imbalance, reduce plant water access and transpiration, and reduce the yield of the plant. Including the plant tissue onion uptake and accumulation toxicity on physiological process caused by long-term effects on perennial plant salt stress, as well as from plant growth and reproduction transfer more energy for resistance and avoidance mechanism [54-55]. Salt-tolerant plants are subjected to mild salt stress increases the amount of dry matter [47]. Effects of salinity and drought plant in a similar way [55], with the salinity or drought soil moisture supply reduction, changed the water status of plants, which affect the short-term and long-term gas exchange and carbon balance [56].

### CONCLUSION

Under salt stress, reduce plant growth usually associated with a variety of physiological and biochemical and molecular features. Most of the plants grown under salt environment in varying degrees in different physiological and biochemical changes so that they can thrive in saline environments [56]. However, the response of plants to salt stress is very complex, because of different species varies, even in the same species, there are also differences. Physiological and biochemical parameters of P. euphratica seedlings can be used as seedling salt tolerance limits of determination of the reference index, the flow characteristics of the present study changes in P. euphratica seedlings treated under different salinity reflects the plants affected by the degree of stress. P. euphratica seedlings can maintain normal growth in the concentration of 50 mM NaCl solution under irrigation, 150 mM is under stress, received severe stress in 300 mM salt water irrigation, drought stress considerably.

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### REFERENCES


CYANOBACTERIAL BLOOM REMOVAL USING FLOCCULANTS MADE OF CALCIUM HYDROXIDE MODIFIED, AUTOCLAVED FLY-ASH BRICK POWDER

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ABSTRACT

Cyanobacterial bloom has become a serious environmental threat throughout the world. In particular, the presence of microcystins (MCs), has become a severe problem. Thus, it is essential to develop technologies for removing cyanobacterial cells and cyanotoxins simultaneously. In the present study, a calcium-hydroxide-modified, autoclaved fly-ash brick powder flocculant was developed. The factors influencing the removal of Microcystis aeruginosa by the modified flocculant, including the calcium hydroxide concentration, the particle size of the autoclaved fly-ash brick powder and additional dosages of the autoclaved fly-ash brick powder, were investigated to determine the ideal conditions for preparation. Under the optimal conditions, the removal efficiency of M. aeruginosa cells by the modified flocculants was 93.98%, and 35.02% of MCs was removed in a 1 mg/L solution of MC at 10 g/L of an additional concentration of the modified flocculant. The results indicate that the calcium-hydroxide-modified, autoclaved fly-ash brick powder flocculants could effectively remove M. aeruginosa cells and microcystins.

KEYWORDS:
Microcystis aeruginosa, microcystin, flocculant, calcium hydroxide, autoclaved fly-ash brick powder

INTRODUCTION

The presence of seasonal algal blooms in water sources, such as lakes and reservoirs, has posed a serious water safety concern to local water industries [1]. In general, many strains of Microcystis aeruginosa produce the toxin microcystin, which is harmful to humans, livestock, and aquatic animals [2]. For example, the algal bloom that occurred in Tai Lake in Jiangsu Province in late May of 2007 contaminated the main supply of drinking water for millions of people [3].

Over the last several decades, significant efforts have been made to develop bloom mitigation strategies around the world [4-9]. Coagulation-flocculation is a proven technique to efficiently remove algae [10-13]. However, the use of chemical coagulants and flocculants (such as polyaluminum chloride and polyacrylamide) have several potential environmental consequences, including (i) an increase in metal concentration in water (which may have human health implications); (ii) the production of large volumes of (toxic) sludge; and (iii) the dispersion of acrylamide oligomers, which may also pose a health hazard to humans [14]. As a result, there is a need for innovative flocculants that are both safe and efficient.

In the current work, calcium hydroxide was used to modify the development of waste from the flocculant and settle M. aeruginosa cells and microcystin (MCs). The factors influencing the removal efficiency, including the calcium hydroxide concentration, the particle size of the autoclaved fly-ash brick powder, the dosage of the autoclaved fly-ash brick powder and the dosage of the calcium-hydroxide-modified, autoclaved fly-ash brick powder, was also studied. The objective of this study was to develop a new, environmental-friendly modification to the method of waste generation to aid in the mitigation of cyanobacterial blooms and microcystin (MCs).

MATERIALS AND METHODS

Algal culture. Axenic unicellular culture of M. aeruginosa was obtained from the Culture Collection of Algae at the Institute of Hydrobiology, Chinese Academy of Sciences. The algal cells were cultured in a sterilized BG11 medium (pH 7.4) at 25 °C and a light intensity of 2500 lux, with a 12:12 h light:dark cycle. The algal cells were cultured for 4 days to the exponential phase at a density of 10⁶ cells/mL and were then used to assay the flocculating property of calcium-hydroxide-modified, autoclaved fly-ash brick powder flocculants. The growth medium for all cultures was BG11 [15].
Preparation of calcium-hydroxide-modified, autoclaved fly-ash brick powder. Autoclaved fly-ash brick was obtained from the construction site of Pingdingshan in Henan Province, China. It was crushed and sieved using an 80-mesh to the 160-mesh screen.

The specific preparation process for the calcium-hydroxide-modified, the autoclaved fly-ash brick powder was as follows. Initially, the calcium hydroxide solution was prepared, and the pretreated, autoclaved fly-ash brick powder was slowly added to the solution. Then, the mixed suspension was slowly stirred for 24 h with a magnetic stirrer. Finally, the suspension was filtered through a qualitative filter paper (10-15 μm), and the filter residue was washed to neutral with distilled water and then dried for 4 h in an oven at 60 °C. The dried filter residue served as the final calcium-hydroxide-modified, autoclaved fly-ash brick powder flocculant.

Removal of M. aeruginosa. The capacity of the calcium-hydroxide-modified, autoclaved fly-ash brick powder flocculant to remove HABs was tested using M. aeruginosa. The calcium-hydroxide-modified, autoclaved fly-ash brick powder flocculant was added into 50 mL of the algal culture in a 100-mL beaker and allowed to settle for 1.5 h. In the control groups, the calcium-hydroxide-modified, autoclaved fly-ash brick powder flocculant was not added. At the end of the settling period, a sample was collected 2 cm below the surface for analysis.

Analytical methods for the concentration of chlorophyll-a. The concentration of chlorophyll-a was measured to indicate the change in the concentration of M. aeruginosa cells throughout the flocculation experiment. The chlorophyll-a concentration was determined using standard methods [16].

The clearance rate of algae (r, %) in each sample based on the chlorophyll-a concentration was determined after a 1.5 h exposure by the following formula:

\[ r = \frac{T_2 - T_1}{T_2} \times 100\% \]  

where \( T_1 \) and \( T_2 \) are the chlorophyll-a concentration after flocculation and the control, respectively.

Removal and measure of microcystin. The solution of MCs was prepared using standard solution (purchased from China standard material net) with concentrations of 1 mg/L. Next, 0.5 g of calcium-hydroxide-modified, the autoclaved fly-ash brick powder was added to 50 mL of the MC solution in a 100-mL beaker, followed by settling for 1.5 h. Following the settling period, the solution was filtered through qualitative filter paper (10-15 μm) and a glass fiber filter membrane (0.45 μm), sequentially. The filtered material was analyzed to determine the MC concentration following flocculation. The content of MCs was determined using a high-performance liquid chromatograph (Thermo Fisher u3000).

RESULTS AND DISCUSSION

Effect of calcium hydroxide on M. aeruginosa cell removal. The flocculation efficiencies for M. aeruginosa cells at varying concentrations of calcium hydroxide were studied (Fig. 1). The removal efficiency of algal cells increased sharply from 48.94% to 96.31% as the calcium hydroxide concentration increased from 0.01 to 0.1 g/L and then decreased gradually with increasing dosages. When 0.05, 0.5 and 1.0 g/L calcium hydroxide was added, 63.68%, 85.79% and 56.84% of the M. aeruginosa cells were removed, respectively. Based on these results, the optimized calcium hydroxide concentration of 0.1 g/L was used for the subsequent flocculation experiments.

Effect of the particle size of autoclaved fly-ash brick powder on M. aeruginosa cell removal. The influence of the particle size of the autoclaved fly-ash brick powder on the algal removal efficiency is shown in Fig. 2. The results indicated that the particle size of the autoclaved fly-ash brick powder significantly affected the algal removal rates. With increasing particle size, the algal removal rates initially increased and then decreased. Hence, the present study suggests an optimum particle size of the autoclaved fly-ash brick powder is 120-μm.

Effect of the addition of autoclaved fly-ash brick powder on M. aeruginosa cell removal. With the calcium hydroxide concentration fixed at 0.1 g/L, 8, 12, 16, 20 and 24 g of autoclaved fly-ash brick powder was added into 100 mL of a calcium hydroxide solution to prepare the modified flocculant. The algal removal efficiencies are shown in
Fig. 3. With increasing dosages, the algal removal rate initially increased and then decreased. The maximum algal removal efficiency reached 99.31% when the dosage of autoclaved fly-ash brick powder was 16 g. However, when the additional dosage was further increased to 20 g, the removal rate dropped to 90.28%. The cause could be due to insufficient reactions of calcium hydroxide and autoclaved fly-ash brick powder.

**FIGURE 2**
Effect of the particle size of autoclaved fly-ash brick powder on the M. aeruginosa cell removal

**FIGURE 3**
Effect of the dosage of autoclaved fly-ash brick powder on M. aeruginosa cell removal

Effect of the concentration of calcium-hydroxide-modified, autoclaved fly-ash brick powder on M. aeruginosa cell removal. Fig. 4 depicts the removal efficiency of M. aeruginosa cells at varying concentrations of calcium-hydroxide-modified, autoclaved fly-ash brick powder. The removal efficiency of algae was increased from 22.29% to 93.98%, as the modified flocculant concentration increased from 2 to 10 g/L. However, the removal efficiency of algal cells decreased as the modified flocculant concentration further increased. Therefore, these results suggest that the optimal modified flocculant concentration is 10 g/L.

In our previous studies, a flocculant composed of autoclaved fly-ash brick and chitosan was also used to remove M. aeruginosa cells [17]. In comparison to this study, the additional dosage of required flocculant was higher than that of the calcium-hydroxide-modified, autoclaved fly-ash brick powder. In addition, the price of chitosan is significantly higher than that of calcium hydroxide, hence, the current results have greater application potential.

**FIGURE 4**
Effect of the calcium-hydroxide-modified, autoclaved fly-ash brick powder concentration on M. aeruginosa cell removal

Effect of the calcium-hydroxide-modified, autoclaved fly-ash brick powder on MC removal. The results indicate that 35.02% of MCs was removed in a 1 mg/L solution of MC at 10 g/L of an additional concentration of the modified flocculant. The reason for low removal efficiency may be due to the high initial concentration of MCs.

The presence of toxic cyanobacteria in surface waters that are used as drinking water sources as well as recreational purposes has received increased attention due to the potential impacts on the ecosystems [18-19]. Chronic exposure to MCs could cause widespread, serious health problems in both animals and humans [20]. For example, the incidence of primary liver cancer in locations in China was found to be related to the presence of MCs in the drinking water [21]. Currently, some technologies have been used in the removal of MCs in water, including microgel-Fe(III) adsorption and UV/H2O2, TiO2 nanotubes, and DMXS degradation [22-25]. In the current study, an autoclaved fly-ash brick from building debris was used as raw material to prepare a modified flocculant, which could simultaneously remove M. aeruginosa cells and MCs.

**CONCLUSIONS**

In the present work, a new modified flocculant was prepared. The preparation process comprises the following steps: 1) waste autoclaved fly-ash brick was crushed and sieved using an 80-mesh to 160-mesh screen; 2) a 0.1 g/L calcium hydroxide solution was prepared, and then 16 g (120 μm) of autoclaved fly-ash brick powder was slowly added...
into the calcium hydroxide solution, followed by slow stirring for 24 h with a magnetic stirrer; and 3) the mixed suspension was filtered through qualitative filter paper (10-15 μm), washing the residue with distilled water to achieve a neutral pH, and then dried. The dried filter residue served as the final calcium-hydroxide-modified, autoclaved fly-ash brick powder flocculant for the study.

Under the experimental conditions, the removal efficiency of the modified flocculant on *M. aeruginosa* cells was 93.98%, and 35.02% of MCs was removed in a 1 mg/L solution of MC at 10 g/L of an additional concentration of the modified flocculant. According to these results, calcium-hydroxide-modified, autoclaved fly-ash brick powder flocculant is effective at removing harmful cyanobacterial blooms and MCs. In addition, the study proposed an innovative utilization of the autoclaved fly-ash brick waste.

ACKNOWLEDGEMENTS

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REFERENCES


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DEW AND RAINWATER CHEMISTRY IN INDUSTRIAL AND AGRICULTURAL AREAS OF TURKEY

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ABSTRACT

The aim of this study was to determine the ionic contents of the dew and rainwater samples around the Luleburgaz region in Turkey. Dew was more acidic than rainwater at both of the sampling locations. The results showed that the ionic contents are lower in rainwater than in dew except of NO₃⁻. It was observed that the most abundant anions were Cl⁻ and SO₄²⁻ and cations were NH₄⁺ and K⁺ in dew and rainwater. PCA analyses results showed that the anthropogenic sources especially industrial activities were an important sources of pollution. These findings point to the fact that the air pollutants caused by the industrial emissions sourced from cement, glass, iron-steel factories and natural gas plant, the emissions from pesticides usage in agricultural land and the marine aerosols were transported to the agricultural land by dew and rainwater in Luleburgaz region.

KEYWORDS:
Dew, Rainwater, Anions, Cations, Luleburgaz

INTRODUCTION

As an initial step for maintaining a clean, habitable environment and to protect it from pollutants, it is important to first define the levels of environmental pollutants and to establish health safety limits for contaminants. A substantial amount of research has gone into the identification and quantification of atmospheric pollutants and their precipitates since the 1990s. Wet precipitates are especially important in the identification of air pollutants and for correctly characterizing region-specific pollution. Condensation of humid air in the atmosphere on a substrate and transformation into liquid water can form dew, fog, clouds and rain. Whereas for natural fog, cloud and rain condensation starts on sub-micron particles in a large atmospheric volume, dew formation is observed on larger surface at ground level [1].

Several experiments on the ecological role of dew in plants growth have also been studied [2, 3, 4, 5, 6]. Water absorption through leaves also extends plants life, improves flowering and fruit quantity, promotes plant growth and increases ground or below-ground biomass [7]. But some studied showed that the wet deposition process is a major pathway to transport particles and gasses from the atmosphere to the biosphere [8]. So the chemical characters of dew water reflect the air quality of the local surface air. Especially in the last study by Xu et al. (2015) showed that the atmospheric fine particulate matters are the condensation nuclei in the process of dew formation [9]. Also dew can absorb and dissolve gasses and aerosols captured by the substrate in the range of 0-2 m above the surface [10]. The aerosols and gasses at this level have a more direct and strong influence on human health than at any other level [9].

Numerous studies conducted in Turkey have focused on the characterization of regional air pollution through precipitation sampling [11, 12, 13, 14]. In Turkey, there is no similar study about the characterization of dew water. Some studies reported that the ion content in dew samples considerably higher than in acid rainwater samples [15, 16, 17]. Dew sampling has not yet been adopted in pollution studies in Turkey despite the fact that it has received considerable attention in studies conducted to determine regional air quality in the rest of the world since 2005 [8, 15, 18, 19, 20, 21].

Eastern Thrace of Turkey is a region suffering from environmental problems that are associated with increasingly intensifying industrialization. Industrialization caused an increase in population in Thrace, consequently increasing the demand for energy. According to the Ministry of the Environment above 2000 industrial plants are concentrated around the Luleburgaz region of Thrace [22]. The Ergene River is located in the area, so this area is also important for agricultural production. The greatest problem for the Ergene Basin and Ergene River is the salinity stemming from the industry. Salinity is the major environmental problem for the products growth, such as limiting of productivity [23]. 56 % of sunflowers, 32 % of rice and 8 % of wheat of the total production in Turkey is provided from this area [23, 24, 25]. Therefore, the quality of air is affected all the industrial, residential and agricultural facilities and also atmospheric deposition effects to the agricultural production.
Air pollution adversely affects humans, forests and agricultural lands. Problems such as decreases in productivity, blasting of leaves and trees as well as damage to fruits have been observed in the agricultural land in and around Luleburgaz. The humidity in the air precipitates as dew on the surface of leaves and fruits at night as the air cools. The dew, which is full of various chemical compounds, has been reported to harm vegetables and fruits [26]. The timeliness of the issue and its possible direct effect on the public’s income and subsistence necessitates a scientific investigation of the air pollution to determine pollutant levels. The ionic compound contents of dew and rainwater samples that were collected from two locations of agricultural activity around Luleburgaz were determined within the present study in order to characterize the air pollution caused by the industrial plants and power plant in the area.

MATERIALS AND METHODS

Eskitaslı and Turgutbey villages in Luleburgaz, Turkey were selected as the locations for sampling dew and the rainwater owing to the intense agricultural activity in the area. Luleburgaz resides in the inlands of the Eastern Thracian steppes on low land (50m). The map of sampling areas, industries and meteorological station in Luleburgaz are given in Figure 1. Terrestrial climate is dominant in the study area with cold and rainy winters and hot and arid summers. Long-term annual average precipitation is 509.6 mm, average temperature is 12.1°C [24]. The dominant winds flow between the north and the east.

Dew and rainwater samples were collected during the period of 1 April-12 August 2012 and 3 October-10 November 2012. 5 plastic washing bowls with 1 m² area were placed onto the table which is 1 m above the ground level to collect the dew and rainwater samples at each sampling stations in Eskitaslı and Turgutbey villages. For dew sampling, plastic sampling bowls were placed into the sampling locations between 23:00 and 24:00 o’clock. Because of the evaporation possibility of dew, the next very early morning around 06:00 – 07:00, dew samples were collected and poured into the bottles. The periods of rain showers are expected according to the weather forecast, sampling bowls were placed to the sampling points. Usually, 10 minutes later from the rain started, the samples were taken. If the rain sample amount were not enough, we waited until that the sample precipitation was sufficient. The samples were taken into the falcon tubes of 50 ml and stored at 4°C in the refrigerator until analysis. The amount of samples was between 30 and 50 ml for rainwater and between 4 and 20 ml for dew. The dew samples below 4 ml were not used for IC analyse because the sample must be at least 4 ml for analysis. We tried to collect samples simultaneously in Eskitaslı and Turgutbey. Some dew and rain water samples were not collected because of the technical problems such as local transportation. A total of 83 dew and 43 rainwater samples were collected from the Eskitaslı village sampling station and 86 dew and 26 rainwater samples from the Turgutbey village sampling station.

The pH values were measured by the pH meter. The ion concentrations of aqueous solutions were determined by Dionex ion chromatography ICS 1100 with Degas and Chromeleon SE for anions and cations respectively. An analytical AS9-HC (4 x 250 mm) column and AG9 guard column (4 x 50 mm) with ASRS-300 (4 mm) suppressor was used in ion-exchange mode in order to determine the water-soluble anions (sulphate-SO₄²⁻, phosphate-PO₄³⁻, nitrate-NO₃⁻, nitrite-NO₂⁻, chloride-Cl⁻, fluoride-F⁻, Bromide-Br⁻). The eluent was 9 mmol Na₂CO₃ and the flow rate of the eluent was 1.0 mL/min. For the determination of water-soluble cations (lithium-Li⁺, sodium-Na⁺, potassium-K⁺, magnesium-Mg²⁺, calcium-Ca²⁺, ammonium-NH₄⁺), an analytical CS16

**FIGURE 1**

Dew and rainwater sampling points, industries and meteorological station in Luleburgaz.
column and a CG16 guard column (both 3 x 50 mm) with CSRS-I (2 mm) suppressor was used in a chemical mode. An eluent of 10 mM methane sulfonic acid was used at flow rate of 1.0 mL/min. The injection volume was 25 μL for all detection runs. Peak identification was confirmed based on a match of the ion chromatograph retention times with the chromatographs of the standard samples. The limit of detection was determined as mean equal to 3 times standard deviation of the field blank value, corresponding to a range of 0.008 to 0.023 ng/L for the anions and to a range of 0.021 to 0.083 ng/L for the cations. In order to obtain blank samples, the bowls were rinsed with distilled water, subtracting the ion concentrations from those of the actual dew and rainwater samples.

Statistical analyses were performed using the SPSS program (version 22.0). The arithmetic mean, median and standard deviation were used to characterize the data distribution. The Pearson correlation coefficient was employed to evaluate the relationship between ions. A paired t-test was used to compare sampling points and deposition ion differences. The criteria for significance in the procedure were p<0.05 and p<0.01. The principal component analysis (PCA) was used for the source identification using independent variables. PCA is a multivariate statistical method which could explain the variability of most of the original data. PCA transforms a set of correlated variables and a set of uncorrelated variables, called principal components. A varimax rotation was applied with an initial eigenvalue above 1.

### RESULTS

**Ionic Composition.** Table 1 shows pH, the mean anion and cation contents of the dew and rainwater samples, correlation and difference values. Also Figure 2 and 3 shows the timely variation of cations and anions in dew and rain samples. Outliers of all ions date were calculated (the mean±2.standard deviation) and above the outliers were excluded. Percentage of excluded date in all is below 5 %. The pH values determined for the all dew and rainwater samples. The pH of dew varied in the range of 5.5 to 7.8 in Eskitasli (mean pH = 6.5) and 5.1 to 8.2 in Turgutbey (mean pH = 6.4). The pH of rainwater was shown to vary in the range of 5.8 to 8.4 in Eskitasli (mean pH = 7.0) and 5.9 to 8.2 in Turgutbey (mean pH = 6.8).

### TABLE 1

Mean, standard deviation and excluded date range values and correlation (R), differences (t) statistics for the ions measured in the dew and rainwater samples collected from Eskitasli and Turgutbey villages (μEq/L).

<table>
<thead>
<tr>
<th>Ions</th>
<th>Eskitasli Dew (N=83)</th>
<th>Turgutbey Dew (N=86)</th>
<th>Eskitasli Rain(N=43)</th>
<th>Turgutbey Rain(N=26)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>mean±stddev</td>
<td>Excluded date range</td>
<td>mean±stddev</td>
<td>Excluded date range</td>
</tr>
<tr>
<td>Na⁺</td>
<td>221±1511</td>
<td>5842-6841</td>
<td>939±641</td>
<td>2944-4315</td>
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<tr>
<td>NH₄⁺</td>
<td>1196±946</td>
<td>3306-3725</td>
<td>1532±1246</td>
<td>5779-6437</td>
</tr>
<tr>
<td>K⁺</td>
<td>924±798</td>
<td>4360</td>
<td>1594±1260</td>
<td>5327-6340</td>
</tr>
<tr>
<td>Mg²⁺</td>
<td>289±194</td>
<td>842-1120</td>
<td>740±423</td>
<td>1904-2646</td>
</tr>
<tr>
<td>Ca²⁺</td>
<td>638±427</td>
<td>1916-3209</td>
<td>1390±795</td>
<td>3444-3948</td>
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<tr>
<td>Li⁺</td>
<td>0.8±0.9</td>
<td>4.3-5.5</td>
<td>1.1±0.9</td>
<td>4.2</td>
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<tr>
<td>NO₃⁻</td>
<td>7.3±10.3</td>
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<td>4.6±15.7</td>
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<tr>
<td>NO₂⁻</td>
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<td>117-173</td>
<td>4.6±11.0</td>
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<tr>
<td>F⁻</td>
<td>15.0±21.9</td>
<td>124-219</td>
<td>17.5±14.5</td>
<td>102-231</td>
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<tr>
<td>Cl⁻</td>
<td>2519±2126</td>
<td>10342-11216</td>
<td>4123±2464</td>
<td>10831-16030</td>
</tr>
<tr>
<td>Br⁻</td>
<td>31±3.5</td>
<td>24-32</td>
<td>1.1±0.7</td>
<td>3</td>
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<tr>
<td>PO₄³⁻</td>
<td>10.8±12.8</td>
<td>52-64</td>
<td>89.4±92.7</td>
<td>290</td>
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<tr>
<td>SO₄²⁻</td>
<td>186±105</td>
<td>480-540</td>
<td>328±252</td>
<td>1015-1558</td>
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<tr>
<td>pH</td>
<td>6.5±0.5</td>
<td>6-6.5</td>
<td>4.0±0.5</td>
<td>7.0±0.7</td>
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</table>

<table>
<thead>
<tr>
<th>Ions</th>
<th>Esk.-dew/Tur-dew</th>
<th>Ratio</th>
<th>R</th>
<th>t</th>
<th>Esk.-rain/Tur-rain</th>
<th>Ratio</th>
<th>R</th>
<th>t</th>
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<tr>
<td>Na⁺</td>
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<td>-0.018</td>
<td>5.914*</td>
<td>0.9</td>
<td>0.142</td>
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<td>1.376</td>
<td>1.4</td>
<td>0.044</td>
<td>2.248*</td>
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<td>2.832*</td>
<td>1.1</td>
<td>0.310</td>
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<td>Mg²⁺</td>
<td>0.4</td>
<td>0.395**</td>
<td>8.972**</td>
<td>0.7</td>
<td>0.530*</td>
<td>0.900</td>
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<tr>
<td>Ca²⁺</td>
<td>0.4</td>
<td>0.452**</td>
<td>7.684**</td>
<td>0.6</td>
<td>0.151</td>
<td>0.967</td>
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<tr>
<td>Li⁺</td>
<td>0.7</td>
<td>0.303*</td>
<td>1.899</td>
<td>1.5</td>
<td>0.196</td>
<td>1.969</td>
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<td>0.303</td>
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<td>0.727</td>
<td>1.0</td>
<td>0.291</td>
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<td>0.105</td>
<td>0.299</td>
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<tr>
<td>Cl⁻</td>
<td>0.6</td>
<td>0.463**</td>
<td>4.966**</td>
<td>1.0</td>
<td>0.105</td>
<td>0.299</td>
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<tr>
<td>Br⁻</td>
<td>2.8</td>
<td>0.076</td>
<td>4.146**</td>
<td>1.0</td>
<td>0.121</td>
<td>0.675</td>
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<td>PO₄³⁻</td>
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<td>0.214</td>
<td>5.970**</td>
<td>0.7</td>
<td>0.198</td>
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<tr>
<td>SO₄²⁻</td>
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<td>0.254</td>
<td>4.221**</td>
<td>0.8</td>
<td>0.148</td>
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<tr>
<td>pH</td>
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<td>0.421**</td>
<td>0.375</td>
<td>1.0</td>
<td>0.641**</td>
<td>0.815</td>
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</table>

**p<0.01, *p<0.05**

6505
Investigation of the anion and the cation contents of the dew and the rainwater indicated that the most abundant anions were Cl\(^-\) and SO\(_4^{2-}\) and cations were NH\(_4^+\) and K\(^+\) in dew and rain. Large NH\(_3\) emissions from fertilizer applications, biomass burning and animal breeding in the near the areas of the sampling sites are responsible for the high NH\(_4^+\) and K\(^+\) concentration [27]. The sampling was carried out in the period of agricultural facilities increase and no domestic heating with fossil fuel. It was not observed any seasonal difference in rain water samples. But we observed an increase in the trend between April and August for some ions (NH\(_4^+\), SO\(_4^{2-}\), Cl\(^-\), Ca\(^{2+}\), Mg\(^{2+}\), K\(^+\)) in dew samples. These chemicals are the components of the primary fertilizers used in crop production [28]. Ammonium sulfate ((NH\(_4\))\(_2\)SO\(_4\)) is a commonly used nitrogen fertilizer because of its stable performance and low price [29].

In the south of the sampling area, there are glass production industry and iron-steel industry and coal is used in the industrial process as fuel and feedstock. So HCl and SO\(_2\) gasses were emitted to the atmosphere and absorbed into rain and dew water. Also Cl\(^-\) might be present to absorption of HCl by airborne liquid droplets followed by deposition of soil and sea derived chloride salts [20].

There are lots of studies in the literature about dew and rain water in various urban and rural areas but the number of studies done in an industrial area is very few. Rain water and dry deposition characterization were investigated near a petrochemical plant by Al-Momani et al. (1995) in İzmir-Turkey. All ion concentrations except K\(^+\) in rain water are comparable to those measured in İzmir. K\(^+\) concentration in this study is higher than measured in İzmir [30].

The mean cation content of the dew samples collected from Eskitaslı and Turgutbey villages was 4 to 20 and 7 to 28 times higher than that of the rainwater samples, respectively. The anion content of dew was 7 (for NO\(_3^-\) and SO\(_4^{2-}\)) to 46 (for PO\(_4^{3-}\)) times higher in Eskitaslı and 3 (for NO\(_3^-\)) to 270 (for...
PO₄³⁻) times higher in Turgutbey than in rainwater samples with the exception of NO₃⁻. On the other hand, nitrate was observed to have 11- to 22-fold higher concentrations in rainwater than in dew. In the similar studies, clearly seen that the dew ion contents were significant level higher than the rain ion. [9, 15, 17, 27, 31]. However, Beysen et al. [10] found that contents of cations and anions for dew were lower than for rain in Bordeaux, France. Furthermore, nitrate dew/rain ratio reported in Bordeaux is 0.13, 0.6 in Delhi, India and 0.8 in Zadar, Croatia [10, 17, 27]. Gaseous pollutants released to the atmosphere from sources near the dew sampling area are important in dew water ionic charge. Additionally, the presence of secondary aerosols formed by gas pollutants in particulate matter increases the amount of ions in dew water resulting from transport, precipitation and dissolution.

**FIGURE 3**
Timely variation of dew and rain anion composition (mg/L).
NO\textsubscript{3}\textsuperscript{-} and NO\textsubscript{2}\textsuperscript{-} nitrate showed an essential behaviour as seen in Table 1. Nitrate in rainwater is approximately 20 times higher than the nitrite but in dew is similar. Similar results found by Yadav and Kumar (2014) in Delhi [27]. The high concentration of HONO during the night has also been occurred by the natural gas power plant near the sampling areas. Combustion of natural gas is the direct source of NO and NO\textsubscript{2} in the urban atmosphere of Luleburgaz which undergo oxidation to produce NO\textsubscript{3} and then absorbed in rain water as nitrate. Therefore, the concentration of NO\textsubscript{3} measured in dew will arise from the dissolution of gas phase HNO\textsubscript{2}, heterogeneous formation of HNO\textsubscript{3} from fine aerosol surface or the dissolution of surface substrates [1, 27, 32, 33]. Yadav and Kumar sampled the dew and rain in winter periods, the concentration is higher than this study. More detailed studies considering more sample and sampling points should be carried in this area in future.

The relationships between the total anion and cation contents of the dew and rainwater samples are presented in Figure 4. \(\sum\text{Anion}/\sum\text{Cation} \) ratio was 0.7±0.8 and 0.8±0.6 for rainwater whereas it was little low as 0.6±0.8 and 0.6±0.5 for dew in Esiktasli and Turgutbay respectively. The good correlation (0.59-0.73, p<0.01) was found between the pH values and the ratio of total anion and cation in the rainwater at both locations. The similar good correlation was found by Lekouch et al. (2010) [17]. The relative anion and cation contents of the rainwater were observed to be proportional to each other whereas the anion content of dew was determined to be relatively low. More cation concentrations into the dew can be from the particulate matter into the flue gas of the cement, glass and iron-steel industry (especially Ca\textsuperscript{2+}, Mg\textsuperscript{2+}, Na\textsuperscript{+}) [34]. The natural gas conversion plant, the industrial facilities and the residential areas in the region release considerable amounts of SO\textsubscript{2}, NO\textsubscript{2} and hydro carbon (H\textsubscript{2}) into the atmosphere. The organic acid bases (HCO\textsubscript{3}, HCOO\textsuperscript{-}, CH\textsubscript{3}COO\textsuperscript{-}) formed by the oxidation of the hydrocarbons (HCs) in the atmosphere by water made an additional contribution to the total ionic charge, causing a relatively lower ratio of anions. We calculated HCO\textsubscript{3} using the pH values and examined the effect on the sum of ionic contents. When we add HCO\textsubscript{3} contents the sum of ions increase between 1-20 % generally and reached 40 % upper 7.3 pH values.

According to the water pollution and control regulation in Turkey, the dew and rain water quality conform to Class 2 and Class 1, respectively (if we ignore NH\textsubscript{4}\textsuperscript{+}). According to the criteria of irrigation water in the same regulation, the rain water conforms to the class 1 (class 1 means it is safe to use) and dew water conforms to the class 2 (class 2 means less damage in use) [35]. This chemical composition is within the standards of the World Health Organization (WHO) for Cl\textsuperscript{-}, Na\textsuperscript{+}, SO\textsubscript{4}\textsuperscript{2-}, NO\textsubscript{3} and pH except for Mg\textsuperscript{2+} [36].

**Statistical Analysis.** As a result of t-test, there is a statistical significant difference (\(t_{\text{Esiktash}}=4.927, t_{\text{Turgutbay}}=3.426, p<0.01\)) between dew and rain pH at these two location. As seen in Table 1, between two location is not statistically different for dew and rain pH. Dew was more acidic than rainwater at both of the sampling locations. The sampling area in this study is a different area in which industry and agricultural activity are intertwined and no similar field study has been found in the literature. In China similar results were observed and Xu et al. (2015) reported dew to be acidic as well [9]. But in India and France, Lekouch et al. (2010), Yadav and Kumar (2014) and Beysen et al. (2006) reported that the rain pH slightly acidic than dew and pH values show a weak maximum in spring and minimum in fall [10, 17, 27]. The pH values may display variability due to the presence of different pollutants and their interactions in the atmosphere in which the dew and rainwater were formed at low elevation and high elevation, respectively. Dew is contaminated only by local ions, in contrast to rainwater that can be contaminated in regions located quite far from the sampling location. Also some acidic gasses (CO\textsubscript{2}, NO\textsubscript{x}, HF and HCl) come from the natural gas power plant and the glass and iron-steel industry located the south-east side and north-west side of sampling points, respectively (Fig. 1). During the nights, the polluted air mass from the sources around the

---

**FIGURE 4**

The variation of the total anion and cation contents of the dew and rainwater samples collected from Esiktasli (full circles) and Turgutbay (open circles).
sampling points cannot be dispersed and this case can be observed in dew samples condensed with falling temperatures. But the pulverized CaO and CaCO₃ particles from the cement plant can assume the nuclei role in the formation of rain droplets in the upper atmosphere. So the weaker alkaline pH for rain than the dew was determined.

Most of the ions in Eskıtaşlı dew are statistically significant differ (p<0.01) from the ions in Turgutbey dew samples, except for NH₄⁺, Li⁺, NO₂⁻, Na⁺ which are higher and Mg²⁺, Ca²⁺ content in dew water in Eskıtaşlı is 2.3 times higher than that of Turgutbey. The Eskıtaşlı sampling point is closer to the cement industry. The lime particles carried with the atmosphere can precipitate and be concentrated in the atmosphere near the ground. This Ca²⁺, carried in the particle, adheres to the water molecules during the formation of raw water in the near atmosphere.

The correlation of same ions in Eskıtaşlı and Turgutbey sampled same times was calculated and given in Table 1. For the rain ions between the stations only Mg²⁺ showed the significant correlation. Furthermore, Mg²⁺, Ca²⁺ and Cl⁻ ions in Eskıtaşlı dew samples were significantly correlate (R= 0.395-0.463, p<0.01) in Turgutbey dew. These ions in dew water in both stations represent to the similar sources. The fact that the small number of rain water samples restricted to calculate the significant correlations.

Comparison to seawater and soil. A comparison of the elemental contents of dew and rainwater with the contents of several elements in seawater and soil is provided in Table 2. In comparison to seawater, the Cl⁻/Na⁺ ratio in dew varied in the range of 1.5-8.6 and in the range of 0.9-1.6 in rainwater samples. Except of Turgutbey dew samples, Cl⁻/Na⁺ ratio was found about 1 and this result can be strongly indicator of the chloride deficiency in the marine aerosols [37, 38]. It is surrounded by their seas to the East, South and West of the study area. This ratio could be associated with the effect of the south-westly winds locally called ‘Iodos’ (from above the Saros Gulf). Also the very high ratio in Turgutbey dew samples and slightly high in the other samples compared to the sea water ratio are clearly shown that the effect of pesticide usage in the agricultural area. This result supported that the high ratio of K⁺/Na⁺. The effect of the salty soil and the industrial-based chlorine emissions in the area should not be underestimated. In Table 2, the ratio of CI⁻/Na⁺ is also very higher than the ratio of soil characterization. CI⁻ and Na⁺ is emitted into the atmosphere by the glass and iron-steel industry processes flue gas [34].

Limited studies were conducted in Turkey about Organochlorine pesticides (OCPs) pollution [39, 40, 41]. OCPs is an important group of persistent organic pollutants (POPs) stayed in atmosphere without any degradation can be transported and deposited to clean surface. Gas phase OCPs is dissolve during the wet deposition. In Turkey, the percentage of particulate phase of pesticides was found the range of 15 % and 32.5 % for total (particle+gas) concentration [39, 40].Coscolla et al. (2014) reported that the most of the pesticide are accumulated in the ultrafine (<1 µm) and coarse (2.5-10 µm) particle size fraction [42]. Fine particulate matter could be absorbed by rain in the upper atmosphere and course particulate matter could be absorbed by dew in the lower atmosphere. As we mentioned in the introduction section, the most problem in this area is salinity [23]. It is clearly seen that one of the main factor of salinity could be air pollution from the agricultural activity.

On the other hand, the unusual value of 0.70 for the Cl⁻/Ca²⁺ ratio in soil and that of dew and rainwater could also have interesting implications. The ratio between Cl⁻ and Ca²⁺ varied in the range of 3.2-19.4 in dew and in the range of 2.3-2.6 in rainwater. The sources of calcium are CaO released as flue gas by the cement production plants, CaCO₃ pulverized into the air from the stone facilities and from the surrounding calcareous soil in the area.

<table>
<thead>
<tr>
<th>Ions</th>
<th>Seawater*</th>
<th>Eskıtaşlı Dew</th>
<th>Turgutbey Dew</th>
<th>Eskıtaşlı Rain</th>
<th>Turgutbey Rain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cl⁻/Na⁺</td>
<td>1.17</td>
<td>1.5±2.5</td>
<td>8.1±17.7</td>
<td>0.9±0.5</td>
<td>1.6±2.9</td>
</tr>
<tr>
<td>SO₄²⁻/Na⁺</td>
<td>0.125</td>
<td>0.2±0.3</td>
<td>0.9±2.5</td>
<td>0.4±0.3</td>
<td>1.0±1.5</td>
</tr>
<tr>
<td>K⁺/Na⁺</td>
<td>0.022</td>
<td>0.7±1.1</td>
<td>12.2±55.2</td>
<td>7.3±5.7</td>
<td>8.9±15.9</td>
</tr>
<tr>
<td>Ca²⁺/Na⁺</td>
<td>0.044</td>
<td>0.7±1.5</td>
<td>2.5±3.7</td>
<td>0.8±1.0</td>
<td>1.5±2.9</td>
</tr>
<tr>
<td>Mg²⁺/Na⁺</td>
<td>0.25</td>
<td>0.2±0.2</td>
<td>1.5±3.7</td>
<td>0.2±0.2</td>
<td>0.4±0.7</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Soil*</th>
<th></th>
<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Cl⁻/Ca²⁺</td>
<td>0.70</td>
<td>19.4±92.0</td>
<td>3.2±1.8</td>
<td>2.6±2.4</td>
<td>2.3±3.6</td>
</tr>
<tr>
<td>SO₄²⁻/Ca²⁺</td>
<td>0.77</td>
<td>2.1±11.5</td>
<td>0.3±0.2</td>
<td>1.2±1.5</td>
<td>1.6±3.1</td>
</tr>
<tr>
<td>NO₃⁻/Ca²⁺</td>
<td>0.10</td>
<td>0.1±0.3</td>
<td>0.00±0.02</td>
<td>2.2±2.7</td>
<td>2.2±4.4</td>
</tr>
<tr>
<td>Mg²⁺/Ca²⁺</td>
<td>0.15</td>
<td>0.6±0.4</td>
<td>0.4±0.3</td>
<td>0.6±1.2</td>
<td></td>
</tr>
<tr>
<td>Na⁺/Ca⁺</td>
<td>0.15</td>
<td>5.8±10.7</td>
<td>0.8±1.5</td>
<td>3.6±3.8</td>
<td>3.6±7.3</td>
</tr>
</tbody>
</table>

*Seawater characterization by Ozsoy, et al. [14] and soil characterization by Singh et al. [15]
There is no meteorological station in the area where dew and rain water are sampled. The nearest meteorological station is Kirklareli-Lüleburgaz Meteorology Station, which is located about 10 km south-west. The wind direction data measured in this station were analyzed and the periods during which the maximum concentration conditions occurred was

**Changing Ions with Meteorological Factors.**

FIGURE 5

Na⁺, Ca²⁺, Cl⁻ and NO₃⁻ maximum concentration vs. wind direction in rain and dew.

(1: Cement plant direction, 2: glass and iron-steel industries direction, 3: power plant direction).
investigated. Figure 5 shows Na⁺, Ca²⁺, Cl⁻ and NO₃⁻ maximum concentration vs. wind direction in rain and dew. The highest values of Na⁺ concentration was observed in rain water during the periods of north east winds dominate, and was observed in dew water when the southern winds dominate. The maximum values measured for Ca²⁺ were transported from the north-east direction to the dew samples while it was transported from the south-east direction to the rain samples. Particulate matter sourced from cement, glass and iron steel factories, precipitate in the atmosphere and cause increasing the cations in the dew formation. The highest concentrations of Cl⁻ were observed when southern winds dominate. The Cl⁻ ion originating from the glass and iron-steel industry is especially evident in Turgutbey, which is close to these plants. A high correlation was calculated between Na⁺ and Cl⁻ concentrations, especially in dew waters (0.84-p<0.01 in Eskitaşlı).

There is an iron-steel plant at the south-east of the study area. Particle emissions from iron-steel plant were investigated by Jiun-Horng et al. (2007) [34]. They observed that Na⁺, K⁺, Ca²⁺, Mg²⁺ and Cl⁻ are major particle elements and SO₄²⁻, NH₄⁺, Cl⁻, Na⁺, K⁺, Ca²⁺ concentrations are high in particle phase. Due to water has dipole molecular structure, it can easily hold the particles. In this study, the dew water includes the particles precipitated in the area. Due to rain water, were observed when north-west winds dominated. In this study, the higher concentration of Na⁺, K⁺, Ca²⁺ reached maximum in the summer.

It is clear that the highest values of NO₃⁻ ions in rain water, were observed when north-west winds dominated. In this study, the higher concentration of NO₃⁻ in rain samples was due to the combustion of natural gas in power plant near the area. The chemical composition of the dew closely related to the air quality of the underlying surface [9]. Dew droplets are smaller than rain droplets and dew is present at the surface. The higher ion concentrations in dew also attributed to higher evaporation effects on dew samples than rain by nocturnal wind [5,9].

Precipitation events affected the concentration of ions in dew. As shown in Figure 2 and 3, continuous precipitation events occurred in 15 to 31 May 2012 (0.5 to 30 mm in daily total rain amount); the ions in dew had lower concentrations in these periods. Also the same results occurred in 1 to 10 November 2012. Precipitation can remove particles and gasses especially effectively after the heavy and continuous rain event [9]. Plentiful rainfall did not occur in June and July, and higher ionic concentrations in dew were detected. We analysed the ratio of after rainy day and general dew mean concentrations and shown in Figure 6. Generally, the ratio is lower; 0.6 in Eskitaşlı, 0.8 in Turgutbey. This means that the ion concentrations that occur in the days after rainfall are well below the general average.

There is a significant effect of humidity and temperature on the pollutant concentrations of the dew waters. In humid atmospheric conditions, pollutants in the air combine with moisture and stick on the surfaces they encounter. This is exactly the inverse in dry and hot weather, and pollutants suspend in the atmosphere. Thus, the average temperature was 15.4 °C and the humidity is 77% in the April-May period when the samples are made, while the average temperature in June-August raised to 25.6 °C and the humidity drops to 50% and below. So generally the concentrations of ions in dew is reached maximum in the summer.

PCA Analysis. The PCA was used for the source identification using independent variables. 13 variables were measured in this study. However, the number of species used in the PCA analyses had to reduce to 11 parameters for the rain samples, because 70 % percent of the total samples were analyzed below the detection limit for the Li⁺, Br⁻ and PO₄³⁻ ions. Bold characters; above 0.7 correspond to the high significant variables for each factor. The PCA results are given in Table 3 and the possible pollutant sources were examined. Factor loads above 0.5 correspond to the moderate statistically significant variables. Four components for the dew samples were extracted explaining 68.2 % in Eskitaşlı and 71.7 % in Turgutbey. Three components for the rain water samples were extracted explaining 67.3 % in Eskitaşlı and 72.5 % in Turgutbey of the total variance.

![FIGURE 6](image-url)

The ratio of after rainy day and general dew mean concentrations.
The first component comprised almost 30% of the variance for the dew and rainwater at two stations has high loading for Cl\(^-\), Na\(^+\) and Mg\(^{2+}\). Ca\(^{2+}\) present the glass and iron-steel factory emissions and marine aerosol component. NaCO\(_3\) and CaCO\(_3\) are used in the glass industry for the smelting of sand. The other have high loading for Cl\(^-\), Na\(^+\) and Mg\(^{2+}\), Ca\(^{2+}\) present the variance for the dew and rainwater at two stations.

The third component loading of PO\(_4^{3-}\) and K for dew has high in Turgutbey and moderate in Eskitaşlı. PO\(_4^{3-}\) is the most limiting nutrient for plant and microbial growth in terrestrial as well as in aquatic ecosystem [43]. Tsukada et al. (2006) found that about 47% of phosphate in atmosphere was contributed by biogenic particles including those from biomass burning [44]. Also the K is indicator of biomass burning. F\(^-\) loaded high (0.93) comprised the fourth component in Eskitaşlı for dew samples and in Turgutbey for rain samples. The F\(^-\) are emitted into the atmosphere in both gaseous and particulate forms mainly from anthropogenic sources, such as production/manufacture of aluminum, glass, plastics, steel, fertilizers, ceramics and power generation [45, 46]. Industrial activity could be the main sources of F\(^-\) in this study.

### CONCLUSION

In this study, the anions and their compounds were identified in the dew and rainwater samples, which were collected during the spring and summer in 2012 in Luleburgaz. This district is a very noticeable area that the local residents live on agriculture and also have a large industrial area is located around the agricultural land. This study is the first of its kind to determine the extent of air pollution in this area. It was observed that the agricultural land was severely adversely affected by the precipitation of pollutant sourced from the industrial site.

The rain is formed at high elevation so it can be absorbed the air pollutants in the upper air of the study area can be transported from long distance. Furthermore, the dew is formed at lower atmosphere and it consists of the aerosols and dissolves gasses. We observed that the dew contained more ions than rain and clearly reflected on the local air pollution. The impact of natural gas power plant, glass and iron-steel industry and agricultural activity in the region is easily observed by the presence of NO\(_3^-\), Cl\(^-\), and NO\(_2^-\). The region suffering from environmental problems that are associated with increasingly intensifying industrialization. The Ergene River is located in the area, so this area is also important for agricultural production. The greatest problem for the Ergene Basin and Ergene River is the salinity stemming from the industry. In future, it should be studied more in this area related to the dew characterization including the seasonal variation and the element contents.

### TABLE 3

<table>
<thead>
<tr>
<th>component</th>
<th>Eskitaşlı-Dew</th>
<th>Turgutbey-Dew</th>
<th>Eskitaşlı-Rain</th>
<th>Turgutbey-Rain</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>0.03</td>
<td>0.57</td>
<td>0.61</td>
<td>0.06</td>
</tr>
<tr>
<td>Li(^+)</td>
<td>0.18</td>
<td>0.85</td>
<td>0.03</td>
<td>0.07</td>
</tr>
<tr>
<td>Na(^+)</td>
<td>0.79</td>
<td>0.05</td>
<td>0.27</td>
<td>0.19</td>
</tr>
<tr>
<td>NH(_4^+)</td>
<td>0.55</td>
<td>0.13</td>
<td>0.20</td>
<td>0.56</td>
</tr>
<tr>
<td>K(^+)</td>
<td>0.68</td>
<td>0.26</td>
<td>0.49</td>
<td>0.02</td>
</tr>
<tr>
<td>Mg(^{2+})</td>
<td>0.85</td>
<td>0.30</td>
<td>0.13</td>
<td>0.08</td>
</tr>
<tr>
<td>Ca(^{2+})</td>
<td>0.26</td>
<td>0.81</td>
<td>0.13</td>
<td>0.10</td>
</tr>
<tr>
<td>NO(_2^-)</td>
<td>0.13</td>
<td>0.07</td>
<td>0.74</td>
<td>0.05</td>
</tr>
<tr>
<td>NO(_3^-)</td>
<td>0.58</td>
<td>0.32</td>
<td>0.15</td>
<td>0.01</td>
</tr>
<tr>
<td>F(^-)</td>
<td>0.04</td>
<td>0.01</td>
<td>0.05</td>
<td>0.93</td>
</tr>
<tr>
<td>Cl(^-)</td>
<td>0.78</td>
<td>0.04</td>
<td>0.25</td>
<td>0.17</td>
</tr>
<tr>
<td>Br(^-)</td>
<td>0.54</td>
<td>0.21</td>
<td>0.10</td>
<td>0.21</td>
</tr>
<tr>
<td>PO(_4^{3-})</td>
<td>0.48</td>
<td>0.16</td>
<td>0.56</td>
<td>0.12</td>
</tr>
<tr>
<td>SO(_4^{2-})</td>
<td>0.84</td>
<td>0.03</td>
<td>0.02</td>
<td>0.11</td>
</tr>
</tbody>
</table>

% of Var. 31.5 14.7 12.4 9.5 34.4 13.4 12.9 11.1 33.7 15.3 11.1 36.7 37.7 13.3

Cum. % 31.5 46.2 58.6 68.2 34.4 47.7 60.6 71.7 33.7 53.7 67.3 36.7 59.1 72.5
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REFERENCES


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INFLUENCE OF SOME ENVIRONMENTAL FACTORS ON MILK YIELD AND MILK COMPONENTS TRAITS IN JERSEY COWS

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ABSTRACT

This study was conducted to assess the effects of stage of lactation, parity and sampling season on milk yield and milk components in Jersey cows in Karaköy State Farm, Turkey. The data set consisted of 2657 milk records of 204 Jersey cows collected from September 2011 to December 2013. For this purpose, test day milk yield (TDMY) records and milk samples were taken once a month for 10-month period after parturition. The effect of stage of lactation, parity and sampling season on TDMY, fat percentage, protein percentage, fat yield and protein yield were found statistically significant (P<0.01). TDMY were correlated with fat percentage (-0.235) and protein percentage negatively (-0.254) but with fat yield (0.755) and protein yield positively (0.950) (P<0.01).

In conclusion, these results suggest animal breeders that the effects of lactation stage, parity and sampling season may be taken into account in husbandry management to improve the milk yield and milk quality in Jersey cows.

KEYWORDS:
Jersey cow, fat percentage, protein percentage, fat yield, protein yield

INTRODUCTION

Milk yield is of great economic importance, and fat and protein concentrations of milk are the most important factor influencing the quality [1]. Thus, the quality of milk is better explained by fat and protein percentages [2].

Fat and protein percentage, the most important nutrients in milk, are of great importance for the dairy products in the dairy industry [3] and dietary source for humans [4]. Protein and fat percentages can provide information about fat to protein ratio, also the presence of metabolic dysfunctions [5].

Environmental and genetic factors affecting milk yield and quality are very important for developing breeding strategies [6]. Environmental factors may obscure the actual genetic ability of animal. If the genetic effect on a trait is lower, the influence of the environment is expected to be high on that trait and it reflects a larger impact [7]. The environmental variance embraces all variation of non-genetic origin and is a source of error that reduces precision in genetic studies. Therefore, the magnitude of environmental impact should be taken into account when designing the study. Efforts to improve traits that are greatly influenced by the environment should primarily focus on managerial inputs that modify the conditions under which the genotypes are expected to perform [8]. It is thus necessary to pre-adjust data for environmental factors when carrying out genetic evaluations of production traits such as milk production and its constituents [7].

The composition of dairy cow milk, in particular fat and protein percentages, are influenced by several environmental factors, including a parity [9], stage of lactation [10] and season [1]. In order to enhance productivity of a dairy cow, it is definitely necessary to determine and understand the factors effecting milk production and compositions. Because environmental factors tend to obscure true genetic ability. Therefore, a selection within the best environment allows a better gene expression and thus causing an improved selection response [8]. Studies on genetic and environmental factors influencing milk production and milk constituent traits are limited in Turkey dairy cows, especially in Jersey cows. That’s why the purpose of this study was to determine the effects of environmental factors on TDMY and some milk component traits and correlations between these parameters in Jersey cows raised in Black Sea Region of Turkey.
MATERIALS AND METHODS

The study material consisted of the total of 2657 milk and component records, which were collected during 1-7 lactation periods between September 2011 and December 2013 from 204 Jersey cows raised in Karaköy State Farm of Samsun province in Turkey. For this purpose, test day milk yield (TDMY) and milk samples were taken in one-month interval through ten-month period after calving for every cow.

The cows were milked twice a day by the milking machine in the morning and evening. They were kept in free-stall barns during the whole year. The cows were fed a total mixed ration (TMR) and ad libitum twice a day, and grazed on pasture during the grazing season. TMR consisted of concentrate feed, silage (corn and vetch), and hay (grass and wheat straw). The daily milk yield of each cow was automatically recorded on a computer via transponders.

Special sampling containers inserted into each milking cap were utilized when collecting the milk samples from the farm where the study was carried out. About 50 ml of the milk sample was collected and put into sterile tubes. The numbers of the animals were written on the adhesive labels on the sterile plastic tubes, into which the milk samples were taken, thereby preventing the samples from mixing with each other. The milk samples collected were placed between ice molds, brought to the laboratory by means of carriers with lids, and preserved at +4°C; furthermore, the milk analyses were made within 12 hours. The milk samples prepared for measurement were heated in the water bath at 37.5°C, and the samples subsequently cooled to the room temperature were subjected to the milk analyses. Milk samples were analyzed for fat and protein percentages by Funke Gerber LactoFlash. Milk fat yield (TDMY * Fat%) and milk protein yield (TDMY * Protein%) were calculated by the use of the values obtained as a result of the analyses.

The cows were grouped based on the parities from 1st to 7th, the stage of lactation from 1st to 10th month interval through ten-month period after calving for every cow. The environmental factors on test day milk yield (TDMY), fat percentage, protein percentage, fat yield and protein yield were not significantly affected by the stage of lactation. Similarly, Stanton et al. [18] showed that the protein percentages of the milk were not significantly affected by the stage of lactation.

A visual inspection was made to analyze the effects of parity, stage of lactation and sampling season on the examined variables. The means were compared by Duncan’s multiple range test. Pearson-correlation test was applied to determine the phenotypic correlation coefficients between TDMY and milk components. The statistical analyses were performed in SPSS 13.00 package program [11].

RESULTS AND DISCUSSION

The milk yield and milk compositions in different stage of lactation, parity and sampling seasons have been presented in Table 1. Overall mean values of TDMY, fat percentage, protein percentage, fat yield and protein yield were 16.1±4.37 kg, 4.85±0.965%, 3.39±0.298%, 0.77±0.227 kg and 0.54±0.142 kg, respectively. TDMY was found to be considerably high in the second month of lactation (P<0.01) (Table 1). The results of present study were in agreement with the results showed by Sobczuk-Szul et al. [12] and Çobanoğlu et al. [13] who detected that the highest yield was typical of milk from early stage of lactation. Similarly, Erdem et al. [14] reported that milk yield was the highest in second stage of lactation. Ducháček et al. [15] stressed that this decline in milk yield was related to negative energy balance (NEB) during early lactation in dairy. The lowest fat percentage was determined between the third and sixth months of lactation. The lowest protein percentage was displayed in second month of lactation. In generally, both fat and protein percentages increased as the lactation periods progress. This finding was consistent with the results of Gurmessa and Melaku [9], who observed that fat percentage in milk was higher in early and late stages than mid stage of lactation. Moreover, Sobczuk-Szul et al. [12] stated that the lowest protein percentage was found in early lactation stage. Yoon et al. [10] also reported that protein percentage elevated with the progress of the lactation. The on the other hand, the decline in fat and protein percentage in the peak period could be explained with the antagonist relationship between negative energy balance and milk yield [16]. However, the finding disagrees with the report of Gurmessa and Melaku [9], who observed that the protein percentages of the milk were not significantly affected by the stage of lactation.

Fat yield and protein yield are reflecting the changes of milk yield, fat percentage and protein percentage. Thus, the fat yield and protein yield in dairy cows are the most important production traits [17]. In this study, the highest fat yield and protein yield were determined during the first two months of lactation. Similarly, Stanton et al. [18] showed that the highest fat and protein yield were in early
lactation.

TDMY, fat yield and protein yield were detected as the lowest in first parity (P<0.01). The result of this study was closely with the results of Cinar et al. [19] determined that the effect of parity on milk yield was significantly important. In addition, Mostert et al. [20] reported that cows that calved at younger ages in first and second lactations produced less milk than cows calving at older ages over the entire lactation. Sudhakar et al. [6] also determined that the differences observed for the milk percentages and fat yield between different lactation periods were not significant, but protein yield was significantly differ (P<0.05).

TDMY in spring and summer were significantly higher (P<0.05) compared with TDMY in autumn and winter. This finding was not consisted with the results from Yoon et al. [10], who detected the highest milk yield in winter and spring months. On the other hand, Mishra et al. [21] reported that milk yield was highest in the summer season which was in the agreement of current study. Fat percentage was the highest in winter, but the lowest in summer. The highest protein percentage was decreased in hot climates [24]. The lowest fat yield was found in summer, but the highest in winter. Furthermore, the lowest protein yield was calculated in autumn, but there were no differences between the other seasons. Khan and Shook [25] also detected that protein yield was higher in winter than in other seasons.

<p>| TABLE 1 | The means of milk yield and milk components (Least Squares Means ± SE) |
|-----------------------------------------------|</p>
<table>
<thead>
<tr>
<th>N</th>
<th>TDMY (kg/day)</th>
<th>Fat (%)</th>
<th>Protein (%)</th>
<th>Fat yield (kg/day)</th>
<th>Protein Yield (kg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>318</td>
<td>13.9±3.08BC</td>
<td>4.99±0.960A</td>
<td>3.45±0.325A</td>
<td>0.69±0.160B</td>
</tr>
<tr>
<td>2</td>
<td>597</td>
<td>16.0±3.90AB</td>
<td>4.94±0.985AB</td>
<td>3.43±0.336AB</td>
<td>0.78±0.215A</td>
</tr>
<tr>
<td>3</td>
<td>456</td>
<td>16.7±4.73A</td>
<td>4.88±0.951ABC</td>
<td>3.39±0.267ABC</td>
<td>0.80±0.240A</td>
</tr>
<tr>
<td>4</td>
<td>457</td>
<td>16.7±4.57A</td>
<td>4.77±0.916ABC</td>
<td>3.31±0.264ABC</td>
<td>0.79±0.239A</td>
</tr>
<tr>
<td>5</td>
<td>407</td>
<td>16.6±4.38A</td>
<td>4.75±0.946ABC</td>
<td>3.39±0.307ABC</td>
<td>0.79±0.223A</td>
</tr>
<tr>
<td>6</td>
<td>268</td>
<td>16.2±4.66A</td>
<td>4.84±1.001ABC</td>
<td>3.37±0.263ABC</td>
<td>0.77±0.237A</td>
</tr>
<tr>
<td>7</td>
<td>154</td>
<td>13.4±3.63A</td>
<td>5.12±0.889A</td>
<td>3.50±0.304A</td>
<td>0.69±0.211A</td>
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**Parity *:**

<table>
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<th>Protein (%)</th>
<th>Fat yield (kg/day)</th>
<th>Protein Yield (kg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
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<td>13.9±3.08BC</td>
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<td>3.50±0.304A</td>
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</table>

**Sampling Season *:**

<table>
<thead>
<tr>
<th>N</th>
<th>TDMY (kg/day)</th>
<th>Fat (%)</th>
<th>Protein (%)</th>
<th>Fat yield (kg/day)</th>
<th>Protein Yield (kg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
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<td>3.50±0.304A</td>
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</tr>
</tbody>
</table>

**ABCDEF:** The differences between the group means with different letters in the same column are significant (*: P<0.05; **: P<0.01) TDMY: Test day milk yield.

| TABLE 2 | The correlations between test day milk yield and milk components |
|-----------------------------------------------|
| Fat Percentage | Protein Percentage | Fat Yield | Protein Yield |
| TDMY -0.235** | -0.254** | 0.755** | 0.950** |
| Fat Percentage | 0.392** | 0.434** | -0.117** |
| Protein Percentage | 0.022 | 0.047** |
| Fat Yield | 0.791** |

*: P<0.05; **: P<0.01; TDMY: Test day milk yield.
Correlations between TDMY, fat percentage and protein percentage are negatively and significantly important (P<0.01). However, the positive and statistically significant (P<0.01) correlations between TDMY, fat yield and protein yield are shown in Table 2. Similar results were reported by Ikonen et al. [26], who found negative correlations between milk yield and fat percentage and protein percentage in milk. Tsuruta et al. [27] also determined the positive and significantly important correlations between milk yield and fat and protein yields. The current results were also confirmed by Yoon et al. [10] who calculated the correlations between milk yield and fat and protein yields as -0.37 and -0.38, respectively.

CONCLUSION

The results of the present study indicate that milk yield, fat percentage, protein percentage, fat yield and protein yield were significantly influenced by the stage of lactation, parity and sampling season. Further, the positive correlations between the TDMY, fat percentage and protein percentage, and positive correlations between fat yield and protein percentage were observed. To conclude, environmental factors affecting milk yield and milk component traits should be taken into account for the improvement of milk yield and milk quality in Jersey cows.

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REFERENCES


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EFFECT OF GREEN TEA (CAMELLIA SINENSIS L.) AND PARSLEY (PETROSELINUM CRISPUM) DIETS AGAINST CARBON TETRACHLORIDE HEPATOTOXICITY IN ALBINO MICE

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¹Baskent University, Vocational School of Healthcare Services, Pathology Laboratory Technicians, Ankara, Turkey
²Gaziantep University, Faculty of Medicine, Department of Histology and Embryology, Gaziantep, Turkey
³Gaziantep University, Faculty of Science and Arts, Department of Biochemistry and Technology, Gaziantep, Turkey

ABSTRACT

In this study, it has been tried to be searched the effects of green tea (Camellia sinensis L.) and parsley (Petroselinum crispum) that is known their antioxidant features on levels of MDA, GSH and activities of GSH-Px, GST, CAT on mice liver tissue.

In the study, total 32 Swiss albino mice has been used that 8 of them are control and 24 of them are practise. Group I: animals were put on a normal diet and sham-treated with 2 ml/kg distilled water through oral gavage, daily for 8 weeks; this group of animals served as the control. Group II: animals were put on a normal diet and treated with 1.5 ml/kg b.w CCl₄ dissolved in 1.5 ml distilled water through oral gavage. Group III: animals were put on a normal diet and treated with 1.5 ml/kg b.w CCl₄ + 500 mg/kg b.w green tea through oral gavage. Group IV: animals were put on a normal diet and treated with and for CCl₄ + 0.464 g/kg parsley oral gavage daily for 8 weeks. As a results, by increasing antioxidant enzymes levels on groups given green tea and parsley to control group (p<0.05), but in MDA levels has been seen a significant decreasing as statistical (p<0.01). While both Green tea and Parsley were found to have a protective effect against CCl₄ induced damage, Parsley was more protective.

Histopathological analysis revealed that liver tissue appeared normal in control on the other hand, there were degeneration, congestion, cellular infiltration, and necrotic areas in the group administered with CCl₄. Even though frequency of lesions decreased, similar lesions were observed in the group with 1,5 ml/kg b.w CCl₄. Consequently, it was determined that while CCl₄ administration increased oxidative stress, green tea and parsley administration had a protective potential increasing antioxidant capacity. Natural antioxidant substances found in parsley and green tea can be considered as the best green chemical substances to cope with oxidative stress without damaging the nature and the living.

KEYWORDS:
Green tea, parsley, hepatotoxicity, antioxidant enzymes, mice.

INTRODUCTION

Experimental evidence indicates that a number of toxic and carcinogenic processes, induced by physical and chemical agents in the liver and other organs, involve the formation of reactive radical species that can induce autooxidative changes in biomembranes and other cellular components, resulting eventually in cell death [1-3]. Free radical-mediated peroxidation phenomena play an important role in the mechanism of cellular damage caused by carbon tetrachloride. It is well documented that CCl₄ triggers hepatic and renal changes in animals and humans [3, 4].

Natural nutrients are consumed, it is very important for our health. Food containing antioxidant ingredients, such as reactive oxygen and nitrogen species people free radicals cause oxidative damage protect against. Green tea (Camellia sinensis L.) and parsley (Petroselinum crispum) foods are two of these nutrients. More and more beginning to be consumed green plant like antioxidants and contain many useful compounds phenolic substances. These compounds have protective and nourishing properties for our bodies [5, 6].

There are antioxidants in foods naturally and phenolic substances; free radical chelating agents, reduced connectors, metal or singlet oxygen holder shows the effects of antioxidants through mechanisms and the metabolism in a positive way [7, 8]. In recent years, the phenolic matters contents of tea and its effects on human health are the most studied subject. There are chemicals with very different structures and features in the composition of the tea. Most known ones are: enzymes, polyphenols, alcoloids, nitrogen compounds, carbohydrates, pigments, vitamins, organic acids, minerals.

Reactive oxygenes play an important role in the mechanism of cellular damage caused by hepato-
totoxic agents such as carbon tetrachloride (CCl₄) [9]. Hepatotoxic-induced oxidative damage to erythrocytes causes loss of membrane function by enhancing lipid peroxidation (LPO) and altering the erythrocyte antioxidant system [4].

The elevation of plasma and tissue LPO levels is an indicator of membrane disruption in various tissue and organ cells and it is positively correlated with the gravity of the disease. Reduct glutathione (GSH), glutathione peroxidase (GSH-Px), glutathione-S-transferase (GST) and catalase (CAT) are among the major antioxidant defence systems that eliminate lipid peroxides and reactive oxygen radicals [1-3, 10]. Recently, there have been several reports extolling the protective effect of GSH-Px [11] and CAT [12] in preventing peroxidative injury induced by a wide variety of toxic systems. Furthermore, reduced GSH is a potent factor in controlling lipid peroxidation [13]. GSH plays an important role in the detoxication of xenobiotics including CCl₄ [14, 15].

The aim of this study was to investigate if dietary supplementation with green tea and parsley could suppress the formation of both nonenzymatic and enzymatic lipid peroxidation products as induced in experimental hepatotoxicity in mice by CCl₄.

MATERIALS AND METHODS

Animals and Collection of Samples. Three week old, clinically healthy, female Swiss albino mice (n: 32) weighing 22-26 g were used in this study. They were housed 4 mice to a cage, in stainless-steel wire-mesh cages in a temperature-controlled room at 24 ± 2 °C with 55 % relative humidity and a 12 h light-dark cycle. Group I (n: 8): animals were put on a normal diet and treated with 1.5 ml/kg b.w CCl₄ dissolved in 1.5 ml distilled water through oral gavage, daily for 8 weeks; this group of animals served as the control. Group II (n:8): animals were put on a normal diet and sham-treated with 2 ml/kg distilled water through oral gavage, daily for 8 weeks; this group of animals served as the control. Group III (n:8): animals were put on a normal diet and treated with 1.5 ml/kg b.w CCl₄ dissolved in 1.5 ml distilled water through oral gavage. Group IV (n:8): animals were put on a normal diet and treated with and for CCl₄ + 0.464 g/kg parsley [17] oral gavage daily for 8 weeks.

Tissue Preparation. After 8 weeks of exposure, all of the groups were slaughtered, ketamine 90 mg/kg intraperitoneal (i.p.)/xylazine 10 mg/kg i.p. and general anesthesia was performed and euthanasia was performed with cervical dislocation and their livers were immediately excised. The tissues were rinsed with ice cold deionized water and 1g of liver tissue were homogenized in 10 volumes of 0.01 M Tris-HCl (pH: 7.4) with a Potter Elvehjem homogenizer in an ice-bath. The homogenates were centrifuged at 600 g for 1 min and recentrifuged at 13 000 g for 20 min at 4 °C to obtain a postnuclear homogenate and postmitochondrial supernatant fractions.

Histological Analyses. Liver tissue was fixed within bain and 10% formol solutions. 5 μm thick cross sections were taken by microtome embedding the tissues into paraffin after routine tissue processing (grade alcohols, methyl benzoate, and benzole processing) following the fixation. Histopathological changes were examined under light microscope by staining cross sections with hematoxylin - eosin among histological staining methods [18].

Analytical Procedures. Lipid peroxidation in tissues was measured by the thiobarbituric acid reacting substance (TBARS) method of Placer [19], and was expressed in terms of the malondialdehyde (MDA) content, which served as standard of 1,1,3,3-tetraethoxypropane (Sigma, T 9889). Samples assayed for MDA contained 1.0 mM butylated hydroxytoluene, (BHT, Sigma, B 1378) in order to prevent artefactual LPO during the boiling step. Values were expressed as MDA equivalents in nmol/g tissue. The reduced GSH levels of the tissue homogenates were measured spectrophotometrically using Elman’s reagent [20]. Glutathione peroxidase activity was expressed in the presence of GSH and cumene hydroperoxide substrates using an endpoint direct assay. The activity was expressed as loss of reduced GSH/min. Glutathione peroxidase activity was expressed in units (1 unit is the enzyme quantity that oxidizes) and was performed according to the method of Aebi [21], in which the decomposition of H₂O₂ was followed spectrophotometrically at 240 nm. The difference in absorbance (ΔA₂₄₀) unit time was a measure of CAT. Triton X-100 was used in the preparation of the homogenate at a final concentration of 1 %. The enzyme activity was expressed as k/mg prot. (1 μmol H₂O₂ loss /min at 25 °C).

GSH-S transferase, (EC 2.5.1.18) is thought to play a physiological role in initiating the detoxification of potential alkylating agents [14, 22]. These enzymes catalyze the reaction of such compounds with the SH-group of glutathione, creating products that are more water soluble. Enzyme activity towards 1-chloro-2,4-dinitrobenzene was measured by Habig [14]. The activity of the enzymes and GSH were calculated to 1 g protein content of the 10 000 g supernatant fraction which was determined by Folin-phenol reagent with bovine serum albumin as the standard Lowry [12].
Statistical Analysis. Groups II, III and IV were compared with the controls (group I). The results of the experiment were evaluated statistically by the Student’s t-test. The data were analysed by one-way ANOVA technique and means were considered different at p<0.05, p<0.01.

RESULTS

The mean liver malondialdehyde (MDA) values in the control and CCl4 groups respectively were determined as 233.16±6.7 - 442.65±0.16 nmol/g dryw. As can be seen from Table 1 liver MDA level was significantly decreased in the CCl4 + Parsley and CCl4 + Green tea group. The glutathione peroxidase (GSH-Px) activity were found to be significantly increased in the green tea and parsley groups relative to the group II (p<0.01). Liver catalase (CAT) activities were not significantly different in comparisons of CCl4, CCl4 + Parsley and CCl4 + Green tea groups with the controls.

Table 1

<table>
<thead>
<tr>
<th>Organ</th>
<th>Enzymes</th>
<th>Controls</th>
<th>CCl4</th>
<th>CCl4 + Green tea</th>
<th>CCl4 + Parsley</th>
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<tbody>
<tr>
<td>Liver</td>
<td>^MDA</td>
<td>233.16±6.7</td>
<td>442.65±0.16**</td>
<td>324.64±2.3**</td>
<td>332±2.04**</td>
</tr>
<tr>
<td>^GSH</td>
<td>231±0.19</td>
<td>186.11±0.06**</td>
<td>213±0.03**</td>
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</tr>
<tr>
<td>^GSH-Px</td>
<td>35.18±7.00</td>
<td>17.88±4.38**</td>
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<td>28.84±1.17**</td>
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<tr>
<td>^GST</td>
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<td>^CAT</td>
<td>28±2.70</td>
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**p<0.01, * p<0.05  ^ : nmol/g, (dryw),  ^ : U/mg prot (dryw),  ^ : µmol (CDNB-GSH conjugate min/mg prot),  ^ : k/mg prot (dryw)

FIGURE 1

The control group (2 ml kg⁻¹ distilled water), histology of liver section by using hematoxylin and eosin staining (H&E): hepatocytes (arrows), magnification x400.

FIGURE 2

The effect carbon tetrachloride (CCl4 1.5 ml kg⁻¹ b.w), histology of liver section by using hematoxylin and eosin staining (H&E) steatosis in hepatocyte cytoplasm (split arrows), focal necrosis areas (arrows), vascular congestion (asterisk), vp: portal veins, db: ductus biliferi, magnification x400.
FIGURE 3
The effect carbon tetrachloride (CCl₄, 1.5 ml kg⁻¹ b.w), histology of liver section by using hematoxylin and eosin staining (H&E): inflammatory cell infiltration (arrows), regenerative binuclear hepatocytes (arrowhead), cv: central veins, hepatocytes (split arrows), magnification x400.

FIGURE 4
The effect carbon tetrachloride (CCl₄, 1.5 ml kg⁻¹ b.w) + green tea (500 mg kg⁻¹), histology of liver section by using hematoxylin and eosin staining (H&E): inflammatory cell infiltration (arrows), areas of sinusoidal congestion (split arrows), ihc: irregularity hepatic cords, vp: portal veins, magnification x400.

FIGURE 5
The effect carbon tetrachloride (CCl₄, 1.5 ml kg⁻¹ b.w) + parsley (0.464 g kg⁻¹), histology of liver section by using hematoxylin and eosin staining (H&E): inflammatory cell infiltration (arrows), sinusoidal congestion (split arrows), regenerative binuclear hepatocytes (arrowhead), cv: central veins, magnification x400.
Histopathological analysis revealed that liver tissue appeared normal in control on the other hand, there were sinusoidal bleeding foci, degeneration, congestion, cellular infiltration, and necrotic areas in the group administered with CCl₄. Compared with the 1.5 ml/kg b.w CCl₄ applied group, the use of parsley and green tea did not show a significant decrease in lesion severity and in its frequency.

**DISCUSSION**

Free radicals play an important role in their cell’s life and death. These are unstable electrons outermost Shell and may become highly reactive. They affect beneficial metabolic and cellular processes and also play key role in pathological conditions of the body. It is normally balanced by endogenous antioxidant system. Imbalances in redox status may develop cellular oxidative stress. The biologically damaging effects of reactive oxygen species are controlled in vivo by a wide spectrum of antioxidant defence mechanisms. Dietary constituents of antioxidant green plant and other nutrients may play an important role in protecting against oxidant damage.

In this study we investigated the effect of dietary supplementation with green and parsley on non-enzymatic free radical-induced lipid peroxidation in CCl₄-induced hepatotoxicity in mice. Studies evaluating the protection of natural substances against hepatotoxic effects are common. Hepatotoxicity caused by dichlorvos, CAPE used against it has a regulatory effect in terms of both histological and biochemical parameters [2]. Again, CAPE against hepatic injury induced by lead acetate showed a protective potential [23]. Hoşbaş [24] performed hepatic damage with CCl₄ in one study. They found that the extract of Achillea biebersteini Afân. (Asteraceae) plant was hepatoprotective against hepatic damage.

There are two types of Camellia assamica and Camellia sinensis. The three main categories of tea-green, black and oolong- are the result of different processing procedures. In addition White tea is a specific form of tea which is made from buds and young leaves of some varieties of Camellia sinensis. It is generally believed that polyphenols such as theaflavins and thearubigns as well as catechins as major constituents of tea are mainly responsible for antioxidant actions. Studies showed that tea possessed diverse pharmacological properties, which include anti-oxidative, anti-inflammatory, anti-mutagenic, anticarcinogenic, anti-angiogenic, apoptotic, anti-obesity, hypocholesterolemic, anti-arteriosclerotic, anti-diabetic, anti-bacterial, antiviral, anti-aging effects [25-29].

The results suggest that Parsley may have a potential antioxidant activity. Animal studies have shown that antioxidant enzyme levels depend on the availability of antioxidants in food [17, 30, 31].

The results obtained in this study suggest that, at the levels of dietary antioxidant tested, green tea and parsley provided protection in vivo when compared with an antioxidant-deficient diet. The impairment of tissue GSH, GSH-Px, GST and CAT after in vivo treatment with oxidizing agents has been proposed as a specific and sensitive index of tissue damage [3, 32]. Significant variation in the activities of these antioxidant enzymes and in LPO levels according to gender has been demonstrated statistically. In the CCl₄ group, LPO level in the liver increased significantly. The prevailing explanation of CCl₄ toxicity holds that this toxicity is based on lipid peroxidation caused by the trichloromethyl radical. In the present study, we found that CCl₄ administration markedly increased TBARS formation.

Our study shows that the changes in lipid peroxidation were also accompanied by a decrease in the activities of enzymes involved in the disposal of superoxide anions and peroxides, namely and SOD, as well as the levels of GSH and its related enzymes (GST and GSH-Px). From these findings, it appears that the initial changes induced by CCl₄ is due to the formation of LP and toxicity is mediated through antioxidant enzymes as well as GSH metabolism. As already noted, in our study we found that tissue MDA and GSH levels were increased. This could be associated with peroxidation of membrane phospholipids and the accumulation of MDA. On the other hand, high CCl₄ concentrations are likely to inhibit GSH [3, 9, 32].

Manno [32] and Nakagawa [33] demonstrated that CCl₄ inhibited erythrocyte GSH-Px and our results confirm their findings. GSH-Px is an important antioxidant enzyme present in virtually all tissues. GST activities in the green tea and parsley group were significantly increased, while conversely LPO levels decreased (p<0.01). CAT activity in all of these groups was not significantly different when compared with the controls. CCl₄ applied in both studies, increased antioxidant capacity while increasing total oxidant level CCl₄ applied in both studies decreased the antioxidant capacity while increasing total oxidant level. As cells are exposed to apoptosis and necrosis, degenerative areas have formed in the liver tissue. In both studies, the use of tea and parsley increased the antioxidant capacity and therefore the severity and frequency of lesions in the liver tissue decreased. Both studies are different in CCl₄ doses and duration of implementation. This green plants foods played a greater role in reducing the oxidative damage caused by the CCl₄ toxification.

In conclusion, the results of this study indicated that chronic dietary intake of CCl₄ induces oxidative damage in the liver by enhancing the peroxidation of membrane lipids and altering the oxidant
systems of the cells. Against hepatotoxicity caused by CCl₄, both green tea and parsley did not significantly reduce the severity of lesions.

REFERENCES


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HERBAGE, SEED YIELD, AND NUTRITIVE VALUE OF WILD OAT (AVENA FATUA L.) IS INFLUENCED BY DIFFERENT LEVELS OF NITROGEN

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ABSTRACT

Nitrogen (N) is considered as one of the most essential nutrients that influences the leaf growth, herbage yield and protein content of oat as a forage crop. The majority of N absorption by plants are occurred almost immediately before the stem elongation stage for rapid growth, whereas the demand of N is maximum at early to maximum vegetative stage. However, to obtain maximum herbage and grain yield and also to increase N use efficiency, it is important to apply N when crop is physiologically prepared to generate tillers and also crop is at a favourable environmental conditions. In the context, the current research was undertaken to describe the effects of different levels of N on herbage, seed yield and nutrients content of oat at the Agricultural Research Station of İğdır, Turkey during the year 2014. Treatments were four levels of N with a control treatment: 0, 30, 60, 90 and 120 kg N ha⁻¹. After experimentation, it was observed that increasing levels of N improved the plant length, green herbage and seed yield. The herbage yield of oat was increased from 16.0 to 31.1 t ha⁻¹ due to the increase of N levels. Similarly, the crude protein (CP%) was increased from 7.57 to 10.0 % by increasing N levels upto 120 kg ha⁻¹. Although, no significant difference in CP % was observed among the treatments. Numerically, the highest CP% was recorded from 7.57 to 10.0 % by increasing N levels upto 120 kg ha⁻¹. Although, no significant difference in CP % was observed among the levels of N, but numerically the maximum CP % was recorded from the maximum level of N (120 kg ha⁻¹), followed by 60 and 90 kg N ha⁻¹. The neutral detergent fiber (NDF) and acid detergent fiber (ADF %) did not differ significantly among treatments. Numerically, the highest NDF % and the lowest ADF % were recorded from 120 kg N ha⁻¹ treatment. But, dry matter digestibility (DMD), digestible energy (DE) and metabolisable energy (ME) of Oat herbage were influenced significantly by different levels of N. The maximum DMD, DE and ME were recorded from the maximum levels of N (120 kg N ha⁻¹), followed by 60 kg N ha⁻¹. In case of grain yield (GY) and yield attributes, the maximum values were also recorded from 120 kg N ha⁻¹, followed by 90 kg N ha⁻¹ and the lowest values for all parameters and harvest index were recorded from control plot. In conclusion, application of N fertilizers to wild oat at a rate up to 120 kg N ha⁻¹ can improve the yield and nutrient composition of oat herbage with more favorable nutritional facts for livestock.

KEYWORDS:
Nitrogen fertilizer, nutritive value, wild oat, yield.

INTRODUCTION

Avena fatua (L.) is known as the common wild oat under the species of grass and belong to the family Poaceae [1]; Fig. 1. It appearances is a typical, with hollow, erect stems (30-120 cm) during green and tall bearing nodding panicles of spikelets at reproductive stage [2, 3]. It is naturalized in some areas and considered as a noxious weed [4, 5, 6]. It is grown as multipurpose crop such as bread making, flavouring alcoholic drinks, grain, pasture and forage crop, animal feed, human food and various industrial uses across the globe [7, 8].

Recently, people become conscious about food and get higher life standard, owing to this, consumers have searched healthier and safety product. Chernyshova et al. [9] found that the grain of oat contains soluble fiber, β-D-glucan and also has lower serum cholesterol that reduces colon cancer and, the risk of heart disease and also control the diabetes. Another study found that protein content of oat seeds varied from 12.4-24.4 %, fat content from 3.0-11.0 %, β-glucan content from 1.8-7.5 % [10]. While, Mut et al. [11] found that in oat seed, ADF and NDF varied from 13.56 to 18.54 % and 31.35 to 37.57, while protein content varied from 10.28 to 13.70 %, starch 33.45 to 46.28 %, β-glucan 2.17 to 3.40 %,
There it is a great challenge for oat breeders for identifying the genetic make-ups that are superior in seed as well as forage yield. It was observed significant differences among the regenerant oats lines and their parents regarding yield and yield related traits were also noted by [12]. However, variations have been also observed in different agronomic traits as flowering date, plant height, grain protein quantity, flag-leaf area, weight and number of seeds, and GY [13, 14]. The development of agronomic attributes has been the main objective of oat breeders for many years in many parts of the word.

Among the plant growth factors, N is one of the vital nutrient for the crop plants particularly for improving the forage, GY and quality [15]. Bhilare and Joshi [16] found that yield and forage quality including ADF, NDF, cellulose and hemicellulose contents of oats increased with increasing levels of N to 160 kg ha\(^{-1}\). Among the forage cereals, oat is the cheapest source of animal feed if it is properly managed, fertilized and harvested at proper stage of growth [17,18]. In view of these facts, there is an urgent need of increasing good quality forage supply by adopting improved agronomic techniques, among which the improved genotypes and balanced fertilizer use are very crucial. Among plant nutrients, N absorbed by the oat crop the most. However, under unfavorable conditions, N use efficiency was decreased and generating an instability, finally reduced the forage and GY [19]. Therefore, the estimation of optimum nitrogen rate for oat production is consider the most important subject because of its associated to lodging and higher productivity.

The value of oat for the producer is a function of both GY and quality. Therefore, the purpose of this study was to determine the optimum N level for oat production, because of its relation to lodging and higher grain or biomass yield. And, to describe the response of wild oat proximate composition and energy content to the N fertilizer, in order to estimate its value as a potential fodder crop for livestock.

**MATERIALS AND METHODS**

**Experimental site.** The experiment was carried out at the Agricultural Research Station of Iğdır University, Turkey (39°39’ N, 34°15’ E and 850 m sea level) during the growing season of 2014.

**Soil and climatic condition.** The site of experiment consists of soluble salts (EC=1.8 dsm\(^{-1}\)), high CaCO\(_3\) (22.37%), organic matter (1.6 %), and pH value of 7.98. Available phosphorus (P\(_{2}O\(_5\)) was 4.2 kg ha\(^{-1}\) (kg ha\(^{-1}\); potassium (K\(_{2}O\)) was 0.3 (t ha\(^{-1}\)) and soil was clay [20].

The temperature in the experimental site was above 30°C from May to October and the mean temperature was 26.5°C in July, and the minimum relative humidity was 39.7% in July. The lowest precipitation occurred in July, August, and September in the experimental site [21].

**Experimental treatments and design.** The study was carried out with randomized complete block design with 4 replications. Plot size was 7.5 m\(^2\) (1.5 m width and 5 m length). The row to row
distance was 30 cm with a continuous seeding. *Avena fatua* L. var. SAINI were used as the experimental material of this trial. The seed rate was 200 kg ha$^{-1}$.

Treatments were four levels of N with a control treatment: 0, 30, 60, 90 and 120 kg N ha$^{-1}$. Each plot was fertilized with P$_2$O$_5$ and K$_2$O at 50 kg ha$^{-1}$ with top dressing of some portions of the fertilizers. The ratio of weed on the row was too low to be tried, and the rows were destroyed by the hoe.

**Data collection.** Data on plant height (cm) and green herbage yield (kg ha$^{-1}$) were recorded at the vegetative stage. While panicle length (cm), straw, grain and biological yield as well as harvest index were recorded from harvesting samples at maturity.

**Data on nutritive values and their analysis procedure.** Quality properties were performed on oats using the DM yield samples. After drying and weighing 500 g plant samples taken from each parcel, these samples were ground by a centrifugal grinder (Retsch ZM 200) to pass through a 1 mm stainless steel sieve for chemical analysis. The ground samples were analysed by near infrared spectroscopy (NIRS) through using a NIRS-Foss system 5000 Rapid Content at Soil Laboratory, Iğdır University, Turkey. Samples were analysed for N content (%) and digestible dry matter (DMD %) [22].

Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined according to Van Soest et al. [23]; through using ANKOM 200 Fiber Analyzer (ANKOM Technology Corp). The DMD% was calculated as follows by Undersander [24]:

\[
\text{DMD} \% = 88.9 - [0.779 \times \text{ADF} \% \text{ (on a dry matter basis)] [24].}
\]

Crude protein (CP) and metabolisable energy (ME) were measured based on the following two equations (2) & (3) [25].

\[
\text{CP} = \text{N\%} \times 6.25
\]

\[
\text{ME (MJ/kg DM)} = 0.17 \times \text{DMD} - 2 [26],
\]

Dry matter intake (DMI, as % of body weight) = 120/\%NDF [24].

**Statistical analysis.** The obtained data was evaluated separately and combined through using SAS statistical package program. Duncan’s Multiple Range Test (DMRT) was used to compare the difference between the mean [27].

**RESULTS AND DISCUSSION**

**Effect of N on plant height of oat.** Plant height is directly correlated with the fodder yield of crops and it is influenced by levels of applied N. Plant height was varied from 84.5 to 106.9 cm due to different levels of N. But, no significant difference was recorded between 30 to 120 kg N ha$^{-1}$ in respect of plant height. The tallest plant was found from the maximum level of N, and the shortest plant was found from the control plot (Fig. 2). Increased level of N increased the plant height also confirmed by [28], who showed that plant height of oat was 132 cm, in the treatment where maximum level of N was applied. Therefore, it was confirmed that application of different levels of N increased the plant height that ultimately increased the biomass of plant.
FIGURE 3

Green herbage yield (t ha⁻¹) of oat is influenced by levels of nitrogen. ±SE was calculated from four replications for each treatment.

FIGURE 4

Crude protein (%) of oat herbage is influenced by different levels of nitrogen. ±SE was calculated from four replications for each treatment.

Effect of N on green herbage yield of oat. A progressive and significant increase in green herbage yield was recorded with the increased levels of N. The herbage yield of oat was ranged from 16.0 to 31.1 t ha⁻¹. Although, the herbage yield of oat was increased due to the different levels of N, but no significant difference was found between the parcels applied with 60 kg to 120 kg N ha⁻¹ (Fig. 3). Whereas, [29] observed that DM yield and nutritional value of Sorghum (Sorghum sp.) and black oat (Avena strigosa) in southern Brazil were influenced by different levels of N levels and found that 210-280 kg N ha⁻¹ in sorghum and 180 kg N ha⁻¹ in black oat in the crop rotation provided the maximum DM yield with good nutritional quality [29].

Effect of N on crude protein content. In the present study, similar to green herbage, the CP contents were significantly influenced by different levels of N. However, CP content in the treatment of 60, 90 and 120 kg N ha⁻¹ were statistically similar as compared with others. Among the applied N levels, the maximum CP content (10.0%) was observed from 120 kg N ha⁻¹ applied treatment, followed by 60 and 90 kg N ha⁻¹ (9.83 and 9.76%). While the lowest (7.57%) was recorded in control plot (Fig. 4). Our results of CP content in oat herbage were similar to the findings of [30] and [31], who reported that the content of protein in the oat were varied between 9 to 14% due to different levels of N applied, while maximum was from without N applied plot. Similarly, CP content of other forage crops was enhanced by increasing the N levels [32]. Many researchers reported significant differences between genotypes in terms of protein % with a range from 9.9 to 13.7% [33, 34]. While, Naneli and Sakin [35] observed that protein content and its quality in plants’ biomass not only depends on levels of N, but also depends on amount and
distribution of precipitation in crop growing month, temperature, soil characteristics as well as depends on other management practices.

**Effect of N levels on neutral detergent fiber (NDF) and acid detergent fiber (ADF) content.** Generally, NDF = hemicellulose + cellulose + lignin + ash, while ADF = cellulose + lignin + ash [36]. In the present research, the NDF percentages were increased with the increasing levels of N, but did not differ significantly among the different levels of N. The higher NDF % was recorded from 120 kg N ha⁻¹ applied treatment, followed by 90 kg N ha⁻¹, while the lowest was found in the control plot. Similarly to NDF, acid detergent fiber (ADF) did not vary significantly due to increased levels of N. The higher value of ADF was recorded from 30 kg N ha⁻¹, followed by 90 kg N ha⁻¹ applied treatment, while the lowest value of ADF was found from 120 kg N ha⁻¹, indicated that higher level of N decreased the ADF value in herbage of oat (Fig. 5). Forages with higher ADF are lower in digestible energy than forages with lower ADF, indicated that increasing the ADF level decreased the digestible energy content [36,37]. The results of the present study also similar to the findings of Givens et al. [38], who also observed that increasing levels of N increased the yield and quality parameters such as N content, protein, NDF, ADF, oil content and oil composition of oat cultivars. The ADF content increased from 11.0 to 16.4 % and NDF content increased from 29.5 to 37.3 % in the seed of oat due to different N fertilizations as reported by [11].

![Graph showing NDF and ADF content](image1)

**FIGURE 5** Neutral detergent fiber (NDF) and acid detergent fiber (ADF) (%) of oat herbage is influenced by different levels of nitrogen.

![Graph showing dry matter digestibility](image2)

**FIGURE 6** Dry matter digestibility (DMD; %) of oat herbage is influenced by different levels of nitrogen. ±SE was calculated from three replications for each treatment. % DMD = 88.9 - [0.779 × %ADF (on a dry matter basis)] [24].
Effects of different levels of nitrogen on DMD, ME and DMI of oat herbage. In the present study, the maximum DMD (63.50 %) was recorded from the maximum levels of N applied treatment (120 kg N ha⁻¹), which was statistically similar with 60 and without N applied treatment. While the minimum DMD (%) was observed in the 30 kg N ha⁻¹, followed by 90 kg N ha⁻¹ treatments (Fig. 6).

In the present study, similar to DMD (%), the maximum ME was recorded from 120 kg N ha⁻¹ applied treatment, followed by the 30 kg N ha⁻¹ (Fig. 7); while ME was the lowest in 60 kg N ha⁻¹. Dry matter intake (DMI) establishes the amount of nutrients available to an animal for health and production, and fundamentally important in animal nutrition [39, 40].

The predicted DMI% in all levels of applied N were similar (did not differ significantly); but numerically higher DMI was found in control plot, while the minimum at 90 kg N ha⁻¹ (Fig. 8). DMI% is negatively influenced by higher NDF and ADF % in forage [41, 42]. To sum up, although the estimated values of DMD, ME and DMI are useful indices for predicting forage quality, it is not reliable lonely, and information should be collected from animal’s performance and health to precisely determine wild oat nutritional quality. Moreover, further studies are warranted regarding the antinutritional components in the wild oat.

**FIGURE 7**

Herbage metabolisable energy (ME; Mcal kg⁻¹ DM) of oat herbage is influenced by different levels of nitrogen.

±SE was calculated from three replications for each treatment. ME (MJ/kg DM) = 0.17% DMD – 2 [26].

**FIGURE 8**

Dry-matter intake (DMI; %) from oat herbage is influenced by different levels of nitrogen.

Dry matter intake (DMI, as % of body weight) = 120/%NDF [24].
Yield and yield attributes of oat is influenced by different levels of N. The increase in green fodder yield significantly was observed with the increase in N fertilizer rate. In recent studies, optimal GYs were achieved with the adding of moderate levels of N fertilizer (40 to 80 kg N ha⁻¹) for soils containing between 20 and 50 kg NO₃-N ha⁻¹ in the top 60 cm of the soil profile [43]. The increase N rate application enhanced lodging and decreased test weight, kernel weight and kernel plumpness, for that, the optimal N management must balance yield improvement against the reductions in nutritive values [15]. Data on the plant height (132.00 cm), number of plants (91.33 m⁻²), tillers (146.00 m⁻²), leaves tiller⁻¹ (5.66), total dry matter (17.70 t ha⁻¹) and fodder yield (60.90 t ha⁻¹) showed that N application at 150% N of recommended dose with drill sowing proved to be the most cost effective technique for fodder oat production in salt affected soil as compared to other treatments [28]. Similar to the previous findings, the maximum panicle length, GY, straw and biological yield was recorded from the highest levels of nitrogen (120 kg N ha⁻¹) applied plot, followed by 90 kg N ha⁻¹, and the lowest values for all parameters including harvest index were recorded from the control plot (Fig. 9).

CONCLUSION

From the study, it can be concluded that increasing nitrogen rate enhanced the quality and quantity (GY) of oat and improved its crude protein and fiber. The impact of wild oat grown under various levels of N fertilizers and cut at different ages on the productivity and health of livestock remain to be determined.

REFERENCES


AN ARTIFICIAL NEURAL NETWORK MODEL FOR PREDICTING THE GREENHOUSE HEAT REQUIREMENT IN ADANA CLIMATE CONDITIONS

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ABSTRACT

In addition to the application of modern cultivation techniques and technological solutions, plant quality and yield can be increased through heating in a greenhouse during the cold winter months. Within a greenhouse heating system, the greenhouse heat requirement is the most important parameter for efficient operation. Calculations of the heat requirement should take into consideration the long-term average temperature and the regional climatic conditions. Based on these calculations, the greenhouse heating system power and production costs can be predicted.

In this study, an artificial neural network (ANN) model which can be used for planning, feasibility studies, and automation systems was developed to estimate the heat requirement of modern greenhouses. In this model, the performance of the activation and training algorithms was determined with the aim to provide heat requirement estimates that are close to actual consumption values.

The fuel consumption data from a commercial greenhouse operation in Adana for the 2015 production year and climatic data from an official meteorological station were used to test the model. By comparing different activation functions and training algorithms, the most suitable algorithm for the model was able to be determined. A total of eight models were then created and their performances compared statistically. As a result, a model that was able to produce estimates that were very close to the actual fuel consumption was developed.

KEYWORDS:
Artificial neural networks, Greenhouse heating, Greenhouse heat requirement, Greenhouses

INTRODUCTION

Artificial intelligence (AI) can be defined as the mathematical imitation of the learning and decision-making characteristics of the human brain. Various models have been employed in artificial intelligence applications; one of these is artificial neural networks (ANNs), an AI system that provides a mathematical analysis of everyday problems using algorithms based on the human brain. An ANN is a black box model that consisting of input-output parameters and the relationships between them. This model does not consider the input, output, causes. Cognitive scientists and neuroscientists aim to understand the functioning of the brain; to achieve this, they build models of neural networks and conduct simulation studies. However, artificial intelligence is a component of computer science and the primary objective here is to build useful systems, as in any domain of engineering [1].

In its most general form, a neural network is a machine that is designed to model the way in which the brain performs a particular task or function of interest; the network is usually implemented by using electronic components or is simulated in software on a digital computer [2]. Machine learning is the act of programming computers in a way that optimizes a performance criterion using example data or past experience [1]. The main attraction of troubleshooting using ANNs is the ability to generalize learning and learned information. The ability to engage in network capacity learning with a small set of samples and the provision of consistent answers for unknown data is proof that ANN's abilities have gone beyond mapping only the input and output relationships. ANNs can remove information that is not expressly granted by the samples.

In previous applications, ANNs have been employed to generate hourly temperature estimates [3], recognize species of butterflies and insects [4, 5], determine precipitation and spatial distributions [6], predict natural disasters using climate data [7], predict soil temperatures in different regions and at different depths [8], estimate water levels in lakes using hydrological data [9], classify farmland [10], estimate the yield of crops [11], completion of missing data in soil property measurement data [12], spatially model soil salinity using wetness indices [13], control weeds in sugar beet crops using an intelligent spraying robot based on image processing [14], determine the level of underground water [15, 16], predict the number of flowers in fruit trees [17], predict fungal infections in pumpkin plants [18], predict nitrate pollution in groundwater [19], classify the maturity of tomatoes [20], estimate the impact of herbal
production on annual gross national turnover using climate data [21], classify cultivated land based on unmanned aerial images [22], evaluate water and energy cycles through the monitoring of long-term meteorological variables such as precipitation, air temperature, proportional and absolute humidity, air pressure, wind velocity, and shortwave radiation [23], determine sowing patterns using images obtained by remote sensing [24, 25], predict solar radiation [26], estimate evapotranspiration [27], evaluate drought reduction options [28], predict and warn of frost in greenhouses [29], estimate tractor fuel consumption [30], estimate the amount of energy consumed in agricultural production and the production of greenhouse gas emissions [31], and identify soil erosion classes and determine sensitivity to erosion [32, 33].

In the agricultural sector, greenhouses are the farming activity that has the highest energy requirements per unit area. Greenhouses are also desirable to be structurally durable [34]. If there is not enough insulation in a heated greenhouse, more heat energy will be needed during cold periods. For example, the regular heating costs of heat-protected greenhouses on the Mediterranean coastline account for 20% of overall production costs [35].

The greenhouse heat requirement is calculated according to European Union standards depending on the size and type of greenhouse, the equipment used, and the temperature required for the plants. However, there are different methods to determine the heat energy requirement in greenhouses [35]. Canakci, et al. [36] calculated the heat energy requirement for Antalya by taking into consideration the temperature averages at night and the night length, while Damrath and Klein [37] calculated the heat energy requirement for Trier (Germany) using hourly values. Damrath [38] also determined the average of the temperature values over many years to calculate the annual heat energy requirement.

The average temperature is also used to calculate the heat energy requirement in greenhouses. However, during transition periods where average temperature is high and the temperature is kept low in the greenhouse, calculations using average temperature provide incorrect results [39]. For example, if the average outside temperature is 16 °C and the target greenhouse temperature is set at 16 °C, it could be concluded that the greenhouse does not need heating. However, the greenhouse temperature may be below or above the average of 16 °C. Therefore, it can be calculated that heat energy is not needed even though heating is needed at certain times of the day when the temperature is high [35].

Von Zabeltitz [40] reported the heat energy requirement for plastic greenhouses in the Mediterranean countries, using the method reported by Hallaire [41], determining the lowest temperature values and the day length values depending on the latitude of the region and finding that the heat energy requirement of a greenhouse can be calculated most accurately from hourly climatic measures.

Depending on the characteristics of the greenhouse, some solar energy is stored in the greenhouse. Heat energy stored throughout the day causes the temperature to rise in the greenhouse. Therefore, taking into consideration the heat storage properties of a greenhouse in the calculations for the daytime and nighttime, the rise of temperature in the series to be taken into consideration also provides more accurate results [42, 43].

In addition to many different parameters need to be taken into consideration when calculating the greenhouse heat requirement such as energy lost from the greenhouse, heat gain of the greenhouse, energy exchange resulting from the cultural activities. In order to simplify this process, an ANN model can be developed.

In this study, the temperature, relative humidity, and fuel consumption were measured for a commercial greenhouse operation in the province of Adana for the 2015 production year. Meteorological data for the region was used as input for an ANN model. The main objective was to determine the optimal ANN transfer function and to develop a model for the greenhouse heat requirement for Adana climatic conditions.

**MATERIALS AND METHODS**

The research was conducted in a 20,160 m² plastic-covered modern commercial greenhouse in Adana. The roof of the greenhouse was single-layer PE plastic (200 μm) and the side walls were covered with double-layer polycarbonate (PC 10 mm). The dimensions of the greenhouse are given in Table 1.

<table>
<thead>
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<th>Dimensions of the greenhouse used in the study</th>
<th>Value</th>
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</tr>
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<tr>
<td>A_H/AG</td>
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<td>-</td>
</tr>
</tbody>
</table>

The plastic greenhouse where the research was carried out is usually heated by a coal boiler. The greenhouse heating pipes are 51 mm in diameter and located in plant rows and close to the greenhouse base. The fuel consumed during the production period (lignite coal) was recorded daily in kg and, for the energy conversion, the lower heat value of the
coal was 8.14 kWh kg⁻¹. The greenhouse temperature was controlled by regulating the water temperature using three-way distributor valves. In the greenhouse, tomatoes were grown in a soil-free culture at a density of 2.5 seedlings per square meter. Irrigation was conducted automatically using spaghetti drippers for each seedling. XLS 15 thermal screens (Ludwig Svensson) were used for the conservation of heat energy within the greenhouse. The thermal screens were closed when solar radiation was 0 W m⁻² and gradually opened over the course of 30 minutes following sunrise. Outside climatic variables and the temperature, humidity, and solar radiation in the greenhouse were recorded as hourly averages. The greenhouse temperature was held at 16 °C using the control elements based on the measured climatic values. An official meteorological station (Adana 35 E 18; 37 N 01) was used for hourly temperature, solar radiation, and wind velocity measurements.

Traditionally, the greenhouse heat requirement is calculated according to the energy balance of the greenhouse. In other words, the energy gains and losses of the greenhouse are calculated then the amount of heat energy required for the desired internal temperature to be reached is determined. Accordingly, the greenhouse heat energy requirement can be calculated according to Equation 1, given by [35]:

\[
Q = \sum_{n=1}^{N} \left( \left( \theta_{in} - \theta_{Lofi} - \Delta \theta_{sp} \right) \cdot k'_{H} \cdot A_{H} \right) \cdot \left( 1 - EE_{ES} \right) \cdot t_{SI} \tag{1}
\]

where \(Q\) is the heat energy (Wh), \(\theta_{in}\) is the indoor temperature (°C), \(\theta_{Lofi}\) is the actual temperature in the unheated greenhouse (°C), \(\Delta \theta_{sp}\) is the temperature rise due to the features of the greenhouse (°C), \(k'_{H}\) is the overall heat requirement coefficient of the cover material (W m⁻² K⁻¹), \(A_{H}\) is the surface area of the greenhouse cover (m²), \(EE_{ES}\) is the heat savings from the thermal screens, \(n\) is the hours of the year, and \(t_{SI}\) is the time period (1 h).

The temperature rise (\(\theta_{Lth}\)) in an unventilated and unheated greenhouse is calculated according to Equation 2 [39, 44]:

\[
\theta_{Lth} = \frac{q_{ES} \cdot D_{c} \cdot \eta \cdot A_{G}}{k'_{H} \cdot (1 - EE_{ES}) \cdot A_{H}} + \theta_{a}
\tag{2}
\]

where \(\theta_{Lth}\) is the theoretical temperature in non-ventilated and non-heated greenhouse (°C), \(q_{ES}\) is the solar radiation (W m⁻²), \(D_{c}\) is the transmittance of the cover material (%), \(\eta\) is the solar energy conversion factor (standard=0.7), \(A_{G}\) is the greenhouse floor area (m²), and \(\theta_{a}\) is the outside temperature (°C).

While determining the input parameters for the ANN, it is necessary to analyze the effect of the input parameters on the output value of the proposed model [45]. For this purpose, the influences on the heat flux and the greenhouse heat balance [46] are summarized in Figure 1.

\[\text{FIGURE 1}\]

Parameters that affect the indoor temperature of a greenhouse

As shown in Figure 1, the greenhouse indoor temperature is influenced by air exchange, the outdoor air temperature, solar radiation, heating, ventilation, and wind. The ventilation rate is not used as an input parameter in the ANN model because ventilation cannot occur during active heating. Wind speeds were tested as an input parameter for the ANN model because heat losses from the surface of the greenhouse depend on the wind speed. In ANN applications, a multi-layer structure is widely used. The values are passed through an input layer, processed in a hidden layer, and forwarded to an output layer [45]. Without the hidden layer, a sensor can only perform linear tasks.

Figure 2 presents the general structure of the network tested in this paper. In most cases, ANN input and output parameters will have significantly different values. Log-sigmoid and tan-sigmoid activation functions are sensitive within the ranges [0,1] and [-1,1], respectively. Therefore, it is recommended that input and output data be scaled to avoid neuronal saturation. Scaling the data from the original range to the normalized range ((0,1) or [-1,1]) in accordance with the selected neuron transfer function is known as preprocessing. Both the input and output data must be normalized before network training [45].

The data used in the model was normalized according to Equation 3:

\[
X_{n} = \frac{x - x_{min}}{x_{max} - x_{min}} \times r + r_{min}
\tag{3}
\]

where \(X_{n}\) is the value to be normalized, \(x_{min}\) and \(x_{max}\) are the minimum and maximum values of the data set, respectively, \(r\) is the normalization range, and \(r_{min}\) is the initial value of the normalization range.
A multilayer feed-forward neuron network was used in the model. The ANN model parameters are given in Table 2. The MATLAB software package [47] was used to train the ANN model. Demuth and Beale [47] have recommended the use of the sigmoid hidden layer activation function, which produces a linear output. Therefore, in this study, sigmoid activation functions were tested. During the production season, 144 days were heated. Of these, 85 were used to develop the model. In total, 70% of the input data was used to train the ANN model, and 30% was used for verification and testing.

In the ANN model, the estimated fuel consumption is calculated according to Equation 4:

$$Q = \sum_{i=1}^{k} \left( \frac{2}{1 + e^{-2(n_1)}} - 1 \right) \times l_i + \theta, \quad (4)$$

where $k$ is the number of neurons in the hidden layer, $l_i$ is the weight vector of the connections between the hidden and output layer, $\theta$ is the output layer bias (threshold), and $N_i$ is the calculated neuron value.

In this study, sigmoid (logsig) and tangent hyperbolic sigmoid (tansig) activation functions in the hidden layer and a linear (purelin) activation function in the output layer were used. The general form of these activation functions are as follows:

$$f(x) = \frac{1}{1 + e^{-x}} \quad (5)$$

$$f(x) = \left( \frac{2}{1 + e^{-2x}} \right) - 1 \quad (6)$$

$$f(x) = x \quad (7)$$

The tansig transfer function (Equation 6) used in ANN applications is associated with a bipolar sigmoid with an output between -1 and +1, and this is mathematically equivalent to tanh ($x$). Although there are very small numerical differences in the results, this function is faster. Therefore, it is a suitable option for neural networks where speed is more important than the exact form of the transfer function [48].

In an ANN model, the value of the neurons in the hidden layer is determined by adding the bias (threshold) to the weighted totals of the input vectors. This is expressed in Equation 8:

$$N_i = \sum_{j=1}^{n} x_j \times w_{ij} + \theta_i \quad (8)$$

where $n$ is the input parameter number, $w_{ij}$ is the input vector of the connections between the input and hidden layer, $x_j$ is the input vector, and $\theta_i$ is the bias (threshold) of the first neuron in the hidden layer.

Ten different training algorithms for the log-sigmoid and tan-sigmoid activation functions were tested to determine the optimal activation function for the ANN model. In the tests, fuel consumption

![FIGURE 2](image_url)

**FIGURE 2**
The structure of the proposed feed-forward ANN model

**TABLE 2**
The ANN model parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Input</td>
<td>6</td>
</tr>
<tr>
<td>Hidden layer</td>
<td>1</td>
</tr>
<tr>
<td>Number of neurons in the hidden layer</td>
<td>6</td>
</tr>
<tr>
<td>Output</td>
<td>1</td>
</tr>
<tr>
<td>Training algorithm</td>
<td>Levenberg-Marquardt (trainlm)</td>
</tr>
<tr>
<td>Training cycle</td>
<td>2000</td>
</tr>
<tr>
<td>Hidden layer activation function</td>
<td>Hyperbolic tangent sigmoid (tansig)</td>
</tr>
<tr>
<td>Output layer activation function</td>
<td>Linear (purelin)</td>
</tr>
</tbody>
</table>

1- Daily max temperature
2- Daily min temperature
3- Inside temperature
4- Wind speed/Relative humidity
5- Vapor pressure
6- Solar radiation
was used as the output parameter with one hidden layer and six input parameters. The maximum number of iterations was 1000, the error value was 0.00001, and the epoch value was 100.

After determining the optimal activation function and training algorithm, eight different models were created. In these models, climatic parameters such as ambient temperature, wind velocity, proportional humidity, solar radiation, and steam pressure were used in different combinations as input vectors. The training cycle was repeated 2000 times for each model. At the end of the training cycle, the model with the smallest mean square error (MSE) was chosen as the best performing model. The best was chosen as the best performing model. The best performance model was then determined by comparing the root mean square error (RMSE), mean absolute percentage error (MAPE), mean absolute error (MAE), and determination coefficient ($R^2$) for the eight models. Accordingly, the model with a higher $R^2$ (Equation 12) and lower RMSE (Equation 9) and MAE (Equation 12) was chosen as the optimal model.

$$\text{RMSE} = \sqrt{\frac{\sum_{i=1}^{n}(x_{i,A} - x_{i,P})^2}{n}}$$

(9)

$$R^2 = 1 - \frac{\sum_{i=1}^{n}(x_{i,A} - x_{i,P})^2}{\sum_{i=1}^{n}(x_{i,A} - x_{i,M})^2}$$

(10)

$$\text{MAPE} = \frac{1}{n} \sum_{i=1}^{n} \left| \frac{x_{i,A} - x_{i,P}}{x_{i,A}} \right|$$

(11)

$$\text{MAE} = \frac{1}{n} \sum_{i=1}^{n} |x_{i,A} - x_{i,P}|$$

(12)

In the equations above, $n$ represents the number of observations, $x_{i,P}$ is the predicted output value, $x_{i,A}$ is the actual fuel consumption value, and $x_{i,M}$ is the mean of the actual fuel consumption. MATLAB 7.12.0 software was used for the analysis on a computer running the Microsoft Windows 10 operating system with a 2.53 GHz Intel i5 CPU and 6 GB of RAM.

**RESULTS AND DISCUSSION**

In this study, the daily fuel consumption and climatic values for the 2015 growing season were used. According to the fuel consumption data (Figure 3), heating took place on 144 days between November 8, 2014 and March 31, 2015.

The results of the tests performed to determine the optimal activation function for the ANN model are provided in Tables 3 and 4.

For the log-sigmoid transfer function (Table 3), the most suitable training algorithm was the Levenberg-Marquardt (trainlm), which had the lowest RMSE and highest $R^2$. This algorithm is a modified Gauss-Newton algorithm that has been successfully used to solve non-linear smallest squares problems, including neural network training. In each iteration, it significantly outperforms variations with higher computational and memory requirements, basic back-propagation, and variable learning rates (e.g., education accuracy, convergence characteristics, general training period [49].

![Graph of Fuel Consumption](image)

**FIGURE 3**

Fuel consumption for the greenhouse under investigation

**TABLE 3**

The performance of the training algorithms employing the log-sigmoid transfer function

<table>
<thead>
<tr>
<th>Function</th>
<th>RMSE</th>
<th>$R^2$</th>
<th>Duration (s)</th>
<th>Number of Iterations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trainlm</td>
<td>0.20376</td>
<td>0.6752</td>
<td>1</td>
<td>229</td>
</tr>
<tr>
<td>Trainrp</td>
<td>0.21395</td>
<td>0.6065</td>
<td>1</td>
<td>290</td>
</tr>
<tr>
<td>Traindm</td>
<td>0.24856</td>
<td>0.2396</td>
<td>26</td>
<td>25000</td>
</tr>
<tr>
<td>Traincgp</td>
<td>0.21858</td>
<td>0.5866</td>
<td>1</td>
<td>216</td>
</tr>
<tr>
<td>Trainscg</td>
<td>0.22713</td>
<td>0.4157</td>
<td>1</td>
<td>266</td>
</tr>
<tr>
<td>Trainbfg</td>
<td>0.21340</td>
<td>0.5484</td>
<td>1</td>
<td>344</td>
</tr>
<tr>
<td>Traincgb</td>
<td>0.21601</td>
<td>0.5841</td>
<td>1</td>
<td>258</td>
</tr>
<tr>
<td>Trainoss</td>
<td>0.21576</td>
<td>0.5836</td>
<td>1</td>
<td>365</td>
</tr>
<tr>
<td>Traincgf</td>
<td>0.23411</td>
<td>0.5101</td>
<td>1</td>
<td>290</td>
</tr>
<tr>
<td>Traindgx</td>
<td>0.21897</td>
<td>0.5384</td>
<td>1</td>
<td>773</td>
</tr>
</tbody>
</table>
The performance of training algorithms employing the tan-sigmoid transfer function

<table>
<thead>
<tr>
<th>Function</th>
<th>RMSE</th>
<th>R²</th>
<th>Duration (s)</th>
<th>Number of iterations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trainlm</td>
<td>0.11059</td>
<td>0.8350</td>
<td>1</td>
<td>208</td>
</tr>
<tr>
<td>Trainrp</td>
<td>0.11385</td>
<td>0.8239</td>
<td>1</td>
<td>333</td>
</tr>
<tr>
<td>Traingdm</td>
<td>0.13650</td>
<td>0.7491</td>
<td>27</td>
<td>25000</td>
</tr>
<tr>
<td>Traincgp</td>
<td>0.15038</td>
<td>0.6918</td>
<td>1</td>
<td>209</td>
</tr>
<tr>
<td>Trainscg</td>
<td>0.14466</td>
<td>0.7333</td>
<td>1</td>
<td>225</td>
</tr>
<tr>
<td>Trainbfg</td>
<td>0.14239</td>
<td>0.7235</td>
<td>1</td>
<td>232</td>
</tr>
<tr>
<td>Traincgb</td>
<td>0.13313</td>
<td>0.7698</td>
<td>1</td>
<td>242</td>
</tr>
<tr>
<td>Trainoss</td>
<td>0.11556</td>
<td>0.8213</td>
<td>1</td>
<td>326</td>
</tr>
<tr>
<td>Traincgf</td>
<td>0.14502</td>
<td>0.7156</td>
<td>1</td>
<td>222</td>
</tr>
<tr>
<td>Traingdx</td>
<td>0.16217</td>
<td>0.6443</td>
<td>1</td>
<td>334</td>
</tr>
</tbody>
</table>

In Table 4, the performances of 10 training algorithms using the tan-sigmoid transfer function are summarized. It can be observed that the optimal training algorithm is trainlm, which has the lowest RMSE and the highest R². When both transfer functions are evaluated together, the most suitable transfer function is tan-sigmoid, and the most suitable training algorithm is trainlm. The effect of increasing the number of hidden layers on the performance of the model was then tested using the tan-sigmoid transfer function and the trainlm training algorithm; the resulting RMSE and R² values are given in Table 5. It was found that increasing the number of hidden layers had no effect on the performance of the model. The best performance was observed in the single-layer model with six neurons.

The relationship between fuel consumption and the values used as input parameters is very important for the success of the model.

Figure 4 presents the regression graphs used to determine the relationship between the input and output parameters.

The R² for the relationships between fuel consumption and indoor temperature, daily minimum temperature, and atmospheric vapor pressure were 0.43, 0.35, and 0.30, respectively, while the R² for the relationship between fuel consumption and atmospheric relative humidity, solar radiation, and wind speed was 0.0005, 0.0266, and 0.0016, respectively. Descriptive statistics for the data set used for the ANN model are provided in Table 6, while the correlation analysis results for fuel consumption and other input parameters are summarized in Table 7.

According to these values, the climatic parameters that had the most significant effect on fuel consumption were daily minimum temperature, atmospheric vapor pressure, and daily maximum temperature value (P<0.01). The correlations between greenhouse fuel consumption and solar radiation and greenhouse indoor ambient temperature were also significant (P<0.05), while the correlations with outdoor ambient relative humidity and wind speed values were not significant (P>0.05).

The effect of some input parameters on fuel consumption may have been low due to the effective thermal insulation, the use of thermal screens, and the maximum wind speed in the region of 3 m/s. Indeed, Tanat [50] found that, for well-insulated greenhouses, wind has no effect on the heat requirement. In the proposed ANN model, using only the temperature parameters and vapor pressure as input parameters would be sufficient. However, to improve model performance, eight different models were created using all of the climatic parameters mentioned above and their performances compared. The results of these models are summarized in Table 8.
FIGURE 4
Relationships between key input and output parameters

TABLE 6
Descriptive statistics for the data set used in the study

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Average</th>
<th>Std. dev.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daily maximum temperature (°C)</td>
<td>5.20</td>
<td>27.00</td>
<td>17.61</td>
<td>4.52</td>
</tr>
<tr>
<td>Daily minimum temperature (°C)</td>
<td>-3.20</td>
<td>15.10</td>
<td>7.30</td>
<td>4.05</td>
</tr>
<tr>
<td>Indoor temperature (°C)</td>
<td>13.85</td>
<td>22.45</td>
<td>17.21</td>
<td>1.21</td>
</tr>
<tr>
<td>Solar radiation (W/m²)</td>
<td>21.10</td>
<td>329.80</td>
<td>184.11</td>
<td>81.95</td>
</tr>
<tr>
<td>Relative humidity (%)</td>
<td>32.64</td>
<td>91.92</td>
<td>69.02</td>
<td>14.23</td>
</tr>
<tr>
<td>Vapor pressure (hPa)</td>
<td>2.40</td>
<td>14.20</td>
<td>9.73</td>
<td>2.44</td>
</tr>
<tr>
<td>Average wind speed (m/s)</td>
<td>0.80</td>
<td>3.00</td>
<td>1.41</td>
<td>0.46</td>
</tr>
<tr>
<td>Heat consumption (kWh/m²)</td>
<td>0.08</td>
<td>1.24</td>
<td>0.62</td>
<td>0.27</td>
</tr>
</tbody>
</table>

TABLE 7
Correlation analysis for the input and output parameters used in the ANN model

<table>
<thead>
<tr>
<th></th>
<th>Daily maximum temperature</th>
<th>Daily minimum temperature</th>
<th>Indoor temperature</th>
<th>Solar radiation</th>
<th>Relative humidity</th>
<th>Vapor pressure</th>
<th>Average wind speed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pearson correlation coefficient</td>
<td>-0.439**</td>
<td>-0.698**</td>
<td>-0.245*</td>
<td>0.219*</td>
<td>-0.098</td>
<td>-0.640**</td>
<td>-0.005</td>
</tr>
<tr>
<td>Sig. (2-tailed)</td>
<td>0.000</td>
<td>0.000</td>
<td>0.024</td>
<td>0.044</td>
<td>0.373</td>
<td>0.000</td>
<td>0.962</td>
</tr>
</tbody>
</table>
TABLE 8

<table>
<thead>
<tr>
<th>Model</th>
<th>Independent variables</th>
<th>R²</th>
<th>RMSE</th>
<th>MAPE</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1</td>
<td>Daily minimum temperature</td>
<td>0.666</td>
<td>0.2677</td>
<td>25.5</td>
</tr>
<tr>
<td></td>
<td>Indoor temperature</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M2</td>
<td>Daily minimum temperature</td>
<td>0.773</td>
<td>0.2207</td>
<td>21.3</td>
</tr>
<tr>
<td></td>
<td>Indoor temperature</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Vapor pressure</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M3</td>
<td>Daily maximum temperature</td>
<td>0.845</td>
<td>0.1827</td>
<td>15.9</td>
</tr>
<tr>
<td></td>
<td>Indoor temperature</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Vapor pressure</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M4</td>
<td>Daily minimum temperature</td>
<td>0.797</td>
<td>0.2094</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>Daily maximum temperature</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Indoor temperature</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Solar radiation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M5</td>
<td>Daily minimum temperature</td>
<td>0.874</td>
<td>0.165</td>
<td>13.4</td>
</tr>
<tr>
<td></td>
<td>Daily maximum temperature</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Indoor temperature</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Solar radiation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Relative humidity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M6</td>
<td>Daily minimum temperature</td>
<td>0.899</td>
<td>0.1485</td>
<td>13.4</td>
</tr>
<tr>
<td></td>
<td>Daily maximum temperature</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Indoor temperature</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Solar radiation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Vapor pressure</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M7 (*)</td>
<td>Daily minimum temperature</td>
<td>0.945</td>
<td>0.1106</td>
<td>10.7</td>
</tr>
<tr>
<td></td>
<td>Daily maximum temperature</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Indoor temperature</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Solar radiation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Relative humidity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M8</td>
<td>Daily minimum temperature</td>
<td>0.893</td>
<td>0.1534</td>
<td>18.2</td>
</tr>
<tr>
<td></td>
<td>Daily maximum temperature</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Indoor temperature</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Solar radiation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Vapor pressure</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Average wind speed</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

As shown in Table 8, the M7 model, which was built using six inputs and the single output network structure presented in Figure 2, produced the best performance. Although the effects of relative humidity and solar radiation on fuel consumption are statistically very low (Table 7), the use of these parameters increased the performance of the model. The detailed results of the M7 model for the training, validation, and test data sets are given in Table 9, showing that its performance falls within acceptable limits.

The regression graphs of the estimated values found in the testing, training and verification stages of the model and the actual fuel consumption values are presented in Figure 5 and the actual and predicted greenhouse fuel consumption values are presented in Figure 6.

The weights for the input values used to calculate the neuron values between the input layer and the hidden layer are given in Table 10, while the weight values between the hidden layer and the output layer are given in Table 11.
FIGURE 5
ANN model results

FIGURE 6
Actual and predicted greenhouse heat consumption

TABLE 10
Weight values between the input and hidden layers

<table>
<thead>
<tr>
<th>Neurons in the input layer (i)</th>
<th>W1i</th>
<th>W2i</th>
<th>W3i</th>
<th>W4i</th>
<th>W5i</th>
<th>W6i</th>
<th>Bias1</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>5.260</td>
<td>1.967</td>
<td>3.575</td>
<td>-0.488</td>
<td>9.229</td>
<td>-10.402</td>
<td>-2.256</td>
</tr>
<tr>
<td>5</td>
<td>0.840</td>
<td>-0.397</td>
<td>1.165</td>
<td>-0.045</td>
<td>-1.181</td>
<td>0.775</td>
<td>1.090</td>
</tr>
</tbody>
</table>

TABLE 11
Weight values between the hidden and output layers

<table>
<thead>
<tr>
<th>Neurons in the input layer (i)</th>
<th>Weights (Wi)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.3276</td>
</tr>
<tr>
<td>2</td>
<td>-0.2724</td>
</tr>
<tr>
<td>3</td>
<td>-0.2716</td>
</tr>
<tr>
<td>4</td>
<td>0.6206</td>
</tr>
<tr>
<td>5</td>
<td>1.1833</td>
</tr>
<tr>
<td>6</td>
<td>0.7517</td>
</tr>
<tr>
<td>Bias2</td>
<td>0.1856</td>
</tr>
</tbody>
</table>
The weight values in Tables 10 and 11 and the output activations obtained from Equation 8 are given in Equations 13-18. According to these results, the model for estimating fuel consumption is given in Equation 19. The input and output (i.e., fuel consumption estimation) values must be normalized using Equation 3.

\[
N_i = \frac{1}{1 + e^{-2(N_i)}} - 1
\]

\[
N_2 = 10.973X_t - 1.985X_t + 24.011X_t - 3.67X_t + 3.131X_t + 14.297X_t - 4.714
\]

\[
N_3 = 19.052X_t - 2.395X_t - 14.309X_t - 16.908X_t - 20.005X_t + 11.51X_t - 9.05
\]

\[
N_4 = 5.26X_t + 1.967X_t + 3.575X_t - 0.488X_t - 9.229X_t - 10.402X_t - 2.256
\]

\[
N_5 = 0.84X_t - 0.397X_t + 1.165X_t - 0.045X_t - 1.181X_t + 0.775X_t + 1.09
\]

\[
N_6 = 1.554X_t - 4.359X_t - 16.987X_t - 3.415X_t + 2.603X_t - 8.274X_t - 10.906
\]

Equation 19 is obtained by replacing the output activations obtained from Equation 8 with the fuel consumption estimation values in Equation 19. The input and output (i.e., fuel consumption estimation) values must be normalized using Equation 3.

\[
Q = \left(\frac{2}{1 + e^{-2(N_i)}} - 1\right) \cdot 0.3276 + \left(\frac{2}{1 + e^{-2(N_i)}} - 1\right) \cdot -0.2724 + \left(\frac{2}{1 + e^{-2(N_i)}} - 1\right) \cdot -0.2716 + \left(\frac{2}{1 + e^{-2(N_i)}} - 1\right) \cdot 0.6206 + \left(\frac{2}{1 + e^{-2(N_i)}} - 1\right) \cdot 1.8133 + \left(\frac{2}{1 + e^{-2(N_i)}} - 1\right) \cdot 0.7517 + 0.1856
\]

(19)

CONCLUSION

In this study, a prediction model for fuel consumption in a soilless tomato greenhouse was developed for the province of Adana using regional meteorological climatic data. An ANN model, variations of which have been successfully applied in many areas, can be used to estimate the greenhouse heat requirement. By adapting this method to specific greenhouse automation and control systems, it may be possible to both optimize fuel savings and plant climatic requirements. However, depending on the type of input data used, the proposed ANN model may not be valid in greenhouses with various spatial and structural arrangements. Therefore, heat requirement estimation models can be developed for greenhouses of different types or characteristics by following the methodology of this study.

REFERENCES


[42] Rath, T. (1992) Use of knowledge-based systems for modelling and presentation of horticultural expertise using the example of the hybrid expert system HORTEX. Horticultural information (Germany). no. 34. (in German).


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MYOCARDIAL INFARCTION IN PATIENTS WITHOUT DIABETES THE IMPORTANCE OF HEMOGLOBIN A1C

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Vocational School of Health Services, Firat University, Elazig, Turkey

ABSTRACT

In this study, non-diabetic myocardial infarction with ST elevation, representing acute myocardial infarction and non-diabetic patients with no history of myocardial infarction have compared.

Subjects included 180 non-diabetic patients who suffered a myocardial infarction with ST elevation, representing acute myocardial infarction (group 1), 180 non-diabetic patients with no history of myocardial infarction, who were scheduled for coronary artery bypass graft surgery, representing coronary artery disease (group 2), and 100 healthy controls (group 3). Groups 1 and 2 had higher levels of hypertension and HbA1c, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, and fasting plasma glucose (FPG) levels compared to those of group 3. Groups 1 and 2 had higher levels of hypertension compared to group 3.

Groups 1 and 2 had similar FPG and HbA1c levels. FPG and HbA1c levels of groups 1 and 2 were significantly higher than those in the control group (P = 0.001, P = 0.041 and P = 0.001, respectively).

Statically, there was not observed any difference between patients with non-diabetic myocardial infarction and non-diabetic patients with history of myocardial infarction. Prediabetes and diabetes were frequently seen in both groups, but most patients were unaware. Detecting prediabetes and diabetes early is important. HbA1c represents a good choice for screening and follow-up evaluations of prediabetes and diabetes in patients with coronary artery disease.

KEYWORDS:  
Myocardial infarction, hemoglobin A1c, prediabetes, diabetes

INTRODUCTION

According to the International Diabetes Federation, there were 246 million diabetic patients worldwide, and 46% were 40–50 years of age. The number of patients with diabetes is estimated to reach 380 million by 2025 if no precautions are taken [1].

Recent studies suggest that the prevalence of diabetes is increasing worldwide; shows that diabetes-related deaths and health care due to diabetes bring a huge burden on social, financial and health care. Diabetes Mellitus, the most important health care in the world is one of the problems. Mortality and Diabetic Patients long-term micro-morbidity and macrovascular complications. The relationship of these patients with the environment they live in is sometimes problematic. [2]. In recent years, the use of hemoglobin A1c (HbA1c) in the diagnosis of diabetes mellitus has become widespread due to its practicality and its inclusion in the diagnostic criteria of diabetes mellitus by the world health organization and ADA [3]. The amount of carbohydrate to be consumed in main / breaks, weight, paint, when and how much exercise, the type of drug / insulin used and the effect hours (maximum effect), age, cholesterol, triglyceride, microalbuminuria and hemoglobin A1c (HbA1c) values, personal choices vary according to preferences, cultural habits and lifestyle, other comorbidities and weight loss targets.

Elevated free fatty acids accompany increased glucose level [1]. Endothelial dysfunction, hypercoagulability, increases in platelet thrombotic properties, and endothelial dysfunction associated with hyperglycemia are factors responsible for the poor prognosis of patients with coronary artery disease (CAD) [5].

The importance of undiagnosed diabetes or prediabetes in CAD is appreciated when the increasing number of patients with diabetes worldwide is considered [1]. Hemoglobin A1c (HbA1c) measurements are recommended to clinically screen for diabetes mellitus DM [2]. HbA1c is a stable marker of long-term blood sugar and reflects mean blood glucose level for the previous 8–12 weeks. Several meta-analyses and clinical studies have shown that glycated HbA1c has both diagnostic and prognostic value in patients with diabetes and prediabetes [6]. In addition, Rohlfing et al. reported that HbA1c is sensitive and specific to DM diagnosis (84.4%). Type 2 DM is a major health problem and has emerged as an important cause of morbidity and mortality worldwide [7]. However, several studies have shown that patients with type 2 DM are unaware of their illness [8].
In this study, HbA1c levels were compared in patients with non-diabetic myocardial infarction with ST elevation, non-diabetic patients with no history of myocardial infarction CAD, and non-diabetic patients with normal coronary artery anatomy.

**MATERIALS AND METHODS**

This study included 180 non-diabetic patients (127 males and 53 females; mean age, 63.2 ± 12.4 years) who had a myocardial infarction (MI) with ST elevation, representing acute CAD (group 1), 180 non-diabetic patients (119 males and 61 females; mean age, 57.4 ± 15.3 years) with no history of MI who were scheduled for coronary artery bypass graft (CABG) surgery, representing CAD (group 2), and 100 healthy controls (66 males and 34 females; mean age, 54.8 ± 10.5 years). Subjects with a history of type 2 DM and taking antidiabetic agents (oral hypoglycemic agents or insulin) were considered diabetics and thus not included. All patients underwent detailed physical examinations, and their demographic characteristics and cardiovascular risk factors were noted. Patients with systolic blood pressure ≥ 140 mmHg and diastolic blood pressure ≥ 90 mmHg, and those taking anti-hypertensive medication were considered hypertensive patients. Blood samples were obtained for routine hematological and biochemical testing following a 12-h fast. MI was diagnosed according to World Health Organization (WHO) criteria, symptoms, high cardiac enzyme levels, and electrocardiographic changes. Control subjects were chosen from patients who had undergone coronary angiography, had normal coronary artery anatomy, and were non-diabetic. Patients in group 1 were selected from those who had suffered a heart attack but had not undergone an invasive procedure due to CAD. In addition to patients with diabetes, those with kidney or liver dysfunction, a hormone disorder, anemia, taking cardiovascular drugs, diagnosed with a hemoglobinopathy, or any known systemic disorder, as well as pregnant women, and those who had undergone surgery within three months were not included in the study. Patients were informed of the study and written informed consent was obtained. This study protocol was approved by the local ethics committee.

**Plasma glucose and HbA1c measurements.**

HbA1c was measured with an ADAMS™ A1c HA-8160 device (ARKRAY, Inc., Edina, MN, USA). The reference range in our laboratory was 4.0–6.0%. FPG levels were measured using the AU 5800 device (Beckman-Coulter, Brea, CA, USA). The FPG reference range was 74–110 mg/dl (4.10–6.11 mmol/L). Prospective studies have shown that 12–25% of cases with HbA1c levels of 5.5–6% develop diabetes within five years [6-9]. Therefore, the same reference range was used in our study.

**Statistical analysis.** Statistical analyses were performed using SPSS 11.5 software (SPSS Inc., Chicago, IL, USA). The chi-square test was used for categorical variables. Continuous variables are presented as the mean ± standard deviation (SD), and were compared with the paired-sample t-test or Wilcoxon test. A P value < 0.05 or beyond the 95% confidence interval (CI) was considered significant.

**RESULTS**

Preoperative demographic and clinical characteristics are presented in Table 1. Groups 1 and 2, which represented CAD, had a higher hypertension rate and increased levels of low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, HbA1c, and FPG compared to those of the control (group 3). Smoking and family history differed significantly between groups 1 and 2 relative to group 3. No differences were observed with respect to total cholesterol, triglycerides, ura, or creatinine levels.

**TABLE 1**

<table>
<thead>
<tr>
<th>The demographic and clinical characteristics of the groups.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group1</strong></td>
</tr>
<tr>
<td>Age (years)</td>
</tr>
<tr>
<td>Gender (F/M)</td>
</tr>
<tr>
<td>Hypertension (%)</td>
</tr>
<tr>
<td>Fasting glucose (mg/dl)</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
</tr>
<tr>
<td>Triglyceride (mg/dl)</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dl)</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dl)</td>
</tr>
<tr>
<td>Body mass index (kg/m2)</td>
</tr>
<tr>
<td>Cigarette Smoking (%)</td>
</tr>
<tr>
<td>Family history (%)</td>
</tr>
</tbody>
</table>

TABLE 2
HbA1c and FPG levels between groups

<table>
<thead>
<tr>
<th>Group</th>
<th>HbA1c (μmol/l)</th>
<th>FPG (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>6.0 ± 1.3</td>
<td>106.7 ± 19.5</td>
</tr>
<tr>
<td>Group 2</td>
<td>5.7 ± 1.4</td>
<td>104.9 ± 18.4</td>
</tr>
<tr>
<td>Group 3</td>
<td>5.1 ± 0.4</td>
<td>89 ± 19.7</td>
</tr>
</tbody>
</table>

TABLE 3
Comparison of HbA1c and FPG levels between groups

<table>
<thead>
<tr>
<th></th>
<th>*p1</th>
<th>*p2</th>
<th>*p3</th>
</tr>
</thead>
<tbody>
<tr>
<td>HbA1c</td>
<td>0.62</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>FPG</td>
<td>0.87</td>
<td>0.041</td>
<td>0.034</td>
</tr>
</tbody>
</table>

*p1 = Group1 / Group2  *p2 = Group2 / Group3  * p3 = Group1 / Group3

TABLE 4
Numbers and percentages of HbA1c levels in the specified range between groups

<table>
<thead>
<tr>
<th>HbA1c (μmol/l)</th>
<th>Group1 n (%)</th>
<th>Group2 n (%)</th>
<th>Group3 n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 5.5</td>
<td>43 (23.8)</td>
<td>58 (32.2)</td>
<td>57 (57)</td>
</tr>
<tr>
<td>5.5 – 6.0</td>
<td>85 (47.2)</td>
<td>80 (44.4)</td>
<td>29 (29)</td>
</tr>
<tr>
<td>&gt; 6.0</td>
<td>52 (28.8)</td>
<td>42 (23.3)</td>
<td>14 (14)</td>
</tr>
</tbody>
</table>

TABLE 5
Comparison of HbA1c levels between groups in a determined range

<table>
<thead>
<tr>
<th>HbA1c (μmol/l)</th>
<th>*p1</th>
<th>*p2</th>
<th>*p3</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 5.5</td>
<td>0.54</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>5.5 – 6.0</td>
<td>0.72</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>&gt; 6.0</td>
<td>0.62</td>
<td>0.001</td>
<td>0.001</td>
</tr>
</tbody>
</table>

*p1 = Group1 / Group2  *p2 = Group2 / Group3  * p3 = Group1 / Group3

Comparisons of HbA1c and FPG levels are presented in Tables 2 and 3. HbA1c levels did not differ significantly between groups 1 and 2. However, FPG levels were significantly different between groups 1 and 2, and between these two groups and the control (P1 = 0.036/*P2 = 0.041/*P3 = 0.001). Group 1 and group 2 HbA1c levels differed significantly compared to those in the control group (*P3/*P2 = 0.001).

Graft numbers among the 180 patients who underwent CABG surgery were as follows: one for five patients, two for nine patients, and three for 72 patients, four for 84 patients, and five for 10 patients. No correlation was detected between HbA1c level and the number of CABGs.

Comparisons of the levels and percentages of HbA1c within a determined range among the groups are presented in Tables 4 and 5.

DISCUSSION

Whether, in the world, it is emphasized that increasing rate of diabetes will reach to huge numbers (in 2025 380 million). In the forthcoming years, it is accepted that diabetes is in the top risk-level in terms of cardiovascular risk and many different guides states, it is suggested that notably LDL cholesterol patients, to the treatment of other cardiovascular risk factors should approach aggressively [1, 13, 14].

Increased cardiovascular risk in prediabetic patients without overt diabetes but who have impaired glucose tolerance must be assessed seriously, as in patients with diabetes, as a great majority of patients with diabetes die due to cardiovascular disease [14, 15]. A large case series of macrovascular prediabetes in patients with CAD emphasized the need to follow-up to avoid complications [19]. Large-scale epidemiological studies have shown that HbA1c levels correlate with complication rates, even in the presence of non-diabetic FPG levels [17, 18]. Both the Diabetes Control and Complications Trial and The United Kingdom Prospective Diabetes Study (UKPDS) showed that the appearance and progression of microvascular complications can be delayed by regulating blood sugar [20]. In addition, Rohlfing et al. reported that HbA1c is a specific and sensitive marker to detect undiagnosed patients with DM (84.4%) [7].

In this study, HbA1c levels were analyzed at three intervals: < 5.7 mmol/L, 5.5–6.5 mmol/L, and > 6.5 mmol/L. The numbers of patients with HbA1c levels of 5.5–6.5 mmol/L was 85 (47.2%) in group 1 and 80 (44.4%) in group 2. Fifty-two (28.8%) patients in group 1 and 42 (23.3%) in group 2 had HbA1c levels > 6.5 mmol/L.

Anderson et al. [20] reported that 73% of non-diabetic patients scheduled for voluntary CABG surgery are dysglycemic (i.e., diabetes or prediabetes). Similarly, 70% of our group 1 patients and 67.7% of our group 2 patients had HbA1c levels in the DM range or a tendency to develop DM [14,
15]. Those patients were hence referred to the Endocrinology Department. Twenty-nine (16.1%) patients in group 1 and 22 (12.2%) in group 2 were diagnosed with DM, and all other referred patients were followed up routinely.

A number of studies have reported that the incidences of acute and chronic CAD may decrease by decreasing HbA1c level [21-23]. The UKPDS reported a 16% decreased risk of MI in an intensively treated group, and hyperglycemia based on HbA1c level was a modifiable risk factor for cardiovascular disease [22]. A meta-analysis on observational studies showed a linear relationship between cardiovascular disease and HbA1c, and every 1% increase in HbA1c level resulted in an 18% increased risk for cardiovascular disease [20]. The ADVANCE study showed that a 0.61% decrease in HbA1c level resulted in a 24% decrease in mortality due to cardiovascular disease [22]. Many studies have shown that slow onset cardiovascular disease can be avoided with good glycemic and metabolic control [25, 26].

Laslett et al. [27] estimated that 17.3 million people died worldwide due to cardiovascular disease in 2012. Thus, it is important to determine “qualifiable” risk factors in order to decrease mortality, morbidity and healthcare costs. A high HbA1c level in patients without DM is predictive of cardiovascular disease and death [28]. Interestingly, half of patients with type 2 DM are unaware of their disease. In our study, HbA1c levels were 67.7% among all patients. Interestingly, this value was within the range of prediabetic values. In the study of Kathyrin A. Britton and her friends, it is observed that diabetic coronary syndrome patients’ haemoglobin level and mortality of in-hospital. Patients that all of them are diabetes, whether patient with ST elevation or with no ST segment elevation patients are implicated in the study [29]. In the conclusion, it is not detected any significant relations between hemoglobin A1c values and mortality. As to this study if could not be found any difference between level of HbA1c patient with acute with st MI and with non-st MI.

A meta-analysis by Liu et al. [30] reported that HbA1c level is a determinant of cardiovascular mortality in patients hospitalized for CAD and were not diagnosed with DM, but such an effect was not evident in patients with a previous DM diagnosis. We found no relationship between HbA1c level and number of grafts. The number of percutaneous coronary intervention procedures, such as stent implantations, has increased. Three (40%) and four (47%) grafts were used by most patients in our study, representing 87% of all patients. HbA1c levels in non-diabetic patients with CAD who have undergone a stent procedure should be investigated.

We found that most patients were unaware of the risks of diabetes and prediabetes. Most of these patients must be followed up or treated in order to prevent diabetic complications. We found no difference between HbA1c levels of non-diabetic patients with acute CAD and non-diabetic patients with CAD. It would be useful to determine HbA1c levels in non-diabetic patients with CAD for early diagnoses of prediabetes and diabetes. The biggest limitation of this study is that HbA1c is more expensive and there is possibility of error in anaemia and haemoglobin.

REFERENCES


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ANTIBACTERIAL AND ANTIFUNGAL ACTIVITIES OF ESSENTIAL OILS FROM LAURUS NOBILIS L. FLOWERS AND LEAVES GROWN IN THE WEST ANATOLIAN AREA

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²Giresun University, Department of Biology, Faculty of Science and Arts, Giresun, Turkey
³Giresun University, Center Research Laboratory Application and Research Center, Giresun, Turkey

ABSTRACT

In this study, chemical composition determination and in vitro antibacterial and antifungal effects of essential oils of Laurus nobilis flowers and leaves grown West Anatolian ecological conditions were investigated. Antibacterial and antifungal activities were evaluated by Minimum Inhibition Concentration (MIC) method. Extractions were carried out with clevering apparatus and essential oil compositions were determined by Gas Chromatography-Mass Spectrometry (GC-MS). According to the results of the gas chromatography-mass spectrometry analysis, camphene (28.46 %), 1,8-cineole (34.08%) were found to be highest in the essential oil obtained from Laurus nobilis leaves. 3-ethyl-6-(methoxycarbonyl)-2-naphthol (16.10%), camphene (18.14%), were found to be highest in the essential oil obtained from Laurus nobilis flowers. MIC values of essential oils of flower and leave parts of L. nobilis range from 1.5625 μL/mL to 6.25 μL/mL against test bacteria. As a result, it is thought that the essential oil content differs in quantity and composition from the past studies, due to the different geographical and environmental effects of the plant.

KEYWORDS:
Laurus nobilis, essential oil, antibacterial, antifungal, Turkey

INTRODUCTION

The Lauraceae family is by far the largest family of the order Laurales with about 50 genera and over 2000 species distributed throughout tropical to subtropical latitudes especially in Southeast Asia and tropical America [1]. Laurus nobilis L. is an evergreen, dioic plant in form of tree or bush which can grow up to 8-10 cm and belong to Laurus genus of Lauraceae family. It generally spreads along the Mediterranean climate zone; Portugal, Spain, Italy, the former Yugoslavia, Greece, Turkey and the southern coasts of Africa [2]. L. nobilis leaves are widely used for manufacturing essential oil [3, 4]. The essential oil obtained from these leaves is used as preservative and flavorant in the food industry. Furthermore, the essential oil obtained from L. nobilis leaves is also used as preservative in cosmetic products, as insect repellent and for melanoma inhibition, joint and muscle pain relief, treatment of digestive system and skin problems, aromatherapy, and massage products [5, 6, 7]. Aromatic medicinal plants have been used for centuries as treatment for human infectious diseases. They are a rich valuable natural source of biologically active compounds and have been shown to possess antibacterial, antifungal, antiviral, insecticidal and antioxidant properties [8]. Essential oils, are complex mixtures are obtained different from organs of plants by distillation. Most of these compounds are included terpenoids (isoprenoids), monoterpenes and sesquiterpenes. Composition and quantities of essential oils depends on type of plant, climate conditions, different organs of the plant [9]. The aim of the present study was to determine the essential oil contents of L. nobilis flowers and leaves growing in West Anatolian ecological conditions and to investigate their antibacterial and antifungal effect on some strong pathogen bacteria and fungi.

MATERIALS AND METHODS

Plant materials. Laurus nobilis leaves and flowers of the plants were collected as study materials in April and in May 2018, which are their blooming periods from West Anatolian and its surroundings. The collected samples were placed in fabric bags and kept in a room with no sunlight.

Isolation of essential oils and GC-MS analysis. Extractions were carried out with Clevenger apparatus and essential oil compositions were determined by Gas Chromatography-Mass Spectrometry (GC-MS). Characterization of essential oil components was based on the library (Wiley and NIST) comparison with the mass spectra of the injected essential oil samples.
Microorganisms. *Salmonella enterica* ATCC 14028 and *Saccharomyces cerevisiae* ATCC 9763 were obtained from Giresun Province Control Laboratory; *Enterobacter aerogenes* CCM 2531, *Bacillus subtilis* IMG 22, *Proteus vulgaris* FMC 1, *Candida albicans* FMC 17 and *Candida tropicalis* ATCC 13803 were obtained from Fırat University Department of Biology; *Candida parapsilosis* ATCC 22019 was obtained from Giresun University Faculty of Education; *Gordonia rubripertincta* (lab isolate) and *Klebsiella pneumoniae* (lab isolate) were acquired from Yeditepe University Department of Genetic and Bioengineering; *Enterococcus faecalis* ATCC 29212 was obtained from Rıze University Department of Biology.

Antibacterial and Antifungal Activities. Determination of Minimum Inhibition Concentration (MIC) of The Essential Oils. The MIC was defined as the lowest concentration that completely inhibited the growth of microorganisms. For determining of MIC, a micro-dilution broth susceptibility test was utilized. Two-fold serial dilutions (in Dimethyl Sulphoxide (DMSO) were prepared from 0.0122 μL/mL to 25 μL/mL of the essential oils of *Çičili Laurus nobilis* flowers, *Çičili Laurus nobilis* leaves, *Aydınlı Laurus nobilis* flowers and *Aydınlı Laurus nobilis* leaves in a 96-well microplate. Plates were incubated 30 °C for 48 h for fungi and 37 °C for 24 h for bacteria [10, 11, 12].

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Composition of the essential oil of <em>L.nobilis</em> flowers and leaves (Aydınlı and Çičili)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RT</td>
<td>Component</td>
</tr>
<tr>
<td>15.710</td>
<td>1,8-cineole</td>
</tr>
<tr>
<td>25.310</td>
<td>3-cyclohexen-1-ol</td>
</tr>
<tr>
<td>38.304</td>
<td>camphene</td>
</tr>
<tr>
<td>38.629</td>
<td>eugenol</td>
</tr>
<tr>
<td>40.859</td>
<td>β-elemene</td>
</tr>
<tr>
<td>42.141</td>
<td>methyleugenol</td>
</tr>
<tr>
<td>42.422</td>
<td>caryophyllene</td>
</tr>
<tr>
<td>44.504</td>
<td>acetic acid</td>
</tr>
<tr>
<td>51.178</td>
<td>2,2-dibutenyl-3-cyclohepten-1-one</td>
</tr>
<tr>
<td>55.482</td>
<td>ledol</td>
</tr>
<tr>
<td>56.527</td>
<td>transcaryophyllene</td>
</tr>
<tr>
<td>58.164</td>
<td>α-cadinol</td>
</tr>
<tr>
<td>67.105</td>
<td>1-methyl-1,2,3,4-tetrahydro-beta-carbolin-3-carboxylic acid</td>
</tr>
<tr>
<td>70.890</td>
<td>2-naphthalenemethanol</td>
</tr>
<tr>
<td>73.201</td>
<td>t-muurolid</td>
</tr>
<tr>
<td>75.201</td>
<td>tricosane</td>
</tr>
</tbody>
</table>

(components which are ≥1% in total ratio)
RESULTS AND DISCUSSION

Chemical composition of the essential oils. Essential oils consist of volatile substances which are naturally synthesized during the secondary metabolism of plants. Various research have been conducted on plants having medical capacity and these plants have been used to obtain essential oils worldwide. The presence of many alkaloid, phenol, terpene derivatives and other antimicrobial agents leads essential oils to inhibit the growth of pathogenic microorganisms. For this reason, essential oil can be regarded as an alternative to combat pathogenic microorganisms [13]. In our study, as L. nobilis leaves and flowers essential oil composition components were given in Table 1 and The gas chromatogram of the oil is shown in Figure 1. According to the results of the gas chromatography-mass spectrometry analysis, camphene (28.46 %), linalool (8.58%) were found to be highest in the essential oil obtained from L. nobilis population. 3-Ethyl-6-(methoxycarbonyl)-2-naphthol (16.10%), camphene (12.05%), were found to be highest in the essential oil obtained from Laurus nobilis flowers Aydn population. For Çiğli flowers populations, camphene (18.14 %) and α-pinene (8.04%), For Çiğli leaves populations 1,8-cineole (34.08%) and camphene (15.59%) were found to be highest in the essential oil (Table 1). Previous studies flowers and other parts of the L. nobilis detected the highest amounts obtained from essential oils as follows: 1,8 cineole (46.16%) [14], 1,8 cineole (63.92%, 58.13%, 56.85%, 52.65%), sabine (11.65%, 17.15%, 15.20%), α-terpinenyl acetate (10.40%) [15], 1,8 cineole (45.36%), bornylen (17.25%) [16], 1,8 cineole (25.7%, 18.69), sabine (8.7%), β-elemene (8.87%) [17], α-terpinyl acetate (28.43 %), methyl eugenol (19.57 %) [18]. In general, these findings confirmed that essential oil composition of plant can be different in quality and quantities in different geographical and environmental conditions and period of growth of plant [8, 19].

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Çiğli L. nobilis flowers</th>
<th>Çiğli L. nobilis leaves</th>
<th>Aydn L. nobilis flowers</th>
<th>Aydn L. nobilis leaves</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. subtilis</td>
<td>1.5625</td>
<td>3.125</td>
<td>1.5625</td>
<td>6.25</td>
</tr>
<tr>
<td>K. pneumoniae</td>
<td>6.25</td>
<td>3.125</td>
<td>3.125</td>
<td>3.125</td>
</tr>
<tr>
<td>G. rubripertincta</td>
<td>3.125</td>
<td>6.25</td>
<td>3.125</td>
<td>6.25</td>
</tr>
<tr>
<td>E. faecalis</td>
<td>3.125</td>
<td>3.125</td>
<td>3.125</td>
<td>3.125</td>
</tr>
<tr>
<td>P. vulgaris</td>
<td>3.125</td>
<td>3.125</td>
<td>3.125</td>
<td>3.125</td>
</tr>
<tr>
<td>E. aerogenes</td>
<td>3.125</td>
<td>1.5625</td>
<td>1.5625</td>
<td>3.125</td>
</tr>
<tr>
<td>S. enterica</td>
<td>3.125</td>
<td>1.5625</td>
<td>1.5625</td>
<td>3.125</td>
</tr>
<tr>
<td>C. albicans</td>
<td>12.5</td>
<td>12.5</td>
<td>12.5</td>
<td>12.5</td>
</tr>
<tr>
<td>C. tropicalis</td>
<td>0.097</td>
<td>1.5625</td>
<td>1.5625</td>
<td>0.3906</td>
</tr>
<tr>
<td>C. parapsilosis</td>
<td>0.3906</td>
<td>0.7812</td>
<td>0.1953</td>
<td>0.3906</td>
</tr>
<tr>
<td>S. cerevisiae</td>
<td>3.125</td>
<td>1.5625</td>
<td>3.125</td>
<td>1.5625</td>
</tr>
</tbody>
</table>

FIGURE 1
Gas chromatogram of the essential oil of L. nobilis flowers and leaves (Aydn and Çiğli)
Antibacterial and Antifungal Activity. Since microorganisms have recently developed resistance to existing antibiotics, the treatment of the infection caused by these microbes gets increasingly complicated. Some research reports that the therapeutic properties of plants result from the synergistic effect of many active substances instead of a single active substance, therefore herbal substances provide a more effective treatment by showing the resistance to microorganisms that are difficult to kill with a single antibiotic. This situation urges researchers to investigate compounds having inhibitory effect of natural antimicrobial agents of vegetable origin [20]. In the present study, antibacterial and antifungal effects of essential oils obtained from leaves and flowers of *Laurus nobilis* plant collected from Çiğli and Aydın against 11 different microorganisms, being 3 Gram-positive, 4 Gram-negative and 4 yeast were tested via MIC method. It was detected that essential oils have a significant antimicrobial effect on test microorganisms (Table 2). Table 2 presents MIC values of the essential oils which obtained from Çiğli *L. nobilis* leaves, Çiğli *L. nobilis* flowers, Aydın *L. nobilis* flowers and Aydın *L. nobilis* leaves; MIC values of Çiğli *L. nobilis* flowers and Çiğli *L. nobilis* leaves ranges from 1.5625 μL/mL to 6.25 μL/mL against bacteria. MIC values of Aydın *L. nobilis* flowers ranges from 1.5625 μL/mL to 3.125 μL/mL against bacteria. MIC values of Aydın *L. nobilis* leaves ranges from 3.125 μL/mL to 6.25 μL/mL against bacteria. Aydın *L. nobilis* flowers showed better antibacterial activity than Çiğli *L. nobilis* flowers. Moreover, Aydın *L. nobilis* flowers and Çiğli *L. nobilis* flowers had similar antifungal effect against test fungi. Çiğli *L. nobilis* leaves had better antibacterial activity than Aydın *L. nobilis* leaves. On the contrary, Aydın *L. nobilis* leaves had better activity than Çiğli *L. nobilis* leaves towards test fungi. All the tested bacteria were sensitive to the essential oils. *C. albicans* was the most resistant fungi in our study. There are a good number of studies conducted on the antimicrobial activities of essential oils obtained from *L. nobilis* in the literature. For example, in a study conducted by Yılmaz et al. the antibacterial and antifungal effects of *L. nobilis* essential oils collected from Hatay was investigated and the MIC value against *C. albicans* was determined as 250 μg/ml [20]. In the present study, on the other hand, the MIC value was found as 12.5 μg/mL. It is assumed that this difference results from the collection of *L. nobilis* sample from different geographies and the use of different parts of the plant in antimicrobial activity studies. El et al. studied on the antimicrobial effects of essential oils obtained through non-solvent microwave extraction and hydrodistillation methods from *L. nobilis* leaves collected from İzmir and reported that essential oils were effective on all test bacteria (*Staphylococcus aureus, Salmonella typhimurium* and *Escherichia coli*) excluding *Listeria monocytogenes* [21]. In the present study, it was found that the essential oils obtained from the flower and leaf parts of *L. nobilis* samples had an effect on all test bacteria. This finding results from the difference in test microorganisms used in both studies. In a study conducted by Goudjil et al. antimicrobial activity of essential oil obtained from *L. nobilis* collected from Tunisia was investigated and MIC values against *Salmonella enterica, Proteus sp.* and *Klebsiella pneumoniae* bacteria were found as 0.2 mg/ml, 0.33 mg/ml and 0.11 mg/ml respectively [22]. In the present study, MIC values ranged from 1.5625 μl/ml to 3.125 μl/ml against *S. enterica*; was 3.125 μl/ml against *P. vulgaris*; and ranged from 3.125 μl/ml and 6.25 μl/ml against *K. pneumoniae*.

**CONCLUSION**

To conclude, the findings of the present study reveal that the essential oils obtained from the flowers and leaves of *L. nobilis* plant, antibacterial and antifungal activities of which were examined, provide varying degrees of activity on all tested microorganisms. In addition, the antibacterial and antifungal effects of these oils, also indicated that the active ingredients contained in these oils may be served as an alternative to some synthetic antibiotics used in the treatment of infectious diseases.

**REFERENCES**


CADMIUM AND ARSENIC SOIL THRESHOLD IN RICE PRODUCTION AREA OF PEARL RIVER DELTA OF GUANGDONG PROVINCE

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ABSTRACT

Heavy metals are harmful to human body. Soil-plant system is an important way for heavy metals to enter the food chain. In our study, we summarize the data published in CNKI, web of science, Wan Fang database and Wei Pu from 2005 to 2017 about heavy metal contamination in soil and rice crop of the pearl river delta region of Guangdong. The database expose that the pollution of Cd in the soil is serious, followed by Cu, Ni, As, Hg. The contamination of Zn, Pb, Cr, was lower as compare to Cd. Concentration of Cd and Pb in rice grains was serious, followed by As and Cr Hg and Cu. When soil organic matter in tillage layer was >20%, the Cd limited value is 0.96 mg/kg. When the organic matter content in soil tillage layer is <20%, the Cd limited value is 1.23 mg/kg. When soil organic matter in tillage layer was >20%, the fitting equation As limit value is 18.86mg/kg. When soil organic matter in tillage layer was <20%, As limited value is 19.68 mg/kg. National soil environment quality standard (GB15618-2018) the pearl river delta soil have Cd contamination which can produce security rice. As contaminated farmland soil cannot produce safety rice crops, in rice cultivation system.

KEYWORDS:
Heavy metal, Farmland soil, rice, threshold value, Pearl river

INTRODUCTION

Heavy metal pollution is one of the major environmental problems. With the development of industry, various human activities, such as mineral exploitation, industrial production, agricultural production and commerce, soil is contaminated with heavy metals [1]. The arable land contaminated by heavy metal is nearly 2×10⁸m², accounting for about 16.67% of the total cultivated land, of which Cd and As is listed as a typical contaminant including heavy metals [2]. One of the direct consequences of these activities is the deposition of heavy metals in surface of soil. Heavy metals can be enter the body through food chains, cause harmful effects to ecosystems including humans [3]. The ability of crops to uptake heavy metal increases with the increase of heavy metal content in soil [4]. Heavy metals can cause developmental delays, multiple cancers, damage kidneys, endocrine disorders, and have an impact on the immune and nervous systems. Low concentration of heavy metals stimulates plant growth, and high concentration of heavy metals can cause certain damage to plants [5, 6]. By reducing photosynthesis and respiration in plants, Cd competes with Ca, affecting the activity of Ca Regulatory proteins and there by affecting cell division. Cd can bind with negatively charged nucleic acid, damage nucleolus structure, inhibit DNase and RNase activity, and inhibit plant DNA synthesis [6, 7]. Studies have shown that cadmium exposure promotes the synthesis of genes related to MPF, thus slowing down the process of meiosis and reducing fertility [8, 9]. Nephrotoxicity is caused by accumulation of cadmium in the kidney, usually after 40-50 years exposure [10, 11]. In the presence of high concentration of arsenic, plant roots become shorter, and photosynthesis and cell growth are inhibited. Under the treatment of As at 80 and 160 mg kg⁻¹, the gas exchange in leaves was significantly reduced, the chlorophyll fluorescence ratio Fv/Fm was also decreased, the content of chlorophyll and protein was decreased, and typical oxidative stress was formed on oats [12]. The nitrogen assimilation rate of oat rice decreased significantly under high arsenic stress [13]. Therefore, it is great theoretical and practical significance to study the safety threshold of soil agricultural products for improving the grain yield and quality of Guangdong province and reducing the risk of environmental pollution.

The pearl river delta is located in the southeast of Guangdong province. The cultivated land area of
the pearl river delta plain area is 607,200 hectares (910.79 million mu), including Guangzhou, Shenzhen, Foshan, Dongguan, Huizhou, Zhongshan, Zhuhai, Jiangmen and Zhaoqing account for 23.98 percent of the total cultivated land of the province. In 2017, Guangdong province is a major province of rice production, trade and consumption in China. According to statistics, the total grain output was 13.651 million tons, of which the rice yield reached 1.0969 million tons [14]. Rice is the major food crop, and occupies a great share in the dietary structure of Chinese residents, especially in the southern China. The area plays an important role in China’s grain production and agricultural product safety [15, 16]. Therefore, protecting the agricultural ecological environment in the pearl river delta region of guangdong province is of great significance to agricultural products and food security.

MATERIALS AND METHODS

Study Area and Data Collection. Through summarizing and sorting the papers published in CNKI, Web of Science, Wan Fang database and Wei Pu from 2007 to 2017, the heavy metal pollution database of paddy soil in the pearl river delta region of Guangdong and the heavy metal point-to-point pollution database of soil and grain of crops were established. The data samples were collected from the pearl river delta region of Guangdong, a subtropical climate with warm and humid year round. Annual precipitation is above 1500 mm. Rainy season and high temperature season synchronous, fertile soil, river crisscross, beneficial to agriculture. Guangdong is 200 meters above sea level, flat; High temperature and rainy, south subtropical monsoon climate dominated [17].

Technical Means. The critical value of heavy metal in soil to ensure the safe production of rice was established through the model and the safety production procedure of the paddy soil contaminated by heavy metal was established. Pearl river delta in order to make a apply rice production areas of agricultural production safety threshold of soil heavy metal, use the method of statistical analysis, establishment of heavy metals in soil and rice system migration and enrichment of the prediction model, according to the national food safety standards, deduce the rice in China from the viewpoint of safety in production safety critical value of farmland soil heavy metal.

Data Analysis. All the experimental data were analyzed by Microsoft Excel. The correlation between variables and the single association algorithm were used to connect a group of variables close to each other SPSS 19.0 was used for statistical analysis, and origin 2017 was used for chart making.

RESULTS AND DISCUSSION

Heavy metal concentrations in soils. In this study, the content of heavy metals was Zn>Pb>Cu>Ni>As>Cd>Hg. In the pearl river delta region of Guangdong, the average value of the eight heavy metals was greater than NQS (National Quality Standards). The collected data were compared with national soil environmental quality standard (GB15618-2018) [18], and found that about 4.5% Pb, 48.52% Cd, 3.1% Cr, 16.67% Hg, 26.3% Ni, 5.5% Zn, 28.6% Cu, and 24.7% As in the Guangdong Soil exceeded the NQS. Under the rice cultivation system, a total of 646, 589, 426, 338, 411, 326, 437, 417 samples of Cd, As, Pb, Hg, Cu, Cr, Ni and Zn in the Paddy soil were collected, respectively (Table 1). From the perspective of heavy metal pollution sources, the soil genesis in the pearl river delta region is mainly river alluvial and delta sediment, and the soil type is moist soil type. Soil permeability is good, mostly acidic or neutral, saline-base saturation is large, and organic matter content is high. In addition to parent material, rapid economic development and excessive human activities are the main causes of heavy metal pollution in the farmland of the pearl river delta region. Since the reform and opening up, the soil environment in the pearl river delta region has gradually deteriorated due to the gradual transition from traditional agriculture to industrial, the increasing emission of industrial and living pollutants and the lack of corresponding pollution control measures. The large-scale metal mines in the province are mainly sulphide deposits, containing a variety of associated metals, such as As Pb, Zn, Cd, Cu, As, Cr, Hg, etc. [19]. Mining and smelting activities lead to a large number of pollutants into the pearl river delta region. For example, the mining pollution in Dabaoshan makes the low-lying upper village farmland in the downstream seriously polluted, which poses serious health risks to local residents and agricultural products, and also causes serious damage to the local ecological environment [20, 21]. The traffic flow in the pearl river delta is large. Although various types of vehicles mainly use unleaded or low-lead gasoline, the use of leaded gasoline has a long history before the end of 1980s, resulting in high lead content in soil. Heavy metals, produced by burning fuel, rubbing on automobile metal parts and tires, enter the soil in ways such as diffusion and runoff. Soil near the highway is contaminated with heavy metals of different degrees of Pb, cadmium, zinc, copper, chromium and nickel,
with heavy pollution of lead and cadmium and light pollution of zinc, copper, chromium and nickel [22]. Frequent use of chemical fertilizers and pesticides lead to heavy metals accumulation in soil. The traffic flow in the pearl river delta is large. Although various vehicles mainly use unleaded or low-lead gasoline, the use of leaded gasoline has a long history before the end of 1980s, resulting in high lead content in soil. The main pollutant in the soil around Beijing’s highways is Cd, which is mainly from the exhaust emissions of motor vehicles and dust generated by tire wear and brake [23]. There are two main reasons for heavy metal pollution caused by agricultural activities. First, unscientific use of chemical fertilizers and pesticides and excessive use of chemical fertilizers and pesticides often contain various trace elements. Second, sewage irrigation causes heavy metals to enter the soil. Saudi Arabia, A country in the Middle East, heavy metal Cr, Pb, Cd and Mn content are high, and the health risk index (HRIs)>1 of edible agricultural products has potential health risks [24]. The content of cadmium, copper, lead, zinc, nickel, chromium and mercury in the soil irrigated by sewage is much higher than that in the soil irrigated by water [25, 26].

**Heavy Metal Concentrations on Rice.** Table 2 shows the statistical of the contents of heavy metal elements in rice grains in the pearl river delta region of Guangdong province. The samples of heavy metals including Cd, As, Pb, Hg, Cu and Cr are 651, 424, 460, 191 and 460 respectively. The collected data were compared with China’s food contamination limit (GB 2762-2017) (MHPRC, 2017) [27], and found that rice grains contained 56 of Cd, 7.8 of As, 36 of Pb, 0.43 of Hg, and 4.6 % of Cr exceeded the standard, which indicated that the pollution of Cd and Pb in rice grains was relatively serious, followed by As and Cr, and Hg and Cu were relatively light. The average concentration of Cd in the Yangtze river delta region is 0.023mg/kg\(^4\), while the average concentration of Cd in the pearl river delta region is much higher than that in the Yangtze river delta region, while the average concentration of copper is slightly lower than that in the Yangtze river delta region [28]. Studies have shown that, the Pb enrichment capacity of rice grains in soil is low [29], while the over-standard rate of the heavy metal Pb content in rice grains is high as 36% in the data collected. May be caused by the atmospheric deposition and transportation emissions leading to the increase of Pb content in rice grains [30]. The concentration of Hg in rice grains in Hunan was less different from that in the pearl river delta region, while the concentration of Pb was significantly lower than that in the pearl river delta region [31]. It can be conclude that Cd and Pb are seriously polluted in rice grains in the pearl river delta region. Studies have documented that genotypic differences have significant effects on the accumulation of heavy metals in rice grains [32, 33]. Hybrid rice has stronger absorption capacity for Cd than conventional rice, and the order of Pb uptake and accumulation for variety is: two-line hybrid rice>three-line hybrid rice> indica conventional rice. The order of uptake and accumulation for As was: indica rice > three-line hybrid rice > two-line hybrid rice. The order of uptake and accumulation of Hg was: indica rice > three-line hybrid rice > two-line hybrid rice [34]. Zhong reveals that there is no significant difference in the accumulation of As among different rice varieties [35]. Different varieties of rice lead to different morphological structure and physiological characteristics of crops, leading to great differences in the absorption and distribution of heavy metal elements in rice [36, 37].

**Safe and Threshold of Rice.** Rice is one of the most important food crops in China. The number of people living on rice is about 65% of the total population. Therefore, the quality and safety of rice grains directly affects human health [38, 39]. For

### TABLE 1

Descriptive statistics of selected heavy metal contents in the soils (mg/kg)

<table>
<thead>
<tr>
<th>Element</th>
<th>Number of samples</th>
<th>Minimum value</th>
<th>Maximum value</th>
<th>Mean value</th>
<th>Standard deviation</th>
<th>Variable coefficient</th>
<th>Criterion(^\text{a})</th>
<th>Over standard rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cd</td>
<td>646</td>
<td>0.01</td>
<td>1.17</td>
<td>0.38</td>
<td>0.67</td>
<td>1.76</td>
<td>0.4</td>
<td>48.52</td>
</tr>
<tr>
<td>As</td>
<td>589</td>
<td>0.06</td>
<td>442.16</td>
<td>26.46</td>
<td>29.01</td>
<td>1.10</td>
<td>10</td>
<td>24.7</td>
</tr>
<tr>
<td>Pb</td>
<td>426</td>
<td>0.25</td>
<td>268.78</td>
<td>93.37</td>
<td>106.24</td>
<td>1.13</td>
<td>100</td>
<td>4.5</td>
</tr>
<tr>
<td>Hg</td>
<td>338</td>
<td>0.003</td>
<td>1.57</td>
<td>0.29</td>
<td>0.41</td>
<td>1.41</td>
<td>0.5</td>
<td>16.67</td>
</tr>
<tr>
<td>Cu</td>
<td>411</td>
<td>0.21</td>
<td>297.24</td>
<td>43.25</td>
<td>39.02</td>
<td>0.90</td>
<td>50</td>
<td>28.6</td>
</tr>
<tr>
<td>Cr</td>
<td>326</td>
<td>2.01</td>
<td>186.19</td>
<td>81.29</td>
<td>72.63</td>
<td>0.89</td>
<td>250</td>
<td>3.1</td>
</tr>
<tr>
<td>Zn</td>
<td>437</td>
<td>0.94</td>
<td>460.29</td>
<td>112.21</td>
<td>115.26</td>
<td>1.03</td>
<td>200</td>
<td>5.5</td>
</tr>
<tr>
<td>Ni</td>
<td>417</td>
<td>1.37</td>
<td>195.43</td>
<td>39.17</td>
<td>43.05</td>
<td>1.10</td>
<td>70</td>
<td>26.3</td>
</tr>
</tbody>
</table>

\(^a\) The second grade of environmental quality standard of heavy metals for farmland soil in China (MEPPRC, 2018) [18]
the soil-plant system of rice paddies, the effects of soil Cd through the food chain depends on the content of rice Cd. In addition, the average daily Cd intake in rice-fed areas is 20-40 μg/kg [40]. If the content of Cd in rice grains exceeds the NQS, which may cause the deleterious effects on human health. Therefore, it is necessary to study the differences of Cd uptake and accumulation in rice grains in different soil environments to understand the mechanism of Cd enrichment in grains. Soil property is an important factor influencing the migration and transformation of heavy metals in soil [41]. Humic acid in soil organic matter can provide more adsorption sites binding to Cd and reduce its effectiveness, while soluble organic acids such as fulvic acid with relatively low molecules often increase the mobility of Cd in soil [42, 43]. With the decrease of soil pH, the positively charged amount of soil colloids will increase correspondingly, and the deprotonation capacity of the obligatory adsorption point decreases, so the soil’s adsorption capacity for heavy metal Cd decreases, thus improving the bioavailability of cadmium [44, 45].

Compared with other crops, rice has a stronger accumulation of arsenic. The growth of plants with different arsenic concentrations in the pot experiment, and found that low concentration of arsenic promotes plant growth and high concentration of arsenic inhibits plant growth [46, 47]. High concentration of arsenic induces free radicals and reactive oxygen species in plants, leading to increased pressure in plants and exacerbating membrane damage and inhibits photosynthesis and cell growth in plants. As(V) and phosphorus (P) have similar atomic structures, which interfere with plant phosphorus metabolism and even replace P, when P and ATP are synthesized by plant phosphorylation [48]. As (III) has a high affinity, to reduce the plant body a kind of important antioxidants [49, 50]. The content of arsenic in rice increases with the increase of As in soil. In this study, 20% of soil organic matter content was divided into high organic matter content and low organic matter content, and the crop safety thresholds of acidic soil in the pearl river delta region of Guangdong were calculated respectively. Figure 4(A) shows the content of Cd in soil and the content of Cd in rice grains when the organic matter content of the soil layer is >20%. Sample number n=132, fitting equation y = 0.124x + 0.0804, R² = 0.7584. Fig. 4(B) shows the content of Cd in soil and the content of Cd in rice grains when the organic matter content in soil layer <20%. Sample number n=67, fitting equation y = 0.145x + 0.0213, R² = 0.6315. The limit value of the heavy metal cadmium in rice grains in GB 2762-2017 is y = 0.2mg/kg, and the calculated x value is the limit value of the heavy metal pollution in soil corresponding to the safe planting of rice. The calculated organic matter content of >20% soil cadmium in rice producing area is 0.96mg/kg, and the organic matter content <20% soil cadmium in rice producing area is 1.23mg/kg. According to the national soil environment quality standard (GB15618-2018) (MEPPRC, 2018) [19] in the pH<6.5 grading standards for reference, the standard values of Cd in the soil was 0.4 mg kg⁻¹, therefore in rice cultivation system, the pearl river delta of Cd contaminated soil can also produce uncontaminated rice grains. Fig. 2(C) shows the As content in soil and the As content in rice grains when the organic matter content in the soil layer is >20%. Sample number n=78, fitting equation y = 0.0084x + 0.0416, R² = 0.8279. Fig. 2(D) shows the As content in soil and the As content in rice grains when the organic matter content in soil layer <20%. Sample number n=81, fitting equation y = 0.0074x + 0.0544, R² = 0.7673. The limited value of the heavy metal As in rice grains in (GB 2762-2017) is y = 0.2mg/kg, which is substituted into the corresponding regression equation. The calculated x value is the limit value of the heavy metal pollution in soil corresponding to the safe planting of rice. The limited value of 20% soil cadmium in rice producing area is 18.86mg/kg, while the limited value of 20% soil cadmium in rice producing area is 19.68 mg/kg. According to the national soil environment quality standard (GB15618-2018) [19] in the pH<6.5 grading standards for reference, the soil As the standard values of 30 mg kg⁻¹, therefore in rice cultivation system, the pearl river delta As contaminated farmland soil cannot produce safety rice.

### Table 2

<table>
<thead>
<tr>
<th>Element</th>
<th>Number of samples</th>
<th>Minimum value</th>
<th>Maximum value</th>
<th>Mean value</th>
<th>Standard deviation</th>
<th>Variable coefficient</th>
<th>Criterion[(i)]</th>
<th>Over standard rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cd</td>
<td>651</td>
<td>0.01</td>
<td>1.785</td>
<td>0.24</td>
<td>0.08</td>
<td>0.421</td>
<td>0.2</td>
<td>56</td>
</tr>
<tr>
<td>As</td>
<td>424</td>
<td>0.04</td>
<td>0.54</td>
<td>0.15</td>
<td>0.13</td>
<td>0.867</td>
<td>0.2</td>
<td>7.8</td>
</tr>
<tr>
<td>Pb</td>
<td>460</td>
<td>0.01</td>
<td>1.22</td>
<td>0.17</td>
<td>0.064</td>
<td>0.376</td>
<td>0.2</td>
<td>36</td>
</tr>
<tr>
<td>Hg</td>
<td>460</td>
<td>0.002</td>
<td>0.008</td>
<td>0.0043</td>
<td>0.0012</td>
<td>0.279</td>
<td>0.02</td>
<td>0.43</td>
</tr>
<tr>
<td>Cu</td>
<td>191</td>
<td>0.44</td>
<td>9.45</td>
<td>3.94</td>
<td>1.96</td>
<td>0.497</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Cr</td>
<td>460</td>
<td>0.08</td>
<td>1.04</td>
<td>0.38</td>
<td>0.082</td>
<td>0.216</td>
<td>1</td>
<td>4.6</td>
</tr>
</tbody>
</table>

*(Maximum levels of contaminants in Foods (GB2762-2017) [27].)*
CONCLUSION

Through summarizing and sorting the literatures published in CNKI, Web of Science, Wan Fang database and Wei Pu from 2005 to 2017, the heavy metal pollution characteristics database of rice farmland soil in the pearl river delta region of Guangdong and the heavy metal point-to-point pollution database of soil and grain of crops were established. On the base of our result we conclude that the pollution of Cd in the rice farmland soil in the pearl river delta region of Guangdong is relatively serious, with the over-mark rate of 48.52%, followed by Cu, Ni, As, Hg, and the over-mark rate of 28.60%, 26.30%, 24.70% and 16.67% respectively. The pollution of Zn, Pb, Cr, was relatively light, and the overshooting rate was 5.50%, 4.50% and 3.10%, respectively. In the pearl river delta region of Guangdong, the pollution of Cd and Pb in rice grains was relatively serious, with the overshooting rate of 56% and 36%, respectively. In our study, the safe threshold value of heavy metals for rice planting in acidic soil in the pearl river delta region is calculated by establishing the prediction equation, which provides a basis for the decision-making departments to formulate reasonable soil measures. Due to different soil physio-properties pants have different coefficients for Heavy metals. Therefore, it is crucial to distinguish the safe production threshold of rice in different regions. The government and environmental protection organization should establish the safe production threshold of rice according to the soil properties of different areas.

ACKNOWLEDGEMENTS

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REFERENCES


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PHYSICAL CHARACTERISTICS OF COBS AND KERNELS IN SWEET CORN UNDER VARYING PLANTING ENVIRONMENTS

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ABSTRACT

Documenting qualitative and quantitative characteristics of landraces is important for maintaining genetic diversity. Sweet corn landraces are grown in Khyber Pakhtunkhwa, Pakistan for their consumption in local markets. A study was commenced to document characteristics of sweet corn under different planting conditions. Four landraces along with an approved corn variety were planted on five dates at almost one month interval at New Developmental Farm (NDF) of University of Agriculture Peshawar, Pakistan. Randomized complete block (RCB) design with split plots was used. Planting dates and landraces significantly affected physical properties of cobs and kernels. Sweet corn attained maximum cob diameter, kernel length, kernel width, kernel rows cob⁻¹ and number of kernels row⁻¹ when planted in July during both years. Landrace Swabi recorded maximum cob diameter, kernel length, kernel width, kernel rows cob⁻¹ and number of kernels row⁻¹ in both years. Further studies to reveal the underlying reasons for genotypic variations and their interaction with environmental conditions are imperative.

KEYWORDS:
Sweet corn, maize, landraces, planting dates, cob, kernel, environment

INTRODUCTION

Maize (Zea mays L.) is the third most important cereal crop in Pakistan. It is mostly grown for three distinct markets in the world viz., fresh, canned, and frozen [1]. Maize is grown in the widest range of environments all over the world. United States of America, Mexico, Nigeria, France and Hungary are the major producing countries [2]. Sweet corn (Zea mays L. var. saccharata Strul) is one of several types of maize, which also includes flint corn, dent corn, popcorn, flour corn, and pod corn [3]. It is grown, for local markets, in different parts of Khyber Pakhtunkhwa including Swat, Mansehra, Swabi and Parachinar [4]. Great potential is envisaged in sweet corn market and if its yield become at par with field corn the income of the farmers can be increased many fold. Little systematic research has been done on this neglected crop, particularly in Khyber Pakhtunkhwa.

Certain traits, such as grain size, color, and taste were given preference by early farmers which became the bases of selection [5]. This mechanism gave rise to the formation of landraces. It became imperative to collect and preserve landraces throughout the world, particularly in areas where modern cultivars are replacing old germplasm and erosion of genetic diversity is happening with changes in climate. Surveying both qualitative and quantitative traits of existing landraces may be useful in maintaining their genetic diversity and preserving them from genetic erosion [6]. Planting time, environment and variety selection are the key factors significantly affecting cob and kernels of the crops [7, 8, 9, 10, 11]. Microclimate plays an important role in growth and yield of sweet corn. Microclimate can be altered in many ways among which planting date is the most important factor to be considered. To fully explore grain production potential of sweet corn, it is essential to know how plants interact morphologically and physiologically in a community and to realize management practices, which allow them to get the most out of growth resources in their environment [12].

Owing to the importance of indigenous landraces and role of planting time for achieving the potential limitations, this study was initiated to document numerous traits of landraces.

MATERIALS AND METHODS

These experiments were carried out at New Developmental Farm (NDF), University of Agriculture Peshawar, Pakistan during 2007 and 2008.
NDF is located at 34° N, 71.3° E longitude and 350 m above sea level. The soil of the field was analyzed for organic matter [13], pH, EC [14], P and K [15]. It was found that the soil was alkaline (pH 8.2), low in organic matter content (0.87 %), having EC (0.74 dSm⁻¹), total N (0.04 %), AB-DTPA extractable P (1.15 mg P₂O₅ kg⁻¹) and high in exchangeable K (506 mg K₂O kg⁻¹). Temperature, humidity, and precipitation during the period of experiment are reproduced in Table I;

<table>
<thead>
<tr>
<th>Month</th>
<th>Average T (°C)</th>
<th>R. Humidity (%)</th>
<th>Total Rainfall (mm)</th>
</tr>
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<tbody>
<tr>
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<td>17.31 (21.18)</td>
<td>65.50 (47.81)</td>
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</tr>
<tr>
<td>April</td>
<td>25.30 (22.92)</td>
<td>55.38 (52.03)</td>
<td>0.8 (16.8)</td>
</tr>
<tr>
<td>May</td>
<td>27.97 (29.29)</td>
<td>49.61 (41.97)</td>
<td>0.2 (0)</td>
</tr>
<tr>
<td>June</td>
<td>32.42 (32.55)</td>
<td>49.35 (62.73)</td>
<td>0 (16)</td>
</tr>
<tr>
<td>July</td>
<td>31.31 (32.56)</td>
<td>64.73 (72.45)</td>
<td>0 (27)</td>
</tr>
<tr>
<td>August</td>
<td>31.95 (31.29)</td>
<td>65.77 (75.61)</td>
<td>20.2 (150.9)</td>
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<td>22 (16.7)</td>
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<tr>
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<td>46.88 (65.07)</td>
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</tr>
<tr>
<td>November</td>
<td>18.44 (18.68)</td>
<td>73.60 (56.37)</td>
<td>8 (0)</td>
</tr>
</tbody>
</table>

Means were compared using least significant difference (LSD) test [16].

### RESULTS AND DISCUSSION

#### Cob diameter (cm).
Cob diameter is an important trait of a variety which is reported to be positively related with number of rows cob⁻¹ and consequently number of grains cob⁻¹ [17]. Cob diameter of sweet corn was significantly affected by planting dates and landraces during both years (Fig. 1a & 1b). In year I, April and July planted crop produced maximum cob diameter, while August planting resulted in lowest cob diameter. For the year II, data showed that maximum cob diameter observed in July planting; while May planted sweet corn resulted in minimum cob diameter. These results showed May and August plantings produced cobs with lowest diameters. The main reason seemed to be lower leaf area recorded for these plantings. As leaf is the main photosynthetic apparatus and lower leaf area means lower photoassimilate production resulting in lower translocation of photoassimilates to cob. On the other hand higher leaf area production in April and July plantings resulted in more photoassimilate production and translocation to the cob, increasing its diameter. Significant effect of planting date on cob diameter of sweet corn was reported earlier [18]. Oktem et al. [7] reported decrease in cob diameter when planting delayed from late April to late June planting. Further delay of planting to late July increased cob diameter and reached to its maximum value. They attributed lower cob diameters to pollination and fertilization problems observed between April and June plantings.

In year I, Azam noted highest cob diameter followed by landrace SWB while lowest cob diameter was observed in landrace PRC. During year II, Azam produced maximum cob diameter followed by landrace SWB, while PRC and MNG noted minimum cob diameter. Azam is a composite cultivar possessing genetic potential for higher production [19] and because of genetic superiority Azam may have produced cobs with more diameters. Landraces (MNG and PRC) were collected from higher elevations with lower mean temperature; therefore they may have problems in acclimatizing with higher mean temperatures and dry climate of Peshawar valley resulting in poor performance. Significant effect of genotypes on cob diameter was also reported by White [18] and Hefny [20]. Lowest cob diameter in August planting may be attributed...
to lower temperature in the months (October, November) bracketing silking, fertilization and cob growth. Under poor environmental conditions cobs suffer more than other parts of the plant [21].

**Kernel length (mm).** Kernel length of sweet corn was significantly affected by planting dates and landraces during both years (Fig. 2a & 2b). In year I, late July planting gave maximum kernel length followed by mid-June planting while last sowing (August) gave minimum kernel length. In year II, maximum kernel length was observed in late July planting while minimum kernel length observed in May planting. These differences in kernel length among different planting dates may be due to differences in leaf area plant$^{-1}$. Since August planting in year I and May planting in both years produced shorter plants and lower leaf area plant$^{-1}$ resulting in less photoassimilate translocation to the kernels resulting in lower kernel lengths. Tollenaar [22] stated that during grain filling, dry matter accumulation depends mostly upon availability of assimilates from other plant parts (leaves, stem). A shortage of assimilate supply or unfavorable environmental conditions during kernel development affects the potential kernel size [23]. Kernel length of landrace SWB was highest during both years while kernel length of landrace MNS was lowest. This difference in landraces may be due to sink potential of kernels. Revilla and Tracy [24] studied 58 cultivars of sweet corn and found significant effect of cultivar on kernel length. These results showed that under optimum conditions landrace SWB produce kernels with more length. Limited source of photo-assimilates in August and May planted crop may have restricted kernel size.

**Kernel width (mm).** Analysis of the data illustrated significant effect of planting date, landraces and their interaction on kernel width of sweet corn during both years (Fig. 3a & 3b). In year I, maximum kernel width was observed in July planting, while August planting noted minimum kernel width. In year II, sweet corn yielded kernel with maximum width from July planting, while minimum kernel width was observed in May planting. During grain filling stage, dry matter accumulation in kernels depend mostly upon availability of assimilates from other plant parts [22]. A shortage of assimilate supply or unfavorable environmental conditions during kernel development affects the potential kernel size [23]. In the light of these findings, lower kernel widths in August (year I) and May (year II) plantings may be due to lower plant heights and leaf area plant$^{-1}$

During both years, landrace SWB produced maximum kernel width followed by Azam, while landrace PRC recorded lowest kernel width. Higher photoassimilate translocation and sink potential of landrace SWB may be contributed in higher kernel width. Zhan et al. [25] concluded that sink strength of an organ in plant is genetically controlled. Significant differences in kernel length of different genotypes of corn were reported by Kandil and Sharief [26]. Landrace SWB attained maximum kernel width under optimum environmental conditions of late July planting. On the other hand, crop planted in mid-August faced lower temperature and solar radiation, during grain filling period, may have slowed down post-silking crop growth rate [27]. Therefore, landrace PRC attained lowest kernel width.

**Number of kernel rows cob$^{-1}$.** Kernel rows should be given more importance in selection for yield improvement in maize [28]. Number of kernel rows cob$^{-1}$ (KRC) was significantly affected by treatments, during both years (Fig. 4a & 4b). In year I, means for planting dates showed highest KRC in July planting and minimum KRC in August planting. Data regarding year II revealed that March, April and July plantings noted statistically same KRC, while lowest KRC were observed in May planting. Shorter vegetative growth period for August sown sweet corn and then low temperature and incident solar radiation during grain set and filling stage resulted in lowest cob diameter which in turn reduced KRC. Poor performance of August planted crop lead us to omit this sowing date in the 2nd year of experiment. Significant reductions in final kernel number unit area$^{-1}$ of crop were observed when sowing date was delayed [29].

In year I, Azam noted highest KRC followed by landrace SWB. Landrace PRC yielded lowest KRC, which were statistically at par with KRC in MNG. In year II, Azam and SWB yielded highest and statistically same KRC. PRC recorded lowest KRC. Variation in KRC among landraces might be due to genetic differences. Significant variations in KRC for different genotypes have earlier reported [20, 30, 31]. Since Azam is a synthetic variety hence it produced more KRC in favorable environmental conditions compared to landraces.

**Number of kernels row$^{-1}$.** Analysis of the data showed that number of kernels row$^{-1}$ (KPR) of sweet corn was significantly affected by planting dates and landraces, during both years (Fig. 5a & 5b). Means data regarding year I suggested that highest KPR were observed in late July planting followed by April planting; while, lowest KPR were noted in August planting. In year II, maximum KPR were reported in July planting while minimum KPR were reported in May planting. Final grain yield depends on KPR ($r^2 = 0.74$ and $r = 0.86$), while KPR depends on cob length ($r^2 = 0.64$ and $r = 0.80$) and physiological conditions of the crop at flowering. Shorter vegetative growth period for August sown sweet corn and then low temperature and incident solar radiation during grain set and
filling stage are important factors resulted in lowest KPR. Frost at later stages of plants growth may be another reason for lower yield. These results lead us to omit August sowing date in the year II. Lowest KPR in May planting may be due to lower growth rate recorded. On the other hand July planting exhibited highest growth rate and cob length resulting in higher KPR value. Since dry-matter partitioning to cobs is a function of plant growth rate at silking [21]. Significant effect of planting date on KPR [20] and planting date on final kernel number per unit area was also reported by Cirilo and Andrade [29]. Sari et al. reported significant effect of planting date on KPR [8]. They stated that delaying planting date from early April to mid-May decreased KPR.

FIGURE 1
Cob diameter of sweetcorn as affected

FIGURE 2
Kernel length of sweetcorn as affected

FIGURE 3
Kernel width of sweetcorn as affected
In year I, mean KPR of Azam was highest followed by landrace SWB while PRC noted lowest KPR. Same pattern was observed in year II, where Azam attained highest KPR followed by landrace SWB, while lowest KPR observed in PRC. Cultivar Azam topped all the other cultivars by producing more KPR followed by landrace SWB in both years of research. This variation may be due to superior genetic makeup of Azam and its adoptability to the environmental conditions of Peshawar. Landrace SWB followed Azam which also seems to be due to its higher adoptability compared to other landraces. Significant effect of genotypes on KPR was reported earlier [20, 30]. This further elaborates superior genetic potential of Azam under optimum environmental conditions. Earlier, Sari et al. [8] also found significant interaction between planting dates and various varieties of sweet corn. Studies to unravel the variation in response of different genotypes to altered environments will help the researchers to better understand the interaction between genotypes and their growing conditions.

REFERENCES


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EFFECTS OF LEAD ON SEED GERMINATION AND SEEDLING GROWTH IN DIFFERENT SESAME (SESAMUM INDICUM) GENOTYPES

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³Department of Field Crops, Faculty of Agricultural, Van Yuzuncu Yil University, Turkey

ABSTRACT

Lead is considered as an essential potent environmental contaminant. Various ecological, environmental and evolutionary processes in the microsphere are disrupted because of lead toxicity to the microbial community. Based on this important perspective, the effects of increasing doses (control, 100, 200 and 400 mg L⁻¹) of Lead Nitrate (Pb(NO₃)₂) as heavy metal on seed germination and seedling growth of 11 different sesame genotypes were investigated. The research was carried out according to the Completely Randomized Experimental Design (CRD) with three replications at 25 °C (±2) in the Department of Field Crops laboratory, Faculty of Agriculture, Kahramanmaras Sutcu Imam University in 2016. In the experiment, 25 seeds of each genotype were placed to germinate for 14 days, and then, some basic germination and seedling growth determining parameters such as germination percentage, radicle length, plumula length, seedling length, seedling fresh weight, seedling dry weight and vigor index were observed.

Lead treatments have a strong negative influence on the growth of sesame by reducing significantly all the above parameters. The growth of the plants grown with increasing lead levels were reduced in compared to control plants. As a result, the effects of increasing levels of lead nitrate on the seeds germination percentages of sesame genotypes varied among the genotypes. The most tolerant genotype to the increasing levels of lead nitrate was Muganlı-57. The highest values of the other features examined were found to be obtained from the control applications of the genotypes.

KEYWORDS:
Germination, lead nitrate, seed, sesame (Sesamum indicum), vigor index

INTRODUCTION

Heavy metals take roles as fundamental plant micronutrients, however, overabundance concentrations of these substance become poisonous toxins [1]. Beyond acceptable levels, these metals are turned into contaminants in the environment. Additionally, their abundance creates pollution into the atmosphere [2]. They cause toxic effects on animals, plants, and human health [3, 4]. Of these, Cu, Cd, Ni, Zn, Cr, Co and as predominantly Pb, are the most reported metals that are the hazard for the growth and development of plants [4, 5]. The heavy metals that are concentrated in plant tissues are due to the widespread application of phosphate fertilizers, sewage sludge, dust from contaminants, industrial wastes and poor irrigation practices in agricultural land [4, 5]. Among above heavy metals, lead which is considered as one of the major toxic environmental contaminant, has a significant negative effect on the plant metabolic processes [6, 7]. Processes and cycles of various environment, ecology and evolution in the microsphere are deteriorated due to toxic effects of lead on the microbials [8, 9]. Lead has highly toxic effects on living organisms and has no biological function but can cause biochemical, physiological and morphological dysfunctions in all plants [6]. It is not only a toxic element, however can be accumulated in various plant organs and agricultural products [10] and can have toxic effects on animal and human who consumed these sources as food. The main lead content of the soil is derived from the application of tetra-methyl lead as a similar antirust agent from the decomposition of geological rock formations, discharge of lead mines, exhausts of automobile, applications in industrial, operations of smelting, impurities of fertilizer, metal coatings and coatings using lead arsenate [11]. However, there is still a problem as far as the exogenous lead can pass through seeds directly from atmospheric pollution or soil solids in contact with the seeds, and consequently can affect germination [12]. In this case, the effect of lead on germination seems likely to depend on the differences in seed structure of different species, particularly the differences in the struc-
tural of seed coats [12]. As is well known, seed coat protects the embryo from harmful external agents [2]. However, the seed layers have a wide variety of anatomical forms not found in any other organ or tissue.

Seed germination is promoted by enzymatic reactions regulation that activate anabolic and catabolic processes in the storage tissues and embryonic axes, respectively, in the first cycle of life in a plant. In this process, germination is prevented even if a single component of the processes is affected. A known understanding of the effect of heavy metals on plant physiology is that it causes various malnutrition problems, so the storage compounds breakdown is an important event that governs seed germination following water absorption in all plants [13], but the plants show different reactions to these trace elements. However, the effects of heavy metals, especially lead, in this process have not been well documented. To the best of our knowledge, no research has been carried out to monitor the phytotoxic effects of lead on the germination process of different sesame species.

Sesame (Sesamum indicum) is an important oilseed plant, which is an annual, summery, shrub, from the family Pedaliaceae, planted in semi-arid and arid regions of the world [14] including Turkey. It is important to understand the effects of heavy metal intake, distribution and its potential toxic effects on the germination and growth of sesame seeds.

The purpose of this study is to contribute to the understanding of the effects of lead on seed germination of different sesame genotypes. In this work, the lead uptake by different sesame genotype seedlings, and its impact on germination by analyzing morphological and biochemical properties during the first 14 days of seeds germination was presented.

MATERIALS AND METHODS

The seeds of 11 different sesame (Sesamum indicum) genotypes used in the study were sterilized in 5% NaOCl (sodium hypochlorite) solution for 5 minutes. After then, the seeds were rinsed with tap water. Four concentrations levels of Lead Nitrate Pb (NO₃)₂, 0, 100, 200 and 400 mg L⁻¹ were prepared, respectively. The study was carried out according to the Completely Randomized experimental Design (CRD) with three replications at 25 °C (±2) in the Department of Field Crops laboratory, Faculty of Agriculture, Kahramanmaras Sutcu Imam University in 2016. In the experiment, 25 seeds from each genotype were placed to germinate in 132 sterile petri dishes that two filter papers placed in each. Then, 15 ml from the all prepared concentrations were added to each petri dishes during sowing. After then, 10 ml of these solutions were added seven times two days apart for each treatment. The germinations and seedlings growth were monitored for 14 days. And then, some basic germination and seedling growth determining parameters such as germination percentage, radicle length, plumula length, seedling length, seedling fresh and dry weight and seedling vigor index were investigated. The germination percentage, after dividing the count of germinated seeds by the number of seeds, was determined by multiplying by 100. Seedling length was measured with ruler. Similarly, the length of the radicle and plumula was determined by measuring with a measuring tape after both were separated. Then, these two parts were weighed to determine the fresh weight. The samples of these two plant parts were kept at 78 °C for 24 hours and then weighed to obtain seedling dry weight. The seedling vigor index was obtained by multiplying the seedling length by the percentage of germination.

Statistical analysis. The statistical analysis for the observed treatment factors, genotype and lead nitrate concentrations were performed using anova with the Completely Randomized experimental Design procedures of the Costat v. 6.0. Duncan multiple range test was used to evaluate mean separations. Additionally, the Pearson Correlation analysis was performed to monitor the relationship among the observed parameters with IBM Spss v. 22.0.

RESULTS AND DISCUSSION

Lead is one of the most common heavy metal that does not have biological function but can cause physiological, morphological and biochemical dysfunctions in plants. Because of their genotypic differences, plants develop a broad tolerance mechanism in response to lead exposure [6]. Here, the effects of lead on germination and seedling growth of different sesame genotypes were investigated.

Germination percentage. Lead has been various inhibitory effects on seed germination percentages. It has been determined that different sesame genotypes respond differently to increased lead levels due to genotypic differences. Among the studied sesame genotypes, the most tolerant one, which had the highest germination percentage (99.67%) with no significant variation between concentration levels was Mucuni-57 (Table 1). In a similar study, Islam et al. [15] reported that at higher concentrations, lead may speed up germination of Elsholtzia argyi. As shown in Table 1, the lowest germination percentage (80.33%) was found to be Kepsut-99 with the least tolerance to lead nitrate concentrations. This may be due to the interaction of lead with protease and amylase enzymes that
cause germination inhibition [7, 16]. Lead did not show permeability through the seed membrane at the first stage of water absorption. In the second step, germination was delayed because the seed membrane has selective permeability on the lead ions [12]. It can be concluded that the above notification of different explanations and results on germination, are due to changing of the permeability of the membrane from genotype to genotype, even though, it has the same species.

Radicle length. Lead nitrate applications had a significant effect (5%) on decreasing of radicle length in the studied sesame genotypes compared to the controls. The highest radicle lengths were measured in control plots for all genotypes, while the lowest data were observed in plots which treated with fourth concentration levels. For radicle length, the highest tolerant genotype to the increasing lead nitrate levels was Arslanbey and Hatipoglu varieties with 2.90 and 3.72 cm seedling lengths, while the least sensitive one was Kepsut-99 (1.55 cm). The reduction in seedling length can be said to be due to lead exposure in plants that greatly limits the development and sprouting of seedlings [17]. Similar results were reported for *Thespesia populnea* seedling growth that decreased with lead treatments as compared to control [1]. In another previous study, which had parallel statements with the study results, was declared a steady decline in the seedling growth of *Zea mays* was observed with the increase in lead concentration [4].

Seedling fresh weight. Fresh weight of seedling shows the overall growth of any plant species. And also, the effect of lead on the accumulation of fresh and dry biomass of plant species varies according to plant species, plant varieties, plant organs and metabolic processes [4]. Seedling fresh weight of 11 sesame genotypes were subjected to different concentrations of lead nitrate, and the effects of the concentrations were found to be significant. Therefore, the genotypes and concentration levels created different duncan groups within its self. A negative correlation was observed in fresh weight of seedling with lead nitrate concentrations. The minimum seedling fresh weights of all genotypes were measured from fourth concentration (400 mg L\(^{-1}\)), while the maximum seedling fresh weights were obtained from control plots. The highest seedling fresh weight was obtained from Hatipoglu variety as 0.380g, while the lowest seedling fresh weight (0.247g) was measured from Kepsut-99 which had least tolerance to lead nitrate doses. It can be said that the different response of the varieties to the lead nitrate doses due to the genotypic structures caused the interaction between the varieties and the lead nitrate doses to be significant. Islam et al. [15] reported that at higher concentrations, lead may simultaneously induce negative effects on the hypocotyl length in *Elschoitzia argyi*.

Plumule length. According to the study results, plumule lengths of sesame genotypes were negatively affected by application of different lead concentration levels. As can be seen in Table 1, increasing of lead nitrate doses decreased the length of plumule simultaneously. The lowest plumule lengths of all genotypes were obtained from fourth doses (400 mg L\(^{-1}\)) of lead nitrate treatments, while the longest plumule lengths were obtained from control plots. The variations between the genotypes were found to be statistically significant (5%). The highest plumule length was determined from Hatipoglu variety as 1.97 cm. The shortest plumule length (0.74 cm) was measured from Kepsut-99 which had least tolerance to lead nitrate doses. It can be said that the different response of the varieties to the lead nitrate doses due to the genotypic structures caused the interaction between the varieties and the lead nitrate doses to be significant. Islam et al. [15] reported that at higher concentrations, lead may simultaneously induce negative effects on the hypocotyl length in *Elschoitzia argyi*.

Seedling dry weight. From Table 1 it is seen that genotypes react differently to lead nitrate doses. Therefore, a significant variation was observed between genotypes. Seedling dry weights of all genotypes showed a sharp decrease at 400 mg L\(^{-1}\) treatment except Köy-2, Arslanbey, Osmanli-99 and Cumhuriyet-99 compared to controls. The tolerant genotype to lead concentrations was monitored as Cumhuriyet-99 with 0.26g at 400 mg L\(^{-1}\) treatment. The lowest tolerant genotype was determined as Kepsut-99 which had no observed samples at 400 mg L\(^{-1}\) treatment. Similar but partially different results were reported by Hussain et al. [4] for *Zea mays*, were treated with different lead concentrations. It can be said that this difference is caused by the difference of plant species.
The seedling vigor index (SVI) was used as a phytotoxicity index to assess the effect of heavy metal on seedling growth [18, 19]. Seedling vigor is a measure of the damage that accumulates along with a decrease in viability, and damage is accumulated in the seeds until the seeds are not germinated and eventually die [19].

### TABLE 1

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Doses (g/lt)</th>
<th>GP (%)</th>
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<th>PL (mm)</th>
<th>SL (mm)</th>
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**GP:** Germination percentage, **RL:** Radicle length, **PL:** Plumula length, **SL:** Seedling length, **SFW:** Seedling fresh weight, **SDW:** Seedling dry weight, **SVI:** Seedling vigor index

* Means with similar letters within each column for each treatment are not significantly different (5%)
while the lowest tolerant genotype was Kepsut-99. The tolerant genotype was determined as Arslanbey, which are increased (Table 1). According to the Table 1, lead doses served compared to control, while the lead doses were increased (Table 1). According to the Table 1, the tolerant genotype was determined as Arslanbey, while the lowest tolerant genotype was Kepsut-99.

The relationships among the observed characteristics. A correlation analysis was carried out to demonstrate the effects of lead concentrations on the relationships between the properties studied. Correlations among all traits examined are thought to be significant (P <1% and P <5%) (Table 2), due to widespread and complex lead interactions occurring in different plant organs.

As can be read from Table 1, germination percentage presented significant (P<0.01) positive correlations with radicle length (r=0.283), plumula length (r=0.315), seedling length (r=0.306), seedling fresh weight (r=0.322) and seedling vigor index (r=0.337). It was found that the radicle length was adversely affected by lead concentrations, so that other investigated properties decreased in parallel with the decrease in radicle length. Therefore, it was found to have positive correlations between radicle length and all other properties examined, as plumula length (r=0.865), seedling length (r=0.976), seedling fresh weight (r=0.796), seedling dry weight (r=0.277) and seedling vigor index (r=0.977), respectively. Plumula length had significant positive correlations with all related properties, seedling length (r=0.953), seedling fresh weight (r=0.856), seedling dry weight (r=0.189) and seedling vigor index (r=0.945), respectively. Significant positive correlations were observed among seedling length with seedling fresh weight (r=0.849), seedling dry weight (r=0.249) and seedling vigor index (r=0.997). The positive correlations among seedling fresh weight with seedling dry weight (r=0.198) and seedling vigor index (r=0.840) were found to be significant. And also, seedling dry weight had a significant positive correlation with seedling vigor index (r=0.254).

**CONCLUSIONS**

The various environmental, ecological and cyclic processes in microsphere are deteriorated due to the toxic effects of the heavy metals on the microbial community. Lead is considered to be one of the most important environmental pollutants. Therefore, in the present study, it was aimed to determine the effect of lead on seed germination and growth parameters seedlings of 11 sesame (Sesamum indicum L.) genotypes. It has been shown that genotypes react differently to increased lead concentrations. It is concluded that, a significant but negative correlation was observed among lead concentrations and observed properties of all sesame genotypes. Moreover, determination of the effect of lead on germination of sesame seeds on some varieties will be able to offer preference in the selection of sesame seeds in future studies.

**REFERENCES**


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HYDROLOGICAL CONFLICTS RISK ESTIMATION IN VOJVODINA, SERBIA

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ABSTRACT

Hydrological conflicts are potential risks that may occur in water supply due to water consumption in agriculture, industrial production and human population. This problem is particularly pronounced in periods of water shortage which is a result of natural causes or the human impact on the environment. The study analyzes possible conflicts over water use and shows the risk zones of identified conflicts in the territory of the northern province of the Republic of Serbia. The indicators of load on water resources are average water consumption in households, average public consumption, industrial consumption, water consumption in livestock and water consumption for irrigation. Geographic Information System (GIS) was used for spatial display and the analysis of collected data. Based on the data about spatial distribution of potentially harmful events, risk maps have been created i.e. the zones of hydrological conflicts. Research results clearly emphasize the existence of hydrological conflicts as a result of the consumption of underground water which is normally used for irrigation purposes, as well as the fact that half of Vojvodina’s population live under the conflict of water consumption.

KEYWORDS:
Hydrological conflict, water consumption, water supply, Vojvodina

INTRODUCTION

Water is the least regulated natural resource in the world [1]. Its shortage can cause serious socio-economic consequences [2, 3, 4] and negative effects on the environment [5, 6]. Climate change and climatic extremes influence the availability of water [7] and it becomes necessary adequately allocate this resource between various users and business sectors in order to prevent damage.

Over the past years in Europe, an increasing attention has been paid to the issues of water scarcity and the impact of agricultural activities on water resources. Namely, it has been estimated that about 24% of total water is used in agriculture, with a tendency of constant growth [8], although in some parts of southern Europe (Greece) this share could reach up to 80% [9, 10, 11]. Agriculture is the largest consumer of fresh water on the global scale, about 70% of total world waters [12].

In order to supply the population and the economy with water, in Vojvodina (autonomous province in the north of the Republic of Serbia), water supply systems based on groundwater sources have been developed, due to the low availability of their own surface waters of adequate quality. Further, over 50% of irrigation water is taken from underground streams [13] and in addition to a significant amount of water flowing through surface currents in the territory of Vojvodina. Reservoirs of quality water from artesian ones are decreasing in some parts of Vojvodina, since the abstractions are higher than dynamic reserves, and the renewal cycle is relatively slow. About 65% of the estimated groundwater capacity comes from alluvials while in some parts of Vojvodina (Bačka and Banat) excess groundwater is used. The quality of groundwater is also undermined due to inadequate source protection.

Groundwater resources of the sandy terrain of the Danube and Tisa interchange can be restored only from precipitation. Due to the climatic conditions in the aftermath of the extremely dry 1970s, water resources were significantly reduced. A significant decline in groundwater resources has not improved even after extremely humid 2010. These facts indicate that natural climatic extremes can influence extremes in water supply in the territory of Vojvodina. On the other hand, natural conditions are at the same time complemented by numerous social causes that can permanently threat groundwater resources. Bearing this in mind, one should expect the emergence of hydrological conflicts between end users of groundwater resources. In order to locate potential conflict zones, it is necessary to put data on water consumption and availability of water in the spatial context. For this purpose, the problem was solved by using Geographic Information System (GIS) as a decision support system which integrates spatially referenced data into the environment [14]. By linking data on the spatial distribution of potentially harmful events and attributes that are not in relation to the position, it is possible to create risk maps - conflict zones.
MATERIALS AND METHODS

The survey covers the territory of the Autonomous Province of Vojvodina. This region extends over the northern part of the Republic of Serbia and covers an area of 21,506 km², which is 24.4% of the total territory of Serbia. It consists of three regions (Banat, Bačka and Srem), each of them possessing highland arable land, rich water resources, relatively high degree of economic and cultural development, densely populated population and demographic diversity. Out of the total area of Vojvodina, which is 2,150,600 ha, agricultural land occupies 1,747,000 ha, or 81.26%, while arable land occupies 74.6%. The most important watercourses in the territory of Vojvodina are the Danube, Sava, Tisa and Tamis rivers, which all have an international character. In addition to surface waters in the entire region of Vojvodina, under the first or freetal issued, groundwater is provided. For the purposes of irrigation of agricultural land, both underground and surface waters are used.

For the purposes of detecting conflicts related to water management in Vojvodina, the characteristic loads of water consumption as well as the thickness of the aquifer layers were identified in the zone of individual load handling. Indicators of water resource load recognition have been identified: average household water consumption, average public consumption, industrial consumption, water consumption in livestock and water consumption in agriculture for irrigation purposes. The stated consumption is calculated on the basis of the norms of consumption [15], the planned quantities prescribed by the Water Management Fund of the Republic of Serbia, which determine the required capacity of the water supply system. Norms are determined empirically, on the basis of real data on water consumption in the observed area. For the calculation of the total water consumed, data from the Republic Statistical Office on the number of inhabitants and the agricultural census were used.

Water consumption in households. The proposal for a household spending standard for the Vojvodina region is based on previous experiences and trends in consumption in the countries that are in the final phase of transition, the consumption standards applied in the countries of the European Union, as well as the specifics of Vojvodina region (climatic conditions, settlement structure, habits of the population, the condition of the built water supply network in the settlements, etc.). Water consumption in households is considered personal consumption of individual physiological, hygienic and sanitary purposes. It varies depending on the number of inhabitants in the settlement. For a further calculation of water consumption in households, a proposal of norms adopted by the Water and Water Protection Strategy in AP Vojvodina was used [16] (Table 1). For the purpose of calculating annual household water consumption, the coefficient of uneven consumption has not been applied because it refers to daily unevenness and does not affect the accumulated water consumption. The total calculated water requirements in homes for the territory Vojvodina is given in the Table 2. Water consumption for household needs is highest on the territory of municipalities: Novi Sad, Subotica, Pančevo and Zrenjanin.

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<th>Water consumption in households in Vojvodina</th>
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<tr>
<td>Total consumption (m³ per day): 136,686 83,182 41,007</td>
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<td>Total consumption (L s⁻¹): 1,582 963 475</td>
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Public water consumption. Public water consumption is the water consumption that is resulting from activities carried out in the narrower area of the settlement - maintenance of public hygiene in the settlement, fountains and public fountains, public baths and swimming pools, institutions and business facilities in the settlement, children's institutions, schools, health institutions, catering facilities, small business and crafts, trade activities, etc. Therefore, in this sector, water consumption mostly depends on the size of the settlement since the larger settlements, due to higher number of activities, have higher public and other consumption than small settlements.

At the planning stage, public and other consumption is usually determined by the percentage of household spending. The usual values range from 5% to 20% (Table 3).

Total calculated daily water consumption for public demands in the territory of Vojvodina is given in the Table 4. Water consumption for public consumption follows the water consumption in households and is highest on the territory of municipalities: Novi Sad, Subotica, Pančevo, Sombor and Vršac.

Estimated total quantity of water for industry needs in Vojvodina is 45,528 m³ per day (Table 6). Water consumption for the needs of the industry follows the spatial distribution of industry in the territory of Vojvodina.

<table>
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<tbody>
<tr>
<td>Up to 5,000</td>
<td>5% of household consumption</td>
</tr>
<tr>
<td>5,000-10,000</td>
<td>5-10% of household consumption</td>
</tr>
<tr>
<td>10,000-20,000</td>
<td>10-15% of household consumption</td>
</tr>
<tr>
<td>20,000-50,000</td>
<td>15-20% of household consumption</td>
</tr>
<tr>
<td>Over 50,000</td>
<td>20% of household consumption</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Type of settlement</th>
<th>Concentrated water consumption norms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Municipal center</td>
<td>25-50% of household consumption</td>
</tr>
<tr>
<td>All other settlements</td>
<td>10% of household consumption</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Concentrated consumption (C) m³ per day</th>
<th>Public consumption (P) m³ per day</th>
<th>Industry consumption (I=C-P) m³ per day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bačka 45,115</td>
<td>18,698</td>
<td>26,417</td>
</tr>
<tr>
<td>Banat 24,345</td>
<td>9,489</td>
<td>14,856</td>
</tr>
<tr>
<td>Srem 8,776</td>
<td>4,522</td>
<td>4,255</td>
</tr>
<tr>
<td><strong>Total (Vojvodina):</strong></td>
<td></td>
<td><strong>45,528</strong></td>
</tr>
</tbody>
</table>
Water consumption for livestock breeding. The amount of water that needs to be provided for the normal development of the production process on the farm is conditioned by many different factors. In addition to climatic conditions, the amount of consumed water depends significantly on other factors, such as: the content of dry matter in the food, the type of nutrients (protein content, salts, mineral matter), the physiological state of the animal (age, steady state, flushing, health condition), milk production in cows in lactation, water temperature for feeding, etc.

Due to the existence of this number of influences, the daily water consumption norms for livestock breeding are given at wider intervals to include all of these factors. When forecasting the water needs of the farm in Vojvodina, it is calculated with the upper limits of the specified intervals given by Water and Water Protection Strategy in AP Vojvodina. On the basis of the given normative in Table 7, the amount of water that would meet the needs for water in livestock for the territory of Vojvodina is calculated and it amounts to 38,828 m³ per day. Table 8 provides an estimate of the necessary water for livestock needs in the territory of Vojvodina. Water consumption for livestock breeding is highest on the territory of municipalities: Novi Sad, Subotica, Pančevo, Zrenjanin and Sombor.

Water consumption for irrigation. The needs of the crop plants to water depends on the phase of the vegetation growth and development of the air-conditioning and hydrologic conditions, the site of cultivation, and so on. The content of available water in the soil is highly variable. According to the Water Management Basis of the Republic of Serbia, the norms of irrigation for the most represented crops are (in the period April-September) with 80% of the security: wheat (1,750-2,100) m³ ha⁻¹, corn (3,500-4,200) m³ ha⁻¹, sugar beet (4,250-5,100) m³ ha⁻¹, alfalfa (4,450-5,300) m³ ha⁻¹, other crops (3,500-4,250) m³ ha⁻¹ and postural sowing (1,700-2,150) m³ ha⁻¹. Based on these norms of irrigation, sowing patterns, types of sources, ways of water capture, conditions of distribution of water to the system and system (losses 5-15%), the irrigation hydromodule ranges from 0.5-0.6 L s⁻¹ per ha [15].

In order to calculate water consumption for irrigation purposes, the assumption is that the irrigation norm is 0.5 L s⁻¹ per hectare and is irrigated for three months a year (June, July and August). The total estimated amount of water consumed for irrigation in Vojvodina is 226,479,927 m³ per year according to total irrigated area (58,251 ha) and daily consumption of 2,516,444 m³ (Table 9). Water consumption for irrigation is highest on the territory of municipalities: Vrbas, Zrenjanin, Bečej, B.Palanka, Kula and Apatin.

### Table 7

<table>
<thead>
<tr>
<th>Type of livestock</th>
<th>Livestock breeding water consumption norms L/pcs per day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Large livestock (cattle, horses)</td>
<td>50-60</td>
</tr>
<tr>
<td>Small livestock (goats, pigs, sheep)</td>
<td>5-10</td>
</tr>
<tr>
<td>Poultry</td>
<td>0.3-0.5</td>
</tr>
</tbody>
</table>

### Table 8

<table>
<thead>
<tr>
<th>Type of livestock</th>
<th>Livestock breeding water consumption norms m³ per day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Large livestock</td>
<td>8,117</td>
</tr>
<tr>
<td>Small livestock</td>
<td>2,435</td>
</tr>
<tr>
<td>Poultry</td>
<td>17,416</td>
</tr>
</tbody>
</table>

### Table 9

<table>
<thead>
<tr>
<th>Cons. norm (N)</th>
<th>Water consumption for irrigation in Vojvodina</th>
</tr>
</thead>
<tbody>
<tr>
<td>Irrigated land (L)</td>
<td>Consumption (C=L*N) m³ per day</td>
</tr>
<tr>
<td>ha</td>
<td>L s⁻¹</td>
</tr>
<tr>
<td>Bačka</td>
<td>35,673</td>
</tr>
<tr>
<td>Banat</td>
<td>18,923</td>
</tr>
<tr>
<td>Srem</td>
<td>3,655</td>
</tr>
<tr>
<td>Total (Vojvodina):</td>
<td>58,251</td>
</tr>
</tbody>
</table>

### Table 6

<table>
<thead>
<tr>
<th>Irrigated land (L)</th>
<th>Cons. norm (N)</th>
<th>Water consumption for irrigation in Vojvodina</th>
</tr>
</thead>
<tbody>
<tr>
<td>Irrigated land (L)</td>
<td>Consumption (C=L*N) m³ per day</td>
<td></td>
</tr>
<tr>
<td>ha</td>
<td>L s⁻¹</td>
<td></td>
</tr>
<tr>
<td>Bačka</td>
<td>35,673</td>
<td>17,837</td>
</tr>
<tr>
<td>Banat</td>
<td>18,923</td>
<td>9,461</td>
</tr>
<tr>
<td>Srem</td>
<td>3,655</td>
<td>1,827</td>
</tr>
<tr>
<td>Total (Vojvodina):</td>
<td>58,251</td>
<td>29,126</td>
</tr>
</tbody>
</table>

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RESULTS AND DISCUSSION

For the needs of households, public consumption, industry and cattle breeding, high-quality drinking water is obtained from exploitation of groundwater. For the needs of agriculture, water from surface flows is used, but due to the abandonment of the hydrosystem, the poor water quality (pollution of surface flows) and the poor economic situation in which domestic farmers are located, farmers are mostly determined for the exploitation of groundwater in the farms. This has led to a rise in water consumption from underground sources for irrigation purposes.

After indication of individual consumption by categories of water consumers it is necessary to spatially display the cumulative water consumption from underground sources and compare the consumption by categories. Such an overview is given in the Figure 1. The largest total water consumption from underground sources is on the territory of the municipalities: Zrenjanin, Bečej, Vrbas, Kula, B. Palanka. The largest water consumption from underground sources is in the irrigation segment, followed by water consumption for the needs of the population. Therefore, in territories with the largest, total water consumption observed, conflicts may arise among these users. Conflicts are possible during the vegetation period and irrigation season.
However, Vojvodina has excellent natural conditions and sewer infrastructure for land irrigation and drainage of excess water. Hydrological network of Vojvodina is made up of large river flows of Danube, Sava, Tisa and Tamiš and highly branched channel network - hydro system Danube-Tisa-Danube. The hydrological network Vojvodina in regard to available surface water which can be used for irrigation is shown in Figure 2.

Despite the exceptional water resources in Vojvodina, their utilization rate is very low. One of the most important reasons for their minimal use lies in the economic conditions for their exploitation. Only 3.6% of the area under crops are irrigated [13] indicating the urgent need to develop a strategy for the improvement of existing and construction of new irrigation systems. National and provincial funds for agricultural development should focus significant resources for procurement of equipment and systems for irrigation, while fiscal policies should encourage farms that use irrigation systems by tax exemptions.

Based on the thickness of the aquifer layers of available water intakes, we can estimate the amount of water available for exploitation and thus recognize spatial-based conflicts. In the whole region of Vojvodina, under the first or unconfined aquifer whose quality is inadequate for human consumption, there are deep groundwaters. This is the name for all groundwater under high pressure. This aquifer system is divided into the upper and lower zones, i.e. the complexes "A" and "B". In most cases, the "A" complex provides good quality water and the largest number of settlements in Vojvodina are supplied with water from this zone. The thickness of the aquifers in the waterfalls in the territory of Vojvodina is divided into 5 categories: less than 20 m, 20-35 m, 35-50 m, 50-100 m and over 100 m. According to water consumption ratio and the thickness of the aquifer, the biggest conflict is in municipalities located above the aquifer layer that is less than 20 m (Figure 3). The picture clearly shows that the municipalities are: Nova Crnja, Vršac, Alibunar, Indjija, Sremska Mitrovica, Irig and Beočin in the zone of the smallest water supplies. Of the mentioned municipalities, the conflict is especially pronounced in the municipalities of Indjija, Sremska Mitrovica and Vršac. The observed conflict zones lie predominantly on the slopes of Fruska Gora and the Vršac mountains, which are the only elevations in the Vojvodina region. These conflict zones are located above a very thin layer of drinking water and their excessive exploitation can irreversibly lower the water and cause water shortages. Also, the water supplies that are found in the next category of aquifers (from 20 to 35 m), although larger than the ones mentioned above, in spatial relation with the high consumption are also indicated by the conflict of water resources management of a particular cadastral municipality. Figure 4 shows the next level of conflict, a relatively thin aquifer layer, and a large consumption in spatial interdependence. It can be concluded that the municipalities: Subotica, Zrenjanin, Novi Sad and Pančevo in the area of small water supplies, with a high consumption.
Based on the above stated, it can be said that the territorial point of view, about half of the territory of Vojvodina has a conflict of consumption and water reserves. Viewed through the population, it can be said that:

- the number of inhabitants in the zone of accentuated conflict is 179,399;
- the number of inhabitants in the conflict zone is 729,955.

The perceived conflict arises as a result of consumption of water used for irrigation. In spite of considerable quantity of water flowing through the water flows in Vojvodina, over 50% of the irrigation water is abstracted from the groundwater flow [13]. This has a direct impact on reducing the level of underground water, which is exploited to supply the population and industry in Vojvodina, due to the inability of water sources renewal quickly enough. Climatic and hydrological conditions further worsen the current situation.

Groundwater represent virtually the only resource that ensure drinking water for the population and industry of Vojvodina. Despite this fact, neither the scope nor the quality of hydrogeological and hydrodynamic groundwater studies followed the development of water demand and creation of new water sources.

Due to excessive use of groundwater that occurred in some areas (Bačka, Banat) there is a significant drawdown of groundwater in primary aquifer complex (in some areas up to 50 m) which necessarily had to reflect on the concept of long-term supply of these zones. In the future they will have to switch to surface water usage, primarily by ceasing to use groundwater for irrigation and technological needs.

CONCLUSIONS

By manipulating spatial data in the area of interest risk maps were created and found that hydrological conflicts exist, but judging by the trends in consumption and forecasts of meteorological and hydrological conditions in the future, there will be more conflicts. Identified conflicts arise as a result of the water consumption from underground sources used for irrigation purposes. According to the research results, Vojvodina currently has 909,354 inhabitants living under the water consumption conflict, which is about half of the total population of this province.

Excessive use of water from inadequate sources can dry up water sources which has negative impacts on agriculture and irrigation conditions. If in the future irrigation in Vojvodina does not turn to surface water sources, due to the projected development in the field of irrigation, detected conflicts will intensify and the risk zones will be changed and increased.

In the future, quality groundwaters should only be used to supply the settlements and technologies that require water of the highest quality. The concept of sustainable development of water resources involves revitalization of complex regional canal systems that use transit waters, including the canal network Danube-Tisa-Danube.

The lack of quality data on relevant parameters, which should serve to analyze the regime and balance of groundwater as a basis for optimizing long-term water supply of Vojvodina, imposes the need to draw up a detailed program and project system research and monitoring of underground resources.
water in the area. The distribution of aquifers must be determined, as well as the quality of various types of water yield and the ability to recharge aquifers. Within the water management plan it is necessary to make the distribution of groundwater resources. Based on the future needs for water supply for settlements and the predetermined real groundwater resources, the needs to be drawn from surface water resources will be determined. This is a priority task of water management in addressing water supply in the future.

ACKNOWLEDGEMENTS

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REFERENCES

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THE EFFECTS OF OPTIMIZATION METHODS ON THE DETERMINATION OF TOTAL ANTIOXIDANT CAPACITY IN SOME PLANTS

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2Department of Chemistry, Faculty of Science, Istanbul Technical University, Istanbul, Turkey

ABSTRACT

Phytochemical properties and prophylaxis of medicinal plants in alternative medicine applications have been emphasized in many studies. Antioxidants are effective particularly in terminating chain reactions, which are caused by free radicals formed during metabolic activity reactions. The elution of these components is usually carried out with solvent-extraction procedure. In this study, it was aimed to measure total antioxidant capacities (TAC) of Equisetum arvense and Lycopodium clavatum plant species that grown in Turkey, using different methods with infusion, boiling and methanol extracts. It was observed that TAC of the extracts prepared by the magnetic stirrer obtained higher yields compared to other methods but there was no statistically significant difference. In temperature studies, the most efficient results for TAC values were determined at 100°C degrees and statistically significant differences were determined (p<0.001). It was observed that the amount of TAC decreased statistically after 10 minutes in the optimum time determination study (p<0.001). For this reason, extractions were carried out at 100°C for 10 minutes. Consequently, it can be said that the amount of TAC of these plant species using alternative medicine and agriculture is better with the applied optimization methods. These methods can be used in agricultural, enviroment and pharmaceu-tical active substance studies.

KEYWORDS:
Agriculture, Equisetum arvense, Lycopodium clavatum, medicinal plants, optimization, total antioxidant capacity

INTRODUCTION

According to the World Health Organization, 65-80% of the world's healthcare practices involve the use of traditional medicine (TM), often referred to as complementary and alternative medicine. Today, it is known that TM is an indispensable part of our health management. Traditional medical practices cover a wide range of therapies and implications, ranging from culture to culture and country to country. From ancient times to modern history, alternative medicines have played an important role in health care [1]. Many countries in Africa, Asia and Latin America prefer traditional herbal medicines for basic health care. Medical plants are also very important because they have therapeutic phytochemicals that enable the development of a new drug. Phytochemicals obtained from plant sources such as phenolic compounds and flavonoids have been reported to have positive health effects such as cancer prevention [2]. The high content of phenolic compounds and flavonoids in medical plants has been associated with antioxidant activities that play a role, particularly in preventing age-related oxidative stress based diseases. Antioxidant-oxidant balance is disturbed in our body by effects such as stress, aging and environmental pollution. As the amount of free radicals increases, in the body occurs damage, including aging, cell death, tissue damage and destruction of the brain vessels [3]. For this reason, antioxidant supplementation is needed exogenously. Equisetum arvense, one of the most commonly used and studied plants, is grown in wetlands in the northern hemisphere. Equisetum arvense is used as a herbal alternative drug in the treatment of kidney and bladder disorders [4]. Recent research shows that not only does the plant have high antioxidant capacity; it also has anti-inflammatory, anti-microbial, anti-cancer, sedative and anti-convulsant effects [5]. Another plant that has been investigated in medicine is Lycopodium clavatum grown in Anatolia, Europe and North America. Lycopodium clavatum is used for aneu-rysms, constipation, chronic lung and bronchial disorders, febrile diseases. They have also been shown to facilitate digestion, help in the treatment of chronic kidney disorders and stomach inflammation in several studies [6]. The search for medical plants begins with extraction procedures, an important step in the processing of bioactive components from plant material. In some studies traditional methods such as maceration and soxlet extraction have been widely used. Modern extraction methods such as microwave, ultrasound assisted extraction and supercritical fluid extraction (SFE) also play an important role in obtaining active compounds from
medical plants. With these methods, active pharmaceuti-
cal ingredients are obtained with lower cost and higher efficiency. Extraction efficiency is in-
fluenced by many factors such as solvent type, extraction method, pH, temperature and time [7, 8]. Equisetum arvense and Lycopodium clavatum plants have been used for the treatment of many disease pathogenesis due to their phenolic com-
pounds and flavonoids [9]. In this study the best conditions and apply methods commonly used by
considering the shape of people’s consumption of these plants in Turkey was examined. In the study, total antioxidant capacities of these plants were examined using different assay methods and the effect of extraction conditions on TAC was investi-
gated. It is also aimed to reveal the utility of alternative antioxidant capacity by optimizing the ex-
traction time, temperature and measurement method to maximize total antioxidant capacity yield.

MATERIALS AND METHODS

Preparation of Equisetum arvense and Ly-
copodium clavatum extracts with temperature
and time studies. Equisetum arvense plant was
gathered from Golcuk Village, Gediz (southwest-
ern), from Kütahya (latitude: 38.886°, latitude:
29.287°, altitude: 645 meters) in Turkey. Samples
were homogenized into small particles by
grinding with the aid of a shredder and stored at
room temperature in storage containers. As a result
of preliminary studies we made and with searching
some literature studies, the amount of the substance
in the plant samples was determined. In Nagai et al.
study, 5 g dry specimens were prepared [10]. For
the Lycopodium clavatum plant 300 g sample was
used in the study by Orhan et al. [11]. Equisetum
arvense 2 g and Lycopodium clavatum 7 g were
quantified to reduce substance loss. Methanol
(MeOH) extractions were prepared on a shaker in
capped ground joint Erlenmeyer. In the first step, 2 g Equisetum arvense
and 7 g Lycopodium clavatum were immersed in 250 mL of purified
water for 3 minutes, then removed and waited for 2
minutes so process carried out 5 minutes in totally.
The infusion solutions were freshly prepared every
day. During this process the plants were treated at
25 °C, 40 °C, 60 °C, 80 °C and 100 °C for the purpose
of examining the effect of temperature. While
the MeOH extracts were being prepared, the plants
were extracted on a shaker in capped ground joint
Erlenmeyer. In the first step, 2 g Equisetum arvense
and 7 g Lycopodium clavatum were mixed with 20
mL of 80% MeOH for 60 min, in the second step
20 mL of 80 mL MeOH for 45 min, lastly 20 mL of
80% MeOH for 15 min at 320 rpm on a shaker. After
all three steps, the extracts were filtered and the filtrates were combined to 50 mL with 80% MeOH solution. Measurements were made at time intervals of 5, 10, 20, 40 and 60 minutes (min) to
determine the optimum boiling time of the extrac-
tion by boiling method.

Total antioxidant capacity assignment
methods. CERAC (Cerium reduction antioxi-
dant capacity) method. 0.2-0.1 mL of the trolox
solution (1x10-3 M) was added to 1 mL of Ce (IV)
(2x10-3 M) and 7 mL of Na2SO4 (1M) mixed solu-
tions and distilled water was added to make a total
volume of 10 mL final solution. After, the reaction
was allowed to proceed for 30 minutes. Absorbance
measurements were performed at 320 nm wave-
length which maximum absorbance of Ce (IV),
against distilled water solution. Since the initial
absorbance of Ce (IV) decreased after interaction
with plant extracts the difference between the initial
and post-reaction measured absorbance of Ce (IV)
was calculated as a measure of the total amount of
antioxidants (TAC) in the plants [12]. The equation
used in the TAC calculation of plant samples in
CERAC method is shown in Eq. (1).

\[
\text{TAC (mmol trolox/g): } \left( \frac{[\text{Ce (IV)}]}{[\text{trolox}]} \right) \times \text{VF} / m \tag{1}
\]

\[
A_0: \text{The initial absorbance of Ce (IV) at 320 nm, } A_1: \text{Measured absorbance, } \text{VF: } \text{The molar absorption coefficient of trolox (mol}^{-1}\text{L cm}^{-1}, \text{VF: The final volume the absorbance is measured (mL),}
\]

\[
\text{VS: Volume sample (mL), DF: Dilution factor, VE: Last volume of extracts or infusions (mL), m: Sample weight (g)}
\]

Spectrofluorometric CERAC method. 0.2-
0.1 mL of the trolox solution (1x10-3 M) was added
to 1 mL of Ce (IV) (2x10-3 M) and 7 mL of Na2SO4
(1M) mixed solutions and distilled water was added
to make a total volume of 10 mL final solution.
After, the reaction was allowed to proceed for 30
minutes. Measurements were taken at 256 nm exci-
tation and 360 nm emission wavelength using the
fluorescence property of the reducing Ce (IV) to Ce
(III) [13]. The equation used in the TAC calculation
of plant samples in the method is shown in Eq. (2).

\[
\text{TAC (mmol trolox/g): } \left( \frac{\text{[Ce (IV)]}}{\text{[trolox]}} \right) \times \text{VF} / m \tag{2}
\]

\[
\text{VF: The final volume the absorbance is measured (mL), VS: Sample volume (mL), DF: Dilution factor, VE: Last volume of extracts or infusions (mL), m: Sample weight (g)}
\]

CUPRAC (Cu (II) ion reducing antioxidant
capacity) method. 1 mL of 1x10-2 M CuCl2 + 1
mL of 7.5x10-3 M neocuproine and 1 mL of 1 M
NH4Ac solution was added to 0.2-0.8 mL of plant
extracts and distilled water was added to give final
RESULTS AND DISCUSSION

Statistical analysis. Normal distributions of all parameters were examined by Kolmogorov-Smirnov and Shapiro-Wilk tests. The One Way ANOVA test was used for independent and normal variables. Probability values of p<0.05 were considered significant. All data analyzes were done with IBM SPSS Statistics 21 package programs.

Determination of appropriate extraction devices. Table 1 shows the calculated mmol trolox / g TAC values of Equisetum arvense plant by CERAC method. Values were expressed as mean ± standard deviation (n = 3).

<table>
<thead>
<tr>
<th>Extract</th>
<th>Magnetic stirrer</th>
<th>Shaker</th>
<th>Ultrasonic bath</th>
</tr>
</thead>
<tbody>
<tr>
<td>Equisetum</td>
<td>0.0153±0.00</td>
<td>0.0144±0.00</td>
<td>0.0140±0.00</td>
</tr>
<tr>
<td>arvense</td>
<td>2</td>
<td>.001</td>
<td>2</td>
</tr>
</tbody>
</table>

There was no statistically significant difference when extractions devices were compared (p>0.05).

However, in the analysis of the extracts prepared by magnetic stirrer, it was observed that the highest amount of TAC was obtained in terms of mmol trolox / g dry sample. For this reason, magnetic stirrer extraction system was used for further processing. Since there was no statistical difference between the methods, only one plant was studied. Some studies have shown that the evaluation and selection of extraction methods depends on the study objectives, examples and target compounds [15]. Theoretically, the optimal extraction method should be simple, safe, reproducible, inexpensive and suitable for industrial [16]. In a study made by Oniszczuk’s colleagues, it was shown that for most of the compounds analyzed, the highest yields were obtained by ultrasound-assisted extraction method [17]. In comparison between different extraction methods developed by other researchers [18], some investigators have demonstrated the superiorit of ultrasound-assisted extraction [17,19,20]. Our data showed that the magnetic stirrer extraction system provided higher efficiency and gave different results than the above mentioned literature studies.

Determination of optimum temperature by infusion method. As the some medicinal plants in agricultural areas among the people are generally used with infusion method, optimum temperature studies in this method have been carried out among the two plant species. Also, the lowest amount of TAC in the preliminary experiments is obtained from infusion method compared to other methods, this method has been chosen in order to optimize the changes in optimum temperature activity. The plant extracts prepared were analyzed by CERAC method. The results are shown in Figure 1, which the TAC values are calculated in terms of mmol trolox / g.

According to the results in Figure 1, as the temperature increased, the TAC values increased statistically significantly (p<0.001). Because the solvent environment is pure water, it was not possible to work at higher temperatures and the appropriate extraction temperature was chosen at 100 °C. These results are similar with the results of Maeda et al. [21] work in which the high temperature suggested can destroy the cell wall to release components in larger amounts, or thermal chemical reactions can produce stronger radical scavenging components. In another study, it was reported that antioxidants could be released due to heat treatment in a similar way [22]. Oniszczuk et al. showed the highest efficiency at 60°C in his study [23]. Equisetum arvense stems are rich in silicic acid, which makes them compact and rigid. This compact structure makes it difficult to diffuse the solvent into the material. For this reason, the most efficient results were obtained at a temperature of 100 ° C, as hard extraction conditions were required, likewise studies in literature [24]. Optimum temperature studies

volume of 4.1 mL. After, the reaction was allowed to proceed for 30 minutes. Absorbance measurements were made at a wavelength of 450 nm with maximum absorbance of Cu (II)–neocuproine chelating agent. This spectrophotometric method involves mixing the antioxidant solution with copper (II) chloride solution, neocuproine with alcohol solution and pH 7 ammonium acetate aqueous buffer solution, and then reading the absorbance of the resulting Cu (II)–neocuproine chelate at 450 nm [14]. The equation used in the TAC calculation of plant samples in CUPRAC method is shown in Eq. (3).

$$\text{TAC (mmol trolox/g):} \frac{((A)/\text{trolox}) \times \text{VF} \times \text{VS}}{\text{x DF} \times \text{VE} / \text{m}} \quad (3)$$

A: The absorbance measured after addition of the sample to the Cu (II) neocuproine solution, trolox: The molar absorption coefficient of trolox (mol⁻¹ L cm⁻¹), VF: The final volume the absorbance is measured (mL), VS: Sample volume (mL), DF: Dilution factor, VE: Last volume of extracts or infusions (mL), m: Sample weight (g)
are also shown in the extraction studies in different plant species in our country and in the world. In a study on Turkish black tea, the highest yield was observed in water extract of 90-95 degrees [25].

FIGURE 1
Optimum temperature graph for *Equisetum arvense* (A) and *Lycopodium clavatum* (B)
a: compared with 25°C results p <0.001 b: compared with 40°C results p <0.001 c: compared with 60°C results p <0.001 d: compared with 80°C results p <0.001.

Determination of optimal time for boiling method. Preliminary studies have shown that the best conditions for determining the optimum temperature are boiling. 2 g *Equisetum arvense* and 7 g *Lycopodium clavatum* plant sample were mixed in 250 mL distilled water at 100 °C for 5, 10, 20, 40 and 60 minutes (min) at 320 rpm in a magnetic stirrer. The obtained solutions were filtered on a black banded filter paper of Whatman and complete with pure water to give a total volume of 250 mL. Prepared plant extracts were analyzed by CERAC method. The results are shown in Figure 2, in which the TAC values are calculated in terms of mmol trolox / g.

As in results, it was observed that the amount of TAC decreased after 10 minutes statistically (p<0.001). For this reason, it was concluded that it would be sufficient to prepare the extracts by heating the sample solutions for 10 minutes at the highest yielding temperature of 100 °C. In some studies, it was observed that the higher efficiency determined as 30 minutes in different extraction methods [26, 17]. However, it can be said that the amounts of TAC in the extracts obtained in 10 minutes according to our data are more efficient than the studies mentioned in terms of time. After the optimization studies, the amount of TAC in the *Equisetum arvense* and *Lycopodium clavatum* plants was calculated by three different extraction methods and three different analysis methods.

Evaluation of total antioxidant capacity determination methods to plant samples. The total amount of antioxidant capacity of the *Equisetum arvense* and *Lycopodium clavatum* plants in terms of mmol trolox / g dry sample is shown respectively in Table 2 and Table 3 with three different extraction methods and three different analysis methods. The values were expressed as mean ± standard deviation (n = 3).

Due to the polar structures of polyphenols in plant species, maceration extracts with 80% MeOH solubility gave statistically significant results with higher yields than the other methods (p<0.001). For the *Lycopodium clavatum* plant, values of 11.3 ± 1.64, 44.1 ± 5.94, 26.6 ± 0.70 and 30.3 ± 0.15 were obtained respectively from the extracts of petrolether, chloroform, ethanol and methanol according to the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method [11]. According to the results of the study, the efficacy of extracts made with methanol solvent on antioxidant activity is similar to our data. When the analysis methods were compared, it was observed that the spectrofluorometric data were obtained higher yield than the spectrophotometric methods. It can be said that the more sensitive and specific of the fluorometric methods is because of the fact that Ce (IV) used in methods and the
antioxidants in the medicinal plants do not show fluorescence characteristic in the working wavelength, which is more advantageous than the other methods. In the literature, the total antioxidant capacity of Equisetum arvense plant was reported as 39 ± 4 μmol TEAC μmol g⁻¹ dry plant weight and is consistent with the results in this study [27]. At the same time, the highest values were obtained with the spectrofluorimetric CERAC method.

**CONCLUSION**

In conclusion, plant extracts are known to have a mixed of structurally different components. For this reason, the most appropriate research methods should be selected for the most effective biological activity. Extraction procedures, temperature, time and measurement methods of plant materials can affect the active components of plants both quantitatively and qualitatively. Therefore, all the optimization studies in medicinal plant research are equally important. In this study, Equisetum arvense and Lycopodium clavatum plant species TAC quantities were determined by different methods to be used in alternative medicine. It was observed that the magnetic stirrer provided higher efficiency than the other methods in the extraction system studies, but no statistically significant difference was found. In the optimum temperature and time studies, it was observed that 100 °C temperature in the infusion method and 10 minutes in the boiling method gave the highest amount of TAC. When the extraction methods were examined with three different analysis methods for both plant species, the highest TAC values were obtained from the maceration extracts with 80% MeOH solvent for CERAC, CERAC and CUPRAC methods. According to the measurement methods of both plants, the lowest amount of TAC was observed in the infusion method. The highest TAC amount was obtained in the maceration extracts obtained with the 80% MeOH solvent in the magnetic stirrer at 100 °C and 10 min time of the spectrofluorimetric CERAC analysis method.

**REFERENCES**


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CYANOBACTERIAL CELLS AND TOXIN MICROCYSTIN-LR REMOVAL BY SODIUM HYDROXIDE-MODIFIED SHRIMP SHELL ADSORBENT

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ABSTRACT

Cyanobacterial blooms have become a serious environmental threat throughout the world. In addition, many cyanobacteria can produce microcystins (MCs), which can threaten human and ecosystem health. Therefore, the removal of Microcystis aeruginosa cells and MCs is essential. In the present work, a modified shrimp shell adsorbent was developed for the removal of M. aeruginosa cells and MCs. The influencing factors of the modified adsorbent on M. aeruginosa removal, including the sodium hydroxide amount, the reaction time and the reaction temperature, were investigated. Under optimal conditions, the removal efficiency of the modified adsorbent toward M. aeruginosa cells was 79.73%, and the adsorbing capacity of the modified adsorbent toward MCs was 100 μg/g. Therefore, the modified shrimp shell adsorbent is an effective technology and has the potential to remove M. aeruginosa cells and MCs from surface water.

KEYWORDS:
Microcystis aeruginosa, microcystin, sodium hydroxide, modified shrimp shell, adsorbent

INTRODUCTION

The harmful effects on fish, birds, mammals, and water resources caused by frequent and intensive cyanobacterial blooms lead to large economic losses [1], which have been reported worldwide [2-7]. Microcystins (MCs) generated by Microcystis aeruginosa can produce toxins that damage the nervous system or liver; hence, these MCs are harmful to animal and human health [8]. The literature contains a multitude of processes for MCs removal, including adsorption and degradation [9-12]. Nevertheless, most of these studies have focused on algal cell removal or MC removal, but reports of the simultaneous removal of cyanobacterial cells and MCs are rare. Therefore, establishing how to control and eliminate cyanobacterial blooms has become an urgent ecological issue that needs to be solved.

Shrimp shells can be used to prepare chitin or chitosan for use as flocculants or adsorbents [13-16]; however, the preparation process is complicated. Shrimp shells are an attractive and inexpensive alternative for the adsorption removal of cyanobacterial blooms. In the present study, a new modified adsorbent was prepared using sodium hydroxide-modified shrimp shells, and the preparation method and removal characteristics of the modified shrimp shells on M. aeruginosa cells and MCs were also investigated.

MATERIALS AND METHODS

Algal culture. Axenic unicellular M. aeruginosa was obtained from the Culture Collection of Algae at the Institute of Hydrobiology, Chinese Academy of Sciences. The algae were cultured in sterilized BG11 medium (pH 7.4) at 25 °C under a light intensity of 2500 lux with a 12:12 h light: dark cycle. The algae were cultured for 4 days to the exponential growth phase at a density of 10⁶ cells/mL and were then used to assay the adsorption properties of the modified shrimp shells adsorbent. The growth medium for all cultures was BG11 [17].

Preparation of modified shrimp shells. Shrimp shells were obtained from the supermarket of Pingdingshan in Henan Province, China. The shells were washed with tap water and then by deionized water to remove debris, after which they were then dried on trays in an oven at 60 °C for 4 h. After drying, the shells were crushed and then sieved using an 80-mesh screen.

The specific preparation process of sodium hydroxide-modified shrimp shells is as follows. First, the sodium hydroxide solution was prepared. Then, 15 g of pretreated shrimp shells was slowly added to the solution, and 200 mL of the mixed suspension was slowly stirred with a magnetic stirrer for 0.5-1.5 h. In the end, the suspension was filtrated with qualitative filter paper (10-15 μm);
the modified shrimp shells were washed to a neutral pH with distilled water and then dried for 4 h in an oven at 65 °C to obtain the final dried modified shrimp shell adsorbent.

**Removal of M. aeruginosa.** The ability of the sodium hydroxide-modified shrimp shells to remove HABs was tested using *M. aeruginosa*. Sodium hydroxide-modified shrimp shells were added to 50 mL of algal culture in a 100-mL beaker and left to stand for 1.5 h. In the control groups, sodium hydroxide-modified shrimp shells were not added. At the end of the settling period, a sample was collected 2 cm below the surface for analysis.

**Analysis methods for the concentration of chlorophyll-a.** The concentration of chlorophyll-a was measured as an indicator of the change in the concentration of *M. aeruginosa* cells during the adsorption experiment. The chlorophyll-a concentration was determined using standard methods [18].

In every sample, the removal of algae (r, %) based on the chlorophyll-a concentration was determined after a 1.5 h exposure by the following formula:

\[ r = \frac{T_1 - T_2}{T_1} \times 100\% \] (1)

where *T*₁ and *T*₂ are the chlorophyll-a concentration after adsorption and the control, respectively.

**Removal and measure of Microcystin.** The MC solution was prepared using a standard substance (purchased from China Standard Material Net) with a concentration of 1 mg/L. Next, 0.5 g of sodium hydroxide-modified shrimp shells were added to 50 mL of the MC solution in a 100-mL beaker and left to stand for 1.5 h. At the end of the settling period, the solution was filtrated with qualitative filter paper (10-15 μm) and a glass fiber filter membrane (0.45 μm). The filtrate was used to measure the MC concentration after adsorption. The content of MCs was determined using high performance liquid chromatography (Thermo Fisher u3000).

**Data analysis.** All statistical analyses were performed using SPSS 19.0 and Origin 8.0 software.

**RESULTS AND DISCUSSION**

**Effect of sodium hydroxide addition.** To explore the influence of sodium hydroxide on *M. aeruginosa* cell removal by the modified shrimp shell adsorbent, five different amounts of sodium hydroxide were evaluated. As shown in Fig. 1, the *M. aeruginosa* cell removal efficiency increased rapidly from 36.72% to 62.50% when the amount of sodium hydroxide increased from 2 g to 5 g, respectively. However, the *M. aeruginosa* cell removal efficiency decreased when the amount of sodium hydroxide further increased. As such, 5 g was chosen in subsequent experiments.

![Figure 1](image1.png)

**FIGURE 1**

Effect of the sodium hydroxide addition amount on *M. aeruginosa* cell removal efficiency

![Figure 2](image2.png)

**FIGURE 2**

Time of modified reaction on *M. aeruginosa* cell removal

Sodium hydroxide as a modifier has been reported [19-21], which can enhance the adsorption effect. Feng et al. [19] found that the surface morphology of orange peel is different from that of modified orange peel by scanning electron micrographs. After being treated with sodium hydroxide, modified orange peel has a more irregular and porous structure than orange peel, and therefore more specific surface area. This surface characteristic will substantiate the higher adsorption capacity.
Effect of modified reaction time. The influence of the modified reaction time on the *M. aeruginosa* cell removal is presented in Fig. 2. The *M. aeruginosa* cell removal efficiency increased markedly with increasing reaction time. We found that the removal efficiency increased 54.55% when the reaction time increased from 0.5 h to 1.5 h, and then, after a plateau, the removal rate dropped. Hence, the optimal reaction time is 1.5 h.

Effect of modified reaction temperature. Fig. 3 presents the *M. aeruginosa* cell removal efficiencies using modified shrimp shells at different modification reaction temperatures. We can see that the removal efficiencies first increased and then decreased. The maximum value of the removal efficiency was 70.00% when the modification reaction temperature was 60 °C. Hence, a modification reaction temperature of 60 °C was chosen for subsequent experiments.

4. When the addition of modified shrimp shell was 0.4 g, the removal efficiency was the lowest; the removal rate first increased and then decreased with increasing amounts of modified shrimp cell. The *M. aeruginosa* cell removal efficiency using modified shrimp shells at 1.0 g was 79.73%, representing the highest efficiency and the optimal amount of modified shrimp shell.

Effect of sodium hydroxide-modified shrimp shells on MC removal. The result shows that 46.38% of MCs was removed in a 1 mg/L MC solution with a 10 g/L concentration of modified shrimp shells. That is, 0.05 mg of MCs was absorbed on 0.5 g of modified shrimp shells. Compared to the results reported in the literature [22], the adsorbing capacity of the modified flocculant was significantly higher than that of immobilized Fe (III) in the soil (52.8 μg/g).

In recent years, there has been considerable interest in the use of biological materials including agricultural by-products and residues as adsorbents to remove cyanobacterial blooms from water body by adsorption [23]. The waste generated during the industrial processing of shrimp is approximately 40-50% of the total weight of the shrimp [24]. Its improper disposal causes serious environmental problems [25]. In fact, numerous high-value by-products, such as chitin, could be recovered from shrimp wastes [26]. Fabbricino and Pontoni [27] found that the removal rates of textile dye obtained using shrimp shells are higher than those using the same dose of pure chitin due to the increased adsorption capacity of carbohydrate fibers interlaced with proteins. In the present work, sodium hydroxide was employed for shrimp shell modification, and the process is simpler than previously demonstrated methods. Although the algae removal capacity of shrimp shells was lower than that of natural and chemical sorbents, using shrimp shells as a sorbent is less expensive and could have increased benefits for shrimp waste recycling.

**CONCLUSIONS**

A new modified adsorbent was designed for the removal of *M. aeruginosa* cells and MCs and resulted in high removal efficiency. The optimum preparing conditions were also obtained. The preparation process comprises the following steps: 1) 5 g of sodium hydroxide solution was dissolved in 200 mL of ultrapure water; 2) 15 g of pretreated shrimp shells was slowly added to the solution, after which the mixed suspension was slowly stirred with a magnetic stirrer; and 3) the suspension was filtered with qualitative filter paper (10-15 μm), after which the modified shrimp shell was washed with distilled water and then dried for 4 h in an oven at 65 °C to obtain the final dried modified shrimp shell adsor-
bent.

Under the optimal conditions, the *M. aeruginosa* cell and MC removal efficiencies of the modified shrimp shell were 79.73% and 46.38%, respectively. The equilibrium time for the removal of *M. aeruginosa* cells was 1.5 h. Our research has shown that the use of modified shrimp shells for the removal of *M. aeruginosa* cells and MCs is viable.

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REFERENCES


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DETERMINATION OF GENOTOXIC EFFECTS OF SOME FOOD ADDITIVES WITH THE HELP OF CBMN TECHNIQUE

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ABSTRACT

The genotoxic effects of ascorbic acid, benzoic acid, citric acid, and sorbic acid have been searched in vitro with CBMN technique in human peripheral blood lymphocytes. The purpose of this study was investigated micronuclei, nucleoplasmic bridges and nuclear buds formations, which were caused by test materials in lymphocyte cells of which preparation was made, have been analyzed and cytokinesis-block proliferation index values have been calculated. Lymphocyte cultures have been treated in 24 and 48 hours period with 1000, 250 and 100 µg/ml material doses of ascorbic acid, benzoic acid, citric acid and sorbic acid. According to the results that have been obtained from CBMN, doses of 1000 µg/ml of benzolic acid and citric acid and 1000 and 500 µg/ml doses of sorbic acid are cytotoxic effect. All doses of ascorbic acid have been observed no cytotoxic and genotoxic effect. 500 and 250 µg/ml concentrations of citric acid, benzoic acid and 250 µg/ml concentrations of sorbic acid induced micronucleus formation statistically significantly at 24 h and 48 h, and 100 µg/ml concentrations of citric acid-induced micronucleus formation statistically significantly at 48 h, when compared with solvent control values. Ascorbic acid, benzoic acid, citric acid, and sorbic acid decreased the cytokinesis-block proliferation index depending on the increase in concentration; however, this reduction in cytokinesis-block proliferation index values was not statistically significant. Also, it has been reached to the inference that nuclear buds and nucleoplasmic bridges formation values were found to be not statistically important. It is concluded that high dose of benzoic acid, citric acid and sorbic acid may indicate cytotoxic and genotoxic effect in vitro human blood lymphocytes when they are taken as food additives.

KEYWORDS:
Ascorbic acid, Benzoic acid, Citric acid, Sorbic acid, Genotoxicity, Cytotoxicity

INTRODUCTION

Food additives are one of the most vital functions of food production. They are intentionally added to food products because of numerous reasons. Additives provide use in a variety of fields, including the technological aim in the manufacture, treatment, processing, preparation, packaging, transfer or storage of foods [1]. Besides, other various classes on additives named antimicrobials and antioxidants, emulsifiers, preservatives, stabilizers, preservatives, sweeteners, waxes, colors, waxes, gums, and several indirect additives are covered [2].

Ascorbic acid, benzoic acid, citric acid, and sorbic acid are used as additives in food products. Ascorbic acid (E-300), generally known as vitamin C, has a very important role in the food formulation that acts as a nutrient and nutritional and functional antioxidant. This vitamin also is a high oxygen scavenger substance vitamin C used in various foodstuffs. Due to its high oxidation potential, it renews oxidized phenolic oxidants and tocopherols. Ascorbic acid is especially important for stabilizing liquids and oils, but can also be used in other matrices [3, 4]. EFSA has collected a scientific opinion on ascorbic acid and emphasized that it is not a risk to its consumption not defining an ADI [5]. Several in vitro and in vivo research have shown that ascorbic acid has the antioxidant and anti-mutagenic effect [6-8]. Despite the anticlastogenic and antimutagenic properties of vitamin C, this vitamin has been shown to have clastogenic and mutagenic effects. Shamberger [9] has demonstrated the genotoxic effects of vitamin C in various test systems. Vitamin C has a mutagenic activity that causes DNA strand breakage and chromosome aberration, which increases the sister chromatid changes the frequency in cells and increases the number of somatic mutations. In contrast, Vitamin C is not an active mutagen. Oxyradicals, which occur during vitamin oxidation, cause toxic effects of vitamin C in vitro. Vitamin C did not show genotoxicity in vivo experiments. The mutagenic effects of this vitamin have been demonstrated in all in vitro tests. The results of in vitro research showed that vitamin C can be cytotoxic and mutagenic [9]. Ascorbate
provides a dose-dependent increase in sister-chromatid changes in human lymphocytes [10]. Anderson et al. [11] state that the effects of antioxidants on DNA damage caused by oxygen radicals in human lymphocytes were investigated using COMET assay. Antioxidant vitamin C has created a response by increasing dose-dependent DNA damage [11]. Antunes and Takahashi et al. [12] showed that ascorbic acid at 1000 μg/ml concentration produced clastogenic effects in human lymphocyte cultures [12]. High concentrations of vitamin C have a genotoxic effect, such as sister chromatid changes [13] and chromosome aberrations [14] induced in Chinese hamster ovary cells. The separated lymphocytes in vitro are exposed to large doses of vitamin C than 200 μM, did not provide protection, but stimulated strand breakage [15].

Benzoic acid (E-210) is added range between 150 and 1,000 mg/kg, like as, syrup, juice, pickle, margarine, ketchup, biscuit, waffle, cake and cream to protect from yeast, mold, and bacteria, widely used as an antimicrobial agent in many food products. Although the epidemiological studies of food additives are important in assessing the toxicological risks for humans, it is difficult to do so since exposure to these food additives cannot be accurately assessed. Therefore, risk evaluation is widely dependent on laboratory toxicity studies [16, 17]. Mutagenicity of benzoic acid and its salts with different test systems were studied. Although positive results were found in Drosophila melanogaster [18], human peripheral blood lymphocytes [19] and A. sativum root tip cells [20], negative results were found in Salmonella/microsomal test, Chinese hamster fibroblast cell line and in brain, stomach, colon, bladder, lung, liver, and bone marrow cells of mice [16].

Citric acid (E-330) is frequently preferred in the preparation of soft drinks, wines and frozen fruits; cheese and other various dairy products; sweets, jams, candies, gelatinous food products; canned seafood; Additionally, citric acid is used with the artificial aromas of dry compound materials such as tablets and powder, it is used as an antioxidant and acidifier, pH regulator, protective buffer, emulsifier and stabilizer in various food products to prevent the chemical in oils and oils [20].

Several studies have reported genotoxicity of citric acid. In some test systems, the genotoxicity of citric acid was investigated. Some researchers have demonstrated the genotoxic-cytotoxic effect of citric acid; human dental cells [21], Tinca tinca erythrocytes [22], Allium cepa root tip cells [20], Allium sativum root tip cells [23] and cultured human peripheral lymphocytes [24]. In contrast, Ishidate et al. [25] suggested that citric acid has a negative effect on Salmonella microsome test and Chinese hamster ovary cells [25].

Sorbic acid (E-200) is generally used in cheeses, bakery products, fresh products, wines, cold meat, and shellfish. Sorbic acid is used to protect meat due to its natural antibiotic properties. Due to its antifungal properties, sorbic acid is also used in canned products such as prunes, maraschino cherries, figs, prepared salad, and pickles. Sorbic acid at concentrations up to 0.2% is used as a preservative. Many studies have been conducted on the carcinogenicity and genotoxicity of various food additives in mice, rats, plants, and human peripheral blood lymphocytes [26, 27]. Studies with sorbic acid have shown that this additive has potential genotoxic and mutagenic effects. Sorbic acid was increased micronuclei and sister chromatid exchanges formations in bone marrow cells of mice [27]. Similar results for the harmful effects of sorbic acid salts potassium sorbate were obtained in vitro in human lymphocytes [1, 28]. When the genotoxic effect of sorbic acid and sodium and potassium was evaluated, it was found that sorbic acid was less genotoxic than sodium sorbate [29, 30]. Another study has shown that; potassium salt and sorbic acid were not genotoxic in vivo and in vitro [31]. Sorbic acid and potassium salts, using the Comet test has been seen to cause DNA damage in many organs [16]. No chromosomal damage was observed in bone marrow cells of mice given 15 mg of sorbic acid/kg bw through the stomach tube for 30 days [32]. Sorbic acid, potassium sorbate, and a fresh sodium sorbate solution were evaluated using micronucleus in SHE fibroblast cells, and SHE cell transformation tests showed negative genotoxicity [33].

MNIs are derived from chromosome fragments or whole chromosomes, except for the main nucleus during cell division; Thus, the formation of the micronuclei shows a clastogenic (causing chromosome aberrations) or a eugenic (causing chromosome number aberrations) activity of the analyzed mutagen [34]. CBMN technique may provide the measurement of genotoxicity and cytotoxicity such as chromosome loss, chromosome breakdown, cell division inhibition, chromosome rearrangement (nucleoplasmic bridges), necrosis and apoptosis using simple morphological criteria [35].

CBPI is used as a measure of cytotoxicity and/or inhibition cyto-B-blocked cell proliferation, resulting in the large extent of the ratio of the divided cells to undivided cells. Micronucleus, which is among the available cytogenetic techniques, is considered to be an indicator of genetic toxicology and the decrease in CBPI is accepted to be an indicator of cytostatic or cytotoxic effect. The MN test detects clastogenic and aneugenic effects and the simplicity and sensitivity of this test has been very promising in genetic damage studies. In the current study, we aimed that micronuclei, nucleoplasmic bridges and nuclear buds formations, which were caused by food additives such as ascorbic acid, benzoic acid, citric acid, and sorbic acid in lymphocyte cells of which preparation was made, have been analyzed and cytokinesis-block proliferation
index (CBPI) values have been evaluated.

**MATERIALS AND METHODS**

**Chemicals.** Food additives such as ascorbic acid, benzoic acid, citric acid, and sorbic acid were used as test substances. These food additives were supplied from Sigma–Aldrich. The chemical formula of ascorbic acid (CAS no.: 50-81-7) is C6H8O6 and its molecular weight is 176.12 g / mol. The chemical formula of benzoic acid (CAS no.: 65-85-0) is C6H5COOH, its molecular weight is 122.12 g / mol. The chemical formula of citric acid (CAS no.: 77-92-9) is C6H8O7 and its molecular weight is 192.13 g / mol. The chemical formula of sorbic acid (CAS no.: 110-44-1) is C6H5COOH, its molecular weight is 112.13 g / mol.

Cytochalasin B (Cyt-B, Cas. No. 14930-96-2) were ensured from Sigma. DMSO (Cas. No: 67-68-5), were obtained from Applichem. Mitomycin-C (MMC, Cas. No. 50-07-7) was obtained from Sigma. Other chemicals used were Chromosome Medium B (Biochrom 5025), Giemsa (Fluka 48900) (CAS-No: 51811-82-6), KCl (0.4%), the fixative (1:3 methanol/glacial acetic acid).

**Collection of blood samples, lymphocyte cultures, and treatments.** CBMN tests were performed by obtaining blood samples from two individuals (one male and one female) between 20-30 years of age. In order to ensure the reliability of the experiment, it is acceptable to select individuals who do not have alcohol and drug intake, non-smoker, no ionizing radiation exposure, no new history of viral infection and no medical treatment. It was taken from peripheral venous blood donors and rapidly transferred to tubes containing sodium heparin as the anticoagulant and was processed in after collection.

The water solubility or insolubility of the test substance used in the study were tested. In order to achieve the desired concentrations, ascorbic acid and citric acid were dissolved in distilled water and benzoic acid and sorbic acid were dissolved in DMSO. The cultures were treated with ascorbic acid, benzoic acid, citric acid, and sorbic acid at doses 1000, 500, 250 and 100 µg/ml. An untreated culture, a negative control: DMSO for benzoic acid and sorbic acid were dissolved in distilled water and citric acid and sterile distilled water for ascorbic acid and citric acid, a positive control (mitomycin C) and four cultures treated with different concentrations of this food additives were applied to each donor. The cultures were kept under the same conditions.

**Cytokinesis-block micronucleus assay.** A comprehensive system for measuring DNA damage as cytostasis and cytotoxicity is a CBMN test. DNA damage status is scored mainly in once-divided binucleated cells and includes nucleoplasmic bridges, nuclear buds and micronucleus. Cytostatic potency is measured via the proportion of mono-, bi- and multinucleated cells and cytotoxicity via necrotic and/or apoptotic cell ratios [36-38].

The CBMN test has been one of the standard cytogenetic tests for genetic toxicology testing in human and mammalian cells, due to its reliability and good reproducibility [38, 39]. Micronucleus assay was generated as described by Fenech [35].

Blood taken from healthy male and female individuals between 20-30 years of age, 6 drops (0.2 ml), was added to 2.5 ml chromosome medium B under sterile conditions. The cultures were incubated in incubators at 37 °C for 72 hours. The cells were treated with ascorbic acid, benzoic acid, citric acid, and sorbic acid at 1000, 500, 250 and 100 µg/ml concentrations for 24 h and 48 h. Each experiment also had a solvent control, DMSO (20 µg/ml) as a negative control and Mitomycin C (0.3 µg/ml) as positive controls were also added. Cytochalasin B (Cyt-B) at a concentration of 6 µg/ml was added to each tube at after 44 hours from the beginning of the culture period. Then the cells were harvested after 72h treated with a hypotonic solution (0.4% KCl) and fixed three times with a fixative (methanol/glacial acetic acid). The slides were air dried an stained with 5 % Giemsa. The frequency of nuclear buds, nucleoplasmic bridges and micronucleus were determined by analyzing 1000 for each treatment. Cytokinesis-block proliferation index (CBPI) was calculated as follows: \((B + 2P)/(M + B + P)\), where \(M\), \(B\) and \(P\) are the numbers of cells that have not yet entered the first mitosis (\(M\), mononucleated), and cells that have divided once (\(B\), binucleated) and twice (\(P\), multinucleated), respectively. \((M + B + P)\) represents a total of at least 1000 cells scored.

**Statistical analysis.** In the evaluation of the CBMN test, The Statistical Package for Social Sciences for Windows (SPSS) was used for statistical analysis and data were evaluated using one-way ANOVA followed by the Dunnett test. The statistical differences between the nucleoplasmic bridges, nuclear buds and micronucleus of the treated cells and the nucleoplasmic bridges, nuclear buds and micronucleus of the solvent controls were calculated with Dunnett test in ANOVA. Differences between two means were considered statistically significant at \(p<0.05\) and this difference was symbolized by asterisks (*). The results were expressed as mean ± SD (standard deviation).

**RESULTS**

The number of micronuclei and cytokinesis-block proliferation index in cytokinesis-blocked lymphocytes treated with ascorbic acid is shown in
TABLE 1
The frequency of micronucleus and cytokinesis-block proliferation index of cultured human lymphocytes treated with ascorbic acid

<table>
<thead>
<tr>
<th>Test substances</th>
<th>Treatment time (h)</th>
<th>Concentration (µg/ml)</th>
<th>MN ± SD</th>
<th>CBPI ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negeative control</td>
<td>24</td>
<td>0.00</td>
<td>4.0 ± 0.00</td>
<td>0.70 ± 0.02</td>
</tr>
<tr>
<td>Positive control (MMC)</td>
<td>24</td>
<td>0.3</td>
<td>23.0 ± 1.41***</td>
<td>0.27 ± 0.01***</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>24</td>
<td>1000</td>
<td>6.5 ± 0.70</td>
<td>0.56 ± 0.08</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>500</td>
<td>5.0 ± 1.41</td>
<td>0.59 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>250</td>
<td>4.0 ± 1.41</td>
<td>0.68 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>100</td>
<td>3.5 ± 0.70</td>
<td>0.83 ± 0.04</td>
</tr>
<tr>
<td>Negeative control</td>
<td>48</td>
<td>0.00</td>
<td>5.5 ± 0.70</td>
<td>0.67 ± 0.04</td>
</tr>
<tr>
<td>Positive control (MMC)</td>
<td>48</td>
<td>0.3</td>
<td>35.0 ± 2.82***</td>
<td>0.22 ± 0.02***</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>48</td>
<td>1000</td>
<td>7.0 ± 2.82</td>
<td>0.62 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>500</td>
<td>6.5 ± 0.70</td>
<td>0.64 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>250</td>
<td>6.0 ± 1.41</td>
<td>0.72 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>100</td>
<td>5.0 ± 1.41</td>
<td>0.77 ± 0.04</td>
</tr>
</tbody>
</table>

MMC: Mitomycin C; CBPI: Cytokinesis-block proliferation index; MN: Micronucleus; ± SD: Standard Deviation.
*Significantly different from the control p<0.05 (t-test)
**Significantly different from the control p< 0.01 (t-test)
***Significantly different from the control p<0.001 (t-test)

Table 1 and the number of nuclear buds and nucleoplasmic bridges in cytokinesis-blocked lymphocytes treated with ascorbic acid is shown in Table 5.

As shown in data (Table 1), the ascorbic acid data obtained as an MN number result of 24 and 48-hour treatment with MMC used as a positive control were found to be significant compared to negative control data (p<0.001). In the ascorbic acid treated lymphocytes, the frequency of micronucleus was found to be close to the control group and increased due to the increase in dose-depending, but the differences were not statistically significant. CBPI has been used as a measure of cytotoxicity and/or inhibition of cyt-B-blocked cell proliferation, largely expressing the ratio of divided cells to undivided cells. We observed that CBPI is significantly reduced in high concentrations of ascorbic acid-treated lymphocytes. According to ascorbic acid data (Table 1), CBPI increased dose-dependent in lymphocytes treated with ascorbic acid in all concentrations at 24 and 48 h treatment periods. None of the applied concentrations of periods was statistically significant from the negative control group. However, the positive control values were statistically significant in both treatment periods. As shown (Table 5), at 24 and 48 h of ascorbic acid the formation of nuclear buds and nucleoplasmic bridges were found close to the negative control, while it was found to be high in positive control. Nuclear buds and nucleoplasmic bridges number were found to be not statistically important. Nuclear buds and nucleoplasmic bridges formation number with all concentration of ascorbic acid obtained as a result of 24 and 48 h treatment with MMC used as a positive control were found to be significant compared to negative control value (p<0.01).

Table 2 shows the number of micronuclei and cytokinesis-block proliferation index, and table 5 shows the number of nuclear buds and nucleoplasmic bridges in cytokinesis-blocked lymphocytes treated with benzoic acid. As shown in benzoic acid data Table 2, benzoic acid was examined for genotoxic effect in healthy lymphocyte cultures. To investigate the effects of benzoic acid on micronucleus in human lymphocytes, human lymphocytes were treated with concentrations of 1000, 500, 250
and 100 μg/ml of this food additive for the 24 and 48-h treatment periods. The effects of benzoic acid on the formation of micronuclei in human peripheral blood lymphocytes are shown in Table 2. High concentrations of benzoic acid (1000 μg/ml) caused a cytotoxic effect on human lymphocytes. Our results showed that; compared to the negative control, benzoic acid produced significant micronuclei formation on peripheral lymphocytes at concentrations of 500 and 250 μg/ml in all treatment periods. Benzoic acid-induced micronucleus frequency was found to be statistically significant at doses of 500 and 250 μg/ml at 24 (p <0.01) and 48 (p <0.01). As seen in Table 2, CBPI was increased in all doses of benzoic acid-treated lymphocytes in a dose-dependent manner during treatment periods of 24 and 48 h. None of the applied concentrations of both was statistically significant from the negative control group. However, the positive control values were statistically significant in both treatment periods. Nuclear buds and nucleoplasmic bridges formation number with the concentration of benzoic acid obtained as a result of 24 and 48 h treatment with MMC used as a were found to be significant compared to negative control data (p<0.01). Nuclear buds and nucleoplasmic bridges values of benzoic acid were found to be not statistically important at 24 and 48 h.

Table 3 shows the micronucleus and cytokinesis-block proliferation index number and Table 5 shows the number of nuclear buds and nucleoplasmic bridges in cytokinesis-blocked lymphocytes treated with citric acid. The results showed that the exposed cells to 500 and 250 μg/ml concentrations of citric acid at 24-h and the exposed cells 500, 250, 100 μg/ml concentrations of citric acid at 48-h induced MN formation statistically significant, compared to solvent control values (Table 3). 100 μg/ml concentrations of citric acid did no induced MN formation statistical significantly when compared with solvent control values at 24 h. This micronucleus induction was performed in 24 h depending on concentrations. High concentrations (1000 μg/ml) of the citric acid-induced cytotoxic effects in human lymphocytes at 24 and 48 h. As shown in citric acid data (Table 3), obtained as a result of 24 and 48 h treatment with MMC used as a positive control were be significant when compared to negative control data (p<0.05). On the other hand, MMC used as a positive control caused an in the micronucleus number (P<0.001) compared with solvent control for both treatment periods. We also evaluated the CBPI for cytotoxicity of citric acid in human lymphocytes and these values are summarized in Table 3. According to the CBPI result, the citric acid reduced the number CBPI, but this decrease was not statistically significant. 1000 μg/ml dose of this additive was toxic and dividing cells were observed. Nuclear buds and nucleoplasmic number with at all concentration of citric acid obtained as a result of 24 and 48 h treatment with MMC used as a positive control were found to be significant by comparing with negative control data (p<0.01). Nuclear buds and nucleoplasmic bridges values of citric found to be not statistically important at 24 and 48 h compared to solvent control values.

Table 4 states micronuclei and cytokinesis-block proliferation index number and table 5 states the number of nuclear buds and nucleoplasmic bridges in cytokinesis-blocked lymphocytes treated with sorbic acid. The data obtained for the micronucleus test after 24 and 48 h of sorbic acid treatment in human blood peripheral lymphocyte cultures are as follows; sorbic acid has cytogenetic activity, induced dose-dependent micronucleus frequency, showed statistically significant differences from the control group, excluding 100 μg/ml dose at 24 and 48 hours (p> 0.05). The increase in the number of micronucleates indicates that sorbic

<table>
<thead>
<tr>
<th>Test substances</th>
<th>Treatment time (h)</th>
<th>Concentration (μg/ml)</th>
<th>MN ± SD</th>
<th>CBPI ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control</td>
<td>24</td>
<td>0.00</td>
<td>4.0 ± 0.00</td>
<td>0.70 ± 0.02</td>
</tr>
<tr>
<td>Positive control (MMC)</td>
<td>24</td>
<td>0.3</td>
<td>23.0 ± 1.41***</td>
<td>0.27 ± 0.01***</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>1000</td>
<td>Toxic</td>
<td>Toxic</td>
</tr>
<tr>
<td>Citric acid</td>
<td>24</td>
<td>500</td>
<td>11.0 ± 1.41**</td>
<td>0.65 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>250</td>
<td>8.5 ± 0.70*</td>
<td>0.69 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>100</td>
<td>6.5 ± 0.70</td>
<td>0.78 ± 0.08</td>
</tr>
<tr>
<td>Negative control</td>
<td>48</td>
<td>0.00</td>
<td>5.5 ± 0.70</td>
<td>0.67 ± 0.04</td>
</tr>
<tr>
<td>Positive control (MMC)</td>
<td>48</td>
<td>0.3</td>
<td>35.0 ± 2.82***</td>
<td>0.22 ± 0.02***</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>1000</td>
<td>Toxic</td>
<td>Toxic</td>
</tr>
<tr>
<td>Citric acid</td>
<td>48</td>
<td>500</td>
<td>12.5 ± 0.70*</td>
<td>0.51 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>250</td>
<td>13.0 ± 1.41*</td>
<td>0.55 ± 0.07</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>100</td>
<td>12.0 ± 1.41*</td>
<td>0.61 ± 0.07</td>
</tr>
</tbody>
</table>

MMC: Mitomycin C; DMSO: Dimethylsulfoxide; CBPI: Cytokinesis-block proliferation index; MN: Micronucleus; ± SD: Standard Deviation.

*Significantly different from the control p<0.05 (t-test)
**Significantly different from the control p< 0.01 (t-test)
***Significantly different from the control p<0.001 (t-test)
### TABLE 4

The frequency of micronucleus and cytokinesis-block proliferation index of cultured human lymphocytes treated with sorbic acid

<table>
<thead>
<tr>
<th>Test substances</th>
<th>Treatment time (h)</th>
<th>Concentration (μg/ml)</th>
<th>MN ± SD</th>
<th>CBPI ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control (DMSO)</td>
<td>24</td>
<td>20</td>
<td>4.0 ± 0.00</td>
<td>0.70 ± 0.02</td>
</tr>
<tr>
<td>Pozitive control (MMC)</td>
<td>24</td>
<td>0.3</td>
<td>23.0 ± 1.41***</td>
<td>0.27 ± 0.01**</td>
</tr>
<tr>
<td>Sorbic acid</td>
<td>24</td>
<td>1000</td>
<td>Toxic</td>
<td>Toxic</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>500</td>
<td>Toxic</td>
<td>Toxic</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>250</td>
<td>8.5 ± 0.70*</td>
<td>0.60 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>100</td>
<td>6.0 ± 0.00</td>
<td>0.74 ± 0.02</td>
</tr>
<tr>
<td>Negative control (DMSO)</td>
<td>48</td>
<td>20</td>
<td>5.5 ± 0.70</td>
<td>0.67 ± 0.04</td>
</tr>
<tr>
<td>Pozitive control (MMC)</td>
<td>48</td>
<td>0.3</td>
<td>35.0 ± 2.82***</td>
<td>0.22 ± 0.02**</td>
</tr>
<tr>
<td>Sorbic acid</td>
<td>48</td>
<td>1000</td>
<td>Toxic</td>
<td>Toxic</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>500</td>
<td>Toxic</td>
<td>Toxic</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>250</td>
<td>11.5 ± 0.70*</td>
<td>0.57 ± 0.07</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>100</td>
<td>9.0 ± 1.41</td>
<td>0.62 ± 0.04</td>
</tr>
</tbody>
</table>

MMC: Mitomycin C; DMSO: Dimethylsulfoxide; CBPI: Cytokinesis-block proliferation index; MN: Micronucleus; ± SD: Standard Deviation.

*Significantly different from the control p<0.05 (t-test)

**Significantly different from the control p< 0.01 (t-test)

### TABLE 5

The frequency of nuclear buds and nucleoplasmic bridges of cultured human lymphocytes treated with ascorbic acid, benzoic acid, citric acid, sorbic acid

<table>
<thead>
<tr>
<th>Test substances</th>
<th>Treatment time (h)</th>
<th>Concentration (μg/ml)</th>
<th>NBuds ± SD</th>
<th>NPBs ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control</td>
<td>24</td>
<td>20</td>
<td>2.5 ± 0.70</td>
<td>0.0 ± 0.00</td>
</tr>
<tr>
<td>Pozitive control (MMC)</td>
<td>24</td>
<td>0.3</td>
<td>18.0 ± 1.41***</td>
<td>5.5 ± 0.70**</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>24</td>
<td>1000</td>
<td>3.0 ± 1.41</td>
<td>1.5 ± 0.70</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>500</td>
<td>3.5 ± 0.70</td>
<td>2.0 ± 1.41</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>250</td>
<td>2.5 ± 0.70</td>
<td>1.5 ± 0.70</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>100</td>
<td>3.0 ± 1.41</td>
<td>0.0 ± 0.00</td>
</tr>
<tr>
<td>Benzoic acid</td>
<td>24</td>
<td>1000</td>
<td>Toxic</td>
<td>Toxic</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>500</td>
<td>6.5 ± 0.70</td>
<td>2.0 ± 1.41</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>250</td>
<td>5.0 ± 1.41</td>
<td>1.0 ± 0.70</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>100</td>
<td>4.0 ± 1.41</td>
<td>0.5 ± 1.41</td>
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<tr>
<td>Citric acid</td>
<td>24</td>
<td>1000</td>
<td>Toxic</td>
<td>Toxic</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>500</td>
<td>7.0 ± 2.82</td>
<td>2.5 ± 1.41</td>
</tr>
<tr>
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<td>6.5 ± 0.70</td>
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</tr>
<tr>
<td></td>
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<td>100</td>
<td>6.0 ± 0.00</td>
<td>1.0 ± 1.41</td>
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<tr>
<td>Sorbic acid</td>
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<td>1000</td>
<td>Toxic</td>
<td>Toxic</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>500</td>
<td>Toxic</td>
<td>Toxic</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>250</td>
<td>5.5 ± 0.70</td>
<td>1.5 ± 0.70</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>100</td>
<td>4.5 ± 0.70</td>
<td>1.0 ± 1.41</td>
</tr>
<tr>
<td>Negative control</td>
<td>48</td>
<td>20</td>
<td>4.0 ± 1.41</td>
<td>1.5 ± 0.70</td>
</tr>
<tr>
<td>Pozitive control (MMC)</td>
<td>48</td>
<td>0.3</td>
<td>25.5 ± 2.12***</td>
<td>6.0 ± 1.41*</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>48</td>
<td>1000</td>
<td>5.5 ± 0.70</td>
<td>2.0 ± 1.41</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>500</td>
<td>5.0 ± 1.41</td>
<td>2.5 ± 0.70</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>250</td>
<td>4.5 ± 0.70</td>
<td>1.0 ± 0.70</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>100</td>
<td>4.0 ± 1.41</td>
<td>0.5 ± 1.41</td>
</tr>
<tr>
<td>Benzoic acid</td>
<td>48</td>
<td>1000</td>
<td>Toxic</td>
<td>Toxic</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>500</td>
<td>7.5 ± 0.70</td>
<td>2.5 ± 1.41</td>
</tr>
<tr>
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<td>250</td>
<td>6.0 ± 1.41</td>
<td>1.5 ± 0.70</td>
</tr>
<tr>
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<td>48</td>
<td>100</td>
<td>5.0 ± 1.41</td>
<td>1.0 ± 1.41</td>
</tr>
<tr>
<td>Citric acid</td>
<td>48</td>
<td>1000</td>
<td>Toxic</td>
<td>Toxic</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>500</td>
<td>7.5 ± 2.12</td>
<td>3.0 ± 1.41</td>
</tr>
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<td>8.5 ± 0.70</td>
<td>1.5 ± 0.70</td>
</tr>
<tr>
<td></td>
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<td>100</td>
<td>8.0 ± 1.41</td>
<td>1.5 ± 0.70</td>
</tr>
<tr>
<td>Sorbic acid</td>
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<td>1000</td>
<td>Toxic</td>
<td>Toxic</td>
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<tr>
<td></td>
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<tr>
<td></td>
<td>48</td>
<td>250</td>
<td>7.0 ± 1.41</td>
<td>2.5 ± 1.41</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>100</td>
<td>5.5 ± 0.70</td>
<td>1.5 ± 0.70</td>
</tr>
</tbody>
</table>

NBUDs: nuclear buds; NPBs: nucleoplasmic bridges; MMC: Mitomycin C; ± SD: Standard Deviation.

*Significantly different from the control p<0.05 (t-test)

**Significantly different from the control p< 0.01 (t-test)

***Significantly different from the control p<0.001 (t-test)
acid can cause genotoxic damage. As shown in ascorbic acid data (Table 4), the data obtained as a result of 24 and 48-h treatment with MMC used as a positive control were found to be statistically significant when compared to negative control data (p≤0.05). Sorbic acid has toxic effects as a result of 24 and 48 h of dosing at 1000 µg/ml and 500 µg/ml. It showed a decrease in the number of micronuclei with decreasing dosage in 24 and 48 hours applications. These increases were dose-dependent. As shown in Table 5, Sorbic acid decreased the number of the cytokinesis-block proliferation index depending on increasing the concentration, this decrease was not statistically important. As shown in table 5, Nuclear buds and nucleoplasmic bridges values of sorbic acid were found to be not statistically significant compared to solvent control values at 24 and 48 h. Nuclear buds and nucleoplasmic bridges formation number with all concentration of sorbic acid obtained as a result of 24 and 48 h treatment with MMC used as a positive control were found to be significant compared to negative control value (p≤0.01).

When the micronucleus result of four food additives is evaluated, benzoic acid, citric acid, and sorbic acid are toxic on 1000 mg doses and the highest toxicity is sorbic acid. Ascorbic acid with the lowest toxicity is not toxic at 1000 mg. Toxic levels of benzoic and citric acid are similar. Both of which are toxic at doses of 1000 mg for 24 and 48 h. Sorbic acid, which has the highest toxicity in the materials, are toxic on the doses of 1000 and 500 mg in 24 and 48 h doses are toxic. All doses of ascorbic acid have been observed no cytotoxic and genotoxic effect at 24 and 48 h.

**DISCUSSION**

Genotoxicity studies were much more preferable in determining the carcinogenic properties of various food additives. Therefore, we investigated the genotoxic effects of ascorbic acid, benzoic acid, citric acid, and sorbic acid with micronuclei, nucleoplasmic bridges and nuclear buds frequency in blood lymphocytes and Cytokinesis-block proliferation index values were calculated.

In human lymphocytes treated with ascorbic acid, it was found that the number of micronuclei did not change compared to the control group and the values were not statistically significant. We also observed that Cytokinesis-block proliferation index values decreased in human lymphocytes due to increased dose depending on ascorbic acid, but these decreased were not statistically significant. Ascorbic acid takes part in various biological processes, including free radical scavenging, and the results of treatment with ascorbic acid have been shown to significantly reduce the genotoxicity of some mutagens [40, 41]. Abosova et al. [42] indicated that; micronuclei were calculated in the buccal epithelium of each individual before and after treatment of vitamins A and C. It has been observed that micronuclei cells and cytogenetic disorders significantly reduced after the course of vitamins and confirmed their antimutagenic effects [42]. Significant increases in both sister chromatid exchange and micronucleus frequency were observed in TiO-treated cultures. However, co-administration of ascorbic acid and TiO contributed to decreases in sister chromatid exchange, sister chromatid exchange and micronucleus ratios compared with the group treated with titanium alone [43]. Another study, the antigenotoxic effect of ascorbic acid was studied on cultured human lymphocytes against the genotoxic damage induced by IMA using chromosomal aberration and sister chromatid exchange as genetic endpoints. The results of this study showed that dose-dependent increases in chromatid exchange and sister chromatid exchange frequencies were observed in cultures treated with IMA. The incidence of chromatid exchange and sister chromatid exchange decreased when the non-genotoxic ascorbic acid alone was treated in combination with IMA [8]. Siddique et al. [44] reported that Hydrogen peroxide was a dose-dependent increase in micronucleus frequency. When H2O2 was given with ascorbic acid separately at a different dose, a significant decrease in micronucleus frequency was observed in human lymphocytes depending on the dose [44].

In most food products, benzoic acid is widely used as an antimicrobial agent. In our study, benzoic acid significantly increased micronucleus formation at 500 and 250 µg/ml dose in human lymphocytes at 1000 dose of benzoic acid induced cytotoxic effects in human lymphocytes at 24 and 48 h. There was a decrease in CBPI values due to the increase in benzoic acid concentration but this change was not significant. In similar studies, sodium benzoate, potassium benzoate, and benzoic acid were investigated for their potential to cause sister chromatid exchange, chromosomal aberration and micronucleus formation in human lymphocytes. The sister chromatid exchange chromosomal aberration and micronucleus frequency were increased in (200 and 500 µg/ml) doses of benzoic acid [19, 45]. Sodium benzoate was found to increase micronucleus frequency at 2.0, 1.5 and 1.0 µg/ml concentrations [46]. Another study with benzoic acid indicates that the decrease in the percentage of living as the concentration increases and the increase in total mutation indicate that the benzoic acid is both toxic and mutagenic according to SMART Test in the Drosophila [18]. Yilmaz et al. [19] stated that benzoic acid significantly increased chromosomal aberration and reduced the mitotic index at Allium sativum root tips [19]. Pandir has shown that; benzoic acid has the DNA damaging effect, especially to its highest concentration, but does not achieve
similar effects in human male germ cells at 50, 100 and 200 μg/ml doses [47]. Different concentrations of benzoic acid may have a significant genotoxic effect in the wing somatic mutation and recombination test (SMART) of Drosophila melanogaster [48].

Citic acid significantly increased the micronucleus value at 500 and 250 μg/ml concentrations in human lymphocytes. 1000 μg/ml dose of induced cytotoxic effects in human lymphocytes at 24 and 48 h. CBPI decreased with increasing citric acid concentration, but this decrease was not significant. Similar results obtained in the study showed that the micronucleus frequency of citric acid increased at concentrations of 250 and 500 μg/ml in human lymphocytes [24]. It is emphasized that citric acid is not genotoxic, but is cytotoxic depending on the dose [49]. The activation of iron and also citric acid has an important role in making soluble to cause free radical formation during these complex interactions, really of this article, the issue of whether there is a safe additive, as stated by the international authority delivers the engrossing size.

Genotoxic effects showed that citric acid is most likely due to free hydroxyl radicals due to iron citrate complexes [50]. The treatment of five food preservatives, including citric acid, was evaluated on root tips of Allium cepa. Mitotic index values were reduced with increasing concentrations and longer treatment times. All abnormal mitotic figures were observed in all mitotic phases and this aberrations (anaphase bridges, micronuclei, C-mitosis, stickiness, lagging, breaks, and unequal distribution) increased with increasing concentrations of these preservatives [20]. Kocak et al. [22] stated that micronucleus frequency by the citric acid increase in peripheral erythrocytes of Tinca tinca [22]. The in vitro genotoxic effects of citric acid and phosphoric acid and their combinations and benzoic acid and calcium propionate were studied using alkaline single-cell gel electrophoresis on human lymphocytes. As a result, it was found that food additives caused DNA damage and citric acid showed the least toxicity [51]. Citric acid significantly increased chromosomal aberrations, sister chromatid changes, and micronucleus frequency in human lymphocytes [24].

Also, sorbic acid is well known antimicrobial material which is using for preserving foods. Sorbic acid significantly increased the micronucleus value at 250 μg/ml dose in human lymphocytes. 500 and 1000 μg/ml dose of sorbic acid induced cytotoxic effects in human lymphocytes at 24 and 48 h. It was found that the cytokinesis-block proliferation index values of the increased concentration of sorbic acid in human lymphocytes decreased, but these values were not statistically significant. Sorbic acid, together with the potassium salt specified as the genotoxic agent, is not harmful to the higher concentration of sorbic acid and its ADIs are fixed at 25 mg/kg body weight by JECFA [52]. Sasaki et al. [16] investigated the genotoxicity of 39 chemicals used as a food additive by the comet assay on the liver, glandular stomach, colon, brain, kidney, lung, urinary bladder and bone marrow in mice. Potassium salts and sorbic acid did not show a statistically significant increase in DNA damage in any of the examined organs [16]. The highest dose of sorbic acid caused a significant increase in micronuclei formations. Sodium nitrite alone has caused a significant number of micronuclei in all doses. Despite these values, a combination of half of the sorbic acid and sodium nitrite concentration produced synergistic effects attributable to the formation of certain genotoxic compounds in vivo to bone marrow cells of mice [27]. Jung stated et al. [31] stated that oral administration of sorbic acid did not trigger sister chromatid changes or micronuclei formations in bone marrow cells of mice. These results indicate; sorbic acid and potassium salt have no genotoxic effect in vivo or in vitro [31]. Mpountoukas et al. [1] observed that sorbic acid salts potassium sorbate exhibited a weak genotoxic effect at 4 and 8 mM concentrations in human lymphocytes using the sister chromatid changes assay [1].

NPB is caused by dicentric chromosomes which may be caused by misrepair of DNA breaks, fusions, and telomere, and may also be observed when the faulty separation of sister chromatids occurs in anaphase due to failure of decatenation. NBUD includes the process of elimination of amplified DNA, DNA repair complexes and possibly excess chromosomes from aneuploid cells [53]. Some investigators consider nuclear buds in lymphocytes as indicators of genotoxicity [54]. In a study, the effect of folate deprivation on the frequency of micronuclei and nuclear buds was also evaluated. Folic acid deprivation resulted in an increase in nuclear buds and micronuclei with terminal agent fragments. This result indicates that adequate folate protects against both structural and numerical chromosome changes [55]. In our study, high doses of benzoic acid, citric acid and sorbic acid, are of cytotoxic effect. All doses of ascorbic acid have been observed no cytotoxic and genotoxic effect. 500 and 250 μg/ml concentrations of benzoic acid, citric acid, and 250 μg/ml concentrations of sorbic acid induced the formation of micronucleus as a statistical significance at 24 and 48-h when compared to the solvent control values. 100 μg/ml concentrations of citric acid-induced micronucleus formation statistically significantly at 48 h. The values of the cytokinesis-block proliferation index decreased, depending on the increase in concentrations of ascorbic acid, benzoic acid, citric acid, and sorbic acid, but this result shows that it is not statistically significant. Also, it has been reached to the inference that nucleoplasmic bridges and nuclear buds formation values are close to the negative control value. No significant difference was found.
in nucleoplasmic bridges and nuclear buds formation values when compared with solvent control values. The results of the study show that when taken as a food additive, high doses of benzoic acid, citric acid, and sorbic acid are genotoxic and cytotoxic in vitro human peripheral blood lymphocytes.

REFERENCES


[5] EFSA Scientific Opinion. (2015a) Scientific opinion on the re-evaluation of ascorbic acid (E300), sodium ascorbate (E301) and calcium ascorbate (E302) as food 726 additives. EFSA Journal. 13, 4087.


[33] Schiffmann, D. and Schlatter, J. (1992) Genotoxicity and cell transformation studies with sorbates in Syrian hamster embryo fibroblasts. Food and Chemical Toxicology. 30(8), 669-672.


THE LIFE TABLE AND BIOLOGY OF CASSIDA RUBIGINOSA FED ON CYNARA SCOLYMYLUS

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ABSTRACT

Cassida rubiginosa Muller (Coleoptera: Chrysomelidae) is a polyphagous species and is widely distributed across the world. This species is regarded as fed on weeds by many researchers, while it is also pest of some cultivated plants. In this study, the biological parameters of C. rubiginosa fed on Cynara scolymus L. (Asteraceae) were investigated. Average periods of egg, 1st instar, 2nd instar, 3rd instar, 4th instar, 5th instar, pre-pupa, pupa and total development of C. rubiginosa fed on C. scolymus were 6.54; 2.60; 2.84; 2.57; 4.69; 5.29; 2.11; 6.91 and 32.68 days, respectively. Life table parameters were calculated as Intrinsic rate of increase: 0.017; Net reproductive rate: 533.25 and Mean generation time: 351.05 for the populations which used C. scolymus as the host, respectively. Weibull frequency distribution was used to determine the curve that best describes the survival ratio of C. rubiginosa fed on C. scolymus. In the host plant it is a type I survival curve, as parameter c, is >1. Depend on this results, population of C. rubiginosa fed on C. scolymus had an increased population.

KEYWORDS:
Artichoke, pest, Weibull distribution

INTRODUCTION

Artichoke (Cynara scolymus L. (Asteraceae)), a Mediterranean originated plant species, is widely used in human nutrition and pharmaceutical industries. Artichoke in Turkey has been intensively produced in the provinces of Izmir, Bursa, Aydin, Antalya and Adana [1]. There are some pests fed on artichoke [2-5]. Cassida rubiginosa Muller (Coleoptera: Chrysomelidae) is one of the these pests [5-8].

C. rubiginosa is a polyphagous species that is widely distributed across the world [6, 9,10]. This species, a known natural enemy of weeds also feed on weeds belonging to Asteraceae (=Compositae) family causing yield reduction in many pastures and harm to many animals feeding on these pastures [11-15]. [14] were reported that C. rubiginosa has one generation in a year. The biology of this species which is both harmful and biological control agent is not known exactly. Indeed, [15] stated that population characteristics of this species are not well understood.

The purpose of this study was to determine the biological parameters of C. rubiginosa fed on C. scolymus (artichoke) and also its life table.

MATERIALS AND METHODS

C. rubiginosa (Coleoptera: Chrysomelidae) individuals used in this experiment were collected from C. scolymus plants in Adana (Turkey). Mating males and females of C. rubiginosa collected separately from these plant were brought to the laboratory and placed in cages (12 × 8 × 7 cm) covered with gauze to lay eggs. Wetted sponges were placed in the cages to provide sufficient moisture. Young foliated plants which collected from field about 10 cm in height were placed in the cages and their roots were continuously immersed in water.

Females that had recently laid eggs were transferred to a new cage with food to obtain eggs. Hatching time of eggs was recorded to determine larval development time and pupal period. The first instar larvae emerging from the eggs were transferred to new culture cages with a soft brush and plastic culture cage labeled by marker. The larvae were fed with fresh plants every two days until pupation. Larval stages, and pre-pupal and pupal periods of these individuals were recorded daily.

The new adult females were removed and transferred individually into another cage containing test plants. The cages were formed in two parts. Pods (10 × 20 cm) were placed on the bottom part of the cage and a plastic jar (25 cm height × 8 cm width) was placed on it. For ventilation, holes 10 cm long and 8 cm wide were opened on both sides of the jar and covered with gauze. Finally, these cages including adult C. rubiginosa individuals were placed in the field. Depending on temperature, feeding adults moved to plant roots for aestivation and wintering periods. After this period, adults
came up to soil surface and started to feed on plants. When necessary, each cage was supplied with a new plant. For mating of these females, male individuals were collected from the field and placed in cages after marking of their elytra. Leaves with eggs deposited by female were removed daily from the plants and egg numbers were counted.

The pots were watered every 2 days. The period of *C. rubiginosa* on the plant roots was determined under field conditions; the other periods were determined under laboratory conditions of 25±1 °C, 65% ± 5 relative humidity and 16 hours light (4000 lux) and 8 hours dark. The experiments were checked every 8 hours (three times per day) to obtain the life table parameters.

**Life tables.** Age-specific life table parameters of *C. rubiginosa* on the host plant were calculated according to the Euler-Lotka equation [16]. All parameters were obtained by using RmStat-3 [17].

These parameters were:

- Age-specific survivor (\( l_t \)) and fecundity rates (\( m_t \)) [16].
- Net reproductive rate, \( R_0 = \sum l_t m_t \) [16].
- Intrinsic rate of increase (\( r_m \)), \( \sum \theta e^{(r_m)} l_t m_t = 1 \) [16].
- Mean generation time, \( T_0 = \ln R_0 / r_m \) [16].
- Gross reproduction rate, \( GRR = \sum m_t \) [16].
- Finite rate of increase, \( \lambda = e^{r_m} [16] \).
- Doubling time, \( T_2 = \ln 2 / r_m \) [18].
- Reproductive value, \( V = \sum l_t e^{r_m} m_t \) [19].
- Life expectancy, \( E = \frac{\sum l_t + l_{st}}{l_t} \) [20-21].
- Stable age distribution, \( C = \sum l_t e^{r_m} \sum l_t e^{r_m} \) [16].

Life table statistics were calculated for the populations on host plant. The differences in \( r_m \), \( R_0 \) and \( T_0 \) values were tested for significance by estimating the variance using the jack-knife method [22-24]. The mean values of (n-1) jack-knife pseudo-values for mean growth rate in each treatment were subjected to analysis of variance followed by SPSS, (ver. 17; P<0.01) and JMP (ver. 9).

The Weibull frequency distribution was chosen as a statistical model to summarize the *C. rubiginosa* survivorship data of all individuals on host plant [25].

\[
S_p(t) = e^{-\frac{(t/t_0)^c}{b}}
\]

Where \( S_p(t) \) represents the probability of surviving to a given age, \( b \) is the parameter that describes the scale, \( c \) is shape of the curve and \( t \) is time. The shape of parameters \( c>1 \), \( c=1 \) and \( c<1 \) correspond to type I, II and III survivorship curves, respectively [25-26]. Statistical analyses were done with CurveExpert pro (ver. 1.6.8), SPSS (ver. 17), MS Excel (ver. 2011).

The number of age-specific eggs laid by a female during the oviposition period was described by the Enkegaard equation:

\[
F(x) = a x e^{(b-x)} [27-29].
\]

where \( F(x) \) is the daily age-specific fecundity rate (eggs/female/day), \( x \) is the female’s age in days, \( a \) and \( b \) are constants. Day 1 is the first day of oviposition period. Analyses were done with CurveExpertPro (ver., 1.6.8), JMP (ver. 9), MS Excel (ver. 2011) and SPSS (ver. 17).

**RESULTS AND DISCUSSIONS**

Development time, preoviposition, oviposition and post oviposition periods of *C. rubiginosa* fed on host plant are given in Table 1.

In field studies in Erzurum Province (Turkey) by [30]. conducted under temperatures of 20-28 °C, the egg period of *C. rubiginosa on Cirsium arvense* (L.) Scop. was 7-8 days. In the same study, larval stages and pupal period were 3-4, 5-6, 5-7, 7-8, 7-8 and 5-7 days. *C. rubiginosa* under field conditions and with two hosts (Canada thistle and Musk thistle) had egg, 1st, 2nd, 3rd, 4th, 5th instar, pre pupa and pupal development times of 5.7, 5.9; 3.2, 3.1; 2.9, 3.1; 2.6 and 2.9; and 3.1, 3.1; 2.8, 2.7; 1.9, 1.8; 6.1, 6.5 days, respectively [31]. Development times of *C. rubiginosa* were found very close each other in all research.

The total developmental time on *C. scolymus* was 32.68 days [31]. reported that the total developmental time of *C. rubiginosa* was 27.4 and 28.5 days on musk and Canada thistle under field conditions, respectively. *C. rubiginosa* in the laboratory at constant temperatures had development times of 20, 26, 41 and 60 days at 32.5, 26.6, 21.1 and 17.8 °C, respectively [32]. All study results for the total developmental time were very similar.

Finally, these cages including adult *C. rubiginosa* individuals were placed in the field. Depending on temperature, feeding adults moved to plant roots for aestivation and wintering periods. After this period, adults came up to soil surface and started to feed on plants. Three male released with each female for mating. Preoviposition, oviposition and postoviposition periods and longevity of *C. rubigi-
and 500.62 days, respectively (Table 1). [33] reported that it started to lay eggs 3-7 days after mating, depending on temperature, photoperiod, rain and wind, and the duration of oviposition was 12 weeks. *C. rubiginosa* laid from 36.0 to 61.4 eggs per individual [34].

**Figure 1**

Survival rate (*l*<sub>x</sub>) and fecundity (*m*<sub>x</sub>) of *Cassida rubiginosa* fed on *Cynara scolymus*

Weibull frequency distribution was used to determine the curve that best describes the survival ratio of *C. rubiginosa* fed on *C. scolymus* (Figure 2).

The curve shape parameters are given in Table 3. Survivorship data can be effectively summarized using the shape and scale parameters of the Weibull frequency distribution (Pinder et al. 1978). In host plant it is a type I survival curve, as parameter c, is >1. Depend on this results, populations of *C. rubiginosa* fed on *C. scolymus* had an increased population.

**Table 1**

Mean development time (days ± SE) of *Cassida rubiginosa* fed on *Cynara scolymus*

<table>
<thead>
<tr>
<th>Stages</th>
<th>n</th>
<th>Cassida rubiginosa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eggs</td>
<td>37</td>
<td>6.54±0.06</td>
</tr>
<tr>
<td>1&lt;sup&gt;st&lt;/sup&gt; Larvae</td>
<td>37</td>
<td>2.60±0.09</td>
</tr>
<tr>
<td>2&lt;sup&gt;nd&lt;/sup&gt; Larvae</td>
<td>37</td>
<td>2.84±0.18</td>
</tr>
<tr>
<td>3&lt;sup&gt;rd&lt;/sup&gt; Larvae</td>
<td>33</td>
<td>2.57±0.09</td>
</tr>
<tr>
<td>4&lt;sup&gt;th&lt;/sup&gt; Larvae</td>
<td>33</td>
<td>4.69±0.43</td>
</tr>
<tr>
<td>5&lt;sup&gt;th&lt;/sup&gt; Larvae</td>
<td>31</td>
<td>5.29±0.19</td>
</tr>
<tr>
<td>Prepupa</td>
<td>31</td>
<td>2.11±0.09</td>
</tr>
<tr>
<td>Pupa</td>
<td>31</td>
<td>6.91±0.39</td>
</tr>
<tr>
<td>Total development time</td>
<td>31</td>
<td>32.68±0.56</td>
</tr>
<tr>
<td>Preoviposition</td>
<td>16</td>
<td>291.56±1.63</td>
</tr>
<tr>
<td>Oviposition</td>
<td>16</td>
<td>85.94±4.97</td>
</tr>
<tr>
<td>Postoviposition</td>
<td>16</td>
<td>90.44±10.41</td>
</tr>
<tr>
<td>Longevity</td>
<td>16</td>
<td>500.62</td>
</tr>
</tbody>
</table>

The intrinsic rate of increase (*r*<sub>m</sub>), the net reproductive rate (*R*<sub>n</sub>) and mean generation time (*T*<sub>n</sub>) which is a basic parameter for an insect population [16], of *C. rubiginosa* fed on *C. scolymus* were given Table 2. The survivorship curve (*l*<sub>x</sub>) and age-specific fecundity rate (*m*<sub>x</sub>) of *C. rubiginosa* fed on *C. scolymus* are shown in Figure 1. The survivorship curve showed that the mortality rates of *C. rubiginosa* fed on *C. scolymus* was zero up to 365<sup>th</sup> day, and then the survival rate started to decrease dramatically, reaching zero by the 562<sup>nd</sup> day. Similarly, age specific fecundity rates (*r*<sub>m</sub>) of *C. rubiginosa* fed on *C. scolymus* started to increase by the 315<sup>th</sup> days, peaked at the 338<sup>th</sup> days, and declined in a gradually decreasing trend (Figure 1).

**Table 2**

Life table parameters of *Cassida rubiginosa* fed on *Cynara scolymus*

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Cassida rubiginosa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intrinsic rate of increase, <em>r</em>&lt;sub&gt;m&lt;/sub&gt;</td>
<td>0.0178±0.000072</td>
</tr>
<tr>
<td>Net reproductive rate, <em>R</em>&lt;sub&gt;n&lt;/sub&gt;</td>
<td>533.252±1.742</td>
</tr>
<tr>
<td>Mean generation time, <em>T</em>&lt;sub&gt;n&lt;/sub&gt;</td>
<td>351.047±0.152</td>
</tr>
<tr>
<td>Gross reproduction rate, GRR</td>
<td>545,997</td>
</tr>
<tr>
<td>Doubling time, <em>T</em>&lt;sub&gt;2&lt;/sub&gt;</td>
<td>38.753</td>
</tr>
<tr>
<td>Finite rate of increase, <em>λ</em></td>
<td>1.0180</td>
</tr>
</tbody>
</table>

Weibull frequency distribution was used to determine the curve that best describes the survival ratio of *C. rubiginosa* fed on *C. scolymus* (Figure 2).

The curve shape parameters are given in Table 3. Survivorship data can be effectively summarized using the shape and scale parameters of the Weibull frequency distribution (Pinder et al. 1978). In host plant it is a type I survival curve, as parameter c, is >1. Depend on this results, populations of *C. rubiginosa* fed on *C. scolymus* had an increased population.
Fecundity of *C. rubiginosa* was determined via the Enkegaard equation, and the calculated parameters are presented in Figure 3.

The relationship between days and fecundity was well described by the model (for *C. scolymus*, $R^2 = 0.83$, $a = 0.96$, $b = 0.04$, respectively). Most of the eggs were laid within the first half of the oviposition period (Figure 3).

<table>
<thead>
<tr>
<th>TABLE 3</th>
<th>Weibull parameters for survival curves of <em>Cassida rubiginosa</em> fed on <em>Cynara scolymus</em> (mean ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parameters</td>
<td><em>Cassida rubiginosa</em></td>
</tr>
<tr>
<td>b</td>
<td>494.77±0.45</td>
</tr>
<tr>
<td>c</td>
<td>13.68±0.21</td>
</tr>
<tr>
<td>Type</td>
<td>1</td>
</tr>
<tr>
<td>$R^2$</td>
<td>0.97</td>
</tr>
</tbody>
</table>

**CONCLUSION**

Besides reports on biological control of weeds with *C. rubiginosa* [10-11, 30-31, 35-36], there have been studies of feeding activities on the cultivated plant *C. scolymus* used in the current study [6-8]. The feeding activity of *C. rubiginosa* on sugar beet (*Beta vulgaris*) was also observed [7].

As seen in the present study, *C. rubiginosa* fed on the important meadow pest and also on the cultivated plant, *C. scolymus*. This insect could be used as a biological control agent for host weeds in grassland areas but it should also be considered that this insect can cause significant losses in agricultural areas, especially in globe artichoke and sugar beet areas.
REFERENCES


THE EFFECTS OF VARIOUS DRYING METHODS ON THE NUTRIENT COMPOSITION OF ALFALFA VARIETIES

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ABSTRACT

This study was carried out to reveal the effects of drying some alfalfa varieties using various drying methods on their nutrient composition. To this end, four drying methods (drying in the sun, shade, oven and microwave) were implemented on sixteen alfalfa varieties (Alsancak, Verdur, Gea, Gözlü 1, Özpınar, Bilensoy, Kayseri, Ömerbey, Magnum, Nimet, Sunter, Verko, Magma 601, Elçi, Savaş and Başbağ). The crude protein, crude ash, ADF (acid detergent fiber), NDF (neutral detergent fiber), DDM (digestible dry matter), DMI (dry matter intake), and RFV (relative feed value) levels of the dried alfalfa varieties were measured. According to the results, differences in the mean values in terms of alfalfa varieties, drying methods and the interaction between the variety and the drying method were found to be significant (P<0.01). The levels of crude protein, crude ash, ADF, DDM and RFV were significantly affected by the variety factor and the drying methods that were used (P<0.01).

KEYWORDS:
Alfalfa, drying method, crude protein, crude ash, ADF, NDF

INTRODUCTION

Alfalfa is an important forage legume that can adapt to various climatic conditions [1]. It also ranks first in meeting the coarse forage needs of livestock. When it is mown during its early flowering stage, it contains energy in the same amount as and crude protein twice as much as other meadow-grass varieties [2]. Besides its high protein levels, the fact that it contains sufficient amounts of amino acids apart from cysteine makes it even more important [3]. Alfalfa may be used for animals’ consumption in dried, fresh and ensiled forms. Dry alfalfa contains 12.11-20.26% crude protein, 1.47-2.33% crude fat, 24.71-30.62% crude cellulose, 8.74-10.57% crude ash, 33.52-39.64% ADF and 8.26-9.92% ADL (acid detergent lignin), though the values may vary according to quality and variety [4]. Like other legumes, alfalfa is categorized as a variety of forage that is difficult to ensile due to its high protein-mineral and low water-soluble carbohydrate contents [5].

Drying is one of the methods applied to alfalfas to meet the quality needs in coarse forage during periods when fresh grass cannot be found due to the difficulty of ensiling. In Turkey, practices of drying and providing alfalfas for animals to consume are more common than other methods. However, significant nutrient loss occurs during the drying process [2]. The nutrient loss that occurs while drying green grass is directly proportionate to the duration of drying. As the duration is lengthened, the loss increases through oxidation of existing carbohydrates and beta-Carotenes, and the nutritional value of the dried grass decreases. Therefore, it is of utmost importance that green grass varieties are recovered with minimal nutrient loss [6].

Such a loss is much lower in artificial drying systems compared to natural ones since artificial systems involve a drying process that takes place rapidly and indoors [2]. With artificial drying systems, the nutrient compositions of plants are affected less, and their nutritive values are protected better [3].

The objective of this study is to investigate the effects of drying sixteen alfalfa varieties with four different methods (drying in the sun, shade, oven and microwave) on their nutrient compositions.

MATERIALS AND METHODS

The alfalfa varieties that were used in the study were Alsancak, Verdur, Gea, Gözlü 1, Özpınar, Bilensoy, Kayseri, Ömerbey, Magnum, Nimet, Sunter, Verko, Magma 601, Elçi, Savaş and Başbağ, and they were harvested when they were 10% through their flowering stage. The harvested alfalfas were dried with the four aforementioned
drying methods.

The alfalfas were divided into four groups after being harvested and weighed. The plants in the first group were laid over a place where they could receive sunlight throughout the day, while the second group plants were laid over a place that did not receive any sunlight throughout the day, and all of them were dried until gaining a stable weight. The plants in the third group were dried within an oven at 70°C until they gained a stable weight, while the plants in the fourth group were dried in a microwave at 600 W for 3 minutes. After the drying process, the alfalfa samples were sifted through a 1-mm sieve, and their nutrient analyses were carried out. For crude ash analysis, the alfalfa samples were exposed to fire in a 550°C ash furnace, and their crude ash contents were calculated. The protein levels of the alfalfa samples were determined through the Kjeldahl method and calculated by multiplying their nitrogen content by AOAC (1990) [7]. Their NDF and ADF values were calculated by using an ANKOM 200 Fiber Analyzer (ANKOM Technology Corp. Fairport, NY, USA) [8]. Using the calculated NDF and ADF levels, digestible dry matter [DDM = 88.9 – (0.779 x % ADF)], dry matter intake (DMI = 120 / % NDF) and relative feed value [RFV = (DDM x DMI) / 1.29] levels were calculated [9].

The data obtained in the study were analyzed by using the SAS statistical software according to the randomized block and factorial experiment designs, while Duncan's test was used to determine the differences among the mean values.

RESULTS AND DISCUSSION

Table 1 shows the crude protein contents of the alfalfa varieties dried by using different methods. Within the scope of the study, the factors of variety, drying method and interaction between variety and drying method significantly affected the crude protein levels of the samples (P<0.01).

Among the varieties, the highest crude protein content was found in Magnum as 21.9%, followed by Ömerbey, which was in the same group as the former, with a crude protein level of 21.6%. The lowest crude protein content was observed in the Alsancak variety as 17.7%. On the basis of the methods that were applied, while the highest crude protein level was achieved with the sun-drying method as 21.0%, the lowest crude protein level was obtained by the microwave method as 18.6%.

Ball et al. [10] reported that the protein level difference among varieties differs based on genetic structures, pectioles, duration of maturation, temperature and fertilization procedures. The reason why the lowest crude protein level was obtained with the microwave drying method may be considered to be loss of ammonia and other non-protein nitrogen compounds due to temperature [11].

In terms of the interaction between he variety and the drying method, the highest protein content belonged to the sun-dried Ömerbey variety with 24.4%, followed by the same variety that was dried by using the oven drying method as 23.7% and the shade-dried Özpınar variety as 23.5%. The lowest crude protein content was obtained from the shade dried Alsancak variety as 14.8% (Table 1).

The results regarding the protein contents bore similarities to those in studies carried out by Canbolat and Karman [12], Jančík et al. [13], Kamalak et al. [14] and lantcheva et al. [15] (as 17.84%, 16.6-21.7%, 15.05-21.39% and 18.2%, respectively), while they were found to be lower than those reported by Çaçañ et al. [16] and Yavuz [17] (as 25% and 22.1%, respectively).

<table>
<thead>
<tr>
<th>Varieties</th>
<th>Sun (Shade)</th>
<th>Oven</th>
<th>Microwave</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alsancak</td>
<td>19.8 h-t</td>
<td>18.9 v-a1</td>
<td>17.2 c-d1</td>
<td>17.71</td>
</tr>
<tr>
<td>Verdur</td>
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<td>20.6 j-p</td>
<td>17.6 b-c1</td>
<td>18.8 G</td>
</tr>
<tr>
<td>Gea</td>
<td>22.7 b-d</td>
<td>20.1 n-s</td>
<td>19.8 o-t</td>
<td>20.6 DC</td>
</tr>
<tr>
<td>Gözlü 1</td>
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<td>21.7 f-i</td>
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</tr>
<tr>
<td>Özpınar</td>
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<td>15.4 g-h1</td>
<td>21.0 h-n</td>
<td>19.6 FG</td>
</tr>
<tr>
<td>Bilensoy</td>
<td>22.3 c-f</td>
<td>20.4 m-q</td>
<td>20.9 j-n</td>
<td>20.1 DEF</td>
</tr>
<tr>
<td>Kaysori</td>
<td>19.3 x-x</td>
<td>19.8 o-t</td>
<td>16.3 d-f1</td>
<td>19.1 GH</td>
</tr>
<tr>
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<td>23.7 a</td>
<td>16.4 d-f1</td>
<td>21.6 AB</td>
</tr>
<tr>
<td>Magnum</td>
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<td>22.6 e-c</td>
<td>20.9 h-n</td>
<td>21.9 A</td>
</tr>
<tr>
<td>Nimet</td>
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<tr>
<td>Sunter</td>
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<td>19.2 r-z</td>
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</tr>
<tr>
<td>Magma 601</td>
<td>22.3 c-f</td>
<td>19.3 s-x</td>
<td>21.4 s-g</td>
<td>21.1 BC</td>
</tr>
<tr>
<td>Elçi</td>
<td>20.4 n-r</td>
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<td>18.3 a-b1</td>
<td>19.1 GH</td>
</tr>
<tr>
<td>Savaş</td>
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<td>19.5 r-w</td>
<td>20.4 l-q</td>
<td>20.5 DC</td>
</tr>
<tr>
<td>Başbağ</td>
<td>20.2 n-r</td>
<td>22.3 c-f</td>
<td>18.3 n-b1</td>
<td>19.8 EF</td>
</tr>
<tr>
<td>Average</td>
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<td>19.9 C</td>
<td>20.2 B</td>
<td>18.6 D</td>
</tr>
</tbody>
</table>
In terms of crude ash ratios, the differences among the mean values of the alfalfa varieties, drying methods and interaction between variety and drying method were found to be statistically significant (P<0.01) (Table 2).

Among the alfalfa varieties in the study, the highest crude ash ratio belonged to Elçi as 8.9%, followed in the same group by the Ömerbey variety with the value of 8.7%. The lowest crude ash ratio was obtained from the Kayseri variety a 7.4%. On the basis of the methods that were applied, the highest crude ash ratio was achieved with the sun-drying method as 8.7%, while the lowest was obtained by the oven drying method as 7.8%. When evaluated in terms of the interaction between variety and drying method, the shade dried Nimet variety achieved the highest crude ash ratio (9.86%), followed in the same group by the sun-dried Alsancak and Elçi varieties (9.75% and 9.55% respectively). The lowest crude ash ratio was found in the oven-dried Magma 601 variety (6.86%).

The values that were obtained regarding the crude ash ratios differed from those found in studies carried out by Canbolat and Karaman [12], Iantcheva et al. [15], Jančík et al. [13] and Kamalak et al. [14] (as 5.75%, 9.3%, 11% and 10.33-11.65%, respectively).

Differences among the ADF ratio mean values of the alfalfa varieties, drying methods and interaction between variety and drying method were found to be statistically significant (P<0.01) (Table 3).

<table>
<thead>
<tr>
<th>Varieties</th>
<th>Sun</th>
<th>Shade</th>
<th>Oven</th>
<th>Microwave</th>
<th>Average</th>
</tr>
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<td>Alsancak</td>
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<td>8.95 d-f</td>
<td>8.29 k-o</td>
<td>7.36 w-z</td>
<td>8.6 B</td>
</tr>
<tr>
<td>Verdor</td>
<td>8.17 m-p</td>
<td>8.85 d-g</td>
<td>7.56 u-x</td>
<td>7.18 y-a1</td>
<td>7.9 E</td>
</tr>
<tr>
<td>Gea</td>
<td>8.77 e-h</td>
<td>6.99 z-l</td>
<td>7.77 q-v</td>
<td>8.16 m-p</td>
<td>7.9 E</td>
</tr>
<tr>
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<td>8.37 j-n</td>
<td>7.47 v-y</td>
<td>8.15 m-p</td>
<td>7.66 t-w</td>
<td>7.9 E</td>
</tr>
<tr>
<td>Özpınar</td>
<td>8.07 m-q</td>
<td>8.74 e-i</td>
<td>7.19 x-a1</td>
<td>7.85 p-u</td>
<td>8.0 E</td>
</tr>
<tr>
<td>Bilensoy</td>
<td>8.66 f-k</td>
<td>7.18 y-a1</td>
<td>7.14 y-a1</td>
<td>7.46 v-y</td>
<td>7.6 F</td>
</tr>
<tr>
<td>Kayseri</td>
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<td>7.48 v-y</td>
<td>7.26 x-z</td>
<td>7.56 u-x</td>
<td>7.4 F</td>
</tr>
<tr>
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<td>9.18 cd</td>
<td>8.66 f-j</td>
<td>8.44 h-m</td>
<td>8.57 g-l</td>
<td>8.7 AB</td>
</tr>
<tr>
<td>Magnum</td>
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<td>8.76 e-h</td>
<td>8.17 m-p</td>
<td>8.35 j-o</td>
<td>8.6 B</td>
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<tr>
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<td>8.16 m-p</td>
<td>7.69 r-w</td>
<td>8.5 BC</td>
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<tr>
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<td>8.14 m-q</td>
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<td>7.65 t-w</td>
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<td>8.3 CD</td>
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<td>Magma 601</td>
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<td>8.81 d-g</td>
<td>6.86 a1</td>
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<td>7.9 E</td>
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<tr>
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<td>8.67 f-j</td>
<td>8.9 A</td>
</tr>
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<td>8.05 n-s</td>
<td>7.06 z-a1</td>
<td>7.98 o-t</td>
<td>7.9 E</td>
</tr>
<tr>
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<tr>
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<td>8.3 B</td>
<td>7.8 C</td>
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<table>
<thead>
<tr>
<th>Varieties</th>
<th>Sun</th>
<th>Shade</th>
<th>Oven</th>
<th>Microwave</th>
<th>Average</th>
</tr>
</thead>
<tbody>
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<td>Alsancak</td>
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<td>35.33h-m</td>
<td>39.39 a-c</td>
<td>32.7 E-G</td>
</tr>
<tr>
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<td>32.69 s-x</td>
<td>30.66 v-y</td>
<td>33.40 n-s</td>
<td>35.67 h-j</td>
<td>32.9 E-G</td>
</tr>
<tr>
<td>Gea</td>
<td>31.54 t-x</td>
<td>34.38 j-o</td>
<td>31.95 q-w</td>
<td>31.45 u-x</td>
<td>32.3 F-H</td>
</tr>
<tr>
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<td>30.56 v-y</td>
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<tr>
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<td>27.33bc1</td>
<td>39.45 ab</td>
<td>37.49 d-g</td>
<td>34.4 CD</td>
</tr>
<tr>
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<td>33.36 n-s</td>
<td>34.61 i-a</td>
<td>32.57 p-u</td>
<td>33.3 D-F</td>
</tr>
<tr>
<td>Kayseri</td>
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<td>33.55 n-q</td>
<td>35.45 h-l</td>
<td>38.54 b-e</td>
<td>35.7 AB</td>
</tr>
<tr>
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<td>31.33 u-y</td>
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<tr>
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<td>23.56 d1</td>
<td>33.89 k-p</td>
<td>39.41 a-c</td>
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<td>Sunter</td>
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<td>28.57 alb1</td>
<td>34.66 i-n</td>
<td>35.63 h-k</td>
<td>33.9 DE</td>
</tr>
<tr>
<td>Verko</td>
<td>28.74 z-b1</td>
<td>32.30 p-v</td>
<td>30.41 w-z</td>
<td>29.68 y-a1</td>
<td>30.3 I</td>
</tr>
<tr>
<td>Magma 601</td>
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<td>31.76 s-x</td>
<td>39.57 ab</td>
<td>34.50 i-n</td>
<td>36.6 A</td>
</tr>
<tr>
<td>Elçi</td>
<td>38.59 b-d</td>
<td>32.59 p-u</td>
<td>36.22 f-i</td>
<td>33.77 l-p</td>
<td>35.3 BC</td>
</tr>
<tr>
<td>Savaş</td>
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<td>33.35 n-s</td>
<td>34.75 i-n</td>
<td>35.83 g-j</td>
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</tr>
<tr>
<td>Başbağ</td>
<td>38.73 b-d</td>
<td>33.69 m-q</td>
<td>28.77 z-b1</td>
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<td>34.2 CD</td>
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<td>Average</td>
<td>34.6 A</td>
<td>30.6 C</td>
<td>33.6 B</td>
<td>34.4 A</td>
<td></td>
</tr>
</tbody>
</table>
ADF is not desired to be on high levels since its digestion in the rumen is slow [17, 18]. Among the sampled alfalfa varieties, the Magma 601 variety had the highest ADF ratio as 36.6%, followed in the same group by the Savaş variety with the value of 36.1% and the Kayseri variety with the value of 35.7%. The lowest ADF ratio was observed in the Ömerbey variety as 28.7%. Güney et al. [19] reported that, as the ADF and NDF ratios increase, forage quality decreases. On the basis of the methods that were applied, the highest ADF ratios were obtained with the sun-drying method as 34.6% and the microwave drying method as 34.4% in the same group, while the lowest ADF ratio was achieved by the shade drying method as 30.6%. This result was in parallel with the report by Pelletier et al. [20] that the ADF ratios of forage samples dried at high temperatures may increase as a result of the Maillard reaction.

When the results are evaluated in terms of the interaction between variety and drying method, it is observed that the sun-dried Savaş variety had the highest ADF ratio (40.54%), followed by the sun-dried and oven-dried Magma variety (40.50% and 39.57% respectively), the oven-dried Özpınar variety (39.45%), the microwave-dried Nimet variety (39.61%) and the microwave-dried Alsancak variety (39.39%). The lowest ADF ratio was observed in the shade dried Alsancak variety (21.67%).

While the results regarding the ADF ratios of the alfalfa varieties dried by using different methods bore similarities to those reported by Jančík et al. [13] (27-39.7%), they were observed to be higher than those reported by Çaçan et al. [16] and Canbolat and Karaman [12] (20.4% and 28.87%, respectively), and lower than those put forward by Yavuz [17] (41.8% and 37.3%, respectively).

The NDF ratios of the alfalfa varieties dried by using different methods are shown in Table 4. They were observed to be significantly affected (P<0.01) by variety factor, drying method and interaction between variety and drying method.

High NDF ratios in forage slow digestion down and affect forage consumption negatively since the NDF ratio is related to forage fermentation [17, 18].

In terms of NDF ratios, the Magma 601 variety had the highest level as 46.3%, while the lowest level was observed in the Ömerbey variety as 37.2%. On the basis of the methods that were applied, the highest NDF ratio was obtained by the oven drying method (45.6%), while the lowest level was achieved by the sun drying method (38.71%). This result was in parallel with the result reported by Parissi et al. [21] that oven drying increases NDF and ADL ratios by causing water-insoluble tannin-protein polymers to appear in forage.

To evaluate the NDF ratios in terms of the interaction between variety and drying method, the sun-dried Nimet variety had the highest NDF ratio (54.48%), while the lowest NDF ratios were observed in the shade-dried Magnum (32.73%) and Nimet (33.74%) varieties, which were in the same group as the former.

The values regarding the NDF ratios of the alfalfa varieties dried through different methods bore similarities to those reported by Jančík et al. [13] (33.8-47.9%), while they were found to be higher than those reported by Çaçan et al. [16] (29.1%) and lower than those put forward by Yavuz [17] and Canbolat and Karaman [12] (46.7% and 42.51%, respectively).

The variety, drying method and interaction between variety and drying method were evaluated in terms of DDM ratios, as a result of which the differences between the groups were found to be statistically significant (P<0.01) (Table 5).

### TABLE 4

The results regarding the NDF ratios (%) of the alfalfa varieties dried by using different methods

<table>
<thead>
<tr>
<th>Varieties</th>
<th>Sun</th>
<th>Shade</th>
<th>Oven</th>
<th>Microwave</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alsancak</td>
<td>41.44 j-n</td>
<td>39.48 r-t</td>
<td>49.33 cd</td>
<td>48.47 d</td>
<td>44.4 BC</td>
</tr>
<tr>
<td>Verdo</td>
<td>41.22 j-n</td>
<td>36.81 u-w</td>
<td>44.58 gh</td>
<td>42.50 i-k</td>
<td>41.3 FG</td>
</tr>
<tr>
<td>Gea</td>
<td>40.64 n-p</td>
<td>42.36 i-l</td>
<td>46.31 ef</td>
<td>40.37 n-q</td>
<td>42.4 FG</td>
</tr>
<tr>
<td>Gözüllü 1</td>
<td>46.49 e</td>
<td>40.58 n-p</td>
<td>41.66 j-n</td>
<td>40.73 m-p</td>
<td>42.4 FG</td>
</tr>
<tr>
<td>Özpınar</td>
<td>42.55 ij</td>
<td>34.59 xy</td>
<td>51.34 b</td>
<td>44.48 gh</td>
<td>43.2 D-F</td>
</tr>
<tr>
<td>Bilecikoy</td>
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<td>42.54 ij</td>
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<td>40.75 m-p</td>
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<td>Kayseri</td>
<td>42.26 i-m</td>
<td>40.92 k-o</td>
<td>49.56 cd</td>
<td>44.56 gh</td>
<td>44.3 B-D</td>
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<td>40.45 n-p</td>
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<td>41.48 j-n</td>
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<td>44.9 B</td>
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<td>38.65 r-t</td>
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<td>40.66 n-p</td>
<td>44.6 C-E</td>
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<td>40.76 m-p</td>
<td>38.82 q-s</td>
<td>43.68 hi</td>
<td>42.4 FG</td>
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<tr>
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<td>43.8 B</td>
<td>45.6 A</td>
<td>41.6 C</td>
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</table>
TABLE 5

Results regarding the DDM ratios (% of the alfalfa varieties dried by using different methods)

<table>
<thead>
<tr>
<th>Varieties</th>
<th>Sun</th>
<th>Shade</th>
<th>Oven</th>
<th>Microwave</th>
<th>Average</th>
</tr>
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<tbody>
<tr>
<td>Alsancak</td>
<td>62.08 q-v</td>
<td>72.02 s</td>
<td>61.37 s-x</td>
<td>58.21 cl-ct</td>
<td>63.4 D-F</td>
</tr>
<tr>
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<td>64.21 h-m</td>
<td>65.01 g-j</td>
<td>62.88 m-r</td>
<td>61.11 v-x</td>
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<td>64.33 h-l</td>
<td>62.12 q-v</td>
<td>64.01 i-o</td>
<td>64.40 h-k</td>
<td>63.7 C-E</td>
</tr>
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<td>59.54 z-cl</td>
<td>64.14 h-n</td>
<td>65.09 g-j</td>
<td>62.78 n-r</td>
<td>62.9 E-G</td>
</tr>
<tr>
<td>Özpınar</td>
<td>62.97 l-r</td>
<td>67.61 cd</td>
<td>58.17 cl-cl</td>
<td>59.70 y-b1</td>
<td>62.1 GH</td>
</tr>
<tr>
<td>Bilensoy</td>
<td>63.53 k-p</td>
<td>62.91 m-r</td>
<td>61.94 r-w</td>
<td>63.53 k-p</td>
<td>63.0 E-G</td>
</tr>
<tr>
<td>Kayseri</td>
<td>61.33 s-s</td>
<td>67.72 o-r</td>
<td>61.29 t-x</td>
<td>58.88 al-dl</td>
<td>61.1 JJ</td>
</tr>
<tr>
<td>Ömerbey</td>
<td>65.24 f-i</td>
<td>65.43 e-h</td>
<td>67.31 cd</td>
<td>68.17 c</td>
<td>66.5 A</td>
</tr>
<tr>
<td>Magnum</td>
<td>63.41 k-q</td>
<td>64.69 g-k</td>
<td>64.50 g-k</td>
<td>64.97 g-j</td>
<td>64.4 C</td>
</tr>
<tr>
<td>Sunter</td>
<td>60.24 x-al</td>
<td>66.65 de</td>
<td>61.90 r-w</td>
<td>61.15 u-x</td>
<td>62.5 FG</td>
</tr>
<tr>
<td>Bilensoy</td>
<td>65.51 d-f</td>
<td>63.74 j-p</td>
<td>65.21 t-s</td>
<td>65.78 t-g</td>
<td>65.3 B</td>
</tr>
<tr>
<td>Magma 601</td>
<td>57.35 e-i</td>
<td>64.16 h-m</td>
<td>58.07 cl-cl</td>
<td>62.03 r-w</td>
<td>60.4 I</td>
</tr>
<tr>
<td>Elçi</td>
<td>58.84 b1-cl</td>
<td>63.51 k-p</td>
<td>60.68 w-z</td>
<td>62.59 p-t</td>
<td>61.4 HJ</td>
</tr>
<tr>
<td>Savaş</td>
<td>57.32 e1</td>
<td>60.92 m-r</td>
<td>61.83 r-w</td>
<td>60.99 v-y</td>
<td>60.8 IJ</td>
</tr>
<tr>
<td>Başbağ</td>
<td>58.73 b1-dl</td>
<td>62.66 o-s</td>
<td>66.49 d-f</td>
<td>61.27 t-x</td>
<td>62.3 GH</td>
</tr>
<tr>
<td>Average</td>
<td>61.9 C</td>
<td>65.0 A</td>
<td>62.7 B</td>
<td>62.1 C</td>
<td>62.3 GH</td>
</tr>
</tbody>
</table>

Regarding the alfalfa varieties, the highest DDM ratio was measured in the Ömerbey variety (66.5%), while the lowest ratios were determined to be in the Magma 601 (60.4%), Savaş (60.8%) and Kayseri (61.1%) varieties, which were in the same group as the former. On the basis of the methods that were applied, the highest DDM ratio was obtained with the shade drying method (65%), followed by the oven drying method (62.7%), microwave drying method (62.1%) and sun drying method (61.9%).

To evaluate the DDM ratios in terms of the interaction between variety and drying method, the highest DDM ratio was obtained in the shade-dried Alsancak variety (72.02%), while the lowest ratios were found in the sun-dried Savaş (57.32%) and Magma 601 (57.35%) varieties.

The results that were obtained bore similarities to those reported by Canbolat and Karaman [12] (59.5-66.8%), while they were lower than those put forward by Çaçan et al. [16] (73%) and higher than those stated by Yavuz [17] (59.7%).

The variety, drying method and interaction between variety and drying method significantly affected (P<0.01) the DMI ratios of the dried alfalfa varieties (Table 6).

To evaluate the dried alfalfas in terms of their varieties, the highest DMI ratios belonged to the Ömerbey (3.24%) and Magnum (3.22%) varieties, both of which were in the same group. The lowest DMI ratios were measured in the Magma 601 (2.61%) and Savaş (2.68%) varieties, likewise in the same group. On the basis of the methods that were applied, the varieties dried through the shade drying method had the highest DMI ratio as 3.12%.

TABLE 6

Results regarding the DMI ratios (% of the alfalfa varieties dried by using different methods)

<table>
<thead>
<tr>
<th>Varieties</th>
<th>Sun</th>
<th>Shade</th>
<th>Oven</th>
<th>Microwave</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alsancak</td>
<td>2.90 k-p</td>
<td>3.12 e-h</td>
<td>2.43 z-b1</td>
<td>2.48 x-a1</td>
<td>2.73 EF</td>
</tr>
<tr>
<td>Verdor</td>
<td>2.91 k-p</td>
<td>3.26 d</td>
<td>2.69 r-u</td>
<td>2.83 o-q</td>
<td>2.92 C</td>
</tr>
<tr>
<td>Gea</td>
<td>2.95 i-l</td>
<td>2.83 n-q</td>
<td>2.59 t-w</td>
<td>2.97 k-t</td>
<td>2.84 D</td>
</tr>
<tr>
<td>Gözütü 1</td>
<td>2.58 i-x</td>
<td>2.96 i-k</td>
<td>2.88 k-p</td>
<td>2.95 i-m</td>
<td>2.84 D</td>
</tr>
<tr>
<td>Özpınar</td>
<td>2.82 o-q</td>
<td>3.47 bc</td>
<td>2.34 b1-cl</td>
<td>2.70 t-r</td>
<td>2.83 D</td>
</tr>
<tr>
<td>Bilensoy</td>
<td>2.90 k-p</td>
<td>2.82 o-q</td>
<td>2.68 r-v</td>
<td>2.95 i-n</td>
<td>2.84 D</td>
</tr>
<tr>
<td>Kayseri</td>
<td>2.84 l-q</td>
<td>2.93 j-o</td>
<td>2.42 z-b1</td>
<td>2.69 r-u</td>
<td>2.72 EF</td>
</tr>
<tr>
<td>Ömerbey</td>
<td>3.47 j-c</td>
<td>3.13 e-h</td>
<td>2.97 i-k</td>
<td>3.38 c</td>
<td>3.24 A</td>
</tr>
<tr>
<td>Magnum</td>
<td>2.94 j-n</td>
<td>3.67 a</td>
<td>2.89 k-p</td>
<td>3.38 c</td>
<td>3.22 A</td>
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<tr>
<td>Savaş</td>
<td>2.54 j-1</td>
<td>3.56 j-p</td>
<td>2.57 t-y</td>
<td>2.63 t-y</td>
<td>2.65 EF</td>
</tr>
<tr>
<td>Başbağ</td>
<td>2.60 t-w</td>
<td>3.06 h-i</td>
<td>2.64 s-v</td>
<td>3.03 h-j</td>
<td>2.83 D</td>
</tr>
<tr>
<td>Average</td>
<td>2.77 C</td>
<td>3.12 A</td>
<td>2.65 D</td>
<td>2.91 B</td>
<td>2.84 D</td>
</tr>
</tbody>
</table>
followed by the microwave drying method (2.91%), sun drying method (2.71%) and oven drying method (2.65%). In terms of the interaction between variety and drying method, the highest DMI ratios belonged to the shade-dried Magnum (3.67%) and Nimet (3.56%) varieties. The lowest DMI ratios belonged to the Ömerbey variety as 160.9, while the lowest values were obtained from the shade-dried Özpınar variety generated the highest RFV (175.6 c-e). In terms of the interaction between variety and drying method, the highest RFV rises above 100 [24].

The variety, drying method and interaction between variety and drying method factors. The RFV levels of the alfalfa varieties (Table 7).

The results regarding the RFV levels of the alfalfa varieties dried by using different methods were observed within the scope of this study. Different drying methods affected the chemical and nutrient compositions of the alfalfa varieties. The crude protein, crude ash, ADF, DDM and RFV levels of the dried alfalfa varieties were determined to be significantly affected (P<0.01) by the variety and drying method factors.

The sun-dried and oven-dried Ömerbey variety and the shade-dried Özpınar variety had the highest crude protein content. The lowest ADF ratio was observed in the shade-dried Alsancak variety, while the lowest NDF ratios belonged to the similar shade-dried Magnum and Nimet varieties. In the light of all these results, it is concluded that alfalfas must be shade-dried for the sake of preserving their nutritional values and digestibility.

CONCLUSION

Changes in the nutritional values of some alfalfa varieties dried by using different methods were observed within the scope of this study. Different drying methods affected the chemical and nutrient compositions of the alfalfa varieties. The crude protein, crude ash, ADF, DDM and RFV levels of the dried alfalfa varieties were determined to be significantly affected (P<0.01) by the variety and drying method factors.

The sun-dried and oven-dried Ömerbey variety and the shade-dried Özpınar variety had the highest crude protein content. The lowest ADF ratio was observed in the shade-dried Alsancak variety, while the lowest NDF ratios belonged to the similar shade-dried Magnum and Nimet varieties. In the light of all these results, it is concluded that alfalfas must be shade-dried for the sake of preserving their nutritional values and digestibility.

REFERENCES


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IMPROVED BIOLOGICAL DENITRIFICATION METHOD FOR WASTEWATER TREATMENT

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ABSTRACT

Domestic wastewater featured with high ammonia nitrogen content and low C/N ratio is detrimental for urban water environment. Various strategies have been developed to well address the impending issues, while conventional processing technologies are less efficient for denitrification. In this work, the improved biological denitrification method with merits in easy operation, low cost and high efficiency is developed, optimal processing parameters which finally render a high ammonia-nitrogen removal rate of 99.3% are determined. The overall findings may pave a way for the optimization of operation parameters using biological denitrification method and its practical applications in wastewater treatment, considering the beneficial attributes particularly in shortening technological process and decreasing floor area, simplifying operational management.

KEYWORDS:
Biological denitrification, wastewater treatment, nitrogen removal, technological improvement

INTRODUCTION

The past decades have witnessed the steady social progress and rapid economic development, while the achievements are accompanied with increasingly serious eutrophication of natural rivers and lakes, which are generally the ultimate outlets for domestic wastewater [1-4]. Nitrogen element roles as one of the important plant nutrient elements, the higher value of which in domestic wastewater represents super eutrophication and therefore goes against for maintaining and improving urban water environment, further resulting in the deterioration of water quality, the decrease of biodiversity as well as ecosystem imbalance [5-6]. In this regard, extensive efforts have been devoted towards the control of nitrogen contamination, among which various efficient denitrification processes have been developed into mature technical system [7-11].

The conventional biological denitrification technology mainly includes ammonification, nitrification and denitrification, that is, the organic nitrogen in water firstly converts into liquid ammonia with aid of ammonifiers, which further converts into NO3-/NO2- using nitrosobacteria and nitrobacteria under aerobic condition. The nitrogen of nitrite and nitrate finally converts into N2 through denitrifying bacteria under anoxic condition [12]. Currently, the biological denitrification technologies are featured with enormous energy consumption and high processing cost. In this regard, the key issue lies in the improvement of denitrification process, as well as shortened technological process and simplified operational management, upon which the quality of outlet water and ecological environment are anticipated to be elevated to a large extent.

Herein, improved biological denitrification technology with easy operation, low cost and high efficiency is developed in this work to well address the above-mentioned deficiencies in wastewater treatment. The highly operative method is believed to exert great impact on both social and environmental benefit in terms of practical applications.

MATERIALS AND METHODS

The wastewater was guided into settling pond and subjected to precipitation for 12-24 h, upon which the supernatant liquid was extracted and was successively filtered using No.1 circular-hole sieve (pore diameter: 1mm) and No. 2 circular-hole sieve (pore diameter: 0.05 mm).

The as-filtered wastewater was guided into ammoniation pond and subjected to 1st aeration treatment with a duration time of 5-10 h. Note that the concentration of dissolved oxygen after 1st aeration was higher than 4 mg/L. Subsequently, lime powder was added to adjust the pH value of wastewater to 7 after aeration. The ammoniation process was completed in ammoniation pond by adding mixed bacteria solution of ammonifiers with a concentration higher than 109/mL, which was comprised of bacillus subtilis (10-20 batches),
bacillus subtilis; B: bacillus pumilus; C: penicillium; D: bacillus steaothermophilus

As shown in Table 1, the parameters involved in present work are varied for optimizing biological denitrification effect. To begin with, the physical treatment including precipitation and two-round filtration are designed for the removal of solid contaminated matter in suspended status. The initial steps are believed to be beneficial for decreasing the loading of post-treatment processes and improving overall treatment effect. Although the ammonia nitrogen (NH₃-N) content fails to fully reflect the removal effect of solid matter, but to some extent it represents the precipitation effect with different duration time. Obviously, the decreased monotonously with increasing precipitation time and the decreasing tendency gets less pronounced with further increasing time (Figure 2). Considering the removal effect and time-saving issue, a precipitation time of 20 h is enough for the initial physical pre-treatment process.

RESULTS AND DISCUSSION

As shown in Table 1, the parameters involved in present work are varied for optimizing biological denitrification effect. To begin with, the physical treatment including precipitation and two-round filtration are designed for the removal of solid contaminated matter in suspended status. The initial steps are believed to be beneficial for decreasing the loading of post-treatment processes and improving overall treatment effect. Although the ammonia nitrogen (NH₃-N) content fails to fully reflect the removal effect of solid matter, but to some extent it represents the precipitation effect with different duration time. Obviously, the decreased monotonously with increasing precipitation time and the decreasing tendency gets less pronounced with further increasing time (Figure 2). Considering the removal effect and time-saving issue, a precipitation time of 20 h is enough for the initial physical pre-treatment process.
The aeration would enhance the supply of dissolved oxygen in wastewater and help to the oxidation of detrimental ammonia nitrogen into nitrites and nitrates [13]. As clearly shown in Figure 3(a), the dissolved oxygen (DO) monitored by DO tester indicates that DO concentration increases with prolonging 1st aeration time, an optimal aeration of 6 h is favorable for oxygen supply. Additionally, natural/biological flocculation induced by aeration enables the aggregation of small particles in wastewater and contributes to precipitation and separation. Other advantages lie in the oxidation of reductive species and blowing off the dissolved volatile matter [14].

To evaluate the conversion of nitrogen-containing compounds as a whole, the concentration of total nitrogen (TN), NH\textsubscript{3}-N and NO\textsubscript{x}-N have been examined after 1st aeration and shown in Figure 3 (b). Note that the use of term “NO\textsubscript{x}-N” actually means that the conversion into NO\textsubscript{3}-N is not 100%, the co-existence of residual NO\textsubscript{2}-N should be taken into consideration. The TN content almost keeps unaltered with varying aeration time, while evident change NH\textsubscript{3}-N is detected when compared to the sample after precipitation. The decrease of NH\textsubscript{3}-N, together with the increase of NO\textsubscript{x}-N manifests that substantial NH\textsubscript{3}-N has been successfully oxidized into nitrites and nitrates. Note that prolonging the aeration period is not always helpful, the subtle increase of NH\textsubscript{3}-N content with an aeration time of 10 h indicate that appropriate aeration period is crucial for efficient conversion. Typically, longer aeration time would probably result in the accumulation of NH\textsubscript{3}-N, while shorter aeration may give rise to incomplete environmental change from aerobic to anaerobic one and further lead to the systematic nitrogen removal effect induced by the weakened microbial ability during nitrification and denitrification processes. After aeration, the addition of lime powder helps to tailor the pH value of wastewater and provides adequate carbon source.

It is also well acknowledged that ammoniation refers to the conversion of organic nitrogen-containing compounds (such as protein, nucleic acids) into NH\textsubscript{3}-N, in which process various kinds of ammonifying bacteria is involved [15]. In this work, the mixed bacteria solution comprised of aerobe, which includes bacillus subtilis, bacillus pumilus, penicillium and bacillus stearotherophilus with different amounts is utilized for ammoniation. An optimal addition shown in Entry 1 in Table 1 is detected, that is, appropriate amount of aerobic bacteria including bacillus subtilis (12 batches), bacillus pumilus (10 batches), penicillium (8 batches) and bacillus stearotherophilus (8 batches) facilitate the ammoniation process, as verified by the monotonous increase of NH\textsubscript{3}-N and decrease of organic-N content (Figure 3c).

Prior to further nitrification treatment, 2nd aeration was carried out to improve the organic degradation and NH\textsubscript{3}-N environment, facilitate nitrification and suppress the consumption of carbon source, in addition to the efficient water-air contact, stirring-mixing effect as well as the retained suspension status of mixed solution. The nitrification process is typically accompanied with the conversion of NH\textsubscript{3}-N into NO\textsubscript{2}-N and finally into NO\textsubscript{3}-N. Figure 3(a) shows that 2nd aeration specifically renders DO concentration of >6 mg/L. The temperature of wastewater is found to exert significant impact on NH\textsubscript{3}-N conversion. With a fixed pH value of ~7 in wastewater and constant DO concentration of 6.9 mg/L after 2nd aeration for

FIGURE 3
(a) The relationship between 1st (or 2nd) aeration time and dissolved oxygen; (b) The variation of TN, NH\textsubscript{3}-N and NO\textsubscript{x}-N in wastewater after 1st aeration treatment; (c) The variation of organic-N and NH\textsubscript{3}-N in wastewater after adding different mixed bacteria solution for ammoniation.
4 h, the temperature effect is typically evaluated in this regard. As shown in Figure 4(a), the removal efficiency of NH$_3$-N can be higher than 92%. The elevation of temperature would be helpful for the increase of nitrification bacteria within a certain range and therefore beneficial for the rapid conversion of NH$_3$-N. An optimal temperature for nitrification is determined to be 30 °C in this work.

In this condition, the effect of DO concentration on NH$_3$-N removal shown in Figure 4(b) also indicates that lower DO concentration (<6.2 mg/L) gives rise to NH$_3$-N removal efficiency of only 20%. Further increase of DO contributes to high NH$_3$-N conversion efficiency up to 99%, as indicated by the ultra-low remnant NH$_3$-N concentration in wastewater [16]. Notably, the optimal NH$_3$-N removal efficiency is accompanied with low nitrite-N (<5mg/L) and high nitrate-N (>200mg/L). Also, the sludge concentration is an important factor that affects the nitrification efficiency [17]. Higher sludge concentration indicates higher concentration of nitrification bacteria and higher reaction rate.

The nitrification can be maintained at desirable level with elevated sludge concentration even when the DO value is relatively low, as indicated in Figure 4(c).

After nitrification, the co-existence of nitrite-N and nitrate-N would be reduced to gas-state nitrogen during denitrification process. In this work, the nitrification room and denitrification room are installed in series within a fluidized bed, which are featured with low space occupation and easy and continuous operation in practical application. In a typical nitrification process, the DO or molecular oxygen roles as electron acceptor. The demand for DO in nitrification and denitrification are contrary, anaerobic environment is of necessity for conventional denitrification process, as both DO and NO$_3^-$ can function as electron acceptor, upon which competitive behavior occurs. The existence of DO would not only suppress the synthesis of nitrate reductase and its activity, but also endows denitrification bacteria preferentially use DO as electron acceptor to degrade organic matter. While the limit
of oxygen delivery in practical technical process may give rise to partial anaerobic environment within sludge floc, that is, denitrification reaction would happen even if there is a certain concentration of DO in aeration pond [18-20]. In this work, it is found that if DO in anoxic pond is controlled to be lower than 0.5 mg/L, favorable denitrification effect can be achieved. Figure 4(d) shows the final NH$_3$-N concentration as a function of sludge concentration during denitrification process, the overall NH$_3$-N removal efficiency can be high up to 99.3%.

The combined findings described above signify that the biological denitrification in this work typically renders high efficiency. The pre-treatment parameters, 1st and 2nd aeration, DO, temperature and sludge concentration are optimized to generate high NH$_3$-N removal rate up to 99.3%, as summarized in Table 2.

**CONCLUSION**

The biological denitrification method developed in this work is featured with easy operation, high efficiency and favorable effect. The use of mixed bacteria solution of ammonifiers for ammonification of wastewater treatment helps to degrade the nitrogen-containing organics (proteins, nucleic acids, etc) into simple nitrogen-containing compounds, such as polypeptides, amino acids and amino sugars, which further convert into NH$_3$ during the deamination process and greatly advance the conversion efficiency. The lime powder adopted in present study is capable of tailoring the pH value of wastewater and providing adequate carbon sources for conversion. NH$_3$-N would convert into nitrate anion when the wastewater passes through nitrification room, which was then poured into denitrification room, the nitrate anion would be further converted into nitrogen gas with high efficiency. The low space occupation of both nitrification and denitrification room which are installed in fluidized bed reactor is of great convenience for continuous operation.

**ACKNOWLEDGEMENTS**

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**REFERENCES**


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EFFECT OF BORON FOLIAR APPLICATION AT THE DIFFERENT DOSES AND GROWTH STAGES ON YIELD AND YIELD COMPONENTS OF COTTON

(Gossypium hirsutum L.)

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ABSTRACT

This study was aimed to assess the effect of boron (B) foliar application at the different doses and different growth stages on the yield and yield components of cotton. The study was carried out at the experimental field of the Faculty of Agriculture Harran University in Eyyubiye in 2014 and 2015 growing seasons a randomized complete block in a split plot with three replications was employed. Stoneville 468 cotton cultivar was used as a plant material. The application three periods (square initiation period, beginning of flowering period and peak flowering period) placed in main plots and four boron doses (control, 1000, 2000 and 3000 ml ha⁻¹) in sub-plots. It was concluded that the B application had no significant effects on earliness ratio, ginning outturn and seed index. The highest seed cotton yield, number of sympodial branch, number of bolls and boll seed cotton weight were obtained from the interaction of flowering peak period x 1000 ml ha⁻¹. It was concluded that prior sowing B treatment is recommended at the presence at low soil B content and the complementary B must be given at the peak flowering.

KEYWORDS:
Cotton, boron, growth stages, application doses, yield

INTRODUCTION

Cotton is an important raw material of various branches of industry. Therefore, both farmers and other stakeholders are concerned. The industry requests high fiber quality, ginning outturn and seed oil ratio, while the farmer consider high yielding with good fiber quality, and resistant to the pest and diseases. All these demands depend on genetic potential variety and farmer practices. Plant nutrition is one of the factors affecting the increase of seed cotton yield in the unit area. One of the important micronutrients in cotton is boron.

All plants need B for their growth. Agricultur-
trol in seed cotton yield and fiber yield [6]. In one study the foliar B treatment provides an increase in the quality of fiber as well as the cotton yield [7], but on the contrary, some other studies reported that the additional B application did not have a positive effect on the cotton yield [8-10]. B treatment to cotton also increased the use of nitrogen [11]. Cotton is more sensitive to B deficiency the generative stages comparing the vegetative stages, especially at the flowering, fruiting, seed setting and seed yield [12]. Foliar B application can be more effective than the application to the soil, especially in the conditions where the amount of B is low; 1.1 kg B ha⁻¹ as foliar application increased yield by 11% compared to the control [13]; B application increased the number of bolls on the plant was reported [14]. B applications increased seed cotton yield (15.5 %), number of bolls and fiber yield [15]. B application increased seed cotton yield by 11.69 % and increased number of bolls and boll weight, but had no significant effects on fiber length, fiber fineness and fiber strength [16]. Foliar application with dose of 1000 g B ha⁻¹ increased seed cotton yield by 25 % [17].

This study was carried out to determine the effect of B applications on yield and yield components of cotton at the different doses and periods under Harran plain conditions.

MATERIALS AND METHODS

The field trials were carried out at the trial site of Agricultural Faculty of Harran University in Eyyubiye Campus (37°07'19.8"N, 38°49'01.4"E) in 2014 and 2015 growing seasons. The trial was laid out as randomized complete block design in a split plot with three replications. Growth stages (beginning of square initiation, beginning of flowering and peak flowering periods) were main plots and the B treatments (control, 1000, 2000 and 3000 ml ha⁻¹ of B) were sub-plots. Stoneville-468 (Gossypium hirsutum L.) cotton cultivar was used. Active ingredient B material of was B ethanol amin, which commercially name is SUPABOR (water soluble (8 %) % w/w). B treatments were applied as foliar application. B treatments were practiced at the beginning of square initiation (1-2 square per 1 meter), beginning of flowering period (1-2 square per 1 meter) and peak flowering period (8-10 flowers per 1 meter or 3-5 flowers per plant) [18]. The B was given to every plot by the back pump during the cool hours after 19.00 pm in the evening. Control plots were sprayed with water only.

Soil Properties. The soil samples were taken before experimental area sand analyzed for physical and chemical composition. The main material of the trial site was alluvial, deep profile. Whole profile had a high rate of lime and potassium, whereas beneficial phosphorus was poor [19]. The soil of the trial area was clay and the lime content was high. In addition, the pH was slightly alkaline and the B level was lower than 0.5 ppm, which was the critical limit for cotton cultivation normal i.e. (0.24 ppm and 0.22 ppm) (Table 1).

Sanliurfa is hot and dry in summers and moderate in winters. During the growth of cotton (April-November) the average temperature varied from 12.1 °C to 32.5 °C in 2014, from 15.7 °C to 33.2 °C in 2015 and the long term average ranged from 12.7 °C to 31.9 °C; maximum temperatures ranged from 22.8 °C to 43.5 °C in 2014, from 24.3 °C to 42.8 °C in 2015, and the long term average ranged from 29.4 °C to 46.8 °C; minimum temperatures ranged from 4.8 °C to 20.3 °C in 2014, from 4.7 °C to 22.1 °C in 2015, and the long term average ranged from -2.7 °C to 16 °C; the average rainfall amount were from 0 to 78.6 mm in 2014, from 0 to 58.8 mm in 2015, and long term average ranged from 0.7 to 46.6 mm; the average relative humidity varied from 26.4% to 53.9% in 2014, from 30.5 % to 50.5% in 2015, and the long term average ranged from 30.5 % to 60.2 % [21].

The trial areas were ploughed deeply in the autumn, and tilled in the spring by a cultivator. And then the clods were crushed by disc harrow. Sowing was practiced on 2th May in 2014 and on 22th April in 2015. Each plot was arranged in 4 rows of 10 m long, inter-row spaces were 70 cm and intra-row spaces were 20 cm. 80 kg ha⁻¹ of N and P (20-20-0) fertilizers were applied as basal at the sowing time and 80 kg ha⁻¹ pure N (33 % Ammonium Nitrate) was given just before first irrigation by lister plough. Considering the economic loss thresholds in plants during the plant growth; a single insecticide with the active ingredient Zetacypermethrin was used at a dose of 1250 ml ha⁻¹ for thrips tabaci and once against bollworm in 2014, no chemical was applied in 2015. No chemical was applied for diseases in both years. Since there was not enough moisture in the soil after planting, sprinkler irrigation was practiced to ensure a good germination in both years. In 2014: 4 times sprinkler, 7 times drip, in 2015; 3 times sprinkler, 7 times drip irrigation were applied.

While the first hand harvested on 27th September and the second hand harvested on 23th October in 2014; the first hand was harvested on 29th September and the second hand harvested on 27th October in 2015. Harvesting was made in the middle 2 rows of each plot by throwing 1 meter from the beginning and end of two rows (8 m x 1.4 m = 11.2 m²). The characteristics under study were scored according to Worley et al. (1976) [22].

The data obtained from the research were analyzed via JMP 11 (SAS Institute Inc.) statistical program as randomized complete block design in a split plot with three replications. Means were grouped according to Fisher’s LSD (p≤0.05) test.
RESULTS AND DISCUSSION

Prior to combined ANOVA perform the homogeneity of error variance were tested through F max test and they were not found homogenous. Therefore, a combined ANOVA was not performed.

F values obtained from variance analysis of the properties examined in the experimental years are given in Table 2.

### Table 1: Physical & chemical composition of experimental area at pre-planting

<table>
<thead>
<tr>
<th></th>
<th>Total Salt (%)</th>
<th>pH</th>
<th>Lime (%)</th>
<th>Sand (%)</th>
<th>Clay (%)</th>
<th>Silt (%)</th>
<th>Organic Matter (%)</th>
<th>P2O5 (kg ha⁻¹)</th>
<th>K2O (kg ha⁻¹)</th>
<th>Fe (ppm)</th>
<th>Zn (ppm)</th>
<th>B (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2014</td>
<td>0.098</td>
<td>7.70</td>
<td>5.4</td>
<td>24.16</td>
<td>53.84</td>
<td>22.00</td>
<td>1.23</td>
<td>35</td>
<td>1093</td>
<td>2.11</td>
<td>0.46</td>
<td>0.24</td>
</tr>
<tr>
<td>2015</td>
<td>0.089</td>
<td>7.65</td>
<td>5.6</td>
<td>25.13</td>
<td>54.61</td>
<td>20.26</td>
<td>1.26</td>
<td>34</td>
<td>1393</td>
<td>2.18</td>
<td>0.39</td>
<td>0.22</td>
</tr>
</tbody>
</table>

### Table 2: Variance analysis results of the properties examined in the experiment (F values).

<table>
<thead>
<tr>
<th>Factors</th>
<th>Seed Cotton Yield (kg ha⁻¹)</th>
<th>Earliness Ratio (%)</th>
<th>Plant Height (cm)</th>
<th>Number of sympodial branches (no. plant⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factor A</td>
<td>15.94 **</td>
<td>23.29 **</td>
<td>4.56 ns</td>
<td>0.10 ns</td>
</tr>
<tr>
<td>Factor B</td>
<td>52.84 **</td>
<td>68.70 **</td>
<td>2.53 ns</td>
<td>0.17 ns</td>
</tr>
<tr>
<td>A x B</td>
<td>25.73 **</td>
<td>17.39 **</td>
<td>1.11 ns</td>
<td>0.14 ns</td>
</tr>
</tbody>
</table>

Factor A: Growth Stages  
Factor B: Application Doses  
A x B: Interaction  
ns: not significant

* (p<0.05)  ** (p<0.01)

### Table 3: Average of seed cotton yield (kg ha⁻¹), earliness ratio (%), plant height (cm) and number of sympodial branch (no. plant⁻¹), LSD and CV% values

<table>
<thead>
<tr>
<th>Growth Stages</th>
<th>Seed Cotton Yield (kg ha⁻¹)</th>
<th>Earliness Ratio (%)</th>
<th>Plant Height (cm)</th>
<th>Number of sympodial Branch (no. plant⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SI</td>
<td>439.52 b*</td>
<td>489.20 b</td>
<td>86.00</td>
<td>86.08</td>
</tr>
<tr>
<td>BF</td>
<td>442.26 b</td>
<td>493.04 b</td>
<td>86.67</td>
<td>86.00</td>
</tr>
<tr>
<td>PF</td>
<td>543.81 b</td>
<td>617.69 b</td>
<td>85.92</td>
<td>85.67</td>
</tr>
<tr>
<td>LSD</td>
<td>7.46</td>
<td>12.24</td>
<td>ns</td>
<td>ns</td>
</tr>
</tbody>
</table>

LSD: Square Initation  
BF: Beginning of Flowering  
PF: Peak Flowering

*Within a column, means followed by the same letter are not significantly different at p<0.05  
**Factor A: Growth Stages  
**Factor B: Application Doses  
**A x B: Interaction  
ns: not significant

### Table 4: Average of seed cotton yield (kg ha⁻¹), earliness ratio (%), plant height (cm) and number of sympodial branch (no. plant⁻¹), LSD and CV% values

<table>
<thead>
<tr>
<th>Growth Stages</th>
<th>Seed Cotton Yield (kg ha⁻¹)</th>
<th>Earliness Ratio (%)</th>
<th>Plant Height (cm)</th>
<th>Number of sympodial Branch (no. plant⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SI X Control</td>
<td>437.38 de</td>
<td>474.84 d</td>
<td>86.33</td>
<td>85.33</td>
</tr>
<tr>
<td>SI X 1000 ml</td>
<td>464.52 b</td>
<td>530.84 bc</td>
<td>86.33</td>
<td>85.33</td>
</tr>
<tr>
<td>SI X 2000 ml</td>
<td>438.81 de</td>
<td>476.85 d</td>
<td>85.33</td>
<td>85.33</td>
</tr>
<tr>
<td>SI X 3000 ml</td>
<td>417.38 fg</td>
<td>474.42 d</td>
<td>86.00</td>
<td>85.33</td>
</tr>
<tr>
<td>BF X Control</td>
<td>405.47 g</td>
<td>437.97 e</td>
<td>86.67</td>
<td>85.33</td>
</tr>
<tr>
<td>BF X 1000 ml</td>
<td>459.52 bc</td>
<td>529.70 bc</td>
<td>86.67</td>
<td>86.33</td>
</tr>
<tr>
<td>BF X 2000 ml</td>
<td>431.19 ef</td>
<td>468.63 d</td>
<td>86.67</td>
<td>86.33</td>
</tr>
<tr>
<td>BF X 3000 ml</td>
<td>428.85 b</td>
<td>536.25 b</td>
<td>86.67</td>
<td>86.00</td>
</tr>
<tr>
<td>PF X Control</td>
<td>458.33 bc</td>
<td>523.09 bc</td>
<td>86.67</td>
<td>86.00</td>
</tr>
<tr>
<td>PF X 1000 ml</td>
<td>498.81 a</td>
<td>557.46 a</td>
<td>87.00</td>
<td>85.67</td>
</tr>
<tr>
<td>PF X 2000 ml</td>
<td>409.28 g</td>
<td>473.69 d</td>
<td>85.00</td>
<td>85.33</td>
</tr>
<tr>
<td>PF X 3000 ml</td>
<td>448.81 cd</td>
<td>516.50 c</td>
<td>85.00</td>
<td>85.67</td>
</tr>
<tr>
<td>LSD</td>
<td>14.91</td>
<td>19.04</td>
<td>ns</td>
<td>ns</td>
</tr>
</tbody>
</table>

CV %: 6.20  7.32  5.08  2.19  4.81  8.14  17.10  9.33

*Within a column, means followed by the same letter are not significantly different at p<0.05  
SI: Square Initation  
BF: Beginning of Flowering  
PF: Peak Flowering
The average seed cotton yield (kg ha⁻¹), earliness ratio (%), plant height (cm) and sympodial branch (no. plant⁻¹) from the experimental years, LSD and CV % values are given in Table 3, the averages number of bolls (no. plant⁻¹), boll seed cotton weight (g), ginning outturn (%) and seed index (g). LSD and CV % values are given in Table 4.

### Seed Cotton Yield (kg ha⁻¹)
Table 3 showed that, BF period (453.81 and 517.69 kg ha⁻¹), according to the application doses, 1000 ml application dose increased seed cotton yield by 10.66 kg ha⁻¹ and also 1.0 kg ha⁻¹ of foliar application increased seed cotton yield by 25 % [17].

**Earliness Ratio (%).** Earliness of the crop maturity is important in the avoidance of frost damage, insect and disease build up, soil moisture depletion and weathering of the open cotton.

Statistically no significant differences were found between growth stages and B doses in both years of the trial in terms of earliness ratio (Table 3). These results indicate that B applications have no effect on earliness ratio.

**Plant Height (cm).** In the first year of the trial, PF period (82 cm) and the dose of 1000 ml ha⁻¹ (81.28 cm) gave the highest plant height values, SI (103.12 cm) and FF periods (102.82 cm), 1000 ml ha⁻¹ as the application dose in the second year. In the interactions of growth stages x application doses, PF x 1000 ml ha⁻¹ interaction (498.81 and 557.46 kg ha⁻¹) gave the highest seed yield. It was reported that cotton is less sensitive to B deficiency in the vegetative growth period compared to the generative growth period [23]. B deficiency in flowering and fruiting periods can significantly increase flowers shedding and decrease fiber yield in cotton production [24]. The results indicated that, when the B was poor in the soil 1000 ml ha⁻¹ of B to the foliar application during PF period may increase the seed cotton yield. Foliar application of micronutrients during the flowering and boll growth stages was reported to be effective in the efficient use of nutrients by cotton, thus reducing boll falling and increasing cotton yield [25].

### Table 4

<table>
<thead>
<tr>
<th>Growth Stages</th>
<th>Number of Bolls (no plant⁻¹)</th>
<th>Boll Seed Cotton Weight (g)</th>
<th>Ginning Outturn (%)</th>
<th>Seed Index (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SI</td>
<td>14.96</td>
<td>12.41</td>
<td>3.90</td>
<td>4.54</td>
</tr>
<tr>
<td>BF</td>
<td>14.72</td>
<td>11.58</td>
<td>3.92</td>
<td>4.50</td>
</tr>
<tr>
<td>PF</td>
<td>15.09</td>
<td>12.48</td>
<td>3.95</td>
<td>4.57</td>
</tr>
<tr>
<td>LSD</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
</tbody>
</table>

**Application Doses**

<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>14.42 c*</td>
<td>11.67 c</td>
<td>3.91 bc</td>
<td>4.43 c</td>
<td>41.78</td>
<td>42.17</td>
</tr>
<tr>
<td>1000 ml</td>
<td>16.17 a</td>
<td>13.14 a</td>
<td>3.94 ab</td>
<td>4.72 a</td>
<td>41.54</td>
<td>41.30</td>
</tr>
<tr>
<td>2000 ml</td>
<td>13.74 c</td>
<td>11.27 d</td>
<td>3.86 c</td>
<td>4.40 c</td>
<td>41.85</td>
<td>41.87</td>
</tr>
<tr>
<td>3000 ml</td>
<td>15.36 b</td>
<td>12.54 b</td>
<td>3.98 a</td>
<td>4.60 b</td>
<td>41.68</td>
<td>42.21</td>
</tr>
<tr>
<td>LSD</td>
<td>0.73</td>
<td>0.33</td>
<td>0.06</td>
<td>0.08</td>
<td>ns</td>
<td>ns</td>
</tr>
</tbody>
</table>

**Interactions**

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>SI X Control</td>
<td>15.37 bcd</td>
<td>12.27 c</td>
<td>4.07 a</td>
<td>4.47 de</td>
<td>41.86</td>
<td>42.09</td>
</tr>
<tr>
<td>SI X 1000 ml</td>
<td>15.17 bcede</td>
<td>12.80 bc</td>
<td>3.90 bc</td>
<td>4.67 bc</td>
<td>41.52</td>
<td>41.36</td>
</tr>
<tr>
<td>SI X 2000 ml</td>
<td>14.00 efgh</td>
<td>12.30 c</td>
<td>3.79 d</td>
<td>4.57 cd</td>
<td>41.94</td>
<td>41.36</td>
</tr>
<tr>
<td>SI X 3000 ml</td>
<td>15.30 bcd</td>
<td>12.27 c</td>
<td>3.85 cd</td>
<td>4.47 cd</td>
<td>41.89</td>
<td>41.67</td>
</tr>
<tr>
<td>BF X Control</td>
<td>12.87 h</td>
<td>10.20 e</td>
<td>3.81 cd</td>
<td>4.23 f</td>
<td>41.50</td>
<td>42.17</td>
</tr>
<tr>
<td>BF X 1000 ml</td>
<td>16.37 ab</td>
<td>12.73 bc</td>
<td>3.86 cd</td>
<td>4.67 bc</td>
<td>41.77</td>
<td>40.40</td>
</tr>
<tr>
<td>BF X 2000 ml</td>
<td>13.47 gh</td>
<td>10.43 e</td>
<td>3.91 bc</td>
<td>4.35 ef</td>
<td>41.72</td>
<td>41.49</td>
</tr>
<tr>
<td>BF X 3000 ml</td>
<td>16.17 abc</td>
<td>12.93 b</td>
<td>4.08 a</td>
<td>4.77 ab</td>
<td>41.73</td>
<td>43.05</td>
</tr>
<tr>
<td>PF X Control</td>
<td>15.03 cdef</td>
<td>12.53 bc</td>
<td>3.84 cd</td>
<td>4.59 cd</td>
<td>41.97</td>
<td>42.26</td>
</tr>
<tr>
<td>PF X 1000 ml</td>
<td>16.97 a</td>
<td>13.90 a</td>
<td>4.07 a</td>
<td>4.83 a</td>
<td>41.34</td>
<td>42.13</td>
</tr>
<tr>
<td>PF X 2000 ml</td>
<td>13.77 fgh</td>
<td>11.07 d</td>
<td>3.88 cd</td>
<td>4.27 f</td>
<td>41.89</td>
<td>42.76</td>
</tr>
<tr>
<td>PF X 3000 ml</td>
<td>14.60 def</td>
<td>12.43 bc</td>
<td>4.00 ab</td>
<td>4.57 cd</td>
<td>41.42</td>
<td>42.92</td>
</tr>
<tr>
<td>LSD</td>
<td>1.27</td>
<td>0.57</td>
<td>0.11</td>
<td>0.14</td>
<td>ns</td>
<td>ns</td>
</tr>
</tbody>
</table>

**CV %**

|               | 8.89 | 8.87 | 2.96  | 4.26  | 2.94  | 2.49  | 5.36 | 5.48 |

*Within a column, means followed by the same letter are not significantly different at p≤0.05
SI: Square Initiation  BF: Beginning of Flowering  PF: Peak Flowering
height [16].

**Number of Sympodial Branch (no. plant$^{-1}$).** SI period (13.5 no. plant$^{-1}$) in 2014, PF period (12.50 per plant) in 2015, 1000 ml ha$^{-1}$ dose (12.59 and 13.20 no. plant$^{-1}$) in both years as the B doses formed the most number of sympodial branches [26]. In growth stages x application doses interactions in both years, PF x 1000 ml ha$^{-1}$ (15.60 and 13.93 no. plant$^{-1}$) interaction gave the highest number of sympodial branches (Table 3). The highest values from the PF x 1000 ml ha$^{-1}$ interaction in both years showed that this application resulted in the highest number of sympodial branches (15.60 and 13.93 no. plant$^{-1}$). The number of sympodial branches is one of the factors indirectly affecting the plant yield. Because the plant yield is related to the number of bolls on it. Too much sympodial branches on the plant increases the probability of more bolls and thus more yield.

**Number of Bolls (no. plant$^{-1}$).** No significant differences were found between B growth stages in terms of the number of bolls. However, 1000 ml ha$^{-1}$ application dose (16.17 and 13.14 per plant) gave the highest number of bolls in both years. In the growth stages x application doses interactions, PF x 1000 ml ha$^{-1}$ (16.97 and 13.90 per plant) interaction can be observed in table 4 which is the highest number of bolls in both years. The higher number of bolls per plant and the higher weight of seed cotton bolls are directly related to the high yielding from the unit area. Similar findings were suggested by some researchers that B application increases the number of bolls [14-16].

**Boll Seed Cotton Weight (g).** Growth stages were no statistically significant on the boll seed cotton weight. 3000 ml ha$^{-1}$ (3.98 g) in 2014, 1000 ml ha$^{-1}$ (4.72 g) in 2015 gave the highest boll seed cotton weight. The highest boll seed cotton weight was obtained from the interaction of PF x 1000 ml ha$^{-1}$ (4.07 and 4.83 g) in both years (Table 4). Taking the highest boll seed cotton weight from the same application in both years reveals that PF x 1000 ml ha$^{-1}$ interaction increases the seed cotton weight. As a matter of fact, taking the highest seed cotton yield from the interaction of PF x 1000 ml ha$^{-1}$ in the number of bolls and number of sympodial branches reveals that the cotton plant responses well to 1000 ml ha$^{-1}$ B application at the flowering peak period.

**Ginning Outturn (%) and Seed Index (g).** Statistically no significant differences were found between B growth stages, application doses and growth stages x application doses interactions in both years. This shows that B growth stages and application doses do not have any effect on ginning outturn and seed index.

**CONCLUSION**

It was concluded that no significant effect of B application was observed on the earliness ratio (%), ginning outturn (%) and seed index (g). Peak flowering (PF) x 1000 ml ha$^{-1}$ interaction yielded the best results in terms of seed cotton yield (kg ha$^{-1}$), number of sympodial branches (no plant$^{-1}$), number of bolls (no plant$^{-1}$) and boll seed cotton weight (g). B is necessary for the growth of all plants. In cotton, especially during flowering and boll growth period, B deficiency result in boll shedding and thus decrease in yield. In this study, it was also concluded that when B level was low in soil prior to sowing, additional B application was needed with 1000 ml ha$^{-1}$ at the peak flowering period.

**ACKNOWLEDGEMENTS**

This study was a part of (Cemile Ceylan) M.Sc. Thesis entitled "Effect of Boron Foliar Application at Different Doses and Different Growth Stages on Yield and Yield Components of Cotton (Gossypium hirsutum L.)", and was partially supported by the Harran University Scientific Research Board.

**REFERENCES**


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ABSTRACT

The citrus juice industry produces a huge amount of remains that are used as feedstuff ingredients. There is a constant concern to valorize the citrus by-products in terms of milk quality and quantity, environment, and economic aspects. The citrus products that are processed and evaluated are bitter orange, orange, grapefruit, and lemon; totally production amount is about 4 million tons in Turkey. After the citrus is processed to fruit juice, approximately 50-60% of pulp occurs. This amount shows that citrus pulp has significant economic potential for reuse and avoid environmental pollution. This study aims to evaluate the drying of orange pulps by different drying methods such as hot air drying (HAD), Infrared Radiation Drying (IRD) and combination of hot air and infrared radiation drying (HAD+IRD). The drying parameters, some macro, and minor elements, and organic acids of orange peels were explored in detail.

The drying time of orange pulps were 10; 13; 21, 26; 20; 17 hours for infrared radiation drying, hot air drying, a combination of them at 70 and 80 °C respectively. The macro elements found in orange pulps were obtained higher at 70 °C IRD comparing by other drying methods. Organic acids compositions were affected individually from drying type and temperatures. The whole study is donated by the Research Council of Turkey (TUBITAK) by 101R114 numbered project.

KEYWORDS:
Citrus pulp, Hot air, Infrared, Drying

INTRODUCTION

The orange (Citrus sinensis) is a fruit originating from Asia. It provides edible fruits through the whole tropical and subtropical lands [1]. Orange fruits are consumed as fresh, processed into juice and dried fruits. Moreover, citrus juice production is a source of dried pulp and molasses, fiber-pectin, cold pressed oils, essences, d-limonene, juice pulps, and pulp wash, ethanol, seed oil, pectin, ascorbic acid, limonoids, and flavonoids [2, 3]. Orange juice is one of the major consumed beverages today.

Furthermore, 50–60% of the processed fruit is turned into citrus peel waste [4]. Increased juice extraction or peeled fruits for various consumptions have increased the environmental risk, and huge economic loses from the remains which could be possible use as feedstuffs (BPF) as an alternative feeds for ruminant [5]. These components are in use of BPF either individually or in various combinations. The dried pulp is considerably additives for feed products. It is used extensively in rations for dairy cattle and has a high energy and protein content (4 – 7%) [2].

In addition to these orange peels contains the 10-12 percent of essential oil which contains the D-limonene and pectin that is used in an organic farm, food and medicinal, in cleaning agents and aromatic therapy area. On the other hand, Şahan [6] evaluated the use of essential oil of orange peel as an ingredient of feed additive in vitro condition. Orange peel essential oil improved the digestion of DM, OM, NDF (dry material, organic material, and neutral detergent fiber) respectively.

According to the statistical database of USDA, the global orange production forecast is about 53.4 million metric tons for 2016/2017 (USDA 2017). In the Global orange juice production for 2016/17 is forecast to surge to 2.0 million metric tons (Figure 1). Moreover, Turkey is the 7th rank in the world in orange producer countries (USDA, 2017). This data leads both researcher and investor to valorize and optimize the orange pulp usage. Therefore this study was conducted to evaluate the drying process of orange pulps. The drying process is a crucial step to achieve high-quality food and low operation cost, which must be optimized and evaluated.

Some researchers evaluated the citrus peel drying earlier. Some of the examples are; citrus peel drying kinetics in a fluidized bed with inert material was investigated [7], different drying temperatures (50, 60, 70, 80, 90 and 100 degrees C) on changes in the flavonoid, phenolic acid and antioxidative activities of citrus fruit (Citrus sinensis (L.) Osbeck) peels [8]; Ultrasound application and hot air drying was
applied to identify the ultrasound effect on dried orange peels [9]. Erdem et al. were dried the orange peel by microwave energy [10]. Hot air assisted microwave drying of orange peels were evaluated by [11]. Microwave drying of orange peel was investigated by Miller and Braddock [3]. On the other hand, the effect of dried orange peel in farm animals was explored by earlier researcher such as; Alzawqari et al. [12], Abbasi et al. [13], Alefzadeh et al. [14].

In this part of the study, the drying parameters of orange pulps and its macro and minor elements and organic acids were evaluated and optimized for hot air, infrared radiation and combination of hot air and infrared radiation drying at 70 and 80°C.

**MATERIALS AND METHODS**

Orange (Citrus sinensis L. Osbeck) fruits were used in this study and purchased from a local market. The whole samples were stored at 4±0.5 °C before the trial to slow down to physiological and chemical changes. Prior to drying, samples were taken out of the storage, and orange juice was extracted. After the extraction process, orange peels were sliced as 10x10 mm sizes. Drying applications were chosen as Infrared drying (IRD), hot air drying (HAD) and combination (HAD+IRD) of them. Drying parameters, organic acids, macro, and microelements were explored in detail.

**Drying equipment.** The whole trial was performed in the laboratory by using an infrared heater installed on hot air oven. Determination of mass decrease was obtained using a digital balance with 0.01 g accuracy (Sartorius GP3202, Germany). According to drying conditions, moisture losses were recorded at 1-hour interval during drying with a sample on the digital balance periodically [15].

**Drying procedure.** Infrared drying was done in hot air oven with installed Infrared heater on the top. Infrared heater output power was 800W. The experiments were performed at 70 and 80 °C. Before the drying initial moisture content was determined by using moisture analyzer (Ohaus Mb45). To set up the temperature for IRD, the thermocouple and PID were adjusted to the system to ensure the temperature in pulps. The temperatures of orange pulp slices were controlled with thermocouple and PLC devices due to infrared drying. The samples were dried under the 10% of wt basis moisture content of itself. The moisture ratio of orange pulps was calculated using the following equation:

\[
(MR) = \frac{M - M_e}{M_0 - M_e}
\]

Where, \(MR\), \(M\), \(M_0\) and \(M_e\) are the moisture ratio, moisture content at any time, initial and equilibrium moisture content, respectively.

**Macro and Micro Element Analyses.** ICP-MS (Agilent, 7500a) was used. To the sample preparation, 0.5 g (dry mass) of fruit sample was weighed into the digestion vessels. Then 10 ml of concentrated HNO3 (Merck) decomposed at CEM Mars 5 model microwave oven (CEM Corporation Mathews, NC, USA). The fruit samples were digested according to the following optimized program (power in W / time in min): 1200/28, ventilation 10 min. The internal temperature was limited to 180°C during the last step and ventilation. After cooling the entire digest were transferred into 50 ml plastic bottles and diluted to 50 g with double deionized water and centrifuged at 4000 rpm in 30 min. Reagent
blanks were prepared similarly to the samples. All sample solution was clear and diluted 10 times before analysis.

**Organic acid Analyses.** Analyses of organic acids (malic acid, citric acid, succinic acid, fumaric acid) in pulp samples According to the method developed by Bozan et al. [16], UV detector and Pre-vail organic acid column with 3 HPLC methods (Agilent HP 1100 series) (150 mm x 4.6 mm, 5 µ). The organic acid contents in the samples were quantitatively and qualitatively determined at 242 nm wavelength according to the calibration curves generated using the external standard and according to the retention time of the standard.

**Statistical Analyses.** The experimental datum was subjected to the statistical analyses by using SPSS 21.00 software program for One-Way ANOVA and Multivariate method.

### RESULTS AND DISCUSSIONS

Drying time and methods affect food quality and energy consumption. Orange pulps dried at infrared drying methods decreases the drying time and energy consumption. The drying process lasted 10-13 hours at 70 and 80 °C respectively. Hot air drying of orange pulps had prolonged drying time by 26 hours at 70 °C and 21 hours at 80 °C. The results demonstrated that the use of hot air assisted infrared radiation had relatively short drying time by comparing hot air drying with the result of 20 and 17 hours at 70 and 80 °C drying temperatures respectively (Table 1).

Mass decreases of orange pulps were recorded with one-hour interval, and MC and MR slopes versus drying times are illustrated in Fig 2 and Fig 3.

Drying is conventional methods in food preservation, and it is a problematic food process which causes undesirable changes in the quality of the dried product [18]. So that drying times and the energy consumption is not only parameters to determine the best drying applications. Although some other parameters are in consider to choose the drying applications. To valorize the orange pulps as BPF some macro and minor elements and organic acid compounds were explored to optimize the drying operation (Table 2). Statistical analyses were performed at SPSS programs with One-Way ANOVA (Duncan) methods. Also drying methods and temperature effect on macro and minor elements on orange peels were found significantly important (p<0.05) depend on multivariate analysis.

#### TABLE 1

<table>
<thead>
<tr>
<th>Type of Drying</th>
<th>Temp °C</th>
<th>Initial MC (%)</th>
<th>Final MC (%)</th>
<th>Drying Time (hour)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infrared Drying (IRD)</td>
<td>70 °C</td>
<td>73.32</td>
<td>4.43</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>80 °C</td>
<td>76.06</td>
<td>0.49</td>
<td>10</td>
</tr>
<tr>
<td>Hot Air Drying (HAD)</td>
<td>70 °C</td>
<td>77.68</td>
<td>1.77</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>80 °C</td>
<td>76.46</td>
<td>5.88</td>
<td>21</td>
</tr>
<tr>
<td>Hot Air Assisted Infrared Drying (HAD+IRD)</td>
<td>70 °C</td>
<td>78.65</td>
<td>6.60</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>80 °C</td>
<td>76.06</td>
<td>4.74</td>
<td>17</td>
</tr>
</tbody>
</table>

**FIGURE 2**

MC and Drying time of dried samples under different conditions
From the table, it can be concluded that IR drying of orange peels has higher C; N; P; K; Mn elements compared to the other drying procedures (p<0.05). The Macro and minor elements of orange peel were affected by temperature and drying type. When the temperature was increased, macro and minor elements, the amount of orange peel was decreased. Combination of IR and HA applications had the worst effect on macro and minor elements of orange peel.

The Macro and minor elements found in Turkish orange peel were also investigated by Ozcan et al. [18]. They reported that 0.83% P; 1.65% of K found in orange peels. In our trial, P amounts were found between 0.80-1.16%, and K amounts were found between 1.04-1.51%. The value of N in our trial was highest at IR drying with the value of 1.16% lowest at HAD+IRD 70 °C with a value of 0.81%.

The effects of different drying methods (IRD, HAD and IRD+HAD) and the different temperatures (70 °C and 80 °C) on the organic acid content of orange pulps are given in Table 3. The data shows that malic acid (P <0.05) and L-ascorbic acid (P < 0.05) values were affected by the drying methods used and the highest value was observed in the IR drying method. Sakai and Mao [19] reported that the loss of vitamin C and β-carotene by IR and chlorophyll and color degradation in vegetable drying is less than the conventional drying methods, which is consistent with the results of the present study.

<table>
<thead>
<tr>
<th>Sample</th>
<th>C (%)</th>
<th>N (%)</th>
<th>P (%)</th>
<th>K (%)</th>
<th>Fe (mg/L)</th>
<th>Mn (mg/L)</th>
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</thead>
<tbody>
<tr>
<td>IRD 70 °C</td>
<td>61.2</td>
<td>1.16</td>
<td>1.6</td>
<td>1.51</td>
<td>5.01</td>
<td>0.43</td>
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<tr>
<td>IRD 80 °C</td>
<td>60.4</td>
<td>1.08</td>
<td>1.1</td>
<td>1.44</td>
<td>1.06</td>
<td>0.19</td>
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<tr>
<td>HAD 70 °C</td>
<td>58.7</td>
<td>1.09</td>
<td>0.9</td>
<td>1.08</td>
<td>5.57</td>
<td>0.39</td>
</tr>
<tr>
<td>HAD 80 °C</td>
<td>58.2</td>
<td>1.02</td>
<td>0.75</td>
<td>1.04</td>
<td>1.99</td>
<td>0.39</td>
</tr>
<tr>
<td>IRD+HAD 70 °C</td>
<td>57.3</td>
<td>0.81</td>
<td>0.82</td>
<td>1.25</td>
<td>2.40</td>
<td>0.34</td>
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<tr>
<td>IRD+HAD 80 °C</td>
<td>57.6</td>
<td>0.89</td>
<td>0.80</td>
<td>1.19</td>
<td>2.65</td>
<td>0.29</td>
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<td>0.00</td>
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<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>

**FIGURE 3**

MR and Drying time of dried samples under different conditions

**TABLE 2**

Macro and micro elements of dried samples

**TABLE 3**

The effects of different drying methods (IRD, HAD and IRD+HAD) and the different temperatures (70 °C and 80 °C) on the organic acid content of orange pulps
Proper Drying applications play an important role in product quality. For the orange peel, the IRD application was better than HAD and a combination of them in terms of drying time, energy demand, and product quality.

CONCLUSION

The main objective of this study was to optimize the drying methods and temperature for the recovery of orange waste. As a result, IR drying methods were better than HAD and a combination of them in terms of drying time, organic acid content, and some macro and minor elements.

IR drying is one of the new methods, which are very easy to install and maintenance. IR drying technologies are very suitable for belt dryer and cabinet dryer. Moreover, it could be even used for drum drying. IR has the advantage of direct and indirect heating. This property is a handy hand tool to design different drying and heating system.

On the other hand, there is a huge amount of biomass source after agricultural, forestry, and industrial process, which includes products, by-products, residues, and wastes. Orange pulp is an important source of recovery products in Turkey. Therefore it needs to evaluate and optimize the recovery process due to drying to ensure feed quality and sustainable agriculture and environment.

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REFERENCES


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SIMULTANEOUS SCREENING OF TOTAL AFLATOXINS (B₁, B₂, G₁, G₂) AND OCHRATOXIN A (OTA) IN COFFEE SAMPLES

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ABSTRACT

The aim of this survey was to determine the occurrence of total aflatoxins (B₁, B₂, G₁, and G₂) and ochratoxin A (OTA) from different coffee brands and types in the southeastern region of Turkey. Coffee samples (deep-roasted coffee (it is called ‘mirra’ in Turkey), powdered Turkish coffee, green coffee beans and instant coffee) were collected from supermarkets, retail coffee shops, cafes and touristic bazaars. Total aflatoxins and OTA were microbiologically detected by solid phase direct ELISA. The survey included 90 coffee samples. Survey results demonstrated that 39 (43%) were positive for the presence of total aflatoxins and 36 (%40) out of the 90 samples were positive for the presence of OTA. Total aflatoxin concentrations were found in the range 0.08 – 42.81 μg kg⁻¹, only 11 of them exceeded the maximum limit of 10 μg kg⁻¹ which is allowed by the European Union for total aflatoxins. OTA levels were range from 0.10 to 41.28 μg kg⁻¹; only 9 of the samples exceeded the maximum allowed a limit of 5.0 μg kg⁻¹, which is set by the Turkish Food Codex and the European Union. The mean relative humidity level of coffee samples was 5.35. The results of this study has shown the significance of government control programmes in coffee production and sale. This study assessed for occurrence of total aflatoxins and OTA in coffee from different types and brands of coffee.

KEYWORDS:
Coffee, ELISA, ochratoxin A, total aflatoxins, Turkey

INTRODUCTION

Coffee is the most widely traded agricultural product infusion from beans of coffee plant which belongs to the genus of *coffee rubiaceous*. The most commercial species are *coffee arabica*, coffee robusta, and *coffee liberica* [1]. Different types of coffee improved like toasted coffee which is roasted without any additives, roasted coffee that is processed with sugar and instant coffee which is the extract of coffee by drying or dehydration [2]. Turkish coffee is a kind of drink offered by the Turks with different preparation and cooking methods. This coffee is obtained by grinding coffee beans very tiny after roasting on the heavy coal fire [3]. Deep-roasted coffee is a kind of coffee obtained from the roasting and the beating of the beans in mortar with thinning very much and its name is derived from the Arabic word ‘murdan’ which means bitter and called ‘mirra’ in Turkey. Instant coffee is the coffee extract obtained by drying the coffee or removing the water [4]. According to the ICO, coffee is a widely traded agricultural commodity with a total production of 143.371.000 bags by all exporting countries. Global exports of coffee are 8.94 million bags in January 2016 in the world, and coffee export of Turkey is 103.000 bags in November 2015 [5]. Irrigation from the air, collection of products from the ground, use of contaminated water during product washing increase mycotoxin contamination in coffee production. Mycotoxins are secondary metabolites of fungi. Aflatoxins have been prevalently reported as contaminants of food in the Mediterranean region of the world [6]. Aflatoxins (AFs) are produced by various strains of *Aspergillus*, generally, *A. flavus*, *A. parasiticus*, *A. nomius* and *A. Tamarii* [7]. In cool climates, OTA is produced by penicillium species and in tropical or subtropical climates, OTA is produced by *Aspergillus* species [8]. *Aspergillus ochraceus*, *Aspergillus westerdijkiae*, *Aspergillus niger* and *Aspergillus carbonarius* are the main mycotoxin species that cause OTA in coffee [9]. Although, the development of OTA-producing fungi occurs in water activity below 0.80, the toxin production does not occur [10]. In coffee beans, water activity should be 0.80-0.95 for OTA toxin production and 0.81-0.87 for aflatoxin production [10, 11]. In many products, the relative humidity content should be above 9% for fungi, especially *Aspergillus* species in order to be able to produce toxins [12].

In the Coffee Guide published by the International Trade Center, although varies from country
to country, the level of relative humidity in all coffee varieties was generally set as 11% In coffee trade, products having over 12.5% relative humidity are not allowed to be loaded on the ship [13]. According to Turkish Food Codex-Coffee Communique, the level of relative humidity in ground coffee products was determined as 4% [14]. Processes for reducing moisture and roasting cause the reduction of mycotoxin amount in coffee beans.

Aflatoxins and ochratoxins are mycotoxins, which found as a contaminant in different food and food products such as cereals, nuts, grapes, beer, poultry meat, grape juice, cocoa and coffee [4, 6, 11]. International Agency for Research on Cancer (IARC) classified aflatoxins as OTA as possibly group 1 and group 2B human carcinogen, respectively [15, 16]. European Food Safety Authority (EFSA) [17] and Joint Expert Committee on Food Additives (JECFA) [18] have set a provisional tolerable daily intake of aflatoxins and ochratoxins (PTDI) levels of 17 ng kg^{-1} bw/day and 14 ng bw/day respectively. Aflatoxins and ochratoxins are a carcinogenic, nephrotoxic, immunotoxic, teratogenic, fetotoxic and hepatotoxic agents [12, 19]. The European Union has set maximum level as 5 ng g^{-1} for OTA in roasted coffee and coffee beans. The limit for instant coffee is 10 ng g^{-1} [20]. However, the maximum limit has not been determined for aflatoxin in roasted and instant coffee [20].

ELISA is a simple, rapid and easy detection of aflatoxins and ochratoxins in manipulation and ELISA levels has been well developed [19]. With regard to roasted coffee, powdered Turkish coffee, instant coffee many surveys have been researched in the worldwide [4]. Although there are several studies to determine the amount of aflatoxins and ochratoxins in coffee varieties in the world [1, 21-27]. There is limited number of studies on coffee in Turkey [3]. The aim of this study was to determine the occurrence of total aflatoxins (B1, B2, G1, G2) and ochratoxins in coffee from different types and brands collected in the southeastern region of Turkey during January-April 2016.

MATERIALS AND METHODS

Samples. A total of 90 coffee samples (deep-roasted coffee, powdered Turkish coffee, green coffee bean, and instant coffee) were obtained from supermarkets, retail coffee shops, cafes and touristic bazaars located in Şanlurfa province of Turkey. Twenty-four packaged coffee types (4 different brands) and 45 unpacked coffee types (11 different brands) randomly purchased from supermarkets, retail coffee shops, and touristic bazaars. Twenty-one instant coffee types (6 different brands) purchased from cafes. Forty-one samples were granulated, five samples were green coffee beans, twelve samples were mirra, thirty-two were powdered Turkish coffee. The coffee samples were measured out into 100-200 g samples and brought to the laboratory at 4 °C for analysis.

Method for total aflatoxins. The concentrations of total aflatoxins were determined using a commercial test kit (Helica Biosystems Inc. Total Aflatoxin Assay CAT. NO.941AFL01M-96) with a solid phase direct competitive enzyme immunoassay. The Helica Total Aflatoxin Assay test kit included microtiter plates coated with antibodies, dilution wells, aflatoxin standards (1.5 mL/vial each 0.0 ng/mL, 0.2 ng/mL, 0.5 ng/mL, 1.0 ng/mL, 2.0 ng/mL, 4.0 ng/mL), peroxidase-conjugated AFB1, substrate (stabilized tetramethylbenzidine) and stop reagent (acidic acid). Methanol was purchased from Merck (Germany).

Firstly extraction solution (70% methanol) was prepared by adding 30 mL of distilled water to 70 mL of methanol (reagent grade) for each sample to be tested. Then a 20 g ground portion of the sample weighed out and 100 mL of the extraction solvent (70% methanol) was added. So the ratio of sample to extraction solvent was 1:5 (w/v). All samples were mixed by shaking in a sealed container for 5 minutes. 5-10 mL of the extracted samples were filtered through a Whatman #1 filter paper. One dilution well was placed in a microwell holder for each standard and sample to be tested. And also an equal number of antibody-coated microtiter wells were placed in another microwell holder. 200 mL of the conjugate was dispensed into each dilution well. Using a new pipette tip for each, 100 mL of each standard and sample were added to appropriate dilution wells containing conjugate and were mixed by priming pipettor at least 3 times. Wells were incubated at room temperature for 15 minutes. After incubation, contents were decanted into a discard basin, the microwells were washed by filling each with distilled water for a total of 5 washes. 100 mL of substrate reagent was added to each microwell. Incubated at room temperature for 5 minutes and covered to avoid direct light. 100 mL of stop solution was added to each well and optical density (OD) of each microwell were read with a microtiter plate reader using a 450 nm filter.

Method for ochratoxin A. The concentrations of Ochratoxin A was determined using a commercial test kit (Helica Biosystems Inc. Ochratoxin A in coffee, cocoa, and spices, CAT. NO.961OCH01COF-96) with a solid phase direct quantitative enzyme immunoassay. The Helica Ochratoxin A Assay in coffee, cocoa and spices test kit included microtiter plates coated with antibodies, mixing wells, Ochratoxin A standards (1.5 mL/vial each 0.0 ng/mL, 0.2 ng/mL, 0.5 ng/mL, 1.0 ng/mL, 2.0 ng/mL, 4.0 ng/mL), Ochratoxin A HRP-conjugate, substrate (stabilized tetramethylbenzidine), wash buffer (PBS-T), assay...
diluent and stop reagent (acidic acid). Methanol was purchased from Merck (Germany). Firstly extraction solution (70% methanol) was prepared by adding 30 ml of distilled water to 70 ml of methanol (reagent grade) for each sample to be tested. Then a 10 g ground portion of the sample weighed out and 50 ml of the extraction solvent (70% methanol) was added. So the ratio of sample to extraction solvent was 1:5 (w/v). All samples mixed by shaking in a sealed container for 5 minutes. 5-10 ml of the extracted samples filtered through a Whatman #1 filter paper. Each clarified extract diluted with 70% methanol in distilled water at 10:1 ratio. One dilution well placed in a microwell holder for standards and samples to be tested. And also an equal number of antibody-coated microtiter wells placed in another microwell holder. 200 ml of the assay diluent dispensed into each mixing well. Using a new pipette tip for each, 100 ml of each standard and sample added to appropriate mixing well-containing assay diluent and mixed by priming pipettor at least 3 times. Wells incubated at room temperature for 30 minutes. After incubation contents decanted into a discard basin, the microwells washed by filling each with distilled water for a total of 3 washes. 100 ml conjugate added to each microwell. Incubated at ambient temperature for 30 minutes and covered to avoid direct light. The microwells washed by filling each with distilled water for a total of 3 washes again. 100ml substrate reagent added and incubated for 10 minutes at ambient temperature. Stop solution added to each well and read the optical density (OD) of with a microtiter plate reader using a 450 nm filter using an air blank.

Detection of moisture. Moisture determinations of coffee samples were carried out in duplicate by following the instructions of the MOC63 Shimadzu (Japan) humidity measuring device.

Validation of the method. The recovery rate of OTA was detected in powdered Turkish coffee and instant coffee at a concentration of 0.2 μg kg⁻¹ in three parallel tests. The recovery rates of OTA from Turkish coffee and instant coffee were 87% and 93% respectively.

RESULTS AND DISCUSSION

Detection of aflatoxins and ochratoxins by ELISA is rapid and reliable. Thirty-nine samples contained total aflatoxins ranged from 0.08 to 42.81 μg kg⁻¹ with the mean level of 19.03 μg kg⁻¹ as shown in Table 1. Thirty-six samples contained OTA ranged from 0.10 to 41.28 μg kg⁻¹ with the mean level of 11.03 μg kg⁻¹ as shown in Table 2.

Of the 90 coffee samples, nine samples (10%) were found to be contaminated with OTA, which surpassed the levels established as guideline levels (10.00 μg kg⁻¹) in Turkey (Table 2). According to the Turkish regulations, ochratoxin A limits were set as 5.00 μg kg⁻¹ for coffee beans, powdered coffee (including all types) and ochratoxin A limits were set as 10.00 μg kg⁻¹ for instant coffee. 43% of the samples were found to contain total aflatoxins with the levels between 0.00 and 42.81 μg kg⁻¹ with the mean level of 12.74 μg kg⁻¹ as shown in Table 1. Eleven samples (12%) were found to be contaminated with total aflatoxins which exceed the levels (10.00 μg kg⁻¹) established in European Union Commission guideline levels for coffee. It was determined that the level of relative humidity in the analyzed coffee samples, except green coffee beans, was over 4%, which was set for the ground coffee in the Turkish Food Codex - Coffee Communiqué (Table 3) [14, 20].

| TABLE 1 | The occurrence and the distribution of total Aflatoxins levels in coffee samples |
| Sample (n) | Percentage of contamination | Range of contamination (μg/kg) | Mean (SD) (μg/kg) | Positive sample n (%) |
| Powdered Turkish coffee (32) | 10 (11%) | 1.50-22.50 | 7.00 (8.11) | 2 |
| Deep-roasted coffee (12) | 1 (1%) | 0.00-0.20 | 0.040 (0.05) | 0 |
| Green coffee bean (5) | 0 (0%) | 0.00-0.00 | 0.00 | 0 |
| Instant coffee (41) | 28 (31%) | 2.65-42.81 | 12.62 (10.98) | 7 |
| Total (90) | 39 (43%) | 0.00 – 42.81 | 12.74 (13.04) | 11 |

| TABLE 2 | The occurrence and the distribution of Ochratoxin A levels in coffee samples |
| Sample (n) | Percentage of contamination | Range of contamination (μg kg⁻¹) | Mean (SD) (μg kg⁻¹) | Positive sample n (μg/kg) |
| Powdered Turkish coffee (32) | 10 (11%) | 0.46-22.50 | 7.00 (8.11) | 2 |
| Deep-roasted coffee (12) | 0 (0%) | 0.00 | 0.00 | 0 |
| Green coffee bean (5) | 0 (0%) | 0.00 | 0.00 | 0 |
| Instant coffee (41) | 26 (25%) | 0.48-41.28 | 13.64 (11.26) | 7 |
| Total (90) | 36 (40%) | 0.00 – 41.28 | 19.03 (10.74) | 9 |
Coffee is frequently consumed however it is contaminated with various pests and fungi of which mycotoxin contamination is on a large scale. This contamination has significant in the interest of health and economy [28]. Coffee quality directly related to the quality of coffee beans and processing influences. Coffee beans are contaminated by fungi during harvesting, preparing, transporting, storage and shelf-life. Most determined fungie along coffee production are *Penicillium*, *Aspergillus* and *Fusarium spp*. International Agency for Research on Cancer [15] classified these fungie’s mycotoxins that aflatoxins are declared carcinogenic and the other less toxic [29]. Countries such as European Countries, Brazil, Indonesia, Iran, Cuba, and Singapore have regulations for coffee. The European Union has set maximum level as 5 ng g⁻¹ for OTA in roasted coffee and coffee beans, and 10 ng g⁻¹ in instant coffee (EC [20]). In this study, OTA was determined above the legal limits (2/32, 6.25%) in powdered Turkish coffee and (7/41, 17.1%) in instant coffee. The separation of the coffee core from the OTA-containing shell, the isomerization of C3 in the less toxic diastereomer and the removal of the possible humidity by heat can lead to a reduction in the amount of OTA in the process of obtaining roasted coffee from green coffee beans. In many studies, it has been reported that OTA content is degraded between 50 and 100% during roasting [30, 31]. In the present study, aflatoxin and OTA were not found in green coffee seeds and deep-roasted coffee samples.

In the coffee samples examined, the values above the relative humidity of 9% required for the toxin production of Aspergillus species were determined only in the instant coffee samples. In the instant coffee samples, Aflatoxin and OTA were detected more than the other analyzed coffee types. Al-Abdalall and Al-Talib found that the total amount of aflatoxin in Turkish coffee at 2.342, respectively, by keeping them at room temperature for 30 days at 45, 25 and 10% relative humidity [27]. In this study, relative humidity (4.92%) and total aflatoxin content (7.00 μg kg⁻¹) are lower than the relative humidity and aflatoxin content determined by Abdullah Al-Abdalall and Al-Talib [27]. Soliman found 15.70 μg kg⁻¹ total aflatoxin content in 30 green coffee bean samples [22]. In our study, total aflatoxins were not found in the samples of green coffee beans. In the present study, the absence of total aflatoxin in green coffee beans may be due to the small amount of moisture (1.34%).

A number of studies have been carried out in the world to determine the presence of OTA in green coffee, roasted coffee and soluble coffee (Table 4). As seen in Table 4, the amount of OTA in green coffee and deep-roasted coffee samples analyzed in our study is below the values found in other countries. However, the amount of OTA in

### TABLE 3
The relative humidity levels in coffee samples

<table>
<thead>
<tr>
<th>Sample (n)</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean (SD)</th>
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</thead>
<tbody>
<tr>
<td>Powdered Turkish coffee (32)</td>
<td>2.89</td>
<td>8.96</td>
<td>4.92 (5.03)</td>
</tr>
<tr>
<td>Pee-roasted coffee (12)</td>
<td>2.24</td>
<td>7.14</td>
<td>4.62 (4.68)</td>
</tr>
<tr>
<td>Green coffee bean (5)</td>
<td>1.22</td>
<td>1.82</td>
<td>1.34 (0.83)</td>
</tr>
<tr>
<td>Instant coffee (41)</td>
<td>4.45</td>
<td>16.54</td>
<td>10.62(10.45)</td>
</tr>
<tr>
<td>Total (90)</td>
<td>1.22</td>
<td>16.54</td>
<td>5.35 (4.04)</td>
</tr>
</tbody>
</table>

### TABLE 4
The detection of OTA in green coffee, roasted coffee and soluble coffee in the World.

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Country</th>
<th>Total Sample/Positive Sample</th>
<th>Range (μg/kg)</th>
<th>References</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Green coffee</td>
<td>Japan</td>
<td>47/14</td>
<td>0.1-17.4</td>
<td>[23]</td>
<td>1997</td>
</tr>
<tr>
<td></td>
<td>USA</td>
<td>19/9</td>
<td>&gt;0.03</td>
<td>[33]</td>
<td>1999</td>
</tr>
<tr>
<td></td>
<td>Italia</td>
<td>162/106</td>
<td>0-48</td>
<td>[34]</td>
<td>2000</td>
</tr>
<tr>
<td></td>
<td>Colombia</td>
<td>42/6</td>
<td>8.4-13.9</td>
<td>[4]</td>
<td>2004</td>
</tr>
<tr>
<td></td>
<td>Egypt</td>
<td>45/27</td>
<td>&lt;5-5.66</td>
<td>[35]</td>
<td>2007</td>
</tr>
<tr>
<td></td>
<td>Argentina</td>
<td>5/5</td>
<td>0.2-20.30</td>
<td>[1]</td>
<td>2013</td>
</tr>
<tr>
<td></td>
<td>Spain</td>
<td>21/4</td>
<td>4.90-37.73</td>
<td>[36]</td>
<td>2014</td>
</tr>
<tr>
<td></td>
<td>Turkey</td>
<td>5/0</td>
<td>0.00</td>
<td>In this study</td>
<td>2017</td>
</tr>
<tr>
<td>Roasted coffee</td>
<td>ABD</td>
<td>13/9</td>
<td>&gt;0.03</td>
<td>[33]</td>
<td>1999</td>
</tr>
<tr>
<td></td>
<td>Brazil</td>
<td>34/23</td>
<td>0.3-6.5</td>
<td>[21]</td>
<td>2000</td>
</tr>
<tr>
<td></td>
<td>Egypt</td>
<td>30/19</td>
<td>&lt;5-8.35</td>
<td>[35]</td>
<td>2007</td>
</tr>
<tr>
<td></td>
<td>Argentina</td>
<td>24/13</td>
<td>0.11-5.78</td>
<td>[1]</td>
<td>2013</td>
</tr>
<tr>
<td></td>
<td>Turkey</td>
<td>12/0</td>
<td>0.00</td>
<td>In this study</td>
<td>2017</td>
</tr>
<tr>
<td>Soluble coffee</td>
<td>Canada</td>
<td>30/20</td>
<td>0.1&lt;</td>
<td>[37]</td>
<td>2002</td>
</tr>
<tr>
<td></td>
<td>Colombia</td>
<td>5/5</td>
<td>0.9-19.4</td>
<td>[4]</td>
<td>2004</td>
</tr>
<tr>
<td></td>
<td>Argentina</td>
<td>22/17</td>
<td>0.22-13.66</td>
<td>[1]</td>
<td>2013</td>
</tr>
<tr>
<td></td>
<td>Turkey</td>
<td>41/26</td>
<td>0.48-41.28</td>
<td>In this study</td>
<td>2017</td>
</tr>
</tbody>
</table>
soluble coffee samples was higher than the other studies. The consumption of coffee per capita in Turkey in 1997-2011 is approximately 400 grams/year (average 415 bags). In Turkey, 83.9% of coffee is consumed as instant coffee and 16.1% as roasted coffee [32]. The amount of OTA in the analyzed powdered Turkish coffee samples was determined as 7 µg kg⁻¹ in average and 13.64 µg kg⁻¹ in instant coffee. According to these results, the daily OTA exposure amounts were calculated as 0.0077 µg and 0.015 µg for Turkish coffee and instant coffee respectively. The specified amount is below the daily PTDI of 0.539 µg (Turkish coffee) and above 1.05 µg (instant coffee) PTDI for a person weighing 70 kg.

CONCLUSION

In conclusion, various commercial coffee varieties presented in Turkey are influenced by various factors such as moisture, contamination grade, storage, heat in mycotoxins contamination, and this study has revealed problems and their solutions in coffee presented for consumption in Turkey. The levels reported in this study point out that it is essential to evaluate the effect of technological and culinary practices to detect the human exposure.

ACKNOWLEDGEMENTS

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REFERENCES


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ANNUAL RUNOFF MODELLING OF KIZILIRMAK BASIN BY ARTIFICIAL INTELLIGENT TECHNIQUES

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⁴Civil Engineering Department, Faculty of Engineering and Architecture, Gaziantep University, 27000, Gaziantep, Turkey

ABSTRACT

Estimation and modelling of meteorological parameters are very important while management and planning of water resources are being design. In this study, the equations which can be used for modeling the annual values of the meteorological data of Kizilirmak Basin is derived from ANN (Artificial Neural Network), GEP (Gene Expression Programming) and Regression analysis program (Datafit). In this regard, measuring data is used to associate on flow for a better understanding of the agreement among testing model fit. The aim of this study is to acquire the formulations which can be used in the flow estimation under influence of different meteorological parameters for Kizilirmak Catchment. The figures were considered from the nonlinear regression analysis. During the analysis, precipitation, humidity and temperature were used as input parameters and discharge was used as output parameter. Also Mean Square Error (MSE), Root mean square error (RMSE), Coefficient of Determination (R²) and Adjusted coefficient of Determination (AdjR²) parameters were calculated for each methods (ANN, GEP and Datafit). The obtained equations were evaluated for each models respect to Meteorological and flow data. Overall, the study demonstrated a good capturing skill of GEP driven flow estimations relative to observation data and model results. The applied approaches developed in this study can motivate future studies over basins study storm event analysis beyond hydrological modelling.

KEYWORDS:
Annual runoff prediction, Kizilirmak watershed, ANN, GEP, Datafit, Meteorological parameters.

INTRODUCTION

Water is very important for humans and the life of the various living organism on the surface of the Earth. “The importance of water is increasing day by day. A water supply is vital for human life, habitat for aquatic biota but too much water causes natural disasters such as flood in some cases”. It is necessary to obtain the flood discharge to protect the environment [1]. So the observation of meteorological parameters that effects the discharge in a basin is very important. The data which obtained from the hydro-meteorological station value are used in the design of water structures (such as bridges, culverts, spillways and irrigation channels). Precipitation, temperature, humidity, evaporation etc. are very important and effective on discharge. So the relationship between other parameters and discharge should be known. It is necessary to determine the flow or the amount of water in the design of hydraulic structure. Because construction cost and flood risk are important for water structure.

In recent years, the modeling of precipitation-flow relations is usually prepared with closed box models where the physical aspect of the event is not taken into account and the details of process is not elaborated. Models are registered as mathematical functions established between input and output variables. In this regard, improved models benefit from the delays between the occurrence of precipitation and the flow occurring at the river stream outlet. In addition, these models are generally based on classical stochastic time series, classical regression analysis and artificial neural networks which recently increase in importance [2].

There are various methods used to estimate the relationship between rainfall and flow in the literature. However, these methods are not separate for each basin. Furthermore, since the characteristic features of each basin are different, applying the same function to all basins may cause different error rates. Soil properties, land slope, vegetation, precipitation type affect the amount of occurring flow in a basin. If there are some meteorological parameters and flow rate belonging to a specific period range for a basin, it is possible to establish a relation between these parameters and the flow rate in the basin. The methods which used for basin flow models are based on the momentum, mass and energy conservation laws of physics. These law-
based equations are used to determine the relationship between the characteristic features of the basin [3].

According to a review [4] was demonstrated a flow and a precipitation station on the Aksu stream in the Giresun region, in Turkey and evaluated their data. Five input models were designed using daily total precipitation and daily average flow data. In these models, Levenberg-Marquardt (LM), Quasi Newton (QN) and Logarithmic Sigmoid (LS) were used as training algorithms in ANN (Artificial Neural Network). In addition, two different functions have tried hidden transfer numbers. These functions are Hyperbolic Tangent Sigmoid (HTS) and Logarithmic Sigmoid (LS). The study demonstrated that provided results are in the acceptable range respect to observations. However, YSA-QN-HTS has the best Correlation Coefficient (R), Root Mean Square Error (RMSE) and Nash-Sutcliffe Coefficient (E).

ANN models were developed on Sarada River basin using rainfall - runoff hydrological modelings by [5]. The data between (2001-2007) and (2008-2010) were used for the calibration (validation) purpose. Thiessen polygon was drawn using Arc-GIS on the basin map. RMSE, R², ENS (Nash-Sutcliffe coefficient efficiency) and MAE (Mean absolute error) were calculated for the basin. The best R² value was calculated as 0.783 and 0.877 using 3-year and 7-year data period, respectively.

The effects of runoff can be investigated in different time periods. In hydrological researches on this subject, monthly, annual or seasonal behaviours of data can be examined. Studies which are determined on a monthly data are important in order to examine the changes of meteorological data in different periods. Monthly data were evaluated for 10 different watersheds by [6]. Precipitation and runoff were then plotted as 12 points that were connected in the chronological monthly sequence to obtain a polygon for each catchment. For this reason that as the amount of rainfall and flow in the season changed, they were examined more clearly on a monthly periods.

In this study, the relationship between flow and meteorological parameters (precipitation, temperature, humidity) was estimated by using different artificial intelligence methods in Kizilirmak River Basin.

MATERIALS AND METHODS

ARC-GIS and Study area. The Kizilirmak River Basin is located between 37°58' N - 41°44' N latitude and 32°48' E - 38°22' E longitude in the eastern part of Central Anatolia. The Kizilirmak is the longest river situated entirely within Turkey, with a total drainage area of about 78180 km². The river flows for a total length of 1355 km, rising in Eastern Anatolia around 39.8°N - 38.3°E, at an altitude of around 2000 m above sea level. The watershed generally reflects the Central Anatolian continental climate, while a small section is the effect of climate of the Black Sea. The river reaches its highest level of the water regime in April, while it flows in the lowest water level between February and July [7].

Arc-GIS is a software that provides mapping, geographic analysis, data update, data management and image processing with integrated interfaces. Different layers can be added on satellite image with the help of Arc-GIS 10.1 software and Arc-Hydro toolbar which is a geographical information system. The basic science of hydrology, which deals with the formation, distribution, movement and properties of water on earth and underground, constitutes the water resources engineering. Geographic information systems software enables users to produce, manage and analyze geographic information and to present them to people through network databases. Areas of application of GIS to water resources engineering; Surface water and groundwater hydrology, water supply for municipalities and irrigation, wastewater and storm water, floodplains, water quality, monitoring and warning, river basins [8, 9].
In this study, using the Digital Elevation Model (DEM) of Turkey, Kizilirmak watershed boundary was drawn by Arc-GIS program. The basin location is shown on Digital Elevation Model (DEM) of Turkey in Figure 1. After the basin boundary was determined, the rainfall, temperature, humidity and flow stations were placed according to their coordinates on it. The rainfall, temperature and humidity stations are shown in Figure 2a and the flow stations in Figure 2b on the basin. The information is given about the stations which located on Kizilirmak basin in Table 1 and Table 2.

![Image](image_url)

**FIGURE 2**

a. Rainfall, temperature and humidity stations’ location and their thiessen polygons.
b. Flow stations’ location Red colors are closed station. Greens are open stations [10]

### TABLE 1

Meteorological stations on Kizilirmak

<table>
<thead>
<tr>
<th>Station Code</th>
<th>Station Name</th>
<th>Observation range</th>
<th>Precipitation height (mm/year)</th>
<th>Latitude</th>
<th>Longitude</th>
<th>Elevation (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>17756</td>
<td>Kaman</td>
<td>1975-2005</td>
<td>471.38</td>
<td>39.3652</td>
<td>33.7064</td>
<td>1075</td>
</tr>
<tr>
<td>17760</td>
<td>Bogazliyan</td>
<td>1975-2005</td>
<td>374.97</td>
<td>39.1897</td>
<td>35.2532</td>
<td>1070</td>
</tr>
<tr>
<td>17802</td>
<td>Pınarbaşı</td>
<td>1975-2005</td>
<td>412.08</td>
<td>38.7224</td>
<td>36.3924</td>
<td>1542</td>
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<tr>
<td>17734</td>
<td>Divriği</td>
<td>1975-2005</td>
<td>391.16</td>
<td>39.3618</td>
<td>38.1142</td>
<td>1121</td>
</tr>
<tr>
<td>17684</td>
<td>Suşehri</td>
<td>1976-2005</td>
<td>420.33</td>
<td>40.1623</td>
<td>38.0752</td>
<td>1164</td>
</tr>
<tr>
<td>17086</td>
<td>Tokat</td>
<td>1975-2005</td>
<td>445.71</td>
<td>40.3312</td>
<td>36.5577</td>
<td>611</td>
</tr>
<tr>
<td>17084</td>
<td>Çorum</td>
<td>1975-2005</td>
<td>447.08</td>
<td>40.5461</td>
<td>34.3962</td>
<td>776</td>
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<tr>
<td>17080</td>
<td>Çankırı</td>
<td>1975-2005</td>
<td>402.05</td>
<td>40.6086</td>
<td>33.6102</td>
<td>755</td>
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<td>17606</td>
<td>Bozkurt</td>
<td>1975-2005</td>
<td>1260.22</td>
<td>41.9597</td>
<td>34.0377</td>
<td>167</td>
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<td>17622</td>
<td>Bafrada</td>
<td>1975-2005</td>
<td>795.05</td>
<td>41.5515</td>
<td>35.9247</td>
<td>103</td>
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<td>17160</td>
<td>Kırşehir</td>
<td>1975-2005</td>
<td>384.43</td>
<td>39.1639</td>
<td>34.1561</td>
<td>1007</td>
</tr>
<tr>
<td>17135</td>
<td>Kırkkale</td>
<td>1975-2005</td>
<td>377.53</td>
<td>39.8433</td>
<td>33.5181</td>
<td>751</td>
</tr>
<tr>
<td>17192</td>
<td>Aksaray</td>
<td>1975-2005</td>
<td>344.52</td>
<td>38.3705</td>
<td>33.9987</td>
<td>970</td>
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<tr>
<td>17730</td>
<td>Keskinköy</td>
<td>1977-2005</td>
<td>420.8</td>
<td>39.6682</td>
<td>33.6118</td>
<td>1140</td>
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<tr>
<td>17140</td>
<td>Yozgat</td>
<td>1975-2005</td>
<td>604.05</td>
<td>38.8205</td>
<td>34.8159</td>
<td>1301</td>
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<td>17196</td>
<td>Kayseri</td>
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<td>38.6687</td>
<td>35.5</td>
<td>1094</td>
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<tr>
<td>17193</td>
<td>Nevşehir</td>
<td>1975-2005</td>
<td>417.2</td>
<td>38.6163</td>
<td>34.7025</td>
<td>1260</td>
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<tr>
<td>17732</td>
<td>Çiçekdağ</td>
<td>1977-2005</td>
<td>357.74</td>
<td>39.6067</td>
<td>34.4235</td>
<td>900</td>
</tr>
<tr>
<td>17712</td>
<td>Sorgun</td>
<td>1984-2005</td>
<td>453.99</td>
<td>39.8016</td>
<td>35.1805</td>
<td>1116</td>
</tr>
<tr>
<td>17835</td>
<td>Urgup</td>
<td>1975-2005</td>
<td>384.2</td>
<td>38.6218</td>
<td>34.9144</td>
<td>1068</td>
</tr>
<tr>
<td>17981</td>
<td>Karataş</td>
<td>1975-2005</td>
<td>781.17</td>
<td>38.6895</td>
<td>35.3894</td>
<td>22</td>
</tr>
<tr>
<td>17090</td>
<td>Sivas</td>
<td>1975-2005</td>
<td>444.44</td>
<td>39.7437</td>
<td>37.002</td>
<td>1294</td>
</tr>
<tr>
<td>17162</td>
<td>Gümüşcü</td>
<td>1975-2005</td>
<td>393.06</td>
<td>39.185</td>
<td>36.0805</td>
<td>1182</td>
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<tr>
<td>17837</td>
<td>Tomarza</td>
<td>1975-2005</td>
<td>398.95</td>
<td>38.4522</td>
<td>35.7912</td>
<td>1402</td>
</tr>
<tr>
<td>17762</td>
<td>Kangal</td>
<td>1975-2005</td>
<td>405.17</td>
<td>39.2428</td>
<td>37.389</td>
<td>1521</td>
</tr>
<tr>
<td>17085</td>
<td>Amasya</td>
<td>1975-2005</td>
<td>448.42</td>
<td>40.6668</td>
<td>35.8353</td>
<td>409</td>
</tr>
<tr>
<td>17083</td>
<td>Merzifon</td>
<td>1975-2005</td>
<td>436.18</td>
<td>40.8793</td>
<td>35.4585</td>
<td>754</td>
</tr>
<tr>
<td>17620</td>
<td>Boyabat</td>
<td>1975-2001</td>
<td>553.65</td>
<td>41.463</td>
<td>34.7853</td>
<td>350</td>
</tr>
<tr>
<td>17074</td>
<td>Kastamonu</td>
<td>1975-2005</td>
<td>486.67</td>
<td>41.371</td>
<td>33.7756</td>
<td>800</td>
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<tr>
<td>17128</td>
<td>Esenboğa</td>
<td>1975-2005</td>
<td>404.89</td>
<td>40.124</td>
<td>32.9992</td>
<td>959</td>
</tr>
<tr>
<td>17646</td>
<td>Cerkes</td>
<td>1975-2005</td>
<td>400.05</td>
<td>40.815</td>
<td>32.8831</td>
<td>1126</td>
</tr>
<tr>
<td>17664</td>
<td>Kızılıcahamam</td>
<td>1975-2005</td>
<td>568.33</td>
<td>40.4729</td>
<td>32.6441</td>
<td>1033</td>
</tr>
<tr>
<td>17650</td>
<td>Tosya</td>
<td>1975-2005</td>
<td>477.52</td>
<td>41.0132</td>
<td>34.0367</td>
<td>870</td>
</tr>
<tr>
<td>17648</td>
<td>Ilgaz</td>
<td>1975-2005</td>
<td>458.73</td>
<td>40.9156</td>
<td>33.6258</td>
<td>885</td>
</tr>
<tr>
<td>17618</td>
<td>Devrekani</td>
<td>1976-2005</td>
<td>536.81</td>
<td>41.5996</td>
<td>33.8345</td>
<td>1050</td>
</tr>
</tbody>
</table>
Rainfall stations are located on or around the basin and these stations are visualized on DEM data. Thiessen polygons of these precipitation stations were plotted. These data were drawn in order to determine the effects of the stations over the catchment. Although the stations that were located outside the basin boundaries their effect on the basin were also included in the analysis. Annual total meteorological data and annual average runoff values were used in the analyzes.

Gene expression programming. GEP was developed by [11]. In Gene Expression Programming the main principles of genetic algorithms and genetic programming are used. The methodology of gene expression programming likes a biological evaluation. It uses character linear chromosomes composed of genes structurally organized in a head and a tail. The problems are encoded in linear chromosomes of fixed-length as a computer program.

A gene is consist of two parts. These are head and tail. The head of a gene includes main variables used to code the any mathematical expression such as some functions, variables and constants. In the head of the gene trigonometric and arithmetic functions takes part such as (+, -, *, /, \sqrt{ }, \sin, \cos, \tan). The tail includes exclusively variables and constants which may be required for additional terminal symbols, in case the variables in the head are incompetent to encode a function. In the gene tail there are constants and independent variables of the problem, like (1, a, b, c) [11-14].

By using GEP, a mathematical function is defined as a chromosome with multi genes and developed using the data presented to it [11].

In this study, the demo version of Gene X Pro Tools software is used for generating Gep models.

**TABLE 2**

<table>
<thead>
<tr>
<th>Station Code</th>
<th>Station Name</th>
<th>Observation range</th>
<th>Average flow (m³/sec)</th>
<th>Average flow height (mm)</th>
<th>Latitude</th>
<th>Longitude</th>
<th>Elevation (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E15A001</td>
<td>Yamula</td>
<td>1975 – 2005</td>
<td>65.03</td>
<td>136</td>
<td>38.890</td>
<td>35.2586</td>
<td>995</td>
</tr>
<tr>
<td>E15A003</td>
<td>Yahşiyan</td>
<td>1975 – 2005</td>
<td>72.75</td>
<td>81.2</td>
<td>39.8433</td>
<td>33.4816</td>
<td>670</td>
</tr>
<tr>
<td>E15A017</td>
<td>Şefaatlı</td>
<td>1975 – 2005</td>
<td>11.18</td>
<td>39.6</td>
<td>39.5038</td>
<td>34.7475</td>
<td>895</td>
</tr>
<tr>
<td>E15A023</td>
<td>Boğazköy</td>
<td>1975 – 1981</td>
<td>5.94</td>
<td>76.9</td>
<td>38.7538</td>
<td>35.3122</td>
<td>1025</td>
</tr>
<tr>
<td>E15A024</td>
<td>Kuylu</td>
<td>1975 – 1998</td>
<td>15.95</td>
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<td>E15A036</td>
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<td>Çadirhöyük</td>
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<td>1997 – 2005</td>
<td>50.36</td>
<td>113</td>
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<td>Purtulu</td>
<td>1999 – 2005</td>
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<td>E15A046</td>
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<td>1999 – 2005</td>
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<td>38.7788</td>
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<td>E15A048</td>
<td>Karaköyüköprüsü</td>
<td>2003 – 2005</td>
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<td>41.0716</td>
<td>34.505</td>
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Gene X Pro Tools. Gene X pro Tools is a flexible tool modeling software designed for Regression, Logistic Regression, Classification, Time Series Prediction, and Logic Synthesis. Gene X pro Tools is very easy to use and create a model. It is possible analysis data, generate models by Gene X pro Tools. Gene X pro Tools can be translate the model results up to 19 different programming languages (Ada, C, C++, C#, Excel VBA, Fortran, Java, JavaScript, Matlab, Octave, Pascal, Perl, PHP, Python, R, Visual Basic, VB.Net, Verilog, and VHDL) [15]. Gene X pro Tools can process datasets with tens of thousands of variables and effortlessly extract the most significant features and their relationships. GeneXproTools is also a very user-friendly application simplifying the access to all types of data stores from raw text files to databases and Excel spread sheets.

RESULTS AND MODEL DEVELOPMENTS

This paper aimed to generate three models for prediction the discharge of Kizilirmak Basin. Three GEP models were generated for predicting the discharge.

In GEP Model I, precipitation, temperature and humidity are input and discharge is output,

In GEP Model II, precipitation and temperature are input and discharge is output,

In GEP Model III, precipitation is input and discharge is output parameter.

In all models, Y is the depth of rainfall (mm), S is temperature (°C), N is humidity (%) and Q is runoff (m³/s). The mathematical functions are generated for define the relationship between meteorological parameters. The model equations and $R^2$ values are given below:
The test results from the GEP Models are compared with observed results in Figure 3 a-c. R² value of the GEP Models are 0.986, 0.981, 0.988 respectively. They can be seen from the figures that there are a high correlation between predicted and measured values. Also high R² values are supported the results. Test results show that desired performance is provided with GEP models.

FIGURE 3
Graphs of predicted and measured discharge values for GEP Model I (a), Model II (b) and Model III (c)
Regression analysis. In this study, Datafit, a program that simplifies graph drawing, regression analysis (curve fitting) and statistical analysis, has been used. The feature of Datafit that distinguishes from similar curve fitting and regression programs is that it is easy to use. Datafit uses the Levenberg-Marquardt method with double precision to perform nonlinear regression. As the regression models are solved, they are automatically sorted according to the goodness of the specified eligibility criteria (Residual Sum of Squares, Correlation Coefficient, Corrected Correlation Coefficient or Standard Er-
While many sub-basin characteristic features (precipitation, temperature and humidity) were analyzed as independent variables, the annual flow quantity was dependent variable in the multiple regression equations. A variable selection algorithm is also required to help determine the combination of independent variables that provide the best estimates of the dependent variable in the multiple regression equations [17].

Multiple regression analysis was performed in three different combinations. In the first combination rainfall, temperature, humidity are input parameters and flow rate is the output parameter. Rainfall, temperature are the input parameter and flow rate is the output parameter, in the second combination. In the third combination input parameter is rainfall and the output parameter is flow rate. The formula and the graphs of these 3 combinations and graphs of these combinations (Figure 4) are shown in below.

The first combination:
\[ Q = 0.35*Y-8.59*S-0.12*N-53.27 \] (4)

The second combination:
\[ Q = \text{-}36.6+0.48*Y-0.15*Y^2+10.8-2.63*Y^4+10^{-12}-10.57*Y^6+10^{-17}-14.28*Y^8+10^{-19}+0.26*S^{-2}+8.53*S^4+10^{-6}-1.25*S^5+10^{-8} \] (5)

The third combination:
\[ Q = \text{-}2.32*Y^{10}+10^{-38}+2.40*Y^{10}+32.15+Y^{10}+10^{23}+6.27*Y^{10}+10^{-19}+5.64*Y^{15}+3.13*Y^{14}+10^{-11}+1.05*Y^{13}+10^{-7}+0.2*Y^{12}+10^{-3}+0.31*Y^{-75}+23 \] (6)

In these formulas Q (m$^3$/s) is runoff, Y (mm) is depth of rainfall, N (%) is humidity and S (ºC) is temperature.

Artificial Neural Networks (ANNs) are the major and well known computing information processing method that emulate human brain during the learning and adapting to new phenomena and conditions. ANNs are designed and copied according to biological nervous system to have capability to learn like a human. They are typically parallel computing devices to obtain a computer model of the brain. Architecture of conventional ANNs consist of layers, neurons and weighted connections between neurons and layers. In a typical ANN, there are three layers called as input, hidden and output layers. Number of hidden layer is chosen as one in most of applications. But it can be two or more. The weighted connections between neurons in the layers can be adjustable during the learning level according to training algorithms. This level is also known as training of neural networks. After that desired outputs for target variables are generated by input parameters. ANNs have been used in the solving of several engineering such as estimation, control, modeling and etc. [18-20].

In this study, there types of ANN models are designed and trained according to variables shown in Table 3. In all of these ANNs, runoff is estimated according to data generated by experimental setup. In first model, designed ANN models have three input like ambient temperature, rainfall and humidity. In second one, ambient temperature and rainfall are used as input parameter. In last one, only rainfall is input. In all simulations, multi-layer forward type neural network models are used and results are given in Table 3 and Figures 5 a-c.

### TABLE 3

<table>
<thead>
<tr>
<th>Input: Temperature, rainfall, humidity.</th>
<th>ANN R$^2$</th>
<th>REGRESSION ANALYSIS R$^2$</th>
<th>GEP R$^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Output: Flow</td>
<td>0.9994</td>
<td>0.9986</td>
<td>0.9867</td>
</tr>
<tr>
<td>R$^2$</td>
<td>7396.297</td>
<td>310.518</td>
<td>20174.062</td>
</tr>
<tr>
<td>MSE</td>
<td>86.001</td>
<td>17.621</td>
<td>46.626</td>
</tr>
<tr>
<td>RMSE</td>
<td>0.9993</td>
<td>0.9985</td>
<td>0.9852</td>
</tr>
<tr>
<td>Adj R$^2$</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| Input: Temperature, rainfall.         | R$^2$     | 0.9995                    | 0.9977    | 0.9814 |
| Output: Flow                          |           |                           |           |
| R$^2$                                  | 6432.201  | 49.794                    | 1948.138  |
| MSE                                   | 80.201    | 7.056                     | 44.137    |
| RMSE                                  | 0.9994    | 0.9996                    | 0.9800    |
| Adj R$^2$                             |           |                           |           |

| Input: Rainfall.                      | R$^2$     | 0.9991                    | 0.9977    | 0.9883 |
| Output: Flow                          |           |                           |           |
| R$^2$                                  | 6083.725  | 51.338                    | 7777.868  |
| MSE                                   | 77.998    | 7.165                     | 88.192    |
| Adj R$^2$                             | 0.9990    | 0.9996                    | 0.9878    |
The coefficient of multiple determination ($R^2$) means a statistical measure of how close the data are to the fitted regression graph. Although the higher the $R^2$, the better model fits your data, low $R^2$ values are not always bad and high $R^2$ values are not always good. For a good model, a high $R^2$ value can be obtained for a model that does not fit a low $R^2$ value or data. Therefore, other parameters and model-estimation graphs are examined and evaluated as a result of statistical analysis.
MSE (Mean Square Error) and RMSE (Root Mean Square Error) are statistical terms. RMSE is an index used to assess the performance of models in statistical analysis (Artificial neural networks, regression analysis, etc.). A MSE and RMSE value are calculated to find the failure quantity between a statistical method applied from the request data and the estimated data. If a RMSE value approaches zero, it is concluded that the model is close to the fact. The calculation of MSE and RMSE are shown in equation 7, 8 and 9.

$$N_j - M_j = e_j$$  \hspace{1cm} (7)

$$MSE = \frac{1}{n} \sum_{j=1}^{n} e_j^2$$  \hspace{1cm} (8)

$$RMSE = \sqrt{\frac{1}{n} \sum_{j=1}^{n} e_j^2} = \sqrt{MSE}$$  \hspace{1cm} (9)

In this study, where 3 different methods and 3 different models are used and all of them have provided successful AdjR² values results. The results of these 3 models obtained from 3 different methods are given in Table 3. In all methods R² values very close to each other and to 1. But RMSE and MSE values are low in regression analysis method (Datafit) to other methods. According to Table 3, Regression analysis method can be used successfully to predict the discharge in Kizilirmak basin.

CONCLUSION

Water is the most important building block of living life. However, more or less quantities of water causes many catastrophes and damages like as drought, scarcity, decrease in the quality of life standards, flood and landslide etc. These disasters are important problems which are affected the life and biogeography. Water management should be planned well in order to prevent and find solutions to these problems. Factors, such as climate change or lack of water planning cause these problems. Therefore water management is very important. Meteorological and flow data play an important role in water management.

This paper indicates that to predict the discharge values in Kizilirmak basin. For this aim three Artificial Intelligent Methods (ANN, GEP, Datafit) are used and three different combinations are generated. In the first combination; rainfall, temperature, humidity are input parameters and flow rate is the output parameter. Rainfall, temperature are the input parameter and flow rate is the output parameter, in the second combination. In the third combination input parameter is rainfall and the output parameter is flow rate. In all combinations Y is precipitation, S is temperature, N is humidity and Q is discharge.

GEP. Three GEP models were generated. In all models there was a high correlation between predicted and measured values. R² value of the GEP Models are 0.986, 0.981, 0.988 respectively. Also RMSE values are 46.626, 44.137, 88.192 and MSE values are 20174.062, 1948.138, 7777.868 respectively.

ANN. Three ANN models were generated in Kizilirmak river basin. In all models a high R² values was obtained as 0.999, 0.999 and 0.999 respectively. RMSE values are 86.001, 80.201, 77.998 and MSE values are 7396.297, 6432.201, 6083.725 respectively.

Datafit. Three different combination were obtained by using Datafit. There were high correlations in all combinations. In combination I; R² was 0.998, RMSE was 17.621 and MSE was 310.518, combination II; R² was 0.999, RMSE was 7.056 and MSE was 49.794, combination III; R² was 0.999, RMSE was 7.165 and MSE was 51.338.

R² values are very high in all methods but R² is not enough to determine the statistical performance of a model. Therefore RMSE, MSE and AdjR² values performed for this techniques. When 3 methods results compare, Regression analysis method (Datafit) gives the best performance for predicting the flow rate in Kizilirmak basin.

REFERENCES


BIOCHEMICAL CHARACTERIZATION OF THE CRUDE CHITIN DEACETYLASE SUPERNATANT FROM BACILLUS CEREUS

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ABSTRACT

Chitosan, produced by thermochemical deacetylation of chitin, is a unique polymer. This chemical process causes insoluble and impure chitin/chitosan derivatives. Therefore, the methods such as enzymatic bioconversion have recently been investigated for the yielding of novel polymers. It is aimed to purification and biochemical characterization of chitin deacetylases from Bacillus cereus in the marine environment. In this research, the biochemical characterization of the crude chitin deacetylase supernatant from Bacillus cereus was analyzed. The specific activity was calculated as 0.274 U/mg. The optimum activity of supernatant was determined at 50°C and pH 7.0. After the pre-incubation of enzyme at 50°C for 20 min, the activity increased up to 115%. Acetic acid (105%), glyceral (123%), CoCl2 (113%), NaCl (137%) and CaCl2 (102%) in 5 mM concentration stimulated to the enzyme activity. EDTA (81.12%) and Triton X-100 (75.5%) behaved as the inhibitory agent on activity. In the presence of SDS, 2-mercaptoethanol, MgCl2 and MnCl2, this activity preserved as 97, 89, 64 and 83% respectively. In SDS-PAGE analysis, two protein bands in 39 and 29.60 kDa molecular weight belonging to chitin deacetylase were estimated. According to our biochemical results, the crude CDA supernatant can suggested to use in several potential applications such as biological control of fungal diseases in human, plant pathogens, some pest insects and food protection.

KEYWORDS: Bacillus cereus, chitin deacetylase, deacetylation of chitin.

INTRODUCTION

Chitin, a linear polysaccharide of β-(1-4) linked N-acetyl-D-glucosamine (GlcNAc), is commonly found in the outer skeleton of insects and crustaceans like shrimp, crabs and lobster, in marine diatoms and algae as well as in the cell walls of certain fungal [1-4]. This homopolymer that has a chemical structure similar to cellulose, is one of the most abundant after cellulose. [2-7]. Chitin, a highly crystalline structure, is translucent, resilient and insoluble in aqueous solutions and organic solvents, low reactivity in its extracted form [3-4]. These properties of chitin, its industrial application areas are limited [8].

Chitosan obtained by the thermochemical alkaline deacetylation of chitin to remove the acetyl groups at present, is an N-deacetylated derivate [2]. Chitosan has biological properties such as chemical resistance, low toxicity, adsorption, chelate formation with metal ions, antitumor, antioxidant and antimicrobial activity, creating film, antibiosis and biocompatibility [9-10]. Due to these unique characteristics, chitosan is widely used in industrial applications including food, treatment technologies, cosmetic, pharmaceutical, biomedical, agriculture, textile and health. Especially, it is evaluated in medical applications such as drug delivery systems, solid polyelectrolytes, surfactants, implants, the creation of membrane for ultra-filtration, osmosis and evaporation [11-12].

Traditional alkali process of extracting chitin converted into chitosan has some disadvantages such as environmentally unsafe, not easily controlled, poor quality heterogeneous of the resulting production and high energy consumption [5-7]. To overcome most of these problems, enzymatic process has recently performed as alternative to chemical NaOH pyrolysis used in deacetylation step [13-14]. The chitin deacetylase (CDA, EC 3.5.1.41) catalyses the bioconversion of chitin to chitosan by hydrolysis acetamido groups of N-
acetyl-D-glucosamine, thus producing glucosamine residues and acetic acid [15-18]. This enzyme, firstly identified and partially purified from the cell wall of *Mucor rouxii* in 1975, is belongs to the carbohydrate esterase family 4 [16, 19].

**MATERIALS AND METHODS**

**Production of Colloidal Chitin.** 100 ml concentrated HCl was slowly added in a 5 L glass flask containing 5 g of powdered chitin. The mixture was stirred by stirring at 4°C for overnight at a high speed and then added 2 L cold ethanol 95% then incubated overnight at 25°C. This mixture was centrifuged at 7454 g for 10 min at 4°C. The collected pellets were resuspended in distilled water after washed twice. pH of this homogenizations were adjusted to 7.0 by 0.01 N NaOH, then centrifuged once again in same condition. The acquired precipitates were incubated into watch glass for 1-2 h at room temperature and kept at 4°C in the dark for further [20].

**Isolation and Identification of Chitin Deacetylase Producing Bacteria.** The soil samples were taken from Mersin and Adana Karatas beaches in Turkey. Diluted fresh soil samples transferred onto agar plates including medium comprised of (grams per liter): (NH₄)₂SO₄ 7; K₂HPO₄ 1; NaCl 1; MgSO₄.7H₂O 0.1; yeast extract 2; tryptone 1; colloidal chitin 10; 4-nitroacetanilide 0.5; agar 15, pH at 7.0. After incubation at 30°C for 3 days, CDA producing bacteria colonies were screened by color reactions of 4-nitroacetanilide [6].

The selected strains were inoculated to liquid culture including 1 g yeast extract, 0.4 g (NH₄)₂SO₄ and 0.15 g KH₂PO₄ (pH 8). Following incubation at 25°C for 24-48 h, 2 mL of this culture was transformed to the test tubes containing diagnostic disc, saturated with 4-nitroacetanilide by dissolved in ethanol. And then was incubated at 25°C for 12-24 h. Both of liquid cultures and discs were screened by the yellow color formation [21].

Bacterial strains were distinguished by applying microbiological methods (Gram staining). According to gram staining, VITEK 2 compact system (microbial identification system) was used for identification.

**The cell free CDA Supernatant.** The bacterial cells were inoculated in liquid fermentation medium including (NH₄)₂SO₄ 7; K₂HPO₄ 1; NaCl 1; MgSO₄.7H₂O 0.1; yeast extract 2; tryptone 1; colloidal chitin 10, pH at 7.0 for enzyme production [8]. The cells were grown aerobically with shaking (180 rpm) at 37°C for 3 days. To achieve crude supernatant, the culture was centrifuged at 7454 g for 45 min at 4°C. The CDA supernatant was kept at 4°C for activity and biochemical characterization analyses.

**CDA Activity.** Chitin deacetylase activity was performed by using 0.1% (w/v) glycol chitin prepared in 50 mM Na tetraborate/HCl buffer (pH 7.0), as a substrate. Activity analyses were initiated by the addition 150 µL substrate and 150 µL crude enzyme preprate, incubated at 30°C for 30 min. The reaction was terminated by adding 200 µL of 33% (v/v) acetic acid solution. The glucosamine residues deacetylated by the enzyme were colorized by attaching 200 µL of 5% (w/v) NaNO₂ in mixture, vortexed 10 min at room temperature. After that, 200 µL 12.5% (w/v) ammonium sulfamate was added and shaken 30 min at room temperature. The mixture was boiled by the addition 800 µL HCl 5% and 80 µL indole 0.1% in absolute ethanol for 5 min. Then cooling, the suspension was spectrophotometrically measured at 492 nm [22-23]. For calculation of enzyme activity, a standard curve was prepared by using standard D-glucosamine solution in different concentrations. One unit of chitin deacetylase activity was described as the amount of the enzyme required to produce 1 µmol glucosamine per minute.

**Determination of Total Protein Concentration and SDS-PAGE Analysis.** The total protein amount was calculated by using bovine serum albumin (BSA) as standard [24]. Molecular weight of the crude chitin deacetylase was determined according to the Laemmli (1970) procedure [25]. ABM (Applied Biological Materials) opti-protein G252 was used as reference marker. Protein bands were become visible by staining Coomassie Brilliant Blue R-250.

**Biochemical Characterization of Bacterial CDA.** The optimal pH of the enzyme was performed by measuring the enzyme activity at different pH values (2.4-10.6) in the following buffers: glycine/HCl (pH 2.4-2.8), citrate (pH 3.0-5.8), citrate phosphate (pH 6.2-6.6), Na/phosphate (pH 7.0), Tris/HCl (pH 7.4-9.0) and carbonate/bicarbonate (pH 9.4-10.6). 0.1% (w/v) glycol chitin substrate in above-stated buffer was prepared. 150 µL of each substrate solution and 150 µL crude enzyme preprate were mixed and activity assay mentioned section 2.4 was performed.

The optimum temperature and thermal stability for CDA activity were determined by using substrate in pH buffer observed the highest enzyme activity. The optimum temperature for activity was detected at temperatures ranging from 20-120°C. 150 µL of substrate and 150 µL crude enzyme preprate were incubated in water (20-80°C) and oil-bath (90-120°C). Thermal stability of CDA was detected by mixing 150 µL of substrate (prepared in optimal pH buffer) and 150 µL of pre-incubated CDA at opti-
mal temperature for 5-10-15-20-25-30 min.

Crude enzyme was pre-incubated in the presence of metal ions at 5 mM final concentration (CaCl2, CoCl2, MgCl2, NaCl and MnCl2), inhibitors (EDTA, acetic acid and glycerol) and detergents (SDS, Triton X-100 and 2-mercaptoethanol). For these, chemical agent and CDA were stirred and pre-incubated at 37°C for 1 h. After pre-incubation, the effects of chemical reagents on activity were tested at the determined optimal pH and temperature. All spectrophotometric analyses were performed in triplicate.

RESULTS AND DISCUSSION

The occurrence of yellow zone around the colonies indicated the enzymatic bioconversion colloidal chitin to chitosan. Accordingly, one of strains isolated from marine environment formed yellow zone on agar plate (Fig. 1). This strain was evaluated as chitin deacetylase producing bacteria. And this strain was identified as Bacillus cereus by using VITEK 2 microbrial identification system, with 95% probability.

The crude CDA supernatant obtained from Bacillus cereus was analyzed. Based on the incubation period at 37°C for 3 days, the specific and total activity was calculated as 0.274 U/mg; 0.181 U/mL/min. Ischaidar et al. (2014) reported that the activity was calculated as 0.274 U/mg; 0.181 U/mL [23]. In another bacterial studying, total activity of CDA produced by immobilized and suspended cells of Alcaligenes sp. ATCC 55938 within fermentation time 18 h was recorded as 0.38 U/mL [27]. Kim et al. (2008) determined a maximum activity (0.6 U/mL) of extracellular chitin deacetylase from Mortierella sp. DY-52 following 3 d fermentation [5].

For extracellular chitin deacetylase by a soil bacterium Bacillus amyloliquefaciens, a maximum of 17.84 U/mL activity reported by Zhou et al. (2010) [6]. According to stated activity in literature, our enzyme activity obtained without optimization studies a remarkable result.

According to the SDS-PAGE analysis, two protein bands located at 39 and 30 kDa for the crude CDA supernatant, was shown (Fig. 2). These estimated weights were nearly similar to the molecular masses reported for other CDAs. Molecular weights of CDAs purified from various microorganisms were notified as follows: 32 and 30 kDa for B. cereus [28], 35 kDa for E. coli [13], 33 kDa for Pichia pastoris GS115 [17], 19.5 kDa for Aspergillus nidulans and 24.2 kDa after cloning to E. coli and expression [29], five isoenzymes ranging between 12.17 and 48.1 kDa for Uromyces viciae-faba [15], two protein bands weighted at 31.5 and 33 kDa for C. lindeuthiumatum [30], 75 kDa for M. rouxii [31].

The optimum pH and temperature for maximum enzyme activity were determined to be 7.0 and 50°C, respectively (Fig. 3, 4). pH values for the optimum activities of extracellular and intracellular CDAs are between 7.0 and 12.0; 4.5 and 6.0, respectively [16]. In general, Zhou et al. (2010) reported to be 50 and 60°C of the CDAs optimal temperature. In comparison with literatures, our results regarding the optimal pH and temperature were showed almost similarity to previous studies. After cloning the extracellular CDA purified from B. cereus to E. coli pLysS cell, the optimal pH acquired by expression of gene was detected to be 7.0 [28]. For the maximum enzyme activity, optimum pH of the extracellular CDAs of Rhizopus nigricans, Aspergillus nidulans, Flammulina velutipes and Macor racemosus were detected as 7.0 [15]. Li et al. (2007) stated that the optimum activity of chitin oligosaccharide deacetylase produced by soil bacterium Bacillus amyloliquefaciens, a maximum of 17.84 U/mL activity reported by Zhou et al. (2010) [6]. According to stated activity in literature, our enzyme activity obtained without optimization studies a remarkable result.

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from *Vibrio cholerae* showed at pH 7.0-7.5 [32]. For CDA activity obtained from *E. coli*, the optimal temperature was reported to be 50°C [13]. In another study, the maximum specific activity of CDA from *Bacillus* PT2-3 was calculated at 60°C [23].

For fungal chitin deacetylase activities from various species such as *Mucor rouxii*, *Absidia coerulea*, *C. bertholliae* and *A. nidulans*, the optimal temperature was recorded as 50°C [15, 31].

![Figure 3](image3.png)

**FIGURE 3**
Effect of pH on CDA activity at 30°C

![Figure 4](image4.png)

**FIGURE 4**
Effect of temperature on CDA activity at pH 7

<table>
<thead>
<tr>
<th>Reagents</th>
<th>Residual Relative Activity (%) 5 mM concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>CoCl₂</td>
<td>113±0.96</td>
</tr>
<tr>
<td>EDTA</td>
<td>91±0.17</td>
</tr>
<tr>
<td>SDS</td>
<td>97±0.78</td>
</tr>
<tr>
<td>Triton X-100</td>
<td>24±0.58</td>
</tr>
<tr>
<td>2-mercaptoethanol</td>
<td>89±0.69</td>
</tr>
<tr>
<td>MgCl₂</td>
<td>64±0.31</td>
</tr>
<tr>
<td>NaCl</td>
<td>137±0.06</td>
</tr>
<tr>
<td>CaCl₂</td>
<td>102±0.54</td>
</tr>
<tr>
<td>MnCl₂</td>
<td>83±0.45</td>
</tr>
<tr>
<td>Glycerol</td>
<td>123±0.60</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>105±0.95</td>
</tr>
</tbody>
</table>
The thermal stability of crude supernatant was examined by pre-incubating of enzyme at 50°C for 5-10-15-20-25 and 30 min. Accordingly to the results of this analysis, 18, 30 and 26% of enzyme activity were lost following pre-incubation for 5, 10 and 15 min, respectively. The enzyme possessed a high thermal stability and continued full activity, incubated for 20 min. However, the remaining CDA activity was detected to be 67 and 51% after incubation for 25 and 30 min (Fig. 5). Gauthier et al. (2008) declared that native and recombinant enzyme exhibited high thermal stability (almost 80-150%) after incubation at 0, 37 and 50°C; for 0, 2, and 4 h. In same study, residual activity of recombinant CDA after incubation for 2 and 4 h at 50°C, was determined above 120 and 140%, respectively [33].

Tsigos and Bouriotis (1995) reported a remarkable thermostability (about 90%) after incubation at 50°C for 50 h, similar to our thermostability data [1]. In addition to, Gao et al. (1995) indicated that this enzyme possessed a high thermal stability after incubation at 50°C for 60 min [34]. In another study, it was determined that less than 30% decrease in activity was observed after incubation at 50°C for 23 h [35]. Thermostability property of crude CDA supernatant is an important advantage for industrial application. In literature, thermal stability results of CDAs from S. cerevisiae, A. coerulea and V. cholerae are similar to our data [32, 34, 36].

In addition, EDTA and Triton X-100 were showed a strongly inhibitor effect on CDA activity. Effect of inhibitors, detergents and metal ions on the CDA activity was showed as residual relative activity (Table 1). CDA activity was moderately inhibited with a loss of low than 40% in the presence of SDS, 2-mercaptoethanol, Mn²⁺ and Mg²⁺. As well as the inhibition effect on activity, simultaneous agents such as Co²⁺, Na⁺, Ca²⁺, glycerol and acetic acid was determined. Similar findings were displayed for S. cerevisiae, E. coli and Mortierella sp. DY-52 CDA that emphasized activation in the presence of Co²⁺ [1, 5, 7, 28, 36]. In another studies for CDA from Bacillus sp. PT2-3, S. cerevisiae and C. lindeimthium, the inhibition in enzyme activity was showed as compared to Ca²⁺ in our study [1, 22, 36]. These results can be explained that CDA is a metalloenzyme and its catalytic activity especially influenced by cations [16].

**CONCLUSION**

So far, it has been reported to chitosan and their derives by deacetylated of chitin material in insects, crustaceans and the cell wall of fungus. As well as limited, the production of bacterial CDAs were investigated in literature [8, 14]. Biochemical characterization of the crude CDA supernatant from B. cereus is declared in present study. Therefore, this article aimed at CDA production from bacteria, is the priority work. According to our biochemical results, the crude CDA supernatant can suggested to use in several potential applications such as biological control of fungal human, plant pathogens, some pest insects and food protection.

**ACKNOWLEDGEMENTS**

The chemical demands of CDA producing bacteria isolation and identification researches of this study and enzyme activity analysis were supplied from 113Z569 and 2209/A numbered project supported. For this reason, we thank TUBITAK.

The all authors of this manuscript state that there is no conflict of interest.
REFERENCES


TAGUCHI BASED GREY RELATIONAL ANALYSIS TO OPTIMIZE CEPHALARIA SYRIACA BIODIESEL PRODUCTION AND KINEMATIC VISCOSITY

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Sakarya University, Department of Environmental Engineering, 54187, Sakarya, Turkey

ABSTRACT

Cephalaria syriaca (Turkish Pelemir) seed oil is edible oil which is not used as cooking oil our country. The current work it was aimed to maximize the yields of biodiesel production from Pelemir seed oil (PSO) by using the grey based taguchi method and at the same time to keep the kinematic viscosity values between the nominal limits. This study presents joined effect of four input parameters (factors) such as catalyst concentration (A), molar ratio of oil to methanol (molar ratio) (B), reaction temperature (C) and reaction time (D) in controlling two significant performance parameters (responses) yields of biodiesel (y1) and kinematic viscosity (y2). Orthogonal array was used for the design of experiments on the principle of L9. Taguchi technique was coupled with grey relational analysis to obtain a grey relational grade for evaluating multiple outputs. Grey relational analysis in the Taguchi technique was employed for determining the best combination values of input parameters. It was found that A1B1C2D1 (0.5%, 12:1, 60°C, 80 min) optimal parametric combination.

KEYWORDS:

Pelemir oil, biodiesel, optimization, Grey based Taguchi Method

INTRODUCTION

The ozone layer is very important for the existence of ecosystems on the planet. Ozone layer is damaged due to increased pollution in the atmosphere. It has become known as the greatest threat in the world as a direct result of the global warming and increasing gas imbalance in the atmosphere [1-3].

Crude oil is a mixture of hydrocarbons and plants living millions of years ago. Crude oil is also a fossil fuel. Biodiesel is the raw material of raw vegetables, animal fats and waste oils. Biodiesel has positive effects on pollutant emissions that cause climate change. Biodiesel causes much less damage than diesel fuel if spilled into the environment. Biodiesel is a non-toxic and biodegradable fuel compared to the diesel fuel. When biodiesel combustion particulate matter, carbon monoxide, sulfur dioxide and hydrocarbons are released less [2-4].

Cephalaria is a genus of about 65 species of flowering plants in the family Caprifoliaceae, native to southern Europe, western and central Asia, and northern and southern Africa. In Turkey, the number of taxa Cephalaria was found to be 41 (39 species, 1 subspecies, and 1 variety) [5]. Many Cephalaria species have been used traditionally for different purposes in various part of the world. Cephalaria syriaca, an important oil yielding plant, has partially been domesticated in Turkey and other countries, although it has been considered as weeds grown in wheat fields [6, 7]. These species have medical and economic importance. For example, in addition to being used as an additive to increase the strength of the dough, it also has antimicrobial, antifungal, herbicide, antioxidant and pesticide [5-7]. Pelemir is one of the plants grown in arid regions. It can be used as an alternative to oil seed plants. 13-21% crude protein and amino acid are found in the seeds of this plant while the oil ratio is 22-28%. This oil is rich in linolenic, myristic, oleic and palmitic fatty acids [5].

Taguchi method based grey relational analysis is improved used for optimizing of procedure with plural performance parameters. Many researchers have successfully used optimization technique to discover optimal rate of input parameters for better output parameters. In the literature review, there are several studies for optimization of multiple performance with biodiesel production. Mahamuni and Adewuyi (2010) produced biodiesel from soybean oil using high frequency ultrasound method and provided optimization using Taguchi method. The authors' parameters for a 92.5% biodiesel yield and a reaction time of less than 30 minutes as follows 0.75% (w/w) KOH catalyst at 6:1 molar ratio, 581 kHz ultrasound frequency and 143W [8].

In another study, Kumar et al. (2015), using the Taguchi optimization method Manilkara zapota (L.) seed oil for biodiesel production. Optimal production conditions was determined 50 °C reaction temperature, 6:1 molar ratio, 1% catalyst concentration and 90 minutes reaction time [9]. Adewale et al. (2017) studied the optimal process parameters for biodiesel production from crude tall oil in presence of...
lipase catalyst was optimized using Taguchi method. 16 h reaction time, 40 °C reaction temperature, 1:1.5 alcohol to oil molar ratio, 16 h reaction time and 1.0 wt% enzyme dose [10]. Similarly Dhawane et al. (2017) studied the use of Taguchi approach to optimization of biodiesel synthesis from rubber seed oil using iron doped carbon catalyst. Optimal process parameters was decided as a maximum yield of biodiesel 97.5% at a catalyst loading of 4.5 wt%, molar ratio of 9:1, temperature of 60 °C and agitation speed of 1250 rpm [11]. Jadhav et al. (2018), Kamalar et al. (2018) and Gadhave and Gawande (2018) used Taguchi method to optimize biodiesel production in their studies [12-14]. Prasada Rao and Appa Rao (2017) applied Grey relation analysis to find out optimal combination was done on the IDI engine using diesel fuel, Mahua biodiesel and methanol additive blends this fuel [15]. Sangram and Madhukar (2016) optimized six input parameters on compression ignition engine (CI) operated on Mangifera indica biodiesel blend by applying Grey based Taguchi technique [16].

Taguchi technique has been at a premium for parameters optimization in design of experimental data. Taguchi’s parameter design is adopted; not only to reduce the number of experiments but also to identify the influencing factors and their interactions. The conventional Taguchi method can effectively determine optimal parameter settings for a single performance characteristic but this approach is not enough when several performance characteristics are considered [16, 17]. Grey based Taguchi method can be replaced by Taguchi desirability method. This method transforms multiresponse optimization problem into a single response optimization condition together with the objective function of the product or process. It is the most effective method available to reduce product cost and improve quality. Design of Experiments (DOE) is a strong statistical technique. DOE using Taguchi approach can economically satisfy the needs of problem solving and product/process design optimization projects. Thus, DOE using the Taguchi approach to ensure the highest possible performance for combination of design factors.

**Materials and Methods**

**Materials.** Experimental studies were performed with pelemir seeds harvested in Ankara (Turkey) in 2016. The Merck brand, potassium hydroxide (KOH), n-hexane (96% analytical grade) and methanol were purchased from the chemical store in Germany. BUCHI brand rotary vacuum evaporator (Rotavapor R-210), electric grinder, soxhlet extractor, Gerhardt Soxtherm System ST40 and NUVE centrifugal machine were used as device for laboratory experiments.

**Biodiesel Production Procedure from Pelemir Seed Oil.** Before oil extraction process, the seeds of pelemir were dried for 1 hour at 100 °C. Then the dried seeds were ground to fine particles a diameter of 0.5 mm with the aid of the electric grinder. Crude oil was obtained from the ground seeds using n-hexane solvent with soxhlet extractor according to the AOAC (Association of Official Analytical Chemists) official method 963.15 [18]. The oil content of the seeds was measured using Gerhardt Soxtherm System ST40.

Laboratory scale experiments were conducted to convert the pelemir oil into biodiesel (methyl ester). At the end of the process, the PSO was separated from the n-hexane solvent using a rotary vacuum evaporator. Free fatty acid (FFA) content in pelemir seed oil was found to be less than 0.5%, so alkaline transesterification was performed as the biodiesel production method.

In the second phase, transesterification process was performed in a rotary evaporator using an alkali-catalyst (KOH). The biodiesel obtained after the transesterification process was taken into the separation funnel and washed with warm pure water four times. In the production of biodiesel using different catalyst concentration (0.5, 0.7, 1% ), molar ratio (8:1, 10:1, 12:1), reaction temperature (40, 50, 60 °C) and reaction time (40, 60, 80 min) yields of biodiesel and kinematic viscosity values were measured. At the end of the experiments the PSOB obtained was analysed using gas chromatography (GC) in order to determine POB biodiesel physico-chemical properties.

**Taguchi Design.** Robust Design method, also called the Taguchi method, promoted by Dr. Genichi Taguchi, widely advances engineering productivity. Taguchi method focuses on improving the basic function of the product or process. It is the most effective method available to reduce product cost and improve quality. Design of Experiments (DOE) is a strong statistical technique. DOE using Taguchi approach can economically satisfy the needs of problem solving and product/process design optimization projects. Thus, DOE using the Taguchi approach to ensure the highest possible performance for combination of design factors.

DOE is performed in accordance with the choosed orthogonal array (OA). An orthogonal array is a significant component used in the Taguchi design. Selection of appropriate OA is depending on the number of factors and their levels and requires a
calculation of the total degrees of freedom (DOF). In this study dof is 8 and the nearest OA is L9. There are nine set of experiment with four factors with their three levels and response values. These responses are respectively yields of biodiesel and kinematic viscosity.

**Grey-based Taguchi Method.** Grey relational analysis is used to research the multiple responses in the process operations. Grey based Taguchi method identify the relationship between real and desired experimental data [19-24]. Grey relational analysis based on grey system theory can be used for solution the complex interrelationships among the multiple responses [23-27]. Grey based Taguchi technique was used in this study to performed a correlation between input and performance parameters. Figure 1 shows the four input parameters used to optimize the multiple performance characteristics of the PSO's biodiesel production.

In order to optimize the multiple performance characteristics for biodiesel production, four input parameters (factors) which are compatible with the literature studies and their three levels for each parameter were selected. Each of these factors and their levels, as shown in Table 1.

In this study, optimization of multiple performance characteristics in biodiesel production was made according to grey relational grade. The multiple responses measures considered here are yields of biodiesel and kinematic viscosity. In this study, biodiesel yield response need to be individually maximized whereas at the same time kinematic viscosity response need to be nominal value.

Grey based Taguchi method is given as follows. Responses, factors and its levels can be chosen based on the prior authority’s opinions and research work in literature. OA is subdivision of chosen combinations of multiple factors at multiple levels. Suited array is selected considering to number of factors and their levels.

Experimental data according to the type of response is normalized (in the range between 0 and 1). It is used S/N ratios of responses in calculation. If the target value of the response is infinite, it has a “larger is better” characteristic and can be normalized as follows (Eq. 1):

\[ X'_i = \frac{X_i - min_{i=1}^n X_i}{max_{i=1}^n X_i - min_{i=1}^n X_i} \]

If a defined target value \( X_d \), exists, the response can be normalized as follows (Eq. 2):

\[ X'_i = 1 - \frac{|X_i - X_d|}{max_{i=1}^n X_i - min_{i=1}^n X_i} \]

If the response is aimed to minimize, its characteristic smaller is better and it was normalized as follows (Eq. 3):

\[ X'_i = \frac{max_{i=1}^n X_i - X_i}{max_{i=1}^n X_i - min_{i=1}^n X_i} \]

where \( X'_i \) is the value after grey relational generation (normalized value), \( X_i(j) \) and \( X_d(j) \)are the largest and smallest values of for \( X_i \) the ith response, respectively. The normalized values are ranged between zero and one. Larger normalized result means to the better performance and it should be equal to 1.

It is calculated grey relational coefficient to denote the relationship between the ideal and the actual experimental results. Grey relational coefficient \( \gamma_{0i}(j) \) can be calculated as (Eq. 4):

\[ \gamma_{0i}(j) = \frac{min_{j=1}^m \min_{j=1}^m \delta_{0i}(j) + \max_{j=1}^m \max_{j=1}^m \delta_{0i}(j)}{\delta_{0i}(j) + (\max_{j=1}^m \max_{j=1}^m \delta_{0i}(j))} \]

**FIGURE 1**

**Interrelations between input and performance parameters**

**TABLE 1**

<table>
<thead>
<tr>
<th>Factors</th>
<th>Code</th>
<th>Levels</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catalyst concentration (%)</td>
<td>A 0.5</td>
<td>0.7</td>
</tr>
<tr>
<td>Molar ratio</td>
<td>B 8:1</td>
<td>10:1</td>
</tr>
<tr>
<td>Reaction temperature (°C)</td>
<td>C 40</td>
<td>50</td>
</tr>
<tr>
<td>Reaction time (min)</td>
<td>D 40</td>
<td>60</td>
</tr>
</tbody>
</table>
where $\Delta_0(j)$ is the deviation sequence of reference sequence $X'_0$ and comparability sequence $X'_i(j)$ (Eq. 5).

$$\Delta_0(j) = |X'_0(j) - X'_i(j)|$$  \hspace{1cm} (5)

$\zeta$ is the distinguishing coefficient and issued to adjust the difference of the relational coefficient.

It takes a value between 0 and 1.

Grey relational grade is calculated as Eq. 6 and it shows the relationship among the series.

$$\gamma_{oi} = \frac{1}{n} \sum_{j=1}^{n} \gamma_{oi}(j)$$ \hspace{1cm} (6)

where $n$ is the number of response. Since higher multiple responses are desirable, the larger is better S/N quality characteristic are adopted for grey relational grade. Higher grade relational grade corresponds to the closer experimental value to the ideal normalized value. Thus, higher grey relational grade shows that the corresponding factor combination is closer to the optimal. The highest grey relational grade is assigned an order of 1.

RESULTS AND DISCUSSION

Characterization of Pelemir Seed Oil. PSO fatty acid analysis was tested that the saturated PSO content is 57.6% and unsaturated PSO content is 42.4%. The largest share of saturated fatty acids belongs to myristic acid with 24.62% while the largest share of unsaturated fatty acids belongs to linoleic acid with 42.4%. Total oil content of pelemir seeds were detected by Gerhardt Sothxen System ST40 as 21.14%.

The Effect of Statistical Analysis on the Factors by Grey based Taguchi Method. Experimental studies for the production of biodiesel from pelemir seed oil by transesterification method were carried out using four input parameters (catalyst concentration, molar ratio, reaction temperature and reaction time) and three levels of these parameters, each experimental study was carried out using 50 grams of crude pelemir oil. Biodiesel yields and kinematic viscosity were measured after each experimental study. If a full factorial experiment is desired, the number of experiments for the 4-factor and 3-level experiments is $(3^4)$ 81, whereas the number of experiments decreases to 9, because L9 is used for the orthogonal array.

In order to determine the best combinations of biodiesel yields and kinematic viscosity based on a grey relational analysis, the following steps were applied respectively.

Step 1: Responses were normalized.

Step 2: Suitable grey relational coefficients were calculated.

Step 3: Grey relational grades were calculated.

Step 4: Optimal levels for the biodiesel production process parameters were calculated.

Equations 4, 5 and 6 were used to normalize the responses. The grey relational coefficients was calculated by using 7 and 8 th equations. Grey relational grades were calculated by using 9 th equations by MINITAB Package. The different combinations were made for the four factor variables and the two responses performance and orthogonal array was formed. Biodiesel yield is larger is better and kinematic viscosity is nominal is best quality characteristics have been used for calculating the S/N ratio for these responses using Equation 2 and 3 respectively.

Experimentally measured amount of performance parameters shown in Table 2. In the grey relation generation formation, for each experimental data, it was normalized by maximizing the biodiesel yields while keeping the kinematic viscosity within the nominal value range. For this aim, range was set from 0 to 1 using Eq. (4) and (5).

Grey relational coefficients was calculated for each performance parameters. Finally, the grey relational grade was obtained by taking the average values of the grey relational coefficient. Table 3 shown grey relational coefficient and grey relational grade.

<table>
<thead>
<tr>
<th>Experiment number</th>
<th>OA Factors</th>
<th>Performance Parameters (responses)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>1</td>
<td>0.5</td>
<td>8:1</td>
</tr>
<tr>
<td>2</td>
<td>0.5</td>
<td>10:1</td>
</tr>
<tr>
<td>3</td>
<td>0.5</td>
<td>12:1</td>
</tr>
<tr>
<td>4</td>
<td>0.7</td>
<td>8:1</td>
</tr>
<tr>
<td>5</td>
<td>0.7</td>
<td>10:1</td>
</tr>
<tr>
<td>6</td>
<td>0.7</td>
<td>12:1</td>
</tr>
<tr>
<td>7</td>
<td>1</td>
<td>8:1</td>
</tr>
<tr>
<td>8</td>
<td>1</td>
<td>10:1</td>
</tr>
<tr>
<td>9</td>
<td>1</td>
<td>12:1</td>
</tr>
</tbody>
</table>
Each input parameter, the suitable level value for the maximum grey relational grade was certain as optimum points. The best combination values of input parameters (factors) were also investigate main effects plot graph for S/N ratios of grey relational grade (Figure 2). A higher rate of grey relational grade judge a greater impact of the particular parameter at that level.

In this study the highest grey relational grade value was achieved in third experiment as shown in Table 3 (0.8257). Out of Table 3 and Figure 2, the best combination of input parameters was determined. The best combination values of input parameters were catalyst concentration (A) of 0.5%, molar ratio of oil to methanol (B) of 12:1, reaction temperature (C) of 60 °C and reaction time (D) of 80 min and represented as A1B3C3D3. In this study, the maximum yields of pelemir biodiesel of 98% and the kinematic viscosity as 3.7 mm²/s was measured using determined optimal levels of the input parameters by statistical analysis.

**Characterization of Pelemir Seed Oil Biodiesel.** After the best combination of factor level (A1B3C3D3) was determined pelemir seed oil biodiesel and it was produced according to this combination. Fuel properties of PSOB results compared with No.2 diesel fuel, EN 14214 and ASTM D 6751 biodiesel standards. Table 4 shows fuel properties of PSOB. It was seen that the analysis results satisfied all the values of specified in EN 14214.

<table>
<thead>
<tr>
<th>Experiment number</th>
<th>Grey relational coefficient (y1) (%)</th>
<th>Grey relational coefficient (y2) (mm²/s)</th>
<th>Grey relational grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.7142</td>
<td>0.4128</td>
<td>0.5635</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>0.4639</td>
<td>0.7319</td>
</tr>
<tr>
<td>3</td>
<td>0.8333</td>
<td>0.8181</td>
<td>0.8257</td>
</tr>
<tr>
<td>4</td>
<td>0.500</td>
<td>1</td>
<td>0.7500</td>
</tr>
<tr>
<td>5</td>
<td>0.8333</td>
<td>0.6164</td>
<td>0.7248</td>
</tr>
<tr>
<td>6</td>
<td>0.7142</td>
<td>0.5696</td>
<td>0.6419</td>
</tr>
<tr>
<td>7</td>
<td>0.3333</td>
<td>0.6924</td>
<td>0.5128</td>
</tr>
<tr>
<td>8</td>
<td>0.6252</td>
<td>0.3333</td>
<td>0.4791</td>
</tr>
<tr>
<td>9</td>
<td>0.4545</td>
<td>0.6716</td>
<td>0.5630</td>
</tr>
</tbody>
</table>

**FIGURE 2**  
S/N ratio for grey relational grade levels of factors.
### Table 4

<table>
<thead>
<tr>
<th>Analysis</th>
<th>Unit</th>
<th>Method</th>
<th>PSOB</th>
<th>EN14214</th>
<th>ASTM D 6751</th>
<th>Diesel No. 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ester content</td>
<td>%(m/m)</td>
<td>EN 14103</td>
<td>98</td>
<td>min 96.5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Monoglyceride</td>
<td>%(m/m)</td>
<td>EN 14103</td>
<td>0.50</td>
<td>max 0.80</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Diglyceride</td>
<td>%(m/m)</td>
<td>EN 14103</td>
<td>0.48</td>
<td>max 0.80</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>%(m/m)</td>
<td>EN 14103</td>
<td>0.14</td>
<td>max 0.20</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Density, 15 °C</td>
<td>g/cm³</td>
<td>EN ISO 3675</td>
<td>0.89</td>
<td>0.86 - 0.90</td>
<td>0.82-0.9</td>
<td>0.82 – 0.86</td>
</tr>
<tr>
<td>Kinematic viscosity</td>
<td>mm²/s</td>
<td>EN ISO 3104</td>
<td>3.7</td>
<td>3.5-5</td>
<td>1.9-6.0</td>
<td>2.5 – 3.5</td>
</tr>
<tr>
<td>Methanol content, % (m/m)</td>
<td></td>
<td>EN 1410</td>
<td>0.16</td>
<td>max 0.20</td>
<td>max 0.20</td>
<td>-</td>
</tr>
<tr>
<td>Iodine value</td>
<td>g iodine/100</td>
<td>EN11411</td>
<td>89.3</td>
<td>max 120</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Pour point °C</td>
<td></td>
<td>ASTM D 97</td>
<td>-8</td>
<td>max 0</td>
<td>0</td>
<td>-33</td>
</tr>
<tr>
<td>Flash point °C</td>
<td></td>
<td>ASTM D 93</td>
<td>135</td>
<td>min 120</td>
<td>93</td>
<td>&gt;55</td>
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<tr>
<td>Heating value MJ/kg</td>
<td></td>
<td>ASTM D 240</td>
<td>38.86</td>
<td>min 35</td>
<td>-</td>
<td>42.7</td>
</tr>
<tr>
<td>Copper band corrosion (3 hours at 50 °C)</td>
<td>-</td>
<td>EN ISO 2160</td>
<td>1a</td>
<td>1a</td>
<td>No.3</td>
<td>-</td>
</tr>
<tr>
<td>Total contamination mg/kg</td>
<td></td>
<td>EN12662</td>
<td>20</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total sulfur content mg/kg</td>
<td></td>
<td>EN14105</td>
<td>0.02</td>
<td>max 0.25</td>
<td>0.24</td>
<td>-</td>
</tr>
<tr>
<td>Sulphur content % (m/m)</td>
<td></td>
<td>EN ISO 20846</td>
<td>0</td>
<td>max 10</td>
<td>500 ppm</td>
<td>350</td>
</tr>
<tr>
<td>Water content mg/kg</td>
<td></td>
<td>EN ISO 3987</td>
<td>0.01</td>
<td>max 0.02</td>
<td>0.02</td>
<td>-</td>
</tr>
<tr>
<td>Phosphorus mg/kg</td>
<td></td>
<td>EN ISO 12937</td>
<td>100</td>
<td>max 500</td>
<td>max 500</td>
<td>&lt;200</td>
</tr>
<tr>
<td>Water content mg/kg</td>
<td></td>
<td>EN 14107</td>
<td>3.2</td>
<td>max 4</td>
<td>max 10</td>
<td>-</td>
</tr>
</tbody>
</table>

### CONCLUSIONS

This study revealed that the Taguchi method connect with grey relational analysis can be effectively used for the research of multiple-performance characteristics of pelemir seed oil biodiesel production. Owing to this statistical method used, the optimal combination were found as 0.5% catalyst concentration, 12:1 molar ratio of oil to methanol, 60 °C reaction temperature and 80 min reaction time for input parameters. When these levels of the input parameters were used, the highest yields of biodiesel as 98%, while the kinematic viscosity value was measured as 3.7 mm²/s remaining within the nominal limit values. According to the best combination, the results of the fuel properties of pelemir seed oil biodiesel were provide EN 14214 and ASTM D 6751 biodiesel standards.

### REFERENCES


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REVISIT INTENTION OF TOURISTS BY DEMOGRAPHIC PROFILES: THE CASE OF BARTIN

Deniz Celik
Bartin University, Vocational High School, Programme of Landscape and Ornamental Plants, Bartin, Turkey

ABSTRACT

Revisit intention of tourists, there is a link between destination natural and cultural landscape values, holiday satisfactions, transportation alternatives, climate conditions and etc. In this context, the aim of this study is to determine whether revisit intention of tourists depending on demographic profiles. Within the purposes of this study; Bartın province, which is located in the Western Black Sea region of Turkey and has rich natural and cultural landscape, was selected as the study area. The survey was conducted with tourists in Bartın. The Simple Random Sampling Method was used in the survey, and 17 closed-ended and 19 other questions were asked to the participants. The reliability, frequency, arithmetic mean (arit. mean), standard deviation (SD) calculations were made, and the t-test and ANOVA analyses were performed. As a result of the study; except for the gender variable, other demographic profiles such as age, education, occupation, and income groups were not determined as the effective factors in revisit intention. So, the revisit intention of tourist will shape according to the characteristics of the destination regardless of the demographic features of tourist. Therefore, it has been suggested that the improvement of physical infrastructure and superstructure, historical buildings, traditional food and handicrafts, alternative tourism activities, destination image, and promotion and marketing activities in Bartın province.

KEYWORDS: Revisit intention, satisfaction, tourism potential, attraction center, Bartın

INTRODUCTION

Many researchers examine whether there is a relationship between the natural and cultural etc. characteristics of the destination and the socio-cultural characteristics of the tourists [1, 2, 3, 4, 5, 6]. In addition, the literatures stated that regarding the revisit intention of tourists, there is a link between destination resources, landscape values, activity opportunities, satisfaction and tourist motivation [7, 8, 9]. At the same time, natural, cultural and physical potentials, sufficient infrastructures and superstructures (such as accommodation, transportation, tourist activities, shopping centers, and attraction centers), regional conditions [10], transportation alternatives, diversity of information and communication resources, work and social environment [11] and climate conditions also play roles on revisit intention [12, 13]. Other than the demographic profiles of tourists, their previous holiday satisfactions and travel motivations may directly affect their preferences on paying a visit to the same destination; in other words, they show visitor’s loyalty in their next touristic trip [14, 15, 16]. In visitor’s profile and behavior; however, the image of a destination may also have an effect on preferability along with that destination’s nearness and farness [17, 18, 19]. Also, being an attraction center, having facilities, service quality and accessibility play significant roles in choosing a place as a destination [20].

In this context, the aim of this study is to investigate whether there is a difference between revisit intention of tourists and their demographic profiles in Bartın. Thus, it will be possible to find answers to the following questions such as: What are the potentials of Bartın province perceived by tourists? What are the most suitable tourism activities for the use of these potentials? What are the tendencies and expectations of tourists from Bartın province?

MATERIALS AND METHODS

The main material of this study is the tourists themselves coming to Bartın province. In addition, the Bartın city itself, the obtained survey data, and literature related to the subject and area were also considered as study material. The study was carried out within the provincial borders of Bartın (Figure 1). Bartın province is located between the latitude of 41° 37’ north and altitude of 32° 22’ east. Two main creeks, namely Kocaçay and Kocanazçay, pass through the city center. The historical Amasra (Sesamos), Kurucaşile (Kromna) and Çakraz (Erythinoi) antique cities in the ‘Paphlagonia’ region is located within Bartın provincial borders. Amasra, Inkumu, and Çakraz, which are located nearby Bartın city center, are the most preferred rural settlements for
the sea tourism. In addition, the region has a touristic importance regarding its natural landscapes such as National park of Kure Mountains, Ulu, Ardiç and Gezen Plateaus, and Gurcuoluk Cave located around Bartın province [21]. Bartın province was chosen as the study area since it has a strong attractiveness for tourists with its rich natural and cultural landscape potentials.

![Localization of the Bartın City in the Turkey Map.](image)

The method and survey questions for the study were developed by reviewing current literature [22, 23, 24, 25, 26]. The simple random sampling method was used in the questionnaires. While determining the sample size, both 95% confidence interval and 10% margin of error were taken into account. Hence, the number of persons to be surveyed was calculated as 96 tourists (Table 1) [27, 28]. The number of national tourists visiting Bartın province was realized as 368,479 in 2014 [21].

The survey was conducted for tourists between May and August 2017. This process was carried out by interviewers via face to face surveying method. With the intent of determining the profile of participants, questions related to gender, age, educational status, occupation, income status, and geographical regions were asked in the survey. For tourist participating in the survey, there were also questions regarding their ‘number of visits, duration of visit, reasons for destination choice, tourism activities made, revisiting tendencies, recommend to others, expectations, and tendencies for Bartın city’. There were 17 closed-ended questions and 19 questions with Likert scale. The tourists participating in the survey were given six propositions related to “Bartın’s potentials” and 13 “Propositions related to Bartın city”, and they were asked to classify these propositions based on the five-point Likert scale between ‘1 disagree and 5 agree’. The SPSS version 20.0 and Excel programs were used to evaluate the surveyed data.

The reliability, frequency, arithmetic mean (arit. mean), standard deviation (SD) calculations were made, and the t-test and ANOVA analyses were performed with the answers given to the prepared questions to examine whether the revisit intention of tourists show variations in accordance with demographic profiles of participants such as gender, age, education level, occupation, and monthly income status. These results were summarized below.

**RESULTS AND DISCUSSION**

The reliability analysis was performed to the survey questions, which were prepared based on five-Likert scale. The reliability analysis, which was performed to measure internal consistencies of the propositions, resulted in a Cronbach Alpha value of 0.814 for the “Potentials of Bartın city”; and 0.897 for the “Propositions of Bartın city”. In the event that the Cronbach Alpha value is within the interval of 0.00 < x < 1.00 and even closer to 1.00, then the survey was considered to be significantly reliable [29, 30, 31, 32]. Of all the tourist attended to the survey, 35.4% stated that they visited Bartın province two to three times, 27.1% visited for the first time; however, 25.0% visited more than six times. For the accommodation, 78.1% of the tourists stated that they preferred staying in Amasra county, 16.7% in Bartın city, and 3.1% in İnkumu county. About 61.5% of the participants specified that they also visited Bartın city center. Of all the participants, 51.0% expressed that they travelled with their families, 41.7% with their friends, and 7.3% with a tour organized. When the participants were listed in accordance with their holiday time duration, the highest ratio of 42.7% was obtained from the time duration between two to four days. Following that, 31.3% of the visitors had one-day duration, and 16.7% had five to seven days of duration, respectively. About 57.3% of the tourists expressed that they visited Amasra, Çakraz, and İnkumu counties due to potential sea tourism of these settlements; however, they travelled to Bartın due to its natural beauties. Furthermore, about 55.2% of the participants stated that they also visited Bartın since there was easy transportation, and it was nearby their settlements; similarly, about 18.8% of the participants visited Bartın due to its handicrafts and foods, and 12.5% for its historical houses.
TABLE 1
Calculation of sample size of tourists

\[ n \geq \frac{Z^2 \times N \times p \times q}{N \times D^2 + Z^2 \times p \times q} \]

Where: 
- \( n \) : Sample size,
- \( Z \) : Reliability coefficient (\( Z = 1.96 \)),
- \( N \) : Main group size (\( N = 368.479 \)),
- \( p \) : Rate of presence of feature on demand in main group (0.5),
- \( q \) : Rate of absence of feature on demand in main group (1-p=0.5),
- \( D \) : Acceptable sample error margin (%10).

TABLE 2
The descriptive statistics of propositions for Bartın city potentials

<table>
<thead>
<tr>
<th>Variable</th>
<th>Propositions</th>
<th>Percentage Distribution of Answers</th>
<th>Arith. Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bartın city potentials</td>
<td>to be nearby the major destinations such as Amasra, Inkumu, Cakraz</td>
<td>4.2 2.1 8.3 14.6 70.8</td>
<td>4.46</td>
<td>1.025</td>
</tr>
<tr>
<td></td>
<td>to be nearby the major attractions such as Safranbolu, Yedigöller</td>
<td>2.1 3.1 9.4 20.8 64.6</td>
<td>4.43</td>
<td>0.937</td>
</tr>
<tr>
<td></td>
<td>passing of a river through the city</td>
<td>1.0 6.3 21.9 26.0 44.8</td>
<td>4.07</td>
<td>1.008</td>
</tr>
<tr>
<td></td>
<td>to be in close proximity to Ankara and Istanbul cities' having natural beauties such as</td>
<td>2.1 3.1 10.4 13.5 70.8</td>
<td>4.48</td>
<td>0.951</td>
</tr>
<tr>
<td></td>
<td>Kure Mountains National Park and Ulukaya waterfall</td>
<td>2.1 5.2 20.8 16.7 55.2</td>
<td>4.18</td>
<td>1.066</td>
</tr>
</tbody>
</table>

1 disagree, 2 moderately disagree, 3 undecided, 4 moderately agree, 5 agree

When the tourists participating in the survey were classified in accordance with their activity variables, which were actualized during a holiday period, about 78.1% of the tourists were determined to prefer the activities of ‘hiking and resting in locations where they accommodated such as Amasra and Inkumu.’ Then, 52.1% of the participants stated that they preferred to visit diverse places and to take photos; 41.7% preferred to visit Bartın city center; 27.1% preferred to see natural wonders such as the National Park of Kure Mountains, Ulukaya Waterfall, and Arıt Plateau; and 19.8% preferred to do hiking in natural areas. Of all the surveyed tourists, 95.8% expressed that they would advise their friends to pay a visit to Bartın for a holiday, and 93.8% stated that they would come to Bartın city again. In addition, participants stated that the most important factor to prefer Bartın city was the following reasons: 64.6% due to their friend’s recommendation, 27.1% via internet, and 6.3% by a tour agency. Regarding the transportation preferences, 62.5% of the tourists participating in the survey had their own vehicle; however, 29.2% used intercity bus trip.

The frequency, arithmetic mean and standard deviation analyses, which were performed based on the answers provided by the tourists regarding their revisit intention variables, were presented in the Table 2.

With an arithmetic mean of 4.24, the surveyed tourists expressed that they found Bartın city as a safe place to travel. In the event that the other propositions were listed with their arithmetic means, the following statements and values were enumerated: availability of gift markets and shopping centers (4.16) and tourist attraction potential of historical buildings (4.08). In the last rank; it was stated that there were sufficient facilities (3.57) as superstructures (garden, parking area, bank, post office, health center, social facility, etc.) in Bartın city.

The “Bartın city potentials” and “Propositions for Bartın City” variables included in the survey were separately subjected to normality tests, and the Skewness and Kurtosis values for the two variables were identified between +1.5 and (-1.5). Since the significance level in statistical analyses was \( p < 0.05 \), it was accepted that the survey showed normal distribution [33].

Regarding the reason for revisit intention of surveyed participants, the ‘independent samples t-test’ was performed to determine whether their perceived significance level differ based on demographic profiles. In this context, the \( t \)-test results as regard to gender variable were presented in Table 3. The \( t \)-test conducted between the Bartın’s potential and participant’s gender resulted in \( p < 0.02 \). However, the \( t \)-test conducted between participant’s gender and propositions for Bartın city was determined as \( p < 0.00 \). Since the analysis was resulted within the \( p < 0.05 \) interval, the difference among participants’ means was regarded as statistically significant. Females scored the propositions higher than males.
Therefore, it was postulated that the components related to the reasons for preference of Bartın varied in accordance with genders of participants.

Among the surveyed tourists to test whether there was a difference between demographic profile variables age, education, occupation, and income groups, which might be effective in revisit intention such as “Potentials of Bartın city”, ”Propositions about Bartın city”, the one-way ANOVA tests were performed using Post Hoc multiple comparison criteria. These results were given in Table 4, 5, 6 and 7. Since the p-value, which was stated in the tables, was \( p < 0.05 \), no difference was determined among all variables. Bartın was preferred as a holiday destination in a homogenous way by all age groups, education levels, occupations and income groups.

It is observed that the concepts such as natural and cultural landscape potentials of a destination, touristic activities, facilities, infrastructure services, image, etc. interact both with each other and with the demographic structures of tourists. Demographic profiles of tourists such as gender, age, income status, education level, place of residence, and marital status can usually be effective in the revisit intention. In the literature review, it was determined that reliability, frequency, mean and factor analysis, \( t \)-test and ANOVA analysis were performed when investigating whether there was a change in the revisit intention according to the demographic variables. Similarly, analyses for reliability, frequency, mean, \( t \)-test and ANOVA were also performed in this study.

<table>
<thead>
<tr>
<th>Gender</th>
<th>Total</th>
<th>Arit. Mean</th>
<th>SD</th>
<th>( t ) value</th>
<th>Significance Value (p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bartın city potentials</td>
<td>Female</td>
<td>45</td>
<td>4,50</td>
<td>0,55</td>
<td>2,34</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>51</td>
<td>4,17</td>
<td>0,80</td>
<td></td>
</tr>
<tr>
<td>Propositions for Bartın City</td>
<td>Female</td>
<td>45</td>
<td>4,14</td>
<td>0,58</td>
<td>2,90</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>51</td>
<td>3,73</td>
<td>0,76</td>
<td></td>
</tr>
</tbody>
</table>

\( p < 0.05 \)

<table>
<thead>
<tr>
<th>Age</th>
<th>N</th>
<th>Arit. Mean</th>
<th>SD</th>
<th>Levene Value (p)</th>
<th>F value</th>
<th>Significance Value (p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bartın city potentials</td>
<td>15-25</td>
<td>7</td>
<td>4.11</td>
<td>0.75</td>
<td>0.12</td>
<td>1.67</td>
</tr>
<tr>
<td></td>
<td>26-35</td>
<td>24</td>
<td>4.15</td>
<td>0.64</td>
<td></td>
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<tr>
<td></td>
<td>36-45</td>
<td>31</td>
<td>4.24</td>
<td>0.86</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>46-55</td>
<td>15</td>
<td>4.65</td>
<td>0.52</td>
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</tr>
<tr>
<td></td>
<td>56-65</td>
<td>11</td>
<td>4.66</td>
<td>0.57</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>66+</td>
<td>8</td>
<td>4.29</td>
<td>0.54</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Propositions for Bartın City</td>
<td>15-25</td>
<td>7</td>
<td>4.02</td>
<td>0.88</td>
<td>0.24</td>
<td>1.21</td>
</tr>
<tr>
<td></td>
<td>26-35</td>
<td>24</td>
<td>3.79</td>
<td>0.80</td>
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<tr>
<td></td>
<td>36-45</td>
<td>31</td>
<td>3.75</td>
<td>0.75</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>46-55</td>
<td>15</td>
<td>4.11</td>
<td>0.59</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>56-65</td>
<td>11</td>
<td>4.18</td>
<td>0.44</td>
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<tr>
<td></td>
<td>66+</td>
<td>8</td>
<td>4.17</td>
<td>0.56</td>
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<table>
<thead>
<tr>
<th>Education Level</th>
<th>N</th>
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<th>SD</th>
<th>Levene Value (p)</th>
<th>F value</th>
<th>Significance Value (p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bartın city potentials</td>
<td>Primary education</td>
<td>3</td>
<td>4.44</td>
<td>0.96</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Secondary education</td>
<td>9</td>
<td>4.53</td>
<td>0.59</td>
<td>0.82</td>
<td>0.31</td>
</tr>
<tr>
<td></td>
<td>High school</td>
<td>32</td>
<td>4.28</td>
<td>0.69</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>University</td>
<td>52</td>
<td>4.31</td>
<td>0.74</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Propositions for Bartın City</td>
<td>Primary education</td>
<td>3</td>
<td>3.84</td>
<td>0.13</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Secondary education</td>
<td>9</td>
<td>3.97</td>
<td>0.58</td>
<td>0.12</td>
<td>2.48</td>
</tr>
<tr>
<td></td>
<td>High school</td>
<td>32</td>
<td>4.18</td>
<td>0.63</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>University</td>
<td>52</td>
<td>3.76</td>
<td>0.76</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
It was stated that vacation factors perceived by Japanese tourists varied depending on both socio-demographic profiles of tourists and travel types [23]. At the same time, it was expressed that touristic activities made in natural areas depended on destination distance and characteristics, and participation in recreational activities varied according to the socio-economic status. Being in employment, high income, education level, and nationality were effective factors in making a decision to participate in recreational activities or revisit intention [22]. In the Bartın and its surroundings, there are natural landscape potentials such as Kure Mountains National Park and Ulukaya, and especially in Amasra, there are historical places for cultural tourism. However, except for gender factor, it was determined that other demographic profiles of the surveyed tourists were not effective in revisit intention. Regardless of age, education, occupation and income groups, tourists preferred both destinations and recreational activities in natural and cultural environments.

In a study was emphasized that attraction centers, potentials and distance of a destination play a significant role in selection of a destination or revisit intention. Also, gender, age and marital status of tourists were the effective factors in selection of tourist activities [34]. Indeed, in the current study, it was determined that the proposition Bartın city being within a close-range to Ankara (capital city), Istanbul, other important destinations, and attraction centers was identified as a significant potential to choose Bartın as a destination point. Therefore, it can be

### TABLE 6
Comparison for destination choice variables by occupation

<table>
<thead>
<tr>
<th>Occupation</th>
<th>N</th>
<th>Arit. Mean</th>
<th>SD</th>
<th>Levene Value (p)</th>
<th>F value</th>
<th>Significance Value (p)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bartın city potentials</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Student</td>
<td>8</td>
<td>4.3125</td>
<td>0.65730</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Private sector</td>
<td>28</td>
<td>4.1964</td>
<td>0.76046</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Public sector</td>
<td>22</td>
<td>4.4091</td>
<td>0.62090</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Self-employment</td>
<td>7</td>
<td>3.8095</td>
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### TABLE 7
Comparison for destination choice variables by income status

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concluded that geographical distance of a destination (Bartın) can be a reason of revisit intention.

Crouch et al. [14] stated in their study that age and wealth were more effective in revisit intention; however, gender was less effective. They specified that previous experiences could be effective in revisit intention as well. They also indicated that preference of older tourist groups for the next destination was made in accordance with art and nature-based experiences; however, youth wanted to engage in more entertaining activities. Furthermore, they emphasized that the tourists with high income wished to participate in different activities such as food, art, and recreational activities. Our study also demonstrated resemblance with the literature studies in terms of the fact that gender might be an effective factor in revisit intention. Similarly, tourists were in tendency to revisit Bartın. About 65% of the tourists were 45 years old or below ages. Nevertheless, while 25% of the tourists desired to visit natural areas and do recreational activities, the remaining tourists were in tendency to make more passive recreations such as walking in the city and taking photos. Therefore, the current study differed negatively from other literatures in terms of age and income status to be effective in revisit intention.

CONCLUSION

Personal choices can also be effective in preference of a destination along with the features of that destination such as tourism potentials, facilities, services, and accessibility. These can be enumerated as travel cost and distance, touristic activities, as well as age, gender, education and income status of visitors. Satisfaction of tourists in this matter is also important. The quality service is of importance on satisfaction. The higher the satisfaction of tourists, the higher they are inclined to revisit intention. In addition, factors such as political conditions [35] and climate conditions may also be effective in revisit intention [36].

In this context, the literature review revealed that revisit intention might vary from country to country, province to province or even period to period. The tourist intention may also change in time due to technological progress, diversification of information and communication resources, increase in transportation alternatives, and diversification of tourist activities.

In our research, a significant relationship was determined between revisit intention and gender of tourists. No significant relationship was identified between revisit intention and among other demographic profiles such as age, education, occupation, and income groups.

Within the scope of this study, only domestic tourists were surveyed. The survey questions were prepared for Bartın citywide. These two elements were considered the limiters of the study. For the next study; therefore, it will be planned to conduct surveys with both local and foreign tourists and specific to open-green areas.

Finally, this research shows that the revisit intention of tourist will shape according to the characteristics of the destination regardless of the demographic features of them. In this scope, it is essential to in revisit intention of tourist to improve and increase physical infrastructure and superstructure, to diversify products, to generate alternative activities, and to establish a brand identity and image. It is thought that the research will contribute to the literature to emphasize the importance of this issue.

ACKNOWLEDGEMENTS

This article relied on scientific research project No. 2016-FEN-A-001, entitled ‘Assessment of Balkamba Natural Park Landscape Potential in terms of Ecotourism’. The floristic research in the project was completed by Assoc. Prof. Zafer Kaya and Asst. Prof. Cevdet Gumus. I thank them for their contributions and participation in the project. Also, I would like to express my thanks to Bartın University Scientific Research Projects Coordination Unit for financial supporting this project.

REFERENCES


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CLIMATE CHANGE PERSPECTIVE IN MOUNTAIN AREA: IMPACTS AND ADAPTATIONS IN NALTAR VALLEY, WESTERN HIMALAYA, PAKISTAN

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4Department of Applied Mathematics, Chung Yuan Christian University, Taoyuan, Taiwan

ABSTRACT

Change in climate and its adverse impacts are rewarding progressively more obvious in fragile ecosystems of Gilgit-Baltistan. Due to geomorphological, topographic, and ecological conditions mountainous landscapes of Hindukush, Karakoram and Himalaya (HKH) is more vulnerable to climate change effects. A detailed questionnaire survey was conducted in eastern Himalaya (Naltar Valley) to admit people’s perception regarding climate change scenario, its impacts on the livelihoods, adaptation and coping measures. Results depicted that people were aware of change in climate, as majority of respondents believed that increase in temperature with decreasing rainfall in winters. They perceived that temperature got more or less extreme as compared to past. Perceptions regarding weather patterns were checked through trend of climate data (1951-2013). Peoples have experienced profusion of issues and foreseeable diseases both in humans and animals, and facing more climate related hazards like landslide, floods and avalanches consequently impacted their social and economic life very badly. The community also professed the climate change associated hazards severely impacted the amount of pastures as the amount of pastures have decreased as compared to past; and people reduced their number of livestock with due reason. Some of them identified some species in pastures and agricultural lands are increasing. Owing to change in climate they identified some coping strategies and adaptation measure to tackle such issues.

KEYWORDS:
Climate change, Karakoram region, Mountain life, Perception, Pastures, Adaptation

INTRODUCTION

Climate change has a great impact on the pastures and livelihood of high altitude. The Hindu Kush and Himalayan ranges are more vulnerable to climate change [1], so there is need to maintain the proper ecosystem services for better livelihoods of mountain communities. The three main drivers of environmental change in HKH region are climate change, land use change and population dynamics [2]. Large amount of variability in the climatic condition in Gilgit Baltistan were observed which are characterized as low annual precipitation, a great range of mean monthly temperature, and harsh frost in winter seasons [3]. Variability in climate change has many adverse environmental effects [2] which cause flash floods, landslides, river bank erosion and flooding of fields [4]. The average increase in temperature of earth surface for this century is 0.61 °C with a threshold level of 2 °C on a reference period of 1986 to 2005 [5]. The records from the past years have shown an increase in mean temperature all over the globe [6] with the global mean temperature increase at the rate of 0.007 °C decade-1 over the last century [7]. This change in scale of temperature has very adverse effects on ecosystem services, and to all communities who take benefit from natural resources [8]. Temperatures are likely to increase more in the high mountain areas than elsewhere [9]. Deforestation leads increase in surface temperature on global scale, thus it change whole global climatic scenario [10].

Warming of earth’s lower temperature gives the potential for certain severe weather events which affect human health directly or indirectly (National Academy of Sciences). Vector borne diseases and water borne diseases are the indirect effects of climate change. According to [11] endemic morbidity and mortality due to diarrheal diseases associated with floods and droughts are expected to rise in across Asia. Increase in the frequency of high intensity rain fall often leading to flash floods and landslides which ultimately affects the human health and livelihood [12]. Pakistan is highly vulnerable to Climate change, hence large floods and droughts are expected in future [13]. Malaria mosquito is observed in high altitudes of HKH region [14]. Rangelands are the second largest land use type encompassing about 2.91 million hectares is
the most important terrestrial ecosystem in Gilgit-Baltistan [15] which are severely affected by climate change and other anthropogenic effects [16]. Vegetation productivity in the HKH region has significantly affected by climate change [14] which is also been observed in Gilgit Baltistan [17]. Farmers in Northern Pakistan also perceived the adverse effects of climate change on pastures and pasture productivity [18]. High altitude areas of HKH region due to unpredictable events such as snowstorms resulting in lack of forage have always led to sudden losses of Livestock in the region [19]. Forage productivity of all rangelands except alpine pastures is three times less than their potential [16]. Mountain ecosystem cover about one fifth of the earth’s continental areas [20] and so called storehouses of global biodiversity [21]. Rapid changes in climatic conditions are therefore likely to change the geographic extent of species distribution, resulting in latitudinal and altitudinal shifts and contraction of species ranges [22].

Climate change is a clear reality and is a greatest challenge of the today’s world. Developing countries are more susceptible for climate change due to lack of resources and capacities to protect against climate related hazards. Most people in the eastern Himalayas perceive climate change as threat [23]. The specific objective was to investigate the climate change impacts on weather patterns, human health, livelihood, pastures and livestock. General herding and seasonal direction with vertical dimension of Pastoralism were also mapped.

**MATERIALS AND METHODS**

**Study Area.** Naltar is 42 km from Gilgit, linked by a jeep road, one of the rainiest valleys in north of Pakistan. Road to Upper Naltar opens year round but beyond Kheot, discarded during winter. Naltar is truly a spectacular valley, covered with pine forests, summer meadows, snowy peaks trekking routes, small lakes, glaciers and above all home to winter skiing [24].

On the basis of landscape Naltar is divided in two parts Naltar Paeen (Lower Naltar) and Naltar Bala (Upper Naltar). Area between Nomal and Taurbat, Kanchli is known as lower Nalter (Figure 1). This area has dry mountains. Livestock development is not so important in this area due to the dry landscape and bare foliage in the mountains. In winters herders bring their sheep’s and goats in this area. The area between Taurbat, Kanchli to Naltar Pass is known as Naltar Bala. The physical environment of upper Naltar is quite different from lower Naltar as lakes, morains and glaciers can easily be seen [25].
Data collection and Analysis. The study is based on people’s perception and field survey conducted in August 2017 in Nagare and Dulan (Figure 1). The survey was done through questionnaire with both open and close ended questions. The survey questionnaire was divided into different sections which are perception on climate change, impacts on health and livelihood, impacts on pastures and adaptation measures. Stratified random sampling was employed. The sample population comprised of people aged more than 40-50. No female was interviewed due to the cultural values and norms. Some of the data and real information was collected through personal observation and participation, especially the areas affected by climate change related hazards like flashflood/landslide etc. to gather all the necessary and relevant information, personal observation and participation has been done, as sometimes respondents do not answer clearly about the question being asked. Further secondary data related to agriculture, livestock, socioeconomic and climate data was analyzed. Supplementary review was made from published and unpublished data to cross check the findings. Meteoro logical data was acquired from Pakistan Meteorological Department (PMD) from 1951-1999 and Data from 1999-2013 was acquired from Gilgit Baltistan Environmental Protection Agency (GB-EPA) to check long term changes in overall previous climate of the area including temperature and rainfall to cross check questionnaire data. The data and information collected were tabulated in the MS-Excel-2007. Regression analysis was applied to see the trends in temperature and rainfall.

RESULTS

Impacts of Climate Change on weather. The increase in temperature has mainly affected high mountainous ecosystems of Gilgit-Baltistan; past records show that the late twentieth century has highest rate of increase in temperature [26]. In the current study respondents were asked about the change in temperature. They were also asked about the months in which they feel more temperature change. About 79% respondents perceived that climate change is happening and they told that temperature is changed from the last 10-15 years and 68% of the respondents are having the view of increase in temperature. There is an increasing trend of temperature in the last two decades from 1980 to 2006 in Gilgit Baltistan [27]. Some of them identified that the temperature got more or less extreme. They also told that June and July are the hottest time of the year. When asked about the rainfall about 46% respondents are having the view that rainfall has decreased but 23% of the respondents told that the intensity of the rain has increased. Majority of the respondents also identified decrease in winter rainfall. About 32% respondents identified decrease in snowfall while there was a mix response regarding the number of days of snowfall. The perceived responses were cross checked with climate data. Climate data shows an increasing trend in annual temperature from 1951-2013. Climate data shows an increasing trend in both temperature and precipitation (Figure 3, 4). Analysis of PMD shows that the precipitation seems to be an increasing trend in Gilgit-Baltistan [28].
**Perceived Impacts on human health and livelihood.** Farmers have experienced skin related diseases like ringworm, measles, and prickly heat in Nepal due to heat waves [29]. When asked about the impacts of climate change on human health 63% respondents said that they observed new diseases in the local area, some of them identified some diseases which are Typhoid, Heart attack, Cancer, Asthma and increase in blood pressure. Climate hazards such as flood followed by heavy intense rainfalls, landslides and windstorms greatly impacted on human causalities and injuries [30]. They also told that there is a small hospital in the valley with no medicines in it, the doctor doesn’t available all the time in the hospital so they have to travel to Gilgit taking their patients for further treatment which is a two hour travel from Naltar Bala to Gilgit city.

Climate Change has always manifested itself through extreme events such like the floods, droughts, storms and GLOF [15]. Rapid melt of snow and glacial lake outburst floods which also enhance landslides [15]. People of Naltar valley are aware of the hazards related to climate change. When asked about climate related hazards they faced in last 5-10 years they identified drought, flood, landslide, snowstorm and avalanches and they discussed that in the last ten years they have faced some of the different hazards and 80% believe that the frequency of such hazards like landslide and floods are increasing. They also feel threatened from the increasing amount of such hazards. Increased risk of natural hazards like floods, landslides extreme weathers land degradation outbreak of pests and food shortages [23]. In mountainous areas like Naltar valley it is very difficult to store enough food owing to the economic problems of the people. Majority of the people belong to lower class so they cannot afford too much and they cannot afford such good homes. When asked about reserves of food from their agricultural input around 78% people said no. They told that the food they get from their farmland can be only utilized for only 4-5 months only and they also cannot afford good homes which can give them shelter in the extreme weather conditions in winters. 72% of the respondents identified that such hazards are badly impacting the local economy.

Wheat and maize don’t give them enough amount of money as compared to potato so they grow potato which gives them enough and profitable amount of money that can be used to spend months from their livelihood. Majority of the people in Naltar valley grow potato instead of wheat and maize. 84% of the respondents identified the occurrence of pest in farmland and they are having the view that potato crop is mostly effected by it. A mix response of soil erosion in farmland was observed some of the respondents are of the view that fast rain also leads to soil erosion. When asked about landslides and around 68% respondents feel increase in such events as compare to the past, they also told that when any such event occurs the local government of Gilgit Baltistan helps them. In Himalayan region 30% of lakes and marshes have disappeared due to increasing climate variability during the past few decades [23]. Stream water is the major source for drinking purpose in Naltar valley and majority of the respondents agree with the fact that water is enough for their household, farmland and grazing land and no water conflicts were observed in the community over water, although 28% respondents observed decrease in amount of water flow in streams. Peoples in Himalaya Uttarakhand, India experienced scarcity of water in the region, it’s perceived to be responsible for reduced duration of snow in the region [31].

There is an increase of pests and outbreak of new diseases to the livestock in the Himalaya Uttarakhand, India [31]. Livestock is a major source of income in mountain livelihood. Around 69% respondents identified new diseased in livestock. When asked about new diseases in livestock some of the respondents identified new diseases in cattle, Shee’s, goats and cows which are Black Quarter and Foot and Mouth Disease (FMD). Peoples have experienced losses of livestock due to flood, outbreak of new diseases and lack of forages in Western Terai, Nepal [30].
Perceived Climate Change Impacts on Pasture. Rangelands are suffering from desertification, degradation and soil erosion due to climate change and anthropogenic factors [16]. Pastoralism is directly related to farmers, land and their herds. Pastoralism is an ancient profession in Naltar valley. When asked about the current and previous amount of pastures around 81% respondents believe that the amount of pastures has decreased as compared to the past. Around 66% respondents believe that events like lightening, floods and sliding is increased from the past and these events cause significant impacts on forest and grazing land like leaves disorder and grass disturbance. Majority of the respondents believe that events like rainfall, drought, cloudburst and land slide disturbs the rangeland, farmland, livestock and house hold properties.

Alitudinal shifts of some valuable plant species and degradation of pastures and pasture resources is observed in HKH [2]. Decreases in grassland productivity, spreading of invasive species changes in distribution and species composition of plant communities are increases due to climate variability [32]. Around 32% respondents perceived that some of the species in the area have increased in some areas of Naltar valley. A little number of respondents are having the view that increase in
these species are the result of temperature change. The increased identified species are Circium vulgans, Canovolus arvensis, Amaranus viridus, Chenopodium botrys, Artemisia annua, Rubus iruteus and Rumex nepaleusis. 7% of the respondents thought that Circium vulgans is increased in the grazing land. None of the respondents identified any increase in the natural forest, forest fires and the occurrence of any insect pest in the forest and grazing land.

**General Herding and Seasonal Pattern.** Rangelands are those areas of the earth in which, due to physical limitations, such as low and erratic precipitation, rough topography or cold temperatures, are unsuited for cultivated agriculture and are source for forage for wild and domestic animals of the mountain region” [34]. Pastoralism is directly linked to different ecological zones and the peoples have opted different agro-pastoral systems. In summers pastoralists move to high altitude pastures like the shani meadows while in spring and autumn they move down and stay in Naltar bala. In winters all the herders move to Naltar Paeen (lower Nalter) (Figure 7). Climate change is predictable threat to species of Himalayas, environment of these mountains are strongly affected by climate, due to their vertical (altitudinal) dimension [35]. Pastoralism is one major economic source of life in the Hindukush, Karakoram and Himalayan (HKH) mountain livelihood. Majority of the people of Naltar valley believe that the general pattern of moment to different pastures at different altitudes is slightly changed as compared to previous 15-20 years. They move to the spring and summer pastures earlier as compared to the past years, but no one was sure regarding the approximate number of days. They use Indigenous knowledge while moving to the different pastures at different seasons of the year (Figure 7).

**DISCUSSION**

Consistent increase in annual temperature is observed in Pakistan [11]. Mountainous areas in the Northern Pakistan are facing more increase in temperature as compared to lower elevations and sharp increase in temperature is seen in the last two decades as compared to the previous decades [13]. Significant increase in annual maximum temperature from 1980-2006 is recorded in all the stations of Gilgit except Bunji with the average increase of 0.38 °C per decade [27]. Temperature increase is also seen in Bagrot Valley of district Gilgit with shorter winters and warmer summers [3]. It is also clear from the temperature data from 1951-2013 that there is an increase in temperature of 0.0024 °C per decade (Figure 3) and the people’s perception (Figure 2) is well-matched with the previous and current findings. Significant trend of winter precipitation is seen in Gilgit [27]. Precipitation pattern from 1951-2013 shows the amount of precipitation is also increasing with a trend of 0.0652 mm per decade (Figure 4). There is a mismatch in the perception and the precipitation trend of climate data regarding the increase in precipitation; although the previous literature shows an increasing trend in the precipitation which is compatible with the climate data of PMD and GB-EPA from 1951-2013. It was observed from the interviews that community is aware of changing weather patterns and also aware of the threats from the extreme weather events (Figure 2).

Natives feel threat from the climate related hazards. Significant amount of climate extreme events like drought, heat wave, cold spell and heavy rainfall have been increased in the Hindukush, Karakoram and Himalayan region [1]. The only thing they did to overcome such hazards to live away from hazardous areas. There is no early warning system available for the people from such hazards and there is no mobility from such extreme events. It is been observed in the Himalayan region that increase in frequency of high intensity rainfall leads to flash floods and landslides [36]. Mountain communities in Upper Kuhi, Nepal identified some hazards that affect the livelihood more which are erratic rainfall, pest in agriculture, livestock disease and droughts, people related the variation of temperature is linked with risk of pest while erratic rainfall destroys crops and increase flood risks [37]. Mountain communities rely mainly on agriculture and livestock. Shortage of water in streams is attributed to temperature increase and reduced snowfall is major reasons of current changes [37]. Climate change is affecting the socioeconomic life of the mountain communities. Variation in climate and extreme weather events is likely to impact the resource dependent societies, affecting both assets and livelihood in the global south [36]. Climate change has directly affected the amount and quality of water like decrease in river flow [1]. After the initial increase in the river flow in the past 2-3 decades, the amount of water is projected to decrease substantially which will affect the irrigation system in Pakistan [38].

A large number of people in the study area depend upon livestock to meet their basic needs. As majority of them believe that the amount of pastures is decreased as compared to past which they think that climate change could be a major reason of the issue, although many respondents claimed that climate related hazards are also effecting the pastures in some areas. Karakoram mainly possess arid climate. Increase in the temperature can have negative impacts on pastures in the arid and semiarid regions [39] as the duration of the growing season mainly effects the pasture production [40]. The decrease in the grass of different pastures in Gilgit
Baltistan is linked with climate change and the local people of the study area seems correct. As some of the respondents are having the view that some of the grass species in the area has increased. After the specie identification of the collected sample from the study area it is found that all the grass species are less palatable in the area which might be a major reason of their increase.

**Adaptation and Coping Measures.** From results and interviews it is cleared that people of Naltar valley are aware of threats regarding changing climate scenario and they are also trying to keep away from vulnerable areas. Owing to current position they have opted different methods of adaptation strategies to deal with changing weather patterns and climate extreme events. On household level they have made different steps to deal with harsh environment; economically stable families has made sheet homes and people who are poor and cannot afford sheet in their houses use plastics in their roofs and put a little layer of soil on it so that rain water cannot seep through. People who live around vulnerable areas have made protected walls to avoid any sort of flood and water flow into their lands and houses. To deal with economic problems majority of population grow potato instead of wheat or maize as potato gives them a handsome amount of money which other crops cannot. Majority of the people perceive no change in the sowing time of crops. Owing to the current development in the technology all the respondents cleared that they have shifted from traditional farming systems to advance systems like use of tractors and machinery as in the past times people use to plow their fields through animals. Due to current amount of flow of water in streams they have made an irrigation management system. Although many of them believe that there is no proper mechanism for soil conservation, as a large number of people were unaware of the mechanism related to soil management and conservation. Many people are opting different occupations what their ancestors and fore fathers were carrying. They have different opinions regarding the financial issues of their family; instead of selling land or any other material majority of them said that they use to sell their livestock or instead of taking money from any friend or relative.

**CONCLUSION**

As compare with others mountainous areas of the world climate change is also taking place in Gilgit-Baltistan, change in climate and its adverse impacts are rewarding progressively more obvious in fragile ecosystems of Gilgit-Baltistan. Gilgit Baltistan is a mountainous and glaciated landscape, where Karakoram, Himalayas and Hindu Kush – the world’s three great mountain ranges meet. Due to their geo-morphological, topographic, and ecological conditions mountainous landscapes are more vulnerable to climate change effects. A little increase in temperature may cause rapid glacier melting which ultimately causes extensive floods and other associated hazards like soil erosion, deforestation, loss of agricultural lands and infrastructure leading to loss of lives and livelihoods. Agriculture may also affect due to floods and change in precipitation patterns as the solid precipitation has been shifted to liquid precipitation from winter to spring. The sowing and growing seasons have also been shifted forward. Productivity of agriculture and rangelands has been affected by climate change patterns. Composition of species and their habitat is also mismatching. All these sectors are being affected so adaptation and mitigation measures are necessary to cope with these changes. Thus extensive research is needed to identify and manage these challenges.

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**REFERENCES**


INDUCTION OF SYSTEMIC RESISTANCE AGAINST MELOIDOGYNE INCognITA BY DIFFERENT CHEMICAL AND BIOLOGICAL INDUCERS IN TOMATO PLANTS

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ABSTRACT

Root-knot, caused by Meloidogyne incognita, is a soilborne disease that causes severe damage and large losses in tomato production worldwide. For this reason, biological and chemical elicitors of induced resistance were examined for their ability to protect tomato from root-knot disease under glasshouse conditions. Treatment of tomato roots with B. thuringiensis as a soil drench resulted in the highest reduction in the number of galls in root system, while the nematicide Rugby® 20% CS and salicylic acid (SA) showed the highest reduction in egg masses and nematode populations. Moreover, pre-treatment of tomato roots with silica nanoparticles (SINPs) exhibited the highest increase in the shoot fresh and dry weights. The activities of defense related enzymes, peroxidase (POX) and polyphenol oxidase (PPO), were significantly increased in treated tomato plants, SA and ascorbic acid treatments showed the best results in this respect. The best transcription level of SA-inducible gene PR2 was found in SA treated tomato plants followed by the Allium sativum extract treatment. Therefore, B. Thuringiensis, SA and SINPs could provide new alternatives for chemical pesticides in integrated pest management strategies against root-knot diseases that cause synergistic yield losses.

KEYWORDS:
Quantitative RT-PCR, Meloidogyne incognita, Bacillus thuringiensis, Bacillus subtilis, Trichoderma viride, tomato.

INTRODUCTION

Root-knot disease, caused by Meloidogyne incognita, occurs worldwide and affects hundreds of plant species [1]. Root-knot nematodes are considered the most widespread nematode pests restricting tomato production all over the world [1, 2, 3]. It causes severe losses and serious damage to crop production particularly in infested sandy soils [4]. Nematicides could be useful for disease management. Disease incidence, disease severity and gall index of root-knot nematode were significantly decreased in eggplants treated with Rugby 10% G [5]. However, their effects are in general short-lived, so growers are in need for other facilities to reduce nematode infection. Biological control was suggested to be a potential method for disease management. Application of bioagents against nematodes could provide further alternatives for managing the damage caused by root-knot nematodes [6]. The mobility of M. incognita juveniles was stopped at 1 day after treatment with Bacillus thuringiensis [7]. Similarly, culture filtrate treatment with B. cereus resulted in increased mortality of juveniles and decreased egg hatching [8]. Moreover, nematode population was significantly decreased due to treatments with P. fluorescens, B. subtilis and T. viride [9]. The egg hatching of M. javanica was significantly decreased due to treatment with Bacillus spp. [10]. The maximum inhibition of disease symptoms was obtained in cowpea plants treated with B. subtilis. Plant growth characters such as root length, fresh and dry weights were considerably increased in plants treated with all Bacillus species compared with the control.

The nematicidal activity of plant extracts was reported in several plant species [11]. The severity of root-knot nematode, Meloidogyne incognita, was significantly reduced in plants treated with extracts of neem [12]. The nematicidal activity of pumpkin oil was also found by Ayaz et al. [13]. Disease symptoms of the northern root-knot nematode Meloidogyne humpa were significantly reduced and the fruit yield was highly increased in plants treated with watercress oil [14].

Systemic resistance was stimulated in different plants by several biotic and abiotic elicitors [15]. Synthetic chemicals such as salicylic acid (SA) and benzothiadiazole (BTH) inhibited root-knot nematode. Disease resistance to root-knot nematode, M. incognita, was enhanced in plants treated with SA as a soil drench treatment [16]. Nematode reproduction and root galls were significantly reduced in plants subjected to root dipping application of SA [17].

Therefore, this study was carried out to evalu-
ate the potential of some bioagents such as \textit{B. subtilis}, \textit{B. thuringiensis} and \textit{T. viride}, some plant extracts such as \textit{A. sativum} and \textit{P. granatum}, some plant oils such as \textit{A. sativum} and \textit{E. globule}, some synthetic chemicals such as SA and ascorbic acid and some pesticides such as Rugby\textsuperscript{®} 20\%CS and Abamectin 1.8\%EC to protect tomato from root-knot nematode.

**MATERIALS AND METHODS**

**Greenhouse experiment.** Pot experiments were carried out in sterilized pots (30 cm in diameter) filled with 8 kg/pot of sterilized sandy clay loam soil (2:1, v/v). Pots were transplanted using one tomato seedling per pot. Tomato plants were treated by pipetting the suspensions to 4 holes around the base of the seedlings. Induction treatments were done with different biological and chemical inducers at 1 week before nematode inoculation.

Dry plant parts (powder of dried husk of \textit{Punica granatum}, cloves peel of \textit{Allium sativum}, leaves of \textit{Eucalyptus globule} and fruits for the oil extract of \textit{A. sativum}) were used to prepare water extracts by soaking (50 gm) plant materials in 1 L distilled water (DW) at 60°C. While dry cloves peel of \textit{Allium sativum} were bended in DW for 6 min. All extracts were then filtered through filter paper (whatman No. 1). All filtrates were used as a standard solution and stored in the refrigerator until use. Plant extracts and oils were applied as soil drench treatments at the rate 200 ml/pot.

Bacterial isolates (\textit{Bacillus subtilis} and \textit{B. thuringiensis}) were prepared by growing on nutrient broth at 28°C, shaking incubated for 4 days (160 rpm) and adjusted to a concentration of 1x10\textsuperscript{8} cfu/ml. \textit{T. viride} was cultured on potato dextrose agar (PDA) for two weeks at 25°C. To prepare the fungal inoculum, about 15 ml DW were added to the growing colony on PDA medium in a petri dish. The spores were suspended in distilled water using a sterile glass rod and applied gently on the surface of the colonies. The number of spores per ml was adjusted to 1x10\textsuperscript{9} spores/ml. Bioagents were applied as soil drench treatments at the rate of 100 ml/pot. Silica Nanoparticles (SiNPs) were kindly provided by Plant Protection Research Institute, Agriculture Research Center, Egypt. SiNPs were added to the soil as a soil drench at the rate of 100 ml/pot. Silica Nanoparticles (SiNPs) were kindly provided by Plant Protection Research Institute, Agriculture Research Center, Egypt. SiNPs were added to the soil as a soil drench at the rate of 100 ml/pot. All filtrates were used as a standard solution and stored in the refrigerator until use. Plant extracts and oils were applied as soil drench treatments at the rate 200 ml/pot.

Biochemical assays of oxidative enzymes. For enzyme assays in plants, 1g of leaf samples were homogenized separately at 0- 4°C in 3 ml of 50mM Tris buffer (pH 7.8), containing 1mM of 50mM EDTA-Na\textsubscript{2} and 7.5\% Polyvinylpyrrolidone at 30 days after inoculation. The homogenates were centrifuged (10000 rpm, 15 min, 4°C) [20]. All measurements were carried out at 25°C using the model UV-160A spectrophotometer (Shimadzu, Japan).

**Peroxidase assay (POX).** Peroxidase enzyme activity was determined according to the methods described by Srivastava [21] by measuring the oxidation of pyrogallol to purpurgallin in presence of H\textsubscript{2}O\textsubscript{2}. The sample cuvette contained 0.5 ml of 0.1m sodium phosphate buffer at pH 7.0, 0.3 ml enzyme extract, 0.3 ml of 0.05 pyrogallol (C\textsubscript{6}H\textsubscript{4}COH\textsubscript{3}) and 0.1 ml of 10\% H\textsubscript{2}O\textsubscript{2} in a total volume 3ml. The absorbance was measured at 425 nm using spectrophotometer (L-5000, Germany).
### TABLE 1

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward primer</th>
<th>Reverse primer</th>
<th>Size</th>
<th>Accession number</th>
</tr>
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<tbody>
<tr>
<td>LePR2</td>
<td>GGACACCCTTTCGCCCT</td>
<td>TGTTCCTGCCTCTCC</td>
<td>81</td>
<td>M80604</td>
</tr>
<tr>
<td>LeUB13</td>
<td>TCCATCTCGCTCCTCC</td>
<td>TTTCGAACCTTTCCAGTGTCATCAACCT</td>
<td>144</td>
<td>X58253</td>
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</table>

### TABLE 2

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Plant height (cm)</th>
<th>Shoot weight (g)</th>
<th>Fruits weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control infested</td>
<td>45.00 ± e</td>
<td>54.90 ± h</td>
<td>14.31 ± d</td>
</tr>
<tr>
<td>B. thuringiensis</td>
<td>69.16 ± e</td>
<td>151.97 ± b</td>
<td>59.50 ± b</td>
</tr>
<tr>
<td>B. subtilis</td>
<td>57.33 ± h</td>
<td>138.29 ± e</td>
<td>39.80 ± c</td>
</tr>
<tr>
<td>T. viride</td>
<td>76.83 ± d</td>
<td>111.89 ± d</td>
<td>29.10 ± e</td>
</tr>
<tr>
<td>Allium sativum (extract)</td>
<td>82.50 ± b</td>
<td>79.89 ± f</td>
<td>19.58 ± f</td>
</tr>
<tr>
<td>Allium sativum (oil)</td>
<td>86.33 ± a</td>
<td>67.89 ± g</td>
<td>15.91 ± f</td>
</tr>
<tr>
<td>Eucalyptus globule (oil)</td>
<td>79.16 ± c</td>
<td>120.21 ± d</td>
<td>36.33 ± cd</td>
</tr>
<tr>
<td>Salicylic acid</td>
<td>84.00 ± b</td>
<td>104.86 ± e</td>
<td>27.81 ± e</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>76.50 ± d</td>
<td>111.60 ± de</td>
<td>28.80 ± e</td>
</tr>
<tr>
<td>Silica (SiNPs)</td>
<td>70.13 ± e</td>
<td>100.73 ± e</td>
<td>26.90 ± e</td>
</tr>
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<td>Abamectin 1.8% EC</td>
<td>71.33 ± e</td>
<td>178.15 ± a</td>
<td>67.38 ± a</td>
</tr>
<tr>
<td>Rugby 20% CS</td>
<td>70.83 ± e</td>
<td>105.04 ± e</td>
<td>27.91 ± e</td>
</tr>
<tr>
<td>Punica granatum</td>
<td>76.33 ± f</td>
<td>121.42 ± d</td>
<td>33.76 ± d</td>
</tr>
<tr>
<td>Allium sativum (oil)</td>
<td>79.16 ± c</td>
<td>120.21 ± d</td>
<td>39.80 ± c</td>
</tr>
<tr>
<td>Salicylic acid (oil)</td>
<td>84.00 ± b</td>
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<tr>
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<td>105.04 ± e</td>
<td>27.91 ± e</td>
</tr>
<tr>
<td>Rugby 20% CS</td>
<td>70.83 ± e</td>
<td>121.42 ± d</td>
<td>33.76 ± d</td>
</tr>
</tbody>
</table>

**Polyphenol oxidase assay (PPO).** The enzyme was determined according to the methods adopted by Matta and Dimond [22]. The reaction mixture contained 1.0 ml of 0.2 ml sodium phosphate buffer at pH 7.0, 10.0 ml of 0.001M catechol (C₆H₄(OH)₂) and 3.0 ml distilled water. The absorbance was measured at 495nm using spectrophotometer (L-5000, Germany).

**Analysis of defense related genes expression.** Tomato plants were treated with induction treatments and inoculated with *M. incognita* as described previously. RNA extraction was carried out at 2 days after pathogen inoculation from tomato leaves using RNA Purification Kit (Thermo Scientific, Fermentas, #K0731). Complementary DNA (cDNA) was synthesized using Reverse Transcription Kits (Thermo Scientific, Fermentas, #EP0451). Quantitative RT-PCR (qRT-PCR) with SYBR Green was utilized to measure the expression of target genes (PR2), with *LeUB13* (Table 1) as an internal reference following the manufacturer protocol (Thermo scientific, USA, # K0221). The quantities critical thresholds (Ct) of target genes were normalized with quantities (Ct) of housekeeping gene (*LeUB13*) by used the 2^−ΔΔCt method [23].

**Statistical analysis.** Data collected were statistical analyzed using the completely randomized block design. Average were compared according to Dancan’s multiple rang test [24]. The analysis was performed using XLSTAT PRO (statistical analysis software, Addinsoft).

### RESULTS

**Effect of induction treatments on tomato growth characters in soil infested with *M. incognita*.** Data in Table (2) showed that all the tested bioagents and the nematicide Rugby20% CS significantly increased plant height, shoot fresh and dry weights in tomato plants infested with *M. incognita* compared to the control treatment. The highest increase of tomato height was recorded in *Punica granatum* (86.33 cm) followed by extract of *Allium sativum* and oil of *A. sativum* (82.50 and 79.1 cm) compared to the control (45.00 cm). On the other hand, all bioagents and the commercial pesticide Rugby20%CS showed a significant increase on shoot fresh and dry weights of tomato plants infected with *M. incognita* compared with the untreated control. Fruits weight was significantly increased in all treatments compared with the control. The effect of the synthetic nematicide Rugby showed the best results in this respect (2310.88 g) followed by ascorbic acid (2023.85), abamectin (1963.53) and salicylic acid (1689.40 g) compared with 584.06 g in the untreated control.

**Control of *M. incognita* by induction treatments in tomato plants.** The effects of different inducers were positive in terms of reduced numbers of galls, egg-masses and juveniles in root system (Fig. 1 and Table 3). Treatment of *B. thuringiensis*, Rugby and *B. subtilis* gave the lowest numbers of...
galls (26.33, 32.33 and 34.66, respectively) compared with untreated control (243). Rugby treatment was the most effective against development of egg-masses in root system of tomato plants when applied as a soil drench treatment before inoculation recording 68.63% reduction in egg-masses followed by salicylic acid and abamectin (75.71 and 72.17 %, respectively) compared with untreated control. The number of J2/250g of soil was significantly reduced in all treatments compared with the infested control plants (Table 3). Results indicated that all treatments and the pesticides decreased the final nematode populations. The results showed that the most effective treatments against nematode populations were rugby treatment followed by treatment with abamectin (90.81 and 84.99%) compared to the untreated control infested with *M. incognita*. While, *E. globule* treatment exhibited the lowest reduction in the final population (58.47%) compared to the untreated control.

### TABLE 3

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Conc. Description</th>
<th>No. of galls/root system</th>
<th>Reduction (%)</th>
<th>No. of egg-masses/root system</th>
<th>Reduction (%)</th>
<th>No. of J2/250cm³ soil</th>
<th>Reduction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control infested</td>
<td>0</td>
<td>243.00 a</td>
<td>0.00</td>
<td>197.66 a</td>
<td>0.00</td>
<td>257.66 a</td>
<td>0.00</td>
</tr>
<tr>
<td><em>B. thuringiensis</em></td>
<td>10⁷ cfu/ml</td>
<td>26.33 i</td>
<td>89.11</td>
<td>73.00 d</td>
<td>63.17</td>
<td>69.00 g</td>
<td>73.22</td>
</tr>
<tr>
<td><em>B. subtilis</em></td>
<td>10⁷ cfu/ml</td>
<td>34.66 hi</td>
<td>85.67</td>
<td>68.00 d</td>
<td>65.43</td>
<td>56.33 h</td>
<td>78.13</td>
</tr>
<tr>
<td><em>T. viride</em></td>
<td>1⁰ spores/ml</td>
<td>67.33 d</td>
<td>72.17</td>
<td>84.33 c</td>
<td>57.33</td>
<td>78.66 ef</td>
<td>69.47</td>
</tr>
<tr>
<td><em>A. sativum</em> (extract)*</td>
<td>50g / l</td>
<td>47.00 fg</td>
<td>80.57</td>
<td>114.00 b</td>
<td>42.32</td>
<td>74.33 f</td>
<td>71.15</td>
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<tr>
<td><em>P. granatum</em></td>
<td>50g / l</td>
<td>94.00 c</td>
<td>61.15</td>
<td>71.33 d</td>
<td>63.91</td>
<td>135.00 b</td>
<td>47.60</td>
</tr>
<tr>
<td><em>A. sativum</em> (oil)</td>
<td>18ml / l</td>
<td>60.00 de</td>
<td>75.20</td>
<td>70.33 d</td>
<td>64.41</td>
<td>77.66 ef</td>
<td>69.85</td>
</tr>
<tr>
<td><em>E. globale</em> (oil)</td>
<td>18ml / l</td>
<td>49.33 ef</td>
<td>79.61</td>
<td>108.00 b</td>
<td>45.36</td>
<td>107.00 c</td>
<td>58.47</td>
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<tr>
<td>Salicylic acid</td>
<td>5Mm</td>
<td>52.00 ef</td>
<td>78.51</td>
<td>48.00 fg</td>
<td>75.71</td>
<td>91.66 d</td>
<td>64.42</td>
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<tr>
<td>Ascorbic acid</td>
<td>2000PPM</td>
<td>115.00 b</td>
<td>52.34</td>
<td>64.33 de</td>
<td>67.45</td>
<td>79.00 e</td>
<td>69.33</td>
</tr>
<tr>
<td>Silica (SiNPs)</td>
<td>10ml / l</td>
<td>52.00 ef</td>
<td>78.51</td>
<td>63.66 de</td>
<td>67.79</td>
<td>46.00 i</td>
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</tr>
<tr>
<td>Abamectin</td>
<td>1.8% EC</td>
<td>0.12ml / kg</td>
<td>37.33 gh</td>
<td>84.57</td>
<td>55.00 ef</td>
<td>38.66 j</td>
<td>84.99</td>
</tr>
<tr>
<td>Rugby20% CS</td>
<td></td>
<td>32.33 hi</td>
<td>86.83</td>
<td>43.33 g</td>
<td>78.07</td>
<td>23.66 k</td>
<td>90.81</td>
</tr>
</tbody>
</table>

### FIGURE 1

Symptoms of root-knot nematode disease on tomato roots treated with *B. thuringiensis* and non-treated control plants.
Effect of induction treatments on the activity of peroxidase enzyme. Peroxidase activity was increased in tomato leaves after induction treatments. All treatments exhibited significant increase compared to control plants (Fig. 2). The highest increase in peroxidase activity was reported in plants treated with SA followed by ascorbic acid, SiNPs, B. thuringiensis and abamectin. On the other hand, no significant difference was found between B. thuringiensis, SiNPs and abamectin treatments in the activity of peroxidase.
FIGURE 4
Effect of biological and chemical elicitors on the expression levels of PR2 gene.
Columns represent mean values. Bars indicate standard errors. Different letters above columns indicate significant differences by Fisher’s LSD test at significant values of P ≤ 0.5.

Effect of induction treatments on the activity of polyphenol oxidase enzyme. The activity of polyphenol oxidase was increased in treated tomato leaves treated with different inducers at 7 days after the infection with nematode (Fig. 3). The treatment with ascorbic acid achieved the highest increase in polyphenol oxidase activity in treated plants compared to control plants. Treating the soil with oil of A. sativum and SiNPs treatments gave the same increasing activities of polyphenol oxidase compared to the controls. Rugby treatment achieved the lowest increasing activity of polyphenol oxidase compared with the control.

Expression of PR2 gene in tomato leaves treated with induction treatments. The transcription levels of PR2 were significantly increased in all induction treatments (Fig. 4). The best result was achieved in SA treated plants followed by the extract of A. sativum. No significant difference was found between abamectin and A. sativum (oil) treatments. The expression levels of PR2 gene in P. granatum treated plants were similar to E. globule treated plants. T. viride treated plants showed the lowest expression levels of PR2 gene compared with other treatments and control plants (Fig. 4).

DISCUSSION
Root-knot, caused by Meloidogyne incognita, is a dangerous disease in different regions of the world [25, 26]. M. incognita is complex of species, highly diverse and has a wide range of host plants causing yield losses in severely infested fields [25, 27].

Biological control is mostly suitable because it is sustainable, eco-friendly, cost-effective and should be included in the management strategies. In this study, different biological and chemical elicitors of induced resistance were evaluated for controlling root-knot nematode, Meloidogyne incognita, in tomato plants under greenhouse conditions. The tested inducers were used as soil drench treatments at 1 week before nematode inoculation. The obtained results showed significant reductions in the numbers of galls, egg-masses and juveniles in root system compared with the infested control. Among biological inducers, B. thuringiensis achieved the best inhibition effects against the infection with root knot nematode, M. incognita. On the other hand, the nematicide Rugby ® 20% CS (cadusafos) was the best among chemical and pesticides treatments. The effect of SA was much higher than the effect of ascorbic acid. Similarly, suppress-
sion of *M. incognita* infection in tomato plants was achieved using cadusafos [4, 28]. Additionally, it was found that the production of ascorbic acid and salicylic acid was motivated after nematode attack [29]. The numbers of root galls and eggs masses were decreased in tomato plants infected with *M. incognita* after treatment with SA [30]. The number of galls and nematode populations were significantly reduced in cowpea and okra plants sprayed with SA and inoculated with *M. incognita* [31]. The activities of CAT and APX were reduced due to SA treatment [30]. The number of galls per root system. High percentages of reduction in nematode populations was reported in plants treated with garlic extract which emphasizes the role of garlic extract and oil as inducer for resistance against the root-knot nematode [40].

The activities of peroxidase and polyphenol oxidase enzymes and the expression of PR2 gene were increased in tomato plants treated with induction treatments. The treatment with the SA achieved the highest increase in the activity of peroxidase and the expression of PR2 gene in infected plants compared to the control. In pot experiments, soil drench or leaf spray treatments with 5 mM SA reduced the diameter and number of *M. javanica* galls and egg masses and also increased the activity of enzymes and phenolic compounds in tomato roots [41]. Additionally, the maximum induction of polyphenol oxidase in eggplant roots infected with *M. incognita* was found in plants treated with salicylic acid [42]. SA has been reported as an endogenous signal for the activation of certain plant defense responses by expression of pathogenesis-related gene (PR-1) and enhanced resistance to the pathogens [43].

In conclusion, the control potentials of *B. thuringiensis* and *B. subtilis* were significantly reduced nematode populations and symptoms making these isolates promising candidates for biocontrol of *M. incognita* under field conditions.

**REFERENCES**


DETERMINING THE CRITERION AND BIOTECHNICAL STRUGGLE METHODS AGAINST FORFICULA AURICULARIA L. (DERMAPTERA: FORFICULIDAE) HARMING IN APRICOT ORCHARDS IN TURKEY

Mehmet Kaplan*
Department of Plant Protection, Faculty of Agriculture, Siirt University, Siirt, Turkey

ABSTRACT

This study was carried out to determine the damage and to investigate biotechnical struggle methods of Forficula auricularia Lin. (Dermaptera: Forficulidae) that damage the apricot orchards of Malatya province in 2014-2016.

As a result of the studies, it was determined that F. auricularia spent as adult and different nymph periods under the soil, rocks and the shells of the old trees in the apricot orchards and in the surrounding areas during the winter. Adults, eggs and nymphs of F. auricularia were first found under rocks beneath the tree bark of old trees and in various sheltered places in nature between the end of March and the third week of April. It had been determined that the density of F. auricularia has been increasing since May in the follow-ups of cardboard traps set up in trees in the apricot orchards.

It was determined that F. auricularia has been fed on the fruits and formed the highest population during the period until harvest from the second week of June when the fruit began to be sweetened in observations made. Along with that, cardboard traps, pit traps and biological insecticide studies have been conducted to develop an alternative struggle against pests.

KEYWORDS:
Apricot, Forficula auricularia L., Biological criteria biotechnical, Turkey

INTRODUCTION

Apricot is a fruit with a very high nutritional capacity in addition to being able to be processed into several types of products and having a tasty nature. Apricot is a type of fruit belonging to the Prunophora subgenus of the Prunus genus in the subfamily of Prunoideae in the family of Rosaceae. The vast majority of the varieties of apricot that are grown globally belong to the species of Prunus armeniaca L. (Armeniaca vulgaris Lam.) [1]. The mainland of apricot, which is a significant type of fruit that is grown for economic reasons, includes Iran, Turkistan, Afghanistan, Central Asia and Western China [2].

In the world, an annual total of 4,257,241 tons of fresh apricot is produced, while approximately 985,000 tons of this is provided by Turkey. With this degree of production, Turkey is at the first place in the world’s apricot production by a share of about 23%. It is followed by Uzbekistan, Italy, Iran and Pakistan [3].

Turkey is prominent in the world’s production of fresh and dried apricots. The reason for this is that the varieties of apricot in Turkey have high potential due to their high-quality and ecological superiorities. The province of Malatya, which is in the Eastern Anatolia Region in Turkey, has approximately 8 million apricot trees, while it matches approximately 68% of the apricot production and 85% of the dried apricot production in the entire country. Almost all apricot products that are produced in the province of Malatya are dried and exported [3].

There are several pests in apricot orchards that directly or indirectly affect quality and yield negatively in terms of the issue of protecting the plants. One of these pests is Earwig causing a significant amount of product and quality loss, feeds on the fruits of these trees and creates harm and is known as Forficula auricularia L. (Dermaptera: Forficulidae).

The population of the pest in question is increasingly higher in the apricot fields in Malatya, there is no product for protecting plants against this pest, and producers of apricot complain about causing decrease in amount of product and quality loss, feeds on the fruits of these trees caused by F. auricularia. In this context, this study was carried out with the purpose of, in addition to controlling this pest, proposal of some biotechnical precautions to be taken in controlling weeds in the period of 2014-2016.

As a result of this study, alternative control methods were determined against F. auricularia and weeds, and fundamental data were obtained for both producers and technical professionals who...
deal with apricot production in both Malatya and Turkey in general.

MATERIALS AND METHODS

The main material of the study consisted of the apricot orchards in the districts of Battalgazi and Kale in the province of Malatya, the pest *Forficula auricularia* L., Azadirachtin, cardboard, pitfall trap with fish oil, insect collection tool and laboratory equipment.

Determining the Biologic Criteria for Combating *Forficula auricularia* L. In this method, fortnight and weekly observations have been made respectively during the winter months and other months at the study orchards. During the observations, certain places such as beneath tree shells, plant residues and other similar places have been checked. Their nests have been sought in the soil and shelter traps have been set on five different trees to create a spot for them to hide. This way, the egg laying period, nymph periods and maturing periods of the pest have been examined to reveal its biology in nature.

Searching for Opportunities to Counter *Forficula auricularia* L. a- Use of Carton Shelter Trap and Pitfall Trap Methods. *F. auricularia* is nocturnal and it looks for shelter in the mornings. This characteristic was the reason behind developing the carton shelter trap method [4] *F. auricularia* is reported to release a wavy grouping pheromone [5]. When *F. auricularia* individuals are removed from the trees thanks to this artificial shelter-grouping pheromone, it will reportedly become possible to establish control against it [6]. Carton shelter trap and pitfall trap study has been conduct- ed in two apricot orchards, one each in Battalgazi and Kale districts, where 9 trees comprised a parcel. In carton shelter tarp, one trap has been set on each tree, and in pitfall trap two traps each with a 15 cm diameter and containing fish oil have been set on both sides of the trunk of a tree.

Traps have been set at a time when apricots started to get their taste and the pest began to be seen commonly (20-30 adults.traps in average) and they have been replaced with a new one in every three days until harvest. Traps have been set in orchards on 16 June 2015 and kept until the harvest on 2 July, and during 2016, they have been set on 21 June 2016 and used until harvesting on 12 July.

b-Bioinsecticide trial Against *Forficula aurricularia* L. Suitable intervals have been used for planting during the study and the selected orchards were the same age, yielding products normally and harmed by *F. auricularia* a year ago. Before the practise, attention has been paid to see whether the pest had sufficient density and was homogeneously distributed. Plant characteristics (cultivation technique, plant size, crown width, inter-row and above-row distance etc.) have been recorded during the trial. The trial has been based on randomized blocks test pattern. The trial has been set with 4 repetitions, in a manner to form the characters of the trial, preparation to be tested, sub-dosages and proof and each parcel contained 3 x 3 = 9 trees. A trial has been similarly set in proof parcel.

Three different dosages of Azadirachtin have been used (500, 400, 300 ml/100 lt water) as bio-insecticide in the study. All components of the trees have been well sprinkled during the study, and as this pest is nocturnal, proceedings took place at evening times. Use of insecticides started once the amount of individuals inside the trap reached 20 and fruits started to mature.

With the purpose of establishing the damage caused by *F. auricularia* on fruits at the end of the study, 1,000 fruits have been counted per tree and visually inspected at each parcel during harvest. Results have been assessed over the harmed fruit ratios by using Abbott (%) formula and the impacts of the practise have been defined.

Statistical Methods Used For Assessment Purposes. With regards to the analyses during the statistical assessment of the obtained results, 21.0 version of SPSS Statistical Package Program has been used and One-Way Anova Univariate has been applied for LSD and DUNCAN multiple comparison tests hav been applied for the inter-group comparisons.

RESULTS AND DISCUSSION

The assessments regarding the wintering behaviour of *F. auricularia* held at the end of this study revealed that it spends the winter during adult and different nymph periods inside the shells of old trees and underneath rocks, and also within the fissures and cracks of soil. [7], reported that the pest spends winter under fallen leaves, inside the cracks in soil, [8. 9.10] reported it spends the winter as a adult, [11] reported it spends the winter as a mature inside the cracks in tree branches and trunks, underneath shells and rocks and other shelters such as wall cracks.

Adult individuals of *F. auricularia* have been spotted for the first time on 09 April 2015 and 29 Mart 2016 in various protected places in non-processed areas such as pastures outside the apricot orchards along with mature female individuals and eggs (each set containing 35-45 eggs) on 16 April 2015 and 05 April 2016, and nymphs have been spotted in nature on 21 April 2015 and 14 April 2016. Female individuals of *F. auricularia* have been observed to lay their eggs 3-5 cm below soil
Individuals of *F. auricularia* have been sighted for the last time on 29 April 2015 and 02 May 2016. [12] Reported that *F. auricularia* lays its eggs in 2 sets, the first laid eggs hatch on mid-April and nymphs start to appear on surface during late April and early May; [13], the pest is laying its eggs into 5 cm-deep cracks close to the soil surface in sets consisting of 30-60 eggs; [8.9], *F. auricularia* spends the winter as a adult, eggs are stored towards the end of winter and hatch during May and adult in August, each nymph period took place in 12 days when average temperature in laboratory conditions was 15-20 °C; [14], females laid 50 to 90 eggs and laid them about 5-8 mm deep from the soil surface; [10], mature females have been obtained through sampling from April to October, while male individuals have been obtained from August to October, matures were able to mate after wintering and lay their eggs once or twice until the end of May; [11], *F. auricularia* is the sole incubating insect that looks after the new-born and a female lays 20-80 eggs (2-3 eggs at a time); [15], reported nymphs hatching from the eggs laid by female of *F. auricularia* during mid-April started climbing to the soil surface by late April and early May, and adult by late June or early July; [16], reported adult females lay around 30-50 eggs in their underground nests.

The first adults and nymphs of the pest in nature have been observed in apricot orchards in Malatya province on 05 May 2015 and 11 May 2016, adult density kept increasing in the following weeks, it started feeding-off the apricots when they started gaining taste (16 June 2015 and 21 June 2016), reached a great density during the period leading to harvest (02 July 2015 and 12 July 2016) and caused damage in apricot fruit at a ratio ranging between 33.9% and 47.75%; [17], reported that *F. auricularia* can cause harm in apricot and nectarine orchards to a degree ranging between 10% and 40%; [18], reported the harm caused by *F. auricularia* in organic peach orchards is over 20%, harm occurs a few days before harvest as the pest opens wide holes inside the ripe fruits and lead to significant economic harm in peach. [19], reported that *F. auricularia* density increases in traps when approaching to harvest during June and July and the harm caused in apricots is between 5% and 14%.

Adult density kept diminishing following harvest and the last adults in apricot orchards have been spotted during October (28 October 2014, 12 October 2015 and 04 October 2016). The observations made led to the conclusion that the adults moving from apricot orchards into non-processed areas, such as pastures, outside the apricot orchards started mating during the month of October and the adults and first eggs were spotted on 28 October 2015 and 11 November 2016, then the first nymphs were spotted on 17 November 2015 and 23 November 2016 and afterwards the matures and nymphs went into seclusion for wintering. [20], reported that *F. auricularia* reached a high population level in July but it kept diminishing in the following months and by September around half of the population left the orchard. It has also been reported that Adult individuals of *F. auricularia* in early October and moved into the soil but a small amount of individuals stayed on trees until early November.

With regards to the corrugated carton traps set for developing alternative countering methods against *F. auricularia*; the fruit ratio in the apricot orchard trees harmed by *F. auricularia* in Battalgazi and Kale districts in 2015 was 10.62% and 8.75%, proof was 43.75% and 36.50%, efficiency was 76.75% and 80.33%, and in 2016 harmed fruit ratio was 10.00% and 11.87%, proof was 40.00% and 46.25% and efficiency was 80.19% and 72.87% (Table 1). [21], reported that *F. auricularia* can be found in orchards and can be caught if a single-wall fibreboard is attached on a tree branch during autumn; [22], reported that carton shelter traps can be effective if replaced on a daily basis or twice a week when combating *F. auricularia*; [16], reported that they have used carton traps because *F. auricularia* is nocturnal and gathers in dark and sheltered places during daytime and also that they replaced the traps every 2-3 days.

In terms of pitfall trap method, the fruit ratio in the apricot orchard trees harmed by *F. auricularia* in Battalgazi and Kale districts in 2015 was 36.25% and 40.00%, proof was 45.00% and 46.25% and efficiency was 18.53% and 15.78%, and in 2016 fruit ratio harmed by *F. auricularia* was 38.12% and 35.00% respectively, proof was 47.75% and 38.5% and efficiency was 17.29% and 17.65% (Table 2). [15], Conducted a study in Southern Washington state in USA surveying *F. auricularia* in pitfall traps set in bushland. The months of April and May witnessed the highest amount of non-adult individuals caught in pitfall traps while mid-July was the time when the highest amount of adult individuals have been caught. [16], Conducted a study on the pest in USA, monitoring its population and catching with traps, and used carton traps as well as pitfall traps containing oil fish for population monitoring during summer months and replaced the traps in every 2-3 days.

The ratio of harmed fruits during the bioinsecticide (Azadirachtin 500-400-300 ml/100 lt. water) trials in apricot orchards in Battalgazi and Kale districts in 2015 was 14.85% and 6.40% respectively, proof ratio was 40.62% and 33.90% and efficiency was 79.75% and 81.11%, and in 2016 harmed fruit ratio was 7.81% and 7.03% respectively, proof was 46.25% and 41.10% while efficiency was 82.75% and 80.75% (Table 3, 4).

Looking at the Variance Analysis Tables provided in table 3 and 4, there is a meaningful difference of 95% reliability between the averages of 300
ml and 400 ml, 300 ml-500 ml dosages, but to the contrary, there is no meaningful difference between the 400 ml and 500 ml dosages. As such, the conclusion reached was that 400 ml dosage of the insecticide can be recommended against adult individuals *F. auricularia* in apricot orchards. [23], reported that Neem extract (Azadirachtin) reduces nymph population of *F. auricularia* on peach trees by 70%. [16]. Conducted a study on pest-management in peach orchards and recommended Azadirachtin when combating earwig.

The efficiency at 400 and 500 ml dosages in both districts was statistically placed within the same group while 300 ml dosage has been placed in a different group. In terms of effect (%) during the trials, the pesticides and traps yielded the same outcomes in both locations and in both years. Variance analysis yielded a statistical difference of 0.01 between practises but no differences have been observed between locations.

500ml and 400ml dosages of Azadirachtin and also corrugated carton traps yielded the greatest efficiency against *F. auricularia* in apricot orchards and the fruits on trees have not been harmed much. But in contrast, 300 ml dosage and pitfall trap not only yielded the lowest effect but the harm on fruits was at a higher level. Therefore, when combating adult individuals of *F. auricularia* in apricot orchards, use of 400 ml dosage of Azadirachtin or corrugated carton traps, one of the biotechnical methods, can be recommended.

### TABLE 1

<table>
<thead>
<tr>
<th>Date</th>
<th>Number of examined fruit</th>
<th>Medicated (a) Control (b)</th>
<th>Loss Rate in Fruits (%)</th>
<th>Efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Battalgazi Kale Battalgazi Kale</td>
<td>Battalgazi Kale Battalgazi Kale</td>
<td>Battalgazi Kale Battalgazi Kale</td>
</tr>
<tr>
<td>02.07.2015</td>
<td>1000</td>
<td>a</td>
<td>10.62</td>
<td>76.75</td>
</tr>
<tr>
<td></td>
<td></td>
<td>b</td>
<td>43.75</td>
<td>36.50</td>
</tr>
<tr>
<td>12.07.2016</td>
<td>1000</td>
<td>a</td>
<td>10.00</td>
<td>80.19</td>
</tr>
<tr>
<td></td>
<td></td>
<td>b</td>
<td>40.00</td>
<td>72.87</td>
</tr>
</tbody>
</table>

### TABLE 2

<table>
<thead>
<tr>
<th>Date</th>
<th>Number of examined fruit</th>
<th>Medicated (a) Control (b)</th>
<th>Loss Rate in Fruits (%)</th>
<th>Efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td>Battalgazi Kale Battalgazi Kale</td>
<td>Battalgazi Kale Battalgazi Kale</td>
<td>Battalgazi Kale Battalgazi Kale</td>
</tr>
<tr>
<td>02.07.2015</td>
<td>1000</td>
<td>a</td>
<td>36.25</td>
<td>18.53</td>
</tr>
<tr>
<td></td>
<td></td>
<td>b</td>
<td>45.00</td>
<td>15.78</td>
</tr>
<tr>
<td>12.07.2016</td>
<td>1000</td>
<td>a</td>
<td>38.12</td>
<td>17.29</td>
</tr>
<tr>
<td></td>
<td></td>
<td>b</td>
<td>47.75</td>
<td>17.65</td>
</tr>
</tbody>
</table>

### TABLE 3

<table>
<thead>
<tr>
<th>The name of the drug</th>
<th>Dosage/100lt water</th>
<th>Loss Rate in Fruits (%)</th>
<th>Efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azadirachtin</td>
<td>500</td>
<td>8.12</td>
<td>79.75 a</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>8.90</td>
<td>79.50 a</td>
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<td></td>
<td>300</td>
<td>14.85</td>
<td>63.46 b</td>
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<tr>
<td>Control</td>
<td>40.62</td>
<td>33.90</td>
<td>-</td>
</tr>
</tbody>
</table>

### TABLE 4

<table>
<thead>
<tr>
<th>The name of the drug</th>
<th>Dosage/100lt water</th>
<th>Loss Rate in Fruits (%)</th>
<th>Efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azadirachtin</td>
<td>500</td>
<td>7.81</td>
<td>82.75 a</td>
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<tr>
<td></td>
<td>400</td>
<td>9.37</td>
<td>79.18 a</td>
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<tr>
<td></td>
<td>300</td>
<td>12.81</td>
<td>72.64 b</td>
</tr>
<tr>
<td>Control</td>
<td>46.25</td>
<td>41.10</td>
<td>-</td>
</tr>
</tbody>
</table>
CONCLUSION

In conclusion, it has been observed that *F. auricularia* is becoming populated in orchards, feeding off apricots as they start to get tasty and is harming them until harvest time. The density of pests and the amount of harm done in these areas varies per year.

Use of chemical pesticides needs to be avoided at the beginning to maintain environmental health and natural balance and biotechnical and cultural methods need to be employed instead. Apricot is a product that is both consumed domestically and is being exported abroad therefore it is necessary to prioritize biotechnical methods and cultural measures to ensure no residues are left on the product.

ACKNOWLEDGEMENTS

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REFERENCES


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ASSESSMENT OF WATER QUALITY AND FISHERIES ACTIVITIES IN UPPER AKCAY RIVER IN DENIZLI-MUGLA PROVINCE

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ABSTRACT

Upper Akcay River (Denizli-Mugla, Turkey) is located in Mugla and Denizli province from Buyuk Menderes Basin, in Southern part of Turkey. Kemer Dam Lake separates the river from the downstream and prevents the fish passages. Akcay River is important for aquaculture activities, irrigation and fisheries activities. In upper Akcay River have many threats which affect the study area these are pollution of agricultural and domestic activities, fishing pressure, habitat degradation, over-abstraction of water and barriers.

The study was conducted to assess the physical and chemical water quality parameters and fisheries activities of upper Akcay River. The upper Akcay River goes through Kemer Dam Lake, in this river fisheries and aquaculture are practice. This study was carried out between June 2012 and May 2013. Water samples were taken in three stations from upper Akcay River and were analyzed for water temperature, pH, dissolved oxygen, electrical conductivity, ammonium nitrogen, nitrite nitrogen, nitrate nitrogen, and orthophosphate. In this study, fish samples were collected by electrofishing and cast net from tree station from the river.

River water quality is important because it is used for domestic purpose, drinking, irrigation and aquatic activity. The upper Akcay River is one of the most prominent and a sensitive region that is not too exposed to human effects in Buyuk Menderes Basin. The river can play a vital role in contributing to social economic and development.

This study describes the water quality assessment of upper Akcay River in the Southwestern Anatolia. The mean values of overall water quality parameters results have been observed as water temperature (15.15 °C), pH (7.58), conductivity (555.25 μS/cm), TDS (371.44), dissolved oxygen (8.56 mg/L), ammonium (0.09 mg/L), nitrate (3.84 mg/L), orto-phosphate (0.04 mg/L). The economic species caught in the river are Onchorynchus mykiss, Capoeta bergamea, Alburnus escherichii, Vimba mirabilis, Chondrostoma meandrense, Squalius fellowesi, Barbus pectoralis and Carassius gibelio.

According to the WPRA, surface water quality of the upper River was classified as level I. At the end of the analyses, it was revealed that the areas is suitable for trout aquaculture and fisheries. The results show that, aquaculture activities has no negative affect in upper Akcay River

KEYWORDS: Akcay River, Aquaculture, Environmental effects, Water quality, Fisheries, Denizli, Mugla

INTRODUCTION

Upper Akcay river is located in Southern part of Aegean Region (Turkey) in the Buyuk Menderes Basin. The River area is a private wetland and is very important for the livelihood to offer several economic benefits in region. The upper Akcay River occurs a significant and original ecosystem in terms of conservation of natural resources and biological diversity, the study area is also rich in fish and bentic fauna [1, 2].

Büyük Menderes River basin is among the 76 most important international wetlands in Turkey [3]. Kemer Dam is within the boundary of Bozdoğan District of Aydin province for irrigation, flood control and energy. The normal water level of the Dam Lake area is 14.75 km² and with volume 544 hm³ volume. Degirmendere, Delicay, Bagdere, Yenidere, Mortumadere and Keklik Streams are other sources that feed the Dam lake [4].

Water quality is the most essential resource of all nutrients required by animals. The water body is plays a role anabolic and catabolic mechanism. The water mineral require for fishes are effected by some factors, such as, quality of water source, nutrient composition and environment factors [5].

The behavior and impact of contaminants in aquatic ecosystem are complex. They may involve, adsorption, precipitation–solubilization, filtration, excretion, sedimentation, resuspension and biological uptake [6]. Many contaminants, imprudent water management practices and destructive land uses threaten aquatic systems in the world. Also, it has been shown that good water quality is a critical component for sustainable socio-economic development [7].
Water pollution is defined as the destruction of natural structure of water sources. The physical, chemical and biological aspects of water quality in the determination of pollution and its effects in water is very important in terms of providing information about the currently situation of water [8]. It is possible to determine whether the water is suitable for the purpose of fish farming in lakes and rivers, by detecting the physical and chemical parameters using appropriate method [9].

The Upper Akcay River comprises 15 different fish species and 76 species bentic invertebrates. Also, the river has a high fish larvae breeding potential which is an important river ecosystem for native fish [1, 2]. However, the river started to get affected by human activity such as agriculture and domestic pollution from the close village. To date, improvement work to acquire new land for agriculture, illegal fisheries and juveniles, uncontrolled cutting and burning of reed, implementation of fish farming and increasing of sedimentation in Kemer Dam lack of water management and increasing aquaculture activity were the main disturbances in upper Akcay River ecosystem.

Anatolia is a large peninsula surrounded by Mediterranean Sea, Aegean Sea, Black Sea and Marmara Sea. Turkey has a very rich water resource potential in both marine and inland waters with 8333 km of coastline, 175 thousand km of rivers, 1 million hectare of natural lakes, 170 thousand hectares of dams, and 7 hunred small dams, which are used for local needs activity. Turkey has also a rich inland lentic waters (200 lakes, 159 dam lakes, 750 small dam lakes) and lotic systems (33 rivers) with fisheries and aquaculture potential [10].

The climate, water resources and topography along the coasts line and these occur many advantage is essential for aquatic life and fisheries, because it is important for significant capability in fish breeding distribution and fisheries of the upper Akcay River. This study was to assess the water quality of the upper Akcay River and evaluate of fisheries activities.

MATERIALS AND METHODS

The Study Area. Upper Akcay River is separated from Kemer Reservoir, which was built for the General Directorate of State Hydraulic Works (DSI) with a 180.50 m-high dam in 1954-58. This Dam is used for irrigation, flood control, hydroelectric purposes and fisheries activities. The hydroelectric station was constructed with an expected capacity of 143 GWh/year. This dam lake is separated from downstream and upper Akcay River created private ecosystem from the lower part of Buyuk Menderes River part is a high number of pollution due to industrial and agriculture activity and human activity.

The study was conducted at three stations in a river from South-western part Anatolia as upper Akcay River, between June 2012 and May 2013. Akcay emerges from the mountains in the North-east of Mugla. Akcay is a high-flowing stream, which is derived from the Bozdağ and Sandraz mountains from Beyagac district of Mugla province. Beyagac (Eskere) Plain passes through other sources. Tavas takes the Yenidere Stream, which is located near Kızılabölük and feeds the Kemer Dam in Aydin, Bozdoğan district.

Akcay joins Yenidere Stream from the slopes facing Tavas Plain. It begins to flow rapidly in narrow and deep valleys. Near the Bozdoğan, be-
fore the lowland level and feeds Kemer Dam in Aydın, Bozdoğan District. It crosses the valley between Karkinçalıdağ and Madran towards Northwest of Yenipazar. Akcay reaches a length of approximately 116 km. Upper Akcay River flows in the area that is not so much exposed to excessive population pressure [28].

**Sampling Methods.** Water samples were collected from 3 stations (Esencay, Goktepe and Camoluk) from the upper akcay River (Figure 1). Esencay (Station 1) as used a reference station because it is not effected from human activities. 1st station coordinates are 37°24’ N and 28°27’W. This area is the main freshwater input. The coming water to the river in the early summer is used for fields irrigation, because of the water level to decrease in summer. The first station is little sloping, and the water flow is slow from late spring to winter. The bottom of 1. Station is covered with sandstone and gravel.

Goktepe (Station 2) is a branch of the river that passes near a small village, with a small trout farm in the upper part. The 2nd station coordinates are 37°27’ N and 28°35’W. The bed flow of the river is sloping and covered with gravel and small rocks, and this make water flow faster.

Camoluk (Station 3) is located after the Goktepe and Esencay junction points, in the spring the water level of Kemer Dam reach to the station with excessive rainfall. The 3rd station coordinates are 37°20’ N and 28°22’W. Some part of Camoluk is covered with clay and mud bottom.

Sampling were carried on monthly between June 2012 and May 2013. For every sampling; temperature, pH, electric conductivity, TDS and dissolved oxygen (DO) (mg/L) were measured directly on the field with MultiProb (YSI 556 MPS). A sample of water was collected at 10-20 cm below the surface, in a 1 liter cleaned plastic Niskin bottles. After the water samples were transported to the laboratory, they were filtered through 0.45 μm pore size membrane filters and partitioned into separate aliquot. All samples were then stored at 4 °C until analysis [29].

![FIGURE 1](image)

*Study Area of Upper Akcay River*

<table>
<thead>
<tr>
<th>TABLE 1. Water quality criteria according to WPCR in freshwaters [30]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parameter</td>
</tr>
<tr>
<td>-----------</td>
</tr>
<tr>
<td>Temperature (°C)</td>
</tr>
<tr>
<td>pH</td>
</tr>
<tr>
<td>Dissolved oxygen (mg/L)</td>
</tr>
<tr>
<td>Ammonium Nitrogen (mg/L)</td>
</tr>
<tr>
<td>Nitrite Nitrogen (mg/L)</td>
</tr>
<tr>
<td>Nitrate Nitrogen (mg/L)</td>
</tr>
<tr>
<td>Ortho-phosphate phosphorus (mg/L)</td>
</tr>
</tbody>
</table>
Analytical procedure. Ammonium nitrogen, nitrite nitrogen, nitrate nitrogen and orthophosphate (the Anions) were analyzed by spectrophotometry with a Hach Lange Dr3900 UVVIS Spectrophotometer.

Water parameters were measured in the laboratory by, SM standard method [29]: Ammonium nitrogen by Phenate method, SM 4500-NH₃-F; Nitrite nitrogen by Colorimetric method, SM 4500-NO₂-B; Nitrate nitrogen by Hydrazine reduction method, SM 4500-NO₃-H and Orthophosphate by Stannous chloride method, SM 4500-PD

The quality criteria of inland water resources according to relevant categories of the Water Pollution Category Registration (WPCR) are given in Table 1 [8, 30].

Statistical Analysis. The monthly mean parameter values for all stations were calculated and WKHDQQXDOWUHQGWRRZDVDQ¶VUKR test was used to assess the correlation among the parameters. The analysis Variance (ANOVA) followed by the post-hoc Tukey test was carried on to assess the differences between the values of the measured parameters among stations. A value of P<0.05 was chosen for significance.

Fisheries Data. Fish species were captured using electro-fishing and scoop net from all three station. Fish were fixed by 4% formaldehyde solution in the field, before taking to the laboratory. We measured fork length (FL), total weight (WT). Fish fauna was determined the status and rates of the stations are given. Fisheries data were determined by the records of provincial directorates of the Ministry of Food, Agriculture and the data obtained from fishermen throughout the year [28].

RESULTS

In Upper Akcay River, the average, minimum and maximum ranges of water qualities parameters were determined below together with the results of ANOVA and post-hoc Tukey test (Table 2). The mean values of temperature, pH, dissolved oxygen, electrical conductivity, TDS, nitrate nitrogen and orthophosphate did not differ among the stations (P<0.05).

Temperature. The mean of water temperatures were 16.61; 14.24 and 15.07 °C at stations 1, 2 and 3, respectively. The minimum water temperature was 4.65 °C in January at station 3; the highest temperature measured 26.35 °C in August at station 1. During the study period, the mean water temperature was 16.15 °C (Table 2; Fig. 2).

According to one year data retrieved in upper Akcay River, it indicates that there is no thermal pollution. According to WPCR the water quality can be classified as I class.

pH. The pH values of all stations in upper Akcay River changed between 5.96 and 8.56. The mean pH was 7.58 and the annual mean pH for stations were given below station 1, 7.56; station 2, 7.59; station 3, 7.58 (Table 2; Fig. 3).

According to the values, the upper Akcay River water is slightly alkaline, also reflects the geological features of the lake surrounding area. According to WPCR the water quality of the Lake in terms of pH is classified as level I (Table 1).

Dissolved oxygen. The average dissolved oxygen values of the station were determined as follows; station 1, 8.63 mg L⁻¹; station 2, 8.57 mg L⁻¹; station 3, 8.47 mg L⁻¹. Minimum value was

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Unit</th>
<th>1.Station Mean (Range) (Min-Mak)</th>
<th>2.Station Mean (Range)</th>
<th>3.Station Mean (Range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature</td>
<td>°C</td>
<td>16.61 (6.87-26.35)</td>
<td>14.24 (4.80-23.69)</td>
<td>15.07 (4.65-25.50)</td>
</tr>
<tr>
<td>pH</td>
<td></td>
<td>7.56</td>
<td>7.59</td>
<td>7.58</td>
</tr>
<tr>
<td>Dissolved Oxygen</td>
<td>mg L⁻¹</td>
<td>8.63 (6.49-8.56)</td>
<td>8.57</td>
<td>8.47</td>
</tr>
<tr>
<td>Electrical conductivity</td>
<td>µS cm⁻¹</td>
<td>676.83 (445-786)</td>
<td>527.00</td>
<td>561.83</td>
</tr>
<tr>
<td>TDS</td>
<td>mg L⁻¹</td>
<td>375.25 (286-511)</td>
<td>362.50</td>
<td>376.58</td>
</tr>
<tr>
<td>Ammonium nitrogen</td>
<td>mg L⁻¹</td>
<td>0.05 (0.01-0.14)</td>
<td>0.11</td>
<td>0.12</td>
</tr>
<tr>
<td>Nitrate nitrogen</td>
<td>mg L⁻¹</td>
<td>3.86 (0.50-5.20)</td>
<td>3.88</td>
<td>3.78</td>
</tr>
<tr>
<td>Orthophosphate</td>
<td>mg L⁻¹</td>
<td>0.06 (0.01-0.30)</td>
<td>0.03</td>
<td>0.05</td>
</tr>
</tbody>
</table>

*All stations in the same row are not significantly different at Tukey test (P>0.05)
seen July at station 3 (3.36 mg L\(^{-1}\)), whereas the maximum was 14.45 mg L\(^{-1}\) in January at station 1 (Table 2; Fig. 4).

Average dissolved oxygen values of all stations for monthly was 8.56 mg L\(^{-1}\). Dissolved oxygen was found to be lower in the summer. When water temperature of the river decreased, dissolved oxygen levels increased suitable level. In summer, the river water used for irrigation activity and water flow decreases. The amount of dissolved oxygen in the water depends on the water temperature, atmospheric pressure, mineral concentrations and water pollution level. The mean Dissolved oxygen is classified as class I to WPCR.

**Electrical conductivity.** Electric conductivity changes between 430-936 μS/cm. The lowest value was 430 μS/cm in May from station 2, the highest
value was 936 μS/cm in November from station 3 (Fig. 5). The annual mean electrical conductivity value was 555.25 μS/cm.

**Total Dissolved Solids (TDS).** TDS of upper Akcay River was measured as the lowest 280 mg/l in May and again as the highest 590 mg/l in November at 2. Station (Table 2). The mean Total Dissolved Solids values follow: station 1, 375.25 mg/L; station 2, 362.50 mg/L; station 3, 376.58. The mean value of all stations was 371.44 mg/l (Fig. 6).

Water quality in terms of TDS values in upper Akcay River was II. class. Klein (1992) has reported that the excess amount of TDS in water disturbed ecological balance and cause distribution of aquatic biota.

**FIGURE 5**
Electrical conductivity (μS/cm) according to stations in upper Akcay River

**FIGURE 6**
TDS (mg/L) value in upper Akcay River

**FIGURE 7**
Ammonium nitrogen (mg/L) in upper Akcay River
Ammonium nitrogen (NH₄-N mg/L). Ammonia nitrogen values vary between 0.01 and 0.40 mg/L. The mean ammonia nitrogen values follow: Station 1, 0.05 mg/L; station 2, 0.11 mg/L; station 3, 0.12 mg/L (Fig. 7).

Annual mean ammonium value was 0.09 mg/L. According to Anonymous (2015), this is classified as class II (Table 1).

Nitrate nitrogen (NO₃-N mg/L). Changes in nitrate nitrogen from all stations were found between 0.5-5.3 mg/L. The average nitrate nitrogen concentrations were 3.86 mg L⁻¹ for station 1; 3.88 mg L⁻¹ for station 2; 3.78 mg L⁻¹. The mean nitrate in all stations is 3.84 mg L⁻¹ (Fig. 8). According to Anonymous (2015), this is classified as class I (Table 1).

The nitrite (NO₂-N mg/L) level of the River was close to zero. No significant difference in nitrate and nitrite levels throughout the year.

Orthophosphate (PO₄-P mg/L). Orthophosphate phosphorus (PO₄-P) change between 0.01-0.30 mg/L. Average values from stations were; 0.06 in station 1; 0.03 mg/L in station 2 and 0.05 mg/L in station 3. The mean annual value of orthophosphate was 0.04 mg/L (Fig. 9). According to Anonymous (2015), this is classified as class II (Table 1).

Statistical analysis. According to the Spearman’s rho test, negative correlation was observed between the temperature values and dissolved oxygen values; also between TDS and annual pH levels. In addition, there was a positive correlation between the water temperature values and pH, electrical conductivity, orthophosphate.

There was no significant relationship between pH and electrical conductivity values or between nitrate and orthophosphate values but there was a positive correlation between nitrate and orthophosphate.
Distribution of Economic Fish and Fisheries. The economic species caught in the river during a year survey included *Onchorynchus mykiss*, Capoeta bergamae, Alburnus escherichii, Vimba mirabilis, Chondrostoma meandrense, Squalius fellowesii, Barbus pectoralis and Carassius gibelio. *C. gibelio*, an exotic fish and *O. mykiss*, an introduced fish of stations were the least caught, and *V. mirabilis* and *S. fellowesii*, endemic and native fish, were the most dominant species in upper Akcay River (Table 3).

According to the data obtained during the research in the region: A small trout farm is occur in the 2nd station on the Akcay River (Yemisendere) with 9 ton, while the aquaculture produces 5 trout farms in Kemer Dam with 4584 ton. The most common fish catchment for fishery in upper Akcay River was *C. bergamae* and *S. fellowesii*. Yield production of fisheries is 10.5 ton (Table 4).

DISCUSSION

Water quality is affected from natural processes. Also, there are anthropogenic impacts, such as man-induced point and non-point sources, xenobiotics and alteration of water quality due to water use and river engineering projects as irrigation, damming. Water quality monitoring refers to the acquisition of quantitative and representative information on the physical, chemical, and biological characteristics of a water body [31, 32].

The mean water temperature was 16.15 °C, according to WPCR (Table 1) the water characteristic was classified as class I. The water quality is suitable for Aquaculture and fish cultivation such as trout. Water temperature is an important climatic factor, it increases biological activities and effect oxygen saturation [15]. In this study, the temperatures of all stations and the reference station did not vary significantly (P>0.05).

The mean values of pH was ranged between 7.56 and 7.59. According to the values of pH was found to be fairly alkaline in upper Akcay River. The pH is the scale of intensity of acidity and alkalinity of water and measures the concentration of hydrogen ions. The biological processes and biochemical reactions effect pH [33]. Accepted water quality criteria indicate a pH of less than 5.5 units may be harmful to many species of fish [32]. The pH value of 6.5 to 9.0 units would be optimal for the protection of aquatic habitats [34].

According to the EPA [32], the mean values of pH in upper Akcay River were normal. When water pH level is high, ammonium and nitrogen compounds have adverse effects. The value of pH, indicates that growing of fish is appropriate [29]. The pH value in stations were not different (P<0.05).

The mean values of dissolved oxygen was ranged between 8.47 mg/l and 8.63 mg/l in upper Akcay River. Dissolved oxygen is one of the most important parameter for water quality, because it shows the biological and physical processes prevailing in water. In freshwater ecosystems, the

### TABLE 3

<table>
<thead>
<tr>
<th>Economic Fish species</th>
<th>Status of Fishes</th>
<th>Catchment Percentage (%)</th>
<th>Occurrence in Stations</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Onchorynchus mykiss</em></td>
<td>Translocated</td>
<td>0.30</td>
<td>2, 3</td>
</tr>
<tr>
<td>Capoeta bergamae</td>
<td>Endemic-NT</td>
<td>12.08</td>
<td>1, 2, 3</td>
</tr>
<tr>
<td>Alburnus escherichii</td>
<td>Native</td>
<td>10.58</td>
<td>2, 3</td>
</tr>
<tr>
<td>Vimba mirabilis</td>
<td>Native-VU</td>
<td>25.38</td>
<td>1, 2, 3</td>
</tr>
<tr>
<td>Chondrostoma meandrense</td>
<td>Endemic-VU</td>
<td>11.78</td>
<td>1, 2, 3</td>
</tr>
<tr>
<td>Squalius fellowesii</td>
<td>Endemic-VU</td>
<td>34.75</td>
<td>1, 2, 3</td>
</tr>
<tr>
<td>Barbus pectoralis</td>
<td>Native</td>
<td>4.83</td>
<td>2, 3</td>
</tr>
<tr>
<td>Carassius gibelio</td>
<td>Exotic</td>
<td>0.30</td>
<td>3</td>
</tr>
</tbody>
</table>

### TABLE 4

<table>
<thead>
<tr>
<th>Economic Fish species</th>
<th>Local name</th>
<th>Fishery activity</th>
<th>Locality</th>
<th>Yield (Kg) (x1000)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Onchorynchus mykiss</em></td>
<td>Alabalik</td>
<td>Aquaculture</td>
<td>Kemer D.</td>
<td>4584</td>
</tr>
<tr>
<td>Capoeta bergamae</td>
<td>Siraz</td>
<td></td>
<td>Yemisendere</td>
<td>9</td>
</tr>
<tr>
<td>Alburnus escherichii</td>
<td>Cay balgai</td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Vimba mirabilis</td>
<td>Ulubat balgai</td>
<td></td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Chondrostoma meandrense</td>
<td>Kababurun</td>
<td>Fishing rods, scope nets</td>
<td>Upper Akcay</td>
<td>1.1</td>
</tr>
<tr>
<td>Squalius fellowesii</td>
<td>Tatilis kefali</td>
<td>and cast nets</td>
<td>River</td>
<td>1.5</td>
</tr>
<tr>
<td>Barbus pectoralis</td>
<td>Biyikli balik</td>
<td></td>
<td></td>
<td>1.2</td>
</tr>
<tr>
<td>Carassius gibelio</td>
<td>Gümüşsazan</td>
<td></td>
<td></td>
<td>0.2</td>
</tr>
<tr>
<td>Silurus glanis</td>
<td>Yayn</td>
<td></td>
<td></td>
<td>0.5</td>
</tr>
</tbody>
</table>
minimum dissolved oxygen may not be less than 5.0 mg/l [35]. The values of dissolved oxygen were normal in upper Akcay River for aquatic life. According to classification continental inland water sources of Water Pollution Control Regulation in River was found I (Table 1). For trout cultivation and fishes, amount dissolved oxygen need to be higher than 5.00 mg L⁻¹ [34]. The mean dissolved oxygen in water is 8.56 mg L⁻¹. Dissolved oxygen is classified as class I for the WPCR. In station III, the minimum dissolved oxygen value is 3.36 mg L⁻¹ in early summer, because it is highly use for irrigation and solid pollution from village.

The mean electrical conductivity value obtained in this study was 555.25 μS/cm. The conductivity value was observed in stations was no significantly different (Table 2). Conductivity level increased in accordance to water temperature and in total dissolved solids. Electrical conductivity of ions in water changed with the total concentration and depends on the change of temperature [36]. Decrease in electrical conductivity, means a reduction of total dissolved solids content [35].

The mean ammonium nitrogen values to stations changed between 0.05 (I. station) to 0.12 mg L⁻¹ (III. station) and the values obtained in the stations are no significantly different. Ammonium should be less than 1 mg/l in clean water [34]. NH₄-N, which is the waste material of aquatic organisms, may be absorbed by organisms. Non-toxic ammonium depending on temperature and high pH (>8.5) could transform into ammonia and become toxic for fish and other aquatic biota [37]. According to Anonim WPCR of the value it is considered to be classified as level II [30].

The nitrite was below 0.001 mg/l all stations in upper Akcay river. According to classification continental inland water sources of the WPCR in river water is I (Table 1). The nitrite nitrogen of the water samples is an intermediate product of biological oxidation of ammonium to nitrate. The concentration of nitrite in natural lake water is generally low [35].

The mean value nitrate was ranged between 3.78 mg/l and 3.88 mg/l in upper Akcay River. The Nitrate nitrogen (NO₃-N), the minimum nitrate nitrogen concentration was 0.50 mg/L in January at station I, while the maximum value was 5.30 L⁻¹ in July at station II. Because of the waste product coming from farming and organic agricultural. Pollution can effect some variable characteristics regarding ammonium and nitrate in reservoirs and rivers [35], According to WPCR, the average annual value of nitrate was about 3.84 mg/L, with this value in the River it could be categorized as I. class.

Dissolved organic phosphorus or suspended organic phosphorus found in natural water and the total phosphorus content in Akcay River was between 0.01-0.30 mg/L. The main source of phosphate is ortho-phosphate. The mean value of orthophosphate phosphorus (PO₄-P) at stations in upper Akcay River was determined to be 0.04 mg/L. The highest orthophosphate value was seen in station I around 0.30 mg/L in June. In terms of orthophosphate phosphorus in upper Akcay River is classified to be level II [30].

According to WPCR, water containing 0.02 mg/L total phosphorus is classified to be at level I and is suitable for fish and fisheries. Phosphorus levels higher than 0.30 mg/L are considered as signal of pollution. The total phosphorus concentration in natural waters depends on the morphometry of basin, the chemical content of the region, and the existence of organic substances and organic metabolism in the water [34]. The orthophosphate concentrations at stations did not differ significantly (P<0.05).

In Upper Akcay River, the water quality parameters were compared with other studies reports. There are intensive fish aquaculture activities in Southwestern part of Turkey such as Esen River, Yuvarlakcay River, Bereket Dam Lake (Table 5). Bafa lake is a lagoon lake and many marine fish breeding activities. The inland water resources in southwestern part of Anatolia are suitable for culturing trout and fisheries. The concentrations of nitrite nitrogen, nitrate nitrogen, and orthophosphate reported for other freshwaters were not significantly different than our result in this study. Bereket Dam Lake’s the nitrate and the phosphate values were higher because of intensive aquaculture activities (Table 5).

<table>
<thead>
<tr>
<th>Locality</th>
<th>T  (°C)</th>
<th>Salinity (%)</th>
<th>pH</th>
<th>EC (μS/cm)</th>
<th>DO (mg/l)</th>
<th>NO₂-N (mg/l)</th>
<th>NO₃-N (mg/l)</th>
<th>PO₄-P (mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yuvarlakcay [10]</td>
<td>19.3</td>
<td>8.1</td>
<td>400</td>
<td>8.50</td>
<td>0.01</td>
<td>0.23</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Aglasun Deresı [38]</td>
<td>13.03</td>
<td>-</td>
<td>484</td>
<td>8.33</td>
<td>5.80</td>
<td>0.39</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hasan River [39]</td>
<td>15.72</td>
<td>0.15</td>
<td>8.51</td>
<td>9.48</td>
<td>2.3</td>
<td>0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bereket D. Lake [40]</td>
<td>15.42</td>
<td>7.93</td>
<td>0.71</td>
<td>6.76</td>
<td>6.45</td>
<td>0.91</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gazizey D. Lake [41]</td>
<td>13.85</td>
<td>8.1</td>
<td>798</td>
<td>9.9</td>
<td>0.002</td>
<td>0.30</td>
<td>0.014</td>
<td></td>
</tr>
<tr>
<td>Akso River [42]</td>
<td>20.49</td>
<td>8.38</td>
<td>714.53</td>
<td>6.27</td>
<td>2.75</td>
<td>0.52</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bafa Lake [43]</td>
<td>22.21</td>
<td>12.95</td>
<td>8.26</td>
<td>7.52</td>
<td>2.10</td>
<td>0.19</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Esen River [44]</td>
<td>14.74</td>
<td>7.7</td>
<td>357.71</td>
<td>8.32</td>
<td>3.33</td>
<td>0.075</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Akcay River</td>
<td>16.15</td>
<td>0.25</td>
<td>7.58</td>
<td>8.56</td>
<td>0.01</td>
<td>3.84</td>
<td>0.30</td>
<td></td>
</tr>
</tbody>
</table>
Many threats of freshwater biota underline about many changes in habitat or community structure, such as pollution, flow regulation, loss or change of aquatic habitat, over-exploitation and introduction of exotic species. These reasons have all contributed through the critical situation facing many freshwater fish species in South-western Anatolia as *Vimba mirabilis* and *Chondrostoma meandrense*. *Vimba mirabilis* is known by the original description from Buyuk Menderes and threatened due to water abstraction. This species extinct from Bafa Lake which connection was banned with Buyuk Menderes River artificial barrier [19]. The decline in fish species and around reservoir is the largest documented loss of biodiversity caused by humans and alien species in an ecosystem. *Carassius gibelio* are potential competitors for the food of numerous endemic and native fish species.

The Akcay River threatened mainly by draining of water as agricultural irrigation, prevention of natural water flows to wetlands by dam constructions, urban development on wetlands and surroundings (urban and industrial utilization), chemical contamination due to the industrial as tar, olive oil activities, household wastes, introduction of exotic species, unsustainable fishing and plants collecting. In the future, the establishment of a hydroelectric power plant on the upper Akcay river is discussed. This study is important because it contributes to the determination of the current situation.

**CONCLUSIONS**

Recommendations with the result obtained on the analyses on water quality of the river can be sustained by the biota and habitat. There is no deterioration in water quality in the stations in upper Akcay river. It has a unique and sensitive habitats as the Buyuk Menderes basin. Therefore, these areas should be protected and monitoring should be carried out.

There is no excessive fish farming in the upper Akcay River. Aquaculture activities is seems very intensive in Kemer Dam Lake to affect the entire dam lake. Clear definition of the receiving environment will facilitate implementation of legal and technical measures to sustain and preserve development. Agricultural activities and domestic wastes should be checked and removed so as not to cause pollution.

The river should be protected from the richness and evenness of riparian vegetation.

The variety of native Fish should be protected. Native and endemic species such as *Vimba mirabilis* and *Chondrostoma meandrense* should be preserved. Exotic species such as *Carassius gibelio* damaged the environment.

It must be a strict implementation of the policies endemic species. illegal fishing should be controlled. The river should be protected from exotic vegetation and fauna.

It should be a community-based management of river by monitoring the area regularly. These are recommended in order for the quality of the stream and could be preserved for the next generation to see and experience the richness of upper Akcay River.

**ACKNOWLEDGEMENTS**

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**REFERENCES**


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EVALUATION OF THE ADAPTABILITY AND STABILITY OF MAIZE CULTIVARS THROUGH GGE BIPLOT ANALYSIS

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2Hebei Provincial Key Laboratory of Crops Drought Resistance Research, Hengshui 053000, China
3College of Agronomy, Qingdao Agricultural University, Qingdao 266109, China

ABSTRACT

This study evaluated the yield performance of 27 maize (Zea mays L.) genotypes from 2016 to 2017 in 80 trial environments in Huang-huai-hai summer maize growing area of China during summer by using a randomized complete block design (RCBD) with three replications. Grain-yield data obtained from the regional trials of the Kechuang Union were analyzed with a genotype-and-genotype-by-environment (GGE) biplot. Analysis of variance (ANOVA) of grain-yield data showed that the effects of environment, genotype, and genotype-by-environment interaction were significant, accounting for 45.27%, 9.18%, and 27.39% of the sum of treatment combinations in 2016, as well as 53.97%, 9.43%, and 19.81% of that in 2017, respectively. Results showed that the best performance and candidate genotypes for the 2016–2017 multi-environment trials were D56, J118, and H321, as well as S617, D205 and D56, respectively. Among the 80 test sites, the environments TA and XJ in 2016 and the environment JX in 2017 were included in the ideal environment due to their excellent discrimination and representativeness.

KEYWORDS:
GGE-biplot, maize, multi-environment trials, stability

INTRODUCTION

Maize (Zea mays L.), the most widely grown crop in the worldwide, is also an important feed, economic, and bioenergy crop [1]. For the first time in 2012, the output of maize exceeded rice and wheat, becoming the largest food crop in China [2]. Maize is widely planted in China, mainly in the Northeast China spring-planting maize region, the Huang-Huai-Hai summer-planting maize region, and the Southwest maize region. Among them, the Huang-Huai-Hai summer-maize region is the largest concentrated maize-planting area in China. The annual sowing area of maize is approximately 10 million hectares, accounting for 30% of the total area planted in the country. The annual output is more than 45 million tons, accounting for approximately 40% of the national total [3]. Huang-Huai-Hai summer maize has a high overall level of production, and the significant differences between rainy and hot seasons in the provinces are conducive to maize production. Although the production conditions in this region are conducive to increasing the yield of summer-maize cultivars, its potential in the Huang-Huai-Hai region has not yet been fully realized. In recent years, natural phenomena such as wind, rain, drought, fluctuating high and low temperature, and high solar radiation occur frequently in the Huang-Huai-Hai summer-maize area, and the corn yield is unstable during the single-year season. The actual yield of maize cultivars in the field is far from the genetic yield [4]. Thus, increasing the maize-grain yield by screening new cultivars with high yield, stability, and adaptability is necessary. Multi-environment trials (METs) are very important in crop breeding and agronomy and can be used to study yield stability and predict genotype yield performance in different environments [5]. The yield performance of the same cultivar in different environments often significantly vary, and the fluctuation patterns of different cultivars also vary. These kinds of fluctuations are genotype-by-environment interactions (GEIs), which are affected by different environmental conditions. This interaction alters the stability of cultivars, indicating that increased GEI corresponds to decreased stability. Accordingly, cultivar stability has become a research hotspot [6-9]. Moreover, due to the different environments and test-site scrapings, evaluating the indicators of new cultivars objectively is difficult. Thus, the representativeness of the test site and its ability to distinguish cultivars, as well as the stability and adaptability of the tested lines, are new analysis prospects [10, 11].

Researchers have proposed several stability-analysis methods to deal with GEI and to determine the genotypes that perform well and produce high
yields in different environments. These methods include statistical analysis for linear regression [12], variance analysis [13], principal component analysis (PCA) [14], and singular-value decomposition [15]. The additive main effects and multiplicative interaction (AMMI) model proposed by Zobel et al. [16] and the genotype-and-genotype-by-environment (GGE) model proposed by Yan et al. [17] have been the most effective statistical-analysis methods in recent years. The AMMI model combines ANOVA and PCA to add the interaction of product forms to the additive model of genotype and environment. This model often used for GEI analysis or cultivar-stability analysis. The ground has a simultaneous analysis of the high yield and stability of the cultivar, so it is less practical in the comprehensive evaluation of the high yield and stability of the cultivars [18, 19]. Based on the original data, the GGE biplot explains the genotype and GEI effect ratios simply and intuitively, thereby providing information on the special adaptability of crop cultivars and can consider interannual differences. In the GGE biplot, only the genotype effect of product forms to the additive model of genotype and environment. The information of these cultivars and their test sites is shown in Tables 1 and 2, respectively.  

**MATERIALS AND METHODS**

**Description of the cultivars and test sites.** The research data were derived from the grain yield results of the summer-maize regional trial of the Huang-Huai-Hai Kechuang Union in the group of the “Hengyu 321” in 2016–2017. In the 2016 and 2017 trials, the numbers of participating cultivars were 13 and 14, respectively, and 40 test sites per year. Among them, Zhengdan 958 was the control cultivar. The information of these cultivars and their test sites is shown in Tables 1 and 2, respectively.

**TABLE 1**

<table>
<thead>
<tr>
<th>Cultivars</th>
<th>Code</th>
<th>Plant type</th>
<th>Breeding institute</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jiyu 518</td>
<td>J518</td>
<td>Compact</td>
<td>Institute of Cereal and Oil Crops, Hebei Academy of Agriculture and Forestry Sciences</td>
<td>2016</td>
</tr>
<tr>
<td>Jiyu 196</td>
<td>J196</td>
<td>Compact</td>
<td>Institute of Cereal and Oil Crops, Hebei Academy of Agriculture and Forestry Sciences</td>
<td>2016</td>
</tr>
<tr>
<td>Hanyu 9112</td>
<td>H9112</td>
<td>Compact</td>
<td>Handan Academy of Agriculture Sciences, Hebei</td>
<td>2016</td>
</tr>
<tr>
<td>Zhongnongda 626</td>
<td>Z626</td>
<td>Semi-compact</td>
<td>Beijing Zhongnongdakang Technology Development Co., Ltd.</td>
<td>2016</td>
</tr>
<tr>
<td>Sudan 510</td>
<td>S510</td>
<td>Compact</td>
<td>Suzhou Academy of Agriculture Sciences, Anhui</td>
<td>2016</td>
</tr>
<tr>
<td>Luyu 36</td>
<td>L36</td>
<td>Flat</td>
<td>Shangxi Luyu Seed Industry Co., Ltd.</td>
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<td>Tangshan Academy of Agriculture Sciences, Hebei</td>
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<td>PT1212</td>
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<td>2017</td>
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Experimental design and field management. The experiment adopted a Random Complete Block Design with three replications, with a length of 6.7 m, a row spacing of 0.6 m, and 5 rows in plots of 6.7x3 m². Sowing dates of each site ranged from 15 June to 20 June based on local conditions, and the planting density was 60 000 plants ha⁻¹. Field management was carried out in accordance with local farming management practices. Three intermediate rows from each location were harvested from October 1 to October 15, and grain yield was adjusted to 13% grain moisture content.

Data analysis. Variance analysis of yield data was performed using Genstat 64-bit Release [27] to study genotype effects (G), environment effects (E), and GEI effects (G×E), and a GGE biplot was drawn. Notably, the GGE model is also called the PCA of environmental centralization. After subtracting the original data from the environmental mean, G was integrated into the interaction item for singular-value decomposition. The grain yield in the METs was expressed by the following formula:

\[ Y_{ger} = \mu + \beta y + \sum \lambda y_{m} + \rho_{ge} + \varepsilon_{ger} \]
where $Y_{gen}$ is the grain yield value of the rth repeat of genotype $g$ in the environment $e$, $\mu$ is the population mean, $\beta_e$ is the main effect of environment $e$, $\lambda_n$ is the singular value of the nth principal component, $\gamma_{ge}$ is the nth principal component scores of genotype $g$, $\delta_{ge}$ is the nth principal component scores of environment $e$, $\rho_{ge}$ is the residual of genotype $g$ in environment $e$, and $\epsilon_{ge}$ is the total error. Considering the eigenvalue assignment in the focus environment, the parameters and are defined as the first principal component scores of genotype $g$ and the environment $e$, respectively, referred to as PC1. The analogy of the second principal component (PCA2) is analogous. The GGE biplot is generated based on the following: (a) location of the most suitable area for the genotype; (b) the GGE biplot can visually show the discriminative power and representative-ness of each environment; and (c) the tested cultivars and sites were ranked for the ideal genotype and ideal environment, respectively.

**RESULTS**

**Analysis of variance of grain yield.** A multi-environment combined variance analysis of the yield of the Huang-Huai-Hai Kechuang Union trials in 2016–2017 showed that G, E, and G×E were highly significant (P<0.01), accounting for 9.18%, 45.27%, and 27.39% of the total sum of squares in 2016 (Table 3), respectively. In 2017, the total sum of squares were 53.97% for E, 19.81% for G×E, and only 9.43% for G (Table 4). Notably, E was the main source of maize yield variation, but it has no effect on genotypic evaluation. The GGE biplot can eliminate E to facilitate the scientific evaluation of genotypes. Meanwhile, the contribution rate of G×E to the treatment variation in the two-year ANOVA was greater than that of G. Thus, further analyzing the interaction between genotype and environment and scientifically dividing the suitable planting areas of each tested cultivar were necessary.

**Grain yield analysis of maize cultivars and test sites in Kechuang Union trial.** The grain yields of maize genotypes and environments are shown in Tables 5 and 6. In 2016, the average grain yield of tested maize cultivars ranged from 8.6633 kg ha$^{-1}$ in L36 to 10.2411 kg ha$^{-1}$ in H321. The average grain yield of the tested genotypes H321, DK56, J118, H9112, S510, and I196 were 5.52%, 5.48%, 5.07%, 1.85%, 1.46%, and 1.04% higher than Z958 (check cultivar), respectively. For the average yield of the test sites, the ranged of change was between 7.3148 kg ha$^{-1}$ in GC and 11.9256 kg ha$^{-1}$ in HXN over 40 test sites. In terms of Kechuang Union trial in 2017, 5 of the 14 tested cultivars were reduced compared with the grain yield of control cultivar. The highest and lowest average grain yields were S617 and PT1212, respectively, which increased yield by 11.23% and reduced yield by 5.82%. Among the 40 test sites, the average grain yield was between 7.3548 in XGG and 11.8585 kg ha$^{-1}$ in XX.

**Adaptability analysis based on GGE biplot.** The suitable planting area for each cultivar was divided by the “Which-won-where view” of the GGE biplot. In the GGE biplot, the icon at the outermost periphery was connected in turn to form a polygon, and all the cultivars of icons can be enclosed in the polygon. From the origin of the double plot to the perpendicular of each side of the polygon, the polygon was divided into different sectors, and the environment within the same sector constitutes one environment combination. The cultivar of each sector located on the top of the polygon corner was the best performing cultivar in each environment in the sector, called the “winning cultivar”, which was the best performing cultivar in the environmental combination area. By contrast, genotypes located inside the polygon and close to the origin of the biplot were cultivars not sensitive to environmental changes [28].

**TABLE 3**

<table>
<thead>
<tr>
<th>Source of variance</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>Percentage of total SS /%</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Block</td>
<td>80</td>
<td>340858.87</td>
<td>4280.74</td>
<td>1.70*</td>
<td>0.02**</td>
</tr>
<tr>
<td>Environment (E)</td>
<td>39</td>
<td>6858184.86</td>
<td>175850.89</td>
<td>45.27</td>
<td>2.87**</td>
</tr>
<tr>
<td>Genotype (G)</td>
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<td>1391510.97</td>
<td>115959.25</td>
<td>9.18</td>
<td>46.17**</td>
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<tr>
<td>Genotype*environment (GxE)</td>
<td>468</td>
<td>4149268.40</td>
<td>8865.96</td>
<td>27.39</td>
<td>3.53**</td>
</tr>
<tr>
<td>Error</td>
<td>960</td>
<td>2411128.93</td>
<td>2511.59</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
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<td>15150952.04</td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: DF, degrees of freedom; SS, sum of squares; MS, mean square ** indicate significant difference at the 0.01 level, the same as below.

**TABLE 4**

<table>
<thead>
<tr>
<th>Source of variance</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>Percentage of total SS /%</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
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<td>469657.71</td>
<td>5870.72</td>
<td>1.39*</td>
<td>0.02**</td>
</tr>
<tr>
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<td>101.49**</td>
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<td>53.18**</td>
</tr>
<tr>
<td>Genotype*environment (GxE)</td>
<td>507</td>
<td>3562433.01</td>
<td>7026.49</td>
<td>19.81</td>
<td>2.87**</td>
</tr>
<tr>
<td>Error</td>
<td>1040</td>
<td>2549571.14</td>
<td>2451.51</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1679</td>
<td>17980469.03</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
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Fresenius Environmental Bulletin

TABLE 5
Average grain yields of maize cultivars over 40 test sites in 2016
Sit
es
G
C
H
D
SZ
W
Q
BT
Z
X
G
Y
Y
Q
SX
JS
Y
C
LC
LZ
JN
Z
Q
W
F
D
Z
JX
PD
L
Q
L
YI
H
Z
T
A
X
Z
SQ
Y
Y
N
Y
X
X
JZ
Z
K
X
Y
G
YI
Z
M
D
L

H91
12
7.55
55
9.87
45
9.09
75
10.5
105
8.88
00
9.82
95
10.8
195
9.32
40
9.19
95
10.4
970
10.3
140
10.0
755
11.6
220
11.2
335
6.43
50
10.2
360
10.1
325
9.55
20
8.03
25
9.41
25
11.0
970
10.9
530
12.3
945
9.79
35
10.9
200
8.97
60
8.40
45
10.0
830
10.2
720
10.7
205
9.03
45
9.03
45

D56

8.10
75
8.35
95
8.89
50
11.3
085
10.9
560
11.4
945
11.8
395
9.60
90
9.22
65
9.41
10
9.55
50
10.1
565
10.4
055
10.7
910
8.57
40
9.63
00
9.53
55
10.0
080
8.64
30
9.56
25
10.3
440
10.1
100
11.4
645
8.06
25
9.79
05
8.99
10
7.92
00
9.75
00
9.88
50
10.0
815
9.66
90
9.66
90

H32
1
7.48
20
8.48
25
10.0
185
11.9
700
11.0
745
11.1
420
11.8
650
9.37
95
9.11
25
11.2
410
10.1
280
10.8
030
11.9
640
10.6
920
9.07
65
9.63
30
11.9
055
9.94
05
11.3
115
9.74
40
10.3
440
12.8
340
11.4
345
9.01
50
11.5
335
7.84
35
8.71
50
9.49
65
11.7
195
9.83
40
8.07
30
8.07
30

Grain yield (t ha-1)
Cultivars
Z696
Z626
S510

7.96
35
9.26
25
9.31
95
11.5
830
10.7
220
10.1
820
11.6
475
9.97
35
9.86
10
11.1
915
10.3
995
9.82
20
10.7
805
11.8
980
8.84
10
9.87
45
9.96
30
11.6
190
9.31
05
8.52
15
9.28
50
11.9
400
10.9
455
9.78
60
11.0
805
9.00
15
7.94
85
9.50
25
11.4
810
11.7
675
9.08
85
9.61
65

7.305
0
9.202
5
9.745
5
10.12
50
9.246
0
10.07
10
10.34
40
9.133
5
9.627
0
10.27
95
9.487
5
8.230
5
10.31
40
10.33
80
7.497
0
10.20
45
10.02
75
9.580
5
8.004
0
8.854
5
10.06
05
10.73
85
9.967
5
9.132
0
7.548
0
7.378
5
7.759
5
9.024
0
10.52
25
10.00
65
8.799
0
8.799
0

6.946
5
9.454
5
9.024
0
10.22
25
10.43
55
10.46
70
10.54
20
9.922
5
9.408
0
10.45
80
9.871
5
8.347
5
11.07
15
9.526
5
8.122
5
9.751
5
10.14
45
10.98
60
8.532
0
8.013
0
11.80
65
9.708
0
10.81
95
9.835
5
9.435
0
8.593
5
8.265
0
8.521
5
10.92
00
10.38
60
8.259
0
8.860
5

6.29
55
10.2
045
8.64
45
9.26
85
8.67
60
8.33
25
9.31
65
11.5
065
9.90
00
10.3
935
10.3
920
9.37
50
11.6
640
10.3
470
6.70
20
9.91
05
10.1
220
11.3
895
12.0
900
9.09
60
11.6
700
9.51
00
11.5
125
11.0
670
6.99
30
9.03
45
8.64
60
9.12
45
10.5
195
11.0
730
9.30
45
9.30
45

11.1
945

10.0
965

10.3
170

11.2
500

11.5
590

10.18
50

9.688
5

9.09

10.5

10.0

9.05

9.13

9.255

9.096

J518

J118

J196

7.159
5
8.043
0
9.589
5
10.89
75
8.431
5
10.29
45
10.81
05
8.725
5
9.618
0
11.57
25
8.896
5
8.839
5
11.05
50
10.43
55
8.086
5
10.19
10
9.646
5
9.924
0
9.561
0
10.49
10
9.219
0
10.45
20
11.05
80
8.065
5
9.354
0
8.715
0
7.798
5
9.268
5
9.771
0
10.20
15
8.841
0
8.841
0

9.92
25
9.24
30
9.74
55
11.1
555
11.5
200
11.4
930
12.4
455
9.15
30
9.61
05
10.9
050
10.3
800
10.1
385
11.0
310
10.7
445
9.24
60
10.7
310
9.31
05
10.4
055
12.5
625
9.57
15
10.5
750
10.4
115
11.4
450
9.21
90
11.1
105
9.01
35
8.01
00
9.12
75
10.9
050
11.0
940
7.18
35
8.09
85

9.771
0
9.936

6723

L36
7.25
10
9.32
40
8.32
05
10.3
185
7.24
80
8.96
70
9.55
20
8.97
90
8.39
55
8.86
50
10.5
585
9.03
00
10.9
890
11.9
760
6.02
85
8.40
45
7.40
10
8.13
75
8.03
25
8.76
90
8.96
25
10.3
350
6.89
55
4.98
60
11.0
970
7.21
80
7.79
25
7.76
85
10.6
140
8.77
95
7.51
20
7.51
20

L150
2
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0
8.049
0
9.513
0
9.238
5
9.196
5
9.772
5
9.939
0
8.625
0
8.316
0
10.46
40
10.37
40
8.941
5
11.21
40
10.10
25
8.140
5
8.851
5
8.443
5
9.054
0
11.47
80
9.324
0
8.919
0
10.39
35
10.52
55
6.706
5
9.580
5
7.629
0
7.887
0
8.373
0
9.901
5
8.922
0
8.398
5
8.398
5

T667
8
6.958
5
9.033
0
10.03
05
10.46
10
10.36
35
9.259
5
10.72
50
7.860
0
8.356
5
9.523
5
9.960
0
8.691
0
10.84
65
10.14
45
7.710
0
8.668
5
9.952
5
9.405
0
10.81
05
10.41
15
8.929
5
11.37
75
10.51
20
6.306
0
9.141
0
8.724
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8.890
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50
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7.879
5

Z958
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11.42
85
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9.354
0
10.73
85
10.38
00
9.712
5
11.87
25
10.12
80
8.613
0
9.994
5
10.44
90
9.610
5
11.51
40
8.844
0
10.24
95
11.59
80
11.09
40
8.737
5
10.99
20
6.934
5
8.242
5
7.662
0
9.577
5
10.60
35
8.307
0
8.307
0

10.7
280

10.7
940

10.38
60

10.88
10

9.366
0

9.64

8.10

8.157

7.962

9.184

Mean

7.3148u
9.0042o
pqrs
9.3010l
mnopq
10.6319
bcdef
9.7140ij
klmno
10.1402
efghij
10.8673
bcd
9.2774l
mnopq
9.2296m
nopqr
10.4262
cdefghi
10.0536
fghijk
9.3972kl
mnop
11.1407
bc
10.6428
bcdef
7.9287tu
9.6985jk
lmno
9.7718hi
jklmn
9.9702f
ghijkl
9.9909f
ghijkl
9.2781l
mnopq
10.1124
efghijk
10.7970
bcde
10.7745
bcde
8.5163rs
t
9.8904g
hijklm
8.3117st
8.1339t
8.9686p
qrs
10.5726
bcdefg
10.2186
defghij
8.4456st
8.6457q
rst
10.4782
cdefgh
9.1695n


### TABLE 6
Average grain yields of maize cultivars over 40 test sites in 2017

<table>
<thead>
<tr>
<th>Sites</th>
<th>Grain yield (t ha⁻¹)</th>
<th>Cultivars</th>
</tr>
</thead>
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<td></td>
<td>D56</td>
<td>H321</td>
</tr>
<tr>
<td>Y</td>
<td>0</td>
<td>810</td>
</tr>
<tr>
<td>H</td>
<td>10.38</td>
<td>11.0</td>
</tr>
<tr>
<td>B</td>
<td>60</td>
<td>385</td>
</tr>
<tr>
<td>H</td>
<td>11.61</td>
<td>12.2</td>
</tr>
<tr>
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<td>75</td>
<td>760</td>
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<td>Q</td>
<td>75</td>
<td>225</td>
</tr>
<tr>
<td>X</td>
<td>0</td>
<td>65</td>
</tr>
<tr>
<td>L</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>G1</td>
<td>1bced</td>
<td>976a</td>
</tr>
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</table>

Note: Different letters indicate significant difference at P < 0.05 level, the same as below.
The polygon of maize cultivars under different test sites is shown in Fig. 1A. The first two principal components (PC1 and PC2) account for approximately 56% (38% and 18% by PC1 and PC2, respectively) of GEI. The largest sector included genotypes S510, Z696, Z626, H9112, and J518 with environments YQ, XZ, XNG, LYI, JX, ZK, XY, HD, GYI, XY, SX, and HXN. S510 was the vertex genotype in this sector. This finding showed that S510 was the winner cultivar in the above test sites. The second largest sector involved genotypes L36, T6678, and L1502 with environments YC and JN. The vertex genotype in this sector was L36. This result indicated that L36 was the best genotype at YC and JN. The third largest sector contained H321, J118, Z958, and J196 with environments EY, BT, ZQ, HZW, GC, LC, PD, LQ, SZ, HB, and JN. The vertex genotype in this sector was L36. This finding showed that L36 was the winner cultivar in the above test sites. The second largest sector involved genotypes L36, T6678, and L1502 with environments YC and JN.
The PC1 and PC2 explained the 57.52% of the G+GE, accounting for 42.66% and 14.86%, respectively (Fig. 1B). The 40 test sites also segregated into five distinct sectors, and the vertex genotypes were PT1212, J757, S617, H6105, and Z111. The largest sector displayed PT1212 and H5177, having no winner in any environment. The second largest sector represents environments, such as JX, LYI, PD, YY, XX, HB, CG, XT, XY, XP, GM, YL, DY, MZ, TA, SN, CP, and HD with genotypes S617 and D205 as the most favorable. The third largest sector revealed environments ZB, LYG, LZ, YQ, HXN, and SZ with H6105 as the best genotype. The smallest sector top angle cultivar was Z111. This sector had no environment, indicating that genotype Z111 performs poorly in all environments. According to the Huang-Huai-Hai Kechuang Union summer-maize trials in 2017, genotypes, such as S617, D205, and H6105, were widely adaptable cultivars, and the most suitable planting area covers most of the Huang-Huai-Hai summer broadcasting area.

Analysis of representativeness and discrimination of each environment based on GGE biplot. As shown in Fig.2, the angle between the line segment and the length of the line segment itself is an important parameter in creating a line segment from the center to each environment. The cosine of the included angle between the two environmental line segments was their correlation coefficient, and the included angle was less than 90°, thereby indicating a positive correlation. Thus, two environments had a similar sorting of cultivars, and a value greater than 90° suggested a negative correlation, showing that the two environments had opposite sorting of cultivars. They also had an angle of 90°, implying that the two environments were unrelated. The small angle between the two environmental line segments indicated that the E of the two test sites on the cultivars were consistent [29]. The length of the environmental segment corresponds to the ability of the test point to distinguish cultivars. Long segment means strong discrimination ability. The angle between the test point vector and the average environment vector measures its representativeness to the target environment. Small angle means strong representativeness of the plots, and great angle indicates weak representativeness of the plots [30].

In the 2016 MET, these 40 testing sites had similarities in the yield evaluation of the tested cultivars because 40 testing sites with an included angle of less than 90° between the environmental line segments were positively correlated. A close positive correlation was observed between sites, such as JN and YC, WQ and GY, ZX and GC, LC and PD, SHQ and YL, JS and DZ, GYI and LYI, and SX and HXN. A positive correlation was observed between these plots, indicating that these testing sites had similar evaluation information in terms of the biomass yield of the cultivars. SQ and XZ had better discrimination capabilities than the other testing sites, and the environmental line segments of YC and JN sites were shorter than those of the other testing sites. Thus, the two plot sites had a poor ability to discriminate the cultivars (Fig. 2A).

In the 2017 MET, except ZB, LYG, LZ, XGG, and LQ, a positive correlation was observed between the remaining test points. A significantly positive correlation between XP and XY, HD and SN, TA and CP, DY and MZ, DY and MZ, HY and LX, and XH and XT were observed. The discrimination of PD, LYI, HXN, and SZ sites were stronger than that of the other sites, and XGG, LQ, GM, WF, and XP sites had poorer discrimination than the others (Fig. 2B).
Performance of high yield and stability of tested maize cultivars. The GGE biplot was used to analyze the high yield and stability of the cultivars of Kechuang Union summer-maize regional test in 2016–2017. The results are shown in Fig.3. The average environmental axis (AEA) represents the total average yield of the test cultivars, and the arrow direction is positive. The vertical line of each cultivar to AEA represents the average yield and stable yield of all cultivars at all test sites. Short length of the line segment means good stability, and closeness to the right side of the AEA indicates high yield. The solid line perpendicular to AEA is the average of the yield, and the genotype yield on the left side is lower than the mean performance on the whole. In addition, the genotype yield on the right side is higher than the mean performance [31].

We can see from Fig. 3A that the genotypes ranking in terms of yield performance was H321>J118>D56>J196>H9112>S510>Z958>J518>Z626>Z696>L1502>T6678>L36. In Fig. 3B, S617 was the highest yield of all tested cultivars, whereas genotypes D205, D56, J902, J757, H321, J118, and H6105 were arranged in order. However, genotypes Z111, L1611, T6461, H5177, and PT1212 were on the left side of AEA, indicating below average yield. Genotypes, such as D56 and L1502, were the most stable cultivars because the line distance between these cultivars and AEA was short. Genotypes S510, Z626, and Z696 had a large contribution to GEI, and the distance between them and AEA were long, indicating the poorly stable cultivars. Given that J118 and D56 had a small contribution to GEI and had the smallest distance from
the AEA, they were the most stable cultivars across the 40 test sites. By contrast, PT1212 was the most unstable genotype in 2017. Comprehensive analysis showed that genotypes D56 and J118 were relatively good cultivars with high yield and stability, and H321 was a cultivar with high yield and general stability. Conversely, genotypes T6678, L36, PT1212, and H5177 were among the cultivars with poor yield and stability.

**Evaluation of ideal genotype (environment) based on GGE biplot.** The GGE biplot makes it easy to find the ideal genotype and ideal environment. The ideal genotype refers to the highest average yield and the most stable yield of all test sites. The ideal environment refers to the environment with the strongest resolution of the cultivar and can generally represent all test sites. Taking the ideal genotype (environment) as the center and making a number of concentric circles, depending on the proximity to the ideal genotype or environment, the genotype or environment can be directly sorted, and the genotype or environment close to the center of the circle is good [32].

**FIGURE 4**
Choice of ideal genotype view of the GGE biplot for grain yield data from the Kechuang Union regional trials in the Huang-huai-hai valley in 2016 (A) and 2017 (B)

**FIGURE 5**
Choice of ideal environment view of the GGE biplot from the Kechuang Union regional trials in the Huang-huai-hai valley in 2016 (A) and 2017 (B)
Genotypes D56, J118, and H321 are closest to the center of the smallest concentric circle, indicating that the overall performance of yield and stability is good. L36 is farthest from the center of the smallest concentric circle, indicating that its yield and stability are the worst among all varieties (Fig. 4A). Genotype S617 is closest to the center of the concentric circle, indicating that S617 has the best high yield and stability, and the genotypes D205, D56, J118, J757, H321, and H6105 perform well. The distance from the smallest concentric circle of PT1212 is farther than other varieties, indicating that PT1212 has the worst overall performance (Fig. 4B). Environments TA and XJ are located on the arc of the innermost concentric circle, which is close to the center of the smallest concentric circle. These two sites have good discriminative power and are ideal environments. SQ, LQ, JN, HD, and YC are located on the outermost concentric circle, i.e., far from the center of the smallest concentric circle, indicating that these areas have poor discrimination against the cultivars (Fig. 5A). JX is closest to the center of the smallest concentric circle, signifying that this area is the ideal environment for cultivar evaluation. Environments CG and YY are ideal for cultivar evaluation, and the environments LZ, LYG, ZB, and XGG sites have poor overall performance (Fig. 5B).

**DISCUSSION**

Crop yields are influenced significantly by G, E, and GxE in METs. Among them, E is the main source of variation followed by GxE and G [33-35]. The results of this research showed that the E and GxE were 4.93 times and 2.98 times higher than G, respectively, in the 2016 MET. The same conclusion was found in the 2017 MET, and E and GxE were 5.72 and 2.10 times higher than G, respectively. This result indicates that before the selection of summer-maize cultivars, the advantages of the natural environment of each area should be fully considered. At the same time, the interaction between the genotype and environment should be emphasized, and the cultivars suitable for local environmental cultivation should be selected according to the local ecological environment [36]. Several studies have confirmed that GEI significantly affects maize-grain yield and is the key factor influencing the stability and adaptability of cultivars [37-40]. Only by fully studying the use of reproducible GEI can the selection efficiency of crop breeding be improved [41].

In recent years, researchers have provided increasing attention to the analysis of cultivar stability. GEI has become a hot spot for people to study. AMMI and GGE biplot have become the most commonly used statistical methods for analyzing GEI. The AMMI model tends to ignore the high-yield but low-stability varieties when evaluating the stability of the variety. Meanwhile, the high-yield but low-stability varieties are promising for a particular region. Some cultivars with high stability have no promotion value because of low yield. Thus, the use of the AMMI model has certain limitations [42]. The GGE biplot combines genotype major effects and genotype with environment interaction effects to visually demonstrate the high yield and stability of the cultivars. The simultaneous evaluation and selection of the high yield and stability of the cultivars are the most practical and effective statistical method to evaluate the high yield and stability of the cultivars [43]. This study comprehensively displays the information results in the two-way data table by constructing the GGE biplot, visually reveals the various relationships between the environment and the genotype, and provides further explanations for the original data. The high yield and stability of each cultivar can be displayed at the same time, and the representativeness and discriminability of the test sites are shown.

The stability and adaptability of cultivars and the identification of test sites are important indicators for the promotion of crop applications. In general, the ideal cultivars are produced in a different natural environment, and their production is characterized by high yield and stability and a wide variety of cultivars. This study found that the combination of high yield and stability is D56 through the two-year METs by GGE biplot analysis, followed by H321 and J118. At the same time, each test site is well-received and adapts only to the special adaptation cultivars of the region. For example, S617 has the highest average yield in the 2017 METs, but its stability is general. S617 performance in JX, LYI, PD, YY, XX, HB, CG, XT, XY, XP, GM, YL, DY, MZ, TA, SN, CP, and HD is outstanding. The same reason exists in S510 and L36 in 2016 METs and D205 and H6105 in 2017 METs. The most important goal of crop breeding is high yield and stable yield, but these two goals are difficult to combine perfectly. Only stable cultivars under the premise of high yield can be widely promoted. However, for low-yield cultivars, even if the stability is good, it cannot be promoted. For high-yield but poorly produced cultivars, suitable areas can be selected for targeted planting, and only those with high production performance and adaptability can bring high benefits to production.

Discrimination and representativeness are two important indicators for evaluating test sites. Test sites without distinguishing ability are useless. Discriminative but non representative test sites can be used to eliminate unstable cultivars but cannot be used to select good cultivars. Test points with distinguishing ability and representativeness can be utilized to effectively select high-yield products [44, 45]. The different test points in this study have a large difference in the discriminating power of
CONCLUSIONS

Maize-grain yield performance was affected most by E, whereas G had the least impact. Genotypes D56, J118, and H321 performed well in the 2016 METs and were ideal cultivars. Genotypes S617, D205, and D56 were ideal cultivars in the 2017 METs. In the 2016 METs, environments TA and XJ belong to the site with good resolution and representativeness, which were ideal environment. In the 2017 METs, JX was an ideal environment.

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REFERENCES


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PHYTOTOXIC EFFECTS OF TiO$_2$-NPs IN TWO MACROPHYTES FOR CONSTRUCTED WETLANDS

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ABSTRACT

This study aimed to investigate nanometer titania (TiO$_2$-NPs) toxicity in two species of plants commonly used in constructed wetlands: Ceratophyllum demersum and Phragmites australis. The seed germination, plantlet development and physiology were evaluated by exposure experiments. Moreover, Ti distribution and accumulation were determined through TEM-EDX and ICP-MS measurement. Results showed no significant differences in germination rates among plant seeds treated with low-level ($\leq 500$ mg L$^{-1}$) TiO$_2$-NPs. By contrast, exposure to high-level ($\geq 1000$ mg L$^{-1}$) TiO$_2$-NPs showed different results, the germination rates and seedling mass for Ceratophyllum demersum or Phragmites australis seeds decreased obviously to 30% and 0.77 g or 31.67% and 0.36 g, respectively, when the seeds were exposed to 2000 mg L$^{-1}$ TiO$_2$-NPs for 6 d. The growth of Ceratophyllum demersum and Phragmites australis was inhibited during the TiO$_2$-NPs exposure to the plants. TiO$_2$-NPs displayed phytotoxicity with reduced chlorophyll contents increased Superoxide Dismutase (SOD) activity, and Malondialdehyde (MDA) contents in both plant species. TiO$_2$-NPs could enter into the Ceratophyllum demersum and Phragmites australis, and higher concentration could result in more entrance. However, it was difficult for TiO$_2$-NPs to transfer from Phragmites australis roots to stems and leaves. Ti accumulation reached 866 and 829 $\mu$g g$^{-1}$ d.wt. in Ceratophyllum demersum and Phragmites australis roots, respectively. By contrast, only 28 $\mu$g g$^{-1}$ d.wt of Ti was translocated to the Phragmites australis stems, and no Ti was detected in Phragmites australis leaves during 12 d of treatment.

KEYWORDS:

TiO$_2$ nanoparticles, phytotoxicity, Ceratophyllum demersum, Phragmites australis, accumulation

INTRODUCTION

Nanotechnology is one of the rapidly developing and important research fields in the twenty-first century. The commercial use of nanomaterials for various novel applications has been increasing drastically [1]. Conservative market estimates for the production of manufactured metal oxide nanomaterials (MONMs) in 2020 were 1.663,168 tons, which was a significant increase from 270,041 tons in 2012 [2]. Among these MONMs, TiO$_2$-NPs are currently attracting great interest because of their unique properties, such as the high-efficiency photocatalysis and electronic conductivity, biological safety, and strong tolerance to photochemical corrosion [3]. Such properties have led to the use of TiO$_2$-NPs for a wide variety of applications, including the medicine, food industry, personal care products, catalysis, water purification, inactivation of pathogens in water and numerous building materials, such as roof tiles, paint and plaster [4,5], and for energy conversion and storage [6]. Commercial production of TiO$_2$-NPs between 2006 and 2010 has been estimated at 5,000 metric tons per year, more than 10,000 metric tons per year between 2011 and 2014 [7], and is expected to reach approximately 2.5 million metric tons by 2025 [8]. Such widespread use of nanosized TiO$_2$ could cause a significant release of TiO$_2$-NPs into the environment, thereby leading to a potential for increased environmental exposure to TiO$_2$-NPs [9,10]. NPs may pose novel health and environmental risks that cannot be predicted by current knowledge of the behavior of macroscopic particles [11]. Therefore, the safety of NPs must be established when considering the further development and applications for nanotechnology.

Over the past years, knowledge on aggregation and biological effects of TiO$_2$-NPs has developed significantly beyond that summarized by Sharma [5]. Generally, the biotoxicity of TiO$_2$-NPs against organisms is species-dependent. For example, under simulated solar irradiation, TiO$_2$-NPs provoked cytotoxicity against Bacillus subtilis and Aeromonas hydrophila, whereas growth of Arthrlobacter sp. and Klebsiella sp. was stimulated [12]. Yeung et al. [13] reported that the TiO$_2$-NPs caused toxicity to organisms by producing reactive oxygen species (ROS) upon the interaction with ultraviolet light and leading to cell membrane damage. Yu et al. [3] found that the TiO$_2$-NPs had low toxicity
to Pichia pastoris, thereby contributing to cell membrane damage, vacuolar membrane permeabilization, and cell wall damage-related ROS accumulation. The toxicological effects of nano-TiO$_2$ on algae have been summarized in several recent research papers [14-16]. However, some scholars reported that TiO$_2$-NPs had a positive effect on organisms. Lei et al. [17] reported that nanoanatase TiO$_2$ promoted photosynthesis, improved spinach growth, and promoted the vigor of aged seeds and chlorophyll biosynthesis in spinach. Yu et al. [3] found that synthesized TiO$_2$-NPs stimulated the production of unsaturated fatty acids (UFAs) to fight against oxidative stress, and is associated with enhanced lipid droplet formation and up-regulation of UFAs synthesis-related genes.

Plants provide a potential pathway for the transport of nanoparticles (NPs) to the environment and serve as an important route for their bioaccumulation into the food chain. TiO$_2$-NPs are widely implemented in various fields; therefore, their safety and toxicity have been the subject of detailed investigation [18,19]. However, information with regard to the interaction of TiO$_2$-NPs with plants is still lacking [20]. Although both the positive and negative effects of MONMs on plant growth have been reported in a few published studies [21-23], the interactions between plant species used for constructed wetlands and TiO$_2$-NPs are still unknown and deserve research.

*Ceratophyllum demersum* and *Phragmites australis* are the two most common plants used in constructed wetlands. This work aimed to study the toxicity of TiO$_2$-NPs on these plant species. The characteristics of TiO$_2$-NPs were analyzed using zeta potential analysis, transmission electron microscopic (TEM), and X-ray diffraction (XRD). The seeds and plantlets of the two plants were exposed to TiO$_2$-NPs at different concentrations to evaluate the nanotoxicity of TiO$_2$-NPs. Then, the germination rates, seedling masses of the seeds, SOD activity, MDA content, and chlorophyll of the plants were measured. TEM was used to investigate the TiO$_2$-NPs distribution in plants. In consideration of the relationship between magnesium (Mg) and chlorophyll, the Ti and Mg contents of plant tissues were determined by using inductively coupled plasma mass spectroscopy (ICP-MS).

**MATERIALS AND METHODS**

**Materials.** TiO$_2$-NPs (AEROXIDE TiO$_2$ P25, aerosol, ≥99.5%, anatase: rutile = 71: 29, 21 nm particle size) were purchased from Beijing Entrepreneur Science & Trading Co., Ltd. TEM analysis (JEM-2100, JEOL, Japan, coupled with an energy dispersive X-ray spectrometer, EDS) was carried out to investigate the morphology and confirm the actual size of the particles. The crystal phase was determined by XRD (BruckerD8 Diffractometer, Germany). A Zetasizer (Zetasizer 2000, Malvern, UK) was used to determine the zeta potentials of the particles after sonication (120 W, 40 kHz, 30 min) into 25% strength Hoagland nutrient solution at 25°C and pH 6.5 with a particle concentration of 1 to 500 mg L$^{-1}$.

Seeds of *Ceratophyllum demersum* and *Phragmites australis* were purchased from Nanjing Jingxiangyuan Co., Ltd. The plants of *Ceratophyllum demersum* and *Phragmites australis* were obtained from Old Summer Palace in China. Prior to analysis, all the plants were cultured at 25°C for 15 d in a 50×40×35 cm aquarium with a 25% strength Hoagland nutrient solution prepared according to the following chemical composition: 0.75 mM K$_2$SO$_4$, 1×10$^{-3}$ mM ZnSO$_4$·7H$_2$O, 0.65 mM MgSO$_4$·7H$_2$O, 1×10$^{-3}$ mM MnSO$_4$·H$_2$O, 2.0 mM Ca(NO$_3$)$_2$, 0.25 mM KH$_2$PO$_4$, 0.1 mM KCl, 1×10$^{-3}$ mM H$_2$BO$_3$, 5×10$^{-6}$ mM (NH$_4$)$_6$Mo$_7$O$_{24}$, and 0.1 mM Fe-EDTA [9]. All these analytical grade chemicals were procured from Sinopharm Chemical Regent Beijing Co., Ltd.

**Seed germination test.** Seeds of *Ceratophyllum demersum* and *Phragmites australis* were sterilized for 10 min in 10% sodium hypochlorite solution before application [24]. The sterilized seeds were then rinsed with sterile distilled water until no sodium hypochlorite odor remained.

To investigate the solution effects on seed germination, the seeds were soaked in TiO$_2$-NPs solutions at concentrations of 0, 20, 100, 200, 500, 1000 and 2000 mg L$^{-1}$ for 48 h (dark condition, room temperature) with gentle shaking in an orbital shaker at 150 rpm [25]. Then, the seeds were washed thoroughly with distilled water (DW) and transferred into 100 mm Petri dishes with one piece of filter paper (90 mm) and 5 mL of DW. The seeds were tested for germination in a growth chamber (MGC-350BP-2, Yiheng, China) under controlled conditions: temperature: 24°C, humidity: 70±25%, photoperiod: 18 h light, light intensity: 300 µE m$^{-2}$ s$^{-1}$ with protection from drying. A total number of 20 seeds were sowed per Petri dish with three replicates. The germination rates and seedling masses were measured after 6 d.

**Plant culture and exposure.** After being cultured for 15 d in the aquarium, four *Phragmites australis* plants (approximately 40 cm high) and *Ceratophyllum demersum* (approximately 10 g) were transplanted into 1000 mL beakers (3 replicates) filled with a 25% strength Hoagland nutrient solution that contained TiO$_2$-NPs at concentrations of 0, 1, 10, 100, 200 and 500 mg L$^{-1}$, respectively. All experiments were carried out in a growth chamber under controlled conditions: day/night temperature (26±2°C), day/night photoperiod (16/8h), and 100 µE m$^{-2}$ s$^{-1}$. Plants were exposed to TiO$_2$-
NPs for 10 d (Ceratophyllum demersum) and 12 d (Phragmites australis) for accumulation and translocation experiments, as well as to investigate the effect of TiO2-NPs on plant development.

**Effect of TiO2-NPs on plant development and physiology.** To assess the effect of TiO2-NPs on plant development, the SOD, MDA content, and chlorophyll were detected. For SOD activities, plant samples (0.1 g) were frozen in liquid nitrogen, and 50 mM phosphate buffer (pH 7.2) was used for extraction. Then, the SOD activities were detected with a reagent box obtained from Nanjing Jiancheng Bioengineering Institute. The MDA concentrations were determined following the method reported by Buege and Aust [26]. Chlorophyll contents were determined by using a DMSO extraction method [27]. In this study, the MDA and chlorophyll were measured every two days while the SOD activity was measured only at the end of the exposure cultivation.

**Analysis of Ti distribution and accumulation.** TEM-EDS was used to confirm the presence of TiO2-NPs in plantlets. At the end of the experiments, plants were removed and washed with DW, and the Phragmites australis were divided into roots, stems, and leaves. For TEM observations, Ceratophyllum demersum and portions of Phragmites australis (5 mm long) were fixed at room temperature in 3% (vol./vol.) glutaraldehyde, 1% (vol./vol.) paraformaldehyde prepared in 0.1 M cacodylate buffer (pH 7.4), post-fixed in 2% (vol./vol.) OsO4, and finally dehydrated in graded concentrations of ethanol to be embedded in Spurr’s resin [19]. Ultrathin sections (70 nm) were prepared, deposited on coated copper grids (Agar Scientific), and observed on a JEOL JEM-2100 TEM operated at 80 kV.

Subsequently, Ti contents in the plantlets were quantified by ICP-MS on digested plant samples. For ICP-MS observations, plant samples were washed thoroughly with DW to remove TiO2-NPs from the surface. Then, samples were oven dried at 70°C for 5 d. Dried plant samples were ground and digested by open-vessel digestion using nitric acid for ICP analysis [28]. Then, a 1.0 g sample was moved into Pyrex tubes, and 10 mL of nitric acid (analytical grade) was added and left for a cold soak for approximately 2 h. Then, the tubes are placed in a heating block. Temperature was slowly increased to 600 °C and heated for 4 h with a minimum supply of DW to prevent total vaporization during heating. Then, the tubes were slowly cooled to room temperature. Cooled digests were diluted to 20 mL with DW and filtered with a filter paper, and finally diluted to 40 mL (Song et al. 2013). Subsequently, the digested plant tissues were analyzed by ICP-MS (ICPS-1000IV, Shimadzu, Japan) for Ti and Mg content.
Statistical analysis. All experiments were conducted in triplicate. Tests to determine statistical differences between treatments were carried out by comparing the critical value through one-way analysis of variance (ANOVA). Comparisons were considered significantly different at \( p < 0.05 \). All statistical analyses were carried out using SPSS statistical software package (SPSS, Version 17.0).

RESULTS AND DISCUSSION

TiO\(_2\)-NPs characterization. TEM images and the physical properties of TiO\(_2\)-NPs used in this study are shown in Fig. 1. Fig. 1a showed that some particles of TiO\(_2\) were spherical and others were ellipsoidal, and the aggregates were composed of fine primary particles. Moreover, the nanoparticles showed uniform single crystals. As shown in Fig. 1b, the average size of TiO\(_2\)-NPs was approximately 20 nm.

The XRD patterns of TiO\(_2\)-NPs used in this study are shown in Fig. 1c. Diffraction peaks at 25.24°, 37.70°, 48.0°, 55.04°, 62.76°, 70.22°, and 75.06° were observed. The peaks among 25.24° and 48.0° were the distinct ones. The XRD pattern of TiO\(_2\)-NPs matched that in the database of Joint Committee on Powder Diffraction Standards card file No. 21-1272, thereby confirming that the anatase phase was predominant among the TiO\(_2\)-NPs.

Fig. 1d showed the zeta potential of the different TiO\(_2\)-NPs concentrations in the 25% strength Hoagland nutrient solution. The zeta potentials of TiO\(_2\)-NPs in all concentrations were negative, ranging from \( \pm 17.27 \pm 0.21 \) mV at 1.0 mg L\(^{-1}\) to \( \pm 27.03 \pm 0.38 \) mV at 500 mg L\(^{-1}\).

Seed germination. The results of germination rates and seedling masses are presented in Fig. 2. No significant differences in germination rates (Fig. 2a, c) were found among plant seeds that were exposed to low-level concentrations (\( \leq 500 \) mg L\(^{-1}\)) of TiO\(_2\)-NPs. Moreover, the low TiO\(_2\)-NPs concentration could boost the germination rate of Phragmites australis seeds (Fig. 2c). However, exposure to high concentrations (\( \geq 1000 \) mg L\(^{-1}\)) of TiO\(_2\)-NPs reduced the germination rates of both Ceratophyllum demersum and Phragmites australis seeds. With exposure to 2000 mg L\(^{-1}\) of TiO\(_2\)-NPs, the germination rates of Ceratophyllum demersum and Phragmites australis decreased to 30% and 31.67%, respectively. The seedling masses of Ceratophyllum demersum and Phragmites australis exhibited the same tendency as the germination rates (Fig. 2b, d). These results indicated that TiO\(_2\)-NPs showed phytotoxicity against seeds germination at high concentrations. In the experiment of Song et al. [29], the germination rates of tomato seeds exposed to any concentration from 0 to 5,000 mg L\(^{-1}\) TiO\(_2\)-NPs or Ag-NPs were not affected. This result was different from our study and could be ascribed to the sensibility discrimination to NPs among the plant seeds.

FIGURE 2
Germination rates and seedling masses of Ceratophyllum demersum (a, b) and Phragmites australis (c, d) seeds after TiO\(_2\)-NPs treatment. Different letters above the error bars indicate statistically significant differences between the treatments (\( P < 0.05 \))
Plant growth. Morphology. The different morphologies of *Ceratophyllum demersum* cultured under different TiO$_2$-NPs concentrations are presented in Fig. 3. The growth of *Ceratophyllum demersum* was obviously inhibited at high TiO$_2$-NPs concentrations. The *Ceratophyllum demersum* grew well with a green color and flourishing shoots at 0 mg L$^{-1}$ TiO$_2$-NPs, whereas the plantlets became withered and yellow, and turned into sparse branches at 500 mg L$^{-1}$ TiO$_2$-NPs.

Fig. 4 shows the different morphologies of *Phragmites australis* cultured at 0 and 500 mg L$^{-1}$ TiO$_2$-NPs. As shown in Fig. 4, the *Phragmites australis* plantlets at 0 mg L$^{-1}$ TiO$_2$-NPs had green and lush leaves, and many new roots had grown. By contrast, the plantlets were withered and exhibited growth retardation with atrophic leaves and roots at 500 mg L$^{-1}$ TiO$_2$-NPs.

* indicates a significant difference between the treatment and the control groups (P < 0.05). Different letters above the error bars indicate statistically significant differences between the treatments (P < 0.05).
FIGURE 6
Chlorophyll and Mg content of Phragmites australis cultured under different TiO2-NPs concentrations: (b) leaves; (c) stems; (d) roots.

* indicates a significant difference between the treatment and the control groups (P < 0.05). Different letters above the error bars indicate statistically significant differences between the treatments (P < 0.05).

Chlorophyll and Mg content. The effect of TiO2-NPs on the total chlorophyll and Mg content of Ceratophyllum demersum and Phragmites australis is shown in Fig. 5 and Fig. 6, respectively.

As shown in Fig. 5a, Ceratophyllum demersum treated without TiO2-NPs showed no differences in chlorophyll content during 10 d of cultivation. However, at the end of the cultivation, the chlorophyll content in Ceratophyllum demersum treated with TiO2-NPs decreased as NPs concentration increased gradually from 1 to 500 mg L⁻¹. These results coincided with the morphological changes of Ceratophyllum demersum and indicated that exposure to TiO2-NPs stressed the plants.

Fig. 5b showed the Mg content of the Ceratophyllum demersum after 10 d of exposed cultivation under different TiO2-NPs concentrations. A decrease in Mg content was observed with the increase of TiO2-NPs concentration. This finding indicated that the aforementioned Mg decrease was concentration dependent and corroborated the chlorophyll observation in Ceratophyllum demersum.

Fig. 6a showed the chlorophyll changes in Phragmites australis after 12 d of exposed cultivation under different TiO2-NPs concentrations. A significant decrease in chlorophyll content was observed when the TiO2-NPs concentration was above 100 mg L⁻¹. From 0 d to 12 d cultivation at 500 mg L⁻¹ TiO2-NPs, the chlorophyll content decreased from 2.67 mg g⁻¹ to 1.83 mg g⁻¹. These phenomena implied that TiO2-NPs exposure could stress the Phragmites australis.

The Mg contents in different tissues of Phragmites australis after 12 d of exposed cultivation under different TiO2-NPs concentrations are shown in Fig. 6b-d. As the TiO2-NPs concentration increased from 0 to 10 mg L⁻¹, the Mg contents in leaves, stems, and roots exhibited some decrease. As the TiO2-NPs concentration increased further, the Mg contents significantly decreased accordingly.

SOD activity and MDA content. SOD activities and MDA content of Ceratophyllum demersum and Phragmites australis roots are presented in Fig. 7. SOD is a reliable indicator of stress and is known to increase in plants exposed to heavy metals. As shown in Fig. 7a, the SOD activities of Ceratophyllum demersum increased rapidly when the TiO2-NPs concentrations were below 100 mg L⁻¹. A significant decrease in SOD activity was observed as the TiO2-NPs concentration rose above 100 mg L⁻¹. These high TiO2-NPs concentrations probably caused the functional degradation of Ceratophyllum demersum and prevented the production of SOD.
As indicated in Fig. 7b, the MDA content of the *Ceratophyllum demersum* treated with TiO$_2$-NPs increased with the increase of NPs concentration and became more obvious at higher TiO$_2$-NPs concentration. In addition, at 500 mg L$^{-1}$ TiO$_2$-NPs, the MDA content of the treated *Ceratophyllum demersum* was three times more than that treated without TiO$_2$-NPs. As reported by Nielsen et al. [30] and Niki [31], MDA was contained among secondary products of lipid peroxidation and could be taken as a potential biomarker for oxidative stress and imbalance. For *Ceratophyllum demersum* treated with TiO$_2$-NPs, the oxidative stress and imbalance became increasingly serious as the NPs concentration increased, and its development was restrained accordingly.

In comparison with *Ceratophyllum demersum*, with the rise in the TiO$_2$-NPs concentration, the changes in SOD activity and MDA content in *Phragmites australis* roots were coincident in Fig. 7c–d. At higher TiO$_2$-NPs concentrations, the increment rates of these two indicators improved significantly. Similar to *Ceratophyllum demersum*, the growth of *Phragmites australis* was also restrained by TiO$_2$-NPs.

**FIGURE 7**

*SOD activity and MDA content of Ceratophyllum demersum (a, b) and Phragmites australis roots (c, d).*

* indicates a significant difference between the treatment and the control groups (P < 0.05). Different letters above the error bars indicate statistically significant differences between the treatments (P < 0.05)

**Ti accumulation and distribution in Ceratophyllum demersum and Phragmites australis.**

The TEM-EDS observation of ultrathin sections of *Ceratophyllum demersum* and *Phragmites australis* treated with 500 mg L$^{-1}$ TiO$_2$-NPs is presented in Fig. 8. In the TEM images of Fig. 8a–d 100 to 1,000 nm clusters were observed, which contained chemical elements with a higher atomic number than the surrounding matrix. Coupled with the EDS in Fig. 8e, Ti showed the highest content among these chemical elements, thereby indicating that the cluster contained the TiO$_2$-NPs.

Moreover, in *Ceratophyllum demersum* exposed to TiO$_2$-NPs diluted in Hoagland medium, the clusters appeared freely in vacuoles of the cell (Fig. 8a) and adsorbed on the walls of cortical parenchymal cells (Fig. 8b). For *Phragmites australis* exposed to TiO$_2$-NPs, the clusters occurred in roots (Fig. 8c) and stems (Fig. 8d), and did not appear in leaves (Fig. 8f). These TEM images showed that the clusters were scattered in the cell walls (Fig. 8d), the Casparian band (Fig. 8c), and in the cell (Fig. 8c). The clusters were either entrapped in membranous structures which looked like endosomes, or appeared freely in vacuoles or in cell nuclei (Fig. 8c).
FIGURE 8
TEM analysis of TiO$_2$-NPs distribution in plants exposed to 500 mg L$^{-1}$ of TiO$_2$-NPs: *Ceratophyllum demersum* (a, b); roots of *Phragmites australis* (c); stems of *Phragmites australis* (d); leaves of *Phragmites australis*; EDS analysis of the bright zone in (b), showing the presence of Ti (e).

Ti contents measured by ICP-MS in the plantlets exposed to TiO$_2$-NPs are shown in Fig. 9. Fig. 9a showed that the Ti uptake by *Ceratophyllum demersum* exposed to TiO$_2$-NPs diluted in Hoagland medium increased significantly as NPs concentrations increased from 1.47 μg g$^{-1}$ at 0 mg L$^{-1}$ TiO$_2$-NPs to 866 μg g$^{-1}$ at 500 mg L$^{-1}$ TiO$_2$-NPs. Combined with the TEM observations, the taken up TiO$_2$-NPs could accumulate in *Ceratophyllum demersum* plantlets, and some TiO$_2$-NPs could penetrate into their cells. These findings were consistent with previous research reported by Lin et al. [32], in which Fullerene C70 nanoparticles were demonstrated to transmit to the progeny through the rice seeds of first generation. These nanoparticles might enter the rice roots via both osmotic pressure and capillary forces, then enter through the cell wall pores and translocate through intercellular plasmodesmata.

As presented in Fig. 9b and Fig. 9c, the Ti content in both of the roots and stems of *Phragmites australis* increased significantly with the increasing TiO$_2$-NPs concentration. At the same TiO$_2$-NPs concentration, the root could accumulate approximately 30 times more Ti than the stem. Moreover, the Ti content in *Phragmites australis* leaves was below the detection limit when treated with TiO$_2$-NPs, which was in agreement with TEM observation. These results indicated that the uptake of TiO$_2$-NPs by the *Phragmites australis* roots...
were difficult to transport and accumulate in the stems and leaves during 12 d of treatment. In a study by Larue et al. [19], the TiO$_2$-NPs with a primary diameter lower than 140 nm could accumulate in the wheat roots, which could be translocated to the leaves if their primary diameter was smaller than 36 nm, but accumulation was below the detection limit in wheat leaves. Evidently, TiO$_2$-NPs accumulation in Phragmites australis roots coincided with the observation of Larue et al. [19] in wheat roots.

**FIGURE 9**

ICP-MS observations of Ti accumulation in Ceratophyllum demersum (a) and Phragmites australis: (b) roots; (c) stems. Different letters above the error bars indicate statistically significant differences between the treatments (P < 0.05).

**CONCLUSIONS**

For wetland plants Ceratophyllum demersum and Phragmites australis, the TiO$_2$-NPs showed acute toxicity on seed germination at the high concentration (>500 mg L$^{-1}$) with a significant decrease in germination rates and seedling masses. Moreover, TiO$_2$-NPs exposure could inhibit the growth of Ceratophyllum demersum and Phragmites australis. The corresponding phytotoxicity of TiO$_2$-NPs in the plants was demonstrated by the reduction in chlorophyll contents and the increase in the SOD activity and MDA contents. TEM and ICP-MS analysis results indicated that TiO$_2$-NPs could be uptaken by Ceratophyllum demersum and Phragmites australis roots, and a small amount of TiO$_2$-NPs was translocated to the Phragmites australis stems. However, further translocation to Phragmites australis leaves did not occur with 12 d of treatment with TiO$_2$-NPs.

**ACKNOWLEDGEMENTS**

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**REFERENCES**


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SEROPOSITIVITY FOR BARTONELLA HENSELAE IN CATS FROM THE KARS PROVINCE AND ITS VICINITY

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ABSTRACT

This study was aimed at determining Bartonella henselae seropositivity in cats from the Kars province and its vicinity. The study material comprised 100 male and female cats that were aged 1-8 years, of various breeds, and from the city centre of Kars, surrounding villages and the Akyaka, Arpaçay, Selim and Susuz districts. The cats were sampled for blood only once, and the extracted sera were applied the indirect fluorescent antibody test (IFAT) to investigate the presence of anti-B. henselae IgG antibodies. IFAT results demonstrating the presence of these antibodies at titers of 1/64 and above were considered to be positive. Accordingly, the overall seropositivity for B. henselae in cats from the Kars province and its vicinity was ascertained as 29.0%. Seropositivity rates differed between the two sexes and were determined to be 28.9% in the male cats and 29.1% in the female cats. The seropositivity rates determined in the different age groups were as follows: 33.3% in the cats aged 1-2 years, 30.4% in the cats aged 3-4 years, and 20.8% in the cats aged 5-6 years. The differences observed between the different age groups and sexes were statistically insignificant (P>0.05). The mean seropositivity rates determined for the different locations were as follows: 27.0% for the Kars city centre, and 27.8%, 16.7%, 7.1%, 14.3% and 61.1% for the Akyaka, Arpaçay, Sarikamış, Selim and Susuz districts, respectively. The differences observed between the study locations for seropositivity were statistically significant (P<0.05).

As a result, in this study, which has serologically confirmed the presence of B. henselae in cats from the Kars province and its vicinity for the first time, the overall seropositivity rate for the bacterium in the cat population of this region was determined as 29%.

KEYWORDS: Bartonella henselae, cat, IFAT, Kars

INTRODUCTION

Bartonella species are intracellular Gram-negative facultative cocccobacillary bacteria characterized by intraerythrocytic localization. These bacteria are opportunistic zoonotic pathogens, which manifest different clinical pictures particularly in immunocompromised individuals, children and elderly people [1, 2]. More than 22 species have been identified under the genus Bartonella [3]. Although B. henselae, B. bacilliformis and B. quintana are known to be the primary species that infect humans, in recent years, B. clarridgeiae, B. elizabethae, B. vinsonii subsp. berkhoffii, B. grahamii and B. ancashi have also been reported to be pathogenic for mankind [4, 5, 6].

Research has shown that the most common Bartonella species observed in cats throughout the world is B. henselae [2, 7, 8, 9]. B. henselae invades feline erythrocytes and establishes chronic asymptomatic relapsing bacteraemia in cats. In humans, this species may cause cat scratch disease (CSD), bacillary angiomatosis (BA), bacillary peliosis, fever, and endocarditis, as well as neurological syndromes particularly in human immunodeficiency virus (HIV)-positive cases [1, 6, 9, 10, 11, 12, 13].

Cats acts as natural reservoirs in the transmission of Bartonella henselae to humans [14]. The major vector of B. henselae, involved in the cat-to-cat transmission of the bacterium, is the cat flea (Ctenocephalides felis). Humans contract the disease from infected cats mostly by being scratched or bitten, but transmission to humans may also occur by means of cat fleas [1, 3, 15].

While seroepidemiological research has shown that the occurrence of B. henselae is common in cats throughout the world [7, 16, 17, 18, 19, 20, 21], there are only very few reports on the presence of B. henselae in cats from Turkey [22, 23, 24, 25]. In view of the scarcity of information available on the seroprevalence of this bacterium in Turkey, this study was aimed at determining the seropositivity of cats from the Kars province and its vicinity for the zoonotic pathogen B. henselae.
MATERIALS AND METHODS

The study material comprised 100 male and female cats that were aged 1-8 years, of various breeds, and from the city centre of Kars, surrounding villages and the Akyaka, Arpaçay, Selim and Susuz districts. Blood samples (3 mL) collected by aseptic procedure from the jugular vein of each cat included in the study. For the extraction of serum, the blood samples were centrifuged at 3000 rpm for 10 minutes in a cooled centrifuge. The extracted sera were stored at -20°C until being used.

Ethics Committee Approval was received for this study from the Animal Care Committee of Kafkas University, Faculty of Veterinary Medicine, Approval no: 2015/046.

For serological analysis, the indirect fluorescent antibody test (IFAT) was used. The commercial Houston-1 (ATCC 49882) strain of B. henselae, coated on slides (VIRCELL-Granada, Spain), was used as the antigen for IFAT. Anti-feline IgG antibodies (H+L) (Kirkegaard & Perry Laboratories, Gaithersburg, MD-USA), produced in goats and marked with fluorescein isothiocyanate (FITC), were used as the conjugate in IFAT. The test was performed in accordance with the manufacturer’s instructions. Homogenous bacterial distribution, displaying green-yellow fluorescence on a black background, when observed under a fluorescent microscope at 40X magnification in a dark room, was scored subjectively. Samples displaying ++ fluorescence at dilutions of 1/64 and above were considered to be positive. Samples displaying weak or no fluorescence were considered to be negative.

The data obtained in this study was statistically analysed with Pearson’s chi-square test and Fisher’s exact test using the SPSS 20 software package.

RESULTS

Of the 100 serum samples, 29 (29.0%) were determined to contain anti-B. henselae IgG antibodies at titers of 1/64 and above. The highest positive titer was detected at a dilution of 1/1024 in three (3%) of the serum samples. The majority of the positive titers were detected at the 1/256 dilution in 14 (14%) of the serum samples. The distribution of the positive IgG titers is presented in Table 1.

Among the different study locations, IgG seropositivity for B. henselae was highest in the Susuz district (37.9%) and lowest in the Sarıkamış, Selim and Arpaçay districts (3.4%) (Table 2). The higher seropositivity detected in the Susuz district was determined to be of statistical significance (P<0.05).

### Table 1

**B. henselae seropositivity according to IgG antibody titers (n=29)**

<table>
<thead>
<tr>
<th>Dilution Rates</th>
<th>Number and Percentage of Positive Samples</th>
<th>Number and Percentage of Negative Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>(+) (%)</td>
<td>(-) (%)</td>
<td></td>
</tr>
<tr>
<td>1/64 dilution</td>
<td>2 (2.0)</td>
<td>98 (98.0)</td>
</tr>
<tr>
<td>1/128 dilution</td>
<td>4 (4.0)</td>
<td>96 (96.0)</td>
</tr>
<tr>
<td>1/256 dilution</td>
<td>14 (14.0)</td>
<td>86 (86.0)</td>
</tr>
<tr>
<td>1/512 dilution</td>
<td>6 (6.0)</td>
<td>94 (94.0)</td>
</tr>
<tr>
<td>1/1024 dilution</td>
<td>3 (3.0)</td>
<td>97 (97.0)</td>
</tr>
</tbody>
</table>

### Table 2

**Anti-B. henselae IgG seropositivity in the different study locations**

<table>
<thead>
<tr>
<th>Study Locations</th>
<th>Number of Animals Sampled</th>
<th>In-group Rate (%)</th>
<th>Percentile Share (%) in Total Number of Positives (n=29)</th>
</tr>
</thead>
<tbody>
<tr>
<td>City centre and surrounding villages</td>
<td>37</td>
<td>10</td>
<td>27.0</td>
</tr>
<tr>
<td>Sarıkamış district</td>
<td>14</td>
<td>1</td>
<td>7.1</td>
</tr>
<tr>
<td>Selim district</td>
<td>7</td>
<td>1</td>
<td>14.3</td>
</tr>
<tr>
<td>Susuz district</td>
<td>18</td>
<td>11</td>
<td>61.1</td>
</tr>
<tr>
<td>Akyaka district</td>
<td>18</td>
<td>5</td>
<td>27.8</td>
</tr>
<tr>
<td>Arpaçay district</td>
<td>18</td>
<td>1</td>
<td>16.7</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>29</td>
<td>29.0</td>
</tr>
</tbody>
</table>

P=0.02, b. Seropositivity was highest in the Susuz district and significantly differed from the seropositivity rates detected in the other study locations.

### Table 3

**B. henselae seropositivity in the different sexes (≥1/64 dilution)**

<table>
<thead>
<tr>
<th>Sex</th>
<th>n</th>
<th>In-group Rate (%)</th>
<th>Percentile Share (%) in Total Number of Positives (n=29)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>55</td>
<td>16</td>
<td>29.1</td>
</tr>
<tr>
<td>Male</td>
<td>45</td>
<td>13</td>
<td>28.9</td>
</tr>
</tbody>
</table>

P=0.982; Odds ratio=0.60 (0.58-0.62)
Of the 100 cats included in this study, 55 (55%) were female and 45 (45%) were male. No statistically significant difference was observed between the two sexes for anti-\textit{B. henselae} IgG seropositivity (P>0.05) (Table 3).

Of the 100 cats included in this study, 30 (30%) were aged 1-2 years, 46 (46%) were aged 3-4 years and 24 (24%) were aged 5 years and older. The distribution of seropositivity in the different age groups is presented in Table 4. No statistically significant difference was observed between the different age groups for anti-\textit{B. henselae} IgG seropositivity (P>0.05).

\textbf{DISCUSSION}

Seroepidemiological and bacteriological studies conducted in different parts of the world have demonstrated varying positivity levels for \textit{B. henselae} in cats. Depending on the geographical location and environmental conditions, seroprevalence ranges from 5% to 80% in cats [26]. \textit{B. henselae} seropositivity rates previously reported in cats were 27.9% in North America [16], 8.8% in Japan [7], 32% in Jordan [17], 29.6% in Spain [27], 23% in the Tehran city of Iran [28], 31.3% in Southern India [21], and 15% in Germany [18]. While anti-\textit{Bartonella spp.} antibodies were detected at a percentage of 25.7% in Pennsylvania-USA [19], polymerase chain reaction (PCR) results revealed the presence of \textit{Bartonella spp.} at a rate of 17.02% in Brazil [20].

In Turkey, anti-\textit{B. henselae} IgG seropositivity in cats was detected for the first time in cats from the vicinity of the Ankara province at a rate of 18.8% [22]. In a study conducted by Güzel et al. [23] using feline serum samples collected from different parts of Turkey, an overall seropositivity rate of 27.9% was determined. Diren Siğırçi and Ilgaz [24] reported the seroprevalence of \textit{B. henselae} in cats from the vicinity of the Istanbul province as 28.1%. In another study carried out by Diren Siğırçi et al. [25] in the Istanbul province, a positivity rate of 67% was detected in blood samples taken from 81 pet and stray cats. In the present study, the overall seropositivity rate determined for \textit{B. henselae} in feline serum samples collected in the city centre of Kars and several districts of this province was 29.0%. This seropositivity rate is in agreement with the results of several previous studies.

In their research on the investigation of \textit{B. henselae} positivity in cats with respect to sex, Bergmans et al. [29] determined that positivity rates were higher in male cats, while Sander et al. [30] ascertained that positivity rates were higher in female cats. On the other hand, Maruyama et al. [7] and Al-Majali [17] reported not to have observed any statistically significant difference between males and females for positivity rates. In similar research conducted in Turkey, Çelebi et al. [22] determined anti-\textit{B. henselae} IgG seropositivity at a rate of 20.8% in females and 15% in males. In their research on feline serum samples from various provinces of Turkey, Güzel et al. [23] detected \textit{B. henselae} seropositivity as 28.1% in males and 26.1% in females, and determined the difference between the two sexes to be statistically insignificant. Similarly, in the present study, \textit{B. henselae} seropositivity rates in cats from the Kars province and its vicinity were determined as 29.1% in females and 28.9% in males, and the difference between the two sexes for seropositivity was found to be statistically insignificant (P>0.05).

In previous serological research, Maruyama et al. [7] reported seropositivity rates of 8.2% in cats under the age of 1 year, 11.5% in cats aged 1-2 years, and 10% in cats older than 2 years of age. In their study conducted in Spain, Pons et al. [27] reported the prevalence of \textit{B. henselae} as 26.3% in cats that were aged 1 year and younger, and as 31% in cats older than 1 year of age, and indicated that the difference between the two sexes for prevalence was statistically insignificant. Çelebi et al. [22] determined anti-\textit{B. henselae} IgG seropositivity rates of 14.2% in cats aged 2-11 months, 17.8% in cats aged 12-23 months, and 22% in cats older than 24 months of age. In the present study, although the different age groups did not significantly differ for seropositivity, it was observed that the rate of \textit{B. henselae} seropositivity was higher in the young cats.

In view of the impact of climatic conditions on the prevalence of bartonellosis, it should be noted that in Turkey, the coastal regions are characterized by a damp and temperate climate during most of the year, the central region is characterized by cold winters and dry summers, and the eastern regions
are cold and cool during most of the year. Due to the varying climatic conditions of the different geographical regions, it is most probable that the prevalence of bartonellosis might also vary among these regions. Given that continental climatic conditions prevail in the Kars province with cold winters and dry summers, compared to the B. henselae seropositivity rate of 29.0% determined in this province and its vicinity, seropositivity would be expected to be at a higher rate in the coastal regions and at a lower rate in the eastern regions. Indeed several literature reports have been published, which indicate high B. henselae seropositivity rates in regions with damp and temperate climatic conditions and low seropositivity rates in cold and dry regions [16, 22, 23]. The underlying reasoning of the differences observed with varying climatic conditions is the impact of climate on the reproduction of fleas, which act as vectors in the transmission of B. henselae among cats [16]. The impact of climate on the reproduction of the vector brings about varying seroprevalence rates of B. henselae even in cats from different regions of a single country [16, 31]. In fact, Güzel et al. [23] reported varying positivity levels in various provinces of Turkey, which were determined as 41.3% in Bursa, 33.9% in Adana, 32.3% in Aydın, 27.5% in Burdur, 12.5% in İstanbul, and 17.9% in Kayseri. These researchers attributed the high Bartonella seropositivity rates they determined in cats from the Adana, Burdur, Aydın and Bursa provinces to the temperate and rainy climate prevailing in the Marmara, Aegean and Mediterranean regions, and the low seropositivity rate they determined in the Kayseri province to the continental climate of Central Anatolia. Furthermore, the lower seropositivity rate detected in İstanbul was attributed to all of the cats included in the study from this province being pet cats with no outdoor access, and Istanbul being situated more to the north and thus, having a colder climate in comparison to the other provinces.

CONCLUSIONS

In view of the present study being the first report on B. henselae seropositivity in cats from the Kars province and its vicinity, and seropositivity being detected at a high rate, and owing to the zoonotic and vector-borne nature of B. henselae, it is considered that further research is required in particular for the identification of risk factors associated with infection.

REFERENCES


CARBON DYNAMICS IN SALT-AFFECTED CALCAREOUS SOILS IN SOUTH EAST TURKEY

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ABSTRACT

Secondary salinity is a significant problem in irrigated agricultural lands. Investigation of the amount of the CO2-C (g kg-1 day-1) quantity released to atmosphere through the application of different carbon resources into soil that is under the effect of secondary salinity is highly important in terms of global warming. This study has been conducted in a laboratory environment to measure the amount of CO2-C (g kg-1 day-1) released to atmosphere for a period of 15 weeks by soil under the effect of different electrical conductivity (EC) (EC:0-2-4-6 dS m-1) and applied with corn biochar (CBC) and corn stalk wastes (CSW). Both on a daily basis and cumulatively, when compared with the control group (C0) at the end of the study, the amount of CO2-C released by soil applied with corn biochar and salt concentration (CBC+EC) has been observed to be lesser than that released by soils applied with corn stalk wastes and salt concentration (CSW+EC). When the amount of CO2-C released to atmosphere is compared with C0 with the increase in EC values, a reduction has been observed in the emission level of soils applied with both CBC and CSW. This reduction has been observed at a higher level in soils applied with CBC+EC. As a result of the application of CBC+EC, dosages, weeks and CBC+ECx week interaction are observed to have a significant effect on CO2-C emission, on the basis of repeated measures analysis of variance result (p<0.05). Following the CSW+EC application, weeks are observed to have a significant effect on CO2-C emission (p<0.05), while CSW+ECx week interaction is observed to have no significant effect (p>0.05). According to CBC+EC salt application dosages and in terms of the effect on CO2-C emission, and based on Tukey HSD multiple-comparison test, CBC+EC0 dose (2.05±0.19a) has been observed to be highest, while CBC+EC 6 (1.58±0.13b) dosage has been observed to be the lowest (p<0.05). CSW+EC4 (4.18±0.41a) the highest dosage, while CSW+EC0 (3.63±0.43b) the lowest result dosage yielded (p<0.05). Looking at Tukey HSD multiple-comparison tests; CO2-C release from the CBC+EC applied soil was highest on 3rd week (3.12±0.16a), while the lowest figure was on the 15th week (0.21±0.02g) (p<0.05). Then in soils applied with CSW+EC, the highest figure has been observed on the 3rd week (7.07±0.11A), while the lowest has been observed on the 15th week (0.46±0.07D) (p<0.05).

KEYWORDS:
Salt-affected soil, electricity conductivity, corn biochar, corn stalks waste, salt doses

INTRODUCTION

Soils with high soil organic carbon content have a higher level of water holding capacity, biologic activity, cation exchange capacity and nutrient content. Being one of the important health (quality) parameters in soil, microbial biomass makes-up 0.6-3.0% of soil organic carbon. Even though microbial biomass is in low amounts in soil, it is assuming the role of an indicator for the separation and splitting soil organic carbon (SOC).

About half of the irrigated lands in the world are under the effect of groundwater, saltiness and alkalinity [1]. In Turkey the existing arid lands make-up 2% of the total land area of the country, 5.48% of the total cultivated agricultural areas, and 17% of the 8.5-million-hectare land that can be economically irrigated. Of all the arid land in Turkey, 74.2% consists of salty, 25.5% consists of saline-alkaline and 0.5% consists of alkaline soil [2]. In line with the increase in saltiness in Turkey, it is believed that within the next 25 years 30% of the sustainable agricultural land will be harmed and by the mid-21st century 50% of it will be harmed [3]. The SOC content in harmed and degraded land is directly affected by the degradation in plant vegetation. Reduction in biomass input brings along a decrease in SOC accumulation [4, 5, 6].

The effect of the sheep manure (SM) and sheep manure biochar (SMBC), applied to soil, have been analysed to see the level of carbon storing in soil and SMBC has been reported to cause less C emission. SM has been reported to lead to more CO2 emission as it goes through a normal
process and becomes mineralized [7]. Based on this, it becomes clear that it is important for both soil and atmospheric CO₂ for organic wastes to be applied to soil after being turned into biochar (BC). Biochar is a highly stable material and due to its C storage capacity, it has the potential to balance CO₂ emission. It can thus be proposed as a strategy to mitigate global warming.

During studies where sheep manure and sheep manure biochar have been applied to calcareous soil in different salt concentrations to determine the effect on carbon emission, it has been observed that CO₂ emission from soil was lower, depending on the increasing EC values. However, this reduction is reportedly not only related to the increasing salt content in soil but also related to the BC added to soil. It has been observed that in short term BC is slowly dissolved in soil, is more resistant against separation, and helps to store carbon in soil as it stays longer in soil. BC is reported to reduce the separation and splitting of OC in soil and to have a potential effect on C storage [8-9].

In this study, the soil has been analysed in a laboratory environment for the following: (i) carbon emissions, (ii) carbon dynamics of soil affected by salt, (iii) effect on carbon emission by different salt concentrations, (iv) effect on carbon emission by corn stalk biochar and corn stalk wastes.

**MATERIALS AND METHODS**

The soil samples used in this study have been collected from a land located between 37° 10′ 14″ N latitudes and 39° 00′ 15″ E longitudes and the elevation of the land is 507 m above sea level. The climate in the area is dry and warm in summer and during winter the temperature is moderate with low precipitation. Average annual precipitation is 448.11 mm and the highest average temperature has been recorded in July by 41.12 °C, while the lowest temperature has been recorded in February by 2.41 °C. The average annual relative moisture has been recorded as 92.32% at highest and as 33.29% at lowest.

With soil texture Bouyoucos method [10] soil reaction (pH) and electrical conductivity (EC) have been respectively measured as 1:2.5 (w/v) and 1:5 (w/v) with de-ionised water [11]. Soil organic carbon (SOC) has been defined through potassium dichromate oxidation method [12]. Soil samples have been observed as clay textured (clay content 41%), neutral or close to neutral (pH:7.25), salt-free (EC:79 μS cm⁻¹), over-calcareous (25%), low organic carbon content (0.79%) and high cation exchange capacity (35.12 cmol kg⁻¹).

Soil samples collected from the field have been dried in laboratory, grinded and then filtered through a 2 mm sieve. In order to define the water content of soil samples, 800 g of soil has been placing into 9.6 L vases with an open bottom and covered by a filter, then saturated, covered with nylon and kept for 72 hours. 500 g of soil has been placed into 2 L plastic containers and then corn stalk waste (CSW) has been added on a sample with 5 g C/kg soil and corn stalk biochar (CSBC) has been added on the other sample again with 5 g C/kg soil and then they have been mixed. And a similar sample has been prepared for controlling purposes, without containing any organic materials. These soil samples have been kept in different EC values prepared by NaCl (EC= 0 dS m⁻¹, EC= 2 dS m⁻¹, EC= 4 dS m⁻¹ and EC= 6 dS m⁻¹) at 50% of soil water content. Samples have been subjected to incubation in laboratory at a temperature of 25.0±2.0 °C for 15 weeks. The CO₂-C content found in the soil samples have been measured on a weekly basis with three repetitions and by using NaOH method [13] and calculated by the use of Equation 1,

\[
E_{CO_2-C} = \frac{V_B - V_H}{P \cdot h}
\]

Where \(E_{CO_2-C}\) is the total CO₂-C content during the incubation process (g CO₂-C week⁻¹); \(V_B\) = volume of HCl used in the blank (mL); \(V_M\) = volume of HCl used in the sample (mL); \(P\) = weight of dry soil sample (g); \(h\) = incubation time.

**Statistical Analysis.** The whole data analysis has been performed by using SPSS 24.0 [14] for Windows software. Data have been statistically analysed by using Levene’s test and Shapiro Wilk test for variance homogeneity and normal dispersion assumption, respectively (P< 0.05). Afterwards, data have been analysed again by using GLM (General Linear Model) for dual repetitive measurements variance analysis and by using Tukey HSD multiple comparison test for determining the difference between groups. Descriptive statistics have been presented for data. Significance has been taken as P <0.05 for all tests.

**RESULTS AND DISCUSSIONS**

Following the measurements, the CO₂-C content in C₀ group samples has been defined as 3.87 g CO₂-C kg⁻¹ day⁻¹. When C₀ group is compared to soil; CO₂-C content released from the soil applied with CBC+EC and CSW+EC has been observed to be lower throughout the trial (3.15 g CO₂-C kg⁻¹) (Fig. 1 a, b). As biochar is splitting slowly in soil and is more resistant against separation, BC applications are reported to lessen the splitting/separation duration of OC and has a positive effect on storing C in soil [15-16].
FIGURE 1
Evaluation of CO$_2$ emissions a. CBC+EC and b. CSW+EC

FIGURE 2
Evaluation of cumulative CO$_2$ emissions a. CBC+EC and b. CSW+EC
When the resulting CO$_2$-C contents are compared on the basis of different salt (EC=0, 2, 4, 6) applied to soil samples, the CO$_2$-C content emitted in connection with the increasing EC values have decreased in both of the samples applied with CBC and CSW (Fig 1 a, b). In their study Sakin and Yanardag [7] reported a reduced amount of CO2 released from soils with different salinity levels and applied with sheep manure (SM) and sheep manure biochar (SMBC), when compared to control group. They have observed a greater reduction in soils applied with SMBC. According to Parton et al. [17], the carbon release from soil is a result of the microorganisms in soil separating and splitting SOC. Metabolic carbon dioxide (CO$_2$) is being measured as a respiration of microbial biomass in soil as a result of the stress exerted by microorganisms population in soil. CO$_2$ release from soil occurs as a result of microbial stress in unit area and unit time and the quantity keeps rising [18].

Looking at the Tukey HSD multiple comparison tests of CBC+EC practises on a weekly basis, the highest outcome has been observed on the 3rd week (3.12±0.11a), while the lowest has been observed on the 15th week (0.21±0.02g) (p<0.05). The highest yield in corn stalk has been observed on the 3rd week (7.07±0.11a), while the lowest one has been observed on the 15th week (0.46±0.07d) (p<0.05) (Table 3). The study has concluded that the CO$_2$-C release from the soil was fixed, it has rapidly increased from the 3rd week and started to reduce after the 8th week. Many studies have reported a stable CO$_2$-C emission during the initial couple of weeks, followed by an increase following the 3rd and 4th weeks while becoming stable again during the last weeks [18-19].

In our study, CO$_2$-C emission was high during the initial weeks, depending on the increasing salt content in soil, but it started to decrease during the following incubation periods. In this regard, Sakin and Seyrek [8] reported that the level of saltiness has an impact on carbon (C) mineralization in soil and it could suppress it.

At the end of the study, the cumulative CO$_2$-C content in C$_0$ group samples applied with CBC+EC has been observed as 30.71 g CO$_2$-C kg$^{-1}$ soil. When compared to C$_0$ group soils; CO$_2$-C emission from soils applied with CBC+EC has been observed to be lower as the ratio of saltines increased during the trial (CBC+EC$_2$: 25.46, CBC+EC$_4$: 24.47 and CBC+EC$_6$: 23.69 g CO$_2$-C kg$^{-1}$ soil) (Fig. 2a).

The cumulative CO$_2$-C content in C$_0$ group samples applied with CSW+EC has been measured as 51.57 g CO$_2$-C kg$^{-1}$ soil. The cumulative CO$_2$-C content has been observed to be higher when compared to C$_0$ group soils (CSW+EC$_2$: 59.84, CSW+EC$_4$: 52.29 and CSW+EC$_6$: 54.72) (Fig. 2b). When both groups were compared within themselves, the lowest CO$_2$-C release has been observed in CBC+EC samples. CBC, applied to soil, has been observed to be more stable against separation and splitting when compared to CSW. This is an indication that the organic optimizers applied to soil have different effects in soil to CO$_2$ emission [20]. The CO$_2$-C emission amount in soil not only dependent to the amount of existing carbon content in soil but also several other factors such as the amount and type of the optimisers applied to soil [7-21]. When organic optimizers are applied without being subjected to any treatment, separation and splitting shall be taking place under normal conditions.

Organic material turned into biochar is more resistant against the splitting and separation activities of microorganisms. Ordinary organic optimizers on the other hand, can split and separate naturally in the long term without being subjected to any thermal treatment [22]. Furthermore, it is leading to more CO$_2$ release as it contains C that can separate more easily [7].

Having an extreme amount of salt accumulated in soil has a negative impact on the physical, chemical and biologic structures of soils [23]. This negatively affects the mineralization of carbon and prevent mineral matters to surface. It has been reported that as EC values of soil increases, soil organic matter (SOM) is rapidly split and this is related to the microorganisms in soil [24]. In connection with the increase in electrical conductivity, the structure of SOM is degraded and its dissolubility increases. Finding excess amounts of carbon in soil, microorganisms rapidly split them, and this is believed to be reason behind the high level of CO$_2$-C emission during the initial weeks.

With regards to the effect on CO$_2$-C emission, and following the application of CBC+EC and the outcome of repetitive measurement variance analysis, it has been observed that dosages, weeks and CBC+EC x week interaction have a statistically meaningful effect with an error ratio of 5% (p<0.05). With regards to the effect on CO$_2$-C emission following the application of CSW+EC and the outcome of repetitive measurement variance analysis, it has been observed that dosages and weeks have a statistically meaningful effect with an error ratio of 5% (p<0.05). But CSW+EC x week interaction has been reported to have no significant effect (p>0.05) (Table 1).

As corn biochar is more resolute in soil, it has been observed to have more effect when compared to corn stalk waste. These effects have continued throughout the study and the resolution continued. And because the splitting and separation of corn stalk wastes continues in a normal process in soil, there is no sign of such resolution. Sakin and Yanardag [7] obtained similar results in ANOYE repeated measurements in their study on SMBC and SM salt interaction.
TABLE 1
CBC+EC and CSW+EC Repeated measures ANOVA

<table>
<thead>
<tr>
<th>Source</th>
<th>SD</th>
<th>KT</th>
<th>KO</th>
<th>F-Value</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CBC+EC</td>
<td>3</td>
<td>6.02</td>
<td>2.01</td>
<td>12.86</td>
<td>0.000   ***</td>
</tr>
<tr>
<td>Weeks</td>
<td>14</td>
<td>156.23</td>
<td>11.16</td>
<td>71.5</td>
<td>0.000   ***</td>
</tr>
<tr>
<td>CBC+ECxH</td>
<td>42</td>
<td>13.34</td>
<td>0.32</td>
<td>2.04</td>
<td>0.001   ***</td>
</tr>
<tr>
<td>Error</td>
<td>120</td>
<td>18.73</td>
<td>0.16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>179</td>
<td>194.32</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CSW+EC</td>
<td>3</td>
<td>8.4</td>
<td></td>
<td>2.8006</td>
<td>3.49    0.018 *</td>
</tr>
<tr>
<td>Weeks</td>
<td>14</td>
<td>1135.55</td>
<td>81.1105</td>
<td>101.2</td>
<td>0.000   ***</td>
</tr>
<tr>
<td>CSW+ECxH</td>
<td>42</td>
<td>34.21</td>
<td>0.8145</td>
<td>1.02</td>
<td>0.458</td>
</tr>
<tr>
<td>Error</td>
<td>120</td>
<td>96.18</td>
<td>0.8015</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>179</td>
<td>1356.95</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

TABLE 2
Tukey HSD multiple comparison test table of the CO2-C content released by CBC+EC and CSW+EC dosages

<table>
<thead>
<tr>
<th>CBC+EC application</th>
<th>Ort±Sh</th>
<th>CSW+EC application</th>
<th>Ort±Sh</th>
</tr>
</thead>
<tbody>
<tr>
<td>CBC+EC0</td>
<td>2.05±0.19a</td>
<td>CSW+EC4</td>
<td>4.18±0.41a</td>
</tr>
<tr>
<td>CBC+EC2</td>
<td>1.70±0.15b</td>
<td>CSW+EC2</td>
<td>4.15±0.42ab</td>
</tr>
<tr>
<td>CBC+EC4</td>
<td>1.63±0.14b</td>
<td>CSW+EC5</td>
<td>3.67±0.39ab</td>
</tr>
<tr>
<td>CBC+EC6</td>
<td>1.58±0.13b</td>
<td>CSW+EC0</td>
<td>3.63±0.43b</td>
</tr>
</tbody>
</table>

TABLE 3
Tukey HSD multiple comparison test table of CO2-C gas released per week by CBC+EC and CSW+EC application

<table>
<thead>
<tr>
<th>Weeks</th>
<th>CBC+EC application ±Se</th>
<th>CSW+EC application ±Se</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>3.12±0.16a</td>
<td>7.07±0.11a</td>
</tr>
<tr>
<td>2</td>
<td>2.81±0.25ab</td>
<td>6.89±0.43a</td>
</tr>
<tr>
<td>6</td>
<td>2.74±0.14ab</td>
<td>6.55±0.28a</td>
</tr>
<tr>
<td>1</td>
<td>2.64±0.10ab</td>
<td>6.27±0.18a</td>
</tr>
<tr>
<td>4</td>
<td>2.61±0.24ab</td>
<td>5.95±0.54ab</td>
</tr>
<tr>
<td>5</td>
<td>2.31±0.17b</td>
<td>5.93±0.39ab</td>
</tr>
<tr>
<td>7</td>
<td>2.30±0.14b</td>
<td>5.91±0.34ab</td>
</tr>
<tr>
<td>8</td>
<td>1.62±0.13c</td>
<td>4.82±0.32b</td>
</tr>
<tr>
<td>9</td>
<td>1.48±0.07cd</td>
<td>2.73±0.21c</td>
</tr>
<tr>
<td>10</td>
<td>1.30±0.09cde</td>
<td>1.37±0.08d</td>
</tr>
<tr>
<td>11</td>
<td>1.03±0.11def</td>
<td>1.35±0.07d</td>
</tr>
<tr>
<td>12</td>
<td>0.80±0.12ef</td>
<td>1.10±0.07d</td>
</tr>
<tr>
<td>13</td>
<td>0.61±0.07fg</td>
<td>0.89±0.05d</td>
</tr>
<tr>
<td>14</td>
<td>0.51±0.08fg</td>
<td>0.71±0.06d</td>
</tr>
<tr>
<td>15</td>
<td>0.21±0.02g</td>
<td>0.46±0.07d</td>
</tr>
</tbody>
</table>

In terms of the effect on CO2-C emission, on the basis of CBC+EC salt application dosages and Tukey HSD multiple comparison test, CBC+EC0 dosage yielded the highest (2.05±0.19a) figure, while CBC+EC2 (1.70±0.15b), CBC+EC4 (1.63±0.14b) and CBC+EC6 (1.58±0.13b) dosages were in the same group and yielded the lowest results statistically with an error level of 5% (p<0.05). This study has concluded that CBC is highly resistant against the splitting and separation of microorganisms in soil and thus it can stay for longer in soil.

With regards to the effect on CO2-C emission, on the basis of CSW+EC salt application dosages and Tukey HSD multiple comparison test, CSW+EC0 (4.18±0.41a) dosage has been observed to be at the highest level while CSW+EC0 (3.63±0.43b) dosage has been observed to be at the lowest level statistically with an error level of 5% (p<0.05) (Table 2). CSW has been separated at a higher level in soil due to the level of saltiness, as an addition to the splitting and separation under ordinary conditions. Hence it led to a higher amount of CO2-C released from soil. In this study, it has been observed that with the increase in soil saltiness, the CO2-C content released from soil, decreased by CBC and increased by CSW.

Looking at the Tukey HSD multiple comparison tests of the CO2-C content released per week in the CBC+EC application, the highest CO2-C release has been observed on the 3rd week (3.12±0.16a), while the lowest has been observed on the 15th week (0.21±0.02g) (p<0.05). The highest CO2-C release of CSW+EC practise on a weekly basis base on the Tukey HSD multiple comparison tests has been observed on the 3rd week (7.07±0.11a) while the lowest has been observed on the 15th week (0.46±0.07d) (p<0.05). The weekly CO2-C content of the CBC+EC application results, based on the Tukey HSD multiple comparison test and with a statistical error level of 5% revealed the highest result in CBC+ECx2 (3.87±0.17a) interaction while CBC+ECx15 (0.20±0.00t), CBC+ECx15 (0.20±0.00t) and CBC+ECx15 (0.20±0.00t) inter-
actions were at the same level and yielded the lowest result (p<0.05) (Table 3).

Anderson and Domsch [25] added organic material to soil and reported a stable CO$_2$-C release from the soil in the following hours. They reported a peak value in the following period, which then started to become lower and in the following weeks it followed a linear value. During the initial weeks, the dry soil was brought to 50% of the field capacity, which led to the manure-soluble carbon to be easily split by microbes, which in turn led to more emission. With the reduction in the amount of variable carbon during the following weeks, the remaining, more stubborn, carbon is slowly separated by microorganisms and this led to lower emission levels. It has been reported that in case biochar is added to soil for a long period of time, soil will be storing more carbon when compared to other organic wastes, hence contributing to lessening the effects of global warming [26].

In both heat maps Ward's minimum variance clustering and Manhattan distances have been used to draw a two-way hierarchical cluster diagram, where diagram a belongs to CBC and diagram b belongs to CSW. Heat map indicates CBC, CSW dosages and the weeks that they these have been applied, as well as some clusters indicating the differences within groups. In this map, the associated dosage and value in each sample of the weeks are being described with an interval given in the top left part of the figure.

The hierarchical cluster analysis made for CBC and given in diagram a indicates that dosages are divided into three clusters while weeks are divided into four clusters (Figure 3). On the condition that it consists of all weeks, cluster I included 2.1, 2.2, 4, 4.2 and 6.2 dosages for CBC; cluster II included 4.1, 6 and 6.1 dosages; while cluster III included all control dosages and dosage 2. This, in this context, indicated that the control group (with highest yield) caused an obvious difference when compared to other groups and that it conformed to Table 2 (for CBC+EC). In terms of weeks, cluster I contains weeks 2, 3, 4 and 6; cluster II contains weeks 1, 5, 7 and 8; cluster III includes weeks 9-12; while cluster IV includes weeks 13-15. Weeks are basically distributed into two clusters and of these clusters, the first one is between weeks 1 and 8, while the second one is between weeks 9 and 15 and Table 3 (for CSW+EC) is supporting this conclusion with slight differences.

The heat map distributions of the CBC+EC and CSW+EC applied to soil samples are provided in Figure 3.

The hierarchical cluster analysis performed for CSW found in diagram b indicates that dosages are divided into three clusters, while weeks are divided into four clusters (Figure 3). On the condition that it consists of all weeks, cluster I included all three control dosages; cluster II consists of 4.2, 6, 6.1 and 6.2 dosages; while cluster III consists of 2, 2.1, 2.2, 4 and 4.1 dosages for CSW. Based on this, it has been concluded that the control group makes a distinct difference (with the lowest yield) when compared to other groups and is consistent with Table 2 (for CSW+EC). In terms of weeks, cluster I includes weeks 2, 3, 4 and 6; cluster II includes weeks 1, 5, 7 and 8; cluster III includes weeks 9-12; while cluster IV includes weeks 13-15. Weeks are basically distributed into two clusters and of these clusters, the first one is between weeks 1 and 8, while the second one is between weeks 9 and 15 and Table 3 (for CSW+EC) is supporting this conclusion with slight differences.

**FIGURE 3**

Heat map for corn biochar and stalk
a: MBC, b: MSA

**CONCLUSION**

The amount of CO$_2$-C, released following the separation and splitting period after the application of organic materials in their natural form, is higher. However, it has also been observed that organic materials turned into biochar through pyrolysis treatment has a higher resistance against splitting and separation after being applied to soil. This practice has reduced the amount of CO$_2$-C released from soil. Soil carbon management in arid and semi-arid regions is believed to be important in
terms of making use of agricultural wastes, both for
the protection of sustainable environment and pre-
vention of global warming.
This study has indicated that the increase in
soil salt content leads to a reduction in CO$_2$-C re-
leased from soil. In fact, however, this reduction is
caused by the salt supressing the living conditions
of the microorganisms in soil. This may seem like a
positive result in terms of global warming, but it is
not so positive for the fertility of soil. Secondary
saltiness is a significant problem in irrigated areas.
Different products may be grown based on the level
of saltiness. Studies to be conducted in such areas
on soil carbon dynamics are highly important with
regards to the sustainability and fertility of soil.
There is a need for such studies to be conducted in
the future.

REFERENCES

Hand book of plant and crop stress. Marcel
Dekker, New York, 3–11.
Management Research and salty soils. Salinity
ish)
differences and screening parameters in terms
of tolerance to salt stress in Okra (abelmoschus
esculentus L.) Republic of Turkey Ministry of
Agriculture and Forestry Bati Akdeniz Agricul-
tural Research Institute. 28(2), 55-70. (in Turk-
ish)
Murphy, B.W. (2010) Soil carbon dynamics in
saline and sodic soils: a review. Soil Use Man-
age. 26, 2–11.
[5] Sakin, E., Celik, A., Dogan, Z., Yalcin, H. and
and Some Soil Quality Parameters of Soils in
Organic and Conventional Agriculture Land.
and Human Friendly Energy System in Turkey:
Application of Sheep Manure and Its Biochar
on Carbon Emissions in Salt Affected Calcare-
ous Soil in Sanliurfa Region Se Turkey.
plication of biochar and different salt concen-
tration to soil on CO$_2$ emissions. In: Arap-
girlioglu, H., Atik, A., Elliott, R.L., Turgeon,
E. (eds.) Researches on Science and Art 21$^{st}$
Century Turkey. Gece Kitapligi. Chapter 15,
88.
different land uses on soil organic carbon and
carbon emission in Harran Plain SE Turkey. In:
Arapgirlioglu, H., Atik, A., Elliott, R.L., Tur-
geon, E. (eds.) Researches on Science and Art
21$^{st}$ Century Turkey. Gece Kitapligi. Chapter
135-143.
analysis. In: Klute, A. (ed.) Methods of soil
analysis. Part 1. Physical and mineralogical
methods. Agronomy Monograph No. 9. 2nd ed.
Am Soc Agron/ Soil Sci Soc Am, Madison, pp
383–411.
bon, organic carbon, and organic matter. In:
Page, A.L. (ed.) Methods of Soil Analysis. 2nd
Ed. ASA Monogr. 9(2). Amer Soc Agron Mad-
ison, WI, 539–579.
Page, A.L. (Ed.) Methods of Soil Analysis,
Chemical and Microbiological Properties. Part
2, American Society of Agronomy, Madison,
WI, USA, 831–871.
24.0, SPSS Inc., Michigan Ave., Illinois, USA.,
Chicago.
[15] Lu, W., Ding, W., Zhang, J., Li, Y., Luo, J.,
Bolan, N. and Xie, Z. (2014) Biochar sup-
pressed the decomposition of organic carbon in
a cultivated sandy loam soil: A negative prim-
ing effect Soil Biology and Biochemistry. 76,
12-21.
[16] Weiwei, L., Weixin, D., Junhua, Z., Yi, L.,
suppressed the decomposition of organic carbon in
a cultivated sandy loam soil: A negative priming
effect. Soil Biology and Biochemistry. 76,
12-21.
[17] Parton, W.J., Schimel, D.S., Cole, C.V. and
Ojima, D.S. (1987) Analysis of factors controll-
ing soil organic matter levels in Great Plains
Grasslands 1. Soil Science Society of America
Journal. 51(5), 1173-1179.


EFFECT OF QUERCETIN ON SELECTED MICRO ELEMENTS IN RAT LIVER TISSUE DURING CARBON TETRACHLORIDE EXPOSURE

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ABSTRACT
The aim of this study was to investigate the effects of carbon tetrachloride and quercetin on selected trace element levels such as Cr (Chromium), Mn (Manganese), Fe (Iron), Cu (Copper) in rats. In the study, 28 male Wistar albino rats (200 ± 300 g) were used. Rats were divided into 4 groups as Control, CCl₄ (carbon tetrachloride), Qu (quercetin), Qu + CCl₄ (quercetin + carbon tetrachloride) (n=7 in each group). When Cr (µg/g) amount was examined an increase was observed in Qu administered group (p <0.01) compared to the control. It was increased in Qu (p <0.05) and CCl₄ + Qu (p <0.001) treated groups compared to CCl₄ group. Mn decreased in CCl₄ and CCl₄ + Qu groups compared to control. Fe (µg / g) content was increased in CCl₄, CCl₄ + Qu and Qu (p<0.01) groups compared to Control. An increase in Qu treated group compared to CCl₄ applied group (p<0.05) was observed. The CCl₄ + Qu group decreased compared to the Qu group (p<0.01). Cu content of CCl₄ and Qu groups showed an increase whereas CCl₄ + Qu applied showed a decrease compared to control. As a result; It is thought that quercetin may have an effect on important trace element levels such as Cr (Chromium) Mn (Manganese), Fe (Iron), Cu (Copper) in the case of oxidative stress caused by carbon tetrachloride.

KEYWORDS:
CCl₄, Quercetin, Trace element, Liver

INTRODUCTION
Trace elements have important effects and roles in life-critical processes. Different studies have shown that trace elements are associated with many common diseases [1]. Elements are needed for the proper functioning of human metabolism. Lack of these elements cause serious metabolic abnormalities and their increase toxicity. In some diseases such as chronic kidney, liver and lung diseases, trace element levels were studied and significant results were obtained [2]. Carbon tetrachloride (CCl₄) is one of the most frequently used hepatotoxins in the experimental study of liver diseases [4]. The organ affected mainly by experimentally induced intoxication with CCl₄ is the liver. Many organs such as kidney, spleen, pancreas, thymus, lymph nodes, lungs, heart, and the system in which they are involved are directly or indirectly affected by cirrhosis resulting from toxicity [5]. The toxicity of CCl₄ probably depends on the formation of the trichloromethyl radical (CCI₃) which interacts with it to form the more toxic trichloromethylperoxyl radical in the presence of oxygen [6]. CCl₄ may react with a variety of biologically important cellular molecules such as proteins, lipids and nucleic acids. This affects the critical cellular processes leading to hepatocellular damage, leading to impaired liver function [7]. Quercetin (3,5,7,3',4'-pentahydroxylflavone) is one of the most widely distributed flavonoids found in fruits, vegetables and many other dietary sources [8]. Quercetin is a bio-flavonoid that directly cleanses free radicals, inhibits bio-molecule oxidation and modifies antioxidant defense pathways in vivo and in vitro [9]. In addition, it has a wide variety of pharmacological activities such as anti-inflammatory, anti-oxidant, anti-tumor, immunomodulator, anti-ulcer and vasodilator effects [10]. Evidence suggests that quercetin can protect the liver from injury caused by hepatoxins [11]. In our study; The aim of this study was to determine the effect of carbon tetrachloride and quercetin on some trace element levels in rat liver tissues such as Cr (Chromium), Mn (Manganese), Fe (iron), Cu (Copper).

MATERIALS AND METHODS

Animals and Treatment. Twenty-eight adult male Wistar albino rats were used in the study (200 - 300 g body weight). Rats were obtained from the Experimental Research Center of Bingöl University (BUHADYEK). They were kept in a controlled room with 20 ± 2°C constant temperature and twelve (12h) hour light-dark cycle (light:07:00-19:00 dark 19:00-07:00); The rats were randomly divided into four groups of 7 in each group and given commercial pellet and tap water ad libitum.
1. Group: Control: The rats in this group received an equal volume of olive oil for 3 days.  
2. Group: Carbon tetrachloride (CCl₄): Rats in this group injected with 1 ml / kg body weight CCl₄ (i.p.) for 3 days [12].  
3. Group: Quercetin (Qu): Rats in this group were injected with 25 mg / kg Qu (i.p.) for 3 days. [13].  
4. Group: Carbon tetrachloride (CCl₄) + Quercetin: The rats in this group were injected with 1.0 ml / kg body weight CCl₄ (i.p.) dissolved in olive oil and 25 mg / kg Qu (i.p.) for 3 days. At the end of the experiment, liver tissue samples were collected from the rats and kept until analysis.

ICP-MS sample preparation and analysis method. In the study, approximately 0.5 grams of rat liver tissue samples were weighed and transferred to Teflon cups of MARS 6 ONE TOUCH (USA) microwave oven. 10 mL of concentrated nitric acid was added to each sample. The mouth was tightly closed and kept at 210 °C for 15 minutes. Teflon tubes opened under fume hood. It was then taken into glass flasks using 10 mL of ultra-pure water and filtered. NexION® 2000 (PerkinElmer® Inc., USA) brand ICP-MS device was used for elemental analysis of the samples. ICP-MS calibration solutions were prepared by diluting commercially available multi-element standards with %1 (nitric acid-ultra-pure water) (Table 1). ICP-MS calibration was performed before each measurement. 100 ppb ⁴⁵Sc internal standard was used for control of element analyzes. [14, 15, 16]. ICP-MS NexION instrument software was used to control the instrument, including calibration, interferences, data collection and data analysis. In addition to argon gas, helium gas was used to prevent interference.

Statistical Analysis. Statistical analysis was performed using SPSS 20.0 (Statistical Program Software System) program. The results were expressed as mean X ± SEM. Statistical significance between the mean values was performed using One-way Analysis of Variance (ANOVA).

RESULT

Cr (Chromium), Mn (Manganese), Fe (iron), Cu (Copper) levels (μg / g) were determined in the study. When Cr (μg/g) amount was examined an increase was observed in Qu administered group (p <0.01) compared to the control. It was increased in Qu (p <0,05) and CCl₄ + Qu (p <0,001) treated groups compared to CCl₄ group (Figure 1). When the Mn content was evaluated, a decrease was observed in CCl₄ and CCl₄ + Qu groups compared to the control group (Figure 2). In this study, Fe (μg / g) content, which is among the important trace elements for metabolism, was also evaluated. It was seen that in CCl₄, CCl₄ + Qu and Qu (p <0.01) groups an increase was observed compared to control. An increase in Qu administered group compared to CCl₄ administered group (p<0,05), a decrease in CCl₄ + Qu administered group compared to Qu administered group (p<0,01) was observed (Figure 3). When Cu content was evaluated, it was found that CCl₄ and Qu groups increased compared to the control group and CCl₄ + Qu group decreased (Figure 4).

TABLE 1

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TABLE 2

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<th>Operation conditions of ICP instrument</th>
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<td>Discriminator threshold</td>
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<td>Alternating current (AC) rod offset</td>
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FIGURE 1
Chromium trace element concentrations in rat liver tissue
a, x: p < 0.001  b: p < 0.01  c: p < 0.05

FIGURE 2
Manganese trace element concentrations in rat liver tissue

FIGURE 3
Iron trace element concentrations in rat liver tissue
b, y: p < 0.01  c: p < 0.05
DISCUSSION

There are 92 elements in nature and hundreds of isotopes that have many effects on human health. These elements, which have a biological function in our body, are considered to be trace elements with an amount less than 100 mg / kg in the body. Elements such as zinc, copper, iron, chromium, selenium, manganese, molybdenum, cobalt and iodine are trace elements [17]. Trace elements are inorganic substances which are incorporated into many important events in the organism and have catalytic, enzymatic and structural activities. They are found in food and water and must be taken from outside via nutrition. Trace elements entering the organism are bound to various blood proteins and distributed to all tissues [18]. Trace elements play a very important role in human health even if they are found in very small amounts. Trace elements are important for enzyme reactions that facilitate the transformation and withdrawal of substrate molecules into certain end products. They release or bonds electrons in redox reactions that are of primary importance in the production and use of metabolic energy. Some have structural roles and are responsible for the stability of important biological molecules. Some trace elements have important effects during biological processes [1]. Trace elements serve the basic metabolic functions in the liver and diaphragm muscles, and the liver plays a central role in the placement of most trace elements. Excessive studies are needed to define the optimal criteria for trace element adequacy and the functional outcomes of deficient functions [19].

Trace elements play an important role in the structure of proteins, enzymes and complex carbohydrates to participate in biochemical reactions [20]. Chromium (Cr) is an essential element for body structural elements with important functions in carbohydrate, lipid and protein metabolism [17]. The daily intake of Cr is 50-200 μg / day [21]. Cr (III) is its basic form of nutrients used by the body. Cr⁶⁺, which has the most stable oxidation state, forms the basis of its chemical properties in biological systems. Chromium, which has important functions in carbohydrate, lipid and protein metabolism, is one of the main nutrients for mammals [22]. Manganese (Mn) is an essential element commonly found in liver, bones, and kidneys, which is the cofactor of important enzymes such as arginase, cholinesterase, phosphoglucomutase, pyruvate carboxylase, mitochondrial superoxide dismutase [17]. Mn is an essential element for trace amounts of health [23]. High levels of Mn can have a toxic effect on multiple organs, thus adversely affecting the functions of the liver, cardiovascular, reproductive system, immune system, and central nervous system [24]. Mn is a trace element related to the transport and absorption of Fe and the levels of these two elements exhibit a positive correlation. Mn also plays an important role in the immune system, interacting with neutrophils and macrophages, protecting the body against oxidative stress [25]. Iron (Fe), oxygen transport and storage, electron transport, oxidative metabolism, cell growth and growth, essential reactions used in the catalysis of life is an indispensable trace element for life. The total amount of iron in the body is about 3-5 grams, and a large majority is in the structure of the hemoglobin molecule [17]. Although excess or lack of Fe is an important nutrient, it can cause oxidative DNA damage [26]. Copper which is a transition metal is an important trace element for humans and animals [27]. In the human organism, copper is found in two forms - most of the copper in the human organism is the first and second form of oxidation. Most of it is found in the second form. The ability of copper to bind and receive electrons easily explains the importance of oxidative reduction processes and the removal of free radicals from the
organism [28]. Cu takes an important role in iron metabolism. The deficiency of copper disrupts iron absorption and severe copper deficiency is accompanied by anemia. Ceruloplasmin, the largest copper-containing protein in plasma, has ferrooxidase activity which oxidizes ferric iron to ferric state before binding to plasma transfer [29]. Cr (Chromium), Mn (Manganese), Fe (iron) and Cu (Copper) levels were evaluated in the study. When the amount of Cr (µg / g), which is one of the important trace elements, was examined, an increase (p <0.01) was observed in the Qu group compared to the control. An increase was determined in the Qu (p <0.01) and CCl4 + Qu (p<0.001) group compared to control. An increase was determined in the Qu group compared to the control group. However, Cu content in liver increased compared to the Qu group (p<0.01). When Cu content was evaluated, it was found that CCl4 and Qu groups increased compared to control group and CCl4 + Qu group decreased. When different studies are evaluated, it was found that CCl4 and Qu groups increased compared to control group and CCl4 + Qu group decreased. When different studies are evaluated in the literature search, it was found that effects of carbon tetrachloride on the copper (Cu), zinc (Zn), manganese (Mn) and cadmium (Cd) microelements were investigated in serum, erythrocyte and liver tissues of rats. Serum copper level was increased in CCl4 treated group compared to control. In contrast, rat erythrocyte Cu levels decreased in CCl4 treated group compared to control group. However, Cu content in liver increased in CCl4 group compared to control [30]. The effect of quercetin on Mg and Ca levels was evaluated in diabetic rats with STZ. Ca and Mg levels were decreased in the diabetic group and mineral contents of quercetin group were found to be close to control [31]. In a different study using carbon tetrachloride, selected rat serum microelements such as serum Ca2+, Fe, Mg2+, K, Se2+, Zn2+ and Na+ were evaluated. Compared to the control group, the amount of Na, Mg, K, Ca, Zn and Se decreased, and the amount of Fe increased compared to the control [32]. Cu, Zn, Fe and Mn contents of liver cirrhosis caused by carbon tetra chloride were evaluated. Mn levels decreased while Cu, Fe and Zn levels were increased in low protein diet + CCl4 treated rats compared to low protein diet fed rats [33]. In our study, it was observed that rat liver trace element (Cr, Mn, Fe, Cu) levels measured using ICP-MS following CCl4 and quercetin application were consistent with the evaluated literature studies.

CONCLUSIONS

As a result; It is thought that quercetin may have an effect on important trace element levels such as Cr, Mn, Fe, Cu in case of oxidative stress caused by carbon tetrachloride.

ACKNOWLEDGEMENTS

Bingöl University approved the experimental procedures used during this study by the Local Ethics Committee on Animal Experiments (BUHADEK: 04.10.2018-2018/08-08 / 01).

The authors declare that there are no conflicts of interest.

REFERENCES


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ANALYSIS OF HYDROLOGICAL CONNECTIVITY DYNAMICS OF THE WETLANDS IN HONGHE NATIONAL NATURE RESERVE

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ABSTRACT

Due to the intense agricultural development and other human activities in the Nongjiang catchment in the 1980s, the runoff of Nongjiang River was cut off. The hydrological connectivity of wetlands in the Honghe National Nature Reserve (HNNR) has been changed remarkably. Maintenance of a good connectivity is one of the key factors to protect the biodiversity and sustain the ecosystem stability and integrity. However, quantitative studies on hydrological connectivity of wetlands and corresponding restoration measures are rarely. Therefore, the aim of our research was to quantify hydrological connectivity of wetlands and propose effective recovery measures. Instead of single quantitative approach, based on the remote sensing image of wetlands in HNNR from 1975 to 2006, we applied the integral index of connectivity (HC), probability of connectivity (PC), and lacunarity index to analyze the changes in wetland connectivity. The results showed the declining trends of landscape connectivity measured by the integral index of connectivity (HC) and probability of connectivity (PC) from 1975 to 2006. The importance of connectivity in each wetland patch varied with the increment of dispersal distances, and some important habitat patches, which exhibit a potential to enhance landscape connectivity, should be given more attention. The results also suggested that lacunarity index analysis shows that the spatial heterogeneity of wetlands varied in different scales, and the scale transformation could reflect the degradation process of wetlands which was consistent with wetland hydrological connectivity analysis. The result of this study provided basic data and a scientific guidance for future wetland restoration.

KEYWORDS:
Integral index of connectivity (HC), Probability of connectivity (PC), Important patches, lacunarity index, HNNR

INTRODUCTION

Global climate changes as well as human activities will have a profound effect on wetland water cycle [1]. Wetland functional decline has raised concerns among experts and scholars [1, 2], and recovery of wetland hydrological function has already been put on the agenda [3, 4]. Connectivity, an important indicator to measure the pattern and function of landscape, refers to the degree of convenience or hinder caused by landscape to the ecological flow [5, 6]. The importance of hydrological connectivity to wetland ecosystem is mainly reflected in wetland ecological environment, water quality, aquatic animal resources, flood control and water resource utilization [7, 8]. Hydrological connectivity not only maintain the integrity of wetland ecosystem but also affect the water quality in water functional areas [9, 10, 11]. In addition to benefit for biological diversity of wetland ecosystem, water quality also be influenced: the better the hydrological connectivity is, the stronger the water self-purification ability in carrying pollutants capacity will be [12]. It is essential to consider connectivity as a basis for conservation planning and landscape change analysis [13, 14]. But before integrating it in operational decision-making it is extremely important to be aware of how connectivity should be measured in this respect.

In the literature, several connectivity approaches and indices have been suggested, so far, a variety of metrics have been invented for the calculation of wetland landscape connectivity, but defects exist in one kind or another [14, 15, 16, 17]. Among all the research in the field of measuring connectivity, despite recent efforts in this respect, there is still a great need for research on the specific properties and measurement abilities of many connectivity metrics, which are essential to select the most appropriate indices with an objective and sound basis, nearly no research put forward a method to restore connectivity of wetlands [17, 18, 19]. Therefore, the purpose of this study was to quantify hydrological connectivity of wetlands and propose effective recovery measures. To achieve this goal, more specific objectives were to select the integral index of connectivity (HC), probability of connectivity (PC), and
lacunarity index to quantify connectivity of wetland and to put forward a method of selecting important patches in the wetland restoration, accordingly determining the selection of important patches at the beginning of the wetland restoration and restoration order.

**MATERIALS AND METHODS**

**Study Area.** Honghe National Nature Reserve (HNNR) located in the hinterland of the Sanjiang Plain—the largest freshwater wetlands in China (Figure 1), is selected as our study area. With the area of 218.36 km², this area has a temperate humid continental monsoon climate, with an annual average temperature of 1.9°C, total evaporation of about 900mm, and average annual precipitation of about 585mm, most of which falls between June and September. The effective accumulated temperature ranges from 2300–2580°C, with the highest temperature of 37°C, and the lowest temperature of -40°C. The frost-free period is 125–140D, the frozen soil layer about 2 m deep. The annual average wind speed is 317m/s [20, 21]. HNNR is a vast plain. There are two rivers in HNNR. The Nongjiang River flows through the nature reserve near the northern boundary, and the Wolan River is located in the central core area. Low gradient areas are favorable for wetland. Widespread inundation by floods is facilitated by a network of cross channels on low-terrain slopes (average slopes less than 1:10,000). Wetland account for about 76.6% of the total area. There are 16 orders, 43 families, and 174 species of waterfowl, including 10 rare and endangered waterfowls and 1012 species of plants, including 6 national endangered plants. As a typical inland wetland and freshwater ecosystem in the northern temperate zone, HNNR was listed as Ramsar Wetland in 2002.

As an international important wetland, this reserve has experienced heavy disturb from regional agricultural development. Now the reserve has been surrounded by agricultural landscape and isolated by three ditches in the west, south and east of the reserve. In 1980, a canal was constructed in the west of HNNR, and the runoff of Nongjiang was cut off, which was once the important surface water supply for HNNR. Therefore, the reserve has suffered serve water shortage after that, which caused in the continuous dropout in surface water and groundwater. As a result, hydrological connectivity among wetlands and the river has decreased remarkably, which resulted in the degradation of wetlands in the reserve [22, 23].

**Material.** The primary data for the Honghe Nature Reserve used in this study come from three land-use maps and Auxiliary data (included 1:100,000 scale topographic maps and Quick Bird images on May, 2004, which spatial resolution panchromatic band is 0.61m). The land-cover maps were interpreted from a series of Landsat Multispectral Scanner (MSS) images and Landsat Thematic Mapper (TM) images. An MSS image was used to obtain the 1975, and two TM images were acquired for the land-use map in 1989 and 2006. All of the images were free of clouds and were obtained during the same season. The applied classification method was a supervised maximum likelihood classification approach with ENVI (Version 5.3, Exelis Visual Information Solutions, Harris Corporation, USA) and the...
The results of the classification were revised based on visual interpretation and field surveys. To confirm the accuracy of the classification of the images, land-use status maps (1:100,000 scale, from 1975 to 2006) developed by the Heilongjiang Department of Land and Resources were used as references to evaluate the classification and accuracy of these images. Auxiliary data (including 1:100,000 scale topographic maps and Quick Bird images on May, 2004, which spatial resolution panchromatic band is 0.61m) were also applied as references for the geometric rectification, classification, and estimation of the accuracy of the images. The assessment of the accuracy of the 2006 images was conducted by comparing the map with ground control points specified by a global positioning system (GPS), and the land-use types at these points (patches) were identified. The overall accuracies of the image classifications for 1975, 1989 and 2006 were 92.33%, 92.60% and 90.14%, respectively. To minimize the classification errors from the differences in the resolution of the remote sensing images, all of the transformed images were exported to ArcGIS (Version 10.3, Environmental Systems Research Institute, Redlands, California, USA) in a grid format, and then convert these new polygon themes using a shape file format. Polygons with the small area in the digitized data were eliminated by merging these small polygons with neighboring polygons.

The land-use maps were classified into six landscape types: marsh, river, forest, meadow, paddy field and dry land. River bogs mainly include rivers with open water surface and various artificial and natural bubbles. The marsh distribution maps were used to analyze the land cover dynamics of the wetlands and the importance of connectivity (Figure 2).

Method. Index of connectivity. Connectivity index is calculated by using Conefor Sensinode 2.6 developed by Duke University [20]. The specific operation is in ArcGIS 10.3, according to the input points and the area vector files of each patch, using the plug-in module Conefor Input for ArcGIS10.3 to calculate and output the resistance distance between the patches in a tabular way, then input the resistance distance in Conefor Sensinode 2.6 software, and finally set the related parameters such as the total area of matrix, threshold distance, etc., to automatically calculate and transport. The HC, PC, dHC and dPC equivalents of each point are given [24, 25]. Extraction of wetland patches as habitat patches, the study area as the background landscape. Whether patches connected or not depend on the scale of the different ecological process, such as habitat availability and the dispersal potential of animal and plant species. Therefore, you need to specify the landscape of the habitat patches connected distance threshold. 6 distance thresholds (0.1, 0.5, 1.0, 2.0, 4.0, 8.0km) were selected to compute the integral index of connectivity. We set to 0.5 the probability of dispersal corresponding to the threshold dispersal distances in order to compare PC and HC.

HC ranges from 0 to 1 and increases with improved connectivity. It is given by the following expression:

\[
HC = \frac{\sum_{i=1}^{n} \sum_{j=1}^{n} \frac{a_i a_j}{\Delta A_i}}{A_L^2}
\]

Where \(a_i\) and \(a_j\) denote the area of patches I and j, \(n_i j\) are the numbers of links in the shortest path (topological distance) between patches \(i\) and \(j\), \(n\) is the total number of patches in the landscape, and \(\Delta A_i\) denotes the total area of the whole landscape (wetland patches and non-wetland patches). HC is based on the binary connection model. Within the range of distance thresholds, patches are connected. Outside the range of the distance threshold, patches are not connected. That is to say, there are only two cases for any two patches in the landscape: connected or disconnected. Among them, \(HC = 0\) means that there is no connection between habitat patches; \(HC = 1\) means that all habitat patches are connected, and the whole landscape is habitat patches.

FIGURE 2
The land-cover maps from 1975 to 2006.
The probability of connectivity index (PC) considers a richer connection model than HC and it is not affected by the presence of adjacent habitat patches or cells in the analyzed datasets [24, 25, 26]. It is given by the following expression:

\[
PC = \frac{\sigma_i \sigma_j}{\sigma_i + \sigma_j}
\]

(2)

Where \(a_i\) and \(a_j\) are the attributes of nodes i and j, \(n\) denotes the total of patches in the landscape, \(\sigma_i\) is the total area of the whole landscape, and \(p_{ij}\) is defined as the maximum product probability of all possible paths between patches i and j (including single-step paths). Here, the \(p_{ij}\) values were characterized by a negative exponential as a function of the inter patch effective distances selected. The product probability of a path is the product of all the \(p_{ij}\) belonging to each step in that path. If patches i and j are close enough, the maximum probability path will simply be the step between patches i and j (\(p_{ij}^* = p_{ij}\)). If patches i and j are more distant, the best path would probably comprise several steps through intermediate stepping stone patches yielding \(p_{ij}^* > p_{ij}\) [26, 27]. We calculated \(p_{ij}\) according to the following expression:

\[
p_{ij} = e^{-k \cdot d_{ij}}
\]

(3)

where \(d_{ij}\) indicates the distance between patches i and j. The constant \(k\) is a species-specific constant set to cause the function to match the probability distance values. PC is based on the possibility model, mainly expressing the possibility of connectivity between habitat patches, which is negatively correlated with the distance between patches [26, 27, 28, 29].

Important value of the landscape patches.

HC and PC not only calculate the connectivity of the landscape, but also calculate the important values of patches for landscape connectivity. Important values of patches indicate the importance of patches in maintaining landscape connectivity. According to the above HC and PC calculation, the wetlands landscape connectivity index and the low wetlands landscape connectivity index in the study area are also the preferred areas for wetlands restoration. In order to analyze which important wetland patches should be given priority in wetland landscape restoration and which wetland patch restoration is the most important to increase the connectivity of the wetlands. The importance value of patches (dI) was selected to measure the importance of each patch for increasing wetland landscape connectivity. According to the connectivity index (HC, PC), the importance dI of each patch was calculated. The important value of each patch was different according to the selected index. The importance of an existing node for maintaining landscape connectivity (dI) according to a certain index (I) is calculated in CS22 as a percentage given by:

\[
dI = \frac{I_{\text{remove}}}{I} \times 100\%
\]

(4)

Where I represents the HC and PC index in wetland landscape, dI represents the change value of dHC and dPC index, I_{\text{remove}} is the connectivity index value of the landscape after removing patch I from the landscape. The important values of each patch are calculated. Therefore, using an adequate landscape-level connectivity index is critical. So, the important values are very useful for decision-making in landscape conservation planning, since they allow identifying the most critical nodes (e.g. habitat patches or cells) for the maintenance or improvement of landscape connectivity, on which conservation or restoration efforts should concentrate. The results of these analyses may largely depend on the selected connectivity index [27, 28, 29].

Lacunarity analysis.

Lacunarity analysis is a multi-scale method for determining textures related to species locations or habitat type. It can be used for binary and quantitative data of one, two and three dimensions. Although this method was originally developed for fractal objects, it is more general and can be easily used to describe non-fractal and multi-fractal patterns. In addition, this method is easy to implement on computer and provides easily interpreted graphical results. Patterns can be detected even in very sparse occupied maps.

Mandelbrot [30] gives a general method for calculating the clearance. Allain and Cloitre (1991) [31] described an algorithm for directly calculating deterministic and random fractal uncertainties. We demonstrate the sliding box algorithm of Allain and Cloitre (1991) through Apack [31]. Space is usually measured at different resolutions by covering boxes of different sizes on landscape maps. Apack allows the box sizes to move one column to the right and then calculate the box mass. This process is repeated over all rows and columns producing a frequency distribution of the box masses, the number of boxes of size \(r\) containing S occupied sites is designated by \(n(S, r)\) [32]. If the map is of size M, then

\[
N(r) = (M - r + 1)^2
\]

(5)

This frequency distribution is converted into a probability distribution Q(S, r) by dividing by the total number of boxes:

\[
Q(S, r) = \frac{n(S, r)}{N(r)}
\]

(6)

The first and second moments of this distribution are now determined:

\[
Z^{(1)} = \sum S \cdot Q(S, r)
\]

(7)

\[
Z^{(2)} = \sum S^2 \cdot Q(S, r)
\]

(8)

The lacunarity for this box size is now defined as:

\[
L(r) = \frac{Z^{(2)}}{(Z^{(1)})^2}
\]

(9)
generally related to the distance thresholds. The probability of connectivity index (PC) values successively decreased from 1975 to 2006 (Table 1). The overall connectivity (HC) and the possible connectivity index (PC) increased with the increase of distance thresholds from 0.1 to 8 km in three years of 1975, 1989, and 2006 (Figure 3). In addition, enhanced connectivity for the same wetland patches was associated with a longer distance in each period. Among the six distance thresholds, the HC and PC value was the highest at a distance threshold of 8 km (Figure 3). This indicates that the resultant prioritization of wetland patches based on HC and PC values is also generally related to the distance thresholds.

The distribution of important wetland patches. According to the distribution of wetland landscape in the study area, using GIS software, the patches with the important value for landscape connectivity can be found and extracted from the landscape patch map. Patches with top 5 important values under different distance thresholds were overlapped together and the distribution of patches which the important value lie in the top 5 under different distance threshold from 1975 to 2006 were listed (Figure 4). Due to the spatial pattern changes from 1975 to 2006, the result showed there were many differences with the top 5 important values on wetland patches in three years (Figure 4). In 1975, there were 5 patches which played an important role in connectivity of wetlands in the research area, arranged by their function from high to low, the areas are 95.9, 48.1, 26.5, 2.2 and 0.43 km², accounting for 21.3%, 21.1%, 13.2%, 6.9% and 0.2% of the total study area respectively. In 1989, there were 5 patches which played an important role in connectivity of wetlands in the research area, arranged by their function from high to low, the areas are 46.6, 46.1, 28.8, 15.1 and 0.4 km², making up the proportion of the total study area were 13.2%, 11.4%, 5.5%, 4.1% and 0.2% respectively. In 2006, the areas of top 5 important wetland patches were 46.5, 24.9 and 11.9, 8.9, 3.6 km², making up the proportion of the total study area were 21.3%, 11.4%, 5.5%, 4.1% and 1.7% respectively. What’s more, certain differences existed between the dHC and dPC results. Compared with the result of dHC, the dPC had more small patches, which also illustrated that PC can better show the importance of small patches.

### TABLE 1

Values of connectivity indices of wetlands in HNNR under different distance thresholds

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<tr>
<th>Distance thresholds (m)</th>
<th>Integral index of connectivity</th>
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<td>100</td>
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<td>0.335</td>
</tr>
<tr>
<td>500</td>
<td>0.347</td>
<td>0.338</td>
</tr>
<tr>
<td>1000</td>
<td>0.366</td>
<td>0.339</td>
</tr>
<tr>
<td>2000</td>
<td>0.378</td>
<td>0.342</td>
</tr>
<tr>
<td>4000</td>
<td>0.390</td>
<td>0.349</td>
</tr>
<tr>
<td>8000</td>
<td>0.401</td>
<td>0.352</td>
</tr>
</tbody>
</table>

### FIGURE 3

Change of connectivity indices of wetlands in HNNR from 1975 to 2006

**RESULTS**

**Hydrological connectivity analysis.** In this study, the patches attribute was area (wetland patch area), and A was the total landscape area (area of the analyzed region, comprising both habitat and non-habitat patches) and HC=1 when all the landscape was occupied by wetlands. Due to numerous wetland patches with large areas, the landscape connectivity was relatively higher in 1975 than the results in 1989 and 2006. The overall connectivity (HC) and the possible connectivity index (PC) values successively decreased from 1975 to 2006 (Table 1). The overall connectivity (HC) and the possible connectivity index (PC) increased with the increase of distance thresholds from 0.1 to 8 km in three years of 1975, 1989, and 2006 (Figure 3). In addition, enhanced connectivity for the same wetland patches was associated with a longer distance in each period. Among the six distance thresholds, the HC and PC value was the highest at a distance threshold of 8 km (Figure 3). This indicates that the resultant prioritization of wetland patches based on HC and PC values is also generally related to the distance thresholds.
The key wetland patch showing a potential to enhance connectivity, important habitat patches played an important role in improving wetland landscape connectivity and nature reserve management, and these key habitat patches should be given more attention. In addition, the location and area of patches have profound effects on the connectivity assessment.

**Lacunarity index analysis.** In order to analyze the difference of spatial heterogeneity of each landscape type with scales, the lacunarity was calculated for box sizes ranging from $r = 2$ to 512. A log-log plot was then made of lacunarity versus box size from 1975 to 2006 (Figure 5). In 2006, the values and curve of the lacunarity index of each landscape type changed greatly compared with 1975 and the lacunarity index order and curve shape of each landscape type were different, which indicated that the spatial heterogeneity of landscape pattern had changed greatly in the past 20 years (Figure 5).

On the smaller scale, the lacunarity index of paddy field, dry land, forest, marsh, river, meadow and in 2006 ranked from large to small, while that of dry land, meadow, forest, river, marsh in 1975, which was consistent with the proportion of landscape types in 2006 and 1975, that is, the smaller the proportion, the larger the lacunarity index of landscape types. Because at a smaller scale, the lacunarity index is mainly affected by the distribution area of landscape type. The smaller the area is, the higher the index is. The larger the area is, the smaller the index is (Figure 2, 5).

On the small scale, the lacunarity index of wetlands in 2006 was higher than that in 1989 and 1975 (Figure 6). Mainly because the area of wetlands in 2006 (marshes and river swamp occupied a small area in the study area accounted for about 53.7%) was significantly reduced compared with that in 1975 (accounted for about 96.3%). The wetlands started to degrade to meadow, paddy field and dry land leading swamps become more scattered with less area. However, after exceeding a certain scale, the lacunarity index in 2006 was lower than that in 1989, until it reduced to zero, indicating that on the larger scale, the distribution of wetlands in 2006 was more uniform than that in 1989, and the difference was reduced (Figure 6). Therefore, lacunarity index can well reflect the wetlands degradation process by scale transformation and plays an important role in the wetlands change law research.

The decrease of wetland area and the dispersion of spatial distribution lead to great changes in the hydrological connectivity of wetlands. This result was consistent with the wetland landscape connectivity index decline analysis.

**FIGURE 4**
The distribution of patches which the important value lies in the top 5 under different distance threshold from 1975 to 2006 (legend: 1-5 display the important value of this patches from 1 to 5)

**FIGURE 5**
Log-log plot of lacunarity versus box size for maps for different landscape types from 1975 to 2006
DISCUSSION AND CONCLUSIONS

As mentioned in the introduction, it was important to measure connectivity of wetlands. So, this study quantified hydrological connectivity of wetlands and proposed effective recovery measures. This study indicated that the integral index of connectivity (HC) and probability of connectivity (PC) made effective evaluation on connectivity of wetlands. The integral index of connectivity (HC) and probability of connectivity (PC) successively decreased from 1975 to 2006. Owing to human activities around the 1980s like the dike and floodgates, the upstream of Nongjing River got its closure, which makes the water once passing through the reserve discharge directly, leading the degradation of the water shortage in the reserve, and ends up with a continuous decrease of wetland landscape connectivity in the research area.

The result of lacunarity index analysis suggested that under a continuous intense human disturbance, the wetland was constantly occupied by meadow, farmland and the dry land, which slightly improved the heterogeneity degree, and also led the decrease of wetland area and the dispersion of spatial distribution. This result was consistent with the wetland landscape connectivity index decline analysis.

We got wetland patches which played an important part in connectivity of wetlands in the research area from 1975 to 2006. The result showed that the top five wetland patches had a larger area and strip type, function as the connective corridors, and almost all located in the channel of the research area. Based on the research results, it is suggested to give priority to the protection of important wetland patches in HNNR. At the same time, small patches should be built between the giant patches to form a connectivity corridor and improve the connectivity of wetlands.

All of the above indicated that it is significant to maintain and recover the wetlands connectivity. The method of this study is operability and practical, not only quantifying connectivity of wetlands by combining HC, PC and lacunarity index, but clearly pointing out the important of each patch on the wetland landscape connectivity in the wetlands restoration, at last deciding the wetlands restoration important patches at the beginning of the selection and the order of restoration. In addition, the paper analysis wetlands spatial heterogeneity change with scales of lacunarity index, to find out the reasons of Wetlands degradation and provide a basic data and scientific guidance of this study for the wetland restoration.

ACKNOWLEDGEMENTS

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REFERENCES


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INDICATOR SPECIES ANALYSIS OF BIRD OF PREY; CASE STUDY FOR FARMLAND IN ATABEY

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Isparta University of Applied Sciences, Faculty of Forestry, Department of Wildlife Ecology and Management, Isparta, Turkey

ABSTRACT

This study was carried out in order to determine aves and cultivated plants as indicator species of bird of prey in Atabey province. 60 sample sites were taken in the course of the study. Presence/absence data for aves and cultivated plants were recorded at each sample site. The data (in first stage to see the relationship aves and bird of prey, then in the second stage to see the relationship cultivated plants and bird of prey), Chi-square test was performed with SPSS. Indicator species and relationship orientation of these species were determined by using interspecific correlation analysis. As a result of study, negative and positive indicator species of bird of prey determined. In the first stage indicator bird species of bird of prey and in the second stage indicator cultivated plant species of bird of prey has been detected. Detrended correspondence analysis (DCA) was applied with the Past program to examine the situation of aves and cultivated plants between bird of prey with sample sites.

KEYWORDS:
Bird of prey, Indicator species, Interspecific correlation analysis, Detrended correspondence analysis, Farmland, Aves

INTRODUCTION

Biological indicators are often used in research and environmental management as diagnostic tools. Plant and animal species have been used for indicators of air and water quality; agricultural and range conditions. The terms of indicator, indicator species, signal species, bio-indicator, bio-monitor, keystone, umbrella, and focal species tend to have different and sometimes overlapping meanings [1-4].

Importance of indicator species to land managers to has possibility to manage a whole community or ecosystem by focusing on the needs of one or a few species. Indicator species used as represent of biodiversity should be different among for different environments. Different species should be expected as surrogate of biodiversity in different landscape, characterized by dissimilar structure and land use composition [5]. If managers have information about indicator species, they could determine management plans accordingly biodiversity, habitat status and relationship between species. In this way they can/could do sustainable management and good agricultural practices in target location.

In the study was aimed to find in the first stage indicator bird species of bird of prey and in the second stage indicator cultivated plant species of bird of prey. Indicator species are also use to assess the effects of agricultural practices on habitats. Bird of prey species, can use as indicators of population trends in other species. This study findings can also use to assess the effects of agricultural practices on habitats and bird species. And results could use for conservation biology studies for bird species.

MATERIALS AND METHODS

The study was carried out in Isparta, Atabey Plain 15 km northeast of Isparta province and located between 30° 27' 43" - 30° 39' 02" with eastern longitudes 37° 50' 32" - 37° 58' 19" northern latitudes (Figure 1). Study area is 20217 ha and has continental climate. According to average temperature values of 2014-2017, annual average temperature 12 °C, highest temperature 22,3 °C in July, lowest temperature 2 °C in January and annual total precipitation 560 mm in area [6]. Traditional and classical farming methods are applied in the field.

During this study a total of 60 sample sites were surveyed. The selection of sample sites 300x300 m was driven by the results of previous studies performed for aves in cultivated and farmland areas [7-10]. 60 sample sites were observed and in order to prevent the occurrence rerecord of bird species, the necessary distance is left between the sample sites [11]. In the sample sites, direct observation technique was applied and during 5 min visible individuals and singers were counted [12, 13]. Species identification was made according to Porter et al. [14]. Plants species of sample sites were described. The survey of birds was conducted between April and August 2016 [11, 15]. In the form of repeated observations in the same areas. Observations were carried out especially in breeding and hatching months that included the best bird watching time [12]. All
sample points were visited at least three times each month, between 06:00-10:00 h and 16:00-20:00 h for 10 min in sunny conditions. Observations were made in the period close to the enlightenment and darkening times of the air, in other words, when the birds were active [16-20]. All birds detected visually and acoustically within a sample site were recorded.

During the study 99 bird species were recorded in Atabey. The species whose frequency percentage is less than 5% [21] are not included (forty seven of the 99 recorded species) in the analysis.

Interspecific correlation analysis was conducted in order to identify the correlation between aves and cultivated plants between bird of prey. Chi-square test was performed with SPSS 22.0 software for analyzing data. The direction of the relationship was identified using C3 formula based on this data [22-27]. To examine the situation of birds, cultivated plants and bird of prey with sample sites, Detrended correspondence analysis (DCA) were applied with the Past3 program [28, 29]. As a result of study, negative and positive indicator species of bird of prey determined.

RESULTS AND DISCUSSION

Results a total of 99 bird species were recorded in the whole study area. Forty seven of the 99 recorded species were excluded from the analysis because their occurrence frequencies were <0.5%. Forty five bird species (Apus apus, Calandrella brachydyactyla, Carduelis carduelis, Clamator glandarius, Columba palumbus, Corvus cornix, Cyanistes caeruleus, Dendrocopos syriacus, Emberiza caesia, Emberiza calandra, Emberiza cerula, Emberiza melanocephala, Ficedula albicollis, Ficedula hypoleuca, Fringilla coelebs, Galerida cristata, Garrulus glandarius, Hirundo rustica, Lanius collurio, Lanius minör, Lanius senator, Luscinia megarhynchos, Melanocorypha calandra, Merops apiaster, Monticola saxatilis, Motacilla alba, Motacilla flava, Oenanthe hispanica, Oenanthe isabellina, Oenanthe oenanthe, Oriolus oriolus, Parus major, Passer domesticus, Passer hispaniolensis, Passer montanus, Phylloscopus collybita, Phylloscopus sibilatrix, Pica pica, Streptopelia decaocto, Streptopelia turtur, Sturnus vulgaris, Sylvia atricapilla, Sylvia melanocephala, Turdus merula, Upupa epops) and seven bird of prey species (Accipiter nisus, Athene noctua, Buteo rufinus, Circus cyaneus, Circus macrourus, Falco naumanni, Falco tinnunculus) were included in analysis, their occurrence frequencies were >0.5% (Table 1 and Table 2).

Results a total of 17 cultivated plant species (Amygdalus communis, Hordeum vulgare, Juglans regia, Malus domestica, Medicago sativa, Papaver somniferum, Populus nigra, Prunus avium, Prunus cerasus, Prunus domestica, Prunus persica, Rosa damascena, Salix viminalis, Triticum aestivum, Vicia sativa, Vitis vinifera, combination various fruit trees) were recorded in the whole study area. All recorded species were included in analysis, their occurrence frequencies were >0.5% (Table 3).

In this study it was found correlation between bird of prey and 13 bird species (C. glandarius, F. coelebs, H. rustica, L. collurio, L. senator, M. apiaster, M. calandra, M. flava, O. hispanica, O. oenanthe, P. major, P. pica, U. epops) of the 45 bird species. Found to correlation between bird of prey and 5 cultivated plant species (P. cerasus, P. somniferum, R. damascen, V. vinifera, combination various fruit trees) of the 17 cultivated plant species.

Chi-square ($x^2$), significance level (p) and correlation direction coefficients ($r_c$), which were the result of the interspecific correlation analysis, used for firstly bird of prey and birds, secondly bird of prey and cultivated plant species are shown in Table 4.
TABLE 1
Frequency of bird species

<table>
<thead>
<tr>
<th>Species</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. nipalensis</td>
<td>23</td>
</tr>
<tr>
<td>C. ceylonica</td>
<td>8</td>
</tr>
<tr>
<td>C. corvus</td>
<td>40</td>
</tr>
<tr>
<td>C. crassirostris</td>
<td>8</td>
</tr>
<tr>
<td>C. cinnclidia</td>
<td>48</td>
</tr>
<tr>
<td>C. glandarius</td>
<td>8</td>
</tr>
<tr>
<td>C. pallidus</td>
<td>25</td>
</tr>
<tr>
<td>E. cucculus</td>
<td>53</td>
</tr>
<tr>
<td>E. calandra</td>
<td>12</td>
</tr>
<tr>
<td>E. urinae</td>
<td>83</td>
</tr>
<tr>
<td>E. corvinus</td>
<td>7</td>
</tr>
<tr>
<td>E. oenanthe</td>
<td>33</td>
</tr>
<tr>
<td>F. albicollis</td>
<td>15</td>
</tr>
<tr>
<td>F. coelebs</td>
<td>45</td>
</tr>
<tr>
<td>F. hypoleucos</td>
<td>7</td>
</tr>
</tbody>
</table>

TABLE 2
Frequency of bird of prey species

<table>
<thead>
<tr>
<th>Species</th>
<th>A. nipalensis</th>
<th>A. noptua</th>
<th>B. macrourus</th>
<th>B. rufinus</th>
<th>C. Cyaneus</th>
<th>F. tinunculus</th>
<th>F. naumanni</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequency (%)</td>
<td>10</td>
<td>15</td>
<td>5</td>
<td>45</td>
<td>7</td>
<td>15</td>
<td>7</td>
</tr>
</tbody>
</table>

TABLE 3
Frequency of cultivated plants species

<table>
<thead>
<tr>
<th>Species</th>
<th>A. commutata</th>
<th>H. sativa</th>
<th>J. regia</th>
<th>M. sativa</th>
<th>P. argum</th>
<th>P. cerasus</th>
<th>R. damascena</th>
<th>P. napetorum</th>
<th>P. persica</th>
<th>V. vinifera</th>
<th>V. sylvatica</th>
<th>V. scalpellum</th>
<th>F. tinunculus</th>
<th>Combination various fruit area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequency (%)</td>
<td>35</td>
<td>38</td>
<td>18</td>
<td>33</td>
<td>7</td>
<td>38</td>
<td>7</td>
<td>20</td>
<td>26</td>
<td>12</td>
<td>8</td>
<td>8</td>
<td>12</td>
<td>62</td>
</tr>
</tbody>
</table>

According to results, *P. cerasus* (p<0.006) was found to be positive indicator species for *A. nipalensis*. *L. colurio* (p<0.022), *O. oenanthe* (p<0.020), *U. epops* (p<0.043) were positive indicators for *A. noptua*.

*P. major* (p<0.011) negative and *P. somniferum* (p<0.022) positive indicator species for *B. rufinus*.

*L. senator* (p<0.006), *M. calandra* (p<0.000), *M. flava* (p<0.000), and *P. pica* (p<0.001) was negative indicators for *C. Cyaneus*.

*H. rustica* (p<0.01) was negative indicator species for *C. macrourus*.

*C. glandarius* (p<0.002), *M. apiaster* (p<0.000), *O. hispanica* (p<0.000) were positive indicators for *F. naumanni*.

*C. glandarius* (p<0.003), *F. coelebs* (p<0.032), *R. damascena* (p<0.047), *V. vinifera* (p<0.028), combination various fruit trees (p<0.008) were positive indicators for *F. tinunculus*.

Wild animals preferred some habitat for nutrition, breed, forage, hiding, nesting and shelter according to their biology. In addition to all these, bird of prey prefers habitats suitable for wingspan and where they can fly comfortably. And they prefer areas, where can find their prey easy and abundant. It should be noted that in all of the study area is found in rodent and other small prey species.

According to DCA results, compared to other bird of prey, *A. nipalensis, A. noptua, B. rufinus* and *F. tinunculus*, which have more frequency than other bird of prey species, were at the center of the societies formed by sample sites (Figure 2). As it is seen in the Figure 2. *C. Cyaneus, C. macrourus* and *F. naumanni* they are seen as distant species both from each other and from other species of bird of prey.

In study are with to irrigation systems, water channels and streams, wild animals can meet their water needs from these areas easily [30]. Where settlements are located, the presence of vegetable gardens and poultry give offers easy food supply for various wild animals. The study area supply with the habitat requirements of various birds and mammals species [27, 31, 32]. During the study farmers stated that they had break up bird nests in order not to birds damage on agricultural crops. Because of these reasons birds has changed their habitats, reproduction and migration areas. This is the main reason for decreasing the frequency of bird presence in agricultural areas. Bird species has been decreased cause of increased agricultural intensification in farmland.
### TABLE 4

<table>
<thead>
<tr>
<th>Bird of prey species</th>
<th>A. hispanica</th>
<th>A. nissus</th>
<th>B. rufinus</th>
<th>C. cyaneus</th>
</tr>
</thead>
<tbody>
<tr>
<td>x²</td>
<td>p</td>
<td>c3</td>
<td>x²</td>
<td>p</td>
</tr>
<tr>
<td>C. glandarius</td>
<td>0.606</td>
<td>-0.436</td>
<td>0.107</td>
<td>0.744</td>
</tr>
<tr>
<td>F. coelebs</td>
<td>0.067</td>
<td>0.795</td>
<td>0.040</td>
<td>0.582</td>
</tr>
<tr>
<td>H. rustica</td>
<td>0.015</td>
<td>0.904</td>
<td>-0.010</td>
<td>0.126</td>
</tr>
<tr>
<td>L. collario</td>
<td>0.247</td>
<td>0.619</td>
<td>-0.059</td>
<td>5.272</td>
</tr>
<tr>
<td>L. senator</td>
<td>0.741</td>
<td>0.389</td>
<td>-0.059</td>
<td>0.015</td>
</tr>
<tr>
<td>M. apiaster</td>
<td>0.351</td>
<td>0.554</td>
<td>-0.027</td>
<td>0.832</td>
</tr>
<tr>
<td>M. calandra</td>
<td>1.071</td>
<td>0.301</td>
<td>0.052</td>
<td>0.756</td>
</tr>
<tr>
<td>M. flava</td>
<td>1.071</td>
<td>0.301</td>
<td>0.052</td>
<td>0.756</td>
</tr>
<tr>
<td>O. hispanica</td>
<td>1.910</td>
<td>0.167</td>
<td>0.059</td>
<td>0.832</td>
</tr>
<tr>
<td>O. oenanthe</td>
<td>1.071</td>
<td>0.301</td>
<td>-0.157</td>
<td>5.378</td>
</tr>
<tr>
<td>P. major</td>
<td>0.278</td>
<td>0.598</td>
<td>0.077</td>
<td>0.768</td>
</tr>
<tr>
<td>P. pica</td>
<td>0.000</td>
<td>1.000</td>
<td>0.000</td>
<td>0.235</td>
</tr>
<tr>
<td>U. epops</td>
<td>2.584</td>
<td>0.108</td>
<td>0.216</td>
<td>4.104</td>
</tr>
</tbody>
</table>

### FIGURE 2

Indicator species distribution of bird of prey (obtained by sample sites) according to DCA
Anthropogenic activities on farmland transform ecosystems at many levels, which are likely to lead to a homogenous or heterogeneous of habitat structure and composition. Some bird species prefer homogenous habitat structure some of them prefer heterogeneous habitat composition [33]. According to DCA results, A. nisus, A. noctua, B. rufinus and F. timmunculus, were at the center of the species formed by sample sites. This species A. nisus, A. noctua, B. rufinus and F. timmunculus has a large distribution in area. And in DCA results distant species from each other C. cyanus, C. macrourus and F. naumanni has special distribution. In my another study which has not been publish yet, relationship between agricultural landscape diversity and birds species diversity have done to explain flesh DCA results out. Our findings correspond Law and Dickman [34], to the requirement by many species for multiple habitats suggests that their conservation is most effective in a mosaic environment like in this study A. nisus, A. noctua, B. rufinus and F. timmunculus species.

In this study results (Figure 2.) C. cyanus, C. macrourus and F. naumanni they are seen as distant species from each other and from other species of bird of prey we can explain that species prefer different habitats from each other. According to this indicator species determination study results, the relationship of animal species with each other it was observed that it gave more information than the results of the relationship between animal species and habitat elements.

CONCLUSIONS

Our findings can be useful for ecological planning. Our results can useful to modelling the distribution of bird species and biodiversity. Our findings suggest to widen the choice of potential bio indicators when working on farmland, not only focusing on plants, habitats but also other animal species.

Some studies reported that, the farmland bird indicator has fallen by about half over the last forty years and decline of farmland specialists is a common theme in Europe [11, 15, 35]. By the cause of changing in agricultural practices in United Kingdom wild birds farmland species decreased -47% between 1970–2008 years and decreased -4% between 1998–2008 years [36].

As a result, we argue that the farmland bird indicator is a useful surrogate for trends in other elements of biodiversity in this habitat.

REFERENCES


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EFFECT OF CARBON TETRACHLORIDE (CCl₄) AND ELLAGIC ACID ON RAT ERYTHROCYTE G6PD, 6PGD, GR, GST AND TrxR ENZYME ACTIVITIES

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Siirt University, Faculty of Veterinary Medicine, Department of Basic Sciences, Siirt, Turkey

ABSTRACT

In presented study, in vivo effects of the important polyphenol ellagic acid and CCl₄ compound known for its toxic effects on metabolic activity levels of some rat erythrocyte enzymes such as G6PD (glucose 6-phosphate dehydrogenase), 6PGD (6-phosphogluconate dehydrogenase), GR (Glutathione reductase), TrxR (Thioredoxin reductase) and GST (Glutathione S-transferase) were investigated. G6PD enzyme activity meaning rised in CCl₄ group and ellagic acid+CCl₄ group compared to the control group (p<0.001). 6PGD enzyme activity decreased in CCl₄ applied group compared to control group (p<0.05). The enzyme activity level increased in ellagic acid (p<0.05) and CCl₄+ellagic acid (p<0.01) applied groups. An increase was observed in ellagic acid (p<0.001) and CCl₄+ellagic acid (p<0.001) applied groups compared to CCl₄ administered group. GST enzyme activity decreased in CCl₄ applied group compared to control group (p<0.01). GR enzyme activity decreased significantly in CCl₄ and ellagic acid groups compared to control group (p<0.05 and p<0.01). TrxR enzyme activity decreased in CCl₄ group and ellagic acid administered group compared to control group (p<0.001). A decrease in CCl₄+ellagic acid group compared to control was also observed (p<0.001).

KEYWORDS:
Carbon tetra chloride, Ellagic acid, Metabolic enzyme

INTRODUCTION

Carbon tetrachloride (CCl₄) is a colorless, clear and volatile liquid and is widely used chemical in the manufacture of petroleum products, varnishes, polishes, resin solvents, and organic compounds. It is frequently used in dry cleaning, fire extinguishing, grain disinfection and insect control [1]. Carbon tetra chloride (CCl₄) can be taken to the body through respiration, digestion and skin. It is first accumulated in the liver and than is distributed to tissues such as the brain, kidney, muscle, lung and testis [2]. It is stated that there is a 0.1 μg CCL₄ input to the human body per day. Excretion from the body is primarily via exhalation and in very small amounts through feces and urine [3]. CCl₄ is a picky hepatotoxic chemical compound. CCl₄-induced reactive free radicals start cell injury, causing two distinct covalent binding mechanisms to the membrane proteins and lipid peroxidation [4]. CCl₄ trichloromethyl radicals, which to cause lipid peroxidation and following tissue damage, detent the formation of CCl₄ and trichloromethyl peroxy radical CCl₄OØ [5]. CCl₄-induced toxicity varies hinge on dose and time of exposure. At low doses, it leads to effects for instance bereavement of Ca²⁺ sequestration, deterioration of lipid homeostasis, release of harmful or helpful cytokines, and regeneration after apoptotic events. Exposure to high doses causes more serious effects for instance lipid degeneration, fibrosis, cirrhosis and also cancer. Fatal hepatic failure occurs in acute toxic doses [6]. It is also known for long that the inhalation of the vapor of this compound can reduce central nervous system activity and cause corruption of kidneys with a harmful and toxic effect to cells and organs [7]. Physiological effects of food and beverages for instance wine, fruits, juices, tea, vegetables, coffee, olive oil and chocolate, that are known to be rich in polyphenols are gaining interest recently as dietary sources of valuable antioxidants for human health [8]. Plant polyphenols play a significant role in human alimentation and are associated with a number of biological properties including antioxidant, anti-inflammatory, anticancer, and anti-atherosclerotic activities [9]. Ellagitannins (ETs) are bioactive polyphenols that are rich in some fruits and nuts such as pomegranate, raspberry, blackberry, strawberry, walnut, and almond. ETs belonging to the hydrolyzable tannin class of polyphenols are complex reproduction of ellagic acid (EA) [10]. Ellagic acid is a dimeric reproduction of gallic acid found in ligneous plants, fruits, nuts [11]. Antioxidant, anticarcinogenic, antifibrosis, antiplazmodial, chemopreventive, antimutagen, anti-inflammatory, and cardioprotective activities of ellagic acid were reported [8,12]. Studies also show that antioxidant-containing natural extracts protect against increased levels of lipid peroxide from CCl₄ and degradation in hepatic conditions [13].
available study, the effects of hepatotoxic compound CCl4 and antioxidant properties ellagic acid on the metabolic activity levels of some rat erythrocytes enzymes such as the G6PD (glucose 6 phosphate dehydrogenase), 6PGD (6-phosphogluconate dehydrogenase), GR (Glutathione reductase), TrxR (Thioredoxin reductase), and GST (Glutathione S-transferase) were evaluated.

### MATERIALS AND METHODS

**Preparation of Hemolysate.** Fresh blood examples were placed in EDTA tubes. The tubes were then centrifuged for 15 min (2500×g) and plasma and leukocytes were thrown. The packaged red cells were washed three times with KCl solution (0.16 M). Blood samples were centrifuged at each time (2500×g) and supernatants were thrown. The obtained erythrocytes were hemolysed using 5-fold distilled water. To remove cell membranes, the examples were centrifuged at +4 °C (10000×g) for 30 min. After discarding the cell membranes, the supernatant was removed and stored for use in enzyme activity measurements [14].

**Determination of metabolic enzyme activities.** The activity of G6PD and 6PGD enzymes was evaluated spectrophotometrically at 340 nm [15]. In the activity measurement of GR enzyme, the maximum absorbance value of NADPH reacted at 340 nm was used. The GR enzyme causes NADPH to decrease in the catalyzed reaction. The enzyme activity was determined while monitoring this decrease spectrophotometrically at 340 nm [16]. The DTNB process was used to measure the activity of thioredoxin reductase (TrxR) enzyme. In this process, thioredoxin reductase is based on NADPH-dependent catalysis of the reduction of disulfide bonds in DTNB [17-18]. GST enzyme activity was evaluated according to the proposed process defined by Habig et al [19].

### RESULTS

The G6PD enzyme activity (Fig.1) increased significantly in the CCl4 group compared to the control group (p<0.001). The enzyme activity in the ellagic acid group was very close to control group. In ellagic acid+CCl4 group, enzyme activity was decreased compared to CCl4 group but rised compared to control group and this rise was statistically meaning (p<0.001).

The decrease observed in the CCl4 applied group compared to the control group was significant (p<0.05) for the 6PGD enzyme activity (Figure 2). The increase in enzyme activity was statistically significant in the groups with ellagic acid (p<0.05) and CCl4+ellagic acid (p<0.01) compare to the control group. It was determined that the increase in ellagic acid (p<0.001) and CCl4+ellagic acid (p<0.001) groups was significant when compared with the CCl4 group.

Compared to the control group, a statistically meaning decrease in the GST enzyme activity was observed in the CCl4 group (p<0.01). In the ellagic acid group, the enzyme activity decreased compared to the control group. The change in the enzyme activity was statistically meaning (p <0.001). In the CCl4+ellagic acid group, a statistically non-important increase in enzyme activity was estimated compared to the control group. Again, the observed increase in the GST enzyme activity of CCl4+ellagic acid group (p<0.001) was found to be significant in comparison to CCl4 group (Figure 3).

**FIGURE 1**

Influence of Ellagic acid and CCl4 on G6PD Activity (a-a1: p<0.001)
A statistically significant decrease in the GR activity level (Fig. 4) was observed in CCl₄ and ellagic acid groups compared to the control group (p<0.05 and p<0.01). The enzyme activity of CCl₄+ellagic acid group was close to the control group. The GR enzyme activity was found to be close to each other in CCl₄ and ellagic acid treated groups.

In the CCl₄ group and in the ellagic acid treated group, there was a statistically significant decrease in the TrxR enzyme activity (Figure 5) compared to the control group (p<0.001). The decrease of CCl₄+ellagic acid group compared to control was found to be significant (p<0.001).
DISCUSSION

Free radicals lead to more than a hundred health problems in humans such as atherosclerosis, arthritis, ischemia, many tissue reperfusion damages, AIDS, central nervous system damage, gastritis and cancer. In addition to the factors such as environmental pollutants, radiation, chemicals, toxins, deep-fried and spicy foods, the physical stress oriented free radicals can also cause depletion of immune system antioxidants, and change in gene expression [20]. Antioxidants provide protection from the damage caused by uncontrolled ROS production and associated lipid peroxidation, protein damage, and DNA strand breaks [21]. Natural antioxidants found in plants are described as cleaning agents for the harmful free radicals from the human body. Plant polyphenolic compounds such as flavonoids are described as cleansers of reactive oxygen species [22]. Thus, the locution “plant phenolics” includes simple phenols, phenolic acids, coumarins, flavonoids, condensethannines, and lignins. Recently, the ability of phenolic substances containing flavonoids and phenolic acids to act as antioxidants has been extensively studied [23]. Ellagic acid is a phenolic compound naturally occurring in many plant species and can be found in fruits and nuts including raspberries, strawberries, and walnuts [24]. It has been reported to inhibit some carcinogen-induced factors and also to have chemopreventive properties [25]. In the present study, an herbal phenolic, ellagic acid, and a highly effective toxin, CCl₄, were used to evaluate the change in the metabolic activities of some the rat erythrocyte enzymes such as G6PD, 6PGD, GR, TrxR and GST. Of these metabolic enzymes, the G6PD (E.C.1.1.1.49) and the 6PGD (E.C.1.1.44) are the regulating enzymes that catalyze the first and third stage reactions of the oxidative reactions of the pentose phosphate pathway [26]. GR, belongs to a flavin family containing pyridine nucleotide-disulfide oxidoreductase. The main function of these homologous dimeric proteins is to catalyze the conversion of GSSG to GSH using NADPH as a coenzyme. The GR plays a key role in maintaining the antioxidant capacity of cells by maintaining high GSH/GSSG ratios [27]. Glutathione and Glutathione reductase (GR) are considered to be important components in clearing reactive oxygen molecules in cells [28]. GST is present in mammals, insects, fish, birds, and many microorganisms. The enzyme is most commonly present in cytosol and membrane of many organs, especially that of liver, small intestine, colon, kidney, lung, breast, muscle, spleen, testis, and placenta [29]. Glutathione S-transferases are a significant part of detoxification enzymes. They catalyze the conjugation of decreased glutathione (GSH) to some compounds containing an electrophilic and/or hydrophilic group [30]. The thioredoxin system found in both prokaryotes and eukaryotes occurs of thioredoxin (Trx), thioredoxin reductase and NADPH [26]. Together with the glutathione system, the thioredoxin system is considered to be the main regulator of the intracellular redox environment, to manage the redox regulation of several cellular processes as well as to control the cellular redox state and antioxidant defense. The system is included in the direct arrangement of many metabolic pathways including DNA synthesis, glucose metabolism, selenium metabolism, and vitamin C recycling [31]. When the results of the study were evaluated in general, we observed that the G6PD enzyme activity was rised in the CCl₄ group and the CCl₄+ellagic acid applied group compared to the control group. The 6PGD enzyme activity decreased in the CCl₄ group and increased in the CCl₄+ellagic acid applied group compared to the control group. The 6PGD enzyme activity was lower in the CCl₄ treated groups compared to the control group. In addition enzyme activities was found higher in the CCl₄+ellagic acid applied group compared to the CCl₄.
group. It was determined that the CCl₄ application caused a decrease in metabolic enzyme activities and that the ellagic acid had an increasing effect on decreased enzyme activity levels. In various studies reported earlier, ellagic acid has been reported to causes an important reduction in the number of NEMA (N-nitrosobenzylmethylamine) induced tumors in rats [32]. It was reported that SOD, CAT, and GST enzyme activities increased significantly by the addition of ellagic acid in CS-A-induced liver injury in a study where ellagic acid was used as a preservative [33]. In a different study conducted on cabontetrachloride which is known to be important in the liver toxicity, the liver GST, GSSG-reductase GSHPx, and SOD enzyme activities decreased due to cabontetrachloride application. The study also reported that with the use of an antioxidant phenolic compound ellagic acid in an oral administration reversed the decreased activity values and ellagic acid exhibited hepatoprotective activity against carbon tetrachloride both in vitro and in vivo [34]. A different study indicated that ellagic acid has a protective effect against oxidative stress [35]. The use of ellagic acid was indicated to be effective in preventing ulcerative colitis formed using dextran sulfate sodium [36]. Similarly, ellagic acid-enriched pomegranate extract was shown to reduce the development of chronic experimental colitis [37]. The prominent protective role of ellagic acid was reported against the CPM-induced kidney damage, DNA damage, and genotoxicity [38]. It was also demonstrated that the ellagic acid addition may play a preventive role against cisplatin-induced oxidative stress in rat heart and liver [39]. Based on the observed anti-glycative and anti-inflammatory effects of ellagic acid, supplementation of ellagic acid reported to help in the prevention or attenuation of diabetic renal diseases in a different study [40]. One study reported that ellagic acid protected against oxidative stress more vigorous than the vitamin E [41]. Recent researches have demonstrated that ellagic acid is better than quercetin for chemoprevention [42]. Ellagic acid has been demonstrated to rise radiation-induced oxidative stress and following cytotoxicity in tumor cells in vitro and in vivo. On the other hand, ellagic acid has been reported to protect normal cells against radiation damage [43].

**CONCLUSIONS**

In conclusion, application of ellagic acid which is known to have important antioxidant effects and CCl₄ have an effect on the activities of main metabolic enzymes such as G6PD, 6PGD, GR, GST, and TrxR. The decreased activity levels of 6PGD, GR, GST, and TrxR enzymes due to CCl₄ application was compensated with the ellagic acid application. We indicated that the antioxidant ellagic acid is effective in reversing the CCl₄-induced effects.

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**REFERENCES**


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IDENTIFICATION OF XANTHOMONAS SPP. DISEASE AGENT/S AND THE EFFECT OF CHEMICAL SEED TREATMENTS TO CONTROL BACTERIAL SPOT OF PEPPER

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ABSTRACT

Bacterial leaf spot caused by the xanthomonads is a destructive disease of both tomato and peppers. This paper aimed to identify the causative bacterial spot agent/s in Kayseri Province of Turkey. Plants of pepper with leaf lesions picked up among pepper plants in fields. Xanthomonad-like yellow pigmented 12 bacterial strains obtained from infected leaf samples. These strains were identified using phenotypic features like gram reaction, oxidative metabolism, growth at 40°C and on YDC medium, amylolytic and pectolytic activities. The molecular identification completed with species specific primers. All strains were gram negative, oxidative metabolism and grew at 40°C, produced yellow mucoid colonies on YDC medium and showed strong amylolytic activity, while all did not have any pectolytic activity. All strains characterized as Xanthomonas euvesicatoria according to phenotypic and molecular characterization. The study concluded that X. euvesicatoria is the prevalent causal agent of pepper bacterial spot in Kayseri province. The second aim of the study was to evaluate the potential use of chemical seed treatments including sodium hypochlorite, cupric acetate, streptomycine sulphate, acetic, citric and lactic acid in the elimination of pepper bacterial spot disease. Two experimental groups were thus designed: 1) efficacy of treatments on the survival of X. euvesicatoria on pepper seeds was observed 2) the effects of seed treatments on germination and disease development on seedlings at chamber room conditions were evaluated. The treatments were used in both experiments negative (with water only) and positive (only pathogen) control, immersion seeds into sodium hypochlorite (3%, 1, 3 or 5 min), streptomycine sulphate (0.02 g per liter), cupric acetate (0.2%, 1, 3 or 5 min), acetic and citric acid (1%, 1, 3 or 5 min) and lactic acid (2%, 1, 3 or 5 min). All treatments were statistically significant. The efficacies of treatments on disease incidence and severity were greater than 89%. Additionally, these treatments had no adverse effect on seed germinations. The study recommended seed treatments with sodium hypochlorite (3%, 3 and 5 min), copper acetate (0.2%, 3 and 5 min), acetic and citric acid (1%, 1, 3 and 5 min) and lactic acid (2%, 1, 3 and 5 min) as the most suppressive treatments to control bacterial spot disease symptoms on pepper. The seed treatments have potential to control the disease in nurseries.

KEYWORDS:
Pepper, control, seed, occurrence, plant disease

INTRODUCTION

Pepper (Capsicum annuum) is grown on over 47,466 ha in Turkey with a total annual production of 1,528 million tons [1]. In Turkey, pepper is consumed as fresh vegetable and processed pepper paste and powder for cooking or spicing foods. Bacterial spot, caused by Xanthomonas spp., is distributed one of the main diseases of pepper around the world [2]. It occurs predominantly during the rainy season with high temperatures [3]. The presence of the disease has been reported from all continents of the world including Africa [4], America [5], Asia [6], Australia [7] and Europe [8]. In Turkey, disease has been reported in tomato [9, 10] and pepper [11] producing regions. Based on DNA-DNA hybridization, four genetically distinct species, namely Xanthomonas euvesicatoria, X. vesicatoria, X. perforans and X. gardneri are reported as the causal agent of bacterial spot of pepper and tomato [5]. In Turkey, Xanthomonas euvesicatoria and X. perforans have been reported on both pepper and tomato [12].

Plant debris, infected seeds and volunteer plants may serve as inoculum sources of the disease [13]. The bacterial spot pathogens can infect all aboveground parts including pepper leaves, petals, stems and fruit stalk. The spots are water-soaked during rainy periods. Yield reductions can result from defoliation, leaf abortion and fruit damage [14]. In several countries including Turkey, coppermancozeb combinations are widely used in disease control. Chemical control of the disease is a challenge due to development of copper and streptomy-
cin resistant bacterial spot pathogen populations ([15, 16], high variability of the causative agent/s and presence of races [5]. Since the pathogen is seed-borne, survives in infected plant debris, presence of copper-resistant Xanthomonas spp., populations, bacterial spot management is collapsed. Cultural practices do not provide a sufficient reduction of the disease. Thus, a range of disease management strategies must be integrated [17].

Three single dominant genes designated as Bs1, Bs2 and Bs3 have been identified for resistance to the pathogen [18]. None of these genes confers resistance to all Xanthomonas strains in pepper. In Turkey, plant breeding based attempts are increased, however, most of newly developed pepper varieties in the market are susceptible to bacterial spot. Rotation of fields may help to avoid transmission of inoculum on volunteer plants and crop residue. Disease is getting common in nurseries both on pepper and tomato as a result of high humidity, a large amount of seedlings have to be destroyed due to bacterial spot infection. The critical control measurement in nurseries is to use pathogen-free seeds or reduce pathogen inoculum on/in seeds.

For decades, seed treatments are highly recommended and employed to eradicate or reduce pathogen transmission [19]. Natural antimicrobial compounds can be used for seed disinfection as an alternative treatment. Essential oils and/or extracts of some plants like Allium sativum, Eucalyptus sp., Rosmarinus officinalis, Satureja hortensis, S. spicigera, Origanum onites and O. rotundifolium have a potential to control seed infestation of Xanthomonas spp. in pepper and tomato [20, 21]. The organic acids, acetic acid and lactic acid were effective for control of wheat common bunt in reducing infection levels [22, 23]. Bacteriophages, biological control agents and SAR inducers have been successfully used for managing tomato plant bacterial diseases [24, 25, 26, 27, 28].

In the first aim of this study, researcher has identified the causal agent/s of pepper bacterial spot in Kayseri Province of Turkey using phenotypic and molecular test procedures.

The use of healthy seeds is the most important factor for controlling the seed-borne diseases. Therefore, this paper has assessed the efficacy of chemical seed treatments against Xanthomonas spp. both on pathogen development and seed germination.

MATERIALS AND METHODS

Bacterial strains and culture conditions. The 12 strains of Xanthomonas spp. were obtained from pepper producing fields in Kayseri Province, Turkey during the years of 2016 and 2017. Strains were grown on King’s Medium B (KB) and incubated at 28 °C for 48 h. For long periods, strains were kept in 30% glycerol (v/v) at -80 °C.

Phenotypic and molecular characterization of Xanthomonas spp. strains. The putative xanthomad strains were subjected to standard biochemical and physiological tests [3, 5, 29]. All strains were tested for gram reaction, oxidative metabolism, growth at 40°C and on YDC medium, pectolytic and amylolytic activities. All the biochemical tests were repeated once.

DNAs of bacterial strains were extracted from a freshly grown bacterial culture on NA medium for 48 h at 28 °C. DNA was isolated with the aid of DNeasy Blood &Tissue Kit (Qiagen, GmbH Germany) according to manufacturer’s instructions. Molecular characterization was determined by PCR with species-specific primer pairs Bs-XeF/Bs-XeR, Bs-XVF/Bs-XvR, Bs-XgF/Bs-XgR and Bs-XpF/Bs-XpR for X. euvesicatoria, X. vesicatoria, X. gardneri and X. perforans, respectively [30]. PCR reactions were performed in a final volume of 25 µl containing 12.5 µl of DreamTaq master mix (THERMO Fisher Scientific, Vilnius, Lithuania), 1 µl of each primer (10 pmol/ µl), 1 µl genomic DNA and 9.5 µl Milli-Q water and the PCR amplification programmes were based on [31].

Pathogenicity tests. Pathogenicity tests were conducted on local pepper cultivar Bursa Yaglik 016 and tomato cultivar H-2274 plants. For each strain, three tomato and pepper plants were inoculated. The bacterial suspension (1x10⁶ CFU/ml) was prepared from 48-h-old culture grown on YDC medium. Suspension was sprayed to the pepper and tomato leaves, petioles and stems until runoff. Sterile distilled water was used as negative control. All inoculated plants were subsequently incubated at 27±3°C and with 85–95% relative humidity in a chamber room to favour symptom development. The pathogenicity tests were repeated once.

Pathogen, growth conditions and inoculum preparation. Xanthomonas euvesicatoria strain SH-5 was used in this study for seed inoculations. The strain was grown on KB and incubated at 25 °C for 48 h. X. euvesicatoria suspension was generated in saline buffer (NaCl 0.85%) and the concentration was adjusted to A₆₀₀=0.2 OD with a spectrophotometer (Shimadzu UV-120-01, North America). The bacterial suspension was ten-fold serially diluted with saline buffer and 100 µl aliquots were spread onto KB. The concentration of the bacterial inoculum was estimated to be 3.6x10⁹ CFU ml⁻¹ and this concentration was used in further studies.

Inoculation of pepper seeds and chemical seed treatments. Pepper seeds of the susceptible cultivar BT Bursa Yaglik 016 (Bursa Seed, Bursa, Turkey) were immersed into 250 mL of a X. euvesicatoria strain SH-5 suspension for 30 min and fil-
tered through double-layered cheesecloth. Seeds were air dried at room temperature (25±2°C) for 24 h in a laminar flow and divided into three lots (100 seeds/lot) before seed treatments. The chemical treatments applied to pepper seeds and the exposure times were as follows (per 0.1 liter sterilized water): streptomycin sulphate, 0.02 g for 5 min; cupric acetate, 0.2% for 1, 3, or 5 min; sodium hypochloride, 3% for 1, 3, or 5 min; acetic acid 1% for 1, 3, or 5 min; citric acid 1% for 1, 3, or 5 min and lactic acid 2% for 1, 3, or 5 min. The solutions were freshly prepared before immersion of pepper seeds. Each sample (n=300 seeds) was rinsed as previously described and treated seeds transferred to dry in a laminar flow hood for 24 h at room temperature.

**Efficacy of treatments on the survival of X. euvesicatoria on pepper seeds.** The colonization ability of X. euvesicatoria was compared on treated and untreated seeds. Initially, X. euvesicatoria strain SH-5 was gradually maintained resistant to rifampicin antibiotic (100 mg/l). Pepper seeds (n=17 g) were inoculated with pathogen suspension as previously described. Two days after inoculation, each sample (n=1 g) of pathogen-inoculated seeds was treated with the chemicals as described above. Negative control pepper seeds (n=1 g) were treated with sterile distilled water. All treated pepper seeds were shaken on a shaker at 200 rpm for 30 min and air-dried in a laminar flow cabinet for 24 h. Each seed sample was put into glass tubes containing 9 ml sterilized nutrient broth and shaken at 250 rpm for 3 h. Each treatment was ten-fold serially diluted with nutrient broth including 1/10, 1/100, 1/1000 and 1/10 000 serial dilutions. Aliquots of 100 µl were spread onto KB medium supplemented with rifampicin, 100 mg/l, and incubated at 25°C for 4-6 days. Bacterial colonies were counted and the results were compared with positive control. Mean bacterial populations were calculated using the plate-count technique (number of colonies/dilution of sample*10).

**Efficacy of seed treatments at chamber room conditions.** Artificially inoculated and treated pepper seeds (n=150 seeds per treatment) were sown in small plastic pots 20x12x3 cm (lengthxwidthxheight) containing sterilized soil and incubated under climatic room with the following conditions: 25-28°C, 75% humidity and 16 h of light alternating with 8 h of darkness. Twenty days after planting, each cotyledon and true leaves were examined for spot symptoms. Bacterial spot seedling transmission (number of seedlings displaying typical bacterial spot symptoms divided by the number of seedlings that germinated*100) was recorded. Disease severity was evaluated visually and scored using a modified range of 0 to 3 (0 shows a healthy-looking plant; 1 signifies 1-2 spot on cotyledon; 2 signifies 3-4 spots; and 3 signifies more than 5 spots), as previously described [32]. Efficacy of seed treatments on seed germination percentage (number of germinated seedlings divided by the number of seeds that sown*100) were also determined. After germination, re-isolations completed from disease-infected seedlings to reveal the causative agent as X. euvesicatoria.

**Experimental design and statistical analysis.** The seeds subjected to chemical seed treatments were randomly placed in a growth chamber. Each treatment was replicated three times and each plastic pot containing fifty seeds was used per replicate. Data from each pot was combined and the entire experiment was analyzed by analysis of variance (ANOVA) at P≤0.05. The efficacy of treatments was evaluated using the Abbott formula (control-treated/control*100). All experiments were repeated twice in controlled conditions.

**RESULTS**

**Phenotypic and molecular characterization of Xanthomonas spp.** The 12 strains of Xanthomonas spp. from pepper were gram negative, had oxidative metabolism and grew at 40°C. All of them produced yellow mucoid colonies on YDC medium and showed strong amylolytic activity, while the strains did not have any pectolytic activity. PCR results showed the amplification of a DNA fragment of 173 bp with primers BS-XeF/BSXeR, specific for X. euvesicatoria, for all characterized strains. There was no amplification for the primers BS-XvF/BS-XvR, BS-XpF/BS-XpR and BS-XgF/BSXgR specific for X. vesicatoria, X. perforans and X. gardneri, respectively. Based on these results, it was concluded that X. euvesicatoria is the prevalent causal agent of pepper bacterial spot in Kayseri province.

**Pathogenicity tests.** The plants were periodically monitored for symptom development up to 4 weeks. Typical bacterial spots developed on both tomato and pepper plants three weeks after inoculations. Koch’s postulates were accomplished by performing re-isolations of the inoculated strains on KB medium, and their identity was checked using species-specific polymerase chain reaction (PCR) primers. No symptoms appeared on control plants.

**Efficacy of treatments on the survival of X. euvesicatoria on pepper seeds.** The forming colonies on plates were recorded after five days of the seed treatments. In control plates, 102 mean bacterial colonies were counted on three ten-fold diluted petri dishes. Seed treatments conducted with sodium hypochlorite (3%, 1 min), copper acetate (0.2%, 1 min), and streptomycine sulphate reduced bacterial colonization on seed compared to control plates.
(Table 1). No bacterial growth was observed on the plate dilutions of sodium hypochlorite (3%, 3 and 5 min), copper acetate (0.2%, 3 and 5 min), acetic acid, citric acid and lactic acid treated pepper seeds.

**Efficacy of seed treatments at chamber room conditions.** All chemical seed treatments significantly reduced bacterial spot development on pepper seedlings (Table 1). Seed treatments; sodium hypochlorite (3%, 3 and 5 min), copper acetate (0.2%, 3 and 5 min), acetic acid (1%, 1, 3 and 5 min) citric acid (1%, 1, 3 and 5 min) and lactic acid (2%, 1, 3 and 5 min) were totally suppressed bacterial spot growth in both experiments. Disease incidence and severity were 62.50% and 56.10% in positive control seedlings. Foliar disease incidence in treated seeds ranged from 0.00% to 6.48% and severity ranged from 0.00% to 4.08%, respectively. The efficacies of treatments on disease occurrence and severity were greater than 89%. All treated seeds displayed 91.33% to 100% germination and incidence were 62.50% and 56.10% in positive control seedlings. Foliar disease incidence and severity were 62.50% and 56.10% in positive control seedlings. All treated seeds showed 91.33% to 100% germination and treatments did not significantly reduce seed germinations (Table 1). According to both studies, seed treatments with sodium hypochlorite (3%, 3 and 5 min), copper acetate (0.2%, 3 and 5 min), acetic acid (1%, 1, 3 and 5 min), citric acid (1%, 1, 3 and 5 min) and lactic acid (2%, 1, 3 and 5 min) were the most suppressive treatment reaching to 100% both in bacterial colonization and seed treatments (150 seeds per treatment).

**DISCUSSION**

This study reports the occurrence bacterial spot of pepper in Kayseri province and characterization of the causal agent, *X. euvesicatoria* for the first time. Like our study [12] characterized this pathogen in her MSc thesis as the prevalent agent among eight out of 10 strains tested. Some phenotypic characteristics of the pepper strains were not similar to the overall description of the species. The amylolytic and pectolytic activity features were different. All tested strains have shown amylolytic activity, but no pectolytic activity. The type strain of *X. euvesicatoria* isolated from pepper in the USA [5] was weakly amylolytic and pectinolytic, while most of the pepper strains from Grenada [33] and Brazil [34] did not have any amylolytic and pectinolytic activity. However, [35] and [3] reported that some *X. euvesicatoria* strains could hydrolyse starch similar to the results obtained in our study. The absence of pectolytic activity of *X. euvesicatoria* strains was previously reported also from Brazil [34], Iran [6] and USA [5]. Contrary to this study, few *X. euvesicatoria* strains isolated from pepper in Grenada showed pectolytic activity [33]. All those results conclude that *X. euvesicatoria* strains are characterized by a high phenotypic diversity, thus, these features are not helpful to fulfill the identification of the strains.

Results of pathogenicity tests showed that pepper strains can infect tomato plants as described previously [5]. This has a great importance for epidemiological aspects since tomato and pepper are widely produced in Turkey and in the same area for years. Also *X. euvesicatoria* can survive on other crops like common bean and nightshade plants which can serve as an important alternative hosts for the pathogen [6]. For this reason, common bean is not suitable for a rotation and cultural practices are so important in/around production areas.

**TABLE 1**

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Cells/g seed</th>
<th>Incidence%</th>
<th>Efficacy%</th>
<th>Severity%</th>
<th>Efficacy%</th>
<th>Germination%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive Control</td>
<td>1.04*10^6</td>
<td>56.10a</td>
<td>62.51a</td>
<td>94.67a</td>
<td>100a</td>
<td></td>
</tr>
<tr>
<td>Streptomycin sulphate</td>
<td>2*10^3</td>
<td>0.48b</td>
<td>99.15</td>
<td>6.48b</td>
<td>89.63</td>
<td>92.00a</td>
</tr>
<tr>
<td>Cupric acetate, 0.2% 1 min</td>
<td>3.9*10^3</td>
<td>0.97b</td>
<td>98.26</td>
<td>6.02c</td>
<td>90.37</td>
<td>91.33a</td>
</tr>
<tr>
<td>Cupric acetate, 0.2% 3 min</td>
<td>0.00</td>
<td>0.00c</td>
<td>100</td>
<td>0.00d</td>
<td>100</td>
<td>98.67a</td>
</tr>
<tr>
<td>Cupric acetate, 0.2% 5 min</td>
<td>0.00</td>
<td>0.00c</td>
<td>100</td>
<td>0.00d</td>
<td>100</td>
<td>94.67a</td>
</tr>
<tr>
<td>Sodium hypochloride 3% 1 min</td>
<td>1*10^3</td>
<td>2.81b</td>
<td>94.99</td>
<td>6.17b</td>
<td>90.12</td>
<td>94.00a</td>
</tr>
<tr>
<td>Sodium hypochloride 3% 3 min</td>
<td>0.00</td>
<td>0.00c</td>
<td>100</td>
<td>0.00d</td>
<td>100</td>
<td>97.33a</td>
</tr>
<tr>
<td>Sodium hypochloride 3% 5 min</td>
<td>0.00</td>
<td>0.00c</td>
<td>100</td>
<td>0.00d</td>
<td>100</td>
<td>100.00a</td>
</tr>
<tr>
<td>Acetic acid 1% 1 min</td>
<td>0.00</td>
<td>0.00c</td>
<td>100</td>
<td>0.00d</td>
<td>100</td>
<td>96.00a</td>
</tr>
<tr>
<td>Acetic acid 1% 3 min</td>
<td>0.00</td>
<td>0.00c</td>
<td>100</td>
<td>0.00d</td>
<td>100</td>
<td>92.00a</td>
</tr>
<tr>
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<td>0.00</td>
<td>0.00c</td>
<td>100</td>
<td>0.00d</td>
<td>100</td>
<td>94.00a</td>
</tr>
<tr>
<td>Citric acid 1% 1 min</td>
<td>0.00</td>
<td>0.00c</td>
<td>100</td>
<td>0.00d</td>
<td>100</td>
<td>98.00a</td>
</tr>
<tr>
<td>Citric acid 1% 3 min</td>
<td>0.00</td>
<td>0.00c</td>
<td>100</td>
<td>0.00d</td>
<td>100</td>
<td>96.67a</td>
</tr>
<tr>
<td>Citric acid 1% 5 min</td>
<td>0.00</td>
<td>0.00c</td>
<td>100</td>
<td>0.00d</td>
<td>100</td>
<td>95.33a</td>
</tr>
<tr>
<td>Lactic acid 2% 1 min</td>
<td>0.00</td>
<td>0.00c</td>
<td>100</td>
<td>0.00d</td>
<td>100</td>
<td>95.33a</td>
</tr>
<tr>
<td>Lactic acid 2% 3 min</td>
<td>0.00</td>
<td>0.00c</td>
<td>100</td>
<td>0.00d</td>
<td>100</td>
<td>96.00a</td>
</tr>
<tr>
<td>Lactic acid 2% 5 min</td>
<td>0.00</td>
<td>0.00c</td>
<td>100</td>
<td>0.00d</td>
<td>100</td>
<td>98.00a</td>
</tr>
</tbody>
</table>

* Means followed by the same letters within each column are not significantly different (P≤0.05) according to Duncan’s Multiple Range Test
PCR results showed the amplification of 173 bp DNA fragment with \emph{X. euvesicatoria} specific primers. Most of the researchers have the similar results indicating that the pathogen is predominant [34, 6]. Since the first report of pepper bacterial spot in Turkey [11], there is no comprehensive study about all \emph{Xanthomonas} species. No other tomato and pepper infecting \emph{Xanthomonas} species observed in this study. It can be explained that the strains were isolated from a restricted area, thus, future studies with strains from other cities of Turkey and worldwide will help for an extensive genetic diversity.

Bacterial diseases of tomato are the major economic threat to this crop worldwide. Bacterial spot is seedborne and seriously affect both tomato and pepper crop yield and quality. Since the seeds are the primary inoculum sources, seed treatments have gained importance to control the disease. In this study, all tested chemical seed treatments significantly reduced bacterial spot development on pepper seedlings. Citric acid is used to control yeasts and bacteria in human health [36, 37], plant pathogenic fungi [38] and some tomato infecting pathogenic bacteria like \emph{Clavibacter michiganensis subsp. michiganensis} and \emph{Pseudomonas syringae pv. tomato} [39]. Recently, [40] evaluated the bactericidal activity of a commercial compound, Den-tamet, containing zinc and copper complexed with citric-acid hydrazides against the bacterium \emph{Xylella fastidiosa subsp. pauca} in Italy. The authors suggest to include this product as spring and summer spray treatments to the integrated management to reduce severity of the pathogen. No published paper is in hand about the inhibition potential of citric acid on bacterial spot of pepper. The present study have revealed that citric acid at the ratio of 1% have a potential to control bacterial spot as seed treatment in nurseries and non phytotoxic. Citric acid is in hand about the inhibition potential of citric acid (0.25% or 0.5%) eradicated \emph{Cnm} from the tomato seeds and soaking seed at 52 °C for 20 minutes with acetic acid (0.25% or 0.5%) resulted in the best reduction of saprophytic bacteria on/in tomato seeds. The scientists recommended this treatment (0.5%) as seed treatment because commercial seedlots may vary in their response to the treatments, that’s why a small sample should be tested in order to check the adverse effect on the germination [41]. Since acetic acid treatment (1%) did not have any adverse effect to pepper germination and eliminated pathogen from pepper seeds, efficacy of cupric acetate (%0.2) has varied. This can be explained with the low dosage of cupric acetate, bacterial resistance to copper compounds [16].

Organic acids like acetic acid, citric acid and lactic acid were effective in reducing infection levels caused by plant pathogenic fungi and bacteria [22, 42, 43]. Like our study, [44] found no significant effect on bacterial count on seeds with the tested organic acids and the reduction was more than 99%. Moreover, seed germination qualities were not significant [45] tested apple and raisin vinegars (acetic acid) as organic acids to control bacterial wilt infected tomato seeds. The authors recommended these seed treatments to be used in organic and conventional farming systems. Our study advised all seed treatments except streptomycin to be used in both growing systems to eliminate bacterial spot from pepper seeds.

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**REFERENCES**


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EVALUATION OF ENVIRONMENTAL BACKGROUND VALUE OF SURFACE SOIL BY SPATIAL AND STATISTICAL ANALYSES

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ABSTRACT

Evaluation of the local environmental background is essential for the environmental management. In this study, statistical and spatial analyses have been applied for the aluminum and lead concentrations of 127 surface soil samples in the farmland of the urban-rural joint area of Suzhou, Anhui province, China, for the evaluation of environmental background. The results show that these soil samples have average enrich factors lower than 1 for both aluminum and lead, together with their low values of coefficient of variation and p-values of normal distribution test, indicating that there is no and slightly anthropogenic contributions for the aluminum and lead, respectively. Some hotspots and anomaly areas have been identified based on the spatial distribution and the spatial autocorrelation analysis, and the transportation related gasoline burning is identified to be the main source for releasing lead into the environment. The environmental background values have been calculated to be 2.18-3.27% and 7.74-18.4 mg/kg for aluminum and lead based on the spatial autocorrelation analysis, respectively, similar to the results obtained by statistical analyses, however, the former based on the hypothesis of normal distribution of background concentrations.

KEYWORDS:
Environmental background, spatial autocorrelation analysis, statistical analysis, heavy metals, soil pollution.

INTRODUCTION

Geochemical background, as defined by Hawkes and Webb [1], was the normal abundance of an element in barren earth material, which reflects natural processes unaffected by human activities [2-4], which was namely environmental background in environmental studies. However, such a background no longer exists due to human influence, such as agriculture, industry and urbanization [5], and the current situation of the environment is a “mixture” of environmental background plus human contribution.

The environmental background is essential for environmental studies, as it is the basis for environmental quality evaluation, and the goal of environmental restoration. Environmental background can be defined and quantified in several ways with different outcomes [5], and numerous scientists have attacked the problem by generating various statistical procedures. Some methods assume normality, leading to trimming or normalization procedures, while others are based on more generally applicable non-parametric methods [4]. Reimann et al. [5] compared three statistical approaches and concluded that only the cumulative probability plot, or the Q-Q plot provide a clearer answer to the background question. Moreover, it is more realistic to view environmental background as a range of values rather than an absolute value because of it changes both regionally with the basic geology and locally with the type and genesis of overburden [3]. For example, the soils originated from weathering of basalts have higher concentrations of Cr and Ni than soils from granite weathering.

Heavy metal pollution is a worldwide concern currently [6], because the pollutions of heavy metals have affected the human health through the food chain, and lead to bad effects for the growth of people (e.g. the cadmium causes a degenerative bone disease, and the mercury and lead damage the central nervous system), although they occurred in water and soil primarily. And therefore, a large number of studies related to the heavy metal pollution have been processed [7-10].

China has undergone fast development during the last three decades. However, coupled with the lack of pollution controls, human activities associated with these developments have caused significant impacts on the local environment [11]. An increase in contaminant emission may have a substantial impact on local agriculture, as chemical materials may enter and accumulate in soils through agricultural activities and atmospheric deposition, which could enhance the risk of chemical contamination of the food chains [12]. Therefore, a series of studies related to toxic elemental pollution of soils, including evaluation of pollution degree, source
identification of metals, as well as remediation of soils have long been carried out [13, 14]. Among these studies, the pollution degree evaluation is essential. However, most of the studies use environmental background value of China as a reference because of the lack of local background values, and which can lead to higher or lower estimating of the pollution degree because of the inhomogeneous of background levels.

Suzhou is a “black-gold” city because coal and agriculture are dominant industry, and therefore, pollution of heavy metals is important for the sustainable development of the city, and a series of studies have been carried out and focused on the pollution degree evaluation and source identification of heavy metals in the soils [15, 16]. However, there is no official local background values have been reported, but all of these studies use the environmental background of China for comparison, which is also unreasonable.

Taking into account the lacking of the soil environmental background value of heavy metals in the city, a total of 127 surface soil samples in the farmland of the urban-rural joint area of Suzhou have been collected and measured for their aluminum and lead concentrations, and then analyzed by spatial (autocorrelation analysis) for the evaluation of environmental background of lead. The result was then compared with the results obtained by previous reported methods (e.g. model based objective method, QQ plots and regression methods), which can provide a new approach for the evaluation of the soil environmental background.

**MATERIALS AND METHODS**

**Sampling and analysis.** Suzhou is located in the northern Anhui province, eastern China with its longitude between 116°09’ and 118°10’, and the latitude is between 33°18’ and 34°38’ (Fig. 1). The length from east to west is 195 km and the width from south to north is 151 km, the total area of the city is 9787 km². The city is located in the transition zone of warm and subtropical climate, and belongs to warm temperature monsoon climate. The city’s land area is 7.8 million acres and the plant area is 15.6 million acres with 200% multiple cropping index. The main crops in the area include wheat, corn, soybean, cotton, potato, rapeseed, peanuts and fruit etc.

A total of 127 surface soil samples (less than 10 cm depth) located in the urban-rural joint area of Suzhou (east to the city) have been collected. All of the samples were collected from the farm land randomly in June, 2014 (Fig. 1). Samples were firstly air-dried in natural conditions, and the debris of animals and plants had been removed by hands. Then, the samples were powdered to 200 meshes (<0.075mm) after parching for 24 h at 80°C in a dryer. Samples were made into tablets by using a 30t condenser, and then analyzed by XRF (Innov-X Explorer 9000 SDD, USA) for measuring the concentrations of Al and Pb in the Engineering and Technological Research Centre of Coal Exploration, Anhui Province, China. National standard sediment sample of China (GBW07307) was analyzed simultaneously for calibration (once per ten samples), and the relative standard derivation is less than 10%.

**Data treatment.** All of the data were firstly processed for statistical analysis by the Mystat 12 software, and the minimum, maximum, mean, standard deviation, coefficient of variation and the p-value of the normal distribution test have been obtained. And then, the contour map of the lead concentrations was plotted by the Surfer 11 software (with natural neighbor grid method). Finally, the spatial autocorrelation analysis processed by the Geoda 1.8.3 software, for obtaining the significant map and the spatial clustering map.

**FIGURE 1**

Location of the study area and the sample distributions.
Previous studies revealed that the environmental background value is the concentrations or range of concentrations of the elements in the relative clean area (with low or no contribution from the anthropogenic activities) [1]. Currently, there are two kinds of methods for determine environmental background values, including the direct (geochemical) and indirect (statistical) methods [4]. Among these two kinds of methods, the direct method is often criticized as having subjective sample selection criteria, high costs, and heavy laboratory workload, and therefore, the application of the direct method is limited. Comparatively, the indirect (statistical) methods have been used more frequently, which used not only for assessing background concentrations, but also for the separation of geochemical anomalies from geochemical background. The methods include regression analysis always applied for soil environmental background calculation [17], the fractal method (C-A and S-A) for identification of anomaly during mineral exploration [18] and the probability plots applied for environmental back- ground calculation of the nitrate in the groundwater [19].

Although some of the studies prefer to use normal or lognormal distribution as the basis for the calculation of the environmental background value [5], it has long been demonstrated to be not true [20]. In this study, two kinds of methods have been applied for the evaluation of the environment background value:

1. Traditionally: the box plot by the Mystat 12, which based on the assumption that the environmental background value in line with the normal distribution. After processing, the samples outside of the lower and upper hinge of the box plot were removed (repeated until no abnormal samples), and then calculate the mean and standard deviation of the rest of the samples. The environmental background value was then calculated to be mean ± 2*standard deviation.

2. Spatially: based on the spatial autocorrelation analysis, the basis is that there is no significant change of the concentrations in the sample relative to its nearby ones, or namely “no mutation”. Therefore, only the “non-significant” samples (not belong to the high-high, low-low, high-low and low-high clusters) [21] after spatial autocorrelation analysis have been considered to be environmental background samples, and the environmental background value was also calculated by the mean ± 2*standard deviation of them.

For comparison, some of the commonly used methods, including the iterative standard deviation, distribution function method [22] and QQ plot [19], as well as the regression methods [17] have also been applied for comparison.

RESULTS AND DISCUSSIONS

Concentrations of aluminum and lead. The concentrations of the aluminum and lead are synthesized in the Table 1. As can be seen from the table, the aluminum and lead of the soil samples are 1.48-3.33% (mean = 2.78%) and 7.00-21.0 mg/kg (mean = 13.1 mg/kg). Previous studies revealed that AEF value (Average Enrich Factor = AC/BC, where AC and BC are the average concentrations of sample and background, respectively) is a good indicator for monitoring the degree of pollution, and 4 degrees had been subdivided: <1 means light pollution, 1-3 means moderate pollution, and >3 means considerable pollution [23]. In comparison with the soil environmental background value of China [24], both aluminum and lead have AEF lower than 1, which indicates that there is no pollution of these two elements. However, a comparison with the local background values is considered to be more meaningful as the background values all over China are heterogeneous [24].

Coefficient of variation (CV=standard deviation/mean) is an index can be used for identifying the anthropogenic contribution degree for the pollution in the environmental studies [25]. Previous studies revealed that when CV < 0.10 and > 0.90 mean low and high anthropogenic contributions, respectively. In this study, the CVs of the aluminum and lead concentrations of the soil samples are 0.096 and 0.208, respectively, which indicates that the soil samples have not or, only slightly influenced by anthropogenic activities. However, the p-values of the normal distribution test for aluminum and lead are > and <0.05, respectively, implying that the aluminum concentrations in this study can pass the normal distribution test (p > 0.05), whereas the lead cannot, which also suggests that there is no anthropogenic contribution for the aluminum, but slightly contribution for the lead.

Spatial distribution. The contour map has long been used for environmental studies because of the visualization of the pollution [15]. As can be seen from the contour map of the aluminum and lead concentrations in Fig. 2, there is only one small area with relative high aluminum can be found in the southwest of the study area, whereas there are four areas with relative high lead concentrations in the west, southwest, center and north-center of the study area. Our observation during sampling confirmed no special for the high aluminum area, but the four high lead areas are consistent with the distribution of the areas with high density of transportation: they are corresponding to the parking lot, factory, traffic light intersection and driving school, respectively. And therefore, the high aluminum area might be a natural anomaly, whereas the high lead areas are transportation related, and the burning of gasoline is the main source.
for releasing lead into the soil environment [25].

**Spatial autocorrelation analysis.** According to the classification of Moran’s index in the local spatial autocorrelation (LISA) [21], all of the samples can be subdivided into two major categories after calculation: “not significant” and “significant”. Samples classified into the former are considered as no “mutation” relative to its nearby samples, and the samples classified into the latter can be divided into four sub categories: high-high, low-low, low-high and high-low, which represent the relationship between a sample and its surrounding ones. The first two are called as “hot spot” and “freezing spot”, respectively, which reflects the regional anomaly, such as the surface pollution, whereas the low-high and high-low samples are abnormal ones, which may be related to the influence of other factors (e.g. point pollution). The results of spatial autocorrelation analysis are shown in Fig. 3.

As can be seen from the figure, 83 samples are classified to be “non-significant” samples for aluminum, whereas the sample numbers classified to be high-high, low-low, low-high and high-low clusters are 13, 13, 1 and 17, respectively. As for the lead, 106 samples are classified to be “non-significant” samples for lead, whereas the sample numbers classified to be high-high, low-low, low-high and high-low clusters are 8, 3, 9 and 1, respectively. Such results indicate that there is one hotspot (square) of aluminum in the northwest, and three anomaly areas (triangle in southwest, southeast and north-center) in the study area. Comparatively, there is also one hotspot of lead in the southwest, and one anomaly area in the north-center of the study area.

<table>
<thead>
<tr>
<th>Element</th>
<th>N</th>
<th>Min</th>
<th>Max</th>
<th>Mean</th>
<th>SD</th>
<th>CV</th>
<th>p-value</th>
<th>BC</th>
<th>AEF</th>
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<tr>
<td>Al</td>
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<td>1.48</td>
<td>3.33</td>
<td>2.78</td>
<td>0.266</td>
<td>0.096</td>
<td>0.148</td>
<td>6.62 %</td>
<td>0.22-0.50</td>
</tr>
<tr>
<td>Pb</td>
<td>127</td>
<td>7</td>
<td>21</td>
<td>13.1</td>
<td>2.73</td>
<td>0.208</td>
<td>0.013</td>
<td>26.0 mg/kg</td>
<td>0.27-0.81</td>
</tr>
</tbody>
</table>

**FIGURE 2**

Spatial distributions of Al (%) and Pb (mg/kg) concentrations.
Environmental background value calculation. Based on the box plots (Fig. 4) of the aluminum and lead concentrations, there is one sample with aluminum concentrations lower than 2% was identified to be outlier, and there is no outlier has been identified in the box plot of lead concentrations. After the removal of the one outlier, the remaining 126 samples were calculated and the mean value was 2.80% for aluminum (with standard deviation = 0.24%). Therefore, the environmental background value calculated by the statistical method was 2.31-3.27%. As for the lead, the environmental background value calculated by the statistical method was 7.64-18.5 mg/kg (Table 2). Based on these values, one and four samples can be classified to be polluted by aluminum and lead, respectively. However, the box plot method based on the hypothesis that the samples without anthropogenic contribution were follow the normal or lognormal distribution, which has not been, and cannot be demonstrated [20].

Another commonly used method for the environmental background evaluation of soil is regression method [17]. Based on the regression analysis, the relationship between aluminum and lead is calculated to be Pb(mg/kg)=1.52×Al(%)+8.86 with 95% confidence interval. According to previous studies [17], aluminum (or iron) is an element without anthropogenic contribution, and therefore, considering with the concentrations of aluminum (1.48-3.33%), the environmental background of lead can be calculated to be 11.1-13.9 mg/kg (Table 2), and there are 51 samples with lead concentrations higher than the background. However, the

**TABLE 2**

<table>
<thead>
<tr>
<th>Method</th>
<th>Aluminum (%)</th>
<th>Lead (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Box plot</td>
<td>2.31-3.27</td>
<td>7.64-18.5</td>
</tr>
<tr>
<td>Regression analysis</td>
<td>1.48-3.33</td>
<td>11.1-13.9</td>
</tr>
<tr>
<td>Spatial autocorrelation</td>
<td>2.18-3.27</td>
<td>7.74-18.4</td>
</tr>
<tr>
<td>Iterative standard deviation</td>
<td>2.40-3.20</td>
<td>8.60-17.2</td>
</tr>
<tr>
<td>Distribution function</td>
<td>2.20-3.40</td>
<td>8.40-17.6</td>
</tr>
</tbody>
</table>
hypothesis of the regression method is that the trace elements are well correlated with aluminum in the natural condition [17]. Although there is only low degree of anthropogenic contribution for lead as revealed by its CV, p-value of normality test and the above calculated results (only four samples have been classified to be polluted), the relationship between aluminum and lead in this study is not significant with \( r=0.15 \) (\( r_a=0.17, \alpha=0.05 \)), which suggests that the result might be unreliable.

As to the spatial autocorrelation analysis, the above mentioned 83 (aluminum) and 106 (lead) samples belong to the “non-significant” category were calculated, and their mean values were 2.72% (with standard deviation = 0.27%) and 13.1 mg/kg (with standard deviation = 2.66 mg/kg), respectively. Therefore, the background values calculated by the spatial autocorrelation method were 2.18-3.20% for aluminum and 7.74-18.4 mg/kg, respectively (Table 2). Based on these values, one and four samples can be classified to be polluted by aluminum and lead, respectively, consistent with the results obtained by above statistical analysis.

For comparison, the iterative standard deviation, distribution function method [22] and QQ plot [19] have also been applied for calculation the environmental background. The results show that the environmental background values of aluminum and lead in the soil are 2.40-3.20% and 8.60-17.2 mg/kg (iterative standard deviation), respectively, and 2.20-3.40% and 8.40-17.6 mg/kg (distribution function), respectively (Table 2). The results are also similar to the results obtained by box plot analysis. Moreover, as can be seen from the QQ diagrams (Fig. 5), both aluminum (except for the lowest one sample) and lead are well following a straight line, which imply that there is no obvious anthropogenic contributions and all of them can be considered to be background samples, and therefore, the environmental background values for the aluminum and lead are at least between their minimum and maximum values, and they are 2.28-3.33% and 7.00-21.0 mg/kg, respectively. However, although the plot is easy to use, but the identification of the inflection point is subjective, which can influence the reliability of the analysis.

CONCLUSIONS

Based on the statistical and spatial analyses of the aluminum and lead concentrations of 127 surface soil samples in the farmland of the urban-rural joint area of Suzhou, Anhui province, China, the following conclusions have been made:

(1) The soils have average enrich factors lower than 1 for both aluminum and lead when comparing with the soil environmental background value of China, along with their low values of coefficient of variation and p-values of normal distribution test, implying that there is no anthropogenic contribution for the aluminum, but slightly contribution for the lead.
(2) Some hotspots and anomaly areas have been identified based on the spatial distribution and the spatial autocorrelation analysis of the concentrations, in combination with the field observation during sampling, transportation has been considered to be the most important anthropogenic contribution for the lead pollution.

(3) The environmental background values have been calculated to be 2.18-3.27% and 7.74-18.4 mg/kg for aluminum and lead, respectively, similar to the results obtained by statistical analyses. However, these two kinds of methods have different basis, the former based on the “no mutation” relative to its nearby samples, whereas the latter based on the hypothesis of normal distribution of background concentrations.

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REFERENCES


QUALITY EVALUATION AND ITS CONTROLLING FACTOR ANALYSES OF SHALLOW GROUNDWATER IN THE URBAN AREA OF SUZHOU, ANHUI PROVINCE, CHINA

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ABSTRACT

Groundwater is an important source for drinking and irrigation purposes in northern Anhui province, China, and management of this resource is significant to meet the increasing demand of water. In this study, forty-three groundwater samples collected from the First (FA, 20 samples) and Second (SA, 23 samples) loose layer aquifers in the west of Suzhou city, north Anhui Province, China, and their major ion concentrations have been analyzed by a series of chemical indexes (including the Nemerow pollution index, water quality index, sodium adsorption ratio as well as sodium percent) for the evaluation of pollution status, drinking and irrigation suitability. Then the mechanisms controlling the groundwater chemistry have been analyzed. The results indicated that the groundwater samples are neutral to slightly alkaline, 75% and 100% of the FA and SA samples have TDS contents similar to the fresh water (1000mg/L), and the hydrochemical facies of the groundwater samples are classified to be Na-HCO3, to a lesser extent, Ca-HCO3 and Mg-HCO3. Based on Nemerow pollution index, 55% and 26% of samples from the FA and SA are suffered litter pollution, respectively. Water quality index indicated that all of samples can be used for drinking proposes, and approximately 40% of the samples from the two aquifers are good for irrigation according to the analysis of sodium adsorption ratio and sodium percent. Furthermore, the Gibbs diagram, chloro-alkaline indices as well as the relationships between Na+, Ca2+, Mg2+ and HCO3- indicated that the weathering of silicate minerals and ion exchange are the main process controlling the groundwater chemistry.

KEYWORDS:
shallow groundwater, water evaluation, major ions, loose layer aquifer, hydrochemistry

INTRODUCTION

Groundwater is the most important resource in the world because of the function for domestic, agricultural and industrial use, and therefore, it is important for human life and economic development [1]. In recent years, surface water pollution has become increasingly serious, and water quality has been difficult to meet the requirements of use. Previous study indicated that near 56% of the water supply for more than 100 million people is provided by groundwater in the North China Plain [2].

Suzhou is a representative city dependent on groundwater resources. According to the Water Resources Bulletin of Suzhou in 2015, the total water consumption of the Yongqiao District was 313.9 million m³ per year, of which the groundwater consumption was as high as 312.9 million m³. As one of the major water supply sources in Suzhou, the water source of west Suzhou city can supply water to nearly 60 million people in the urban areas with more than 54 million m³ per year. In recent years, a series of studies have been carried out for the surface water [3-5], but the groundwater quality has not received corresponding attention in Suzhou, northern Anhui province, China.

Therefore, in this study, forty-three shallow groundwater samples have been collected from the First (20 samples) and Second (23 samples) loose layer aquifers in the water source of west Suzhou city, northern Anhui province, China, and the major ion concentrations have been analyzed by a series of methods for getting the information about: (1) the characteristics of major ion concentrations and hydrochemical facies; (2) evaluations of pollution status, and the suitability of drinking and irrigation and (3) identifying the mechanism controlling the groundwater chemical compositions.
MATERIALS AND METHODS

Study area. Suzhou is located in the northern part of Anhui Province with the geographical coordinates at 116°09'-118°10' and 33°18'-34°38' (Fig. 1). The climate in the area is warm and semi-humid with an average annual temperature of 14.6°C. The average annual rainfall is 865mm, most of which concentrated among June, July and August.

Previous investigations indicated that there are three aquifer systems in the study area from shallow to deep: the loose layer aquifer system, the clastic rock aquifer system and the Carboniferous limestone aquifer system. Among them, the loose layer aquifer system is located between 0-150m depth, and can be further divided into three secondary aquifers from shallow to deep: the first (FA), the second (SA) and the third (TA) aquifer. Moreover, in the study area, the groundwater from FA and SA is the main source for domestic and agricultural purpose, and the details of the two aquifers are as follows:

(1) FA: The thickness of the aquifer is near 30 m, and the lithology is dominated by silt, followed by fine sand, sub-sand, and local fine sand and silt. In the natural state, the recharge methods mainly include atmospheric precipitation infiltration replenishment, surface water infiltration replenishment and irrigation backflow replenishment.

(2) SA: Buried deep of this aquifer is between 30-100m, and the lithology is dominated by fine sand, followed by medium fine sand, silt, and local coarse sand. In the natural state, the SA can accept the overflow recharge of the upper aquifer and the lateral runoff recharge of the interlayer.

Sampling and analysis. A total of 43 groundwater samples were collected from the water source of west Suzhou city, northern Anhui Province, China. Samples F1-F20, S1-S23 were collected from the first and second loose layer aquifers, respectively. All samples were collected using pre-cleaned polyethylene bottles and then transported to the laboratory, stored in a refrigerator at a temperature of <4°C and analyzed within one week. Concentrations of major ions (Na⁺, Ca²⁺, Mg²⁺, Cl⁻, SO₄²⁻ and HCO₃⁻) have been analyzed following the methods bellow: Na⁺, Ca²⁺, Mg²⁺, Cl⁻ and SO₄²⁻ were analyzed by Ion Chromatography (ICS-600-900), whereas HCO₃⁻ were analyzed by acid-base titration. All analytical processes were conducted in the Engineering and Technological Research Center of Coal Exploration, Anhui Province, China. Moreover, the analytical results were calculated by Eq (1) and indicated that analytical errors were below 5%, which coincide with the international standard.

$$\text{AE}\% = \frac{\Sigma \text{cations} - \Sigma \text{anions}}{\Sigma \text{cations} + \Sigma \text{anions}} \times 100\%$$ (1)
RESULTS AND DISCUSSION

Major ion concentration. In this study, major ions concentrations of the groundwater samples from the FA and SA are shown in the Table 1. As can be seen from the table, the Na+, Ca2+, Mg2+, Cl-, SO42- and HCO3- concentrations from the FA range from 28-252, 20-94, 14-67, 5-95, 14-203 and 302-853 mg/L, respectively. The mean concentrations of major ions occur in order of HCO3- (519mg/L) > Na+ (118mg/L) > SO42- (72mg/L) > Ca2+ (54mg/L) > Cl- (45mg/L) > Mg2+ (42mg/L). TDS concentrations of the FA samples range from 532-1328mg/L (mean=847mg/L), and 75% of the samples have TDS lower 1000mg/L, similar to the fresh water [6, 7]. Moreover, the pH value varied from 7.1-8.1 (mean=7.7), indicated that the FA samples are neutral to slightly alkaline.

Comparatively, the groundwater samples from the SA have higher mean concentration of Ca2+, Cl- and HCO3-, but lower Na+, Mg2+, SO42- and TDS, and the Na+, Ca2+, Mg2+, Cl-, SO42- and HCO3- concentrations from the SA range from 51-202, 35-116, 25-68, 11-230, 14-110 and 437-869 mg/L, respectively. The mean concentrations of major ions occur in order of HCO3- (534mg/L) > Na+ (110mg/L) > Ca2+ (63mg/L) > Cl- (53mg/L) > SO42- (44mg/L) > Mg2+ (37mg/L). TDS concentrations of TA samples range from 427-816mg/L (mean=563mg/L), all of the them have TDS lower the fresh water (1000mg/L) [6, 7]. As to the pH value, the samples are varied from 7.1-8.1 (mean=7.7), similar to the FA samples.

Hydrochemical facies. Piper diagram [8] can provide information for understanding the hydrological evolution of study area, which is the most popular method for classifying the hydrochemical facies [9]. Taking for instance, during the groundwater flows from the recharge to the discharge zone, the dominant anion species of water will change from HCO3-, SO42- to Cl- [10, 11]. In this study, classification of groundwater hydrochemical facies is based on the concentration of major cations and anions by using Aquachem (version 3.70) and Piper diagrams (Fig. 2). As can be seen from the figure, all of the groundwater samples are classified to be HCO3- facies, including Na-HCO3 (account for 72%, 14 and 17 samples from the FA and SA, respectively), Ca-HCO3 (account for 21%, 5 and 4 samples from the FA and SA, respectively) and Mg-HCO3 (account for 7%, 1 and 2 samples from the FA and SA, respectively).

<table>
<thead>
<tr>
<th>Aquifer</th>
<th>Range</th>
<th>Na+ (mg/L)</th>
<th>Ca2+</th>
<th>Mg2+</th>
<th>Cl-</th>
<th>SO42-</th>
<th>HCO3-</th>
<th>TDS</th>
<th>pH</th>
<th>WQI</th>
</tr>
</thead>
<tbody>
<tr>
<td>FA (20)</td>
<td>Max</td>
<td>252</td>
<td>94</td>
<td>67</td>
<td>95</td>
<td>203</td>
<td>853</td>
<td>1328</td>
<td>8.1</td>
<td>27.1</td>
</tr>
<tr>
<td></td>
<td>Min</td>
<td>28</td>
<td>20</td>
<td>14</td>
<td>5</td>
<td>14</td>
<td>302</td>
<td>532</td>
<td>7.1</td>
<td>89.6</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>118</td>
<td>54</td>
<td>42</td>
<td>45</td>
<td>72</td>
<td>519</td>
<td>847</td>
<td>7.7</td>
<td>54.7</td>
</tr>
<tr>
<td>SA (23)</td>
<td>Max</td>
<td>202</td>
<td>116</td>
<td>68</td>
<td>230</td>
<td>110</td>
<td>869</td>
<td>816</td>
<td>8.1</td>
<td>30.6</td>
</tr>
<tr>
<td></td>
<td>Min</td>
<td>51</td>
<td>35</td>
<td>25</td>
<td>11</td>
<td>14</td>
<td>437</td>
<td>427</td>
<td>7.1</td>
<td>79.0</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>110</td>
<td>63</td>
<td>37</td>
<td>53</td>
<td>44</td>
<td>534</td>
<td>563</td>
<td>7.7</td>
<td>45.0</td>
</tr>
</tbody>
</table>

![Piper diagram](FIGURE2)
**Pollution assessment.** The Nemerow pollution index method is one of the most commonly used methods for calculating the comprehensive pollution index. In this paper, six factors of Na⁺, Ca²⁺, Mg²⁺, Cl⁻, SO₄²⁻, and TDS have been selected for pollution evaluation. The specific calculation formula are as follows:

\[ P_X = \frac{C_X}{S_X} \]  
\[ P_N = \sqrt{\frac{P_{\text{max}}^2 + P_{\text{avg}}^2}{2}} \]

Eq (2): \( P_X \), \( C_X \) and \( S_X \) are the pollution index, measured concentration and standard concentration of the factor \( X \), respectively. (The \( S_X \) value are: Na⁺ 200mg/L, Ca²⁺ 300mg/L, Mg²⁺ 30mg/L, Cl⁻ 250mg/L, SO₄²⁻ 250mg/L, TDS 1000mg/L) [7]

Eq (3): \( P_N \) is the Nemerow pollution index, \( P_{\text{max}} \) and \( P_{\text{avg}} \) are the maximum and average values of \( P_X \), respectively.

Based on the \( P_N \) value, the water quality can be divided into five classes (Table 2). As we can see from the table, all of the samples are classified to be class I and II. Comparatively, the samples from SA have better quality than FA, 55% and 26% of samples from the FA and SA are suffered litter pollution, respectively. This situation is due to the fact that the FA is closer to the surface and is more susceptible to pollution (surface sewage infiltration, irrigation water back seepage, etc.), and some of the pollutants can pass through the layers to the SA.

### Quality evaluation for drinking.

The water quality index (WQI) [12] has been applied for the drinking water quality evaluation. Based on the standard of WQI, five classes have been classified: excellent <50, good 50-100, poor 100-200, and very poor 200-300 and unsuitable >300 [13]. The calculation formulas are as follows:

\[ Wi = \left( \frac{1}{ \sum_{i=1}^{n} wi} \right) W_i \]  
\[ Q_i = 100 * \frac{C_i}{S_i} \]  
\[ WQI = \sum_{i=1}^{n} Wi * Q_i \]

Eq (4): \( Wi \) is the relative weight, \( wi \) is the weight of each parameter, and \( n \) is the number of parameters.

Eq (5): \( Q_i \) is the quality rating, \( C_i \) is the concentration of each parameter (mg/L), and \( S_i \) is the World Health Organization standard (Na⁺ 200mg/L, Ca²⁺ 300mg/L, Mg²⁺ 30mg/L, Cl⁻ 250mg/L, SO₄²⁻ 250mg/L, TDS 1000mg/L) (WHO, 2017).

From the Table 1, the samples from FA have WQI value range from 27.1-89.6 (mean=54.7), indicating that they are excellent and good for drinking (Table 3). The samples from SA have WQI value range from 30.6-79.0 (mean=45.0), and 83% and 17% of them are excellent and good for drinking, respectively.

### Quality evaluation for irrigation.

There are several chemical indexes have been used for the suitability evaluation of water quality for irrigation, such as sodium adsorption ration (SAR), sodium percent (%Na⁺), residual sodium carbonate (RSC), permeability index (PI), Kelly’s ratio, magnesium ration, as well as by comparing water quality parameter with the guidelines proposed by FAO (Food and Agriculture Organization) [14]. In this study, the most popular indexes (SAR and %Na⁺) have been chosen for water quality evaluation, and the results are shown in Table 4.

SAR. SAR proposed by Richards can be used for determining the relative activity of sodium ions in the exchange reactions with the soil [15]. As can be seen from Table 4, all of the samples are excellent for irrigation.

\[ \text{SAR} = \frac{Na^+}{\sqrt{(Ca^{2+} + Mg^{2+})/2}} \]  

### Quality evaluation for irrigation based on SAR and %Na⁺.

<table>
<thead>
<tr>
<th>Indices</th>
<th>Categories</th>
<th>Ranges</th>
<th>FA (20)</th>
<th>SA (23)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAR</td>
<td>Excellent</td>
<td>&lt;10</td>
<td>20</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>Good</td>
<td>10-18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>%Na⁺</td>
<td>Excellent to Good</td>
<td>20-40</td>
<td>6</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Good to Doubtful</td>
<td>0-20</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Doubtful to Permissible</td>
<td>20-40</td>
<td>6</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Permissible to Suitable</td>
<td>40-60</td>
<td>13</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Suitable to Unsuitable</td>
<td>60-80</td>
<td>4</td>
<td>1</td>
</tr>
</tbody>
</table>

Note: SAR sodium adsorption ratio, %Na⁺ sodium percentage

%Na⁺. When the Na⁺ has high concentration in irrigation water, Na⁺ tend to be displace Ca²⁺ and Mg²⁺ in clay particles, and this phenomenon will reduce the permeability of the soil [14, 16, 17]. Based on the %Na⁺ values (Table 4), 10%, 30%, 40% and 50% of the water samples from FA are excellent to good, good to permissible, permissible...
to suitable and suitable to doubtful for irrigation. Besides, for SA, 39% of the samples are good to permissible for irrigation and the rest of the samples are located in permissible to suitable category (except for 1 sample is grouped within suitable to doubtful category).

\[
\%Na^+ = \frac{Na^+}{Na^++K^++Ca^{2++}+Mg^{2+}} \times 100\% \quad \text{(unit: meq/L)}
\]

(8)

**Process controlling the groundwater chemistry.** Gibbs diagram [18] can be used to understanding the mechanism controlling the groundwater chemistry, and the calculation functions of Gibbs ratios are Gibbs I = Cl⁻/(Cl⁻+HCO₃⁻) and Gibbs II = (Na⁺+K⁺)/(Na⁺+K⁺+Ca²⁺) (unit: meq/L). The Gibbs diagram can be divided into three regions: precipitation, evaporation and water rock interaction dominance [19]. In this study, the Gibbs ratio I and Gibbs ratio II are range from 0.01-0.45 (mean=0.13) and 0.20-0.90 (mean=0.60), respectively. As shown in Fig.3, most of the samples from FA and SA are located into the water rock interaction region, which suggesting that the water rock interaction plays an important role in controlling the groundwater chemistry of the study aquifers.

According to Schoeller, the ion exchange can be studied through chloro-alkaline indices (CA I and CA II), and the calculated formulas are CA I = Cl⁻-(Na⁺+K⁺)/Cl⁻ and CA II = Cl⁻-(Na⁺+K⁺)/(SO₄²⁻+HCO₃⁻+CO₃²⁻+NO₃⁻) (unit: meq/L) [20]. The positive or negative values for the CA I and CA II were obtained, exchange of Na⁺/K⁺ from rock with Ca²⁺ and Mg²⁺ in water have taken place or the reverse. The calculated results show that all of the samples from FA and SA had negative CA I and CA II values, indicating that the host rock are the main sources for the chemical compositions in the groundwater, besides, the prevalence of base exchange reaction.

Moreover, it can also be seen from Fig.4 that the groundwater samples from FA and SA have Ca²⁺/Na⁺ ratios range from 0.11 to 3.91 and 0.25 to 1.45, respectively, and Mg²⁺/Na⁺ ratios range from 0.26 to 3.18 and 0.38 to 1.56, respectively, and suggesting that weathering of silicate minerals. It is also further confirmed by the correlation between Ca²⁺/Na⁺ and HCO₃⁻/Na⁺. The Fig.4 shows that almost all the samples grouped into the silicate weathering area, which means the groundwater samples from FA and SA are mainly controlled by weathering of silicate minerals.

![FIGURE 3](image1)

Gibbs diagrams.

![FIGURE 4](image2)

Ca/Na-Mg/Na and Ca/Na-HCO₃-Na diagrams.
CONCLUSIONS

Based on the analyses of major ion concentrations of shallow groundwater from the water source of west Suzhou city in northern Anhui province, China, the following conclusions have been made:

(1) The groundwater samples are neutral to slightly alkaline, and 75% of samples from the FA and all of samples from the SA with TDS similar to the fresh water (1000mg/L), the hydrochemical facies are classified to be Na-HCO₃ (account for 72%), to a lesser extent, Ca-HCO₃ (account for 21%);

(2) Based on Nemerow pollution index, 55% and 26% of samples from the FA and SA are suffered litter pollution, respectively. Water quality index indicated that all of samples can be used for dinking proposes, and according to the analysis of sodium adsorption ratio and sodium percent, approximately 40% of the samples from the two aquifers are good for irrigation.

(3) The Gibbs diagram, chloro-alkaline indices as well as the relation between Na⁺, Ca²⁺, Mg²⁺ and HCO₃⁻ indicated that the weathering of silicate minerals and ion exchange are the main process controlling the groundwater chemistry.

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REFERENCES


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BIOINFORMATICS ANALYSIS OF HIGH-CHLOROPHYLL RICE GENE

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ABSTRACT

A rice mutant with light-green leaves was discovered from a transgenic line of Oryza sativa. The mutant has reduced chlorophyll content and abnormal chloroplast morphology throughout its life cycle. Genetic analysis revealed that a single nuclear-encoded recessive gene is responsible for the mutation, here designated lgl1. ORF Finder software analysis is studied. Phylogenetic analysis of LGL1 and its homologous proteins is inspected. An isolated a light-green leaf mutant lgl1 from a transgenic rice line. Fine mapping showed that the lgl1 gene was located within a 76.5 kb region between the INDEL markers L5 and L6 within BAC OSJNBA0037L20 on chromosome 12. Hydrophilic analysis revealed that the gene-encoded protein is a hydrophilic protein, which may be related to the ability of the gene to increase chlorophyll in rice.

KEYWORDS:
Gene, Plant development, Rice, High-chlorophyll, Bioinformatics Analysis

INTRODUCTION

Chlorophyll molecules, crucial for photosynthesis, capture light energy from the sun and convert it to chemical energy, giving photosynthetic plants their green color. Chlorophyll is arranged in and around the thylakoid membranes of chloroplasts. The fine-tuned control of chlorophyll metabolism is required for chloroplast development and maintenance [1]. Mutant plants with altered leaf color have been found in many plant species and extensively used to explore chlorophyll metabolism and chloroplast development [2].

To date, more than 10 genes involved in yellow-green (or chlorina) mutations have been identified in rice. OsCAO1, encoding chlorophyll a oxygenase, plays a major role in chlorophyll b biosynthesis [3]. Ygl1 encodes chlorophyll synthase, which catalyzes conversion of chlorophyllide a into Chlorophyll a to complete the last step of Chlorophyll a biosynthesis [4]. Cde1(t) encodes glutamyl-tRNA synthetase, which is required for chlorophyll synthesis [2]. OsDVR encodes a 8-vinyl reductase, which is involved in the conversion of divinyl chlorophyllide (ide) a to monovinyl chlorophyllide (ide) a [5]. Yvl encodes plastid caseinolytic protease P6 subunit [6]. Ygl2 encodes heme oxygenase 1, which catalyzes heme degradation [7]. YGL138(t), encoding a putative signal recognition particle 54 kDa protein, might be involved in the translocation of chloroplast proteins [8]. Chl1/Ygl3/YGL7 and Chl9 respectively encode the ChlD and ChlII subunits of Mg-protoporphyrin IX chelatase, which catalyzes the conversion of protoporphyrin IX to Mg-protoporphyrin IX [9-11]. Ygl6 encodes a putative 3-β-hydroxysteroid dehydrogenase/isomerase family protein that might play a role in the synthesis of brassinosteroids [12]. YGL8, encoding a UDP kinase, catalyses the phosphorylation of UMP to UDP [13]. In addition, transgenic rice plants with RNAi construct of OsHAP3A or OsNOA1 displayed the yellow-green leaf phenotype [14, 15]. OsHAP3A encodes a HAP3 subunit of a CCAAT-box binding complex, which functions in regulating chloroplast development [14]. OsNOA1 encodes a circularly permuted GTPase, which is implicated in chloroplast ribosome assembly [15]. Among these genes mentioned above, only Ygl6 was located on chromosome 12.

In this study, we isolated a novel light-green leaf mutant, from lines of rice (Oryza sativa L.) carrying a transfer DNA (T-DNA) insertion. Bioinformatics analysis of high-chlorophyll rice gene is studied.

MATERIALS AND METHODS

Quantitative real time PCR analysis. Total RNA was extracted and purified from various tissues using Qiagen RNeasy Plant Mini Kit and RNase-free DNase Set (Qiagen, Hilden, Germany). First-strand cDNAs were synthesized using Transcriptor High Fidelity cDNA Synthesis Kit (Roche, Indianapolis, USA) with an oligo dT primer. The real time PCR was performed using the 2x SYBR Green PCR Master Mix (Applied Biosystems, Warrington, UK) on the Applied Biosystems 7900HT Real Time PCR System. The relative expression levels of each transcript were normalized to the
OsACT1 gene using the comparative CT method. PCR was carried out as follows: preheating at 95°C for 10 min, followed by 40 cycles of denaturation at 95°C for 15 s and annealing/extension at 60°C for 1 min.

RESULTS AND DISCUSSION

**ORF Finder software analysis.** The results showed that the gene consisted of a complete and continuous open reading frame. Conserved domain database analysis showed that the gene has two highly conserved regions, from amino acids 281 to 335 and amino acids 401 to 441. The mutation site occurs in the first highly conserved region.

**Phylogenetic analysis of LGL1 and its homologous proteins.** The homologous proteins of the LGL1 protein were retrieved by a BLASTP search. CSP41b proteins were widely distributed among photosynthetic eukaryotes, including Chlamydomonas reinhardtii and Physcomitrella patens, which suggested an early origin of the CSP41b protein. There is only one copy of the CSP41b protein in all these organisms. Phylogenetic analysis indicated that the rice LGL1 has a closer phylogenetic relationship to CSP41b from grass family including maize and sorghum than it does with dicotyledonous species (Fig. 1). LGL1 shares considerable identity (68%, 221/324) with the CSP41b protein from Chlamydomonas reinhardtii, suggesting that this gene has been evolutionarily conserved among photosynthetic eukaryotes.

![Phylogenetic tree of LGL1 and its homologous proteins](image1)

**FIGURE 1**
Phylogenetic tree of LGL1 and its homologous proteins

![Surface Plot of Chlorophyll content (mg/g)-wild vs D, C](image2)

**FIGURE 2**
Surface Plot of Chlorophyll content (mg/g)-wild vs D, C
Temperature-chlorophyll biosynthesis is a series of enzymatic reactions, which are affected by temperature. The lowest temperature for chlorophyll formation is about 2°C, the optimum temperature is about 30°C and the highest temperature is about 40°C. The yellowing of leaves in autumn and the whitening of seedlings after cold wave in early spring are related to the inhibition of chlorophyll formation by low temperature. The decomposition of chlorophyll is greater than that of synthesis at high temperature, so green leafy vegetables turn yellow in summer less than one day (Fig.2-4). On the contrary, the decomposition of chlorophyll is slow at low temperature, which is one of the reasons for low temperature preservation. A kind of formation of nutrient element chlorophyll must have certain nutrient elements. Nitrogen and magnesium are the components of chlorophyll, while iron, manganese, copper and zinc have catalytic or other indirect roles in chlorophyll biosynthesis. Therefore, lack of these elements can cause chlorosis, especially nitrogen, which can be used as a marker to measure the level of nitrogen in plants.

Light is the main factor affecting chlorophyll formation. It takes light to transform chlorophyll esters from protochlorophyll esters, and when light is too strong, chlorophyll will be destroyed by photooxidation. The seedlings growing in darkness are yellow-white, and the stems and leaves shaded or buried in the soil are yellow-white. This phenomenon, which affects chlorophyll formation and leaves yellowing due to lack of certain conditions, is called etiolation. There are exceptions, such as algae, bryophytes, ferns and coniferous plants, which can synthesize chlorophyll in darkness, and the amount of chlorophyll is certainly less than that formed under light; the cotyledons of Citrus Seeds and the germs of lotus seeds can also form chlorophyll in the absence of light, presumably there are biomass in these plants that can replace visible light to promote chlorophyll synthesis (Fig.5-7).
FIGURE 5
Surface Plot of Chlorophyll content (mg/g)-wild vs D, A

FIGURE 6
Surface Plot of Chlorophyll content (mg/g)-wild vs C, A

FIGURE 7
Surface Plot of Chlorophyll content (mg/g)-wild vs B, A
Water shortage not only affects chlorophyll biosynthesis, but also accelerates the decomposition of the original chlorophyll, so the leaves are yellow-brown in drought.

The light-green leaves (lgl1) mutant was first found among T1 transgenic rice lines in the “Nipponbare” background. The lgl1 mutant could be clearly distinguished from wild type during the entire growth period (Fig. 8–10). Chlorophyll and carotenoid contents of the latest full expanded leaves isolated from the mutants and wild type plants at different growth stages were measured. The results indicated that leaves from the mutants contained less chlorophyll a and chlorophyll b than those from wild type plants at different growth stages (Fig. 11). The lgl1 mutants also had a lower chlorophyll a to chlorophyll b ratio and reduced carotenoid contents at the seedling stage (Fig. 12). However, at the maturity stage, the lgl1 mutants had a higher chlorophyll a to chlorophyll b ratio and higher carotenoid content (Fig. 13).

Hydrophilic analysis revealed that the gene-encoded protein is a hydrophilic protein, which may be related to the ability of the gene to increase chlorophyll in rice. Because the synthesis of chlorophyll is a process that requires water to participate, the hydrophilicity of the gene product is increased, which is conducive to the transport of water, thereby accelerating the biosynthesis of chlorophyll.
FIGURE 10
Surface Plot of Chlorophyll content (mg/g) vs D, A

FIGURE 11
Surface Plot of Chlorophyll content (mg/g) vs D, C

FIGURE 12
Surface Plot of Chlorophyll content (mg/g) vs D, B
CONCLUSION

In this study, we isolated a light-green leaf mutant lgl1 from a transgenic rice line. Fine mapping showed that the lgl1 gene was located within a 76.5 kb region between the INDEL markers L5 and L6 within BAC OSJNBa0037L20 on chromosome 12. Hydrophilic analysis revealed that the gene-encoded protein is a hydrophilic protein, which may be related to the ability of the gene to increase chlorophyll in rice. Because the synthesis of chlorophyll is a process that requires water to participate, the hydrophilicity of the gene product is increased, which is conducive to the transport of water, thereby accelerating the biosynthesis of chlorophyll.

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REFERENCES


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EFFECTS OF SLUDGE ADDITION AMOUNT ON SOIL PHYSICOCHEMICAL PROPERTIES AND GROWTH OF MAIZE SEEDLINGS IN ROCKY DESERTIFICATION AREAS OF GUIZHOU PROVINCE

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ABSTRACT

The effects of different proportion of sludge (sludge: soil is 0:1, 1:1, 1:2, 1:3) on soil physicochemical properties and growth of maize seedlings were analyzed through a pot experiment. The results showed that the addition of sludge could significantly improve the soil fertility and promote the growth of maize seedlings in rocky desertification areas of Guizhou Province. Contents of the soil organic matter, water-holding capacity, available N, available P and the heavy metal were all positively correlated with the amount of sludge added in the mixed-substrate. When the sludge addition ratio is 50%, the indexes were the highest. However, the total amount of Cu, Cd, Pb and Zn of each treatment did not exceed the national secondary standard of “Environmental quality standards for soils” (GB 18918-2008, China). On the other hand, the addition of sludge increased the maximum root length, plant height, biomass, protein, chlorophyll and β-carotene content of maize seedlings. Plants grew best when the proportion was between 25% and 33%, while a high proportion (50%) of the sludge inhibited the growth of maize seedlings due to the high content of heavy metal. After adding sludge, the dominant root microorganism of maize seedlings changed, Glutamicibacter and Citrobacter were the dominant bacterial communities, while Trichosporon and Fusarium were the dominant fungal communities.

KEYWORDS:
Sludge, rocky desertification, soil restoration, mixed-substrate, maize seedlings

INTRODUCTION

The soil erosion of Guizhou Province is extremely serious, where the rocky desertification area has approximately reached 5×10⁸ km² [1], thus making it the largest and most inferior province of all rocky desertification areas in China [2]. The soil types and erosion characteristics there are highly representative in China and even in karst areas of the whole world [3]. Due to the shallow soil and low productivity, coupled with years of unreasonable exploitation, the fragile ecological environment in rocky desertification areas has been further degraded [4]. Soil poverty and economic backwardness form a vicious cycle, leading to the dual challenges of ecology and poverty in many rocky desertification areas, which urgently need to be managed and changed. The restoration and reconstruction of soil in rocky desertification areas has become a common requirement for improving ecological environment, developing regional economy and lifting people out of poverty [5]. At the same time, with the development of urban economy and the growth of population, sewage treatment plants have become more and more popular, and the production of sludge has increased sharply as well. At present, China’s annual sludge production has exceeded 40 million tons [6, 7]. However, due to the lagging development of sludge treatment facilities, the amount of improperly disposed sludge has been more than 30 million tons per year [8]. Most of the sludge is directly disposed as the solid waste, causing serious environmental pollution. Sludge is rich in nitrogen, phosphorus and organic matters that are required by plants [9]. But if not properly handled, it will not only pose a serious threat to the ecological environment, but also cause a great waste of resources [10]. The application of sludge to the ecological restoration of barren and eroded soil can increase the soil nutrients, improve the soil properties and promote the growth of plants. This method not only disposes the sludge, but also restores the ecological environment, which is one of the best technical routes currently.

However, because of the problems of heavy metals and persistent organic pollutants [11], the sludge disposal is still dominated by landfill at present, and the effective land use of sludge has been progressing slowly. Recently, the research [12] showed that the heavy metal content of urban sludge in China is generally lower than that in Eu-
rope, America and other countries. Especially, the heavy metal content of sludge in Guizhou Province was far lower than that of the national average. Therefore, some previous research suggested that long-term application of urban sludge may lead to the heavy metal pollution of soil, which is not in line with China’s reality. But the risk might be not as terrible as people thought [13, 14]. With a lot of important environmental, ecological, social and economic benefits, using urban sludge to improve soil properties and prevent soil erosion in rocky desertification areas is not only possible but also necessary. Under the background that Guizhou Province has fully implemented the comprehensive management project of rocky desertification and a large amount of sludge has not been properly disposed till now, the application of sludge to soil restoration in rocky desertification areas can achieve the dual purpose of both improving soil properties and recycling sludge, which is of great practical research significance indeed.

In this study, the soil of Guanling County, a typical rocky desertification area in Guizhou Province, was taken as the research target. By adding different proportions of sludge, the effects of sludge on soil physical-chemical properties and the growth of maize seedlings were analyzed, hence providing some scientific foundation for the feasibility of land use of sludge in rocky desertification areas.

**MATERIALS AND METHODS**

**Sewage sludge and soil collection.** De-watered sludge with about 80% water content was collected from Xiaoha Municipal Wastewater Plant in Guiyang City, Guizhou Province. Soil in three plots were taken from a small catchment in a typical rocky desertification region located in Xiangle Town, Guanling County, Guizhou Province, China. Each sample was comprised of five cores taken from 0–20 cm soil depth. The soil for the subsequent experiment was mixed by the samples collected in the three plots mentioned above. Pieces of gobbet, visible plant materials and fine root were removed. Both sewage sludge and soil were sieved through a 2 mm mesh screen after air drying.

**Experiment design.** Four experiments were conducted in a greenhouse in Guiyang University. PVC pots with 25 cm diameter and 30 cm height were filled with 3.0 kg sludge/soil mixtures with three different ratios 1:1, 1:2 and 1:3, designated as T1, T2, and T3 respectively. Besides, the soil without sludge addition was set as the control treatment, labelled as CK. Fifteen replicates were included in each treatment. The soil mixtures were maintained at the natural water holding capacity with deionized water. The experiment was allowed to equilibrate for 30 days. After 45 days’ incubation and stabilization, five replicated samples for each treatment were for measurement of soil physico-chemical properties. Maize was grown for 60 days in the other ten pots of each treatment. Before planting, the germination rate of maize was tested and confirmed 100%. Maize seeds were dipped in NaCl solution for disinfection, and then seeded five to soil per pot. When germinated, three seedlings were chosen and saved per pot. Five pots of each treatment were used to monitor the growth status, and the other five pots were used to determine the chlorophyll, carotenoid, carbon (C), nitrogen (N) and phosphorus (P) content in the maize seedlings as well as the microbial community in maize roots.

Experiment for the water-holding capacity was also conducted. Three replicates of 50 g mixed soils of each treatment were filled in plastic cups, which were pricked with small holes at the bottom. Each cup was placed in the circle of iron frame stage. Water was dripped to each cup through five burettes at the same time. Then stop to drip water when the first drop of water presented at the cup bottom. Subsequently, record the weight of the cup. The cups were placed indoor at natural temperature, and the cup weight was recorded at the fixed time, and the saturated water-holding capacity of each mixture was calculated as well.

**Soil and plant analysis.** The soil pH was determined in 1:2.5 soil/water suspension by using a digital pH meter (Systronic Scientific Equipments Ltd, India). The soil conductivity was measured by using an electric conductivity meter (Leici, DDS-307, China). The soil organic matter (SOM) was determined by dichromate oxidation and titration with ferrous ammonium sulfate. Available N was measured according to the method of Lu [15]. Available P was extracted with 0.5 mol·L⁻¹

<table>
<thead>
<tr>
<th><strong>TABLE 1</strong></th>
<th>Physical and chemical properties of the sludge and soil used in the experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>pH</strong></td>
<td><strong>SOM</strong> (g/kg)</td>
</tr>
<tr>
<td>Sludge</td>
<td>7.97</td>
</tr>
<tr>
<td>Soil</td>
<td>7.52</td>
</tr>
</tbody>
</table>

*Environmental quality standards for soils*(GB 18918-2008, China) (the national secondary standard)
The maximum capacity set by GB/T 24600-2009, China

SOM soil organic matter, AN available nitrogen, AP available phosphorus, AK available potassium.
NaHCO₃ (pH=8.5) as reported by Olsen and Sommers [16] and determined colorimetrically by the molybdate-ascorbic acid procedure [17]. Total Cu, Zn, Cd, and Pb concentrations were determined by atomic absorption spectrophotometer (AA7000, Shimadzu, Japan) after digestion of the samples with the mixture of nitric, sulphuric and perchloric acid in the ratio of 5:1:1 [18].

The chlorophyll content and carotenoid of maize seedling leaves were measured in the acetone and ethanol extract of fresh leaf tissues [19]. The protein content was determined following the procedure given by Bradford [20]. C in the maize seedlings was determined by the dichromate oxidation and titration with ferrous ammonium sulfate. N and P in the maize seedlings were obtained from a complete digestion for 10 h with the H₂SO₄-H₂O₂ mixture and then determined by a continuous-flow autoanalyzer (AutoAnalyzer III, Bran+Luebbe GmbH, Germany) using the indophenol blue method for N and phosphomolybdic acid blue color method [21] for P. The microbial community was determined by Shanghai Meiji Biomedical Technology Co., LTD. The samples were packaged in sterile bags, frozen in liquid nitrogen for 3–4 h and transported with dry ice. The company conducted the sample processing and total DNA extraction. After amplifying the target regions of 16s rDNA and ITS by PCR, the high-throughput sequencing was performed.

**Statistical analysis.** All statistical analysis were carried out by using SPSS 22.0 for Windows software package, and the accepted significance level was α = 0.05. ANOVA was used to analyze the difference in soil properties, plant growth and physiological parameters. The Pearson’s correlation coefficients between variables were calculated by the Bivariate Correlations procedure. Bioinformatics software was used to analyze the community structure of microbial in maize roots.

**RESULTS AND DISCUSSION**

Effects of sludge addition amount on soil physico-chemical properties. Nutrient properties. With the increase of sludge addition, the pH tended to increase, but the overall change was small (Table 2). The soil acidity and alkalinity could affect a series of physical and chemical reactions including the microbial activities and their living environment in the soil. As pH increased, the amount of cationic metabolism in soil increased, which could provide more active sites for the nutrient exchange and adsorption, thus affecting the soil fertility preservation and plant growth. The soil conductivity also rised with the increase of sludge, which might be ascribed to the high salt content of sludge (Table 2). CK has the weakest conductivity that was 1,250 us/cm while T1 had the strongest conductivity which was 2,063 us/cm. T2 and T3 were respectively 1,807 us/cm and 1,585 us/cm. The higher the conductivity was, the salinity in the mixed substrate was higher. However, the high salinity would destroy the balance between nutrients, cause damage to plant roots and inhibit the absorption of nutrients. In each treatment, adding sludge obviously increased the organic matter, available N and P (Table 2). The nutrient content increased the most in T1, and the organic matter, available N and available P increased 5.2 times, 6.6 times and 2.2 times respectively compared with those in CK. Secondly, the organic matter, available N as well as available P of T2 increased 3.2 times, 4.1 times and 1.1 times respectively compared with those in CK. Thirdly, the organic matter, available N and P of T3 increased 2.1 times, 2.8 times and 0.61 times respectively compared with CK.

In rocky desertification areas, the soil was barren and nutrient deficient, and the land productivity deteriorates rapidly, which restricts the growth of plants and seriously affects their ecological environment [22,23]. The rich N and P content, organic matters and other nutrients in sludge are the nutrients necessary for plant growth, and the soil fertility in rocky desertification areas is greatly enhanced after adding sludge. The application of sludge to soil restoration in rocky desertification areas of Guizhou Province has realized a virtuous cycle of nutrients in soil, plant, city, sewage, sludge and soil, which is considered to be the most attractive and effective disposal method.

**Heavy metals.** Cd and Pb were not detected in CK, which indicated that the background value of heavy metal content in rocky desertification soil was low. With the increase of sludge addition, the heavy metal content in the mixed-substrate increased significantly, but the contents of Total Cu,

<table>
<thead>
<tr>
<th></th>
<th>CK</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
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</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.58±0.05a</td>
<td>7.97±0.02a</td>
<td>7.94±0.02a</td>
<td>7.87±0.02a</td>
</tr>
<tr>
<td>Conductivity</td>
<td>1250±0.4d</td>
<td>2063±7.4a</td>
<td>1807±5.3b</td>
<td>1585±6.5c</td>
</tr>
<tr>
<td>Organic matter</td>
<td>10.3±0.5d</td>
<td>64.3±1.3a</td>
<td>42.8±0.9b</td>
<td>32.1±0.6c</td>
</tr>
<tr>
<td>Organic N</td>
<td>30±4.38d</td>
<td>231.6±27.75a</td>
<td>154.2±18.46b</td>
<td>115.8±18.9c</td>
</tr>
<tr>
<td>Organic P</td>
<td>11.9±0.2d</td>
<td>38.4±1.7a</td>
<td>25.3±1.1b</td>
<td>19.2±0.9c</td>
</tr>
</tbody>
</table>

Values are means ± standard error (n=5). Different letters in the same line indicate significant effects on different treatment (p < 0.05).
Cd, Pb and Zn in each treatment did not exceed the national secondary standard of “Environmental quality standards for soils” (GB 18918-2008) of China (Table 3). Moreover, the contents of heavy metals in each treatment measured were below the limits stipulated in the Control Standard of Pollutants in Agricultural Sludge (GB/T 24600-2009, China). Soil Zn content was slightly higher than the standard limited value, which was attributed to that galvanized pipes were widely used in sewage transportation in China, and Zn was also an element that was easy to migrate. The increasing of Cu concentration was 5.4%–22.7% compared with that of CK. Zn, Cd and Pb concentration was 33.3%–45.5%, 945.45%–1,672.72%, 6.62%–36.46% higher respectively compared with those in CK.

In developed countries, urban sludge disposal has been dominated by land use [24, 25], however, the domestic heavy metal limit has been too strict, and the land use of sludge has been very cautious. Relevant policies, resources and channels have not been released [26]. In the real sense, the proportion of land use was still very limited, and the nutrient resources of sludge have not been fully used [27]. In the long term, the soil fertility will gradually deteriorate and become barren. Recently, the heavy metal content in urban sludge has been greatly reduced because China has adopted more effective sewage treatment technologies and stricter sewage discharge standards [28]. In addition, various organic substances in soils could be complexed with heavy metal elements, and various inorganic substances also had strong adsorption capacity for heavy metal [29,30], so the potential heavy metal risks brought to the environment by sludge land use were even lower. Hence, we should adopt a more scientific, rational and positive attitude to the land use of urban sludge and promote its development in China. So it is necessary indeed to strengthen long-term research on the potential risks of heavy metals, find out the action mechanisms and transport rules of heavy metals in soils and plants, and seek effective measures to passivate heavy metals in sludge. As long as the amount of sludge are added strictly, the risk of heavy metals in the utilization of sludge can be also reduced to acceptable range. This effort can make full use of the environmental capacity without polluting the environment, solve the disposal problem of sludge, and meet the nutrients demand of soil ecological restoration, so as to achieve a “win-win” of the ecological restoration and sludge utilization in rocky desertification areas of Guizhou Province.

**Water-holding capacity.** The saturated moisture content of sludge and soil was 92.4% and 71.5% respectively. After adding sludge, the saturated moisture content of each treatment increased obviously (Table 4). T1 has the highest saturated moisture content and the longest water-holding time, reaching 80.3% and 33 days respectively. The saturated moisture contents of T2 and T3 were 77.2% and 75% respectively, and the water-holding time was 31 and 30 days respectively. CK has the lowest saturated moisture content and the shortest water-holding time, which suggested that the addition of sludge might improve the water-holding capacity of soil. The reason was that sludge could significantly improve the structure and performance of soil, reduce the soil bulk density, increase the porosity, and improve the ventilation and water permeability [31], thus providing favorable conditions for plant growth.

**Effects of sludge addition amount on growth of maize seedlings. Maximum root length and plant height.** After adding sludge, the maximum root length and plant height of maize seedlings increased, but both were negatively correlated with the amount of sludge added, that is, T3 increased the most, T2 was the second, and T1 was the lowest (Fig. 1). The maximum root length of T1, T2 and T3 increased by 0.06cm, 1.76cm and

<table>
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<th>TABLE 3</th>
<th><strong>Heavy metal content of mixed-substrate in different treatments</strong></th>
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<tbody>
<tr>
<td></td>
<td>CK (mg/kg)</td>
</tr>
<tr>
<td>Total Cu (mg/kg)</td>
<td>66.5±6.0d</td>
</tr>
<tr>
<td>Total Zn (mg/kg)</td>
<td>141.0±16.4c</td>
</tr>
<tr>
<td>Total Cd (mg/kg)</td>
<td>0.044±0.01d</td>
</tr>
<tr>
<td>Total Pb (mg/kg)</td>
<td>36.2±2.24d</td>
</tr>
</tbody>
</table>

Values are means ± standard error (n=5). Different letters in the same line indicate significant effects on different treatment (p < 0.05).

<table>
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<th>TABLE 4</th>
<th><strong>Water-holding time and saturated moisture content in different treatments</strong></th>
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<tbody>
<tr>
<td></td>
<td>CK</td>
</tr>
<tr>
<td>Time of water-holding (d)</td>
<td>27</td>
</tr>
<tr>
<td>saturated water-holding capacity (%)</td>
<td>71.5</td>
</tr>
</tbody>
</table>
4.32 cm respectively compared with that of CK. Plant height of T1, T2 and T3 increased by 0.18 cm, 1.05 cm and 1.24 cm respectively compared with that of CK. Therefore, an appropriate sludge addition amount of 25%–33% (T3, T2) could significantly increase the maximum root length and plant height of maize seedlings. However, due to the higher heavy metal content in sludge, a higher sludge addition amount of 50% (T1) might inhibit the efficiency of maize seedlings to absorb nutrients, so the maximum root length and plant height of maize seedlings increased little compared with those of CK.

**Aboveground biomass and protein content.** After adding sludge, the aboveground biomass and protein content of maize seedlings increased (Fig. 2). The aboveground biomass (dry mass) of T1, T2 and T3 increased by 0.68 cm, 0.13 cm and 0.42 cm respectively compared with that of CK, and the protein content of T1, T2 and T3 were higher than that of CK by 16.61%, 41.4% and 27.04%, respectively. After the addition of sludge, the increase of nutrients in the soil promoted the absorption of N, P and other nutrients of maize seedlings, so that the aboveground biomass and protein content of T1, T2 and T3 were higher than those in CK without sludge dosage. Among them, the aboveground biomass of T3 was the highest and the protein content of T2 was the highest. Thus, the mixture ratio of sludge and soil in T2 and T3 could be obtained as the best for maize seedlings to absorb nutrients in sludge.

**Chlorophyll and β-carotene content.** After adding sludge, chlorophyll and β-carotene contents of maize seedlings increased (Fig. 3), among which, chlorophyll-a content of T2, chlorophyll-b content of T3 and β-carotene content of T2 were the highest. Compared with CK, chlorophyll-a of T1, T2 and T3 increased 109.4%, 144.5% and 131.0% respectively, chlorophyll-b increased 122.9%, 202.9% and 244.4% respectively, and β-carotene increased 112.5%, 133.5% and 131.2%, respectively. The higher pigment content of the leaves indicated that the photosynthesis of plants was stronger, and the situation of plant growth and nutrient intake were better. Although the nutrient content of T1 was higher, the heavy metal content was relatively higher, which inhibited the growth of plants comprehensively. On the contrary, the nutrient content of T2 and T3 was relatively lower, heavy metal content was also relatively lower, which was suitable for plant growth and non-adverse effects on plants. Therefore, the optimum sludge: soil ratio was 1:2 (T2) and 1:3 (T3).
Plant restoration was the key and main measure for the ecological restoration in rocky desertification areas of Guizhou Province [32]. The fertilizer effect of sludge was equivalent to the high-quality farmyard fertilizer [33]. After adding sludge, soil nutrients were supplemented, and the nutrient content required for the growth of maize seedlings was sufficient. The maximum root length, plant height, biomass, protein, chlorophyll β-carotene content were all increased, and the plant growth condition was significantly better than that without sludge. In addition, the root system secreted a large amount of organic acids in the process of plant growth, which could improve the activity and biological efficiency of heavy metals.

**Root microbial abundance.** Taxonomic analysis showed that the bacteria in the mixed-substrate belonged to 33 phylums, 74 classes, 145 orders, 264 families and 495 genera, and 20.36%–28.10% of the bacterial communities could not determine their taxonomic status at the genus level (Fig. 3). *Massilia* (15.35%) was the dominant bacterial community of CK, and there were other 20
genera with a relative abundance of >1%. The top five were *Glutamicibacter* (9.18%), *Nocardioides* (6.31%), *Saccharibacteria* (5.58%).

*Brevundimonas* (4.39%), *Ovalobacteraceae* (2.86%). *Glutamicibacter* (14.23%) was the dominant bacterial community of T1, and there were other 21 genera with a relative abundance of >1%. The top five were *Streptomyces* (7.97%).

*Brevundimonas* (4.88%), *norank_o_JG30-KF-CM45* (3.68%), *Roseiflexus* (3.68%), *Sphingobacterium* (3.64%). *Glutamicibacter* (20.24%) was the dominant bacterial community of T2, and there were other 19 genera with a relative abundance of >1%. The top five were *Citrobacter* (7.00%), *Roseiflexus* (5.10%), *Kluyvera* (4.13%), *Streptomyces* (3.94%), *Sphingobacterium* (3.53%). *Citrobacter* (12.73%) was the dominant bacterial community of T3, and there were other 19 genera with a relative abundance of >1%. The top five were *Glutamicibacter* (11.00%), *Kluyvera* (8.78%), *Pseudomonas* (6.63%), *Roseiflexus* (4.71%), *norank_o_Acidimicrobiales* (3.86%). In each treatment, *Glutamicibacter* was the dominant bacterial community, which suggested that *Glutamicibacter* was the characteristic bacterial community of maize root. After adding sludge, a large number of *Citrobacter* were found in T2 and T3 where maize seedlings had great growth performance. Four of the top five bacterial communities were identical in T2 and T3, which were *Glutamicibacter*, *Citrobacter*, *Roseiflexus* and *Kluyvera*, which might play a decisive role in the growth of maize seedlings.

Taxonomic analysis showed that the fungi in the mixed-substrate belonged to 6 phyla, 19 classes, 54 orders, 101 families and 169 genera, and 1.15%~17.97% of the fungal communities could not determine their taxonomic status at the genus level (Fig. 4). *Cladosporium* (13.64%) was the dominant fungal community of CK, and there were other 17 genera with a relative abundance of >1%. The top five were *Humicola* (11.57%), *Mortierella* (7.46%), *Penicillium* (6.25%), *Fusarium* (5.89%), *unclassified_f_Davideliaceae* (4.55%). *Trichosporon* (36.26%) was the dominant fungal community of T1, and there were other 9 genera with a relative abundance of >1%. The top five were *Fusarium* (16.45%), *Chaetomium* (12.69%), *Penicillium* (10.86%), *Gibberella* (7.29%), *unclassified_f_Microascaceae* (5.12%). *Trichosporon* (29.56%) was the dominant fungal community of T2, and there were other 11 genera with a relative abundance of >1%. The top five were *Chaetomium* (12.48%), *Fusarium* (11.28%), *Mortierella* (11.20%), *Gibberella* (9.36%), *Sporobolomyces* (5.38%). *Trichosporon* (24.38%) was the dominant fungal community of T3, and there were other 11 genera with a relative abundance of >1%. The top five were *Gibberella* (23.53%), *Fusarium* (21.95%), *unclassified_f_Nectriaceae* (10.89%), *Mortierella* (2.58%), *Cephalophora* (2.44%). In each treatment, *Trichosporon* was the dominant fungal community except CK, the results showed that the characteristic fungal community changed after adding sludge. The number of *Trichosporon* increased with the increase of sludge addition. Five main maize root fungi that were suitable to live in the mixed-substrate, namely *Trichosporon, Fusarium, Gibberella, Chaetomium* and *Mortierella*.  

**FIGURE 5**

Relative abundances of fungal community at genus level
CONCLUSIONS

This study illustrates the possibility of sludge used for soil remediation in rocky desertification areas of Guizhou Province. Experiment showed that the addition of sludge obviously enhanced the soil fertility and promoted the growth of maize seedlings. When the proportion of sludge was 50%, the increase of nutrient content and heavy metals was the largest. Plants grew best when the proportion was between 25% and 33%. Thus, it is feasible to use sludge for the soil and ecological restoration in rocky desertification areas of Guizhou Province, but the proportion of sludge addition should be paid attention to. As long as the amount of sludge added was strictly controlled, the environmental risk of heavy metals in sludge could be within the acceptable range.

ACKNOWLEDGEMENTS

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REFERENCES


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ANALYSIS OF THE SOURCES OF AGRICULTURAL INFORMATION AVAILABLE TO GREENHOUSE TOMATO GROWERS IN TURKEY

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ABSTRACT

In Turkey, greenhouse cultivation started tentatively at research institutes in 1940s. Commercial greenhouses were constructed in Antalya and Izmir in 1970. Growers were provided with initial information regarding greenhouse cultivation by the agricultural extension practitioners working at the provincial and sub-provincial directorates of the ministry of agriculture. The major reasons why Antalya has become a hub of greenhouse cultivation are its climate and the agricultural extension activities carried out for the producers in the region. The sources of information that producers consult in seeking an answer to their questions about their agricultural activities or a solution to their problems vary depending on their age, experience, educational attainment, land size and the types of crops they cultivate. The present study analyses the demographics of 128 greenhouse tomato growers in Aksu, Antalya and the sources of agricultural information available to them. According to the findings obtained, the growers have been cultivating tomato in greenhouses for 23.6 years in average. The average land size and greenhouse size in the enterprises examined under the study are 15.6 decares and 4.1 decares, respectively. While 68.0% of the growers stated that their initial source of information regarding greenhouse cultivation was their father, 51.6% noted that they consulted pesticide dealers as a source of information. The statistical relationship between the educational attainment of the growers and the answers given was tested by chi-square analysis. Accordingly, it was found that there was a significant relationship between the growers' educational attainment and the sources of information they consulted with respect to economic matters.

KEYWORDS:
Greenhouse Cultivation, Tomato, Sources of Agricultural Information, Agricultural Extension, Input Dealers

INTRODUCTION

The history of agriculture is as old as the history of mankind. It is a sector that should be addressed with a strategic, sociocultural and economic approach based on productivity and people-oriented development. According to the data by the Turkish Statistical Institute, Turkey's total population is 80.8 million, 7.5% of which live in rural areas. 19.4% of the employed population at and above 15 years of age work in the agricultural sector. Turkey has a total agricultural area of 23.4 million hectares. The number of agricultural land sections per enterprise is 5.9 and the average size of agricultural land sections is 12.9 decares [1].

Antalya is one of the prominent touristic and agricultural hubs in Turkey. It is the fifth largest province in terms of population. Its value of agricultural production is 9.5 billion TRY. It accounts for 6.8% of the vegetable production of Turkey in terms of value. Thanks to its fertile soil and favourable climatic conditions, field crops and greenhouse cultivation are the primary means of livelihood of growers. The province is home to approximately 156 thousand growers. In Turkey, the total area of greenhouses has reached 752 thousand decares as of 2017, compared to 85 thousand decares in 1988. The greenhouse vegetable production has reached 7.4 million tons, compared to 2.6 million tons in 1996. Antalya accounts for 1.5% of the total agricultural area, 40.6% of the greenhouse area. 48.9% of the greenhouse vegetable production and 61.7% of the greenhouse tomato production in Turkey [1]. Hence, Aksu, Antalya, where greenhouse tomato cultivation is widespread, was selected for the study.

The subject of the study is the analysis of the sources of agricultural information available to greenhouse tomato growers. The reason underlying the selection of this subject is that it requires a set of skills and knowledge for growers to keep track of the developments in agricultural technologies, draft and implement operating plans and develop marketing strategies. Research has shown that growers consult conventional and modern sources of information with regard to any question they may have throughout the production period [2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12]. Particularly, the primary factor that
enables small family-run businesses to compete with large-scale enterprises is the ability of growers to acquire current and correct information in a timely manner. For this purpose, it would be wise for growers to pay attention to these criteria when selecting the source of information they need. If growers make use of the sources of correct information, their level of income and life satisfaction will be enhanced by the increase in productivity and quality of production. However, the sources of incorrect information may result in the use of unnecessary and wrong inputs, which in turn may lead to production of costly products that are detrimental to the environment and human health.

In Turkey, there are numerous studies that analyse the use of inputs, production techniques and sources of information with respect to economic matters in greenhouse tomato cultivation [13, 14, 15, 16, 10, 17, 18]. Nevertheless, the research aimed at identifying the greenhouse tomato growers' sources of information and assessing them from the viewpoint of agricultural extension is quite limited.

As such, the general purpose of this study is to identify the greenhouse tomato growers' sources of information and assess them from the viewpoint of agricultural extension. In this context, the objectives of the study are to:

- compile the general details of growers;
- find out the reasons why they are engaged in greenhouse cultivation;
- identify the stages of greenhouse tomato production and the inputs used;
- identify the sources of information used by growers regarding greenhouse cultivation, tomato production and economic matters;
- determine the growers' level of confidence in their sources of information and the extent to which they implement the recommendations given by pesticide dealers;
- determine the extent to which the growers participate in the agricultural extension activities carried out in the region; and
- explain the contributions that the findings of the study will make to the growers, agricultural extension practitioners, agricultural advisors, input dealers, researchers and other stakeholders.

**MATERIALS AND METHODS**

The main material of this study is the greenhouse tomato growers in Aksu, Antalya. Furthermore, previous national and international publications on the subject and the data provided by the Turkish Statistical Institute were drawn on.

The villages where the study would be carried out were selected based on the knowledge and experience of the agricultural engineers working at the Sub-provincial Directorate of Food, Agriculture and Livestock in Aksu. The population of the study is all of the greenhouse tomato growers who are registered in the "Greenhouse Registration System" and are actively engaged in greenhouse tomato production. Taking into account the frequency distribution of the population's land size, the enterprises were divided into 3 strata, namely, less than 10 decares, 10-25 decares and above 25 decares. The sample size was found using the Stratified Random Sampling Method and Neyman Allocation at a confidence interval of 95% and with a standard deviation of 5% [19]. The number of enterprises that were included in the sample from each stratum was calculated based on the ratio of each stratum to the population. Accordingly, face-to-face interviews were conducted with 128 growers from 6 villages, namely, Yurtpinar, Soluk, Hısamıye, Boztepe, Kumköy and Kurgulu. The data derived from the questionnaire survey were analysed on SPSS. Initially, the growers were divided into two groups by the level of their educational attainment. Group I was defined as growers that were literate and had a primary school or secondary school diploma, whereas Group II was defined as growers that had a high school diploma or college or bachelor's degree. Then, the growers' educational attainment and their answers were compared. For this purpose, the potential statistical relationship between the expected frequencies and the observed frequencies was tested by chi-square analysis. However, the cells were merged where the expected frequency was lower than 5 [20]. Moreover, mean values, frequencies, percentage distributions and cross tables were used.

**RESULTS**

Grower and enterprise information. The average age of the growers in the sample is 48.5 years. Their average family size is 3.6 persons and average greenhouse experience is 23.7 years. The average size of the lands owned by the enterprises in the sample is 15.6 decares. Greenhouse cultivation accounts for 4.1 decares (26.3%) of this average land size, whereas field cultivation is carried out on 5.7 decares (36.5%) and wheat and, as a secondary product, maize are cultivated on 1.7 decares (10.9%). The remaining 3.21 decares is left unseeded. The enterprises have 2.4 head of dairy cattle in average. 87.2% of the total family income of the growers is generated from agricultural activities, and 12.8% from non-agricultural activities (Table 1). In a study conducted on the greenhouse vegetable growers in Kumluca, Antalya, their average age, greenhouse experience and average greenhouse size were found to be 42.7 years, 13.6 years and 6.1 decares, respectively and their agricultural income accounted for 84.4% of their total income [10]. In another study analysing the profitability of greenhouse tomato production in Antalya, the aver-
age of the growers was found to be 42 years, the average greenhouse experience 15.7 years, the average family size 3.9 persons, the average greenhouse size 4.62 decares and the average number of greenhouses 2.72 [14]. Similar results were found in the other studies focusing on the same subject [13, 21, 22, 16, 23, 24, 18, 25]. The data obtained from the survey indicate that the growers live in a nuclear family, are predominantly engaged in greenhouse cultivation and thus are experienced growing.

It was found that 83.6% of the growers did not have any non-agricultural income; 85.2% did not consider immigrating; 73.4% had a moderate level of income compared to other farmers and spent 71.8% of their income on foodstuff. 90.6% of the growers are not a member of any agricultural cooperative; 52.3% has not borrowed any loan in the last 5 years; and 84.4% has social security from Bagkur (Social Security Organisation for Artisans and the Self-employed). A study examining the profitability of greenhouse tomato production in the central sub-provinces of Antalya as well as Serik and Kumluca found that 50.4% of the growers were a member of the Chamber of Agriculture and Agricultural Credit Cooperative [14]. A study addressing the cooperator-cooperative relationships in the agricultural development cooperatives in Turkey demonstrated that 40.1% of the cooperator enterprises resorted to banks to borrow loans [26]. A study researching the extent to which vegetable growers in Diyarbakır benefit from agricultural extension activities found that 87.5% of the growers were not a member of the producer organisations [14]. Another study revealing the factors that affect the cooperative partnership in the cattle breeding enterprises in the Eastern Anatolia Region showed that as the breeders level of experience and educational attainment increased they were less likely to opt to be a member of the cooperative [27]. According to the data obtained, it may be said that growers opt to resolve their problems on their own or borrow loans from banks, rather than becoming a member of the agricultural cooperatives. However, it is quite important that producers get organised for sustainable agricultural production and rural development.

35.2% of the growers interviewed in the study stated that they were engaged in greenhouse cultivation as this was their father's profession, 25.0% because they found it profitable, and 24.2% because they did not have any better option. The chi-square analysis showed that there was no significant relationship between the educational attainment of growers and the reasons why they are engaged in greenhouse cultivation.

| TABLE 1 | Grower and Enterprise Information |
|-----------------------|-----------------------|-----------------------|-----------------------|
| General Details | Minimum | Maximum | Average |
| Grower's age (years) | 29 | 72 | 48.5 |
| Family size (persons) | 2 | 5 | 3.6 |
| Greenhouse experience (years) | 4 | 45 | 23.7 |
| Total land size (decares) | 2 | 77 | 15.6 |
| -Tomato in greenhouse | 1 | 9 | 3.4 |
| -Other vegetables in greenhouse | - | 4 | 0.7 |
| -Vegetables on open field | - | 30 | 5.7 |
| -Field crops | - | 15 | 1.7 |
| -Orchard | - | 6 | 0.9 |
| -Unseeded land | - | 46 | 3.2 |
| Bovine livestock (head) | 1 | 4 | 2.4 |
| Agricultural income (%) | 40 | 100 | 87.2 |
| Non-agricultural income (%) | - | 60 | 12.8 |

| TABLE 2 | Reasons Why Growers Are Engaged in Greenhouse Cultivation |
|-----------------------|-----------------------|-----------------------|-----------------------|
| Reasons | Group I | Group II | Total |
| | Frequency | % | Frequency | % | Frequency | % |
| It is profitable | 22 | 24.2 | 10 | 27.0 | 32 | 25.0 |
| It is easy | 13 | 14.3 | 7 | 18.9 | 20 | 15.6 |
| I do not have any better option | 26 | 28.6 | 5 | 13.5 | 31 | 24.2 |
| It is my father's profession | 30 | 33.0 | 15 | 40.5 | 45 | 35.2 |
| Total | 91 | 100.0 | 37 | 100.0 | 128 | 100.0 |

\( \chi^2 = 3.339 \quad \chi^2_{0.05} = 7.815 \quad \text{Result = insignificant} \)
Stages of greenhouse tomato production and the inputs used. All of the growers interviewed in the study use seedlings. In response to the question about where they procure the seedlings, 46.9% of the growers replied that they bought them from middlemen, 31.2% from a company producing seedlings, and 21.9% from pesticide dealers (Table 3). A study conducted in Antalya showed that 98.5% of the growers used ready-to-grow seedlings and bought them from input dealers or cooperatives [22]. In another study conducted in Antalya and Izmir, similar data were obtained [21, 23]. In short, almost all growers choose to buy ready-to-grow seedlings, rather than seeds. The reason is that growing from seeds is both more costly and requires more labour. The growers were asked some questions about the stages of greenhouse tomato cultivation. It was found that 67.2% of the growers did not have soil analysis conducted, 91.4% disinfected the soil before each production period (soil solarisation), 75.0% applied soil flushing from time to time, 71.9% sometimes used farm manure, 97.7% did not use green manure, 88.3% always used chemical fertiliser, 64.8% sometimes used foliar fertiliser, 68.8% always applied chemical pesticides, 49.2% watered tomato plants three times a week and 44.5% watered them twice a week. The source of irrigation water used by all of the growers is well water, and the mode of irrigation applied by them is drip irrigation. All of the growers sell their tomatoes to the middlemen in the wholesale marketplace that is closest to them (Table 3). Similar results were obtained from the studies conducted in different regions [21, 22, 23]. The findings suggest that the growers have a certain fund of knowledge regarding greenhouse tomato cultivation.

Sources of Information. Information is the knowledge or facts learned through research and observation. Agricultural information is a significant factor of agricultural production like land, labour, capital and entrepreneurship [28]. Furthermore, it is an exceptionally important resource to producers like soil, water and seeds. The reason is that it is quite difficult for producers to utilise the resources available to them efficiently and effectively without having the technical and economic knowledge of the plant and animal production activities. The information acquired by rural communities can be generated both within and outside the rural communities [29]. Research has shown that growers consult conventional and modern sources of information with regard to any question they may have [2, 3, 4, 6, 8, 9, 10, 11]. Conventional information is knowledge generated, experimented and tested in the rural area in order to resolve the problems faced during agricultural activities and used by family members and other producers. Modern information is the new techniques and technologies developed by universities and other research institutions. Conventional sources of information are the producer’s own knowledge and experience, family members and other producers. Modern sources of information include the agricultural extension practitioners working at provincial/sub-
provincial directorates of agriculture that keep producers abreast of agricultural innovations as well as agricultural advisors working at non-governmental organisations, chambers of agriculture and agricultural consultancy companies or on their own account to provide agricultural advisory services. Additionally, seed, fertiliser and pesticide dealers are also among the modern sources of information that producers consult. 68.0% of the growers interviewed in the study stated that their initial source of information was their father, 21.0% other producers and 10.1% pesticide dealers. Agricultural activity is a way of living. That is, farming is a profession handed down from father to son. The growers interviewed in the study have been engaged in greenhouse cultivation for long years. Hence, it is not surprising that their initial source of information is their father and other growers. However, the chi-square analysis suggests that there is a significant relationship between the educational attainment of the growers and the initial source of information concerning greenhouse cultivation. While 75.8% of the growers in Group I cite their father as their initial source of information, 20.9% other growers and 3.3% the Internet, 48.6% of the growers in Group II cite their father as their initial source of information, 27.0% the Internet and 24.4% other growers. In short, the growers are more likely to draw on the Internet sources as the level of their educational attainment increases (Table 4).

The growers' need for new information concerning their agricultural activities has increased and been diversified in view of the rapidly changing market conditions and developing technologies. Previous research has shown that growers do not draw on a single source of information regarding cultivation, but resort to both conventional and modern sources of information in relation to production techniques such as soil cultivation, sowing techniques, selection of varieties, fertilisation, agricultural pest control and irrigation in an attempt to enhance productivity and quality [3, 30, 13, 4, 31, 21, 32, 15, 14, 6, 8, 9, 23, 33, 11, 34]. The growers interviewed in the study were asked what the primary source of information they drew on concerning greenhouse tomato cultivation was. 51.6% of the growers answered pesticide dealers, 14.8% their own knowledge and experience, 10.9% agricultural advisors, 9.4% other growers, 7.8% officials at the sub-provincial directorate of agriculture and 5.5% the Internet. In Table 5, "my own knowledge and experience" and "other growers" are included in the "conventional sources of information", whereas "pesticide dealers", "agricultural advisors", "agricultural extension practitioner" and "Internet" are included in the "modern sources of information". However, the chi-square analysis showed that there was no significant relationship between the educational attainment of the growers and the sources of information they drew on in cultivating tomato (Table 5). In brief, it was found that there was no significant difference between the sources of information that the growers drew on in cultivating tomato, regardless of their educational attainment.

### Table 4

<table>
<thead>
<tr>
<th>Initial sources of information</th>
<th>Group I</th>
<th>Group II</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Frequency</td>
<td>%</td>
<td>Frequency</td>
</tr>
<tr>
<td>Father</td>
<td>69</td>
<td>75.8</td>
<td>18</td>
</tr>
<tr>
<td>Other growers</td>
<td>19</td>
<td>20.9</td>
<td>9</td>
</tr>
<tr>
<td>Pesticide dealers</td>
<td>3</td>
<td>3.3</td>
<td>10</td>
</tr>
<tr>
<td>Total</td>
<td>91</td>
<td>100.0</td>
<td>37</td>
</tr>
</tbody>
</table>

$\chi^2 = 17.586 \quad \chi^2_{0.05} = 5.991 \quad \text{Result = significant}$

### Table 5

<table>
<thead>
<tr>
<th>Sources of information*</th>
<th>Group I</th>
<th>Group II</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Frequency</td>
<td>%</td>
<td>Frequency</td>
</tr>
<tr>
<td>My own knowledge and experience</td>
<td>12</td>
<td>13.2</td>
<td>7</td>
</tr>
<tr>
<td>Other growers</td>
<td>8</td>
<td>8.8</td>
<td>4</td>
</tr>
<tr>
<td>Pesticide dealers</td>
<td>58</td>
<td>63.7</td>
<td>8</td>
</tr>
<tr>
<td>Independent agricultural advisor</td>
<td>7</td>
<td>7.7</td>
<td>7</td>
</tr>
<tr>
<td>Public agricultural extension practitioner</td>
<td>4</td>
<td>4.4</td>
<td>6</td>
</tr>
<tr>
<td>Internet</td>
<td>2</td>
<td>2.2</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>91</td>
<td>100.0</td>
<td>37</td>
</tr>
</tbody>
</table>

*Conventional: My own experience, Other growers

Modern: Pesticide dealer, Agricultural advisor, Agricultural extension practitioner, Internet

$\chi^2 = 0.861 \quad \chi^2_{0.05} = 3.841 \quad \text{Result = insignificant}$
A study focusing on the profitability of greenhouse tomato cultivation in Antalya found that producers relied on pesticide dealers (86.33%) and agricultural advisors (8.98%) as a source of information [14]. Another study analysing the greenhouse tomato producers’ level of knowledge and the agricultural extension services in Antalya revealed that 39.6% of tomato producers relied on pesticide dealers as a source of information [34]. Similarly, a study addressing the adoption and dissemination of the integrated pest control method in Antalya found that 30.5% of the producers consulted pesticide dealers as a source of information concerning chemical pest control [16]. A study conducted in Kumluca, Antalya highlighted that the sources of information that producers consult with respect to cultivation in greenhouse varied depending on the practices implemented [10]. According to another study, 73.9% of the producers cultivating cucumber in greenhouses in Denre, Finike and Kumluca, Antalya consult technical officials as a source of information concerning application of pesticides [22]. Another study conducted on pomegranate producers in Antalya showed that elderly producers relied on conventional sources of information in relation to soil preparation, sowing techniques, storage, etc., whereas young producers consult both conventional and modern sources of information. In the same study, the chi-square analysis showed that there was no significant relationship between the educational attainment of producers and the sources of information consulted by them [2]. In a study conducted in Gaziantep, it was found that producers relied on conventional sources of information in relation to soil preparation, selection of seeds, fertilisation, irrigation, harvesting and marketing, but on modern sources of information in relation to agricultural pest control [8]. A study conducted in Kahramanmaraş found that 48.5% of the producers consulted pesticide dealers in relation to agricultural pest control and family members in relation to all other agricultural practices [11]. A study assessing the sources of information that the producers in Isparta draw on in relation to the use of chemical fertilisers from the viewpoint of agricultural extension found that the producers relied on their own knowledge and experience (33.7%), officials at the sub-provincial directorate of agriculture (17.4%), recommendations given by fertiliser dealers (15.3%) and advice given by their neighbours and relatives (15.3%) when determining the amount of fertiliser to be applied [9]. According to the findings obtained from the abovementioned studies, the sources of information drawn on by producers vary from one region to another and from one crop to another.

The studies conducted in the regions with characteristics similar to those of the region in which this study was conducted highlight that producers are most likely to rely on pesticide dealers as a source of information. Although the main field of activity of pesticide dealers is selling pesticides, almost all pesticide dealers in Turkey provide producers with advisory services concerning agricultural issues. In short, pesticide dealers are both the physician and pharmacist of producers. Hence, it is of utmost importance that pesticide dealers are knowledgeable about the origin of diseases, deficiency of plant nutrients, distinction between diseases symptoms, diagnosis of pests, etc. A study conducted in Konya found that 63.6% of the pesticide dealers had a sufficient level of knowledge about plant diseases and pests. However, it was found that only 8.1% of the dealers knew what LD50 meant and only 4.0% knew what antidote referred to. Moreover, it was found that 55.0% of the pesticide dealers did not have sufficient knowledge about the origin of diseases and had difficulties in answering questions in this area [35]. These data indicate that pesticide dealers actually have a quite low level of knowledge in pest control. A study conducted in Antalya presented the views of producers in relation to agricultural advisors that they consulted as a source of information. Accordingly, 20.7% of the producers were very satisfied, 67.0% satisfied, 6.2% not satisfied and 1.8% not satisfied at all with the agricultural advisors responding to the diseases and pests in a timely manner. The same study highlighted that 57.1% of the agricultural advisors considered themselves sufficiently competent, and 61.9% consulted the provincial/sub-provincial directorates of agriculture to seek an answer to the questions outside their area of specialisation [36]. A study conducted in Tokat found 60.0% of the producers considered that agricultural extension practitioners responded to plant diseases and pests in a timely manner [37]. A study carried out in Tokat found that 42.9% of the agricultural advisors had an insufficient level of technical knowledge [38]. Consequently, producers should consult more than one source of information to resolve any problem they may have during their agricultural activities. Particularly, it is wrong to rely on dealers of inputs such as pesticide, fertiliser and seed as a source of information, as they are primarily operated to do business and sell products, rather than providing information. In a study conducted in Antalya, 72.2% of the input dealers stated that they provided information in an effort to increase the sale of pesticides to producers. In the same study, 66.7% of the input dealers stated that there were also producers who visited their stores in order to just ask questions and left without buying any input [39]. For this reason, it is of great importance that input dealers pay attention to the provision of correct information to producers without any commercial concerns. Furthermore, public agricultural extension agencies should deliver training courses that will enable producers to analyse whether the information provided by their sources...
is correct and select the most suitable pieces of information, thereby becoming an expert in the area of their own business.

This study focused on the sources of information consulted by growers in relation to economic aspects such as input prices, cost calculation, profit margin, seasonal fluctuations of tomato prices, market price, cooperatives, marketing and export. 59.4% of the growers stated that they relied on pesticide dealers, 29.7% their own knowledge and experience and 10.9% the Internet. While the Internet ranks sixth with a share of 5.5% among the sources of information consulted by growers in cultivating tomato (Table 6), it ranks third with a share of 10.9% among the sources of information consulted by growers in relation to economic matters. According to the chi-square test, it was found that there was a significant relationship between the growers' educational attainment and the sources of information they consulted with respect to economic matters (Table 6). In brief, the growers are more likely to consult the sources on the Internet with respect to economic matters as the level of their educational attainment increases. However, a relationship was found between the growers' educational attainment and the use of the Internet as a source of information [40].

53.9% of the growers stated that they totally trusted the recommendations given by their sources of information and 46.1% stated that they partially trusted them. According to the chi-square test, it was found that there was a significant relationship between the growers' educational attainment and their confidence in the recommendations given by their sources of information (Table 7). In short, the growers are more likely to trust the recommendations given by their sources of information as the level of their educational attainment increases. A study focusing on the activities of organisations supplying agricultural inputs in Antalya from the viewpoint of agricultural extension found that according to the input dealers 66.7% of the producers had full confidence, 22.2% half confidence and 11.1% high confidence in the information provided by them [39].

In recent years, the irresponsible use of pesticides and fertilisers has increased productivity, but also resulted in production of low-quality products that are detrimental to human health [41]. A study conducted on greenhouse vegetable growers in Antalya, Mersin, Muğla and Izmir found that tomato growers used pesticides in an amount that is 36.0% higher than necessary [13]. In another study conducted in Antalya, the producers that were and were not involved in the integrated pest control project were asked whether if they followed the recommendations given by the sources of information they consulted in relation to chemical control. It was found that the producers involved in

---

**TABLE 6**

<table>
<thead>
<tr>
<th>Sources of information</th>
<th>Group I</th>
<th>Group II</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Frequency</td>
<td>%</td>
<td>Frequency</td>
</tr>
<tr>
<td>My own knowledge and experience</td>
<td>25</td>
<td>27.5</td>
<td>13</td>
</tr>
<tr>
<td>Pesticide dealers</td>
<td>61</td>
<td>67.0</td>
<td>15</td>
</tr>
<tr>
<td>Internet</td>
<td>5</td>
<td>5.5</td>
<td>9</td>
</tr>
<tr>
<td>Total</td>
<td>91</td>
<td>100.0</td>
<td>37</td>
</tr>
</tbody>
</table>

\[\chi^2 = 12.157\]  \[\chi^2_{0.95} = 5.991\]  Result = significant

**TABLE 7**

<table>
<thead>
<tr>
<th>Level of Confidence</th>
<th>Group I</th>
<th>Group II</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Frequency</td>
<td>%</td>
<td>Frequency</td>
</tr>
<tr>
<td>Full confidence</td>
<td>42</td>
<td>46.2</td>
<td>27</td>
</tr>
<tr>
<td>Partial confidence</td>
<td>49</td>
<td>53.8</td>
<td>10</td>
</tr>
<tr>
<td>Total</td>
<td>91</td>
<td>100.0</td>
<td>37</td>
</tr>
</tbody>
</table>

\[\chi^2 = 7.614\]  \[\chi^2_{0.95} = 3.841\]  Result = significant

**TABLE 8**

<table>
<thead>
<tr>
<th>Level of Confidence</th>
<th>Group I</th>
<th>Group II</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Frequency</td>
<td>%</td>
<td>Frequency</td>
</tr>
<tr>
<td>1 fully follow</td>
<td>31</td>
<td>34.1</td>
<td>25</td>
</tr>
<tr>
<td>1 partially follow</td>
<td>60</td>
<td>65.9</td>
<td>12</td>
</tr>
<tr>
<td>Total</td>
<td>91</td>
<td>100.0</td>
<td>37</td>
</tr>
</tbody>
</table>

\[\chi^2 = 11.997\]  \[\chi^2_{0.95} = 3.841\]  Result = significant
the project partially followed the advices given by their sources of information in relation to the dose of application, and that the producers that were not involved in the project exceeded the recommended dose [42]. Hence, it is of exceptional importance for productivity and quality that producers first question the information provided by their sources of information and then fully follow the recommendations given by the source in which they have confidence. In the present study, 43.8% of the growers stated that they fully followed and 56.2% stated that they partially followed the recommendations given by their sources of information. According to the chi-square test, it was found that there was a significant relationship between the growers’ educational attainment and the extent to which they follow the recommendations given by their sources of information (Table 8). In short, the growers are more likely to follow the recommendations given by their sources of information as the level of their educational attainment increases.

**Participation in Agricultural Extension Activities.** Agricultural development can be achieved only if the technological innovations introduced based on scientific research findings start to be commonly used by producers. In this context, agricultural extension has been a driving factor and element of social innovation for the development of agriculture for centuries. Agricultural extension is usually carried out by the Ministries of Agriculture and Rural Development across the globe. In addition, universities and non-governmental organisations also take part in agricultural extension activities. The only public institution that is responsible for agricultural extension in Turkey is the Ministry of Agriculture. The primary function of the agricultural extension practitioners working at the sub-provincial units of the Ministry is to keep producers abreast of agricultural innovations and to deliver training courses to ensure that the innovations are adopted by producers. They are also responsible for equipping them with skills to resolve the problems they may face during their agricultural activities [43, 44, 45]. After having fulfilled this function for years, the Ministry drafted the "Regulation of Rearrangement of Agricultural Extension and Advisory Services", which went into effect in 2006, in an effort to privatise the extension. Thus, the agricultural extension and advisory activities were assigned to the agricultural extension practitioners working at provincial/sub-provincial units of the Ministry as well as to agricultural advisors working at non-governmental organisations, chambers of agriculture and agricultural consultancy companies or independently on their own account to provide agricultural advisory services. While independent agricultural advisors are currently not an alternative to the public agricultural extension practitioners, they have become an important option in the regions where agriculture, and in particular, greenhouse cultivation are carried out intensively, such as the Aegean and Mediterranean Regions [46].

A study conducted in Isparta found that 78.6% of the producers had not attended any training or extension activity concerning fertilisation [9]. In a study researching the extent to which vegetable growers in Diyarbakır benefit from agricultural extension activities, 86.3% of the growers stated that no extension activity had been carried out in their village [13]. The participation of the growers in the agricultural extension activities carried out by public agricultural extension practitioners was also addressed in the present study. In this scope, the growers were asked whether any training course about agricultural topics had been delivered in their village in the last 5 years. 51.6% of the growers stated that they had not heard about it, 41.4% answered yes and 7.0% answered no. 62.5% of the growers stated that they had not attended the courses and 37.5% stated that they had attended them. In response to the question on which subjects they would like to see courses being delivered, 46.9% answered production techniques, 21.1% agricultural innovations, 19.5% application of pesticides and fertilisation, and 12.5% other (Table 9). 98.4% of the growers have a smart phone and Internet connection. Thus, they are able to search for and easily find information about any agricultural subject and the problems they face regarding agricultural production. However, information pollution on the Internet is an issue. For this reason, apart from the Internet, growers need to consult public agricultural extension practitioners or independent agricultural advisors that are specialised in that particular area so that they can reach correct and up-to-date information concerning agricultural production.

<table>
<thead>
<tr>
<th>TABLE 9 Participation of Growers in Agricultural Extension Activities</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Agricultural Extension Activities</strong></td>
</tr>
<tr>
<td>Has any training course about agricultural topics been delivered in your village in the last 5 years?</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Have you attended any training course delivered in your village?</td>
</tr>
<tr>
<td></td>
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<td></td>
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<td></td>
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<td></td>
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</tbody>
</table>
CONCLUSION

This study analyses the sources of agricultural information available to 128 greenhouse tomato growers in Aksu, Antalya. According to the data, the initial source of information of 68.0% of the growers is their father. The growers have been cultivating tomato in greenhouses for 23.7 years on average. Hence, 72.7% of the growers consider themselves highly knowledgeable about greenhouse cultivation. Furthermore, it may be said that the agricultural extension and training activities carried out by public and private organisations for long years have contributed to this opinion about themselves. In response to the question "What is your primary source of information in relation to issues concerning greenhouse tomato cultivation?", 51.6% of the growers answered pesticide dealers, 14.8% their own knowledge and experience, 10.9% independent agricultural advisors, 9.4% other growers, 7.8% public agricultural extension practitioners and 5.5% the Internet. In short, the growers opt for pesticide dealers, their own knowledge and experience or independent agricultural advisors, rather than public agricultural extension practitioners as a source of information. According to the chi-square test, there is a significant relationship between the growers’ educational attainment and the sources of information they consult with respect to economic matters. In brief, the growers are more likely to consult the sources on the Internet with respect to economic matters as the level of their educational attainment increases.

In Turkey, the Ministry of Agriculture and Forestry is officially in charge of keeping producers abreast of new agricultural techniques and technologies and inform them of the inputs used in agricultural production. The Ministry has been fulfilling this function for long years. However, the data derived from numerous studies focusing on the issue have shown that the Ministry has not been sufficiently effective in meeting the expectations of producers. Therefore, the opportunities for cooperation among universities, research institutes, public agricultural extension agencies, input dealers, independent agricultural advisors, chambers of agriculture and agricultural credit cooperatives should be enhanced in order to ensure both efficient use of public resources and collective performance of the activities that are currently carried out incoherently. For this purpose, not only producers, but also all stakeholders involved in the agricultural sector should be kept abreast of new agricultural production techniques, technologies and economic matters, with extension programmes being developed and regular short training courses delivered under the coordination of public agricultural extension practitioners at the provincial and sub-provincial units of the Ministry. Information brochures should be distributed to agricultural input suppliers and meetings should be held during the periods when the use of agricultural inputs is critical, such as the diagnosis of diseases and pests and identification of the time for pesticide use. Thus, it can be ensured that producers access the correct information in a timely manner. Research has shown that as the level of educational attainment increases, producers are more willing to attend agricultural extension activities and are engaged in agricultural practices more consciously. Therefore, participation in such activities will result in the enterprises’ productivity and profitability being enhanced in the short-term and contribute to the raising of the living standards in rural areas in the long-term. Additionally, an agricultural communication network with a direct link on the Ministry’s website should be developed so that producers can be kept abreast of agricultural innovations, access the correct information they need in relation agricultural issues immediately and exchange information. Moreover, producers can be provided with advisory services free of charge using Internet tools such as Facebook, WhatsApp, Instagram, Twitter, Skype, Viber, Tango and voice chat websites under the coordination of the Ministry. Thus, producers will be able to access correct information provided by various sources, rather than a single source, in a timely manner.

REFERENCES


STUDY ON ULTRASONIC ASSISTED AND ULTRAMICRO PULVERIZATION TECHNOLOGY FOR EXTRACTING FLAVONOIDS FROM THE HORDEUM VULGARE TENDER SEEDLING AND THEIR ANTIOXIDANT ACTIVITIES

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ABSTRACT

In this experiment, the content of total flavonoids in barley seedlings was determined by ultrasonic synergistic ultrafine pulverization method, and its antioxidant activity was studied. The results showed that the optimum extraction conditions were as follows: extraction with ethanol concentration of 70%, ratio of material to liquid of 1:20 (g/mL) and ultrasonic power of 200 W for 20 min, and the extraction amount could reach 0.3432 mg/g under this condition; The effect is better than the method of ultrasonic and enzymatic hydrolysis, respectively. The antioxidant activity of total flavonoids prepared by this method was studied. The results showed that DPPH free radicals and hydroxyl radicals have strong scavenging ability. According to the stability of the experiment, the precision and the recovery of the standard addition indicate that the reproducibility of this experiment is an effective way to extract the total flavonoids from the Hordeum vulgare tender seedling.

KEYWORDS:
Hordeum vulgare Tender seedling, ultrafine pulverization, ultrasonic, flavonoids, oxidation resistance

INTRODUCTION

Barley (Hordeum vulgare) belongs to the genus Hordeum and Poaceae. The barley seedlings are rich in vitamins, chlorophyll, flavonoids, antioxidant enzymes and protein. [1-2] It is “the king of natural food” and “close to perfect food” [3]. It has been reported that flavonoids contained in barley seedlings have strong ability to scavenge free radicals and resist oxidation. There are many methods for extracting flavonoids, such as hot water extraction, alkaline water or alkaline dilute alcohol extraction and other organic solvent extraction methods, as well as microwave extraction, ultrasonic extraction, supercritical fluid extraction, enzymatic extraction and Semi-bionic extraction method, etc.

In this experiment, the content of total flavonoids in barley seedlings was determined by ultrasonic synergistic ultrafine pulverization method, the process conditions were optimized, and the oxidation resistance was studied.

MATERIALS AND METHODS

Materials and reagents. 15-20 cm barley seedling sample; quercetin standard (analytical grade); absolute ethanol, phenanthroline, sodium dihydrogen phosphate, disodium hydrogen phosphate, ferrous sulfate, DDPH are analytically pure, hydrogen peroxide (30%).


Experimental method. Sample preparation. The harvested fresh barley seedlings are washed, dried, pulverized with an ultrafine pulverizer, and placed in a dry bottle for storage.

Determination of the maximum absorption wavelength. Pipette 0.5 mL of 0.242 mg/mL quercetin standard solution into a 10 mL colorimetric tube, dilute to the mark with 80% ethanol solution, and measure the absorbance of the sample solution at 190-500 nm in a 3 cm absorption cell. The 80% ethanol solution was used as a blank to determine the wavelength corresponding to its maximum
absorption peak. According to the experimental measurement, when the absorption light wavelength is 336 nm, the absorbance of the sample solution is the largest, so the absorption wavelength used when determining the total flavonoid content should be 336 nm.

**Drawing of standard curve.** Preparation of the standard curve: accurately absorb the above standard solution 0.00, 0.20, 0.40, 0.60, 0.80, 1.00, 1.20 in a 10mL colorimetric tube, and make up to volume with 80% ethanol [4,5]. The absorbance (A) value of each standard sample was measured at a wavelength of 336 nm using 80% ethanol as a blank control. Then take the absorbance (A) value as the ordinate and the standard sample concentration as the abscissa to draw the standard curve, draw the working curve, and find the linear regression equation as: A=27.782C+0.001, correlation coefficient R²=0.999.

**Determination of flavonoid content.** A certain amount of the collected liquid was taken, and its absorbance was measured at 336 nm. The concentration of flavonoids in the barley tender powder in the collected liquid was calculated according to the regression equation: A=27.782C+0.001, and the content of flavonoids was calculated according to the following formula.

\[ X = \frac{C \times n \times V}{w} \]

Wherein \( X \) ----flavonoid content, the unit is mg / g;
\( c \) ----the total flavonoid concentration of the test solution calculated from the standard curve, the unit is mg/ml;
\( n \) ----multiple dilution of sample solution;
\( v \) ----the volume of the liquid to be tested, the unit is ml;
\( w \) ----The quality of raw materials, the unit is g.

**Single-factor experiment.** (1) **Effect of ethanol concentration on total flavonoids extraction.** Accurately weighed 1.00g of barley tender seedling powder, a total of 7 parts, respectively, with 20 mL of 0, 10%, 30%, 50%, 70%, 90%, 100% ethanol solution in a conical flask, 100W, ultrasonic extraction time is After 30 min, after extraction, seven different volume fractions of ethanol extract were poured into a 50 mL colorimetric tube, and the volume was adjusted to 50 mL with distilled water. After stabilization, filter paper was used to pipette 0.5 mL and 10 mL colorimetric tubes. Then, the absorbance was measured at a wavelength of 336 nm by using a 80% ethanol solution to a volume of 10 mL.

(2) **Effect of ultrasonic time on the extraction of total flavonoids.** Accurately weighed 1.00g of barley tender seedling powder, 5 parts, respectively, added 20 mL of 70% ethanol, and extracted 10, 20, 30, 40, 50, 60 min under ultrasonic power 50 W respectively. After extraction, six different extraction times of ethanol were extracted. Pour into the 50 mL colorimetric tube, dilute to 50mL with 70% ethanol solution, stabilize with filter paper and pipette 0.5mL and 10mL colorimetric tube, then dilute to 10 mL with 70% ethanol solution. The line was measured for absorbance at a wavelength of 336 nm.

(3) **Effect of ultrasonic power on the extraction of total flavonoids.** Accurately weighed 1.00g of barley tender seedling powder, 6 parts, respectively, added 20mL of 70% ethanol, respectively, with ultrasonic wave time of 30min, respectively, with 100 W, 130 W, 160 W, 200 W, 250 W, 280 W ultrasound, the extraction is completed, six different ultrasonic powers will be Pour the ethanol extract into a 50mL colorimetric tube, and dilute to 50 mL with 70% ethanol solution. After stabilization, filter with a filter paper and pipette 0.5mL and 10mL colorimetric tube, then dilute with 70% ethanol solution. The absorbance was measured at a wavelength of 336 nm up to a 10 mL reticle.

\[ y = 27.782x + 0.0008 \]
\[ R^2 = 0.999 \]
(4) Effect of ratio of material to liquid on extraction of total flavonoids. Accurately weigh 1.00g of barley tender seeding powder, 5 parts, soaked in 1:10, 1:20, 1:30, 1:40, 1:50, 70% ethanol solution, ultrasonic power 100W, ultrasonic extraction time is 30min. After the extraction is completed, six different volumes of ethanol extract are poured into a 50 mL colorimetric tube, and the volume is adjusted to 50 mL with 70% ethanol solution. After stabilization, filter paper is used to pipette 0.5 mL and 10 mL colorimetric tubes. Then, the absorbance was measured at a wavelength of 336 nm by diluting to a 10 mL reticle with a 70% ethanol solution.

Orthogonal experiment. According to the results of single factor test, the main factors affecting the extraction of flavonoids in barley tender powder were determined, orthogonal design and experiment were carried out, and the orthogonal test results were verified to determine the best extraction of flavonoids by ultrafine pulverization and ultrasonic extraction process conditions.

Antioxidant activity test. (1) Determination of the ability to scavenge hydroxyl radicals (·OH) [6-7]. Add 1 mL of 0.75 mol/L phenanthroline solution, 2 mL 0.2 mol/L, pH 7.4 phosphate buffer and 1 mL of distilled water to 10 mL plugged test tube, mix well, add 1 mL 0.75 mol/L FeSO₄ solution, mix well. After the reaction was started by adding 1 mL of 3% H₂O₂, the absorbance A₁ value was measured at 511 nm in a water bath at 37°C for 60 min, and the absorbance A₂ was measured by using 1 mL of the sample instead of 1 mL of H₂O₂. The absorbance A₃ [8] was measured at a wavelength of 532 nm using 1 mL of the sample instead of 1 mL of distilled water.

\[
s = \left( \frac{A_2 - A_1}{A_2 - A_3} \right) \times 100\%
\]

Determination of DPPH free radical scavenging capacity [9]. Take 2 mL of DPPH solution and add 2 mL of the sample dissolved in the same solvent to mix well. The absorbance was measured at 517 nm after standing at room temperature for 30 min. The DPPH free radical scavenging rate is calculated as:

\[
s_i = \left( 1 - \frac{A_i - A_e}{A_j} \right) \times 100\%
\]

Among them, A_i is the absorbance measured by 2 mL of sample solution and 2 mL of DPPH solution (2×10⁻⁴ mol/L); A_e is the absorbance measured by 2 mL of solvent and 2 mL of DPPH solution; A_j is 2 mL of sample solution and 2 mL of solvent the measured absorbance.

RESULTS AND DISCUSSION

Single factor condition determination. Effect of Ethanol Concentration on the Extraction of Total Flavonoids. It can be seen from Fig. 2 that the extraction amount of ethanol solution with different concentrations is much higher than that of water extraction. With the increase of ethanol concentration, the extraction amount increases gradually. When the ethanol concentration is 70%, the extraction amount is the highest. The solvent used for extracting flavonoids must have strong solvency for total flavonoids. Flavonoids can be well dissolved in ethanol and water, both of which can be used as extraction solvents, but flavonoids are more soluble in ethanol, so ethanol is used as a solvent for better extraction. Therefore, it is reasonable to choose ethanol concentration of 70%.

![FIGURE 2](https://example.com/figure2.png)

**FIGURE 2**
Effect of Ethanol Concentration on the Extraction of Total Flavonoids
Effect of extraction time on total flavonoids extraction. It can be seen from Fig. 3 that as the extraction time is prolonged, the amount of sample extraction is gradually reduced. This may be because the solvent is volatilized in a large amount due to long-term treatment, and the extraction time is too long, and the flavonoid compound undergoes a certain chemical reaction. The active ingredient in the medium is destroyed, and the amount of elution of the impurities is increased, which causes inconvenience to subsequent operations. When the ultrasonic extraction time was 10 min, the concentration of the active ingredient reached equilibrium and the total flavonoid content was the highest. Therefore, it is more reasonable to select the ultrasonic time of 10 min.

Effect of Ultrasonic Power on the Extraction of Total Flavonoids. It can be seen from Fig. 4 that the ultrasonic power has a great influence on the extraction rate, the ultrasonic power is continuously increased, and the flavonoid extraction rate first increases and then decreases, because for a certain frequency, the ultrasonic power increases, and the sound intensity increases. Increase. The amplitude of the sound pressure and the pressure in the liquid also increase, and the time required for the collapse of the cavitation bubble is shortened, which is advantageous for increasing the yield. However, as the ultrasonic power continues to increase, the amplitude of the sound pressure increases, so that the cavitation bubble can not collapse within the sonic compression phase, and the cavitation effect is not obtained, and the effect is affected by the oxidation effect [10]. When 130 W is used, the extraction amount of flavonoids is the highest.

Effect of ratio of material to liquid on extraction of total flavonoids. It can be seen from Fig. 5 that the ratio of flavonoids is the highest when the ratio of material to liquid is 1:20. This may be because the solvent can reach a certain volume and the active ingredients in the barley tender powder can be completely extracted, exceeding the optimum volume. When the content of impurities in the extract increases, the content of the active ingredient decreases, which affects the extraction effect. Therefore, it is more reasonable to choose the ratio of material to liquid at 1:20.
Orthogonal test. On the basis of the results of single factor experiment, the orthogonal experiment was carried out by selecting four factors of $L_9(3^4)$ four factors to determine the optimum technological conditions for flavonoid extraction. The factors are shown in Table 1. The experimental results are shown in Table 2.

The range analysis showed that the main order of factors affecting the extraction amount of flavonoids in barley tender powder was: A (ethanol concentration) > B (liquid-to-liquid ratio) > D (extraction power) > C (extraction time), the best The combination is: A2B3C2D3, that is, the ethanol concentration is 70%, the ratio of material to liquid is 1:40 (g/mL), the ultrasonic extraction time is 20 min, and the ultrasonic extraction power is 200 W.

**Comparison of extraction rate of total flavonoids in different crushing methods.** Under the optimal scheme of orthogonal test, the content of total flavonoids in ultrafine and ordinary pulverized barley seedlings was determined, as shown in Table 3 and Table 4.

It can be seen from Table 4 and Table 3 that the content of flavonoids in the ultrafinely pulverized barley seedling powder is significantly higher than that of ordinary pulverization. After ultrafine pulverization, the cell wall is broken, which contributes to the dissolution of flavonoids and accelerates the dissolution rate.
### TABLE 3
Total flavonoids extracted from samples prepared by ultrafine pulverization

<table>
<thead>
<tr>
<th>Test number</th>
<th>Absorbance A</th>
<th>Extraction amount (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.991</td>
<td>0.3563</td>
</tr>
<tr>
<td>2</td>
<td>0.941</td>
<td>0.3383</td>
</tr>
<tr>
<td>3</td>
<td>0.932</td>
<td>0.3350</td>
</tr>
<tr>
<td>Average</td>
<td>0.9547</td>
<td>0.3432</td>
</tr>
</tbody>
</table>

### TABLE 4
Total flavonoids extracted from ordinary pulverized samples

<table>
<thead>
<tr>
<th>Test number</th>
<th>Absorbance A</th>
<th>Extraction amount (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.462</td>
<td>0.1660</td>
</tr>
<tr>
<td>2</td>
<td>0.543</td>
<td>0.1951</td>
</tr>
<tr>
<td>3</td>
<td>0.441</td>
<td>0.1584</td>
</tr>
<tr>
<td>Average</td>
<td>0.482</td>
<td>0.1732</td>
</tr>
</tbody>
</table>

### TABLE 5
Stability test results

<table>
<thead>
<tr>
<th>Times</th>
<th>Absorbance</th>
<th>Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.797</td>
<td>0.287</td>
</tr>
<tr>
<td>10</td>
<td>0.796</td>
<td>0.286</td>
</tr>
<tr>
<td>20</td>
<td>0.797</td>
<td>0.287</td>
</tr>
<tr>
<td>30</td>
<td>0.796</td>
<td>0.286</td>
</tr>
<tr>
<td>40</td>
<td>0.795</td>
<td>0.286</td>
</tr>
<tr>
<td>50</td>
<td>0.796</td>
<td>0.286</td>
</tr>
<tr>
<td>60</td>
<td>0.797</td>
<td>0.287</td>
</tr>
</tbody>
</table>

### TABLE 6
Precision test results

<table>
<thead>
<tr>
<th>Times</th>
<th>Absorbance</th>
<th>Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.771</td>
<td>0.277</td>
</tr>
<tr>
<td>2</td>
<td>0.771</td>
<td>0.277</td>
</tr>
<tr>
<td>3</td>
<td>0.772</td>
<td>0.278</td>
</tr>
<tr>
<td>4</td>
<td>0.772</td>
<td>0.278</td>
</tr>
<tr>
<td>5</td>
<td>0.772</td>
<td>0.278</td>
</tr>
<tr>
<td>6</td>
<td>0.771</td>
<td>0.277</td>
</tr>
</tbody>
</table>

### TABLE 7
Recovery rate of plus standard

<table>
<thead>
<tr>
<th>Sample</th>
<th>Before adding standard solution</th>
<th>Amount of adding standard solution</th>
<th>After adding standard solution</th>
<th>Recovery rate</th>
<th>Average value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.350</td>
<td>0.0</td>
<td>0.350</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0.350</td>
<td>0.2</td>
<td>0.453</td>
<td>76.65%</td>
<td>110.71%</td>
</tr>
<tr>
<td>3</td>
<td>0.350</td>
<td>0.4</td>
<td>0.642</td>
<td>108.57%</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0.350</td>
<td>0.6</td>
<td>0.756</td>
<td>100.62%</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0.350</td>
<td>0.8</td>
<td>0.978</td>
<td>116.74%</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>0.350</td>
<td>1.0</td>
<td>1.366</td>
<td>150.95%</td>
<td></td>
</tr>
</tbody>
</table>

**Precision.** Accurately transfer 0.500mL of test solution to a 10mL colorimetric tube and add 80% ethanol solution to the engraved line. The absorbance was measured at 277 nm. The total parallel measurement was 6 times. The relative standard deviation (RSD) of the test was 0.197%. It shows that the precision of this method is high. The results are shown in Table 6.

**Standardized recovery test.** In the 0.2 mL sample solution of the orthogonal test No. 2 sample, 0.0, 0.2 mL, 0.4 mL, 0.6 mL, 0.8 mL, and 1.0 mL of the standard solution were added to measure the absorbance, and the average recovery rate was 110.71%. The results are shown in Table 7.

**Analysis of antioxidant activity results.** Hydroxyl radical (OH·) scavenging ability. The ability of total flavonoids to scavenge hydroxyl radicals (OH·) is an important indicator for evaluating their antioxidant activity. From the results in Table 8, it can be seen that different concentrations of total flavonoids have a certain ability to scavenge (OH·).

**Clear DPPH free radical ability.** The extracted total flavonoids were formulated into solutions of 0.01, 0.02, 0.03, 0.04, and 0.05 mg/mL to perform DPPH free radical scavenging detection. As can be seen from Figure 6, flavonoids have a strong ability to scavenge DPPH free radicals.
TABLE 8

| No. | $A_1$ | $A_2$ | $A_3$ | Clearance rate |%
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.340</td>
<td>0.266</td>
<td>0.332</td>
<td>89.2</td>
</tr>
<tr>
<td>2</td>
<td>0.336</td>
<td>0.260</td>
<td>0.327</td>
<td>88.2</td>
</tr>
<tr>
<td>3</td>
<td>0.337</td>
<td>0.264</td>
<td>0.329</td>
<td>89.0</td>
</tr>
</tbody>
</table>

Average value 88.8

CONCLUSIONS

(1) Ultrasonic power with appropriate power can effectively increase the extraction amount of total flavonoids in barley seedlings. Ethanol concentration>liquid-to-liquid ratio>extraction power>ultrasonic time. The best combination of process is: ethanol concentration is 70%, ratio of material to liquid is 1:40 (g/mL), ultrasonic extraction time 20 min, ultrasonic power 200 W, under the optimal process conditions, the total flavonoids extracted from barley tender powder was 0.343.

(2) Total flavonoids were extracted from the samples treated by ordinary pulverization and ultrafine pulverization, respectively. The results showed that the ultrafine pulverization method had higher yield than the ordinary pulverization method, and the total extraction was carried out by the ultrafine pulverization method. Flavonoids can greatly save time for simple extraction, so they have obvious advantages.

(3) The precision relative standard deviation of the experiment was RSD=0.197%, the stability relative standard deviation was RSD=0.187%, and it was stable within 60 min. The recoveries were 110.71%.

(4) Determination of flavonoid antioxidant activity: (a) Different concentrations of total flavonoids have certain scavenging ability to (OH*), (b) DPPH-clearing ability: DPPH clearance rate of barley tender seedling powder with increasing flavonoid concentration stronger.

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REFERENCES


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EVALUATION AND ANALYSIS OF THE COORDINATED DEVELOPMENT OF ENVIRONMENT AND ECONOMY

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ABSTRACT

Economic development, accompanying with environmental damage and energy depletion, becomes essential nowadays. This study is aim to build a dynamic model of the economy–environment system, which explicitly explore the interaction of economics and environment and effects of the key influencing factors. Nanjing is selected as a case study to verify our model. Results shows that, current scenario is not sustainable; technology scenario is applicable to economic growth; With the introduction of environment and economic friendly sustainable development strategy, returning farmland to forests, afforestation and other attempts to improve the ecological environment, but the deterioration of ecological environment is slow, and its benefits are difficult to appear in the short term. The environment fitting curve can reflect out, the time series shows fluctuations, and the overall environment is gradually improving.

KEYWORDS:
Coordination assessment, environment, economy, economy–environment system, dynamic model

INTRODUCTION

Sustainable development is a major factor in any social planning and economic programs and environmental issues are a key component of the process. Sustainable development relates to the ability to meet the needs of the current generation without compromising that of future generations [1]. In a complex social-economic-environmental system, the economic subsystem relates to the funding aspects of development while the social and environment subsystems refer to providing necessary human resources and material foundations [2]. Since traditional economic development that is based on unfettered economic growth has degraded the environment and depleted natural resources, finding alternative development paths and changing human behavior is a critical undertaking for policy makers [3, 4]. Further, creating the most suitable development model requires a strong understanding of the key contributors to sustainable development systems as well as their interactions.

As the biggest developing country, China has achieved significant economic growth. There are also a number of issues associated with the current economic development model such as the unreasonable industrial structure, intensive resource consumption and serious environmental pollution [5-9]. For instance, many cities in China are experiencing frequent haze episodes in recent years [10]. The Chinese government has put forward specific goals on energy saving and emission reduction in the 11th and 12th Five-Year Plan [11]. However, some of these goals were not achieved by the end of 11th Five-Year period [12]. This indicates that an effective mechanism of coordination among energy, economy and environment has not been established [13-16]. Although much efforts have been made for energy saving and emission reduction, the energy efficiency level still fell behind. The development of economy and environment are not coordinated. Therefore, it is imperative to explore how economy and environment subsystems interact and consequently investigate how to coordinate their developments.

It is urgent to develop clear information evaluation models to simulate the relationship between ecological environment and social economic development for management and decision-making basis. Three-dimensional model has been gradually applied to the evaluation field, such as the city’s environmental quality, eco-city planning, and the natural capital utilization [17-19]. Few coupled model has been studied, such as Niccolucci et al., [20], Niccolucci et al., [21] proposed the concept of “three-dimensional ecological footprint” for natural capital accounting. Application of three-dimensional model on simulation the relationship between ecological environment and social economic development, especially on carrying capacity of regional ecological environment to social economic development was less reported. Therefore, a three-dimensional evaluation model for the carrying capacity of regional ecological environment to social economic development has been established to evaluate the comprehensive level of regional integrated system. It can clarify the main contradiction between social development stage and sustainable development and can provide management and decision-making basis to achieve economic and
environmental coordination, sustainable development.

This study presents a CCDM method to model the dynamic interactions and future development of the urban system. Comparisons with existing results and policy recommendations are provided in this paper.

**MATERIALS AND METHODS**

Evaluation of the coupling coordination degree. Assessment indicator system for the economy–environment system. We constructed an indicator system based on previous case studies and the structure of our established SD model to synthetically analyse the development level of each subsystem and the coupling relationships of the economy–environment system. Table 1 shows the structure of the indicator system, which contains 9 indicators. The indicator system synthetically reflects economic production and industrial structure in the economy subsystem and environmental pollution and environmental governance in the environment subsystem. Table 2 shows division of the development stages of the economy–environment system.

**RESULTS AND DISCUSSION**

**TABLE 1**

<table>
<thead>
<tr>
<th>Indicator system used to assess CCD of the economy–environment system</th>
<th>Indicator</th>
<th>Direction</th>
<th>Unit</th>
<th>Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subsystem</td>
<td>Indicator</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Economy subsystem</td>
<td>Per capita GDP</td>
<td>+</td>
<td>Yuan/capita</td>
<td>0.3562</td>
</tr>
<tr>
<td></td>
<td>Per capita built-up area</td>
<td>+</td>
<td>Km²/capita</td>
<td>0.2474</td>
</tr>
<tr>
<td></td>
<td>Proportion of the secondary industry</td>
<td>+</td>
<td>%</td>
<td>0.2717</td>
</tr>
<tr>
<td></td>
<td>Proportion of the tertiary industry</td>
<td>+</td>
<td>%</td>
<td>0.1247</td>
</tr>
<tr>
<td>Environment subsystem</td>
<td>Discharge of COD</td>
<td>–</td>
<td>Tons/capita</td>
<td>0.0745</td>
</tr>
<tr>
<td></td>
<td>Discharge of SO₂</td>
<td>–</td>
<td>Tons/capita</td>
<td>0.2122</td>
</tr>
<tr>
<td></td>
<td>Discharge of solid waste</td>
<td>–</td>
<td>Tons/capita</td>
<td>0.2528</td>
</tr>
<tr>
<td></td>
<td>Environmental protection investment</td>
<td>+</td>
<td>Yuan/capita</td>
<td>0.2201</td>
</tr>
<tr>
<td></td>
<td>Pollution Index</td>
<td>–</td>
<td>Dimensionless</td>
<td>0.2404</td>
</tr>
</tbody>
</table>

Notes: ‘+’ and ‘−’ represent the positive and negative indicators, respectively.

**TABLE 2**

<table>
<thead>
<tr>
<th>Value of D</th>
<th>0≤D&lt;0.25</th>
<th>0.25≤D&lt;0.5</th>
<th>0.5≤D&lt;0.75</th>
<th>0.75≤D≤1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Development stages</td>
<td>Seriously unbalanced</td>
<td>Slightly unbalanced</td>
<td>Barely balanced</td>
<td>With superior balance</td>
</tr>
</tbody>
</table>

**FIGURE 1**

Contour Plot of Value of D vs D, C

**FIGURE 2**

Contour Plot of Value of D vs D, B
Fig.3-4 shows that the development speeds of both energy and economic systems are decreasing, implying the slowing down of economic, which could also lead to the decrease of energy demand. At the same time, the environment index is slightly increased and the coordination of environment and economy comprehensive development could be improved. For example, comparing to 2012, the development speeds of both economic and energy systems decreased in 2013, but environment and environment-economy system show an opposite trend.

Fig.5-6 shows that energy and economic systems could be positively correlated. Therefore, besides changing the economic mode of high-energy consumption and low energy efficiency, slowing down the economic development and inhibiting the energy demand could also help to improve the environmental quality.

In the past 10 years, the coupling degree of ecological environment and economic system in Nanjing has fluctuated, which makes it possible for the population of Nanjing to continue to increase and the industrial structure to adjust continuously. With the introduction of ecological environment and economic friendly sustainable development strategy, returning farmland to forests, afforestation and other attempts to improve the ecological environment, but the deterioration of ecological environment is slow, and its benefits are difficult to appear in the short term. The ecological environment fitting curve can reflect out, the time series shows fluctuations, and the overall ecological environment is gradually improving.

CONCLUSIONS

This study is aim to build a dynamic model of the economy–environment system, which explicitly explore the interaction of economics and environment and effects of the key influencing factors. Nanjing is selected as a case study to verify our model. Results shows that, current scenario is not sustainable; technology scenario is applicable to economic growth; With the introduction of environment and economic friendly sustainable development strategy, returning farmland to forests, afforestation and other attempts to improve the ecological environment, but the deterioration of ecological environment is slow, and its benefits are difficult to appear in the short term. The environment fitting curve can reflect out, the time series shows fluctuations, and the overall environment is gradually improving.
ACKNOWLEDGEMENTS

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REFERENCES


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THE EFFECTS OF SOWING DATE ON GROWTH, SEED YIELD AND OIL CONTENT OF SUNFLOWER (HELIANTHUS ANNUUS L.) CULTIVARS UNDER RAINFED CONDITIONS

Ismail Demir*

Kirsehir Ahi Evran University, Faculty of Agriculture, Department of Field Crop, Kirsehir, Turkey

ABSTRACT

This study was conducted at Research Farm of Ahi Evran University in Kirşehir Province of Turkey in 2012 and 2013 to determine effects of sowing date on yield and agronomic characteristics of sunflower hybrid cultivars in rainfed conditions. Five sowing dates 10 days apart set on 10th April, 20th April, 1st May, 10th May and 20th May and 6 hybrid sunflower cultivars (LG-5580, SanayMr, SanbroMr, Sirena, Tarsan and Transol) were used. The study was taken place in both rainy (2012) and dry (2013) warm conditions due to different weather. In this way, the effects of both sowing dates and extreme climatic conditions were tested. Sunflower yield and yield components were higher in 2012 than 2013, except dehulled/hulled seed weight and oil content. When sowing date was delayed, seed and oil yields declined. The highest plant height (151.18 cm), 1000-seed weight (51.72g), crude oil content (46.18%), seed yield (2.55 t ha⁻¹) and oil yield (1.18 t ha⁻¹) were obtained on the second sowing date (20th of April) while the highest ratio of dehulled/hulled seed weight (70.04%) and head diameter (20.49 cm) were obtained on the first sowing date (10th of April) in 2012. However, the first sowing date in early April resulted in higher yield and agronomic characteristics than delayed sowing due to a decrease in rainfall during the growing period of both research years. SanbroMr cultivar in the first year and Transol cultivar in the second year reached the higher yields with best yield components. In conclusion, by considering the spring last frost date, the sowing date of 20th April provided significant improvements in yield and yield parameters due to the longer growing season with suitable soil moisture, allowing sufficient time for vegetative growth and head and achenes filling.

KEYWORDS:
Oil content, rainfed, sowing date, sunflower, yield.

INTRODUCTION

Sunflower is an essential edible oil seed plant, which has been ranked at 3rd after soybean and rapeseed in the world. World sunflower production was 47.3 million tons from 26.2 M ha areas in 2016 [1]. Sunflower is a temperate zone crop, which can easily adapt and perform well under a variety of climate and soil condition [2-4]. Sunflower is an important oilseed crop also to meet the vegetable oil demands of Turkey due to the high drought tolerance and sufficient yield in non-irrigated areas [5-7]. Although sunflower has high yield with great adaptation capacity, the main factors affecting sunflower production in rainfed conditions are irregular and inadequate amount of precipitation during the growing season [8]. Additionally, higher temperatures (and consequently higher evapotranspiration rates), relatively shallow soils and uncertain water storage during winter and growing season restrict plant growth and sunflower yield [9]. With delayed sowing, the generative development is hastened and early harvesting time is observed due to the crops are exposed to higher temperature with dry conditions [10]. As usually known, it is a kind of reproduction instinct of plant to continue its generations. Shortening of growing cycle decreases the amount of radiation intercepted during the growing season and thus total dry matter at harvest [11-13]. When the sowing time is delayed sunflower yield decreases in rainfed conditions [13, 14]. The water availability of soil is usually low during flowering and seed maturation, which are crucial periods in terms of seed filling. Water deficiency during these periods reduces the supplementation of nutrients from the soil for reproductive growth and, consequently, reduces seed yield [15, 16]. The number and weight of seeds per unit of area, which are main yield components of sunflower, are closely related to air temperature and soil water content during critical seed set period [11, 17, 18]. Andrade [11] reported that a delay in sowing date reduced grain yield in sunflower due to decreases in the number of seeds and their weight per square meter. The sowing date had significant effects on the both seed yield and its oil content in sunflower cultivars under different climate conditions [19].
MATERIALS AND METHODS

The field study was conducted in springs of 2012-2013 growing season at the Research Farm of Ahi Evran University in Kırşehir Province. This experimental field is located at 39.15° Northern latitude and 34.11° Eastern longitude at 1014 meters above sea level.

The experimental design was randomized complete block design with split plot arrangement having 3 replicates. Five planting dates (on 10th April, 20th April, 1st May, 10th May and 20th May) were main plots and six varieties (LG-5580, SanayMr, SanbroMr, Sirena, Tarsan and Transol) were subplots. The plot size was 4.2 m x 6.0 m. SanayMr, SanbroMr, Sirena, Tarsan and Transol) were main plots and six varieties (LG-5580, SanayMr, SanbroMr, Sirena, Tarsan and Transol) were subplots. The plot size was 4.2 m x 6.0 m.

According to the climatic data, the experimental site was under arid and semi-arid conditions, the water is the most limiting factor for sunflower production. That is why the early sowing date has a great importance for maintaining reasonable production values in these climate conditions [9]. The productivity in sunflower farming depends on the environmental factors such as temperature, precipitation and rainfall distribution and also sowing date [20-23].

The previous studies [21, 24-27] underlined the importance of the genetic materials and sowing date on sunflower seed yield and its oil content. Therefore, it is crucial to identify effects of sowing date on seed yield and yield components of hybrid sunflower cultivars under rainfed conditions to meet of vegetable oil demand and improve the cultivation of sunflower in arid conditions. The purpose of this research is to examine the changes in seed yield, oil content and yield components, in response to without irrigation, in six hybrid cultivars of sunflower at five different sowing dates (spring), in rainfed conditions.

TABLE 1

<table>
<thead>
<tr>
<th>Texture</th>
<th>pH</th>
<th>EC (mmhos/cm)</th>
<th>Salinity (%)</th>
<th>Available P2O5 (kg ha⁻¹)</th>
<th>CaCO3 %</th>
<th>Available K2O (kg ha⁻¹)</th>
<th>OM (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clayed-Loamy</td>
<td>7.59</td>
<td>0.52</td>
<td>0.02</td>
<td>21.4</td>
<td>27.90</td>
<td>666.2</td>
<td>1.81</td>
</tr>
</tbody>
</table>

TABLE 2

<table>
<thead>
<tr>
<th>Month</th>
<th>Relative Humidity (%)</th>
<th>Precipitation (mm)</th>
<th>Temperature (°C)</th>
</tr>
</thead>
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<tr>
<td>January</td>
<td>83.7</td>
<td>78.7</td>
<td>83.7</td>
</tr>
<tr>
<td>February</td>
<td>79.8</td>
<td>74.5</td>
<td>74.4</td>
</tr>
<tr>
<td>March</td>
<td>68.4</td>
<td>67.6</td>
<td>63</td>
</tr>
<tr>
<td>April</td>
<td>50.3</td>
<td>63.8</td>
<td>63.2</td>
</tr>
<tr>
<td>May</td>
<td>66.5</td>
<td>61.5</td>
<td>50.7</td>
</tr>
<tr>
<td>June</td>
<td>47.7</td>
<td>54.3</td>
<td>41.1</td>
</tr>
<tr>
<td>July</td>
<td>38.8</td>
<td>48.4</td>
<td>41.2</td>
</tr>
<tr>
<td>August</td>
<td>42.7</td>
<td>48.7</td>
<td>39.7</td>
</tr>
<tr>
<td>September</td>
<td>39.4</td>
<td>53.2</td>
<td>50</td>
</tr>
<tr>
<td>October</td>
<td>63.0</td>
<td>63.7</td>
<td>52.9</td>
</tr>
<tr>
<td>November</td>
<td>82.5</td>
<td>73.6</td>
<td>67.1</td>
</tr>
<tr>
<td>December</td>
<td>84.6</td>
<td>78.6</td>
<td>75.7</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>62.23</td>
<td>63.73</td>
<td>58.56</td>
</tr>
</tbody>
</table>
the long-term annual average in 2013. Annual precipitation was 499.40 mm in 2012, which was above the long-term average precipitation values. On the other hand, annual precipitation was 254.70 mm in 2013, which was considerably below the long-term average precipitation values. Total monthly precipitation was observed as irregular during the months of sunflower cultivation. Precipitation was 109.5 mm in April, 2012. Although this level of precipitation might appear to have a positive impact on the total level of annual precipitation, irregular precipitation in the other months and less rain in July having a potential to affect yield adversely. Temperature values during the cultivation period were above the long-term average for the region.

RESULTS AND DISCUSSION

According to the present results, the differences in sunflower yield parameters among sowing dates and cultivars are shown in Tables 3 and 4. Sowing dates and cultivars affected all investigated parameters significantly. The effects of sowing date on plant height, 1000 seed weight, head diameter and ratio of dehulled/hulled seed weight (only at 0.05 significance level in 2013), oil content, seed yield and oil yield were statistically important (at 0.01 significance level) in 2012 and 2013 growing season. On the other hand, cultivar had significant effects on yield and agronomic traits (at 0.01 significance level), except plant height, the ratio of dehulled/hulled seed weight (at 0.05 significance level in 2012), 1000-seed weight and crude oil content (at 0.05 significance level in 2013). However, the interaction of cultivar x sowing date was not significant, except for 1000- seed weight, seed yield and oil yield (Tables 3 and 4).

Plant Height. Significant differences were detected in plant height between two years. The average plant height in 2012 was 12.65 cm higher than that of 2013 due to the changed weather conditions, considerably below the long-term average precipitation values in 2013. When comparing the two years with respect to plant height, the highest plant height was observed in the first year on 2nd sowing date, 20th April (151.18 cm), since the highest amount of rainfall was recorded during this growing season (Table 3). This was evidently associated with warmer and rainy weather conditions prevailing during the early growth stage and flowering, particularly at the first three sowing dates. In contrast, in the second year of study, the lower precipitation and warm weather conditions affected all sowing dates and resulted in stable decrease in plant height. In the second year, the highest plant height (142.37 cm) was observed for the 1st sowing date on 10 April. Depending on the ability of varieties to adapt to environmental conditions, the plant heights were significantly different between cultivars for both years (Tables 3 and 4). In the first year when rainfall was high, SanayMr (145.18 cm) produced significantly higher plants than others. In the second year when rainfall was low, SanbroMr was the

| TABLE 3 |
| Effects of sowing date and cultivars on yield and yield characters of oilseed sunflower grown in 2012. |

<table>
<thead>
<tr>
<th>Analysis of Variance</th>
<th>Df</th>
<th>Plant Height (cm)</th>
<th>Head diameter (cm)</th>
<th>1000 Seed Weight (g)</th>
<th>The ratio of dehulled/ hulled seed weight</th>
<th>Oil content (%)</th>
<th>Seed yield (t ha⁻¹)</th>
<th>Oil yield (t ha⁻¹)</th>
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<td>Replication</td>
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<td>ns</td>
<td>ns</td>
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<td>ns</td>
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<td>ns</td>
<td>ns</td>
</tr>
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<td>Sowing Dates</td>
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<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
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<td>Error1</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Cultivars</td>
<td>5</td>
<td>*</td>
<td>**</td>
<td>**</td>
<td>*</td>
<td>**</td>
<td>**</td>
<td>**</td>
</tr>
<tr>
<td>SDxC</td>
<td>20</td>
<td>ns</td>
<td>ns</td>
<td>*</td>
<td>ns</td>
<td>ns</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Error</td>
<td>50</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CV(%)</td>
<td>4.44</td>
<td>7.17</td>
<td>6.43</td>
<td>3.25</td>
<td>2.82</td>
<td>6.56</td>
<td>6.5</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sowing date</th>
<th>Plant Height (cm)</th>
<th>Head diameter (cm)</th>
<th>1000 Seed Weight (g)</th>
<th>The ratio of dehulled/ hulled seed weight</th>
<th>Oil content (%)</th>
<th>Seed yield (t ha⁻¹)</th>
<th>Oil yield (t ha⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>April 10</td>
<td>150.71a</td>
<td>20.49a</td>
<td>50.04a</td>
<td>70.04a</td>
<td>46.18a</td>
<td>2.06c</td>
<td>0.95b</td>
</tr>
<tr>
<td>April 20</td>
<td>151.18a</td>
<td>19.39b</td>
<td>51.72a</td>
<td>69.79a</td>
<td>46.05a</td>
<td>2.55a</td>
<td>1.18a</td>
</tr>
<tr>
<td>April 30</td>
<td>141.82b</td>
<td>16.85c</td>
<td>47.22b</td>
<td>68.07a</td>
<td>44.10b</td>
<td>2.18b</td>
<td>0.96b</td>
</tr>
<tr>
<td>May 10</td>
<td>137.94b</td>
<td>15.38d</td>
<td>42.82c</td>
<td>64.67b</td>
<td>42.89b</td>
<td>1.84d</td>
<td>0.79c</td>
</tr>
<tr>
<td>May 20</td>
<td>126.48a</td>
<td>14.52d</td>
<td>39.31d</td>
<td>62.90b</td>
<td>42.85b</td>
<td>1.54e</td>
<td>0.66d</td>
</tr>
<tr>
<td>LSD</td>
<td>4.51</td>
<td>1.07</td>
<td>2.54</td>
<td>2.28</td>
<td>1.58</td>
<td>0.97</td>
<td>0.53</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cultivars</th>
<th>Plant Height (cm)</th>
<th>Head diameter (cm)</th>
<th>1000 Seed Weight (g)</th>
<th>The ratio of dehulled/ hulled seed weight</th>
<th>Oil content (%)</th>
<th>Seed yield (t ha⁻¹)</th>
<th>Oil yield (t ha⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lg5580</td>
<td>140.51b</td>
<td>16.38c</td>
<td>38.95d</td>
<td>67.56a</td>
<td>43.51b</td>
<td>1.94c</td>
<td>0.85d</td>
</tr>
<tr>
<td>SanayMr</td>
<td>145.18a</td>
<td>16.69c</td>
<td>47.75b</td>
<td>67.08a</td>
<td>43.22b</td>
<td>1.93c</td>
<td>0.84e</td>
</tr>
<tr>
<td>SanbroMr</td>
<td>141.01b</td>
<td>18.47b</td>
<td>48.44b</td>
<td>67.25a</td>
<td>43.86b</td>
<td>2.33a</td>
<td>1.02a</td>
</tr>
<tr>
<td>Sirena</td>
<td>141.84b</td>
<td>17.89a</td>
<td>45.07c</td>
<td>65.31b</td>
<td>45.44a</td>
<td>1.95c</td>
<td>0.88cd</td>
</tr>
<tr>
<td>Tarsan</td>
<td>141.83b</td>
<td>17.84b</td>
<td>50.13a</td>
<td>67.03a</td>
<td>45.04a</td>
<td>2.04b</td>
<td>0.92c</td>
</tr>
<tr>
<td>Transol</td>
<td>139.37b</td>
<td>15.78d</td>
<td>47.00bc</td>
<td>68.33a</td>
<td>45.40a</td>
<td>2.04b</td>
<td>0.93b</td>
</tr>
<tr>
<td>LSD</td>
<td>4.61</td>
<td>0.875</td>
<td>2.18</td>
<td>1.07</td>
<td>0.919</td>
<td>0.98</td>
<td>0.43</td>
</tr>
<tr>
<td>MEAN</td>
<td>141.62</td>
<td>17.33</td>
<td>46.22</td>
<td>67.09</td>
<td>44.41</td>
<td>2.03</td>
<td>0.91</td>
</tr>
</tbody>
</table>

* , ** significant at the 0.05 and 0.01 level, respectively. For each main effect, values within columns followed by the same letter are not significant. ns, nonsignificant.
highest (132.38 cm). On the other hand, Transol (139.37 and 126.88 cm) had the lowest plant height in all seasons. When the cultivars were compared in two different wet and dry seasons, significant differences were obtained in plant lengths of the cultivars. In wet season SanayMr (14.95 cm high) and Sirena (14.67 cm high) had higher plant height than dry condition whereas SanbroMr (8.63 cm high) was affected by the seasons in a lesser extent. Semiarid, arid and rainfed condition rainfall is very important climate parameter for vegetative growth and in this research the lack of rainfall caused significant reductions in plant height. These observations were also supported by, Baghdadi, et al. [30], Ahmed, et al. [25] and Ozturk, et al. [27] who emphasized that the plant heights of sunflower increased with the early sowings dates but decreased with delayed sowing dates.

**Head Diameter.** The mean head diameters of the sunflower cultivars in 2012 and 2013 were 17.33 and 15.04 cm, respectively. Both high precipitation in 2012 and low precipitation in 2013 affected the size of the head diameter compared to long-term rainfall. The highest head diameter was obtained from the first sowing date and delaying sowing date reduced head diameter in both years. Similar finding was reported by Miller, et al. [31] and Ozturk et al. (2017) and they emphasized that when sowing date was delayed, head diameter, seed yield and crude oil content declined. Comparing the two year head diameter results of cultivars, cultivar Sirena (18.79 cm) in 2012 and cultivar LG5580 (15.91 cm) in 2013 had highest head diameter and cultivars Transol (15.78 cm) in 2012 and cultivar Tarsan (14.01 cm) and Sirena (14.39 cm) had smallest head diameter in 2013. Depending on the genetic characteristics of the cultivars, significant changes were observed in head diameter in rainy and dry periods. The significant difference in the head diameter could be attributed to the availability of adequate moisture, which helps roots to absorb sufficient amount of nutrients for plant growth and development [21].

**Thousand Seed Weight.** Thousand seed weight was significantly affected by sowing date. Planting on 20th April produced heavier seeds while sowing on 20th May produced lighter seed weight in 2012, in which more rain than long-term was observed. In the 2nd year, early sowing date on 10th April, the heaviest seed weights were obtained than other sowing dates. In both two years delaying sowing date decreased 1000-seed weight. The reduction in 1000-seed weights for the late sown sunflower can be explained by increased temperature [32] in rainfed conditions. Overall, 1000-seed weight was between 38.95 to 50.13 g in 2012, and 37.53 and 40.76 g in 2013. Cultivar Tarsan had the heaviest 1000- seed weight (50.13 g) in the first year and LG5580 had the heaviest 1000-seed weight (38.95 g) in rainy weather conditions. In the second year, cultivar SanbroMr had the heaviest 1000- seed weight (40.76g), while SanayMr had the lightest 1000-seed weight (37.53 g). The 1000-seed weights of cultivars were different between the years 2012 and 2013. This is due to the decrease in

![Table 4](image-url)
precipitation and soil moisture which cause negative effect on seed growth period. With respect to 1000-seed weight, Tarsan cultivar was better in the rainy season, whereas SanbroMr cultivar was better in arid conditions. This is due to the genetic capabilities and different reflex of the cultivars for changing environmental conditions. The significant sowing date x cultivar interaction was observed for 1000-seed weight. The interaction indicated that the heaviest 1000-seed weight (60.82 g) was observed for 20th April sowing date in Tarsan cultivar in 2012 while this was 47.60 g for 10th April sowing date in SanbroMr cultivar in 2013 (Figure 1a-b).

The Ratio of Dehulled/Hulled Seed Weight. The ratio of dehulled/hulled seed weight which directly affected crude oil ratio in sunflower is an important quality criterion. The results presented in Table 3 and 4 of the ratio of dehulled/hulled seed weight, indicate both genetic and climatic effects. Comparing two years, the sunflower cultivars were tended to produce a higher ratio of dehulled/hulled seed weight in 2013 (70.76%) than that of 2012 (67.09%). These differences related to the weather condition. The climatic effects revealed by the significant differences for the cultivars in the ratio of dehulled/hulled seed weight highest in 2013 and lowest in 2012. Precipitation was much higher in 2012 than in 2013, throughout the growing cycle, especially after flowering. This can be related to the seed hull more grower than the seed kernel when precipitation is sufficient. This would also explain the difference in the seed yield of cultivars, which was much higher in 2012 than in 2013 (2.03 vs. 1.38 t/ha). Similar findings were reported by Killi and Altunbay [33], Ahmad [34] and Dauguet, et al. [35]. The higher ratios of dehulled/hulled seed weight (70.04%, in 2012) and 72.23%, in 2013) were obtained from the first sowing dates, and at the later sowing dates resulted decreases in both years. Killi and Altunbay [33] reported that sunflower crops did not have sufficient time to fill achenes due to late sowing and, consequently, had higher hull ratio.

Crude oil content (%). In general, the crude oil content of sunflower cultivars seed was significantly changed by year and sowing dates (Tables 3 and 4). For example, the crude oil content of seed in 2012 (44.41%) was significantly lower than that of 2013 (47.50%). These differences related to the

FIGURE 1
The interaction of sowing date and cultivars on thousand seed weight (g) graphics in 2012 (a) and 2013 (b)

FIGURE 2
The interaction of sowing date and cultivars on seed yield (t/ha⁻¹) graphics in 2012 (a) and 2013 (b)
The effects of the interaction between sowing date and cultivars on oil yield (t/ha) graphics in 2012 (a) and 2013 (b)

Seed Yield. The results showed that the higher annual mean seed yield (2.03 t ha⁻¹) was obtained in rainy and warmer season (in 2012) compared to dry and warm season in 2013 (1.38 t ha⁻¹). In general, when sowing dates of two years (2012 and 2013) were compared, 20th April produced maximum seed yield, and lowest seed yield was recorded on 20th May (last sowing date). According to the seed yield data, sunflower cultivars showed the significant differences in both years. SanbroMr (2012) and Transol (2013) were tended to have higher seed yield (2.33 and 1.62 t ha⁻¹, respectively) than other cultivars. Cultivar Sirena gave the lowest seed yield (1.93 and 1.22 t ha⁻¹) in both years. Related studies (Ali et al., 2012; Nasim et al., 2012; Hussain et al., 2015) reported that the cultivars showed wide differences in their agronomic characteristics and seed yield, depending on their genotypes and environmental conditions. Different responses occurred between sowing date and cultivars in both years. The higher seed yields (2.84 and 1.87 t ha⁻¹) were obtained from SanbroMr (2012) and Transol (2013) sunflower cultivars planted on 20th April sowing date. The lowest yields (1.37 and 1.07 t ha⁻¹) were obtained from Sirena cultivar on 20th May (2012) and 10th May (2013) sowing dates (Figure 2a-b).

Seed yield generally decreased with delayed sowing, and this might be attributed to the decrease in yield components [37]. This behavior might be ascribable to the best soil water content in the early stages of plant development [38].

Oil Yield. The oil yield is calculated by multiplying the seed yield by crude oil content. Therefore, oil yield mainly depends on seed yield. The oil yield obtained in this study was significantly changed by sowing date and cultivars in both years. In both years, oil yield generally decreased when sowing was delayed. The highest oil yield (1.29 and 0.92 t ha⁻¹) was obtained from sunflower plants sowed at the end of April (in second sowing date) compared to the early and late sowed plants for both two years. According to the oil yield results, sunflower cultivars were completely different (Tables 2 and 3) between each other. Similar to seed yield and oil content, the oil yield of SanbroMr (1.02 t ha⁻¹) and Transol (0.77 t ha⁻¹) was higher than those of other cultivars in 2012 and 2013. The interaction between sowing dates and cultivars had also a significant influence on oil yield. The highest oil yield was obtained from the SanbroMr (1.29 t ha⁻¹) in 2012 and from Transol (0.92 t ha⁻¹) in 2013 when sown at 20th April (Figure 3a-b). In addition to this, SanbroMr cultivar in rainy and warm season
and Transol cultivars in dry and warm season for had superior oil yields than others due to the height seed yield and crude oil content. Differences among hybrids for seed oil content and seed yield may be attributed to their genetic potential as well as interactive effects of environmental variables during achene development and crop physiological maturity [39].

CONCLUSIONS

Hybrid sunflower commercial varieties produced higher seed yield having higher oil content in sunflower seed production trials. The changes in yield and yield parameters are dependent upon the environmental conditions in which the varieties are cultivated. When we take into account the negative effects of climate change, the choosing of cultivars and the sowing date in arid regions are very important factors affecting the yield and yield parameters. There is a need to conduct further studies on sowing date and cultivars due to the changes in global climate conditions and new hybrid cultivars by advanced breeding studies for different ecological conditions. It is necessary to efficient sunflower production to meet demand of world vegetable oil and this necessity increased the importance of this kind of studies as determining sowing date and cultivars. For this reason, five different sowing dates (spring) and six hybrid sunflower cultivars were compared in order to determine which of them maximize their yield and quality parameters in the present study.

The results of the study showed that the yield and yield components in rainfed conditions generally tend to be decreased in both two years (2012 and 2013) with delayed sowing. Sunflower cultivars showed yield performance in terms of sowing date. This was a distinctive factor for both years in the present study. Also, the changes in production traits might be related to the annual weather differences. The research also showed how the cultivars and sowing dates effect yield and yield parameter of sunflower in both dry and wet conditions. To conclude, the early planting (on 20th April) of sunflower cultivars provided significant advantage in yield and yield parameters due to the long growing season with convenient soil moisture and sufficient time to fill head and achenes.

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REFERENCES


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THE EFFECT OF ORGANIC WASTES ON THE HORIZONTAL AND VERTICAL WETTING DISTANCE IN SOIL

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2Bingol University, Faculty of Agriculture, Department of Biosystem Engineering, 12000, Bingol, Turkey
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4Bingol University, Faculty of Agriculture, Department of Landscape Architecture, 12000, Bingol, Turkey

ABSTRACT

The growing world population has made establishing new agricultural areas and increasing the fertility of current areas necessary. In terms of yield and productivity, sandy soils are generally poor in terms of crop production and require good management in terms of plant production. Due to their large pores, they possess high water conduction capacities, however their water holding capacity is low. Water movement in these soils is formed as a result of directing by gravity and adhesive forces. The aim of this study was to determine the effect of walnut shell biochar (WS), farm manure (FM) and worm manure (VC) on the water movement in the horizontal and vertical direction in the soil. Soil was mixed with soil organic matter (SOM) at different doses (0%, 1%, 2%, 4% or 8%). Following the incubation period, water movement was observed in the soil irrigated with a constant debit. As a result of the research, it was determined that FM and VC applications increased the wetting distances in horizontal direction. In addition, increased doses of SOM increased the horizontal wetting distance (p <0.001). Similarly, increased doses of SOM were determined to decrease the wetting distance in the vertical direction.

KEYWORDS:
Wetting Distance, Irrigation, Water movement, Infiltration, Organic matter

INTRODUCTION

The most important factor limiting the plant growth is the lack of beneficial water in the plant root area in arid areas and areas with limited water resources. The usability of the soil water in the root area of the plant is limited to the extent allowed by the physical and hydraulic properties of the soils. Information on the hydraulic properties of the soil is necessary to solve problems involving irrigation, drainage, protection of water, infiltration and control of surface flow [1]. Most of these problems are closely related to the water movement in the soil and, therefore, the wetting area. Wettability is a parameter that affects the physical and chemical properties of soil. According to [2], the depth of the wet soils is related to the depth of the root system, while the width is related to the design of irrigation systems. The capacity of any soil to absorb and retain water depends on the type of clay fraction that forms the soil, the level of organic matter it contains, the compression state of the soil, the moisture content and other physical characteristics [3, 4, 5, 6, 7, 8].

Whether in liquid or vapor form, water is almost always in motion in soil. The water movement in the soil is generally affected by two forces. The first one is gravity while the second is adhesive force. Adhesive forces are higher in clayey soils due to the number and the structural properties of the pores. Therefore, water movement is slower in clayey soils than that in sandy soils [9]. Soil organic matter (SOM) positively affects the formation of aggregates in the soil due to its physical and chemical properties and therefore directly or indirectly affects the water movement and wettability in the soil. In numerous studies, it was determined that SOM levels had a positive effect on soil-water relationship [10, 11, 12]. Demir and Dogan Demir [13] found that increased SOM levels in sandy soils increased the water holding capacity and reduced the hydraulic conductivity. Pandey and Shukla [14] stated that composting application on sandy soils increased water retention in the soil, preventing the water loss in soil. Stamatiadis et al. [15] reported that SOM addition reduced the rate of infiltration in sandy soil.

The aim of the present study was to investigate the effect of the level of SOM on the horizontal and vertical water movement in the soil. Accordingly, in a sandy soil with different levels of SOM addition, time dependent motion and wetting distance of the soil water in horizontal and vertical directions were examined.
MATERIALS AND METHODS

The research was carried out in Bingöl University Soil Science and Plant Nutrition Department Laboratories. The study was carried out in the laboratory to minimize the environmental adverse factors and to control the incubation of SOM added to the soil. The mean temperature in the laboratory was 25±3 °C while the mean moisture was 65±3%. The general properties of the experimental materials, soil and SOMs are given in Table 1. Walnut shell (WS) biochar, farm manure (FM) and vermicompost (VC) used in the study were provided by a commercial company.

Texture analysis of soils was carried out according to the Bouyoucos hydrometer method [16]. The pH and electrical conductivity values were measured in a saturated soil medium according to [17]. Organic matter content in the soil was determined according to the Smith Weldon method [18]. The lime content of the soil was determined according to [19] using the ‘Scheibler Calcimeter’. The cation exchange capacity was determined using sodium acetate as described by [20]. Volume weights were determined as described by [21]. Porosity was determined according to [22].

The SOMs were sieved through a 0.5-mm-diameter sieve and added to the soil at 0% (control), 1%, 2%, 4% or 8% weight/weight (w/w) ratios. The mixtures were prepared with three replications and placed into the transparent glass earthenware containers (Figure 1). The experimental setup consisted of a glass earthenware container (10 cm x 50 cm x 50 cm), an inoffcone funnel for setting the water flow, a stopwatch and a camera. The bottom of the soil container was drilled for free water movement. The soil-SOM mixture added to the test cups was incubated under laboratory climatic conditions at the field capacity level and incubated for three months. The quality of irrigation water used in the study was classified as C1S1 according to [23].

### TABLE 1

The general properties of the materials

<table>
<thead>
<tr>
<th>Properties</th>
<th>Soil</th>
<th>WS</th>
<th>VC</th>
<th>FM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clay (%)</td>
<td>17.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Silt (%)</td>
<td>24.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sand (%)</td>
<td>58.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soil texture class</td>
<td>Sandy Loam</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Great Soil Group</td>
<td>Xerorthent</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>7.0</td>
<td>7.4*</td>
<td>6.7*</td>
<td>6.5*</td>
</tr>
<tr>
<td>EC (µS/cm)</td>
<td>176.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CaCO₃ (%)</td>
<td>0.1</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Bulk density (g/cm³)</td>
<td>1.49</td>
<td>0.57</td>
<td>0.38</td>
<td>0.32</td>
</tr>
<tr>
<td>Porosity (%)</td>
<td>42.7</td>
<td>68.0</td>
<td>72.0</td>
<td>77.0</td>
</tr>
<tr>
<td>Organic Matter (%)</td>
<td>2.3</td>
<td>77</td>
<td>46</td>
<td>69</td>
</tr>
<tr>
<td>CEC</td>
<td>31.6</td>
<td>33.4</td>
<td>48.2</td>
<td>47.1</td>
</tr>
<tr>
<td>C:N</td>
<td>9:1</td>
<td>78:1</td>
<td>15:1</td>
<td>22:1</td>
</tr>
</tbody>
</table>

*1:2 H₂O, WS: Walnut shell, VC: Vermicompost, FM: Farm manure, EC: Electrical conductivity, CEC: Cation exchange capacity, C:N: Rate of carbon on nitrate

### FIGURE 1

The experimental design (soil box was 0.10 m in width, 0.5 m in length and 0.5 m in height)
Following the incubation process, irrigation was ended and the soils were left to dry under room conditions. At the beginning of the trial, the application debit of the water to the soil was set to 2 L/h. At this stage, the amount of water that will be given to the soil was adjusted by means of a valve and the horizontal and vertical wetting distance in the soil was photographed at the 5th, 10th, 20th and 40th minutes. The obtained images were transferred to the Autocad program in scale and the wetting distances were recorded horizontally and vertically in millimeters. The Autocad software was used for a more precise measurement. The statistical evaluation of the data obtained in the research was carried out JUMP 5.01 statistical software.

RESULTS AND DISCUSSION

The horizontal and vertical wetting distances were observed in SOM-added soils drip-irrigated with a specific debit. The point where the water was dripped on the soil and the wetting distances at the 5th, 10th, 20th and 40 minutes are given in Figures 2, 3 and 4. The SOM dose had an effect on the wetting distance in the horizontal direction. It was observed that the soil wetting distance in the soil increased with the increase in SOM level. This result was observed in all three SOM types. The 8% WS application in the soil had a higher effect on the horizontal water movement compared to the soil to which no SOM application was carried out. Similar results were also obtained in for FM and VC applications.

The vertical movement of the water was also affected by the SOM application. The soil water under the effect of gravity has a tendency to drain to the lower layers through large pores in sandy soils. However, the transformation of these large pores into smaller geometric shapes by the SOMs added to the soil slows down the downward movement of the water. Furthermore, materials with greater adhesive strength added to the soil affect the water movement in the soil. As seen in Figure 2, water movement in the vertical direction in a sandy soil was affected by the SOM added to the soil.

FIGURE 2
The Effect of organic matter level (control, 1%, 2%, 4%, 8%) the water movement in the soil at a given period of time in terms of wetting distance/time (cm / min).
Figure 3 and Figure 4 shows the effects of SOM types on wetting distances according to their doses. In general, the wetting distance in the vertical direction according to the reference surface (soil surface) was observed in soils with no SOM treatment. On the other hand, the minimum wetting distance was obtained in the WS application at the 5th minute, and in the VC application at the 10th, 20th and 40th minutes.

**FIGURE 3**
The effect of organic matter level (control, 1%, 2%, 4%, 8%) on water movement in soil in terms of wetting distance/dose at the 5 and 10 minutes

**FIGURE 4**
The effect of organic matter level (control, 1%, 2%, 4%, 8%) on water movement in soil in terms of wetting distance/dose at the 20 and 40 minutes
The effect of SOMs on the wetting distance of soil water was evaluated statistically and the results are given in Table 2. The 4% and 8% doses of FM as the SOM type had statistically significant effects on the wetting distance in the horizontal direction (p < 0.01). Although the lowest wetting distance value in the vertical direction in the same period was determined in the WS application, it was statistically located in the same group as the VC application (p < 0.01). As shown in Table 2, the SOM had no statistically significant effect on the wetting distance in the vertical direction at the 5th minute. Examining the wetting distance of the water in horizontal and vertical directions at the 10th minute, FM had a significant effect on the horizontal direction compared to other SOM applications (p < 0.001). In other words, the wetting distance of the sandy soil with FM application for 10 minutes was higher than those of the other SOM applications. The application doses 2%, 4% and 8% were significantly different from the control and 1% applications and located in the same group. The wetting distance in the vertical direction was the same as that measured at the 5th minute. In the 10th minute, WS and VC applications were more effective than FM application in the horizontal wetting distance. The lowest wetting distance in the vertical direction was obtained in 4% dose (p < 0.001).

In the measurements made in the 20th minute, it was determined that VC application yielded the highest horizontal wetting distance value in the soil. In the same period, the highest horizontal wetting distance value was obtained in soils containing 8% SOM (p < 0.001). The lowest distance in vertical wetting distance was obtained in VC applications. It was found that the highest mean wetting distance in the vertical direction at the 20th minute was determined at 8% dose. In this case, 8% SOM application to a sandy soil significantly reduced the wetting distance in the vertical direction at the 20th minute compared to the control application. It was determined that the highest wetting distance in horizontal direction was determined in WS application at the 40th minute and the highest mean dose effect was determined in 4% and 8% applications. SOM addition at the 40th minute had no effect on the wetting distance in the vertical direction. The mean application dose in the vertical direction was obtained in 8% application.

The water entering into the soil starts to move in time due to various forces and forms a wetting area. The dimensions of this area are important in designing drip irrigation projects, determining the dripper intervals and intra-row distances between plants [24, 25, 26, 27]. The wetting pattern and the horizontal and vertical wetting distances of this pattern are affected by some physical and chemical properties of the soil [28, 29]. Since SOM is associated with many properties of soil, it also has the ability to affect the water movement in the soil. In this study, horizontal and vertical water motion was visually examined by adding SOM to sandy soils with limited properties in terms of yield and productivity. As a result of the research, it was determined that WS, FM and VC applications had an effect on horizontal and vertical water movement in the soil. Similarly, application doses of these materials were found to be effective.

In the study, the wetting distance in the horizontal direction in SOM-added soils was higher than that in nontreated soils. On the other hand, the wetting distance in the vertical direction was lower in the SOM-added soils. SOM can change the soil pore structure and, therefore, can have an effect on water movement. Soil texture and especially pore geometry determine the proportional importance of capillary and gravitational forces [30]. Sandy soils have larger pores than those of clayey soils. Therefore, the capillarity is very weak in sandy soils. Hachum et al. [31] found that the gravitational force was limited in fine-grained soils, that the horizontal and vertical wetting distances were equal to each other whereas the vertical wetting distance in coarse-grained soils was higher. Sahin et al. [32] determined that the wetting distance values were lower in the horizontal and vertical directions in perlite-added soil compared to that in pumice.

<table>
<thead>
<tr>
<th>SOM Dose (%)</th>
<th>Horizontal Distance</th>
<th>Vertical Distance</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>6.0c</td>
<td>9.4</td>
</tr>
<tr>
<td>1</td>
<td>5.7d</td>
<td>10.2</td>
</tr>
<tr>
<td>2</td>
<td>6.6b</td>
<td>9.5</td>
</tr>
<tr>
<td>4</td>
<td>7.2a</td>
<td>9.1</td>
</tr>
<tr>
<td>8</td>
<td>7.2a</td>
<td>9.7</td>
</tr>
</tbody>
</table>

(WS: Walnut Shell, FM: Farm Manure, VC: Vermicompost)
In this study, it was found that WS, FM and VC materials increased the wetting distance in vertical direction and decreased it in vertical direction in sandy soil. This result is important in terms of keeping the soil water in the plant root area. Today, approximately 13% of the earth’s surface is covered by Entisols (psamments), also known as sandy soils [33]. In the management of these soils, it is very important to prevent water drainage from the soil surface or by infiltration directly to the groundwater [34]. SOMs and other soil enhancers are often used in the management of problematic soils. According to FAO data, as of 2016, annual world organic fertilizer production reached 124 million tons. Adding these materials to sandy soils is important for the protection of soil water. WS, which has a particularly high active carbon content, has high suction power [35]. Therefore, WS plays an important role in the retention of water in the soils with high permeability. Water draining rapidly to the lower layers in sandy soils with the effect of gravity can be kept in the soil pores with the help of WS. In this study, the maximum effect on wetting distance in vertical direction was determined in WS application and this result can be associated with the high suction power of this material.

REFERENCES


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STABILITY EVALUATION OF BREAD WHEAT GENOTYPES UNDER VARYING ENVIRONMENTS BY AMMI MODEL

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1Kiziltepe Vocational School, Mardin Artuklu University, Mardin, Turkey
2Department of Field Crops, Faculty of Agriculture, Dicle University, Diyarbakir, Turkey

ABSTRACT

Stable and high yield varieties identification under various conditions prior to release as a variety is the main steps for breeding program. In order to exploit narrow and broad adaptability of genotypes and assess their effects, environment and GE interaction, 12 spring bread wheat genotypes were grown at four various experimental locations during 2013-14 and 2014-15 growing seasons. The stability and superiority of genotypes were identified by the AMMI (additive main effect and multiplicative interaction) and GGE (genotype, genotype x environment) biplot analysis. The AMMI analysis showed that the variance of genotype, environment and GE interaction were significant and the major treatment sum of squares were significantly affected by environments (85.47%), genotypes (8.51%) and GE interaction (6.07%). On the other hand, the first principal component axes (PCA 1) distributed to the complete interaction as 62.56%, and the second PCA 2 axes 37.44%. The GGE biplot analysis indicated that the total variation PC (principle component) was 83.09%, and PC1 was accounted as 63.69%, PC2 only 19.40%. The AMMI analysis showed that C11 was quite stable as well as the highest yielder among test genotypes, while C7 and C8 were unstable and low yielding across environments. The GGE biplot indicated that it was detected in two mega-environments, and the first mega-environment covered three environment (E1, E2 and E3), and the second mega-environment covered only E4. The genotypes C11 and C12 remained superior under ME I, while genotypes C1, C3 and C5 were for ME II. Among the genotypes, the genotype C1 may be recommended to be developed and released as an approved cultivar for being comparatively more stable and the highest yielder. Therefore the AMMI and GGE biplot models have an opportunity to determine the best genotypes under multiple environments considering on adaptability and stability concentrating on overall performance for screening superior genotypes.

KEYWORDS: Yield, interaction, superiority, environment.

INTRODUCTION

Bread wheat (Triticum aestium L.) has covered the most cultivation area and its product is the most widely used products in the world and of primary importance for human nutrition [1, 2]. The yield of bread wheat should be increased in parallel with the increasing population [3]. It is a mandatory food crop and provides as a staple food in different parts of the world [4]. Wheat is grown, mostly under rain-fed conditions in Turkey. It is a major food grain in Turkey, therefore the primary objective of wheat specialists is to increase the grain yield [5, 6, 7]. This is possible through the development and application of new cultivation techniques and also by the development of more efficient and high yielding cultivars [5]. For this purpose, many different methods have been used in order to characterize the behaviour of genotypes under changing environmental conditions.

Wheat genotypes identification with varied adaptation to dissimilar conditions, multi - location, resulting in the empirical identification of superior varieties [8]. The yield trials in different environments give information about the interaction between environment and genotypes, when analysed by traditional methods. However, it is so difficult to notice the influence of GEI in area which the environments are fluctuated, because in these conditions the effect of factors (G, E, GEI) are not clear. Several ecological and agronomic problems are restricting the grain yield of genotypes, so it is requested to use various models to overcome these problems and definite the best genotypes in across environments [9]. For this purpose, GE, GEI and AMMI have been built up in order to characterize the behaviour of genotypes under various environmental conditions. Therefore, many researchers have been used in different conditions, years and environments [10, 11]. GxE interaction leading to reduction the relationship between phenotypic and genotypic values and thus, a genotype that produce well performance in a given environment may not essen-
tially respond well in other environment. So if environments are sufficiently variant, GxE interaction can result in variant yield ranking of assessing genotypes [12]. The superiority of genotypes depends on their stability across locations, years or environments. If the genotypes performance changes in different environments, then the interaction of the genotype x environment is an important factor to improve new cultivars. Therefore, analysis of any variance combined can measure GEI and identify main effectiveness, however, it is not enough to declare the GEI effectiveness. A convenient analytic pattern-like the AMMI can cure both the additive main interactions and multiplicative interaction constituent like the AMMI can cure both the additive main and stable yield performance across various environments (s) by two biplot models (AMMI and GGE) (ii) to analyses the effect of GEI on grain yield of twelve spring bread wheat genotypes [14]. The GGE biplot exploits the PCA approach to use the ANOVA (analysis of variance) and IPCA (Interaction Principal Components Analysis) [13, 14]. The GGE biplot and stable yield performance across various environments, and stable yield performance across various environments. If environments are sufficiently variant, GxE interaction can result in variant yield ranking of assessing genotypes and (iii) to clarify the associations and variations among yield-stability to measure GE interaction impacts to identify high productivity and stable genotypes (iv) to detect appropriate genotype(s) for every location or environment.

MATERIALS AND METHODS

The field experiments were performed in (2013-2014 and 2014-2015) cropping season at various locations in Turkey. In this study, the yield performance, stability and genotypes environmental interactions several of twelve bread wheat genotypes were evaluated in four environmental conditions across two successive years. The information of genotypes and locations showed in Table 1. The information meteorological about test location is given in Table 2. Plant materials were organised in the form of a randomized complete block design with four replications.

Each parcel was set in a six row plot of 6 m length and 1.2 m diameter with plant spacing of 20 cm. Sowing of trials were done in autumn and sowing density was used 500 seeds in per m-2. The

### TABLE 1

<table>
<thead>
<tr>
<th>Environment code</th>
<th>Location</th>
<th>Latitude</th>
<th>Longitude</th>
<th>Altitude (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E1</td>
<td>Sanliurfa/Siverek</td>
<td>37°32'40.41&quot; N</td>
<td>39°24'14.78&quot; E</td>
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<td>E2</td>
<td>Diyarbakır</td>
<td>37°53'23.04&quot; N</td>
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<td>Mardin/Derik</td>
<td>37°12'36.58&quot; N</td>
<td>40°03'43.47&quot; E</td>
<td>483 m</td>
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<tr>
<td>E4</td>
<td>Diyarbakır</td>
<td>37°53'23.04&quot; N</td>
<td>40°16'34.42&quot; E</td>
<td>669 m</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Genotype Code</th>
<th>Name of cultivars</th>
<th>The name of developing institutions</th>
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</thead>
<tbody>
<tr>
<td>C1</td>
<td>DZ13-1</td>
<td>Dicle University, Faculty of Agriculture, Department of Field Crops</td>
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<tr>
<td>C2</td>
<td>CEMRE</td>
<td>GAP International Agricultural Research and Training Center</td>
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<tr>
<td>C3</td>
<td>DZ13-2</td>
<td>Dicle University, Faculty of Agriculture, Department of Field Crops</td>
</tr>
<tr>
<td>C4</td>
<td>PEHLIVAN</td>
<td>Trakya Agricultural Research Institute</td>
</tr>
<tr>
<td>C5</td>
<td>DZ759</td>
<td>Dicle University, Faculty of Agriculture, Department of Field Crops</td>
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<td>C6</td>
<td>RUMELI</td>
<td>Trakya Agriculture Company</td>
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<td>AVARIC</td>
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<td>C10</td>
<td>HAKAN</td>
<td>Çukurova University, Faculty of Agriculture, Department of Field Crops</td>
</tr>
<tr>
<td>C11</td>
<td>CEYHAN 99</td>
<td>Eastern Mediterranean Agricultural Research Institute</td>
</tr>
<tr>
<td>C12</td>
<td>27 SAWSN-3104</td>
<td>CIMMYT</td>
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### TABLE 2

<table>
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<tr>
<th>Environment /Year</th>
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<th>November</th>
<th>December</th>
<th>January</th>
<th>February</th>
<th>March</th>
<th>April</th>
<th>May</th>
<th>June</th>
<th>Total/ average</th>
</tr>
</thead>
<tbody>
<tr>
<td>E1-2013-14</td>
<td></td>
<td>78.6</td>
<td>55.4</td>
<td>82.5</td>
<td>100.8</td>
<td>79.0</td>
<td>24.3</td>
<td>10.3</td>
<td>0.7</td>
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<td>E2-2013-14</td>
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<td>53.8</td>
<td>50.4</td>
<td>43.0</td>
<td>60.6</td>
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<td>48.8</td>
<td>21.4</td>
<td>334.9</td>
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<tr>
<td>E3-2014-15</td>
<td>-</td>
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<td>100.4</td>
<td>60</td>
<td>111.0</td>
<td>149.9</td>
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<td>49.7</td>
<td>3.7</td>
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<td>69.2</td>
<td>55.6</td>
<td>29.0</td>
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<table>
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<th>December</th>
<th>January</th>
<th>February</th>
<th>March</th>
<th>April</th>
<th>May</th>
<th>June</th>
<th>Total/ average</th>
</tr>
</thead>
<tbody>
<tr>
<td>E1-2013-14</td>
<td>11.7</td>
<td>9.6</td>
<td>6.0</td>
<td>8.1</td>
<td>11.3</td>
<td>14.9</td>
<td>22.4</td>
<td>26.9</td>
<td>13.9</td>
</tr>
<tr>
<td>E2-2013-14</td>
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<td>11.4</td>
<td>3.4</td>
<td>3.4</td>
<td>10.8</td>
<td>14.7</td>
<td>19.7</td>
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<td>7.5</td>
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<td>10.1</td>
<td>14.5</td>
<td>22.5</td>
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<tr>
<td>E4-2014-15</td>
<td>9.8</td>
<td>3.9</td>
<td>1.1</td>
<td>7.9</td>
<td>9.7</td>
<td>15.7</td>
<td>19.9</td>
<td>26.8</td>
<td>12.6</td>
</tr>
</tbody>
</table>
fertilization of 60 kg N ha⁻¹ and 60 kg P ha⁻¹ was done sowing time, and 60 kg N ha⁻¹ was applied to each plot at tillering time. The grain yield was recorded from each plot after harvesting of crops and measured to ton/ha and was considered in to analysis.

Statistical analysis (AMMI and GGE). Statistical computations and estimation were done by GenStat version 14.1 [15] Copyright program.

RESULTS

The AMMI analysis. Genotype, location and genotype by location interactions were assessed by the additive main effect and multiplicative interaction (AMMI) model (Table 3). The AMMI analysis showed that variance of genotype, environment, GEI and IPCA had significant (p<0.01 and p<0.05) effect on the grain yield of 12 bread wheat genotypes tested in four environments (Table 3). The variance analysis of AMMI for wheat production detected positive influences for genotype, location and genotype by location interaction. The main effect of environment accounted for 85.47% of total variation, compared with 8.51% for genotype and 6.07% for GE interaction effects in the analysis of combined variance (Table 3). The quantity of genotypic effect was higher than the GEI effect, which indicates different possible presence of MEs in the METs data. The significant variations between the circles influenced the average of squares and caused the grain yield of each environment. The multiplicative variance of the treatment sum of squares due to interaction was partitioned into the two interaction principal components and the only one of them significant effect. The first PCs was significant and accounted for 62.56%, the second for 37.44% of the variability in grain yield of the 12 genotypes tested at four environments (Table 3).

Additionally, the AMMI model indicated the stability of genotypes across environment by IPCA scores which x-axis describes the genotypes and environment main impact and y-axis describes the influence of interaction (Fig. 1). The IPCA2 scores which closer to the zero means that they are stable genotypes across examined environments. Therefore, C11 was the most stable cultivar, followed, C2 and C9. On the other hand, C7 and C12 were the most unstable genotypes (Fig.1 and Table 4). Also IPCA 1 scores show the GEI effect and the highest IPCA1 scores and supported the substantially to GEI. Hence, E1 and E3 were highest yielding, while E2 and E4 were the low yielding among test environments. The environment located above the y-axis mean that they are desirable and high-yielding, whereas the environments, which are located under of y-axis mean that they are unfavorable and low-yielding. In the AMMI indicated that grain yield of the E3 (7598 kg/ha) is higher than the other environments (Fig.1). When assessed the environment conditions based on grain yield average, E1 and E3 were located above average (y axis), while E2 and E4 were located under average (y axis) in across environments (Table 5). The AMMI (Fig. 1) also showed the superiority of genotype based on mean grain yield of different environments. The genotypes located on the right of the y-axis mean that they are high yielding and favorable, whereas the genotypes which are located left side of y-axis mean that they are unfavorable and low-yielding. Accordingly, C1, C3, C5, C9, C11, and C12 were yielding and favorable, while C2, C4, C7, C8 and C10 are unfavorable genotypes across test environments (Fig.1 and Table 4). Thus, the influence of environments was responsible for the variation by genotype and genotype by environment interaction.

The GGE Biplot analysis. The mean grain yield across two environments of 12 spring bread wheat genotypes were showed in Table 4. This information was used to generate Fig. 2 - Fig. 4. The GGE bi-plot analysis indicated that the total variation PC (principles component) was 83.09%, and PC1 was accounted as 63.69%, PC2 only 19.40%, respectively (Fig.2-Fig.4). The GGE biplot provides beneficial explanation of the pattern, concerning first two IPCs. The high rate of environmental impacts indicated the positive variances were observed among the environments for grain production.

### TABLE 3

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>G+E+GE SS explained (%)</th>
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<td>3154634</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Treatments</td>
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<td>522499843</td>
<td>11117018</td>
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<tr>
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<td>44458777</td>
<td>4041707</td>
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<td>Environments</td>
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<td>706455</td>
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<td>Interactions</td>
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<td>960772</td>
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<td>IPCA 1</td>
<td>13</td>
<td>18108559</td>
<td>1392966</td>
<td>2.57**</td>
<td>62.56</td>
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<td>IPCA 2</td>
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<td>984916</td>
<td>1.82ns</td>
<td>37.44</td>
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<td>Error</td>
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<td>71557710</td>
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</tbody>
</table>

df - degrees of freedom; SS - sum of squares; MS - mean square; ns; not significant, **; p<0.05, **; p<0.01; G - genotypes; E - environment
### TABLE 4
AMMI-estimates per environment yield (t/ha) across years and locations

<table>
<thead>
<tr>
<th>Cultivars (E1)</th>
<th>Yield</th>
<th>(E2)</th>
<th>Yield</th>
<th>(E3)</th>
<th>Yield</th>
<th>(E4)</th>
<th>Yield</th>
<th>Mean</th>
<th>IPCAg [1]</th>
<th>IPCAg [2]</th>
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<tr>
<td>C1</td>
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<td>C1</td>
<td>4475</td>
<td>C1</td>
<td>8784</td>
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<td>4789</td>
<td>C12</td>
<td>6224</td>
<td>-7.023</td>
</tr>
<tr>
<td>C2</td>
<td>5906</td>
<td>C2</td>
<td>3355</td>
<td>C10</td>
<td>7358</td>
<td>C11</td>
<td>3487</td>
<td>C10</td>
<td>5027</td>
<td>-0.783</td>
</tr>
<tr>
<td>C3</td>
<td>6391</td>
<td>C6</td>
<td>3943</td>
<td>C4</td>
<td>8378</td>
<td>C9</td>
<td>4285</td>
<td>C5</td>
<td>5749</td>
<td>-9.195</td>
</tr>
<tr>
<td>C4</td>
<td>5690</td>
<td>C4</td>
<td>3827</td>
<td>C9</td>
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<td>C8</td>
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<td>C4</td>
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<td>C11</td>
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<td>C8</td>
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<td>2897</td>
<td>C2</td>
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<td>C2</td>
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<tr>
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<td>C7</td>
<td>3891</td>
<td>C8</td>
<td>5352</td>
<td>3.435</td>
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<tr>
<td>C10</td>
<td>4505</td>
<td>C3</td>
<td>3926</td>
<td>C12</td>
<td>6749</td>
<td>C1</td>
<td>4119</td>
<td>C1</td>
<td>4804</td>
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<tr>
<td>C11</td>
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<td>C9</td>
<td>3935</td>
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<td>7672</td>
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<tr>
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<td>C5</td>
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<tr>
<td>Mean</td>
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<td>3743</td>
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<td>4052</td>
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### TABLE 5
The first four AMMI selections for per environments, variance and IPCA scores

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<th>Env</th>
<th>Mean</th>
<th>Score 1</th>
<th>Score 2</th>
<th>Score 3</th>
<th>Score 4</th>
<th>IPCAg[1]</th>
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<th>Variance</th>
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<td>C3</td>
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<td>C12</td>
<td>C11</td>
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<td>2.231,741</td>
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<td>0.41</td>
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<td>C3</td>
<td>C5</td>
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<tr>
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<td>4052</td>
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<td>C1</td>
<td>C11</td>
<td>C3</td>
<td>-2.335,038</td>
<td>477.005</td>
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</tbody>
</table>

### FIGURE 1
The AMMI shows the stability of genotype based on mean grain yield of multiple environments.

The genotypes located near the x-axis and on the right of the y-axis mean that they are stable and high-efficient, whereas the genotypes, which are located far from x-axis and left side of y-axis mean that they are unstable and low-yielding. E1-E2 are environment codes for 2013-14, E3-E4 for 2014-15 growing season. C1-C12 is the codes for cultivars, respectively.

The lines which were pointed from centre of figure to the edge of figure called a sector line and the distance between two sectors line is called “sector” (Fig. 2). As a result of the study, a total of 6 sectors were formed, including genotypes and environments. The vertex of the most suitable genotypes is determined for each sector by connecting straight lines and rest of genotypes fall inside the polygon. The vertex genotypes per sector were C12, C1, C3, C2, C8, C7 and C10. These genotypes are superior to conditions which positioned in same sector or poorest genotypes for across environments because they are extreme farther from the origin of biplot except C4, C6 and C11. On the other hand, it was occurred in two mega-environment which are located in different sectors. The first MEs winning C12 and C11 genotypes and the second MEs winning the C1, C3, and the genotypes positioned at the vertex we can say that C12 is winning genotype for sector I(E4), C1 for sector II (E1, E2 and E3). The stability of genotypes showed in Fig.3, and generated by GGE biplot. The AEC is showing the stability genotypes based on across environment. Therefore C1 has very small length of projection line, therefore it is stable across environments and C3 are favorable for specific environments. The similar results indicated on Fig.4, and C1 and G3 are favorable genotypes because they located near of centre ideal for biplot. The biplot indicated that the selection of genotypes can be done both on specific environments and across environments.
Which-won-where for genotypes and environments.
The polygon view of genotype-environment interaction for bread wheat disomic addition lines over four test environments. The vertex genotype in each sector is the best genotype at environments whose markers fall into the respective sector. E1-E2 is environment codes for 2013-14, E3-E4 for 2014-15 growing season. C1-C12 is the codes for cultivars, respectively.

The ranking model of GGE biplot shows the stability of genotypes in across environments.
E1-E2 is environment codes for 2013-14, E3-E4 for 2014-15 growing season. C1-C12 is the codes for cultivars, respectively.

The scaling for comparison model of GGE-biplot demonstrates the favorable genotypes based on the ideal genotype.
E1-E2 is environment codes for 2013-14, E3-E4 for 2014-15 growing season. C1-C12 is the codes for cultivars, respectively.
DISCUSSION

Recently, researchers are using variance statistical models to define GEI and to suggest the superior genotype for appropriate environmental condition. Using AMMI stability value and mean yield indicates that, the low value of this traits produce appropriate genotypes with high productivity and stability [16]. The different statistical models (AMMI, GGE, GE and GEI) aim to identify stable and superior genotypes for multiple environmental conditions, because of the unpredictable environmental factors [17, 18]. The findings of grain production and stability provide the breeders to propose the appropriate conditions from genotypes [5]. These methods are still used in breeding studies, and were developed to elucidate the effect of genotype, environment or interaction [19]. In addition, the GGE biplot and AMMI models have been used for a long time by many researchers to understand the influence of genotype, environment and GEI based on grain production [18, 20, 21]. The AMMI analysis is used to identify the stability and superiority of genotypes, favorable and yielding environments based on multiple environments [10, 22]. Thus interaction of the 12 wheat genotypes with 4 environments was best predicted by the first two principal components of GEI. Thus, to explain the performance of genotypes across four locations, the findings are showed in some sections. In the current investigation, AMMI analysis indicated that C1 was yielding genotypes, while C3 and C11 were stable in tested environments (Fig. 1). On the other hand, the results of GGE biplot indicated that the selection of genotypes can be done both on stability and superiority. Two mega-environments were occurred in the test environments and C11 and C12 were the best yielding in the poorest environments or years, and C3 for yielding environments, while C1 is stable for across test environments (Fig 2-4). The genotypes or locations (environment) on the right side of the midpoint of the perpendicular line produce higher productions than those on the left Sid [23]. These findings were in agreement with Kadhem and Bakrash [24], Muhammad et al. [25], and described the greatest genotype needs to combine better grain production and stable performance across a range of production conditions. On the other hand, the study showed that the fluctuation of grain yield is depended on environmental conditions [17, 26]. In addition, the environments (E1 and E2) have proven to be high yielding, some other environments (E3 and E4) have proven to be low-yielding because of these environment conditions. These findings were according to Yan et al. [26] described mega conditions as a cluster of locations that constantly share the similar greatest variety. The results of this study indicated that the main environmental influence was higher than genotype and GE interaction effects. Naroui Rad et al. [17] defined the magnitude of environmental effect which was higher than genotypic effect. Moreover, the study showed that the multiplicative variance of the treatment sum of squares due to interaction was partitioned into the two interaction principal components and only one of them showed significant effect. The results were in corroboration with the previous research results that showed similar higher magnitude of GEI variance obtained by the first two principal components of GEI [27, 28]. The adjusted yield of 12 genotypes examined across four locations were exposed to GGE biplot to explain GEI. The first two interactions principal component axis best clarifies the interaction sum of squares [29]. Both AMMI and GGE were able to efficiently explore the variability present in MET data due to genotype-environment interaction and both models proved to be approximately equivalent, leading to substantially the same conclusions about the genotypes with the greatest productivity and stability [23, 30]. Although, the ideal environment and genotype could not exist while, these could be used as references to identify genotype in multi-environmental data [25, 31]. Further, Mehari et al. [23], Muhammad and Mohammad, [31] recorded the same findings regarding the stability of grain production of wheat genotypes across multi environments. Considering the ideal environment as the centre, concentric circles were drawn to provide visualise the distance between each environment and the ideal environment [26]. The findings of the AMMI and GGE biplot models had close results of grain yield of 12 bread wheat genotypes across four examined environments.

CONCLUSION

The findings of additive main effect and multiplicative interaction indicated that grain yield of spring bread wheat was highly influenced by environmental effect followed by genotypic influences, and GEI contributed the least. This study also revealed that AMMI model was found to be effective for discriminant the ability and representativeness of the examined conditions along with assessing the stability of spring bread wheat genotypes. According of the study, C12 and C3 showed specific ability, while C1 and C11 general adaptability across test environments. Moreover, C1 can be recommended for mega-environment II (E1, E2, E3), while C12 for mega-environment I (E4). On the other hand, C1 can be registered as variety for study area, because this genotype is quite stable and high yielding among genotypes under investigation in multiple environments. Consequently, the GGE and AMMI model is a useful tool for visual elucidation of the complex GE interaction and yield stability in breeding programs.
ABBREVIATIONS

G: Genotype; E: Environment; ME: Mega-Environment; GGE: Genotype, Genotype x Environment; GEI: Genotype-by-environment interaction, PCA: Principle Component Analysis, AMMI: Additive Main effects and Multiplicative Interaction

REFERENCES


EVALUATION OF THIRTEEN DURUM WHEAT (*TRITICUM DURUM* DESF.) GENOTYPES SUITABLE FOR MULTIPLE ENVIRONMENTS USING GGE BI PLOT ANALYSIS

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1Kiziltepe Vocational School, Mardin Artuklu University, Mardin, Turkey
2Department of Field Crops, Faculty of Agriculture, Dicle University, Diyarbakir, Turkey

ABSTRACT

The present study was undertaken to identify the best durum wheat genotypes suitable for the South-Eastern Anatolia Region of Turkey with desirable grain yield and quality. In the context, thirteen spring durum wheat genotypes were evaluated in four environmental condition of the target region in consecutive two growing seasons in the year 2013-14 and 2014-15. The stability and superiority of genotypes, and favorable testing environments were described by using ANOVA and GGE biplot analysis (genotype, genotype x environment). Genotype, environment and GEI (genotype x environment interaction) was found to be highly significant for multiple traits. The total variation of PCI (princes component) and PC2 was calculated 90% for ETI (environment trait interaction), 57.35 for GTI (genotype trait interaction), and 87.5% for GE interaction. The results of total variation of ETI was found higher than GTI and GEI. On the other hand, the biplot analysis showed that four mega-environments occurred among ETI and three environments (E1, E2 and E3) correlated with different traits, while environments E4 did not correlated with any traits. The environment E1 was found the best for Grain yield, test weight, thousand kernel weight and starch content, E2 for L*, b*, SPAD, and E3 for zeleny sedimentation, protein content and wet gluten content. In the biplot analysis, the environments divided three sector based on traits. Among the genotypes, the genotype G8 was performed the best in all tested environments, while G9 was found the best based on all traits. The results of the study showed that GGE biplot analysis can be used as a good tool to identify of the best genotypes for future breeding program.

KEYWORDS:
GGE biplot, protein, stability, interaction, semolina colour.

INTRODUCTION

In the world, durum wheat (*Triticum durum* Desf.) is usually use to make food products such as pasta, couscous, noodles and bulgur [1] and its demand is increasing day by day due to the demand of first foods in worldwide. It production is about 40-50 million tons per year in the world, while nearly 20% is produced in EU countries and about 17% in Canada [2]. In Turkey, it is also an important cereal and produces nearly 10% of world production per year, where half of the durum wheat is produced in the South-Eastern Anatolia Region of Turkey [3]. The agro-ecological conditions of this region are suitable for cultivation of durum wheat production [5].

Nowadays, the consumers using the durum wheat products prefer especially high quality products. Therefore, durum wheat producers prefer high quality durum wheat varieties which give high yield per unit area. For this reason, durum wheat breeders continue their breeding activities with an effort to determine the high quality, stable and high yielding genotypes through breeding programs [5]. Generally, the durum wheat genotypes shows narrow adaptability and also yield fluctuations across environments than bread wheat. Hence, it is very important to develop good quality, high yielding and stable durum wheat varieties [6]. Furthermore, the strategy for breeding durum wheat needs to definite the genotypes which are stable based on multi traits and multi environments and unpredictable climatic conditions [7].

The candidate and new varieties need to be favorable, stable and high-yielding in across environments. In order to characterize the varieties under different environmental conditions, many different methods have been developed [8]. The yield of trials across environments gives information about the stability of genotype based on different statistical methods. Assessments of genotypes have been with stand two major problems. The first is the negative interaction between the GE and the second is the basic traits [9]. Therefore, it is important to identifying genotypes which are unaffected by the GEI (genotype x environment interactions) and stable. For this purpose, GGE biplot
conception, have been developed to see the stability of genotypes across environmental conditions [10]. Therefore, this method have been used by many research in durum wheat multiple environments [11, 12]. On the other hand, the candidates could give best outputs (high yield and quality) in different environmental conditions (drought and heat stress, resistant to diseases, and frost risk, irregular rainfall) [13]. The real is that it is very difficult for researchers to improve the best varieties in terms of all traits in across environments [14, 15].

The aims of this study were to evaluate the durum wheat genotypes across environment based on multiple traits, to see the stability of genotypes across environments, to see the best genotypes based on multiple traits, and to find out the favorable environments based on multiple traits for a specific genotype.

MATERIALS AND METHODS

Thirteen durum wheat genotypes (Table 1) were evaluated under different environments in South Easter Anatolia of Turkey. These genotypes were conducted at the three locations during 2013-2014 and 2014-2015 growing season. Environment code and four environments are shown in the Table 2. The study was design a randomized complete block design replicated four replications. The seeds were sown in November at all locations during both years of investigation. Each experiment parcel consisted 4 m long 20 cm spaced six rows. Fertilizers were applied 60 P kg ha⁻¹ and 60 N kg ha⁻¹ with before sowing and applied 60 N kg ha⁻¹ at tillering stage. Chlorophyll content index (SPAD) was measured by using SPAD meter at heading stage. Protein content, starch content, wet content, Zeleny sedimentation test weight were analyzed using near infrared transmittance spectroscopy (FOSS, The Infratec™ 1241). Grain color (L*, b*) was measured with HunterLab ColorFlex, A60-1010-615

Statistical analysis. The GGE biplot was used to see the stability of genotypes based on multiple traits across four environments, and this method was used as recommended by Yan and Thinker (16), to identify the favorable environments and superior genotypes based on multiple traits. On the other hand; the data were analyzed by using SPSS and GenStat analysis programs. The all graph generated by GGE biplot software, and this method used for 1) the which-won-where based on genotype environment interaction, 2) the stability of genotypes across environments, 3) which-won-where based genotype trait interaction, 4) the ranks genotypes based on multiple environments, 5) which-won-where based on environment trait interaction, 6) definite favorable environment based on multiple traits.

RESULTS

The analysis of variance (ANOVA) indicated that locations were found to be highly significant (P<0.01, P<0.05) for multiple traits (SPAD, TWK, TW, PC, SC, WG, L* and b* value, whereas there was no differences among locations for GY and ZS (Table 3). The genotypes were found to be highly significant for SPAD, GY; and the GEI effects was significant for all traits, except SPAD and L* value of semolina colour. The results of analysis indicated that genotypes were differed considerably based on multiple traits in across environments (Table 4, Table 5, Table 6). These results were supported by generated different GGE biplot figures (Fig. 1, Fig. 6).

TABLE 1
Code and name of durum wheat genotypes used in the investigated

<table>
<thead>
<tr>
<th>Genotype code</th>
<th>Genotype name</th>
<th>Origin</th>
<th>Genotype code</th>
<th>Genotype name</th>
<th>Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>Sena</td>
<td>Turkey</td>
<td>G8</td>
<td>FIRAT 93</td>
<td>Turkey</td>
</tr>
<tr>
<td>G2</td>
<td>CESARE</td>
<td>Italy</td>
<td>G9</td>
<td>GUNEY YILDIZI</td>
<td>Turkey</td>
</tr>
<tr>
<td>G3</td>
<td>CEYHAN 95</td>
<td>Turkey</td>
<td>G10</td>
<td>HAT 300</td>
<td>Turkey</td>
</tr>
<tr>
<td>G4</td>
<td>DZ7-27</td>
<td>Turkey</td>
<td>G11</td>
<td>PITAGORA</td>
<td>Italy</td>
</tr>
<tr>
<td>G5</td>
<td>DZ7-34</td>
<td>Turkey</td>
<td>G12</td>
<td>URFA 2005</td>
<td>Turkey</td>
</tr>
<tr>
<td>G6</td>
<td>DZ7-51</td>
<td>Turkey</td>
<td>G13</td>
<td>ZUHRE</td>
<td>Turkey</td>
</tr>
<tr>
<td>G7</td>
<td>DZ7-52</td>
<td>Turkey</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

TABLE 2
Informational of the experimental area

<table>
<thead>
<tr>
<th>Environment code</th>
<th>E1</th>
<th>E2</th>
<th>E3</th>
<th>E4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Location</td>
<td>Sanliurfa/Siverek</td>
<td>Diyarbakır</td>
<td>Mardin/Derik</td>
<td>Diyarbakır</td>
</tr>
<tr>
<td>Soil texture</td>
<td>Clay loam</td>
<td>Clay loam</td>
<td>Clay loam</td>
<td>Clay loam</td>
</tr>
<tr>
<td>Latitude</td>
<td>37°32’40.41”N</td>
<td>37°53’23.04”N</td>
<td>37°12’36.58”N</td>
<td>37°53’23.04”N</td>
</tr>
<tr>
<td>Longitude</td>
<td>39°24’14.78”E</td>
<td>40°16’34.42”E</td>
<td>40°03’43.47”E</td>
<td>40°16’34.42”E</td>
</tr>
<tr>
<td>Altitude (m)</td>
<td>812 m</td>
<td>669 m</td>
<td>483 m</td>
<td>669 m</td>
</tr>
<tr>
<td>Total precipitation</td>
<td>431.6</td>
<td>334.9</td>
<td>616.6</td>
<td>431.6</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>6.0</td>
<td>-3.4</td>
<td>5.2</td>
<td>1.1</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>Maximum</td>
<td>26.9</td>
<td>26.5</td>
<td>28.5</td>
</tr>
</tbody>
</table>
TABLE 3
ANOVA for SPAD, grain yield and related traits of tested durum wheat genotypes across four environments

<table>
<thead>
<tr>
<th>V.K.</th>
<th>D.F.</th>
<th>SPAD</th>
<th>GY</th>
<th>TWK</th>
<th>TW</th>
<th>PC</th>
<th>SC</th>
<th>WGC</th>
<th>ZS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype</td>
<td>12</td>
<td>58.70**</td>
<td>1571885.30</td>
<td>151.03**</td>
<td>25.62**</td>
<td>2.26**</td>
<td>1.94**</td>
<td>14.79**</td>
<td>31.35</td>
</tr>
<tr>
<td>Environment</td>
<td>3</td>
<td>431.91**</td>
<td>103926642*</td>
<td>1954.10**</td>
<td>210.19**</td>
<td>256.06**</td>
<td>174.31**</td>
<td>2478.07**</td>
<td>1749.31**</td>
</tr>
<tr>
<td>Gen x Env</td>
<td>36</td>
<td>9.01</td>
<td>650342.27**</td>
<td>8.92*</td>
<td>8.63*</td>
<td>1.94*</td>
<td>0.70*</td>
<td>10.05*</td>
<td>16.67**</td>
</tr>
<tr>
<td>Error</td>
<td>153</td>
<td>9.98</td>
<td>793171.00</td>
<td>5.33</td>
<td>3.98</td>
<td>1.05</td>
<td>0.46</td>
<td>5.91</td>
<td>11.02*</td>
</tr>
<tr>
<td>CV (%)</td>
<td></td>
<td>6.31</td>
<td>16.80</td>
<td>5.88</td>
<td>2.41</td>
<td>1.08</td>
<td>0.75</td>
<td>6.70</td>
<td>1.97</td>
</tr>
</tbody>
</table>

*, ** %5, %1 respectively, level significantly. GY: Grain yield, TWK: Thousand kernel weight, PC: Protein content, SC: Starch content, WGC: Wet gluten content, ZS: Zeleny sedimentation

TABLE 4
Chlorophyll content index, grain yield, thousand kernel weight and test weight of tested durum wheat genotypes across four environments

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Chlorophyll content index (SPAD)</th>
<th>Grain yield (kg ha⁻¹)</th>
<th>Thousand kernel weight (g)</th>
<th>Test weight (kg hl⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>G2</td>
<td>G3</td>
<td>G4</td>
<td>G5</td>
</tr>
<tr>
<td>G6</td>
<td>G7</td>
<td>G8</td>
<td>G9</td>
<td>G10</td>
</tr>
<tr>
<td>G11</td>
<td>G12</td>
<td>G13</td>
<td>Mean</td>
<td></td>
</tr>
</tbody>
</table>

FIGURE 1
Polygon view of GGE Biplot (which—won—where) showing the G×E interaction effect of tested durum wheat genotypes across four environments.
The stability of genotypes based on GEI by which-won-where and ranking biplot models. The biplot of GEI based on across environment data is visualize the polygon of which-won-where/what (Fig. 1). The figure divided by thick axis from center figure to each zone separated by two thick lines is referred to as the “mega environment” or “sector”, starting from the lower right part of the graph, and if the genotypes and environments located in the same sector it means that they are closely related each other [5, 16]. The results showed that five sectors were occured and the environments E2, E3 and E4 located in first sectors and correlated with G1, G6, G7, G8 and G11 and only E1 located in third sector and correlated with G2 and G4. The other genotypes located in different sector without environments. The G1 won the sector 1 because this genotype locates the vertex polygon of this sector. On the other hand, the G4 won the sector 3 with vertex polygon in this sector. Consequently, two mega environments were occurred and G1 G4 and G3 were desirable for tested environments Therefore; the best genotype can be definite for mega environments by which-won-where analysis.

The ranks genotypes based on across environments shows the stability of genotypes (Fig. 2). The figure is interpreted genotypes based on two

### TABLE 5
Protein content, starch content, wet gluten content and zeleny sedimentation of tested durum wheat genotypes across four environments

<table>
<thead>
<tr>
<th>Genotype</th>
<th>E 1</th>
<th>E 2</th>
<th>E 3</th>
<th>E 4</th>
<th>Mean</th>
<th>E 1</th>
<th>E 2</th>
<th>E 3</th>
<th>E 4</th>
<th>Mean</th>
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<tbody>
<tr>
<td>G2</td>
<td>16.95</td>
<td>15.84</td>
<td>14.94</td>
<td>14.03</td>
<td>15.84</td>
<td>14.94</td>
<td>14.03</td>
<td>14.03</td>
<td>14.03</td>
<td>14.03</td>
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<tr>
<td>G5</td>
<td>16.46</td>
<td>15.35</td>
<td>14.45</td>
<td>13.54</td>
<td>15.35</td>
<td>14.45</td>
<td>13.54</td>
<td>13.54</td>
<td>13.54</td>
<td>13.54</td>
</tr>
<tr>
<td>G6</td>
<td>16.97</td>
<td>15.86</td>
<td>14.96</td>
<td>14.05</td>
<td>15.86</td>
<td>14.96</td>
<td>14.05</td>
<td>14.05</td>
<td>14.05</td>
<td>14.05</td>
</tr>
<tr>
<td>G9</td>
<td>16.50</td>
<td>15.40</td>
<td>14.51</td>
<td>13.60</td>
<td>15.40</td>
<td>14.51</td>
<td>13.60</td>
<td>13.60</td>
<td>13.60</td>
<td>13.60</td>
</tr>
</tbody>
</table>

### TABLE 6
L* value and semolina colour (b*) of tested durum wheat genotypes across four environments

<table>
<thead>
<tr>
<th>Genotype</th>
<th>E 1</th>
<th>E 2</th>
<th>E 3</th>
<th>E 4</th>
<th>Mean</th>
<th>E 1</th>
<th>E 2</th>
<th>E 3</th>
<th>E 4</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>G2</td>
<td>15.61</td>
<td>14.60</td>
<td>13.60</td>
<td>12.60</td>
<td>14.60</td>
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<td>12.60</td>
<td>12.60</td>
<td>12.60</td>
<td>12.60</td>
</tr>
<tr>
<td>G3</td>
<td>15.02</td>
<td>14.01</td>
<td>13.01</td>
<td>11.01</td>
<td>14.01</td>
<td>13.01</td>
<td>11.01</td>
<td>11.01</td>
<td>11.01</td>
<td>11.01</td>
</tr>
<tr>
<td>G5</td>
<td>13.84</td>
<td>12.83</td>
<td>11.83</td>
<td>10.82</td>
<td>12.83</td>
<td>11.83</td>
<td>10.82</td>
<td>10.82</td>
<td>10.82</td>
<td>10.82</td>
</tr>
<tr>
<td>G7</td>
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<td>10.65</td>
<td>9.64</td>
<td>11.65</td>
<td>10.65</td>
<td>9.64</td>
<td>9.64</td>
<td>9.64</td>
<td>9.64</td>
</tr>
<tr>
<td>G8</td>
<td>12.07</td>
<td>11.06</td>
<td>10.06</td>
<td>9.05</td>
<td>11.06</td>
<td>10.06</td>
<td>9.05</td>
<td>9.05</td>
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</tr>
<tr>
<td>G10</td>
<td>10.89</td>
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<td>8.88</td>
<td>7.87</td>
<td>7.87</td>
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<td>7.87</td>
</tr>
<tr>
<td>G11</td>
<td>10.30</td>
<td>9.29</td>
<td>8.29</td>
<td>7.28</td>
<td>9.29</td>
<td>8.29</td>
<td>7.28</td>
<td>7.28</td>
<td>7.28</td>
<td>7.28</td>
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<tr>
<td>Mean</td>
<td>9.71</td>
<td>8.70</td>
<td>7.70</td>
<td>6.69</td>
<td>8.70</td>
<td>7.70</td>
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<td>6.69</td>
<td>6.69</td>
<td>6.69</td>
</tr>
</tbody>
</table>
The ranking biplot model shows the stability of genotypes across environments.

The polygon of GGE biplot (which-won-where) based genotype trait interaction across four environments.

The relationship genotype by trait based on interaction by which-won-where and ranking biplot models. The relation among genotype and traits shows by different biplot models. The relationships between genotype by trait interaction based on across environments data visualize the performance each genotype on traits by which-won-where biplot model (Fig. 3). This model already was described in detail above. The results showed that five sectors were occurred and G9 located in first sector correlated with PC, G5 and G8 located in the second sector and correlated with WG, TW, ZS, TKW traits, G3, G6, G7 and G10 located in third sector and correlated with only SC, and G1, G4 and G11 located in sector 4 and correlated with...
L*, GY and SPAD, and, G2, G12 and G13 located in sector 5 and correlated only with b* value of semolina color. E2, E3 and E4 located in first sector and correlated with G1, G6, G7, G8 and G11 and only E1 located in third sector and correlated with G2 and G4. The other genotypes located in different sector without environments. The G1 won the sector 1 because this genotype locates the vertex polygon of this sector. On the other hand, the G4 won the sector 3 with vertex polygon in this sector. Consequently, five sectors were occurred and G2, G5, G6, and G11 were desirable for group traits. Moreover, there were high correlation between GY and SPAD, L*, while negative correlation between GY and other traits. Therefore, the best genotype can be definite for general or special traits by which-won-

where analysis.

The ranking model shows relationship among genotypes and traits based on across environments (Fig. 4). This model already was described in detail above. The results showed that five genotypes (G2, G5, G8, G9 and G12) have high mean value, the G9 was best stable genotype, G5 was favorable, but unstable genotype based on multiple traits across environments. The other genotypes were unfavorable for across environments, because they locate the under mean line. Moreover the effect variation of majority quality traits was high, while GY effect was low in the ranking biplot model. Therefore, the stable and favorable genotypes based on multiple traits can definite across environments by ranking biplot model.

![FIGURE 4](image1)
The ranking biplot model shows stability genotypes based on multiple traits and across four environments

![FIGURE 5](image2)
The polygon view of GGE biplot (which-won-where) shows the interaction of environment trait based on thirteen genotypes
The relationship trait by environment based on interaction by which-won-where and ranking biplot models. The relation between trait and environment shows by different biplot models. The relationships between trait and environment based on thirteen durum wheat genotypes data visualize the performance each environment based on multiple traits by which-won-where biplot model (Fig. 5). This model already was described in detail above. The results showed that four sectors were occurred and each environment located different sector and separated each other based on traits group. Moreover, E1 located in first sector and correlated with TW, GY, TKW and SC, E2 located in the second sector and correlated with SPAD, b* (yellowness) and L* (brightness) of semolina color, E3 located in third sector and correlated with ZS, PC and WGC, while E4 located in sector 4, but did not correlated with any traits. It means that E4 is a poor environment to study traits and GY of durum wheat. The G1 won the sector 1 because this genotype locates the vertex polygon of this sector. On the other hand, the G4 won the sector 3 with vertex polygon in this sector. Consequently, five sectors were occurred and G2, G5, G6, and G11 were desirable for group traits. Moreover, there were high correlation between TW, GY, TKW and SC, while negative correlation between these traits to ZS, PC and WGC traits. Therefore, it is very important to know the environment condition for study quality, physiology or GY and other agronomy application and the study can be definite the general, special, weak and good environment based on traits by which-won-where analysis.

The ranking model shows relationship among traits and environments based on genotypes data (Fig. 6). This model already was described in detail above. The results showed that two environments (E1 and E2) have high mean value; E4 was stable environment, while this environment located under mean of data. On the other hand, E3 was very poor environment, but it was correlated special quality traits (ZS, PC and WGC). The ranking results showed that there is not very stable environment for all traits, while we can study for special traits in E1, E2, and E3 environments Therefore, the favorable environments based on multiple traits can definite to study genotypes by ranking biplot model.

**DISCUSSION**

The determination of the best suitable testing environments provides the opportunity to identification superior genotypes for releases new varieties. Therefore, majority genotypes tested in multi-location and examined based multiple traits. The methodology GGE biplot has given us the opportunity to explore the interactions between genotype by environment, genotype by traits or environment by traits [3, 17, 18]. González-Ribot et al. [19], due to high GEI, obtaining high yielding genotypes of durum wheat for different environments, especially Mediterranean rain-fed areas (rainy, cold winters and dry, hot summers), is considered difficult. For this reason, the researchers have proposed that genotype selection under Mediterranean rain-fed conditions may be improved by explore the interactions between genotype by environment, genotype by traits or environment by traits [3, 17, 18], Gonzalez-Ribot et al. [19], due to high GEI, obtaining high yielding genotypes of durum wheat for different environments, especially Mediterranean rain-fed areas (rainy, cold winters and dry, hot summers), is considered difficult. Therefore, the methodology GGE biplot used to evaluate the interaction among examined genotypes, traits and locations [20]. The total variation of PCI (principles component) and PC2 was calculated 90% for ETI (environment trait interaction), 57.35 for GTI (genotype trait interaction), and 87.5% for GE interaction. The results of total variation of ETI was high.
than GTI and GEI. The GGE biplot analysis establishes a framework for classifying target test environments that differ between genotypes which are stable and yielding (Fig. 1). If the effect of genotype in the variation (G) is quite large (50.6%), PC1 scores will be highly correlated with G and PC2 (36.9%) is controlled by GE interaction [16, 21, 22]. This identification can be said for variation in GTI (genotype x trait interaction) and ETI (environment x trait interaction) too. Karimizadeh et al [18] said that the GGE biplot facilitate a meaningful grasp of GE interaction and enables the explanation of relationships among genotypes and test environments. Yan [23], understanding the relationship between crop performance and environment has long been a key issue for plant breeders and geneticists. In the crop performance, GEI is important when different genotypes respond to different environments and researchers agree that GE is important only when it causes significant changes in genotype ranking in different environments [24]. The GGE biplot defines the relationships between all circles based on the general model of MET data, whereas the simple correlation coefficients define only the relations between the two environments [25].

On the other hand, the analysis of variance indicated that locations were found to be highly significant for majority traits whereas there was no difference among locations for GY and ZS (Table 3). The genotypes were found to be highly significant for SPAD, GY; and the GEI effects was significant for all traits, except SPAD and L* value of semolina colour. The results of analysis indicated that genotypes were differed considerably based on multiple traits in across environments (Table 4 and Table 5). In order to be considered as a successful candidate of durum wheat cultivation, it is necessary to grow in the most suitable environment and to have the highest yield and quality. Therefore, in this study, firstly the genotypes were described based on multi environment and multi traits (Table 4, Table 5, Fig. 1, Fig. 3). The second favorable environments were described based on genotype and multi traits (Fig.1 and Fig. 5). The unstable candidate can be registered for specific environment where they can attain a high performance with regard to high quality or resistant to morphological traits in depended of seasonal effects [26]. The stability of genotypes or across environment based on both quality, grain yield and morphological traits should be an important registration of new varieties [27, 28]. Therefore, the study concentrated the stability of genotypes based on across environment and multiple traits.

In the study, the results showed that there was high correlation among WG, TW, ZS, TKW and PC quality parameters and G5 was the best for these traits, while G11 was the best for L*, GY and SPAD. On the other hand, SC and b* were independendent did not correlated with other quality parameters, grain yield and SPAD. The result also showed that there was high correlation between grain yield and SAPD. The result study of Yildirim et al. [29] and Kendal [3], had same output and pointed that high values of SPAD support to GY positively in Southeastern Anatolia Region of Turkey, and Mustoriti et al. [30] said that the cultivar specific SPAD value at heading can provide a more accurate estimate of the final yield in wheat. Moreover, the study showed that the environments (E1, E4) which have high personification and low temperature during season have low quality value, while the environments (E2, E3) which have low personification and high temperature during season have high quality of durum wheat (Table 2). In order to have good quality characteristics, durum wheat genotypes require low temperature and high rainfall before heading time and high temperatures moderate precipitation after heading time and before harvesting. The study demonstrated that, it is possible to determine the best registration candidate of durum wheat and favorable environments based on multiple quality parameters in Southeastern Anatolia Region of Turkey with GGE biplot method and this study can be used successfully for other crops.

**CONCLUSION**

The stability and superiority of genotypes, and favorable testing environments based on multiple traits were described by using GGE biplot analysis. The results indicated that interaction between the traits with the genotypes, and with the environment were quite complex, but making the objective assessment of the genotypes comfortable. The quality parameters and SPAD varied based on variation in the interactions, both between the each other and grain yield. The genotype (G1 and G9) with highest productivity exhibited also highest variability under the specific environments based on multiple traits, with the exception of G8, which behaved as the most universal variety in this study. Furthermore, the results of study showed that GGE biplot was a good tool to identity of the most suitable environment in terms of all the characteristics affecting the stability of a genotype and can increases the success in breeding studies.

**REFERENCES**


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GENETIC PARAMETER ESTIMATIONS OF BAYESIAN HIERARCHICAL LINEAR AND NONLINEAR GROWTH CURVES IN JAPANESE QUAILS

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Isparta University of Applied Sciences, Faculty of Agricultural Sciences and Technologies, Department of Animal Sciences, 32260, Isparta, Turkey

ABSTRACT

Growth curve estimates of livestock can be used as a criterion for genetic breeding. This study investigated the possibility of using the hierarchical linear and non-linear growth curves model parameters as a selection criterion in the Japanese quail (Coturnix coturnix japonica). The growth curves of the quails were estimated using the hierarchical linear and hierarchical non-linear models according to the Bayesian approach. Then, the variance components of the posterior values of the growth curve model parameters were estimated by means of univariate and multivariate models and the genetic parameters were obtained. \( \beta \) and \( \alpha \) posterior averages of hierarchical linear model were 4.03 and 132.6 whereas \( \Omega_1 \), \( \Omega_2 \) and \( \Omega_3 \) parameters of hierarchical non-linear model posterior averages were found to be 212.7, 15.8 and -0.14, respectively. The heritability of 0.49, 0.30 and 0.28 were obtained for \( \Omega_1 \), \( \Omega_2 \) and \( \Omega_3 \) parameters, respectively. High phenotypic and genetic relationships between the parameters of both models and the observations were determined. In the experiment it can be concluded that the hierarchical linear and hierarchical non-linear model parameters could be the criteria of selection for the genetic breeding of quails.

KEYWORDS:
Quail, Bayesian, hierarchical linear, hierarchical non-linear, growth curve, genetic parameters

INTRODUCTION

Growth is a time-dependent process functioned by combination of genetic potential and the effect of the environment [1]. Growth curves of organisms are obtained from mathematical functions. In general, non-linear functions are used to model the growth of living organisms since growth performances of them conform to “S” type sigmoidal curves [2, 3]. However, the analysis of non-linear functions is much more complicated than linear functions [4]. Growth curves can be used as an efficient tool for various purposes such as breeding parameters [1, 2, 5-8] to determine the most appropriate slaughtering age of animals, in the production productivity indices, preparation of feeding programs [9] and quantifying the environmental effects on animal performances. Quail is a model organism for poultry research as well as being used in meat and egg production [10, 11]. Thus, growth curve estimations for quails were performed by many researchers [7, 12-14] by using least squares and maximum likelihood approaches. Both methods assume that the error is normally distributed as the number of samples is high, and that the parameter distributions have an approximately normal distribution [9]. In recent years, Bayesian approach has also been utilized in the growth curve estimations. For example, the growth curve of the Gompertz, Brody, logistic and Von Bertalanffy growth curve models using Bayesian approach has been frequently used by many researchers in the last decade [4, 6, 7, 15, 16]. Bayesian approach has been preferred due to its advantages of using preceding previous information and avoids asymptotic inferences [9]. This approach computes model parameters in more flexible, robust and accurate way [9, 15]. Pre-existing information about the variable can be included by the prior probability distribution. Thus, it can obtain posterior estimates more consistent with the actual value of the variable of interest. The Bayesian paradigm does not set strict rules for choosing the proper prior distributions. This paradigm makes possible to differentiate different states of knowledge on prior information. When there is lack of an informative prior or any non-informative prior can be selected which can be a flat or uninformative prior.

In this study, hierarchical linear and hierarchical non-linear growth curves’ estimations were obtained by Markov Chain Monte Carlo technique (MCMC) under a Bayesian approach by using weekly weights of quails. Hierarchical non-linear growth curve was obtained using logistic model. Subsequently, the possibilities of the hierarchical linear and hierarchical non-linear growth curve parameters as selection criteria were evaluated.
MATERIALS AND METHODS

The study was carried out in the Quail Unit of Isparta University of Applied Sciences, Faculty of Agricultural Sciences and Technologies, Turkey. 60 male and 60 female quails were randomly selected from the population and they were matched using 1:1 sex ratio. From each of these 60 full-sib families, chicks of three groups were obtained from the weekly collected eggs for three incubation periods. A total of 533 quails were used in the study. The birds were feed with a ration containing 24% of crude protein and 3100 kcal/kg metabolic energy.

The access of birds to feed and water during the trial period has been provided as ad-libitum. The hatching weights of the quails and then the weekly weights were determined. In this way, the hatching weights of the chicks (HW) and their subsequent weekly weights (1-8th weeks) were measured with a balance sensitive to 0.1 g and recorded with their parental numbers to collect a pedigree data-set.

Bayesian linear and non-linear growth curves. Growth is a time-dependent biological function. The growth of living organisms can be measured at differing time intervals to obtain a longitudinal data. When the data of the quail eggs and their weekly growth weights are converted into the longitudinal data set, \(y_{ij}\) is the weight of the quail \(i\) at week \(j\). \(y_{ij}\) consists of a data-set with normal distribution and quantitative phenotype class. And this data can be expressed with two parameters \((\eta_{ij}, \tau)\) as follows:

\[y_{ij} \sim Normal(\eta_{ij}, \tau)\]

The parameters of the hierarchical linear growth curve estimation can be written in more detail [15] as:

\[y_{ij} \sim Normal(\alpha_i + \beta_i(x_j - \bar{x}), \tau)\]

\(i = 1, ..., 533\) and \(j = 1, ..., 8\)

(1)

Where the normally distributed data-set with average and variance \(\alpha_i \sim Normal(\alpha_m, \tau_\alpha)\) and \(\beta_i \sim Normal(\beta_m, \tau_\beta)\). In the study, non-informative priors were determined for these parameters, assuming there was no preliminary information about the structure of the probability distributions of the parameters:

\[\alpha_m \sim Normal(0.10E^{-6} - \delta)\]

and

\[\beta_m \sim Normal(0.1.E^{-6})\]

\[\tau_\alpha = Gamma(0.001, 0.001)\]

\[\tau_\beta = Gamma(0.001, 0.001)\]

\[\tau = Gamma(0.001, 0.001)\]

Standard deviations of these parameters are:

\[\sigma_\alpha = 1/sqrt(\tau_\alpha), \sigma_\beta = 1/sqrt(\tau_\beta)\]

The longitudinal data of the quails were also analysed using a hierarchical non-linear model. The \(y_{ij}\) estimates of the logistic growth model were obtained using the following general equation [18]:

\[y_{ij} \sim Normal(\eta_{ij}, \tau)\]

When the equation is extended as,

\[y_{ij} \sim Normal\left(\frac{\theta_{i1}}{1 + e^{-\theta_{i2}(x_j - \bar{x})}}, \tau\right), i = 1, ..., 533\]

and \(j = 1, ..., 8\)

(2)

Model parameters \(\theta_{i1}, \theta_{i2}\) and \(\theta_{i3}\) can be calculated from the equations below:

\[\theta_{i1} = log(\theta_{i1}), \quad \theta_{i2} = log(\theta_{i2} + 1)\]

and \(\theta_{i3} = log(-\theta_{i3})\)

The assumption of normal distribution prior was accepted for \(\theta_{i1}, \theta_{i2}\) and \(\theta_{i3}\) model parameters:

\[\theta_{i1} \sim Normal(\mu_{i1}, \tau_{i1}), \quad \theta_{i2} \sim Normal(\mu_{i2}, \tau_{i2})\]

and \(\theta_{i3} \sim Normal(\mu_{i3}, \tau_{i3})\)

Non-informative priors were assigned for \(\tau_{i1}, \tau_{i2}, \tau_{i3}\) and \(\tau\) as:

\[\tau_{i1,2,3} = Gamma(0.001, 0.001)\]

and \(\tau = Gamma(0.001, 0.001)\)

The WinBUGS (Windows-based Bayesian Inference Using Gibbs Sampling) computing program [19] was used for hierarchical linear (1) and non-linear (2) Bayesian analysis. The program uses the Markov Chain Monte Carlo (MCMC) technique in the Bayesian analysis of complex statistical models. Posterior estimates obtained using both models were calculated after discarding 30,000 initial updates. The simulation was carried out with 30,000 updates after the initial burn. By this way, it was possible to decrease the Monte Carlo error term below 5% of the respective sample standard deviation in the cases. In addition, Kernel density of each parameter was maintained typical normal distribution.

Estimation of Genetic Parameters. In this study, the variance components were estimated by using the posterior values of the growth curve parameters \((\alpha_i, \beta_i, \bar{\theta}_{i1}, \bar{\theta}_{i2}, \bar{\theta}_{i3})\) obtained using Bayesian models of the hatching weights (HW) and weekly body weight (WK1,...,8) data. This process has been applied even to the original data of the quails. The variance components were estimated using the Restricted Maximum Likelihood (REML)
method using the ASREML program [20] based on univariate and multivariate models. The mathematical expression of the univariate model is as follow:

\[ y = Xb + Za + e \]  

(3)

Where, \( y \) vector of observations (HW, WK1,...,8 and \( \alpha, \beta, \theta_1, \theta_2, \theta_3 \)), \( b \) vector of fixed effects (incubation period and sex), \( a \) vector of random animal effects or random parameters effects, \( e \) vector of random residual effects.

The multivariate model and its elements are as follows.

\[
\begin{bmatrix}
\mathbf{y}_1 \\
\mathbf{y}_2
\end{bmatrix} =
\begin{bmatrix}
X_1 & 0 \\
0 & X_2
\end{bmatrix}
\begin{bmatrix}
b_1 \\
b_2
\end{bmatrix}
+ \begin{bmatrix}
Z_1 & 0 \\
0 & Z_2
\end{bmatrix}
\begin{bmatrix}
\alpha_1 \\
\alpha_2
\end{bmatrix}
+ \begin{bmatrix}
e_1 \\
e_2
\end{bmatrix}
\]  

(4)

Where, \( \mathbf{y}_{1,2} \) vector of observation for 1 and 2nd traits, \( b_{1,2} \) vector of fixed effects (incubation period and sex) for 1 and 2nd traits or 1 and 2nd parameters, \( \alpha_{1,2} \) vector of random animal effects for 1 and 2nd traits or 1and 2nd parameters, \( e_{1,2} \) vector of random residual effects for 1 and 2nd traits.

RESULTS AND DISCUSSION

Table 1 shows the weekly weight averages of male and female quails and the Tukey test results of these averages. Multiple comparisons of weekly averages were also included. Results indicated that the body weight of male and female birds became different for statistical means. These results are consistent with reported sexual dimorphism in the literature [7, 13, 21, 22]. In addition, weekly body weights consistently increased to the highest level at 7 weeks of age. Measured weekly body weight averages (8.04, 32.2, 69.5, 116.5, 185.9, 202.2, 210.3 and 210.8 g) were highly in agreement with those reported by Narinc et al. [12], Barbieri et al. [23], Karadavut et al. [24] and higher than the ones determined by Kaplan et al. [14] and Finco et al. [15].

The posterior averages of the hierarchical linear and non-linear model parameters obtained using the Bayesian approach and the standard deviations for these averages and Markov Chain Errors (MCE) were given in Table 2. The highest posterior density (HPD) and confidence limits along with median values were also presented in the Table.

### Table 1

<table>
<thead>
<tr>
<th>Traits</th>
<th>Male Mean±SE</th>
<th>Female Mean±SE</th>
<th>Total Mean±SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>HW</td>
<td>8.05±0.05A</td>
<td>8.02±0.05A</td>
<td>8.04±0.04H</td>
</tr>
<tr>
<td>WK1</td>
<td>31.7±0.4A</td>
<td>32.8±0.4A</td>
<td>32.2±0.3G</td>
</tr>
<tr>
<td>WK2</td>
<td>68.3±0.6B</td>
<td>70.8±0.7B</td>
<td>69.5±0.5F</td>
</tr>
<tr>
<td>WK3</td>
<td>114.9±0.8B</td>
<td>119.0±0.9A</td>
<td>116.9±0.6E</td>
</tr>
<tr>
<td>WK4</td>
<td>157.7±0.9B</td>
<td>165.7±1.1A</td>
<td>161.5±0.7D</td>
</tr>
<tr>
<td>WK5</td>
<td>175.5±0.8B</td>
<td>197.3±1.3A</td>
<td>185.9±0.9F</td>
</tr>
<tr>
<td>WK6</td>
<td>184.0±0.9B</td>
<td>222.3±1.4A</td>
<td>202.2±1.2B</td>
</tr>
<tr>
<td>WK7</td>
<td>191.2±1.0B</td>
<td>231.3±1.5A</td>
<td>210.3±1.2A</td>
</tr>
<tr>
<td>WK8</td>
<td>192.3±1.1B</td>
<td>221.2±1.5A</td>
<td>210.8±1.2A</td>
</tr>
</tbody>
</table>

1 t test between gender averages, 2 The Tukey test between week averages. The same letters indicate non significance at p>0.05.

The MCE values of all posterior means obtained were less than 5% of the standard deviations of the samples. This can account for the accuracy of the estimates. Linear and non-linear growth functions were quite different from each other. In addition, it was not reasonable to compare parameters other than asymptotic weight parameter in different growth curve models [4]. The estimated asymptotic weight parameter (\( \theta_1 \)) can be compared with the literature. The asymptotic posterior average value (212.7) found in this study was comparable with the ones reported by Firat et al. [4], Kaplan and Gürcan [13], Narinc et al. [12], Barbieri et al. [23], Karadavut et al. [24] and higher than the ones determined by Kaplan et al. [14] and Finco et al. [15].

The estimated values of HL and HNL models along with the observed body weights were given in Table 3. The differences between the estimates and measured body weights (Residual) were also tabulated. The residual values indicated that the HNL estimates were almost closer to the actual values comparing with the HL estimates in the entire growth period. Therefore, it can be said that the HNL model had better estimates than the HL model.

### Table 2

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Mean</th>
<th>SD</th>
<th>MCE</th>
<th>HPD</th>
<th>2.5%</th>
<th>Median</th>
<th>97.5%</th>
</tr>
</thead>
<tbody>
<tr>
<td>HL</td>
<td>( \beta )</td>
<td>4.03</td>
<td>0.29</td>
<td>1.03E-4</td>
<td>3.47</td>
<td>4.03</td>
<td>4.59</td>
</tr>
<tr>
<td></td>
<td>( \alpha )</td>
<td>132.6</td>
<td>6.67</td>
<td>0.013</td>
<td>119.5</td>
<td>132.6</td>
<td>145.7</td>
</tr>
<tr>
<td></td>
<td>( \theta_1 )</td>
<td>212.7</td>
<td>4.89</td>
<td>0.16</td>
<td>203.3</td>
<td>212.6</td>
<td>222.5</td>
</tr>
<tr>
<td></td>
<td>( \theta_2 )</td>
<td>15.8</td>
<td>0.87</td>
<td>0.03</td>
<td>14.1</td>
<td>15.8</td>
<td>17.6</td>
</tr>
<tr>
<td>HNL</td>
<td>( \theta_1 )</td>
<td>-0.14</td>
<td>0.006</td>
<td>2.1E-04</td>
<td>-0.16</td>
<td>-0.14</td>
<td>-0.13</td>
</tr>
</tbody>
</table>

1SD standard deviation, 2 MCE Monte Carlo Error, 3HPD highest posterior density, 4HL hierarchical linear model, 5HNL Hierarchical non-linear model.
TABLE 3
Observed and estimated body weights from HL and HNL models

<table>
<thead>
<tr>
<th>Traits</th>
<th>Obs.</th>
<th>HL1</th>
<th>HNL2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean±SD 5</td>
<td>HPD 3</td>
</tr>
<tr>
<td>HW</td>
<td>8.05</td>
<td>23.7±0.2</td>
<td>3.66</td>
</tr>
<tr>
<td>WK1</td>
<td>31.7</td>
<td>47.9±0.3</td>
<td>30.3</td>
</tr>
<tr>
<td>WK2</td>
<td>68.3</td>
<td>76.1±0.3</td>
<td>60.9</td>
</tr>
<tr>
<td>WK3</td>
<td>114.9</td>
<td>104.4±0.4</td>
<td>90.7</td>
</tr>
<tr>
<td>WK4</td>
<td>157.7</td>
<td>132.6±0.4</td>
<td>119.5</td>
</tr>
<tr>
<td>WK5</td>
<td>175.5</td>
<td>160.8±0.5</td>
<td>147.2</td>
</tr>
<tr>
<td>WK6</td>
<td>184.0</td>
<td>189.1±0.5</td>
<td>173.8</td>
</tr>
<tr>
<td>WK7</td>
<td>191.2</td>
<td>217.3±0.5</td>
<td>199.7</td>
</tr>
<tr>
<td>WK8</td>
<td>192.3</td>
<td>245.5±0.7</td>
<td>225.1</td>
</tr>
</tbody>
</table>

1Hierarchical linear model, 2Hierarchical non-linear model, 3Highest posterior density, 4Average of weekly observation values, 5Average of weekly estimation values.

Figure 1 shows the growth curve graphs drawn using the weekly posterior mean estimates of the weekly body weight values of the quails obtained from the HL and HNL models given in Table 3. It can be seen that the estimations obtained by the HNL function were close to the actual values. Being “S” shaped (sigmoidal) growth curve in most of the organisms allows better estimation of non-linear models than in the linear model [25 - 27].

Phenotypic and genetic correlations and heritability (h²) of the original data of the quails' hatching weight (HW) and weekly body weights (WK1-WK8) were estimated using univariate (3) and multivariate (4) models (Table 4).

TABLE 4
Heritability (on the diagonal), genetic correlation (below the diagonal) and phenotypic correlation (above the diagonal) for body weight

<table>
<thead>
<tr>
<th></th>
<th>HW</th>
<th>WK1</th>
<th>WK2</th>
<th>WK3</th>
<th>WK4</th>
<th>WK5</th>
<th>WK6</th>
<th>WK7</th>
<th>WK8</th>
</tr>
</thead>
<tbody>
<tr>
<td>HW</td>
<td>O.E. 1</td>
<td>0.30**</td>
<td>0.25**</td>
<td>0.20**</td>
<td>0.17**</td>
<td>0.12**</td>
<td>0.08</td>
<td>0.10</td>
<td>0.12**</td>
</tr>
<tr>
<td>WK1</td>
<td>0.19</td>
<td>0.43</td>
<td>0.88**</td>
<td>0.67**</td>
<td>0.49**</td>
<td>0.33**</td>
<td>0.28**</td>
<td>0.30**</td>
<td></td>
</tr>
<tr>
<td>WK2</td>
<td>0.14</td>
<td>0.89</td>
<td>0.31</td>
<td>0.73**</td>
<td>0.57**</td>
<td>0.38**</td>
<td>0.34**</td>
<td>0.34**</td>
<td></td>
</tr>
<tr>
<td>WK3</td>
<td>0.09</td>
<td>0.78</td>
<td>0.99</td>
<td>0.87**</td>
<td>0.70**</td>
<td>0.49**</td>
<td>0.43**</td>
<td>0.42**</td>
<td></td>
</tr>
<tr>
<td>WK4</td>
<td>0.08</td>
<td>0.65</td>
<td>0.92</td>
<td>0.96</td>
<td>0.89</td>
<td>0.52**</td>
<td>0.42**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WK5</td>
<td>0.08</td>
<td>0.46</td>
<td>0.79</td>
<td>0.83</td>
<td>0.95</td>
<td>0.44</td>
<td>0.39</td>
<td>0.84**</td>
<td></td>
</tr>
<tr>
<td>WK6</td>
<td>0.06</td>
<td>0.27</td>
<td>0.59</td>
<td>0.65</td>
<td>0.79</td>
<td>0.44</td>
<td>0.89</td>
<td>0.44</td>
<td></td>
</tr>
<tr>
<td>WK7</td>
<td>0.06</td>
<td>0.23</td>
<td>0.55</td>
<td>0.63</td>
<td>0.72</td>
<td>0.44</td>
<td>0.87</td>
<td>0.43</td>
<td></td>
</tr>
<tr>
<td>WK8</td>
<td>0.07</td>
<td>0.13</td>
<td>0.42</td>
<td>0.47</td>
<td>0.66</td>
<td>0.83</td>
<td>0.99</td>
<td>0.41</td>
<td></td>
</tr>
</tbody>
</table>

1Over estimated.
TABLE 5
Phenotypic and genetic relations between observation values with growth curve parameters, heritability estimates of HL and HNL growth curve parameters

<table>
<thead>
<tr>
<th>Phenotypic correlation</th>
<th>Genetic correlation and heritability</th>
</tr>
</thead>
<tbody>
<tr>
<td>HL1</td>
<td>HNL2</td>
</tr>
<tr>
<td>( \beta )</td>
<td>( \alpha )</td>
</tr>
<tr>
<td>HW</td>
<td>0.05</td>
</tr>
<tr>
<td>WK1</td>
<td>0.13**</td>
</tr>
<tr>
<td>WK2</td>
<td>0.19**</td>
</tr>
<tr>
<td>WK3</td>
<td>0.31**</td>
</tr>
<tr>
<td>WK4</td>
<td>0.45**</td>
</tr>
<tr>
<td>WK5</td>
<td>0.73**</td>
</tr>
<tr>
<td>WK6</td>
<td>0.93**</td>
</tr>
<tr>
<td>WK7</td>
<td>0.95**</td>
</tr>
<tr>
<td>WK8</td>
<td>0.95**</td>
</tr>
</tbody>
</table>

\( \beta \)          | 0.47                                | 0.48            |
\( \alpha \)          |                                      | 0.49            |
\( \varphi_1 \)       |                                      | 0.30            |
\( \varphi_2 \)       |                                      | 0.28            |

1Hierarchical linear model, 2Hierarchical non-linear model, 3Below the diagonal, 4Over estimated.

The heritability estimates of weekly body weights ranged from 0.31 to 0.44. These estimates were found similar to those reported by Silva et al. [28] and Resende et al. [29]; lower than the ones given by Narinc et al. [12] and higher than those determined by Saatci et al. [30]. Genetic correlation coefficients (when the HW feature was not considered) were estimated to be 0.13-0.99 and phenotypic correlations in the range of 0.10-0.88. Genetic correlation coefficients were higher than the phenotypic coefficients. The predicted genetic and phenotypic correlation coefficients were consistent with the estimates reported in the literature [12, 28 - 30].

Phenotypic, genetic correlation coefficients and heritability between the HW and WK1-WK8 characteristics in the original data-set and posterior averages of the parameters belonging to HL and HNL models \((\alpha, \beta, \varphi_1, \varphi_2, \varphi_3, \varphi_4)\) computed from the equation 1 and 2 were presented in Table 5.

High phenotypic and genetic relationships were determined between the posterior parameters \((\beta, \alpha, \varphi_1, \varphi_2, \varphi_3)\) of HL and HNL models and the body weight observation values. The heritability of the parameters was estimated as 0.47 and 0.48 for the parameter \((\beta)\) and \((\alpha)\) of HL model and 0.49 for the \(\varphi_1\) parameter of the HNL model. These estimates were very close to the 0.30-0.44 values obtained from the observation values. High phenotypic and genetic relations (except for HW) determined between the asymptotic posterior value \((\varphi_4)\) of HNL model and weekly body weight observations were also reported in the literature [7, 12, 14] that are consistent with the current findings. This result is important because it showed that \(\varphi_4\), which is the asymptotic parameter of \(\beta, \alpha\) and HNL model, could be a replacement criterion instead of weekly body weights. The fact that the predicted heritability of the parameters of the model equations were very close to the computed \(h^2\) predictions from the observation values also strengthens the hypothesis that the growth curve parameters can be used as breeding criteria.

CONCLUSION

It was determined that the hierarchical non-linear model estimates of Bayesian approach were closer to the observation values comparing to the hierarchical linear model estimates. The high phenotypic and genetic correlation of the parameters of the HL and HNL models with the observation values indicated that they can be used as criterion in the selection programs of these parameters (especially the HNL asymptotic weight parameter). This condition was supported by the agreement between the heritability of the HL and HNL model parameters, and the heritability of the observation values. In addition, Bayesian approach can be successfully used for growth curves estimation.

REFERENCES


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ABSTRACT

Study of the mechanism of porosity evolution and diagenesis is a hot and difficult spot in the field of petroleum geology. The diagenesis and pore evolution of the Triassic Chang 6 oil layer in the Huangling area of the Ordos Basin, China were quantitatively analyzed, by using thin sections, X-ray diffractions and scanning electron microscopy. The quantitative calculation of porosity evolution showed that the initial average porosity of the Chang 6 sandstone was 38.89%. During the diagenesis process, the average porosity loss was 25.59% and 9.81% due to compaction and cementation, respectively. The dissolution of minerals such as feldspar and rock debris in the studied sandstone was strong, and it increased the porosity of the sandstone by 4.63%. According to the criteria for diagenetic strength division, four types of diagenetic facies were identified in the Chang 6 tight sandstone. It was found that the strong compaction, medium cementation, strong dissolution facies is the most favorable diagenetic facies.

KEYWORDS:
Ordos Basin, diagenesis, porosity evolution, Yanchang Formation, quantitative analysis

INTRODUCTION

The Upper Triassic Yanchang Formation in the Ordos Basin develops tight sandstone reservoirs with enormous potential for oil and gas resources [1-8]. How to describe the diagenesis and pore evolution of the tight sandstone reservoir has become a hot and difficult spot in current researches [3-10]. However, the research on the mechanism of porosity evolution and diagenesis is still insufficient [11-15].

In this paper, taking the Chang 6 member tight sandstone in the Huangling area as an example, the diagenesis characteristics of the tight sandstone were systematically analyzed by using the thin sections, particle size analysis, cathodoluminescence, X-ray diffractions and scanning electron microscopies. Finally, the coupling effect between the diagenetic types and the porosity evolution was quantitatively characterized.

RESULTS

Petrological characteristics. The Chang 6 reservoir are dominated by feldspar sandstones, followed by lithic feldspar sandstones, while the feldspar lithic sandstones are occasionally seen (Fig. 1a). The content of the debris particles is 54.0 to 96.0%, with an average value of 88.4%. The Chang 6 sandstone has a high feldspar content and low maturity. The interstitials of the Chang 6 reservoir are mainly matrix and cement (Fig. 1b) with a content ranging from 4% to 46%, and with an average value of 11.5%.
The Chang 6 reservoir mainly develops residual intergranular pores, dissolution pores, intercrystalline micropores and micro-fractures. The pore combination type of the samples is a “dissolution pores - residual intergranular pores - intercrystalline micropores” combination, and the average face ratio is 1.03%.

Porosity and permeability. According to the petrophysical property test results of 51 samples collected from 5 wells. The porosity of the rock samples is between 0.9 and 10.8%, with an average of 7.1%. The permeability of the rock samples is distributed in the range of $0.01 - 0.23 \times 10^{-3} \mu \text{m}^2$, and the average value is $0.04 \times 10^{-3} \mu \text{m}^2$.

According to the relationship between porosity and permeability, when the porosity is less than 7%, the permeability does not change significantly with the increase of porosity. However, when the porosity is greater than 7%, the permeability shows a significant growth trend as the porosity increases (Fig. 2).

DISCUSSION

Diagenesis. Compaction. The results of microscopic observations showed that the plastic components such as biotite were significantly deformed by compression. The rigid mineral particles had an obvious directional arrangement tendency, and the contact relationship of the particles was dominated by point contact (Fig. 3a). Some quartz and feldspar particles were shear-ruptured due to influence of local overburden loading [23-24].

Cementation. The types of cements of the target layer include carbonate, clay and siliceous cements. The carbonate cements are mainly composed of ferrocalcite and ferrodolomite, and the content is between 0 and 25%, with an average value of 3.36%. Ferrocalcite usually forms a continuous crystalline cement (Fig. 3b). While ferrodolomite is mostly developed along the edge of the particles (Fig. 3c) [25-26]. Chlorite is the main authigenic clay mineral in the target layer, while illite and kaolinite are less abundant (Fig. 3d-e). The chlorite is mostly characterized by particle envelope; while the illite is mostly fibrous and hair-like filled in the pore throat of the reservoir [27]. The siliceous cementation is mainly characterized by quartz overgrowth and autogenous quartz particles (Fig. 3f) [28-29]. The content of siliceous cement is between 0 and 2% with an average of 0.42%. Quartz overgrowth generally grows in the form of a thin film along the edge of the quartz particles (Fig. 3g).

Dissolution. The dissolution of the target layer is dominated by feldspar dissolution, which occurs along the cleavage seams and edges of the particle
Notes: (a) S180 well, 1 263.28 - 1 263.43 m, strongly deformed biotite; (b) S171 well, 1 560.5 m, ferrocalcite basement cement; (c) S177 well, 1 478.86 m, monocristalline rhomboid ferrodolomite; (d) S177 well, 1 475.9 m, intergranular pores filled with filamentous illite, ×1 500; (e) S177 well, 1 479.6 m, intergranular pores are filled with authigenic quartz particles, and chlorite film covers the surface of the authigenic quartz particles, ×908; (f) S180 well, 1 232.49 - 1 232.66 m, intergranular dissolution pores, feldspar intragranular dissolution pores, microfractures and ferrocalcite cement; (g) S177 well, 1 478.86 m, intergranular pores are filled with authigenic quartz, and a small amount of filamentous illite is developed, ×1 278; (h) S171 well, 1 572.41 m, feldspar dissolution pore, ×1 600. Qtz- quartz; Chl- chlorite; III- illite.

As the dissolution strength increases, the feldspar dissolution pores are irregularly semi-connected (Fig. 3f). Some feldspar particles suffered a strong dissolution and nearly completely being dissolved (Fig. 3h).

**Diagenetic sequence and porosity evolution.**

**Diagenetic sequence.** The porosity of the Chang 6 reservoir was continuously reduced during the compaction process. The quartz overgrowth occurred only outside the contact sites of the quartz particles, indicating that quartz overgrowth occurred after intense compaction [33-35]. The chlorite film is distributed over the authigenic quartz particles (Fig. 3e), indicating that chlorite film cementation occurred after silicic cementation. After the formation of chlorite film, precipitation of early carbonates and clay minerals occurred in the residual intergranular pores [36]. As the burial depth increased, the thermal evolution of organic matter increased and organic acids were produced, the unstable components such as feldspar and rock debris were dissolved [37]. Some of the secondary dissolution pores are filled with ferrocalcite and ferrodolomite. This indicates that the carbonate cementation continues to occur after dissolution [38].

**Porosity evolution model.** By counting the original intergranular pore face rate, the secondary dissolution pore face rate and the volume fraction of various cements, the porosity occupied by the cement ($\phi_{oc}$) and the secondary porosity generated by the dissolution ($\phi_{dis}$) can be calculated.
After obtaining the initial average porosity ($\phi_1$) and the current average porosity ($\phi_{ave}$) of the reservoir, the loss of porosity ($L_1$) during compaction can be calculated:

$$L_1 = \phi_1 - \phi_{ave} \cdot \phi_{ave}$$  \hspace{1cm} (1)

In summary, the main considerations for porosity evolution are porosity loss ($L_1$) by compaction, porosity loss ($L_2$) by cementation, and porosity increase ($\phi_3$) by dissolution. The main parameters such as face rate and cement content were used to calculate the influence of diagenesis on reservoir porosity (Table 1) [39-44].

### Initial porosity recovery

The initial porosity of 50 sandstone samples was recovered by the unconsolidated sandstone porosity model (Eq. (2)) [41].

$$\phi_1 = 20.91 + 22.90 / S_o$$  \hspace{1cm} (2)

where $\phi_1$ is porosity of the unconsolidated sandstone; $S_o$ is Trask's Sorting Coefficient.

The initial porosity of the Chang 6 sandstone was recovered by the particle size analysis data and the Eq. (2) (Table 2). The Trask Sorting Coefficient ($S_o$) of the Chang 6 sandstone is between 1.16 and 1.32, with an average value of 1.28. The initial porosity was between 38.17% and 40.61%, with an average value of 38.89%.

### Calculation methods of porosity evolution in different diagenesis stages of the Chang 6 reservoir in Huangling area.

<table>
<thead>
<tr>
<th>Diagenesis</th>
<th>Sorting factor, $S_o$, (%)</th>
<th>Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>Residual porosity</td>
<td>$S_o = (D_25/D_75)^2$</td>
<td>$\phi_2 = 20.91 + 22.90 / S_o$</td>
</tr>
<tr>
<td>Loss of porosity</td>
<td>$L_1 = \phi_1 - \phi_{ave} \cdot \phi_{ave}$</td>
<td></td>
</tr>
<tr>
<td>Porosity loss rate</td>
<td>$f_1 = L_1 \times 100 / \phi_1$</td>
<td></td>
</tr>
<tr>
<td>Residual porosity</td>
<td>$\phi_3 = \phi_{ave} \cdot \phi_{ave}$</td>
<td></td>
</tr>
<tr>
<td>Loss of porosity</td>
<td>$L_2 = \phi_2 - \phi_3$</td>
<td></td>
</tr>
<tr>
<td>Porosity loss rate</td>
<td>$f_2 = L_2 \times 100 / \phi_1$</td>
<td></td>
</tr>
<tr>
<td>Generated porosity</td>
<td>$\phi_4 = \phi_{ave} \cdot \phi_{ave} / \phi_{ave}$</td>
<td></td>
</tr>
<tr>
<td>Porosity increase</td>
<td>$f_3 = \phi_3 \times 100 / \phi_1$</td>
<td></td>
</tr>
</tbody>
</table>

Notes: $D_25$—particle diameter corresponding to 25% of the particle content cumulative curve, mm; $D_75$—particle diameter corresponding to 75% of the particle content cumulative curve, mm; $\phi_{ave}$—current cement content, %; $\phi_{ave}$—intergranular pore face rate in cast, %; $\phi_{ave}$—measured average porosity of rock sample, %; $\phi_{ave}$—total face rate in the cast, %; $\phi_{ave}$—dissolution face rate in the cast, %.

### Statistical results of initial porosity recovery parameters of the tight sandstone in the Chang 6 member of the study area.

<table>
<thead>
<tr>
<th>Well name</th>
<th>Number of samples</th>
<th>Maximum value</th>
<th>Minimum value</th>
<th>Average value</th>
<th>Maximum value</th>
<th>Minimum value</th>
<th>Average value</th>
</tr>
</thead>
<tbody>
<tr>
<td>S180</td>
<td>13</td>
<td>1.32</td>
<td>1.16</td>
<td>1.27</td>
<td>40.61</td>
<td>38.22</td>
<td>38.96</td>
</tr>
<tr>
<td>S183</td>
<td>12</td>
<td>1.33</td>
<td>1.23</td>
<td>1.26</td>
<td>39.58</td>
<td>38.17</td>
<td>39.03</td>
</tr>
<tr>
<td>S1048</td>
<td>3</td>
<td>1.32</td>
<td>1.27</td>
<td>1.30</td>
<td>38.92</td>
<td>38.20</td>
<td>38.57</td>
</tr>
<tr>
<td>S171</td>
<td>10</td>
<td>1.32</td>
<td>1.23</td>
<td>1.28</td>
<td>39.48</td>
<td>38.22</td>
<td>38.78</td>
</tr>
<tr>
<td>S177</td>
<td>12</td>
<td>1.32</td>
<td>1.23</td>
<td>1.28</td>
<td>39.48</td>
<td>38.29</td>
<td>38.80</td>
</tr>
</tbody>
</table>

### Analysis of calculation results

In the early sedimentation stage, the particle size and sorting coefficient of the clastic particles of the Chang 6 reservoir were similar, therefore, the difference in initial porosity of the reservoir was small. With the increase of burial depth, the average porosity losses during compaction and cementation were 25.59% and 9.81%, respectively. The dissolution of feldspar, rock debris and other particles increased the porosity of the reservoir by an average value of 4.63% (Fig. 4).

### Application of diagenetic facies identification

The relationship between diagenesis intensity and porosity change in the target layer is shown in Table 3. According to the above criteria for diagenetic strength division, four types of diagenetic facies were identified, namely, the “strong compaction, medium cementation, strong dissolution” facies, the “strong compaction, medium cementation, medium dissolution” facies, the “medium compaction, strong cementation, medium dissolution” facies, and the “strong compaction, medium cementation, weak dissolution” facies (Table 4) [45-46]. The strong compaction, medium cementation, strong dissolution facies represented by the S177 well is the most favorable diagenetic facies (Table 4). Although the strong compaction resulted in an
average porosity loss of 69.56% of the initial porosity, the secondary pores are relatively developed by strong dissolution. The medium compaction, strong cementation, medium dissolution facies represented by the well S183 is a diagenetic facies with poor porosity development. Although strong cementation mitigates the effect of compaction on porosity, the precipitation of a large number of cements further densifies the reservoir, which leads to the weakening of the pore fluid activity and inhibits the dissolution. The average porosity of the reservoir under the control of this diagenetic facies is only 6.9%.

CONCLUSIONS

(1) The Chang 6 reservoir has the pore combination type of “dissolution pores - residual intergranular pores - intercrystalline micropores”. Compaction, cementation and dissolution all play an important role in controlling reservoir porosity.

(2) The initial average porosity of the Chang 6 reservoir was 38.89%. With the increase of burial depth, the average porosity losses during compaction and cementation were 25.59% and 9.81%, respectively. The dissolution of feldspar, rock debris and other particles increased the porosity by an average value of 4.63%.
(3) According to the criteria for diagenetic strength division, four types of diagenetic facies were identified in the Chang 6 tight sandstone.

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REFERENCES


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IMPACT OF HONEY COLOR FROM JORDANIAN FLORA ON TOTAL PHENOLIC AND FLAVONOIDS CONTENTS AND ANTIOXIDANT ACTIVITY

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ABSTRACT

The total phenolics and flavonoids contents and antioxidant activities of twenty different floral types of locally produced honey were determined and correlated with their colors. Dark multifloral honey showed the highest total phenolics, flavonoids contents and reducing power activity (15.84 mg GAE/100g, 5.74 mg QE/100g and 574.5% vitamin C equivalent, respectively), while light multifloral honey was the lowest in total phenolics and flavonoids contents (3.77 mg GAE/100g and 1.52 mg QE/100g, respectively). The antioxidant activities evaluated by DPPH free radical test were the highest (IC50 = 68.7 mg/ml) in the multiflora honey, while the lowest (IC50=302.6 mg/ml) was in the multiflora honey sample 1. Strong correlation was found between honey’s color redness and reducing power activities (r = 0.88), total phenolic content (r = 0.62) and total flavonoids content (r = 0.86), while yellowness was positively correlated with total flavonoids contents (r = 0.67).

KEYWORDS:
Phenolics, Honey color, Flavonoids, DPPH

INTRODUCTION

Honey is one of nature’s wonders and has been used since ancient times; it has been utilized by humanity for thousands of years for nutrition and therapeutic purposes. The simple sugars in honey are responsible for its sweetness, hygroscopicity, energy value and other physical properties, such as high grade viscosity, stickiness, relatively high density and immunity [1]. It exhibits functional properties [2], and its significance in traditional medicine has been recognized in various cultures [3] and in holy Quran (honey is “… the healing for mankind”).

Honey is the natural substance produced by honey bees. It is a supersaturated solution of sugars, mainly composed of fructose (38%) and glucose (31%), containing also minerals, proteins, free amino acids, enzymes, phytochemicals and vitamins [4] which makes it a potent energetic food with antibiotic properties, that reduce the risk of heart disorder, cancer and enhance the immune system [5]. In addition to its high energy potential, honey can be used as a nutritive food. Although they are present in small quantities, honey has enzymes, amino acids, and phenolic compounds that make it much healthier than sucrose [6].

Honey has anti-inflammatory, immune boosting property, and exhibits broad spectrum antibacterial activity. Much of the therapeutic properties of honey are due to the high sugar concentration, low pH and due to hydrogen peroxide generated from the oxidative conversion of glucose to gluconic acid by glucose oxidase upon dilution [7,8]. Honey applied to wounds, burns promotes faster healing by clearing infections, through promotion of tissue growth and regeneration, and preventing dehydration of the infected site, with little or no formation of scars [9].

The composition of honey and its antioxidant activity vary greatly depending on the floral source and external factors such as the season and environment [10]. Honey is an extremely variable food, even if it is made from the same floral origin or the same bee species, it can vary in texture, color, and composition depending on the geographical origin, soil, weather conditions, and even the age of the bees, which greatly affects its therapeutic properties [6]. Honey is often eaten as an energy food. It has simple sugars that are absorbed directly into bloodstream without digestion. The moisture absorbing quality of honey helps breads, cakes, cookies and candies stay fresh longer [11].

Honey color is one of the factors determining its acceptability by the consumers. Most of the floral markers in honey are flavonoids or phenolic acids, which come from the nectar or pollen of specific plants. The identification of these compounds in honey can be an important tool for the recognition of the honey’s floral type and its therapeutic activities [12]. This study may contribute to consumers’ knowledge of choosing honey with therapeutic properties as indicated from its color.
MATERIALS AND METHODS

Chemicals. Gallic acid, ferric chloride (FeCl₃), Aluminum trichloride (AlCl₃), Quercetin and L-ascorbic acid were purchased from Sigma-Aldrich (Steinheim, Germany). Folin-Ciocalteau reagent and trichloroacetic acid were purchased from AppliChem GmbH (Darmstadt, Germany). Potassium dihydrogen phosphate (KH₂PO₄·2H₂O) was purchased from Fluka-Garanite (Buchs, Switzerland). 1,1-diphenyl-2-picrylhydrazyl (DPPH) was purchased from ICN Biomedicals Inc. (South Chillicothe Road Aurora, Ohio). Sodium carbonate (Na₂CO₃), and potassium ferricyanide (K₃[Fe(CN)]₆) were purchased from E. Merck (Darmstadt, Germany). Other chemicals were of reagent grade and purchased from local companies.

Honey sample. Twenty honey samples (n =3) of citrus and multifloral types of honey were collected from bee-keepers from Amman and Jarash areas (Jordan) during 2017. The samples were stored at room temperature in the dark until analysis within 2 weeks.

Preparation of honey samples. Homogenized honey sample was diluted with distilled water in the ratio of 1:10 (w/v) with the aid of ultrasonic bath at 50 °C (Model Elmasonic S, Singen, Germany). Samples then filtered through Whatman No.1 filter paper and the obtained filtrate was used for chemical and color analysis in triplicate [13].

Determination of total soluble solids (Brix). The total soluble solids were determined as described by ISO 2173 (1998). A portable hand refractometer (Model RHB-82 ATC, China) with ranges of 45-82 * Brix was used to take measurements at 25°C. A drop of honey sample was placed on the refractometer prism and the reading was measured as percentage of total soluble solids (%) in triplicate.

Determination of total phenolic content. The total phenolic compounds present in water extracts of the selected honey samples was determined using Folin-Ciocalteau reagent [14]. Briefly, 1 ml of each plant extract (10g/100ml) was transferred into a 10 ml volumetric flask, followed by addition of 2.5 ml of distilled water, then, 250 µl Folin-Ciocalteau reagent was added, followed by mixing thoroughly for 3 minutes and after that 0.5 ml of 10% sodium carbonate (10g/100ml) was added then complete until 10 ml with distilled water. The absorbance was measured at 760nm by spectrophotometer (Model UVD-2950, Labomed, Inc.). Gallic acid was used as the standard for a calibration curve. The total phenolic compound contents (mg/100g) were expressed as Gallic acid equivalent (GAE) and determined from regression equation based on the established calibration curve (Appendix 1) Y = 0.077x, R² = 0.994, Where Y is the absorbance and X the Gallic acid concentration. All measurements were done in triplicate.

Determination of total flavonoids content. The contents of flavonoids were determined by the Miliauskas method [15]. Briefly, 1 ml from each sample (10g/100ml) was mixed with 400 µl of 2% AlCl₃ in 10 ml volumetric flask and diluted with distilled water to 10 ml. The absorption at 415 nm was taken after 40 minutes at 20°C (Model UVD-2950, Labomed, Inc.). The absorption of Quercetin standard solutions will be measured under the same conditions to establish standard curve. The amount of flavonoids in water extracts of the honey samples as quercetin equivalents (QE) was determined using regression equation based on the established calibration curve (Appendix 2): Y = 0.068x , R² = 0.994, Where Y is the absorbance and X the Quercetin concentration. All measurements were done in triplicate.

Determination of antioxidant activities. DPPH free radical scavenging assay. DPPH (1,1-diphenyl-2-picrylhydrazyl) was used to determine the free radical scavenging activity in the water extracts of the selected samples using the method of Hatano [16]. Briefly: 0.5, 1, 1.5, and 2 ml of each honey sample (10g/100ml) was complete with distilled water to reach 2 ml, then mixed with 2 ml of a methanolic solution of DPPH (6X10⁻³M), vortex and the absorbance of each extract, control (2 ml of a methanolic solution of DPPH, 2 ml of methanol) was measured at 517 nm (Model UVD-2950, Labomed, Inc.). After 30 minutes.

The scavenging activity of the extracts was calculated as follows:

\[
\text{% DPPH radical scavenging} = \frac{\text{Control absorbance} - \text{Sample absorbance}}{\text{Control absorbance}} \times 100
\]

IC₅₀ is defined as the concentration of extract in mg/ml needed to scavenge 50% of the DPPH radical. The IC₅₀ for each honey sample extract was calculated from its concentration-response curve.

Reducing power activity. The reducing powers of water extracts of the honey samples were determined using the Yildirm method [13]. One ml of each sample was mixed with 2.5 ml phosphate buffer (0.2M, pH 6.6) and 2.5 ml (1g/100ml D.H₂O) potassium ferricyanide. The mixture was then incubated at 50°C for 30 minutes. An aliquot of 2.5 ml trichloroacetic acid (10g/100ml D.H₂O) was then added and the mixture then centrifuged at 1650 x g for 10 minutes. After that 2.5 ml of upper layer solution was taken and mixed with 2.5 ml ferric chloride (0.1g/100 ml D.H₂O). The absorbance was measured at 700 nm for the water extracts of each of the selected samples and standard of
ascorbic acid (30µg).

The Reducing power activity of the extracts was calculated as follows:
Reducing power activity (%) = \[ \frac{\text{Sample absorbance} \times 100}{\text{Ascorbic acid absorbance}} \]

Honey color measurement. Lovibond colorimeter. Color of honey samples was determined using Lovibond PFXi-series (Tintometer limited, Wilts, UK). The reflectance was recorded in the CIE L*a*b* color system. The Lovibond spectrophotometer was calibrated throughout the study by remote calibration via internet and the zero baseline was adjusted before measurement according to the instrument instruction manual. Honey samples (10%) were placed in 50 ml Lovibond chamber and measurements for L*a*b* were recorded. L* represents lightness; a* represents the red/green axis; and b* represents the yellow/blue axis.

Color intensity (ABS 560 nm). The direct color absorbance of aqueous filtrate (10%) of each honey sample was measured at 560 nm using UV-Visible spectrophotometer (Model UVD-2950, Labomed, Inc.) against blank of distilled water in triplicate [13].

Statistical analysis. Antioxidant activity, phenolics and flavonoids was performed in triplicate for each of honey samples. Statistical calculations were performed using statistical analysis system, SAS program, 2000 (SAS Institute Inc., Cary, NC, USA). Significant differences among means of treatments were determined using LSD test. Differences at P≤0.05 will be considered significant. Correlation coefficient between color measurements and phenolic and flavonoids contents, and antioxidant activity was determined using Microsoft Excel.

RESULTS AND DISCUSSION

Table 1 shows the average values of some physiochemical characteristics determined in twenty samples of citrus and multifloral types of honey from Jarash and Amman cities in Jordan.

Total soluble solids (TSS). The results of total soluble solid (TSS %) of analyzed honey were ranged from 78-82% (Table 1). All investigated honey samples found to contain total sugar content higher than the recommended level (> 60%) by European Commission Directive (2002). The highest TSS (%) was found in sample 7 (82%). Sugar is the main component of honey, and the reducing sugars (mainly fructose and glucose) are the main sugars responsible for its keeping ability and sweetest taste. The results obtained are in agreement with the reported values of TSS from neighboring countries (Saudi Arabia and Egypt) [18]. They reported that the total sugar content values of 23 samples of Saudi honey were ranged between 70 to 85%, while Egyptian tested honeys had average TSS value of 77.5%. This study shows that there is negative correlations between TSS (%) with Abs, phenolic content, flavonoids content, IC50, reducing power activity, redness, and yellowness (r=-0.22, -0.04, -0.33, -0.07, -0.19, -0.28, -0.07, respectively) as shown in Table 3.

Color intensity: ABS560nm. The color of honey usually ranges from light yellow to amber, dark amber and black [19]. The tested honey samples in this study were observed to have differences in their color intensity (Table 1). The color intensity of honey as represented by the absorbance at 560 nm indicates the presence of different substances that imparts different color for the studied honeys, such as carotenoids, minerals, phenolics and flavonoids [20]. Significant differences were existed between the studied samples; the highest absorbance (0.271) was found in multifloral sample 18, while the lowest absorbance was found in samples 13, 1 and 12 (0.06, 0.072 and 0.073, respectively). Several factors affecting honey color have been reported like the use of old wax honey combs for honey production, floral origin, exposure to heat and light, contamination with heavy metals and minerals content [21, 22, 23, 39]. Table 3 shows strong positive correlation between the color intensity of honey at 560 nm with total flavonoids content, reducing power activity, and redness of the studied honeys (r=0.81, 0.75, 0.88, respectively). Similar results were reported by Moniruzzaman et al. [22]. In general, from this study we could find that citrus and light multifloral honeys showed lower absorbance than dark multifloral honeys.

Total phenolic content. Phenolic compounds are aromatic secondary metabolites which are responsible for the color, sensory and antioxidant properties of food [8, 24, 40]. The total phenolic compounds content of the studied twenty honey samples from different floral type were varied significantly and ranged from 3.77- 15.84 mg GAE/100g (Table 1 and Figure 1). This result was in agreement with those obtained by Lachman et al. [25] who reported that the total phenolics in selected Czech honey were ranged between 3.92–16.71 mg GAE/100g.

As shown in Table 1, considerable variation was found in phenolic compounds content for different honey samples; dark multifloral honey sample (14) had the highest total phenolic compounds (15.84 mg GAE/100 g) followed by the multifloral honey samples 17 and sample 1 (14.68 and 14.24 mg GAE/100g, respectively). Citrus honey of samples 15, 4 and 10, in general had the lowest total
phenolic compound values (5.36, 5.71, 7.7 mg GAE/100g). These results were in agreement with values reported by different studies of different floral type honeys from different origins showing that citrus honey had lower phenolic contents than dark colored honeys [26, 27, 28] obtained by Rababah et al. [29] study was 0-400 mg/L gallic acid and such curve is linear up to 10 mg/L in our study.

**TABLE 1**

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>English Name</th>
<th>Latin Name</th>
<th>TSS %</th>
<th>Abs.560nm</th>
<th>Total phenolic content mg GAE/100g</th>
<th>Total Flavonoids content mg QE/100g</th>
<th>IC50 mg/ml</th>
<th>Reducing power activity %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Multifloral</td>
<td>Thymbra vulgaris + Camaraea iberica</td>
<td>80</td>
<td>0.072±0.00071</td>
<td>14.2±2.27</td>
<td>1.81±0.087</td>
<td>302.6±13.4</td>
<td>155.9±0.3</td>
</tr>
<tr>
<td>2</td>
<td>Multifloral</td>
<td>Ziziphus spinachristi</td>
<td>78</td>
<td>0.153±0.00071</td>
<td>5.45±1.04</td>
<td>2.21±0.145</td>
<td>219.8±7.9</td>
<td>235.7±0.3</td>
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<tr>
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<td>Ziziphus spinachristi</td>
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<td>0.199±0.00071</td>
<td>10.52±0.91</td>
<td>4.71±0.145</td>
<td>180.3±3.3</td>
<td>338.6±0.5</td>
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<tr>
<td>4</td>
<td>Citrus</td>
<td>Citrus</td>
<td>80</td>
<td>0.093±0.00071</td>
<td>5.71±0.60</td>
<td>1.67±0.224</td>
<td>147.3±10.7</td>
<td>113.8±0.6</td>
</tr>
<tr>
<td>5</td>
<td>Multifloral</td>
<td>Prosopis farcata</td>
<td>79</td>
<td>0.165±0.00035</td>
<td>8.66±0.27</td>
<td>3.68±0.145</td>
<td>165.4±31.8</td>
<td>287.4±0.3</td>
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<td>Prosopis farcata</td>
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<td>1.76±0.145</td>
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<td>Thymbra vulgaris + Camaraea iberica</td>
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<td>12.56±1.37</td>
<td>3.24±0.145</td>
<td>102±19.2</td>
<td>329.7±0.3</td>
</tr>
<tr>
<td>8</td>
<td>Multifloral</td>
<td>Thymbra vulgaris + Camaraea iberica</td>
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<td>0.076±0.00014</td>
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<tr>
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<td>Citrus</td>
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<td>3.77±0.13</td>
<td>1.52±0.087</td>
<td>210.9±9.9</td>
<td>166.8±0.6</td>
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<tr>
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<td>Citrus</td>
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<td>0.249±0.00071</td>
<td>15.84±0.47</td>
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<td>105.9±7.2</td>
<td>574.5±1.1</td>
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<td>Citrus</td>
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<td>0.104±0.00014</td>
<td>5.36±0.33</td>
<td>2.7±0.087</td>
<td>113.2±21.8</td>
<td>236.6±0.3</td>
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<td>16</td>
<td>Multifloral</td>
<td>Citrus</td>
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<td>0.092±0.00014</td>
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<td>91.2±12.2</td>
<td>251.7±0.6</td>
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<tr>
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<td>Ziziphus spinachristi</td>
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<td>0.189±0.00071</td>
<td>14.68±1.25</td>
<td>4.41±0.295</td>
<td>104.3±10.7</td>
<td>360.4±3.4</td>
</tr>
<tr>
<td>18</td>
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<td>Ziziphus spinachristi</td>
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<td>0.27±0.00071</td>
<td>14.07±0.33</td>
<td>4.02±0.224</td>
<td>104.4±15.1</td>
<td>347.3±0.8</td>
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<tr>
<td>19</td>
<td>Multifloral</td>
<td>Ziziphus spinachristi</td>
<td>78</td>
<td>0.13±0.00014</td>
<td>11.6±1.05</td>
<td>3.14±0.224</td>
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<tr>
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<td>Multifloral</td>
<td>Ziziphus spinachristi</td>
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<td>0.13±0.00014</td>
<td>10.65±0.34</td>
<td>3.29±0.224</td>
<td>122.8±41.2</td>
<td>284.5±0.6</td>
</tr>
</tbody>
</table>

**FIGURE 1**

Standard curve of Gallic acid for the total phenolic compound contents determination

Ranged from 33.7 to 86.3 mg GAE/100g, were higher than what is reported in this investigation by almost ten times. The higher reported values was attributed to improper establishment of gallic acid standard curve, since the concentration used to produce the curve in Rababah et al. [29] study was 0-400 mg/L gallic acid and such curve is linear up to10 mg/L in our study.
Table 3 showed strong positive correlation of the studied honey samples between total phenolic contents and total flavonoids content, reducing power activity, and redness ($r=0.64$, $0.60$, $0.62$, respectively), while negative correlation was found between total phenolic content and lightness ($r=-0.59$).

The variation and the significantly differences in total phenolic contents among honey samples are mainly due to the diverse floral sources that contain different phenolic acids, location and existence of other substances that affect the phenolic content [30, 31].

**Total Flavonoids content.** Table 1 shows the concentration of total flavonoids compounds in twenty honey samples collected from different floral type in Jordan. The results were varied significantly and ranged from 1.52 mg QE/100g in light multifloral sample 13 and multifloral sample 9 to 5.74 mg QE/100g in dark multifloral sample 14 (Figure 2). The variation and the significant differences in total flavonoids contents between our samples are mainly due to the variation in the floral sources from which the honey collected; for example the multifloral (Jujube Christ thorn) and multifloral (Jujube Christ thorn + Syrian mesquite) showed high total flavonoids contents (4.71 and 4.41 mg QE/100g, respectively), while samples 4 (citrus honey) and 6 (multifloral honey, Syrian mesquite) showed low flavonoids contents (1.67, 1.76 mg QE/100g, respectively). Our result is in consistent with values obtained from Meda et al. [32] who reported that the total flavonoids content of South African honey was ranged between 0.17–7.13 mg QE/100 g and with the values obtained by Rababah et al. [29] for flavonoids of Jordanian honey (0.9-4.6 mg QE/100g). However, different results were obtained from Italian honey, which ranged from 0.45-1.01 mg QE/100 g [33], and from Czech honey which ranged from 0.53-1.23 mg QE/100 g [8, 25].

The dark multifloral sample (14) shown to have the highest total flavonoids and total phenolic contents, while light multifloral sample (13) shown to have the lowest total flavonoids and phenolic contents. This means that the darker the color of honey is the higher in its phenolics and flavonoids contents than the lighter honey color. This result in agreement with the finding of Lachman et al. [25] who reported that the dark colored kinds of honey have the higher values of total phenolics and total flavonoids contents in comparison with the light colored kind of honey.

Table 3 showed strong correlation between total flavonoids content with reducing power activity, IC$_{50}$, redness and yellowness ($r=0.84$, $0.82$, $0.86$, $0.67$, respectively). This high correlation values indicate that the total flavonoids content has more positive correlation with honey color and antioxidant activities than phenolic contents.

**Antioxidant activity.** The antioxidant activities of honey samples were determined by both DDPH free radical-scavenging activity and reducing power activity.

**DDPH Radical Scavenging Activity.** DDPH is a free radical, which is stable at room temperature with a deep purple color. When tested samples possessing antioxidant activity added to this free radical, the DDPH radical decolorized, due to the donation of H ions from antioxidant compounds and this decolorization effect is measured at 517 nm. The antioxidant activity expressed as IC$_{50}$ which is the concentration of honey in mg/ml needed to scavenge 50% of the DDPH radical.

The DDPH IC$_{50}$ values for different honey samples were presented in Table 1. Significant differences among honey samples IC$_{50}$ values were noted. The lower the IC$_{50}$ value of the sample, the higher is in its antioxidant activity. The DDPH IC50 values of different honey samples ranged between 68.7 and 302.6 mg/ml. The highest DPPH scavenging activity was found in multifloral honey samples 12, 9, and 19 with IC$_{50}$ values of 68.7, 81.8, and

![Standard curve of Quercetin for the total flavonoids contents](image-url)
The lowest IC50 values found for multifloral (thyme + Iberian star-thistle) honey sample (1) followed by multifloral honey sample 2 with values of 302.6 and 218.9 mg/ml, respectively and these two high figures reflect poor antioxidant activity against DPPH free radical. The DPPH results were in agreements with the IC50 values ranges reported by krpan et al. [34], on acacia honey and with Ferreira et al. [35] on Portuguese honeys. Rababah et al. [29] reported the DPPH scavenging activities for different Jordanian honeys, the IC50 value was 65 mg/ml for multifloral sample and this result was in agreement with our result for multiflora sample 68.7 mg/ml at the studied concentration levels.

These results demonstrate that the variations in antioxidant activities of honey samples and their ability to scavenge the free radicals are a function of different floral sources of nectar [36]. So the antioxidant activities varied significantly between samples due to the variation in the floral sources from which the honey collected.

Reducing power activity. The reducing power activity measures the reducing potential of an antioxidant to reduce Fe3+ to Fe2+. As shown in Table 1, the reducing power activity of different tested honey samples were ranged between 574.5-155.9%, expressed as 30 µg ascorbic acid (Vitamin C) equivalent.

The reducing power activities varied significantly between samples due to the variation in the floral sources, the highest reducing power activity indicating that the sample had a strong antioxidant activity comparable to ascorbic acid.

The highest reducing power activity was found in dark multifloral honey sample (14) with reducing power activity of 574.5%. This high activity was related to the highest phenolic and flavonoids, and to the expected synergistic activity between phenolic compounds and other plant constituents which may influence their antioxidant activities [30]. The lowest reducing power activity was found in multifloral (thyme + Iberian star-thistle) honey sample (1) with reducing power activity of 155.9%.

The reducing power activity (expressed as 30 µg vitamin C equivalent) (%) for the honey samples showed a strong correlation with the flavonoids content (r=0.84), with color (redness) (r = 0.88) and with color intensity absorbance at 560 nm. In general, the darker the color of honey sample is the higher in the reducing power activity.

Color Measurements. The lightness (L*), redness (a’), yellowness (b’) values of honey samples are shown in Table 2. The lightness, redness, yellowness varied significantly among different floral sources. Honey color could be classified to be light in color if the L* >50 according to Gonzalez-Miret et al. [20] classification. Light multifloral honey sample (13) had the highest lightness value (79.2). This value was in agreement with Rababah et al. [29] who reported values for multifloral 44% citrus spp. honey (76.4) honey sample, however the multifloral honey sample 18 shown to have the lowest lightness value (24.1).

Dark multifloral honey sample 14 had the highest redness value (10.4), this value was in agreement with the values reported by Bertoncelj et al. [37] for forest honey with highest redness value of 10.14. Multifloral honey sample 16 had the lowest redness value (1.32) and the highest yellowness value (44.68) these values are in consistent with the reported values by Bertoncelj et al. [37] for forest honey who reported that the highest yellowness value is 46.45 for multifloral honey sample. Multifloral honey sample 1 (thyme + Iberian star-thistle) had the lowest yellowness value (21.51).

The reducing power activity % expressed as 30µg ascorbic acid (Vitamin C) equivalent and was considered to be 100%.
In this study we found that the dark multifloral honey sample 14, which had the highest redness value, had the highest total phenolic content, the highest total flavonoids content and also had the highest reducing power activity. This means there is a strong correlation between the color of honey and phenolic, flavonoids content and reducing power activity and the darker the honey color is the highest in its phenolic and flavonoids contents, and reducing power activity.

Light multifloral honey sample 13, which had the highest lightness value, also had the lowest total phenolic and flavonoids contents. This means the lighter the color of honey is the lowest in its phenolic and the flavonoids contents.

**Correlation coefficients (r) among different color components and bioactive compounds.** The correlation results among the investigated honey samples parameters are presented in Table 3. Lightness is negatively correlated with redness (r = -0.93), total phenolic content (r = -0.59), total flavonoids (r = -0.82), and reducing power activity (r = -0.84). While redness is positively correlated with total phenolic content (r = 0.62), total flavonoids content (r = 0.86), and reducing power activity (r = 0.88). The yellowness was positively correlated with total flavonoids contents (r = 0.67), and reducing power activity (r = 0.57).

Strong correlation was found between total flavonoids content and reducing power activity (r = 0.84), between total phenolic content and total flavonoids (r = 0.64), between total phenolic contents and reducing power activity (r = 0.6). Rababah et al. [29] reported a positive correlation between total phenolic and flavonoids contents (r = 0.57), and between redness and total flavonoids contents (r = 0.59), which is consistent with our results.

<table>
<thead>
<tr>
<th>Sample number</th>
<th>Sample source</th>
<th>L* (lightness)</th>
<th>a* (redness)</th>
<th>b* (yellowness)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Multifloral (thyme + Iberian star-thistle)</td>
<td>68.2±0.12</td>
<td>2.03±0.02</td>
<td>21.51±0.0071</td>
</tr>
<tr>
<td>2</td>
<td>Multifloral</td>
<td>46.2±0.12</td>
<td>4.02±0.01</td>
<td>25.91±0.01</td>
</tr>
<tr>
<td>3</td>
<td>Multifloral (Jujube Christ thorn)</td>
<td>36.5±0.13</td>
<td>8.42±0.11</td>
<td>35.15±0.21</td>
</tr>
<tr>
<td>4</td>
<td>Citrus</td>
<td>61.7±0.1</td>
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<td>27.24±0.01</td>
</tr>
<tr>
<td>5</td>
<td>Multifloral</td>
<td>43.6±0.14</td>
<td>5.8±0.1</td>
<td>33.64±0.02</td>
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<tr>
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<td>Multifloral (Syrian mesquite)</td>
<td>62.6±0.13</td>
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<tr>
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<tr>
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<td>Multifloral (thyme + Iberian star-thistle)</td>
<td>67.1±0.16</td>
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<td>Multifloral (Jujube Christ thorn + Syrian mesquite)</td>
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<td>5.08±0.01</td>
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* Values (mean± SD) followed by different letters within column are significantly different at P<0.05.

<table>
<thead>
<tr>
<th>correlation factor between Parameters</th>
<th>TSS</th>
<th>Abs</th>
<th>Total phenolic content</th>
<th>Total flavonoids content</th>
<th>IC50</th>
<th>Reducing power activity</th>
<th>L* (Lightness)</th>
<th>a* (Redness)</th>
<th>b* (Yellowness)</th>
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<td>0.18</td>
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<td>-0.07</td>
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<td>0.16</td>
<td>0.75</td>
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<td>0.88</td>
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<td>0.57</td>
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<td>L* (Lightness)</td>
<td>1</td>
<td>-0.93</td>
<td>-0.52</td>
<td>0.48</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a* (Redness)</td>
<td>1</td>
<td>0.48</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>b* (Yellowness)</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
CONCLUSION

This study evaluated twenty honey samples from different floral sources in Jordan for phytochemicals and colors. The results indicated that honey samples from each floral source had different amounts of total phenolics, flavonoids and antioxidant activities. The lightness, redness, yellowness values of honey samples varied among different floral type. Significant differences existed in different honey sample from different source in Jordan. Strong correlation was found between redness and reducing power activity (r=0.88), between redness and total flavonoids content(r=0.86), and a between reducing power activity and total flavonoids content(r=0.84). In general, we can say darker color honey (redness) is rich in flavonoids content and reducing power activity, strong correlation between reducing power activity and total flavonoids content.

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REFERENCES


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COMPARISON BETWEEN DEVELOPED IPM PROGRAM AND CONVENTIONAL CONTROL PRACTICES FOR THE TOMATO LEAFMINER, *Tuta absoluta* (MEYRICK) (LEPIDOPTERA: GELECHIIDAE) IN JORDAN

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ABSTRACT

The tomato leafminer, *Tuta absoluta*, is a serious pest of tomato that was introduced into Jordan in 2010. This study aimed to compare the efficacy of a conventional control (spraying deltamethrin) commonly practiced by Jordanian farmers against an IPM program for *T. absoluta*. The tested IPM program consisted of using a least susceptible cultivar, a most effective insecticide, muslin barriers, sticky traps and trap plants. The experiment was conducted in Al-Karamah in Jordan Valley farm. The IPM program consisted of planting the least susceptible tomato cultivar (Dafnis), one insecticide application of the most effective insecticide (Avaunt®), yellow sticky traps, muslin barriers and trap plants. Evaluation was based on counting number of larvae at weekly basis, yield of tomato and infestation % of *T. absoluta*. The yield under the IPM program (3135 kg) was significantly higher than the yield under conventional treatment (2480 kg). The infestation % was significantly lower under IPM (1.77%) as compared to the conventional treatment (6.78%). The number of larvae found in the plastic house under IPM program was 484 larvae compared to the conventional plastic house (1422 larvae). This proved that the used IPM program was effective in controlling this pest and, therefore, it is recommended for controlling the insect under plastic houses in Jordan.

KEYWORDS:

Tomato leafminer, Tomato, Conventional control, IPM program, Jordan

INTRODUCTION

Tomato, *Solanum lycopersicum* Mill is a vegetable crop of economic importance throughout the world. Its annual production accounts for 163 million metric tons grown in more than 4.8 million hectares [1]. Tomato is one of the most important strategic, economic and cash-crop in Jordan. It is grown around the year in open fields and under plastic houses. More than 145 thousand donums (each donum equal to 1000 m²) are grown to tomato and produce approximately 744 thousand metric tons with more than 517 thousand tons exported to Arab region and Europe [2]. It is one of the main agricultural labor sectors where thousands of local families completely depend on its revenue. As an economic and food security crop in Jordan and many other countries as well, decline in the tomato yield and planted areas due to any reason is considered a frightening case.

The tomato leafminer, *Tuta absoluta* (Meyrick) is a devastating pest of tomato, originating from South America. After its initial detection in eastern Spain in 2006, it has rapidly invaded various other European countries and spread throughout the Mediterranean Basin. Larvae produce large galleries in leaves, burrow into stalks, apical buds and green and ripe fruits [3]. The larvae feed on leaf parenchyma tissue and on tender portions of the stems. In developing and mature fruits, the insect causes bud drop, fruit malformation, fruit rot, and a drastic reduction in leaf area [4]. Tomato plants can be attacked at any developmental stage, from seedlings to mature stage [5, 4].

If no control measures are taken, the pest may cause up to 80-100% yield losses in tomato crops in recently invaded areas and may pose a threat to both greenhouse and open-field tomato production [6, 3, 7, 8]. Six years ago, the pest has suddenly appeared in Jordan (Gawr As Safi) as a destructive pest in most of the planted areas and caused a significant loss that reached up to 100% in Al-Mafraq Governorate. Thereafter, it became a limiting factor to tomato production in Jordan [9, 10, 14, 26, 27, 28, 29, 30].

The management tactics in most countries are chemicals control. organophosphates were initially used for *T. absoluta* control, then during 1970s were replaced by pyrethroids and in 1980s Cartap (Nereis toxin) were used, which was alternated with pyrethroids [11]. Resistance has been developed and reported against organophosphates, pyrethroids and Cartap in Chile, Brazil and Argentina [11, 12,
MATERIALS AND METHODS

Components of the IPM program. The IPM program consists of the following components: i) transplanting the least susceptible tomato cultivar (Dafnis) obtained from a previous susceptibility experiment, ii) one insecticide application of the most effective insecticide (Avautant®) obtained from previous pesticides experiment, iii) using yellow sticky traps, iv) using muslin barriers, and iv) using trap plants.

Conventional control practices by Jordanian farmers. Jordanian farmers commonly use deltamethrin as an insecticide spraying every 2 weeks to control T. absoluta. This was considered for comparison between what Jordanian farmers practice for controlling the pest and the used IPM program. The cultivar, distance between rows and plants were used the same as above. No muslin barriers were used.

Statistical analysis (means and analysis of variance: ANOVA) was performed using SAS 9.4 software (SAS Institute, Inc., Cary, NC). An ANOVA model was used for individual treatment comparisons at P<0.05 and means were separated by the Least Significant Difference (LSD).

RESULTS AND DISCUSSION

Population densities of larval stage of Tuta absoluta. Figure (1) shows the population density of larvae on the cultivar Dafnis under IPM program and conventional practices (control). In the IPM treatment, larvae were first observed on the 11th of March 2015, after 62 days of transplanting. The average number of larvae per leaf increased from 0.03 larva to a peak of 2.6 larvae per leaf on the June 3, 2015. In the traditional treatment, the first observed larva was on the 25th of February 2015, after 49 days of transplanting. The average number of larvae per leaf increased from 0.08 larvae to a peak of 4.02 larvae. The total number of larvae on the IPM treatment was much lower than in the control treatment (Fig. 2).

MATERIALS AND METHODS

Study locations. The experiment was carried out in AlKaramah in Jordan Valley (JV) (35°57043E, 31°92895N). The soil texture was clay loam. It was covered by black plastic mulch and irrigated by drip irrigation during the period of the experiment.

Environmental conditions at Al Karamah location. The average annual rain fall in Al Karamah was about 200 mm/year, and the minimum temperature was 5°C usually in January to maximum of 38°C usually in August. The RH ranges from 32% to 90% [15].

The temperature and relative humidity (%) during the experimental period (24/12/2012 to 29/4/2013) was recorded inside the plastic house using Thermo-Hygrometer (HTC-103 CTH). The average daily temperature ranged from 11°C to 31°C with an average of 23°C. The RH% ranged from 49% to 72% with an average of 63.4%.

The layout of this experiment was Complete Randomized Design. Two plastic houses were used in Al Karamah Area in Jordan Valley in 2012, one for implementing the IPM program and one for conventional control practices. Evaluation was based on (i) counting number of larvae at weekly basis without removing the leaves and (ii) yield of tomato and (iii) infestation % of T. absoluta.
Total tomato yield and infestation%. The total yield of tomato produced in the IPM treatment (3135kg/plastic house) was higher than the one produced in the control treatment (2480kg/plastic house). The infested fruits in the IPM treatment (297) was lower than the control treatment (927). The infestation % was lower in the IPM program compared to the conventional practices (Table 1).

![Graph showing average number of larvae per tomato leaf on the IPM program compared to the conventional practices at Al Karamah, Jordan Valley between February and June, 2015.](image1)

**FIGURE 1**
Average number of larvae per tomato leaf on the IPM program compared to the conventional practices at Al Karamah, Jordan Valley between February and June, 2015.

![Graph showing total numbers of the larvae of T. absoluta for the IPM and control treatments at Al Karamah, Jordan Valley during 2015.](image2)

**FIGURE 2**
Total numbers of the larvae of *T. absoluta* for the IPM and control treatments at Al Karamah, Jordan Valley during 2015.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Yield (kg/plastic house)</th>
<th>Fruits</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Number of fruits</td>
</tr>
<tr>
<td>IPM</td>
<td>3135</td>
<td>16815</td>
</tr>
<tr>
<td>Control</td>
<td>2480</td>
<td>13687</td>
</tr>
</tbody>
</table>

Means within the same column sharing the same letter do not differ significantly at 5% level using LSD test.
Different IPM programs were developed against *T. absoluta* since its spread in many parts of the world. Abbès and Chermiti [16] used water traps with sexual pheromone, massive release of *Nesidiocoris tenuis* and selective insecticides (Avaun®, Oberon®, *B. thuringiensis*) in Tunisia obtained successful results compared to control (no use of bio-agents and pheromone traps). Harbi et al. [17] controlled successfully the pest by using sex pheromone water traps, insect-proof screens on doors and two sprays with indoxacarb under greenhouses conditions in Tunisia. Taha et al. [18] developed an IPM program based on the use of insecticides (Thiamethoxam+chloranlaniprole, clothianidin, *Bacillus thuringiensis*) together with pheromone-bated water traps in Egypt. The results were successful in reducing the damage of *T. absoluta*. No direct comparison between the results of these IPM programs and the results of the present IPM program is possible because of the differences of the components used in each program. In addition to other factors such as the used tomato cultivars, the chemicals used, the environmental conditions and used cultural practices. Generally all used IPM programs has proved to be more effective than depending only on insecticides.

*Tuta absoluta* has a high risk of evolving resistance to insecticides due to the large number of generation per year as many cases have been reported to date for this pest [19, 20, 21]. *Tuta absoluta* demonstrated a great genetic plasticity a long 35 years of its introduction in Brazil, developing resistance with relative ease to many insecticides used to control [22].

The immediate consequence of the introduction of tomato leaf miner into Jordan was the sudden increase in insecticide use in tomato fields by farmers which was mainly deltamethrin. Salazar and Araya [12] reported resistance to deltamethrin, metamidophos, lambda-cyhalothrin and mevinphos in Chilean populations of *T. absoluta*.

The population of *T. absoluta* larvae was much lower when the developed IPM program (484 larvae) was used compared to the conventional methods (1422 larvae). The harvested tomato yield was also higher in the plastic house where the IPM program was implemented (3135kg) compared to the yield in the conventional plastic house (2480kg). In addition, the infestation% (1.77%) was lower in the IPM plastic house compared to 6.78% in the conventional plastic house. Harbi et al. [17] applied an IPM program in Tunisia consisting of the use of pheromone traps and insect proof screen at the entrance of the greenhouse. They found that the fruit loss was 144kg, representing 5% damage compared to 1134kg infested fruits representing 31.4% damage.

Cherif et al. [23] found in their study in northeastern Tunisia under greenhouse conditions that the mean number of *T. absoluta* larvae on leaves during the 4-month study period (from mid-January to early May) was significantly lower (in the greenhouse equipped with insect proof than the one without insect-proof screens. The weekly number of larvae found did not exceed 20/week in the greenhouse without insect-proof screens. These findings showed that the use of insect-proof screens significantly reduced density of *T. absoluta* larval populations on tomato leaves. Accordingly, this control tool could be successfully incorporated in an integrated pest management program against the tomato leafminer. The obtained results in the present study agreed to a great extent with the findings of Cherif et al. [23].

The combination of pheromone water traps and insect-proof screens was used in reducing tomato damage by *T. absoluta*. According to Harbi et al. [17], the use of one sex pheromone water trap combined with insect-proof covering of doors and aeration openings was sufficient to provide good crop protection level against tomato leafminer infesting greenhouse tomato crops in Teboulba, Tunisia.

Several authors reported tolerance of *T. absoluta* to pesticides, for instance in Argentina two population of *T. absoluta* has become tolerant to deltamethrin and one population to abamectin [11]. In Brazil, since 1999 significant resistance of *T. absoluta* to acephate and deltamethrin was reported by Branco et al. [24]. Vais et al. [25] found that the mutation reduced the sensitivity of the sodium channel to deltamethrin by ten folds and could confer more than 10,000 folds resistance to deltamethrin when inserted in the wild type.

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REFERENCES


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ABSTRACT

The tomato leafminer, *Tuta absoluta*, is a serious pest of tomato that was introduced into Jordan in 2010. Thus, this study aimed to investigate the susceptibility of three tomato cultivars and the efficacy of three insecticides in controlling the immature stages and in developing an IPM program. Experiments were conducted in Al-Karamah Al Balqa governorate in the middle of Jordan valley during 2013 to 2015. The first tested experiment was the susceptibility of Dafnis, Newton and Shams cultivars to *T. absoluta*. The second tested experiment was Avaunt®, Belt® and Phytomax® for controlling the pest on cultivar Dafnis. The third test was the least susceptible cultivar and the most effective insecticide in developing an IPM program and compared it to conventional control. The IPM program used cultivar Dafnis which was the least susceptible cultivar to *T. absoluta* in the Alkarameh location. The total number of larvae was the lowest in cultivar Dafnis compared to Newton and Shams (1786, 2252, 2421) in Al Karamah. The insecticide Avaunt® was also used which was the most effective insecticide. The total number of larvae was the lowest using Avaunt® compared to Belt®, Phytomax® and control (216, 498, 757, 895) in Al Karamah.

KEYWORDS:
Broad tomato leafminer, Tomato, Cultivars, Susceptibility, Chemicals, Jordan

INTRODUCTION

Tomato, *Solanum lycopersicum* Mill is a vegetable crop of economic importance throughout the world. Its annual production accounts for 163 million metric tons grown in more than 4.8 million hectares [1]. Tomato is one of the most important strategic, economic and cash-crop in Jordan. It is grown around the year in open fields and under plastic houses. More than 145 thousand donums (one donum is equal to 1000mm²) are grown to tomato and produce approximately 744 thousand metric tons with more than 517 thousand tons exported to Arab Region and Europe [2]. It is one of the main agricultural labour sectors where thousands of local families completely depend on its revenue. As an economic and food security crop in Jordan and many other countries as well, decline in the tomato yield and planted areas due to any reason is considered a frightening case. The tomato leafminer, *Tuta absoluta* (Meyrick) is a devastating pest of tomato, originating from South America. After its initial detection in eastern Spain in 2006, it has rapidly invaded various other European countries and spread throughout the Mediterranean Basin [3, 4]. It has been reported in many countries and several regions; in South America in Venezuela, Colombia, Bolivia, Argentina, Peru, Uruguay, Brazil, Costa Rica, Ecuador, Chile, Panama and Paraguay [3, 4, 5, 6, 7, 8]. It is also reported from Europe in Spain, France, Italy, the Netherlands, Albania, Portugal, Malta, the UK, Slovenia, Crete, Bulgaria, Cyprus, Germany, Hungary, Bosnia, Croatia, Denmark, Greece, Kosovo, Lithuania, Romania, Russia, Switzerland, Serbia and Mayotte island [3, 4, 9, 10, 11, 12]. In Africa, it is reported from Algeria, Morocco, Tunisia, Egypt, Libya [3, 4, 10, 11]. In Asia and Middle East it is reported from India, Bahrain, Iraq, Kuwait, Saudi Arabia, Syria, Turkey, Iran, Qatar, Oman, Yamen and Jordan [3, 4, 11, 13, 14, 15, 16, 17, 18, 19].

If no control measures are taken, the pest may cause up to 80-100% yield losses in tomato crops in recently invaded areas and may pose a threat to both greenhouse and open-field tomato production [3, 4, 6, 14, 20]. Six years ago, the pest has suddenly appeared in Jordan (South Ghor, Safi) as a destructive pest in most of the planted areas and caused a significant loss that reached up to 100% in
Al-Mafraq Governorate. Thereafter, it became a limiting factor to tomato production in Jordan [3, 4, 8]. The exceptional distribution and extent of *T. absoluta* invasion have called the growers in Jordan and many other countries for intensive applications of chemicals which added more economic costs and intensified hazardous problems. *Tuta absoluta* is a species of moth classified under order Lepidoptera and family Gelechiidae and known in different names; tomato borer, tomato leafminer, broad tomato leafminer [3, 4, 8], tomato leafminer moth, south American tomato pinworm and south American tomato moth. According to several workers [3, 4, 14] the insect can also feed, develop and reproduce on other cultivated Solanaceae plants such as eggplant, potato, sweet pepper, and tobacco. In addition, it can attack wild Solanaceae plants like *Datura querescifolia* and *D. ferox*. It’s also reported that *T. absoluta* can attack some Fabaceae plants like common bean (*Phaseolus vulgaris*) [9]. The adult female lays up to 260 eggs on the aerial parts of their host plant [3, 4, 18] and eggs deposited singly, or rarely in batches. On the average, the development period was estimated to be 23.8 days at 27.1°C. The Adult lifespan ranges between 10-15 days for female and 6-7 days for males [3, 4, 14]. *Tuta absoluta* may be able to complete 12 generations per year [9] or 13 per year [3, 4]. The larval stage consists of four instars and completed in 12-16 days. The 4^th^ larval instar usually drops to the ground on a silk threat and pupates in the soil or also on the surface of the leaves. The developmental period of pupa ranges from 7-9 days to give adults. Larvae produce large galleries in leaves, burrow into stalks, apical buds and green and ripe fruits [3, 4, 20]. The larvae feed on leaf parenchyma tissue and on tender portions of the stems. In developing and mature fruits, the insect causes bud drop, fruit malformation, fruit rot, and a drastic reduction in leaf area [3, 4, 22, 25, 26, 27, 29, 30].

The primary *Tuta absoluta* management tactic in most South American countries is relying on chemical control [23]. Organophosphates were initially used for *T. absoluta* control, which were gradually replaced by pyrethroids during the 1970s. During the early 1980s, Cartap (Nereis Toxin), which alternated with pyrethroids and thiocyclam, proved highly efficient in controlling pest outbreaks [23]. In the 1990s, novel insecticides were introduced, such as abamectin (Avermectins), acyurela IGR, spinosad (Spinosyns), tebufenozide (Diacylhydrazines), chlorfenapyr (Clorfenapyr) and Coragan (phthalic acid). Recently in Brazil, 10 new molecules of pyrethroids proved to be effective in controlling *T. absoluta*, with different toxically effects, and in some cases, up to 100% larval mortality was achieved [30]. Valchev et al. [31] showed that Avaunt® and Coragan had the best activity against larvae of *T. absoluta*. Santos et al. [32] also found that Avaunt® caused 96.1 and 93.6% reduction in the infestation of *T. absoluta* at 3 and 7 days after application. Resistance development has been reported against organophosphates, pyrethroids, abamectin and Cartap in Chile, Brazil, and Argentina [24, 25, 23]. Therefore, this study aimed to investigate the susceptibility of three different tomato cultivars and the efficacy of three chemicals in controlling the broad tomato leafminer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) in the region of middle Jordan valley in Jordan to develop IPM programme useful to control this devastating insect pest to minimize environmental contamination.

**MATERIALS AND METHODS**

The study location. A field experiment was carried out in Jordan. The first location was in Al-Karamah in the middle of Jordan valley (35° 57043'E, 31° 92895'N) in Jordan. The location belong to Al Balq'a governorate. The soil texture in the location was clay loam. It was covered by black plastic mulch and irrigated by drip irrigation during the period of the experiment.

Environmental conditions at Al Karamah location. The average annual rain fall in Al Karamah was about 200 mm/year, and the minimum temperature was 5°C usually in January to maximum of 38°C usually in August. The RH ranges from 32% to 90% [28]. The temperature and relative humidity (%) during the experimental period (24/12/2012 to 29/4/2013) was recorded inside the plastic house using Thermo Hygrometer- HTC-103 CTH. The average daily temperature ranged from 11°C to 31°C with an average of 23°C. The RH% ranged from 49% to 72% with an average of 63.4%.

Population densities of immature life stages of *Tuta absoluta* on tomato cultivars. The layout of this experiment was Complete Randomized Block Design (CRBD), with three treatments and three replicates. The treatments represented two new cultivars used by farmers (Dafnis and Shams), in addition to, the widely cultivated cultivar, Newton. The cultivars were grown in Al Karamah location in a plastic house (56 X 9m), divided into three blocks and separated from each other by a distance of 1m. By excluding border areas, the area remaining for each block was 15X7m. In each replicate, the assigned transplants of each of the three cultivars were planted in January 2013 in the Al Karamah location in rows 120cm apart and at a distance of 40cm among plants. A total of 67 plants were used for each cultivar and for each replicate. Plants were fertigated and weeded as necessary. No insecticides were applied. For detecting early infestation of *T. absoluta*, a yellow pheromone-water trap per plastic house was placed outside the plastic house at the time of transplanting, 0.5m above ground and...
about 5m from the front end of the plastic house. After the first catch of *T. absoluta* males, the pheromone trap was removed and data recording was started. Three parameters were used to assess the susceptibility of the tested cultivars. These were population densities of the immature life stages of *T. absoluta* during the growing season on the location. This was done by counting the egg and larval stages of *T. absoluta* on tomato cultivars inside the plastic house by randomly tagging 7 plants for each replicate with a specific cloth-colour. From each tagged plant, 3 leaves were taken in a plastic bag from the upper, middle and lower parts of the plant at 3-day intervals, until the end of harvesting the crop. The leaves were then inspected under an binocular dissecting microscope for eggs, and by naked eye for larvae and pupae of *T. absoluta*. Egg and larval numbers were recorded for each part of the plant separately (upper, middle, and lower parts). However, pupae were collected and counted wherever seen on the plant regardless the plant part. Infestations % in tomato harvested fruits of the different cultivars were calculated by recording the total number of fruits and total number of infested fruits to determine the infestation% at the harvesting period. The total harvested yield was weighed and recorded until the termination of the experiment.

Efficacy of three chemicals in controlling *Tuta absoluta*. The experiment design of this experiment was CRBD, with 4 treatments and 3 replicates. The treatments were: Belt® (flubendiamide) (Bayer), Phytomax® (azadirachtin) (Russell IPM), and Avaunt® (indoxacarb) (Dupont) (Table 1), and the 4th treatment was control. The control was sprayed with water only. Each replicate consisted of 25 tomato plants. The layout of the experiment was as the susceptibility experiment. The three chemicals were chosen because they represented three newly used different groups of chemicals each which a different mode of action with the exception of Azadirachtin which has unknown mode of action (Table 1) [29].

### RESULTS

**Population density of eggs.** Figure (1) shows that oviposition of *T. absoluta* started on the selected cultivar (Dafnis) on the 13th of February 2013, after 43 days of transplanting (2/1/2014). The average number of eggs per leaf increased from one egg to a peak of 4 eggs per leaf on the date of the experiment termination. The total numbers of eggs were 312, 215, 190, 596 in the four treatments (Belt®, Avaunt®, Phytomax® and the control), respectively (Table 1). Means of eggs were significantly higher in the upper part of the plant in all treatments. Mean of eggs of the control treatment was 312, 215, 190, 596 in the four treatments (Belt®, Avaunt®, Phytomax® and the control), respectively (Table 1). Means of eggs were significantly higher in the upper part of the plant in all treatments. Mean of eggs of the control treatment was significantly the highest while the lowest was when treated with Phytomax® (Fig.2, Table 2).

<table>
<thead>
<tr>
<th>Trade name</th>
<th>Common name</th>
<th>Chemical group</th>
<th>Formulation*</th>
<th>Rate of application ml/L</th>
<th>Chemical name (IUPAC)#</th>
</tr>
</thead>
<tbody>
<tr>
<td>Belt®</td>
<td>Flubendiamide</td>
<td>Neonicotinoids</td>
<td>SC 480%</td>
<td>0.1</td>
<td>3-iodo-N’-{2-methyl-1,1-dimethyl-ethyl}-N-[(1,2,2,2-tetrafluoro-1-(trifluoromethyl)-ethyl]-0-tolyl]phthalimide methyl (S)-N-[7-chloro-2,3,4a,5-tetrahydro-4a-methoxycarbonylindeno[1,2-e][1,3,4]oxadiazin-2-ylcarbononyl]-4’-(trifluoromethoxy)carbanilate dimethyl(2aR-2aalpha,3beta,4beta(1aR *,2S *,3aS *,6aS *,7aS *,7S *,7aS *)alphahalbeda</td>
</tr>
<tr>
<td>Avaunt®</td>
<td>Indoxacarb</td>
<td>Oxadizine</td>
<td>SC 14.5%</td>
<td>0.25</td>
<td>3-iodo-N’-{2-methyl-1,1-dimethyl-ethyl}-N-[(1,2,2,2-tetrafluoro-1-(trifluoromethyl)-ethyl]-0-tolyl]phthalimide methyl (S)-N-[7-chloro-2,3,4a,5-tetrahydro-4a-methoxycarbonylindeno[1,2-e][1,3,4]oxadiazin-2-ylcarbononyl]-4’-(trifluoromethoxy)carbanilate dimethyl(2aR-2aalpha,3beta,4beta(1aR *,2S *,3aS *,6aS *,7aS *,7S *,7aS *)alphahalbeda</td>
</tr>
<tr>
<td>Phytomax®</td>
<td>Azadirachtin</td>
<td>Tetranaortriterpenoids</td>
<td>EC 3%</td>
<td>0.33</td>
<td>3-iodo-N’-{2-methyl-1,1-dimethyl-ethyl}-N-[(1,2,2,2-tetrafluoro-1-(trifluoromethyl)-ethyl]-0-tolyl]phthalimide methyl (S)-N-[7-chloro-2,3,4a,5-tetrahydro-4a-methoxycarbonylindeno[1,2-e][1,3,4]oxadiazin-2-ylcarbononyl]-4’-(trifluoromethoxy)carbanilate dimethyl(2aR-2aalpha,3beta,4beta(1aR *,2S *,3aS *,6aS *,7aS *,7S *,7aS *)alphahalbeda</td>
</tr>
</tbody>
</table>

FIGURE 1
Average number of eggs per tomato leaf on cultivar Dafnis for all treatments at Al Karamah, Jordan Valley between February and May, 2014.

TABLE 2
Total numbers of eggs, larvae and pupae of *T. absoluta* for cultivar Dafnis when treated with three different insecticides in Al Karamah, Jordan Valley between February and May, 2014.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>No. of eggs</th>
<th>No. of larvae</th>
<th>No. of pupae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Belt®</td>
<td>312</td>
<td>498</td>
<td>131</td>
</tr>
<tr>
<td>Avaunt®</td>
<td>215</td>
<td>216</td>
<td>59</td>
</tr>
<tr>
<td>Phytomax®</td>
<td>190</td>
<td>757</td>
<td>138</td>
</tr>
<tr>
<td>Control</td>
<td>596</td>
<td>895</td>
<td>188</td>
</tr>
</tbody>
</table>

FIGURE 2
Average number of eggs or larvae per tomato leaf on cultivar Dafnis among the three pesticides and the control at Al Karamah, Jordan Valley during 2014.
Population density of larvae. Figure (3) and Table (2) show the population density of larvae on cultivar Dafnis. Larvae were first observed on 20th of February 2014, after 48 days of transplanting and 5 days of laying eggs. The average number of larvae per leaf increased from one larva to a peak of 4 larvae per leaf on the 19th of April 2014, then it declined until the termination of the experiment. Means of larvae were higher on the middle part of the plant for all treatments. The means of larvae were the highest in the control and Phytomax® treatment. However, it was the lowest on plants treated with Avaunt®. The effect of Belt® was intermediate and differed from all treatments.

The total number of larvae on cultivar Dafnis treated with Avaunt® was the lowest followed by Belt®, Phytomax® and the control (Table 2).

Population density of pupae. Pupae started to appear on the 19th of March 2014 after 72 days of transplanting, 34 days of laying eggs and 27 days of the first sampled larvae (Fig. 4). During the entire experiment period in 2014 tomato plants treated with Avaunt® had the lowest total number of pupae. This may indicate that Avaunt® was more effective in controlling the larvae of *T. absoluta* (Table 2).

FIGURE 3
Average number of larvae per tomato leaf on cultivar Dafnis among the three insecticides and the control at Al Karamah, Jordan Valley between February and May, 2014.

FIGURE 4
Average number of pupae per tomato plant on cultivar Dafnis for all treatments at Al Karamah, Jordan Valley between March and May, 2014.
The obtained results of this study where azadirachtin and organophosphorus compounds, high resistance by *T. absoluta* has been developed [33]. Salazar and Araya [24] in Africa and Chile found that deltamethrin was the least toxic to *T. absoluta* in which deltamethrin was the mostly commonly used insecticide against adult *T. absoluta* in Jordan when it was first appeared in 2010.

During the present study, three different insecticides were used with two known mode of action and the third one is unknown. Belt® targets and disrupts the Ca²⁺ balance (Ryanodine receptor modulators). Avaunt® works as a sodium channel blocker, and Phytomax® with unknown mode of action [29]. The pesticide efficacy experiments were conducted under plastic houses at Al Karamah in Jordan Valley.

The experiments were conducted to study the efficacy of the three mentioned insecticides on the population of eggs, larvae and pupae in cultivar Dafnis which was the least susceptible cultivar based on the result of the susceptibility experiment. The results showed that Phytomax® had the highest effect on the eggs compared to the other insecticides on the selected cultivar, Avaunt® had more effect on the larval and pupal stages with a significant difference compared to other insecticides. Azadirachtin was reported to impair larval development in lepidopterous pests including some leafminer species [34]. Azadirachtin caused egg-laying avoidance and also had an effect on the crawling by larvae, but not on the leaf mining, which indicated the potential direct effect of this insecticide on *T. absoluta* [34]. This was clear in the obtained results of this study where azadirachtin showed significant effect on the number of eggs in the tested cultivar compared to other used insecticides. This agreed with the results found by Brunherotto et al. [35]. This indicates that azadirachtin delayed development leading to high insect mortality and allowed few insects to reach the pupal stage compared to the traditional insecticides. Hafsi et al. [36] found that Azadirachtin caused 43.8% egg mortality while several other insecticides showed low ovicidal activity against *T. absoluta*. Santos et al. [32] reported that indoxacarb (Avaunt®) caused 96.1% reduction of *T. absoluta*. Both studies agreed with the obtained results in the present study that indoxacarb showed the most effect on *T. absoluta* larvae with the same indication for the pupal stage.

In a study conducted on *T. absoluta* control by several insecticides in Turkey, the highest insecticide resistance was found against indoxacarb compared to chlorantraniliprole, metaflumizone, spinosad and azadirachtin [37]. Results of the present experiment did not agree with the above study, probably because the used insecticides for comparison were different and might be that the Turkish strain of *T. absoluta* differed from the Jordanian strain. In addition, the history of pesticide use in Jordan was supposed to be different from that in Turkey. Valkov et al. [31] showed that Avaunt® and Coragen had the best activity against Larvae of *T. absoluta*. Santos et al. [32] also found that Avaunt® caused 96.1 and 93.6% reduction in the infestation of *T. absoluta* at 3 and 7 days after application. Both results agreed with the results of the present study. Mohamoud et al. [38] found that the effect of tested insecticides on the number of tunnels in the infested plants by *T. absoluta* were 22, 25.67, 21.67 and 22.33 tunnels in control, Avaunt®, Coragen, Aljambo and Superlambda treatment, respectively. They indicated that there was no significant difference among the different treatments. Furthermore, they found that Avaunt® was the less effective against the larval stage and on egg laying when compared to the other used insecticides. However, in the present study it was clear that Avaunt® had a significant effect on the number of larvae of *T. absoluta*.

Results of the above study was in general agreement with the result of the present study in which Avaunt® was the most effective followed by Belt® and Phytomax®.

**DISCUSSION**

Chemical insecticides are one of the main tools in the management of tomato leafminer *T. absoluta*, particularly in greenhouse crops. Several insecticides have been used for the control of *T. absoluta*. In Brazil, due to heavy use of pyrethroids and organophosphorus compounds, high resistance by *T. absoluta* has been developed [33]. Salazar and Araya [24] in Africa and Chile found that deltamethrin was the least toxic to *T. absoluta* in which deltamethrin was the mostly commonly used insecticide against adult *T. absoluta* in Jordan when it was first appeared in 2010.

**TABLE 3**

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Total yield (kg/plot)</th>
<th>Total number of fruits</th>
<th>Number of infested fruits</th>
<th>Infestation %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Belt®</td>
<td>560.3</td>
<td>3198</td>
<td>116</td>
<td>3.6</td>
</tr>
<tr>
<td>Avaunt®</td>
<td>590.6</td>
<td>3369</td>
<td>91</td>
<td>2.7</td>
</tr>
<tr>
<td>Phytomax®</td>
<td>542.1</td>
<td>3092</td>
<td>201</td>
<td>6.5</td>
</tr>
<tr>
<td>Control</td>
<td>423.8</td>
<td>2414</td>
<td>270</td>
<td>11.1</td>
</tr>
</tbody>
</table>

The highest yield of cultivar Dafnis was recorded when plants were treated with Avaunt® compared to the other treatments (Table 3). The infestation was 3.6%, 2.7%, 6.5% and 11.1% for Belt®, Avaunt®, Phytomax® and the control, respectively (Table 3).
In is difficult to compare results of insecticide applications in different experiments conducted in different countries. These differences among different used insecticides could be due to the effect of climate conditions such as RH, light and temperature and in addition to the different application methods which used by different researchers. Another reason might be due to the different cultural practices in each experiment like spacing, weeding, pruning, and application volume, canopy coverage, spraying nozzles, irrigation and fertilization.

CONCLUSIONS

In conclusion, the results of the present study showed that cultivar Dafnis was the least susceptible to *T. absoluta*. At the same time the insecticide (Avaunt®) showed that it was the most effective insecticides among the used ones. *T. absoluta* had a preference to lay eggs on apical leaves, compared to medium or basal leaves of tomato plants. The muslin barriers were effective in reducing infestations. To set up a successful IPM program, it is very important to start monitoring with pheromone traps, choosing the least susceptible cultivar for planting, using the most effective insecticide, and using other control methods such as muslin barriers, trap plants and yellow sticky traps at the entrance of the plastic house. Implementing a successful IPM program will significantly reduce the number of insecticides applications, increase yield as compared to conventional control methods, decrease the infestation level and preserve natural enemies of this distractive pest.

RECOMMENDATIONS

Testing a large number of tomato cultivars that can be planted in Jordan in plastic houses as well as in the open field to find out the least susceptible. Testing a large number of insecticides, belonging to as many as different groups, to found out the most effective one in controlling the different stages of *T. absoluta*, and to determine the least effective insecticide against the natural enemies of the pest. Searching for predators and parasitoids of *T. absoluta* from different parts of Jordan and studying their impact on the population of the pest in order to choose the most effective ones in the IPM programs. Studying the morphological and/or chemical factors found in the different tomato cultivars that affect the resistance. Studying the effect of mass trapping using pheromones on the population of the pest in a wide area. Studying the survival strategy of the pest during the months in which no tomato plants are available. This might include surveying its wild hosts and determining whether the insect has a diapause period or not. Conducting periodical evaluations of the insecticides frequently used against the pest in Jordan for early detection of resistance development.

ACKNOWLEDGEMENTS

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REFERENCES


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EXPERIMENTAL PREPARATION AND CHARACTERIZATION OF A NOVEL NONIONIC DENDRITIC MACROMOLECULAR DERIVATIVES USING FOR WASTE WATER BASED DRILLING FLUID TREATMENT

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ABSTRACT

Drilling fluids are essential for oil and gas drilling operations. Water-based drilling fluids will inevitably generate large amounts of drilling waste fluid during recycling. Therefore, timely and efficient disposal of waste water-based drilling fluids is essential for environmental protection. At present, the commonly used disposal methods for waste drilling fluids generally have problems of low processing efficiency, incomplete treatment, high cost, and inadequate environmental protection. In this paper, we proposed an experimental preparation method for a novel nonionic dendritic macromolecular derivative, based on the waste water-based drilling fluid treatment. Firstly, amino dendrimers PATAM-0, PATAM-1 and PATAM-2 were prepared based on the highly regular spatially symmetric structures of dendritic macromolecules, monomer optimization and optimized preparation techniques. Then, the corresponding non-ionic dendritic macromolecular derivative PATAM-AM was developed by end group modification and addition reaction. Meanwhile, we quantitatively analyzed its structure and physicochemical properties. Acrylamide (AM) was preferred as a nonionic monomer for terminal modification of dendritic macromolecular PATAM-1 and PATAM-2, and PATAM has been modified with an aqueous solution polymer. The results showed that PATAM-1 is more suitable as the core of flocculated macromolecules. The prepared PATAM-AM conforms to the basic requirements of the molecular structure of the dendritic macromolecular derivatives. Moreover, we also determined the optimal preparation conditions of PATAM-AM-1 by orthogonal tests.

KEYWORDS: Nonionic flocculant, dendritic macromolecular derivatives, environmental requirements, experimental characterization, waste water-based drilling fluid

INTRODUCTION

Water-based drilling fluids are the most commonly used fluids to ensure safe, high-quality, and efficient drilling operations. A large amount of drilling waste liquids will be produced during the drilling process. As one of the main pollutants in the petrochemical industry, if the waste liquid is not effectively and harmlessly treated, it will pose a serious threat to the ecological environment [1-3]. The waste water-based drilling fluid is a multi-phase steady-state colloidal suspension system containing clay, weighting materials, chemical treatment agents, sewage, oil pollution and cuttings. The main components of the hazardous materials are hydrocarbons, salts and various polymers, lignosulfonates, certain metal ions (mercury, copper, arsenic, chromium, zinc, and lead) and impurities in barite [4-8]. These substances are difficult to decompose naturally. If the degraded waste is retained in the natural environment for a long time, it will seriously damage the local ecological environment and even endanger the life safety of the organism. Strict environmental regulations require that drilling waste fluids be properly and harmlessly treated to reduce environmental pollution and ecological damage.

According to the composition, treatment and discharge of waste liquid, the current treatment methods for drilling waste liquid are also various, such as curing treatment, conversion of waste drilling fluid into cement slurry (MTC technology), chemical strengthening solid-liquid separation technology, mechanical dehydration method, incineration method, spray drying method, recycling method, and microbial method [9-15]. However, these commonly used disposal methods for waste drilling fluids generally have problems of low processing efficiency, incomplete treatment, high cost, and inadequate environmental protection.

Based on the basic principles of reduced input, significant effectiveness, and reusability during the field drilling process, the waste water-based drilling
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Compared with linear macromolecules, this
type of derivative has the following advantages as a
flocculant (Fig. 1): (1) The branches in the molecular structure are separated from each other, and the
entanglement between the molecular chains is not
easy to occur. (2) The density of the terminal group
is extremely large, so a large amount of a desired
functional group can be introduced through the
reaction. (3) In the case where the molecular weight
is equivalent, the dendritic macromolecules have a
low viscosity, so the viscosity of the liquid phase is
not excessively high. Therefore, in this paper, we
proposed an experimental preparation method for a
novel nonionic dendritic macromolecular derivative, based on the waste water-based drilling fluid
treatment. This method can provide a reference for
similar researches worldwide [19-22].

fluids should be reused as much as possible [1618]. According to the types of recyclable products,
the recycling method can be divided into: (1) the
processed product is divided into two parts, a solid
phase and a liquid phase, which can be reused; (2)
the drilling waste liquid is completely converted
into a harmless solid, and the liquid phase is no
longer discharged separately. Field experiences
show that the method of converting all drilling
waste liquids into a harmless solid phase requires a
large amount of water resources, so it cannot be
applied on a large scale at the drilling field. In contrast, the first type of method can greatly reduce the
volume of the harmless solid phase, and the separated liquid phase can also be reused, thus achieving environmental protection and resource conservation.
At present, the solid-liquid separation method
of drilling waste liquid mainly adopts a flocculation-settlement method based on chemical additives. Dendritic macromolecules are new types of
polymer macromolecules that have received extensive attention in recent years. This type of macromolecule has a highly regular spatially symmetrical
structure, and consists of a core molecule as the
initial core, and an iterative reaction forms a repeating branching unit that is radially connected to the
initial core.

MATERIALS AND METHODS
Flocculants based on dendritic macromolecules have been used in waste water treatment in
recent years and have achieved good application
results. Therefore, in order to enhance the solidliquid separation ability of the waste water-based
drilling fluid, a suitable dendritic macromolecular
can be prepared and introduced as a gel-flocculant
to replace the existing linear organic polymer. At
the same time, the best separation effect can be
achieved by selecting an inorganic flocculant as a
sedimentation aid.
First, a terminal amino group dendritic macromolecular is prepared, and then, a terminal group
modification and an addition reaction are used to
introduce a plurality of monomers having different
functional groups into the dendritic macromolecules. A flocculant of a nonionic dendritic macromolecular derivatives was formed by Michael addition and radical polymerization. Finally, the fabric
and physical and chemical properties of the newly
developed flocculant were evaluated.
The main materials used in the tests include
pentaerythritol (PTA), AR, laboratory-made; acryloyl (AM), amine methyl acrylate, methanol, ethyl
acetate, petroleum ether, sodium bicarbonate, all
AR, Saen Chemical Technology (Shanghai) Co.,
Ltd. The experimental instrument is the DF-101S
magnetic stirrer of Shanghai Dongyu Refrigeration
Instrument Co., Ltd.; DZF-6050 vacuum rotary
evaporator, Japan Rikakikai company; Suplecosil
LS-Si normal phase silica gel column, Shanghai
&KXGLQJ $QDO\WLFDO ,QVWUXPHQW &R /WG ;¶3HUW
Pro MPD X-ray Multi-Function Powder Diffractometer, PANalytical, The Netherlands; WQF-520
infrared spectrometer, Beijing Ruili Analytical
Instrument Co., Ltd.
In addition, the dendritic macromolecular with
pentaerythramine as the core was named PATAM.
Unless otherwise specified, the PATAM appearing

FIGURE 1
Flocculant failure of linear polymers and
function of dendrimer derivatives flocculant

6924


in the study refers to a macromolecular with pentae-
rythramine as the core.

RESULTS AND ANALYSIS

Preparation of PATAM-0. Preparation. A
certain amount of pentaerythritol and methanol
were placed in a three-necked flask equipped with a
magnetic stir bar, a reflux condenser, and a ther-

ometer, stirred at a predetermined temperature,
and a quantitative amount of methyl acrylate was
added dropwise using a dropping tube. After a
certain period of time, a vacuum distillation was
carried out at 25 °C under a pressure of 133 Pa to
remove the solvent methanol and excess raw mate-
rial pentaerythritol, and then purified by chroma-
tography on a silica gel column to obtain a pale
yellow viscous solid (i.e., PATAM-0 finished prod-
uct). The 3D molecular structure of PATAM-0 is
shown in Fig. 2.

| FIGURE 2 |
| 3D molecular structure of PATAM-0. |

Structure Characterization. The \(^1\)HNMR
tests of PATAM-0 dissolved in chloroform (CDCl\(_3\))
was conducted using a Bruker A400 NMR spec-
trometer. The results are as follows: \(^1\)HNMR (500
MHz, CDCl\(_3\)), d (TMS, ppm): 2.22 (s, 8H, 4 C-
CH\(_2\)-N), 2.49 (s, 16H, 8 C-CH\(_2\)CO), 3.61 (s, 24H,
8-CH\(_3\)), 3.76 (s, 16H, 8 N-CH\(_2\)-C), the molecular
structure of the product met the expected results,
indicating that the desired product was obtained by
this experiment.

Considering the effect of reactant ratio, reac-
tion temperature and reaction time on the yield, the
preparation conditions of PATAM-0 were opti-
mized based on the orthogonal test method: tem-
perature 30 °C, time 24 h, molar ratio of PTA to
MA = 1: 15.

Preparation of PATAM-1. Preparation. A
certain amount of PATAM-0 and methanol were
placed in a three-necked flask equipped with a
magnetic stir bar, a reflux condenser, and a ther-

ometer, stirred at a predetermined temperature,
and a quantitative amount of pentaerythritol was
added dropwise using a dropping tube. After a
certain period of time, a vacuum distillation was
carried out at 72 °C under a pressure of 266 Pa to
remove the solvent methanol and excess raw mate-
rial pentaerythritol, and then purified to obtain a
pale yellow viscous solid (i.e., PATAM-1 finished prod-
uct). The 3D molecular structure of PATAM-1 is
shown in Fig. 3.

| FIGURE 3 |
| 3D molecular structure of PATAM-1 |

It can be seen from Fig. 3 that the molecular
structure of PATAM-1 is relatively loose, and the
spatial configuration is not close to the spherical
shape, but the pentaerythrine in the outer structure
is a small molecule of a body shape, which gives a
large spatial blocking effect between the branches.
Affected by this, the spatial distribution of the sur-
face amine groups of PATAM-1 is relatively uni-
form, which is favorable for the uniform distribu-
tion of linear molecular segments introduced later
in space.

Structure Characterization. The \(^1\)HNMR
tests of PATAM-1 dissolved in chloroform (CDCl\(_3\))
was conducted using a Bruker A400 NMR spec-
trometer. The results are as follows: \(^1\)HNMR (500
MHz, CDCl\(_3\)), d (TMS, ppm): 1.47 (s, 48h, 24-
NH\(_2\)), 2.22 (s, 8H, 4 C-CH\(_2\)-N), 2.49 (s, 16H, 8 C-
CH\(_2\)CO), 3.61 (s, 16H, 8 N-CH\(_2\)-C), 3.76 (s, 8H,
8 NCH\(_2\)CH\(_2\)), 8.01 (s, 8H, 8 -NH-), the molecular
structure of the product met the expected results,
indicating that the desired product was obtained by
this experiment.

Considering the effect of reactant ratio, reac-
tion temperature and reaction time on the yield, the
preparation conditions of PATAM-1 were opti-
mized based on the orthogonal test method: tem-
perature 30 °C, time 36 h, molar ratio of PATAM-0
to PTA = 1: 15.
Preparation of PATAM-2. According to the above experimental methods and procedures, PATAM-1.5 was successfully prepared, and PATAM-2 was prepared by using PATAM-1.5 as a raw material.

Preparation. A certain amount of PATAM-1.5 and methanol were placed in a three-necked flask equipped with a magnetic stir bar, a reflux condenser, and a thermometer, stirred at a pre-determined temperature, and a quantitative amount of pentaerythritol was added dropwise using a dropping tube. After a certain period of time, a vacuum distillation was carried out at 72 °C under a pressure of 266 Pa to remove the solvent methanol and excess raw material pentaerythritol, and then purified to obtain a pale yellow viscous solid (i.e., PATAM-2 finished product). The 3D molecular structure of PATAM-2 is shown in Fig. 4.

As can be seen from Fig. 4, the molecular spatial configuration of PATAM-2 is obviously similar to the spherical morphology. Compared with PATAM-1, the molecular density and the number of terminal amine groups of PATAM-2 are significantly increased. The regular spatial configuration of PATAM-2 facilitates the uniform distribution of the end groups in their molecular surface layers.

Structure Characterization. The $^1$HNMR tests of PATAM-2 dissolved in chloroform (CDCl$_3$) was conducted using a Bruker A400 NMR spectrometer. The results are as follows: $^1$HNMR (500 MHz, CDCl$_3$), d(TMS, ppm): 1.47 (s, 288H, 144 NH$_2$), 2.22 (s, 8H, 4 C-CH$_2$-N), 2.36(s, 48h, 24 C-CH$_2$-N), 2.49(s, 48h, 24 C-CH$_2$CO), 2.53(s, 288H, 144 C-CH$_2$-N), 3.06(s, 96H, N-CH$_2$-C), 3.61(s, 16H, 8 N-CH$_2$-C), 3.32(s, 8H, 8 C-NH-C), 3.64(s, 112H, 56-CH$_2$-), 3.76(s, 16H, 8 N-CH$_2$-C), 8.01(s, 48h, 48-NH-), the molecular structure of the product met the expected results, indicating that the desired product was obtained by this experiment.

Considering the effect of reactant ratio, reaction temperature and reaction time on the yield, the preparation conditions of PATAM-2 were optimized based on the orthogonal test method: temperature 30 °C, time 24 h, molar ratio of PATAM-1.5 to PTA = 1: 70.

The yield of PATAM-2 is further lowered as compared with the yield of PATAM-1.5 because the molecular structure of the product is further complicated, the number of iterative reactions required is increased, and the molecular weight is significantly increased. The limitations of the purification and impurity removal processes, as well as the structural defects in the product, limit the yield of PATAM. In addition, the surface end groups of PATAM-1 and PATAM-2 are primary amine groups, which have a more spherical structure and a larger end group density.

Determination of nonionic graft modified monomers. The prepared PATAM-1 and PATAM-2 are the same as the integer generation PAMAM dendritic macromolecules, and the surface end group is a primary amine group. Therefore, PATAM-1 and PATAM-2 can have Michael addition reaction with vinyl monomer containing an $\alpha$, $\beta$-unsaturated double bond such as acrylamide, acrylic acid, 2-acrylamido-2-methylpropanesulfonic acid, dimethyl diallylammonium chloride under appropriate conditions. Its product is centered on PATAM dendritic macromolecules. Due to the wide variety of such monomers that meet the requirements, it is desirable to optimize and determine the optimal monomer desired.

Commonly used non-ionic monomers for modification are acrylamide, acrylonitrile, methyl acrylate and methyl methacrylate. Among these monomers, acrylamide (AM) is most widely used because of its low toxicity, low cost, solubility in water, and acting water as a reaction solvent. Therefore, acrylamide is preferably a nonionic monomer for preparing a flocculant in this experiment.

Preparation of nonionic dendritic macromolecular derivatives. Synthesis of dendritic macromolecular derivatives. A certain amount of PATAM-1 dendritic macromolecules was dissolved in deionized water, and the pH of the system was adjusted to 7 with NaHCO$_3$, and then added it into a 500 mL three-necked flask with a funnel and a reflux condenser, and the temperature was raised to a predetermined temperature in an N$_2$ atmosphere. After the reaction was continued for a predetermined period of time, it was taken out, and the solvent was removed by rotary evaporation under reduced pressure. Then, the obtained product was purified by precipitation with absolute ethanol to obtain a crude product, which was then subjected to dialysis in an ultrafiltration membrane for 96 hours in distilled water to remove oligomers and unreact-
ed monomers in the product. Finally, the product was placed in a vacuum oven and dried under vacuum at 60 °C for 48 h to form a pale yellow viscous solid, which was the final product after purification, and it was named PATAM-AM-1. The synthetic route of PATAM-AM-1 is shown in Fig. 5.

PATAM-AM-2 can be further prepared based on the preparation of PATAM-2 by the same technical method.

**Physical and chemical performance characterization.** (1) **Elemental analysis.** In the preparation of PATAM dendritic macromolecules, if the density of the terminal groups is too large, many end groups are vacant due to the steric hindrance effect between the modified segments. At this time, the excessive end group density of PATAM-2 may result in the terminal amine group cannot be linked to the AM.

To verify whether this is true or not, elemental analysis was performed on PATAM-AM-1 and PATAM-AM-2. If most of the end groups of the two generations of PATAM dendritic macromolecules can undergo an addition reaction with AM, the ratio of the elements of the product should be close to a certain theoretical value.

Elemental analysis of the product was conducted using the Vario EL III CHNOS elemental analyzer. The selected products were the reaction products of PATAM-1 and PATAM-2 with AM which is $5 \times 10^3$ times their own molar amount. The content of each element in the prepared product was measured, and the difference between the theoretical value and the measured value was compared. The experimental results are shown in Table 1.

It can be seen from Table 1 that the measured values of the four elements of PATAM-AM-1 are close to the theoretical values, and the difference between the measured value of PATAM-AM-2 and the theoretical value is large, especially the determination of C and O elements. This indicates that the actual molecular structure of PATAM-AM-2 differs greatly from the theoretical expectation, which is due to the fact that too many, too dense end groups cannot participate in the modification reaction.

Due to the low molecular weight of AM, the steric hindrance effect is small, and its reaction activation energy is lower than that of AMPS and DMDAAC, then PATAM-2 has a lower probability of effective reaction with AMPS and DMDAAC. Therefore, PATAM-2 is not suitable as the core of the dendritic macromolecules for the preparation of flocculant.

(2) **FTIR infrared spectroscopy.** The purified PATAM-AM-1 powder was treated with potassium bromide and subjected to structural analysis using a WQF-520 infrared spectrometer. The results are shown in Fig. 6.

### TABLE 1

<table>
<thead>
<tr>
<th>Element</th>
<th>C</th>
<th>H</th>
<th>N</th>
<th>O</th>
</tr>
</thead>
<tbody>
<tr>
<td>PATAM-AM-1</td>
<td>Theoretical value (%)</td>
<td>50.71</td>
<td>7.11</td>
<td>19.77</td>
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<tr>
<td></td>
<td>Measured value (%)</td>
<td>48.73</td>
<td>6.89</td>
<td>21.21</td>
</tr>
<tr>
<td>PATAM-AM-2</td>
<td>Theoretical value (%)</td>
<td>50.73</td>
<td>7.12</td>
<td>20.01</td>
</tr>
<tr>
<td></td>
<td>Measured value (%)</td>
<td>46.34</td>
<td>6.91</td>
<td>21.97</td>
</tr>
</tbody>
</table>
FIGURE 6
Infrared spectrum analysis results of PATAM-AM-1.

TABLE 2
Synthesis conditions of PATAM-AM-1 and its relationship with molecular weight.

<table>
<thead>
<tr>
<th>No.</th>
<th>Temperature (°C)</th>
<th>Time (h)</th>
<th>m(PATAM): m(AM)</th>
<th>$M_n$ (theoretical)</th>
<th>$M_n$ (actual)</th>
<th>$M_w$</th>
<th>FDI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>50</td>
<td>24</td>
<td>3.51</td>
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<td>3.79x10^5</td>
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</tr>
<tr>
<td>2</td>
<td>50</td>
<td>36</td>
<td>1:10 000</td>
<td>7.12x10^5</td>
<td>5.54x10^5</td>
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<td>1.31</td>
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<td>3.55x10^5</td>
<td>1.33</td>
</tr>
</tbody>
</table>

Notes: Distribution coefficient $FDI=M_w/M_n$.

It can be seen from Fig. 6 that 3 363 cm⁻¹ is the characteristic peak of the polymer N-H chain, 1 645 cm⁻¹ is the C=O bond vibration absorption peak in the AM amide group, and 1 548 cm⁻¹ is the amide group N-H bond stretching vibration absorption peak. 1 498 cm⁻¹ is the C-N bond stretching vibration peak in AM, 1 444 cm⁻¹ and 967 cm⁻¹ are the vibration absorption peaks of methylene group in polymer.

The results of infrared spectroscopy indicated that the product contained characteristic peaks of the AM polymer chain, and therefore, the structures of the prepared product conformed to the expected results.

(3) FTIR molecular weight test. The molecular weight of the product largely determines the properties of the flocculant. Taking the molecular weight of purified PATAM-AM-1 as the evaluation standard, the effects of temperature, reaction time and feed ratio on the molecular weight of the product were investigated by orthogonal level experiments. The specific results are shown in Table 2.

As is clear from Table 2, the number average molecular weight ($M_n$) and the weight average molecular weight ($M_w$) of PATAM-AM-1 differed depending on the reaction conditions. When the reaction temperature is 50 and 55 °C, the difference between the actual number average molecular weight of the product and its theoretical value is smaller than the difference in molecular weight of the product at a temperature of 60 °C, and the molecular weight distribution coefficient ($FDI$) of the product at the first two temperatures is also less than $FDI$ at 60 °C, indicating that its molecular weight distribution is more concentrated.

When the feed molar ratio of PATAM-1 to AM is 1: 15 000, the difference between the actual molecular weight of the product and its theoretical value is greater than the difference between the molecular weights of the other two feed ratios. The actual molecular weight of the product differs little from the actual molecular weight of the product at a feed ratio of 1: 10 000. In contrast, the effect of reaction time on the molecular weight of the product is relatively limited.

The experimental results show that the AM monomer cannot be fully integrated into the PATAM-1 dendritic macromolecules due to the steric hindrance effect. Therefore, increasing the amount of AM is not conducive to the preparation of high molecular weight products. When the reaction tem-
perature is 50 and 55 °C, the difference between the actual molecular weight and the theoretical value is small, and at the same time, the molecular weight distribution is concentrated. This may be due to the higher grafting efficiency of the AM monomer in this temperature range, and the lower probability of occurrence of adverse reactions such as disproportionation and the termination of chain growth.

Based on the above analysis, the optimum reaction conditions for PATAM-AM-1 were as follows: reaction temperature 55 °C, reaction time 48 h, m (PTATM-1): m (AM) = 1: 10 000. And the actual number average molecular weight of the product was 5.91×10^5, the weight average molecular weight was 7.51×10^5, and the FDI coefficient of the product was only 1.27, indicating that the molecular weight distribution was very concentrated.

CONCLUSIONS

(1) In this paper, four dendritic macromolecules, PATAM-0, PATAM-1, PATAM-1.5 and PATAM-2, were prepared by using self-prepared pentaerythritol as the initiation nucleus, and using pentaerythritol and methyl acrylate as repeating structural units, and using methanol as a reaction solvent. Analysis of the molecular structure indicated that these macromolecular products met the expected requirements.

(2) The difference between the structure and elemental composition of PATAM-1 with the theoretical expectation is small. Therefore, PATAM-1 is preferred as the core of the dendritic macromolecules for preparing flocculant. The acrylamide with low toxicity, low price and water solubility was preferred as the monomer, and the end group modification of PATAM-1 was conducted by means of aqueous solution polymerization, and finally the PATAM-AM-1 dendritic macromolecular derivative was prepared. PATAM-AM-1 has a regular and compact structure that is close to spherical and can be used as a flocculant for the separation of waste drilling fluids.

ACKNOWLEDGEMENTS

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REFERENCES


OPTIMIZATION OF SYNTHESIS PROCESS OF POLYETHYLENE GLYCOL MODIFIED POLYLACTIC ACID BY ORTHOGONAL EXPERIMENT

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School of Materials and Chemical Engineering, Bengbu College, Anhui Bengbu 233030, China

ABSTRACT

Polyactic acid polyethylene glycol copolymers (PLEG) were synthesized using stannous octoate as catalyst via lactic acid pre-polymer, two-stage temperature controlling. The synthesis process of PLEG were optimized by L25(5)^6 orthogonal experiment. The influence of stannous octoate addition (a), temperature for LA pre-polymerization (b), temperature for PEG-LA low-temperature polymerization (c), duration of PEG-LA low-temperature polymerization (d), temperature for PEG-LA high-temperature polymerization (e), duration of PEG-LA high-temperature polymerization (f) were studied. The results showed that the optimal technologies were that stannous octoate addition was 0.8%, lactic acid pre-polymer temperature was 120 °C, PEG-LA low-temperature polymerization temperature was 110 °C, polymerization time at low-temperature was 4h, PEG-LA high-temperature polymerization temperature was 180 °C, polymerization time at high-temperature was 7h. The PLEG was characterized by IR, the tensile tests and contact angle testing. The results demonstrate that the flexibility of the composites can be effectively proved with the addition of PEG. Compared with poly (L-lactic acid), the hydrophilicity properties of PLEG were distinctly improved.

KEYWORDS
Polylactic acid, Polyethylene glycol, Modified, Orthogonal experiment, Synthesis.

INTRODUCTION

In recent years, there is a growing interest in studying biodegradation of high molecular materials both at home and abroad. Polylactic acid (PLA) is characterized by excellent biocompatibility and biodegradability. As the end products of the degradation are CO₂ and H₂O, it presents good processability, and can be used to manufacture fiber by melt spinning. The raw material LA can be prepared from starch by fermentation. As a renewable resource, PLA has become one of the most important biodegradable materials, and is widely used in industries of biomedical engineering, textile, packaging, etc. [1-5] With the advancement of research and the continuous expansion of PLA application, application of pure LA homopolymer has been greatly limited by its deficiencies, including high synthesis cost, strong hydrophobicity and high fragility [6-8], and it can no longer satisfy practical demand. Therefore, synthesis of modified PLA has become a hot research topic.

Polyethylene glycol (PEG) is a commonly used modifier. Regarding existing methods, LA monomer and PEG are added at once for polymerization under raised temperature [9-11]. It is suggested in relevant studies that PEG might suffer from transfer reaction with growing active chain end during copolymerization, and that the addition of PEG enhances the viscosity of the reaction system, which affects the copolymerization reaction. Therefore, the viscosity average molecular weight of the resulting copolymer PLEG is relatively low. Besides, polymerization temperature is also an important factor affecting copolymerization reaction. It is generally believed that polymerization temperature should not be too high, as PEG is inclined to oxidation under a high temperature. Therefore, polymerization is usually conducted under a temperature of no higher than 150 °C. Consequently, there are deficiencies of low reaction efficiency and low molecular weight of PLEG. Furthermore, the viscosity of the system increases with reaction time. It is difficult to remove water generated during late polymerization reaction, and the continuity of polymerization reaction is compromised. Therefore, how to optimize experimental process and to obtain ideal copolymerization products is the main research direction of PEG-modified PLA.

Based on single addition and raised-temperature-based polymerization, polymerization process of PLEG is modified in the paper. Firstly, LA is preliminarily pre-polymerized to form short chains of PLA. Then, PEG is added as a modifier. Through N₂ protection and low-temperature polymerization during a middle stage, high-temperature oxidation of PEG is prevented. High-temperature polymerization is applied during late-stage reaction, while a water separator is used for
backflow and water removal so as to solve the problems concerning increased system viscosity during late reaction and the difficulty of water elimination. By pre-polymerization of raw materials, addition of a modifier in middle stage and stage-based temperature control, PLEG copolymer with a relatively high viscosity average molecular weight is obtained. Meanwhile, an orthogonal experiment is used and a 6-factor 5-level L25(5^6) plan is adopted to design and optimize the synthetic process and seek out optimal reaction conditions [12-13].

MATERIALS AND METHODS


**Experimental Method.** Take 50 mL LA solution (mass fraction of 85%); add a% stannous octoate as the catalyst. PLEG is synthesized through the abovementioned polymerization process, namely pre-polymerization of raw material, addition of a modifier in middle stage and stage-based temperature control. In the experiment, the stannous octoate addition (a), the temperature for LA pre-polymerization (b), the temperature for PEG-LA low-temperature polymerization (c), the duration of PEG-LA low-temperature polymerization (d), the temperature for PEG-LA high-temperature polymerization (e) and the duration of PEG-LA high-temperature polymerization (f) affect copolymerization. A total of 5 levels (5 values) are selected for each of the above factors. The levels of
### TABLE 1
Levels of the Experimental Factors

<table>
<thead>
<tr>
<th>Factor</th>
<th>Level 1</th>
<th>Level 2</th>
<th>Level 3</th>
<th>Level 4</th>
<th>Level 5</th>
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<td>a (Stannous octoate addition /%)</td>
<td>0.5</td>
<td>0.8</td>
<td>1.0</td>
<td>1.5</td>
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<tr>
<td>b (Temperature for LA pre-polymerization /°C)</td>
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<td>100</td>
<td>110</td>
<td>120</td>
<td>130</td>
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<tr>
<td>c (Temperature for PEG-LA low-temperature polymerization /°C)</td>
<td>90</td>
<td>100</td>
<td>110</td>
<td>120</td>
<td>130</td>
</tr>
<tr>
<td>d (Duration of PEG-LA low-temperature polymerization /h)</td>
<td>3</td>
<td>4</td>
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<td>7</td>
</tr>
<tr>
<td>e (Temperature for PEG-LA high-temperature polymerization /°C)</td>
<td>150</td>
<td>160</td>
<td>170</td>
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<td>190</td>
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<td>f (Duration of PEG-LA high-temperature polymerization /h)</td>
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### TABLE 2
Direct Analysis of the L_{25}^{(5^6)} Orthogonal Experiment Results

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<th>a/%</th>
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<th>c/°C</th>
<th>d/h</th>
<th>e/°C</th>
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<td>Average k4</td>
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<td>890.40</td>
<td>1889.20</td>
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### TABLE 3
Variance Analysis of the L_{25}^{(5^6)} Orthogonal Experiment Results

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<th>DOF</th>
<th>F ratio</th>
<th>F Critical Value</th>
<th>Significance</th>
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<td>a/%</td>
<td>108974385.20</td>
<td>4</td>
<td>42.354</td>
<td>6.390</td>
<td>*</td>
</tr>
<tr>
<td>b/°C</td>
<td>6660344.40</td>
<td>4</td>
<td>2.589</td>
<td>6.390</td>
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</tr>
<tr>
<td>c/°C</td>
<td>10511748.80</td>
<td>4</td>
<td>4.085</td>
<td>6.390</td>
<td></td>
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<tr>
<td>d/h</td>
<td>2572952.00</td>
<td>4</td>
<td>1.000</td>
<td>6.390</td>
<td></td>
</tr>
<tr>
<td>e/°C</td>
<td>14214480.00</td>
<td>4</td>
<td>5.525</td>
<td>6.390</td>
<td></td>
</tr>
<tr>
<td>f/h</td>
<td>19571115.867</td>
<td>4</td>
<td>7.606</td>
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<td>Error</td>
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<td></td>
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</table>

The experimental factors [14-15] are shown in Table 1.

Analysis of Experiment Result. According to Table 1, an orthogonal table L_{25}^{(5^6)} is used to design the experiments and to determine viscosity average molecular weights of the reaction products. The direct analysis of the results is shown in Table 2. The duration of PEG-LA low-temperature polymerization (d) is taken as the error term, and the variance analysis with a=0.05 is shown in Table 3.
The R values in Table 2 are ranges of the experimental factors, and reflect variations of product experimental indexes under different factor levels. The larger the R value is, the index is more significantly affected by the factor, and the factor plays a more important role in the experimental process [16-17]. It can be viewed from Table 2 that, regarding to the influences on PLEG viscosity average molecular weight, the factors are ranked from the most to the least significant influence as the stannous octoate addition (a), the duration of PEG-LA high-temperature polymerization (c), the temperature for PEG-LA low-temperature polymerization (f), the temperature for PEG-LA high-temperature polymerization (c), the temperature for PEG-LA low-temperature polymerization (d), and the duration of PEG-LA pre-polymerization (b), and the duration of PEG-LA low-temperature polymerization (d). According to the variance analysis presented in Table 3, the stannous octoate addition (a) and the duration of PEG-LA high-temperature polymerization (f) both pose significant influences on PLEG viscosity average molecular weight. It is indicated by k value analysis in Table 2 that the optimal combination condition is \(a_2b_4c_3d_2e_4f_4\).

### Analysis of Influence Trends of the Factors.

The results of the orthogonal experiment suggest the law concerning the influences of the factors on the experimental result. The following figures, Fig 1(a) ~ (f), are mapped with the five levels of the 6 factors as a horizontal axis and PLEG viscosity average molecular weight (Mv) as a vertical axis.

It can be viewed from Fig 1(a) that the stannous octoate addition affects PLEG viscosity average molecular weight significantly; with the increase of catalyzer dosage, PLEG viscosity average molecular weight increases and then decreases; when the dosage of the catalyzer is 0.8%, the molecular weight reaches a peak value. Through analyzing Fig. 1(a), when the catalyzer addition is relatively low, the catalytic effect is not obvious; with the increase of the catalyzer addition, the catalytic effect is enhanced and the PLEG viscosity average molecular weight grows constantly. The PLEG viscosity average molecular weight decreases when the addition of the catalyzer is above a certain level, since the increase of the catalyzer addition, to some extent, promotes more side reaction and some de-polymerization and goes against the forward reaction, resulting in a decrease of PLEG molecular weight [9]. During the pre-polymerization of the raw material, as shown in Fig. 1(b), the temperature for LA pre-polymerization shows no significant impact on PLEG viscosity average molecular weight.

During the stage of PEG-LA low-temperature polymerization, as indicated in Fig 1(c) and (d), the polymerization temperature poses relatively significant impact on PLEG viscosity average molecular weight, while the influence of the duration is not obvious. With the rising of the polymerization temperature, the PLEG viscosity average molecular weight presents an increasing-decreasing trend. The increase is attributed to an accelerated reaction rate under a higher temperature. While, under an even higher temperature, more adverse reactions within the system go against copolymerization [9].

For the PEG-LA high-temperature polymerization stage, as indicated in Fig. (f), the duration significantly affects PLEG viscosity average molecular weight, which increases firstly and starts to decline after reaction for 7 hours. Because a water separator is used for backflow and water removal during the stage, the reaction duration is extended, and water generated within the reaction system can be eliminated in an improved way, leading to the increase of the PLEG viscosity average molecular weight. With the extension of the reaction time, the system viscosity increases with adverse reactions (decomposition of oligomers, carbonization of the products and darkening), which prevent elimination of water and byproducts generated by the reaction system, hinder forward reaction, and result in the decline of the product viscosity average molecular weight. Viewed from Fig. 1(e), PLEG viscosity average molecular weight is significantly affected by the polymerization temperature and reaches a peak level under the temperature of 180°C, because adverse reactions increase and product degradation rate is accelerated under an excessively high temperature, leading to the decline of the PLEG viscosity average molecular weight.

### Determination of Optimal Reaction Conditions.

By integrating above experimental analysis, the experiment obtains PLEG with a relatively high molecular weight and ideal chaining, and overcomes problems encountered by existing copolymerization modification technique. Based on the k values presented in Table 2 and the influences of the factors on PLEG viscosity average molecular weight shown in Fig 1, the optimal reaction condition is \(a_2b_4c_3d_2e_4f_4\). Namely, the stannous octoate addition is 0.8%; the temperature for LA pre-polymerization is 120°C; the temperature for PEG-LA low-temperature polymerization is 110°C; the duration of PEG-LA low-temperature polymerization is 4 h; the temperature for PEG-LA high-temperature polymerization is 180°C; and the duration of PEG-LA high-temperature polymerization is 7 h.

### Analysis of Structure and Performance.

Copolymer PLEG is synthesized under the optimal condition, and PLLA homopolymer is synthesized under same condition as reference for the property test.
Influences of Different Factors on PLEG Viscosity Average Molecular Weight

Infrared Analysis. The infrared spectrograms of PLLA and PLEG are presented in Fig. 2. Based on analysis of the PLEG infrared spectrogram, there is a strong peak near 1754.9 cm⁻¹, and it is a stretching vibration peak of C=O radical group; a strong peak exists near 3484.7 cm⁻¹ and is a stretching vibration peak of O-H; two absorption peaks exist near 2996.8 cm⁻¹ and 1618 cm⁻¹, respectively corresponding to a stretching and a flexural vibration peaks of C-H in CH₃; near 2946.7 cm⁻¹ and 1382.7 cm⁻¹, there is one peak each, being the a stretching and a flexural vibration peaks of C-H in CH₂; absorption peaks exist near 1276.6 cm⁻¹, 1190 cm⁻¹ and 1091.5 cm⁻¹ and are antisymmetric and symmetric vibration peaks of C-O, suggesting containment of O-C=O radical group. Comparing with PLLA, the peak area of the absorption peak at 2946.7 cm⁻¹ and the absorption strength of the absorption peak are both larger in the PLEG spectrogram, suggesting containment of CH₂ radical group in the copolymer PLEG as well as introduction of PEG -CH₂- structure in the PLLA.

Mechanical Property and Hydrophilic Performance Analysis. According to the national standard GB1040-79, both PLEG and PLLA are processed into sample bars and tested for tensile strength and elongation at break. PLEG and PLLA are dissolved in chloroform respectively; before measuring the contact angles, the solutions are slowly dripped onto glass slides under dust-free environment and dried under 30°C for 48 h to volatize chloroform. Refer to Table 4 for relevant data.

As shown in Table 4, PLEG has a lower tensile strength and a significantly higher elongation at break than PLLA. The fragility deficiency of pure PLLA is avoided, and it is expected to be applied for improvement of polylactic acid material toughness. The test of contact angle is one of the important indicators measuring material hydrophilic performance. A small contact angle indicates strong hydrophilic performance. It is suggested by comparing the contact angles shown in Table 4 that PLEG shows enhanced hydrophilic property. The PLEG copolymer synthesized by addition of PEG improves flexibility and hydrophilic property of polylactic acid material.
CONCLUSION

Based on one-time addition of LA monomer and PEG and raised-temperature-based polymerization, polymerization process of PLEG is modified by pre-polymerization of raw materials, addition of a modifier in middle stage and stage-based temperature control to obtain PLEG with a relatively high viscosity average molecular weight.

The stannous octoate addition \((a)\), the temperature for LA pre-polymerization \((b)\), the temperature for PEG-LA low-temperature polymerization \((c)\), the duration of PEG-LA low-temperature polymerization \((d)\), the temperature for PEG-LA high-temperature polymerization \((e)\) and the duration of PEG-LA high-temperature polymerization \((f)\) are main influencing factors. With PLEG viscosity average molecular weight as the evaluation indicator, the plan of 6-factor 5-level \(L_{25}(5^6)\) is adopted to design the experiments and to optimize reaction conditions.

It is concluded that, regarding influences upon PLEG viscosity average molecular weight, the factors are ranked as \((a)\), \((f)\), \((c)\), \((e)\), \((b)\) and \((d)\) from a higher degree to a lower degree. The optimal conditions are as follows. The stannous octoate addition is 0.8%; the temperature for LA pre-polymerization is 120°C; the temperature for PEG-LA low-temperature polymerization is 110°C; the duration of PEG-LA low-temperature polymerization is 4 h; the temperature for PEG-LA high-temperature polymerization is 180°C; and the duration of PEG-LA high-temperature polymerization is 7 h.

The PLEG copolymer synthesized by addition of PEG effectively enhances the flexibility of polylactic acid material, and improves its crystallization property as well as its hydrophilic performance.

ACKNOWLEDGEMENTS

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REFERENCES


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EXPERIMENTAL STUDY ON THE CHARACTERISTICS OF HIGH SALINITY FRACTURING BACKFLOW FLUID IN SHALE WELLS

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ABSTRACT

Fracturing is one of the main measures to increase production of oil and gas Wells. Fracturing backflow fluid usually contains oil, formation water, polymer, etc. If it is not treated effectively in time, it is easy to cause greater environmental pollution. Fracturing backflow fluid usually has the characteristics of high salinity and weak alkaline pH value. Different types of fracturing fluids will form fracturing backflow fluids with different properties. Therefore, it is necessary to analyze the characteristics of fracturing backflow fluid to provide references for the ground environmental protection treatment. Aiming at the characteristics of high salinity and weak alkaline pH value of fracturing backflow fluid, two shale wells were chose and carried out experimental study. The oil-water adsorption rate was tested by oil-water adsorption experiment. The composition of rock samples was tested by Energy Dispersive Spectrometer (EDS) analysis. Through Conductivity test (EC), cation exchange capacity test (CEC), ion concentration test, pH value and oxygen concentration test, the main characteristics of fracturing backflow fluid are analyzed in depth. The results show that the dissolution and leaching of minerals in clay is one of the main reasons for the high salinity of the fracturing backflow fluid. And the surface to volume ratio exhibits an important influence on the adsorption process of water and the dissolution and leaching of minerals in clay, and the larger the surface to volume ratio, the higher the ion concentration in the aqueous solution.

KEYWORDS:
Fracturing backflow fluid, high salinity, environmental protection, characteristics analysis, shale wells

INTRODUCTION

Low permeability shale gas reservoir is a hotspot of shale gas development nowadays, and hydraulic fracturing is the main method in the process of shale gas development [1-3]. At present, water-based fracturing fluid is the main widely used fluid type. The waste liquid produced after fracturing mainly comes from two aspects: one is the waste liquid produced by using active water to wash wells before and after the construction; the other is the fracturing gel fluid returned from the borehole after the fracturing construction and the remaining raw gel fluid. Different types of fracturing fluids will form different characteristics of fracturing backflow fluids [4-7]. Field fracturing fluid test results show that the salinity of backflow fluid is quite different from that of fracturing fluid. The salinity of fracturing fluid is lower and that of backflow fluid fluid is higher, and it is increasing with time. Meanwhile, the pH value of fracturing backflow fluid is usually neutral and weak alkaline. Domestic scholars often neglect this phenomenon in the process of shale fracturing research, and usually interpret the characteristics of formation water itself [8-10]. However, the cause of high salinity and neutral pH value in the backflow fluid is one of the important factors affecting the evaluation of hydraulic fracturing process. Therefore, it is of great significance to analyze the cause and characteristics of high salinity and neutral pH value in the backflow fluid for the fracturing development of shale wells. Furthermore, it is of great value to study the composition and characteristics of fracturing backflow fluid for guiding the ground environmental protection treatment [11-13].

Aiming at the problem of high salinity and weak alkaline pH value of fracturing backflow fluid compared with injected liquid, two representative shale wells (1 # and 2 #) in a shale gas field were studied by experimental analysis. Laboratory experiments on oil-water adsorption were carried out, and the sources of high salinity of fracturing back-
flow fluid were deeply studied by EDS test, EC test and CEC test combined with theoretical analysis.

MATERIALS AND METHODS

Experimental materials preparation. In order to obtain accurate experimental results as far as possible, a large number of rock samples are collected from the target wells. Meanwhile, in order to study the influence of the surface to volume ratio on oil-water adsorption, core samples with different surface areas ($A_{sp,low}$, $A_{sp,medium}$, $A_{sp,high}$) were prepared.

Mineral composition of core samples. In order to clarify the main mineral composition of the samples, XRD experiments were used. The experimental results are shown in Table 1.

<table>
<thead>
<tr>
<th>Mineral composition</th>
<th>1# (wt.%)</th>
<th>2# (wt.%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quartz</td>
<td>77</td>
<td>32</td>
</tr>
<tr>
<td>Potash feldspar</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>Plagioclase</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>Calcareous spar</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>Ferrodolomite</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Dolomite</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Troilite</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Illite/montmorillonite</td>
<td>5</td>
<td>14</td>
</tr>
<tr>
<td>Illite/mica</td>
<td>11</td>
<td>19</td>
</tr>
</tbody>
</table>

As shown in the Table 1, 1# rock samples are mainly composed of 77% quartz and 16% clay minerals, while 2# rock samples are mainly composed of 32% quartz and 33% clay minerals. Quartz content in 1# rock sample is more than twice that of 2# rock sample, while clay content in 2# rock sample is almost twice that of 1# rock sample. In addition, 2# rock sample contains 10% calcite and 5% pyrite, and the content of these components in 1# rock sample can be neglected. Clay components in rock samples can participate in ion exchange reaction during and after hydraulic fracturing. The clay components of the two shale samples are mainly composed of illite, mica and montmorillonite. The cation exchange capacity (CEC) of illite and mica ranges from 10 to 40 cmol/kg, and CEC value of montmorillonite ranges from 60 to 150 cmol/kg [14]. Therefore, it is easier for montmorillonite to participate in cation exchange reaction than illite and mica before and after the process of hydraulic fracturing.

RESULTS AND ANALYSIS

Oil-water adsorption experiment. The variation of water absorption and oil absorption with time of 1# and 2# rock samples under $A_{sp,low}$ conditions was shown in Fig. 2. It can be found from the Fig. 2 that the oil absorption rate of 1# and 2# rock samples is lower than that of water absorption. This is because capillarity is not the only factor affecting the adsorption process on rock surface. Other factors, such as permeability effect, natural cracks, cracks induced by adsorption process and clay adsorption on liquid, may also affect the absorption of liquid by rock samples. Compared with 1# rock sample, the difference of water absorption and oil absorption of 2# rock sample is greater because of the higher content of clay components in 2# rock sample.
The variation of water absorption and oil absorption of 1# and 2# rock samples with time under different \( A_{sp} \) values was shown in Fig. 3.

As shown in Fig. 3, the change of \( A_{sp} \) has a significant effect on water absorption, and the effect on oil absorption can be neglected. Higher \( A_{sp} \) value significantly increases water absorption of rock samples, which is due to the strong dependence of water absorption on water/rock interface. At the same time, the higher surface area to volume ratio also increases the possibility of contact between clay and water, which further promotes the absorption of water. Among 2# rock sample, the difference of water absorption caused by different \( A_{sp} \) values is more obvious, because the clay component in 2# rock sample is obviously higher than that in 1# rock sample.

**Conductivity test.** The conductivity test results of 1# and 2# rock samples in deionized water under different \( A_{sp} \) conditions was shown in Fig. 4.

The results show that the conductivity of aqueous solution increases with time. This is because in the process of water absorption, when water penetrates into rock, ions will diffuse into the water, and the conductivity of solution will increase with the increase of ion concentration. Meanwhile, for 1# and 2# rock samples, the conductivity is higher for the samples with higher \( A_{sp} \) value. Moreover, for 2# rock sample, the difference of conductivity between samples with different \( A_{sp} \) values is more obvious.
values is more obvious. It means that larger surface area to volume ratio promotes ion exchange reaction, that is to say, larger surface area to volume ratio makes deionized water better connection with clay, thus promoting cation leaching from clay [15-17].

Cation exchange capacity test. The cation exchange capacities of 1# and 2# rock samples were tested as shown in Fig. 5.

![Figure 5](image)

**FIGURE 5**
Results of cation exchange capacity for different rock samples

It can be found from the Fig. 5 that the CEC value of 2# rock sample is higher than that of 1# rock sample, which indicates that 2# rock sample has more exchangeable cations than 1# rock sample. According to Table 1, the clay content of 2# rock sample is almost twice that of 1# rock sample, and there are exchangeable cations between clay mineral layers. Therefore, the high clay content of 2# rock sample may be one of the reasons why its CEC value is higher than that of 1# rock sample. Meanwhile, because the CEC of 2# rock sample is higher, its sub-exchange reaction rate is faster and ion transfer rate is faster, which is consistent with the test results of Fig. 4.

Na/Cl molar ratio test. The results of Na/Cl molar ratio in the process of deionized water adsorption was shown in Fig. 6.

As shown in Fig. 6, the molar ratio of Na/Cl increases firstly and then decreases under \( A_{sp, \text{low}} \) conditions, while the molar ratio of Na/Cl decreases continuously with the increase of time under \( A_{sp, \text{medium}} \) and \( A_{sp, \text{high}} \) conditions. According to Table 1, plagioclase is the only non-clay sodium mineral in the rock samples. However, the dissolution of plagioclase in water adsorption experiments at room temperature is very slow, so the high Na/Cl molar ratio at the initial stage is due to the rapid leaching of exchangeable sodium from clay components, while the decrease of Na/Cl molar ratio at the later stage is due to the dissolution of chlorine-containing components. In addition, during the process of water adsorption, water will permeate into the pore structure of rock samples [18]. Chloride salts (such as halite and potassium chloride) which may precipitate in pore space can be dissolved in water and diffused back into solution. And the diffused chloride ions can reduce the molar ratio of Na/Cl in solution.

Measurement of Ferric and Sulfate concentration. The results of iron and sulfate concentration were shown in Fig. 7.

It can be seen from the Fig. 7 that in the water adsorption experiment, the sulfate concentration initially increased sharply, and the increasing trend slowed down with the adsorption process. Ferric ions show a trend of increasing firstly and then decreasing, which is due to the formation of sulfur-iron compounds resulting in precipitation [19-20].

pH value and Oxygen concentration test. The results of pH value and Oxygen concentration of the fracturing backflow fluid were shown in Fig. 8. From the test results, it can be found that the pH value of the backflow fluid is between 8 and 8.5, which is weak alkaline. Most scholars in China have ruled out that mineral dissolution is one of the reasons for the high salinity of the backflow fluid,
based on the fact that the pH value of the backflow fluid is non-uniform. However, the pH value between 8 and 8.5 is not enough for the dissolution of positive minerals to be negligible. In 1 # shale well and 2 # shale well, the oxidation of pyrite and the dissolution of calcite will increase the salinity of the backflow fluid, while maintaining a weak alkaline pH value. Pyrite reacts with oxygen and water contained in the injected fracturing fluid to form sulfate and acid. The presence of calcite and dolomite can dissolve with the acid produced by pyrite, which not only increases the ion content in the solution, but also increases the pH value of the solution, thus constituting the pH buffer system of calcite-dolomite [21-23].

The dissolution and leaching of minerals in clay is one of the main reasons for the high salinity of the fracturing backflow fluids. And the molar ratio of Na/Cl increases with time and then decreases. Surface area to volume ratio plays an important role in the adsorption process of water and the dissolution and leaching of minerals in clay. The larger the surface area to volume ratio, the higher the ion concentration in aqueous solution. The binary buffer system composed of calcite and dolomite can not only maintain the weak alkalinity of the backflow fluid, but also increase the salinity of the backflow fluid to a certain extent. The viewpoints obtained in this study can preferable guide the preparation of field fracturing fluid system and the ground environmental protection treatment of backflow fluids.

CONCLUSIONS

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REFERENCES


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EXPERIMENTAL STUDY ON THE AFFECTING FACTORS OF ELECTRICAL PARAMETERS FOR THE TOTAL DIAMETER CARBONATE RESERVOIRS

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2Drilling & Production Technology Research Institute, Liaohe Oilfield Company, Panjin 124010, China

ABSTRACT

In order to improve the prediction accuracy of reservoir parameter logging, carbonate rocks with different degree of pore development in a block are selected. Then the self-developed SCAR-II full diameter measuring instrument was used to measure the resistivity of cores under different water saturation confining pressure conditions. On this basis, the electrical parameters a, b, m, n of the carbonate rocks in this area are obtained by using the classical Archie formula. The changes of resistivity, cementation index m and saturation index n with porosity and confining pressure were studied. The effect of porosity and confining pressure on resistivity is also discussed. The experimental results show that the resistivity of saturated water core increases with the increase of confining pressure, the resistivity of unsaturated water core decreases first and then increases with the increase of confining pressure. The dried rock resistivity increases greatly but decreases with the increase of confining pressure. Besides, the saturation index n decreases with the increase of confining pressure, The saturation index n decreases with the increase of confining pressure, When the confining pressure is high, the variation range of m and n gradually decreases.

KEYWORDS: Full-sized carbonate reservoir, Archie formula, resistivity, water saturation, confining pressure, rock electrical parameters

INTRODUCTION

Archie's formula establishes the link between resistivity measurement and porosity measurement [1]. And the theoretical basis for quantitative evaluation of original oil (gas) saturation using electric logging data is established [2]. The petroelectric parameters are affected by many factors. At present, researches on the petroelectric parameters of carbonate rocks mainly focus on pore structure, wettability, temperature, confining pressure and formation water salinity [3]. Carbonate reservoir pores are very well developed and the pore structure is extremely complex, which makes the petroelectric parameters have very complex characters. Wang Jian [4] et al. believed that formation factor F and saturation index n were weak to temperature sensitivity and sensitive to effective stress. Tian Suyue [5] et al. found that the more complex the fracture and cave structure of fractured carbonate rock is, the smaller the saturation index n will be. Wang Li [6] et al. studied the petroelectric parameters under different solution salinity conditions and improved Archie model, but they did not consider the influence of confining pressure on the petroelectric parameters. Chen Chunyu [7] et al. analyzed the relationship between core resistivity and saturation index with different water saturation degrees and changes of confining pressures, and discussed the influence mechanism of confining pressures on core resistivity. Towle [8] obtained the value range of cementing index m of the pore reservoir through numerical simulation, and found that the cementing index m of the pore reservoir was significantly higher than that of the fractured reservoir, but this model was established on the basis of matrix porosity of 0. Wang Yong [9] et al. found that the m value increased while the n value decreased under the formation conditions, and it was necessary to classify the pore structure before obtaining the parameters m and n.

At present, researches on the electrical parameters of carbonate rocks at home and abroad mainly focus on the influence of rock wettability, temperature and formation water salinity on resistivity. Fractures and cavities in carbonate reservoirs are very well developed, and the pore structure is extremely complex, so that the resistivity has a very complex change rule. There is no consistent conclusion about the impact of extrusion stress on the resistivity, and the results are not only the same due to different core properties and test means. Due to the great difficulty in coring and the limitation of experimental equipment, almost all the researches on rock resistivity adopt the standard rock column with a diameter of 25mm, and the researches on full-size core are rarely reported [10-11]. The full-size core contains more formation information such
as holes and fractures, which can better reflect the real information of the reservoir. Therefore, it is necessary to carry out the change rule of the resistivity of the full-size core with different pore types along with the compressive stress. In this paper, full-size carbonate rocks with different degree of pore development are divided into three categories. The petroelectricity experiments under confining pressure and different water saturation are carried out to study the variation mechanism of resistivity and petroelectricity parameters.

MATERIALS AND METHODS

Full diameter core. The both ends of the core were cutted flat, and in strict accordance with the relevant requirements of engineering rock mass test method standard (GB/T 50266-99), water is used to cool the blade in the process of grinding, slicing and polishing the end face of the core column. Nine carbonate rock cores were selected, as shown in Fig. 1.

As can be seen from Fig. 1, most cores are more or less developed with various types of fractures. From the origin of the cracks, there are mainly weathering cracks, dissolution cracks and sutures. From the Angle of the crack, it includes all kinds of angles from horizontal to vertical. In terms of the degree of mineral filling, it includes various fractures such as unfilled, semi-filled and filled [12-13]. Therefore, compared with the standard rock column, the fractures in the full-diameter core are obviously more developed and have more types of fractures. Each core is distributed with various types of holes, including intergranular, karst and gravel holes, etc., and the size of the holes on the full diameter core is larger. The porosity and permeability parameters of the cores in each group are shown in Table 1. The cores are divided into three groups. The karst caves and fractures in the core of group I are well developed with large pore sizes. Groups II and III were medium and low porosity samples, respectively.

Establishment of core saturation. According to the structural characteristics of fracture-vuggy carbonate rocks, the advantages and disadvantages and adaptability of five different saturation methods, namely gas flooding method, air drying method, self-priming water increasing method, centrifugal method and semi-permeable partition method, are combined to establish the method [14]-[15]. On the premise of achieving the experimental purpose, Water saturation establishment method suitable for fractured and cavernous carbonate rocks is selected from the perspective of economy and efficiency [16-18].

<table>
<thead>
<tr>
<th>Groups</th>
<th>Core number</th>
<th>Porosity (%)</th>
<th>Permeability (mD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>L-3</td>
<td>10.69</td>
<td>2.2390</td>
</tr>
<tr>
<td></td>
<td>J-6</td>
<td>10.10</td>
<td>0.8504</td>
</tr>
<tr>
<td></td>
<td>M-1</td>
<td>9.75</td>
<td>2.0210</td>
</tr>
<tr>
<td>II</td>
<td>L-2</td>
<td>5.98</td>
<td>0.9979</td>
</tr>
<tr>
<td></td>
<td>M-2</td>
<td>5.19</td>
<td>0.3401</td>
</tr>
<tr>
<td></td>
<td>S-1</td>
<td>3.95</td>
<td>0.1114</td>
</tr>
<tr>
<td>III</td>
<td>S-3</td>
<td>2.83</td>
<td>0.0322</td>
</tr>
<tr>
<td></td>
<td>J-4</td>
<td>2.62</td>
<td>0.0530</td>
</tr>
<tr>
<td></td>
<td>J-3</td>
<td>2.57</td>
<td>0.0082</td>
</tr>
</tbody>
</table>

FIGURE 1
The total diameter carbonate rocks

TABLE 1
The permeability and porosity of rocks
Firstly, the matrix of fractured and cavernous carbonate rocks is abnormally dense, with weak water conduction and water absorption capacity, and the size distribution range of fractures and pores is large. Its pore-throat channel is much larger than that of tight sandstone, resulting in smaller capillary force and poor water absorption performance of capillary. Due to the large diameter of the full-diameter core, the water can only be absorbed at the surface when adopting the self-suction water-increasing method. The water absorption in the core is very small and cannot be fully saturated. Thus, the water saturation is at a very low level and cannot reach a high water saturation. Therefore, the self-priming method is not suitable for the fractured and cavernous carbonate rocks.

Secondly, fractures in fractured and cavernous carbonate rocks are well developed, and all kinds of fractures in the core are interwoven together. In places where fractures are developed, the degree of cementation is often weak, which makes the core easily broken and dropped when subjected to force or vibration during the experiment, and it is difficult to ensure the integrity of the core. If the block drops, the subsequent calculation of water saturation and the accurate measurement of resistivity will have a great impact. However, in the process of establishing water saturation by centrifugal method, high speed rotary dehydration is required, which is easy to shake and damage the core. Therefore, the centrifugal method is not applicable to fracture-vuggy carbonate rocks.

Thirdly, the permeability of the fractured cavernous carbonate rocks is generally low, and the gas flooding effect is poor. It usually takes a long time to get the required water saturation. For some cores with very low permeability, it is even difficult for gas to penetrate the core. However, the core with high permeability is generally developed with fractures through the core. In such a core, although the gas can pass directly through the core along the fracture during gas flooding, it can only displace the water in the fractures. However, the water in the pores far away from the fractures cannot be effectively displaced, resulting in poor displacement effect, so gas flooding method is not suitable for fractured and cavernous carbonate rocks. Although the semi-permeable partition method is effective, due to the wide distribution of porosity and permeability in fractured and cavernous carbonate rocks, a large number of separators with different properties are required and the cost is relatively high, so it is not adopted.

The air drying method can avoid problems such as difficulty in water absorption, core dropping and slow desaturation. Therefore, in this paper, air drying method is selected to establish water saturation for the fractured cavernous carbonate rocks [19-22]. According to the experimental purpose and the nature of original formation water, the simulated formation water of corresponding strata was prepared in the laboratory. In order to make the core fully saturated with formation water, the core was placed in the formation water container for 24h after drying and draining. Then 100%, 60%, 50%, 20% and 10% water saturation were obtained by natural drying method, and the test confining pressures were 0, 10, 20, 40, 50 and 60MPa, respectively. The resistivity of different cores was obtained at different water saturation and confining pressure.

**Laboratory equipment.** The multi-parameter measuring instrument for full-diameter core was shown in FIG. 2, which can comprehensively measure gas porosity, gas permeability, crosswise wave time difference and core resistivity of full-diameter core.
The equipment is also equipped with SCAR-II monitoring software system, which can directly monitor each sensor and automatic control valve of the instrument through the software interface, as shown in Fig. 2.

RESULTS AND ANALYSIS

Affecting factors of resistivity. There are a large number of fractures and karst caves of different sizes developed in the core and surface of fractured and cavernous carbonate rocks. Under a certain confining pressure, these fractures and karst caves will deform, so that the distribution of simulated formation water in the core is different in the petroelectric experiment, resulting in the difference in core resistivity test results. At the same time, due to the differences in the degree of deformation and water saturation of fractures and karst caves of different sizes, the distribution of formation water in the core under different confining pressures and water saturation is also different, and shows complex rules.

The variation law of core L-2 and core J-4 resistivity with confining pressure were shown in Fig. 3 and Fig. 4 respectively. The experimental results show that the core resistivity increases linearly with the confining pressure when the water saturation is 100%. When core pores and fractures are fully filled with formation water, with the increase of confining pressure, core fracture closure, pore volume reduction, current path blocking or conductive area reduction. Therefore, the core resistivity increases with the increase of confining pressure. When the core is not fully saturated with formation water, the resistivity decreases rapidly with the increase of confining pressure. Then it increases slowly, and the cores of different pore types show the same change. The author believes that when the confining pressure is 0MPa, the formation water is in a discontinuous state due to low content and water phase trap, etc. A smaller compressive stress can overcome the surface tension between the fluid and the solid, causing the formation water to flow.
and stay connected. Therefore, core resistivity decreases rapidly after confining pressure is applied. When the confining pressure increases to a certain value, the mechanism is the same as that when the water content is 100%, and the current flow area decreases as a result of fracture closure and hole volume reduction. So the resistivity increases gradually.

Due to the strong compressibility of cores, even if the confining pressure continues to increase, the core pore volume changes very little and the current flow area changes very little. So the resistivity increases slowly. During the experiment, it was also found that the resistivity under the same confining pressure was measured after cyclic loading. The variation law is complex and some core fluctuation ranges are large. The reason may be that the distribution of formation water in the core changes, resulting in a complex law of resistivity. The core resistivity varies greatly due to the difference in the distribution of fluid and pore, even though its water saturation is the same. The variation of core resistivity with porosity under different confining pressures is shown in Fig. 5 and Fig. 6.

Under different water saturation and confining pressure conditions, core resistivity changes remain consistent. The porosity decreases with increasing porosity. This is because when the core contains a conductive fluid, the greater the porosity, the larger the core conductive area, and therefore the lower the resistivity.

According to the resistivity of full-diameter carbonate rocks saturated with water in groups I, II and III, the formation factors for the three groups of cores are obtained. The changes of formation factors of carbonate reservoir selected in the experiment with porosity and confining pressure are shown in Fig. 7 and Fig. 8.

As can be seen from Fig. 7, under different confining pressures, formation factors all decrease with the increase of core porosity. When the core is saturated with formation water, holes and fractures will be filled with formation water with low resistivity. Moreover, the resistance of formation water is far less than that of core skeleton, and formation water is the main channel of current. Therefore, the
The stratigraphic factors and porosity scatter plots under different confining pressures

![FIGURE 7](image)

The changing rule of formation factors of different porosity with confining pressure

![FIGURE 8](image)

TABLE 2

The table of a and m under different confining pressure

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Confining Pressure</th>
<th>0MPa</th>
<th>10MPa</th>
<th>20MPa</th>
<th>40MPa</th>
<th>50MPa</th>
<th>60MPa</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td></td>
<td>3.6555</td>
<td>3.2610</td>
<td>3.4383</td>
<td>3.5765</td>
<td>3.6022</td>
<td>3.5910</td>
</tr>
<tr>
<td>m</td>
<td></td>
<td>1.8410</td>
<td>1.8610</td>
<td>1.8870</td>
<td>1.9660</td>
<td>2.0080</td>
<td>2.0550</td>
</tr>
<tr>
<td>R²</td>
<td></td>
<td>0.8009</td>
<td>0.8272</td>
<td>0.8281</td>
<td>0.8358</td>
<td>0.8381</td>
<td>0.8422</td>
</tr>
</tbody>
</table>

more developed the core is, the larger the current channel, i.e. the conductive cross-sectional area, will make \( R_0 \) decrease. Since \( F = \frac{R_0}{R_w} \) is defined as the formation factor, the \( R_w \) does not change, so the formation factor \( F \) increases.

As can be seen from Fig. 8, With the increase of confining pressure, formation factors increase. With the increase of confining pressure, pore volume is compressed and some pore channels are cut off, effective conductive cross-sectional area is reduced, and core formation factors are gradually increased. With the change of confining pressure, the formation factor of core J-4 (phi =2.62%) increases greatly, \( F \) increases from 1000 to 4000, and the formation factor of core L-3 (phi =10.69%) and M-2 (phi =5.19%) changes little with the change of confining pressure, \( F \) increases from 500 to 900 and 1100 to 1800, respectively. Within the confining pressure range of 0~60MPa, the change rate of formation factor of group I sample is only 21%, and that of group III sample is as high as 71.7%. Because the full-diameter core hole is large, the increase of formation factors does not slow down with the continuous increase of confining pressure.
This phenomenon is different from the rock electricity experiment of standard core, and the core stratum factor with smaller porosity is more sensitive to the change of confining pressure.

**Analysis of variation law of electrical parameters.** In order to study the difference of rock electrical characteristic parameters under different confining pressures. Based on Archie theory, the lithology coefficient formation a and the cementation index m of each core were obtained by statistical regression with respect to the saturated water core formation factor F and porosity of different confining pressures under logarithmic coordinates, and the correlation coefficient $R^2$ of a, m and $f\cdot\phi$ were calculated. The results are shown in Table 2.

According to the experimental results, it can be found that the lithology coefficient a basically remains unchanged with the increase of confining pressure, and the cementing index m gradually increases with the increase of confining pressure. The variation rule of lithology coefficient a is consistent with the previous conclusions, which is determined by lithology and close to a certain value. According to the test results of this experiment, if a value is 3.52, the variation law of cementing index m and confining pressure is studied. The change of cementing index m with confining pressure is shown in Fig. 9.

As can be seen from Fig. 9, the cementing index m increases with the increase of confining pressure, and basically maintains a linear relationship. Similarly, the water saturation $S_w$ of each core and the formation resistivity increase coefficient I calculated by the corresponding resistivity $R_t$ were statistically regressive according to Archie’s formula, and the corresponding saturation index n and the lithology correlation coefficient b were obtained.

The varying curves of b and n for different porosity of cores with different confining pressure were shown Fig. 10 and Fig. 11. The lithological correlation coefficient b of cores with different porosity is not consistent with the variation of confining pressure. When the porosity is small, such as the core L-3 ($\phi$=2.57%), S-3 ($\phi$=2.62%), J-4($\phi$=2.83%), J-3($\phi$=5.98%), the lithology correlation coefficient b decreases with the increase of confining pressure and tends to be constant. However, when the porosity is large, such as L-3 ($\phi$=10.69%), M-1 ($\phi$=9.75%), with the increase of confining pressure, the lithology correlation coefficient b decreases and then increases. At high confining pressure, it is the same as the core with small porosity and tends to a certain value. It can be seen from the above analysis that different distribution ranges of core porosity selected in the experiment will lead to different variation rules of lithology correlation coefficient b with confining pressure [21-24]. When the confining pressure increases to 20MPa, the lithology correlation coefficient b basically tends to remain unchanged. This parameter is similar to the lithology coefficient a and is less affected by external factors.

Fig. 10 also shows that when the confining pressure is 60MPa, the lithological correlation coefficient of the core with small porosity is higher than that of the core with small porosity. The average lithology correlation coefficient b of group I is 1.5, while the average lithology correlation coefficient of group iii is 0.8. Therefore, the value of coefficient b should be classified and processed according to the core porosity to obtain its saturation index n. Fig. 11 shows the change of saturation index n with confining pressure in core L-3 (phi $=10.69\%$), L-2 (phi $=5.98\%$) and S-3 (phi $=2.83\%$). The saturation index n of carbonate rocks with different porosity types decreases with the increase of confining pressure, and the change of n becomes smaller and smaller when the confining pressure is higher.
CONCLUSIONS

(1) It is difficult to carry out the petroelectricity experiment because of the small capillary force of carbonate rocks, low permeability or the development of fractures through the core, while natural drying method can avoid problems such as difficulty in water absorption, core dropping and slow desaturation. Therefore, air drying method is selected to establish the water saturation of carbonate reservoir.

(2) When water saturation is 100%, core resistivity increases linearly with confining pressure. When the core is not completely saturated with water, the resistivity decreases rapidly with the increase of confining pressure, and then slowly increases, and the cores with different pore types show the same change.

(3) Under different confining pressures, the formation factors decrease with the increase of core porosity and increase with the increase of confining pressure. The increase of formation factors does not slow down with the increase of confining pressure. The smaller the porosity is, the more sensitive the formation factor is to the variation of confining pressure.

(4) Lithology coefficient $a$ is basically unaffected by external factors. The mean value of 3.52 measured in this paper is larger than that of conventional test results. At 0MPa, the lithology correlation coefficient $b$ is relatively large and slightly increases with the increase of confining pressure. The cementation index $m$ increases linearly with the increase of confining pressure. The saturation index $n$ decreases with the increase of confining pressure, and the decreasing amplitude decreases to a certain value.
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REFERENCES


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APPLICATION OF WELL TEST CONSTRAINED BY FULL HISTORY PRESSURE IN COMPLEX CARBONATE RESERVOIR

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ABSTRACT

As a special reservoir type, fractured-vuggy carbonate reservoir has strong heterogeneity, various boundary and complicated fracture and cave association, which brings great difficulty for well test interpretation using conventional workflow, of which only the build-up pressure is used. In order to improve the reliability of well test interpretation in fractured-vuggy carbonate reservoir and reduce the multiplicity of interpretation results, an advanced workflow is developed by converting limited pressure test data and the daily well head pressure to the full history flowing pressure, except the the flow pressure gradient test data and well head pressure, the daily production rate and ratio are also used to ensure the accuracy of flow pressure conversion. Finally, the advanced well test interpretation workflow with production process history constraint is established to realize improvement of the dynamic prediction. The advanced workflow of this paper can greatly improve the reliability of well test interpretation results for a typical carbonate clean gas well, of which the permeability, skin, gas in place and forecast production volume have difference range 11.9%~85.4% comparing the results of conventional well test method. Another two examples of carbonate condensate gas well also show good interpretation results using this advance workflow.

KEYWORDS:
Fractured-vuggy carbonate reservoir, numerical well test, full history pressure, production forecast

INTRODUCTION

The efficient development of fractured-vuggy carbonate rocks requires a high-level, multi-disciplinary, reservoir description. Gas reservoir dynamic description technology based on well test and modern production decline analysis is one of the key technologies for scientific and rational development of carbonate gas fields [1-6]. Gas reservoir dynamic description means comprehensively and accurately interpreting gas reservoir to obtain gas well and gas reservoir parameters by utilizing dynamic data such as pressure, production, fluid and so on, which is based on modern well test analysis, material balance and production performance analysis [7-12]. In recent years, the combination of short-term well test and long-term production performance has been studied at home and abroad.

Liu G. [13] studied the seepage law of carbonate reservoirs by analyzing physical model, and established an ideal reservoir seepage model to identify the spatial structure of fractured-vuggy carbonate reservoirs; Chen L. et al. [14] studied the seepage characteristics of the carbonate reservoir in Halahatang Oilfield by combining static data with production characteristics and concluded three combination models of fractures and caves, namely bead type (fracture, fracture+cave, bead), composite type and interference type.

Chen L. et al. [14-15] have studied the relationship between stratum parameters, reservoir model and production dynamic characteristics to analyze the relationship between well test and production characteristics of carbonate reservoir in Harahatang. Four reservoir models are established, namely constant volume cave model, constant volume fracture cave model, non-constant volume unproductive model and non-constant volume ready-to-use reservoir model. Liu Z. et al. [16-17] combined analytical well test and digital well test describing the distribution and development range of carbonate reservoir obtained the boundaries range of connected reservoirs, the seepage characteristics and reservoir volume in different seepage areas. About the problem of seepage characteristics of carbonate reservoir after acid fracture, Chen L. et al. [18] studied acid fracture curve, fracture net pressure fitting and unstable well test curve after acid fracture by using unstable well test analysis method, and obtained different types of reservoir seepage characteristics, namely radial recombination, bead and crack injuries. Zhang H. et al. [19-20] discussed the complexity of well test interpretation in fractured-vuggy carbonate reservoirs,
the limitations of conventional well test interpretation and numerical well test techniques. In addition, seepage characteristics of reservoirs in different area and reservoir boundary of carbonate reservoirs in Harahatang were studied. As a result, large well-controlled area and small well-controlled area in multifractured cave body were summarized, and corresponding stimulation measures were put forward. Wang H. et al. [21] discussed the limitations of numerical well test interpretation of iso-thickness mode. By combining qualitative model with numerical well test technology, they studied the numerical well test interpretation of non-iso-thickness and variable porosity geological model. This research proves that the numerical well test reservoir thickness model can describe the real situation of complex carbonate reservoir more effectively, obtain more reasonable interpretation results, and solve the well test interpretation with the influence of adjacent wells [21].

However, there is little paper about dynamic description of similar fractured-vuggy carbonate gas reservoirs. Especially some wells show the characteristics of compound model with supplement in production performance. After a period of production, the outer zone begins to replenish energy to the inner zone [22-26]. On the other hand, "diversity, multi-solution and complexity" often occur in well test interpretation. Therefore, it is necessary to develop more robust workflow of well test to improve interpretation results for reservoir characterization and dynamic performance analysis. This paper demonstrates that using an advanced workflow of well test, which is constrained of whole history pressure data, can greatly maximize the data utilization, reduce the multi-solution of well test interpretation, and more accurately estimate the dynamic reserves and recovery. The improvement of this well test interpretation workflow or technology can provide guidance for further well test study of complex fractured-vuggy carbonate gas reservoirs.

In this paper, we firstly introduce the basic concepts of conventional well test, and then the advanced workflows or the technology of analytical and numerical well test constrained by full history pressure was demonstrated respectively. Finally, the applications of the proposed workflows or technology on 3 typical wells, of which two are condensate gas well and one is clean gas fractured horizontal well, are studied and discussed.

**MATERIALS AND METHODS**

**Well test analysis model and interpretation chart.** Unsteady well testing is a widely used method in the exploration and development of oil and gas fields. The process is to change the working system of oil, gas and water wells, such as opening a well from shut-in state or closing a production well instantaneously, to cause pressure redistribution in formation, and to measure the change of bottom-hole pressure with time [8-10]. According to this change, the characteristic parameters of test wells and test layers in the range of pressure influence are studied combined with the production and oil and gas properties of the well. These parameters mainly include formation permeability $K$, flow coefficient $K\omega/\mu$, formation pressure $P_r$, skin factor $S$ after completion, and internal and external boundary characteristics. Well test analysis model refers to the reappearance of oil and gas seepage process in actual formation by physical or mathematical methods.

When the well test analysis model is used to interpret the actual formation parameters, that is, to solve the inverse problem, the typical analysis model which is drawn as a log-log graph is called the well test interpretation chart. Different strata have different maps.

**Dimensionless quantity and pressure derivatives in well test interpretation charts.** In the previous version, the vertical and horizontal coordinates are dimensionless, also known as dimensionless [7, 12-15]. General physical quantities have dimensions. For example, the length dimension expressed by $m$ is $[L]$, the area dimension expressed by $m^2$ is $[L^2]$, the gas production dimension expressed by $m^3/d$ is $[L^3/t]$. However, there are also some dimensionless quantities, such as gas saturation $S_g$, porosity $\phi$, skin factor $S$. For the convenience of operation, dimensionless quantities are often used. For example, time $t$ is denoted as $t_0$ after dimensionless, and its expression is as follows:

$$t_D = \frac{3 \cdot 6 \times 10^{-3} K}{\phi \mu C_t R_w} \cdot t$$

(1)

Where, it includes not only time $t$, but also permeability $K$, porosity $\phi$, viscosity $\mu$, compression coefficient $C_t$ and bottom-hole radius $R_w$. If the unit of time is $h$ (hour), then the units of $K$, $\phi$, $\mu$, $C_t$ and $R_w$ in the formula are exactly $h^{-1}$ after operation. In this way, $t_D$ becomes a dimensionless quantity.

The method of defining dimensionless quantities of physical quantities is not unique. People often define the same dimensionless quantity with different formulas according to different needs. It should be emphasized that the definition of pressure derivative in well test analysis is that pressure differentiates logarithmic time. The characteristic of pressure derivative is very important for well test analysis. Its expression is:

$$\Delta p' = \frac{d\Delta p}{d \ln t} = \frac{d\Delta p}{dt} \cdot t$$

(2)

Further conversion yields the following formulas:

$$p_D' = \frac{dp_D}{d \ln t_D} = \frac{dp_D}{dt_D} \cdot t_D$$

(3)
Analytical well test analysis constrained by full history pressure. Firstly, the short-term build-up pressure data and long-term production data are sorted out, and the whole pressure history is converted with the measured flow pressure gradient as the constraint. The short-term and long-term pressure historical data are obtained by screening, sorting and splicing. The production rate data of the main phase can also be prepared using similar way. Secondly, the theoretical dynamic model is determined by applying the data mentioned to well test interpretation and verifying the interpretation results by fitting the whole pressure history. Finally, the dynamic model is applied to predict and analyze the production performance.

Numerical well test analysis constrained by full history pressure. The numerical well test is a new well test interpretation technology developed in recent years, which draws on the technology of reservoir numerical simulation in describing complex reservoir attributes.

Describing the change of fluid properties, reservoir thickness, the heterogeneity of permeability conditions and the special shape of reservoir outer boundary. In addition, the pressure data recorded by high-precision pressure gauge are adopted as the reference of model fitting test.

The essence of numerical well test technology is the fine numerical simulation of a well group or flow unit. Compared with analytical well test technology, it has the characteristics of fewer assumptions, wider description range and considering the influence of adjacent wells. Therefore, it accords more with the actual development of oil and gas reservoirs and seepage flow characteristics. It can better solve the problems of well test data interpretation that cannot be solved by analytic well test such as multiphase flow, multi-well interference, complex boundary and plane heterogeneity, and can determine the stratum pressure, reservoir parameters and saturation distribution under multi-well system.

Firstly, the short-term build-up pressure data and long-term production data are sorted out, and the whole pressure history is converted based on the measured flow pressure gradient. The short-term and long-term pressure historical data are obtained by screening, sorting and splicing, and the production data of all phase, including oil, gas and water, are also sorted out; Secondly, the above data are used to analyze well test interpretation, and the theoretical dynamic model is determined by fitting the whole pressure history to verify the interpretation results; Thirdly, on the basis of geological knowledge, structural analysis, logging interpretation, drilling data, combined with analytic well test interpretation results and seismic carving attributes, a multi-zone composite numerical model is established, which is applied to well test curve fitting and pressure history fitting analysis, and a relatively reasonable numerical dynamic model is obtained. Finally, the dynamic model is applied to predict and analyze the production dynamics.

RESULTS

Example 1: Comparing the results between conventional and advanced workflow. Well PG13 is a fractured horizontal clean gas well with 13 stages, which with carbonate reservoir depth of 2850 m, an average porosity of 3.3%, and an average net pay thickness of 6.3 m. Although only 3 flow pressure gradient tests are used as constraints, the calculated flowing pressure of total life period is considered accurately.

As the low quality build-up pressure data in the early time, the derivate plot is abnormal and it’s difficult to get skin value. Meanwhile, there is no boundary response on the pressure data as the low permeability, which is difficult to estimate the gas in place and recovery. Two interpretation analyses are conducted to compare the different results of the advanced workflow of this work and conventional one, and the Log-Log plots are shown in Fig. 1. The pink lines are the model results only using the build-up pressure and the black lines are the model results constrained by full history pressure data. Both of the analyses are acceptable in the Log-Log plots, but the pressure math results show that the black one is more reasonable as the good match of full history pressure, shown in Fig. 2. Finally, the Fig. 3 shows the forecast productions are calculated based on the interpreted parameters, such as $K_h$, $S$ and GIIP, and these input parameters, listed in Table 1. The comparison of Table 1 demonstrates that the conventional one may get unreal formation parameters and overestimate the GIIP and the production volume subsequently. It’s observed that the advanced workflow using full history pressure constraints can dramatically reduce the uncertainty of the results and get more reasonable forecast production.

Example 2: Analytical well test constrained by full history pressure. Well ZG43 is a vertical gas well with reservoir depth of 5157.09 m, an average porosity of 3%, and an average net pay thickness of 11.09 m. 17 flow pressure gradient tests are carried out, and the whole flow pressure history is calculated under the constraint of flow pressure gradient, as shown in Fig. 4. Pressure build-up test time splices the short-term pressure recovery data with the whole process pressure history after conversion to the uniform altitude depth, the whole process pressure history and flow history are established, shown in Fig. 5.

The conventional well test interpretation is carried out by using the vertical well + radial compound + closed boundary model, and then the whole pressure history is match for verification. The double
logarithmic (Log-Log) plot is shown in Fig. 6. The full pressure history match curve in shown in Fig. 7, in which the red line is the model calculated pressure value. Results show that formation transmissibility $Kh$ is 1030 mD·m, permeability $K$ is 39.5 mD, skin factor $S$ is 5.26, composite radius is 421 m, fluidity ratio $M$ is 400, and dispersion ratio $D$ is 6.
TABLE 1
Well test interpretation results for PG13

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Conventional</th>
<th>Full history constraint</th>
<th>Difference (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model type</td>
<td>Fractured horizontal well + square boundary</td>
<td>Fractured horizontal well + square boundary</td>
<td>/</td>
</tr>
<tr>
<td>Skin, S</td>
<td>0</td>
<td>0.05</td>
<td>/</td>
</tr>
<tr>
<td>Transmissibility, Kh (m.mD)</td>
<td>0.2262</td>
<td>0.20223</td>
<td>11.9</td>
</tr>
<tr>
<td>Permeability, K (mD)</td>
<td>0.0359</td>
<td>0.0321</td>
<td>11.9</td>
</tr>
<tr>
<td>Gas in place, GIIP (MMm³)</td>
<td>191.28</td>
<td>103.17</td>
<td>182.3</td>
</tr>
<tr>
<td>Forecast Cumulative Gas, (MMm³)</td>
<td>19.6</td>
<td>15.8</td>
<td>24.1</td>
</tr>
</tbody>
</table>

FIGURE 4
Full history production and pressure curves for ZG43

FIGURE 5
Full flowing pressure history of ZG43 well
FIGURE 6
Log-Log plots of ZG43 well

FIGURE 7
Full history pressure match plots for ZG43 well

FIGURE 8
Full history production and pressure curves of ZG14-1 well
Taking well ZG14-1 as an example, the Ordovician carbonate reservoir is drilled. The reservoir depth was 6215.7 m, with temperature 139.8°C, initial gas production being 10.5×10^4 m^3, daily oil production being 36.5 t. This well has no water production and average gas oil ratio is 2900 m^3/t. 15 flow pressure gradient tests have been carried out in this well. The pressure history of the whole process is calculated by RTA software and converted by the measured flow pressure gradient constraint, shown in Fig. 8.

Firstly, the conventional analytical well test interpretation is carried out, and the radial composite model + variable well storage + vertical well model are used for interpretation, well test curve analysis and pressure history fitting. Well test curve analysis and pressure history fitting show in Fig. 9 that the stratum coefficient is 6540 mD·m, skin factor is -2, fluidity ratio is M = 100, dispersion ratio is D = 0.225, and composite radius are 356 m.

As shown in Fig. 10, the well is near to well ZG14-3H, well ZG14-H4 and well ZG14, with staggered faults. According to the structure, fault recognition and analytic well test results, a grid plane composite numerical model is constructed as shown in Fig. 11.

Through the numerical well test analysis, the double-pair curve fitting and pressure history fitting are better. As shown in Fig. 12, the analysis results show that ZG14-1 well is not connected with its adjacent well, the fault is closed, and the pressure does not spread to ZG14 well. The model can be used for pressure prediction analysis.
The decline trend of wellhead gas production is predicted by production analysis, and the decline trend of stratum pressure is predicted by substitution model. The abandoned pressure of the gas reservoir is 17.6 MPa. It is predicted that cumulative gas production is $0.2 \times 10^8$ m$^3$ and pressure drop is 4.4 MPa after 800-day continuous production, shown in Fig. 13. The recoverable reserves of the well are predicted to be $1.1 \times 10^8$ m$^3$ and recovery rate is 62.9%.

CONCLUSIONS

(1) The advanced workflow of whole life cycle well test interpretation is based on the combination of short-term pressure data and long-term production performance data. Due to the "diversity, multi-solution and complexity" in well test interpretation, the well test analysis technology based on full history matching can greatly improve the utilization rate of data and reduce the multi-solution of well test interpretation results.

(2) Conventional well test workflow only using build-up pressure and ignoring full history production process can lead error interpretation results, such as getting unreal formation parameters and overestimating the GIIP and the production volume subsequently. It’s strongly suggested that the full history pressure should be used as constraint when conducting well test.

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REFERENCES


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APPLICATION OF IMPROVED NEWBERRY MODEL IN GEOSTRESS PREDICTION OF COAL MEASURE TIGHT SANDSTONE FORMATION

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ABSTRACT

Current geostress evaluation using logging data can provide a basis for fracturing stimulation and optimization of horizontal well trajectories in tight sandstone reservoirs. In this paper, taking the upper Paleozoic coal measure tight sandstone reservoirs in the Ordos Basin of China as an example, the rock mechanics properties and geostress of tight sandstones were comprehensively evaluated. Firstly, the dynamic and static mechanical parameter conversion relationship was obtained according to the rock mechanics experiments. Then, the geostress was calculated by the hydraulic fracturing method. The calculation results showed that \( \sigma_h \) is mainly distributed in 24-70 MPa; \( \sigma_t \) is mainly distributed in 22-43 MPa; and \( \sigma_v \) is mainly distributed in 34-55 MPa. The variation of the geostress in the longitudinal direction is very large, which is affected by the strong anisotropy of the formation. Geostress state of the target layer satisfies \( \sigma_h < \sigma_H < \sigma_v \), however, some local geomechanical layers exhibit a high tectonic stress environment. Geostress obtained by the Newberry model can be introduced by introducing the correction factor \( C^* \) into the Newberry model. At the same time, considering the anisotropy of the geostress, a non-equilibrium structural factor \( U_b \) was introduced, and the maximum horizontal principal stress \( \sigma_H \) was predicted. The prediction results of the geostress have a well agreement with the measured results, which proves that the method is effective in predicting the geostress of strong anisotropic formation.

KEYWORDS:
Tight sandstone formation, geostress, rock mechanics, newberry model

INTRODUCTION

"Coal measure" generally refers to coal-bearing strata, and coal measure tight sandstone reservoirs are an important oil and gas exploration and development field in the world [1-2]. The Carboniferous-Permian in China generally develops thick coal measure strata, which has a very large potential for exploration of tight sandstone gas. Coal measure tight sandstone reservoirs have strong heterogeneity and anisotropy, and their effective development generally requires the use of hydraulic fracturing and horizontal well development [3-6]. Current geostress evaluation can provide a basis for fracturing design and horizontal well network optimization [7-10].

The occurrence and activity of reservoir fluids in the underground are affected by the current geostress and pore pressure of the formation [10-12]. The current geostress can be determined by experimental test methods, log interpretation and finite element simulation [11-14]. The prediction results of the geostress obtained by logging and simulation methods still need to be corrected by the measured geostress results. At present, only the differential strain test and the hydraulic fracturing method can accurately determine the current geostress value of the formation [15-17]. While the geostress determined by other methods, such as acoustic emission and imaging logging, can only be used as a reference. The experimental test can only determine the geostress of certain intervals in the formation, while the logging evaluation method has lower cost and can establish continuous longitudinal ground stress distribution results.

At present, the major current geostress evaluation models in the world generally include three categories: Mohr-Coulomb criterion model, uniaxial strain model and stratigraphic anisotropy model [14-17]. For strong heterogeneous coal measure strata, the stratigraphic anisotropy model is more suitable. Because this type of model better considers the variation of geostress in all directions.

There are multiple tight sandstone gas layers in the Upper Paleozoic in the Block X of the Ordos Basin. For the same gas layer, the effect of reservoir modification is quite different, and the research on the reformability of tight sandstone reservoir has not been conducted at present. Therefore, the current geostress evaluation of the Upper Paleozoic gas reservoirs in this area can provide some refer-
ences for proposing reasonable development plans for the study anisotropic sandstone. In this paper, taking the upper Paleozoic coal measure tight sandstone reservoirs in the Block X, Ordos Basin, China as an example, the rock mechanics properties and geostress of the tight sandstones in the target layer were comprehensively evaluated. A current geostress prediction method which is suited for coal measure tight sandstone formation was proposed. The method in this research can provide reference for similar studies.

MATERIALS AND METHODS

Rock mechanics experiments. The study area is located in the Block X of the Ordos Basin. Its tectonic position is located between the Yishan Slope and the Jinxi Flexing Belt in the Ordos Basin. The terrain is high in the east and low in the west, high in the north and low in the south. There are many sets of tight sandstone gas reservoirs in the Upper Paleozoic of this area, and the gas-bearing strata are mainly distributed in the wide depth range of 1,200 m-2,200 m.

The rock mechanics parameter experimental instrument adopts the MTS petrophysical test system. The complete set consists of a high temperature and high pressure triaxial chamber, a confining pressure system, an axial compression system, an ultrasonic transducer, and an ultrasonic pulse emission-receiving control box. The maximum confining pressure of the triaxial chamber is 200 MPa, which can accommodate a rock sample with a diameter of 50 mm. The internal design of the autoclave has a compensating function for the confining pressure during the loading process, which can offset the plunger topping force generated by the confining pressure. Therefore, for the triaxial experiments, the longitudinal pressure exerted by the press on the rock sample is equal to the differential stress of the rock sample. The confining pressure and axial pressure of the triaxial chamber are all pressurized by the electro-hydraulic servo control system. The axial, transverse and axial loads of the rock samples during the test were measured by sensors mounted in the autoclave. All data signals are ultimately transmitted to the TESTSTAR automatic acquisition control system.

The instrument’s pressure sensor has an error of less than 1% and its displacement resolution is 0.000 1 mm. 15 groups of experimental test samples were taken from the Upper Paleozoic strata in the study area. The rock mechanics parameters tested included dynamic and static parameters. The dynamic parameters are the longitudinal wave velocity (P-wave velocity) and the shear wave velocity (S-wave velocity) of the rock samples, and the static parameters are the Young’s modulus and Poisson’s ratio of the rock samples. The test results of the dynamic and static elastic parameters of the collected rock samples are shown in Table 1.

Hydraulic fracturing. In sedimentary basins, rocks are affected by both skeletal stress (σ) and formation pressure (Pf), and the resultant force is the effective stress (σe) [18-20]. For medium and strongly consolidated rock, the pore fluid does not actually bear the full formation pressure, considering the strong compaction and cementation of the interior of the particle supported rock [21]. Therefore, Biot proposed the theory of effective stress [10]. The tight sandstone of the Upper Paleozoic coal measure in the study area has strong compaction characteristics, and the β (effective stress coefficient) value is closely related to the porosity (φ). The β value can be quantitatively characterized by the Eq. (1) [13]. The Upper Paleozoic gas reservoir in the study area is basically in a normal pressure state with a pressure coefficient of 1.

\[
\beta = 1 - (1 - \phi)^{0.8}
\] (1)

<table>
<thead>
<tr>
<th>Well name</th>
<th>Depth (m)</th>
<th>Density (g·cm⁻³)</th>
<th>Young’s modulus (GPa)</th>
<th>Poisson’s ratio</th>
<th>P-wave velocity (m·s⁻¹)</th>
<th>S-wave velocity (m·s⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
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<td>2.49</td>
<td>28.45</td>
<td>0.279</td>
<td>4,039</td>
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</tr>
<tr>
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<td>3,982</td>
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</tr>
<tr>
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<td>26.17</td>
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</tr>
<tr>
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<td>4,543</td>
<td>2,651</td>
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<td>13.84</td>
<td>0.162</td>
<td>4,173</td>
<td>2,273</td>
</tr>
</tbody>
</table>
Small-scale hydraulic fracturing tests can accurately capture the current geostress of the fractured formation [16-17]. During the complete fracturing process, the fracturing curve clearly records information such as pressure changes in the formation. The minimum horizontal principal stress ($\sigma_h$) is the crack closure pressure ($P_c$). After the $P_c$ value is determined, the maximum horizontal principal stress ($\sigma_H$) of the formation can be obtained according to the following formula (Eq. (2)).

$$\sigma_H = 3P_c - P_f - P_p + T$$

Where $\sigma_H$ is maximum horizontal principal stress, MPa; $P_c$ is crack closure pressure, MPa; $P_f$ is fracture pressure, MPa; $P_p$ is formation pressure, MPa; $T$ is tensile strength of the rock, MPa.

RESULTS

Conversion of dynamic and static rock mechanics parameters. The Young’s modulus and Poisson’s ratio of the rock samples were calculated according to the rock mechanics test results and the following physical equations (Eqs. (3-4)).

$$E_d = \frac{3\rho_b\Delta t_s^2 - \Delta t_p^2}{\Delta t_p^2 - \frac{\Delta t_s^2}{2}}$$

$$\nu = \frac{1}{2} \left(1 - \frac{\Delta t_s^2 - 2\Delta t_p^2}{\Delta t_p^2 - \Delta t_s^2}\right)$$

Where $E_d$ is dynamic Young’s modulus, GPa; $\rho_b$ is rock density, g·cm$^{-3}$.

Magnitude of current geostress. The current geostress of the Upper Paleozoic tight sandstone reservoir in the study area was calculated by hydraulic fracturing method (Table 2). The calculation results showed that $\sigma_H$ is mainly distributed in 24-70 MPa; $\sigma_h$ is mainly distributed in 22-43 MPa; and $\sigma_v$ is mainly distributed in 34-55 MPa. It could be found that the variation of the geostress in the longitudinal direction is very large being affected by the strong anisotropy of the formation.

As the depth of burial increases, the principal stresses in all three directions gradually increase. Overall, the geostress state basically satisfies $\sigma_h < \sigma_H < \sigma_v$. However, some data show a stress state of $\sigma_h < \sigma_v < \sigma_H$, which indicates that the local formation stress environment is complex and has strong tectonic stress values [26-30]. As the depth of burial increases, the horizontal differential stress ($\sigma_H - \sigma_h$) also exhibits a tendency to increase gradually (Table 2).

Logging evaluation of current geostress. (1) Logging interpretation model. At present, the current geostress interpretation models in the world mainly include Mohr-Coulomb failure model, uniaxial strain model and anisotropic formation model [14-17]. Although the Mohr-Coulomb failure model has a certain physical basis, it assumes that the maximum in-situ shear stress of the formation is determined by the shear strength of the formation, so it does not have universal significance. This model is more suitable for soft shale formations. The uniaxial strain model assumes that only the vertical strain occurs in the sedimentary stratum, and the horizontal stress is completely induced by the vertical stress. Therefore, the principal stresses in the horizontal direction are equal. This type of model is generally only applicable to strata with weak tectonic movements, such as strata in the...
TABLE 2

<table>
<thead>
<tr>
<th>Well name</th>
<th>Fracturing depth (m)</th>
<th>(\rho_p)</th>
<th>(P_r) (MPa)</th>
<th>(P_c) (MPa)</th>
<th>(\beta) (MPa)</th>
<th>(\sigma_h) (MPa)</th>
<th>(\sigma_H) (MPa)</th>
<th>(\sigma_v) (MPa)</th>
<th>(\sigma_H-\sigma_h) (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-1</td>
<td>1.564.9</td>
<td>1.01</td>
<td>38.60</td>
<td>29.73</td>
<td>0.173</td>
<td>39.73</td>
<td>38.25</td>
<td>40.69</td>
<td>8.52</td>
</tr>
<tr>
<td>A-2</td>
<td>1.933.2</td>
<td>1.01</td>
<td>46.30</td>
<td>39.30</td>
<td>0.177</td>
<td>39.30</td>
<td>41.64</td>
<td>42.23</td>
<td>1.82</td>
</tr>
<tr>
<td>A-4</td>
<td>1.624.4</td>
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<td>23.14</td>
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<td>24.96</td>
<td>42.23</td>
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<tr>
<td>A-5</td>
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<td>38.86</td>
<td>0.177</td>
<td>38.86</td>
<td>44.44</td>
<td>43.37</td>
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<td>0.97</td>
<td>34.09</td>
<td>27.45</td>
<td>0.352</td>
<td>27.45</td>
<td>36.05</td>
<td>37.44</td>
<td>8.60</td>
</tr>
</tbody>
</table>

Notes: \(\rho_p\) is formation pressure coefficient; \(P_r\) is fracture pressure; \(P_c\) is crack closure pressure; \(\beta\) is effective stress coefficient; \(\sigma_h\) is maximum horizontal principal stress; \(\sigma_H\) is minimum horizontal principal stress; \(\sigma_v\) is vertical principal stress; \(\sigma_H-\sigma_h\) is differential stress.

Hydraulic fracturing parameter values and current geostress calculation results of the study tight sandstone reservoir.

abdomen of the basin. The anisotropic formation model well considers the non-uniformity of the horizontal geostresses, and its calculation accuracy is high. While its main problem is that it has many undetermined coefficients, and the calculation process is cumbersome.

In order to simplify the calculation process and improve the prediction accuracy, different calculation models were compared. It was found that the introduction of the correction coefficient \(C^*\) to the Newberry model can better predict the minimum horizontal principal stress \(\sigma_h\), and the expression is shown in Eq. (5).

For the maximum horizontal principal stress \(\sigma_H\), considering the anisotropy of formation stress, a non-equilibrium structural factor \(U_b\) was introduced, and the expression of \(\sigma_H\) is given by Eq. (6).

\[
\sigma_H = \sigma_{\Delta} \times U_b
\]

\[
\sigma_{\Delta} = \frac{\nu}{1-\nu} \left[ \sigma_b - \alpha P_r (1 + C^*) \right] + \frac{P_r (1 + C^*)}{C^*}
\]

(5)

(6)

In the above formula, \(U_b\) can be obtained by using double caliper data (Eq. 7).

\[
U_b = 1 + k \left[ 1 - \left( \frac{D_{\text{max}}}{D_{\text{min}}} \right)^{0.5} \right] \frac{E}{E_{\text{min}}}
\]

(7)

In the above formula, \(D_{\text{max}}\) is maximum diameter of wellbore, cm; \(D_{\text{min}}\) is minimum diameter of wellbore, cm. \(E\) is rock Young’s modulus, GPa; \(E_{\text{min}}\) is rock skeleton Young’s modulus, GPa. \(k\) is scale factor.

The vertical principal stress is determined by the gravity gradient of the overlying stratum and can be integrated by the density log (Eq. 8).

\[
\sigma_v = \int_0^H \rho(z) g dz
\]

(8)

Where \(H\) is the buried depth of the formation; \(\rho(z)\) is the density of the formation rock at the buried depth \(z\).

(2) Evaluation results. The comparison between the test and interpretation results of the geostress of the rock samples in the study area is shown in Table 3. The compliance of the predicted results is high. From the prediction results, the absolute error of \(\sigma_H\) is distributed between 0.75 MPa-10.49 MPa, and the average absolute error is 5.91 MPa; the absolute error of \(\sigma_h\) is distributed between 0.42 MPa-6.82 MPa, and the average absolute error is 3.13 MPa. Overall, the logging interpretation scheme is highly accurate and can meet the engineering needs.

In this paper, a current geostress prediction method which is suited for coal measure tight sandstone formation was proposed. The method in this research can provide reference for similar studies.
TABLE 3
True measured current geostress results and the comparison with the calculated geostress results based on the improved Newberry model.

<table>
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<tr>
<th>Well name</th>
<th>Fracturing depth (m)</th>
<th>Measured results (MPa)</th>
<th>Calculation results (MPa)</th>
<th>Absolute error (MPa)</th>
</tr>
</thead>
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<tr>
<td></td>
<td>( \sigma_h )</td>
<td>( \sigma_m )</td>
<td>( \sigma_H )</td>
<td>( \sigma_h )</td>
</tr>
<tr>
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<td>29.73</td>
<td>42.96</td>
</tr>
<tr>
<td>A-2</td>
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<td>55.66</td>
<td>39.30</td>
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</tr>
<tr>
<td>A-4</td>
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<td>24.96</td>
<td>23.14</td>
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</tr>
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<td>48.62</td>
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<tr>
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<td>30.80</td>
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</table>

CONCLUSIONS

(1) In this paper, taking the upper Paleozoic coal measure tight sandstone reservoirs in the Ordos Basin of China as an example, the rock mechanics properties and geostress of tight sandstones were comprehensively evaluated. The dynamic and static mechanical parameter conversion relationship was obtained according to the rock mechanics experiments. Meanwhile, the geostress was calculated by the hydraulic fracturing method.

(2) The calculation results showed that \( \sigma_h \) is mainly distributed in 24-70 MPa; \( \sigma_m \) is mainly distributed in 22-43 MPa; and \( \sigma_v \) is mainly distributed in 34-55 MPa. The geostress state of the target layer satisfies \( \sigma_h < \sigma_m < \sigma_v \), however, some local geomaterial layers exhibit a high tectonic stress environment.

(3) The prediction of the minimum horizontal principal stress (\( \sigma_h \)) of tight sandstone was achieved by introducing the correction factor \( C \) into the Newberry model. At the same time, considering the anisotropy of the geostress, a non-equilibrium structural factor (\( U_b \)) was introduced, and the maximum horizontal principal stress (\( \sigma_H \)) was predicted. The prediction results of the geostress have a well agreement with the measured results.

REFERENCES


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NUMERICAL SIMULATION OF DISPLACEMENT EFFICIENCY OF ECCENTRIC ANNULUS UNDER DIFFERENT WELL DEVIATION ANGLES

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ABSTRACT

In cementing engineering of petroleum industry, how to improve the displacement efficiency of cement slurry displacement drilling fluid is a prerequisite to ensure the quality of cementing. To effectively improve the displacement efficiency, we conducted numerical simulation of the displacement efficiency of eccentric annulus under different well deviation angles based on FLUENT software. This method allows for a more intuitive observation and understanding of displacement by analyzing volume of fluid (VOF). Through the research, the relationship between different displacement speed, eccentricity and positive density difference and displacement efficiency under constraints of different well deviation angles was obtained. The experimental results showed that under different well deviation angle conditions, the displacement efficiency of eccentric annulus increases first and then decreases with the increase of displacement velocity, and it has the highest efficiency under certain eccentricity. At the optimal displacement speed, the displacement efficiency has a decreasing trend with the increase of the well deviation angle, but when the eccentricity is 0.3, the displacement efficiency value is relatively high. With the increase of positive density difference and the well deviation angle, the decrease of the displacement efficiency is more obvious at large angles, and the displacement efficiency is a relatively stable high value when the positive density difference is 400-600 kg/m³.

KEYWORDS:
Drilling fluid, cement paste, displacement efficiency, density difference, well deviation angle, displacement velocity, eccentricity

INTRODUCTION

In the cementing engineering of the petroleum industry, how to improve the displacement efficiency of drilling fluid displacement using cement slurry is a prerequisite for ensuring the quality of cementing. The inner and outer well walls of an ideal wellbore environment are concentrically equidistant, and at a good injection rate, the cement slurry pushes the drilling fluid out of the wellbore [1-3]. But in fact, the effect of gravity will cause eccentricity and well angle deviation of the inner and outer well walls, which will affect the displacement efficiency in cementing.

The quality of cementing is directly related to the service life of oil and gas wells and whether it can increase production. The study of displacement efficiency is of great significance for cementing [4-7]. For vertical wells, displacement efficiency typically decreases with increasing eccentricity and well angle. However, for inclined wells, as the displacement speed of cement slurry increases, the change in displacement efficiency becomes complicated. At the same time, the density difference between the drilling fluid and the cement slurry is also an important cause of the change in displacement efficiency [8-9]. Moreover, in the actual construction process, the factors affecting the displacement efficiency may be more complicated.

Numerical simulation can save material costs and instrument losses relative to experimental methods. And the simulation results can provide a more intuitive visual effect. Therefore, a numerical simulation study of the effect of eccentricity, displacement speed and density difference on displacement efficiency under different well deviation angle conditions was performed based on FLUENT software.

MATERIALS AND METHODS

Mathematical model. The eccentric annulus considered is an axially constant eccentric form of the eccentric borehole (Fig. 1). After mathematical operations, the eccentric gap width considering the angular coordinates can be expressed as [10-12]:

\[ h = (R_2^2 - e^2 \sin \phi)^{1/2} - R_1 + e \cos \phi \]  

(1)
Where $R_1$ and $R_2$ are the outer radius of the casing and the inner radius of the wellbore, respectively; $e$ is the eccentricity of the casing; $\psi$ is the angular coordinate.

![Cross section of eccentric annulus.](image)

**FIGURE 1**

The eccentric gap width regardless of the angular coordinates can also be expressed as $[10-11]$:

$$h = e(R_1 - R_2)$$  \hspace{1cm} (2)

For the axially constant form of the eccentric wellbore, the non-Newtonian flow and the two-phase flow theory were combined to study the one-dimensional two-phase flow of the drilling fluid displacement using cement slurry $[13-14]$. Then, the limit width of the cement slurry displacement flow was obtained:

$$(R_1^2 - e^2 \sin \phi)^{1/2} - R_1 + e \cos \phi = 2(\tau_0 / \eta - \tau_0 / \eta_1) / h$$

$$((1 / \eta - 1 / \eta_1)) (\Delta P / L - \rho g)$$  \hspace{1cm} (3)

When there is no residual mud at the narrowest gap of the eccentric annulus, the suitable displacement flow pressure gradient is:

$$\Delta P / L = \rho_i g + 2(\tau_0 / \eta - \tau_0 / \eta_1) / (R_2 - R_1)(1 - e)(\eta_1 - \eta)$$  \hspace{1cm} (4)

Where $\tau_0$ and $\tau_0$ are the yield values of cement slurry and drilling fluid respectively; $\rho$ is the density of cement slurry; $\eta_1$ and $\eta$ are the plastic viscosity of cement slurry and drilling fluid respectively; $\Delta P$ is the pressure drop within length $L$.

The model used the $k$-$\varepsilon$ model. The turbulent energy $k$ and turbulent dissipation of the standard $k$-$\varepsilon$ model are transported as follows $[13, 15-16]$: 

$$\frac{\partial (\rho k)}{\partial t} + \frac{\partial (\rho u_i k)}{\partial x_i} = \frac{\partial}{\partial x_i} \left[ \left( \mu + \frac{\mu_t}{\varepsilon} \right) \frac{\partial k}{\partial x_i} \right] + G_k + G_b - \rho \varepsilon - Y_M + S_k$$  \hspace{1cm} (5)

$$\frac{\partial (\rho \varepsilon)}{\partial t} + \frac{\partial (\rho u_i \varepsilon)}{\partial x_i} = \frac{\partial}{\partial x_i} \left[ \left( \mu + \frac{\mu_t}{\varepsilon} \right) \frac{\partial \varepsilon}{\partial x_i} \right] + C_{1e} \frac{\varepsilon}{k} (G_k + C_{3e} G_b) - C_{2e} \rho \frac{e^3}{k} + S_{\varepsilon}$$  \hspace{1cm} (6)

$$G_k = \mu_t S_d^2$$  \hspace{1cm} (7)

$$G_b = \beta g_1 \frac{\mu_t}{\rho_t} \varepsilon T$$  \hspace{1cm} (8)

$$Y_M = 2 \rho e M_e^2$$  \hspace{1cm} (9)

$Y_M$ is the dissipation rate in the turbulent pulsation expansion to the global flow in the compressible flow; $S_d$ and $S_c$ are user-defined turbulent energy term and turbulent dissipation source term, respectively.

**Model parameter.** This paper establishes a three-dimensional model of the wellbore. The wellbore model is an annular three-dimensional space with an outer diameter of 121 mm, an inner diameter of 91 mm and a length of 10 m. The dimensionless eccentricity of the inner and outer cylinders under different conditions is 0, 0.1, 0.2, 0.3, 0.4 and 0.5, respectively. The parameters of this model are as follows: drilling fluid density 840 kg/m$^3$, drilling fluid viscosity 6 mPa·s, cement slurry density 1 150 kg/m$^3$, cement slurry viscosity 23 mPa·s. The grid uses hexahedral mesh, and the size of each grid is 2 mm, the total number of grids is 2.1×10$^9$.

The displacement model uses the volume of fluid (VOF) model. In terms of boundary conditions, the inlet is velocity, and the velocity uses 0.2 m/s, 0.4 m/s, 0.6 m/s, 0.8 m/s, 1.0 m/s, 1.5 m/s, and 2.0 m/s, respectively; the outlet is pressure, and the gauge pressure is zero. The wall speed is processed according to the no-slip boundary condition, and the initialization model uses the standard initialization method. The time step is affected by different displacement speeds, ranging from 0.005 s to 0.05 s, and the number of iteration steps is 2 000 steps.

**RESULTS AND ANALYSIS**

**Effect of displacement speed on displacement efficiency.** In a vertical well, no matter how severe the interface is out of balance, the displacement efficiency under high displacement speed must be higher than under low displacement speed at the equidistant length. For a more intuitive understanding of displacement efficiency, this example analyzes the displacement efficiency at a displacement rate of 2 m/s (Fig. 2). It can be clearly seen from Fig. 2 that the influence of different displacement speeds on the displacement interface when the eccentricity is 0.2 and the well deviation angle is 45°.

It can be seen from Fig. 3 that the displacement efficiency increases rapidly with the increase of the velocity in the range of 0.2 m/s to 0.6 m/s regardless of the well deviation angle. However, in the range of 0.6 m/s to 0.8 m/s, the displacement efficiency rapidly decreases as the speed increases. This is because, although the eccentric annulus has an increasing tendency with the increase of the well deviation angle, the displacement efficiency in the speed range of 0.6 m/s to 1.5 m/s is relatively high, and when the speed reaches 2 m/s, although the displacement efficiency is not high, it is very stable. The displacement before 2 m/s is laminar.
displacement, and the displacement at 2 m/s is turbulent displacement. Since the difference in flow velocity between the wide and narrow gaps in the turbulent displacement is smaller than the difference in the velocity of the laminar flow at a higher speed, thus, the flow velocity of the entire annulus is relatively uniform, and the displacement efficiency is stable.

![Image](image1)

**FIGURE 2**

Influence of different displacement speed on displacement interface
(The eccentricity is 0.2 and the well deviation angle is 45°)

![Image](image2)

**FIGURE 3**

Displacement efficiency under different well deviation angles and displacement speeds
(The eccentricity is 0.2).

![Image](image3)

**FIGURE 4**

Influence of different well deviation angles and eccentricities on the displacement interface.
Notes: e is eccentricity; DA is well deviation angle.
Influence of eccentricity on displacement efficiency. In the actual wellbore, the inner and outer walls of the wellbore cannot form equidistant concentric circles, but there is a certain degree of eccentricity, thus forming an eccentric annulus, so the displacement efficiency will be affected [17-19].

In this paper, the displacement efficiency when the eccentricity is increased from 0.1 to 0.5 is numerically simulated under different well deviation conditions. Fig. 4 shows the effect of eccentricity on the displacement interface under partial well deviation angle conditions. With the increase of the well deviation angle and eccentricity of the well, when the well deviation angle is 30°-45° and the eccentricity is 0.2-0.3, the displacement liquid level is relatively stable and the displacement efficiency is high.

As can be seen from Fig. 5, the displacement efficiency is gradually reduced as the well deviation angle of the well increases. When the well deviation angle is greater than 45°, the displacement efficiency first increases and then decreases with the increase of the eccentricity. At the same time, it can be seen from Fig. 5 that when the eccentricity is 0.3 and 0.4, the displacement efficiency does not change significantly with the increase of the well deviation angle. And the displacement efficiency is relatively high. This is because there is a wide and narrow gap in the eccentric annulus. In the wellbore, as the displacement progresses, there is a difference in the resistance between the wide and narrow gaps, resulting in a difference in axial velocity, which in turn makes the wide gap liquid surface higher than narrow. The gap forms a situation in which the liquid level is unbalanced, and the displacement efficiency is lowered. As the well deviation angle increases, the eccentricity also increases, which makes the imbalance between the wide and narrow liquid surfaces smaller. However, when the eccentricity is too large, the liquid surface will protrude in other positions of the annulus, and the displacement interface will once again lose balance.

Effect of positive density difference on displacement efficiency. Previous studies on the factors affecting displacement efficiency suggested that the density difference between cement slurry and drilling fluid also has a great impact on displacement efficiency. Especially when the density of the cement slurry is greater than the density of the drilling fluid, the cement slurry replacement interface is stable and the displacement efficiency is high [20-21]. The reason is that the buoyancy caused by the positive density difference is one of the main driving forces for cement slurry displacement.

The ideal displacement interface is the liquid level balance at the wide gap and the narrow gap without any spurt. However, under actual conditions, due to the wide and narrow gaps of the eccentricity, the velocity of the cement slurry replacing the drilling fluid is not uniform due to the effect of the annulus resistance effect and the difference in gravity caused by the density difference. Therefore, it leads to the imbalance of the displacement liquid level. At the same time, due to the problem of the well deviation angle, the force component of gravity also has a certain influence on the displacement interface of cement slurry and drilling fluid with density difference in the eccentric annulus. When the wide gap protrudes due to the density difference, the force component of gravity increases with the change of the well deviation angle, and the annulus resistance increases, so that the narrow gap accelerates the flow, and the liquid level imbalance of the wide and narrow gap is reduced. Only by designing a reasonable density difference according to different well deviation angles, we can get a relatively stable displacement interface and improve the displacement efficiency.
a) DA=30°, Δρ=400 kg/m³  b) DA=45°, Δρ=600 kg/m³  c) DA=60°, Δρ=400 kg/m³  d) DA=60°, Δρ=600 kg/m³

**FIGURE 6**
Influence of different well deviation angles and positive density differences on the displacement interface.
Notes: DA is well deviation angle.

**FIGURE 7**
Displacement efficiency under different well deviation angles and positive density differences
(The eccentricity is 0.3 and injection speed $v=0.4$ m/s).

CONCLUSIONS

1. The displacement efficiency of eccentric annulus increases first and then decreases with the increase of displacement velocity, and it has the highest efficiency under certain eccentricity.

2. At the optimal displacement speed, the displacement efficiency has a decreasing trend with the increase of the well deviation angle, but when the eccentricity is 0.3, the displacement efficiency value is relatively high.

3. With the increase of positive density difference and the well deviation angle, the decrease of the displacement efficiency is more obvious at large angles, and the displacement efficiency is a relatively stable high value when the positive density difference is 400-600 kg/m³.

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REFERENCES


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SEASONAL CHANGES OF ANTIOXIDANT ACTIVITY AND DNA DAMAGE PROTECTION POTENTIAL OF FONTINALIS ANTIPYRETICA HEDW. AND HYPNUM CUPRESSIFORME HEDW.

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ABSTRACT

In this study, antioxidant activity was evaluated by selected mosses DPPH, β-carotene /Linoleic acid test, ABTS, and CUPRAC methods. This is the first time DNA damage protection effect of seasonal differences Fontinalis antipyretica Hedw. and Hypnum cupressiforme Hedw. The amount of total phenolic and flavonoids were high in F. antipyretica methanol extracts in spring season. The amount of phenolics content and flavonoids were high in F. antipyretica methanol extracts in spring season than others. F. antipyretica methanol extracts in winter showed better DPPH free-radical scavenging assay and β-carotene /Linoleic acid assay. The highest CUPRAC and ABTS antioxidant activity was seen in winter and spring season H. cupressiforme chloroform and methanol extracts, respectively. The protection against DNA damage activity of extracts were studied by agarose gel electrophoresis method. It was observed that spring season chloroform extract of H. cupressiforme completely disintegrate DNA in 1 μM concentration.

KEYWORDS:
Fontinalis antipyretica, Hypnum cupressiforme, Phytochemical screening, DPPH, CUPRAC, ABTS, pBR322 DNA.

INTRODUCTION

Turkey due to its geological location, the Euro-Siberian, located in three different genes, including Iran-Turan and Mediterranean belt. It also has a rich flora due to its climatic condition. Our study material is consisting of mosses, liverworts and hornworts are small, low-growing plants and constitute the phylum Bryophyta, which is phylogenetically placed between vascular plants and algae. Bryophyta have more than 23,000 members all over the world, and nearly 3000 bryophytes are reported to have medicinal value and therefore, the members of this unique division in the plant kingdom are now increasingly used as new sources of pharmaceuticals. Bryophytes are being therapeutically used worldwide, especially in Indian and Chinese cultures for the treatment of hepatitis and skin disorders due to their antibiotic, anti-inflammatory, and diuretic properties [1-3]. Bryophytes biological activities can be presented as antibacterial, antifungal, cytotoxic, antitumoral, vasopressin (VP) antagonist, allergy - causing, insecticidal. Some latest results also predict the beneficial influence of bryophytes in AIDS therapy (some bibenzyls of liverworts). The secondary metabolites identified from mosses belong to terpenoids, flavonoids, and bibenzyls, but they are also rich in other compounds; fatty acids, acetophenone etc. [4]. Plants including fruits, vegetables, and medicinal herbs contain a variety of antioxidants, for example phenolic compounds, nitrogen compounds, vitamins, and terpenoids. The protective effects of antioxidants against oxidative DNA damage and cancer have been proven in many [5-6]. These studies have shown that antioxidants can reduce cancer induction or growth and that DNA damage induced by reactive oxygen species can be controlled by these phytochemicals [7]. Fernandes et al. (2011) have been suggested that the moss Sanionia uncinata has potentially promising qualities for photo protection and medical and cosmetic applications. Their results showed that the aqueous (AE) and hydro alcoholic (HE) of this moss had protective effects against plasmid DNA cleavage by ROS [8]. Ceratodon purpureus and Bryum argenteum are used to cure fungal infections of horses. Several medical uses seem promising, such as anti-leukemic properties and anticancer agents in Germany. India and nearby people of Kumaon Himalaya use Marchantia polymorpha and Marchantia palmata to cure burns, abscesses and to reduce pus formation, while pasting of Riccia spp. is applied to the ringworm disease of the skin and Plagiochasma appendiculatum for the cure of burns, boils, and blisters of skin [2]. Chemical composition, antimicrobial, antioxidant and anthocyanin activities of Cinclidotus fontinaloides (Hedw.) P. Beauv. and Palustriella commutata (Hedw.) Ochyra) were investigated by Yayintas et al. (2017b) and they have found that the
extract of these mosses has great potential to be used in medicine, cosmetics and pharmaceutical applications as well as food and agricultural use [9]. The volatile components in extracts from Thuidium tamariscinum (Hedw.) Schimp. and Platyhypnidium riparioides (Hedw.) Dixon, collected from Kazdağları (Kalkım-Yenice, Çanakkale, Turkey), were isolated by solid phase micro extraction technique and identified by mass selective detector gas chromatography (GC-MS) and antioxidant capacities of these species were determined by CERAC and CUPRAC methods and phenolic contents by Folin-Ciocalteu method [10]. These mosses shown good antioxidant capacity. Karim et al. (2014) studied phytochemical, anti-oxygen and antiproliferative properties of five different species of mosses while using total phenolic and flavonoid values with Folin-Ciocalteu and aluminum chloride methods; FRAP, ABTS, and DPPH methods were used to determine the antioxidant properties of mosses such as Pogonatum cirratum subsp. fascatum and Sphagnum cuspidatum were potential anti-cancer agents [11]. Bhattarai et al. (2009) indicated the potential of Antarctic mosses Sanionia uncinata and Polytrichastrum alpinum to be used as antioxidants the medicinal and cosmetic purpose [12]. The antioxidant property, scavenging activities and phenolic content of the aqueous extract of Brachythecium rutabulum, Calliergonella cuspidata and Hypnum mammillatum have investigate and B. rutabulum showed the higher phenolic property than other species [13]. Methanol and ethyl acetate extracts of M. polymorpha have also show the antioxidant property. Bryophyte could be the source of many antioxidants which could be used for novel drug discovery [14-18].

MATERIALS AND METHODS

Plant material and preparation of extracts. Moss samples were collected from Mount Ida (Kazdağları), Canakkale in January and May 2018. Fontinalis antipyretica Hedw. and Hypnum cupressiforme Hedw. specimens were identified by Dr. Ozlem Tonguc Yayintas and deposited in Canakkale Onsekiz Mart University, School of Applied Sciences in Turkey. Collected mosses were cleaned carefully by removing small stones, soil, dead remains, and lichens. The dried samples were converted into flour by a grinder for experimental analyses. 10 g from each powdered plant materials were extracted with 300 mL of methanol and chloroform for 12 hours using soxhlet equipment (Wisd, Wise Therm). After filtering with Whatman filter paper (#1), all extracts were concentrated by rotary evaporation to dryness in vacuum at 45 °C and stored at the -20 °C [19].

In this study, phytochemical screening, total phenol and flavonoid, DPPH, β-Carotene /Linoleic Acid, CUPRAC, ABTS and DNA protection potential were investigated in chloroform and methanol extracts of F. antipyretica and H. cupressiforme species in winter and spring seasons. Each values expressed are means ±S.D. of three parallel measurements.

Phytochemical screening. All the two extracts were subjected to various tests in order to detect the presence of different phytochemicals such as coumarins, cardiac glycosides, terpenes, phlabotannis, quinones, flavonones, anthocyanins and proteins. Phytochemical analysis was carried out for all the extracts using standard methods [20-21]. The qualitative results are indicated that (+) for the presence and (-) for the absence of phytochemicals.

Determination of total phenolic compound. The total amount of phenolic compounds of the extracts was determined by Folin-Ciocalteau reagent and according to the method developed by Slinkard and Singleton (1977) [22]. Gallic acid (GA) was used as the standard phenolic compound. The samples were recorded by spectrophotometer at 760 nm (Shimatsu) To calculate the total amount of phenolic compounds of the extracts, the following equation from the standard curve was used and the results were calculated by arranging mg gallic acid / g extract.

\[ y = 0.001x + 0.067 \]

\[ y = \text{Absorbance value}; x = \text{Gallic acid content (mg)} \]

Determination of total flavonoid compound. The total amount of flavonoid material of the extract was determined by Matejić et al. (2013) was developed according to the method [23]. Quercetin was used as a standard flavonoid compound. The absorbance value of the mixture was determined by spectrophotometer at 415 nm. The total amount of flavonoids contained in the extracts was determined using the following equation obtained from the standard curve and the results were expressed in mg quercetin / g extract.

\[ y = 0.010x + 0.104 \]

\[ y = \text{Absorbance value}; x = \text{Quercetin amount (mg)} \]

DPPH free-radical scavenging assay. The antioxidant activity of the extracts was measured by using 2,2-diphenyl-1-picrylhydrazyl (DPPH) as a free radical model [24] with a few modifications. Extracts to be tested or BHT solution to be used as standard were prepared to be 1 mg / ml and taken to test tubes in different amounts (10, 20, 40, 60, 80 μL). Then, methanol was added so that the total volume in each tube would be 2 mL 0.5 ml of a 20 mg/L DPPH solution was added to the samples and mixed vigorously. The mixture was incubated in the dark at 20°C for 30 min. The absorbance values of the samples were measured at 517 nm. Results are calculated as % reduction.
Scavenging of DPPH (\%) = \left[ \frac{(A_0 - A_1)}{A_0} \right] \times 100

where \(A_0\) is the absorbance of control sample and \(A_1\) is the absorbance of tested extract or standard. IC\(_{50}\) values for the percentage of DPPH radical scavenging were estimated.

**β-carotene/Linoleic acid test.** This method is the free radical chain reaction of linoleic acid by heat and air oxidation based on the monitoring of beta-carotene color expression by the resulting alkyl peroxides. The technique developed by Marco (1968) [25] and modified by Miller (1971) [26]. The absorbance was measured at 470 nm and compared with BHT, a synthetic antioxidant. Compared with BHT, a synthetic antioxidant.

**CUPRAC assay (Cupric ion reducing antioxidant activity method).** 1 mL 10 mM cupric chloride, 1 mL 7.5 mM neocuproine, 1 mL 1M amonium acetate buffer (pH 7) and 1 mL water were mixed. 0.1 mL bryophyte extracts were added in this mixture. The samples were incubated for half an hour at room temperature, absorbance against a reagent blank was measure at 450 nm. All procedures were repeated in triplicate. The results were expressed as means (±SD) mmol trolox per gram dry bryophytes [27].

**ABTS Method.** ABTS [2,2]-azinobis (3-ethylbenzothiazoline 6-sulfonate) assay was determined by Miller et al. (1993) [28]. This method is achieved by measuring the decrease in absorbance of the ABTS radical in solution in the presence of antioxidants. ABTS mixture is allowed to stir in the dark for 6 hours. The absorbance of the solution was measured at 734 nm. ABTS scavenging activity was calculated according to the following formula:

\[
\% \text{ Scavenging} = \left( \frac{(A_0 - A_1)}{A_0} \right) \times 100
\]

where \(A_0\) is the absorbance of control sample and \(A_1\) is the absorbance of tested extract.

**DNA damage protection potential.** DNA damage protection potential was performed using pBR322 DNA plasmid by agarose gel electrophoresis. pBR322 DNA in Tris-Cl buffer (10 mM, pH 7.2) treated with the extracts of F. antipyretica and H. cupressiforme at 37°C for 3h. To determine the mechanism of damage protection potential H\(_2\)O\(_2\) was added to mixture as an oxidizing agent. After incubation loading buffer was added and samples were electrophoresing for 1 hour at 60V on 1% agarose gel in TAE buffer (40mM Tris-acetate, 1mM EDTA at pH 8.2) according to Russo et al. 2001 [29] with some modifications. Then, the DNA bands were visualized under UV light and photographed (Quantum ST4 gel imagining system, Vilbar Lourmat).

**RESULTS AND DISCUSSION**

Oxidation is the binding of the oxygen molecule to an element and causes damage to the human body. In addition, oxidation that causes food spoilage directly affects human health due to food safety. Antioxidants have oxygen retention properties because they react with oxygen in closed systems. They also prevent the poisoning, cell deformation and cancer caused by free radicals in the human body. Antioxidants can be divided into two classes as major synthetic and natural antioxidants. During the recent years, research on natural antioxidants has gained importance and has been expanded since there has been a lot of discussion about the toxicity and side effects of synthetic antioxidants.

Bryophytes, the oldest land plants are able with various bioactive compounds such as terpenoids, phenolics, lignins, tannins, flavonoids, quinones, coumarins, alkaloids, amines, betalains, and other metabolites which are verified antioxidants. Latest studies have shown that many of these phytochemicals possess anti-inflammatory, anti-atherosclerotic, antitumor, anti-mutagenic, anti-carcinogenic, antibacterial, and antiviral activities and will reveal novel new molecules, some of which are not synthetizable by higher plants. The biosynthesis and degredation of these chemicals play important roles in the ecology and physiology of them. The phytochemistry of bryophytes has been ignored for a long time because they are small and difficult to collect in large amounts as pure samples [30-31]. Dazy et al. (2008), was used as an aquatic mosses F. antipyretica and they were observed that antioxidative enzymes superoxide dismutase (SOD), catalase, ascorbate peroxidase (APX) and glutathione peroxidase increased when exposed to F. antipyretica type chromium nitrate and potassium bicromate [32].

With this study, it is now possible to find new sources of raw materials for natural antioxidants in the food and pharmacology field, so that F. antipyretica and H. cupressiforme can be used as a source of natural antioxidants. In addition, the current study will form and shed light on new work to be done on mosses.

**Determination of total antioxidant capacity.** The total antioxidant capacity of mosses from chloroform and methanol extracts determined using DPPH, CUPRAC, and ABTS methods. The results of the analysis have been made 3 times parallel repetition and they are calculated mmo/g dry bryophytes and given in Table 1. Phytochemical screening of whole plant extract of F. antipyretica and H. cupressiforme showed the presence of various bioactive compounds like coumarins, cardiac glycosides, phlabotannins, quinones, flavonones, anthocyanins, and proteins.
As can be seen in Table 1, phytochemical data except anthocyanin and phlabotanine were observed in winter; in the spring period, these values were observed to be negligible. The presence of coumarin, cardiac glycosides, flavonoids and saponins as part of *F. antipyretica* chemical constituent is an indication that the plant has some pharmacological potential.

**Determination of total phenolic and flavonoid capacity.** Phenolic and flavonoid compounds are known to have a correlation with antioxidant activities. Compared to methanol and chloroform extracts, it is seen that the values in methanol are higher. Therefore, it was concluded that methanol is a more suitable solvent for the extraction of these species. They have a vital role in absorbing and neutralizing free radicals and decomposing peroxides [33]. The results showed that total phenol and flavonoid concentrations in aquatic bryophytes were the highest (FMS: total phenol 67.62±0.58 mg GAE/g; total flavonoid 27.36±0.43 mg QE/g), while those of epiphytic bryophytes the lowest (HCW: total phenol 21.20±0.26 mg GAE/g; total flavonoid 5.33±0.13 mg QE/g) (Figure 1-2). This situation can be explaining spring period data higher than winter period because of climatic changes and plant growth factors. Look over the plenty literature on total flavonoid concentrations in plants, the range for spermatophyte species was from around 0.095 mg/g to 25.01 mg/g and the total flavonoid content of most pteridophytes was reportedly greater than 50.0 mg/g [34]. Our results were clearly higher than the other researched plant groups. The total flavonoid contents of bryophytes were similar to those of spermatophytes, but far less than those of pteridophytes. This result may be explaining the differences in evolutionary status in these plants [35].

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Phytochemical screening of <em>F. antipyretica</em> and <em>H. cupressiforme</em>.</th>
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<td></td>
<td>FCW</td>
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<td>Coumarins</td>
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<td>Cardiac glycosides</td>
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<tr>
<td>Phlabotannins</td>
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<td>Quinones</td>
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<td>Flavonanes</td>
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<td>Anthocyanins</td>
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<td>Proteins (Biuret test)</td>
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**Figure 1**

FIGURE 2
Total flavonoid content of *F. antipyretica* and *H. cupressiforme* chloroform and methanol extracts.

FIGURE 3
DPPH free scavenging activity of *F. antipyretica* and *H. cupressiforme* chloroform extracts

DPPH free radical scavenging activity. DPPH is another radical scavenging evaluation method for bryophytes and the results were given in terms of IC$_{50}$ values which represent the amount of bioactive compound needed to reduce to 50% of original DPPH reagent. When we compared the inhibition (%) value obtained DPPH methods for two extracts; based on the IC$_{50}$ values, *F. antipyretica* methanol extract in winter and *H. cupressiforme* chloroform extract in spring 5.21 and 5.61 μg/mL, respectively, were the IC$_{50}$ values of BHT is 1.49 μg/mL. Among all the other extracts *H. cupressiforme* methanol extract in winter had the highest IC$_{50}$ value (13.66 μg/mL) which indicated that poor scavenging activity. Free radical scavenging activity increased with increasing extract concentrations (Figure 3 and 4). The free radical removal percentages of the *F. antipyretica* plant of the chloroform extract tested during winter and spring seasons were 39.06 ± 0.23 and 31.52 ± 0.09; *H. cupressiforme* was 38.56 ± 0.22 and 30.74 ± 0.11, respectively (Figure 3).
For the *F. antipyretica* species of the methanol extract, the percentages of free radical removal in the winter and spring seasons were 49.25 ± 0.27 and 32.24 ± 0.14; for the *H. cupressiforme* species it is 25.35 ± 0.19 and 25.35 ± 0.09, respectively (Figure 4). The free radical removal capacity of the BHT was 84.46± 1.27 %.

**Antioxidant capacity by CUPRAC and ABTS method.** Antioxidant capacities of the crude extracts and the fractions of the *F. antipyretica* and *H. cupressiforme* were determined according to their radical scavenging potential with CUPRAC and ABTS method.

The maximum and the minimum antioxidant capacity (52.63±4.85; 23.45±2.62) was observed by CUPRAC for the chloroform and methanol extracts of *H. cupressiforme* collected from in winter season. CUPRAC values for winter and spring respectively; BHT>HC>FC>FM>HM; BHT>HM>HC >FC>FM (Figure 5). The maximum and minimum antioxidant capacity (27.69±0.23; 12.58±0.21) was calculated by ABTS for the methanol extract of *H. cupressiforme* and *F. antipyretica* collected from in spring season. ABTS values for winter and spring respectively; BHT>HM>FC>FM>HC; BHT>HM>HC >FC>FM (Figure 6).

**FIGURE 4**
DPPH free scavenging activity of *F. antipyretica* and *H. cupressiforme* methanol extracts.

**FIGURE 5**
Reductive antioxidant capacity of Cu (II) ion of *F. antipyretica* and *H. cupressiforme* extracts.
Yayintas et al. (2017a) were studied chemical composition antioxidant activities of Oxytegus ten-uirostris, Eurhynchium striatum and Rhynchostegium murale. They reported the maximum antioxidant capacity was observed by CUPRAC for the chloroform extract of Eurhynchium striatum than other extracts and moss species [36]. Another study from Yayintas et al. (2017b) reported that total antioxidant activities of C. fontinaloides and P. commutata were determined by ABTS method, and Trolox equivalent value (TEAC) of mosses was calculated as 26±0.32 and 10±0.22 mg/g [9]. The amounts of the flavonoid compounds were calculated as mg gallic acid (mg GAE /g extract). Gallic acid equivalent value of mosses was calculated as 587±0.55 and 496±0.22 mg/g. These results are the proof that C. fontinaloides and P. commutata extract possesses potent antioxidant activity. Karim et al. (2014) reported that the aqueous, methanolic and ethanolic extract of the moss Pogonatum cirratum subsp. fuscatum possess strong ABTS radical scavenging activity [11]. Ertürk et al. (2015) were investigated eight mosses [37]. Especially, H. sericeum and E. striatulum showed the best antioxidant activity. The highest value 8.21±0.04 mg GAE/100 g sample was determined for H. sericeum. E. striatulum showed the best antioxidant activity. The screening for the antioxidant property of the aqueous extract of the three moss namely B. rutabulum, C. cuspidate and H. mammillatum in context of their ABTS cation scavenging activities and phenolic content have known to show some positive response. Out of the three extracts, B. rutabulum have shown the highest of the phenolic content which further suggested potential of this extract in search of many other novel antioxidant compounds in this moss. Apart from this methanol and ethyl acetate extract of M. polymorpha have also shown antioxidant property [3].

Climate is the most important ecological factor that determines the bryophyte types and the distribution areas of the bryophytes associations. The most common effects of the climate factors (heat, humidity, rain, and light etc.) have important role in formation of the bryophyte vegetation of a place. Depending on these factors, antioxidant amounts in our species vary both within themselves and between species.

**β-carotene / Linoleic acid method.** When β-carotene / linoleic acid test results were examined, it was determined that the seasonal factors were important on the antioxidant activities of the extracts. It was determined that the extract type and duration were important in antioxidant activity. According to the results of β-carotene / linoleic acid test, it was determined that the highest antioxidant activity was FMW (39.85±0.52%) and the lowest was HMS (7.53± 0.05%). It is concluded that there exist seasonal variations in total phenolic content, flavonoids, antioxidants in all the presently studied species of two mosses which might be due to fluctuations in temperature, precipitation, conditions of their habitat, the duration and intensity of sunshine as well as the photoperiod.

**Protection against DNA damage.** DNA protective activities of the chloroform and methanol extracts obtained from the mosses used in the study were investigated on the basis of visualization of the plasmid DNA by agarose gel electrophoresis in the presence of H2O2. One way to examine DNA breakdown is supercoiled DNA (superfold annular DNA; no fracture; Form I), open-circular (single-chain fracture-containing DNA; broken in one of the DNA chains; Form II) or linear (linear DNA; broken one or more in two chains; Form III) to observe the transformation of the form to the broken).
In the presence of an oxidizing agent (H₂O₂), *F. antipyretica* in the spring and *H. cupressiforme* winter season extracts, showed no protective activity. *F. antipyretica* at the concentrations of 0.5 and 1 μM of methanol extract in winter and in the all concentrations of *H. cupressiforme* spring season extracts Form III were observed. In addition, it was observed that spring season chloroform extract of *H. cupressiforme* completely disintegrate DNA in 1 μM concentration (Figure 7).

### CONCLUSION

The need for natural antioxidants is increasing day by day as cancer risks, one of the biggest problems in the world. Due to the increasing population density and the decrease in arable lands, it is very important to reduce stress in our world where food shortages may be experienced in the future. In the light of technological developments in this aim; understanding the defense mechanisms of plant species resistant to stress factors will be a very important step in minimizing product losses. Naturally occurring antioxidant compounds are identified and can be obtained purely by working on mosses. These ingredients can be used in the pharmaceutical, cosmetic, agricultural, and food industries. Thus, we can obtain new and more effective compounds that enhance cellular defense. Many future studies with mosses can be promising in the formation, progression and treatment of many diseases. This is the first time that the DNA protection activity of *F. antipyretica* and *H. cupressiforme* have been studied. The high antioxidant property shown by the plant is mainly due to the presence of considerable amount of terpenoids. Further studies are necessary to isolate the potential lead substance and to evaluate its biological properties.

### REFERENCES


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EFFECTS OF COVER CROP TREATMENTS ON SOME SOIL QUALITY PARAMETERS AND YIELD IN A KIWIFRUIT ORCHARD IN TURKEY

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ABSTRACT

Effects of different cover crops on some soil quality parameters and yield of a kiwifruit orchard located in Samsun province of Turkey were investigated. For this purpose, Festuca rubra subsp. rubra (FRR), Trifolium repens (TR), Festuca arundinacea (FA), T. repens (40%) + F. rubra rubra (30%) + F. Arundinacea (30%) mixture (TFF), Vicia villosa (VV) and Trifolium meneghinianum (TM) were used as cover crops in a kiwifruit orchard with loamy soil. Experiment also included a plot mechanically cultivated (MC), herbicide treatment (HC) and bare control plot (BC). Soil samples were taken from two different depth (0-20 and 20-40 cm) and samples were subjected to analyses for some soil quality parameters. It was observed that cover crop treatments improved soil quality attributes. TR treatment compared with the other treatments showed the highest increases in OM of 83.73%, SSI of 9.38%, F of 14.60% and the largest decrease in BD of 13.04%. Cover crop treatments increased organic matter contents in the following order; HC (1.50%) < BC (1.59%) < MC (1.72%) < TM (2.29%) < FRR (2.34%) < FA (2.37%) < TFF (2.43%) < VV (2.83%) < TR (2.94%). While the highest mean kiwifruit yield (130.71 kg/ha) were found in the TM, the lowest mean kiwifruit yield (36.20 kg/ha) were obtained with in the MC. It was concluded based on current findings that cover crops may be incorporated into cropping systems to improve soil quality parameters and may have significant contributions to sustainable soil management.

KEYWORDS: Cover crops, soil quality, chemical and physical parameters, kiwifruit orchard, loamy soil.

INTRODUCTION

Increasing fertilizer uses may have various negative influences on both environment [1] and human health [2]. Excess use of chemical fertilizers result not only in in environmental pollution and ecological damage, but increase the production costs [3]. Cover crops can reduce the use of external inputs such as fertilisers and can improve and maintain soil fertility. Establishing cover crops has also an important effect on improving soil physical, chemical, and biological properties and hence on increasing the yields of successive row crops [4]. They are used in some cases as green manure to improve soil quality. They are commonly used to prevent soil erosion and nutrient leach outs from the soils [5]. Nutrient cycles can also be improved and thus efficient nutrient use can be provided by cover crops [6]. Cover crops can enrich soil organic matter contents, and depending on organic matter contents, improve soil quality parameters, nutrient cycles. Crop residue management is a key element of sustainable crop production. Crop residues have been used for soil and water conservation as mulch. Also crop residues allow to maintain soil organic matter and to return nutrients to. It is important to use cover crops to achieve the objectives of sustainable cropping systems [7]. Therefore, new approaches should be evaluated for environmental protection and human health. While there are many studies on cover crops, studies dealing with effects on soil quality of the Festuca rubra subsp. rubra, Trifolium repens, Festuca arundinacea, T. repens (40%) + F. rubra rubra (30%) + F. Arundinacea (30%) mixture, Vicia villosa and Trifolium meneghinianum in an orchard are very limited. Thus, this study was conducted to investigate the effects of the cover crop treatments on some soil quality parameters and yield of a kiwifruit orchard in Turkey.

MATERIALS AND METHODS

This study was carried out in 2013 and 2014 growing seasons in a kiwifruit orchard located in Samsun province of Turkey. The orchard is located in Middle Black Sea region. Monthly average temperature was 14.5 °C and annual average precipitation was 685.5 mm. The cover crop treatments consisted of Trifolium repens L. (TR), Festuca rubra rubra L. (FRR), Festuca arundinacea (FA), Trifolium repens (40%) + Festuca rubra rubra
(30%) + Festuca arundinacea (30%) mixture (TFF), Vicia villosa (VV) and Trifolium meneghinianum (TM). The species chosen for cover cropping are generally those that are familiar to the grower and are known to perform well in a particular environment, and for which seed can be cheaply and readily obtained [8]. Experiment were conducted in randomized complete block design with 4 replications. A bare control, mechanical control and herbicide control was included as control plots. Soil samples were taken from two different depth (0-20 and 20-40 cm) from each plot. Soil samples were air-dried and passed through 2 mm sieve and made ready for analyses. Hydrometer method was used to determine soil particle size distribution [9]. Soil pH was measured in 1:1 (w:v) soil-water suspension with a pH meter; soil electrical conductivity (EC25°C) was measured again the in the same soil suspension with an EC meter; exchangeable cations were determined with ammonium acetate extraction [10]; soil available P content was measured through extraction with 0.5 M NaHCO3 at pH 8.5 [11]. Modified Walkley – Black method was used to determine soil organic matter (OM) content [12]. Total N contents were determined with LECO. Soil respiration rates were measured in accordance with Isermayer [13] through measuring CO2 productions at 22°C. The CO2 productions were expressed in mg CO2/100g at the end of 24 hours incubation period. The physical and chemical properties of the test soil are provided in Table 1. The results can be summarized as: textural class, loamy; non-saline; pH slightly alkaline; organic matter content, slight [14]. Field capacity (FC) and permanent wilting point (PWP) were determined with a pressure plate respectively at 1/3 and 15 atm pressure. The difference in moisture contents at FC and PWP was taken as available water content (AWC) [15]. Soil bulk density (BD) was determined in accordance with the method specified in Tüzün [16]. Porosity (F) was calculated with the following equation [15];

\[ F = 1 - (BD / 2.65) \]  

### TABLE 1

<table>
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<th>Soil properties De</th>
<th>0-20 cm</th>
<th>Dept, cm</th>
<th>Soil properties De</th>
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### TABLE 2

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<th>BSR, mg CO2/100g soil**</th>
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<td>46.52 ab</td>
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<td>1.50 e</td>
<td>0.098 d</td>
<td>19.99</td>
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<td>0.85 c</td>
<td>0.42 a</td>
<td>36.48 b</td>
<td>12.9 d</td>
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2014

<table>
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<tr>
<th>Treat. (1:1)**</th>
<th>pH</th>
<th>EC25°,</th>
<th>OM,</th>
<th>N,</th>
<th>NH4OAc extractable, mek/100 g</th>
<th>Ca*</th>
<th>Mg*</th>
<th>K*</th>
<th>Na*</th>
<th>P, ppm**</th>
<th>BSR, mg CO2/100g soil**</th>
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<td>7.20 c</td>
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<td>4.42 b</td>
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<td>5.02 ab</td>
<td>1.06 c</td>
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<td>12.6 e</td>
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</tr>
</tbody>
</table>

*Significant at 5% level, **Significant at 1% level.

Trifolium repens L. (TR), Festuca rubra rubra L. (FRR), Festuca arundinacea (FA), T. repens (40%) + F. rubra rubra (30%) + F. Arundinacea (30%) mixture (TFF), Vicia villosa (VV) and Trifolium meneghinianum (TM), a plot mechanically cultivated (MC), herbicide treatment (HC) and bare control plot (BC).
TABLE 3
Effects of different cover crops on chemical soil quality parameters at 20-40 cm soil depth.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>pH (1:1)</th>
<th>EC25°, ds/m</th>
<th>OM, %</th>
<th>N, %</th>
<th>NH4OAc extractable, meq/100 g</th>
<th>P, ppm</th>
<th>BSR, mgCO2/100g soil</th>
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P < 0.01
Trifolium repens L. (TR), Festuca rubra rubra L. (FRR), Festuca arundinacea (FA), T. repens (40%) + F. rubra rubra (30%) + F. Arundinacea (30%) mixture (TFF), Vicia villosa (VV) and Trifolium meneghinianum (TM), a plot mechanically cultivated (MC), herbicide treatment (HC) and bare control plot (BC).

RESULTS AND DISCUSSION

Cover crop treatments had significant effects on soil quality parameters at 0-20 cm soil depth. There were significant increases in electrical conductivity, organic matter, total N, K, P and basal soil respiration values and decreases in pH and Na contents with cover crop treatments. Higher improvement rates were observed in the second year of the experiment (Table 2). Cover crop treatments increased organic matter contents in the following order: HC (1.50%) < BC (1.59%) < MC (1.72%) < TM (2.29%) < FRR (2.34%) < FA (2.37%) < TFF (2.43%) < VV (2.83%) < TR (2.94%). Organic matter plays a great role in soil quality management [5]. Organic matter is a source of nutrient for both soils and plants and significantly improves soil air and water permeability and water retention [21]. Zhang et al. [22] proved that organic or green manure could increase soil C storage. The changes in soil chemical characteristics as compared to bare control treatment is provided in Table 7. Percent increases in soil organic matter content as compared to bare control treatment at 0-20 cm soil depth varied between 43.58% in TM and 83.73% in TR treatment in the second year of the experiment (2014). Carbon inputs [5], thus soil quality is significantly improved by cover crops [23]. Reduced tillage together with cover crops may even maximize soil organic carbon contents [24]. In both years of the experiments, organic matter contents of a plot mechanically cultivated, herbicide treatment and bare control plot at 0-20 cm soil depth were
generally not significant statistical differences (Table 3).

As compared to bare control treatment, increases in nitrogen contents varied between 42.79% in FRR and 71.62% in TR treatment. Nitrogen plays a catalyzer role in soil organic matter formation [25]. Right at this point, cover crops contribute that nitrogen either through scavenging residual N or through N2 fixation with legume crops. Legumes fixate atmospheric nitrogen to the soils [26]. Such fixation rates vary with species, growth stages, management practices and climate conditions. The N release from cover crops varies based on lignin, carbohydrate and cellulose content of the residues [27]. There were significant decreases in soil pH values of the present study with cover crop treatments and such decreases as compared to bare control treatment varied between -3.56% in TFF and -5.92% in TR treatment. Cover crop treatments increased exchangeable cation contents, except for Na. Cover crop treatments significantly decreased exchangeable Na values (from 0.43 me/100g in bare control treatment to 0.29 me/100g in VV treatment). The greatest decreases in Na content (30.47%) were observed in VV treatment. As compared to bare control treatment, increases in K contents varied between 38.2 mg CO2/100g soil in bare control treatment to 67.38 ppm in VV and 38.2 mg CO2/100g soil in TR treatments, respectively (Table 2). While Ca contents varied between 17.98 me/100g in BC and 20.83 me/100g in TFF treatments, Mg contents ranged from 4.42 me/100g in HC to 6.01 me/100g in FA treatments. Except for electrical conductivities, the differences in chemical quality parameters of cover crop treatments were not significant for 20-40 cm soil depth in both years of experiments (Table 3).

Soil physical quality parameters are also influenced by agricultural management practices. There were significant increases in field capacity, permanent wilting point, available water capacity, volumetric water content, total porosity, aggregate stability, structural stability index and saturated hydraulic conductivity values and significant decreases in bulk density values with cover crop treatments (Table 4). While the highest bulk density was found in the HC treatment (1.42 gr/cm3), the lowest bulk density was obtained with the Trifolium repens (TR) treatment (1.22 gr/cm3). The greatest increase in total porosity (14.60%) and the greatest decrease in bulk density (13.04%) was observed in TR year of the experiment (2014). Electrical conductivities is an indicator of soil salinity and used as an essential parameter for monitoring organic matter mineralization [28] since it reflects anion and cation quantities in soil solution [33]. Available P and BSR significantly increased with cover crop treatments (from 38.07 ppm and 12.6 mg CO2/100g soil in bare control treatment to 67.38 ppm in VV and 38.2 mg CO2/100g soil in TR treatments, respectively) (Table 2). While Ca contents varied between 17.98 me/100g in BC and 20.83 me/100g in TFF treatments, Mg contents ranged from 4.42 me/100g in HC to 6.01 me/100g in FA treatments. Except for electrical conductivities, the differences in chemical quality parameters of cover crop treatments were not significant for 20-40 cm soil depth in both years of experiments (Table 3).

### TABLE 4

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Db, gr/cm3</th>
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<th>PWP, %</th>
<th>AWC, %</th>
<th>F, %</th>
<th>(\theta_i), cm³/h</th>
<th>Ks, cm³/h</th>
<th>AS, %</th>
<th>MWD, mm</th>
<th>SSI, %</th>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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*Significant at 5% level. **Significant at 1% level.

Trifolium repens L. (TR), Festuca rubra rubra L. (FRR), Festuca arundinacea (FA), T. repens (40%) + F. rubra rubra (30%) + F. Arundinacea (30%) mixture (TFF), Vicia villosa (VV) and Trifolium meneghinianum (TM), a plot mechanically cultivated (MC), herbicide treatment (HC) and bare control plot (BC).
treatment. Total porosity is an indicator for pore spaces through which air and water move and thus bulk density is inversely proportional to porosity [30]. Plant roots loosen soil structure and thus decreasing bulk density, increasing total porosity and saturated hydraulic conductivity were observed in this study. Crop residues may improve soil aggregation, thus improve porosity, hydraulic conductivity, infiltration rate and water-storage capacity of soils [31]. Bhagat and Verma [32] also reported improved soil structure, water retention capacity, Acharya et al. [33] improved infiltration rates and Khaleel et al. [34] decreased bulk densities with crop residues and green manures. As compared to bare control treatment, the changes in soil physical quality parameters are provided Table 8. While the bare control treatment, the changes in soil physical properties of cover crop treatments were not fond to be significant for 20-40 cm soil depth (Table 5).

Soil organic matter contents of the present study positively correlated with SSI (0.835**), Ks (0.912**) and FC (0.796**). Gülser [38] indicated that organic carbon reduced bulk density and increased total porosity and reported positive correlations between organic carbon and aggregate stability. Increasing field capacity, permanent wilting point, available water capacity, volumetric water content, total porosity, aggregate stability, structural stability index and saturated hydraulic conductivity values and decreasing soil bulk density values were observed with increasing organic matter contents because of cover crops. Saturated hydraulic conductivity had significant positive correlations with SSI (0.812**) and negative correlations with bulk density (-0.914**). Saturated hydraulic conductivity had also significant correlations with EC (0.703**), F (0.743**) and exchangeable Na (-0.816**). Improved organic matter contents had significant effects on various soil physical quality parameters. It was reported in a previous study that organic

| TABLE 5 |
| Effects of different cover crops on physical soil quality parameters at 20-40 cm soil depth. |

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Db, g/cm³</th>
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<th>PWP, %</th>
<th>AWC, %</th>
<th>F, %</th>
<th>θ, %</th>
<th>Ks, cm/h</th>
<th>AS, %</th>
<th>MWD, mm</th>
<th>SSI, %</th>
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<td>0.76</td>
<td>36.05</td>
<td>0.560</td>
<td>43.42</td>
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<td>0.560</td>
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</table>

| 2014       |          |       |        |        |      |     |        |      |        |       |
| TR         | 1.37     | 30.05 | 15.60  | 14.73  | 48.30| 19.45| 0.79   | 35.95| 0.541  | 42.75 |
| FRR        | 1.38     | 29.16 | 15.47  | 14.09  | 47.92| 19.36| 0.78   | 36.02| 0.572  | 42.80 |
| FA         | 1.40     | 30.26 | 15.58  | 14.67  | 47.17| 18.97| 0.79   | 35.15| 0.540  | 42.83 |
| TFF        | 1.38     | 29.77 | 14.90  | 14.87  | 47.92| 19.00| 0.76   | 36.77| 0.558  | 43.06 |
| VV         | 1.38     | 30.09 | 15.44  | 14.55  | 47.92| 19.24| 0.83   | 36.62| 0.576  | 42.36 |
| TM         | 1.38     | 29.36 | 14.96  | 14.40  | 32.83| 18.59| 0.80   | 35.12| 0.544  | 42.80 |
| HC         | 1.40     | 29.44 | 15.53  | 13.91  | 47.17| 17.97| 0.76   | 36.30| 0.538  | 42.30 |
| MC         | 1.40     | 29.98 | 15.43  | 14.55  | 47.17| 18.42| 0.79   | 36.53| 0.560  | 42.79 |
| BC         | 1.39     | 29.57 | 15.28  | 14.29  | 47.55| 18.09| 0.77   | 35.81| 0.557  | 42.09 |

Trifolium repens L. (TR), Festuca rubra rubra L. (FRR), Festuca arundinacea (FA), T. repens (40%) + F. rubra rubra (30%) + F. Arundinacea (30%) mixture (TFF), Vicia villosa (VV) and Trifolium meneghinianum (TM), a plot mechanically cultivated (MC), herbicide treatment (HC) and bare control plot (BC).
residues significantly improved soil structure [39]. Present findings comply with those earlier ones. Significant positive correlations were also observed between OM and total N (0.828**); between pH and EC (0.835**); between BSR and OM (0.913***). Among the chemical quality parameters, significant correlations were observed between OM and K (0.884**), between total N and K (0.847**), between Na and EC (0.556*) and between Na and pH (0.678**). Plant available water capacity [40], infiltration rates [41], aggregate formation and stability [42] and bulk density [43] were considered as the soil quality parameters related to organic carbon contents. It was reported in a previous study that soil fertility decreased with decreasing organic matter contents [44]. Organic matter supplementation to soils may eliminate such destructions in soil quality [45]. Soil fertility is also closely related to microbial activity and such activity is then closely related to mineralization of organic elements (C, N, P and S) [50]. A significant correlation (0.780 at p<0.01) between soil respiration and total porosity in this study confirms that CO2 production correlated directly with aerobic respiration and hence aerobic biological activity [47].

Among the physico-chemical quality parameters, there were significant negative correlations between SSI and Na (-0.630*) and between organic matter and bulk density (-0.862**). It was observed that organic matter, exchangeable Na and electrical conductivity significantly correlated with all physical quality parameters. EC values also positively correlated with SSI (0.482*), F (0.618*), θ (0.625*) and Ks (0.704**) and negatively correlated with BD (-0.697*). Exchangeable Na content had negative significant correlations with F (-0.755**), Ks (-0.816**) and significant positive correlations with BD (0.872**). Present findings revealed that electrical conductivity and exchangeable Na could also be used as significant quality indicators. Several previous researchers reported significant positive correlations between organic carbon and aggregate stability [48]. Oades [42] indicated two primary means of effect of microorganism on soil structural stability as of mechanical binding of soil particles

### TABLE 6

**Effects of cover crops and other treatments on kiwifruit yield**

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Kiwifruit yield, kg/ha</th>
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<th>2014**</th>
<th>Mean**</th>
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<tr>
<td>MC</td>
<td>59.38</td>
<td>c</td>
<td>13.02</td>
<td>c</td>
</tr>
<tr>
<td>BC</td>
<td>162.50</td>
<td>ab</td>
<td>15.72</td>
<td>c</td>
</tr>
</tbody>
</table>

**Significant at 1% level.

*Trifolium repens* L. (TR), *Festuca rubra rubra* L. (FRR), *Festuca arundinacea* (FA), *F. repens* (40%) + *F. rubra rubra* (30%) + *F. Arundinacea* (30%) mixture (TFF), *Vicia villosa* (VV) and *Trifolium meneghinianum* (TM), a plot mechanically cultivated (MC), herbicide treatment (HC) and bare control plot (BC).

### TABLE 7

**Changes (%) in soil chemical quality parameters at 0-20 cm soil depth as compared to bare control treatment**

<table>
<thead>
<tr>
<th>Treatments</th>
<th>pH</th>
<th>EC</th>
<th>OM</th>
<th>N</th>
<th>NH4OAc extractable</th>
<th>Ca</th>
<th>Mg</th>
<th>K</th>
<th>Na</th>
<th>P</th>
<th>BSR</th>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>TR</td>
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<td>71.08</td>
<td>69.72</td>
<td>66.34</td>
<td>3.90</td>
<td>5.93</td>
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<td>-24.15</td>
<td>55.66</td>
<td>76.00</td>
<td></td>
</tr>
<tr>
<td>FRR</td>
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<td>49.88</td>
<td>30.48</td>
<td>34.10</td>
<td>4.85</td>
<td>3.16</td>
<td>12.42</td>
<td>-12.23</td>
<td>31.17</td>
<td>29.60</td>
<td></td>
</tr>
<tr>
<td>FA</td>
<td>-4.20</td>
<td>44.43</td>
<td>31.89</td>
<td>33.44</td>
<td>4.79</td>
<td>8.49</td>
<td>13.99</td>
<td>-20.86</td>
<td>47.38</td>
<td>51.57</td>
<td></td>
</tr>
<tr>
<td>TFF</td>
<td>-3.84</td>
<td>60.02</td>
<td>43.16</td>
<td>57.59</td>
<td>4.29</td>
<td>10.99</td>
<td>10.90</td>
<td>-16.36</td>
<td>58.13</td>
<td>87.27</td>
<td></td>
</tr>
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<td>90.54</td>
<td>72.97</td>
<td>69.26</td>
<td>5.69</td>
<td>4.61</td>
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<td>-17.55</td>
<td>52.07</td>
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<tr>
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<td>64.92</td>
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<td>21.75</td>
<td>5.75</td>
<td>-13.80</td>
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<td>-9.15</td>
<td>1.89</td>
<td>-0.44</td>
<td>4.68</td>
<td>5.60</td>
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<tr>
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<td></td>
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<td></td>
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<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>TR</td>
<td>-5.92</td>
<td>84.23</td>
<td>83.73</td>
<td>71.62</td>
<td>11.92</td>
<td>25.87</td>
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<td>-30.47</td>
<td>74.11</td>
<td>203.17</td>
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</tr>
<tr>
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<td>47.27</td>
<td>46.65</td>
<td>42.79</td>
<td>11.08</td>
<td>23.89</td>
<td>27.60</td>
<td>-21.12</td>
<td>38.08</td>
<td>124.24</td>
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<td>43.92</td>
<td>13.52</td>
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<td>-24.56</td>
<td>38.12</td>
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<tr>
<td>TFF</td>
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<td>45.53</td>
<td>52.10</td>
<td>54.05</td>
<td>15.81</td>
<td>17.86</td>
<td>36.63</td>
<td>-22.08</td>
<td>47.55</td>
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</tr>
<tr>
<td>VV</td>
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<td>77.02</td>
<td>68.69</td>
<td>3.84</td>
<td>17.48</td>
<td>55.01</td>
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<td>167.37</td>
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</tr>
<tr>
<td>TM</td>
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<td>54.43</td>
<td>43.58</td>
<td>56.76</td>
<td>9.26</td>
<td>26.09</td>
<td>49.37</td>
<td>-18.64</td>
<td>49.60</td>
<td>109.52</td>
<td></td>
</tr>
<tr>
<td>HC</td>
<td>-0.49</td>
<td>1.74</td>
<td>5.95</td>
<td>-11.94</td>
<td>6.92</td>
<td>-1.40</td>
<td>-3.61</td>
<td>1.48</td>
<td>-3.46</td>
<td>-5.56</td>
<td></td>
</tr>
<tr>
<td>MC</td>
<td>-1.05</td>
<td>12.41</td>
<td>7.46</td>
<td>14.64</td>
<td>9.94</td>
<td>12.00</td>
<td>12.78</td>
<td>-4.73</td>
<td>1.28</td>
<td>15.08</td>
<td></td>
</tr>
</tbody>
</table>
and production of effective binding agents. In this study, higher aggregate stability and electrical conductivity values were also obtained with increasing organic matter contents. Dexter [49] indicated that organic matter lowered soil bulk density and increased soil aggregate stability.

Generally, cover crop treatments increased mean yield in kiwifruit orchards according to the control (Table 6). These results are supported by Calegari et al. [50] and Harrington et al. [51-52]. While the highest mean kiwifruit yield were found in the TM treatment (130.71 kg/ha), the lowest mean yield were obtained with in the MC treatment (36.20 kg/ha). Generally, planting of mixed legumes and grasses from perennial cover crops also increased kiwifruit yield. Regarding the effect of cover crops on kiwifruit yields, the lowest yield was obtained from MC plots. Since the plough in the orchards damages the tree roots, the mechanical struggle is done in the form of mow. This causes the weeds to germinate again and increase the yield loss.

**CONCLUSIONS**

This study showed that cover crop treatments generally improved some soil quality parameters at 0-20 cm soil depth. The increases in soil organic matter most likely led to improvements in soil structure as shown by decreased bulk density and increases in saturated hydraulic conductivity and volumetric moisture content. Organic matter was higher in soils under TR and VV management systems than under no cover crop. This was due to low input of plant residues into the soil under no cover crop system. All the cover crop treatments had positive impacts on soil quality parameters. Cover crop treatments increased soil organic matter contents and consequently resulted in significant increases in soil structural stability, total porosity, saturated hydraulic conductivity, total N and K contents and considerable reductions in bulk density, pH and Na content as compared to bare control treatments. The greatest impacts on soil physical quality parameters and organic matter contents were also observed in TR and VV treatments. In both years of the experiments, there were not significant differences in soil quality parameters of a plot mechanically cultivated, herbicide treatment and bare control plots. Except for electrical conductivities, the differences in soil quality parameters of cover crop treatments were not significant for 20-40 cm soil depth in both years of experiments. According to the control, cover crop treatments generally increased mean yield in a kiwifruit orchard. It was concluded based on current findings that *Vicia villosa* (VV) and *Trifolium repens* (TR) treatments could be incorporated into cropping systems to improve soil quality and to provide a sustainable soil management.

**ACKNOWLEDGEMENTS**

The authors express their sincere thanks to Turkish Scientific and Technological Research Council (TUBITAK) for financial support provided to present study (with the Project number of 111-O-647).

---

**TABLE 8**

Changes (%) in soil physical quality parameters at 0-20 cm soil depth as compared to bare control treatment

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Db</th>
<th>FC</th>
<th>PWP</th>
<th>AWC</th>
<th>F</th>
<th>Θ</th>
<th>Ks</th>
<th>AS</th>
<th>MWD</th>
<th>SSI</th>
</tr>
</thead>
<tbody>
<tr>
<td>2013</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TR</td>
<td>-11.83</td>
<td>9.83</td>
<td>8.30</td>
<td>10.54</td>
<td>13.45</td>
<td>43.43</td>
<td>86.49</td>
<td>6.45</td>
<td>9.70</td>
<td>9.32</td>
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<td>2.72</td>
<td>8.63</td>
<td>9.88</td>
<td>18.00</td>
<td>75.38</td>
<td>2.14</td>
<td>8.12</td>
<td>6.39</td>
</tr>
<tr>
<td>FA</td>
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<td>10.40</td>
<td>7.72</td>
<td>13.43</td>
<td>10.08</td>
<td>22.90</td>
<td>91.29</td>
<td>4.04</td>
<td>6.36</td>
<td>6.73</td>
</tr>
<tr>
<td>TFF</td>
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<td>9.64</td>
<td>6.82</td>
<td>11.93</td>
<td>11.69</td>
<td>32.30</td>
<td>88.89</td>
<td>3.03</td>
<td>6.99</td>
<td>7.30</td>
</tr>
<tr>
<td>VV</td>
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<td>10.87</td>
<td>8.26</td>
<td>13.28</td>
<td>12.06</td>
<td>45.67</td>
<td>103.00</td>
<td>8.75</td>
<td>8.82</td>
<td>7.94</td>
</tr>
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<td>TM</td>
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<td>4.24</td>
<td>9.10</td>
<td>9.88</td>
<td>23.73</td>
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<td>3.79</td>
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<td>6.61</td>
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<td>1.65</td>
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<tr>
<td>TR</td>
<td>-13.04</td>
<td>12.18</td>
<td>8.65</td>
<td>15.75</td>
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<td>4.02</td>
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<td>5.47</td>
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<tr>
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<td>8.71</td>
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POVERTY REDUCTION AS A FACTOR OF SUSTAINABLE DEVELOPMENT OF RURAL AREAS IN THE REPUBLIC OF SERBIA

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ABSTRACT

Poverty is a multi-dimensional phenomenon that, in addition to insufficient income to meet the subsistence needs of the population, also involves difficult access to labor market, inadequate housing, inadequate access to cultural and educational institutions, social protection and health care institutions, utility services, as well as inability to access important institutions and participate in decision-making processes. Being a largely rural phenomenon, poverty is the result of inadequate management of rural development, which results in long-term stagnation of rural communities’ well-being. The subject of this paper is poverty in rural areas, while its main goal is to identify the most common causes that significantly impair quality of life and motivation of people to stay in their villages. Given the level of development of a country and regional disparities, the paper analyzes the structure of household income, looks at the demographic and socio-economic characteristics and presents the data on absolute poverty in rural areas of the Republic of Serbia. Based on the research results, the paper suggests measures that should be implemented in order to reduce rural poverty.

KEYWORDS:
Sustainable rural development, risk of poverty, rural communities, Republic of Serbia

INTRODUCTION

Although contemporary development processes require the rural development to be implemented as a sustainable process of economic, social, cultural and environmental advancement of rural communities, aimed at improving their long-term well-being [1], such a development concept is very difficult to achieve in practice. The research presented in this paper is motivated by the fact that population in rural areas is more poverty-stricken than that in urban areas, based on data provided by the Survey on Income and Living Conditions (EU-SILC), while the framework of the study includes the concept of relative poverty and definition of rurality as defined by the Eurostat (Statistical Office of the European Communities). Furthermore, the information provided by the Household Consumption Survey, conducted by the Statistical Office of the Republic of Serbia (SORS), is also used. The mentioned survey provides the option to supplement the poverty analysis with the data on the absolute poverty rate, which is of particular importance for the sustainable development of rural areas in the Republic of Serbia and their participation in economic development, since, according to the data published by the SORS, the largest part of the territory of the Republic of Serbia consists of rural settlements. Out of a total number of 6,158 settlements, 5,965 (96.9%) falls into the category “other” - rural settlements [2].

The subject of the research is the poverty in rural areas of the Republic of Serbia, i.e., the situation, trends, policy development and possible directions of strategic management of sustainable rural development in order to reduce the risk of poverty, considering the fact that in rural areas, in 2016, the absolute poverty rate amounted to 10.5%, that is, out of the total population of 2,763,060 people living in these areas 290,607 people were identified as poor [3].

The key hypothesis the paper builds upon is that if the rural areas in the Republic of Serbia, in terms of the majority of strategic documents, are identified as areas with pronounced poverty and significant development constraints, they require priority focus of all social factors of development, as well as recognizing the great responsibility of local communities in the mobilization of resources and building institutional capacity and procedures to create the conditions for reducing social exclusion and poverty of the population in rural areas.

Primary and secondary research, together with the application of quantitative and qualitative analyses, made it possible to include in the study, as well as analyze by applying common scientific and research instruments and tools, the following characteristics: the basic characteristics and the situation in terms of poverty in rural areas in the Republic of
Serbia; structure of income and the at-risk-of-poverty rate of rural households; material deprivation; profile of persons at risk of poverty in rural areas; as well as enabled making recommendations for reducing rural poverty in the Republic of Serbia.

INTRODUCTORY CONSIDERATIONS

The problems of rural communities, which are mostly economic and social in nature, influence the increase in risk of poverty, thus significantly reducing the quality of life and motivation of people to stay in their villages.

The main limitation of the research and statistical analyzes relating to rural areas and settlements refers to the definition of criteria for their identification. Previous studies have shown that there is no universal and/or universally accepted definition of rurality. Population size, population density and number of commuters are the variables that are commonly used in the definition of rural areas. In order to ensure international comparability of data, the OECD (Organization for Economic Co-operation and Development) recommends that rurality should be defined based on the lowest territorial level and the population density, therefore, the settlements with a population density of less than 150 inhabitants per km² are considered rural areas [4]. However, in the Republic of Serbia, for statistical purposes, a typology based on administrative and legal criteria is applied, which classifies settlements in two categories: urban settlements and “other”. The Eurostat in its Statistics on Income and Living Conditions (EU-SILC) classifies different areas based on the degree of urbanization. Since 2011, the typology of areas according to the degree of urbanization is based on population grid cells, therefore, there are three types of areas [5]:

- Densely populated areas are those areas characterized by high-density clusters with population grid square cells of 1 km² which have a density of at least 1,500 inhabitants per km² and a total population of at least 50,000 inhabitants;
- Intermediate density areas or urban clusters are grid square cells of 1 km² which have a density of at least 300 inhabitants per km² and a total population of at least 5,000 inhabitants;
- Thinline-populated areas are grid cells that do not fall in the category of urban clusters.

According to the mentioned typology, densely populated areas correspond to cities/large urban areas, intermediate density areas imply towns and suburbs, while thinly-populated areas are, in fact, rural areas.

POVERTY IN RURAL AREAS OF THE REPUBLIC OF SERBIA

Poverty is more serious issue in rural communities than in urban areas, due to the unfavorable economic structure, remoteness in terms of distance from urban centers, inadequate attitude of the state towards rural areas, socio-economic heterogeneity and etc. The most common causes of higher poverty levels in rural areas, are as follows [6, 4, 2]:

- unfavorable characteristics of agricultural structure;
- insufficient diversification of income and variations in economic activity of the rural population;
- unfavorable age, gender and educational structure of rural population;
- insufficiently stimulating development policy of the country in terms of rural areas and agriculture;
- unfavorable geographic location of some rural areas and underdeveloped infrastructure.

<table>
<thead>
<tr>
<th>Table 1: Structure of income in money and in kind, individual consumption, in rural areas of the Republic of Serbia, 2016 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Household income in money</td>
</tr>
<tr>
<td>---------------------------</td>
</tr>
<tr>
<td>89.3</td>
</tr>
<tr>
<td>Regular salaries and wages</td>
</tr>
<tr>
<td>Other income</td>
</tr>
<tr>
<td>Pensions (old-age, survivors’, disablement and other)</td>
</tr>
<tr>
<td>Other social insurance receipts</td>
</tr>
<tr>
<td>Income from agriculture, hunting and fishing</td>
</tr>
<tr>
<td>External receipts</td>
</tr>
<tr>
<td>Real estate related income</td>
</tr>
<tr>
<td>Donations and awards</td>
</tr>
<tr>
<td>Other receipts</td>
</tr>
<tr>
<td>Household receipts in kind</td>
</tr>
<tr>
<td>Earned receipts in kind</td>
</tr>
<tr>
<td>Natural consumption</td>
</tr>
<tr>
<td>Individual consumption - total, %</td>
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</table>

6999
Different shares of relatively poor areas, depending on their degree of urbanization, calls for analyzing the factors that make the rural population more at risk of poverty. Since income is one of major determinants in measuring the relative poverty, it is important to look at the structure of income in rural areas of the Republic of Serbia.

The basic household income in cash in urban areas in 2016, amounted to 99.1%, while the income in-kind was 0.9%. In rural areas the household income in cash was 89.3% and the income in-kind was 10.7%. Of these, in rural areas (Table 1) [7] the main source of income was from full-time employment earnings (37.1%) and pensions (28.8%), while in urban areas 55.9% of income was generated by full-time employment earners and 32.1% by personal pensions. Other social welfare transfers (i.e., other social welfare payments) in urban areas amounted to 2.8% and in rural areas to 3.4%. This suggests that the risk of poverty is higher in rural than in urban areas.

Poverty is two times higher in rural areas (Figure 1) [3] than in urban ones. The regional overview of poverty indicates striking discrepancies in terms of the Southern and Eastern Serbia region which had the highest poverty rate over the entire observed period (Figure 2) [3].

The Belgrade (Beogradski) region has persistently lowest rate of poverty, while the situation in Vojvodina and Sumadija and Western Serbia (Sumadija i Zapadna Srbija) regions in the observed period from 2006 to 2016, can be considered as a positive one, since it is steadily being improved and thus, almost resembles the situation in the Belgrade region.

![FIGURE 1](image1.png)

**FIGURE 1**
Republic of Serbia poverty rate by settlement type

![FIGURE 2](image2.png)

**FIGURE 2**
Republic of Serbia poverty rate by region
DEMOGRAPHIC AND SOCIOECONOMIC CHARACTERISTICS OF RURAL HOUSEHOLDS IN TERMS OF POVERTY RISK

The demographic structure is very important in terms of the poverty risk. The rural population, which is at higher risk of poverty, is mainly composed of: farmers living in hilly and mountainous areas, elderly and single-person households, internally displaced persons living in rural areas, full-time employed rural population that receives no income from agriculture or people that earn income from agriculture and are not involved in any non-agricultural activities.

The territory of the Republic of Serbia, including Kosovo and Metohija, covers an area of 88,499 km² with 6,158 settlements, of which 193 are urban and 5,965 “other” settlements that are considered rural. On the territory of Sumadija and Western Serbia, there are 2,112 settlements in total, of which 97.5% belong to the category of “other” settlements (i.e., villages). In the region of Vojvodina, from a total of 467 settlements, 88.9% are classified as villages, while in the Southern and Eastern Serbia region (Region Juzne i Istocene Srbije), out of a total of 1,973 settlements, 97.7% fall into category of “other” settlements, that is rural settlements. In terms of the Belgrade region, of 157 settlements, 89.8% are classified as “other” (rural) settlements [7].

The 2011 Census data [8] indicate that the demographic trends in rural areas are becoming increasingly unfavorable. The rural population showed a decline in the period 2002-2011 and was reduced for 311,139 inhabitants (10.9%), that is, it fell below 3 million people; presently rural population makes 40.6% of the total population of the Republic of Serbia. About 1000 villages have a population of less than 100 inhabitants, while the majority of these settlements are located in the southern and eastern parts of the country. Sumadija and Western Serbia is the only region where more people live in rural areas than in urban ones, i.e., 52.6% [9].

### TABLE 2

Age structure of persons in the Republic of Serbia according to the risk of poverty and the level of urbanization of the area (%)

<table>
<thead>
<tr>
<th>Not-At-Risk-Of-Poverty</th>
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<th>Thinly-populated areas</th>
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<td>15 to 24 yrs of age</td>
<td>10.50</td>
<td>10.52</td>
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<td>25 to 44 yrs of age</td>
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<td>over 65 yrs of age</td>
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<td>15 to 24 yrs of age</td>
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<td>15.64</td>
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<td>45 to 65 yrs of age</td>
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<td>over 65 yrs of age</td>
<td>11.11</td>
<td>15.75</td>
<td>21.58</td>
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<tr>
<td>Total</td>
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### FIGURE 3

Age structure of the Republic of Serbia citizens per degree of urbanization of the area and poverty risk (%), based on SORS Income and Living Conditions Survey, 2014
The age structure of the population at risk of poverty, according to the degree of urbanization of the area, is relatively similar. It should be noted that all these areas are characterized by the largest share of the people aged between 45 and 65, who are at risk of poverty (Table 2) [10]. The most striking differences according to the degree of urbanization of the area, are mainly related to the young and persons over 65 years of age. In densely populated areas, the persons between 15 and 24 years of age are at higher risk of poverty than the persons over 65 years of age. In contrast, the population over 65 years of age in rural areas are at higher risk of poverty than the young population between 15 and 24 years of age (Figure 3) [10].

In terms of the gender structure, the point of the degree of the urbanization of the area, both men and women are at risk of poverty, however, in rural areas the number of women at risk of poverty is slightly higher compared to men (Table 3) [10].

Considering the educational structure of persons older than 15 years of age, living in the rural areas of the Republic of Serbia, persons with completed secondary education (37.4%) make the largest share, while illiterate persons or persons that did not complete primary education make 34.6% [11]. Furthermore, the 2011 Census shows that the share of people with some college or university degree in rural population is very low in all regions (Figure 4) [11].

The persons with the high school degree make the largest share of persons at risk of poverty according to the indicators degree of urbanization and poverty risk. Generally, persons living in the households where the head of the household did not complete primary education are 10 times more exposed to poverty in relation to persons living in households where the head of the household has some college or university degree. Persons living in households where the head of the household is unemployed and inactive are 4-5 times more likely to be poor, than people living in households where the head of the household is employed, even though the employment status does not guarantee poverty alleviation. The share of self-employed persons at risk of poverty (15.77%) is higher in rural areas compared to urban ones [3].

| TABLE 3 |
| Gender structure of the Republic of Serbia citizens per risk of poverty and the degree of urbanization of the area (%) |
| Not-At-Risk-Of-Poverty | Densely populated areas | Intermediate density areas | Thiny-populated areas | Total |
| Men | 46.74 | 47.68 | 49.05 | 47.83 |
| Women | 53.26 | 52.32 | 50.95 | 52.17 |
| Total | 100.00 | 100.00 | 100.00 | 100.00 |
| At-Risk-Of-Poverty | Densely populated areas | Intermediate density areas | Thiny-populated areas | Total |
| Men | 48.54 | 49.26 | 49.37 | 49.18 |
| Women | 51.46 | 50.74 | 50.63 | 50.82 |
| Total | 100.00 | 100.00 | 100.00 | 100.00 |

**FIGURE 4**
Educational structure of rural population in the Republic of Serbia by region
Data on material deprivation are also very important in terms of the poverty analysis. The EU Social Protection Committee has drawn up a list which contains specific questions, i.e. items, that are used to define the measure of material deprivation and these relate to the following [12]:

- the ability of households to regularly pay rent, housing loan installments, utility bills and other bills;
- the ability of households to keep the house adequately warm;
- the capacity to face unexpected financial expenses;
- the ability of household to afford a meal with meat, chicken, fish (or vegetarian equivalent) at least every other day;
- the ability of the household to afford one week annual holiday away from home;
- the ability of the household to afford a colour TV, a washing machine, a car and a telephone.

According to the EU-SILC methodology, the three levels of deprivation are measured. The lowest level of deprivation is the material deprivation, which means that a person lives in a household that cannot afford at least three of the items included in the mentioned list. If the household cannot afford at least four items in the list, it is considered to be severely materially deprived, and if the household cannot afford any five or more items in the list, then it falls into the category of the extreme material deprivation [13, 14].

At the national level, about 69% of people at risk of poverty are materially deprived. According to the degree of urbanization, there is a relative homogeneity between rural and densely populated areas in terms of the material deprivation rate - about 28% of extremely deprived persons, respectively; however this rate is slightly lower in intermediate density areas - around 35% [10].

Aging of the population, the decreased percentage of the active workers and modest capabilities for revenue generation, together with the low educational attainment, significantly worsen the situation with respect to the population in rural areas in the Republic of Serbia. Therefore, it is necessary to encourage the creation of new employment opportunities through diversification of the rural non-farm economy, with the aim of reducing poverty in rural areas.

Poverty alleviation and increased and equal distribution of income, are important aspects of sustainable development of rural areas in the Republic of Serbia; therefore, it is necessary to draw attention to the range of self-employment opportunities in rural areas (rural tourism, traditional crafts, handicrafts, organic production, creativity, innovation, SMEs and entrepreneurship, the IT sector, the production of alternative energy, public-private partnerships and the formation of local action groups, acquiring additional knowledge and skills and involvement in the educational processes).

POVERTY ALLEVIATION MEASURES

Budgetary support for rural development measures in the Republic of Serbia is still insufficient, while the contribution of sectoral policies to poverty alleviation and social inclusion is rather limited. Institutional changes aimed at poverty alleviation and social exclusion in rural areas are quite sluggish. The situation is further complicated due to demographic trends in rural areas, which are much less favorable than in urban settlements. In addition, rural areas are characterized by regional development disparities, lack of local initiatives, limited access to educational institutions and computer literacy programmes, which diminishes the competitiveness of the rural labor force in the labor market. Social services are not available in many local communities, while, in terms of their availability, there are striking rural-urban and regional imbalances. The capacities of local governments to take a leading role in supporting the development are generally limited, while the rural population insufficiently participates in the planning and decision-making processes at the local level. Problems of vulnerable social groups are mainly unrecognized and are considered sporadically and not in a comprehensive manner [15].

In terms of poverty alleviation in rural areas, it is necessary to implement different measures, activities and support programmes, such as [16, 17, 18]:

- institutional adjustment and establishment of a more efficient coordination in terms of public policies relevant for rural development;
- strengthening the capacities of the operational structures responsible for the adoption, programming, monitoring, implementation and evaluation of rural development policy;
- support to the establishment of an efficient agriculture and rural development funding system;
- appropriate risk management in agriculture and rural economy;
- support to the establishment of public-private partnerships (PPPs) and participatory decision-making in terms of the of rural development management;
- strengthening the capacity and the role of the local development stakeholders, including the support to the local self-government units which represent the interests of local communities;
- support to networking at all levels (inter-municipal, cross-border, inter-sectoral cooperation, and etc.) and the development of the functional cooperation;
- encouraging horizontal and vertical integration in agribusiness system and rural economy;
- strengthening the capacity and motivation of producers to join relevant associations;
- support to demographic revitalization and diversification of rural agricultural and non-agricultural economic activities;
- realization of the employment programmes in rural areas;
- promoting the development of SMEs and entrepreneurship in the field of agribusiness;
- encouraging tourism activities, through support to the preservation of rural heritage and its creative use;
- permanent educational and training programmes to meet the needs of sustainable rural development, as well as actions in the field of access to education, knowledge and information;
- application of modern information and communication technologies in rural areas, as well as other technologies that are health-safe and environmentally friendly.

An important challenge faced by the policy makers relating to rural development in the Republic of Serbia is how to step up the development of creative industry in rural areas. Here, it is particularly important to provide financial support for the creative sector, redefine system to support the development of small and medium enterprises and rural entrepreneurship, encourage the use of modern knowledge and information and communication technologies and human resource development.

The new rural development strategies, based on the territorial capital and creativity, must be defined depending on the specifics of the area and the unity of natural, cultural, historical and traditional characteristics of the specific environment, in line with the modern needs of the economy and society.

CONCLUSION

Sustainable rural development is one of the economic, social and environmental priorities in modern society. In this regard, it is important to provide a strong support to social and territorial cohesion of the population in rural areas. Given the fact that the rural population of the Republic of Serbia has been faced with numerous structural and socio-economic problems for a number of years, especially poverty and social exclusion, overcoming these problems depends largely on the activities of all key development stakeholders at all levels, particularly the state that has an irreplaceable role in this process.

The key hypothesis the paper builds upon is confirmed in relation to the conclusion that if the rural areas in the Republic of Serbia are identified as areas with pronounced poverty and significant development constraints in most strategic documents, they require priority focus in terms of all social factors of development. In order to appropriately address unavoidable contemporary internal and external challenges, it must be understood that the state and local communities have the significant role and great responsibility in the mobilization of available resources.

Based on the entire research conducted in this paper, it can be concluded that rural poverty represents a serious problem in the Republic of Serbia, which must be addressed immediately. The adoption and implementation of appropriate strategies and policies in practice related to this issue, should be carried out by promoting measures targeting the employment and self-employment of the rural population, organization into cooperatives, facilitating the acquisition of additional knowledge and skills in order to increase the competitiveness of rural population in the labor market. Diversification of the rural economy should be used as a means of improving the situation of rural households and encouraging investments in the sustainable development of rural areas.

REFERENCES


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