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CONTENTS

ORIGINAL PAPERS

EFFECTS OF FRUIT THINNING ON THE QUALITY AND SIZE OF 'WONDERFUL' POMEGRANATE FRUITS

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ABSTRACT

Pomegranate trees are tending to produce more fruits than they can carry. Carbohydrate source of each tree is particular; thus if trees produce more fruits than they can carry, fruits compete with each other for carbohydrates and it results with the reduction of fruit size and quality. Producing high quality fruits is at utmost important to ensure environmental and agricultural sustainability. The aim of this research was to study the effects of fruit thinning on the size and quality of pomegranate cv. Wonderful fruits. Present study conducted with the fruits of a 7-years old pomegranate orchard located in the west of Northern Cyprus. First of all, 50 uniform trees were selected from the orchard, thus all axillary fruits of these trees removed by hand 3-4 weeks after full bloom; and only terminal fruits left on the each spur. Then, trees were divided into 5 groups with 10 trees in each group. The groups are A) 80-89; B) 90-99; C) 100-114; D) 115-134; and E) 135-150 fruits trees^{-1}. Results showed that, number of the fruits on the tree has significant influence on the average fruit weight, fruit size and total yield. Highest total yield was measured as 49.8 kg tree^{-1} from group-B [with average 94.1 fruits tree^{-1}] while the lowest yield was measured from group-A [average 81.9 fruits trees^{-1}] as 39.2 kg tree^{-1}. On the other hand, highest average fruit weight was obtained again from group-B as 456.0 g fruit^{-1} and the lowest obtained from group-E as 425.7 g fruit^{-1}.

KEYWORDS:
Export quality, fruit number, fruit weight, harvest, packaging, total yield

INTRODUCTION

Pomegranate (*Punica granatum* L.) is one of the oldest known edible and cultivated fruits; and is capable of growing in different agro-climatic conditions ranging from the tropical to sub-tropical [1]. However, consumers' preference was low for many centuries due to the hassle of extracting the arils. Since the beginning of 21th century, many scientific works conducted about the health benefits of pomegranates which revealed the efficacy against coronary heart disease, cancer, hypertension, and infectious diseases [2-5]. On the other hand, it was also reported that the bioactive compounds content in pomegranate juice, which is responsible from the health benefits is variable and depends on agronomic factors, genetic and fruit maturity [6-7]. Findings of such studies and changes in the diet of human beings caused a considerable increase in the consumption of pomegranate fruit. Large-sized fruits are of significant importance for premium markets and consumers prefer large-sized fruits with high total aril weight and juice content [8].

Many trees i.e. peach, apricot, plum, etc. including pomegranate have a tendency to produce high numbers of flowers [9]. However, when environmental and other conditions are adequate; and most of these flowers set; then trees have more fruit than they can adequately size to meet market standards [10]. Every individual fruit needs carbohydrates to grow but the carbohydrate source of each tree is particular and can't feed all fruits [11]. Therefore, excessive number of fruit causes fruits to be smaller than the market standards and also cause tree vigor to decline which then reduce the next season's yield too. When excessive fruit set occurs, removal of fruit is necessary in most species to ensure that remaining fruit attain marketable size and quality [10]. Correct fruit thinning allows each fruit to receive more light which improves fruit color and flavor too. Fruit thinning also prevents the occurrence of some pest i.e. mealy bug. Pomegranate tree generally begin to bear fruit in the third year but it is highly variety specific. Main flowering occur during September to October in southern hemisphere and March to April in northern hemisphere. Fruit becomes ready for harvesting in 5 to 7 months after full blossom depending on the cultivar [12]. Both self and cross-pollination are known in pomegranate. Pomegranate trees consist of both hermaphrodite and functionally male flowers. Fruit are derived from hermaphrodite flowers, and male flowers fail to set fruit [13]. Pomegranate fruits are mostly occurring on clusters and the general rule of fruit thinning is to leave alone the terminal fruit, by removing the axillary fruits [12]. Natural flower & fruit drop may occur on pomegranate trees which mean not every flower produce fruits. Therefore, recommended time for fruit thinning is about 3-4

weeks after full bloom when the fruits are about walnut size. Glozer and Ferguson [14] suggested allowing a closer spacing near the base of the branch and a wider spacing near the tip of the branch to avoid the branch bending. As a general statement, when the number of fruits is decreased on the tree, the fruit weight and quality increase [15-16]. However, according to authors' knowledge there are very few studies about the effects of number of fruits on the fruit size and quality on pomegranate. Thus, this research aimed to study the effects of fruit thinning on the size and quality of pomegranate cv. Wonderful fruits.

MATERIALS AND METHODS

The materials of present study were the fruits of 7-years old 'Wonderful' pomegranate tree grown in an orchard located in the west of Northern Cyprus. This cultivar was firstly originated in Florida and now is a leading commercial cultivar throughout the world. The fruits are of deep purple-red color and are large when comparing with most of other varieties. Fruits are known to require about 160-180 day from full bloom to ripening [12].

About 3-4 weeks after the onset of full bloom when terminal fruits on cluster reached about walnut size; fruit thinning was performed in May 2017. First of all, 50 uniform trees were randomly selected in the orchard, thus all axillary fruits (on clusters) of these trees removed by hand and only terminal fruits left on the each spur. After that, trees were grouped into 5 with 10 trees in each group. The groups are A) 80-89 fruits tree^{-1}; B) 90-99 fruits tree^{-1}; C) 100-114 fruits tree^{-1}; D) 115-134 fruits tree^{-1}; and E) 135-150 fruits tree^{-1}. Regular irrigation and fertigation performed for all selected and unselected trees in the orchard until harvest. All trees received same amount of fertilizer and water during this period. When fruits reached commercial maturity (~17% TSS and ~1.80 TA) fruits of selected trees were harvested and fruits of each tree were classified and graded into six different commercial sizes (size: 6, 7, 8, 9, 10 and 12) and an uncommercial category. Then, the total weight of each fruit size on every single tree was measured by using digital scale (sensitive to 0.01 g). After that, one fruit was selected from each size of each tree (total 60 for each group). Arils of selected fruits were extracted by hand carefully, counted and noted. Then, the arils were pressed and the juice volume of each fruit was measured. These data were used to calculate juice percentage of each fruit.

Data of the experiments were subjected to an ANOVA and mean separations were done using Tukey's test at $P<0.05$. Correlation analysis was performed to describe the relationship between number of fruits, fruit weight, number of arils, and juice content. SPSS 20 Software was used for all analysis.

RESULTS AND DISCUSSION

Results showed that number of the fruits on the tree has significant influence on the average fruit weight, number of arils, juice content, fruit sizes and total yield. Highest total yield was obtained from group B (with average 94.1 fruits tree^{-1}) as an average yield of 49.8 kg tree^{-1} while the lowest yield was measured from group A (average 80.7 fruits tree^{-1}) as 39.2 kg tree^{-1}. No significant difference was determined among the other three groups 46.6, 46.7 and 45.7 kg tree^{-1} for group C, D and E, respectively (Figure 1.).

On the other hand, it was determined that groups C, D and E (which has more than 100 fruits tree^{-1}), include some un-commercial fruits while the other groups (A and B) do not include. Most of these fruits were found to be out of the standard sizes where a few of them were cracked. No fruit cracking was noted from the commercial yield, which is an important finding indicating that right fruit thinning may also prevent fruit cracking. Results of present study are in agreement of the findings of Wetzstein et al. [8] but unfavorable with Mohsen and Osman [17] who reported decrease in the total yield when performing fruit thinning. However, there is difference between present study and the study of Mohsan and Osman [17] who performed fruit thinning by leaving axillary fruits on clusters too.

An important result found about the effects of fruit thinning on the average 'Wonderful' fruit weight in present study. Results exhibit that fruit thinning has a significant influence on the average weight of fruits even in the same sizes. Group B (average 94.1 fruits tree^{-1}) found to have the highest average fruit weight for all sizes; and Group E (144.8 fruits tree^{-1}) found to have lowest (Table 1.). According to the results, it can be concluded that leaving more fruits on tree not only reduce the total yield, but also reduce the average fruit weight for all sizes. The results are in conjunction with the findings of Greene et al. [18], Hussein et al. [19] and Mohsen and Osman [17]. In a similar study in Iran, Akbarpour et al. [20] reported that fruit weight of 12 cultivars ranged from 103.4 to 505.0 g in Iran. Moreover, Polat et al. [21] reported that the average fruit weight of 4 different varieties is ranging from 241.1 to 319.8 g in Turkey.

Divergently from these studies, present study showed that different number of fruits on the trees of same variety at same age also induces significant changes on the average weight of fruits. The highest average fruit weight obtained from group B (80.7 fruits tree^{-1}) as 456.0 g fruit^{-1} and lowest as 425.7 g fruit^{-1} from group E (144.8 fruit tree^{-1}).

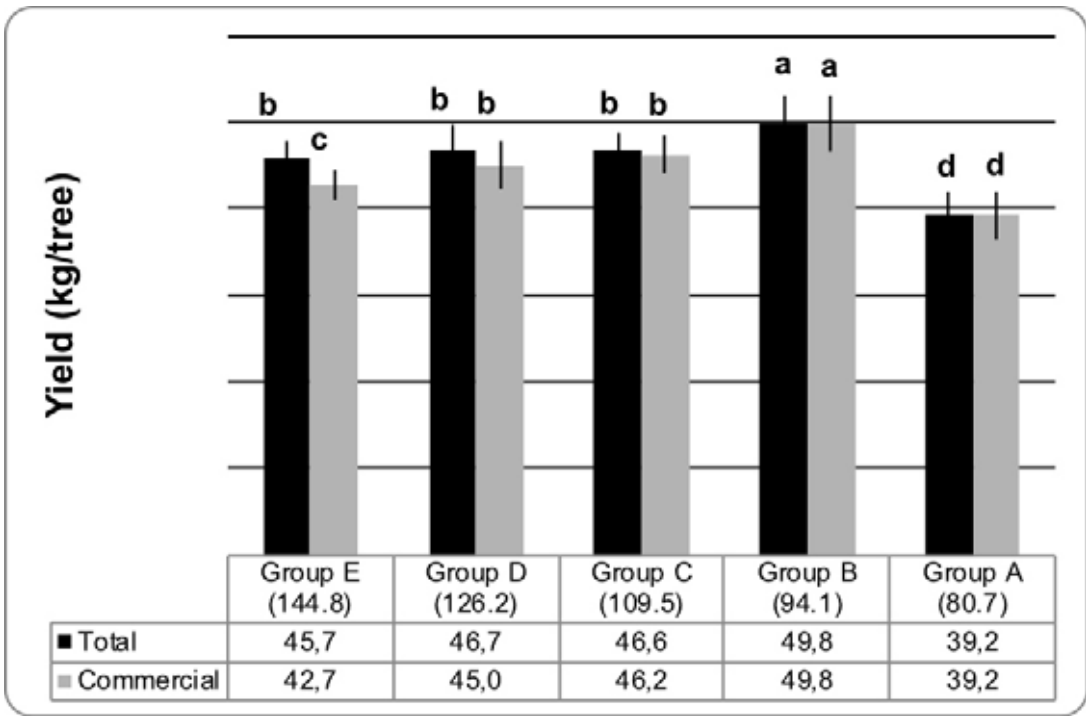

FIGURE 1
Total and commercial yields of 'Wonderful' pomegranate for different fruit thinning practices.

TABLE 1
Effect of fruit thinning on the average fruit weight of 'Wonderful' pomegranate fruit for different commercial fruit sizes.

Fruit Thinning	Average Fruit Weight (g)						
	Size 6	Size 7	Size 8	Size 9	Size 10	Size 12	Average
Group E (144.8)	647.7ab	504.9ab	445.9a	387.0a	318.1c	250.4c	425.7c
Group D (126.2)	654.8a	499.2b	465.8a	416.3a	330.5bc	275.7b	440.4bc
Group C (109.5)	669.1a	542.5a	450.4a	413.0a	349.8ab	278.7b	450.6ab
Group B (94.1)	668.3a	543.8a	456.4a	387.6a	365.1a	314.7a	456.0a
Group A (80.7)	656.6a	535.0ab	456.3a	384.5a	315.0c	264.2bc	435.3c

Distinct letters in the row indicate significant differences according to Tukey's test ($P \leq 0.05$).

Palmer et al. [22] reported that the leaf / fruit ratio has an important influence on fruit weight where more number of leaves is being devoted for each fruit, which support fruit growth and reduce the competition between the fruits. Therefore, fruit thinning increases the available carbohydrates which are responsible for increasing fruit weight and size [23].

Harvested fruits were also graded according to commercial sizes and as un-commercial; thus clear differences determined among the groups (Figure 2.). It was verified from the results that fruit thinning improves fruit size and prevents the occurrence of un-commercial fruits. Un-commercial fruits were only noted from group C, D and E which has more than 100 fruits on same tree.

It is also clear from the Figure 2 that fruit thinning improves fruit size. Size-6 represents the biggest fruits (6 fruits in a box of 40*30cm dimensions) and highest percentage of size-6 fruits measured from group B as 33.3%. Group A followed group B with 29.2% and third best result obtained from group C as only 14.8%. According to the obtained results, group E had only 1.4% size-6 fruits but had the highest percentage of size-12 as 25.3%. It is clear from the results that when the number of fruits on tree is increasing, fruits are becoming smaller and, vise versa. Jafari et al. [16] conducted a study to determine the effects of severity of hand-thinning on fruit size and quality attributes of 'Malase Torshe Saveh' pomegranate and reported that thinning out the fruits from 87 to 66, caused an increase in the average fruit weight from 292 g to 359 g. Similarly El-Mahdy et al. [24] reported that fruit thinning with GA_3 application provides highest number of grade I fruits.

Results showed that fruit thinning (number of fruits on tree) significantly influence the juice percent of fruits even in same size. This result is in accordance with the fruit weight results of present study and with the findings of Wetzstein et al. [8]. The juice content of pomegranate fruit is highly

related with the fruit weight. Similar with the other findings of present study, group B has found to have highest effect on the juice content of the fruits (Table 2). Highest juice percentage of fruits belonging to size-6 was calculated from group B as 41.0% and followed by group A and C as 39.5%. Similar results calculated for all sizes and averages too. Highest average juice content obtained from group B again with 35.5% and followed by group A, C, D and E as 34.3%, 34.2%, 33.8%, and 32.3% respectively.

According to the results, very weak negative correlation (-0.228) exists between the number of fruits and average fruit weight (Table 3.). The relationship was found to be non-significant. It was noted before in Table 1 that number of fruits on the tree influences the average fruit weight. However, the influence was not correlated with the number of fruits in which either more or few fruits significantly reduce the average fruit weight. The relationship between the number of fruits and number of arils was also found to be negative and weak correlation was observed (-0.411**). However, when analyzing the relationship between the number of arils and fruit weight, a strong positive correlation (0.961**) observed. Number of fruits found to have weak correlation with the fruit weight but high negative correlation (-0.806**) with juice percent. On the other hand, a high positive correlation exists between the number of arils and juice percent of the 'Wonderful' pomegranate fruit. Wetzstein et al. [8] reported that fruit size is highly correlated with both total aril and non-aril (pericarp and membrane) weights and suggested that number of arils per fruit is the main factor influencing fruit weight. Similar results obtained from present study, but not limited with the number of arils, but also number of fruits per tree is found to significantly influence fruit weight, number of arils and juice percent.

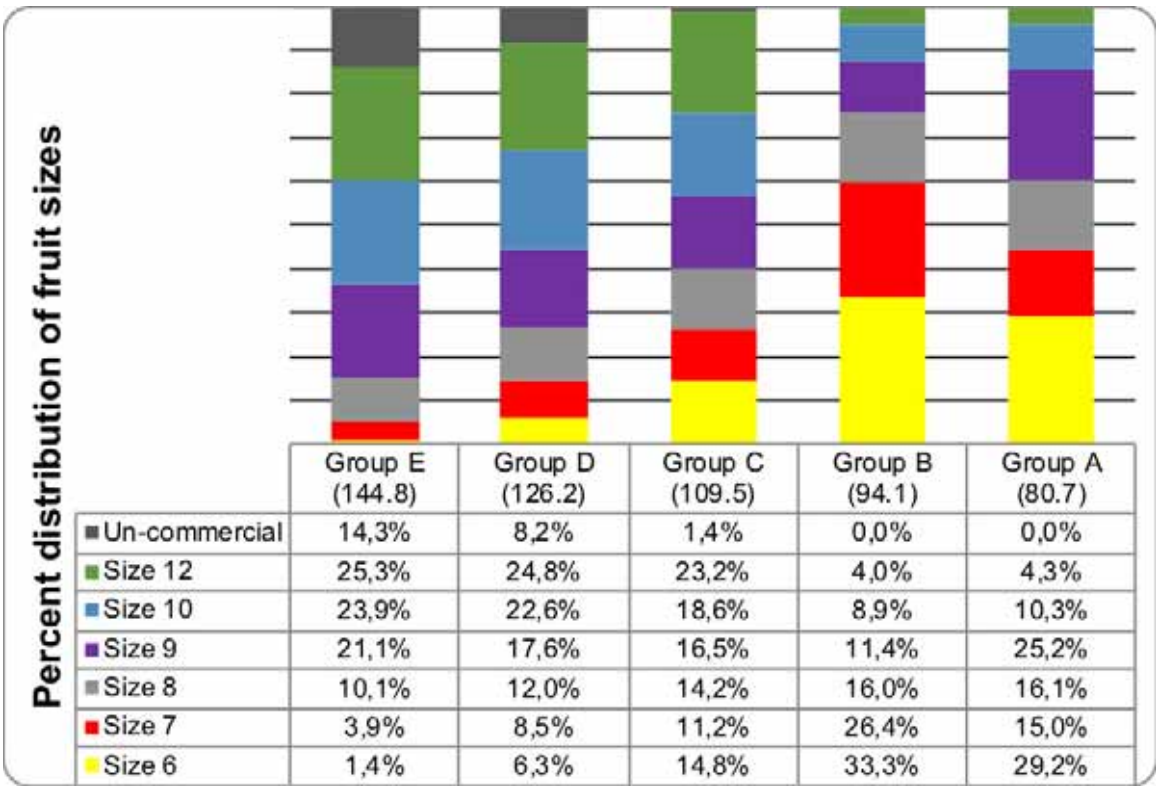

FIGURE 2
Effects of fruit thinning on the percent distribution of 'Wonderful' pomegranate fruit sizes.

TABLE 2
Effect of fruit thinning on the juice content of 'Wonderful' pomegranate fruit for different commercial fruit sizes.

Fruit Thinning	Juice Percent (%)						
	Size 6	Size 7	Size 8	Size 9	Size 10	Size 12	Average
Group E (144.8)	38.1%d	35.2%d	34.7%d	33.2%c	29.9%d	22.8%d	32.3%d
Group D (126.2)	38.9%c	36.2%c	35.6%c	34.6%b	31.4%b	26.0%c	33.8%c
Group C (109.5)	39.5%b	36.5%b	35.8%b	34.5%b	31.2%c	28.0%b	34.2%b
Group B (94.1)	41.0%a	37.5%a	36.3%a	35.4%a	31.8%a	30.8%a	35.5%a
Group A (80.7)	39.5%b	36.5%b	35.8%b	34.5%b	31.2%c	28.3%b	34.3%b

Distinct letters in the row indicate significant differences according to Tukey's test ($P \leq 0.05$).

TABLE 3
Correlation coefficients among the number of fruits, fruit weight, number of arils and juice percent of 'Wonderful' pomegranate fruit.

Parameters	Fruit Weight	Number of Arils	Juice Percent
Number of Fruits	-0.228	-0.411**	-0.806**
Fruit Weight		0.961**	0.563**
Number of Arils			0.741**

**Correlation is significant at the 0.01 level. Fruit n = 50.

CONCLUSIONS

Results of present study showed that the number of fruits on tree significantly influence fruit weight, juice percentage, fruit quality and total yield of 'Wonderful' pomegranate. According to the results obtained, leaving 90-99 fruits on each well-developed tree of 'Wonderful' variety provides highest yield (around 50 kg tree^{-1}). On the other hand, it was found that 90 fruits is critical and leaving less than 90 fruits on tree again ensures fruit quality and size, but significantly reduce tree yield up to around 39 kg. The upper limit for number of fruits tree^{-1} was found to be 100 which cause considerable decline in all of fruit weight, fruit sizes, fruit quality and total yield.

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INFLUENCE OF NITROGEN AND PHOSPHORUS LEVELS ON YIELD AND QUALITY OF QUINOA (*CHENOPODIUM QUINOA* WILLD.)

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ABSTRACT

This study was conducted to determine the effects of different levels of Nitrogen and Phosphorus fertilizer on yield and quality components of cultivar, Altiplano. The agronomic performance and nutritive value of quinoa was analyzed as an alternative dry-season feed for ruminants and food for human during growing seasons of 2016 and 2017 in Ankara. In this study, four different nitrogen were applied in randomized block design with three replicates. We studied herbage (H), hay (DM) and grain yield (GY), crude protein content in DM and grain, acid detergent fiber and neutral detergent fiber levels. The highest average herbage yield (47.940 kg ha^{-1}) was observed in N_3-P_3, the highest average crude protein content of DM (14.93%) was observed in N_3-P_2. The highest average grain yield (1.627,2 kg ha^{-1}) was observed in N_3-P_3, the highest average crude protein content of grain (16.80 %) in N_3-P_3. Our experimental results showed that applying N and P fertilizers significantly increases the yield and guality ($P<0.01$). These findings suggest optimal fertilizer (150 kg ha^{-1} N and 90 kg ha^{-1} P) levels for obtaining higher yield and quality of forage and grain which could be useful in increasing of food for humans and livestock production.

KEYWORDS:
Quinoa, nitrogen, phosphorus, yield, quality

INTRODUCTION

Quinoa (*Chenopodium quinoa* Willd.) is one of the most important economic crops belongs to the family Chenopodiaceae. It has fulfilled various roles in these ancestral cultures, in addition to its role in human and animal nutrition, quinoa had a sacred importance [1]. Quinoa (*Chenopodium quinoa* Willd.), a stress-tolerant species, has been cultivated along the Andes, from about 3000 B.C and is still being cultivated in Peru, Bolivia, Chile, Ecuador, Colombia and Argentina [2]. It is upright, reaching heights varying from 0.60 to 3.00 m, depending on the type of quinoa, genotypes, fertility of soil and environmental conditions where it grows. Beside the plant has high crude protein (14,85%) and low ADF (29,24%) and NDF (39.47%) content as compared to other forage plant that NDF content of sorghum 52%, sudanse 53,28%, sorghum- sudanense 50 % and maize 54%, and 8,69 % crude protein content [3]. Accordingly Altiplano genotype is a favorable plant for animal and human food because of it has high digestibility protein and saponin free of seed.

Nitrogen, phosphorus and potassium, are the primary nutrients. Each of these fundamental nutrients plays a key role in plant nutrition. Because nitrogen and phosphorus are an essential element required for successful plant growth. Nitrogen and phosphorus applications have been inevitable to increase the yield and guality of plant. Although inorganic nitrogen compounds (*i.e.*, NH_4^+, NO_2^-, and NO_3^-) account for less than 5% of the total nitrogen in soil [8]. Quinoa can be successfully grown on marginal soils showing its very low nutrient requirements [4]. The influence of N (calcium ammonium nitrate (27% N)) rate on grain yield was even stronger than for amaranth. Grain yield was enhanced to 94 % at N 120 compared to N_0, Erley et al. [5] informed that the application of 0, 119 and 238 kg N ha^{-1} with biofertilizers led to consistent increase the grain yield per hectare of quinoa as compared with untreated plants (control) over the years. Nitrogen level of 75 kg N ha^{-1} was proved to be optimum level for nitrogen supplementation of soil for quinoa growth and development to harvest maximum economic yield under ecological conditions of Egypt [6]. Kakabouki et al. [7] reported that nitrogen fertilization increased also the grain yield of quinoa under different tillage system.

Thanapornpoonpong et al. [8] explored the effect of different nitrogen rates (0.16 and 0.24 g N kg^{-1} soil) on protein content of seed and amino acid profile of amaranth and quinoa. Nitrogen fertilization effected amino acid content of quinoa and amaranth. Both had rich lysine contents (6.3-8.2 g 100 g^{-1} protein) but low methionine (1.28 g 100 g^{-1}protein). Thus, diets of humans can be improved by maintaining and increasing essential amino acid content and proteins by applying N fertilizer [6]. The protein of quinoa seed is rich in

essential amino acids, particularly methionine, threonine and lysine, which are the limiting amino acids in most cereal grains [9]. Quinoa contains gluten-free high-quality protein, so it can play an important role in the diet of people suffering from celiac disease [10, 11]. Quinoa responds to N and P application not only increase yield but also the quality of forage and grain.

The agronomic performance and nutritive value of quinoa was analyzed to define alternatives to local forages for dry-season feeding of ruminants and for quality food for human being. So, the investigation was carried out to determine the optimum level of nitrogen and phosphorus for getting maimum yield and quality of quinoa genotypes under central anatolia condition.

MATERIALS AND METHODS

The experiment was carried out during growing season (starting mid March) in Ankara at 39. 9334° N, 32. 8597° E and 938 m above the sea level. Experimental area is located in Central Anatolia zone of the country. Dry and hot in summers cold and snowy in winters. The used soil was a silty-clay loam (21,9 % clay, 22,2. % silt, and 20,4% sand) with pH 6.52, 1,16% organic matter, 0.073% salt, 0.128% total N, 1.11 ppm phosphorus and 229 ppm potassium. Total rainfall during the vegetation period of the plant was 182,6 mm, and 171,5 mm. Plants were irrigated two times because of insufficient rainfall (measured quantity about 90 mm).

The experiment was conducted in a completely randomized block design with three replications. In the study, 4 different nitrogen (N_0: 0 kg ha^{-1}, N_1: 50 kg ha^{-1}, N2: 100 kg ha^{-1} ve N3: 150 kg ha^{-1}) and 4 phosphorus levels (P_0: 0 kg ha^{-1}, P_1: 30 kg ha^{-1}, P_2: 60 kg ha^{-1} ve P_3: 90 kg ha^{-1}) were tested on Altiplano cultivar. Seeds were sown by hand into rows 35 cm apart and at a depth of 2-3 cm. Each plot was consisted of 6 rows with 5 m).

We investigated herbage (H), hay (dry matter=DM) and grain yield (GY), crude protein (CP) content in DM and grain, acid detergent fiber (ADF) and neutral detergent fiber (NDF) levels in DM. Dry matter (DM) yield, crude protein (CP) content, CP in experiment. The data obtained were tested to the analysis of variance with the MSTAT-C package program according to the randomized complete blocks experimental design, and differences between averages which were found significant were showed by the LSD test [12].

RESULTS AND DISCUSSION

Herbage (H) and hay (DM) yield. The results are summarized in Table 1,2. Nitrogen and phosphorus level effects were the main sources of variation in all characters tested. The analysis of variance of data revealed effect of nitrogen and phosphorus levels on mean H and DM yield were highly significant differences by a level of significance of of $P<0.01$. H and DM yield of quinoa responded to Nitrogen (N) and phosphorus (P). There were significant differences among fertilization treatments concerning H and DM yields. All fertilization (N and P) treatments resulted in values higher than those of the control. Concerning P levels, the average HY ranged from 42.920-36.980 kg ha^{-1}, while the highest mean yield (42.920 kg ha^{-1}) was recorded in (P_3), and the lowest yield (36.980 kg ha^{-1}) in P_0 applications. Besides, concerning N levels, the average HY ranged from 45.290-33.410 kg ha^{-1}, while the highest mean yield (45.290 kg ha^{-1}) was observed in N3 (150 kg ha^{-1}), and the lowest yield (33.410 kg ha^{-1}) in N_0.

The analysis of variance of data revealed N-P interaction was highly significant differences by a level of significance of of $P<0.01$. According to the mean result of the N-P interaction on herbage yield ranged from 47.940-29.830 kg ha^{-1}. The highest mean HY observed by 47.940 kg ha^{-1} in N_3-P_3, while the lowest yield by 29.830 kg ha^{-1} in N_0-P_0.

HY increase was significantly high (60,07 %) in N_3-P_3 plots. The same situation was recorded in DM yield in N_0-P_0 and N_3-P_3 (7.530 and 12.730 kg ha^{-1} respectively) combination. The enhancement of H and DM yield was up to level of N and P (Table 1, 2).

When the appropriate N and P levels (150 and 90 kg ha^{-1} respectively) are practiced, the average HY and DM yield increased about by 62-61% respectively as compared to N_0-P_0, and also is economical. In this connection, corresponds with studies of Ullah et al. [13] reported that Nitrogen not only enhances the yield but also improves the food quality. Optimum, rate of N increases photosynthetic processes, leaf area production, leaf area duration as well as net assimilation rate [14]. The maximum leaf area (LA) and total leaf biomass of plants are a determinant of higher crop yield [15].

Quinoa responds well to nitrogen fertilization Berti et al. [16], Schooten and van Pin-terhuis [17], and Erley et al. [5] Mahmoud and Sallam [18] reported that the importance of N application N contents increased by 7.9 and by 39.7% in hay over the control when the plants are fertilized by 14.28 and 28.56 g N m^{-2} respectively. Besides, N_2 fertilization at rates of 14.28 and 28.56 g m^{-2} increased the yield of biomass by about 33.5 and 60% more than the control under fresh and 10 dS m^{-1}saline water irrigation. Under irrigation with 20 dS m^{-1}, N applica-

tion by corresponded rates increased the biomass by 57 and 100%, respectively.

The average DM yield ranged from 11.170 9.341 kg ha^{-1}, while the highest mean DM (11.170 kg ha^{-1}) was observed in P_3 (90 kg ha^{-1}) and the lowest (9.341 kg ha^{-1}) in P_0, besides, the mean DM yield ranged from 11.550-8674 kg ha^{-1}, while the highest mean yield (11.550 kg ha^{-1}) was observed in N_3, and the lowest yield (8.674 kg ha^{-1}) in N_0. (Table 3). The analysis of variance of data revealed N-P interaction on mean yield was highly significant differences and ranged from 12.730-7.527 kg ha^{-1}. the highest mean DM yield (12.730 kg ha^{-1}) was observed in N_3-P_3 and the lowest (9.341 kg ha^{-1}) in P_0. In this connection Many researchers informed that nitrogen application enhance vegetative growth as well as the metabolism process in the plant and icrease in dry matter accumulation [13, 14, 15, 19]. Amjed et al. [20] reported that increasing levels of nitrogen significantly influence on grain yield of crop. The maximal grain yield of wheat 3.848 tons $ha^{-1}$was obtained through application of 180 kg N. Our findings are in accordance with those researcher's results. Quinoa responds positively to the nitrogen and phosphorus levels. Schooten and Pin-terhuis [17] reported that N level had a significant effect on DM content.

According to the result the increase in H and DM yield were observed depending on nitrogen and phosphorus levels. These results indicated that quinoa H and DM yield responded to N application. The more nitrogen and phosphorus are appiled, the more H and DM yield are obtained.

Crude protein (CP) content of dry matter. The analysis of variance of data revealed effect of N and P and N-P interaction on average CP was highly significant differences by a level of significance of P<0.01. CP content responded to N and P positively as a result of statistical analysis. According to the mean result of CP content the ranged from 13.98-11.56%. In terms of P effect while the average highest CP content (13,98 %.) was observed in P_3, and the lowest (12,79 %.) in P_0 (fertilizer free). In terms of N effect the average CP ranged from 14.74-11.56 % while the average highest CP (14.74 %) was recorded in N_3 (150 kg ha^{-1}), and the lowest (11.56 %) in "N_0". The analysis of variance of the data showed that N-P interaction effect on CP content was highly significant. According to the mean result of CP content the ranged from 14.93-10.86 %. The highest average CP content (14,93-14,85 %) were recorded in N_3 - P_2 - N_3 - P_3 while the lowest CP ratios were obtained from N_0 - P_1. Because nitrogen essential in the formation of protein, and protein makes up much of the tissues of most living thing, N level had more effect on CP content than P level (interaction p<0.01, Table 3). Schootenand and Pin-terhuis [17] reported that at 70 and 84 growing days there was a substantial effect of N level on CP content.

TABLE 1
Average herbage yield (HY) in different N and P level (kg ha^{-1})

Fertilizer Level	N_0	N_1	N_2	N_3	Average
P_0	29.830 i	34.790 g-i	40.210 d-f	43.100 a-e	**36.980C**
P_1	31.970 hi	36.470 f-h	42.340 b-e	43.730 a-d	**38.630BC**
P_2	34.78 0 g-i	38.370 e-g	44.250 a-d	46.400 ab	**40.950 AB**
P_3	37.040 f-h	40.920 c-f	45.770 a-c	47.940 a	**42.920 A**
Average	**33.410 C**	**37.640 B**	**43.140 A**	**45.290 A**	

LSD1 %

TABLE 2
Average hay (DM) yield in different N and P level (kg ha^{-1})

Fertilizer Level	N_0	N_1	N_2	N_3	Average
P_0	7.527 i	8.893 gh	10.020 df	10.930 b-d	**9.341 B**
P_1	8.217 hi	9.763 e-g	10.550 c-e	10.890 b-d	**9.853 B**
P_2	9.230 fg	10.380 de	11.520 bc	11.660 b	**10.700 A**
P_3	9.723 e-g	10.560 c-e	11.671 b	12.730 a	**11.170 A**
Average	**8.674 C**	**9.900 B**	**10.940 A**	**11.550 A**	

LSD %1

TABLE 3
Average CP content of DM (%)

Fertilizer Level	N_0	N_1	N_2	N_3	Average
P_0	10.92 h	12.89 ef	12.75 fg	14.58 ab	**12.79 C**
P_1	10.86 h	13.17 d-f	14.25 a-c	14.61 ab	**13.22 BC**
P_2	11.88 g	13.37 c-f	13.92 a-d	14.93 a	**13.53 B**
P_3	12.56 fg	13.77 b-e	14.75 ab	14.85 a	**13.98 A**
Average	**11.56 D**	**13.30 C**	**13.92 B**	**14.74 A**	

LSD 1 %

TABLE 4
Average acid detergent fiber levels (ADFL) (%)

Fertilizer Level	N_0	N_1	N_2	N_3	Average
P_0	30.21 b-d	31.11 a-c	29.61 cd	25.78 e	29.18 AB
P_1	29.34 cd	32.40 a	31.86 ab	26.25 e	29.96 A
P_2	30.87 a-d	30.31 b-d	29.15 d	25.23 e	28.89 B
P_3	30.38 b-d	31.70 ab	29.82 cd	25.08 e	29.24 AB
Average	30.20 B	31.38 A	30.11 B	25.59 C	

LSD 1 %

TABLE 5
Average neutral detergent fiber level (NDFL) (%)

Fertilizer Level	N_0	N_1	N_2	N_3	Average
P_0	44.54 a-c	47.12 ab	47.03 ab	39.75 d	44.61
P_1	42.05 cd	49.38 a	46.07 a-c	44.85 a-c	45.58
P_2	42.96 b-d	48.16 a	46.40 a-c	39.79 d	44.32
P_3	45.60 a-c	46.17 a-c	48.62 a	39.47 d	44.96
Average	43.79 B	47.71 A	47.03 A	40.97 C	

LSD 1 %

Acid detergent fiber level (ADFL). Digestibility is the most common nutritive parameter used in feeding standards for ruminants and is the basal unit when evaluating the nutritive value of forage [21]. ADF is insoluble protein, as the ADF level increase, digestible energy levels decrease. The results are summarized in Table 5. The analysis of variance of data revealed effect of N and P levels and N-P interaction on mean ADFLwere highly significant differences by a level of significance of P<0.01. According to the mean result of the ADFL ranged from 29,96-28,89 %, while the highest ADFL (29,96 %) was recorded in P_1, and the lowest (28,89 %) in P_2 levels. Likewise, the average ADFL ranged from 31,38-25,59 %, while the highest ADF (31,38 %,) was observed in N_2, and the lowest (25,59 %) in N_0. The analysis of variance of the data showed that N-P interaction effect on ADFL was highly significant. According to the mean result of ADFL the ranged from 32.40-25.08 %. The highest average ADFL (32.40 %) was recorded in N_1-P_1, while the lowest ADFL (25.08 %) was observed in N_3 - P_3. As the N level increase, ADFL decrease. Forage intake is affected by crude protein, fibre and ash content [22] Acid detergent fibre (ADF) is a major indicator of digestibility, negatively affects feed quality [23]. Corresponds with studies of Kering et al. [24] reported that N fertilization consistently decreased ADF content in berrnuda grass forage.

Kakabouki et al. [7] reported that there were significant differences between fertilization treatments concerning the ADF content. The highest ADF content was found under N2 (200 kg ha^{-1}) treatment. Correspond with study of Balabanlı et al. [25] reported that N fertilization significantly decreased native rangeland ADF content from 46.45 to 39.02% The ADF concentration of fertilized herbage were significantly lower in plots with additions N+P than in plots P free. The ADF concentration were affected by N+K fertilization. Increasing N fertilization decreased cellulose and lignin contents from 29.30 to 24.18 % and 6.85 to 2.77 %. Cellulose and lignin contents decreased from N+P and N+K fertilization. However, application of N, P and K did not affect hemicellulose content of native rangeland. In our study N and P aplications have contributed reducing of ADFL, and increasing of digestibility.

Neutral detergent fiber level (NDFL). The NDF level is one of the most parameters concerning the digestibility for ruminants. The results of NDFL are summarized in Table 5. In the light of information of analysis of variance of data revealed effect of N levels and N-P interaction on average NDFLwere highly significant differences by a level of significance of P<0.01. There were no significant differences among P fertilization treatments concerning the NDF content. That means P levels effect on NDFL was not significant statistically. N levels effect on NDFL was highly significant. According to the mean result of the NDFL ranged from 47.71-40.97 %, while the highest NDFL was recorded in N1 (100 kg ha^{-1}) and the lowest in N_3. (150 kg ha^{-1}). The analysis of variance of the data showed that N-P interaction effect on NDFL was highly significant.

According to the mean result of NDFL the ranged from 49.38-39.47 %. The highest average NDFL was recorded in N_1 and P_1, while the lowest NDFL was observed in N_3-P_3. As the N level increased, NDFL decreased. The NDFL were significantly lower in N_3-P_3 (39,47 %) than N_1-P_1 (49,38 %). In addition, the NDFL was 45,60 % in N0-P3, 39,47 % in N3-P3, and 39,75 in N3-P0. That means as N level increases, NDFL decreases. Corresponds with studies of Balabanlı et al. [25] reported that N and P fertilization significantly decreased native rangeland NDF content from 74.32 to 68.46%. The NDF concentrations were significantly lower in plots with additions N+P than in plots P free. Increasing N fertilization decreased cellulose and

lignin contents from 29.30 to 24.18% and 6.85 to 2.77%.

Grain yield (GY). The results are summarized in Table 6. The analysis of variance of data revealed effect of nitrogen and N-P interaction on mean GY were highly significant differences by a level of significance of of P<0.01. All fertilization (N and P) treatments resulted in values higher than those of the control. But Concerning P levels, the average GY ranged from 1167.41-1105,33 kg ha^{-1}, the effect of P levels may be e-tremely minor. Thus, P level was not statistically significant of P<0.01. In respect of N levels, the average GY ranged from 1.557-911.50 kg ha^{-1}, while the highest average yield was observed in N3 (150 N ha^{-1}), and the lowest yield in N_0.(N free). In our study, the grain yield of quinoa increased with the increasing nitrogen level from 0 to 150 kg N ha^{-1}.

The analysis of variance of data revealed N-P interaction was highly significant differences by a level of significance of of P<0.01. According to the mean result of the N-P interaction on GY from 1.627,20-904,00 kg ha^{-1}. The highest average GY observed in N_3-P_3, while the lowest yield by in N_0-P_3. As N and P levels increased, GY increased. As it was seen in the Table 7, GY increase was significantly high (80 %) in N_3-P_3, as compared to N_0-P_3. In addition to this, GY was observed 950.70 kg ha^{-1} in N_1-P_3, 1188.80 kg ha^{-1} in N_2-P_3, 1.627,20 kg ha^{-1} in N_3-P_3 level. On the other hand GY was observed by 921.80 kg ha^{-1} in N_0-P_0, by 928.20 kg ha^{-1} in N_1-P_0, by 1.085 kg ha^{-1} in N_2-P_0 and by 1.486,20 kg ha^{-1} in N_3-P_0. That is N levels effect on GY was higher than P levels, but according to numeric information the enhancement of GY up to level of N and P (1486,20 kg ha^{-1} in N_3-P_0 -1.627,20 kg ha^{-1} in N_3-P_3). Findings showed that all values of P levels effect were the same in N_3 level, but there were significant differences among other nitrogen treatments ($N_{0\text{-}1\text{-}2}$ and N_3) statistically. The increase of yield is depends on N and P level in soil. The GY increased in optimum N and P treatments.

In this connection, corresponds with studies of Mahmoud and Sallam [18] reported that nitrogen fertilization at rates of 14.28 and 28.56 g m-2 increased the seed yield where the application of nitrogen significantly increased the yield under non-saline and saline conditions of irrigation water. Besides, N contents in seed increased by 15,9 and 36,8% in seeds over the control when the plants are fertilized by 14.28 and 28.56 g N m^{-2}, respectively. Razzaghi et al. [26] and Schulte auf'm Erley et al. [27] reported that nitrogen improved both biomass and seed yield. Geren [29] reported GY in quinoa crops ranged from 867 kg to 3308 kg ha^{-1} there were significant differences between nitrogen treatments concerning the yield and the highest grain yield (3308 kg ha^{-1}) was found in the at 150 kg N ha^{-1} level. Erley et al. [5] stated that grain yield of quinoa was affected by nitrogen fertilization from 0 to 120 kg ha^{-1} being 1790 kg to 3495 kg ha^{-1}. Jacobsen et al. [4] informed that there was a significant grain yield increase when the amount of nitrogen fertilizer was increased from 40 to 160 kg N ha^{-1} and, the yield decreased by 24–1% when the nitrogen supply was reduced from 160 to 40 kg N ha^{-1}, while the yield decrease was 120 kg N ha^{-1} and 2–7% when the nitrogen supply was reduced to 80 and 120 kg N ha^{-1}, respectively. In addition, Shams [30] reported that the increases in quinoa grain yield per hectare with the increase in N fertilizer application from 90 up to 360 kg N ha^{-1} over the control treatment were 518%, 769%, 936% and 1394% in average of both years. Gomaa [30] informed that the application of 0, 119 and 238 kg N ha^{-1} with biofertilizers led to consistent increase the grain yield per hectare of quinoa as compared with untreated plants (control) over the years. Kakabouki et al. [7] reported that nitrogen fertilization increased also the grain yield of quinoa under different tillage system.

Crude protein content of Grain. The analysis of variance of data revealed effect of N, P and N-P on GCP content were highly significant differences by a level of significance of of P<0.01. All fertilization (N and P) treatments resulted in values higher than those of the control. According to the mean result of GCP content the ranged from 15,01-12,04 %. In regard to P, the average GCP content ranged from 15,01-13,27 %, while the highest average GCP content was recorded in (P_3), and the lowest in P_0. Besides, concerning N levels, the average GCP ranged from 15,96-12,04 %, while the highest mean GCP was observed in N3, and the lowest in N_0. The analysis of variance of data revealed N-P interaction was highly significant differences by a level of significance of of P<0.01. According to the mean result of the N-P interaction on GCP ranged from 16,80-11,51%. The highest mean GCP content observed in N_3-P_3, while the lowest in N_0-P_0. GCP increase was significantly high (45,9 %) in N_3-P_3 plots.

TABLE 6
Average grain yield (GY) (kg ha^{-1})

Fertilizer Level	N_0	N_1	N_2	N_3	Average
P_0	921.80 c	928.20 c	1085.10 bc	1486.20 a	1105.33
P_1	910.30 c	941.00 c	1125.50 bc	1564.80 a	1135.14
P_2	910.00 c	933.10 c	1154. 30 bc	1550.00 a	1136.84
P_3	904.00 c	950.70 bc	1188. 80 b	1627.20 a	1167.41
Average	911.50 C	938.30 C	1138.00 B	1557. 00 A	

TABLE 7
Average crude protein content of grain (GCP) (%)

Fertilizer Level	N_0	N_1	N_2	N_3	Average
P_0	11.51f	12.12 ef	13.41 def	16.05 a	13.27 B
P_1	12.21 def	12.89 def	12.94 def	15.28 abc	13.33 B
P_2	12.03 ef	14.09 bcd	13.75 cde	15.71 ab	13.89 AB
P_3	12.41 def	15.48 abc	15.33 abc	16.80 a	15.01 A
Average	12.04 C	13.65 B	13.86 B	15.96 A	

LSD 1 %

In this connection, corresponds with studies of Basra et al. [6] informed that the major fact that determines the grain protein content is nitrogen availability, and quinoa is highly responsive to nitrogen fertilizer and higher CP content, in a crop with high yield, can be obtained just by application of higher nitrogen quantities. The higher protein content at higher nitrogen levels was mainly due to the structural role of nitrogen in building up amino acids [9; 30]. The progressive increase in protein contents of quinoa seed with the increasing nitrogen rates were also reported by Jacobsen et al. [4], Shams, [29]. Erley et al. [5] informed that average CP content of quinoa cultivars (Faro and Cochabamba) increased gradually (12.3% to 14.6%, respectively) with the increasing nitrogen levels from 0 kg N to 120 kg N ha^{-1} and, Miranda et al. [40] reported an average CP content of 18.8% using cold resistance quinoa cultivars (Regalona Baer and Villarrica). Kakabouki et al. [7] also stated that increasing nitrogen level increased CP content of quinoa from 7% to 27% under different tillage system.

CONCLUSION

The study has shown that guinoa has high potential as an alternate forage crop concerning yield and guality, and its grain for human food and also possibilities of quinoa cultivation in arid affected areas in Turkey.

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FIRST REPORT OF THE PREDATORY SPIDER, OXYOPES LINEATUS LATREILLE (ARANEA: OXYOPIDAE) FEEDING ON THE TOMATO LEAF MINER, TUTA ABSOLUTA (MEYRICK) (LEPIDOPTERA: GELECHIIDAE)

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ABSTRACT

The Lynx spider belonging to the family Oxyopidae is an important hunting spider. Members of this family are all predatory in nature, attacking many herbivorous pests. This is the first report on the occurrence of the predatory spider, *Oxyopes lineatus* (Araneae: Oxyopidae) predating on tomato leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) from Iran. *Oxyopes lineatus* was found in both tomato greenhouses and open fields during the summer of 2015. The spider was observed to attack and consume *T. absoluta*. It would appear that this spider can be considered a natural control agent against tomato leaf miner; helping to naturally improve suppression of *T. absoluta* populations.

KEYWORDS:
Tomato leaf miner, spider, predator, natural control.

INTRODUCTION

The tomato leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) has been reported to cause devastating yield losses up to 80-100% in tomato crops [1]. This can pose a potential threat to greenhouse and open-field tomato production systems [2]. The pest originated from South America but has been recently introduced into the Mediterranean region [2]. According to Baniameri and Cheraghain [3], *T. absoluta* was first reported in Iran in 2011 in Uromiyeh (Western Azerbaijan province) in the North West.

Studies related to basic biology and population development of *T. absoluta* are limited and mostly focused in South American countries where environmental conditions are conducive for the development of the pest [4]. *Tuta absoluta* is a multivoltine pest with seven life stages, being most destructive at the larval stage where the pest makes larval galleries in plant parts, including leaves, terminal buds, flowers and fruits [5-7].

Tuta absoluta is an oligophagous pest and although it is inclined to attack tomato plants, it can also infest other Solanaceous crops such as potato, eggplant, tobacco and wild Solanaceous weeds [8]. Since the pest prefers apical buds, flowers or fruits, and forms a black frass, it is easily detectable. The pest only attacks the aerial part of potato plants and never the tubers [8-10].

Over recent years, non-chemical methods, in specific, use of natural enemies or biocontrol agents, have been widely considered as a measure to prevent the development of pesticide resistance [11-13]. Some of the biocontrol agents of tomato leaf miner have been reported to significantly suppress populations, thus maintaining them under economic injury levels [11-13]. Globally, approximately 20 hymenopteran parasitoid species have been documented [14-18]. *Trichogramma acheae*, a potential parasitoid of *T. absoluta* eggs, has already been released in commercial tomato greenhouses in the Mediterranean region of Europe [19]. Other egg parasitoids commonly used in Europe include *Macrolophus pygmaeus* (Ramber) (commercially available as *Macrolophus caliginosus*) and *Nesidiocoris tenuis* Reuter (Hem.: Miridae), with the latter being popular in the Mediterranean region of Europe [19]. Other biocontrol agents of *T. absoluta* are; the mirid *Dicyphus maroccanus* Wagner, the nabid *Nabis pseudoferus ibericus* Remane, two phytoseiidae mite species, *Amblyseius swirskii* Athias-Henriot and *Amblyseius cucumeris* (Oudemans), and *Stenomesius* sp. (Hym.: Eulophidae), a larval parasitoid [19].

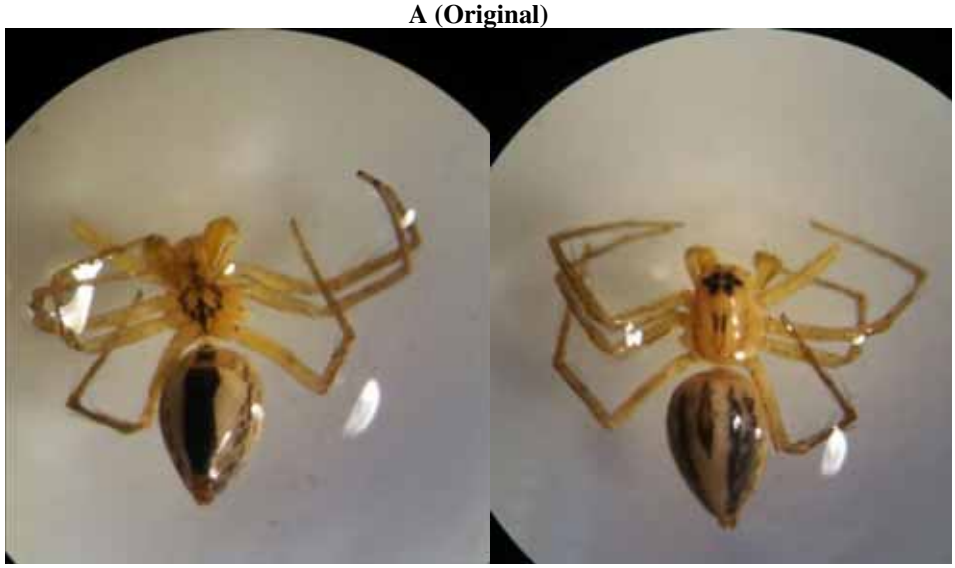
Spiders are naturally occurring invertebrates belonging to a predatory group that has limited information available on their potential to suppress herbivore populations. There are eighty-seven predatory families belonging to Aranea whose role in pest management has not been established [20]. Spiders from the family Oxyopidae are long-legged, diurnal, hunting arthropods with an ability of running rapidly on low vegetation and jumping

on their prey [21]. The genus *Oxyopes* Latreille, 1804, is globally distributed [22] and has been reported not to use silk threads to catch its prey [23]. *Oxyopes lineatus* is a predominantly European spider, present in Portugal, Spain, France, Italy, Slovenia, Belgium, Czech Republic, Slovakia, Switzerland, Turkey, Romania, Ukraine, and southern Russia [24]. The spider is generally found near the ground on small plants, particularly on bushes and grasses, where it chases and seizes its prey with a single leap [25]. *Oxyopes lineatus* preys on Diptera, Hymenoptera, Homoptera, Thysanoptera, Lepidoptera, Orthoptera, Coleoptera, Araneae and Acari [26]. *Oxyopes lineatus* is also a myrmecophagic spider, with roughly 20% of its diet being worker ants [26]. The aim of this study is to report on the predatory behavior of *O. lineatus* on *T. absoluta*.

MATERIALS AND METHODS

This investigation was carried out in both greenhouse (about 200^2) and tomato farms in Hamedan (34˚ 52′N 48˚ 32′E) west of Iran during the summer of 2015. Adult spiders were collected by use of an aspirator inside the tomato fruit worm cages. Specimens were placed in separate vials containing 75% ethyl alcohol and brought back to the laboratory for identification. The identifications and drawings were done by means of a SZX9 Olympus stereomicroscope with a camera lucida (Fig 1). Voucher specimens of *O. lineatus* were deposited in the Department of Medical Entomology, School of public Health, Tehran University of Medicine. Identification was carried out using the identification keys developed by Heimer and Nentwig [27] and Roberts [21].

A (Original)



B (Original)

FIGURE 1

(A) Female and (B) Male *Oxyopes lineatus*

(Original)
FIGURE 2
Oxyopes lineatus* feeding on an adult of *Tuta absoluta

RESULTS AND DISCUSSION

The spider was observed to predate on *T. absoluta* (Figs 2a, b). In this research, about 50-60 spiders were collected from the research greenhouse. The spiders were identified using the key as follows (taken from Heimer and Nentwig [27] and Roberts [21]):

1a. *O. globifer*. Colouring dark brown, with a large and blackish band peripherally and a yellowish colour in the middle part of the sternum; legs are yellowish, have brownish and blackish spots at the articulation; centre section of epigyne dark brown.

1b. *O. heterophthalmus*. Colouring dark brown, dorsally whitish; legs black with bright hairs; centre section of epigyne reddish-brown; male palp has large and conspicuous tibial apophysis.

1c. *O. lineatus*. Colouring yellowish-light brown with white design; in both sexes, a pair of dark stripes usually runs from the anterior median eyes, down over the clypeus, and along the front of the chelicerae.

General description of *Oxyopes lineatus*. *Oxyopes lineatus* is a medium-sized lynx spider that in adult stage measures 6-8 mm and 4-5 for females and males, respectively [25, 28]. The spider is yellowish to light brown in colour with a pattern of white markings. It is sexually dimorphic, with males being conspicuously smaller in size than females [25]. *Oxyopes lineatus* is an ambush-hunting spider that preys on insects and other small animals. The spider has long legs which facilitate rapid movement and does not use a web to trap its prey. The spider attacks its prey by jumping like a cat, hence its name 'lynx' spider. These lynx spiders have a more developed eyesight than spiders in other families, except Salticidae [29], and are capable of locating their prey from a distance of up to 10 cm [30]. They inject venom from their fangs, paralyze and consume their prey [31]. They are diurnal and prefer sunshine where they can be seen running and jumping over leaves and grasses. The spiders have a total of eight eyes; a pair of two large eyes in front, a pair, smaller in size below them, a pair, medium-sized, high up on the side of the head and the last pair is large in size and looks above and backward. With this kind of eye combination, the spider has an almost 3600 views of its surroundings. According to Huseynov [26], the spiders feed during the day and night. Studies done on *O. lineatus* indicate that it is a polyphagous predator feeding on a total of nine arthropod orders [26, 32]. [Fig. 1] [Fig. 2].

Implementation of a successful biological control program requires acquiring knowledge of a suitable biocontrol agent that naturally thrives in the specific region. Although spiders are among the most important naturally occurring biocontrol agents in different habitats [33], their role is less known. Apart from reducing pest density, they stabilize populations due to their top-down effects, microhabitat use, prey selection, polyphagy, functional and numerical responses, and obligate predatory feeding strategies [34]. A number of families of spiders are commonly found in agroecosystems and a majority have been reported to prey on major crop pest species [35-44]. Spiders may be regarded as the most important biocontrol agents of crop pests such as aphids, leafhoppers, plant hoppers, flea hoppers, and caterpillars [45]. However, a spider species that acts as a biocontrol agent in one

location could prey on beneficial insects in another location [34]. It is therefore imperative that further research is done to establish the extent of predation in an array of crops, climatic conditions and management approaches before conclusions concerning their efficiency as biological control agents are validated [36, 46]. There are some agroecosystems that spiders have been shown to capture important pest species. According to a study by Jeyaparvathi et al. [34], in non-commercial cranberry bogs, hunting spiders comprised 61% of total spider fauna; with 87% being lycosids. These spiders were reported to mainly prey on Collembola and small Diptera, which are not cranberry pests. However, there were very few hunting spiders that captured pest insects such as cranberry weevils or Lepidoptera larvae. A majority of these spiders captured their prey near the ground, their microhabitat being on or near the ground surface.

There are three important factors that contribute to spider migrations from an ecosystem. These include: inappropriate climatic conditions, shortage of food resources and disturbance(s) in ecosystems [47]. Although humans have no control on the inappropriateness of climatic conditions, a reduction in disturbance of ecosystems can significantly increase the diversity and density of spiders [48-49]. Pesticide use and crop harvesting are some forms of human disturbances to spider ecosystems or habitats [50]. In spite of this, use of some selected pesticides can be an effective way to protect spiders in agroecosystems [47]. Since these predators are not host-specific, they feed on all small invertebrates including beneficial insects. This situation has led to a limited comprehensive research on their effectiveness as a pest control method. There is need for more research on their role in biological control of invertebrate pests. In particular, further research on *O. lineatus* to determine its potential and efficiency as biocontrol agent for *T. absoulta* is recommended.

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SOME BIOCHEMICAL PROPERTIES OF POLYHENOLOXIDASE FROM SAGE (*SIDERITIS PERFOLIATA* L. SUBSP. *ATHOA* (PAPAN. & KOKKINI) BADEN.)

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ABSTRACT

In this study, a partial purified of polyphenol oxidase (PPO) obtained from sage (*Sideritis perfoliata* L. subsp. *athoa* (Papan. & Kokkini) Baden) was described that PPO is characterization through $(NH_4)_2SO_4$ precipitation and dialysis. The samples obtained from $(NH_4)_2SO_4$ precipitation and dialyses were used for the characterization of PPO. The characterization of PPO was studied in terms of substrate specificity, optimum pH and temperature. It was found that the enzyme had an activity with 4-methylcatechol, catechol, and pyrogallol substrates. Of these three substrates, 4-methylcatechol was the best substrate because of the highest V_{max}/K_M value, followed by catechol and pyrogallol. The optimum pH was at 5.0; 7.0 and 7.5 for 4-methylcatechol, catechol and pyrogallol substrates, respectively. The enzyme had an optimum temperature of 20, 20 and 50 ^{0}C for the 4-methylcatechol, catechol and pyrogallol substrates, respectively. The effect of different inhibitors such as ascorbic acid, glutamic acid and L-cysteine on partially purified polyphenol oxidase activity was investigated spectrophotometrically by using 4-methylcatechol, catechol and pyrogallol substrates. It was found the most effective inhibitor L-cystein using 4-methylcatechol and catechol, the most effective inhibitor ascorbic acid using pyrogallol. Protein amount of the enzyme extract was showed 14.06 mg/100g using by Bradford method.

KEYWORDS:
Polyphenol oxidase, sage, *Sideritis perfoliata* L. subsp. *athoa* (Papan. & Kokkini) Baden, substrat specificity, pH, temperature, inhibition.

INTRODUCTION

Sideritis species grow as herbs or small shrubs, both annual or perennial and aromatic [1]. The genus Sideritis belongs to the Labiateae Family, tribe Lamieae. This genus is distributed in the Northern Hemisphere, from Bahamas to Western China and from Germany to Morocco. Most species are mainly found in Turkey possess the highest number of different species. The genus Sideritis are established in Marmara and Aegean regions predominate in Turkey [1]. Sideritis species are used anti-inflammatory, antioxidant, antiulcerative, antimicrobial, vulnerary, antispasmodic, antibacterial, antifungal, anticonvulsant, analgesic and carminative agents [2].

Polyphenol oxidase (PPO) (EC 1.14.18.1) is a copper containing enzyme [3] which can be found in many living organisms such as fungi, mammals, birds, insects and a diversity of plants [4]. PPO is one of the most common browning agents in nature, affecting fruits and vegetables during handling, storage and processing [5]. Important point is that browning is a prominent problem in fruits and vegetables. The enzyme and substrates may come into contact, leading to rapid oxidation of phenols [6]. The prevention of these undesirable reactions has always been a challenge for food scientists.

However, we have not encountered any report of PPO of *Sideritis perfoliata* L. subsp. *athoa*, even though it is necessary to characterize the *Sideritis perfoliata* L. subsp. *athoa* -PPO for controlling enzymatic browning of plant products. Therefore, the characterization of the enzyme could help to develop more effective methods for controlling browning of plant cheese and products. In this study, we investigate the effects of substrate specificity, heat-inactivation, temperature, pH and inhibitor on PPO activity obtained from Sideritis *perfoliata* L. subsp. *athoa* (Papan. & Kokkini) Baden.

MATERIALS AND METHODS

Plant Material. Leaf of sage has been used as research material in this study. It was collected in Ulus Mountain in Turkey and stored at -80 °C until used in the study. All chemicals used in this study were the best grade available and were used without

further purification as they were obtained from Sigma Chemical Co. (Deisenhofen, Germany). Enzyme assays were measured UV–Vis Spectrophotometer (Perkin Elmer Lambda 35).

Extraction and partial purification of PPO. For preparing the crude extract, 10 g of the plant was homogenized in 100 ml of 0.1 M phosphate buffer (pH 6.5) containing 4 g. polyethylene glycol and 10 mM ascorbic acid by using a Waring blender for 2 min. The crude extract was filtered and the filtrate was centrifuged at 15 000 g for 10 min at 4°C. The supernatant was brought to 80% $(NH_4)_2SO_4$ saturation with solid $(NH_4)_2SO_4$. Inactive proteins were partially removed by ammonium sulfate precipitation. The precipitated PPO was separated by centrifugation at 15000g for 60 min 4°C. The pellet then at was dissolved in a small amount of 0.01 M phosphate buffer (pH 7.0) and dialyzed at 4 °C in the same buffer for 24 h with four changes of the buffer during dialysis. The dialyzed sample was used as the PPO enzyme source in the following experiments [7].

Assay of polyphenol oxidase activity. PPO activity was assayed by measuring the rate of increase in absorbance at a given wavelength using a double beam model a UV-VIS Spectrophotometer, as described previously [2]. The activity was determined using different substrates by measuring the increase in absorbance at 420 nm for 4-methylcatechol and catechol substrates and 320 nm for pyrogallol substrate. Total reaction volume was always maintained at 3.0ml. The sample cuvette contained 0.1ml of the enzyme, 2.3ml of 0.1M buffer solution and 0.6mlof 0.1M substrate solution. The linear portion of the absorbance versus time curve was used to determine the initial rates. One unit of PPO activity was defined as the amount of enzyme causing 0.001 increase of absorbance per minute.

Protein determination. After partial purification step enzyme was determined spectrophotometrically at 595nm according to the Bradford method, using bovine serum albumin as the standard [8].

Effect of pH. The optimum pH values for PPO obtained from leaf of sage was obtained using catechol as a substrate. The reaction mixture contained 0.6 ml of 0.1 M catechol, 2.3 ml of 0.1 M buffer solution and 0.1 ml of enzyme solution [9]. PPO activity was determined at pH values of 4.0, 5.0,6.0, 7.0, 8.0 and 9.0, respectively, using 0.1 M acetate (pH 4-6) and 0.1 M phosphate (pH 6–9) buffer adjusted with 0.1 M NaOH or 0.1 M HNO_3. Each assay mixture was repeated twice.

Effect of Temperature. PPO activity was determined between 20-70 °C. The effect of temperature on the sage PPO activity was assayed by heating the standard reaction mixtures to the appropriate temperature with circulating water-bath before introduction of the PPO. Once temperature equilibrium was reached, PPO was added and the reaction was followed spectrophotometrically at constant temperature at given time intervals. The reaction mixture contained 0.6 ml of 0.1 M catechol, 2.3 ml of 0.1 M buffer solution and 0.1 ml of PPO solution. Each assay mixture was repeated twice [10].

Effect of inhibitors. The inhibition kinetic analysis of sage PPO was determined for the most effective and food compatible inhibitors of PPO without inhibitor and in the presence of inhibitors at three different concentrations. Inhibition of PPO by ascorbic acid, L-cysteine, glutamic acid, benzoic acid and sodium azide as specific inhibitors of enzyme was measured for catechol and 4-methylcatechol and pyrogallol substrate at three different concentrations of inhibitor. Three millilitres of reaction mixture contained the substrate solutions at various concentrations in 100 mM phosphate buffer (pH 6.5), 0.1ml enzyme solution and the inhibitor solution at fixed concentrations. IC_{50} values were calculated from the plots of inhibitor concentration versus percentage inhibition of 4methylcatechol, catechol and pyrogallol oxidations, and inhibition constants (Ki) were deduced from the Lineweaver–Burk plots for each inhibitor.

DISCUSSION AND RESULTS

In our study, PPO obtained from sage was partially purified. PPO was determined substrate specificity, the investigation of substrate affinity, K_M and Vmax values, determination of affinity of enzyme with different inhibitors, determination of inhibition-type and Ki values.

Optimum pH. PPO activity, as a function of pH, was determined in a pH range of 4.5-9.0 in acetate buffer and phosphate. As seen from Table 1 PPO activity was assayed using 4-methylcatechol, catechol and pyrogallol as a substrate. Sage PPO has showed different pH optimums of 5.0, 7.0 and 7.5 using 4-methylcatechol, catechol and pyrogallol as substrates, respectively. In general, most plants show maximum PPO activity at near pH values [11]. The optimum pH for PPO activity differs among fruits. Furthermore, it is also found that the PPOs obtained from various sources have different pH values. For example, it is reported that optimum pH values are 5.0 for mulberry [12], 6.0 for *Ferula* sp. [14] and aubergine [15], 4.5 for strawberry [16] 6.5 for medlar [17]. 3.0 for napoleon grape [18] using 4-methylcatechol as a substrate, 8.0 for quince [19] and 7.0 for mulberry [8], *Ferula* sp. [14], and marula [20], 6.2

TABLE 1
Effect of pH and temperature on PPO activity using three different substrates

Substrates		pH						
		3	**3.5**	**4**	**4.5**	**5**	**5.5**	**6**
4-methylcatechol	**Activity (qmol min^{-1} mg^{-1}))**	265	4097	4187	5170	5373	4554	5172
Catechol		489	460	942	1225	918	1049	3520
Pyrogallol		60	640	925	1035	783	1083	1360
Substrates		**pH**						
		6.5		**7**	**7.5**	**8**	**8.5**	**9**
4-methylcatechol	**Activity (qmol min^{-1} mg^{-1}))**	4261		3528	1436	1110	773	405
Catechol		5150		6116	2958	4063	3271	2259
Pyrogallol		1450		6756	10689	0	200	0
Substrates		**Temperature (°C)**						
		20		**30**	**40**	**50**	**60**	**70**
4-methylcatechol	**Activity (qmol min^{-1} mg^{-1}))**	5373		4322	4413	4430	3340	2321
Catechol		6069		3042	2368	1099	597	0
Pyrogallol		6611		5511	5352	6982	0	0

TABLE 2
Substrate specificities, inhibition type, K_i, IC_{50} of *Sideritis perfoliata* L. subsp. *athoa* PPO (V_{max}(EU mL^{-1}min.$^{-1}$), K_M (mM), V_{max}/K_M(EU mL^{-1} min.$^{-1}$ mM^{-1}))

Substrates	Vmax	Km	Vmx/Km	Inhibitors	[I] (M)	K_i (M)	K_i'(M)	Inhibition type	IC_{50} (M)
4-methylcat-echol	5000	1.5	3333.3	L-Cysteine	1,33 x 10^{-4}	1,4 x 10^{-3}	-	Competitive	2,12x10^{-4}
				Ascorbic acid	2,0 x 10^{-2} 1,0 x 10^{-4} 1,6 x 10^{-4}	2,04 x 10^{-4} 1,01 x 10^{-4} 1,7 x 10^{-3}	- - -	Competitive	5,34x10^{-3}
				Glutamic acid	3,3 x 10^{-3} 1,0 x 10^{-2}	- -	9,9 x 10^{-3} 3,3 x 10^{-3}	Un-Competi-tive	1,36x10^{-2}
Catechol	11111	11	1010	L-Cysteine	3,3 x 10^{-3} 1,0 x 10^{-2}	6,7 x 10^{-6} 6,1 x 10^{-5}	- -	Competitive	9,87x10^{-5}
				Ascorbic acid	3,3 x 10^{-3} 6,7 x 10^{-3}	2,7 x 10^{-3} 2,8 x 10^{-3}	- -	Competitive	1,57x10^{-4}
				Glutamic acid	3,3 x 10^{-3} 6,7 x 10^{-3}	- -	2,7 x 10^{-3} 2,8 x 10^{-3}	Un-Competi-tive	1,25x10^{-2}
Pyrogallol	5000	5.0	1000	L-Cysteine	3,3 x 10^{-3} 1,0 x 10^{-2}	6,66 x 10^{-3} 2,0 x 10^{-2}	5 x 10^{-1} 5 x10^{-1}	Mixed type	5,11x10^{-3}
				Ascorbic acid	1,6 x 10^{-3} 8,3 x 10^{-2}	4,1 x 10^{-4} 2,1 x 10^{-2}	- -	Competitive	3,65x10^{-5}
				Glutamic acid	1,3 x 10^{-3} 1,0 x 10^{-2}	3,33 x 10^{-5} 2,0 x 10^{-2}	2 x 10^{-3} 1 x 10^{-2}	Mixed type	7,43x10^{-3}

for ripe peaches [21, 22], 5.0 for pear [13] using catechol as a substrate, 7.5 for mulberry [23], 7.3 for kiwi [24] and lettuce [27], 8.0 for pear [13] and artichoke [15], 8.6 for Amasya apple [9], 9.0 for *Ocimum basilicum* L. [25] using pyrogallol as a substrate. The differences between the optimum pH is closely related with obtained from different sources presence of various forms of PPO, the maturity status of the enzyme source, the degree of purification of the enzyme and the substrate type [26].

Optimum temperature. The effects of temperatures between 20 and 70 °C were assayed for each substrate and the results are shown in Table 1. As seen in Table 1, optimum temperatures are substrate dependent. The optimum temperature of activity for sage PPO was 20, 20 and 50 °C for 4-methylcatechol, catechol and pyrogallol as substrates, respectively (Table 1). It is reported that optimum temperature values are 20 °C for *Ocimum basilicum* L. [25], 40 °C for *Lactuca sativa* L. [27], 30 °C for olive obtained from Çukurova [28] using 4-methylcatechol as a substrate, 40 °C for *Ocimum basilicum* L. [25], 40 °C for *Lactuca sativa* L. [27], 25 °C for Victorian grape[29] using catechol as a substrate, 50 °C for *Ocimum basilicum* L. [25], 30 °C for *Lactuca sativa* L. [27] using pyrogallol as a substrate.

Substrate specificity. Sage PPO activity was tested for substrate specificity using 4-methylcatechol and catechol as diphenolic substrates and pyrogallol as a triphenolic substrate. In the literature, polyphenol oxidases was showed diphenolase activity in tomato seeds [28], DeChaunac grapes [30], *Solonum tuberosum* [31], Sorghum grains [32] and tri phenolase activity in strawberry [16], apple [33]. In order to investigate of enzyme kinetics, Lineweaver–Burk graphs are formed to calculate the K_M and Vmax values. Kinetics parameters, Vmax, K_M and Vmax/ K_M, calculated for sage PPO were given in Table 2. As seen in Table 2, sage showed different

polyphenol oxidase activity with respect to substrates studied. The Lineweaver–Burk plots, from which the kinetic parameters were derived, are shown in Figure 1. The plots shown are for 4-methylcatechol, catechol and pyrogallol substrates. The Km values for sage PPO; in order of decreasing affinity were 1.5, 11.0 and 5.0 mM, for 4 methylcatechol, catechol and pyrogallol, respectively. However, using Vmax/Km as the criterion for catalytic efficiency, the order of suitability as substrate for sage PPO is 4-methylcatechol>catechol>pyrogallol. In our study, similar results were found for basil with 1.62 mM K_M value [25], 1.33 mM for *Annona cherimola* MİII [34], sage K_M values were lower than 9.8 mM for *Thymus* [35] 11.6 mM for artichoke [15] using 4-methylcatechol as a substrate. Similar results were found for aubergine with 9.3 mM K_M value [10], 12.5 mM for tea, sage K_M values were lower than 34 mM for Amasya apple [9] 18 mM for *Thymus* [11] using catechol as a substrate. In our study, our K_M values were higher than *Lactuca sativa* with 3.0 mM [27]. Our results were lower than tea with 17.8 mM K_M value [36] and Amasya apple with 27 mM K_M value [9] using pyrogallol as a substrate. It has been found that K_M value for PPO activity has varied with the type of substrate, buffer and food sources [35].

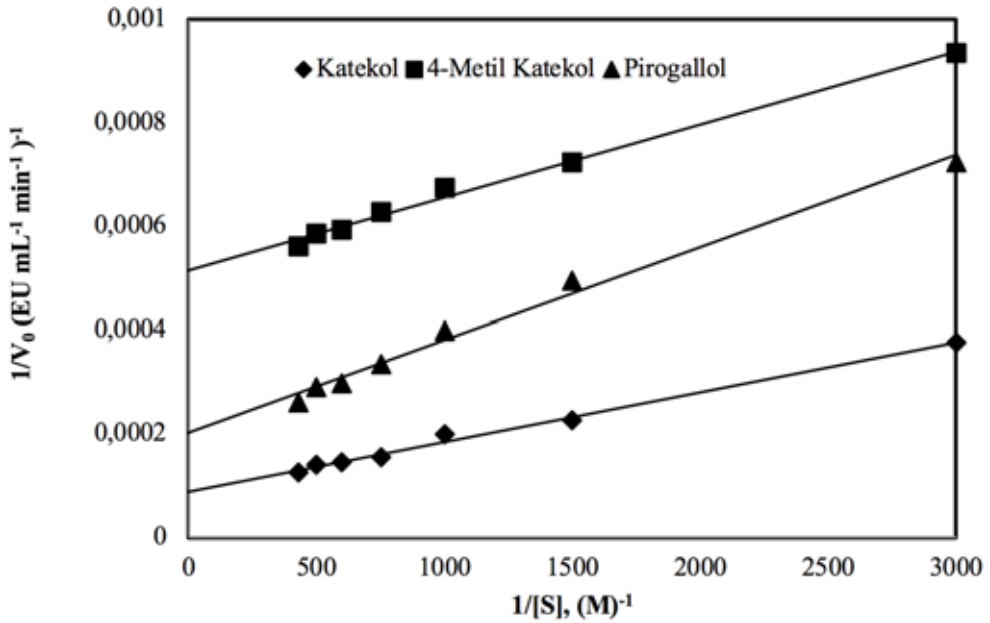

FIGURE 1
Lineweaver-Burk plots for *Sideritis perfoliata* L. subsp. *athoa* PPO

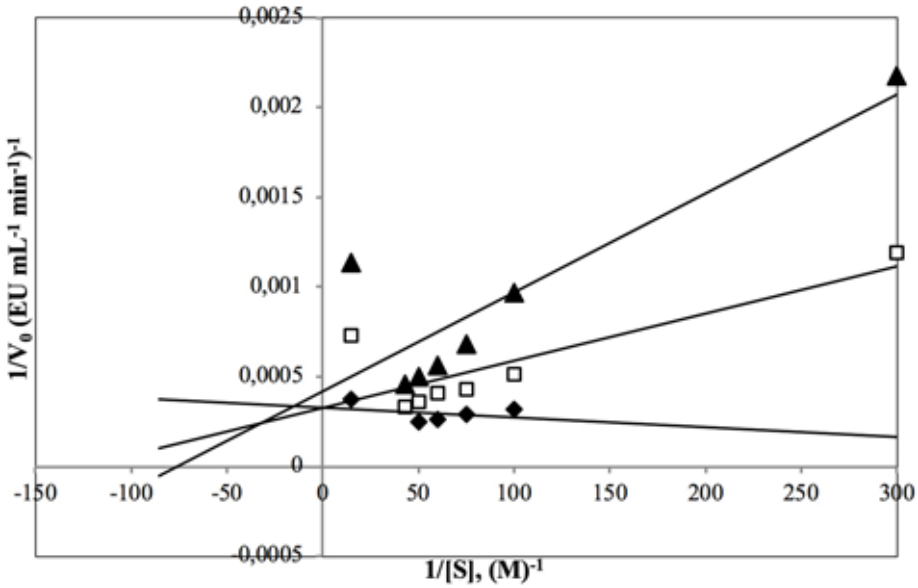

FIGURE 2
Lineweaver-Burk double reciprocal plots showing compatitive inhibition of *Sideritis perfoliata* L. subsp. *athoa* (Papan. & Kokkini) Baden PPO by ascorbic acid inhibitor using pyrogallol as a substrate

Effect of inhibitors. Vegetables, such as the enzymatic browning reactants oxygen and phenolic compounds from the environment by eliminating or PFO using inhibitors may delay or entirely avoidable [11].

The behaviour of sage PPO was examined with general PPOs inhibitors. In this study, glutamic acid, L-cysteine, ascorbic acid, benzoic acid and sodium azide were used as the inhibitors. As seen as in Table 2 the experimental results have showed that no inhibition effect for benzoic acid and sodium azide on sage PPO. Table 2 have showed that the inhibition type is competitive inhibition for L-cysteine and ascorbic acid using 4-methylcatechol and catechol as substrates. The inhibition type for ascorbic acid was determined competitive inhibition using pyrogallol as a substrate (Figure 2). Similar results were found for *Thymus* PPO [11] with glutathione inhibitor using 4-methylcatechol, pyrogallol and catechol as substrates; for field bean seed PPO [37] with L-cysteine, tropolone and ascorbic acid inhibitors using pyrogallol as a substrate, for palmetto PPO [38] with benzoic acid inhibitor using 4-methylcatechol as a substrate. Again, percent inhibition and Ki values for inhibitors used have been given in Table 2 for 4-methylcatechol, catechol and pyrogallol as substrates.

Uncompetitive type inhibition was obtained with glutamic acid inhibitor using 4-methylcatechol and catechol as substrates in Table 2. Similar result was obtained for mushroom PPO [7] with 2,3-diaminopyropionic acid inhibitor using pyrogallol and catechol as substrates, for *Ocimum basilicum* PPO [7, 39] with glutamic acid inhibitor using 4-methylcatechol as a substrate.

A mixed type inhibition was determined with L-cysteine and glutamic acid using pyrogallol (Figure 3) (other figures not shown) as a substrate for sage PPO. It has been reported that the action of cinnamic acid and tropolone inhibitors for potato PPO was found a mixed type inhibition [40]. In the literature, similar results were obtained for lettuce PPO [27] with tropolone and 4-aminobenzoic acid inhibitors using 4-methylcatechol as a substrate; with glutathione and ascorbic acid inhibitors using catechol as a substrate, with ascorbic acid inhibitors using pyrogallol as a substrate [27].

Table 2 shows IC_{50} values of L-cysteine, glutamic acid and ascorbic acid inhibitors using 4-methycatechol, catechol and pyrogallol as substrates. Linear regression method was used to determine whether the experimental data fit with the inhibition equations.

CONCLUSIONS

- The partial characterization and purification of *Sideritis perfoliata* L. subsp. *athoa* PPO is reported for the first time.
- It was found that the enzyme had an activity with 4-methylcatechol, followed by catechol and pyrogallol. Of these three substrates 4-methylcatechol was the best substrate because of V_{max}/K_M value.
- It was found the most effective inhibitor L-cystein using 4-methylcatechol and catechol, the most effective inhibitor ascorbic acid using pyrogallol.
- Protein amount of the enzyme extract was showed 14.06 mg/100g using by Bradford method.

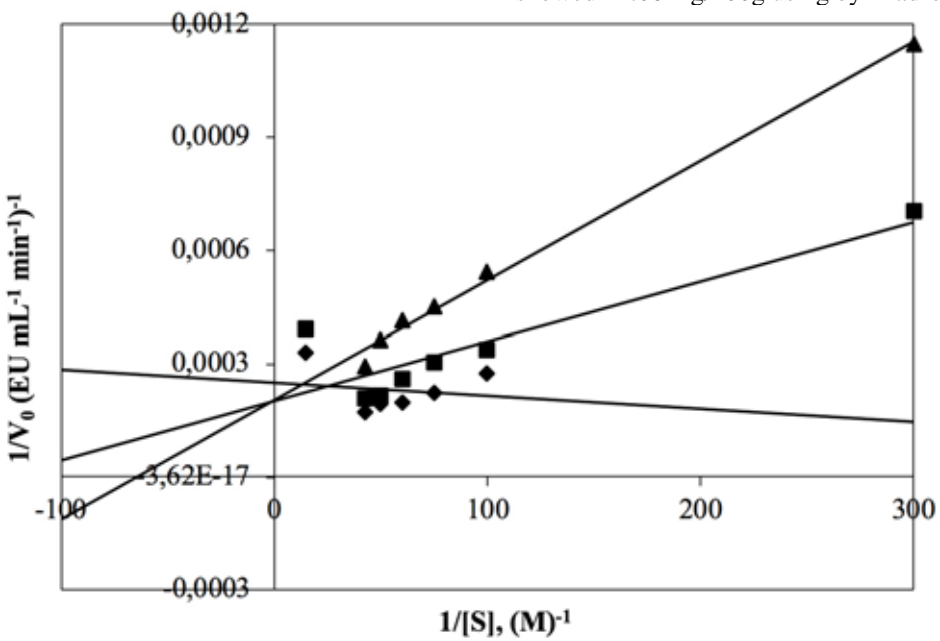

FIGURE 3

Lineweaver-Burk double reciprocal plots showing compatitive inhibition of *Sideritis perfoliata* L. subsp. *athoa* (Papan. & Kokkini) Baden PPO by L-cysteine inhibitor using pyrogallol as a substrate

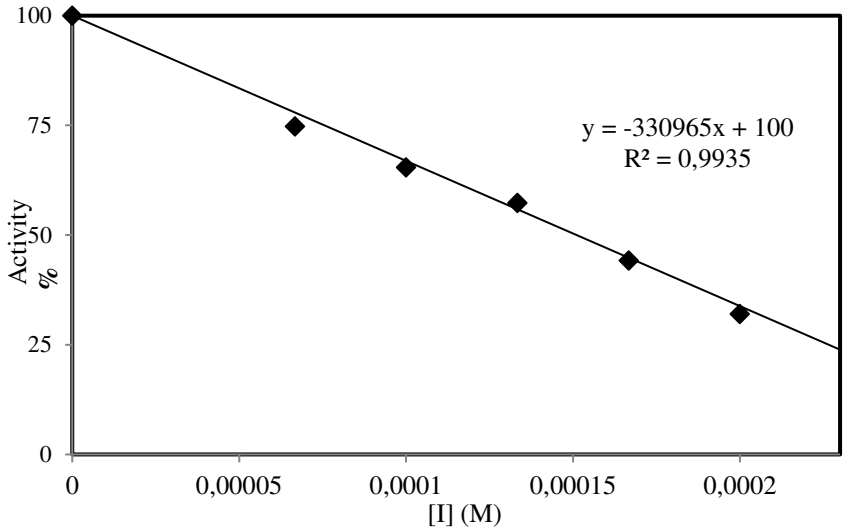

FIGURE 4
Activity % vs. inhibitor concentration curve for *Sideritis perfoliata* L. subsp. *athoa* (Papan. & Kokkini) Baden PPO by ascorbic acid inhibitor using 4-methyl catechol as a substrate

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