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ORIGINAL PAPERS

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MOLECULAR GENETIC DIVERSITY OF SOME *LAURUS NOBILIS* L. (LAURACEAE) POPULATIONS GROWN IN THE AEGEAN REGION/TURKEY

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ABSTRACT

In this study, molecular characterization of *Laurus nobilis* populations distributed in Aegean region was performed using ISSR markers. Genomic DNA isolation from the leaves of the plant samples was carried out using a commercial kit. Ten ISSR primers were employed to determine the molecular characterization between populations. Polymerase Chain Reaction (PCR) was carried out using DNA samples and primers. The PCR products were run on agarose gel electrophoresis and visualized under UV light. All gel images were examined and the presence and absence of polymorphic bands were scored as 0 and 1. A total of 46 bands were obtained from the primers. 22 of these bands were polymorphic and the polymorphism rate was found to be approximately 48%. Phylogenetic trees and genetic distances between populations were calculated using the PAUP 0 4.0b10 analysis program. The PAUP analysis indicated that the closest genetic distance is between the Seferihisar and Yeşilyurt populations with a value of 0.02174 and the farthest genetic distance was found between Aydın and Muğla populations with 0.30435. The phylogenetic tree was obtained using the UPGMA algorithm and the tree was composed of two groups. It was concluded that the ISSR markers polymorphism rate was slightly low among *Laurus nobilis* populations, and more information could be obtained by using different markers (AFLP, SSR, RAPD, etc.).

KEYWORDS:
Genetic diversity, ISSR, *Laurus nobilis*, Turkey

INTRODUCTION

With its geographical location, Turkey has quite a rich floristic structure. One of the reasons for the presence of such a rich floristic diversity in Turkey is that Turkey is located at the intersection of three phytogeographic regions such as Mediterranean, Europe-Siberia and Iran Turan. This situation increases both the diversity of species and the number of endemic taxa as well as the endemism rate and thus a rich floristic structure emerges [1]. Lauraceae, a large pantropic family consisting mostly of large trees or bushes, is composed of approximately 53 genera and 2500-3000 species [2]. *Laurus nobilis* L., belonging to the Lauraceae family, is an evergreen shrub that is indigenous to Europe and the southern parts of the Mediterranean region [3]. Leaves of *L. nobilis* are short and thick and the fresh leaves are yellow which turn into light green with light green veins. They also have a low aromatic odour. The fresh shoots are green, while the following is red, black and hairless. Their maximum length is known as 2 cm [4]. The dried leaves of *Laurus nobilis* are used as spices [5]. The essential oil obtained from *Laurus* leaves is used as a preservative and a flavouring agent in the food industry. In addition, these essential oils are used in melanoma inhibition, joint and muscle pain relief, preservation of cosmetic products, treatment of digestive system and skin problems, aromatherapy, massage products, and as an insect repellent [6].

The morphological features used in the classification of plants are used to determine the diversity and relationships between plant species. However, these properties are not sufficient due to the environmental impacts they are under the influence of. In recent years, molecular markers have been utilized to characterise and distinguish different species in a more explicit way [7,8]. ISSR method manifests itself as a method that is based on the random distribution of nucleotide units such as 2, 3, 4, 5 that replicate on the eukaryotic genomes independent from the locus and with high reproducibility [9-11]. ISSR markers have many advantages over other marker systems. The ISSR technique is simple, fast and less costly, like the RAPD technique. ISSR markers have higher replicability than RAPD primers due to longer primer length [12]. The technique is useful in areas of genetic diversity, phylogenetic studies, gene tagging, genome mapping and evolutionary [13-17].

The aim of this study, we performed a molecular characterization using ISSR marker for some *Laurus nobilis* populations grown in the Aegean region of Turkey.

MATERIALS AND METHODS

Plant Samples and DNA Extractions. *Laurus nobilis* populations used in the study were collected from certain regions in Aegean region (Aydın, Muğla, Denizli, İzmir- Çiğili, Seferihisar, Yeşilyurt, Manisa-Salihli) between July and September, 2018. Total genomic DNA was extracted using DNAeasy Plant Kit (GeneMark). DNA samples were stored at -20°C.

PCR Amplification. In order to visualize gDNA samples, 1.0% standard agarose gel electrophoresis procedure was performed. For ISSR-PCR amplification, eight ISSR primers were used (Table 1). ISSR amplification reactions were carried out in 25 µL volume containing 5 µL master mix (PCR buffer, $MgCl_2$, dNTP, Taq DNA polymerase), 1 µL ISSR primers, 2.0 µL gDNA (around 10 ng/µL), and 17 µL of ddH_2O. PCR amplification was performed with an initial denaturation step of 94 °C for 4 min, followed by 35 cycles of strand denaturation at 94 °C for 1 min, annealing at 50-52 °C for 1 min, and primer extension at 72 °C for 1 min, and a final elongation at 72 °C for 10 min. Amplification products were analyzed by electrophoresis on 1.0% agarose gels buffered with 0.5 X TBE (Tris-Borate-EDTA), stained with ethidium bromide and pictured under ultraviolet light.

Data Analysis. After PCR analyses were completed, DNA bands were scored by giving 1 in case that DNA was present and 0 in case that DNA was absent in the DNA bands. ISSR analyses were run in polymorphic bands by removing monomorphic bands. The genetic relationship of the local *Laurus nobilis* populations used in the study was analysed using the PAUP 4.0b10 [18] program and the UPGMA (Unweighted Pair Group Method of Arithmetic Average) phylogenetic tree of the same program was plotted according to the arithmetic averages of the genealogical trees. At the same time, the distance matrix between populations was determined using the PAUP 4.0b10 program.

RESULTS AND DISCUSSION

Recent advances in molecular biology have paved the way for the development of a new generation of markers to facilitate easier, more efficient and rapid dissection of plant genes. These DNA-based markers are versatile tools with many advantages over traditional phenotypic markers [12]. One of these markers, the ISSR technique, is used extensively in genetic variability studies on organisms [19]. In the ISSR-PCR analysis, a total of 46 bands were detected. 22 bands were polymorphic and the polymorphism rate was 48%. Polymorphic band ratio was highest in UBC-836 (75%) primer and at least in UBC-831 (28%) primer. PAUP 4.0b10 analysis program was used to calculate the phylogenetic trees and genetic distances between populations. According to the PAUP data, the closest genetic distance was 0.02174 between Seferihisar and Yeşilyurt populations, while highest genetic distance was 0.30435 between Aydın and Muğla populations (Table 2).

TABLE 1
Primers used in the ISSR-PCR reactions and their Tm degrees

ISSR Primers	DNA Sequences(5'-3')	Tm	Total bands	Polymorpic bands	Polymorphism rate (%)
UBC-831	5'-CTCTCTCTCTCTCTCTT-3'	50	7	2	28
UBC-830	5'-TGTGTGTGTGTGTGTGG-3'	52	4	2	50
UBC-807	5'-AGAGAGAGAGAGAGAGT-3'	50	5	2	40
UBC-819	5' - GTGTGTGTGTGTGTGTA -3	50	3	2	66
UBC-808	5'-AGAGAGAGAGAGAGAGC-3'	52	5	2	40
UBC-836	5'-AGAGAGAGAGAGAGAGYA-3'	52	4	3	75
UBC-856	5'–ACACACACACACACACYA-3'	52	4	2	50
UBC-853	5' - TCTCTCTCTCTCTCTCRT -3'	52	6	3	50
UBC-855	5'-ACACACACACACACACYT-3'	52	5	3	60
UBC-810	5' -GAGAGAGAGAGAGAGAT-3'	50	3	1	33

TABLE 2
Pairwise distance matrix obtained from ISSR primers

Populations	1	2	3	4	5	6	7
Seferihisar	-	0.15217	0.13043	0.10870	0.21739	0.02174	0.21739
Salihli	7	-	0.06522	0.13043	0.19565	0.17391	0.23913
Çiğli	6	3	-	0.10870	0.21739	0.10870	0.26087
Denizli	5	6	5	-	0.19565	0.13043	0.28261
Aydın	10	9	10	9	-	0.23913	0.30435
Yeşilyurt	1	8	5	6	11	-	0.23913
Muğla	10	11	12	13	14	11	-

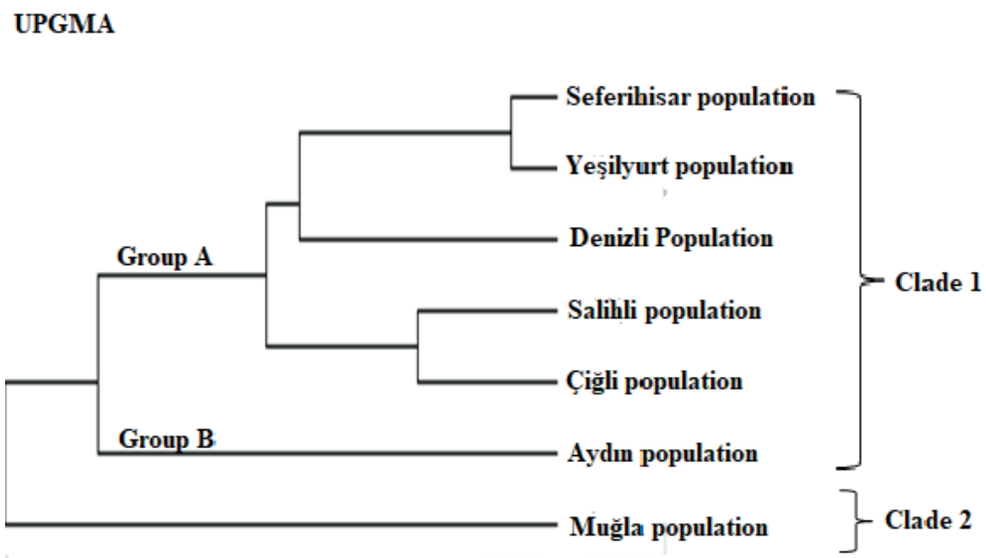

FIGURE 1
The UPGMA tree generated ISSR data of *Laurus nobilis* populations

The phylogenetic tree was obtained using the UPGMA algorithm, and the tree was consisted of two clades. Clade 1 is divided into two groups within itself. In Group A, Seferihisar, Yeşilyurt and Denizli populations formed a group. Within this group, the population of Seferihisar and Yeşilyurt is located in the province of İzmir in the Aegean region, whereas Denizli population is more distant geographically. Salihli and Çiğli populations formed a separate group. The Aydın population was close to these two subgroups. Clade 2 consisted of only Muğla population (Figure 1). Bulut et al. [20] identified the genetic characterization of 95 Laurus nobilis chosen from Turkey/Hatay flora using SSR markers. In the SSR analysis, 6 primers were used and a total of 82 alleles were detected in 5 polymorphic loci with an average of 16.4. Arroyo-García et al. [21] revealed the genetic relationships of 14 Laurus nobilis and Laurus azorica populations from different geographical regions including the Canary and Madeira Islands, France and Italy using the AFLP molecular marker technique. The results of the study showed that AFLPs are very useful in analysing the genetic similarity between the natural populations of Laurus. Arroyo et al. [22] identified the genetic diversity of 63 populations of Laurus nobilis and Laurus azorica species using 20 newly designed SSR markers. They found 196 alleles of 37 genotypes belonging to Laurus nobilis species. Additionally, it has been shown that SSR markers are useful tools for determining genetic diversity the populations of the Laurus species.

CONCLUSION

As a results of, in this study, the genetic relationships of seven *Laurus nob*ilis populations distributed in the Aegean region were revealed by using ten ISSR primers. The results based on ISSR data indicated the genetic distance and phylogenetic relationship between the populations and groups were formed. Also, this study aimed to shed light on the relationship between different populations grown in the region. Undoubtedly, more data with more taxa (e.g., morphological and/or DNA sequencing data) will contribute to the implications and lead to more reliable results.

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EVALUATION OF ESSENTIAL OIL COMPOSITION OF *ORIGANUM ONITES* L. (LAMIACEAE) PLANT AND ANTIFUNGAL ACTIVITY ON SOME STRONG PATHOGEN FUNGI

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ABSTRACT

The Lamiaceae family is widely distributed around the world and contain rich essential oil. The members of this family have medical properties and some of them are commercially important products. In this study, chemical composition and antifungal activity of the essential oil *Origanum onites* L*,* towards some pathogenics fungi were investigated. Extractions were carried out with Clevenger apparatus and essential oil composition was determined by Gas Chromatography-Mass Spectrometry (GC-MS). Specifically, *O.onites* was found to be rich in carvacrol, 37.08%. Essential oil of *O. onites* has no activity against *C. parapsilosis. S. cerevisiae* was the most sensitive fungi against the tested essential oils. Essential oil of *O. onites* exhibited strong antifungal activity even higher than nystatine.

KEYWORDS:
Origanum onites, essential oils, antifungal, Turkey

INTRODUCTION

The Lamiaceae family comprises numerous species that are considered aromatic plants due to their high contentof essential oils [1]. Turkey is regarded as an important gene center for the family Lamiaceae [2]. The aerial material of most aromatic plants belonging to this family, such as members of the genera *Origanum*, *Thymbra*, *Satureja*, *Thymus*, *Sideritis, Salvia*, *Teucrium, Mentha*, etc., are added to foods for their organoleptic properties and are often consumed as herbal teas in Turkey [3,4]. The genus *Origanum*, which is represented by 43 genus, 9 subspecies and 12 hybrids in the world, has a total of 32 taxa in Turkey's Flora. Turkey significantly hosts the genes of many species of the stock *Origanum L.* just like many other species [5]. *Origanum onites* L, thyme is cultured species, most exports among the species that are traded in Turkey are among the species being performed. *Origanum onites* plants and leaves forming a collection of leaves as spices are consumed [6]. The aim of the present study was to determine the essential oil contents of Lamiaceae family belonging to plants*, Origanum onites,* growing in Aydın ecological conditions and to investigate antifungal effect on some strong pathogen fungi.

MATERIALS AND METHODS

Plant materials and Isolation of essential oils. *O. onites* aerial parts of the plant was collected as study materials in July and in August 2015, which are their blooming periods from Aydın/Turkey. Approximately, 100 g of plant samples were used for the essential oil extraction process. Extraction was performed with Clevenger apparatus using water distillation.

GC-MS analysis. Qualitative and quantitative essential oil analysis were conducted at Eskisehir Anadolu University Medicinal Plants, Drugs and Scientific Research Center by Hewlett Packard 5973 Mass Selective Detector System and GC-MS 6890 instrument equipped with an Agilent HP-Innowax colon (60 m X 0.25 mm film, 0.25 µm thickness). Hellium was used as a carrier gas. Conditions were as follows; from 50 ºC to 240 ºC by an increase of 4 ºC / minutes. At 240 °C, 40 minutes of waiting time were implemented. Injection port and detector temperature were 240 ºC and 280 ºC respectively. 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. *Candida albicans* and *Candida tropicalis* were obtained from Fırat University Department of Biology; *Candida parapsilosis* were obtained from Giresun University Faculty of Education, *Saccharomyces cerevisiae* was obtained from Giresun Province Control Laboratory.

Antifungal Activity. The antifungal activity of the essential oils was determined by disc diffusion method. The essential oils were sterilized by filtration through a 0.45 µm membrane filter [7]. The turbidity of fungal suspensions were adjusted with 0.5 Mc Farland standard (10^7 CFU/mL fungi concentration), then, the fungal suspension spread on petri dishes [8]. The discs (6 mm diameter) were put on the inoculated agar and separetely impregnated with 25 µL of essential oils. Nystatine was used as positive control. Plates were kept at 30 °C for 48 h. Antifungal activity was assessed by measuring the diameter of the growth-inhibition zone in milimeters [9].

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 MIC, a micro-dilution broth susceptibility assay was used. Two-fold serial dilutions (in Dimethyl Sulphoxide (DMSO) were prepared from 0,0122 µL/mL to 25 µL/mL of the essential oil of *O. onites* in a 96-well microplate. Plates were incubated 30 °C for 48 h [10,11].

TABLE 1
Essential oil compositions of *O.onites* plant.

O.onites **RT**	**Components**	**Percent (%)**
11.55	*α*- pinene	1.34
11.82	*α*-thujene	1.36
13.75	camphene	0.93
19.09	*β*-myrcene	2.85
19.81	*α*-terpinene	2.45
20.73	limonene	0.59
21.21	sabinene	0.50
23.18	*γ*-terpinene	7.10
24.44	*o*-cymene	10.97
24.84	*α*-terpinolene	0.57
31.57	1 octen 3-ol	0.61
32.24	*β*-terpineol	0.55
35.13	linalool	0.80
35.33	cis-sabinene	0.52
35.58	linalyl acetate	0.55
37.37	terpinene- 4-ol	6.97
40.41	*α*-terpineol	0.70
40.68	borneol	6.08
41.07	germacrene D	0.55
41.40	*β*-bisabolene	5.74
48.70	piperitenone oxide	1.86
49.71	caryophyllene oxide	1.07
54.08	thymol	1.51
55.40	carvacrol	37.08
Total		**93.25**

RESULTS AND DISCUSSION

Chemical composition of the essential oils. Essential oils, are complex mixtures are obtained different from organs of plants by distillation. Most of these compounds are included terpenoids (isoprenoids), monoterpenes and sesquiterpen. Composition and quantities of essential oils depends on type of plant, climate conditions, different organs of the plant [12]. In our study, totally 61 component were detected as *O.onites* aerial parts essential oil composition. 93.25% of the total essential oils in 24 components (components which are ≥0.4% in total ratio) were given in Table 1. The essential oils obtained from the *O. onites* plant were detected to contain carvacrol (37.08%), *o*-cymene (10.97%), *γ*-terpinene (7.10%), 3-cyclohexen-1-ol (6.97%), borneol (6.08%), *β*-bisabolene (5.74%), at most (Table 1). This finding is parallel with the results of other studies conducted by different researchers [13-17]. Carvacrol, the main component of thyme oil and thyme water, is known to have a much higher antioxidant activity than the various synthetic antioxidants. This compound; It is a volatile monoterpene with antimicrobial, antifungal, natural food preservative and retarding properties in mammals. It has also been suggested that carvacrol is a strong antimutagenic and antitumor effect [18,19].

TABLE 2
Inhibition zones of essential oil of *O. onites* (mm)

Fungi	*O. onites*	Nystatine	DMSO
C. albicans	37	30	-
C. tropicalis	35	30	-
C. parapsilosis	-	25	-
S. cerevisiae	42	17	-

TABLE 3
MIC values of essential oil of *O. onites* (µL/mL)

Fungi	*O. onites*
C. albicans	12.5
C. tropicalis	12.5
S. cerevisiae	3.125

Antifungal Activity. There are many synthetic and natural product-based drugs available for treating fungal infections, but they are not consistently effective. Furthermore, the development of resistance in fungi against most of the drugs has now been reported for several years. The use of amphotericin B, known as the "gold standard", is limited because of its infusion-related problems and nephrotoxicity. [20]. Table 2 summarizes antifungal efficiency of essential oil of *O. onites*. Essential oil of *O. onites* has no activity against *C. parapsilosis. S. cerevisiae* was the most sensitive fungi against *O. onites*. The tested essential oils were more active than standard antifungal agent nystatine. DMSO showed any activity towards the fungi. Table 3 shows values of MIC. The values ranges from 3.125 to 12.5 µL/mL for *O. onites*. Essential oils exhibited the lowest MIC value against *S. cereviae*. Essential oils from *Origanum onites* (Turkish oregano) that are rich in phenolic compounds (carvacrol, thymol), possess antifungal properties against pathogenic or nonpathogenic fungi [21-25]. Manohar *et al*, found

that origanum oil effectively inhibits the in vitro growth of *C. albicans*. *Origanum* oil at 0.25 mg/mL was found to completely inhibit the growth of *C. albicans* in culture. Growth inhibitions of 75% and >50% were observed at 0.125 mg/mL and 0.0625 mg/mL level, respectively [26].

CONCLUSION

As a result, essential oil components and antimicrobial effects of *O. onites* plant having a medical importance were questioned as understandable with these studies. In studies made with both plants, essential oils from more plants were isolated, and these are tried on fungi. Our study was on these plants which are similar and grow in West Anatolian ecological conditions. However, considering factors such as different methods, different geographical regions where growing plants are different, different collection periods thereof, used fungi strains and essential oil amount soaked into disc, the fact that different results were obtained than previously conducted studies was regarded as normal. Observation of antifungal effect even in one microorganism shows us that plants containing etheric oil may be used for treatment purposes and be an alternative to synthetic antibiotics.

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DETERMINATION OF THE ANTIFUNGAL ACTIVITY OF *ALKANNA ORIENTALIS* PLANT EXTRACTS ON THE PLANT PATHOGENS

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ABSTRACT

As a result of the negative effects of pesticides used in agricultural areas, the studies regarding finding alternative methods have increased. In this study, antifungal activities of *Alkanna orientalis* methanol and water extracts against *Fusarium oxysporum* f. sp. *Lycopersici* (FOL)(Sacc.) W.C. Snyder and H.N. Hans, Alternaria solani (A.S)(Ell. and G. Martin), *Verticillium dahliae* (VD) *Rhizoctonia solani* (R.S) Kühn.,and *Sclerotinia sclerotiorum* (S.S) Lib De Bary plant pathogens, which cause considerably yield losses in tomatoes, potatoes, and cucumbers in the world and in Turkey were determined.

In order to evaluate the plant extracts used, the mycelium inhibition (MGI) ratios were calculated for comparing with 80% thiram, which is a standard fungicide. As a result of the study, 10000 ppm dose of the water extract used on the plant pathogens prevented the FOL, *A. solani, V. dahlia,* and *S. sclerotiorum* mycelium growth at the rates of 39.58, 37.22, 27.27 and 20.72%; however, it had no effect on *R. solani* mycelium growth. While the methanol extract of the plant showed the highest effect on *V. dahliae* with the rate of 56.43% compared to the negative control, this was followed by 55.22% (FOL), 49.44% (*A. solani*), 29.66% (*S. sclerotiorum*), and 8.45% (*R. solani*). Consequently, currently used natural antifungal bio-pesticides have a potential to control against the plant pathogens because they are cheap and environment-friendly.

KEYWORDS:
Alkanna Orientalis, Antifugal activity, Plant patho-genes

INTRODUCTION

Finding environmental-friendly methods in accordance with the integrated control principles is important in order to replace the chemicals in the control of plant diseases. In addition, in order to sustain agriculture, investigation and application of alternative methods for chemical control appear as a necessity. One of these alternative methods is to determine the plant- origin compounds and to use them in the control of plant diseases, pests and weeds. There are many studies on the fungicidal, herbicidal and insecticidal effects of the compounds and essential oils within the plants and also their biological activities [1-3].

Alkanna genus (Boraginaceae family) is represented by 34 species and 40 taxa, 32 of which are endemic, in Turkey and it has a remarkable feature in the flora of Turkey with an endemism rate of about 80%. Although it grows in many parts of Turkey, it is mostly found in the Mediterranean coast, Central, Western, and Eastern Anatolia. *Alkanna* taxa are known with various local names in Turkey. Although it grows in various parts of Turkey, it is mostly distributed in Mediterranean coasts and in Central and Western Anatolia. *Alkanna Orientalis* is referred with various local names such as 'tosbağaotu, Kanburuyan and kurbağaotu' in Turkey [4-5].

Root extracts of *Alkanna* genera are used as a fiber, cloth, nutrient and colorant in cosmetics, and also as a traditional medicine [6-7]. It is known that there are substances with high antimicrobial properties in the plants belonging to the Boraginaceae family [8-9]. It has also been reported by several researchers that it has some activities such as anti-inflammatory, anti-thrombotic, cytotoxic, and antioxidant activities [5,10-11]. However, it has been not reported that the plant extracts obtained from the *Alkanna orientalis* plant have any effect on the plant pathogens.

In this study, the opportunities to use the herbal substances obtained from wild plants for controlling of *Fusarium oxysporum* f. sp. *lycopersici* (FOL)(Sacc.) W.C. Snyder and H.N. Hans, Alternaria solani (A.S) (Ell. and G. Martin), *Verticillium dahliae* (VD) *Rhizoctonia solani* (R.S) Kühn., and *Sclerotinia sclerotiorum* (S.S) Lib De Bary plant pathogens, which cause considerably yield losses in tomatoes, potatoes, and cucumbers in Turkey were investigated. Thus, the antifungal activities of the extracts obtained by using methanol and a water solvent against five different plant pathogens of the *Alkanna Orientalis* plant were detected.

MATERIAL AND METHOD

Collection of the plant material. The plant parts of *Alkanna Orientalis* were collected from Tokat, Turkey according to the growth periods in 2017. The collected plant parts were washed with sterile distilled water and then dried in the shade at room temperature. Then, each plant segment was milled and divided into small pieces.

Obtaining the Fungus Cultures. Fungi, the plant pathogen used in the study, were obtained from the stock cultures present at Ahi Evran University, Faculty of Agriculture, Department of Plant Protection, Phyclinic laboratories. In the trials, young fungus cultures developed for 7 days at 25±2 ºC in 90 mm petri dishes containing 20ml potato dextrose agar (PDA) among these stock cultures were used.

Obtaining plant extracts. 100 gr of each milled plant materials was weighed and placed in a 1-lt erlenmayer flask. Organic solvent (methanol) was added in such a way to cover the plant parts. They were mixed at 120 rpm in an orbital agitator at room temperature for 72 hours. Then, the extracts were passed through a filter paper and the organic solvent was removed by evaporating at 40 ºC using a rotary evaporator. The remaining dry extracts were solved in 50% aqueous acetone and different concentrations (2500, 5000, 10000 ppm) were obtained in order to be used in the study [12].

Antifungal activity of the plant extract under *in vitro* conditions. Stock solution was obtained by dissolving the obtained substances with acetone-water mixture. Among the unique solutions obtained, they were added to the PDA media that were chilled up to 45-50 oC in order for the final concentration to be 2500, 5000 and 10000 ppm (Onaran and Yılar, 2012). As control, fungi were only inoculated in petri dishes containing PDA. In addition, a fungicide containing a Thiram active substance was used as a positive control in the trials. These PDA media at different doses were poured into petri dishes with a diameter of 60 mm, as 10 ml in each. Mycelium discs with a 5-mm diameter taken from the plant pathogen cultures developed 7-10 days before the trials were inoculated in petri dishes containing extract-added PDA media. Fungus cultures were left for incubation for 7 days at 25±1oC in the growth cabin after inoculation. This trial was repeated 2 times as 4 repetitions. Diameters of the mycelium growing in the petri dishes were measured by a digital caliper device. The inhibition percentage of the mycelium growth in the extracts was calculated according to the following formula:

I:100x(dc-dt)/dc
I:Inhibition percentage of mycelium growth
dc: Mycelium growth in the control
dt: Mycelium growth in the application [13]

Data Assessment. The significance level of the differences between the treatments in the trials was determined by analysis of variance (ANOVA) and the mean scores were compared by using the DUNCAN test. Statistical analyses were performed by using the SPSS computer program.

CONCLUSION AND DISCUSSION

Table 1 shows antifungal activity values of the water extract obtained from *Alkanna Orientalis* plant against FOL, *A. solani*, *V. dahliae, R. solani* and *S. sclerotiorum* (Mycelial growth inhibition=MGI).

The water extract obtained from the *Alkanna Orientalis* plant did not inhibit the mycelium growth of the plant pathogenic fungi at the rate of 100%. However, according to the control, percentage inhibitions occurred depending on the dose increase in the pathogens except for *R. Solani* plant pathogens. Dose of 10000 ppm of the water extract used on the plant pathogens inhibited the FOL *A. solani, V. dahliae* and *S. sclerotiorum* mycelium growth at the rates of 39.58, 37.22, 27.27 and 20.72%, respectively. While FOL is the most susceptible one among the plant pathogenic fungi, the most resistant one is *R. solani*.

TABLE 1
Shows antifungal activity values (MGI) of the water extract obtained from *Alkanna Orientalis* plant against FOL, *A. solani*, *V. dahliae*, *R. solani*, and *S. sclerotiorum*.

Doses (ppm) (water)	*A. solani*	*S. sclerotiorum*	*R. solani*	*F. oxysporm* fs p. *lycopersici*	*V. dahliae*
Control^{+}	100a*±0.00	100a±0.00	100a±0.00	100a±0.00	100a±0.00
Control^{-}	0.00d±0.00	0.00c±0.00	0.00b±0.00	0.00e±0.00	0.00d±0.00
2500	0.00d±0.00	0.00c±0.00	0.00b±0.00	9.21d±2.41	0.00d±0.00
5000	23.40c±2.01	0.00c±0.00	0.00b±0.00	21.22c±2.01	10.11c±4.08
10000	37.22b c±2.60	20.72b±2.28	0.00b±0.00	39.58b±1.80	27.27b±4.40

Means in the same column with the same letter were not significantly different by ANOVA (a = 0.05) MGI: Mycelium growth inhibition

TABLE 2
Shows antifungal activity values (% MGI) of the methanol extract obtained from *Alkanna Orientalis* plant against FOL, *A. solani*, *V. dahliae*, *R. solani*, and *S. sclerotiorum*.

Doses (ppm) (methanol)	*A. solani*	*S. sclerotiorum*	*R. solani*	*F. oxysporm* fs p. *lycopersici*	*V. dahliae*
Control^{+}	100a±0.00	100a±0.00	100a*±0.00	100a±0.00	100a±0.00
Control^{-}	0.00e±0.00	0.00d±0.00	0.00c±0.00	0.00e±0.00	0.00e±0.00
2500	5.25d±1.98	0.00d±0.00	0.00c±0.00	20.85d±1.92	12.59d±2.74
5000	22.01c±1.90	7.85c±1.06	0.00c±0.00	39.05c±2.59	32.76c±1.29
10000	49.44b±1.27	29.66b±1.94	8.45b c±1.60	55.22b±1.89	56.43b±2.88

Means in the same column with the same letter were not significantly different by ANOVA (a = 0.05) MGI: Mycelium growth inhibition

TABLE 3
The antifungal activity of plant extract on the mycelium growth (mm) of plant pathogenic fungi

Water extracts						Methanol extracts				
Doses	S.s	R.s	A.s	Fol	Vd	S.s	R.s	A.s	Fol	Vd
PC	0	0	0	0	0	0	0	0	0	0
NC	60	60	60	60	60	60	60	60	60	60
2500	60	60	60	54.47	60	60	60	56.84	47.50	52.44
5000	60	60	45.96	47,26	53.93	55,27	60	46,74	43.57	40.34
1000	47,56	60	37.22	36.24	43.63	42.20	54.92	30.33	26,93	26.13

Methanol extract obtained from the *Alkanna orientalis* plant inhibited the mycelium growths of all the plant pathogens at varying rates. However, an inhibition of 100% was not observed in the mycelium growth of the plant pathogens used. While the methanol extract of the plant showed the highest effect on *V. dahliae* with the rate of 56.43% compared to the negative control, this was followed by 55.22% (FOL), 49.44% (*A. solani*), 29.66% (*S. sclerotiorum*), and 8.45% (*R. solani*).

When all the disease factors were considered together, the most affected fungus species was *V. dahliae*. This was followed by FOL A.s S.s, and R.s. The highest effect against *V. dahliae* was observed as 56.43% in the methanol extract of *Alkanna orientalis* plant. The highest antifungal effect against A.solani was detected as 49.44% in the methanol extract. Methanol extract showed the highest antifungal effect against *S. sclerotiorum and R. solani*. They showed an antifungal effect of 29.66% and 8.45%, respectively. Thiram 80%, used as positive control in the present study, was used at the dose recommended by the manufacturer and it had an inhibition of 100% all the disease factors. Similarly, as negative control, 50% acetone which was used as a solvent in dry extracts, was added and 0.00% mycelium inhibition was observed.

When the plant extracts used in the present study were evaluated according to mycelium growth of the pathogens, the least affected fungus species was *R.solani*. The mycelium growth of *S. sclerotiorum*, *A. solani, V. dahliae* and *Fol* was between 46.56-42.20, 37.22 -30.33, 43.63 -26.13, and 36.24 mm-26.13 mm (Table 3)

In the previous studies, antibacterial and antifungal activity of the extracts obtained from *Alkanna orientalis* plant was reported. In a study, it was determined that the extracts obtained from *Alkanna orientalis* plant had an antibacterial effect on a gram-positive bacteria type *Bacillus subtilis* (NRS-744), *Staphylococcus aureus* (B-767), and a gram-negative bacteria *Klebsiella pneumoniae* (B-3521), *Escherichia coli* (B-3704), and *Proteus vulgaris* (B-123) and also showed an antifungal activity on *Candida albicans* (Y-477), two *Microsporum canis* and *Trichophyton mentagrophytes* fungi [14]. In a similar study, water and ethanol extracts obtained from the *Alkanna orientalis* plant were tried on the *Pseudomonas aeruginosa ATCC 27853, Bacillus subtilis, Escherichia coli ATCC 25922 and Staphylococcus aureus ATCC 25928* bacteria and it was reported that methanol extract was more effective than the water extract[15]. However, there was no study reporting the effect of the extracts obtained from the *Alkanna orientalis* plant on plant pathogenic fungi such as FOL A.s, S.s, and R.s.

CONCLUSION

It was determined that the plant species used in the study had a noticeable antifungal effect. These differences varied between 56.43% and 0.00% according to the mycelium inhibition ratios and different results depending on the extract type revealed. Different effects were determined depending on the dose increase in the plant extracts used, and as the dose increased, the effect also increased in parallel. Hopeful results are obtained from the plant extracts used compared to the control.

According to these results, the antifungal activities of the plant extracts obtained from the plant species to be used against the plant pathogens (*F. oxysporum f. Sp. lycopersici, V. dahliae, S.*

sclerotiorum, A. solani, and R. solani) used in the present study were determined. Similarly, besides other commercially used herbal pesticides, one of the important results is the presentation of a successful alternative to chemical control. Thus, the disadvantages caused by the chemical control can be minimized.

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FOOD DEMAND ANALYSIS: A CASE OF TURKEY

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ABSTRACT

This article aims to be built a reliable empirical model of food expenditures (FE) due to the dynamics of Gross Domestic Product (GDP) for Republic of Turkey. It is very important to conclude any relationship between food expenditures (FE) and GDP. We will use any secondary data belong to Turkish Statistical Institute (TURKSTAT), The Central Bank of the Republic of Turkey (CBRT), Republic of Turkey Ministry of Development (MOD) and the other related institutions database.

In this study, data of the household budget surveys conducted by the Turkish Statistical Institute for years are used; income, price, and cross price elasticities under different groups are estimated within the framework of the an almost ideal demand system approach for food expenditures; and estimation of household consumers' food demand in Turkey is analyzed.

According to the findings obtained, it is established that a price-bound change would appear in the food demand, and elasticities are calculated. Expenditures by product groups and price elasticities are obtained.

KEYWORDS:
Food expenditures, Gross domestic product, Consumer behaviour, An almost ideal demand system.

INTRODUCTION

The problem in this study stems from the fact that what household demand parameters by commodity groups would be in Turkey. In addition, as for the importance of this study for Turkey, it reveals the expectation that despite advanced technological developments in the globalized world of today, famine, food insecurity and food consumption will continue to be an important problem in Turkey and in the world in the future, just as if it is today.

Due to expenditure and consumption diversities between the households in Turkey, examination of the expenditure items structure represents an issue of great importance. Moreover, price elasticity of expenditure items is significant instruments in the formation of an effective economy policy and the formation of budget policies.

The problem in this study stems from the fact that what household demand parameters by food commodity group would be in Turkey. In this study, calculation of price and expenditure elasticities drawn from the Almost Ideal Demand System was aimed by using expenditure data relevant to the commodity groups included in the Household Budget Surveys (HBS) between 2002 and 2016.

The main goal of this study is to analyze the consumption behavior of the households living in Turkey. As it is known, despite the fact that household consumption expenditures are composed of various commodity groups, budget shares for expenditures represent an important part of them. Within this study, it is possible to monitor, via the price and expenditure elasticities, the commodity groups for which the most and the consumers made the least expenses.

Studies conducted related with the Almost Ideal Demand System in Turkey and out of the country were examined. Elasticities obtained in the other studies carried out, on the other hand, are as follows:

Food price elasticity as 0.07 in the levels model and as 0.22 in the first order difference model [1].

Dynamic-linear an almost ideal demand system, made an estimation of 11 aggregated product groups. Here, the expenditure and price elasticities were obtained as respectively 0.37 and -0.32 for solely food-relevant values [2].

The post-war consumer behavior of the USA with the An Almost Ideal Demand System. Food expenditure elasticity was found as 2.06 with no autocorrelation and as 1.11 with autocorrelation [3].

Meat consumption and socio-demographic data, of the SUSENAS surveys of household food expenditure and consumption of 1990, 1993, and 1996 was used to estimate the meat demand in Indonesia. The estimated prices of beef and the poultry group were observed to be respectively -0.92 and -1.09 [4].

The pooling the data of per capita consumption expenditures in Belgium, the United Kingdom and the United States of America between 1961 and 1978, tested the quadratic expenditure system. In the study conducted, price elasticities for Belgium, the United Kingdom and the United States of America were found as respectively -0.463, -0.08, and -0.71 [5].

In Turkey, on the other hand, there are econometric studies on demand analysis, though in lim-

ited number. Results of some of these were given below:

Data relevant to the food and the food subgroups out of household consumption expenditures[6], [7]. Econometric analysis of red meat and its products demand and supply [6].

The relations between the demands for certain food products which are important in human nutrition and the factors affecting the demand for this foodstuff in Turkey [8].

Household food consumption surveys conducted in eight periods in about two years and the LA /AIDS model, estimating the central demand model of Erzurum province, established the elasticities which are fundamental data regarding food consumption which might be used in the analysis of food policies [7].

Food expenditure elasticities in the distinction of rural and urban areas and the results she found were respectively 0.6316 and 0.7172 on average [8].

AIDS by regions in the rural and urban areas in Turkey, found income, price and savings elasticity estimations, endeavored to analyze consumer behavior patterns [9].

Household income and consumption expenditure surveys conducted by the Turkish Statistical Institute for 1994 and 2003 years were used; income, price, and cross price elasticities under six aggregated product groups were estimated within the framework of the an almost ideal demand system approach for food expenditures; and estimation of household consumers' food demand in Turkey was analyzed. According to the findings obtained, it was established that a price-bound change would appear in the food demand, and elasticities were calculated. Expenditures by product groups and price elasticities were obtained, and the product groups were aggregated as bread and cereals; meat, fish, and poultry; milk and dairy products, oil and egg; vegetables and fruits; various fast food and alcoholic and non-alcoholic beverages [10].

Turkish consumer behaviors models for Turkey, urban and rural districtions are made an analysis by estimating income and price elasticities for food and non-alcoholic beverages group by applying An Almost Ideal Demand System. Household income and consumption expenditure data between 2002-2006 years are used. Expenditure and price elasticities under food and non-alcoholic beverages group are estimated within the framework of the almost ideal demand system approach in Turkey was analyzed. Expenditure elasticities for Turkey, urban and rural were calculated and respectively 0.975, 0.961 ve 0.992. According to Turkey, urban and rural, values of expenditure elasticities are obtained almost the same [11].

MATERIAL AND METHODS

Turkish Statistical Institute (TURKSTAT) has started annual regular budget surveys for 2002. While Household Budget Survey (HBS) for 2002 was conducted on a total of monthly 800 and annually 9.600 sample households, HBS for 2003 was conducted on a total of monthly 2.160 and annually 25.920 sample households for a year period between 1 January and 31 December 2003. 2004, 2005 and 2006 HBS were conducted on a total of monthly 720 and annually 8.640 sample households. That is, HBS sample households structure have been changing steadily since 2002 [12].

The estimation level of the HBS of 2002 is the distinction between whole Turkey, urban and rural areas. Estimations were drawn on the basis of 12 Level-1 regions and 26 Level-2 regions from the distinction made between rural and urban areas in the Statistical Regional Units Classification in the survey 2003. Starting from 2004 on the other hand, in the distinction between whole Turkey and rural and urban areas made via annual survey results, it is possible to make estimations at regional level by combining every year's survey results with those of the previous one [12].

Household consumption expenditure by types of expenditure in Turkey for between 2002 and 2016 is examined. The highest ratio for expenditures is Housing and rent, second group is Food and non-alcoholic beverages group and third group is Transportation. That is, If a household has 100 Turkish Liras in 2002, this household gives 27.3 Turkish Lira for Housing and rent, 26.7 Turkish Lira for Food and non-alcoholic beverages and 8.7 Turkish Lira for Transportation. The same household in 2016, 25.2 Turkish Lira for Housing and rent, 19.5 Turkish Lira for Food and non-alcoholic beverages and 18.2 Turkish Lira for Transportation. In 2002, total of these three expenditure groups is 62.7% and in 2016 total of the same groups is 62.9%. In Turkey, households have to allocate their budget these three expenditure groups. Namely, the remains proportion for the other expenditure groups is approximately 37%. The lowest ratios are Health and Educational services [13].

Proportion of food expenditures in total expenditures in Turkey was calculated to be 26.7% in 2002 and 19.5% in 2016. According to these results, it is understood that a large part of the incomes of Turkish households is allocated to food consumption in 2002 but in 2016 this ratio is lower than in 2002. That is, Turkish households remains income is distributed by necessities to the other commodity groups is higher in 2016 than in 2002 (Figure 1).

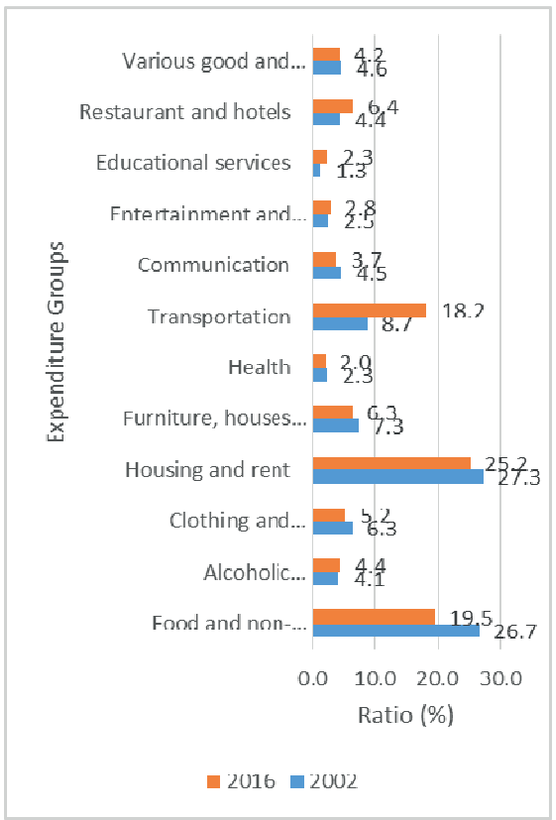

FIGURE 1
Comparing to 2002 and 2016 years for household consumption expenditures by types of expenditure, Turkey

In this study, the HBS data between 2002 and 2016 years, the method of which is given above by the Turkish Statistical Institute (Turkstat) were used. This survey data were organized and changed with relevant to the researcher's objective.

In the data relevant between 2002 and 2016, general sum of total monthly consumption expenditures and expenditure values groups regarding Food and non-alcoholic beverages was taken. In this study, food commodity group was emphasized above and focused.

12 month-consumer prices index figures were used from the Turkstat's Price Statistics database. Price indexes belong to commodity groups were used separately for every commodities groups. After that price data were converted into real price values.

The data set created for study use was distributed by each item group. Moreover, the data which were regions in the cross section and 15 year-observations of 2002 and 2016 in the time section were combined and the panel data set was obtained. Thus, the data set used in the analysis consists of 45 observations.

In this section, how to make an estimation parameter of an Almost Ideal Demand Systems are shown as follows:

If the basic model is written for each commodity, the following equations might be obtained:

$$w_1 = \alpha_1 + \gamma_{1.1}\ln p_1 + \gamma_{1.2}\ln p_2 + \ + \gamma_{1.12}\ln p_{12} + \beta_1 \ln (x/P^*) + u_1$$

$$w_2 = \alpha_2 + \gamma_{2.1}\ln p_1 + \gamma_{2.2}\ln p_2 + \ + \gamma_{2.12}\ln p_{12} + \beta_2 \ln (x/P^*) + u_2 \quad (1)$$

$$\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots$$

$$w_{12} = \alpha_{12} + \gamma_{12.1}\ln p_1 + \gamma_{12.2}\ln p_2 + + \gamma_{12.12}\ln p_{12} + \beta_{12}\ln(x/P^*) + u_{12}$$

In this equation,

w_i : represents the budget share of ith good,

p_j : is price of the jth good,

x : is total expenditure on all goods,

P^* : is price index.

It is possible to generalize and demonstrate the system in the equation 1 with matrices for n number of products and k number of variables:

$y = X\beta + u$

Where,

y: the column vector of the observations (n x1) relevant to y_i dependent variable;

X: (nxk) matrix indicates n observation value of explanatory variables from X_1 to X_k and is known as data matrix. The first column composed of one (1) values indicates the constant term.

β: the column vector of k number of unknown parameters (k x 1)

u: the column vector of n number of u_i error terms (nx1).

Since additivity and negativity constraints are automatically met by the model in the AIDS, no test is done for these constraints.

The Almost Ideal Demand System (AIDS) was selected for the study use for its following advantages. The AIDS model provides arbitrarily the first order approximation for any demand system. It provides definite estimations of axioms of choice. It aggregates consumers perfectly. It has a functional form which is consistent with household budget data. It is easier to make estimations in the form of linear approach. It can be easily used to test homogeneity and symmetry constraints [2].

Price elasticity belongs to AIDS model are calculated as follows: [8].

$$\varepsilon_{ii} = -1 + \frac{\gamma_{ij}}{w_i} - \beta_i \qquad \text{Marshall}$$

(Compensated) Price elasticity

$$\delta_{ii} = -1 + \frac{\gamma_{ii}}{w_i} + w_i \qquad \text{Hicks}$$

(Compensated) Price elasticity

$$\eta_i = 1 + \frac{\beta_i}{w_i} \qquad \text{Expenditure elasticity}$$

RESULTS AND DISCUSSION

The estimation of model parameters was calculated by The Ordinary Least Squares (OLS) method. Estimation of the model was made by using Eviews 7 econometrics package program. The

TABLE 1
An Almost Ideal Demand System Statistics, 2002-2016

Commodity Group	R^2	DW
Food and non-alcoholic beverages	97.82	2.32

TABLE 2
Price And Expenditure Elasticities For Food and non-alcoholic beverages, 2002-2016

Commodity Group	Expenditure Elasticity	Marshall (Compensated) price-demand elasticity	Hicks (Compensated) price-demand elasticity
Food and non-alcoholic beverages	0.58	1.06	1.19

data relevant to the commodity groups which take place in the Household Budget Survey of between 2002 and 2016 were applied to the An Almost Ideal Demand System (Table 1).

In Table 1, R^2 and DW values relevant to the Almost Ideal Demand System were given. According to these values, the R^2 value was in the Food and non-alcoholic beverages with 0,9782 that is 97.82%. DW value relevant to the almost ideal demand system was given in Table 2. For 45 observations at 5% level and two explanatory variables taken out of Durbin-Watson table, d_L = 0,95 and d_u = 1.54. When the DW value was considered, there was no autocorrelation in the group of Food and non-alcoholic beverages (Table 1).

Price (Marshall and Hicks) and expenditure elasticities values belong to Food and non-alcoholic beverages commodity for AIDS model was calculated for estimated parameter values that were as follows in Table 2.

Demands with an elasticity equal to one in absolute value are unit elastic; the demand is smoothly responsive to price changes. According to the AIDS model, price elasticities belong to are suitable for economic theory that is values of price elasticities are negative. Price elasticities (Both of them) of Food and non-alcoholic beverages is elastic.

When the price elasticities of the commodity groups are considered respectively, in case of 1% increase in Food and non-alcoholic beverages, this might be interpreted as demand for Food and non-alcoholic beverages will grow by 1.06%.

Expenditure elasticity reveals how the demanded amounts of the products would differ in the face of income changes. In line with expenditure elasticities, properties of the products are defined. Those with an expenditure elasticity higher than 0 are normal goods (Table 2).

When Table 2 is examined, the expenditure elasticity was found to be 0.58 for Food and non-alcoholic beverages. It is understood that examined commodity is normal goods.

According to the calculated expenditure elasticity belong to the one commodity, assuming all the other variable constant, in case of an average increase of 1% in the household's income, it is possible to say that Food and non-alcoholic beverages expenditures will increase by 0.58%.

CONCLUSION

Price elasticities were found in line with the parameters estimated from the AIDS. Findings for the price elasticities for AIDS model are consistent with economic theory. At that time, price elasticities are negative.

With the help of the studies relevant to the analysis of consumption expenditures, producers will gain knowledge about the structure of consumer demand, while consumers will gain knowledge about learning and determining consumption patterns. In line with this, in the analysis of production units, production decisions, and sector, decision-makers, that are managers, will benefit from these analysis results while they elaborate effective macro-economic policies.

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