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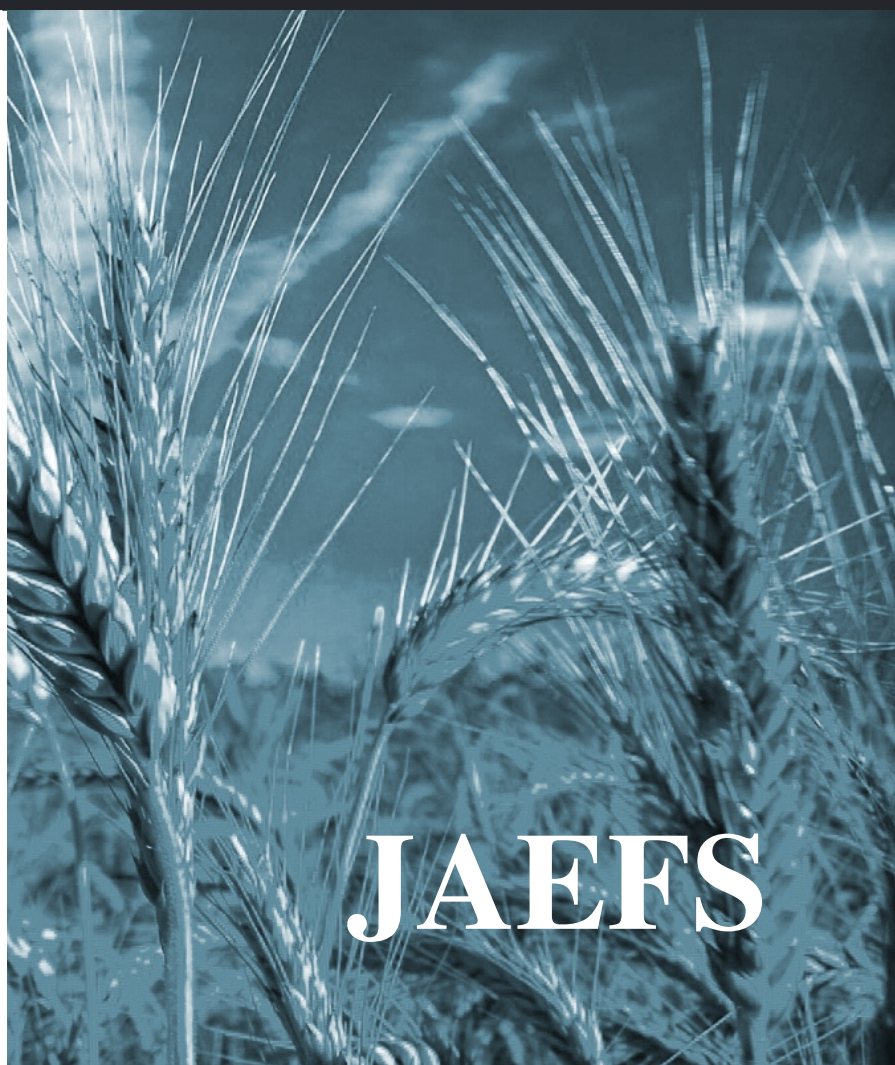

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Research Article

Int J Agric Environ Food Sci 5 (1):1-6 (2021)

Estimation of total tannin and total phenolic content in plant (*Crataegus azarolus* L) by orbital shaker technique

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Abstract

The plant of *Crataegus Azarolus L*, genus that belongs to the Rosaceae family and is a low, dense, spiny tree with a beautiful inflorescence, the phytochemical investigations on genus Crataegus were mainly performed on the leaves, flowers and berries. The objective of this experiment was to determine the ratio of tannin and phenol compound by method orbital shaker extraction. The plant of *Crataegus azarolus L* was collected in hasanbag mountain soran, Kurdistan. The parts of seed, leaf and steam of *Crataegus Azarolus L* was extract with water solvent and orbital shaker and assessed yield extraction, total tannin and total phenol. The highest significant value (p<0.05) of yield extraction was observed from leaf (71.56%), compared with other part of *Crataegus Azarolus L*. On the other hand, the total tannin was showed uppermost in leaf (3.03 kg/mg), and the steam (2.87 kg/mg). Whereas, the smallest total tannin was observed from the seed (1.10%). Furthermore, the total phenolic contents of the plant were observed the lowest value in seed as (0.98 mg GAE/g). Whereas, the total phenol in leaf was observed a higher significantly value (3.68 mg GAE/g) and in steam (1.92 mg GAE/g), with all significant value (p<0.05). This study showed that the leaf and other part of *Crataegus Azarolus L*, rich in total phenolic and total tannin after orbital shaker method was used.

Keywords: Crataegus Azarolus, Tannin, Orbital shaker, Total Phenolic, Leaf

Introduction

Crataegus L. genus that belongs to the Rosaceae family is one of the most important genera concerning the number of species. *Crataegus azarolus L*. The plant is widely distributed in North Europe, temperate regions of Asia, Africa and North America. Eastern North America and europe were proposition to be the most recent common areas for crataegus L. In Tunisia, the Crataegus' fruits are known by their famous name "Zaarou-ra", while in Spain "Azerolier" and in the anglophones' coun-tries by "Azerole Hawthorne". Crataegus azarolus L. is vastly

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distributed in the Northern West, the Cap Bon (in the centre), and the Dorsal Mountain of Tunisian in bioclimatic regions extending from the upper semi-arid to the decrease humid (Khiari et al., 2015).

Crataegus azarolus var. is a low, dense, spiny tree with a beautiful inflorescence up to six m tall and with orange fruit(Christensen, 1992). Phytochemical investigations on genus Crataegus were fundamentally performed on the berries,-flowers and leaves (T Bahorun et al., 1994; Kao et al., 2005; Kumar et al., 2012).The insulated compounds were:bioflavonoid, oligomeric procyanidins, polysaccharides, catecholamines, vitamin C, saponins, cardiotonic amines, tannins,ursolic acid and purine derivatives (Sokół et al., 2007; Hamahameen and Jamal, 2013; Duke, 1992).

The potential of plant *Crataegus azarolu* a source of antioxidants (Th Bahorun et al., 1996; Ljubuncic et al., 2005). Hawthorn flowers and fruit act as diuretics, and can be used to treat kidney problems and "dropsy" (Twaij *et al.* 1987), the dietary and medicinal cost of C. azarolus fruit to assist the exploitation of azarole according to a Few research (Bignami et al., 2001; Koyuncu et al., 2007).

In Palestine, morocco and Tunisia (Ali-Shtayeh et al., 2000), the hawthorn fruit and vegetation are used to deal with cardiovascular disease, sexual weakness, diabetes and cancer(Bignami et al., 2001; Koyuncu et al., 2007).

The tannins (is a tannic acid) are water-soluble polyphenols that are current in many plant foods. Tannins are a various type of compounds and have a number of effects on health. The antimicrobial and antioxidant activities of tannin are well authenticated. They are additionally used as antiseptics and astringents, antioxidant things to do confer upon the anti-mutagenic and anti-carcinogenic properties of tannins. (Chung et al., 1998).

The aim of his study was to evaluate of total tannin and total phenolic in the plant of *Crataegus azarolus L* by a technique of orbital shaker extraction

Materials and Methods

Plant Collection and Preparation of extraction methods

The plant of *Crataegus azarolus* L was collected from 6/ September to 17 September. 2019. In Hasanbag mountain. after that the plant sample was ground by grinder (Model GI, Capacity/hour 10 Kg, Capacity 4 letter, Speed 13000 rpm, and Cycle 500 gr), has been done at the home. after that prepared of powder plant was soaked in a solvent (distilled water) for 24 h. using the Orbital shaker extraction method. After that filtered and evaporated using Fume Hood.

Yield determination

The yield percentage of the extract was determined by using the following formula for each one of the extraction techniques which was given below: (Murugan and Parimelazhagan 2014)(Zhang *et al.* 2009).

Equation 1: extract percentage yield

Where,

X is the oven dry weight of extract (g),

Y is the oven dry weight of the sample (g).

Determination of total condensed tannin

This assay was carried out by Shimadzu UV-vis spectrophotometer. The extraction solution was prepared by mixing 0.05 g of Fe2SO4, 95 ml *N*-butanol and 5 ml HCl (35%). For determining the condensed tannin, 0.01 g of crude plant in a test tube and 10 ml of extraction solution was added and placed in a water bath for heating 1 h. The absorbance was measured at 580 nm wavelength (Karaogul *et al.* 2017),(Makkar and Singh 1995).

Determination of total phenolic compounds

The total phenolic content was estimated by the Folin Ciocalteu method as described by Dewantoet al. An aliquot of the diluted extract was added to 180 mL of distilled water and 20 mL of Folin–Ciocalteu reagent. The mixture was shaken and allowed to stand for 5 min, before the addition of 1.60 mL of a 7% sodium carbonate solution were added. The solution was then adjusted with distilled water to a final volume of 3 mL and mixed thoroughly. After incubation in the dark, absorbance at 760 nm was read versus a prepared blank. The total phenol content of plant parts was expressed mg of Gallic acid/g (GAE/g), from a calibration curve with Gallic acid. All samples were analyzed in three replicates (Dewanto *et al.* 2002).

The TPC was measured using a gallic acid standard and expressed as mg of gallic acid/g (GAE/g). All the experiments were carried out in triplicate.

Statistical Analysis

The plant of Crataegus azarolus L. was analyzed and expressed as values of means ± S.E (standard errors) of triplicate calculated all parameter. The results of the three groups were compared using the analysis of One-way ANOVA-samples F-test with significantly different ($p<0.05$), by (IBM SPSS for Windows (version 20.).

Results and Discussion

In general, the results of the yield extraction, total tannin and total phenol of *Crataegus azarolus L* extract to be prepared by orbital shaker technique and with distilled water solvent. In this study, the yield extraction of plants was observed a highest significant value ($p<0.05$) in leaf (71.56%), compared with

steam (53.41%), and seed (35.64%) respectively, table 1 and figure 2. The effects of solvents polarity on extraction yield both qualitatively and quantitatively was confirmed by (Franco1 *et al.* 2008).

The tannin was calculated by the n-butanol- HCl- iron way to the use of this assessment for the amount of tannin was quantitatively released from the sample. The greatest commonly used standard for n-butanol /HCl assay is mimosa-tannin under normal reaction/condition which calculated using the regression equation (y = 151,96x - 6,9042), R^2 = 0.9978 previously earlier from the linear calibration curve (Figure 1). Comparison of the high amount of tannins found in all root plants species by the percentage of tannin was determined, Calculation: % = A/3m, where A = absorbance value, m = mass weight (Karaogul *et al.* 2017).

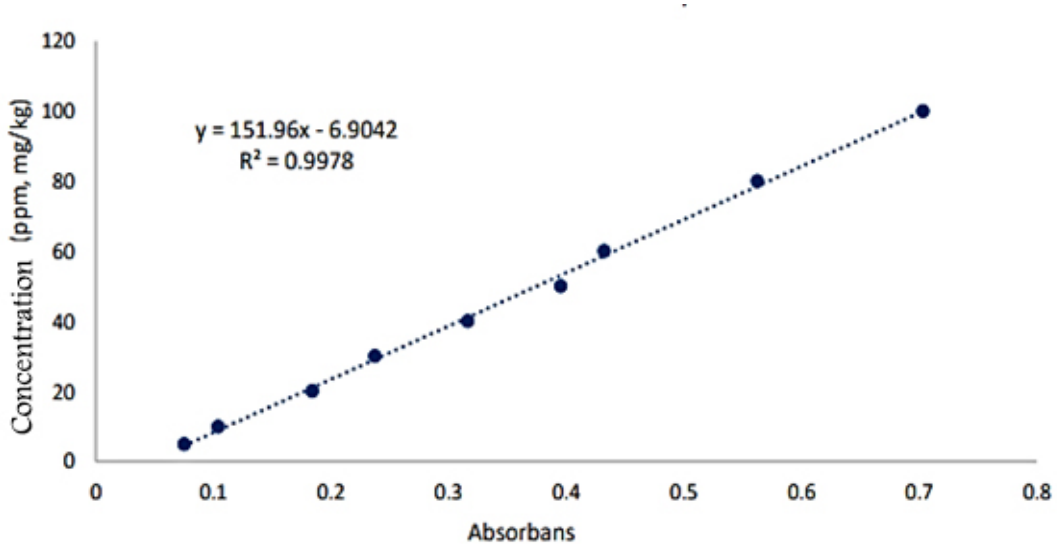

Figure 1. Calibration chart from mimosa tannins

Also, the tannin content determined based on the procedure of (Makkar and Singh 1995), (Karaogul *et al.* 2017), so that the result was expressed as absorbance unit at 580 nm per 1 mg of extract (A580/mg) In this study, the total tannin content was Illustrated the uppermost in leaf (3.03%) mg/kg, and the steam (2.87%) mg/kg. Whereas, the smallest total tannin was observed from the seed (1.10%) mg/kg. Respectively, with all significant value ($p<0.05$), that is shown in (table 1, and figure 3). Many types of research had been studied and reported the importance of tannin and its variation. Their activity is possible because of their capability to connect with extracellular and soluble proteins or combine with the cell wall of fungi. The character of these compounds may disrupt fungal membranes(-Franco1 *et al.* 2008).

Table 1. Extraction yield, total tannin and total phenolic of *Crataegus azarolus L* solvent extracts

	Orbital Shaker Extraction/ D.W solvent		
	Yield extraction	Total tannin(mg/kg)	Total Phenol(mg GAE/g)
plant	Std. Error of Mean	Std. Error of Mean	Std. Error of Mean
seed	35.64±0.881	1.10±0.008	0.98±0.005
Steam	53.41±0.577	2.87±0.008	1.92±0.005
Leaf	71.56±0.881	3.03±0.003	3.68±0.005
F-test	512.324	20724.26	56356.00
(P-value)	0.000	0.000	0.000

Values are Mean ± SE of Triplicate Samples, one-way ANOVA -samples F-test significantly different ($p<0.05$)
OSE: Orbital Shaker Extraction, DW: Distilled Water

Furthermore, the total phenolic of the plant was presented in, table 1 and figure 4. The total phenolic content was observed the lowest value in seed as (0.98 mg GAE/g). Whereas, the total phenol in leaf was observed a higher significantly value (3.68 mg GAE/g) and in steam (1.92 mg GAE/g), respectively ($p<0.05$). Because depend on abilities extract with the plant were found. Indeed, it could be due to the polyphenolic content of the plant being greatly affected by environmental factors as well as edaphic factors like soil type, sun exposure, rainfall, altitude and high tide, soil nutrients. Etc (Manach *et al.* 2004). Our findings are in similar with (Balaky *et al.* 2020; Ismael et al., 2019; Hamahameen and Jamal, 2013; Deliorman Orhan et al., 2012), our results are disagreement with studies by (Rebaya *et al.* 2015)(Kumar *et al.* 2012), because of the method technique and solvents difference.

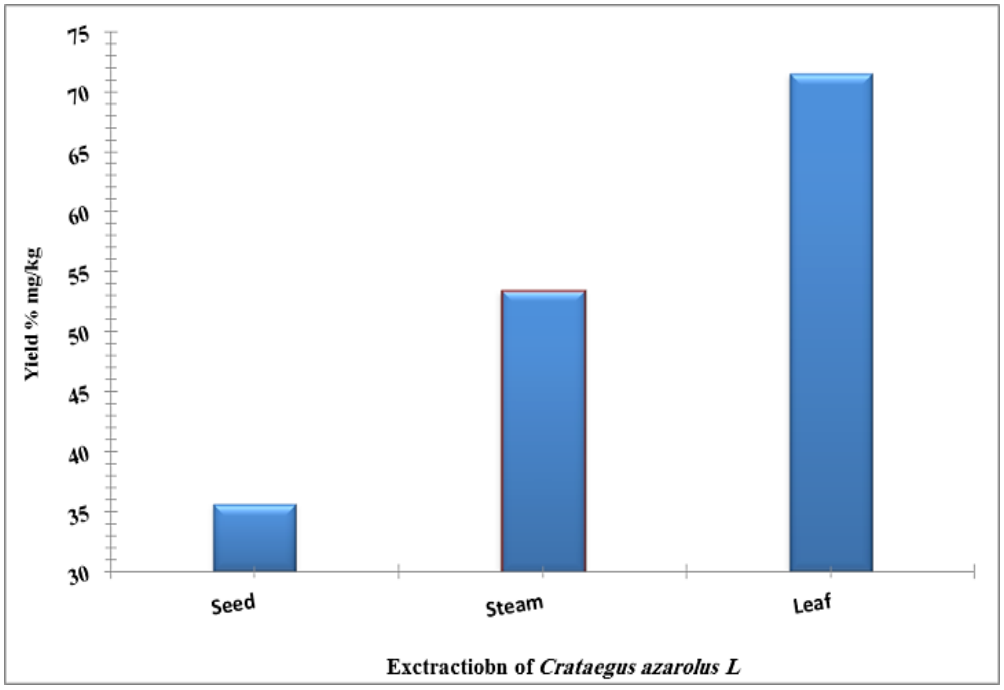

Figure 2. Extraction yield percentage of plant *Crataegus azarolus L*

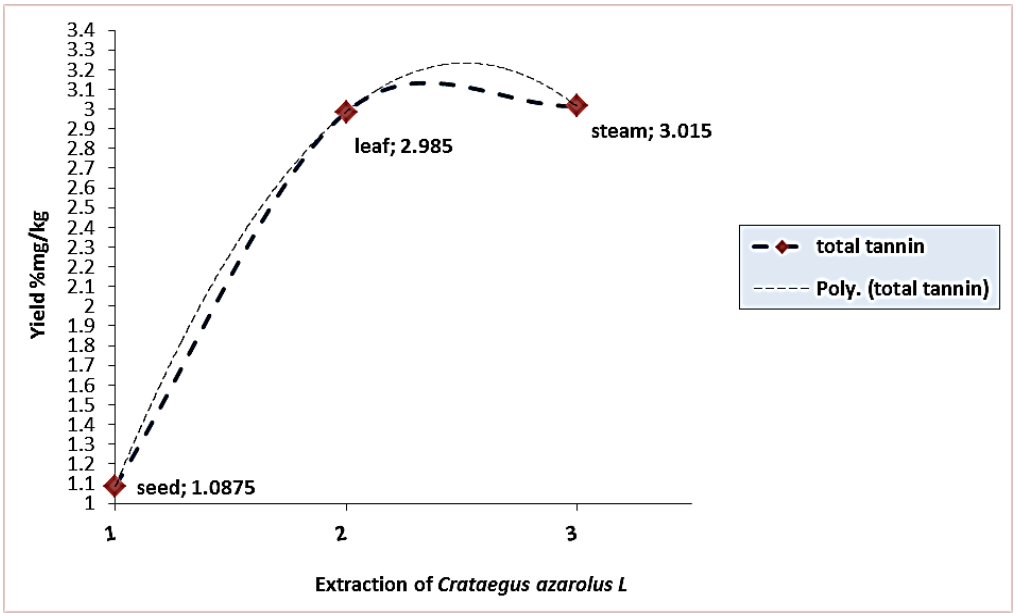

Figure 3. Total Tannin content of *Crataegus Azarolus L* solvent extract

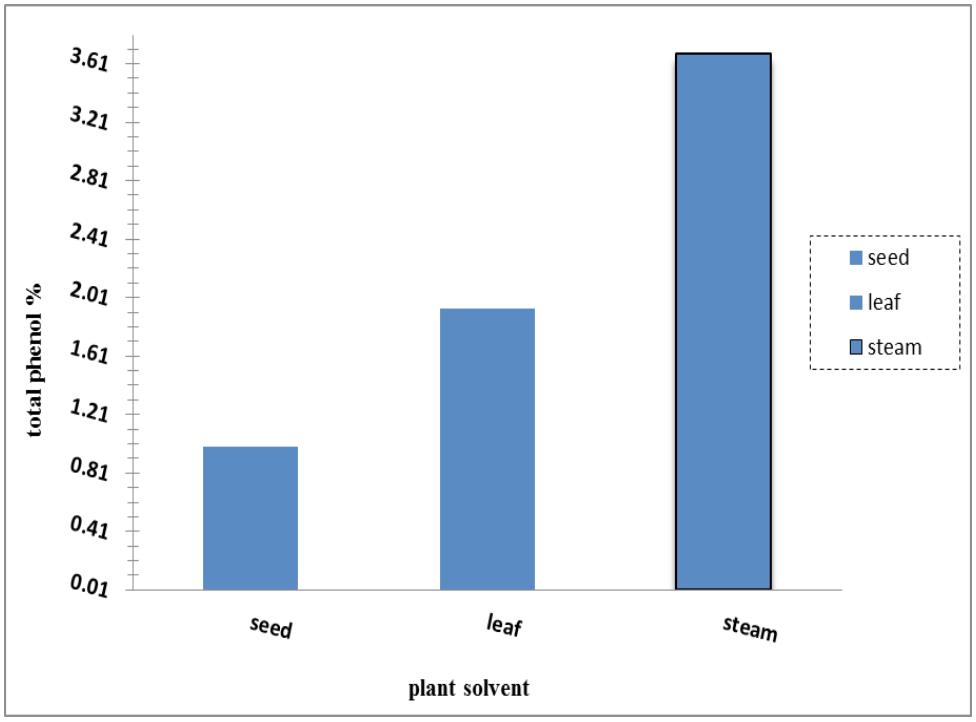

Figure 4. Total Tannin Phenolic content of *Crataegus Azarolus L* solvent extract

Conclusion

This study shows an overview analysis of the solvent of distilled water with the method of orbital shaker extraction in a plant. Those shown all results of the analysis were found as limited value and high significant value ($p<0.05$) of yield extraction (71.56%), total tannin (3.03%), and total phenol (3.68) respectively. Furthermore, the part leaf with all analysis was observed a significantly high value. Our study will be useful to researchers and others and, suggest to researchers who interested in our plant. This study showed that the leaf and other part of plant *Crataegus Azarolus L* that is a good natural edible plant and rich in antioxidant for human consumption after orbital shaker method was used.

Compliance with Ethical Standards

Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

Not applicable.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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Reproductive fitness of five *Pratylenchus thornei* populations from Isparta Province in Turkey on sterile carrot discs, wheat and barley cultivars

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Abstract

In the study, five populations of *Pratylenchus thornei* which obtained from different locations in Isparta Province in Turkey were investigated for their reproductive fitness on carrot discs and wheat varieties of Ikizce and Altay and barley varieties of Tarm92 and Burakbey. Reproductive fitness evaluated as the ratio of the final number of nematodes to the number of nematodes inoculated. There was a significant difference in reproductive fitness among the five *P. thornei* populations on carrot discs. The highest reproductive fitness on carrot discs was obtained with the *P. thornei* populations of SK11 and SK24 whereas pathogenicity of these populations was differentiated among wheat and barley varieties. The highest reproductive fitness was found at SK11 population with all wheat and barley varieties. GD18 and ISP10 populations developed better in barley than in wheat varieties. This study showed that there might be differences in reproduction of populations of the same nematode species isolated from different geographical areas.

Keywords: *Pratylenchus thornei*, Reproductive fitness, Pathogenicity, Monoxenic culture

Introduction

Pratylenchus thornei Sher & Allen, 1953 is the most economically important lesion nematode species on wheat and barley that reduces grain yield and quality (Fanning et al., 2020). It has been reported that the reproduction rates of these nematodes vary on wheat and barley varieties (Sheedy et al., 2007, 2008; Thompson et al., 2008; Smiley, 2009). It is stated that the susceptibility of wheat to root lesion nematodes was higher than barley (Smiley et al., 2004; Vanstone et al., 2008; Smiley and Machado, 2009). Root lesion nematodes were surveyed and identified on wheat cultivation areas in different regions of Turkey (Kasapoğlu et al., 2014; Kasapoğlu-Uludamar et al., 2018; Yavuzaslanoğlu et al., 2012, 2020). *Pratylenchus thornei* and *P. neglectus* were reported to have been found together generally at different population densities in wheat fields in Turkey (İmren et al., 2015). Göze Özdemir (2020) reported that *P. thornei* was the dominant species on cereal areas in Isparta and Burdur Provinces in Turkey and especially it had wide distribution on barley cultivation.

Monoxenic cultures on single sterile carrot discs in homogeneous environmental conditions involving a constant temperature exerted to compare the reproductive fitness of *Pratylenchus* populations (Tuyet et al., 2013). Reproductive fitness and virulence of nematodes are significant indicators of pathogenicity on plants so that, these factors enable the evaluation of nematode damage in plants (Castillo et al., 1998).

There was no relationship between the reproductive fitness of *P. vulnus* populations including the first host plant from which the nematodes were isolated on carrot discs (Pinochet et al., 1994). Mudiope et al. (2004) reported that difference in reproduction rates among the Jinja and the other isolates of *P. sudanensis* on carrot discs. Biological diversity among populations of the same species in *Pratylenchus* genus was reported by Pinochet et al. (1994) and Hafez et al. (1999). Tiyagi

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and Parveen (1992) and Castillo et al. (1995b) have reported that differences in reproductive fitness or pathogenic capability among populations of *P. thornei*.

The main objective of this study is investigating the reproductive fitness of five *P. thornei* populations on carrot discs and wheat and barley varieties which collected from wheat and barley fields in Isparta Province in Turkey.

Materials and Methods

Nematode populations

Totally five populations of *Pratylenchus thornei* one from Isparta centre, one from Yalvaç, two from Şarkikaraağaç and one from Gelendost districts in Isparta Province were used for investigation of their reproduction on sterile carrot discs, wheat and barley varieties. The nematode sampling districts and hosts isolated were given in Table 1. Nematodes extracted from wheat and barley roots using a modified Baermann funnel method (Hooper, 1986) in 2019. Then, *P. thornei* individuals were selected under light microscope and nematodes surface sterilized. Nematodes surface sterilization was performed with 1% streptomycin sulfate and penicillin solution in 35 mm petri dish. Nematodes were exposed to antibiotic solutions for 10 minutes sequentially and then washed three times with sterile distilled water.

Table 1. Origins of five populations of *Pratylenchus thornei* used in the study

Population code	Isparta district	Coordinate	Host
YLVC24	Yalvaç	N: 38˚18'40.7"/E: 031˚08'24.2"	Barley
SK11	Şarkikaraağaç	N: 38˚04'39.4"/E: 031˚27'23.3"	Wheat
SK24	Şarkikaraağaç	N: 38˚05'01.8"/E: 031˚23'26.6"	Barley
ISP10	Isparta center	N: 37˚50'39.6"/E: 030˚31'56.1"	Wheat
GD18	Gelendost	N: 38˚12'40.3"/E: 031˚01'26.9"	Barley

Monoxenic cultures on carrot discs

Carrots used in the study were purchased from the market. Carrots were washed and kept in alcohol for five minutes and burned in a fire for surface sterilization. Then, carrots were peeled aseptically. After sterilization, the carrots were cut into discs and put into 60 mm sterile petri dishes. A single carrot disc was placed in each petri dish (Behmand et al., 2017).

***In vitro* Reproductive fitness of five *Pratylenchus thornei* populations on carrot discs**

The surface sterilized nematodes were selected and transferred to the petri dish containing carrot disc in 60 mm petri dish under the light microscope. For each population of *P. thornei*, 10 replicates were used in a completely randomized plot design. There was only 1 carrot disc in 1 repetition. There were 15 females of *P. thornei* in each repetition. Each population was incubated at 24°C for 6 weeks in an incubator (Castillo et al., 1998).

After 6 weeks, The carrot discs were transferred to 120 mm petri dishes. Then, carrot disc cut into small pieces and water was added into petri dish. After 6-10 hours, nematodes extracted using a modified Baermann funnel method. Each repetition of *P. thornei* populations of nematode suspensions was reduced to 15 ml and taken in centrifuge tubes (Mudiope et al., 2004). Nematode eggs, juveniles and females counted under the light microscope at 10X magnification. Reproductive fitness was calculated by the reproduction rate Pf/Pi (final nematode population /initial nematode population (larvae+female+eggs) per an inoculated disc (Castillo et al., 1998).

Reproductive fitness of five *Pratylenchus thornei* populations on wheat and barley varieties

Reproductive fitness of the five *P. thornei* populations was tested on the 2 wheat varieties (Ikizce and Altay) and 2 barley varieties (Tarm92 and Burakbey). Wheat and barley varieties were obtained from Field Crops Central Research Institute, Ankara, Turkey.

The experiment was carried out at 25±1°C and 65±5% RH, with a 16:8 h L:D photoperiod in a controlled environment chamber. Wheat and barley varieties were planted onto a mixture of 200 g soil (%68 sand, %21 silt, %11 clay) in 250 cc plastic pots sterilized in an autoclave. The experiment was set up with 10 replications according to the completely randomized block design. One wheat seed was sown into each pot. A week after sowing, nematodes were inoculated with 1000 (larvae+female) nematodes in 10mL of sterile distilled water in the holes drilled to a depth of 2-3 cm around the root zone with the help of plastic pipettes. Control plants were treated only with 10mL of sterile water. The experiment was terminated 6 weeks after inoculation. Nematodes extracted from root and soil using a modified Baermann funnel method (Hooper, 1986) and counted light microscope 10X. The evaluation was made using reproductive fitness that was calculated by the reproduction rate Pf/Pi (final nematode population/initial nematode population (larvae+female+eggs))(Castillo et al., 1998).

Statıstical Analyzes

SPSS (version 20.0) program was used for the statistical analysis of the data obtained in the experiments, and analysis of variance (ANOVA) was performed to test the differences between the means. "Tukey" was used in cases where the variances were homogeneous at $p \leq 0.05$ significance level to determine the different group averages.

Results and Discussion

In the study, all nematode populations reproduced on carrot discs well above reproduction factor of 23 times which was obtained with GD18 population. There was statistically significant difference in reproductive fitness on carrot discs among the five *Pratylenchus thornei* populations (Table 2). The highest reproductive fitness on carrot discs were determined at

SK11 and SK24 populations; reproduction factors were 132.8 and 131.7, respectively, these populations were not statistically different each other in terms of reproduction fitness grouped as a according to Tukey test. Although the number of eggs and females of YLVC24 population was lower than the SK11 and SK24 populations, the number of larvae was similar (Table 2). ISP10 population provided statistically significantly higher reproduction (RF: 36.6) than GD18 population (RF:22.4), but their reproduction rate was statistically lower than SK11, SK24 and YLVC24 populations (Table 2).

No male was found in all *P. thornei* populations on carrot discs in the study. For each isolate, larvae density were greater than females and eggs. The number of females of ISP10 and GD18 populations was statistically lower than SK11, SK24 and YLVC24 populations, it is grouped as c according to Tukey test. However, ISP10 population of number of eggs and larvae on carrot discs were higher than GD18 population. In addition, the number of eggs and females of YLVC24 population was lower than SK24 and SK11 populations but the number of larvae was not statistically different in these populations on carrot discs (Table 2).

Table 2. Reproductive fitness of five *Pratylenchus thornei* populations on carrot discs

Nematode Population code	Number of Eggs ±STD error of mean *	Number of Larvae ±STD error of mean	Number of Females ±STD error of mean	Reproductive fitness ±STD error of mean
GD18	76,9±3,5 d	160,6±6,2 c	104,9±3,5 c	22,4±0,7 d
SK11	625,0±7,5 a	966,0±16,7 a	405,2±17,5 a	132,8±1,5 a
SK24	620,3±6,4 a	960,3±13,5 a	401,0±14,5 a	131,7±1,4 a
YLVC24	358,1±8,2 b	912,0±21,3 a	257,6±3,7 b	101,4±1,6 b
ISP10	142,2±3,8 c	271,6±13,1 b	140,4±3,1 c	36,6±0,8 c

* There is no statistical difference between the averages shown with the same letter in the same column ($p \leq 0.05$).

All populations of *Pratylenchus thornei* reproduced on wheat and barley varieties in the study. There were differences in pathogenicity of nematode populations to wheat and barley varieties. The highest reproduction was found with SK11 population in all wheat and barley varieties. While SK11 and SK24 pathogenicity were found close to each other in wheat varieties, it was determined that the pathogenicity of SK24 on barley varieties was statistically significantly lower than SK11. There was no difference between the reproduction fitness of ISP10 and GD18 populations on Ikizce wheat variety. However, the reproduction rate of ISP10 population was found to be statistically significantly lower in Altay wheat variety (Table 3).

The difference between YLVC24 and GD18 populations of reproductive fitness on Tarm92 barley variety were not statistically significant ($p \geq 0,05$). The lowest reproductive fitness was found ISP10 population on Tarm92 barley variety. Pathogenicity of ISP10 population was higher in Burakbey barley variety than Tarm92 barley variety. Interestingly, this population was expected to have higher pathogenicity in wheat because it was derived from wheat roots, but the reverse was seen (Table 3).

Table 3. Reproduction of five *Pratylenchus thornei* populations on wheat and barley varieties

	Reproductive fitness±STD error of mean			
	Wheat varieties*		Barley varieties	
Population code	Ikizce	Altay	Tarm92	Burakbey
GD18	4,2±0,1 c	4,3±0,1 c	6,0±0,1 c	6,5±0,1 d
SK11	12,0±0,1 a	11,6±0,1 a	12,0±0,2 a	12,3±0,2 a
SK24	12,0±0,1 a	12,2±0,2 a	10,4±0,2 b	10,3±0,1 b
YLVC24	8,3±0,1 b	7,3±0,2 b	6,9±0,2 c	7,9±0,2 c
ISP10	3,5±0,1 c	2,7±0,1 d	4,0±0,5 d	7,2±0,1 cd
Control	0,0±0,0 d	0,0±0,0 e	0,0±0,0 e	0,0±0,0 e

* There is no statistical difference between the averages shown with the same letter in the same column ($p \leq 0.05$).

Pratylenchus thornei populations were found to increase 23-133 folds on carrot discs in the study. Verdejo-Lucas and Pinochet (1992) reported that *P. thornei* and *P. neglectus* female populations increased 294 and 40 fold, respectively. Differences were found in reproductive fitness on carrot discs and pathogenicity on wheat and barley of *P. thornei* populations in present study. Unlike, Castillo et al. (1998) found that no significant differences in reproduction rates 40 days after inoculation in axenic carrot disc cultures of 4 populations of *P. thornei* from different locations but differences were observed

of the same population pathogenicity on chickpea genotypes. Biological diversity among populations of the same species in *Pratylenchus* genus was found *P. brachyurus* (Payan and Dickson, 1990), *P. goodeyi* and *P. penetrans* (Hafez et al., 1999) and *P. vulnus* (Pinochet et al., 1994). In the study, *P. thornei* populations of Sarkıkaraagac and Yalvac districts in Isparta were the higher reproductive fitness on carrot discs than Isparta centre and Gelendost districts. Stoffelen et al. (1999) reported that *P. coffeae* population of Honduras which isolated from banana was the higher reproductive fitness than Ghana and Vietnam *P. coffeae* populations on carrot discs. Mudiope et al. (2004) found that on carrot discs all the life stages of *P. sudanensis* Jinja isolate had lower densities than Masaka and Rakai isolates. No male was found in all *P. thornei* populations on carrot discs in the current study. Parthenogenetic reproduction is observed in *P. neglectus* and *P. thornei* species and the populations of these two species are almost entirely composed of females (Castillo and Vovlas, 2007).

The study showed that populations might cause different levels of damage to wheat and barley cultivars due to variation at reproduction rates of populations. This may be related to the host reaction of wheat and barley plants to the nematode. Several factors such as the ability to perceive and attract to the host, penetrate the host contribute to the pathogenicity of a nematode on a particular host (Williamson, 1999). Castillo et al. (1998) found that reproduction of *P. thornei* populations was significantly affected chickpea genotype and enabled to determine the best and poorest hosts. In the present study, Ikizce and Altay wheat and Burakbey and Tarm92 barley varieties were found the host to all *P. thornei* populations. Furthermore, it was determined that the GD18 and ISP10 populations developed better on barley than on wheat varieties. It is seen that the best host for ISP10 population is Burakbey barley variety.

It was interesting that there was high variation in nematode populations from the same province. This result may be due to various factors such as soil type, applied host rotation practices by farmers and culture processes where the samples are taken from, because these affect the population density, they may cause a change in the pathogenicity. In previous studies, researchers have stated that several factors such as cereal type, variety, soil type, pH, organic matter, fallow, alternation times and tillage could alter population density and pathogenicity in fields (Govaerts et al., 2008; Thompson et al., 2008, 2010; Collins et al., 2011).

Conclusion

The pathogenicity of the nematode is related to its reproductive fitness and many other factors especially host susceptibility or resistance which is known to have an effect. This study showed that there might be differences in reproductive fitness between different geographical populations of the same nematode species. Aware of the relationship between nematode and its host is essential for the development of nematode management practices including resistance and crop rotation. Therefore, it is seen that it would be better for breeders and nematologists to work with populations from different geographical regions in screening and breeding programs.

Compliance with Ethical Standards

Conflict of interest

The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Author contribution

The author read and approved the final manuscript. The author verifies that the Text, Figures, and Tables are original and that they have not been published before.

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Research Article

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Long time SST and Chlorophyll-a Pigment concentration of Lake Van Using MODIS

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Abstract

Current work concentrated on long-time SST and Chlorophyll-a pigment concentration of Lake Van using Modis-Aqua acquired data. Investigating the relationship between sea surface temperature (SST) and chlorophyll-a (Chl-a) increases our understanding of marine ecosystem and also provide us to assess the effect on aquatic animals that may arise as a result of changes in the environment. Long term Sea Surface Temperature variability and its relationship with chlorophyll-a pigment of phytoplankton biomass were investigated by using MODIS-Aqua (Moderate Resolution Imaging Spectroradiometer) satellite imagery. SST and Chl-a acquired from MODIS-Aqua showed seasonal, annual and interannual variability of temperature. Monthly variability Chl-a from MODIS 2002 to 2020 also investigated, significant correlation between SST and Chl-a was not found $P<0.05$. First PCA of SST seems to show general average pattern of the lake, on the other hand PCA's of Chl-a could not be calculated due to lack of null pixels within images.

Keywords: Chlorophyll, SST, Lake Van, EOF, MODIS, Climate

Introduction

Lake Van is the largest lake in Turkey, located in the far east of the country in Van district. It is a saline soda lake, receiving water from numerous small streams that descend from the surrounding mountains. Lake Van is also one of the world's largest endorheic lakes (having no outlet). The original outlet from the basin was blocked by an ancient volcanic eruption. Although Lake Van is situated at an altitude of 1,640m with harsh winters, it does not freeze due to its high salinity except occasionally the shallow northern section, (Britannica, 2019).

Lake Van is located with GPS coordinates at 38° 38′ 27″ North and 42° 48′ 45″ East, 119 meters across at its widest point, averaging a depth of 171 meter with a maximum recorded depth of 451 meter. The lake surface lies 1,640 meter above sea level and the shore length is 430 kilometers. Lake Van has an area of 3,755km^2 and a volume of 607 km3 (E. Degens, Wong, Kempe, & Kurtman, 1984).

The western portion of the lake is deepest, with a large basin deeper than 400 m lying northeast of Tatvan and south of Ahlat. The eastern arms of the lake are shallower. The Van-Ahtamar portion shelves gradually, and the maximum depth at which it joins the rest of the lake on its northwest side is about 250 m. The Ercis arm is very shallow, it has a maximum depth of less than 50 m and a maximum of 150 m. (Wong & Degens, 1978) (Tomonaga, Brennwald, & Kipfer, 2011).

Climate change is considered to be one of the most severe threats to ecosystems around the globe (Adrian et al., 2009) (Hassol et al., 2004) (Rosenzweig et al., 2007). Monitoring and understanding the effects of climate change pose challenges because of the multitude of responses within an ecosystem and the spatial variation within the landscape. A substantial body of research demonstrates the sensitivity of lakes to climate and shows that physical, chemical, and biological lake properties respond rapidly to climate-related changes (Hassol et al., 2004) (Rosenzweig et al., 2007).

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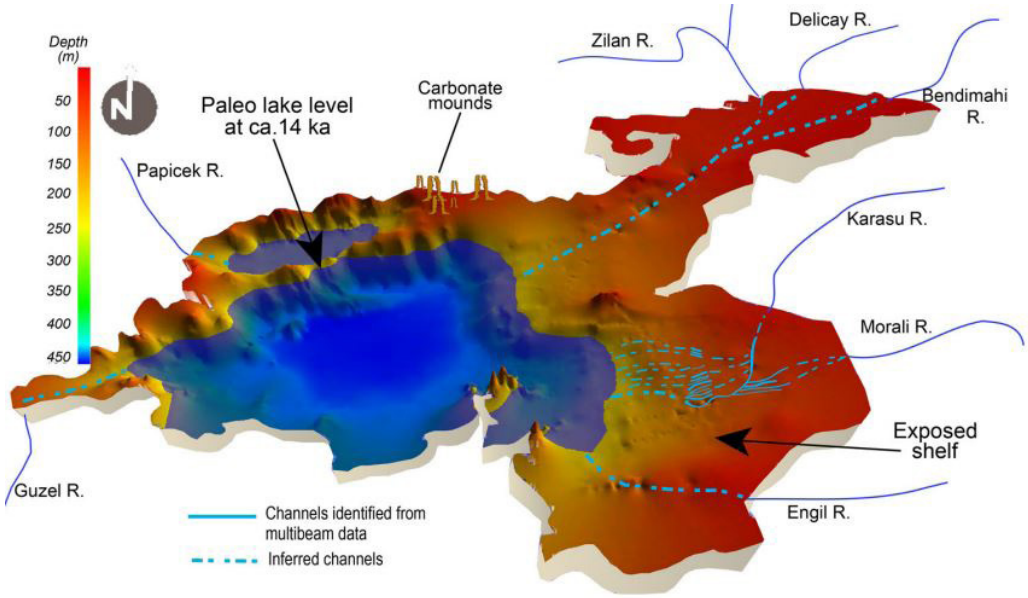

Figure 1. Bathymetry of Lake Van (Cukur et al., 2015).

Previous studies have suggested that lakes are good sentinels of global climate change because they are sensitive to environmental changes and can integrate changes in the surrounding landscape and atmosphere (Carpenter et al., 2007) (Pham, Leavitt, McGowan, Peres-Neto, & Oceanography, 2008; Williamson, Dodds, Kratz, Palmer, & Environment, 2008). One of the parameters, SST, which is important indicator of global warming first, was studied for Lake Van (Sari, Polat, & Saydam, 2000) from February 1998 to January 1999 to map SST with bathymetry and current using remote sensing techniques and by long-term SST (M. T. Kavak & S. Karadogan, 2012). Level change studied (Yıldız & Deniz, 2005). Environmental Geology of Lake Van Basin studied by (Çiftçi, Isık, Alkevli, & Yesilova, 2008). Affect by climate change studied by (Kadioğlu, Şen, & Batur, 1997). Geologically studied by E. T. Degens and Kurtman (1978).

The main producers that make food molecules (organic matter) in the water environment are phytoplankton. All phytoplankton includes at least one form of chlorophyll (chlorophyll-a) and thus can carry out energy photosynthesis, which means they have the ability to turn carbon dioxide and water into energy using sunlight. The role of phytoplankton in global climate change is important. It has been suggested that phytoplankton can reduce global warming by two different mechanisms. One of them is that this phytoplankton takes carbon dioxide from the atmosphere and converts it to organic carbon and send to the sea bottom with the dying organisms. Therefore, the amount of carbon dioxide in the atmosphere decreases and the greenhouse effect is reduced. Another mechanism is dimethyl sulfate gas, which comes out of some phytoplankton groups. This gas is oxidized to sulphate aerosols in the atmosphere and acts as cloud condensation core. It has been noted that this gas can lead to global cooling, as cloud formation will prevent the sun's rays from reaching the Earth. Phytoplankton also produces half of the earth's oxygen. Considering that phytoplankton, which is a direct or indirect food source of marine creatures, affects global warming, the importance of these organisms in the ecosystem is better understood (Develi, 2009).

On the other hand, the damages of phytoplankton should be taken into consideration as well as their benefits. While organic load and organic wastes, which are formed in the lighted layer and produced in connected productions, settle deeper in the water branch, cause the use of oxygen dissolved in water with bacterial breakdown and decrease the oxygen required for life. Chlorophyll-a concentration is considered as phytoplankton biomass and it increases along with eutrophication as a result of higher nutrient concentrations. Eutrophication, which occurs with increasing chlorophyll concentration, may decrease the amount of dissolved oxygen in the water and cause the death of the aquatic ecosystem in the long term, as well as damaging to the species by changing the habitat of aquatic animals like fish.

This study will lead to the assessment of the effect on aquatic animals by determining the chlorophyll concentration levels that are important for the aquatic ecosystem. It will also be a step towards the investigation of the effect of interpretable phytoplankton on global warming depending on the amount of Chl-a. In addition, it is possible to investigate the distribution of aquatic animals by examining the relationship between SST and Chl-a, and there are many articles about it.For example; by investigating the parameters of SST and Chl-a, the abundance of a fish species in a particular region was seasonally examined (Diankha, Sow, Thiaw, & Gaye, 2013). The relationship between sea surface temperature and chlorophyll-a concentration in fisheries aggregation area was investigated by using satellite images (Nurdin, Mustapha, & Lihan, 2013).

Present work concentrated on two parameters SST and Chlorophyll-a also their relationship using MODIS-AQUA data for 18 years which might be useful for scientists to study Lake Van for today and beyond with other parameters such as level change, eco-system, dissolved organic carbon (DOC), regional air temperature, aquatic animals' abundance and

amounts in time an etc.

The aforementioned parameters, available from remote sensing observations, are commonly used to detect the presence of algal blooms: – Chlorophyll-a Concentration (Chl-a) – Chlorophyll-a Concentration Anomalies – Sea Surface Temperature (SST) – Optical Characteristics (absorption, backscattering).

Material and Method

To examine concentrations of chlorophyll-a pigment and SST; monthly average level 3 images of MODIS-AQUA were downloaded from the Ocean Color web site from August 2002 to April 2020 and used. 4µ nighttime SST was chosen as it is less affected from atmosphere during nighttime. 210 images for each parameter, 420 images in total were downloaded (The calendar month data value varies depending on the month; this will be 28, 29, 30, or 31 days.) and processed by using SNAP which was developed by ESA. (European Space Agency) (https://step.esa.int/main/download/snap-download/). Dominant pattern of SST and Chl-a was also studied using an Empirical Orthogonal Function (EOF) analysis, also known as Principal Components Analysis. This analysis has already been used for other regions by (Kelly, 1985); (Lagerloef & Bernstein, 1988; Paden, Abbott, & Winant, 1991); (Fang & Hsieh, 1993) (Gallaudet & Simpson, 1994); (Hernandez-Guerra & Nykjaer, 1997), (Kavak, 2012).

The main interest of EOF analysis is due to its capability of decomposing a data set onto orthogonal (i.e. independent) functions. The functions which contain high variance can generally be related to physical phenomena, while the functions which contain less variance are more difficult to interpret due to the orthogonality constraint and they can be considered as noise. This technique can represent the dominant patterns of residual variance found in large and complex datasets.

Figure 2 depicts the reflectance levels for set of pixels by plotting their positions in what is commonly called band space (in this case, for an image with two spectral bands). Each axis represents the reflectance in the indicated spectral band. Each image pixel can be plotted in this space by placing its location at the intersection of its reflectance level on each band. As can be noted, there is a significant amount of correlation between bands. Since the bands in Figure 2 are correlated, each does not carry independent information. Therefore, there is a good chance of being able to predict the reflectance of a pixel in one band from the reflectance in the other. Basically, principal components analysis creates new axes called band axes along the lines of maximum variance within the data. Therefore once the pixels have been located by their new co-ordinate system (see Figure 2) a band axis PC1 image would contain more information than any other band axis image (Parsons, 1985).

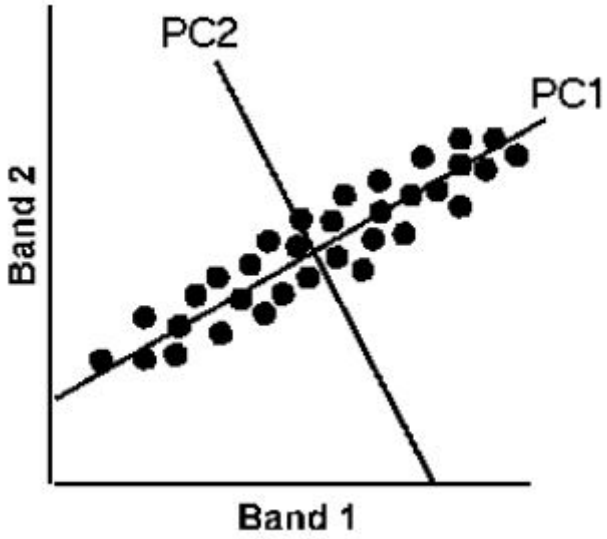

Figure 2. Principal component transforms.

Detailed information about principal component analysis may be found in some textbooks and published papers mentioned above, such as (Jensen, 2005) and (Gallaudet & Simpson, 1994).

Results and Discussions

Long time SST along with Chl-a of Lake Van. The red line represents SST, while the black represents Chl-a. As seen by the time both tend to increase over time (Figure 3).

Cross plot SST and Chl-a (Figure 4) show approximately -0.65% of correlation which were not statistically significant. Contrary to the Black Sea and Caspian Sea, which are two important water bodies close to Lake Van and studied by M.T. Kavak and S. Karadogan (2012) are showed statistically significant correlation. This situation can be attributed to the special conditions of Van Lake and the closed basin in which it is located. As a matter of fact, Van Lake is a closed basin and it is discharged only by karstic underground drainage. The surrounding of the basin is generally composed of volcanic units and has a feature of lake water soda. Perhaps the most important of the environmental conditions is the risk of increasing the pollution level with the city of Van on the lake and the surrounding agricultural elements.

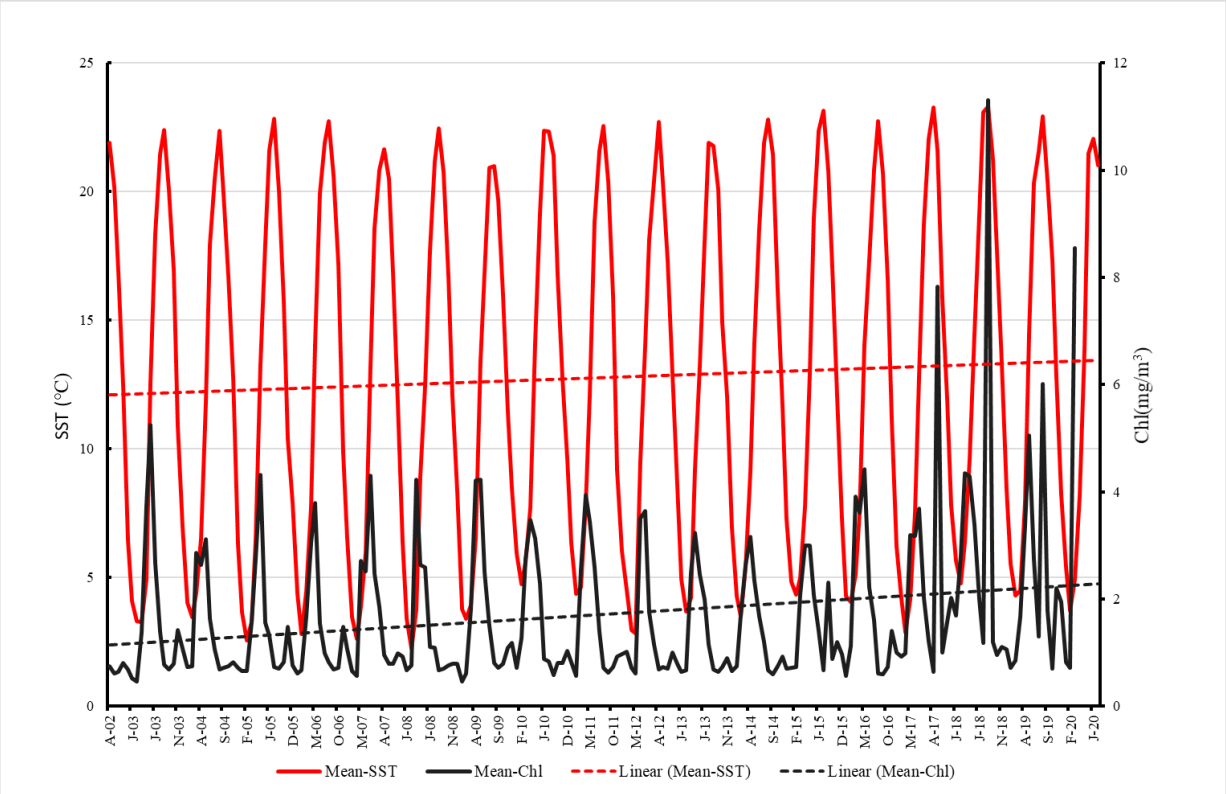

Figure 3. SST (and Clorophyll (mg/m³) variation of Lake Van from August 2002 to April 2020.

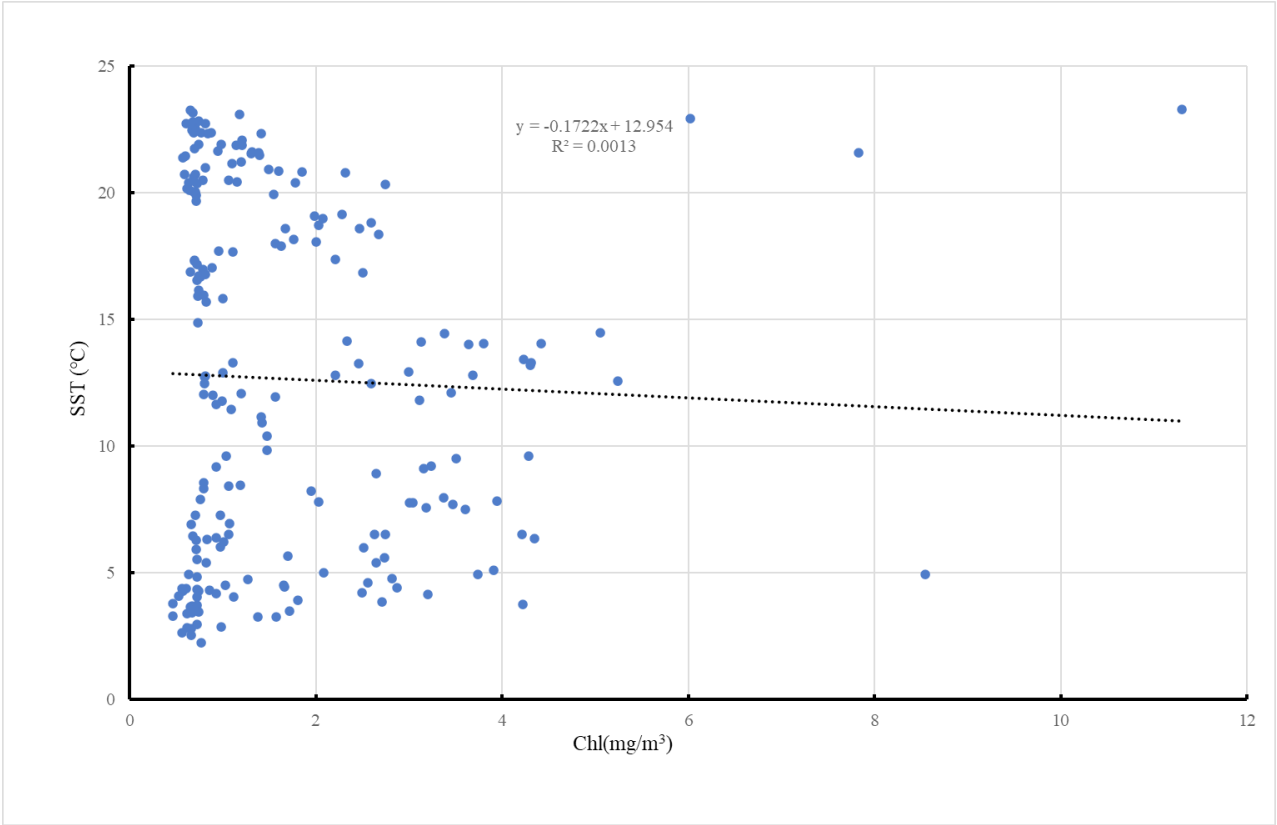

Figure 4. Relationship between SST and Chl-a values from August 2002 to April 2020.

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Looking at the seasonal chlorophyll-a variation of Lake Van (Figure 5), it could be seen that March 2003 and 2012 were below average, and September 2017, August 2018-2019 March 2020 was above the average. Highly remarkable values that were well above the average should be related to urban coastal landscaping and landscape studies.

Likewise, anomalies draw attention in the temperature graph (Figure 6). However, the deviations from the average seen on the temperature graph depend on the meteorological conditions and were in line with the temperature values at the same date.

Figure 6 shows 18 years of monthly distribution of SST values which shows that October, November 2002 and May, June 2003 are slightly off the average.

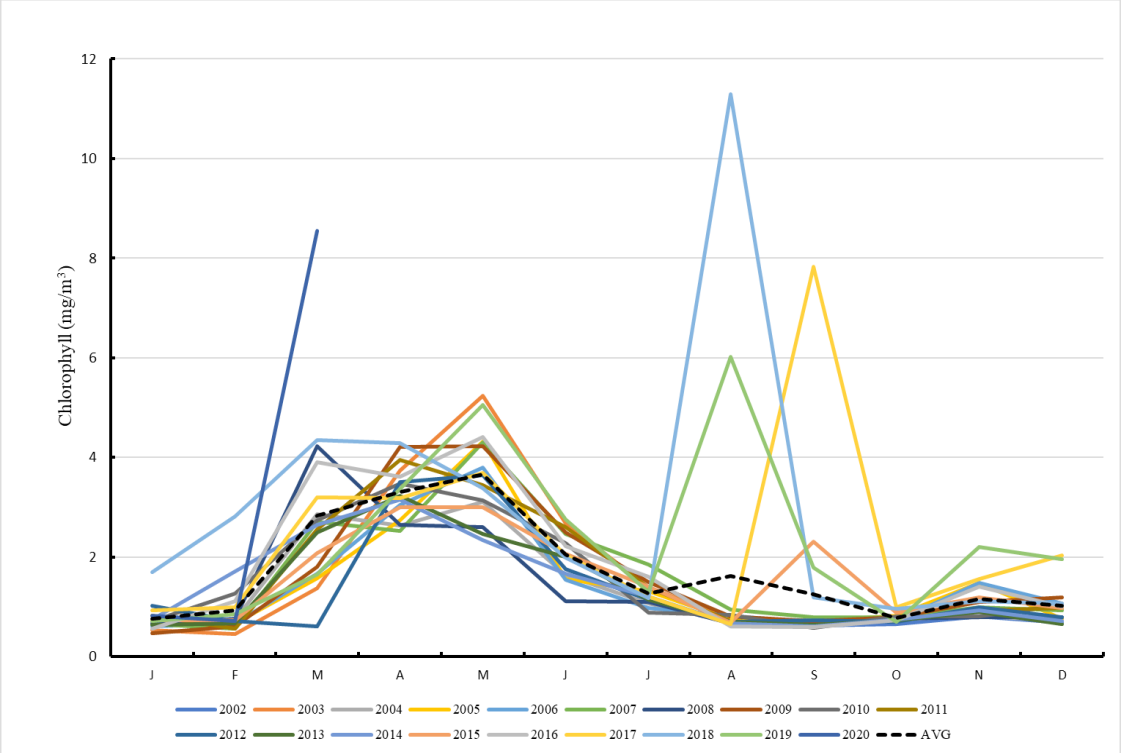

Figure 5. Monthly distribution of chlorophyll-a pigment concentration of lake Van

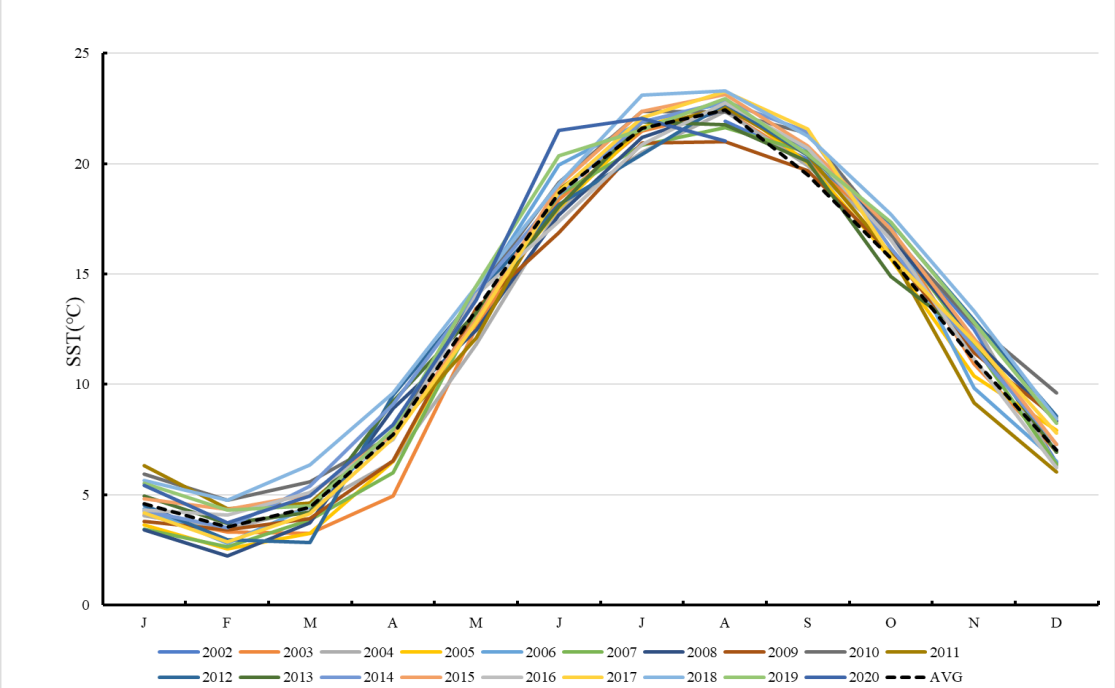

Figure 6. Monthly distribution of SST October, November 2002 and May, June 2003 are off the average.

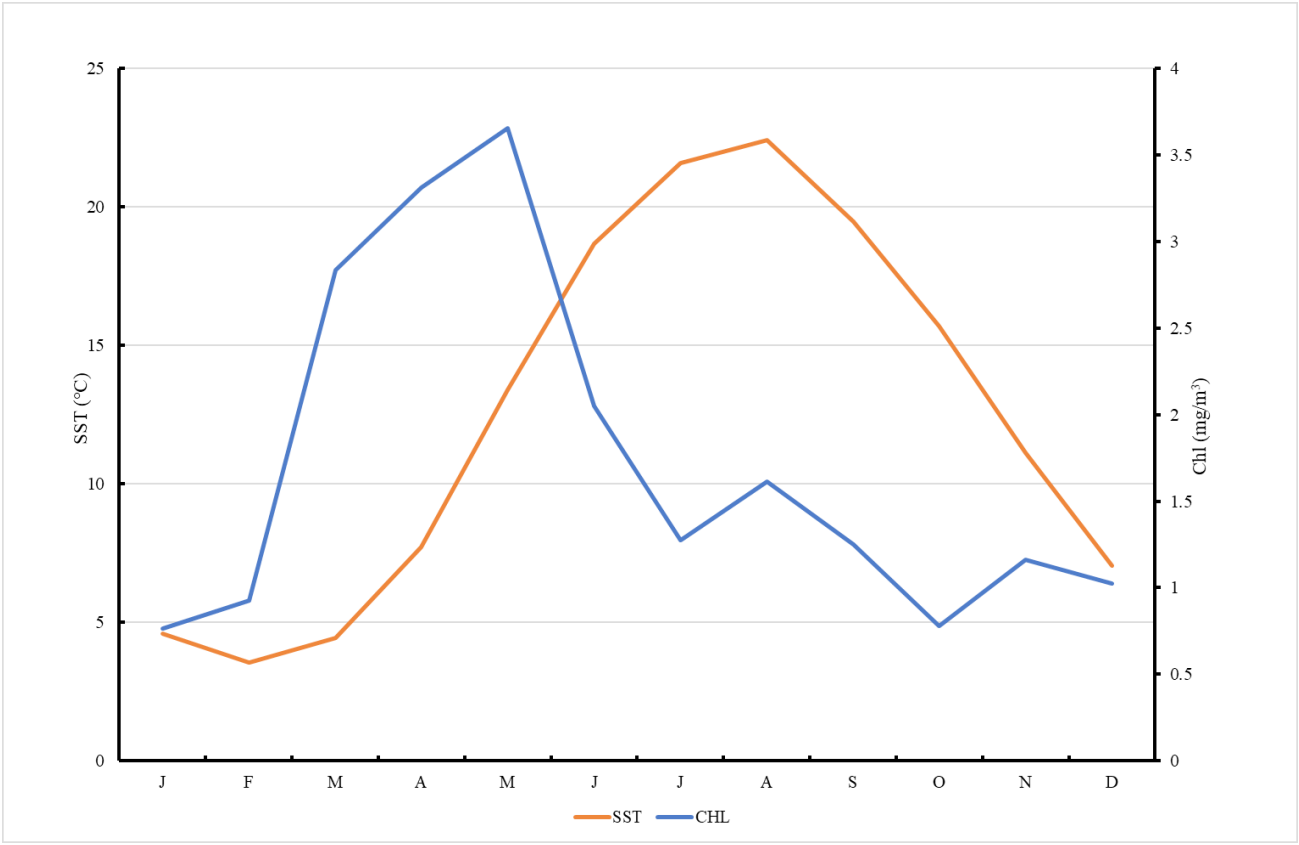

Figure 7. Monthly distribution SST and Chl-a average of Lake Van

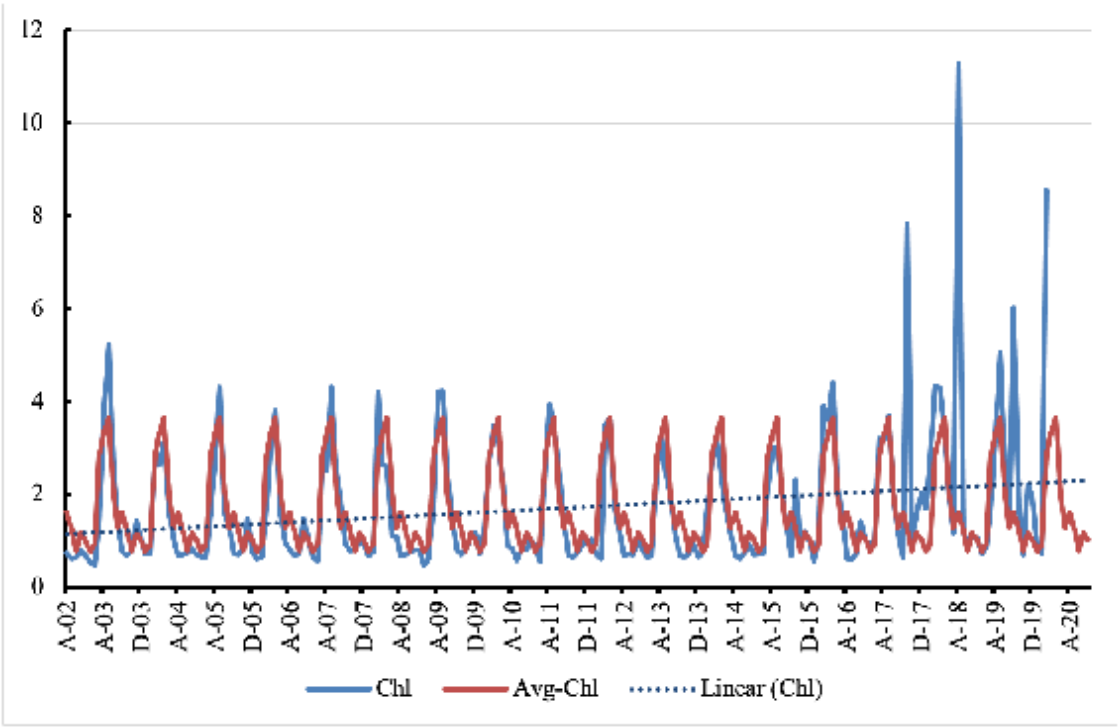

Figure 8. Long term chlorophyll-a pigment concentration along with the average variation of the Lake Van from August 2002 to April 2020.

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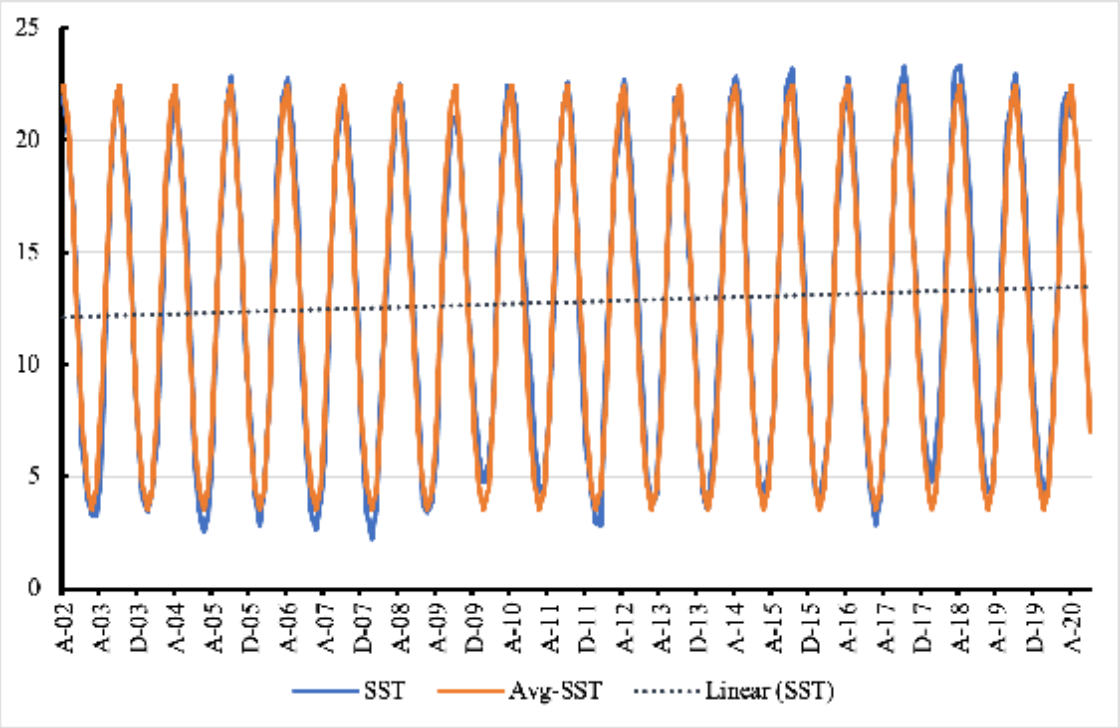

Figure 9. Long-term SST distribution with average and trendline

Although the correlation was not significant, both with a lag behaving similarly, increasing or decreasing (Figure 7).

August 2003, September 2017, March April and August 2018, and May August 2019 were off the average, general trend also showed an increase on Chlorophyll-a pigment concentration.

Long term SST in general behaved normally since it followed a sine curve however, October November 2002 and May June 2003 were off the average (Figure 8).

Long term SST (Figure 9) in general behaved normally since it followed a sine curve however, October November 2002 and May June 2003 were off the average. Increase in trendline may be due to global warming and tectonic activities present at the region.

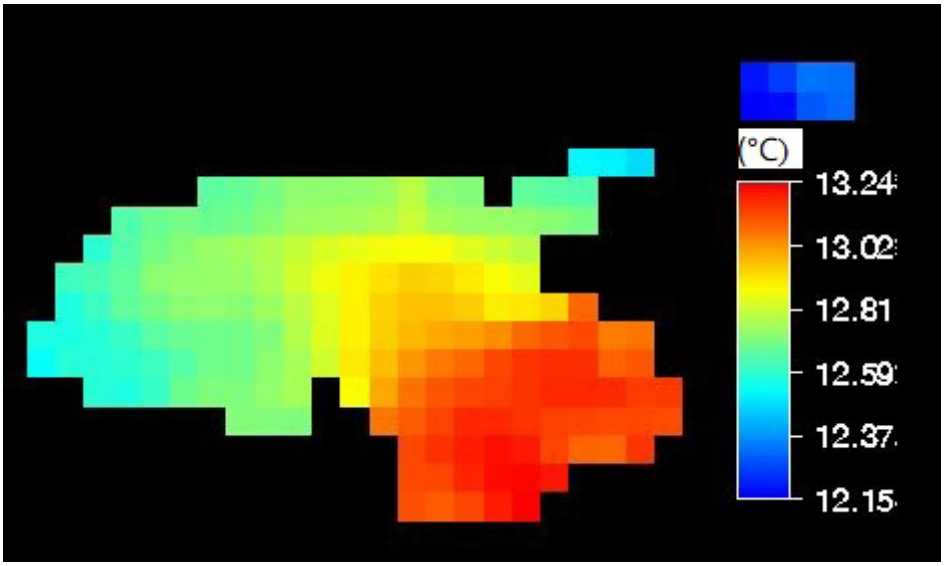

Figure 10. General average of SST of 210 images.

Average of 210 images shows that southeastern side of the lake has higher temperature than the other parts of the lake may be due to stratification caused by lack of wind. North of the lake has lower temperature caused by cold water input and bathymetry.

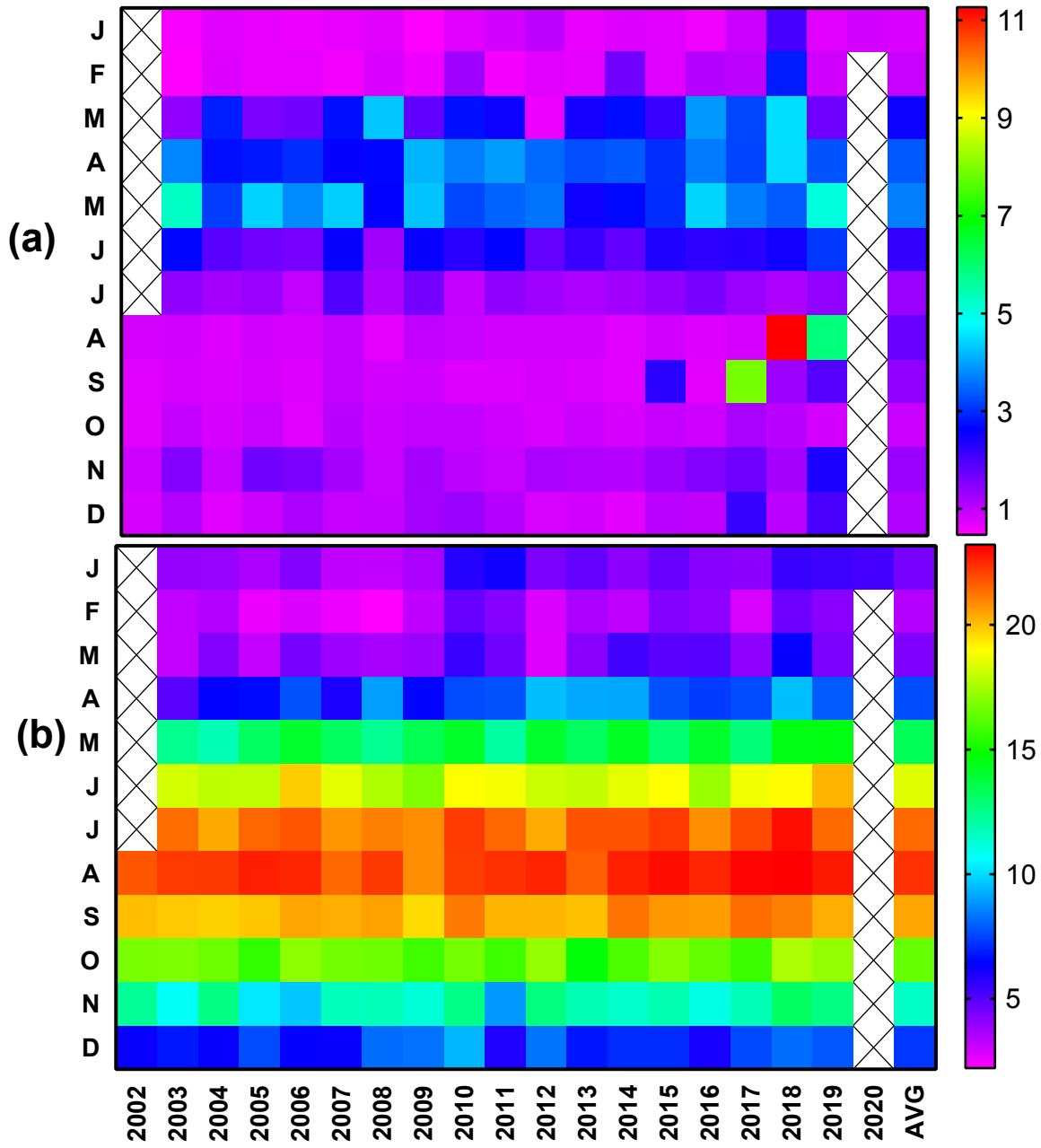

Figure 11. Monthly heatmap of Chlorophyll-a pigment concentration (mg/m^3) (a) and SST (°C) (b).

Apart from 3 extreme values, high pigment concentration generally could easily be seen (Figure 10a) on March, April, May, and June, these may be results of seasonal activities on coastal zone. According to heat map (Figure 10b) August is the warmest month of the year due to less cloud cover which blokes sun light reaching the lake surface.

Table 1. Eigen values along with accumulated variances

Bands	PC1	PC2	PC3	PC4
Cov. Eigenvalues	26.115	12.714	3.558	1.814
% Variance	0.487	0.237	0.66	003
Accumulated Variance	0.48	0,72	0.79	0.82

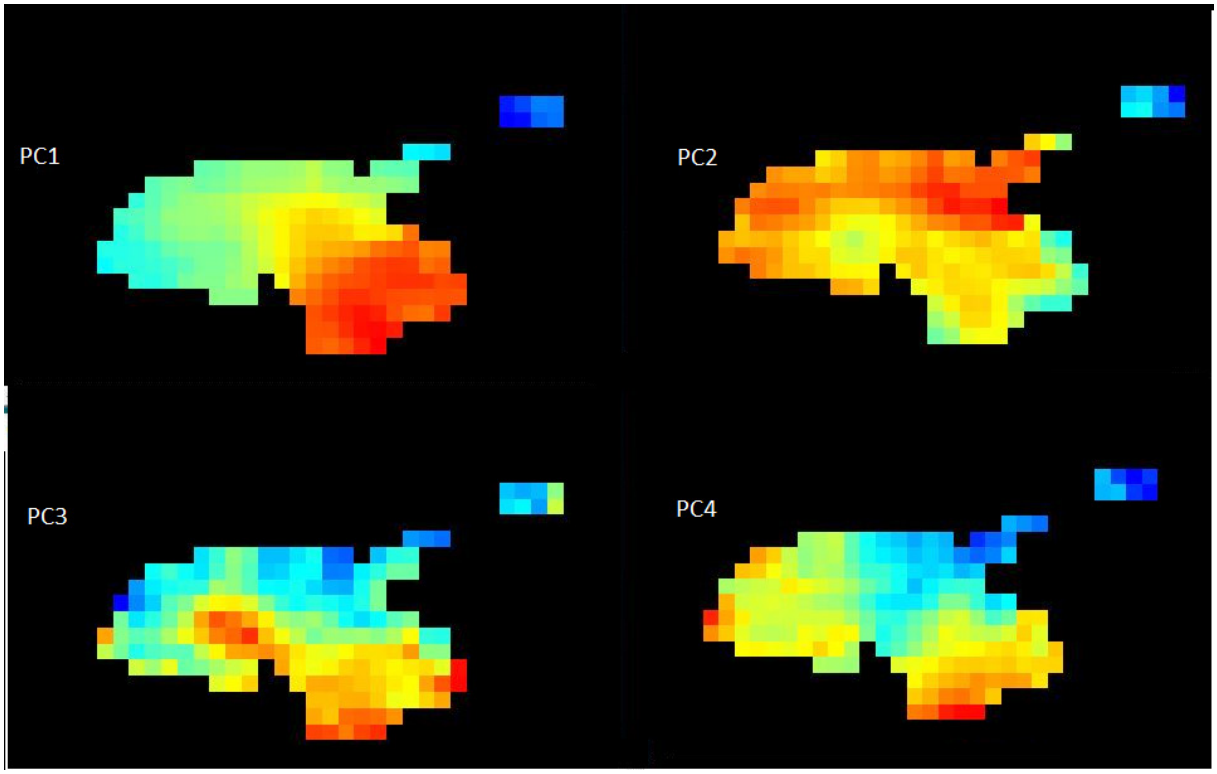

Figure 12. PC1, PC2, PC3 and PC4 all contain 82% of variances.

General Average of 210 images (Figure 11) shows that southeastern side of the lake has higher temperature than the other parts of the lake may be due to stratification caused by lack of wind. North of the lake has lower temperature caused by cold water input and bathymetry.

PC1 value in Table 1 which contains 48% of variance, almost identical to the pattern of general average SST and seems to represent bathymetry of the lake. On the other hand, PC's for Chl-a could not be calculated due do null values within the images. Generated pattern of the components PC1, PC2, PC3 and PC4 can be seen in Figure 12.

PC1 which contains 26.11% of variance, almost identical to the pattern of general average SST and seems to represent bathymetry of the lake. On the other hand, PC's for Chl-a could not be calculated due do null values within the images.

According to the findings obtained:

Contrary to the surrounding water bodies no significant correlation was found between SST and chlorophyll-a concentration. This could be because of the special geographical conditions of Lake Van and the basin in which it is located should be sought. Both in a long time tend to increase but not proportionally to each other. Both lake surface water temperature and Chlorophyll-a concentration show quite different deviations from the average in some periods. The anomalies seen in the chlorophyll-a concentration could be related to the periodic effects caused by the dense urban settlement in the environment.

While chlorophyll-a pigment concentration tended to decrease until 2011, it has been in a marked upward trend in recent years. The results also showed best time to study chlorophyll-a is March, April, May, and June. The amount of Chl-a in 2017 and 2018 deviated from the average and showed a significant upward trend, which may indicate that the habitat of aquatic animals in that area has deteriorated these years. This study can help to reach necessary parameters to investigate the reasons for the reduction of aquatic animals in water and the investigation of phytoplankton effects on global warming, which is the biggest problem of the century. In addition, it is possible to examine the abundance and distribution of aquatic animals using SST and Chl-a parameters.

In addition, it is possible to examine the effect of SST and Chl-a parameters (climate change effect) on fisheries, population parameters (e.g. growth, mortality and reproduction) the abundance and distribution of aquatic animals and fish species (Van fish, Alburnus tarichi) in the Van Lake.

Lake Van is very important in terms of recreation, tourism, and aquaculture for residential areas in the eastern regions as well as for neighboring countries. In addition, the investigation of the properties and habitat of lake water is of great importance for scientific research. For this reason, many studies are carried out by many different disciplines. In this study, lake surface temperature and chlorophyll-a concentration were investigated using satellite data for a long time. This study may be a guide for future research on local discharged water.

Compliance with Ethical Standards

Conflict of interest

The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Author contribution

The author read and approved the final manuscript. The author verifies that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

Not applicable.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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Research Article

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A research on the determination of energy consumption and carbon dioxide emissions in corn production in Harran plain

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Abstract

Due to global climate change, greenhouse gas (GHG) analyzes for agricultural production systems are becoming more common with energy analyzes. In this study, carbon dioxide (CO_2) emissions in the Harran Plain due to fuel consumption in first crop seed corn production were evaluated. A total of 100 face-to-face surveys were conducted with a total of 100 producers in the Harran Plain and data were collected on fuel consumption in production processes. The calculations to determine the CO_2 emissions released as a result of corn production are based on the proposed method of calculation of the fuel-based CO_2 emissions in the Intergovernmental Panel on Climate Change (IPCC). The total fuel and oil consumption per unit production area (ha) for different field applications in corn production is 98 l/ha in the Harran Plain. The total fuel and oil consumption in unit production corresponds to 3645.89 MJ/ha of energy consumption, resulting in a total of 269.6 kg CO_2 emissions per hectare. The specific fuel consumption, the specific energy productivity, the specific CO_2 emission, the specific fuel efficiency, the specific energy consumption and the specific CO_2 efficiency in seed corn production in the Harran Plain were determined to be 8.91 l/t, 3.02 kg/MJ, 24.51 kg_{CO2}/t, 112.22 kg/l, 331.44 MJ/t and 40.80 kg/kgCO_2, respectively.

Keywords: Corn, Fuel consumption, Carbon dioxide emission, Harran Plain

Introduction

Due to its rich nutrients, corn is a very valuable product in terms of both human and animal nutrition and has a variety of uses. Corn is used both directly in human nutrition and as a raw material in starch, glucose, oil and feed industry. Corn demand is increasing in parallel with the development of animal husbandry in our country, depending on the feed demand. Corn grain is a very good source of energy, being rich in starch and high digestibility of starch increase the nutritional value. Corn is also an important roughage used in animal nutrition as green and as silage. In other words, most of the corn production is used as animal feed.

The efficiency and profitability of agricultural production depends on energy consumption. Today, agricultural production technologies are developing rapidly and aiming higher profitability. However, despite all efforts, exhaust emissions from the fuel and motor oil consumption of tractors and other agricultural machinery still exceed the permissible limits. The power and design features of agricultural tools and machines are not selected in accordance with the production processes and the operating conditions are not suitable due to the overloading of the engines, which have negative effects on the environment. In such cases, harmful substances, petroleum products and fumes in the exhaust emissions are released into the atmosphere. These emissions significantly damage natural ecosystems.

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Carbon dioxide (CO_2) comes first among the greenhouse gases and this effect is global. Some pollutants have local characteristics such as acid rains caused by SO_2 emission. Greenhouse gases are released through both natural processes and human activities. The most important natural greenhouse gas in the atmosphere is water vapor. However, human activities increase the atmospheric concentrations of these gases, causing large amounts of greenhouse gases to be released. This situation warms the climate by increasing the greenhouse effect.

The amount of irrigated land in the Şanlıurfa-Harran Plains, which has the most fertile lands of the Southeastern Anatolia Region, is around 142,000 hectares. It is inevitable for maize to take place in crop rotation systems in irrigated areas. Corn, which has a high yield potential in the region, is a plant that can be grown as a second crop after cool climate grains and lentils. Corn is an important field crop that should take part in a crop rotation with legumes, cool climate cereals and other cultivated plants in irrigated areas such as the Harran Plain (Kün 1992). With the implementation of the irrigation project in the Southeastern Anatolia Project (GAP) region, corn cultivation has come a long way over the years. With the full implementation of irrigation investments in the GAP, the plant and animal production pattern has naturally changed. In particular, the local sheep breeds with low yield potential have been replaced with high milk and reproductive fattening and high carcass quality cattle. In crop production, intensive agriculture was introduced with irrigation.

In a study conducted to determine the performances of 10 single hybrid maize varieties under the Harran Plain main and second crop conditions. It has been reported that there are statistical differences in the second crop conditions in terms of flowering time, plant height, cob height, thousand kernel weight, grain cob ratio and grain yield, and the grain yield varied between 682.8 and 966.8 kg/da (Dok 2005). Different researchers (Öktem 2005; Öktem and Öktem 2003; 2009; Coşkun et al 2011a; 2011b) reported that they obtained high grain yields in horse-tooth corn in the second crop conditions of the the Harran Plain. In a study by Çelik and Gülersoy (2013), the change in the agricultural product pattern of the Harran Plain with GAP was examined. There are important changes in the agricultural product pattern of the Harran Plain, which is studied with the help of remote sensing. More than 90% of the Harran Plain is class I lands and is ideal for agriculture. As a matter of fact, the agricultural product pattern of the Harran Plain, which started to be irrigated since 1995 with the GAP, has changed rapidly from dry agriculture to irrigated agriculture. The change in the agricultural product pattern of the Harran Plain was examined periodically and put forward with numerical values.

Unit area yield in seed corn has shown a rapid increase trend in recent years. The main factors in yield increase are the introduction of new varieties with high yield potential and further development of breeding techniques. In recent years, hybrid varieties developed by different commercial companies and research organizations are cultivated in the corn cultivation areas of our country, and the performances of each variety in different regions can also be different. In some studies conducted on this subject, Öktem and Öktem (2009) found that there were statistically significant differences in grain yield, grain moisture at harvest, plant height and first ear height between 26 grain maize genotypes that they examined for two years under Şanlıurfa second crop conditions. Coşkun et al. (2014) found that the yield performances of corn varieties differed in different growing years. Öner et al (2012) stated that the plant height, harvest moisture, stem diameter, number of rows in the cob, number of grains in the row, thousand kernel weight, grain/cob ratio and grain yield per unit area of corn genotypes included in different death groups are affected significantly depending on the locations and different death group. They also reported that its characteristics had a significant effect on 1000 grain weight, harvest moisture, grain yield per unit area, number of rows in the cob and the height of the first cob.

Çoşkun et al (2014) conducted experiments in 2008 and 2009 in order to examine the performance of some horse tooth corn varieties under second crop conditions in the Harran plain. In the experiments, 15 horse tooth corn varieties were used as herbal material. The experiments were carried out in a randomized block design with 4 replications. In the research; grain yield, flowering time, plant height, first cob height, grain moisture at harvest, grain / cob ratio were examined. The grain yield varied between 1173.75 (Rx770) and 1429.00 (ALPAGA) kg/da in 2008. The grain yield varied between 797.25 (ALINEA) and 1107.00 (DKC 6120) kg/da in 2009. Consequently, DKC 6120 variety can be recommended for the Harran Plain second crop conditions.

In this study, it was aimed to determine the total energy consumption (diesel+ motor oil) and CO_2 emissions in first crop seed corn production in the Harran Plain. For this purpose, the processes and fuel consumption in the production of seed corn in the Harran Plain were examined in detail. The diesel consumption values of the tools and machinery used in the production of seed corn were determined by surveys conducted with farmers. During the process of seed corn production, efficiency criteria for total fuel (Diesel + lubrication oil) consumption for the tractor engine in the use of equipment and machinery are defined based on production and consumption and CO_2 emission values. In this study, direct energy use and CO_2 emissions related to fuel and oil consumption in first crop corn production in the Harran Plain were evaluated. In the calculations made to determine the CO_2 emissions released as a result of corn production, the fuel-based CO_2 emission calculation method recommended in the Intergovernmental Panel on Climate Change (Anonymous 1996) was taken into consideration.

Material and Method

Material

Study area description

The region with the largest agricultural land and irrigation systems included in the GAP project is the Harran Plain. The Harran Plain is also having one of Turkey's most frequent irrigation systems with approximately 160 thousand hectares.

The Harran Plain (Figure 1) is surrounded by Şanlıurfa and Germiş Mountains in the north, Tek Tek Mountains in the east, Akçakale and Syria border in the south and Fatik Mountains in the west. The total area of the Harran Plain, which is 65 km in North-South direction, is approximately 225 thousand hectares. As the landforms of the Harran plain, it has a very high inclination in the North-South direction with a length of 50 km and a height of approximately 130 m. The slope values are even higher at the foothills of Tektek in the east of the plain and the Fatik Mountains in the west. This high slope creates a continuous flow of water in the North-South direction and from the mountains to the middle of the plain. While the said water flow increases in terms of drainage capacity for the northern regions of the plain, it decreases the groundwater level in approximately 15-20 thousand hectares of land close to the Syrian border and consisting of low altitude parts of the plain. Groundwater is in very deep parts in the north and approaches the soil surface around Harran and Akçakale in the South. In the South, this situation affects the yield of the plant as the groundwater rises up to the plant root zone and stays in the root zone.

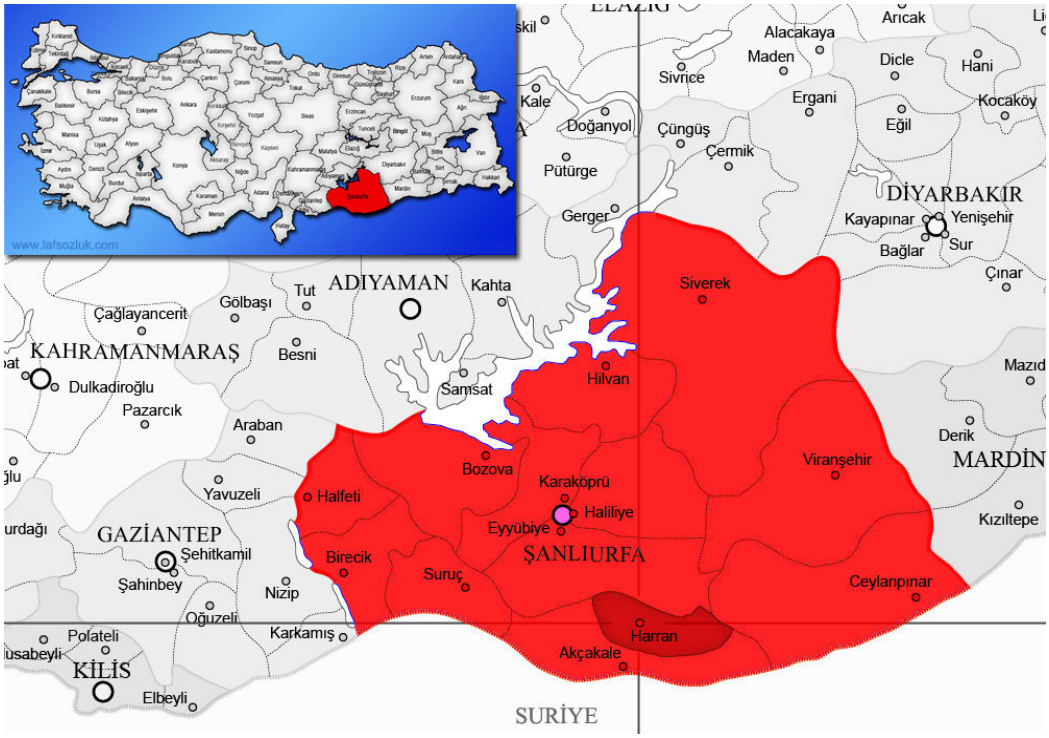

Figure 1. The map of the Harran Plain

A continental climate prevails in the Harran plain. The plain has a continental climate with not too cold winters and high temperatures in summer. Both daily and annual temperature differences are quite high. The climate data characterizing the Harran plain were evaluated using the data of Akçakale station. Temperatures generally show high values in the Harran plain. In the Harran plain, whose annual average temperature is 18 °C, the temperature values do not fall below 5 °C even in winter. In the summer, the temperature values are above 30 °C, showing a feature that almost resembles desert temperatures. Severe high temperatures in summer in the plain accelerate the salinization of agricultural lands. Salinization in the soil increases as high temperatures exacerbate the capillarity in the soil. In the study area, rainfall almost does not fall for about six months from May to October, and severe evaporation is observed in this period due to high temperatures (MGM 2020).

There are 6 different large soil groups in the agricultural areas of Şanlıurfa province. Among these, the ones covering large areas are red brown soils (1 236 366 ha), basaltic soils (431 218 ha), brown soils (167 325 ha). In addition, colluvial soils, brown forest soils and alluvial soils are included in the provincial agricultural areas. Harran series soils, which are widely included in the Harran Plain red brown soil group, are flat and nearly flat sloping soils with alluvial parent material. Typical red-colored profiles are clayey textured. Topsoil middle corner block, then granular; the subsoil is strong, large prismatic, then a strong middle corner block structure. It contains secondary lime pockets with increasing density towards the bottom (Dinç et al 1988).

Method

Method used to determine the sample size

The main material of the study is the primary data collected by making face-to-face surveys with seed corn producers in Harran Plain. In order to determine the number of questionnaires, the sampling size was calculated using the *Neyman* method (Yamane 1967) whose formula was given in equation (1).

$$n = \frac{(\sum N_h S_h)}{N^2 D^2 + \sum N_h S_h^2} \text{.....(1)}$$

where: n- sample volume, d- projected deviation, N- total number of producers, z- standard normal distribution value, N_h = number of producers in the layer. S_h = layer variance and $D = d / z$.

The number of samples to be surveyed in the Harran Plain was determined with 5% deviation from the mean and 95% confidence level. In the Harran Plain (Figure 1); A face-to-face survey was conducted with a total of 100 corn producers and data on fuel consumption in production processes were collected.

Methods used in data analysis

Calculation of total fuel consumption

The diesel and engine oil values per unit production area (da) consumed by tractor during seed corn cultivation processes were evaluated as total fuel consumption.

$$TFC=DC+LOC \text{.....(1)}$$

Where; *TFC is* the total fuel consumption (l/ha), *DC* is Diesel consumption (l/ha) and *LOC* is the lubrication oil consumption (l/ha).

Calculation of lubricant oil consumption

The lubrication oil (lubricant) consumption per hour for tractor used in seed corn cultivation operations was determined as follows, depending on the tractor's highest power take off (PTO_{max}) (Öztürk 2010).

$$LOC=0{,}00059 \times PTO_{max}+0.02169 \text{.....(2)}$$

Where; *LOC* is tractor lubrication oil consumption per hour (L/h) *and* PTO_{max} is tractor's highest *PTO* power (kW).

The maximum tail shaft power (PTO_{max}) for the agricultural tractor used for seed corn cultivation is taken into account as 88% of the tractor rated power (*TRP*, kW) and is determined as follows (Öztürk 2010).

$$PTO_{max}=0.88 \times TRP \text{.....(3)}$$

Where; *TRP* is the rated power of tractor (kW).

Determination of total energy consumption

The total energy consumption (*TEC*, MJ/ha) pertaining to the consumption of diesel and engine oil per unit production area (da) was determined by the tractor used during seed corn cultivation processes are as follows.

$$TEC=DEC+LEC \text{.....(4)}$$

Where; *DEC* is Diesel energy consumption (MJ/ha) *TEC* is the total energy consumption (MJ/ha) and *LEC* is the lubrication oil energy consumption (MJ/ha).

Calculation of Diesel energy consumption

The diesel energy consumption (*DEC*, MJ/ha) related to Diesel consumption consumed per unit production area (ha) by the tractor used during seed corn cultivation processes is determined as follows.

$$DEC=DC \times LHV_D \text{.....(5)}$$

Where; LHV_D is the lower Heating Value of Diesel (MJ/l).

The lower heating value of the Diesel (fuel) consumed by the tractor during agricultural production using agricultural tools and machinery was considered as 37.1 MJ/l (IPCC 1996).

Calculation of lubrication oil energy consumption

The lubrication oil energy consumption (*LEC*, MJ/ha) per unit production area (ha) consumed by tractor used during seed corn cultivation processes was determined as follows.

$$LEC=LOC \times LHV_L \text{.....(6)}$$

Where; LHV_L is the Lower Heating Value of lubrication oil (MJ/l).

The lower heating value of the lubrication oil (LHV_L) consumed by the tractor during the production operations in the field area with agricultural tools and machinery was taken into account as 38.2 MJ/L (IPCC 1996).

Calculation of CO_2 emissions

The CO_2 emissions from all motor vehicles burning fossil fuels can be calculated taking into account the amount of fuel consumed and the distance travelled. In the method of calculating CO_2 emissions taking into account the amount of fuel consumed, the value of fuel consumption is multiplied by the CO_2 emission factor for each type of fuel. This emission factor is developed depending on the thermal value of the fuel and the carbon fraction oxidized in the fuel and the carbon content. This approach is defined as the fuel-based CO_2 emission calculation method as it uses average fuel consumption data. The fuel consumption-based approach can be applied taking into account vehicle effectiveness data and fuel economy factors that enable the calculation of fuel consumption. Distance-based emission factors are taken into account when calculating emissions using the distance-based method. The fuel-based CO_2 emission calculation method is the preferred approach, since data on the fuel consumed is generally more reliable. However, since the uncertainty level in CO_2 estimates can be quite high, the distance based method should be used as a last remedy (IPCC 1996).

Fuel Heating Values and Selection of Emission Factors

Taking into consideration the lubrication oil consumption value of the tractor engine, CO_2 emissions related to oil consumption can also be calculated. The values given in Table 1 are used for the thermal values of diesel fuel and engine oil and CO_2 emission factors depending on the type of fuel.

Table 1. Thermal values and CO_2 emission factors (IPCC 1996)

Fuel	Lower heating value (MJ/l)	CO_2 emission factor ($kgCO_2$/MJ)
Diesel	37.1	0.07401
Lubricant oil	38.2	0.07328

Calculation of total CO_2 emissions

In calculating the CO_2 emissions released in result of seed corn production, the fuel-based CO_2 emission calculation method proposed in the Intergovernmental Panel on Climate Change was taken into account (IPCC 1996). The proposed approach to calculate CO_2 emissions based on fuel consumption is summarized in equations (8) and (9).

The total CO_2 emission (TCO_2E, $kgCO_2$/ha) pertaining to the consumption of Diesel and engine oil per unit production area (da) was determined by the tractor used during seed corn cultivation processes as follows.

$$TCO_2E = CO_2E_D + CO_2E_L \text{……(7)}$$

Where; TCO_2E is the total CO_2 emission ($kgCO_2$/ha), CO_2E_D is the CO_2 emission related to Diesel consumption ($kgCO_2$/ha) and CO_2E_L is the CO_2 emission related to lubricant oil consumption ($kgCO_2$/ha).

Calculation of CO_2 emission related to Diesel consumption

The CO_2 emission (CO_2E_D, $kgCO_2$/ha) for Diesel consumption per unit production area (ha) was determined by the tractor used during seed corn cultivation processes as follows:

$$CO_2E_D = DC \text{x} LHV_D \text{x} EF_D \text{ ……..(8)}$$

Where; CO_2E_D is the CO_2 emission related to Diesel consumption ($kgCO_2$/ha), DC is Diesel consumption (l/ha), LHV_D is the Lower Heating Value of Diesel fuel (MJ/l) and EF_D is the CO_2 emission factor for Diesel fuel (0.07401 $kgCO_2$/MJ).

CO_2 emission calculation related to lubrication oil consumption

The CO_2 emission (CO_2E_L, $kgCO_2$/ha) related to engine oil consumption per unit production area (ha) was determined by the tractor used during seed corn cultivation processes as follows.

$$CO_2E_L = LOC \text{x} LHV_L \text{x} EF_L \text{ ……(9)}$$

Where; CO_2E_L is the CO_2 emission related to lubrication oil consumption ($kgCO_2$/ha), LOC the lubrication oil consumption (l/ha), LHV_L is the Lower Heating Value of lubrication oil (38.2 MJ/l) and $EF_{L=}$ the CO_2 emission factor for lubrication oil (0.07401 $kgCO_2$/MJ)

The specific fuel consumption

The specific fuel consumption for the production of any product indicates how much fuel is consumed per unit amount produced and is defined by equation (10):

$$SFC = \frac{FC}{Y} = \frac{l/ha}{kg/ha} = \frac{l}{kg} \text{.....(10)}$$

Where; SFC is the specific fuel consumption (l/kg), FC is Amount of fuel consumed (l/ha) and Y is the amount of product produced (yield) (kg/ha).

The specific fuel efficiency

The specific fuel efficiency for the production of any product is the inverse of the specific fuel consumption value. It specifies how much product is produced per total amount of fuel consumed in production processes and is defined by equation (11):

$$SFE = \frac{Y}{FC} = \frac{kg/ha}{l/ha} = \frac{kg}{l} \text{.....(11)}$$

Where; SFE is the specific fuel efficiency (kg/l).

The specific CO_2 emission

The specific CO_2 emission indicates the CO_2 emission generated per unit mass of product produced during cultivation processes and is defined by equation (12):

$$SCE_{ems} = \frac{CO_2}{Y} = \frac{kg_{CO_2}/ha}{kg/ha} = \frac{kg_{CO_2}}{kg} \text{.....(12)}$$

Where; SCE_{ems} is the specific CO_2 emission (kg/kg_{CO2}).

The specific CO_2 efficiency

The specific CO_2 efficiency indicates the mass of product produced per unit CO_2 emission generated during cultivation processes and is defined by equation (13):

$$SCE_{eff} = \frac{Y}{CO_2} = \frac{kg/ha}{kg_{CO_2}/ha} = \frac{kg}{kg_{CO_2}} \text{.....(13)}$$

Where; SCE_{eff} is the specific CO_2 emission (kg/kg_{CO2}).

The specific energy consumption

The specific energy consumption related to fuel and oil consumption in the cultivation processes realized in the production of any product indicates how much energy is used per unit amount of the product obtained as a result of production and is defined by equation (14):

$$SEC = \frac{EC}{Y} = \frac{MJ/ha}{kg/ha} = \frac{MJ}{kg} \text{.....(14)}$$

Where; *SEC* is the specific energy consumption (MJ/kg) and *EC* is the amount of energy consumed (MJ/ha).

The specific energy efficiency

The specific energy efficiency (energy productivity) that occurs in the production of any product is the opposite of the specific energy consumption. It specifies how much product is produced per unit energy related to fuel and oil consumption in cultivation processes (Equation 15).

$$SEE = \frac{Y}{EC} = \frac{kg/ha}{MJ/ha} = \frac{kg}{MJ} \quad \text{..... (15)}$$

Where; *SEE* is the specific energy efficiency (kg/MJ).

Results and Discussion

Fuel consumption and CO_2 emissions

In the Harran plain, fuel and fuel energy consumption and CO_2 emission values per unit production area (ha) for different field applications in the production of first crop corn are given in Table 1. In the Harran Plain, 89.73 l diesel fuel is consumed per unit area (ha) in corn production. In response to the amount of fuel used at this value, a total of 3323.98 MJ of fuel energy is consumed per unit area (ha). Among corn production processes, the highest fuel consumption is in disc harrow and roller applications. In disc harrow and roller applications, 28.7 l fuel is consumed, which corresponds to 31.98% of the total fuel consumption. During soil cultivation at 15-20 cm depth with the plow, 18.8 l/ha fuel consumption is realized and this value corresponds to the energy consumption of 20.95% (697.48 MJ/ha) of the total fuel energy (Table 2). In terms of fuel energy consumption in corn production, the plow-soil process is followed by harvesting with corn headers (15 l/ha and 556.5 MJ/ha) and sowing (10.63 l/ha and 394.37 MJ/ha), respectively.

In the Harran Plain, the fuel consumption per unit production area and CO_2 emission values as a result of fuel consumption for different field applications in the production of first crop corn are given in Figure 2. A total of 246.38 $kgCO_2$ emissions are generated per unit production area (ha) in corn production. The highest amount of CO_2 emission occurs in plow deep plowing and disc harrow and slider applications, which are the two processes with the highest fuel consumption. The CO_2 emission per unit production area (ha) has been determined as 1064.77 $kgCO_2$ in disc harrow and roller applications and 697.48 $kgCO_2$ in plowing.

Table 2. Fuel and fuel energy consumption values per unit production area for different field applications in corn production

Cultivation processes	Fuel consumption per hectare (l/ha)	Total energy equivalent (MJ/ha)	CO_2 emission per hectare ($kgCO_2$/ha)	Ratio to total value (%)
Plowing	18.80	697.48	51.62	20.95
Discharrow and roller	28.70	1064.77	78.80	31.98
Sowing	10.63	394.373	29.19	11.85
Hoeing	4.20	155.82	11.53	4.68
Fertilizing	4.20	155.82	11.53	4.68
Spraying	8.20	304.22	22.52	9.14
Harvesting	15.00	556.5	41.19	16.72
Total	89.73	3328.98	246.38	100

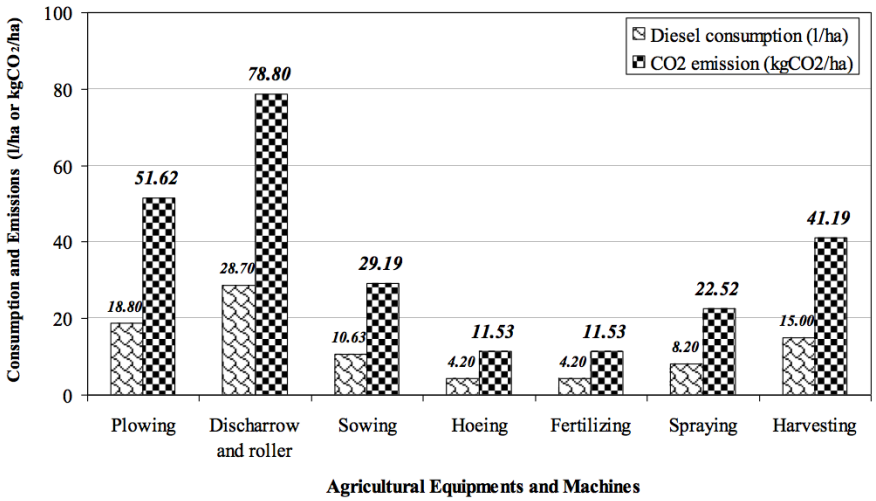

Figure 2. Fuel consumption and CO_2 emissions in corn cultivation processes

Oil consumption and CO_2 emission

Oil and fuel energy consumption and CO_2 emission values per unit production area (ha) for different field applications in corn production in the Harran Plain are given in Table 3. In the Harran Plain, a total of 8.3 liters of lubricating oil is consumed per unit area (ha) in corn production for tractors and combines used in seed corn harvesting. In response to the amount of oil used at this value, a total of 316.91 MJ of fuel energy is consumed per unit area (ha).

Table 3. Oil and oil energy consumption values per unit production area for different field applications in corn cultivation

Cultivation processes	Lubricant oil consumption per hectare (l/ha)	Total energy equivalent (MJ/ha)	CO_2 emission per hectare ($kgCO_2$/ha)
Plowing	1.578	60.28	4.42
Discharrow and roller	1.155	44.12	3.23
Sowing	0.968	36.98	2.71
Hoeing	0.685	26.17	1.92
Fertilizing	0.685	26.17	1.92
Spraying	0.725	27.70	2.03
Harvesting	2.5	95.50	7.00
Total	8.3	316.91	23.22

Among corn cultivation processes, the highest oil consumption (2.5 l/ha) occurs in the harvesting process. 1.578 l/ha of fuel consumption is achieved during tillage at 20-25 cm depth with plow (Table 3). In terms of oil energy consumption in seed corn production, plow soil treatment is followed by harrow and roller applications (1.155 l/ha and 44.12 MJ/ha), sowing (0.968 l/ha and 36.98 MJ/ha), spraying (0.725 l/ha and 27.70 MJ/ha), fertilization and hoeing applications (0.685 l/ha and 16.17 MJ/ha), respectively.

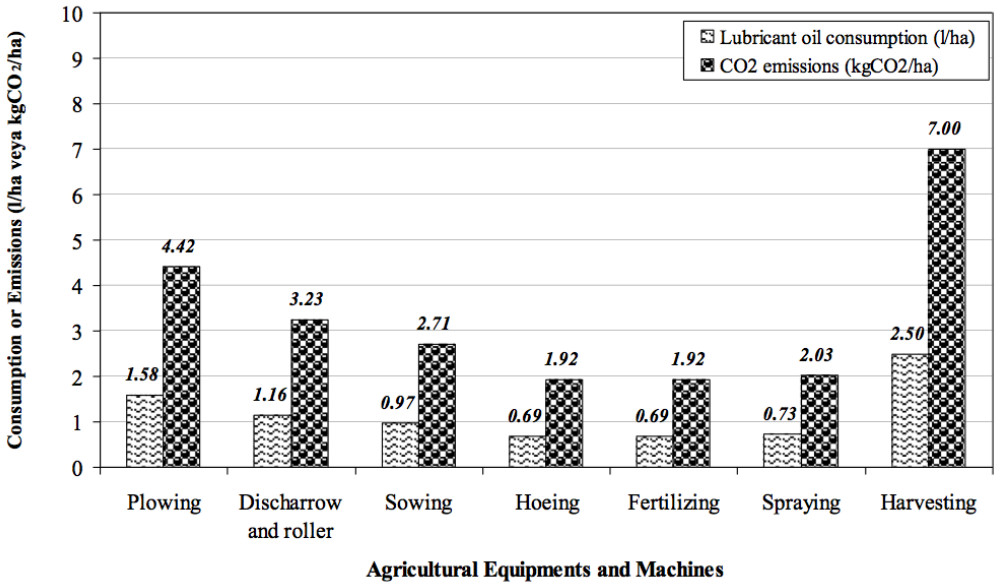

Figure 3. Oil consumption and CO_2 emissions in corn cultivation processes

In the Harran Plain, the lubricant oil consumption and the CO_2 emission values as a result of lubricant oil consumption per unit production area for different field applications in seed corn production are given in Figure 3. The total of 23.22 $kgCO_2$ emission per unit production area (ha) occurs as a result of oil consumption in corn production As with fuel consumption, the highest amount of CO_2 emissions occur in harvesting and plowing, which are the two processes with the highest oil consumption. The unit production area (ha) CO_2 emission realized as a result of engine oil consumption was determined as 7 $kgCO_2$ in the harvesting process and 4.42 $kgCO_2$ in the plowing process.

In the Harran Plain, 98 l total fuel and oil consumption per unit production area (ha) is realized for different field applications in seed corn production. The total fuel and oil consumption in the unit production area corresponds to 3645.89 MJ/ha energy use, resulting in a total of 269.6 $kgCO_2$ emissions per hectare.

The specific variables

Regarding fuel and oil consumption in different field applications in seed corn production in Harran Plain, the specific fuel consumption, the specific energy productivity and the specific CO_2 emissions are given in Table 4. The specific fuel consumption (l/kg) is defined as the ratio of the total amount of fuel consumed in the cultivation processes to the total amount of product harvested. The Specific fuel consumption refers to the amount (l) of fuel consumed to produce a unit quantity (kg or ton) of product. The low specific fuel consumption value means high energy efficiency in production. The specific fuel consumption in seed corn production in the Harran Plain is determined to be 8.91 l/t. In this case, respectively 8.91 l fuel is consumed for 1 t of seed corn production in the Harran Plain.

Table 4. The specific variables for fuel-energy-emission values in seed corn production

The specific variables	Seed yield (11000 kg/ha)
The specific fuel consumption (l/t)	8.91
The specific energy consumption (MJ/t)	331.44
The specific CO_2 emission ($kgCO_2$/t)	24.51
The specific fuel efficiency (kg/L)	112.22
The specific CO_2 efficiency ($kg/kgCO_2$)	40.80
The specific energy efficiency (kg/MJ)	3.02

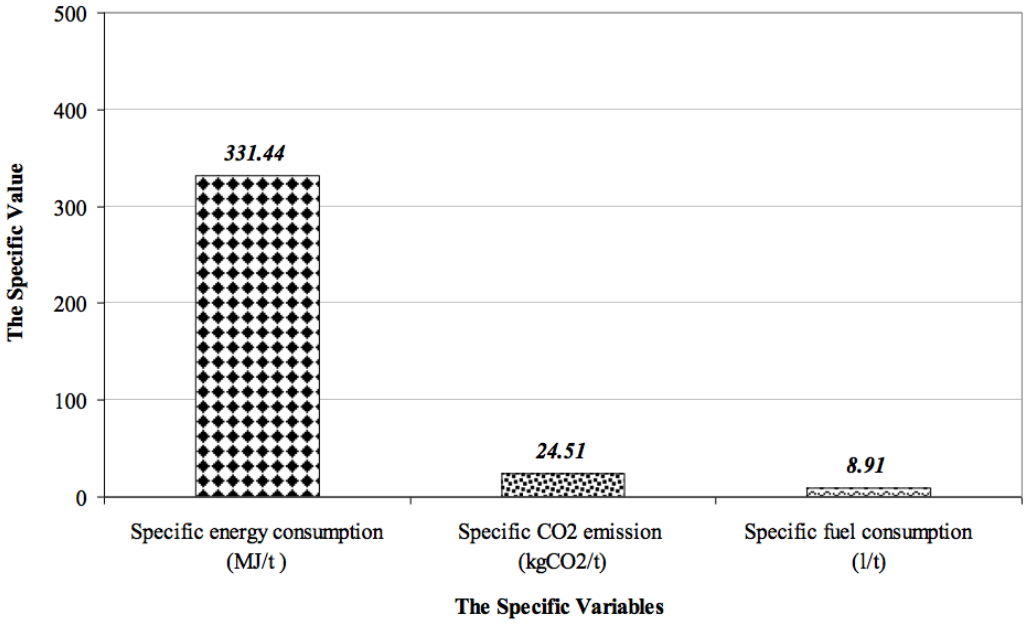

Figure 4. Change of specific variables in corn production

The specific fuel consumption

The specific energy consumption (MJ/kg) is defined as the ratio of the total amount of energy used for fuel consumption in cultivation processes to the total amount of crops harvested. The specific energy consumption value indicates the amount of energy (MJ) consumed in the cultivating processes to produce a unit quantity (kg or t) of product. The low specific energy consumption value means high energy efficiency and environmental efficiency in production. The specific energy consumption in seed corn production in the Harran Plain is determined as 331.44 MJ/t. In this case, 331.44 MJ of fossil fuel energy is consumed for 1 t seed corn production in the Harran Plain (Figure 4).

The specific CO_2 emission

The specific CO_2 emission ($kgCO_2$/kg) is defined as the ratio of CO2 emission realized as a result of the total amount of fuel consumed in cultivation processes to the total amount of harvested product. The specific CO_2 emission refers to the CO_2 emission ($kgCO_2$) value realized as a result of fuel consumption to produce a unit quantity (kg or t) of product. The low specific CO_2 emission value means that the energy efficiency in production is high and the negative effects on the environment are low. The specific CO_2 emission in seed corn production in the Harran Plain has been determined as 24.51 $kgCO_2$/kg. In this case, 24.51 $kgCO_2$ emission is realized for 1 ton seed corn production in the Harran Plain (Figure 4).

The specific fuel efficiency

Regarding the fuel and oil consumption in different field applications in the production of first crop corn in the Harran Plain, the specific fuel efficiency and the specific CO_2 efficiency values are given in Figure 5. The specific fuel yield (kg/l) is defined as the ratio of the total amount of product harvested to the total amount of fuel consumed in the cultivation processes. The specific fuel efficiency is the inverse of the specific fuel consumption (l/kg) value, indicating the amount of product (kg or t) harvested as a result of unit quantity (l) fuel consumption for cultivation processes. The high specific fuel efficiency value means that the energy efficiency of the production is high. The specific fuel efficiency in seed corn production in the Harran Plain has been determined as 112.22 kg/l. In this case, 112.22 kg of seed corn is produced in the Harran Plain as a result of 1 l fuel consumption for corn cultivation processes (Figure 5).

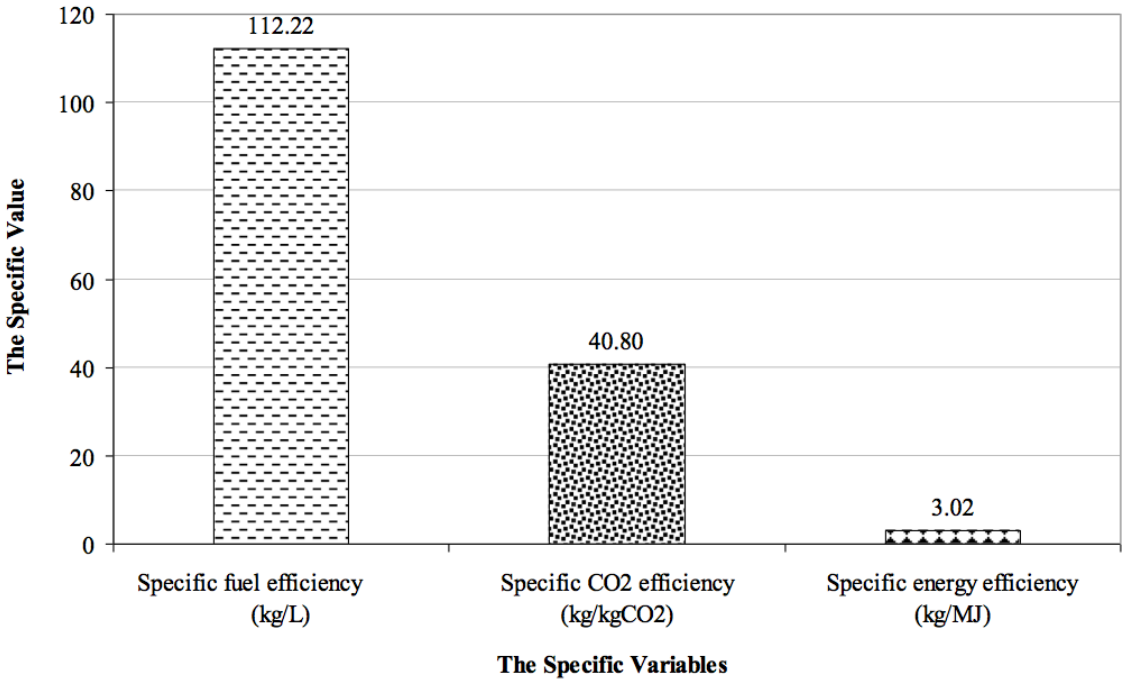

Figure 5. Change of specific variables in corn production

The specific energy efficiency

The specific energy efficiency (energy productivity) (kg/MJ) is defined as the ratio of the total amount of crop harvested to the total amount of energy used in the cultivation processes. The specific energy productivity refers to how much product (kg or t) is produced per unit amount of fuel energy (MJ) used. The high specific energy productivity value means the high energy efficiency in production. The specific energy efficiency in corn production in the Harran Plain has been determined as 3.02 kg/MJ. In this case, 3.02 kg of seed corn is produced in the Harran Plain as a result of 1 MJ of fossil fuel energy consumption for cultivation processes (Figure 5).

The specific CO_2 efficiency

The specific CO_2 efficiency ($kg/kgCO_2$) is defined as the ratio of the total amount of harvested product to the CO_2 emission realized as a result of the total amount of fuel consumed in the cultivation processes. The specific CO_2 efficiency value is the inverse of the specific CO_2 emission value and indicates the amount of product (kg or t) produced per unit CO_2 emission ($kgCO_2$) realized as a result of fuel consumption for cultivation processes. The low specific CO_2 efficiency value means that the energy efficiency in production is high and the negative effects on the environment are low. The specific CO_2 efficiency in seed corn production in the Harran Plain has been determined as 40.8 $kg/kgCO_2$. In this case, when 1 kg of CO_2 emission is relased as a result of fossil fuel consumption for cultivation processes in the Harran Plain, 40.8 kg of seed corn is produced (Figure 5).

Results and Conclusions

With the decrease in the agricultural population and labor force, mechanization practices have replaced human labor in production, and accordingly, production and productivity values have increased. The traditional tillage systems have been replaced by different tillage systems in recent years for various purposes such as reducing field traffic, minimizing production costs, and controlling erosion. Compared to traditional soil cultivation and protective soil cultivation, it requires higher inputs in terms of machinery investment, maintenance-repair and labor. As a result of researches, it has been determined that the conservative tillage and direct

cultivation increase energy efficiency and reduce energy need. In cultivation with protective tillage, it is aimed to realize the production without disturbing the physical, chemical and biological structure of the soil with the least intervention to the soil. The conservative tillage planting is a planting system in which enough plant residues are left on the surface to protect the soil from erosion throughout the year. The direct sowing, zero tillage sowing, the reduced soil tillage sowing, mulch sowing, ridge sowing and permanent ridge sowing methods, which are alternative to the traditional soil tillage using plow, and with less intervention to the soil, are accepted as protective tillage sowing methods. Protective tillage production can be an alternative to the traditional tillage-cultivation system by ensuring the sustainability of agricultural production and efficient use of energy. Thus, ensuring the efficient use of all resources and converting them to the highest output is of great importance for agricultural and environmental sustainability. In addition, since chemical fertilizer applications, which constitute the most important energy input, are the most environmental pollutants, soil analysis should be done well and their applications should be used in accordance with the technique (especially by taking measures to reduce nitrogen fertilizers). Agricultural enterprises should analyze their current mechanization situation well and make their plans according to advanced technology levels. In particular, measures should be taken to reduce the power requirements and fuel consumption of the agricultural machinery used, and agricultural equipment and machinery should be used in accordance with the power source.

Compliance with Ethical Standards

Conflict of interest

The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Author contribution

The author read and approved the final manuscript. The author verifies that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

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Green synthesis, characterization, antimicrobial and antioxidant activities of zinc oxide nanoparticles using *Helichrysum arenarium* extract

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Abstract

Active substance production at the nano-level attracts attention in the scientific world due to its wide application area. Different methods are used for the biosynthesis of nanoparticles. Recent studies have focused on non-toxic, environmentally friendly synthesis methods. Nanoparticles obtained by green synthesis using various biological elements such as plants, microorganisms and proteins have taken part in many scientific studies. Plants, which have an important potential in active ingredient production, are highly preferred in nanoparticle production. Scanning Electron Microscope and Energy Dispersive X-Ray Analysis (SEM / SEM-EDX), Fourier Transform Infrared Spectroscopy (FT-IR), X-Ray Diffraction (XRD) and Ultraviolet visible light absorption Spectroscopy (UV-vis) techniques were used for the structural and morphological characterization of Zn nanoparticles obtained by green synthesis using *Helichrysum arenarium* plant extract and ZnO.The antioxidant capacity of Zn NPs/Ha structures was determined by performing the DPPH test. Antimicrobial effects of zinc nanoparticles on six different pathogens (*Bacillus cereus* ATCC 10876, *Escherichia coli* ATCC 25952, *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATTC 29213, *Pseudomonas aeruginosa* ATCC 27853 and *Candida albicans* ATTC 90028) were investigated. As a result of this studies, it has been observed that it has an inhibitory effect against some pathogen microorganisms. It has also been found that its antioxidant content is at a significant level.

Keywords: Zinc Oxide, Nanoparticle, Antimicrobial, *Helichrysum arenarium*

Introduction

In recent years, the using of nano and microstructures in science and technology has been increasing (Herlekar et al., 2014). It is thought that the nanomaterials produced by Green synthesis can be used as anti-cancer agents and pharmaceutical agents (Hanan et al., 2018; Hay et al., 2017; Dipankar & Murugan, 2012; Bupesh et al., 2016). Understanding the biocompatibility of nanoparticles is a necessary step for biomedical applications (Das et al., 2016). It has quickly gained popularity in different industries such as nanotechnology, bioenergy, and agricultural systems (Rai et al., 2018). Synthesis of metal nanoparticles using plants has been an important way to overcome the limitations of traditional synthesis approaches such as physical and chemical methods (Rajeshkumar & Bharath, 2017; Wu et al., 2017). Research in nanotechnology has yielded environmentally and biologically safe results. Plant-based synthesis of metal nanoparticles was chosen as the best strategy because of its environmental friendliness and ease of synthesis (Khan et al., 2019). *Helichrysum arenarium* (L.) plant is widely available in Asia and Europe. This plant that has been used in traditional therapy, it has a good antioxidant content used in cholecystitis and bile treatments (Tepe et al.,

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2005). It has been determined that the essential oils obtained from the flowers of the *Helichrysum arenarium* plant have an important antimicrobial potential (Rančić et al., 2005). Zinc is an important micro element for humans found in protein and enzymes (Prasad, 2014). Zinc oxide (ZnO) nanoparticles have properties such as antimicrobial and photocatalytic activity (Miri et al., 2019). Zinc Oxide-derived nanoparticles have become a new treatment thanks to their chemical and thermal resistance (Wu, 2019). ZnO nanoparticles obtained by Green synthesis have been shown to inhibit the growth of *Escherichia coli* and *Staphylococcus aureus* pathogens (Ansari et al., 2020).

In our study, nanoparticles were obtained by green synthesis using *Helichrysum arenarium* plant and ZnO. Scanning electron microscopy (SEM/SEM-EDX) image was obtained for the structural and morphological characterization of ZnO nanoparticles. At the same time, FT-IR analysis, X-Ray Diffraction method (XRD) and UV-vis spectrophotometer analyzes were performed to demonstrate the reliability of the obtained nanoparticles. Antimicrobial effects of ZnNPs/Ha structures on six different pathogens were investigated by performing antioxidant content testing.

Materials and Methods

Preparation of plant extract

Helichrysum arenarium plant collected from the mountainous area of Van in Turkey was washed in the laboratory. After drying for 7 days at room temperature, it was pulverized with a grinder. 100 g of the ground sample was taken and left to boil at 80 ° C for 10-12 minutes with 500 mL of distilled water. It was cooled after the color change occurred. Filtration was performed by using Whatmann No: 1 filter paper and the obtained extract was stored at 4 °C (Gün et al., 2011).

Synthesis of ZnO nanoparticles

For the synthesis of ZnO nanoparticles, 1 mM 500 ml of ZnO solution and 100 ml of *Helichrysum arenarium* plant leaf extract were reacted in a 1000 ml flask at room temperature. Color changing in the solution was observed with in 25-40 minutes with the reduction of zinc ions. The resulting solution was centrifuged at 10.000 rpm for 5 minutes and the liquid phase remaining in the upper phase was discarded. The solid phase was washed several times. The obtained particles (Zn NPs/Ha) were left to dry for 72 hours at 45-50 °C in the oven. It was stored at 4 °C for characterization and analysis (Joseph & Mathew, 2015).

Characterization of Zn NPs/Ha

Topography of the samples was taken for scanning electron microscopy (SEM) imaging. In addition, the density of the metal was measured by SEM-EDX (SEM, Zeiss Smart EDX). The crystal structure of Zn NPs / Ha was obtained using XRD technique (Panalytical Emperian diffractometer, 40 mA, 40 kV, k 1.54056). Characteristic absorption values for zinc nanoparticles were investigated using UV-Vis (PEL 750 instrument (measurement range 250-800 nm wavelength)). FT-IR analysis was measured in the wavelength range of 500-4000 cm-1 using the Perkin Elmer Spectrum Two Spectrometer (Aiken & Finke, 1999).

Antioxidant activity (DPPH)

The DPPH quenching activity of Zn NPs/Ha was calculated using the previously method (Blois, 1958; Koçak et al., 2020). BHA and BHT were used as positive controls in this method. The experiment was performed using 0.1 mg/ml DPPH methanol solutions. DPPH and extracts with the same ratio were prepared in 5 different concentrations of 25, 50, 100, 200 and 250 µg/ml. 3 ml of Zn NPs/Ha extract and positive control were taken and DPPH solution was added on them. The mixtures formed in the tubes were left to incubate for 30 minutes in the dark and at room temperature. At the end of this period, absorbance values were measured at 517 nm.

$\% I = [(Akontrol-Aample) / Akontrol] \times 100$

According to the result of this process, a graph of Zn NPs/Ha concentration versus increasing DPPH ethanol concentration was obtained.

Antimicrobial Activity

The antimicrobial activity of *Helichrysum arenarium* plant and zinc nanoparticles was investigated by disk diffusion method (Prabhu et al., 2010). *Bacillus cereus* ATCC 10876, *Escherichia coli* ATCC 25952, *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATTC 29213, *Pseudomonas aeruginosa* ATCC 27853 and *Candida albicans* ATTC 90028 were used as pathogens. Test microorganisms were obtained from Van Yüzüncü Yıl University, Faculty of Science, Department of Biology. Müller Hinton medium was used in this study. Plant extract and Zn NPs/Ha 25 µL were absorbed on blank discs and dried at room temperature. Then the discs and Neomycin (10 µg) were placed in the medium with the positive control group. The prepared petri dishes were kept in the oven for 24 hours at 36.5 °C for incubation. Then the zone measurements were made and the images were recorded.

Results and Discussion

Characterization of zinc nanoparticles

It was determined that ZnNPs subjected to electron by scanning electron microscope (SEM) have a shallow and spherical structure (Figure 1.). The SEM/EDX spectrum of ZnNPs shows the peaks of zinc, carbon, calcium, chlorine and oxygen elements (Figure 1.) (Elumalai & Velmurugan, 2015). The UV-Visible absorbance spectrum used in the studies should show the nanoparticle formation In this context, it was observed that the ZnO nanoparticle peaked at 370 nm (Figure 2). It was observed that ZnO nanoparticles were formed by using *Helichrysum arenarium* extract. It was observed that Zinc oxide analyzed using UV-Vis spectroscopy formed a strong surface plasma peak at 370 nm wavelength (Büyük and Ilıcan, 2018). However, it was seen that the peak formed disappeared because Zn NPs/Ha was reduced to Zn^{+2}- Zn^{0} due to the transition between the electrons (Figure 2). XRD technique was used to determine the crystal structure of zinc nanoparticles. X-ray diffraction (XRD) of synthesized zinc nanoparticles was used to verify the crystal structure of the particles. Figure-3 shows a representative XRD model of zinc nanoparticles synthesized with *Helichrysum arenarium* plant extract after complete reduction of zinc. In the present study, it is seen in Figure 3 that ZnONPs is crystallized as hexagonal wurtzite form. XRD results appear to be in line with studies

in the literature. The XRD results obtained are in parallel with similar studies in the literature. Considering the spectrum, different peaks are seen (Figure 3). It has been determined that Zn NPs have different refractive peaks as 32.05 ° 37.45 ° 47.23 ° and 66.22 ° versus Zn (111), Zn (200), Zn (220), and Zn (311) planes (Anvekar et al., 2017). Figure 4 shows peaks formed by functional groups (glycine, octasiloxane, glycolic acid, flavones, coumarin, glycoside) in the structure of *Helichrysum arenarium*, which is the current plant in the study, in the range of 800-1500 cm^{-1} (Figure 4). It is thought that the peaks in between 3500-2700 cm-1belong to many organic components and the sharp peak at 2900-3000 cm-1 belongs to the C-H organic component (Xiong et al., 2009).

Antioxidant activity (DPPH)

Antioxidants can prevent cell damage by clearing the damaging molecules called free radicals in our cells. In our current study, by determining the antioxidant properties of ZnNPs created using ZnO, the quenching activity of DPPH (2,2-Diphenyl-1-picrylhydrazyl) free radical was determined. DPPH radical is a commercially available free radical and a safe molecule in total antioxidant calculations. This radical is a purple colored solution and provides maximum absorbance at 517 nm (Yu et al., 2002). It is observed that the purple color of DPPH radical, which reacts with antioxidant components, decreases over time and its absorbance value decreases. In our current study, Zn NPs/Ha clusters were compared with the positive control BHA and BHT (Figure 5.). DPPH radical quenching activities for positive control BHA and BHT at 100 µg/ml concentration, respectively; While it is 94.155-89.480%, this value for Zn NPs/Ha is 79.871%. Compared to many studies, Zn NPs/Ha appears to be a good antioxidant (Nunes et al., 2018; Suresh et al., 2015).

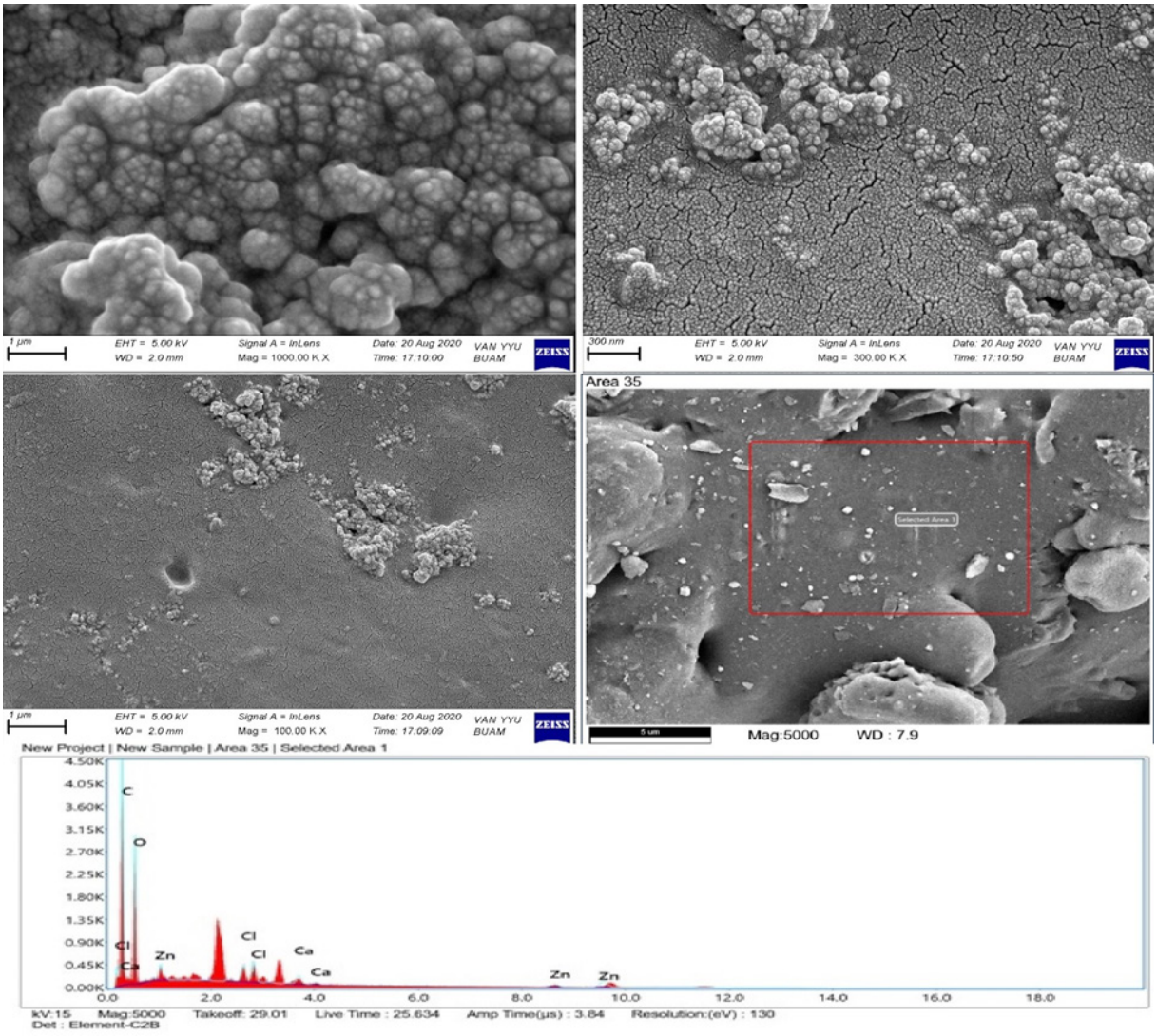

Figure 1. Scanning electron microscope (SEM) and SEM / EDX spectrum image of Zn NPs/Ha.

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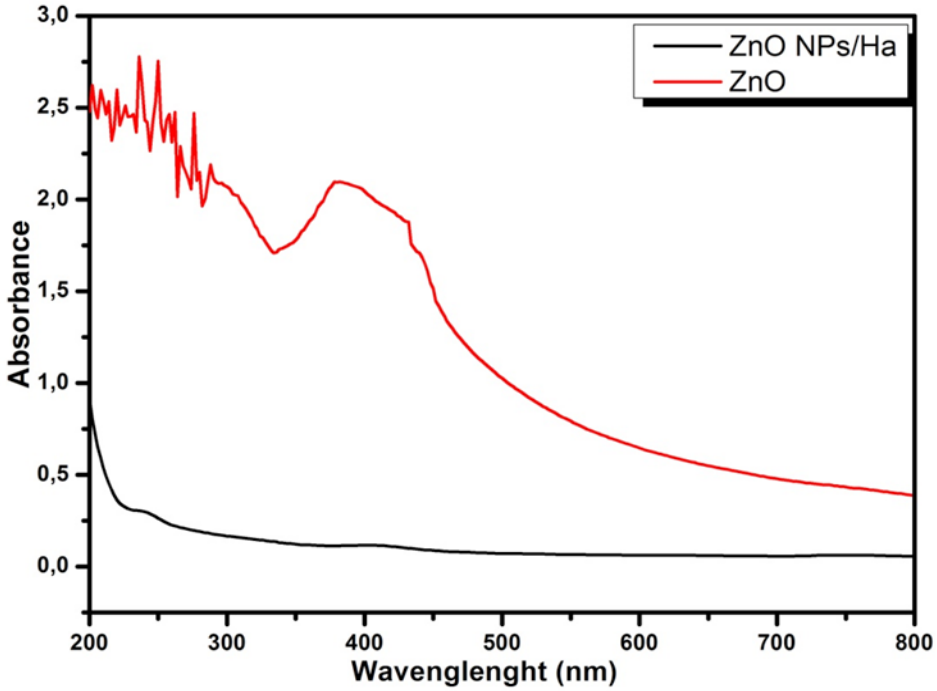

Figure 2.UV-vis spectra of ZnO and Zn nanoparticles

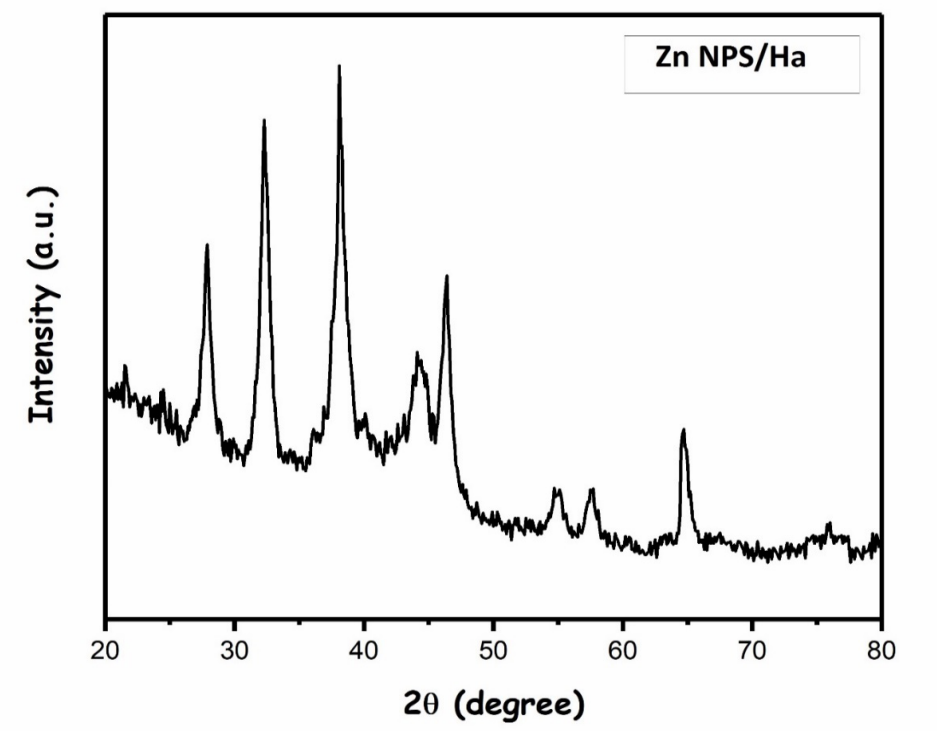

Figure 3. XRD graph of Zinc Oxide nanoparticles

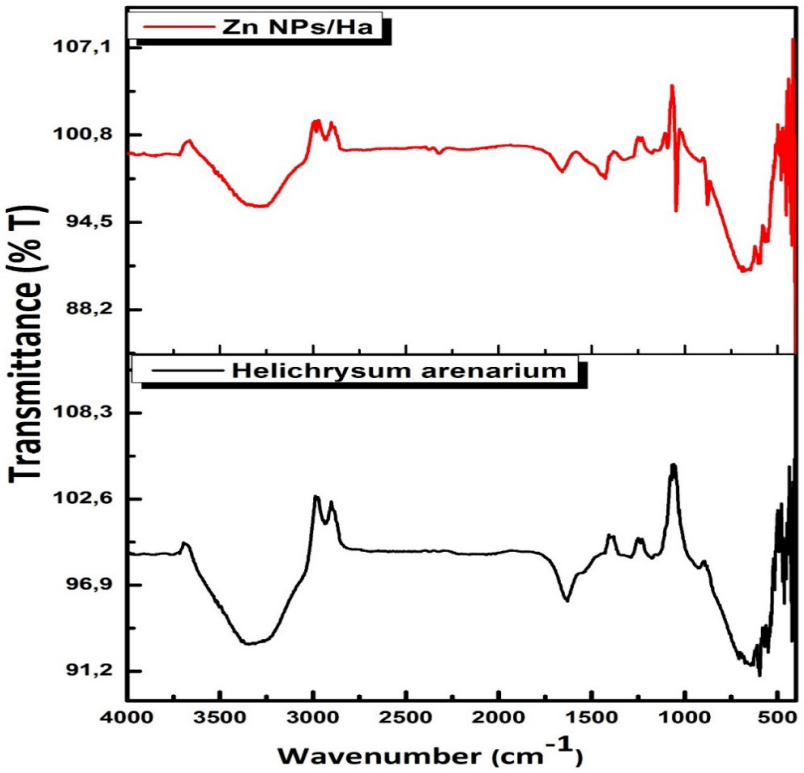

Figure 4. FT-IR spectra of *Helichrysum arenarium* and Zn NPs/Ha samples

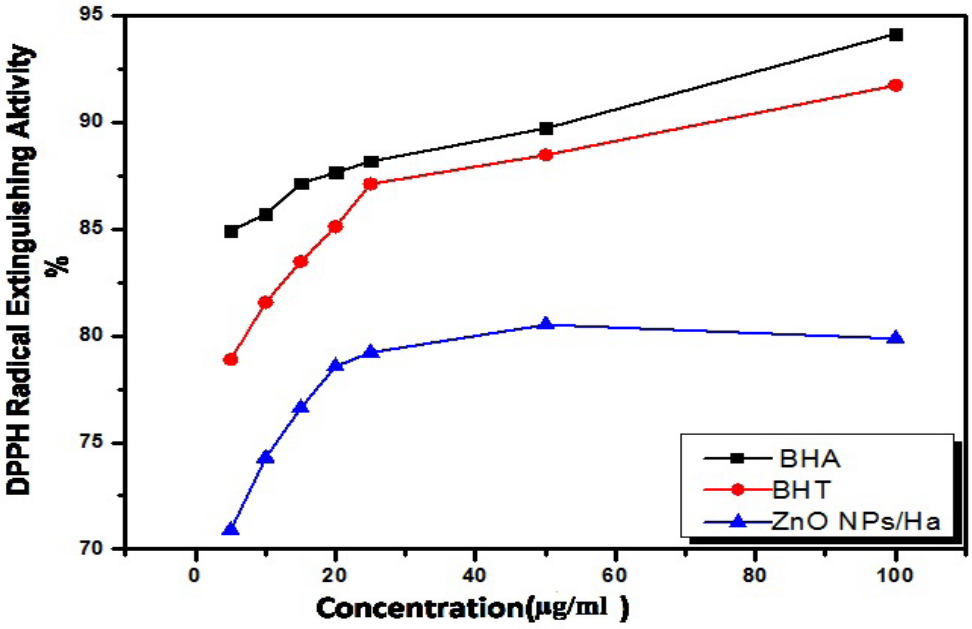

Figure 5. DPPH Radical activity of ZnO nanoparticles.

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Antimicrobial Activity

According to the obtained data, it was determined that *Helichrysum arenarium* plant extract and Zn-NPs/Ha clusters were effective against pathogens. In the antibacterial activity measurements, it was determined that synthesized ZnO nanoparticles provided bacterial activity inhibition in *E. coli* and *S. aureus* (Erdoğan et al., 2019). ZnONPs are known to inhibit the growth of *Campylobacter jejuni, Salmonella enterica, Escherichia coli, Vibrio cholerae* and *Staphylococcus aureus* bacteria (Sirelkhatim et al., 2015). It was observed that extract and ZnO nanoparticles formed an inhibition zone varying between 8.15-11.15 mm against pathogenic microorganisms. Antimicrobial activity results are given in Table 1, the zone rates formed are in Figure 6 and some images obtained as a result of disk diffusion are given in Figure 7.

Table 1. Antimicrobial activity results.

Test Microorganisms	Inhibition zones (mm)		
Bakteria	Extract	Zn NPs/Ha	Neomycin
Bacillus cereus ATCC 10876	9±1.5	9±3.0	20±1.0
Bacillus subtilis ATCC 6633	9±1.0	8±1.5	22±1.5
Escherichia coli ATCC 25952	10±1.0	9±2.5	14±2.5
Pseudomonas aeruginosa ATCC 27853			
Staphylococcus aureus ATTC 29213	10±2.0	9±2.0	17±2.5
Fungus			
Candida albicans ATTC 90028	11±1.5	9±1.0	21±3.0

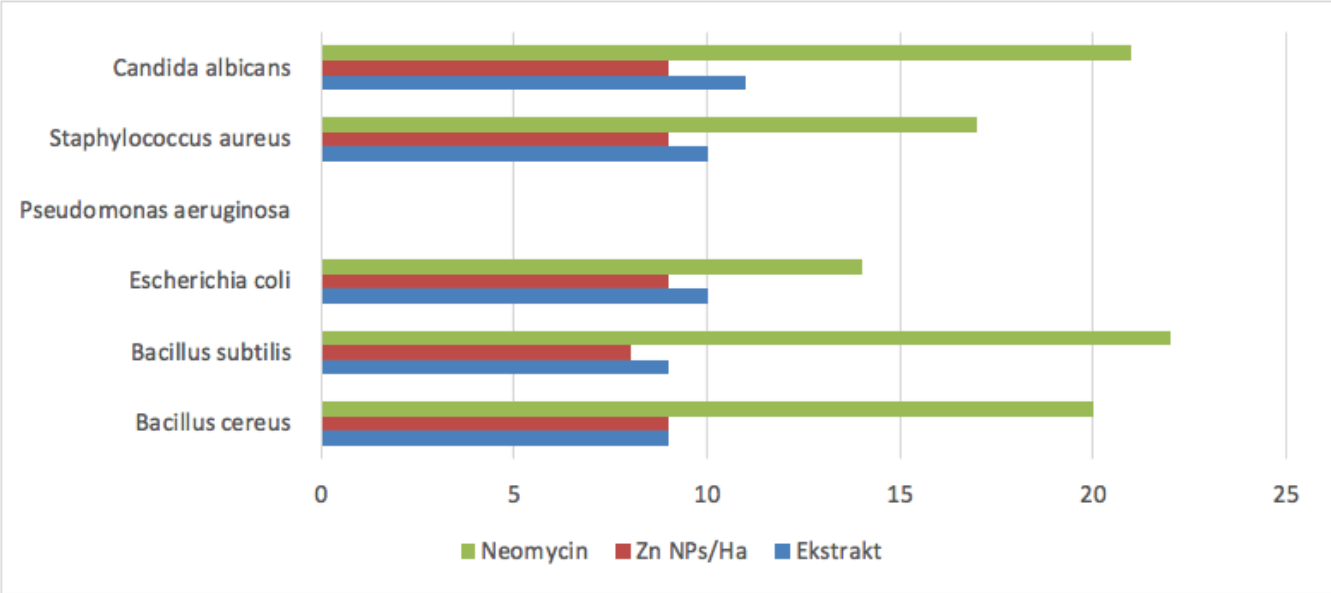

Figure 6. Zone rates resulting from antimicrobial activity

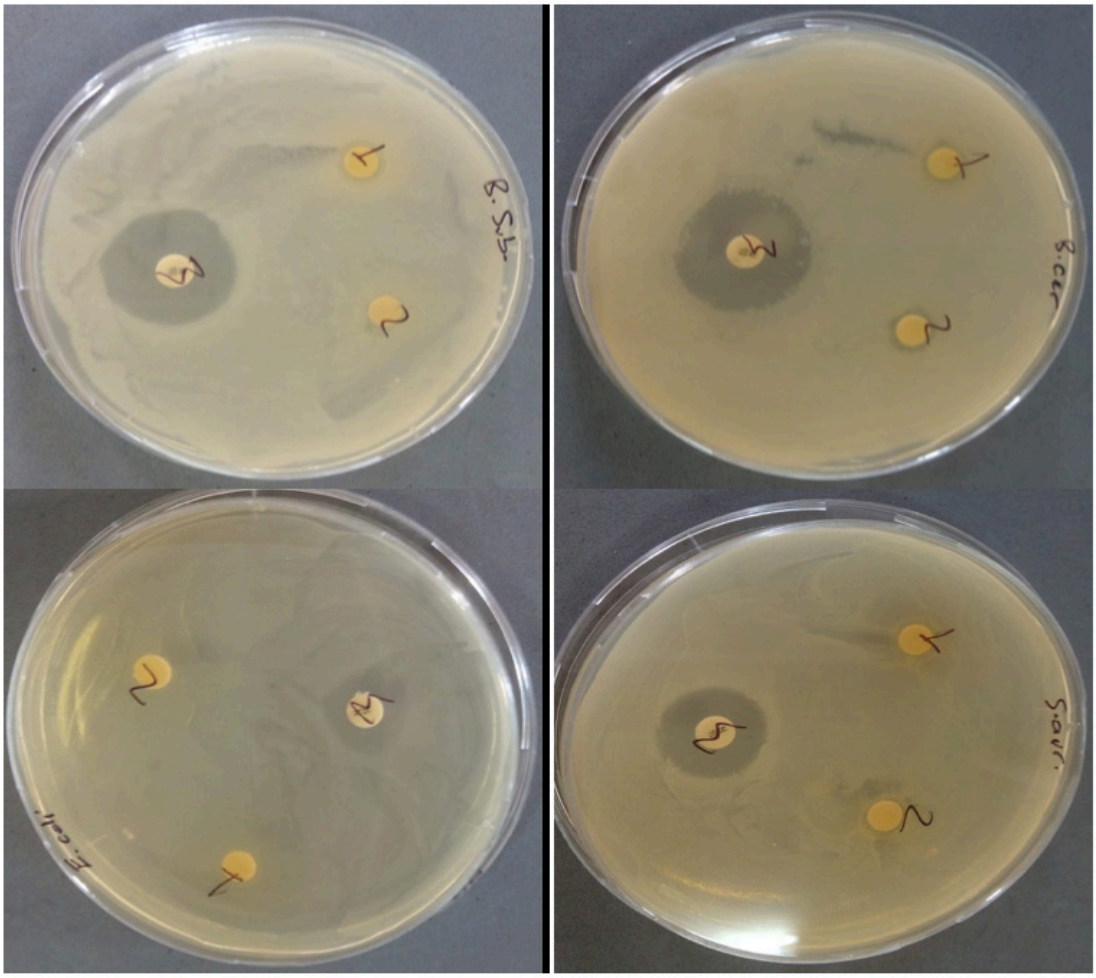

Figure 7. Some images obtained as a result of disk diffusion

Conclusion

Considering the radical quenching activity of ZnO-NPs obtained by using *Helichrysum arenarium* plant, it is understood that it is a powerful antioxidant. In addition, considering the zones formed against harmful microorganisms, it has been determined that it has an antimicrobial effect. It is thought that it can be used as an antimicrobial agent in the fields of medicine and pharmacology by conducting more comprehensive content analysis.

Compliance with Ethical Standards

Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

Not applicable.

Funding

No financial support was received for this study.

Data availability

Not applicable.

Consent for publication

Not applicable.

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Combination effect of hot smoking and vacuum packaging on quality parameters of refrigerated thornback ray (*Raja clavata* Linnaeus, 1758)

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Abstract

In this study, hot smoking process was applied to thornback ray (*Raja clavata*), which is a non-target catch, and the product obtained was vacuum packaged and then stored at refrigerated conditions (4±1 °C) for 120 days. The nutritional, chemical, microbiological, and sensory changes in the products were examined every 15 days. After the hot smoking process applied to thornback rays, significant changes were observed in the nutritional composition of the products ($P<0.05$). Total volatile basic nitrogen (TVB-N), trimethylamine nitrogen (TMA-N), and thiobarbituric acid (TBA) values of smoked thornback rays increased during storage, and these values were determined between 23.11-98.06 mg/100 g, 2.48-7.33 mg/100 g, and 0.1-0.3 mg malondialdehyde/kg, respectively. It was determined that the total aerobic mesophilic bacteria (TAMB) count detected in the fresh sample decreased in smoked products due to the smoking process and the value was found below the detectable limit value (1.47 log CFU/g) during storage. As a result of the sensory evaluation, it was determined that the texture, odor, and flavor criteria of the smoked thornback rays were below the consumable limit value on the 120th day. According to the results, it was determined that the thornback ray vacuum packaged by hot smoking can be consumed safely for 105 days at 4±1 °C. This study shows that discarded thornback rays, which are a high protein food, can be transformed into high value-added products by applying different processing methods such as smoking and so can be evaluated as human food.

Keywords: Thornback ray, Vacuum packing, Hot smoking, Cold storage, Shelf life

Introduction

Due to the rapid increase in the world population and the changing ecological balance, the importance of limited food sources is increasing constantly. This situation causes nutritional problems, which is the most important element of human life, and people demand different foods. Aquatic animal resources are of vital importance in this respect. Efforts are being made to maximize opportunities to exploit the existing potential of seafood around the world. The importance of animal origin proteins in the healthy nutrition of living creatures is increasing day by day. Seafood are a high-value food group thanks to their protein, fat, mineral, and vitamin content (Çağlak and Karsli, 2013). However, seafood are among the foods that spoil very quickly due to their biological structure. In this respect, it is important to keep it under suitable conditions after hunting and to increase the variety with different processing methods (Ayas et al., 2005).

The consumption of seafood in Turkey is generally listed as fresh, frozen, and other processing techniques (salting, canning, smoking, and marinade). While many of the smoked products are consumed as a delicious taste in various parts of the world, there is no great demand for these products in Turkey (İzci

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and Ertan, 2004; Duman and Patır, 2007). Smoked products are products whose durability is increased by reducing the amount of moisture, especially by using wood shavings, and by processing with antioxidant and antimicrobial effects of smoke compounds such as formaldehyde, carboxylic acid, and phenols. Besides, thanks to the smoking process, products are given sensory properties such as special flavors and color (Gökoğlu, 2002; Erkan, 2004). Hot smoking is usually applied at temperatures of 80-100 °C and it is in the forefront of give the products a smoke aroma during cooking (Gülyavuz and Ünlüsayın, 1999). Chemical, microbiological, and sensory changes occur during the storage of smoked products, and these changes depend on the fish species, fat ratio, pre-smoking processes, smoking method, smoking time, smoking temperature, and smoke content. Also, the storage conditions and packaging processes affect maintaining the quality of smoked products (Erkan, 2004). Duyar et al. (2008) reported that the shelf life of Atlantic bonito increased thanks to hot smoking and vacuum packaging.

When the meat structure of thornback ray is examined, it has the potential to be considered as a food source in terms of having rich protein and fatty acid content like other seafood (Turan et al., 2007). There is no commercial hunting on thornback ray in Turkey and it is generally obtained as a discarded product. While the thornback ray, which is not consumed in Turkey, can be considered as an export product in the Aegean region, however, it is not subjected to any evaluation process in the Black Sea region. In this respect, it is economically important to transform this species, which has valuable nutritional components, into high value-added products by applying different processing methods.

In this study, it was aimed to determine the combined effect of hot smoking and vacuum packaging on some quality changes of thornback ray under refrigerator conditions (+4±1 °C) and to create an economic value for discarded this species with different consumption methods.

Materials and Methods

Fish Material

Thornback ray caught as discarded products were obtained from a fishing boat registered in Rize province, Turkey. 40 thornback rays in total were iced immediately after hunting and brought to Recep Tayyip Erdogan University Fish Processing Technology laboratory in a styrofoam box. Following the thornback ray cleaning procedure, the wing parts of the thornback ray were cut and separated. Then, the skins of the cut wings were peeled off and the meat portions were obtained.

Smoking Process

The smoking process was performed using a mechanical smokehouse with an electronic thermostat (0-300 °C) and humidity control according to the method of Çağlak et al. (2015). Rough sawdust obtained from beech wood was used for the smoking process. First, the products were kept in 10% salt solution at +4 °C for 90 minutes and then filtered for 15 minutes to remove the excess water from the products. Afterward, the filtered products were taken to the incense grids and the smoking process was carried out at 30 °C for 30 minutes (pre-drying), at 60 °C for 60 minutes (smoking), and at 90 °C for 30 minutes (cooking) (Figure 1). The products obtained after the smoking process were cooled, vacuum packed, and stored at +4±1 °C. During storage, analyses were carried out every 15 days with 3 randomly selected packages.

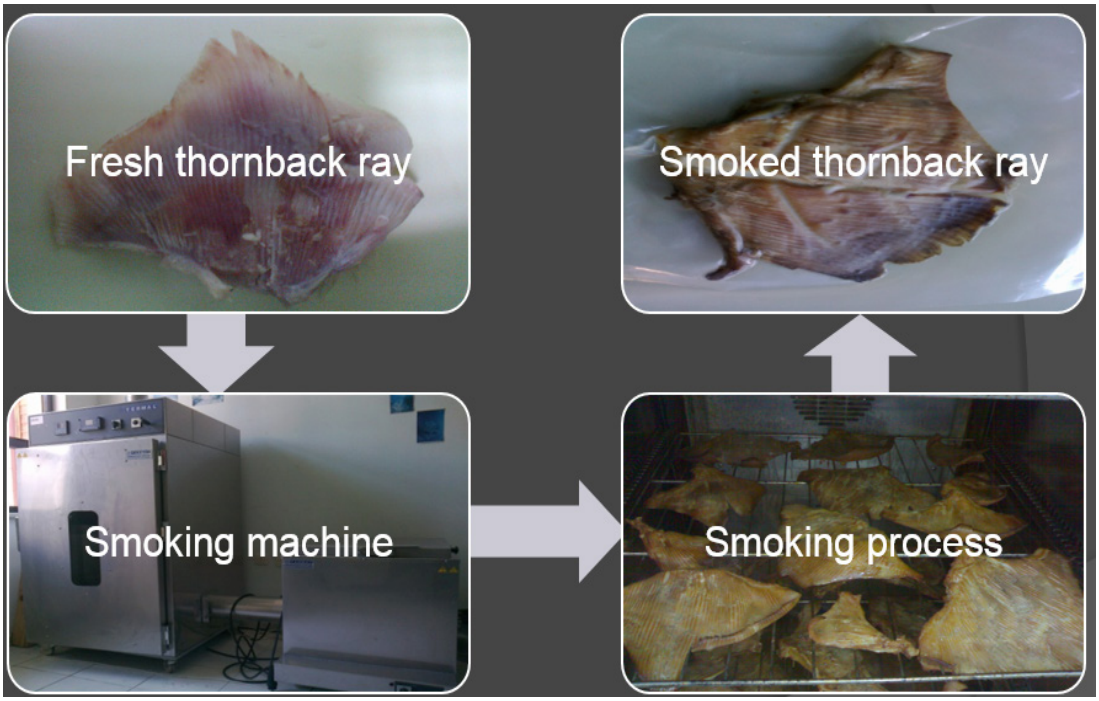

Figure 1. The scheme of product development

Analyses

Meat yield was determined by the ratio of the weight of edible parts to the total fish weight and it was expressed as a percentage (%). The moisture (%), crude ash (%), crude fat (%), and crude protein (%) analyses were performed according to the method of Norwitz (1970). Total volatile basic nitrogen (TVB-N) analysis was carried out according to the Lücke-Geidel method (İnal, 1992; Varlık et al., 1993), trimethylamine nitrogen (TMA-N) analysis was performed according to the method proposed by Dyer (1949) (AOAC, 1990), thiobarbituric acid (TBA) analysis was performed according to Tarladgis et al. (1960) method and pH measurement was conducted according to Curran, et al. (1980).

To determine the microbiological load of the samples, 25 grams of samples were homogenized in sterile physiological saline solution (PSS) of 0.85% and all serial diluting was carried out using PSS. Then, 0.1 ml of the appropriate dilutions were inoculated onto petri dishes. The inoculation process carried out as 48 hours at 37 °C using Plate Count Agar for total aerobic mesophilic bacteria (TAMB) count, 48 hours at 37 °C using Violet Red Bile Agar for total coliform bacteria count, and 3-5 days at 30 °C using Potato Dextrose Agar for the yeast-mold count (Harrigan and McCance, 1976; Halkman, 2005). At the end of the incubation, the results were expressed as log CFU/g.

For sensory analysis, the sensory evaluation form was created by modifying the method reported by Kurtcan and Gonul (1987), Altuğ and Elmacı (2005), and Özer et al. (2012). The products were evaluated by a panelist group of 7 people, considering this form. According to the sensory evaluation form, the appearance, texture, flavor, and odor values of the products were determined using the 10-point hedonic scale (1-2= dislike strongly, 3-4= dislike moderately, 5-6= neutral, 7-8= like moderately, 9-10= like strongly).

Statistical Analysis

All the tests were done in triplicate. Data analyses were performed by one-way ANOVA (analysis of variance) using the JMP software program 5.0.1 (SAS Institute Inc, Cary, NC, USA). Comparison of means was performed using a least significant difference (LSD) method at $P<0.05$ (Sümbüloğlu and Sümbüloğlu, 2000).

Results and Discussions

In the present study, a total of 25135 g of thornback rays were used and the weight of the remaining wing parts after cleaning was determined to be 8762.04 g. The meat yield of fresh thornback rays before the smoking process was calculated as 34.86%. After the smoking process, the weight of the thornback ray wings was determined to be 5502.35 g (37.2% of total raw wing weight). Meat yield in fish may vary depending on fish species, gender, age, size, breeding period, nutritional status, stomach contents, and geographic region. In the present study, the meat yield of thornback ray was 62.8%. Similarly, Yılmaz and Akpınar (2003) reported that the meat yield of common guitarfish (*Rhinobatos rhinobatos*) was 65%.

The moisture, crude protein, crude fat, and crude ash values (%) of fresh thornback ray were determined as 77.12%, 20.24%, 0.85%, and 1.03%, respectively. Changes were observed in the biochemical composition of smoked thornback rays depending on pre-smoking procedures and temperature applications during smoking process. After the smoking process, the moisture, crude protein, crude fat, and crude ash values of smoked thornback rays were found to be 74.33%, 25.23%, 1.17%, and 2.17%, respectively (Figure 2). The changes in the moisture, crude ash, crude fat, and crude protein of smoked thornback rays during storage are shown in Figure 2. Depending on the smoking process, the initial decrease in the moisture values stabilized after the 15th day of storage. During refrigerated storage, the minimum and maximum moisture values of smoked thornback rays were found to be 68.37% and 74.33%, respectively. There was no statistically significant difference among storage days in terms of moisture values ($P>0.05$), however, the fresh thornback ray with the highest moisture content differed statistically from smoked products ($P<0.05$). Due to brine treatment applied to the samples before the smoking process, an increase in ash content of smoked samples was observed, and these values varied between 1.59% and 2.40% during the storage period. The crude ash content of fresh sample was statistically lower than smoked products determined during storage ($P<0.05$). There is an inverse relationship between fat and moisture ratios. A proportional increase in crude fat values was observed as a result of the loss of moisture due to the temperature process applied in the smoking process. The lowest and highest crude fat value during refrigerated storage was found to be 1.17% on day 1 and 2.54% on day 120, respectively. When examining the changes in crude values during storage, it was determined that the fresh product was different from those on other storage days except day 1 ($P<0.05$). Also, statistically significant differences were found in the crude fat values of smoked products depending on the storage period ($P<0.05$). The crude protein value detected in fresh thornback rays increased by 19.78% on day 1 after the smoking process. It was determined that the protein value, which was 25.23% on the 1st day of storage, reached the highest value with 30.64% on the 45th day and this value was found to be 27.64% at the end of the storage period (120th day). The amount of protein detected on the 45th day of storage statistically differed from fresh and 1st day values ($P<0.05$). This proportional increase in the amount of protein after the smoking process is thought to be due to a decrease in moisture loss due to the heat applied in the smoking process.

In general, the biochemical composition of seafood consists of 66-84% water, 15-24% protein, 0.1-22% fat, 0.8-2% mineral substances, and very small amounts of carbohydrates (Jeyasanta and Patterson, 2013; Matsumoto, 1979). In this study, the moisture, crude protein, crude fat, and crude ash values (%) of fresh thornback ray were within these reported values. The biochemical composition of seafood varies from species to species, as well as among individuals of the same species, depending on age, sex, season, and hunting area. (Huss, 1988; Borgstrom, 1961). Ayas et al. (2019) reported that the fat levels in the muscle tissue of the four ray species (*Dasyatis pastinaca*, *Raja radula*, *Raja clavata,* and *Torpedo*

marmorata) were between 0.71-1.90%. They also determined that the fat levels of *R. clavata* were between 0.95-1.38%. Özer et al. (2012) investigated the changes in microbiological, physicochemical, and sensorial quality of vacuum-packed sausage from thornback ray and they reported that moisture, protein, fat, and ash content of thornback ray at the beginning and after the smoking process was 75.08-66.34%, 21.57-22.35%, 0.43%-2.40% and 1.47-3.73%, respectively. Colakoglu et al. (2011) stated that the moisture, protein, fat, and ash values of thornback rays were 76.51%, 18.58%, 3.39%, and 1.1% in fresh and 61.36%, 30.62%, 4.41%, and 2.65% in smoked products, respectively. Turan et al. (2007) reported that the moisture, crude protein, crude ash, and crude fat values of thornback rays were 77.47%, 20.02%, 1.38%, and 0.51%, respectively. In another study, it was reported that the moisture, crude protein, crude fat, and crude ash values of common guitarfish changed between 75.83-79.88%, 16.63-22.63%, 0.2-0.7%, and 1-1.65%, respectively (Yılmaz and Akpınar, 2003). Pastoriza and Sampedro (1994) reported that the protein, fat, ash, and moisture values of *R. clavata* wing meats were 16.87%, 0.8%, 1.1%, and 78.9%, respectively. It was determined that the moisture, crude fat, crude protein, and crude ash values determined in the present study were compatible with the nutritional content obtained from other studies.

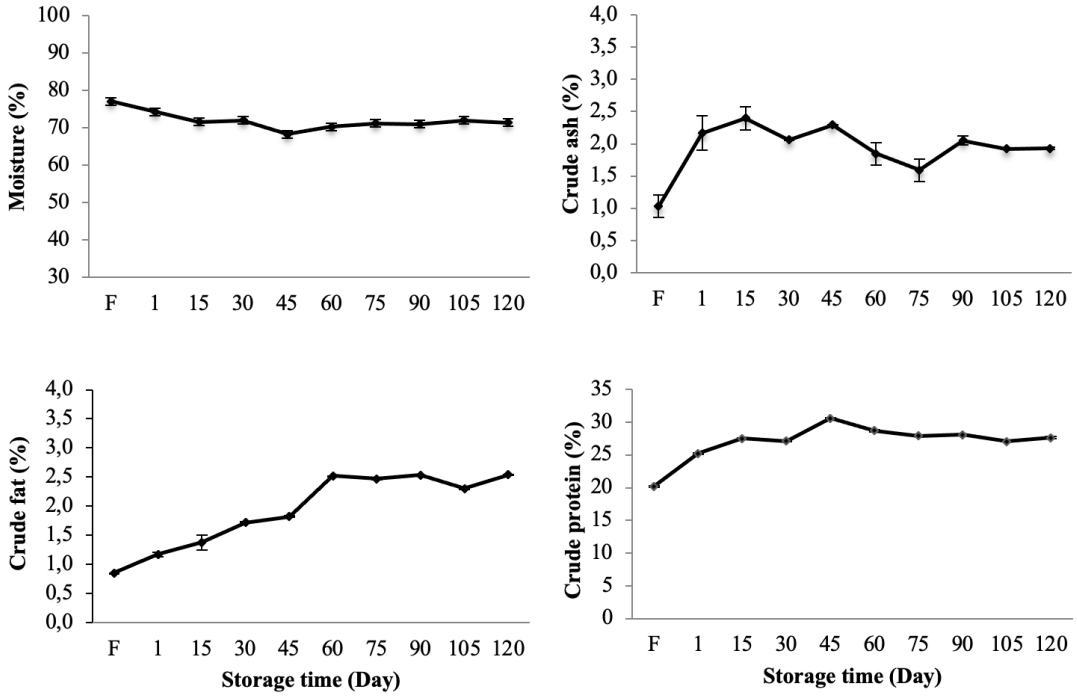

Figure 2. Changes in the biochemical composition of hot smoked thornback ray during storage (F: Fresh).

The change in the pH, TVB-N, TMA-N, and TBA values of the smoked thornback ray during the storage period is shown in Figure 3. In this study, pH was measured as 6.74 in fresh samples. Then this value decreased after the smoking process due to acidic compounds and it is determined to be 5.51 at the end of the 120 days storage period. While a difference was observed between the pH values of the fresh and smoked thornback ray ($P<0.05$), no significant difference was observed among the pH value of the smoked products depending on the storage time ($P>0.05$). It has been stated that the pH value for fresh fish is 6-6.5 and the consumable limit value is 6.8-7 (Connell, 1980; Varlık et al., 1993). Mugica et al. (2008) stated that the initial pH value (6.5) of *Raja clavata* stored in ice increased to 9 levels on the 10th day of storage. Pastoriza and Sampedro (1994) reported that the pH value of *R. clavata* wing muscle, which was initially determined as 6.24, reached 9 at the end of the 15-day ice storage period. In another study, pH values of frozen common guitarfish during the 6-month storage were between 6.5-6.82 (Yılmaz and Akpınar, 2003). Turan and Sönmez (2007) found that the pH values of surimi made from thornback ray fluctuated between 6.9 and 7.4 during storage. Özer et al. (2012) determined that the pH value of the thornback ray was 6.41 at the beginning and 6.18 after the smoking process. They also reported that the pH dropped to 5.8 levels on the 49th-56th days of storage, while it was between 6.1 and 6.4 on other storage days. Compared to the pH data of fresh thornback ray, the data of the present study were similar to the results of Mugica et al. (2008) and Yılmaz and Akpınar (2003), while the findings found by Pastoriza and Sampedro (1994) and Özer et al. (2012) were found to be higher than the data of this study. In the present study, the decreases in the pH values of the thornback ray after the smoking process compared to the fresh sample were also similar to the data reported by Özer et al. (2012).

Thornback ray, which is included in the cartilage fish group, has a higher proportion of urea and nitrogen compounds than other seafood due to their biological structure. In this respect, the TVB-N limit value for cartilage fish is stated as 50-100

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mg/100 g (Varlık et al., 1993; Uyttendaele et al., 2018). The TVB-N content of smoked products varies according to fresh product quality, product packaging type, brine density applied before smoking, storage conditions, and the type of product processed (Bilgin et al., 2007). In this study, the TVB-N value of fresh thornback ray was determined to be 23.11 mg/100 g, however, it reached 98.06 mg/100 g on day 90 (Figure 3). These values determined in fresh and on the 90th day was statistically different compared to other storage days ($P<0.05$). Although fluctuations were observed in the TVB-N value of smoked thornback rays during storage, it generally tended to increase. Considering the TVB-N limit value of 100 mg/100 g, it was observed that the TVB-N value of smoked thornback ray approached the limit value on the 90th day (98.06 mg/100 g). Turan and Sönmez (2007) stated that the TVB-N value of fresh thornback ray is 15.4 mg/100 g. Özer et al. (2012) reported that the initial TVB-N value of sausages obtained from thornback ray increased from 28.7 mg/100 g to 31.5 mg/100 g after the smoking process. In the same study, they also reported that the TVB-N value of thornback ray sausages stored at +4 °C increased to 50 mg/100 g on the 14th day, decreased to 25 mg/100 g on the 21st day, and then again increased to 43 mg/100 g at the end of the storage period. Yılmaz and Akpınar (2003) reported that the TVB-N values of frozen common guitarfish during the 6-month storage period were between 19.87 mg/100 g and 48.62 mg/100 g. The high TVB-N value detected in thornback ray in this study was similar to other literature studies. Similarly, it was determined in other studies that TVB-N values of rays did not exceed the limit value of 100 mg/100 g reported for TVB-N. It is thought that this increase in the TVB-N value of the smoked products is caused by the water loss of the products as a result of the smoking process and the proteolytic activities that continue in the fish during salting and smoking (Günlü, 2007).

The lipids in seafood are more exposed to oxidation than other meats due to their high unsaturation. Depending on the oxidation, fatty acids and peroxides are formed first, and then the oxidation of peroxides creates aldehydes and ketones that cause unpleasant odor and rancidity. Thiobarbituric acid (TBA) value is used to determine the rancidity of lipids (Ramanathan and Das, 1992; Soyer, 1999). It was reported that TBA should be lower than 3 mg malonaldehyde (MA)/kg in very good material, 3-5 mg MA/kg in a good product, and the consumable threshold value is 7–8 mg MA/kg (Schormuller, 1968). TBA value, which is indicative of lipid oxidation, was in the range of 0.1 and 0.3 mg MA/kg during 120 days of refrigerated storage (Figure 3). The TBA value of the fresh sample was statistically different from the values of the smoked products obtained during storage ($P<0.05$). Considering the changes between storage days, it was determined that the TBA value on the 105th and 120th days of storage was statistically different from the other storage days ($P<0.05$). The low levels of TBA during storage are thought to be due to the low fat content of the thornback ray used in this study, the antioxidant properties of the smoke compounds, and the oxidation inhibiting effect of the vacuum packaging application. Aberoumand and Baesi (2020) reported that vacuum packaging treatment had significant effects on delaying lipid oxidation. Turan and Sönmez (2007) reported that the maximum TBA value of frozen surimi thornback ray stored for 6 months was 1.24 mg MA/kg. Özer et al. (2012) reported that the TBA value of vacuum-packed sausage from thornback ray increased regularly during 56 days of storage and it did not exceed 2.5 mg MA/kg at the end of the storage period. As seen in other studies, the increase in the TBA values of the rays during the storage in the present study was limited and the TBA value did not even reach 3 mg MA/kg, which is a very good material value.

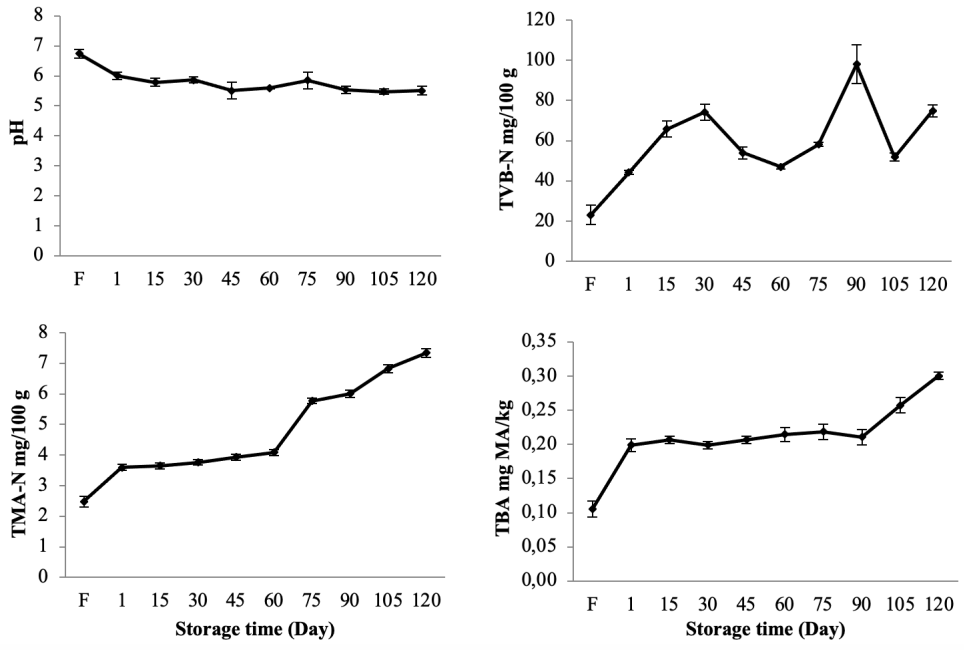

Figure 3. Changes in pH, TVB-N, TMA-N, and TBA values of hot smoked thornback ray during storage (F: Fresh).

TMA-N is degraded by the reduction of trimethylamine oxide (TMAO) by bacteria and mostly forms the breakdown products of proteins. TMAO is a quaternary ammonium compound responsible for osmoregulation in marine fish and its amount in fish may vary depending on the fish species, size, age, seasons, and environmental conditions (Huss, 1995; Koutsomanis and Nychas, 1999). It has been recommended that the TMA-N value should be between 1 and 8 mg/100 g in quality seafood for human consumption (Varlık et al., 1993). In another study, the TMA-N value was reported to be good up to 4 mg /100 g and marketable up to 4-10 mg/100 g (Nickerson and Sinskey, 1972). In the present study, the amount of TMA-N in the fresh thornback ray was found to be 2.48 mg/100 g. The differences observed in TMA-N values among storage days (except for the 1st and 15th days of storage) were found to be statistically significant ($P<0.05$). The TMA-N value of smoked products increased regularly during storage period and reached 7.33 mg/100 g on the 120th day (Figure 3). At the end of the storage period, it was determined that smoked thornback ray preserved their marketable feature according to the limit values reported in terms of TMA-N values. Similarly, Turan and Sönmez (2007) reported that the TMA-N value of fresh thornback ray is 2.08 mg/100 g. Özer et al. (2012) stated that the initial TMA-N value of 0.4 mg/100 g in vacuum-packed sausage from thornback ray increased to 2.8 mg/100 g at the end of the storage period (56 days). This increase in TMA-N was in agreement with the result of the present study, but they found a lower TMA-N value than the present study data at the end of the storage period. This difference is thought to be due to the different initial TMA-N content. In addition, Goulas and Kontominos (2005) reported that the TMA-N value of vacuum-packed smoked chub mackerel increased during 30 day storage periods at 2 °C, however, TMA-N values of salted and smoked products were at very low levels (2.28-3.64 mg/100 g) at the end of the storage.

The maximum microbiological limit value in seafood for consumption has been reported as 6-7 log CFU/g for total aerobic bacteria and 2.0 log CFU/g for total coliform (ICMSF, 1986). Coliform bacteria are not found in fish caught from clean waters, but these bacteria can be found in fish meat as an indicator of contamination in further processing (Patır and İnanlı, 2005). In this study, TAMB, total coliform, and yeast-mold analyses were carried out during the study period. The TAMB count in fresh thornback ray was found to be 2.64 log CFU/g and the value fell below detectable the limit value of 1.47 log CFU/g after the smoking process. Total coliform and yeast-mold values were determined as <1.47 log CFU/g in fresh and smoked products during refrigerated storage. Also, it was determined that TAMB, total coliform, and yeast-mold values did not change during 120 days of storage and these values were below 1.47 log CFU/g. Therefore, data on TAMB counts were not shown in Figure. The reason for this decrease may be the killing or preventing the growth of bacteria with the effect of the temperature and smoke components used in the smoking process. Vyncke (1978) reported that the initial total bacteria of thornback ray treated with sodium tripolyphosphate and citric acid kept under ice increased from 5.8 log CFU/g to 9 log CFU/g at the end of the storage period. In a study, the total bacterial count of *R. clavata* wing muscle was reported as 3 log CFU/g at the beginning and 6.6 log CFU/g at the end of 15 days storage (Pastoriza and Sampedro, 1994). Özer et al. (2012) found that the TAMB count of vacuum-packed sausage from thornback ray increased from 4.09 log CFU/g (initial value) to 7.69 log CFU/g at the end of 56 days storage. They also reported that the total coliform and yeast-mold counts were not detected in samples during storage. The TAMB count in fresh thornback ray determined in the present study was similar to the results reported by Pastoriza and Sampedro (1994). Also, the results of total coliform and yeast-mold count reported by Özer et al. (2012) were consistent with the result of the present study. However, it is seen that the number of TAMB detected in this study is lower than the other study data. Microorganism load in fish is affected by many internal and external factors such as fish species, fishing location, fishing season, and procedures applied (Hussain and Uddin, 1997). In addition, packaging conditions that reduce the amount of oxygen present in the package, such as vacuum package, can inhibited the growth of aerobic spoilage bacteria and extend the shelf life of the product (Jaberi et al., 2019). In this respect, the differences observed in the studies are thought to arise due to these factors.

The results of the sensory analysis performed by using the appearance, texture, flavor, and odor criteria of the thornback ray are shown in Figure 4. Sensory evaluation is one of the most important criteria used in determining the quality of seafood. If a product is sensually undesirable, it cannot be consumed even if it is of good quality in terms of other quality criteria (Dokuzlu, 1997; Özden et al., 2001). Criteria such as appearance, odor, flavor, and texture considered in the sensory analysis are evaluated with human senses. Therefore, considering these sensory criteria in studies on food is of great importance for the consumer in food quality control. In this study, a 10-point hedonic scale was used for the sensory evaluation of the products, and products below 5 points were considered to be inconsumable. At the beginning, the smoked samples were appreciated by the panelist and received a higher score. Then, the sensory scores of the smoked thornback ray evaluated by the panelists showed a linear decrease depending on the storage time. According to the sensory evaluation, it was determined that smoked thornback rays maintained their sensory quality for 105 days, while texture, flavor, and odor criteria of the products were below the consumable limit values (5) on the 120th day of storage. It was determined that the changes in appearance, texture, flavor, and odor criteria, which decreased depending on the storage period, were statistically significant ($P<0.05$). Özer et al. (2012) reported that the average sensory acceptability of vacuum-packed sausage from thornback ray was 5.98 out of 10. Turan and Sönmez (2007) stated that the sensory scores of surimi thornback rays were at moderate and acceptable levels at the end of the 6-month storage period. Yılmaz and Akpınar (2003) reported that the sensory scores (appearance, smell, chewiness, aroma, moistness and overall palatability) of frozen common guitarfish, which were evaluated over 10 points, were 6.0-

7.1 in vacuum products and 5.7-6.4 in non-vacuum products at the end of 6 months storage. Comparing the data of this research with other studies, it has been observed that although the sensory qualities of different products obtained from rays changed depending on the processes performed, they preserve their organoleptic properties for a long time.

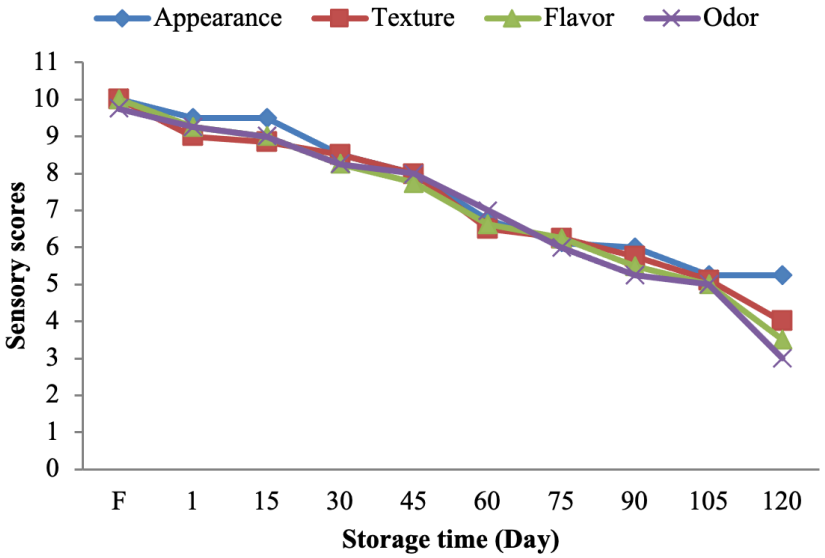

Figure 4. Changes in the sensory scores of hot smoked thornback ray during the storage period (F: Fresh)

Conclusion

In this study, nutritional, chemical, microbiological, and sensory changes of hot smoked thornback ray stored at +4±1 °C for 120 days were examined. According to the results, the chemical quality criteria such as TVB-N, TBA, and TMA-N of smoked thornback rays were within the consumable limit values during the storage period. The smoked thornback ray maintained their sensory quality for 105 days and remained within the consumable limit values. However, the sensorial scores of smoked thornback rays were below the consumable values at the end of the 120 day storage. It is thought that the extension of the shelf life and the improvement of the quality criteria of samples during the storage period is due to the combined effect of hot smoking and vacuum packaging. When it was examined nutritionally, it was observed that thornback ray has high protein content such as anchovy, bonito, horse mackerel, sea bass, sardine fish which are generally consumed. Smoked thornback ray was also sensually appreciated by panelists. According to these results, the evaluation of discard products such as thornback ray used in this study by applying different processing methods such as smoking, marination, and canning will be of great importance in meeting the increasing food demand due to the increasing world population. Thus these applications can contribute to both waste management and the economy.

Compliance with Ethical Standards

Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

Not applicable.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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Physico-chemical characteristics of some Gilaburu (*Viburnum Opulus* L.) genotypes

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Abstract

Gilaburu is an important fruit species in terms of healthy effects, having potential for use as alternative medicine and suitable for industrial product processing. In this context, physico-chemical characteristics of three different gilaburu (*Viburnum opulus* L.) genotypes grown under the ecological conditions of Kayseri, Turkey were determined. In addition, correlations among the investigated characteristics were calculated and their potential to be handled together was revealed. According to results, Fruit width, fruit length and fruit weight characteristics varied between 7.40-7.95 mm, 7.65-8.81 mm and 0.30-0.37 g, respectively. Genotype 3 showed the highest values in terms of pomological characteristics, while the lowest values were obtained from Genotype 1. Among the chemical properties; pH, soluble solid content (SSC) and titratable acidity (TA) values ranged from 3.53 (Genotype 1) to 3.97 (Genotype 2), 10.46 (Genotype 1) to 12.72 (Genotype 2) and 1.56 (Genotype 2) to 2.16 (Genotype 1), respectively. A high level of positive correlation was found between fruit width and fruit length (r = 0.73 ***). Also, these properties have been found to increase fruit weight. While a highly negative correlation was found between titratable acidity and pH (r = -0.95 ***), it was determined that the increase in fruit size and weight decreased dry matter accumulation.

Keywords: European cranberrybush, Fruit, Selection, *Viburnum Opulus* L.

Introduction

Gilaburu (*Viburnum opulus* L.), is a fast-growing, bush-formed berry fruit belonging to the Adoxacea family and commonly grown in Europe and North Africa, while growing naturally in Turkey, especially in Kayseri, Bursa, Konya, Tokat, Ankara and Sakarya (Baytop 1999, Aksoy et al.2004, Sagdic et al.2006; Yıldız and Ekici, 2019). Gilaburu, which performs better in continental climate, has a plant height of 1.5-3.5 m and its fruits are long-living.

In recent years, with the increasing demand for natural products, studies on species with high nutritional value and health importance are increasing (Polat et al., 2018; Gündeşli et al., 2019; Güney, 2020; Okatan, 2020). Gilaburu is one of the remarkable species in this context.

When the studies are examined, it is observed that gilaburu fruits are rich in phenolic compounds such as caffeic acid, chlorogenic acid, ellagic acid, p-coumaric acid, gallic acid, protocatechuic acid, ferulic acid, rutin, syringic acid, quercetin, catechin, epicatechin, cyanidin-3-glucoside, pelargonidin 3-glucoside (Zarifikhosroshahi et al, 2018). Thanks to its rich and diverse biochemical content, it has been demonstrated with different studies that it has high antimicrobial, antioxidant, antimutagenic and pharmacological effects (Bolat and Özcan, 1995; Gerçekçioğlu, 1999; Burnaz et al., 2010; Murathan et al., 2018; Taşkın et al. , 2019; Yıldız and Ekici, 2019). It has been reported that its seeds also show the same effect (Güleşçi, 2019). In addition, within a study conducted on air pollution, it has been emphasized that the production of this

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fruit should increase in areas with heavy industrial production and highways due to its tolerance to sulfur dioxide among 72 species and it has been determined as a potential species that can be used in landscape areas in North America among 38 species (Mapeza, 1986).

It is very important to choose the genotypes that show superior features in gilaburu, which has a future in many different areas such as being consumed dried or fresh, used in the fruit juice industry, used in alternative medicine due to its rich and diverse biochemical content, preventing air pollution and used in landscaping. In this study, it was aimed to determine the pomological and chemical properties of gilaburu (*Viburnum opulus* L.) varieties growing in 3 different locations under ecological conditions of the province of Kayseri. In addition, as a result of the correlation analysis, the relationships between the examined features have also been revealed.

Material and Methods

Material

This study was conducted in 2016 and three different genotypes were used as material naturally grown in Akkışla (Genotype 1), Uluşağ (Genotype 2) and Kesdoğan (Genotype 3) of Kayseri, Turkey. Collected fruits were took into cold chain without losing time. All the analyses were performed in the pomology laboratory of the Isparta Applied Sciences University, Department of Horticulture.

Determination of pomological characteristics

Harvested fruits from the genotypes were immediately transferred to the laboratory. Fruit weight was determined using an electronic scale susceptible to 0.001 g (Vibra, AJH-42OCE) and digital calipers were used to measure fruit width and fruit length at 0.01 mm precision.

Determination of chemical characteristics

Fifty fruit belonging to each genotype were squeezed with a juice extractor and filtered with coarse filter paper to obtain their juices and these juices were used for phytochemical measurements. Soluble Solid Content (SSC) were measured by a digital refractometer (Hanna, HI 96801) and the results were given in percentile values (Karaçalı, 2012). For the determination of titratable acidity, the fruit juices were titrated with 0.1 N sodium hydroxyl solution using phenolphthalein as the indicator and the results were expressed as malic acid % by calculating the formula showed by Karaçalı (2012).

Statistical Analysis

The study was designed according to a randomized plot experimental design. Fifty fruit were used for pomological properties for each genotype. Fruit juices were used for the determination of chemical properties obtained from fifty fruit from each genotype. Results are expressed as mean ± standard deviation. Statistically significant differences among genotypes were determined by the one-way ANOVA procedure in the Minitab-17 program package. The Tukey multiple comparison test was used to reveal differences and correlation analysis was used to reveal the relations between the investigated features. (Zar, 2013).

Results and Discussion

Pomological results of the investigated genotypes in the study are given in Table 1. According to results, among the investigated properties, fruit width, fruit length and fruit weight varied between 7.40-7.95 mm, 7.65-8.81 mm, 0.30-0.37 g, respectively. The highest values in terms of these properties were obtained from Genotype 3, while the lowest values were measured in Genotype 1. While a stable ranking was not observed in terms of color values, the highest L*, C* and hº values were determined as 49.38 (Genotype 1), 46.23 (Genotype 2) and 29.85 (Genotype 3), respectively. Titratable acidity, expressed as the sum of organic acids, was measured highest from Genotype 1 with 2.16% and lowest from Genotype 2 with 1.56%. As expected, contrary to titratable acidity, the highest pH was obtained from Genotype 2 and lowest from Genotype 1. Soluble solid content was determined in the range of 10.46% (Genotype 1) and 12.72% (Genotype 2).

Table 1. Physico-chemical characteristics of investigated gilaburu genotypes

	Genotype 1	Genotype 2	Genotype 3
Fruit length (mm)*	7,40±0,94^{b}	7,48±0,84^{ab}	7,95±0,76^{a}
Fruit width (mm)***	7,65±1,01^{b}	8,29±0,98^{a}	8,81±0,91^{a}
Berry weight (g)**	0,30±0,08^{b}	0,34±0,09^{ab}	0,37±0,09^{a}
L*	49,38±7,81^{a}	44,20±5,55^{b}	46,59±6,89^{ab}
C*	40,23±13,55^{ns}	46,23±10,41^{ns}	44,79±13,64^{ns}
hº	28,39±5,02^{ns}	29,02±4,31^{ns}	29,85±5,73^{ns}
Ph***	3,53±0,04^{c}	3,97±0,04^{a}	3,94±0,04^{b}
TA (%)***	2,16±0,09^{a}	1,56±0,08^{c}	1,78±0,10^{b}
SSC (%)***	10,46±0,42^{c}	12,72±0,42^{a}	11,96±0,52^{b}

ns: non-significant, *,** and ***: significant at P<0.05, P<0.01 and P<0.001, respectively.

Previous studies conducted in different parts of Turkey indicated great variation on fruit weight, soluble solid content, titratable acidity and pH among gilaburu genotypes and were reported between 0.40 - 0.87 g, % 7.81 – 14.37, %1.49-2.85 and 2.13 – 3.90, respectively (Bolat and Ozcan, 1995; Karadeniz et al., 2003; Ozrenk et al., 2011; Gundogar, 2013; Ersoy et al., 2017; Ozrenk et al., 2020). In studies conducted with different gilaburu genotypes, change interval for fruit length was reported between 9.37-11.25 mm (Gündoğar, 2013); 11.83 mm - 12.55 mm (Kara ve ark., 1995; Karadeniz ve ark., 2003.

Similar notifications were made in the range of for fruit width in 9.23 mm and 11.96 mm (Gündoğar, 2013).

When the results of different studies were examined, it was seen that the color values were evaluated using the weighted ranking method. In the study conducted by Gündoğar (2013), 12 and 31 genotypes were reported as light red and red, respectively. Similarly, Ersoy et al. (2017), reported 4 genotypes as light red, 2 genotypes as red and 4 genotypes as dark red.

Although differences in all investigated characteristics were thought to be mainly due to the differences in genotypes examined, differences in climate and soil characteristics, geographical status of the cultivation area, harvesting type and time, storage or processing of the crop, method or periodical differences of the applied cultural processes lead to significant differences in the final shape and content of the products (Li et al., 2012; Tiwari and Cummins, 2013; Mertoğlu et al., 2020; Büyüksolak et al., 2020).

Correlation coefficients between the investigated properties in the study are given in Table 2. A high level of positive correlation was found between fruit width and fruit length (0.73 ***). In plants, after fertilization, firstly an increase in the number of cell is observed then cell expansion is seen. Horizontal and vertical growth progress in parallel during the cell expansion phase and this explains the strong relationship between these two features. Volume increase in cells that make up the fruit, increases the weight also. In this context, a strong positive relationship was detected between fruit weight with fruit width and fruit length, respectively, at 0.87*** and 0.89***. A strong and positive relations between fruit physical characteristics have been reported in different species (Lo Bianco et al., 2010; Sarıdas et al., 2017).

Table 2. Correlation coefficients among the investigated characteristics

	FW	FL	BW	L*	C*	hº	SSC	Ph
FL	0,73***							
BW	0,87***	0,89***						
L*	-0,20ns	-0,25*	-0,17ns					
C*	0,15ns	0,20ns	0,11ns	-0,82***				
hº	0,23*	0,21*	0,16ns	-0,79***	0,92***			
SSC	-0,26*	-0,05ns	-0,22*	-0,21*	0,16ns	0,03ns		
Ph	-0,02ns	0,20ns	0,05ns	-0,24*	0,18ns	0,06ns	0,93***	
TA	0,19ns	-0,03ns	0,13ns	0,23*	-0,16ns	-0,01ns	-0,98***	-0,95***

FL: Fruit length, FW: Fruit width, BW: Berry weight, SSC: soluble solid content, TA: Titratable acidity,
ns: non-significant *P<0.05, **P<0.01, ***P<0.001

Intercellular spaces of the cells, increase with the increase of fruit volume and weight. As a result, soluble solid content accumulation per unit area decreases. Consequently, a negative and significant relationship was found between soluble solid content with fruit width and fruit weight, respectively, at -0.26* and -0.22* in the study. Similar results were reported by Eskimez et al. (2020).

A strong and negative relation (-0.95 ***) was found between the pH properties of TEA, which is the calculated form of total organic acids in terms of dominant acid. Negative relation between pH and TEA was similarly reported by Mertoğlu and Evrenosoğlu (2019) as -0.81*** and by Eskimez et al. (2020) as -0.78***.

Increase of color giving pigments in fruit, makes the fruit darker and dull at the same time. For this reason, it was found that L value were negatively correlated with C and H values and the correlation coefficients between them were -0.82*** and -0.79***, respectively. While there is a positive correlation between fruit sizes and weight with C* and hº values, L* value wasfound in a negative relationship with these characteristics. These relations may have resulted from the fact that color pigments are synthesized as a result during photosynthesis, which also improves the physical properties of the fruit.

Conclusion

In recent years, it has become very important to identify superior genotypes of species with high antioxidant effect, having potential for use as alternative medicine and suitable for industrial product processing. In this context, three different genotypes of gilaburu grown in different province of Kayseri (Akkışla, Uluşağ and Hastane) has potential and were characterized in this study in terms of their physico-chemical characteristics. According to the results, genotype-3 stood out in terms of pomological characteristics, while genotype-2 showed superior characteristics in terms of chemical properties.

Compliance with Ethical Standards

Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

Author contribution

Mehmet Polat designed the study and collected the data with İlknur Eskimez. Kerem Mertoğlu made the statistical analysis and wrote the original draft of the article. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

Not applicable.

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Data availability

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Consent for publication

Not applicable.

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Research Article

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Analysis of knowledge transferred by agricultural field staff to cotton growers and its impact on rural development: evidence from Pakistan

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Abstract

Cotton is the main cash crop in Pakistan and it contributes 0.8% shares in GDP (Gross domestic product). The area of cotton in Pakistan is increased in recent years but its production is decreasing due to unfavorable climatic conditions. COVID-19 outbreak has also an effect on cotton consumption. From August 2019 to July 2020, the COVID-19 pandemic reduced global cotton consumption by 15%. Agricultural extension and advisory activities play an important role in agricultural development and can help to improve the living conditions of farmers. Keeping in view the importance of working in agriculture field staff for cotton, the present study was designed to analyze the quality of knowledge transferred by agriculture field staff. The present study was based on primary data and conducted in the district Muzfargarh becsasue it is one of major cotton producing district of Pujnab, Pakistan. A total of 180 respondents were taken from Tehsil Alipur of Muzfargarh from different union councils through a simple random sampling technique and interviewed through a pretested structured interview schedule. The data collected was analyzed using the Statistical Package for Social Sciences (SPSS). About 58% of the respondents identified that extension meetings are good source of information and 37.7% of the respondent said that agricultural field staff visit them on monthly basis.Impact of improved cotton management practices on health rated satisfactory by 52.77% of respondents. Based on findings it is recommended that the government should work with all stakeholders to implement regular training programs for cotton farmers in all areas.Monitoring of agricultural field staff should also be done on regular basis. Modern ways of communications should be implemented in rural areas for the quality of knowledge transfer among cotton growers.

Keywords: Rural development, Agricultural Field Staff, Cotton

Introduction

In Pakistan, most of the population (65%) lives in rural areas, and their livelihoods directly or indirectly depend on agriculture, where it plays an important role in economic development, food security, poverty reduction, livelihood, rural development, and the environment. The shares of agriculture in GDP(Gross domestic product) is 19.3% and workforce contribution is 45%(Govt. of Pak., 2020).

Cotton is Pakistan's main cash crop and accounts for around 0.8% of GDP and 4.1% of the total value added in

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agriculture. In the period 2019-20, cotton planted on an area of 2,527 hectares, with an increase of 6.5% compared to last year (2018-19,2,373 hectares). Cotton production is estimated at 9.178 million bales, down 6.9% from 9.861 million bales last year(2018-19). Although the total area has increased compared to last year, the overall performance has remained lower due to unfavorable weather and poor water availability during the important stages of plant development, as well as pest attacks (Govt. of Pak., 2020).

Global cotton production in 2019/20 is estimated at 125.8 million bales, 5% incresae (almost 6.5 million bales) compared to 2018/19, and the second-highest production recoreded after 2011/12 which was 127.2 million bales. In 2019/20, cotton production estimates for the major-producing countries are mixed, which is likely to be increased. World cotton harvested area is forecast at 34.7 million hectares (85.8 million acres), 3.5 percent above 2018/19 and the highest since 2011/12; most of the 2019/20 area increase is attributable to the United States, where expected abandonment is reduced considerably from 2018/19 as a result of improved moisture in the Southwest. The 2019/20 global cotton yield is forecast at 789 kilograms/ hectare (kg/ha) (704 pounds per acre), slightly above the previous 3-year average (USDA 2019).

Global cotton production continues concentrated among a few countries. In 2019/20, the top five cotton-producing countries are forecast to account for more than 78 percent of total production, similar to 2018/19 but 2 percentage points above the 2015/16-2017/18 average. India is forecast to be the leading producer in 2019/20, contributing 23 percent of the global crop estimate, while China and the United States are projected to account for 22 percent and 17 percent, respectively. Meanwhile, Brazil is expected to contribute 10 percent, and Pakistan accounts for an additional 6 percent (USDA 2019).

Cotton is grown worldwide and is usually harvested by machines, but sometimes by hand. Around 25 million tonnes of cotton are produced every year worldwide. From August 2019 to July 2020 the COVID-19 epidemic resulted in a 15 percent drop in global cotton consumption(USDA 2020).

Cotton is an important commercial crop in rural areas as it holds a significant potential in economic production (Jayne, 1994; Aboudou, 2019). When it comes to the use of cotton, the country with the world's largest textile industry is the largest importer of cotton lint. In the late 1990s, China's largest textile producer accounted for more than a quarter of the world's cotton production (Baffes, 2005; Kholikova, 2020).

Despite being one of the largest cotton-producing countries, Pakistan's cotton production is lower than other countries. The low cotton production is due to weather conditions, pest attacks, and low response by farmers to scientific and pest control techniques. It is the public judgment that government agencies are building vigorous efforts to stimulate the well-being of the rural community, try to close the gap in cropping crops, and start various agricultural extension programs in Punjab (FAO, 2002) as it is major cotton production province in Pakistan (Memon, 2019; Ur Rahman et al., 2020).

As extension services have a vibrant segment in agricultural development. It brings information for the agricultural community and new skills that can be adopted to recover production, income, and living conditions. Agricultural field staff is responsible for dissemination of latest technologies and information to farmers. The previous failure in the adaptation of latest technology is due to the inefficiency of agriculture extension services. (Khan, 2012; Olorunfemi, 2020).

The researchers believed that developing countries needed to localize their extension schemes and develop other management developments to deal with many of these issues and to recognize rural development goals such as the reasonable distribution of financial development and mitigation.

There is a lot of research done to assess the work efficiency and knowledge transferred by agricultural extension workers to cotton growers. However, none of the research has met the target and does not meet farmers' requirements. Farmers are still looking for satisfaction with field staff performance. The agricultural staff is expected to serve as a solid bridge between the field and research institutions and provide equal services to all farmers, regardless of the social status of the client and their properties. The study was designed to investigate the level of expectations and satisfaction of the agricultural community with the knowledge imparted by agricultural workers to cotton producers.

Keeping in view the importance of working of agricultural field staff concerning cotton crops, the present study is designed to analyze the quality of knowledge transferred by staff. It is hoped that the results of the study will help put efforts in a more effective way of achieving the desired objectives.

Materials and Methods

The present research is based on primary data and data was collected from the cotton growers in the study area.

Study area

Muzaffargarh is the one of the major cotton growing district in Punjab. District consist of 4 Tehsils Alipur, Jatoi, Kot Addu and tehsil Muzaffargarh. The present study was conducted in district Muzaffargarh because this district is convenient for the researcher to collect and analyzes the data.

Sampling and its procedure

Tehsil Alipur is selected through simple random sampling among the 4 Teshils. The Tehsil comprises of 14 union councils. Out of a total of 14 union councils the study was conducted in 4 union councils which were selected using simple random sampling. From 4 union councils, twelve villages were selected by using a random sampling technique. From each village, 15 cotton growers were selected randomly. Thus making a total of 180 cotton growers as a sample for the study.

Study tool

Many considerations can allow the interviewer maximum comfort and minimum confusion during the interview process (Fontana & Frey, 2000). An interview schedule was planned based on the basic objectives of the study in such a way to gather complete and actual information. It was consist of open and closed ended questions. The interview schedule was in English and the interview was given in Urdu and local languages.

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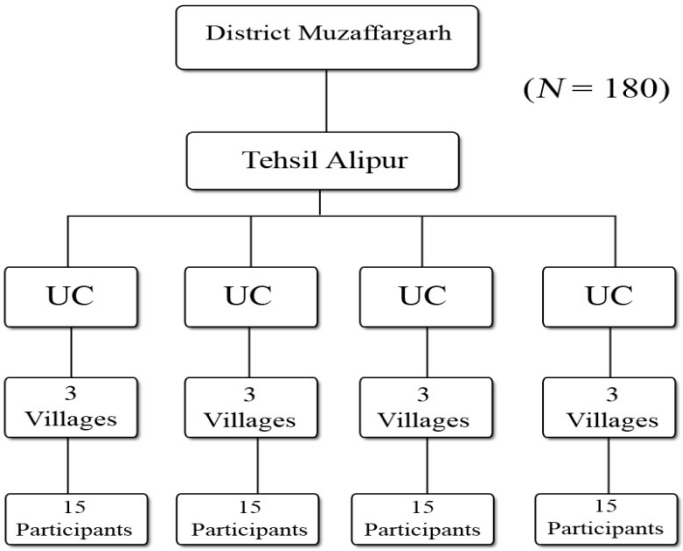

Pre-testing

The research tool was previously tested on 10 randomly selected respondents and the interview schedule was modified with the necessities.

Interviewing the Respondents

The interview was conducted from farmer respondents at their fields. Questions were asked in local language.

Data analysis

The data were analyzed with the Microsoft excel and statistical package for social sciences (SPSS).

Results and Discussion

The present study was conducted to evaluate the knowledge transferred by Agricultural field staff to cotton growers and their impact on rural development The collected data was analyzed to draw a conclusion and to provide a measure that will help to highlight the role of agricultural field staff in providing knowledge to cotton growers in tehsil Alipur.

Farmers' perception regarding sources of information of cotton crop management

Data Plotted in figure 1 shows that the majority of the respondent (90-95%) reported that they get information from Radio, Television and friends/family members and other 62% from newspapers and 58% from the Extension meetings while other also get information from govt. agencies. This showed that farmers mainly rely on agriculture field staff, local people radio, and newspapers and they do not depend on only one source for fulfilling their needs regarding best crop management.

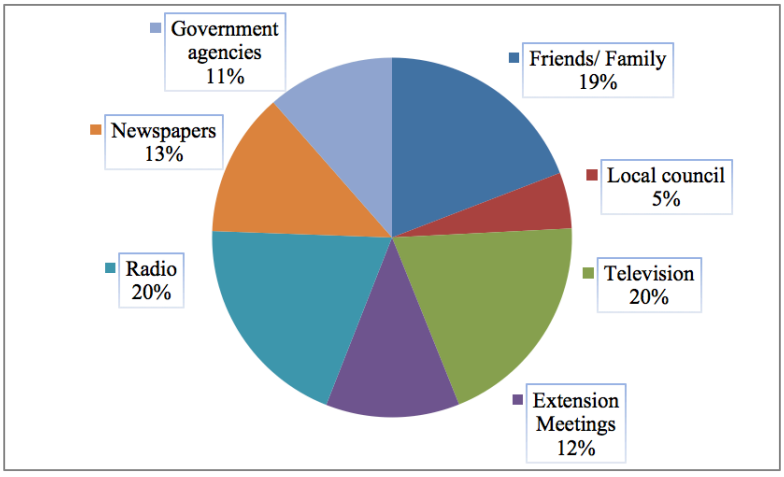

Figure 1. Sources of information regarding Cotton crop management

Locals views about staff visits to the respondents

The respondents were inquired about the frequency of extension visits. More than 41.11% of respondent reported that agriculture field staff visit often fortnightly and 37.7% of the respondent reported that about the monthly visit and 21.11% of respondent reported that agriculture field staff visit on a weekly basis (Figure 2).

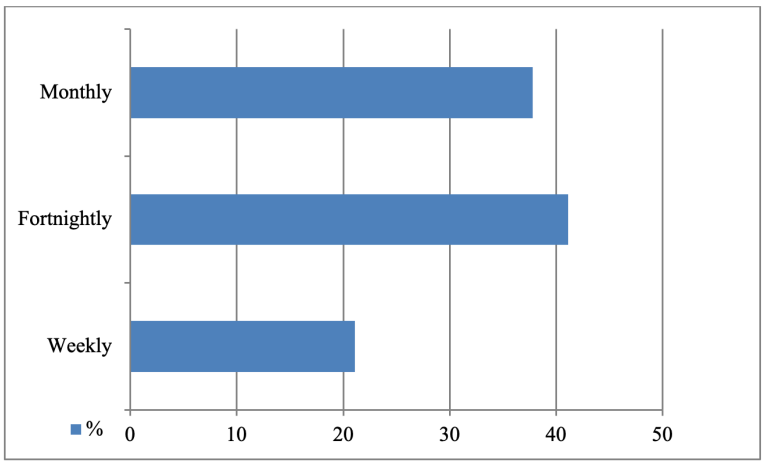

Figure 2. Frequency of visit to field by agricultural staff

Farmer's perception regarding different kinds of extension methods used in the study area

Farmers were asked about which kinds of methods are being used by agriculture statt. Data in figure 3 shows that majority (93.01%) of the respondents said that extension teaching methods used by the extension field staff were farm visit. Less than half (45.56%) of the respondents said that extension field staff did not use audio video cassette as extension teaching method. Majority (91.11%) of the respondents said that extension field staff used pamphlets for teaching method. Farmers (84.44%)also responded that group meetings has been done by private agriculture workers on regular basis. Radio still has the most common source of information for rural people in the remote areas of Pakistan. Farmers (87.77%) responded that agriculture department publish information regarding cotton management but due to lack of education they can't read these information. A local responded that " I can't read and write but when I see the practical demonstration of different agricultural technologies I can easily implement these in my field. He further suggested that these kinds of activities should be on regular basis".

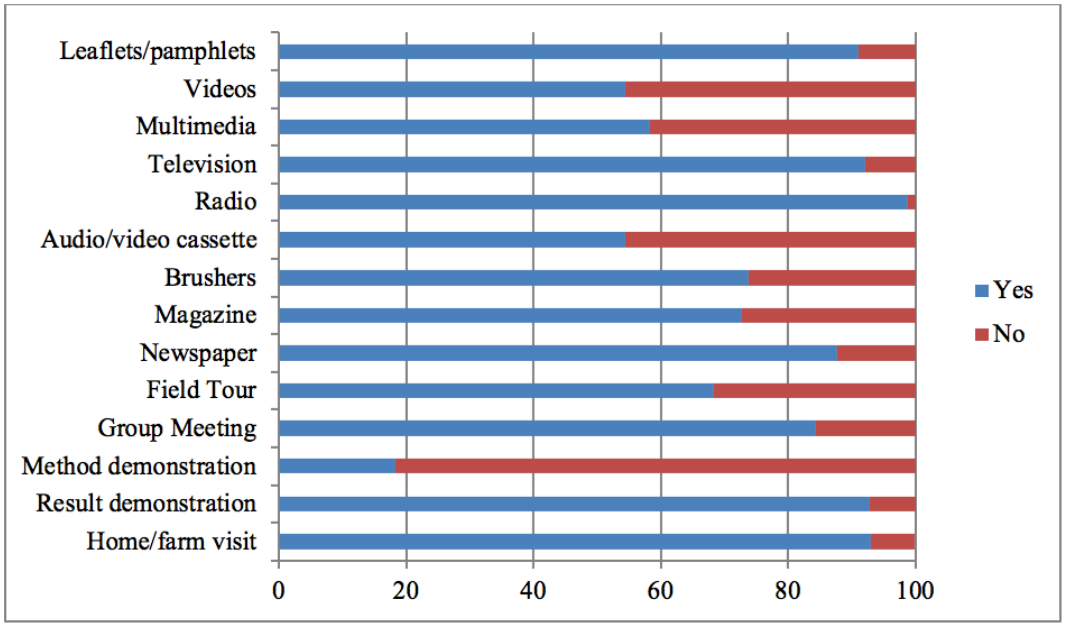

Figure 3. Teaching methods performed by agricultural staff

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Ranking order of teaching methods according to their effectiveness

As data in figure 4 shows the ranking order of the teaching methods according to their effectiveness. Results shows that radio stood first as most effective source of information for farmers with weighted score of 432 and mean value of n=4.32. In this modern era people believes what they see information provided through television found be effective and stood 2nd in the table with weighted score of 389 and mean value of 3.89. A local responded that "I can't read and write but when I see the practical demonstration on television of different agricultural technologies I can easily implement these in my field. He further suggested that these kinds of activities should be on regular basis". Newspaper, leaflets, field tour stood 4th , 5th and 3rd respectively in the ranking table.

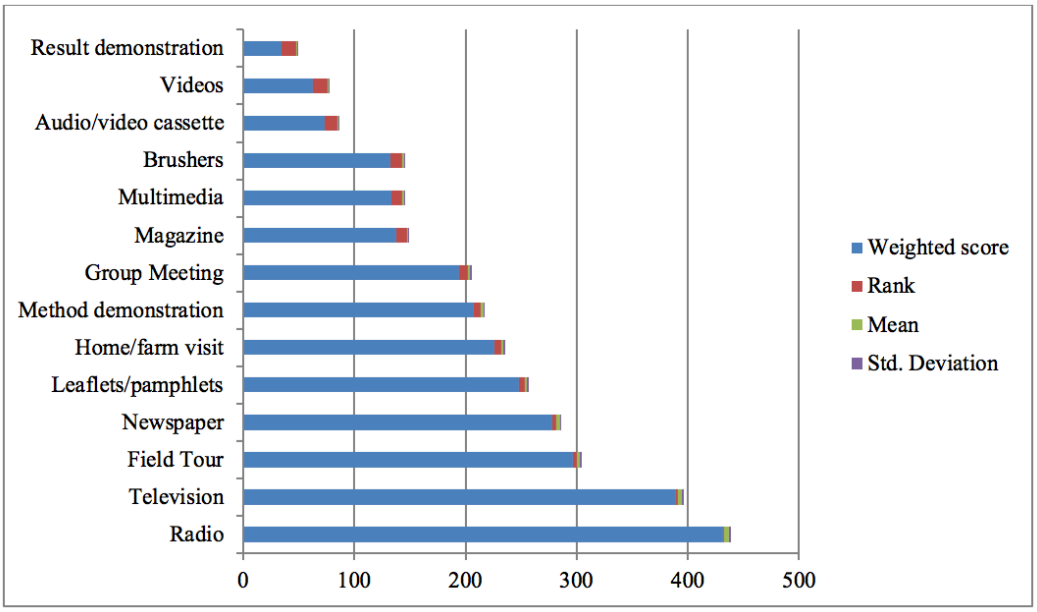

Figure 4. Ranking order of effective methods used in providing services

The ranking order of different agricultural practices given by the agricultural field staff

The table 1 shows that 42.22% of the respondent stated that training and information regarding agronomic practices related to Time of sowings are good and they are getting benefits to some extent in their overall productivity. The given data reported that practices about fertilizer application were rated Satisfactory by 58.8% of the respondent and practices about the selection of varieties rated poor by 2.22% of the respondent and other practices are also satisfactory to some extent and practices regarding Thining and gap-filling fell in between satisfactory and good categories but shows its tendency towards good category (mean = 3.49). Information and training regarding land preparation rated fair by 47.77 percent of the respondents.

Table 1.Agronomic practices suggested by Agricultural field staff

Agronomic Practices	Poor %	Fair %	Satisfactory %	Good %	Excellent %	Mean
Time of sowing	2.22	-	35.55	42.22	20	3.77
Thinning and gap filling	3.33	25.55	15	30.55	25.55	3.49
Seed rate and spacing	-	9.44	45	41.66	3.89	3.4
Picking and harvesting	11.66	-	47.77	22.77	17.77	3.46
Fertilizer application	9.44	-	58.88	13.88	17.77	3.4
Selection of variety	2.22	35	26.11	6.66	30	3.27
Irrigation	1.11	23.88	28.88	40.55	5.55	3.25
Weeding and inter culturing	14.44	5.55	37.22	38.33	4.44	3.12
Land preparation	-	47.77	40	12.22	-	2.64

Source: Own calculation through interview

The ranking order of agronomic practices given by the agricultural field staff

The Figure 5 shows that the time of sowing and thinning and gap-filling stood first and second in the ranked order with a weighted score of 378 and 349 respectively. Time of sowing fell in between satisfactory and good category but shows its tendency towards good category (mean= 3.77). Fertilizer application stood 5th in the ranked order with a weighted score of 331. Fertilizer application fell in between good and satisfactory categories but show it trends towards satisfactory category (mean=3.40). land preparation stood 9th in the ranked order with weighted score of 264.

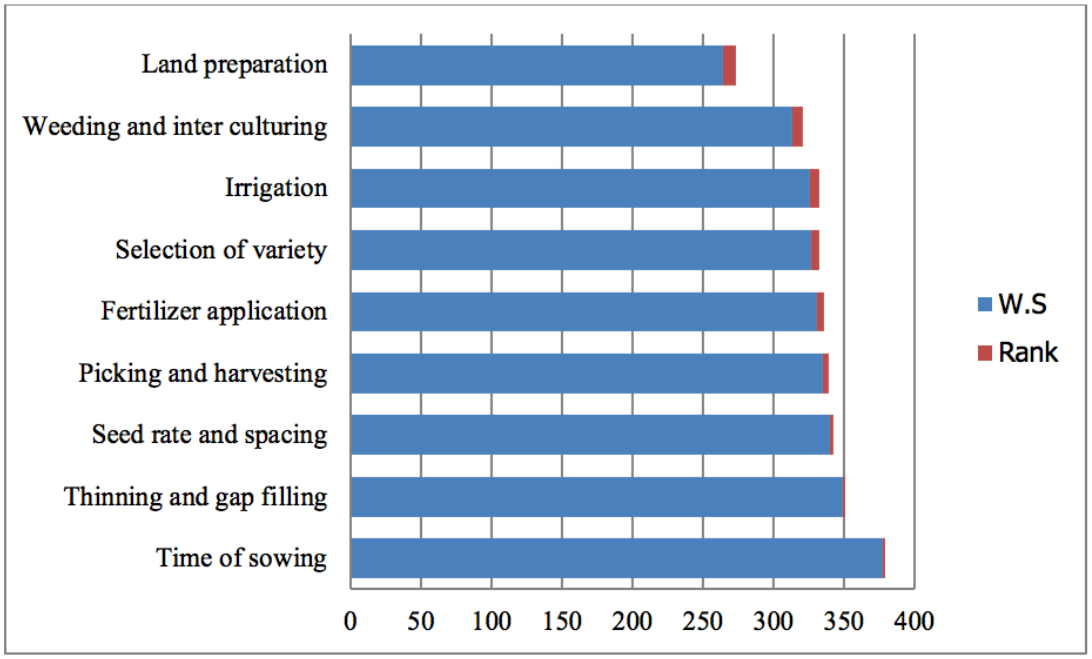

Figure 5. Ranking order of agronomic practiced adapted suggested by

Quality of knowledge transferred by agricultural field staff

As figure 6 shows that 55.5% of the respondent rated the quality of knowledge transferred by EFS as Satisfactory while 30.55% of the respondents rated the quality of knowledge fair and 5% of the respondent rated the quality of knowledge Excellent while 2.22% rated poor.

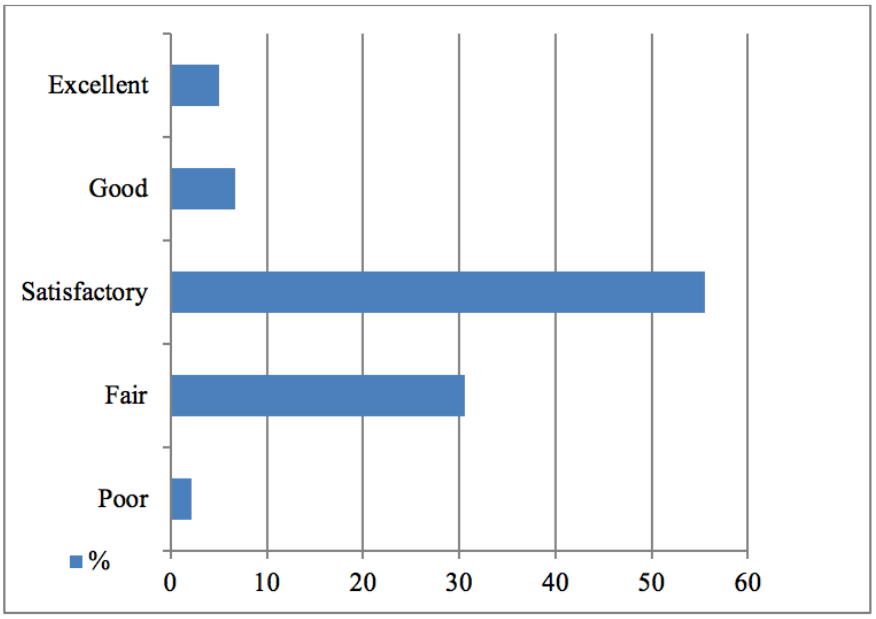

Figure 6. Farmer's response about quality of perceived knowledge

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The role of agricultural field staff in providing information regarding best crop management practices

Berdegue and Escobar (2002) have stated that the efficient supply and use of agricultural extension services have direct and indirect effects on reducing rural poverty. They believe that the direct impact of technological innovation on poverty reduction is the cumulative benefit of farmers who have implemented the changes. Table 2 shows that the practices about soil management regarding the seedbed preparation rated poor by 17.22% of the respondent and practices about furrows management rated good by 58.8% of the respondent and 11.6% Satisfactory about ridges table also reveals that information provided by agricultural field staff about fertilizer management Di Ammonium Phosphate rated Satisfactory by 51.11% of the respondent and FYM rated 23.33% Excellent by the respondent. Surface Irrigation rated good by 40% of the respondent and furrows irrigation rated poorly by 2.22% of the respondent.

IPM practices suggested by agricultural field staff

It is estimated that about 10% of synthetic pesticides and 20–25% of insecticides are used every year in cotton farming. Developing countries use about 50% of the total (EPA, 2002). The frequent and improper use of pesticides has led to the development of insect resistance and pest outbreaks in cotton. (Kranthi et al., 2002).

Cultural practices

The results show that in cultural practices information provider by agricultural field staff about resistant varieties is rated fair by 51.11 % of the respondent and rated Excellent by 6.11% of the respondent while about Earthling up 46.6 % of the respondent rated Satisfactory and about thinning 35.6% is poor.

Table 2. Information regarding best crop management practices

Production management practices	Poor	Fair	Satisfactory (%)	Good	Excellent	Mean
Soil management						2.07
Seedbed preparation	17.22	62.22	16.67	3.88		3.62
Ridges	3.33	5.56	48.89	9.44	32.78	3.43
Furrows	2.22	21.67	11.67	58.88	5.56	3.42
Fertilizer management						
Nitrogen phosphorus potassium	1.11	22.78	35.56	13.88	26.67	2.81
Di Ammonium phosphate	1.67	35	51.11	4.44	7.78	3.12
Farm yard manure	1.11	36.67	29.44	9.44	23.33	3.31
Irrigation management						2.9
Surface irrigation		18.33	36.67	40	5	2.68
Furrows irrigation	2.22	20	68.33	3.88	5.56	3.65
Cultural practices						
Resistant varieties		51.67	33.89	8.33	6.11	3.74
Rotating annual plants	1.11	14.44	25	37.22	22.22	3.19
Intercropping	1.11	4.44	40	27.77	26.66	2.3
Earthling up	1.67	23.89	46.67	8.88	18.88	3.56
Thinning	35.56	18.89	29.44	12.22	3.88	3.46
Hoeing	2.78	8.89	45	15.55	27.77	2.82
Physical practices						
Hand picking	2.22	18.89	32.22	23.88	22.77	2.86
Pruning		39.44	48.33	2.77	9.44	3.33
Mulching	2.78	33.89	40.56	19.44	3.33	2.51
Trapping	17.78	3.33	15	55	8.88	2.94
Biological practice						
Beneficial insect	25.56	25.56	31.67	6.11	11.11	3.38
Microorganisms	2.78	41.67	17.22	35	3.33	3.12
Parasitic nematodes	8.33	6.11	41.11	27.77	16.66	3
Chemical Practices						
Insecticidal soap	6.11	26.11	23.89	36.67	7.22	2.66
Horticultural oils	7.78	26.67	25	38.88	1.66	3.67
Botanical insecticides	16.67	20.56	50	5.55	7.22	3.48
Inorganic fungicides	3.33	7.22	23.88	49.44	16.11	2.8
Inorganic insecticides	3.33	3.33	57.77	12.22	23.33	3.42

Source: Own calculation through the interview

Physical practices

The results show that in physical practices information related to handpicking is rated fair by 18.9% of the respondent and poor by 2.2% of the respondent while information regarding mulching is rated satisfactory by 40.6% of the respondent and about trapping by 3.3% of the respondent rated fair.

Biological practice

The results show that information regarding biological practices delivered by agricultural field staff about Microorganisms rated Excellent by 3.3% of the respondent. Beneficial insect regarding information were rated poor and fair by 25.6% of the respondent

Chemical Practices.

In Chemical practices information spread by agricultural field staff about Horticultural oils is rated poor by 7.78% of the respondent and inorganic fungicides rated good by 49.44% of the respondents.

Impact of improved cotton crop management practices

Table 3 shows that most (63.88%) of the respondents reported that skill factors were good and respondents were satisfied with the skill practices. A few (8.88%) of the respondents said that the impact of improved cotton crop management practices on health was excellent. More than one-fifth (23.88%) of the respondents said that the impact of the improved cotton crop on the environment was very poor.

Table 3. Impact of improved cotton crop management practices

	Poor	Fair	Satisfactory (%)	Good	Excellent	Mean	Std. Deviation
Skill	1.66	18.33	16.11	63.88		3.42	0.84
Management	1.11	12.77	52.77	28.33	5	3.23	0.77
Finance	2.22	5	65	25	2.77	3.21	0.67
Production	5	29.44	52.22	7.22	6.11	2.8	0.88
Health	21.66	5.55	52.77	11.11	8.88	2.8	1.16
Environment	23.88	61.66	8.33	2.77	3.33	2	0.85

Source: Own calculation through the interview

Conclusions and Recommendations

Cotton is an important crop in the southern part of Punjab, but according to recent studies, its production is decreasing in the areas of Pakistan. Different factors are responsible for the decrease in cotton production, the most important factor is climate change and lack of the latest knowledge and skills in cotton management. The present study is conducted to evaluate the quality of Knowledge transferred by the agriculture field staff to the cotton growers and their impact on rural development. The study found that farmers need training in their field activities with the help of the Department of Agricultural Extension through which they can increase their production and quality of life. The availability of modern technology and effective skills are two essential conditions for productivity. There is considerable dissatisfaction in agriculture and related communities regarding the performance of extension services.

Based on the findings it is recommended that the government, in cooperation with all stakeholders, conduct regular training programs for farmers and agricultural workers about good agricultural practices (GAP) of cotton and ensure timely access to information on modern agriculture to improve the quality of cotton growers. Also, it is recommended to implement an effective system for monitoring and evaluating agricultural workers' performance and management practices about cotton crops for the betterment of the agriculture sector of Pakistan.

Compliance with Ethical Standards

Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

Not applicable.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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Somatic mutations and recombination test in *Drosophila melanogaster* used for investigating the genotoxicity of some food additives

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Abstract

In the present study, the effects of several food colorings, namely (Ponceau 4R (E 124), Tartrazine (E 102), and Pea green (E 102-E 133), were investigated in vivo using the wing spot test, SMART (somatic mutation and recombination test), in *Drosophila melanogaster*. Food colorings are the food additives, which are used for improving the appearance of food and beverages. In SMART, multiple wing hair (*mwh*), flare (*flr*^{3}), and beaded serrate (*BdS*) marker genes on the third-largest chromosome of *Drosophila* are used. The genotoxic effects of the food colorings on the imaginal disc cells that will develop into the wing spot cells during the embryonic development of *Drosophila* heterozygous larvae and the genotypic changes caused by mutation or recombination in somatic cells also play a role in the formation of mutant spots in the wings. Classes by mutant clones are as follows: small single spots containing 1-2 *mwh*, large single spots containing ≥3 *mwh* or ≥4 *flr*^{3}, and twin spots containing adjacent *mwh* and *flr*^{3} cells (GRAF et al., 1984). Negative control medium was prepared with distilled water, while positive control medium was prepared with 1 mM EMS (ethyl methane sulfonate). According to results obtained from SMART, Ponceau 4R, Tartrazine, and Pea green demonstrated significant results in trans-heterozygous flies (*mwh*/*flr*^{3}) for inducing the mutant wing spots compared to control groups at 25 mg/ml, 50 mg/ml, and 75 mg/ml exposure concentrations. On the other hand, Ponceau 4R, Tartrazine, and Pea green yielded significant results for inducing the mutant wing spots in balancer-heterozygous flies (*mwh*/*TM3*) at 25 mg/ml, 50 mg/ml, and 75 mg/ml exposure concentrations. The numbers of mutant wing spots were increased by all three colorings depending on the concentration (X^2= df=3, P<0.001). It was also determined that these numbers were significantly higher than the flies in the negative control medium and it suggests that these food colorings have genotoxic effects. However, the numbers of mutant wing spot were less than the flies in the positive control medium; this finding indicates that genotoxic effects of the food colorings were not as much as the EMS.

Keywords: Ponceau 4R, Tartazine, Pea green, *Drosophila melanogaster* Meigen, SMART

Introduction

Nutrition is one of the most important factors ensuring the continuity of life. Nowadays, it is seen that people's eating habits significantly differ as a result of the changes in lifestyle and economic development. Although the food additives play a significant role in the food industry, their effects on human health constitute an important issue. As a result of the increase in ready-made food consumption together with urbanization, potential health risks occur with the body's exposure to more additives (Akbulut, 2011).

Synthetic food colorings, which are commonly used class food additives, can create genotoxic effects, as well as health problems such as allergic reactions, skin rashes, asthma, hyperactivity, and concentration disorder when not used within

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the legal restrictions (Yentür et al., 1996). It is also stated that the food coloring agents cause hypersensitivity, migraine, preterm delivery, salicylate sensitivity, and cancer (Maier et al., 2010). With genotoxicity tests and epidemiological studies on *Drosophila*, mouse, rat, and bacteria, when not used within the limits specified in regulations, the synthetic food colorings have been reported to be carcinogenic when used in order to imitate the high quality (Yentür et al., 1996; Sarıkaya et al., 2010). It has been determined that the level of food colorings used in studies on this subject in our country is well above the statute limits and may have harmful effects in terms of public health (Yentür et al., 1996; Topsoy, 1990; Yaman, 1996).

Many studies on human diseases have shown that *Drosophila melanogaster* Meigen, a down-organized eukaryotic organism, can be used instead of mammals. Because, the biological properties of the imaginal disc cells in *Drosophila* larvae undergoing proliferation and differentiation to form body parts in the adult stage are similar to many cancer-sensitive mammalian cells, and more than 60% of genes identified in human genetic diseases are common with the genes in *Drosophila* genome. *Drosophila* is frequently included as a model organism in SMART test. This test is suitable for detecting mutagenic and recombinogenic activities that are the results of genotoxic and anti-genotoxic effects in somatic cells induced by chemicals. When compared with the other tests, SMART is a quite fast, precise, and economical option (Bernards & Hariharan, 2001). Moreover, this in vivo test method in *Drosophila* may be associated with in vivo genotoxicity tests in mammals (Graf et al., 1984; Graf & Wurgler, 1996). Mutations induced by chemicals in somatic cells of *Drosophila* larvae are transferred to daughter cells through the several cell divisions in wing SMART. Genotypic changes caused by a mutation or recombination in somatic cells may be seen as mutant spots in the wings (Graf et al., 1984; Tripathy et al., 1989).

The synthetic food colorings used in the present study were preferred because, in the literature, there are very few scientific studies similar to the subject of the present research. There is no study carried out on Ponceau 4R (E 124) and Pea green (E 102-E 133) in *Drosophila* by using SMART, and there is only one previous study of Tartrazine (E 102), (Tripathy et al., 1989). There are few studies carried out on *Drosophila* regarding the genotoxicity of these food colorings.

Using SMART, the present research was conducted in order to determine whether Ponceau 4R, Tartrazine, and Pea green at 25 mg/ml, 50 mg/ml, 75 mg/ml concentrations have a detrimental effect on *D. melanogaster* Meigen's *mwh* and *flr*^{3} lines.

Materials and Methods

Culture of *Drosophila* Lines

D. melanogaster Meigen lines used in the present study were obtained from Trakya University and Akdeniz University, Science Faculty, Department of Biology. *Drosophila* flies were cultured in an incubator, where optimum living conditions (25±1 °C and 40-60% relative humidity) were provided and adjusted to 12 hours light-dark cycle.

Experimental Groups

SMART method developed by (Graf et al., 1984) was used in determining the mutagenic and/or recombinogenic effects of Ponceau 4R, Tartrazine, and Pea green on *D. melanogaster* Meigen lines.

Genetic Structure of *Drosophila* Mutant Strains

In *Drosophila* SMART, the *mwh*, *flr*^{3}, and *BdS* (beaded serrate) marker genes on the third-largest chromosome of *Drosophila* are used. In *Drosophila* wing SMART, *flr*^{3}/*TM3*, *BdS* virgin females and *mwh*/*mwh* males are crossed for normal metabolic activity (Lindsley & Grell 1968; Lindsley & Zimm 1992; Garcia-Bellido & Dapena 1974).

Experiments in the present study were simultaneously conducted in the application groups consisting of experimental and control groups. Distilled water was used in the negative control group and, in literature, 1 mM concentration of EMS (Kasımoğlu & Uysal 2016), which was used in the positive control group, was stated to have a mutagenic effect. All of the food colorings used in the experiment groups dissolved in distilled water. Ponceau 4R, Tartrazine, and Pea green were used at 25‰, 50‰, and 75‰ concentrations for 72±4 hours in the experimental groups for *Drosophila* larvae.

D. melanogaster Meigen lines were fed with a standard *Drosophila* medium when kept in stock and in a cross bottle in order to ensure the fertilization and embryogenesis to obtain heterozygous larvae. *Drosophila* instant medium was used for *Drosophila* larvae for 72±4 hours in the experimental and control groups. *Drosophila* instant medium and the food colorings used in the study were purchased commercially. *Drosophila* instant medium was procured from Carolina Biological Supply Company, Ponceau 4R with Pea green from Roha JJT Group Company, Tartrazine from Parshwanath Colour Chem Group, and EMS from Sigma Aldrich GmbH.

Applications in Experimental Groups

Larvae in groups of 100 were left into falcon tubes, where 1.5 g of *Drosophila* instant media were wetted with 5 ml of solutions containing 25‰, 50‰, and 75‰ concentrations of Ponceau 4R, Tartrazine, and Pea green. While edges of wings of the adult flies developed from trans-heterozygous larvae (*mwh*/*flr*^{3}) at each concentration in experimental groups have normal structure, the edges of wings of the balancer-heterozygous flies (*mwh*/*TM3*) are in the form of serrate. In analyses, 40 wings were used for each concentration in each experimental group. Slides of normal and serrate wings were examined using a light microscope under 40×10 magnification in order to detect the presence of mutant clones (Kaya, 2000). Classes by mutant clones are as follows: small single spots containing 1-2 *mwh*, large single spots containing ≥3 *mwh* or ≥4 *flr*^{3}, and twin spots containing adjacent *mwh* and *flr*^{3}cells (Graf et al., 1984).

Wing spot test (SMART)

The SMART assay principle is based on loss of heterozygosity in recessive marker genes; *mwh* and *flr*^{3} on the third-largest chromosome lead to transformation of imaginal disc cells in *Drosophila* heterozygous larvae into mutant wing spots cell by mutation or recombination (GRAF et al., 1984).

Statistical Analysis

Minitab package program was used in the statistical analysis

of the study results. In the data analysis, it was investigated using the Chi-Square test if the total number of mutant spots in the wings of the flies in the positive and negative control groups was statistically different (df=1). Chi-square test (df=3) was also used in the analyses, where the total numbers of mutant spots in the wings of the flies at three different doses in experimental groups were compared separately with the flies fed in the positive control group and negative control group.

As a result of analyses, the total number of wing mutant spots obtained from the study and presented as Fr (frequency) values and the differences below $P<0.05$ were considered to be statistically significant.

Results and Discussion

When the results of the study were evaluated, the total number of mutant wing spots in normal ($X^2=213.22$, df=1, $P<0.001$) wings (in Table 1) and serrate ($X^2=69.06$, df=1, $P<0.001$) wings (in Table 2) of *Drosophila* flies in the positive control group was found to be much more than those of negative control group.

SMART data obtained from experimental group studies of Ponceau 4R were compared with results of positive and negative control groups in both Table 1 and Table 2. The total number of mutant wing spots in the normal wings (in Table 1) and serrate wings (in Table 2) of the *Drosophila* flies grown in the medium containing with 25‰, 50‰, and 75‰ concentrations of the Ponceau 4R were higher than those grown in the medium prepared with distilled water (normal wing: $X^2=22.84$, df=3, $P<0.001$; serrate wing: $X^2=29.82$, df=3, $P<0.001$) but it was found to be less than those grown in medium with EMS addition (normal wing: $X^2=330.87$, df=3, $P<0.001$; serrate wing: $X^2=51.64$, df=3, $P<0.001$).

In Tables 1 and 2, the study results obtained in the experimental group by using different concentrations of Tartrazine and in positive and negative control groups are given comparatively. According to the results obtained, the total number of mutant wing spots in the normal wings (in Table 1) and serrate wings (in Table 2) of the *Drosophila* flies grown in the medium containing with Tartrazine at 25‰, 50‰, and 75‰ concentrations were higher than those of the flies grown in the medium prepared with distilled water (normal wing: $X^2=22.42$, df=3, $P<0.001$; serrate wing: $X^2=29.91$, df=3, $P<0.001$) but it was found to be less than those of the medium EMS added (normal wing: $X^2=324.56$, df=3, $P<0.001$; serrate wing: $X^2=65.19$, df=3, $P<0.001$).

The numbers of mutant wing spots and total mutant spots in normal wings (in Table 1) and serrate wings (in Table 2) of *Drosophila* flies grown in the medium prepared with Pea green at 25‰, 50‰, and 75‰ concentrations were higher than those grown in the medium containing with distilled water (normal wing: $X^2=35.85$, df=3, $P<0.001$; serrate wing: $X^2=24.98$, df=3, $P<0.001$) but it was found to be less than those of the flies grown in the medium with EMS (normal wing: $X^2=277.27$, df=3, $P<0.001$; serrate wing: $X^2=40.45$, df=3, $P<0.001$). In Tables 1 and 2, the study results of the experimental group containing with different concentrations of Pea green are compared with positive and negative control groups comparatively.

Table 1. SMART data with *mwh/flr3* wings obtained with the food coloring agents and positive and negative control groups tested.

Application groups/ Concentration	Number of wing	Small single spots (1-2 cell)		Large single spots (>2 cells)		Twin spots		Total mwh spots		Total spots	
		No	Fr	No	Fr	No	Fr	No	Fr	No	Fr
Distilled water	40	3	(0.08)	5	(0.13)	4	(0.13)	3	(0.08)	12	(0.30)
1 mM EMS	40	49	(1.23)	130	(3.25)	68	(1.70)	99	(2.48)	247	(6.18) a*
Ponceau 4R											
25 mg/ml	40	1	(0.03)	37	(0.93)	0	(0.00)	1	(0.03)	38	(0.95) a* b*
50 mg/ml	40	1	(0.03)	41	(1.03)	0	(0.00)	1	(0.03)	42	(1.05) a* b*
75 mg/ml	40	1	(0.03)	49	(1.23)	0	(0.00)	1	(0.03)	50	(1.25) a* b*
Tartrazin											
25 mg/ml	40	0	(0.00)	40	(1.00)	0	(0.00)	1	(0.03)	40	(1.00) a* b*
50 mg/ml	40	3	(0.08)	44	(1.10)	0	(0.00)	5	(0.13)	47	(1.18) a* b*
75 mg/ml	40	4	(0.10)	42	(1.05)	0	(0.00)	4	(0.10)	46	(1.15) a* b*
Pea Green											
25 mg/ml	40	1	(0.03)	40	(1.00)	0	(0.00)	1	(0.03)	41	(1.03) a* b*
50 mg/ml	40	1	(0.03)	57	(1.43)	0	(0.00)	1	(0.03)	58	(1.45) a* b*
75 mg/ml	40	3	(0.08)	59	(1.48)	0	(0.00)	4	(0.10)	62	(1.55) a* b*

No: Number of mutant clones, Fr: Frequency; X^2: In evaluation with Chi-Square test; a: with Distilled water, b: with EMS, *: $P<0.001$

Table 2.SMART results with *mwh/TM3* wings obtained with the food colorings and positive and negative control groups tested.

Application groups/ Concentration	Number of wing	Small single spots (1-2 cell)		Large single spots (>2 cells)		Twin spots		Total mwh spots		Total spots	
		No	Fr	No	Fr	No	Fr	No	Fr	No	Fr
Distilled water	40	1	(0.03)	6	(0.13)	0	(0.00)	1	(0.03)	7	(0.18)
1 mM EMS	40	22	(0.55)	66	(3.25)	0	(0.00)	71	(1.78)	88	(2.2) a*
Ponceau 4R											
25 mg/ml	40	1	(0.03)	24	(0.93)	0	(0.00)	2	(0.05)	25	(0.63) a* b*
50 mg/ml	40	0	(0.00)	30	(1.03)	0	(0.00)	1	(0.03)	30	(0.75) a* b*
75 mg/ml	40	3	(0.08)	44	(1.23)	0	(0.00)	3	(0.08)	47	(1.18) a* b*
Tartrazin											
25 mg/ml	40	1	(0.03)	18	(1.00)	0	(0.00)	2	(0.05)	16	(0.48) a* b*
50 mg/ml	40	0	(0.00)	26	(1.10)	0	(0.00)	0	(0.00)	26	(0.65) a* b*
75 mg/ml	40	4	(0.10)	40	(1.05)	0	(0.00)	6	(0.15)	44	(1.10) a* b*
Pea Green											
25 mg/ml	40	4	(0.10)	32	(1.00)	0	(0.00)	4	(0.10)	36	(0.90) a* b*
50 mg/ml	40	1	(0.03)	31	(1.43)	0	(0.00)	1	(0.03)	32	(0.80) a* b*
75 mg/ml	40	5	(0.13)	38	(1.48)	0	(0.00)	5	(0.13)	43	(1.08) a* b*

No: Number of mutant clones, Fr: Frequency; X^2: In evaluation with Chi-Square test; a: with Distilled water, b: with EMS, *: P<0.001

Figure 1 illustrates the study data with the types of mutant wing spots and total mutant spots in the Ponceau 4R at 25‰, 50‰, and 75‰ concentrations in experimental groups and in the positive and negative control groups in the normal wings of the *Drosophila* flies.

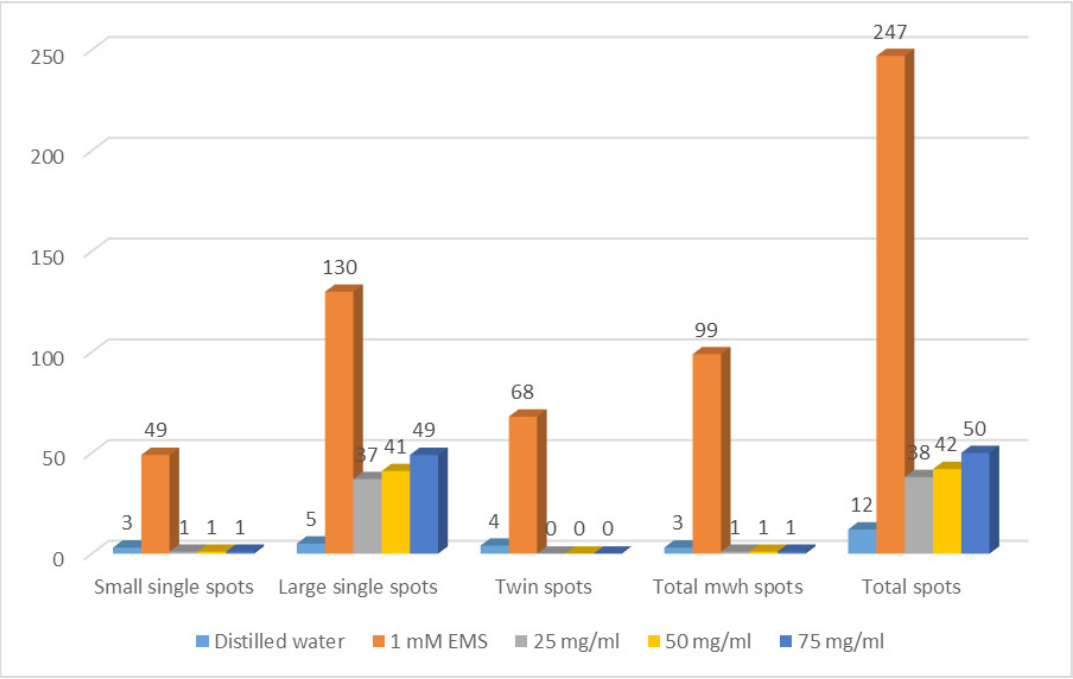

Figure 1. Classification of mutant clones with Normal wings obtained with Ponceau 4R

Figure 2 illustrates the study results with the types of mutant wing spots and total mutant spots in the Ponceau 4R experimental groups in 25‰, 50‰, and 75‰ concentrations and in the positive and negative control groups in the serrate wings of the *Drosophila* flies.

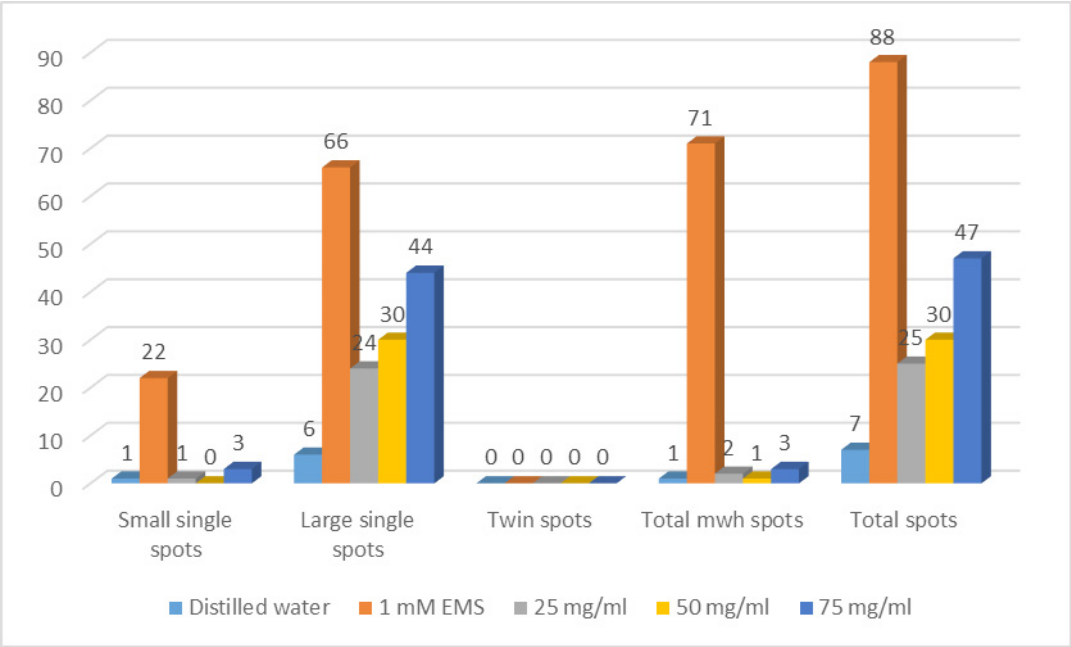

Figure 2. Classification of mutant clones with Serrate wings obtained with Ponceau 4R

The types of mutant wing spots and total mutant spots in the normal wings (in Figure 3) and serrate wings (in Figure 4) of the *Drosophila* flies in the experimental group with 25‰, 50‰ and 75‰ concentrations of Tartrazine and those of the flies in the positive and negative control groups were observed.

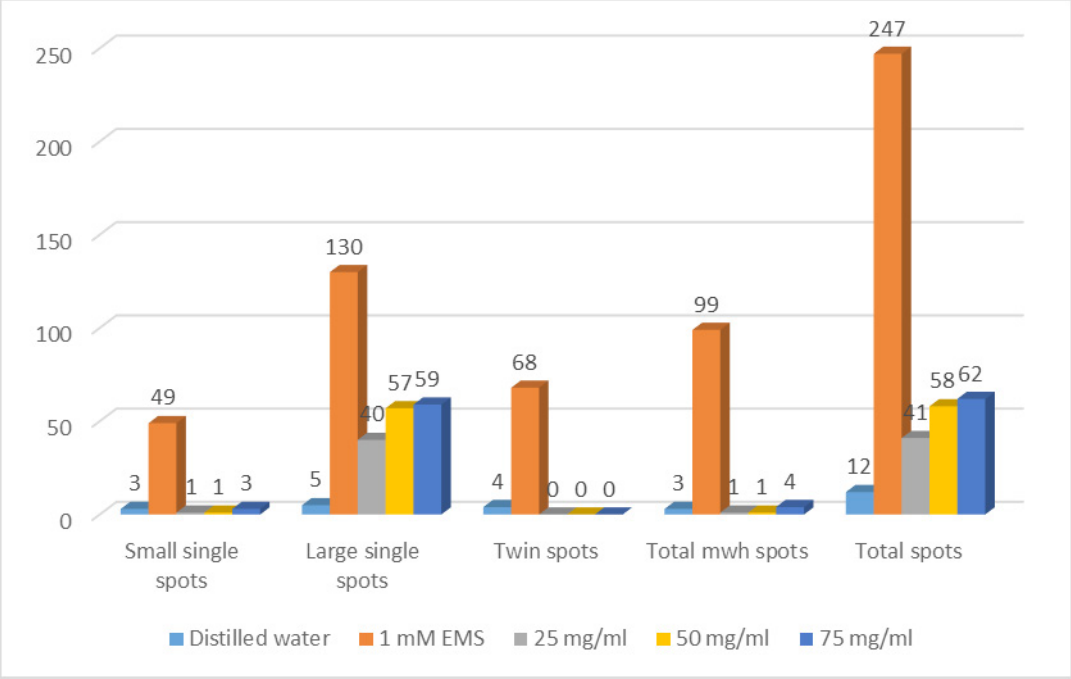

Figure 3. Classification of mutant clones with normal wings obtained with Tartrazine

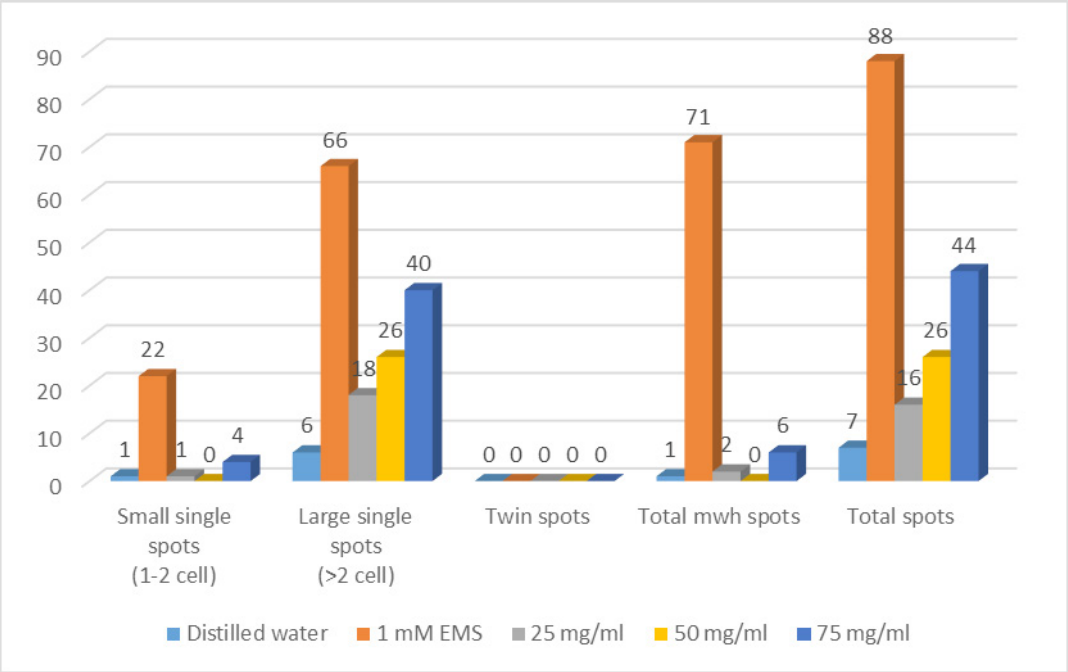

Figure 4. Classification of mutant clones with serrate wings obtained with Tartrazine

Figure 5 shows SMART results with the types of mutant spots and total mutant spots in normal wings of the flies in the Pea green experimental group at 25‰, 50‰, and 75‰ concentrations with those in the positive and negative control groups.

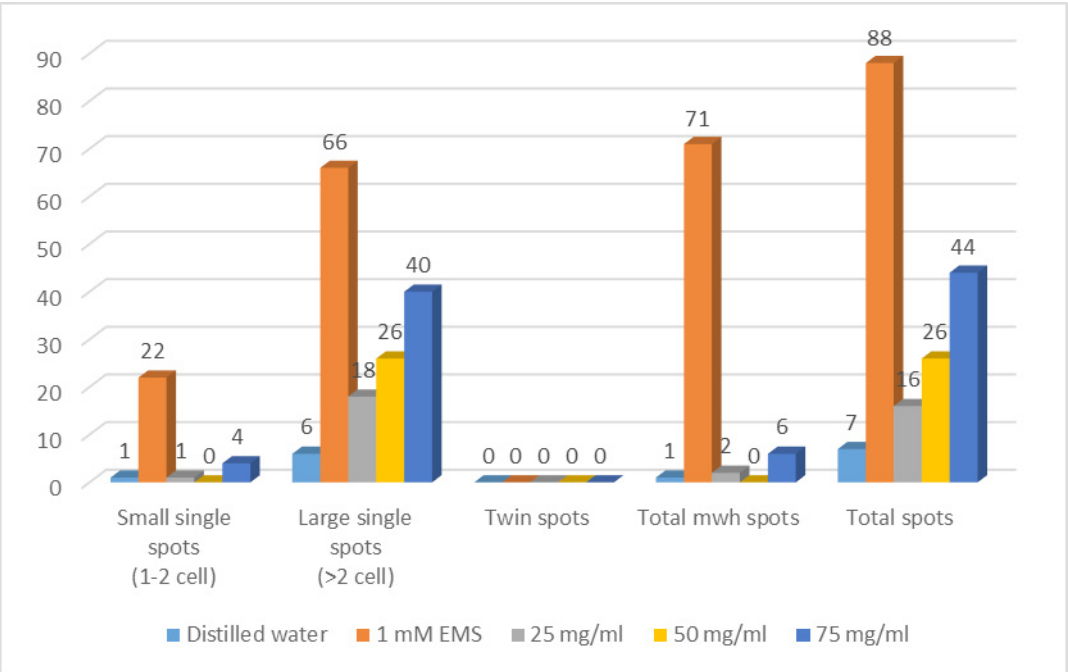

Figure 5. Classification of mutant clones with normal wings obtained with Pea Green

Figure 6 illustrates SMART data with the types of mutant spots and total mutant spots in serrate wings of fruit flies in the Pea green experimental group at 25‰, 50‰, and 75‰ concentrations with those in the positive and negative control groups.

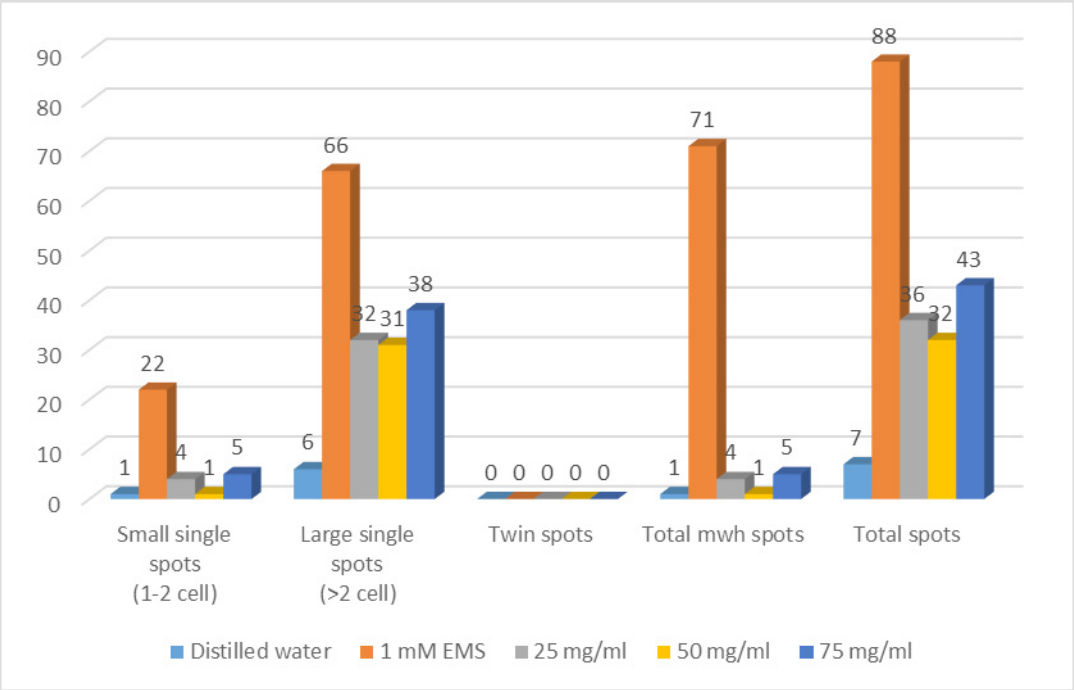

Figure 6. Classification of mutant clones with serrate wings obtained with Pea Green

Discussion

In addition to the nutritional values of consumed food, its safety and effects on human health are nowadays widely discussed (Alkan & Anlas, 2015). With the new technologies used in the food industry and the increasing variety of products, the interest in ready-made foods is increasing day-by-day with consumer demand (Akbulut, 2011). Food additives are frequently found in ready-to-eat foods and are among the potentially important genotoxic agents (Vural, 2005; Alkan & Anlas, 2015).

SMART plays an important role in the present study for examining the synthetic food colorings that cause genotoxic damage on the model organism, *D. melanogaster* Meigen. When the present study data are evaluated using SMART, it becomes clear that Ponceau 4R has a mutagenic effect in normal and serrate wings of *Drosophila* flies in studied concentrations. This finding clearly indicates that the food coloring agent has a genotoxic effect in the imaginal disc cells that will transform into the wing spot cells during the embryonic development of *Drosophila* heterozygous larvae and the genotypic changes caused by mutation or recombination in somatic cells also play a role in the formation of mutant spots in the wings. Serrate wings of the fruit flies contain only clones resulting from mutation, while mutant clones resulting from both mutation and recombination can be seen in normal wings (Kaya et al., 1999; Zordan et al., 1994). The mutant spots induced in *Drosophila* flies with Ponceau 4R are a result of the genotoxic activity of this genotoxic agent depend on its concentration. The data are similar in genotoxicity to the following studies on Ponceau 4R. Uysal & Semerdöken (2011) showed increased toxicity in *Drosophila* larvae at 72±4 hours due to the concentration increase of Ponceau 4R. Semerdoken (2012) reported that it showed an increase in mortality rate of Oregon R wild and Vestigial mutant strains of *Drosophila* larvae (72±4 hours) as the application concentration of Ponceau 4R rise. Turkoglu et al. (2015) published that the longevity of *Drosophila melanogaster* decreased significantly, depending on the different concentrations of Ponceau 4R. Uysal et al. (2017) notified that maximum mean life of Oregon R wild of *D. melanogaster* larvae at 72±4 hours decreased in different concentrations of Ponceau 4R depend on feeding. The researches data are compatible and supports the results of this study.

As a result of Tartrazine application in the experimental groups, it was clearly seen that this synthetic food coloring has a mutagenic effect. It is clearly understood that Tartrazine is effective as a mutagen in the development of wing spot cells during the embryonic development of *Drosophila* larvae and plays a role in increasing the number of mutant spots in the wings. Tartrazine got almost the same results like those of Ponceau 4R. The data obtained show, when exposure concentrations of Tartrazine are applied to the fruit flies, its mutagenic effect is so strong that the numbers of mutant spots in the wings of the flies increases. The data obtained in the present study, which clearly show the genotoxicity of Tartrazine, are supported by the following researches on the genotoxic, histopathological, and carcinogenic effects of this food coloring reported in the literature. Tripathy et al. (1989) reported that Tartrazine had genotoxicity in *Drosophila melanogaster* Meigen at 0.6‰ and 1.2‰ concentrations using the eye mosaic test and the wing spot test, whereas Poul et al. (2009) using micronucleus test revealed that Tartrazine was cytotoxic and not genotoxic in mice. Sasaki et al. (2002) determined that Tartrazine caused DNA damage in the stomach, colon, and bladder of mice depend on the dose, Mpountoukas et al. (2010) published that Tartrazine was cytotoxic for human lymphocyte cells at 1 and

2 mM concentrations, and Paterson & Butler (1982) concluded that Tartrazine applied to Muntiacus muntjac cells between mammalian fibroblast cells at 5-20 µg/ml concentrations caused chromosomal disorders. The researches on the harmful effects of Tartrazine, confirm the results of this study.

Results obtained from Pea green experimental group were very similar to experimental data of Ponceau 4R and Tartrazine. When Pea green applications were evaluated using SMART in normal and serrate wings of *Drosophila* flies, it was clearly seen that this coloring agent has a mutagenic effect. It was understood that Pea green has genotoxic effects in the imaginal disc cells that will develop the wing spot cells during embryonic development of the *Drosophila* heterozygous larvae and the genotypic changes caused by mutation and/or recombination in the somatic cells also play role in increasing number of mutant spots in wings. The present study result is consistent with the researches below, where the toxic effects of Pea green (Tartrazine-E 102 and Brilliant Blue FCF-E 133 combination) are examined. Uysal & Semerdöken (2011) reported increased toxicity in Oregon R of *Drosophila* larvae (72±4 hours) depending on the increase in concentration of Pea green, whereas Semerdoken (2012) issued that Tartrazine increased the mortality rate in Oregon R wild and Vestigial mutant strains of *Drosophila* larvae at 72±4 hours and Uysal et al. (2017) reported that, according to larval mortality and life span, Tartrazine had the highest toxicity in Oregon R wild of *D. melanogaster* larvae at 72±4 hours among other food dyes, Turkoglu et al. (2015) notified that Brilliant Blue FCF caused the biggest decreased in life span of *Drosophila melanogaster* among other food colorants and Kumar et al. (2019) revealed that on exposure to Brilliant Blue, larvae and pre-adult stages were prone to developmental toxicity.

Conclusion

It is thought that the present study with the synthetic food colorings can contribute to various scientific researches on toxicological, histopathological, carcinogenic, and teratogenic effects in/on various experimental organisms and may be a source for similar experiments will be done later. The results obtained from the present study are noteworthy because SMART can be applied in vivo (Graf et al., 1984; Graf & Wurgler, 1996) and down-organized eukaryotic *D. melanogaster* Meigen can be used as a model organism to investigate the genotoxic effects of different chemicals. In addition, the biological properties of the imaginal disc cells in *Drosophila* larvae undergo proliferation and differentiation to form body parts in the adult stage are similar to many cancer-sensitive mammalian cells, and more than 60% of genes identified in human genetic diseases are common with the genes in *Drosophila* genome (Bernards & Hariharan, 2001). These expressions increase the value of the results of the present study even more. As shown in similar studies on food additives, the disuse of the food colorings in the present study in accordance with the regulations may cause toxic effects on people. For this reason, the food additives should be consumed in a more controlled manner in terms of public health. When considering the effects of the synthetic food colorings used in the study, public health should be tried to be protected by changing the nutrition habits. Future studies on these food colorings will be of great importance for the continuity of healthy lives in the coming years.

Compliance with Ethical Standards

Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

Not applicable.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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Application of the Space Syntax method in accessibility studies, Antalya city case

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Abstract

Accessibility is an expression of the space and the quality of the city. Ensuring the free circulation of people with disabilities in urban areas is related to the development of community standards and the accessibility of transportation systems. Therefore, the physical environment and transportation systems should be arranged as accessible. The life quality of disabled people is related to the environment in which they carry out their daily activities. New methods can be used to ensure accessibility along with the developing technological opportunities and fast and accurate solutions can be obtained. Therefore, scientific studies as in all other fields should be open to new methods. It is essential to adapt to this process for occupations in the field of physical planning such as urban design, architecture and landscape architecture. The space syntax method specializes in the study and design of urban development, in particular, the design of pedestrian connections and public spaces. The method makes direct observations on pedestrian and vehicle movements and uses computer programs designed to predict how the new recommendations will have effects on such issues. The space syntax works to define the complex physical structures of cities and provides objective and consistent results using mathematical methods in which the spatial system is represented. In this study, the current situation of street accessibility in Antalya city has been examined by using the space syntax method. As a result, it was revealed that 2 of the 31 axes found in Antalya city center had very low accessibility scores, 17 of them had several problems on accessibility, but accessibility could be achieved with various improvements, and 12 axes had high accessibility scores. At the end of the study new proposals have been revealed to the problem areas.

Keywords: Space Syntax, Antalya city, Accessibility, Barrier-free design

Introduction

Human is a social entity that communicates with the environment and creates their social lives with interactions. People are required to interact with their environment to maintain their social existence. One of the most important socialization opportunities in society is the environment. Public spaces, parks, squares, and shopping centers in the neighborhood where individuals live are places that allow them to communicate with the community.

In societies, it is possible to talk about the diversity of individual depending on gender, age, ethnic origin or racial differences. In this diversity, it is possible to identify disabled people as a social category (Öter, 2018).

Failure to provide appropriate services to disabled people in society is an indicator of the failure of the social organization. One of the basic ways to cope with the negativity disabled people face is to make them visible in social life (Burcu, 2011). The most important detail in this context is making urban spaces accessible to disabled individuals.

Accessibility is an expression of the quality of the space

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and the city. The source of the problem that disabled people experience about accessibility is not their obstacles, social barriers. Consequently, in order to eliminate these problems, the main components that constituent cultural and physical barriers must be regulated. In this process, in addition to point solutions, it is important to obtain a wider perspective and head for technology use.

The most important component in the creation of accessibility is the streets. In this study; the current status of street accessibility in the whole city has been examined by using the Space Syntax method. To determine the usage potential of the pedestrian paths within the research area, the software named Axwoman 6.3, which is installed in the Arc GIS 10.0 program, was used. Accessible axes were identified in the city of Antalya, field studies were carried out and new proposals were introduced at the end of the study.

Materials and methods

In this study, the accessible axes of the Antalya City were defined by the Space Syntax method using through the master plan (13.01.2014 / 36).

Space Syntax is a research method developed by Bill Hillier and Julienne Hanson (1984) and it is based on human movements and perception. The method is used to recognize the cities and internal structures of the buildings (Şıkoğlu and Arslan, 2015). The Space Syntax method is one of the most influential scientific movements in the fields of architecture and urban design as a set of techniques used to study the spatial textures of buildings and cities and as a chain of theories combining space and society (Hillier and Hanson, 1998). The method tries to reveal the interrelationships between the unknown characteristics of the city and the observed functions. These functions are also associated with land use patterns, social-economic performance and crime rates. Space Syntax treats spatial configuration as an independent variable. Studies show that Space Syntax analysis is informative about movement patterns. As the model explained the current situation, it also tests the possible effects of different suggestions on the movement patterns in the design process. New design alternatives are used to investigate, evaluate and predict results (Hillier and Hanson, 1984).

The spatial analysis begins with a re-representation of the urban pattern. Spatial analysis is a method for understanding the social logic of urban fabric, in other words, for reading the potential of physical space to bring people together depending on the movement within (Çil, 2008). This analysis aims to create the hierarchy of the streets in the settlement, from the most used areas to the least used space. The streets that pass through the most are integrated, while the lesser ones are called segregated (Şıkoğlu and Arslan, 2015).

The Space Syntax specializes in the study and design of urban development, in particular, the design of pedestrian connections and public spaces. The method makes direct observations on pedestrian and vehicle movements and uses computer programs designed to predict how the new recommendations will have effects on such issues. The Space Syntax works to define the complex physical structures of cities and provides objective and consistent results using mathematical methods in which the spatial system is represented (Özer, 2006).

According to the method, people entering a settlement walk through the linear axes where the breaks are at least, and they move their movements according to their viewing distances during this walking distance and try to match these lines in mixed spaces. As a result, there is a chain of safe movements and spaces created by people. The points of view, vistas and building features between mixed spaces are in parallel with the movement pattern in urban space (Özbek, 2018). In the method, the size of the settlement is expressed as the number of lines. The axial map of the open space structure of a settlement consists of the least number of straight lines passing through each convex space and forms axial connections. The continuity of the axles with the addition of other axles is important (Özyılmaz, 2009). Several studies on various cities with different backgrounds have shown that the Space Syntax Theory, and its analytical methods, is an efficient way to analyze urban areas in terms of accessibility (Alkamali et all., 2017).

The methods employed in this study sought to compare the degrees of accessibility through the evaluated streets qualitatively and quantitatively. The qualitative analysis was examined with the site studies by measurements and taking photos.

Axial Maps are the primary Space Syntax method to analyze the street network in terms of its integration and accessibility (Kubat et all., 2012). Axial lines correspond to the longest and fewest line extensions possible crossing one or several spaces. A set of these intersecting lines would form an axial map. The axial map is used then to calculate the integration values of each line which measure the degree of depth, or shallowness, of the spatial configuration under study (Al Sayed, 2014).

The axial map is the basis of spatial analysis in settlements (Hillier and Hanson, 1984). The axial line integration analysis says something fundamental about the spatial integration of public green and open spaces. Integrated spaces will, according to the theory of natural movement (Hillier et all., 1993), play a more central role in the urbanity. These spaces will not only be more frequently visited and used, but they will also probably get better known because they are located in more legible places and at the same time within the people's daily movement patterns (Stahle, 2005).

In the next stage of the study, an observation form, designed for this study, consisting of 11 criteria was formed to observe the pedestrian paths which have the highest integration value. Criterias used in observation form defined as; Infrastructure, Pavements, Ramps, Pedestrian way, Distinction borders, Lighting height, Height of crown base, Tree continuity, Tactile paving, Frequency of benches, Social facilities. The form is designed to have a range of 0 to 3 points on various factors. In the study, as improper 0 points, bad 1 point, average 2 points, and excellent option 3 points were determined. Accordingly, the maximum value of the path can be 33, whereas the minimum value is 0. These total values were divided into 3 and were classified as inadequate between 0-11, average between 12-22 and sufficient between 23-33.

As a result of the classification, axles with a value between 0-11 are represented in red, 12-22 in orange, and 23-33 in yellow. Thus, the walkability of accessible roads in the city has been demonstrated.

At the last stage, roads which are suitable for accessibility were specified and new suggestions were made for inappropriate pedestrian paths.

Findings

As a result of the Space Syntax analysis, the integration value map that identifies the accessible axes of the city is shown in Figure 1.

Figure 1. Integration analysis of streets

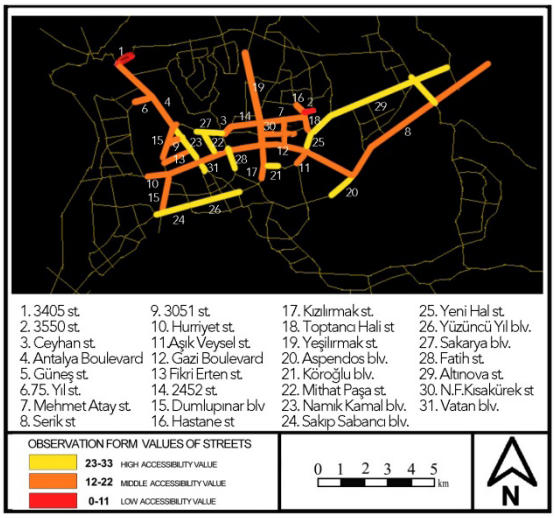

Figure 2. Observation form values of streets

Results and Discussion

In the results of working; The space syntax method is thought to be more effective in detecting areas with a high density of use. Therefore, it is thought to be more appropriate to use this method in the site selection stage to create new green areas. It is foreseen that prioritization of the streets with high integration value will result in faster social integration in the community.

It is a fact that the participation of all individuals with or without a disability to social integration is important to social life. It is necessary to create a barrier-free design for all individuals instead of specially designed areas. For this reason, it is necessary to organize the streets in the city as barrier-free.

As a result of the study, it was observed that there was no distinction between vehicle and pedestrian route in all 31 areas except a small part of Dumlupınar Boulevard. This distinction is essential for visually impaired people. It is stated that a distinction should be made between pedestrian and vehicle roads and if this distinction is to be made with plants, it should be made from non-toxic, thornless and soft textured plants (Yilmaz et al., 2013).

During the study, the positive and negative aspects of space syntax method were observed. To increase the accuracy of the method, it was realized that the master plan should be carefully drawn to the computer and the drawing should be repeated several times. The reason for this is that the breaking point of the drawn axles is effective in the analysis. The axial map can contain some differences since the quality of axes depends on the person who draws the axles (Kaya, 2015). In this study, especially where the pedestrian paths are broken, it was observed that the points where the axle lines end and start again are effective. For this reason, it is emphasized that the repeating of the drawing with a second person or that the person repeats the same drawing will increase the accuracy of the method.

Conclusion

This study primarily aims to show the advantages of using space syntax method in accessibility studies and to show that the process can progress quickly and accurately. The study also revealed that the space syntax method could be used in determining the areas with high pedestrian potential before design and planning applications.

Compliance with Ethical Standards

Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

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Effects of shoot tip colchicine applications on some grape cultivars

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Abstract

Polyploidization can provide changes in vital features such as growth, development, environmental stress tolerance in plants. Colchicine is one of the most commonly used chemicals as a polyploidization agent. In this study, 2-year-old 'Ekşi Kara', 'Gök Üzüm' and 'Trakya İlkeren' (2x, *Vitis vinifera* L.) saplings grown on their own roots were used. When the enforced shoots reached about 15 cm length, colchicine applied (0, 2.5 g L^{-1}, 5 g L^{-1}, 7.5 g L^{-1}) 24 and 48 hours to the lateral shoot tips. The effects of treatments were evaluated by shoot tip viability, stoma size and density, chloroplast counts, and flow cytometry (FC) analysis, and 'Kyoho' (4x) were used as the control. The maximum stomatal variations were determined in Ekşi Kara cultivar at 2.5 g L^{-1} 24-h application. Based on morphological differences, FC analysis was performed only in 'Ekşi Kara' but there was no genomic duplication. Since the morphological differences were not sufficient in the diagnosis of polyploid in grape cultivars, FC analysis should be performed to achieve confirmed results.

Keywords: Grapevine, Cultivar development, Breeding, Chemical mutagen, Autotetraploidy

Introduction

Grape (*Vitis vinifera* L.) is one of the most important fruit species grown globally as producing table grape, wine, raisin and fruit juice. Approximately 36% of the world production, and 56.1% in Turkey's grape production is used as table grape (OIV, 2019). New grape cultivars are needed to ensure high adaptation to changing environmental conditions for sustainable viticulture and to meet market demands.

Polyploidization, is an important tool employed to create new genetic resources in many plant species, to shorten the time needed for breeding and to obtain properties that cannot be achieved through hybridization (Yue et al., 2017). Mutation, in plants can be stimulated with many chemicals and physical mutagens, colchicine is the most commonly used chemical mutagen for this purpose. This antimitotic agent promotes polyploidy in the cells by blocking the mitosis in the metaphase stage (Planchais et al., 2000). In previous studies reported that there is increasing fruit quality and developing the stress tolerance in polyploid grapes (Notsuka et al., 2000; Park et al., 2004; Yamada and Sato, 2016). This method has been used in grape breeding since 1937 and interest has been increasing in recent years (Olmo, 1937). Polyploid grape cultivars are used commercially and cv. Kyoho (4x) constitutes 44% of the total vineyard area in China, which ranks the first in the world grape production (Olmo, 1937).

'Ekşi Kara' and 'Gök Üzüm' are ancient and autochthonous grape cultivars grown extensively in Central Taurus Region (Kara et al., 2017a). In order to meet the pollen needs of 'Ekşi Kara' (Kara et al., 2017b), and to increase its marketability without loss of adaptation ability to the area where traditional cultivar is grown, its fruit quality characteristics must be improved (Kara et al., 2017b). Previous studies conducted on chromosome doubling differ in terms of application doses, durations, tissue types, and application methods for polyploidy

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stimulation (Değirmenci-Karataş et al., 2010; Dhooghe et al., 2011; Kuliev, 2011; Ma et al., 2014; Kara et al., 2018). Studies are needed to determine the proper colchicine application methods with different cultivars, and to obtain cultivars with increased ploidy levels.

In this study, the effects of different doses and durations of colchicine applications on shoot tips were tested in order to provide genomic duplication in 'Ekşi Kara', 'Gök Üzüm' and 'Trakya İlkeren' grape cultivars *in vivo* conditions.

Materials and Methods

Plant Material

In this study, Ekşi Kara and Gök Üzüm autochthonous cultivars which were obtained from Selçuk University Faculty of Agriculture Department of Horticulture, and cv. Trakya İlkeren obtained from Tekirdağ Viticulture Research Institute, were used. 'Ekşi Kara' and 'Gök Üzüm' are well-adapted and the most intensely grown cultivars in Konya-Karaman provinces and Central Taurus Region. Since 'Ekşi Kara' has functional female flowers, 'Gök Üzüm' is used as a pollinator. Both cultivars are used as table and as raisin locally (Kara et al., 2017a). 'Trakya İlkeren' is preferred in early ecology in terms of its early yield, it can also be successful in short-vegetation areas (Köse and Ateş, 2017; Gülcü et al., 2020). Plants obtained from cuttings were used in study. Thermotherapy was applied for 30 minutes to the cuttings that would be used in the study at 50°C before rooting (Waite and Morton, 2007). After thermotherapy, cuttings were rooted in the mixture (peat: perlite 2:1 v:v) in the greenhouse. Rooting plants were planted in pots that containing a 2: 1 peat-perlite mixture. The chloroplast counts in stoma guard cells of plants were compared to 'Kyoho' (4x) (Yamada and Sato, 2016).

Chemical Mutagen Colchicine Applications

Colchicine (Sigma-Aldrich) applications were made to stimulate the polyploidy, which is effective even in low doses (0.5 mM< dose) in plants (Allum et al., 2007). In the present study, 1% dimethyl sulfoxide (DMSO) dose was used as the solvent for colchicine (Yang et al., 2006). 2-year-old potted plants grown on their own roots in greenhouse conditions, pinching was applied in early active growth period, when the shoots reached at 15 cm length. Lateral shoots tips were exposed to colchicine, 3 different doses (2.5 g L^{-1}, 5 g L^{-1}, 7.5 g L^{-1}) and 2 different time which 24 [3 times in 24 hours, (morning, noon and evening)]-48 hours [3 times in every 24 hours, (morning, noon and evening)]. After 24 hours from the first application, shoot tips were washed with sterile water. Control plants underwent washing only with a sterile water.

Determination of plant growth and ploidy level after colchicine applications

Survival rates of shoot tips (%)

Two weeks after the applications, the number of surviving shoot tips was determined by proportioning the number of alive shoot tips to all shoots of treated plants (%) (Kara et al., 2018).

Stoma Length (μm), Stoma Width (μm) and Stoma Density (stoma mm^{-2}) Observations

Two mounts after the treatments, the leaf epidermal traces of the plants that underwent the application were examined at the abaxial side of the fourth leaf from the end on the developing shoots after the application was carried out. The lower epidermis was removed by pasting with transparent nail polish from three different areas, and placed on the slide to determine the width and length of the stoma with a ×400 microscope (Moghbel et al., 2015).

Chloroplast Count (pcs $stoma^{-1}$)

Two mounts after the treatments, the changes in chloroplast counts were examined in stoma guard cells in all treated plants survived shoots. In the leaves that were taken for the stoma sample, the colour of the leaf sections was decolorized with Carnoy's Solution (3-part ethyl alcohol: 1-part glacial acetic acid v/v). The leaf sections that were taken out of the solution were kept in sterile water for 2-5 minutes, and then stained with 1% I-KI for 30 seconds. A total of 30 stoma chloroplast counts were performed in each sample. Chloroplast numbers were detected with ×400 microscope (Yuan et al., 2009), and were compared to diploid parents and tetraploid 'Kyoho'.

Flow Cytometry (FC) Analysis

Fresh leaf samples (3-4 weeks) were taken to a petri dish of 0.5 cm^2 for each application, 500 µL isolation buffer (Partec-Nuclei Buffer Extraction) was added, and the leaf texture was divided into small pieces with razor blades. The samples in the petri dish were shaken for 10-15 seconds, filtered with Partec-CellTrics 30 µm- green filter into the tube (Partec-Sample Tubes, 3.5 ml, 55x12 mm). A total of 1600 µL staining solution [Partec-DAPI (4.6 diamidino-2-phenylinole) Staining Buffer] was added to the tubes and was kept for 5 minutes in a medium isolated from light. Then the samples were analysed with the FC device. Samples were compared based on peak channels formed by diploid parents and tetraploid (4x) control in the FC device (Pazuki et al., 2018).

Statistical Analysis

The experiment was conducted in completely randomized design, with 3 repetitions, and with 10 shoot tips per repeat. The effects of the applications dose and duration interaction were compared in the JMP 13.0 Statistical Program with the Tukey test at $p<0.05$ significance level (Yue et al., 2017).

Results and Discussion

Survival Rates of Shoot Tips (%)

The shoot tip viability rates were varied according to the interaction of the cultivar, dose and duration. The colchicine doses and application times tested in this study affected the survival rates of the shoot tips in varying degrees according to the cultivars. The minimum shoot viability rates in 'Ekşi Kara' (83.67%) as a result of the toxic effect was recorded in 2.5 g L^{-1} 24-h, while in the control all of them were alive. The lowest shoot tip viability rates in 'Trakya İlkeren' and 'Gök Üzüm' were detected in 2.5 g L^{-1} 48-h (86.22%) and 2.5 g L^{-1} 24-h (84.89%) applications, respectively. It was observed that in 'Gök Üzüm' shoot tip viability rates were higher than other cultivars (Table 1).

Since the microtubules are in different tubule compounds in explant sources like shoot tips, the sensitivity levels of explant sources to chemical mutagens might vary. Sekiguchi et al. (1971) indicated that shoots did not grow as a result of the

Table 1. Effects of applications on survival rates of shoot tips (%)*

Time		Ekşi Kara	Gök Üzüm	Trakya İlkeren
24 h.	Control	100.00±0.00	100.00±0.00	100.00±0.00
	2.5 g L^{-1}	83.67±1.53	84.89±2.70	87.62±2.27
	5 g L^{-1}	87.72±1.55	95.03±2.27	88.48±1.34
	7.5 g L^{-1}	84.94±2.84	93.86±1.97	86.27±2.23
48 h.	2.5 g L^{-1}	84.45±0.57	92.88±2.83	86.22±3.36
	5 g L^{-1}	83.69±3.49	90.40±1.20	89.15±1.82
	7.5 g L^{-1}	86.00±3.61	92.85±1.90	89.84±2.64

*Colchicine applications and time interactions are non-significant at $p<0.05$

damage to the shoot tip area due to applications with mutagenic effects in some species. Also, it was reported that the shoot tips dyed at varying rates in antimitotic applications made to different types of tissues in rootstocks and grape cultivars due to the toxic effect of the chemical used (He et al., 2016; Kara et al., 2018). The findings from the present study are similar to these results.

Stoma Length (*µm*), Stoma Width (*µm*) and Stoma Density (stoma mm^{-2}) Results

The effects of the applications varied according to the cultivars, and the increases in stoma lengths were determined. Applications of 2.5 g L^{-1} and 7.5 g L^{-1} doses in 'Ekşi Kara' for 24 and 48 h caused elongation in stoma length compared to the controls (19.73 µm), while 5 g L^{-1} application caused decreases (19.10 µm). Similarly, in 'Trakya İlkeren' the 2.5 g L^{-1} and 7.5 g L^{-1} for 24-h and 48-h applications increased the stoma length. The longest stoma was recorded in the 2.5 g L^{-1} 24-h (26.23 µm) application in 'Gök Üzüm'. In the 'Trakya İlkeren' stoma length was increased in the 7.5 g L^{-1} 24-h (24.17 µm) application.

The stoma widths significantly ($p<0.05$) affected by colchicine applications, varied according to the cultivars. The 7.5 g L^{-1} colchicine for 48-h application in 'Ekşi Kara' and 'Trakya İlkeren' increased stoma width as 17.16 µm, 16.56 µm, respectively. In 'Gök Üzüm', the highest width was achieved in the 2.5 g L^{-1} 24-h (17.61 µm) (Figure 2).

The effects of colchicine dose and time of applications combinations in all grape genotypes in terms of stoma count per unit area were statistically important ($p<0.05$). Stoma densities were decreased depending on colchicine applications in all cultivars. The lowest stoma density values were determined in 'Ekşi Kara' the 5 g L^{-1} 24-h (297.10 stoma mm^{-2}), in 'Trakya İlkeren' the 7.5 g L^{-1} 48-h (408.11 stoma mm^{-2}) and in 'Gök Üzüm' with 5 g L^{-1} 48-h (433.91 stoma mm^{-2}) applications (Figure 3).

Stoma data, ensures approximate identification of genome size for the autopolyploid stimulated genotypes (Yang et al., 2006). The increase in cell size causes depending on the response of the species and cultivars, increasing occur in the shoot diameter, pollen, leaf and stoma sizes in tetraploid plants (Motosugi et al., 2002; Sattler et al., 2016). As a result of the increase in stoma size, decreases are detected in stoma count per unit area ($mm^{2)}$ (Ma et al., 2014; Xie et al., 2015). According to the findings in present study, stoma data can be used for pre-evaluation of the ploidy detection, the stoma data obtained outside the full genome folding might vary, and be affected by environmental conditions.

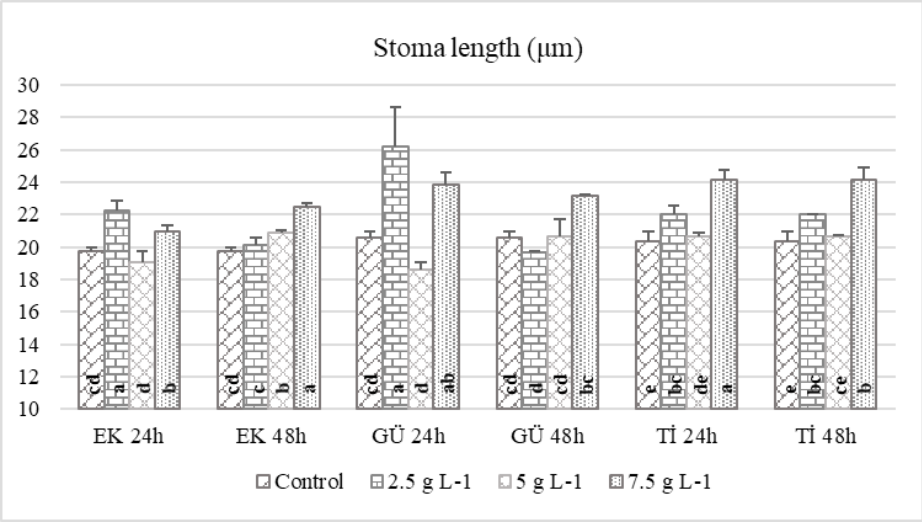

Figure 1. Effects of applications on stomata length (µm) (EK: Ekşi Kara, GÜ: Gök Üzüm, Tİ: Trakya İlkeren)

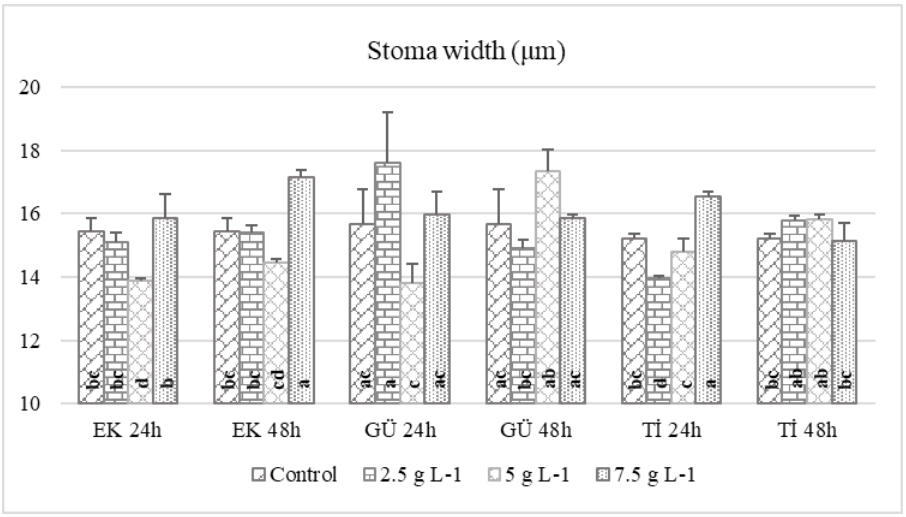

Figure 2. Effects of applications on stoma width (µm) (EK: Ekşi Kara, GÜ: Gök Üzüm, Tİ: Trakya İlkeren)

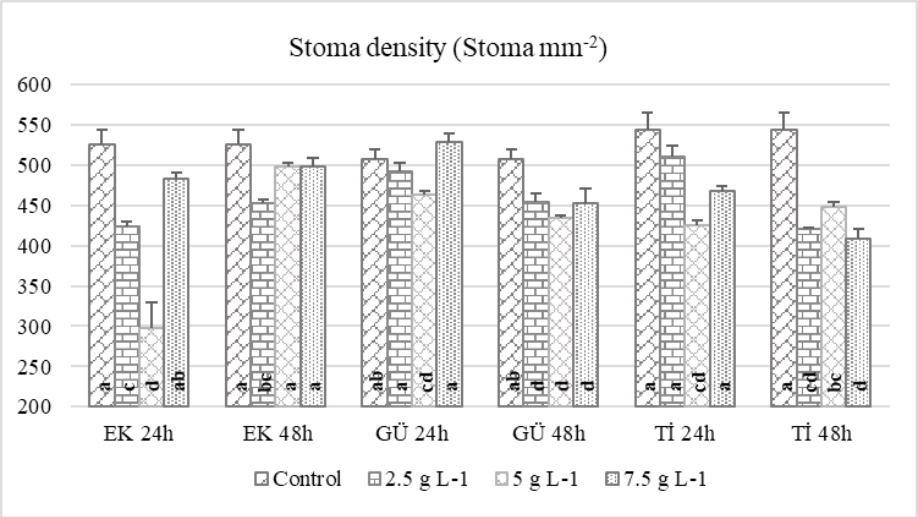

Figure 3. Effects of applications on stomata density (stoma mm^{-2}) (EK: Ekşi Kara, GÜ: Gök Üzüm, Tİ: Trakya İlkeren)

Chloroplast Count (pcs stoma^{-1}) Results

Chloroplast counts of stoma guard cells differed in 'Ekşi Kara' which were colchicine applied, and in control 'Kyoho'. The range of chloroplast count was between 18-28 in mutagen applied grape cultivars, and 38-40 in tetraploid 'Kyoho' (Table 2). The chloroplast numbers of the colchicine applied samples in Trakya İlkeren and 'Gök Üzüm' were similar to those of the controls (18-20); however, that was increased in 'Ekşi Kara' a dose-dependent, and the maximum value was 24.92 in the 2.5 g L^{-1} 24-h application.

The previous studies were reported that there is an association between the chloroplast counts and ploidy levels in stoma guard cells (Chen et al., 2009). Xie et al. (2015) indicated that chloroplast counts were made easier and earlier in stoma guard cells compared to the chromosome counts and FC analysis. In the present study, chloroplast counts increased compared to the original diploids; however, its frequency remained low comparted to the tetraploid control 'Kyoho'.

Flow Cytometry (FC) Analysis Results

Based on the numerical differences occurring in chloroplast counts, FC analysis was performed on plants whose ploidy levels were estimated to be different. As a result of FC analysis, it was determined that the ploidy levels of the samples examined did not change and they maintained their diploid forms (Figure 4).

In previous studies, FC analyses were used to determine the change in the ploidy levels of plants. In our study, FC analysis results were similar to the literature (Yang et al., 2006; Dhooghe et al., 2011; Acanda et al., 2013; Acanda et al., 2015).

Table 2. Effects of applications on chloroplast number (stoma mm^{-2})*

		Ekşi Kara		Gök Üzüm		Trakya İlkeren	
	Number of stoma	Average	Range	Average	Range	Average	Range
Kyoho	30	38.50±0.50 a	38-40	38.33±0.58 a	38-40	38.75±1.09 a	38-40
Control	30	19.73±0.28 d	18-20	20.05±0.23 cd	18-22	20.53±0.42 b	18-22
2.5 g L^{-1} 24h	30	24.92±1.01 b	18-28	19.71±0.25 d	18-22	20.45±0.08 b	18-22
2.5 g L^{-1} 48h	30	20.42±0.05 de	18-22	20.88±0.39 bc	18-22	20.25±0.12 b	18-22
5 g L^{-1} 24h	30	21.34±0.15 d	18-22	21.76±0.48 b	18-22	20.91±0.21 b	18-22
5 g L^{-1} 48h	30	20.26±0.09 de	18-22	20.06±0.26 cd	18-22	20.29±0.15 b	18-22
7.5 g L^{-1} 24h	30	23.11±0.12 c	18-26	20.03±0.20 cd	18-22	20.20±0.00 b	18-22
7.5 g L^{-1} 48h	30	19.58±0.04 d	18-20	20.83±0.15 bc	18-22	19.84±0.01 b	18-22

*Mean separation within columns by Tukey multiple test at, 0.05 level

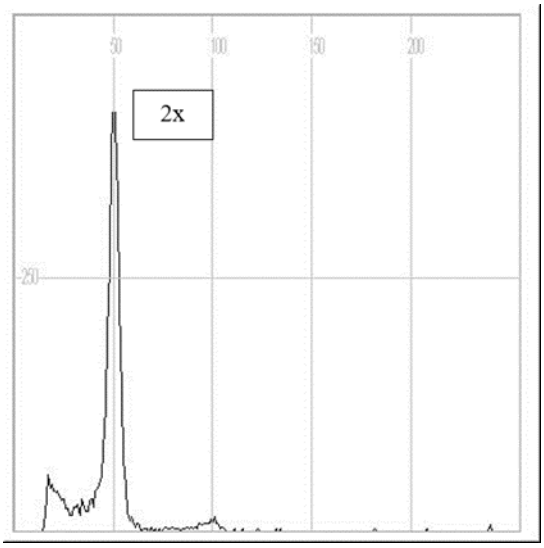

Figure 4. FC analysis result of 'Ekşi Kara' 2.5 g L^{-1} 24-h application (diploid, 2n = 2x)

Conclusion

The polyploidy breeding method is used in the breeding of economically important plants since its provides potentially beneficial results. Although differences were detected in the morphological and stoma sizes of the grape cultivar that underwent colchicine applications, it was determined with the chloroplast counts and FC analyses in stoma guard cells that these changes did not cause differences at the genome level. It was observed in the study that the reactions to *in vivo* applications varied on cultivar basis, and that the colchicine applications had limited effects on the development of grape cultivars that had domestic and regional importance. It is considered that future studies should focus on different cultivars and tissue types, dose and duration combinations.

Compliance with Ethical Standards

Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

Not applicable.

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Data availability

Not applicable.

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Interaction between culture filtrates of *Fusarium culmorum* isolates and some Root lesion nematodes

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Abstract

This study was conducted with root lesion nematodes *Pratylenchus penetrans*, *P. thornei* and *P. neglectus* and three isolates of *Fusarium culmorum* culture filtrates (ISP, B4, FC5) *in vitro* conditions. The culture filtrates were diluted 1/0,1/10, 1/20, 1/30, 1/40, 1/50, 1/60, 1/70, 1/80, 1/90, 1/100 in 1.5 ml microtubes, 250 µl of root lesion nematodes adult+larvae were placed in each microtubes that containing different concentrations of culture filtrate with 50 µl of purified water together with micropipette and incubated at 25±1°C. Nematode mortality were determined after 24 h, 48 h and 72 h. As a result, The mortality effect of culture filtrate of FC5 isolates was determined to be high on three lesion nematode species. *Fusarium culmorum* culture filtrates of B4 and ISP isolates caused more deaths on *P. penetrans* than *P.thornei* and *P. neglectus.* It has been found to mortality rate increase over time *in vitro*. The lowest mortality rates were generally found at concentrations of culture filtrates of 1/100 and 1/90, while the highest mortality rates were found at concentrations of 1/0 and 1/10. No difference on *P. thornei* mortality was detected between the three isolates of *F. culmorum* culture filtrate at each diluted concentration. In the study, the antagonistic relationship between culture filtrates from *F. culmorum* isolates and root lesion nematodes were determined *in vitro* conditions.

Keywords: Antagonism, Culture filtrate, *Fusarium culmorum*, Root lesion nematodes, Interaction

Introduction

Root lesion nematodes (*Pratylenchus* spp.) and *Fusarium culmorum* (W.G. Smith) Sacc. cause significant yield losses in cereal roots. *Pratylenchus neglectus* and *P. thornei* caused significantly wheat yield losses economically in the world (Smiley and Nicol, 2009). *Pratylenchus* spp. have a migratory endoparasitic feeding behavior (Yeates et al., 1993) and cause brown lesions on the plant roots and loss of root function, and consequently, reduce in plant vigor and yield (Jones and Fosu-Nyarko, 2014). Also, root lesion nematodes assist the invasion of soilborne pathogens into plant root tissue and, this interaction increases the importance for such infections (Smiley & Nicol, 2009). In addition, *Fusarium graminearum* (*Gibberella zeae*) and *F. culmorum* are widespread soilborne fungi in cereals root and crown rot diseases, and decrease the yield (Miedaner et al., 2008; Poole et al., 2012). *Fusarium culmorum* has a highly competitive saprophytic capability and as a facultative parasite, it is able to cause foot and root rot (FRR) and Fusarium head blight (FHB) in wheat and barley (Scherm et al., 2013). Root lesion nematode and *F. culmorum* were reported by researchers in wheat in regions of Turkey (Yavuzaslanoğlu et al., 2012, 2020; Yumurtacı et al., 2017; Dolar et al., 2019).

Many experiments have shown that a biological interaction between nematodes and soil-borne fungi are great importance in agriculture (Bhagawathi et al., 2000; Mallaiah et al., 2014).

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While some researchers state nematodes are necessary in interactions for the formation and development of fungal pathogens, Most researchers report that nematodes generally support the pathogenicity due to the changes they make in the host plant as nutrition and migration (Mauza and Webster, 1992; Bowers et al., 1996; Back et al., 2002; LaMondia, 2003; Hoseini et al., 2010; Mallaiah et al., 2014). However, there are studies that report antagonistic relationships between nematodes and fungi (Sankaralingam and McGawley, 1994; El-Borai et al., 2002a, b; Poornima et al., 2007). While the plant is damaged in the synergistic interaction between nematode and fungus, the antagonistic interaction reflects positively on the plant (Back et al., 2002). Taheri et al. (1994) reported that in the presence of *P. neglectus* in wheat, the lesion scale values of *F. oxysporum*, *F. equiseti* and *F. acuminatum* in the roots increased, but the nematode density was lower than the initial inoculation density.

It is not known whether the compounds produced by nematode or fungi are involved in natural interactions between fungi and plant parasitic nematodes. However, many compounds secreted by *Fusarium* species have antagonistic properties in most alive (Eriksen, 1998; Bankole and Adebanjo, 2003; Turkington et al., 2014). *Fusarium* species, which secrete 4,15-diacetylnivalenol and 4,15 diacetoxyscirpenol (DON) compounds that inhibit protein synthesis and activation of defense genes, have toxic effects against plant parasitic nematodes (Rotter et al., 1996; Nitao et al., 2001). Some *Fusarium* species, when grown in laboratory conditions, release toxic compounds against plant parasitic nematodes, and these compounds effect egg laying, viability and mobilization larvae (Nitao et al., 1999;2001). Production of toxic metabolites of endophyte *Fusarium* in the plant not only cause nematode paralysis, but can also inhibit host search and the infection processes (Sikora et al., 2003; Athman et al.,2006). Non-pathogenic *Fusarium* Fo162 reduces invasion of *Meloidogyne incognita* and induces systemic resistance against *M. incognita* by altering the chemical composition of tomato root exudates (Dababat and Sikora 2007a; b). Some *Fusarium* isolates induced systemic resistance in banana against burrowing nematodes *Radopholus similis* (Vu et al., 2006).

Culture filtrates from fungal cultures and their active compounds have the potential to be applied as new nematicides in the control against plant parasitic nematodes. The nematicide DiTera ® (Valent BioSciences Corporation, Libertyville,IL, USA) consist of fungus *Myrothecium* culture filtrates which was originally isolated from *Heterodera glycines* Ichinohe (soybean cyst nematode,SCN) (Meyer et al., 2004). Various non-endophytic *Fusarium* isolates have been shown to produce filtrates toxic to plant-parasitic nematodes *in vitro* (Athman et al., 2006). *Fusarium solani* culture filtrate is composed of long chain alkanes and shows toxic effect on *M. incognita* (Mani et al., 1986). Several commercial mycotoxins produced by *Fusarium* species have been tested against *M. javanica* and have been found to have nematicidal activity even at low concentrations (Ciancio, 1995). *Fusarium* secondary metabolites are thought to be used control of *R. similis* (Dubois and Coyne, 2011). Göze Özdemir et al. (2018) reported that *F. culmorum* spore suspension was able to suppress root lesion nematodes at low levels *in vitro* conditions and this situation changed depending on the isolates.

The objectives of the current study were to screen *F. culmorum* isolates of antagonistic to Root Lesion Nematode species *(Pratylenchus thornei, P. neglectus, P. penetrans*) and to determine the effects of culture filtrate concentrations on mortality of Root Lesion Nematode species *in vitro* bioassay varied from 1/0-1/100 % dilution ratio.

Materials and Methods

Materials

This study was conducted with *Pratylenchus penetrans*, *P. thornei* and *P. neglectus* root lesion nematodes and three isolates of *Fusarium culmorum* culture filtrates. The root lesion nematodes and *F. culmorum* isolates used in the study are identified in a previous study that conducted in Turkey (Söğüt and Devran, 2011; Arıcı 2006; Arıcı et al., 2013).

Preparation of Root Lesion Nematode

Pure, sterile cultures of *Pratylenchus penetrans*, *P. thornei,* and *P. neglectus* were maintained on carrot disks (Zuckerman et al., 1985). Carrot cultures transferred to 12 cm diameter petri dishes and cut into pieces. Sterile purified water is placed on it and steeps for 4-6 hours for nematodes to pass into the water. Then, the nematodes were passed through a 38 and 20 µm sieve and taken to a centrifuge tube. The larvae+adult density counted under a light microscope and taken into tubes in order to use in the experiments.

Preparation of Culture Filtrates

Fungal culture filtrates were obtained from *Fusarium culmorum* isolates B4 (Adana), ISP (ISP) and FC5 (Ankara). Fifty mL of PDB (Potato Dextrose Broth) media was placed in a 250 mL flask and sterilized for 20 minutes at 121 ° C. Seven agar discs (8 mm in diameter) from each fungal isolate were placed in PDB medium and incubated for 21 days at ± 25°C in the laboratory and shaken by hand every day. The fungal suspension was then vacuum filtered with a sterilized paper filter (Whatmann 3MM) and aspirator, and fungal micelles and spores were removed. The pH of the culture filtrates was adjusted to 5.8. Then the culture filtrates were passed through 0.22 µm milipore filters (Badea et al., 1997; Arıcı, 2006).

Antagonism of Diluted Culture Filtrates

The culture filtrates obtained from *Fusarium culmorum* ISP, B4 and FC5 isolates were diluted 1/0, 1/10, 1/20, 1/30, 1/40, 1/50, 1/60, 1/70, 1/80, 1/90, 1/100 in 1.5 ml microtubes, average 250 µL of root lesion nematode adult+larvae were placed in each microtubes that containing different concentrations of culture filtrate with 50 µl of purified water together with micropipette and incubated at 25±1°C. Total volume in each microtubes was 1100 µL. Three replicate microtubes were included for each concentration. Experiments for each isolate and 3 root lesions nematode were established separately. Pure water was used in the control. All experiments were conducted in a completely randomized design with 3 replications. Nematode mortality was determined after 24 h, 48h and 72 hours. The evaluation was made on mortality rate that the percentage of death. Nematodes were considered

dead if they didn't move when investigated with a fine needle (Cayrol et al, 1989).

Statistical analysis

SPSS (version 20.0) program was used for the statistical analysis of the data obtained in the experiments, and analysis of variance (ANOVA) was performed to test the differences between the means. "Tukey" was used in cases where the variances were homogeneous at P≤0.05 significance level to determine the different group averages.

Results and Discussion

In the present study, the culture filtrates of *Fusarium culmorum* isolates have a mortality effect on *Pratylenchus neglectu*s, *P. thornei* and *P. penetrans*, and this effect has been found to increase over time. The effect of culture filtrates of FC5 and ISP isolates on root lesion nematodes were determined higher than B4 isolate and isolate diversity seems to be important factor in terms of their antagonism *in vitro* conditions (Table 1). The mortality rate of *Fusarium culmorum* isolates on root lesion nematodes was evaluated in terms of time. The highest mortality rate was obtained generally after 72 hours, while the lowest mortality rate was determined after 24 h (Table 1). After 24 h, mortality rates were determined to vary between 19.3-58.7% depending on the isolate and nematode species. However, it was observed that mortality rate of nematodes after 48 h were 25.7-73.9%. In the experiment conducted with B4 isolate of *F.culmorum* culture filtrate and *P. penetrans,* mortality rate was found 63.8% while *P. neglectus* and *P. thornei* mortality rates were 32.0% and 45.9%, respectively after 72 h. The culture filtrate of the B4 isolate has a higher lethal effect on *P. penetrans* . After 72 h, mortality rates of *P. neglectus, P. penetrans* and *P. thornei* were found 77.6%, 70.4% and 63.5%, respectively in the culture filtrate of FC5 isolate. There was a statistically significant difference between the mortality rate of the 24, 48 and 72 h only *P. thornei* in culture filtrate of FC5 (P≤0.05) (Table 1). Mortality rate of *P. neglectus* was found 61.4%, while *P. penetrans* and *P. thornei* mortality rates were found 84.8% and 62.4%, respectively in ISP isolate culture filtrate after 72 h. The culture filtrate of the ISP isolate has a higher mortality rate on *P. penetrans* than *P. thornei* and *P. neglectus* (Table 1). There was no statistically significant difference between the mortality rate of 48 h and 72 h in the experiment conducted with the culture filtrate of ISP isolate with *P. neglectus*.and *P. penetrans* (P≥0.05) (Table 1). In addition to difference of isolate, time, and nematode species are also important in the effect of culture filtrate on nematodes *in vitro* conditions.

Differences were found in the mortality effect of different concentrations of culture filtrates on root lesion nematodes *in vitro* conditions. Mortality was found to decrease as the culture filtrate concentrations were diluted. In the study conducted with three root lesion nematodes, the lowest mortality rates were generally detected at 1/100 and 1/90 concentrations of culture filtrates, while the highest mortality rates were found at 1/0 and 1/10 concentrations *in vitro* conditions. However, it seems that even the mortality rates at 1/100 and 1/90 concentrations are higher than control (Table 2). All concentrations of the culture filtrate of the FC5 isolate were showed higher mortality effect on *P. neglectus* than other isolates (Table 2). *Pratylenchus neglectus* mortality rate were found statistically significant between ISP, B4 and FC5 isolate at 1/80, 1/70, 1/60, 1/50, 1/40, 1/30, 1/20, 1/10 and 1/0 culture filtrate concentrations (P≤0.05) (Table 2). ISP isolate is more effective on *P. penetrans* even at low concentrations (Table 2). However, There was no statistically significant difference between *F. culmorum* ISP, B4 and FC5 isolates of mortality rate at the concentrations of 1/50, 1/40, 1/30, 1/20, 1/10 and 1/0 culture filtrate on the *P. penetrans* (P≥0.05) (Table 2). There was no difference between isolates in terms of *P. thornei* mortality rate at all concentrations (Table 2). It was determined that isolate concentrations was significant for mortality on *P. neglectus* but not significant factor on *P. thornei in vitro* conditions (Table 2).

Table 1. Effect of culture filtrates of *Fusarium culmorum* isolates on root lesion nematode

Fusarium culmorum isolate	Hours	Mortality rate (%) ±STD error of mean					
		Root Lesion Nematode					
		Pratylenchus neglectus		*Pratylenchus penetrans*		*Pratylenchus thornei*	
B4 Adana isolate	24	21.2±2.4	b*	35.3±3.6	b	21.3±2.7	b
	48	25.7±2.8	ab	41.2±3.9	b	37.4±4.2	a
	72	32.0±3.1	a	63.8±5.2	a	45.9±3.6	a
FC5 Ankara isolate	24	58.7±5.3	b	38.9±4.8	b	21.2±2.1	c
	48	70.4±5.5	ab	54.1±5.7	ab	47.6±4.7	b
	72	77.6±5.4	a	70.4±5.9	a	63.5±5.4	a
ISP Isparta isolate	24	25.6±2.6	b	34.6±3.1	b	19.3±2.1	c
	48	50.0±5.0	a	73.9±5.3	a	45.3±4.8	b
	72	61.4±5.8	a	84.8±4.7	a	62.4±5.5	a

The effect of each isolate on root lesion nematodes was evaluated within itself.

*There is no statistical difference between the mean shown with the same letter in the same column (P ≤0.05). N:36

Table 2. The effect of culture filtrate concentrations of *Fusarium culmorum* isolates on root lesion nematodes

Fusarium culmorum		Mortality rate (%)±STD error of mean		
		Root Lesion Nematode		
Concentration	Isolate	*Pratylenchus neglectus*	*Pratylenchus penetrans*	*Pratylenchus thornei*
1/100	ISP	9.8±1.3 b*	33.0±5.6 a	8.4±2.1 a
	B4	11.2±0.7 b	15.2±2.3 b	13.5±3.0 a
	FC5	25.5±3.6 a	8.2±1.7 b	12.5±2.3 a
1/90	ISP	19.3±2.9 b	44.9±9.4 a	18.8±4.5 a
	B4	13.7±1.3 b	25.3±4.6 b	15.3±3.3 a
	FC5	37.0±2.8 a	22.8±4.7 b	20.5±4.2 a
1/80	ISP	27.3±3.1 b	63.1±10.0 a	23.9±4.3 a
	B4	15.3±1.3 c	31.2±3.5 b	18.3±3.4 a
	FC5	49.7±2.4 a	29.7±4.6 b	26.2±4.4 a
1/70	ISP	34.3±3.8 b	67.4±10.0a	32.7±5.0 a
	B4	16.3±1.5 c	36.9±3.5 b	23.3±4.3 a
	FC5	69.5±6.3 a	42.4±5.2 b	34.2±4.9 a
1/60	ISP	43.2±4.9 b	71.6±9.6 a	39.1±6.3 a
	B4	19.1±2.1 c	41.6±4.7 b	27.7±4.6 a
	FC5	76.4±5.4 a	56.3±8.0 ab	41.1±6.2 a
1/50	ISP	48.6±5.8 b	75.8±9.9 a	48.1±7.7 a
	B4	23.4±1.7 c	48.3±5.1 a	35.3±4.0 a
	FC5	86.4±5.0 a	64.8±8.3 a	48.7±7.6 a
1/40	ISP	63.2±8.9 b	77.2±9.7 a	56.8±8.7 a
	B4	28.5±1.8 c	57.5±6.1 a	45.9±4.8 a
	FC5	89.3±5.3 a	70.3±7.7 a	58.4±8.5 a
1/30	ISP	66.0±9.4 b	80.1±9.4 a	63.1±9.1 a
	B4	34.7±1.7 c	68.4±6.2 a	50.9±4.2 a
	FC5	94.8±2.6 a	81.5±6.0 a	65.4±9.2 a
1/20	ISP	74.0±9.0 b	85.0±7.4 a	67.6±9.5 a
	B4	45.7±2.5 c	75.4±6.3 a	55.5±4.5 a
	FC5	96.8±1.6 a	87.1±5.2 a	69.7±9.3 a
1/10	ISP	77.8±7.8 b	85.6±7.0 a	71.6±9.6 a
	B4	51.0±2.2 c	78.8±5.3 a	60.0±4.6 a
	FC5	99.5±0.4 a	92.6±3.5 a	72.6±9.3 a
1/0	ISP	83.2±7.1 b	88.3±5.8 a	76.7±8.8 a
	B4	56.1±2.5 c	81.6±4.9 a	70.7±4.6 a
	FC5	100.0±0.0a	96.7±1.6 a	78.0±8.6 a
Control	ISP	1.7±0.2 a	0.9±0.2 a	1.4±0.2 a
	B4	1.7±0.1 a	1.2±0.1 a	2.1±0.2 a
	FC5	1.6±0.1 a	1.3±0.1 a	2.8±0.3 a

The mortality rate of each concentration on root lesion nematodes was evaluated in terms of *Fusarium culmorum* isolates.
* There is no statistical difference between the means shown with the same letter in the same column ($p < 0.05$).N:9
ISP: Isparta isolate,B4: Adana isolate, FC5: Ankara isolate

It was observed that *Fusarium culmorum* culture filtrate obtained from the B4, ISP and FC5 isolates have higher mortality rates on *Pratylenchus thornei, P. neglectus, P. penetrans* with respect to previous study conducted with spore suspension of same *F. culmorum* isolates (Göze Özdemir et al., 2018). Additionally, found that spore suspension of ISP isolate of *F. culmorum* had the highest mortality effect on root nematodes and more effective on *P. thornei* while the lowest mortality effect of isolate was FC5 (Göze Özdemir et al., 2018). However, in this study determined that culture filtrate of FC5 isolate of *F. culmorum* highest mortality rate in the three lesion nematode species. FC5 was found to be the most effective isolate on *P. neglectus* in the study conducted with both spore suspension (Göze Özdemir et al., 2018) and culture filtrate. It has been determined that the application of *F. culmorum* isolates as spore suspension or culture filtrate has significant mortality effect of root lesion nematodes. The antagonistic effect of the culture filtrate appears to be higher than the spore suspension. Also, Mani and Sethi (1984), found that culture filtrates of *F. solani* inactivated 100% *Meloidogyne incognita* juveniles only after 48 h. Non-diluted culture filtrate of *F. roseum vat. arthrosporoides* inactivatied 100% of *M. arenaria* juveniles but a 10% dilution caused no alteration in nematode activity compared to control (Cayrol and Djian, 1990). Hallmann and Sikora(1996), non-pathogenic *F. oxysporum* strain 162 metabolites reduced *M. incognita* mobility within 10 minute of exposure and 98% of juveniles were inactivated at 60 minute, 50% of the juveniles were dead after 5 hours and resulted in 100% mortality at 24 hours. Zaraeen et al. (2001) tested the effects of 10 different *F. solani* strains on *M. javanica in vitro* and under controlled conditions and reported that the strains' culture filtrates varied in terms of parasitism and larval death in the eggs and females of *M. javanica*. Athman et al. (2006) reported that *Radopholus similis* mortality rates after 24 h exposure in culture filtrates ranged from 76.4% to 100% and the length of exposure time to culture filtrates increased the percentage of paralysed nematodes. Mwaura et al. (2010) investigated the lethal effect of 5 endophytic *F. oxysporum* isolates on *P. goodeyi* and found that the percent mortality rate (62.3-72.8) after 48 hours was higher than the control (17.3-34.6%). Yan et al., (2011), reported that among the 294 isolates screened which endophytic fungi from cucumber seedlings, 23 significantly (*Fusarium* (5), *Trichoderma* (1), *Chaetomium* (1), *Acremonium* (1), *Paecilomyces* (1), and *Phyllosticta* (1)) reduced galls formed by *M. incognita* in greenhouse test. Van Dessel et al. (2011) found that 3 different *F. oxysporum* strain culture filtrates caused high mortality rates against *Helicotylenchus multicinctus*, *Radapholus similis* and *P. goodeyi* within 24 hours. In the same study, it was found that *H. multicinctus* was less sensitive than *R. similis* to culture filtrate applications, while *R. similis* was more susceptibly than *P. goodeyi*.

Conclusion

In the study, the antagonistic relationship between culture filtrates from *Fusarium culmorum* isolates and root lesion nematodes were determined *in vitro* conditions. This suggests that *F. culmorum* were able to suppress root lesion nematodes in natural conditions. The highest mortality rate in the three lesion nematode species was also determined in FC5 and ISP isolates. It is not clear whether the compounds produced by *Fusarium* play a role in the natural interactions between fungi and the plant parasitic nematodes. However, the detection of high mortality rates in the culture filtrate suggested that the antagonistic effect might be caused by the enzyme or toxins secreted by *F. culmorum* in this study.

The present study shows that interaction between *F. culmorum* and root lesion nematodes is caused by the ability of isolates from some *Fusarium* spp. to produce toxins. The maximum effect of culture filtrates were observed indicating these isolates can be used as a potential of biocontrol management of lesion nematodes. Therefore, more detailed studies are required, and in particular the determination of the culture filtration content is of great importance. The identification of nematode antagonistic compounds may be a step toward examining such interactions and activities related to antinematode effects in the future. In addition, new nematicides could be developed from active compounds obtained from fungal cultures that have a nematicidal effect on plant parasitic nematodes. As a result of the study, when studying interactions between *Fusarium culmorum* and root lesion nematodes, researchers should take into account that *Fusarium* isolates can produce lethal toxins in nematodes.

Compliance with Ethical Standards

Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

Author contribution

Fatma Gul GOZE OZDEMIR, Bulent YASAR, Serife Evrim ARICI designed the experiments. Fatma Gul GOZE OZDEMIR performed the laboratory works and analysed the data. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

Not applicable.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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filtrate of FC5 isolate and root lesion nematode at International Agriculturel Science Congress, 09-12 May 2018, Van, TURKEY and *F. culmorum* culture filtrate of ISP isolate and root lesion nematode at Agriculture for life, Life for Agriculture,7-9 June, 2018, Bucharest, Romania.

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Effect of inulin concentration on physicochemical properties and antioxidant activity of date powders obtained by hot-air tray dryer

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Abstract

In the present study, it was aimed to produce free-flowing date powders using various levels of inulin as drying-aid agent (10, 20, 30, 40, and 50%) through hot-air drying at 60°C for 24 h. Effects of different inulin ratios on physicochemical properties of date powders were investigated. This is the first report which evaluated the suitability of this prebiotic carbohydrate as drying-aid agent to fabricate date powders. Inulin addition yielded date powders with high flowability. On the other hand, contents of bioactive compounds including total phenolics, flavonoids, and condensed tannins of date powders decreased significantly ($P<0.05$) as the inulin concentration increased from 10% to 50% Accordingly, DPPH-radical inhibition capacities reduced in date powders containing higher levels of inulin. Furthermore, significant correlations were detected between bioactives contents and antioxidant activity of date samples. The results showed that free-flowing date powders with improved prebiotic content may be produced by incorporating inulin up to ratio of 50% and used as sugar substitute in different food products.

Keywords: Condensed tannin, Date palm, Drying, Flavonoid, Inulin

Introduction

Date palm (Phoenix dactylifera L.) is one of the most economically important plants grown in arid and semi-arid regions of the world (Mohamed, Fageer, Eltayeb and Mohamed Ahmed, 2014). Egypt, Saudi Arabia, Islamic Republic of Iran, Algeria, Iraq, Pakistan, Sudan, Oman, United Arab Emirates, and Tunisia are top date-producing countries (Food and Agriculture Organization of the United Nations, 2018). The fruit is composed of flesh (85–90%), and seed or pit (6–12%) along with the skin which covers fleshy part of fruit (Al-Orf et al., 2012). Carbohydrates (mainly glucose, fructose, and sucrose) constitute almost 70% of date fruit, while lower amounts of dietary fibre, minerals (potassium, calcium, magnesium, sodium, and phosphorus), protein, vitamins (B1, B3, B5, B6, and C), and phenolic compounds (chelidonic acid, ferulic acid, dicaffeoyl shikimic acid) are also present (Al-Farsi, Alasalvar, Morris, Baron and Shahidi, 2005; Al-Shahib and Marshall, 2002; Aslam, Khan and Khan, 2013; Baliga, Baliga, Kandathil, Bhat and Vayalil, 2011; Khallouki, Ricarte, Breuer and Owen, 2018; Nadeem et al., 2019; Sola Agboola and Lateef Adejumo, 2013). The moisture contents of fresh date fruits at Tamr stage (fully ripened) vary over a wide range (6.8–39.25%) depending on differences in cultivar, climate, harvesting period, and drying conditions (Hasnaoui, Elhoumaizi, Hakkou, Wathelet, and Sindic, 2010; Saafi, Trigui, Thabet, Hammami and Achour, 2008).

Date palm fruits may be processed into syrup, juice, molasses, paste, stick, jam, vinegar, and liquor, whereas dried fruits of some date cultivars can be directly ground into powder and used as sugar substitute (Ahmadnia and Sahari, 2008; Al-

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Manhal, 2004; Alsenaien et al., 2015; Amin, Abdel Fattah, El kalyoubi and El-Sharabasy, 2020; Barimah, Laryea and Okine, 2015; Dhankhar, Vashistha and Sharma, 2019; Fahloul, Abdedaim and Trystram, 2010; A Hariri, Ouis and Bouhadi 2017; Hariri, Ouis, Bouhadi and Benatouche, 2018; Mohamed, Fawzy, Mohamed, Mostafa, and Zeinab, 2016; Nadeem et al., 2017; Nwanekezi, Ekwe, and Agbugba, 2015; Senthil Kumar and Yaashikaa 2019; Tang, Shi and Aleid, 2013). Moreover, the powder is incorporated in yoghurt and different bakery products including biscuits and pancakes for fortification of products in terms of dietary fibre and antioxidants (Elsharnouby, Al-Eid and Al-Otaibi, 2017; Jrad, Oussaief, Bouhemda, Khorchani and El-Hatmi, 2019; Messaoudi and Fahloul, 2018; Sakr and Hussien, 2017; Sulieman, Masaad and Ali, 2011). However, in general, production of powder from various date cultivars by different drying techniques is challenging due to high amounts of low molecular weight sugars including glucose, fructose, and sucrose found in the fruit (Sablani, Shrestha and Bhandari, 2008). The low glass transition temperatures (Tg) of these carbohydrates lead to stickiness that prevents the conversion of fruit into free-flowing powder form (Bhandari, Datta and Howes, 1997). The most common approach to overcome this problem is the addition of carrier(s) with high molecular weight as drying-aid agent to increase the Tg of the mixture (Bhandari and Roos, 2012). In this context, maltodextrin, liquid glucose, gum arabic, tapioca starch, cashew tree gum, apple pectin, whey protein concentrate, sodium caseinate, and soy protein isolate have been used as drying-aid agent for the production of free-flowing powders from sugar-rich foods (Bazaria and Kumar, 2016; de Oliveira et al., 2009; Jayasundera, Adhikari, Howes and Aldred, 2011; Moreira et al., 2009; Muzaffar and Kumar, 2016; Papadakis, Gardeli and Tzia, 2006; Phoungchandang and Sertwasana, 2010; Sarabandi, Peighambardoust, Sadeghi Mahoonak and Samaei, 2018; Tonon, Brabet, Pallet, Brat and Hubinger, 2009; Vardin and Yasar, 2012).

Inulin is a kind of storage carbohydrate naturally occurring in chicory root, Jerusalem artichoke tubers, banana, asparagus, onion, and garlic (Huber and BeMiller, 2017). It is categorized as potent prebiotic along with fructooligosaccharides, human milk oligosaccharides, galactooligosaccharides, arabinoxylan oligosaccharides, isomaltooligosaccharides, xylooligosaccharides, pectin oligosaccharides, and pyrodextrins (Yang and Xu, 2018). Inulin cannot be hydrolysed by human digestive enzymes. Therefore, it is classified as "nondigestible oligosaccharide" (Flamm, Glinsmann, Kritchevsky, Prosky and Roberfroid, 2001). Furthermore, it reduces the digestion of digestible saccharides and decreases fasting blood sugar level (Roberfroid, 1993). Inulin has been used as drying-aid agent for powder production from Araticum (Annona crassiflora) pulp, blueberry waste extract, and coffee creamer (Botrel, Rodrigues, Souza and Fernandes, 2016; Hedayatnia and Mirhosseini, 2018; Waterhouse, Sun-Waterhouse, Su, Zhao and Zhao, 2017).

Until today, few attempts have been made to obtain date powder using maltodextrin (6, 10, 18, 19, and 20 dextrose equivalent), gum arabic, pectin, and whey protein concentrate through various drying techniques, mainly spray-drying (Dev, Annamalai, Orsat, Raghavan and Ngadi, 2018; Farahnaky, Mansoori, Majzoobi and Badii, 2016; Manickavasagan et al., 2015; Moghbeli, Jafari, Maghsoudlou and Dehnad, 2019, 2020; Sablani et al., 2008; Seerangurayar, Manickavasagan, Al-Ismaili and Al-Mulla, 2017). However, to the best of our knowledge, there is no study on evaluation of inulin as drying-aid agent to obtain free-flowing date powder. Therefore, the aim of this study is to investigate the effects of different ratios of inulin (10, 20, 30, 40, and 50%) on some physicochemical properties (moisture, ash, and colour values, water activity, bulk and tapped density, flowability, solubility, contents of total phenolics, total flavonoids, total condensed tannins, and DPPH-radical scavenging activity) of date powders obtained through hot-air drying technique.

Materials and Methods

Materials and Chemicals

Date paste (Metro Chef) was bought from Metro Cash and Carry (İzmir, Turkey). Inulin and tricalcium phosphate were obtained from Smart Kimya Tic. ve Danışmanlık Ltd. Şti. (İzmir, Turkey). Aluminium (III) chloride, Folin-Ciocalteu's reagent, quercetin, potassium acetate, and methanol were purchased from Merck (Darmstadt, Germany). Distilled water was used to dilute date paste. 2,2-diphenyl-1-picrylhydrazyl (DPPH) reagent, sodium carbonate, and gallic acid were obtained from Fluka (Buchs, Switzerland). Vanillin, 6–hydroxy–2,5,7,8–tetramethylchromane–2–carboxylic acid (Trolox), and (+)-catechin hydrate were purchased from Sigma-Aldrich (St.Louis, MO, USA).

Methods

Drying by hot-air tray dryer

100 g of date paste was diluted with 100 mL distilled water and homogenized by using a hand-blender for 2 min (Tefal Activflow Power Soup 1000 Watt, İstanbul, Turkey). Then, certain amount of inulin (10, 20, 30, 40, or 50% of diluted date paste weight) and 7% of tricalcium phosphate were added to the mixture. Afterwards, the mixture was again homogenized for 3 min. Finally, the diluted date paste was spread on rectangular silicone baking mat and allowed to dry at hot-air tray dryer operating at 60°C for 24 h. Dried product was then ground into powder using a knife-mill (Grindomix GM-200, Retsch GmbH and Co, Haan, Germany).

Moisture and ash contents, water activity (a_w)

Moisture and ash contents of date powders were determined according to official methods of AOAC (AOAC, 2019). Water activity (a_w) was measured by using a digital a_w–meter (Rotronic Hygropalm, Bassersdorf, Swiss).

Bulk and tapped densities of powders

The bulk (ρB) and tapped (ρT) densities of date powders were determined according to the method of Chinta et al. (2009) which was then modified by Quispe-Condori, Saldaña, and Temelli (2011). Briefly, 3 g (m0) of date powders was weighed in a 25-mL graduated cylinder. Then, the powder residues sticking to the wall of cylinder was collected by hitting the cylinder gently. The volume (V0) was recorded and the bulk density was calculated using Eq.1.

$$\rho B(g/mL) = \frac{m_0}{V_0} \times 100 \qquad (1)$$

For determination of tapped density, the powder was tapped until constant volume was achieved. The volume (V_n) was recorded and the tapped density was calculated using Eq.2.

$$\rho T(g/mL) = \frac{m_0}{V_n} \times 100 \qquad (2)$$

Powder flowability

The flowability of powders were evaluated using Carr's Index (CI) and Hausner Ratio (HR) (Quispe-Condori et al., 2011). The Eqs. 3-4 were used for determination of CI and HR values, respectively.

$$CI = \frac{\rho_T - \rho_B}{\rho_T} \times 100 \qquad (3)$$

$$HR = \frac{\rho_T}{\rho_B} \qquad (4)$$

Solubility of powders

Water solubility was determined according to the method of Cano-Chauca, Stringheta, Ramos and Cal-Vidal (2005). 1 g of date powders was homogenized with 100 mL of distilled water using a hand-blender for 5 min, followed by centrifugation at 3000 rpm for 5 min. Then, 25 mL of aliquot of supernatant was taken to pre-weighed petri plate and dried at 105°C till constant weight. Solubility was determined gravimetrically and expressed as "%".

Colour of powders

Colour analysis was carried out with a bench–top colorimeter (Konica Minolta CR–5, Tokyo, Japan). CIE Lab scale was used, and the results were expressed as L^* (lightness), a^* (–a: greenness, +a: redness), and b^* (–b: blueness, +b: yellowness) values.

Total phenolic content (TPC)

Samples (2.5 g) were extracted with 50 mL of methanol/water (1:1, v/v), followed by vortexing for 1 min. After shaking of mixture using a shaking incubator (IKA Ks4000i, Staufen, Germany) at 20°C for 1 h, it was centrifuged at 4000 rpm for 20 min to obtain methanolic extract. TPCs of date paste and date powders were determined spectrophotometrically according to the method described by Singleton and Rossi (1965) and modified by Li et al. (2006). A gallic acid calibration curve (R^2 = 0.9984) was used and the results were expressed as mg gallic acid equivalent (GAE) per 100 g sample.

Total flavonoid content (TFC)

Methanolic extracts previously prepared were used for TFC determination. TFCs of date paste and date powders were estimated spectrophotometrically according to the method developed by Olumese and Oboh (2016) and modified by Aksoylu Özbek, Çelik, Günç Ergönül, and Hepçimen (2020). A quercetin calibration curve (R^2 = 0.9996) was used and the results were expressed as mg quercetin equivalent (QE) per 100 g sample.

Total condensed tannin content (CTC)

Methanolic extracts previously prepared were used for CTC estimation. CTCs of date paste and date powders were determined spectrophotometrically by using the vanillin/HCl method suggested by Broadhurst and Jones (1978). A (+)-catechin calibration curve (R^2 = 0.9996) was used and the results were expressed as mg catechin equivalent (CE) per 100 g sample.

DPPH-radical scavenging activity

Total antioxidant activities of date paste and date powders were determined using the DPPH-radical scavenging assay developed by Brand-Williams, Cuvelier, and Berset (1995) and modified by Singh, Chidambara Murthy, and Jayaprakasha (2002). A Trolox calibration curve was used (R^2 = 0.9987) and the results were expressed as millimoles Trolox equivalent (TE) per 100 g sample.

%inhibition was calculated using Eq.5.

$$\%Inhibition = \left[\frac{Abs_{control} - Abs_{sample}}{Abs_{control}}\right] \times 100 \qquad (5)$$

Statistical analysis

A completely randomized design was used in this study. Inulin ratio was the only factor. All analyses were carried out in triplicate. The results were subjected to one-way analysis of variance (ANOVA) by using General Linear Model (PROC GLM) procedure in SAS (Version 8.2, Sas Institute, Inc., Cary, NC, USA). Differences were compared by Fisher's LSD test. All statistical analyses were conducted at a significance level (alpha) of 0.05. PROC CORR procedure of SAS were used to calculate Pearson correlation coefficients.

Results and Discussion

Influence of inulin amount on physicochemical characteristics of date powders

The physicochemical properties of date powders containing different ratios of inulin were presented in Table 1. Inulin concentration had statistically significant influence on moisture contents. They varied from 1.48% to 3.65% which were in accordance with the recommended maximum moisture levels (3–4%) for food powders (Klinkesorn, Sophanodora, Chinachoti, Decker, and McClements, 2006). Moisture contents increased as the ratio of inulin increased in the formulation. This increase may be explained by the hindrance of water diffusion to the drying air due to high affinity of this carbohydrate for water (Botrel et al., 2016). Additionally, a rapid crust formation caused by inulin may also restrict the water diffusion and evaporation from date paste (Fernandes, Borges, and Botrel, 2014). A similar relationship between inulin concentration and moisture content of beetroot juice powder was previously detected by Carmo et al. (2019). Our results are comparable with the findings of Moghbeli et al. (2019) who reported moisture contents ranging from 1.48% to 3.45% for spray-dried date powders including pectin-whey protein complexes. Similar moisture contents were also

reported by Raza et al. (2019) for maltodextrin/gum arabic mixture-containing date powders obtained through oven-drying at 60°C (1.55–2.59%) and spray-drying (1.47–2.55%). On the other side, our findings are lower than those of Botrel et al. (2016) (5.06–6.78%) who investigated the utilization of different ratios of inulin (20% or 30%) to produce araticum (*Annona crassiflora*) pulp powder using cabinet convective air dryer at 70, 80 or 90°C.

Table 1. Physicochemical properties of date palm fruit powders containing different ratios of inulin

Characteristic	10% IN	20% IN	30% IN	40% IN	50% IN
Moisture, g/100g	1.48 ± 0.11^{e}	3.23 ± 0.02^{d}	3.40 ± 0.01^{c}	3.50 ± 0.05^{b}	3.65 ± 0.01^{a}
Ash, g/100g (dw)	13.99 ± 0.04^{a}	11.80 ± 0.01^{b}	10.25 ± 0.03^{c}	8.89 ± 0.08^{d}	8.09 ± 0.02^{e}
Water activity	0.16 ± 0.01^{d}	0.21 ± 0.001^{c}	0.24 ± 0.01^{b}	0.25 ± 0.01^{b}	0.27 ± 0.01^{a}
Bulk density, g/mL	0.76 ± 0.01^{a}	0.77 ± 0.01^{a}	0.79 ± 0.01^{a}	0.77 ± 0.01^{a}	0.73 ± 0.03^{a}
Tapped density, g/mL	0.87 ± 0.01^{a}	0.89 ± 0.01^{a}	0.91 ± 0.01^{a}	0.88 ± 0.01^{a}	0.83 ± 0.04^{a}
CI	12.50 ± 0.01^{a}	12.50 ± 0.01^{a}	12.50 ± 0.01^{a}	12.50 ± 0.01^{a}	11.81 ± 0.69^{a}
HR	1.14 ± 0.01^{a}	1.14 ± 0.01^{a}	1.14 ± 0.01^{a}	1.14 ± 0.01^{a}	1.14 ± 0.01^{a}
Solubility in water, %	12.53 ± 0.20^{a}	11.68 ± 1.07^{a}	11.29 ± 0.47^{a}	11.65 ± 0.39^{a}	12.62 ± 0.15^{a}
*L**	57 ± 1.05^{d}	61.04 ± 0.65^{c}	63.49 ± 0.86^{b}	67.44 ± 0.49^{a}	67.55 ± 0.26^{a}
*a**	9.23 ± 0.40^{a}	8.15 ± 0.16^{b}	7.34 ± 0.14^{c}	6.32 ± 0.12^{d}	6.19 ± 0.04^{d}
*b**	22.25 ± 0.44^{a}	21.91 ± 0.23^{a}	21.42 ± 0.15^{b}	20.30 ± 0.09^{c}	19.28 ± 0.06^{d}

CI: Carr's Index, HR: Hausner Ratio, IN: inulin

Mean values within in a row with different superscripts are significantly different ($P<0.05$).

Inulin-containing date powders had ash contents of between 8.09% and 13.09% (Table 1). Comparatively low ash contents in date powders (4.73–5.76%) with maltodextrin/gum arabic mixture were declared by Raza et al. (2019). This remarkable difference may be the result of various drying-aid agent concentrations used in these studies and different accumulation of minerals in fruits due to cultivars, climate, growing location, and cultivation practices (organic/conventional) (Boussaa et al., 2020; Kapoulas, Milenković and Mirecki, 2013; Marzouk and Kassem 2011). The amount of inulin increased, the ash contents of date powders significantly decreased in our study. As pure inulin used in our study did not include components comprising ash content, this finding is not surprising. A similar observation was previously reported by Caliskan and Dirim (2013) and Caliskan and Dirim (2016) who examined the effects of different ratios of maltodextrin on the properties of sumac extract powder obtained through spray-drying or freeze-drying.

As shown in Table 1, a_w was significantly influenced by inulin concentration. The a_w values of date powders increased from 0.16 to 0.27 as the ratio of inulin increased from 10% to 50%. In general, a_w values between 0.2 and 0.3 correspond to a suitable moisture content region which ensures maximum shelf-life in dehydrated foods (Labuza and Altunakar, 2007). Similar a_w values were reported for spray-dried date (0.23–0.28), watermelon (0.20–0.29), açai (0.19–0.25), and pitaya powders (0.30–0.35) accompanied with maltodextrin with different dextrose equivalents, gum arabic or tapioca starch as drying-aid agent (Manickavasagan et al., 2015; Quek, Chok and Swedlund, 2007; Tonon et al., 2009; Tze et al., 2012).

The bulk and tapped density values of the date powders ranged between 0.73–0.79 g/mL and 0.83–0.91 g/mL, respectively (Table 1). The inulin concentration did not affect nor bulk density neither tapped density values significantly. Bulk density of the powders with inulin were higher than foam-mat freeze dried date powders containing different ratios of maltodextrin (0.60–0.68 g/cm3) and gum arabic (0.56–0.70 g/cm3) and spray-dried instant date powders with maltodextrin (0.41–0.51 g/cm3) (Nortuy, Suthapakti and Utama-ang, 2018; Seerangurayar et al., 2017). Comparable bulk density values (0.66–0.79 g/cm3) were reported for oven dried maltodextrin-containing date powders (Sablani et al., 2008). The density values of the powders considerably increased (13.70–15.58%) after tapping. This increase may lead to poor flowability (Onwulata, Konstance and Holsinger, 1996). Inulin-containing date powders had higher tapped density values than spray-dried instant date powders (0.54–0.71 g/cm3), while were comparable to foam-mat freeze dried date powders (0.80–0.90 g/cm3 for maltodextrin, and 0.75–0.87 g/cm3 for gum arabic-added powders) (Nortuy et al., 2018; Seerangurayar et al., 2017).

The flowability of any food powder is an essential parameter for various processes including storage, transportation, formulation and mixing, compression and packaging (Teunou, Fitzpatrick and Synnott, 1999). The flowability and cohesiveness of the powders were evaluated through CI and

HR values, respectively. Incorporation level of inulin did not have statistically significant effect on CI and HR. As shown in Table 1, except date powder including 50% inulin (11.81), all powders had a CI value of 12.50. On the other hand, all powders had the same HR value (1.14). Based on CI and HR values, the flowability of powders can be classified as excellent (CI≤10, HR: 1.00–1.11), good (CI: 11–15, HR: 1.12–1.18), fair (CI: 16–20, HR: 1.19–1.25), passable (CI: 21–25, HR: 1.26–1.34), poor (CI: 26–31, HR: 1.35–1.45), very poor (CI: 32–37, HR: 1.46–1.59), and awful (CI>38, HR: >1.60) (Quispe-Condori et al., 2011). Our results showed that inulin-containing date powders had good flowing properties. Inulin as a drying-aid agent yielded free-flowing date powders by increasing Tg and preventing bridging (Juliano and Barbosa-Cánovas, 2010). However, instant date powders containing maltodextrin exhibited poor flowability (CI:20.37–36.06, HR: 1.26–1.57) (Nortuy et al., 2018), while Seerangurayar et al. (2017) reported good flowability for 50% gum arabic containing date powders and fair flowability for freeze dried date powders with 40% or 50% maltodextrin, or 40% gum arabic and intermediate cohesiveness for all samples. In conclusion, inulin enables to produce fruit powders with improved flowability and therefore, it may be suggested as a promising alternative to common drying aids such as maltodextrin and gum arabic.

Solubility values of date powders ranged from 11.29% to 12.62% (Table 1) and inulin ratio did not significantly affected this characteristic. In other studies, highly soluble (66–88.60%) date powders including maltodextrin or gum arabic and maltodextrin/gum arabic mixture were produced through spray-drying or oven-drying techniques (Manickavasagan et al., 2015; Raza et al., 2019). Date powders obtained in the current study had considerably lower water solubility than spray-dried guava (85.87–95.43%), cast-tape- or spray-dried mango powders (77.22–79.17%) including maltodextrin as drying-aid agent (Patil, Chauhan and Singh, 2014; Zotarelli, Durigon, da Silva, Hubinger and Laurindo, 2020). Poor solubility of date powders may be attributed to drying technique (hot-air tray drying) used in our study which leads to a dense structure. This structure reduces the access of water through powder (Caliskan and Dirim, 2016). Furthermore, the poorly water soluble nature of drying-aid agent (inulin) at room temperature and high amounts of insoluble dietary fibres and non-starch polysaccharides as well as other lipophilic substances such as carotenoids found in date palm fruit may also contribute to the limited water solubility of date powders (Elleuch et al., 2008; Kha, Nguyen and Roach, 2010; Kim, Faqih and Wang, 2001; Ozdikicierler, Dirim and Pazir, 2014; Vinita and Punia, 2016).

The colour results summarized in Table 1 show that inulin addition led to increase in L* and decrease in a* and b* values of date powders. These changes were found to be statistically significant. White colour of powdered inulin increased the lightness (L*) of date powders. On the other side, inulin concentration-dependent decreases observed in a* and b* values may be attributed to the dilution of date palm fruit and underestimated perception of date palm fruit pigments, mainly carotenoids as previously stated by Chong and Wong (2017). Similarly, (Michalska-Ciechanowska, Majerska, Brzezowska, Wojdyło and Figiel, 2020) reported increases in L* and decreases in a* and b* values of freeze- or spray-dried cranberry powders depending on increasing inulin levels (15, 25, and 35%). Except a* values, our results (L* and b*) were in accordance with the findings of (Sablani et al., 2008) who recorded higher a* values in oven-dried date powders as the proportion of maltodextrin increased in the formulation.

Influence of inulin amount on bioactives contents of date powders

Total phenolic content

Chelidonic acid, t-ferulic acid, dicaffeoyl shikimic acid derivatives (1, 2, and 3), taxifolin-3-O-rhamnoside, gallic acid, p-coumaric acid, caffeic acid, sinapic acid, syringic acid, ellagic acid, catechin, quercetrin, rutin, isoquercetrin, and quercetin were identified as the main phenolic compounds of date palm fruits (Benmeddour, Mehinagic, Meurlay and Louaileche, 2013; Nizar Chaira et al., 2009; Hamad, 2014; Khallouki et al., 2018; Mansouri, Embarek, Kokkalou and Kefalas, 2005). TPCs of fresh date paste and date powders containing different amounts of inulin were illustrated in Fig.1. The fresh date paste had a TPC of 550.85 mg GAE/100g. Mansouri et al. (2005), Biglari, AlKarkhi and Easa (2008), Chaira, Mrabet and Ferchichi (2009), Saafi, El Arem, Issaoui, Hammami and Achour (2009), Nadeem, Salim-ur-Rehman, Anjum and Bhatti (2011), Al Juhaimi, Ghafoor and Özcan (2014), Louaileche, Hammiche and Hamoudi (2015), Ali Haimoud, Allem and Merouane (2016), Matloob and Balakit (2016) and Nadeem et al. (2019) reported lower TPCs (2.49–8.36 mg GAE/100g, 2.89–4.82 mg GAE/100g (dw), 3.88–9.71 mg GAE/100g, 209.42–447.73 mg GAE/100g, 140.67–296.67 mg GAE/100g, 94–198 mg GAE/100g, 127.97–334.58 mg GAE/100g, 2.06–6.53 mg GAE/100g (dw), 147.60–475.50 mg GAE/100g, and 142.52–298.02 mg GAE/100g, respectively) in ripe and fresh date palm fruits grown in Algeria, Iran, Pakistan, Saudi Arabia, Iraq, and Tunisia. On the other side, comparable TPCs were reported for the fresh fruits of Bahraini (276–342 mg GAE/100g in Tamr stage), Mauritanian (405.50–661.10 mg GAE/100g (dw)), Iranian (298–845 mg GAE/100g (dw)), Moroccan (331.86–537.07 mg GAE/100g (dw)) date varieties (Allaith, 2008; Bouhlali et al., 2017; Mohamed Lemine et al., 2014; Sadeghi, Valizadeh and Shermeh, 2015). Previously, Al-Turki, Shahba and Stushnoff (2010) showed the effects of growing location and variety on TPC of date palm fruits. In addition to these, very different TPCs of date fruits may be explained by climate conditions, and pre/post-harvest factors that greatly influence accumulation of these secondary metabolites in plant and the polarity of solvent used for extraction of phenolic compounds (Kozłowska, Gruczyńska, Ścibisz and Rudzińska, 2016; Mnari, Harzallah, Amri, Dhaou Aguir and Hammami, 2016). A comprehensive research demonstrated that date fruit had the highest TPC (585.52 mg GAE/100g) among 62 different fruits including sweetsop (405.41 mg GAE/100g), guava (194.11 mg GAE/100g), pomegranate (146.94 mg GAE/100g), cherry (114.56 mg GAE/100g), persimmon (112.09 mg GAE/100g), plum (102.43 mg GAE/100g), and pineapple (94.04 mg GAE/100g) (Fu et al., 2011).

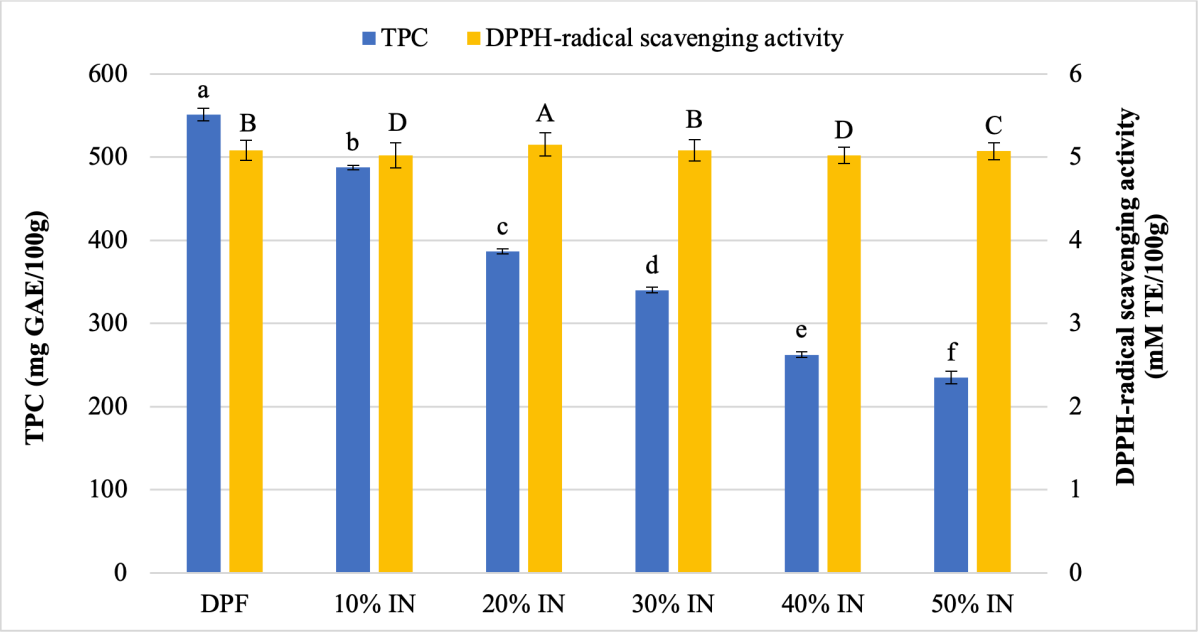

Figure 1. Total phenolics contents and DPPH-radical scavenging activities of fresh date palm paste and date powders containing different ratios of inulin. Different lowercase letters indicate statistically ($P<0.05$) significant differences between TPCs of the samples. Different uppercase letters indicate statistically ($P<0.05$) significant differences between DPPH-radical scavenging activities of the samples. DPF: date palm fruit, GAE: gallic acid equivalent, IN: inulin, TE: Trolox equivalent, TPC: total phenolic content

Increasing amount of inulin significantly reduced TPCs of date powders. TPC of date powder including 10% inulin was 487.42 mg GAE/100g, while that of date powder with 50% inulin decreased to 234.75 mg GAE/100g. Seerangurayar et al. (2017) reported that TPCs of foam-mat freeze-dried date powders were in the range of 84–173 mg GAE/100g and 85–197 mg GAE/100g including 40%–50% maltodextrin or gum arabic, respectively. Another study showed that TPCs of spray-dried date powders with whey protein-pectin complexes varied from 345 mg ascorbic acid equivalent (AAE)/100g to 701.25 mg AAE/100g. However, it should be kept in mind that different drying techniques cause changes in TPC of fruit powders in different ways. For instance, Raza et al. (2019) stated that oven-drying at 60°C for 48h yielded maltodextrin/gum arabic mixture-containing date powder with higher TPC (77.24–438.94 mg GAE/100g) than spray-drying technique (76.92–411.77 mg GAE/100g). The sensitivity of date palm fruit phenolics to high drying temperatures was also proved by Shahdadi, Mirzaei, Daraei Garmakhany, Mirzaei and Ghafori Khosroshahi (2013), Manickavasagan et al. (2015), Shahdadi, Mirzaei and Daraei Garmakhany (2015), İzli (2016) and Moghbeli et al. (2020). In accordance with our findings, Seerangurayar et al. (2017) detected significant decreases in TPCs of date powders as the concentrations of drying-aid agents increased. Higher levels of drying-aid agents lead to the dilution of raw material (date paste) in the formulation which decreases the amounts of phenolics to be extracted as previously reported by Caliskan and Dirim (2013) and Mishra, Mishra, and Mahanta (2014).

Total flavonoid content

Quercetin, luteolin, apigenin, isoquercetrin, rutin, catechin, isorhamnetin, and chrysoeriol are the flavonoids which have been identified in fresh date fruit extracts up to now by some researchers (Amira et al., 2012; Farag, Mohsen, Heinke and Wessjohann, 2014; Hamad et al., 2015). As shown in Table 2, TFC of fresh date paste was 37.59 mg QE/100g. Wide ranges of TFCs for ripe date fruits grown in Tunisia (6.28–54.46 mg QE/100g; 300–2609 mg QE/100g (dw)), Algeria (15.22–299.74 mg QE/100g (dw); 15.89–40.78 mg QE/100g), and Mauritania (39.50–112.50 mg QE/100g (dw)) were reported by several researchers (Benmeddour et al., 2013; Chaira et al., 2009; Lekbir et al., 2015; Masmoudi-Allouche et al., 2016; Mohamed Lemine et al., 2014). Our finding partially agrees with these results. Geographical, climatic and genetic factors which control accumulation of secondary metabolites as well as use of different solvents to extract bioactive compounds may lead to considerable variations in TFCs of plant materials (Kozłowska et al., 2016; Mnari et al., 2016). TFC of fresh palm fruit is higher than mango (5.30–28.10 mg QE/100g), plum (5.40–20.43 mg QE/100g), sweet (14.80 mg QE/100g) and acid (10.30 mg QE/100g) starfruits, passion fruit (12.10 mg QE/100g), pink (11 mg QE/100g) and white (20.90 mg QE/100g) guavas, pineapple (2–15.90 mg QE/100g), banana (5.60–9.90 mg QE/100g), avocado (2.10 mg QE/100g), and papaya (1.50–1.70 mg QE/100g), whereas lower than mangosteen (257.10 mg QE/100g), strawberry (207.60 mg QE/100g), red Chinese guava (71.20 mg QE/100g) and litchi (53.30 mg QE/100g) (Barcelo et al., 2016; Cosmulescu, Trandafir, Nour and Botu, 2015; Luximon-Ramma, Bahorun and Crozier, 2003; Septembre-Malaterre, Stanislas, Douraguia and Gonthier, 2016).

Table 2. Contents of total flavonoids, condensed tannins and DPPH-radical inhibition values of fresh date pulp and date powders containing different ratios of inulin.

	DPF	10% IN	20% IN	30% IN	40% IN	50%IN
TFC, mg QE/100g	37.59 ± 0.86[a]	29.91 ± 0.01[b]	25.85 ± 0.60[c]	20.49 ± 0.25[d]	17.82 ± 0.82[e]	14.21 ± 0.01[f]
CTC, mg CE/100g	101.62 ± 0.41[a]	81.18 ± 1.20[b]	76.15 ± 1.88[c]	62.06 ± 1.58[d]	47.51 ± 0.71[e]	36.23 ± 0.83[f]
DPPH-radical inhibition, %	92.03 ± 1.69[a]	89.55 ± 3.11[a]	89.55 ± 0.26[a]	81.58 ± 0.13[b]	69.75 ± 0.01[c]	61.14 ± 1.30[d]

CE: Catechin equivalent, CTC: condensed tannin content, DPF: date palm fruit, IN: inulin, QE: quercetin equivalent, TFC: total flavonoid content.
Mean values within in a row with different superscripts are significantly different (P<0.05).

TFCs of inulin-containing date powders ranged from 14.21 mg QE/100g to 29.91 mg QE/100g. Due to the reasons explained for TPCs of date powders above, the TFCs also decreased significantly as the ratio of inulin increased in the formulation. Therefore, less flavonoids were extracted from the date powders containing higher levels of inulin as a result of dilution effect. However, this result contrasts with the findings of Alcantara Marte, Alcantara Marte, Tejada and Ros Berruezo (2018) who reported higher TFCs for spray-dried lemon juice powders including increased levels of pulverized mesocarp of Citrus paradisi Macf. as a drying aid due to its ability to protect flavonoids from degradative reactions. Similarly, improved betalains and anthocyanin retention in spray-dried beetroot juice concentrate powder and Jamun pulp powder were obtained by increasing the ratio of drying-aid agents due to preservation of these bioactive compounds effectively against oxidation and/or degradation (Bazaria and Kumar, 2016; Singh, Paswan and Rai, 2019). Our finding may suggest that inulin did not protect the date flavonoids against undesirable reactions during hot air-drying process at 60°C.

Condensed tannin content

Immature date fruit is rich in soluble tannins that give astringency to the fruit. These compounds are converted to their insoluble forms which are known as "condensed tannins (procyanidin polymers)" as the fruit ripens. Therefore, mature date palm fruits are less astringent and more palatable than immature ones (Amira et al., 2012; Hussain, Farooq and Syed, 2020; Myhara, Al-Alawi, Karkalas and Taylor, 2000). Condensed tannins constitute almost 80% of TPC of date fruits at commercial maturity (Tamr stage) (Hammouda, Chérif, Trabelsi-Ayadi, Baron and Guyot, 2013). CTC of fresh date paste was 101.62 mg CE/100g in our study (Table 2). Our finding partially agrees with the CTCs of Algerian (74.22–394.27 mg CE/100g), Tunisian (41.85–102.37 mg CE/100g), and Moroccan (57.56–92.14 mg CE/100g (dw)) date fruits at Tamr stage (Arem et al., 2013; Benmeddour et al., 2013; Bouhlali et al., 2016; Souli et al., 2018). Total amount of condensed tannins in date palm fruit is lower than hawthorn (920–4110 mg CE/100g) and higher than strawberry (2.93–31.55 mg CE/100g), rosehip (2.02–2.34 mg CE/100g), blackberry (14.28–16.59 mg CE/100g), and blackthorn (9.53–10.50 mg CE/100g) (Dou, Leng, Li, Zeng and Sun, 2015; Turker, Kizilkaya, Cevik and Gonuz, 2012).

In our study, the lowest CTC (36.23 mg CE/100g) was obtained from date powder including 50% inulin, whereas 10% inulin addition yielded the date powder with the highest CTC (81.18 mg CE/100g). Increased inulin concentration significantly reduced CTCs of date powders. As explained in TPCs and TFCs of date powders, lower CTCs were determined in samples with more inulin due to dilution of raw material (date paste). Similar observations were reported for lycopene and carotenoid contents of spray or freeze-dried watermelon powders as the drying-aid agent concentration increased in the formulation (Oberoi and Sogi, 2015).

DPPH-radical scavenging activity

DPPH-radical scavenging activities of fresh date paste and date powders were illustrated in Fig.1. DPPH-radical scavenging activity of fresh date paste was 5.08 mM TE/100g, while those of hot air-dried date powders ranged between 5.02 mM TE/100g and 5.15 mM TE/100g. Unlike our study, comparatively low DPPH-radical scavenging activities were determined in fresh date palm fruits grown in U.S.A., Saudi Arabia, Iran and Mauritania (Al-Jasass, Siddiq and Sogi, 2015; Al-Turki, 2008; Al-Turki et al., 2010; Hemmateenejad, Karimi, Javidnia, Parish and Khademi, 2015; Mohamed Lemine et al., 2014). Similarly, İzli (2016) reported lower DPPH-radical scavenging activity (0.14 mM TE/100g (dw)) for methanol/water extracts of fresh date fruits sold in Turkey.

In addition to DPPH-radical scavenging capacities of the date samples in terms of Trolox equivalent, DPPH-radical inhibition ability values were also determined. As shown in Table 2, while the fresh date paste had the highest DPPH-radical inhibition ability (92.03%), date powders with lower antioxidant capacities depending on increasing levels of inulin were obtained in our study. Inulin addition more than 20% led to significant reductions in antioxidant activities of date powders compared to fresh date paste. The methanol/water (50/50, v/v) extract of fresh date sample used in the present study has higher DPPH-radical inhibition capacity than those of methanol/water (80/20, v/v) extracts of date palm fruits (69.50–72.70% in Tamr stage, and 62.50–65% in Rutab stage) grown in Sultanate of Oman, methanol/water (50/50, v/v) and acetone/water (70/30, v/v) extracts of Tunisian date palm fruits (57.54–90.12%, 83.57–88.24% in Tamr stage, respectively (Kchaou, Abbès, Blecker, Attia and Besbes, 2013; Singh, Guizani, Essa, Hakkim and Rahman, 2012). The differences observed for antioxidant activities of date palm fruits in various studies may arise from various factors such

as growing location and variety which influence accumulation of secondary metabolites that are responsible for antioxidant activity as well as the composition and polarity of extraction solvents (Al-Turki et al., 2010; Kchaou et al., 2013).

Correlations between bioactives contents and DPPH-radical inhibition ability of date samples

A correlation analysis was carried out to determine the relationships between bioactives contents including total phenolics, flavonoids and condensed tannins and DPPH-radical inhibition ability of fresh date pulp and date powders (Table 3).

Table 3. Pearson correlation coefficients between total phenolics, total flavonoids, total condensed tannins, and %DPPH-radical inhibition values of date palm samples.

	TPC	TFC	CTC	%Inhibition
TPC	1.000	0.9840 <.0001	0.9763 <.0001	0.8958 <.0001
TFC		1.000	0.9867 <.0001	0.8846 0.0001
CTC			1.000	0.9405 <.0001
%Inhibition				1.000

TPC: Total phenolic content, TFC: total flavonoid content, CTC: condensed tannin content

Extremely high positive correlation coefficients suggest that phenolics, flavonoids and condensed tannins contributed to the DPPH-radical inhibition capacity of date samples to a great extent. The positive relationships between catechin, rutin (dominant flavonoids of date palm fruits) and oxidation inhibition capacity of aqueous extracts of date palm fruits were also approved previously (Saleh, Tawfik and Abu-Tarboush, 2011). Therefore, the very high positive correlation coefficient determined for TFC/DPPH-radical inhibition capacity (r = 0.8846) in our study is not surprising. In accordance with our findings, Odeh et al. (2014) and Hemmateenejad et al. (2015) detected very strong positive correlations for TFC/antioxidant activity (r = 0.935–0.961) and TPC/antioxidant activity (r = 0.97) of Palestinian and Iranian date palm fruits, respectively. However, our findings contrast with the results of Lekbir et al. (2015) who reported very weak correlations between TFC/DPPH-radical scavenging capacity (r = 0.24) and TFC/TPC (r = 0.342) of Algerian date palm fruits. Additionally, positive relationships between TPCs, TFCs, and CTCs of date samples were also confirmed by high correlation coefficients. The great contribution of TFC to TPC of date palm fruits (r = 0.99) was also declared by Abbas, Foroogh, Liong and Azhar, (2008) and Biglari et al. (2008).

Conclusion

The results obtained in this study showed that inulin can be effectively used as a drying-aid agent to produce free-flowing date powders. Stickiness problem occuring during hot air drying of date fruit as a result of its high simple sugar content was completely eliminated by means of inulin addition. This prebiotic carbohydrate may serve as a convenient alternative to common carbohydrate-based drying-aid agents like maltodextrin, gum arabic, and pectin which have been used for fabrication of free-flowing date powders up to now. Inulin concentration (10–50%) considerably affected physicochemical properties including moisture and ash contents, water activity, and colour of date powders. On the other hand, bulk and tapped density values, solubility in water as well as flowability were not influenced by different ratios of inulin. As a general trend, bioactives contents (TPC, TFC, and CTC) of date powders decreased as the inulin amount in the formulation increased. Therefore, date powders with lower DPPH-radical scavenging activities depending on increasing ratios of inulin were obtained in our study. However, date powders including inulin still had quite high DPPH-radical inhibition capacities (61.14–89.95%). In conclusion, free-flowing date powders can be successfully produced by incorporation of this prebiotic carbohydrate (inulin) up to 50% ratio into formulation. Date powders containing inulin may be used as sugar substitute in different food products. Our further study will focus on the determination of glass transition temperatures of these powders to determine the suitable storage temperatures in order to ensure powder stability.

Compliance with Ethical Standards

Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

Author contribution

Zeynep Aksoylu Özbek carried out the data curation, investigation, formal analysis, writing-original draft. Kıvılcım Çelik contributed the investigation, formal analysis, visualization. Pelin Günç Ergönül contributed the supervision, writin-review and editing.

All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

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Research Article

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A biomonitoring study: Using the biomarkers in *Cyprinus carpio* for the evaluation of water pollution in Sapanca lake (Sakarya, Turkey)

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Abstract

This study aims to determine the toxic effects of heavy metal pollution on carp (*Cyprinus carpio*) in Sapanca Lake by biochemical and histological analyses. For this reason, fish and water samples were taken from the lake in 2015. Heavy metal (Cu, Fe, Zn, Pb, Cd) analyzes in the water column and tissues (muscle, liver, gill) were determined by ICP-OES. CAT, GSH and MDA levels, which are oxidative stress bioindicators in tissues, were measured by spectrophotometric methods. Histopathological findings in tissues were determined by Hematoxylin-Eosin staining. As a result, heavy metal concentrations in water were determined as Fe > Zn > Pb > Cu > Cd. The accumulation of Cu, Fe and Cd in the tissues of the fish were liver > gill > muscle, and the accumulation of Zn was gill > liver > muscle. CAT activity, MDA and GSH level of the tissues changed with the water temperature. General signs of destruction were observed in the gill tissues of the fish. Necrotic conditions in hepatocytes were observed. In conclusion, the presence of biochemical and histopathological findings in tissues suggests that the lake is not only affected by heavy metals but also by other pollutants.

Keywords: Heavy metals, Carp, Oxidative stress, Freshwater, Histopathology

Introduction

Due to the pollution in the air, water and soil, which are the basic elements of life, all living beings, especially humans, are damaged and negatively affected (Kahvecioglu et al., 2003; Katalay et al., 2005; Ozyürek, 2016). Increasing environmental problems cause undesirable changes in ecosystems. Industrial activities affect the air, soil and water ecosystem negatively, as well as environmental pollution in the air, not only affects the air negatively but also pollutes the water and soil ecosystems.

Turkey is surrounded by seas on three sides and it has 8333 km coastline, from more than 200 natural lakes, more than 1000 ponds, 706 reservoirs and 177 714 km long streams (Yılmaz, 2014). But when we assess Turkey in terms of the available water catchment area, it appears that Turkey is not a water-rich country. The amount of water is per capita per year in Turkey 1500 m^3. Turkey Statistical Institute (TUIK), in 2040 estimated population of 100 million will be achieved (TUIK, 2018), even in such a case the water of 1000 m^3 figures set for the issue urgently to ensure the necessary work will be important. For this reason, water pollution is one of the most important environmental problems.

Since the lakes from aquatic environments are more stagnant and open to human influence than rivers, they feel the impact of human activities more. The Sapanca Lake, which is an important drinking water resource for the provinces in the Marmara Region, is also an important water resource in terms of industrial water supply and aquaculture production. In recent years, the Sapanca Lake has suffered serious damage as a result of looting and destruction concentrated on and

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around it (Adasu, 2003; Çakır, 2010). In investigating the pollution in the lakes, it is important to evaluate the biological, physical and chemical parameters of the environment and their changes over time. Aquatic organisms that live in contaminated ecosystems and accumulate heavy metals in their tissues are used as bioindicators in biomonitor researches to determine the degree of pollution of their environment and the effects of contaminants (Chen and White, 2004; Sarkar et al ., 2006; Udroiu, 2006; Holt and Miller, 2011; Authman et al., 2015).

Previous studies on Sapanca Lake relates generally only to water quality and pollution (Altuğ and Okgerman, 2008; Altundağ et al. 2019). Unlike other studies, this research aims to determine the effects of heavy metal pollution in the water on heavy metal accumulation in carp tissues and biochemistry and histology of tissues. This study is also of great importance in determining the effects of pollution in the lake on human health through the food chain.

Material and Methods

Study Area, Test Organism and Sample Collection

The perimeter of the Sapanca Lake is 39 km, 26 km of it surround with the borders of Sakarya and 13 km of it surround by the borders of Kocaeli (Figure 1). The long axis of the lake is in the east-west direction and the short axis in the south-north direction. The average depth of the lake is 31–33 m, but its maximum depth is 61 m (Adasu, 2003; Çakır, 2010). A Study conducted in Sapanca Lake in previous years indicates that there are 32 fish species in the lake (Okgerman et al., 2006), but a recent study shows that this number has decreased to 22 (Kuş, 2012).

In this study, in which heavy metal pollution was investigated in Sapanca Lake, carp (*Cyprinus carpio*) was chosen, because of it was hunted heavily due to its economic importance and the best represents the effects of environmental pollutants due to its feeding habits (Singh et al., 2010). The fish samples were collected from Kırkpınar location between January-December 2015. The necessary permissions were obtained from The Republic of Turkey Ministry of Agriculture and Forestry General Directorate of Fisheries and Aquaculture, and Marmara University Animal Experiments Local Ethics Committee. With the help of a professional fisherman, fish samples were caught in the fishnet and brought to the laboratory, and water samples were also taken to the laboratory on ice, in brown bottles. In the laboratory, after measuring of fish's weight and height, muscle, liver and gill tissues were taken from fish samples.

Figure 1. The location of the sampling site (X) on the map of the study area, Sapanca Lake, Turkey.

Heavy metal analyses

Samples stored in the freezer were subjected to wet-burning with the nitric acid (HNO_3) in the Milestone Start D (Italy) microwave oven equipped with a temperature control program. Metal concentrations were determined by Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES; Spectro Arcos, Germany). High Purity- ICP- 200-7-5 brand ICP-OES multi-element standard was used as standard, DORM-3 was used as certified reference material for fish tissues, NW-KEJIM-02 Soft Lake Water was used as reference material for Lake Water. Heavy metal analyzes in water samples taken in brown bottles were determined with ICP-OES by adding 65% HNO_3 at the rate of 10%.

Transfer Factor (TF) analyses

The Transfer Factor (TF) is the ratio between the accumulated concentration of a given pollutant in any organ and its dissolved concentration in water. It gives an indication about the accumulation efficiency for any particular pollutant in any fish organ (Canpolat et al. 2016). TF was calculated according to Aboul Ezz and Abdel-Razek (1991) using the following equation:

$$TF = M_{tissue} / M_{water}.$$

Where; M_{tissue} is the metal concentration in fish tissue µg/kg and M_{water}, metal concentration in water µg/L.

Assessment of oxidative stress parameters

For the purpose of homogenization, the tissues taken from the freezer were thawed on ice. Tissues weighed for the purpose of homogenization of muscle, liver and gill samples were taken

into eppendorf tubes with a 10% (w/v) cooled homogenate buffer and disintegrated in the homogenizer with the help of glass beads. Samples were preserved in ice at all stages of the studies. The homogenate was centrifuged for 20 minutes at 10000 rpm at 4°C. After the centrifuge, the supernatant part was taken and the pellet part was discarded.

Lipid Peroxidation (LPO)

The LPO in the tissue samples were measured using the thiobarbituric acid reaction according to the method described by Ledwozyw et al., 1986. The absorbance was determined at 535 nm and its concentration was expressed as nmol MDA/g tissue.

Catalase Enzyme Activity (CAT)

The enzyme activity was measured following the decrease of absorbance at 240 nm due to hydrogen peroxide (H_2O_2) consumption (Aebi, 1974). The activity was expressed as U/mg tissue.

Total Glutathione (GSH)

GSH concentration was measured with an assay using the dithionitrobenzoic acid (DTNB) recycling method described by Beutler (1975). GSH concentration was expressed as nmol GSH/g tissue.

Total Protein Content

Total protein was determined according to the method of Bradford (1976). The intensity of the developed blue color was measured at 595 nm against the blank. Its concentration was expressed as µg/µL.

Histopathology analysis

The liver tissues were fixed in 10% neutral buffered formalin were dehydrated using a series of graded ethanol solutions (70–100%), cleared in xylene, embedded in paraffin and sectioned at 5 µm. The gill tissues were fixed again in Bouin's solution for 24 h for decalcification. Then the tissues were dehydrated and embedded in the paraffin wax and sectioned at 5 µm thickness and stained with Hematoxylin and Eosin (H&E) for standard histopathological evaluation. For each month, 5 secondary filaments from the inner section on 10 slides were analyzed. Slides were examined under the light microscopy.

Statistical analysis

Statistical analyzes were made using IBM SPSS Statistic 23 computer program. Study findings were expressed as mean ± standard error of mean (SEM). Comparisons between the two groups are the parametric Student's t-test and nonparametric Mann-Whitney U test in unequal variances, and comparisons between more than two groups are one-way ANOVA; in statistically significant results, Tukey's post hoc test was performed to compare the significant difference between groups following ANOVA. In all statistical comparisons, those with a significance level less than $p < 0.05$ were considered significant.

Results and Discussion

The amount of Cu, Fe, Zn, Pb and Cd metals in the water samples taken from Sapanca Lake was investigated in this study. The samples were collected between January and December 2015 (Table 1). The results of the reference material and the sample values were different. For example, Fe was found to be more than the reference in January and March, and quite low in other months. Cu showed an increasing trend throughout the year. In the first study on water pollution between 1978 and 1980 in our study area, Sapanca Lake, the water samples were taken seasonally was examined. It was emphasized that one of the drinking water resources could be lost (Sümer et al., 1996), in the following years, it is stated that the lake faces the danger of pollution (Yalçın and Sevinç 2001). In the study conducted on the streams flowing to Sapanca Lake before and after the Marmara earthquake on August 17, 1999, it was observed that the accumulation of lead and cadmium in Istanbul, Mahmudiye and Kuruçay streams increased after the earthquake (Dündar et al., 2003). In other studies carried out in Sapanca Lake, which is one of the important wetlands (Bakan and Balkas, 1999; Yalçın and Sevinç, 2001; Şişman et al., 2002; Duman et al., 2007), it was reported that the water quality is changing and the pollution in the lake has increased. Heavy metal levels determined in water samples taken from Mogan Lake, Turkey in 2009 were determined as Pb> Fe> Cu≥Zn> Cd (Dostbil, 2010). Zuo et al. (2018) showed that the ranges of the metal concentrations in Taihu Lake's (China) water in the following order Zn> Cu> Cr> Cd. As a result of this study, heavy metal concentrations in the water column of Sapanca Lake were determined as Fe> Zn> Pb> Cu> Cd. Mwamburi (2009) determined that Fe level in the water column of the Victoria Lake is far above the drinking water standards set by WHO and other parameters (Al, Mn, Zn, Cu, and Cr) are in compliance with the standards. When the water quality of the Sapanca Lake was evaluated according to SKKY (Regulation on Control of Water Pollution) (2004), for Fe and Zn were in Class I, for Pb was in Class III, for Cd was in Class II and for Cu was the first six months in Class I, the other months in Class II.

During this experiment, totally 41 *Cyprinus carpio* (25 ♂ and 16 ♀) were caught in the sampling period. The weight, length, fork length and condition factors of fish were shown in Table 2. Heavy metals were bioaccumulated at varying levels and were noticeable in different tissues of *Cyprinus carpio*. Zn and Fe were detected in all tissues every month as a result of heavy metal analysis of carp fish caught from Sapanca Lake (Table 3). Cu was not detected only in the muscle tissues of the fish in some months. Pb was not detected in the liver and gill tissues of carp in all months. Cd was detected in all tissues in some months. All results were found under the specified maximum limit of fish tissues by FAO and TFC. The evaluated heavy metal concentration can be ordered in muscle Fe>Zn>Cu>Cd>Pb, in liver and gill Zn>Fe>Cu>Cd>Pb. Because of not being an active organ, heavy metal accumulation in muscle tissue is thought to be at low levels. Altundağ et al. (2019) found the following levels of Cu< Fe< Zn in muscle tissue of carp caught from the Sapanca Lake. Junianto and Apriliani (2017) analyzed the accumulation of Zn, Cd, Pb and Hg in the muscle tissue of *C. carpio*, *Oreochromis niloticus* and *Pangasianodon hypophthalmus* in Cirata Dam (Indonesia), and determined the Pb value above of the determined standard values in fish. Mustafa, (2020) determined that Pb and Cd content in *Luciobarbus xanthopterus* caught from the Tigris River in

Baghdad and represented the general order of metals levels in different tissues as follows liver> kidney> muscle> gill. According to the analysis data revealed in this study made with carp samples, the organ with the heavy metal accumulation in all fish species was found to be the liver. Then the liver tissue was followed by gill and muscle tissues, respectively. Heavy metals usually accumulate more in non-lethal concentrations in the metabolically active organs of fish, such as the liver (Kargın and Erdem, 1992; Gülcü-Gür and Tekin-Özan, 2017). The high concentration of metal in the gill is due to the metals absorbed by breathing water adhering to the mucus in the gill and staying between lamellae (Heath, 1987; Al-Bairuty, 2013; Stanley and Preethe, 2016). In a study conducted by Uysal (2010), the Zn level in which the highest Zn accumulation in the tissues of the *C. carpio, Carassius carassius* and *Rutilus rutilus* species were found in the gill tissue. They reported that the Zn level in the muscle tissues of all species is lower than the value determined by the Turkish Food Codex (TFC). Likewise, this study found that the concentrations of the heavy metals were lower than the value determined by TFC and FAO.

The concentrations of metals in tissues of carp are much higher than in the water were found. The Transfer Factors (TF) from water to fish in case of *C. carpio* were in the muscle tissue in order of Fe> Zn> Cu> Cd; in the liver tissue in order of Zn> Fe> Cu> Cd; in the gill tissue in order of Zn> Fe> Cu> Cd> Pb (Table 4). The presence of metals in high levels in the fish environment does not indicate a direct toxic risk to fish if there is no significant accumulation of metals by fish tissues (Engin et al., 2016). On the other hand, TF from the water was higher than 1.00 which means that the *C. carpio* accumulated metals from water, especially Fe and Zn values. According to the accumulation of heavy metals in tissues, the TF can be listed as follows: for Cu, Fe and Cd; Liver> Gill> Muscle, for Zn; Gill> Liver> Muscle. This result agrees with many previous studies. Abdel-Baki et al. (2011) calculated TF of five heavy metals from water in Tilapia fish, results indicated that fish accumulated all metals (Pb> Cr> Cu> Hg> Cd) in its tissues from water. Canpolat et al. (2016) determined that the TF of heavy metals in *C. carpio* from Karakaya Dam Lake, Turkey. They were found that heavy metals accumulated in the muscle of *C. carpio* were higher than that in the surrounding water and explained that Fe was the greatest metal accumulated by *C. carpio* from water while the TF of Cu was the lowest. According to a previous study made with *C. carpio* captured from Karacaören (I) Dam Lake, Turkey, the highest organ/water ratio was determined for Zn in the liver (Kır et al. 2016).

Table 1. The heavy metal accumulation in Sapanca Lake water results were expressed as Mean± SEM.

	Cu (μgL^{-1})	Fe (μgL^{-1})	Zn (μgL^{-1})	Pb (μgL^{-1})	Cd (μgL^{-1})
January	15.4±3.72[b]	360.8±24[a]	90.8±18.7	18.4±1.50[b]	1.89±0.4[b]
March	17.9±2.80	303.01±11.37	73.12±12.4	43.4±9.6[a]	3.03±0.72[a]
April	16.2±3.4	41.41±5.4	34.92±5.7	39.14±11.2	3.011±1.04
May	16.82±5.78	27.13±4.3	18.93±3.9	40.7±10.3	2.694±0.47
June	16.27±1.2	21.74±3.75	16.17±3.2[b]	39.26±2.46	2.66±0.08
July	18.41±1.37	12.84±2.9[b]	20.36±1.9	40.45±6.42	2.70±0.9
August	20.46±1.1	68.73±5.8	22.85±2	39.15±7.6	2.84±0.42
September	20.83±4.32	67.31±14.7	57.67±8.45	40.35±13.7	2.75±0.09
October	20.2±1.4	61.94±6.7	60.29±15.3	42.4±9.36	2.94±0.51
November	22.17±6.15	63.75±9.1	108.8±21.3[a]	43.06±11.2	2.88±0.64
December	22.25±1.7[a]	45.44±2.7	82.95±11.7	43.22±7.65	2.94±0.7
NW-KEJIM-02	18.78±0.4	119.3±8.5	19.63±2.84	38.19±0.59	2.6195±0.06

a: Maximum, b: Minimum

Table 2. The parameters results of carp were expressed as Mean± SEM (Standard Error of the Mean).

Months	n gender	Weight (±SE) (min.-max., g)	Total Length (±SE) (min.-max., cm)	Fork Length (±SE) (min.-max., cm)	Condition Factor (±SE) (min.-max.)
January	3 (2 ♂ +1♀)	282± 48.2[b] (200-367)	27± 1.73[b] (24-30)	24.3± 1.2[b] (22-26)	1.41± 0.026 (1.35-1.44)
March	6 (4♂+ 2♀)	623.8 ±77.96 (445-950)	33.83±1.3 (30-39)	31.9±0.63 (29.5-34)	1.57±0.057 (1.37-1.78)
April	2 (2♂)	2710±20 (2690-2730)	73.2±0.2[a] (73-73.4)	70.75±0.25[a] (70.5-71)	0.69±0.0005[b] (0.69-0.691)
May	6 (4♂+2♀)	1192.6±269.2 (188-1884)	40.25±4.48 (25-55)	36.75±4.34 (21-50.5)	1.73±0.34 (1.09-3.36)
June	3 (2♂+1♀)	455.66±92.4 (270-671)	28.83±1.92 (26-32.5)	27±2.08 (24-31)	1.81±0.05 (1.53-1.95)
July	3 (1♂+2♀)	1187±137.99 (949-1427)	43.3±2.18 (39-46)	41.16±2.08 (37-43.5)	1.455±0.086 (1.3-1.59)
August	3 (2♂+1♀)	2328±467.13 (987-4232)	53±8.326 (41-69)	51.16±8.207 (39.5-67)	1.4±0.062 (1.28-1.5)
September	3 (3♂)	2133±290.5 (1600-2600)	50.3±1.85 (48-54)	47±2 (45-51)	1.65±0.122 (1.44-1.86)
October	3 (1♂+2♀)	2897.6±162.54[a] (2643-3200)	53.3±1.85 (57-51)	50.3±1.33 (49-53)	1.915±0.094[a] (1.72- 2.02)
November	5 (2♂+3♀)	562.8±81.7 (322- 759)	33±1.51 (28- 36)	30.9±1.57 (26- 34.5)	1.51±0.06 (1.41- 1.77)
December	4 (2♂+2♀)	557±160.89 (949- 161)	30.5±3.37 (22- 38)	28.5±3.38 (20- 36)	1.76±0.16 (1.51- 2.25)

a: Maximum, b: Minimum

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Table 3. The average concentration of heavy metals in the tissues of *Cyprinus carpio*. Data are represented as mean ± SEM.

MUSCLE					
Months	Cu (µgkg^{-1})	Fe (µgkg^{-1})	Zn (µgkg^{-1})	Pb (µgkg^{-1})	Cd (µgkg^{-1})
January	16.18±3.72	825.5±64	674.91±58.69	BDL*	BDL
March	BDL	200.84±11.37^{b}	288.73±12.39	BDL	BDL
April	6.18±2.4	660.61±53.4	1042.95±95.7	BDL	BDL
May	15.02±3.5	881.4±64.3	587.93±43.9	BDL	BDL
June	BDL	225.6±43.75	299.04±35.2	BDL	BDL
July	3.06±1.2^{b}	689.48±67.9	343.76±41.9	BDL	BDL
August	12.82±5.1	759.12±65.8	575.37±26	BDL	BDL
September	5.14±2.4	360.52±26.7	334.65±45.3	BDL	BDL
October	4.69±1.7	500.79±39.1	268.43±61.3^{b}	BDL	BDL
November	12.92±4.21	329.5±18.6	298.87±28.4	BDL	0.71±0.03
December	462.13±35.2^{a}	1864±142.6^{a}	1344.89±112.3^{a}	BDL	0.96±0.213
LIVER					
Months	Cu (µgkg^{-1})	Fe (µgkg^{-1})	Zn (µgkg^{-1})	Pb (µgkg^{-1})	Cd (µgkg^{-1})
January	1343.49±98.5	5484.9±134.6	8385.5±240.6	BDL	BDL
March	882.3±47.3	3864.1±36.6	4287.6±321.8	BDL	BDL
April	1634.15±97.45^{a}	5220.1±351.3	21083±367.9^{a}	BDL	BDL
May	1559.81±250.6	8423.27±125	8937.5±75.8	BDL	BDL
June	1219.8±69.7	6066.3±305.2	4006.63±203.5	BDL	2.66±0.98
July	1470±109.4	8114.5±264.2	10852.9±409.7	BDL	4.58±1.43^{a}
August	946.65±109.4	8998.3±305^{a}	10854.32±679	BDL	0.65±0.08
September	1347.79±187.1	5707.68±217	12763.76±445	BDL	0.49±0.2^{b}
October	452.64±35.6	4587.65±123.3	3722.87±235.4	BDL	BDL
November	412.25±27.3^{b}	2937±219.2	2677.52±198.5	BDL	BDL
December	679.26±96.4	2428.43±218.4^{b}	2347.21±512^{b}	BDL	3.49±1.4
GILL					
Months	Cu (µgkg^{-1})	Fe (µgkg^{-1})	Zn (µgkg^{-1})	Pb (µgkg^{-1})	Cd (µgkg^{-1})
January	49.51±12.4	2872.34±421	10934.3±563.4	6.77±2.4	BDL
March	36.47±10.3	2418.13±219.3	19025.55±208.4	3.93±0.89^{b}	BDL
April	32.49±12.4^{b}	3248.03±176.4	30261.55±420.9^{a}	BDL	BDL
May	78.59±21.2^{a}	4933.7±101.3	26769.9±451.3	29.49±11	BDL
June	50.42±9.3	3418.39±202.4	8546.42±302.4	10.97±3.2	0.504±0.1
July	36.47±9.6	2583.2±408.2	14745±763.3	BDL	2.01±0.05
August	69.16±4.2	5324.28±191^{a}	27331±947.3	43.04±2.6^{a}	2.9±0.8
September	47.42±21.2	3721.79±219.2	21700.35±739.3	BDL	0.38±0.01^{b}
October	32.67±8.3	1037.18±85.4^{b}	17766.85±594.6	BDL	0.44±0.02
November	39.6±7.4	2254.5±32.6	11034.25±847.4	BDL	1.02±0.4
December	47.27±18.3	2838.69±263.4	8472.92±289^{b}	BDL	2.76±1.35^{a}
DORM-3	752.37±38.06	12252.1±750.6	1986.28±144.94	218.15±28.5	34.7±0.51
FAO(max.)	30.000	-	40.000	500	100
TFC (max.)	20.000	-	50.000	300	50

a: Maximum, b: Minimum

*BDL: Blow Detection Limit

Table 4. Transfer Factors of heavy metals from water to the tissues of *Cyprinus carpio*.

MUSCLE					
Months	Cu	Fe	Zn	Pb	Cd
January	1.05	2.28	7.43	*	*
March	*	0.66[b]	3.95	*	*
April	0.38	15.95	29.86	*	*
May	0.89	32.48	31.06[a]	*	*
June	*	10.37	18.49	*	*
July	0.16[b]	53.69[a]	16.88	*	*
August	0.62	11.04	25.18	*	*
September	0.24	5.35	5.80	*	*
October	0.23	8.085	4.45	*	*
November	0.58	5.16	2.75[b]	*	0.24
December	20.76[a]	41.02	16.21	*	0.32
LIVER					
Months	Cu	Fe	Zn	Pb	Cd
January	87.24	15.20	92.35	*	*
March	49.32	12.75[b]	58.63	*	*
April	100.81[a]	126.05	603.75[a]	*	*
May	92.73	310.48	472.13	*	*
June	74.97	279.04	247.78	*	1.02
July	79.84	631.97[a]	533.05	*	1.69[a]
August	46.26	130.92	475.02	*	0.23
September	64.70	84.79	221.32	*	0.18[b]
October	22.4	74.06	61.75	*	*
November	18.59[b]	46.07	24.6[b]	*	*
December	30.52	53.44	28.29	*	1.18
GILL					
Months	Cu	Fe	Zn	Pb	Cd
January	3.21	7.96[b]	120.42	0.36	*
March	2.04	7.98	260.19	0.09[b]	*
April	2.004	78.43	866.59	*	*
May	4.67[a]	181.85	1414.15[a]	0.72	*
June	3.09	157.23	528.53	0.28	0.19
July	1.98	201.18[a]	724.21	*	0.74
August	3.38	77.46	1196.1	1.09[a]	1.02[a]
September	2.27	55.29	376.28	*	0.14[b]
October	1.61[b]	16.74	294.68	*	0.15
November	1.78	35.36	101.4[b]	*	0.35
December	2.12	62.47	102.14	*	0.93

a: Maximum, b: Minimum

*: BDL values were not taken into account for TF determination.

Aquatic organisms, such as fish, are widely used to assess aquatic environmental quality and are considered as bioindicators of environmental pollution. Various organisms can be used to determine the mechanism of the effect of pollution on specific physiological functions. Güngördü et al., (2012) monitored the pollution in Meriç River Delta temporally and spatially by using carp as a biomonitor and showed that antioxidant enzyme parameters can be used as a useful biomarker in the evaluation of environmental contamination.

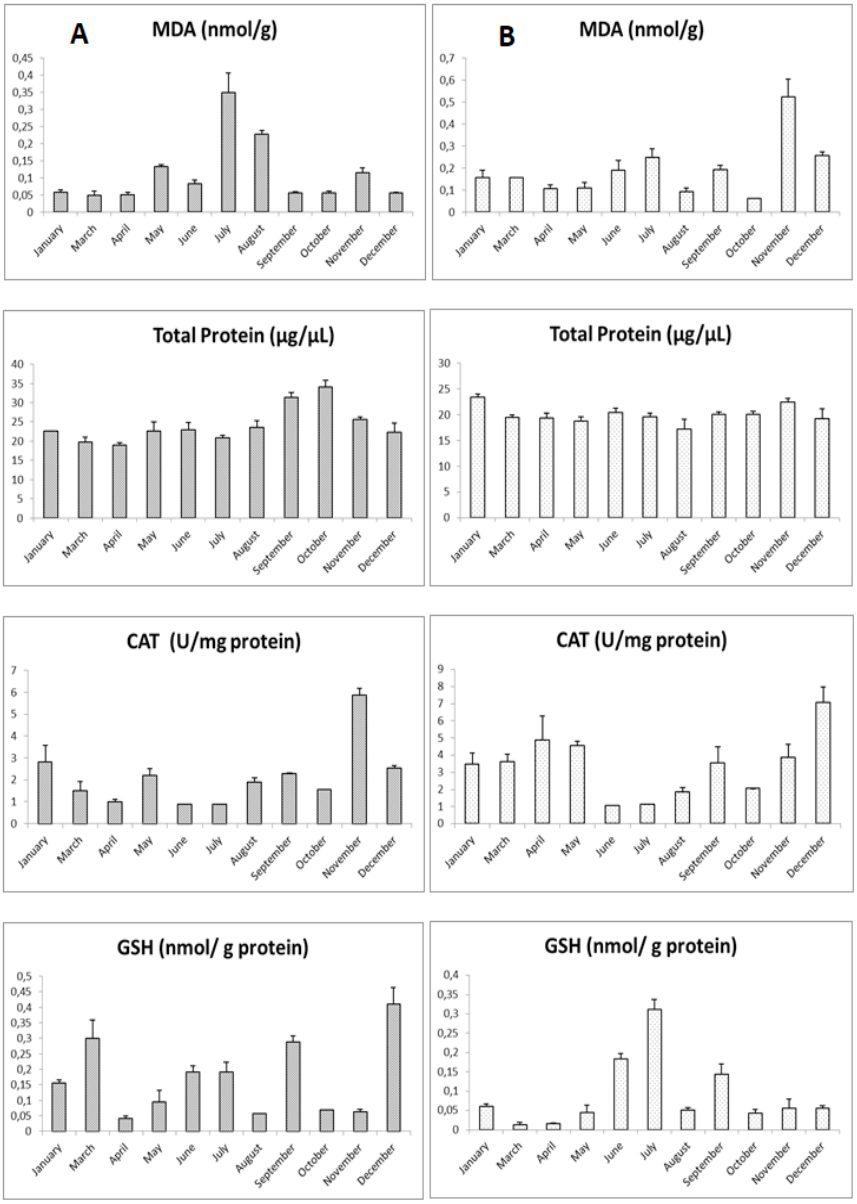

Figure 2. The responses of oxidative stress parameters in tissues (Liver (A) and Gill (B)) of carp. Lipid peroxidation (LPO; MDA), Catalase enzyme activity (CAT), Total glutathione Level (GSH). Data are represented as mean ± SEM.

Especially the temperature and nutrition increase oxygen consumption and cellular oxyradical production and enhance the antioxidant defense system to compensate these oxyradicals (Sheehan and Power, 1999; Javed et al., 2016). In this study, due to the increased sensitivity of fish tissues to oxidative stress, a decrease in the antioxidant defense system was observed in the summer months (Figure 2). Catalase (CAT) is one of the important intracellular antioxidant enzymes in the defense mechanism, and it is one of the peroxidases that provide H_2O_2 detoxification by breaking down the oxygen and water. It was determined that catalase gave different responses in xenobiotic effect (Mahboob, 2013; AnvariFar et al., 2018). It has been reported that excessive O_2 production can reduce CAT activity by its ability to inhibit CAT activity (Pandey et al., 2003). Karadağ et al. (2014) determined that increased CAT activity in carp samples were taken from the polluted area by untreated wastewaters, when compared to the relatively clean areas of Ataturk Dam Lake, Turkey. Padmini et al., (2008) determined the liver oxidative stress status of grey mullets living in heavy-metal-rich polluted Ennore Estuary compared with

unpolluted Kovalam Estuary. CAT was significantly decreased in polluted estuary fish compared with unpolluted estuary fish. This indicates the inability of polluted estuary fish to cope with available antioxidant defense presumably due to the free radicals produced by pollutants. When the production of ROS is increased, the activity of antioxidant enzymes is intensified to reestablish the redox balance and health of the individual. The failure to reestablish this balance causes oxidative damage to biomolecules, compromising the ability of the organism to survive (Valavanidis et al., 2006).

Glutathione (GSH) is important in antioxidant defense as an oxygen radical scavenger. The change in the GSH level is an important indicator of detoxification ability. Although the metal-GSH conjugation process is the desired condition to provides the removal of metals with gall but reduces the antioxidant defense capacity due to the consumption of GSH (Cheung et al., 2001). This might be a factor responsible for the lack of elimination of toxic compounds that enter the fish and thus result in their accumulation, aggravating oxidative stress (Nammalwar, 1992). Fırat and Kargın, (2010) studied the effect of cadmium and zinc on *O. niloticus* was investigated in erythrocyte antioxidant systems, GSH and CAT activities were reported to increase in all groups. Yıldırım et al. (2011) determined decreased GSH levels in the *Capoeta trutta* samples were caught from the contaminated station when compared to the uncontaminated stations in Munzur River, Turkey. Hermenean et al. (2015) observed reduce GSH content the exposure to the polluted waters of the Tur River, Romania, on the *Leuciscus cephalus* compared to fish from reference river areas. In this study, the GSH level started to decrease in tissues due to the warming of the weather and reached the lowest levels in the summer months. Reduced GSH level is thought to serve as an indicator for determining the degree of pollution in fish exposed to pollutants.

Malondialdehyde (MDA) is one of the products formed as a result of lipid peroxidation (LPO) and is a commonly used parameter in demonstrating oxidative damage. The high amount of MDA indicates lipid peroxidation. Lack of lipid peroxidation or low levels is indicative of the protective effects of oxidative enzymes. Şahan et al., (2010) analyzed the levels of CAT, GSH, and LPO from biomarkers in carp to determine the level of agricultural and industrial pollution in the Ceyhan River. CAT activity and GSH levels were observed at high levels in the liver tissues of the fish in the polluted area. Besides, the amount of LPO was found to be quite high in the polluted area. Perez-Coyotl et al., (2019) specified increase LPO level in embryos of *C. carpio* was exposed to the water samples taken in The Madín Dam, Mexico. Rajeskumar et al. (2017) showed that metals exposure caused a time-dependent significant increase of LPO values in the fish exposed to multiple metal mixtures and it was almost double in 30 days group compared to controls. Heavy metals can interact with cell membrane structures and alter the normal physiology by stimulating LPO (Ahmad et al., 2006). In addition, because the typical response to oxidative stress is peroxidative damage to unsaturated fatty acids, an increase in the LPO level has been extensively used as a marker of oxidative damage in organisms. The reactive carbonyls produced during lipid peroxidation may diffuse from the original site of radical production, causing damage to inter- and intra-cellular targets (Yeşilbudak and Erdem, 2014).

Pollutants that enter the aquatic systems directly or indirectly through deliberate disposals or accidents adversely affect the various tissues and organs of living things in these environments. Basic data on these effects are provided by histopathological research, which is still the most reliable method. In addition to respiratory functions, gills that are in direct contact with the aquatic environment play an active role in the regulation of ion balance, osmoregulation and partial removal of nitrogenous wastes (Bury et al., 2003; Simonato et al., 2008; Hadi and Alwan, 2012). These are the structures that are primarily affected by all changes in ambient conditions (İşisağ Üçüncü et al., 2010; Uribe et al., 2011).

It is already well known that the histological structure of the gills shows high sensitivity to toxic chemicals in water (Brand et al., 2001; Rodrigues et al., 2010; Hesni et al., 2011). The results of this study also overlap with this information. In the gill tissues of carp caught from Sapanca Lake, general signs of destruction such as irregularity of lamellae, and hyperplasia were observed (Figure 3). Desquamation (spillage, deformity) in epithelial cells and edema in secondary lamellar tips were seen. Rarely, mutual fusions of secondary lamellae and vacuolization in primary lamellae have been observed. Also, mucus-like accumulation between lamellae has been encountered many times. As noted by Furia (2004) reported that hyperplasia, separation, and lamellar fusion in the lamellar epithelium, by increasing the distance, to some extent delay the passage of the toxic substance into the veins where it will be involved in toxicodynamic processes. The researcher also states that this defense response will cause damage to the respiratory, circulatory and osmoregulation systems, increase the risk of illness and death, and even more and more threaten the population. Hesni et al., (2011) also highlighted that gill histopathologies can reach levels that may affect the population. These general views are confirmed by the results of the presented study. Indeed, hyperplasia, hypertrophy, and even edema strengthen the cell-tissue barrier for chemicals in a biophyscochemical. However, as can be understood from desquamation, the barrier quality of the epithelium, which can be easily worn, is essentially weak. This natural barrier, which is enhanced with the increased mucus secretion, can be easily overcome by chemicals, bacteria, parasites, etc. (Fonseca et al., 2017). Fusion plays a major role at this point; because fused lamellae reduce the exposure surface. The labefaction due to the shortening in the fusion extension in the lamellar sizes can also be compensated by hyperplasia, hypertrophy, and edema (Ossana et al., 2019).

In histopathological studies, the liver and gill are the most appropriate target organs used to determine the effects of pollution. These organs are primary indicators of aquatic pollution. This study confirms all common views that these vital and complex organs are truly biomarkers (Figure 4). Accordingly, there is no lobular arrangement in the parenchyma of the liver, in some instances, regular polygonal-shaped hepatocytes, which are separated from each other by

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sinusoids, have formed clumps. However, hepatocytes and sinusoids have hemorrhage, which is evident with dark pink staining. Hepatopancreas cells contain zymogenic granules. In some samples, erythrocytes were seen in the hepatopancreas, which was separated from its environment by necrotic areas. In some cases, hepatopancreas is dysplasia and atrophic. Kuppfer cells, a type of macrophage specific to the liver, are evident in all samples. In some hepatocytes, nucleus and nucleolus can be easily selected, while in others it is significantly hypertrophic. In some examples, various nucleus dystrophies have been identified in the form of karyolisis and picnosis. As it is known, the main histopathological changes caused by the effects of various chemicals in the teleost liver, which has a special function of detoxification: steatosis, hypertrophy in hepatocytes and destruction in membranous structures; nucleus injuries such as pycnosis, karyorrhexis, karyolysis; necrosis and sometimes cancer. The other elements of the parenchyma are of course also affected by chemicals; dilatation and congestion, hemorrhagia and hyperemia can be observed in the vessels; the inflammatory response can be characterized by lymphocyte infiltration. Various changes in bile pigments can be seen in the extension of hepatocellular atrophy because of the liver's functions within the digestive system. In addition, fibrosis and cystic formations can occur. The melanomacrophage changes are also one of the main indicators of the immune response (Peters et al., 1987; Hinton and Lauren, 1990; Köhler et al. 1992; Hinton et al., 2001; Hinton et al., 2008). The excessive oil accumulation was detected in hepatocytes of *A. anguilla* samples collected from the contamination of the water with polychlorinated biphenyls, organochlorinated pesticides, PAH and heavy metals (Oliveira Riberio et al., 2005). Syasina et al., (2012) observed the large and heavily vacuolated hepatocytes, binuclearity, numerous nuclei of irregular shape, karyopyknosis and the accumulation of large amounts of lipids (lipidosis) in the liver of carp from the Bol'shoi Ussuriiskii Island.

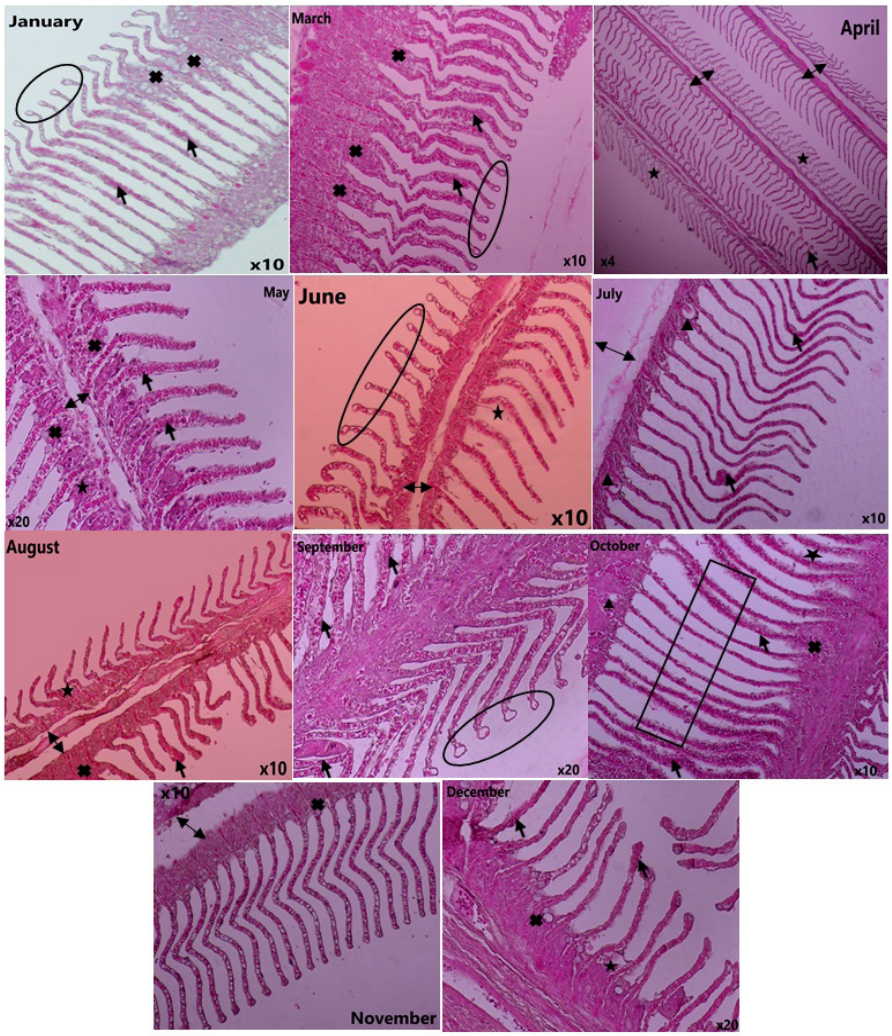

Figure 3. Histopathological results of carp fish gill tissue. Hyperplasia in the secondary lamellae (arrows), separating the primary lamellae (bidirectional arrows), desquamation in secondary lamellae (stars), the mutual fusion of secondary lamellae (rectangular), vacuolization in the primary lamellae (triangle), edema secondary lamella ends (ellipse), mucus-like accumulation (cross) between lamellar. H & E.

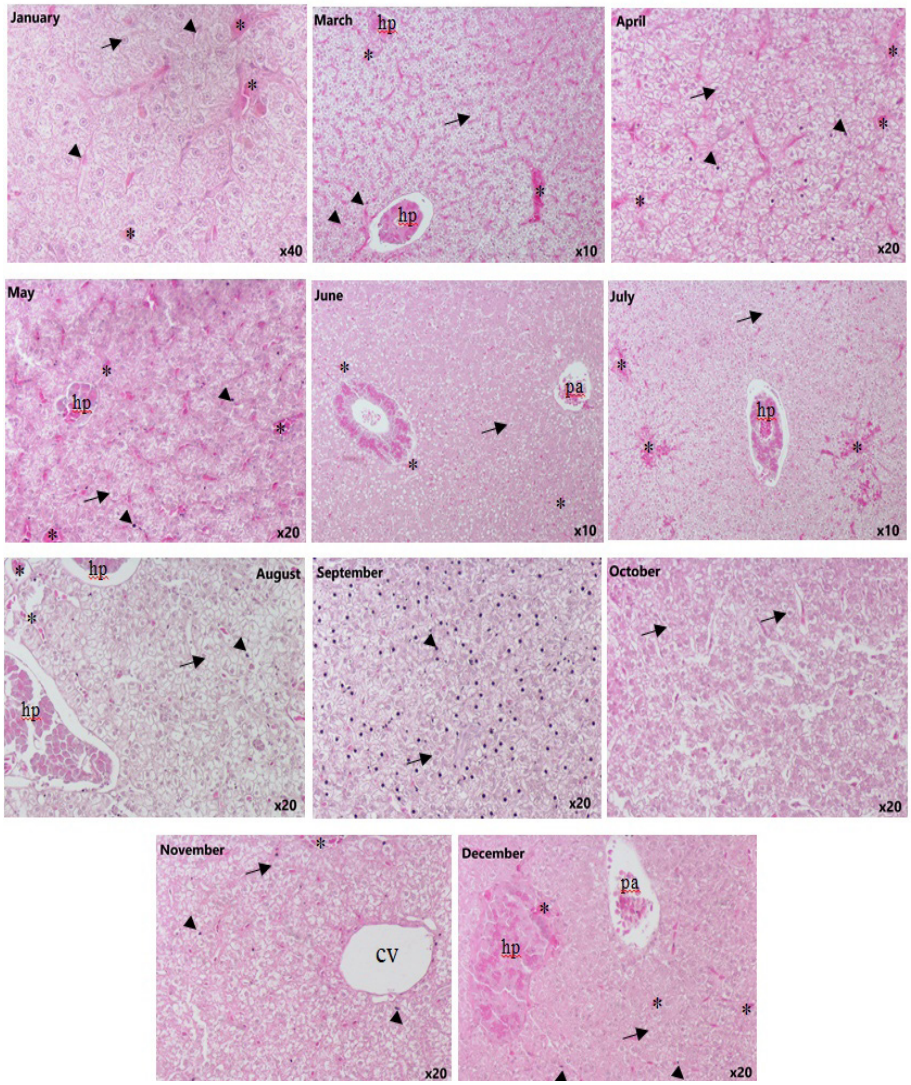

Figure 4. Histopathological results of carp fish liver tissue. Central vena (cv), hepatocytes (arrows), kuppfer cells (arrowheads), hepatopancreas (hp), portal area (pa) and red blood cells (star) in hepatocytes and sinusoids, H&E.

Conclusion

Increasing environmental pollution has become an important problem in the modern world due to industrial and domestic wastes. The recovery of lake waters polluted by the discharge of these wastes is very costly and adds an extra burden to the economy of the countries. Therefore, it is necessary to protect the water ecosystem from pollution and to protect public and environmental health. The histological and biochemical findings of this study showed that there is pollution in the lake, although heavy metal amounts are below specified standards in the analysis of water and fish tissues. These results suggest that Sapanca Lake may be under the influence of not only heavy metal pollution but also other pollutants.

Nowadays, many scientific researchers are conducted on the characterization and remediation methods of contaminated water systems and the sources polluting them, and the results are published. The aquatic ecosystem's organisms, such as fish, are commonly used to assess aquatic environmental quality and are considered as bioindicators of environmental pollution. Continuing to regularly monitor the water quality and biological parameters of the lake will help keep the condition of the lake under control. Future studies should pay more attention to what concentrations of pollution have become dangerous for aquatic organisms and humans. As a result, a clearer prediction should be provided about the measures to be taken by determining the in vivo and in vitro effects of the pollutants in the lake on the organic and inorganic environment.

Compliance with Ethical Standards

Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

Author contribution

Gullu KAYMAK carried out the collecting of samples, analysis, reporting, correction of literature sources, article writing, and publishing procedures. In addition to Nazan Deniz YON ERTUG, who was the thesis advisor, Figen Esin KAYHAN contributed to the reporting, analysis, and statistical analysis of the data, review, and editing of the article.

All the authors read and approved the final manuscript. All the

authors verify that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

Not applicable.

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Data availability

Not applicable.

Consent for publication

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Analysis of vegetable oil demand and its price reform in Iran: using rural and urban household level data

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Abstract

In a group of food types, vegetable oils are the second-largest source of energy in the human diet and their consumption is very important from a health and economic viewpoint. Consumer behavior evaluation can help a lot in adopting the right policies; so, the framework of the Almost Ideal Demand System model for food expenditures was used to examine rural and urban vegetable oil consumer behavior in Iran using household survey data conducted by the Iranian Statistical Institute for the 2018 year. For this purpose, income, price and cross-price elasticities under ten aggregated food groups i.e., Cereal, Meat, Dairy, Butter and Animal Oil, Vegetable Oil, Fruits and Nuts, Vegetables and Legumes, Sugary, Spices and, Dinks were estimated for vegetable oil using the Seemingly Unrelated Regression. The obtained results showed that vegetable oil concerning the positive income elasticities is a necessity goods both rural (0.872) and urban (0.889) consumers. Own-price elasticities revealed that the demand for vegetable oil is less responsive to the increase in the price in urban areas (-0.280) than the rural area (-1.073). Moreover, Butter and Animal Oils food groups are highly substitutable with vegetable oil for rural consumers. Since the absolute value of cross-price elasticities are often less than an entity, consumers of vegetable oil will not have a noticeable change in demand as prices change for other food groups. Due to vegetable oil price reform, per capita compensation payments for a typical rural person would be greater than the urban person. The results suggest that policymakers should adopt different policies about rural and urban consumers of vegetable oil.

Keywords: Almost Ideal Demand System, Demand Elasticities, Households, Iran, Vegetable Oil

Introduction

Nowadays oilseeds constitute the world's second-largest food reserve after cereals (Heydari et al. 2010). The extracted oil from oilseeds has both edible and industrial uses. Between the five components of foodstuffs, oils and fats, after the group of carbohydrates, are the main source of human energy supply, and because of fat-soluble vitamins such as A, D, K, E, and their high saturation, considered as essential and strategic consumer goods. According to the results of the National Nutrition and Food Technology Research, about 21% of the total energy consumed in Iran is provided by vegetable oil. Based on the data of income and consumption expenditure surveys conducted by the Iranian Statistical Center for the 2018 year, per capita vegetable oil consumption is around 14.50 and 13.29 kg (accounting for 4.57% and 4.01% of household food expenditure) for each rural and urban person, respectively (Iranian Statistical Center (ISC), 2019) (Figure 1). Annually, 1.129 million tone vegetable oil is being consumed in Iran.

Various reasons, such as the role of animal oils in heart problems, increasing population, increased consumerism in

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society, changes in villagers' dietary patterns and the cheapness of these oils compared to other oils have led to increasing consumption of vegetable oil in the community.

The sources of vegetable oil supply in Iran highly rely on importing oilseeds, crude oil and extraction from domestic oilseed production. Today, Iran is considered one of the largest importers of vegetable oil. The self-sufficiency coefficient indicates that the vegetable oil supply from domestic sources fluctuated to less than 10% in the 2018 year, implying a high dependency on imports for this essential and strategic commodity (Yuzbashkendi, 2019). Therefore, the supply of vegetable oil in the country can be considered as a significant foreign currency drain. Furthermore, climate change and variability may worsen the condition of food security particularly for water-limited regions such as Iran (Nouri & Homaee, 2020; Paymard, Yaghoubi, Nouri, & Bannayan, 2019; Satari Yuzbashkandi & Khalilian, 2020).

Considering the high consumption of vegetable oil and consequently the high import of this product in Iran, it is important to study and analyze its demand. Demand structure and household consumption patterns analysis was vital and widely used in policy analysis (A. S. Deaton, Ruiz-Castillo, & Thomas, 1989; Sekhampu & Niyimbanira, 2013). Therefore, policymakers and planners use the results to predict the future. It is also important to study the effectiveness of various economic policies, including policies related to market regulation, control or increase of product supply, subsidy management, taxes and price changes on food security and health of the community and consumer welfare (Kalkuhl, von Braun, & Torero, 2016; Pishbahar & Nataj Firoozjah, 2014). Also, producers, food processors and other market players need to forecast demand to plan and design their production and sales, in this regard, demand elasticities are important (Ullah, Jan, Fayaz, Ali, & Shah, 2019).

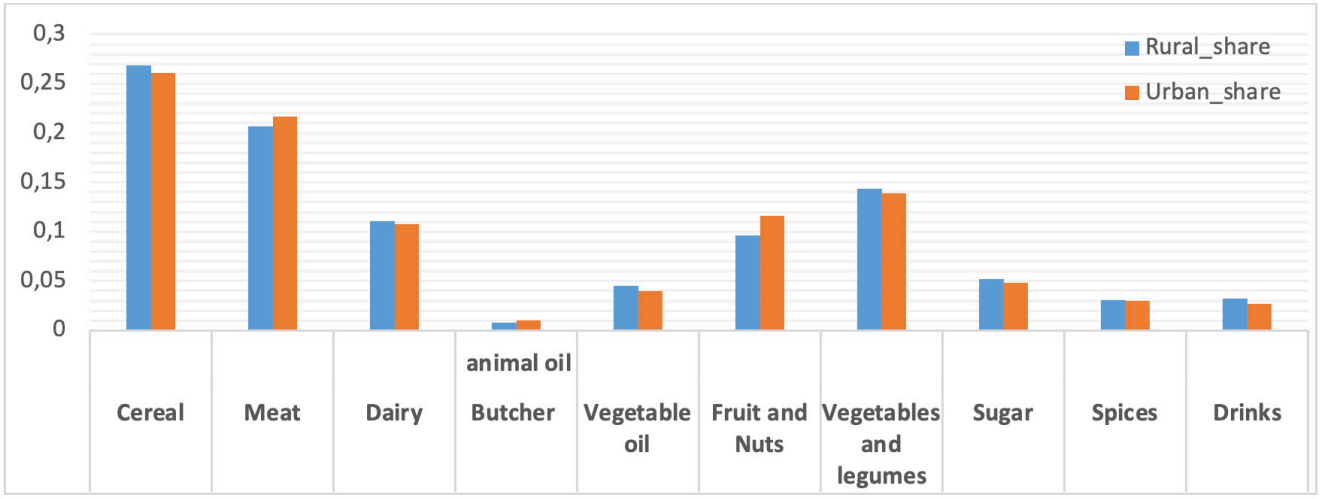

Figure 1. The contribution of different commodity groups from the food expenditure

Given the expenditure and consumption variation among Iranian households, examining the structure of food consumption is an important issue. Thus, the food elasticities of income, expenditure and price can be used as useful tools in the implementation of effective food policy (Şahinli & Fidan, 2012). Some studies have been conducted to analyze food items demand using the AIDS approach in Iran and the world (Alnafissa & Alderiny, 2019; Ataie s & Mohammadi, 2018; Chen, Saghaian, & Zheng, 2018; Hoang, 2018; Hooshmand, Khodadad Kashi, & Khoshnevis, 2017; Pishbahar & Nataj Firoozjah, 2014; Rathnayaka, Selvanathan, Selvanathan, & Kler, 2019; Şahinli & Fidan, 2012; Sekhampu & Niyimbanira, 2013; Yazdani & Sherafatmand, 2013).

In this study, an Almost Ideal Demand System approach was applied to calculation of vegetable oil demand (income and price elasticities) by using the food sub-group expenditure data included in the household income and consumption expenditure surveys conducted by the Iranian Statistical Center for 2018. Given the importance of vegetable oils in the household food basket, this study aimed to evaluate the vegetable oil consumption pattern of the rural and urban households living in Iran. The results can help policymakers as well as non-profit organizations and businesses in adopting appropriate policies.

Materials and methods

Data sources

We have used the 2018 Household Expenditure and Income Survey (HEIS) gathered by the Statistical Center of Iran (SCI). The HEIS is the main annual household survey in Iran. This method is based on a three-stage cluster sampling method with strata and collected for more than fifty years (Akbari, Ziaei, & Ghahremanzadeh, 2013). All the consumed items for a month are recorded by interviewing households. In other words, information about the money spent on each item and the consumption is collected. The 2018 HIES was carried out by a sample of 20350 households in urban areas and 18610 households in rural areas. The raw data of HIES was used instead of published ones. The Classification of Individual Consumption by Purpose (COICOP) data structure in 4 digits was used for organizing and classification of food items. Food

items were classified into ten food groups: Cereal, Meat, Dairy, Butter and Animal Oil, Vegetable Oil, Fruits and Nuts, Vegetables and Legumes, Sugary, Spices, and Drinks. Table 1 shows the distribution of food sub-groups and aggregate group name. In this study, to more accurately assess the vegetable oil demand, the vegetable oil and butter (animal oil and butter) groups were separated from each other. These aggregated food groups constitute almost 100% of the food consumption basket of rural and urban households in Iran. To calculate the budget shares of each aggregated food, the expenditure of each sub-group was divided by total expenditure. The geometric mean with expenditure shares as the weight was used to compute the price indices of aggregated food groupings.

Table 1. Distribution of food sub-groups in Iranian households

Commodity coeds	Food sub-group names	Aggregate group name
1111, 1112, 1114, 1115, 1116, 1117	Rice and Rice flour, Wheat and Wheat flour, Bread, Biscuits, Pastry, Confections and Other Cereal Products.	Cereal
1121, 1122, 1123, 1124, 1131, 1132	Mutton, Beef, Chicken, Fish and other meat products.	Meat
1141, 1142, 1143, 1144	Eggs, Milk and Dairy products except butter.	Dairy
1151, 1152	Animal oil, Fats, Butter	Butter
1153	Vegetable oil	Vegetable Oil
1161, 1162, 1163, 1164, 1165, 1166	Nuts, Treed fruits and other fresh fruits	Fruits and Nuts
1171, 1172, 1173, 1174, 1175, 1176	Fresh vegetables, Dried vegetables, Chickpea, Bean, Split pea, Soybean and other Pulses.	Vegetables and Legumes
1181, 1182, 1183, 1184,	Sugar, Jams, Honey, Molasses, and other Sugary Products	Sugary
1191, 1192, 1193, 1194	Salt, Tomato paste, Ketchup, Lemon juice, Sourness, Pickled Cucumbers and other Spices	Spices
1211, 1221	Tea, Coffee, Cocoa and Non-alcoholic drinks	Drinks

The empirical LA-AIDS model

The almost ideal demand system was first proposed by A. Deaton and Muellbauer (1980), and most commonly applied for demand analysis. This demand system is taken by the cost function introduced by Deaton and Muellbauer that indicates the minimum cost necessary to achieve a certain level of utility U at price vector P as follows:

$$\ln C(U,P) = a_0 + \sum_{1}^{n} a_i lnp_i + \frac{1}{2}\sum_{i}^{n}\sum_{j}^{n} \gamma_{ij}\, lnp_i lnp_j + u\,\beta_0 \prod_i p_i^{\beta_i} \quad (1)$$

n=10 i,j=1,2,3,…,n

where is the natural logarithm of the cost function, , , , and are constant coefficients, i and j are the indexes representing different food groups. By using Shephard's lemma theorem and the first derivative of the cost function (), the compensation demand function is obtained. Finally, we extract the modified version of an AIDS model, in which share of the ith food group expenditure is a function of prices and the related food expenditures. It can be written as:

$$w_i = a_i + \sum_j \gamma_{ij}\, lnp_j + \beta_i \ln\left(\frac{m}{p}\right) \quad (2)$$

where and are the expenditure share and price associated with food groups, respectively, m is the total expenditure on the system of the ten food groups given by , where is the quantity demanded j^{th} group of food, and , , and are parameters to be estimated, p is the price index of food groups.

In order to convert the AIDS model to LA-AIDS model, A. Deaton and Muellbauer (1980) suggested the Stone price index p (Ngui, Mutua, Osiolo, & Aligula, 2011):

$$\ln p = \sum w_i \ln p_i \quad (4)$$

In most empirical studies, the LA-AIDS model is more frequently estimated than the nonlinear AIDS model (Berck, Hess, & Smith, 1997; Chen et al., 2018; Edgerton et al., 1996; Elsner, 2001; Liu et al., 2019).

2.2.1. Demand function restrictions in AIDS

To make LA-AIDS in line with the demand theory, the Eq. (2) must satisfied the adding-up, homogeneity and symmetry conditions which apply on the parameters of the aforementioned equation:

$$\sum_i a_i = 1 \quad \sum_i \gamma_{ij} = 0 \quad \sum_i \beta_i = 0 \quad \forall j \quad \text{"adding} - \text{up" } condition \quad (5)$$

$$\sum_j \gamma_{ij} = 0 \quad \forall i \quad \text{"homogenity"} \quad codition \quad (6)$$

$$\gamma_{ij} = \gamma_{ji} \quad \forall\, i,j \quad and\ i \neq j \quad \text{"symetry" } condition \quad (7)$$

Eq.(5) ensures the expenditure shares always sum up to entity , Eq.(6) guarantees that if all prices and expenditure change at the same rate, the quantities purchased do not change, while Eq.(7) shows the stability of consumer choices.

Expenditure and price elasticities

After estimation of system coefficients, expenditure elasticity , Marshallian (uncompensated) and Hicksian (compensated) own-price and cross-price elasticities can be derived from (2) and (4) as follows (Green & Alston, 1990):

$$\eta_i^E = \frac{\beta_i}{w_i} + 1 \qquad \text{(Expenditure elasticity)} \tag{8}$$

$$\varepsilon_{ij} = -\delta_{ij} + \frac{\gamma_{ij}}{w_i} - \beta_i \frac{w_j}{w_i} \qquad \text{(Marshallian)} \tag{9}$$

where δ_{ij} is Kronecker delta $\delta_{ij} = 1 \; for \; i = j; \quad \delta_{ij} = 0 \; for \; i \neq j,$

$$\varepsilon_{ij}^{*} = -\delta_{ij} + \frac{\gamma_{ij}}{w_i} + w_j \qquad \text{(Hicksian)} \tag{10}$$

LA-AIDS specification

The equations system to be estimated is:

$$\begin{aligned} w_1 &= a_1 + \gamma_{1.1} lnp_1 + \gamma_{1.2} lnp_2 + \cdots + \gamma_{1.10} lnp_{10} + \beta_1 \ln\left(\frac{m}{p}\right) + u_1 \\ w_2 &= a_2 + \gamma_{2.1} lnp_1 + \gamma_{2.2} lnp_2 + \cdots + \gamma_{2.10} lnp_{10} + \beta_2 \ln\left(\frac{m}{p}\right) + u_2 \\ &\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots \\ w_{10} &= a_{10} + \gamma_{10.1} lnp_1 + \gamma_{10.2} lnp_2 + \cdots + \gamma_{10.10} lnp_{10} + \beta_{10} \ln\left(\frac{m}{p}\right) + u_{10} \end{aligned} \tag{11}$$

After applying the constraints into the model, the number of equations in the LA-AIDS model becomes (n-1=9) and the other equations can be estimated using an Iterative Seemingly Unrelated Regressions (ISUR) technique. Furthermore, econometric software Eviews 10 was used. In this study, the Almost Ideal Demand System (AIDS) approach was applied to analyze the vegetable oil demand in the Iranian rural and urban sector for following benefits: a) it applies arbitrarily the first-order approximation to any system; b) it meets the principle of choice; c) it aggregates over consumers perfectly; d) its functional form makes it in consistence with household budget data; e) it can be easily estimated; and f) allows testing of symmetry and homogeneity conditions (Blanciforti & Green, 1983; Şahinli & Fidan, 2012; Satari Yuzbashkandi & Mehrjo, 2020).

Welfare indicators in AIDS system

By changing the vegetable oil price, consumer utility rates may increase or decrease. The Compensating Variation (CV) is often used to determine the impacts of price changes on consumers. The aforementioned index shows the amount of money that is necessary to compensate a consumer as a result of price change so that it achieves the first utility. The CV is represented based on the Compensated Demand Curve, in other words, the Hicksian demand curve (Davoodi, 2010) (Satari Yuzbashkandi & Mehrjo, 2020). Supposed that the price of vegetable oil changes, that way p0≠p1. The change of CV can be written in the form of a difference between two values of the expenditure function after and before the price change (Hicks, 1946; Khalili Araghi & Barkhordari, 2012):

$$CV = E(P1_d, U0) - E(P0_d, U0) \tag{12}$$

Where E and U refer to expenditure and indirect utility functions, respectively. As well as the subscripts of (0) and (1) show the before and after the price change. To measure the welfare effects of rising prices, the compensating variation function for the almost ideal demand system is extracted as follows (Noorollahi, Jabbari, Moradkhani, & Faramarzi, 2017):

$$CV = exp\left[A_1 + \prod_{i=1}^{n} (p_i^1/p_i^0)^{\beta_i} . (logc(u^0, p^0) - A_0)\right] - c(u^0, p^0)$$

$$A_0 = a_0 + \sum_{i=1}^{n} a_i \, logp_i^0 + 1/2 \sum_{i=1}^{n}\sum_{j=1}^{n} \gamma_{ij} \, logp_i^0 logp_j^0$$

$$A_1 = a_0 + \sum_{i=1}^{n} a_i \, logp_i^1 + 1/2 \sum_{i=1}^{n}\sum_{j=1}^{n} \gamma_{ij} \, logp_i^1 logp_j^1 \tag{13}$$

Results and discussion

To explaining the consumer's behavior, the homogeneity and symmetry conditions were tested by Wald test (and (, respectively. The results of the homogeneity test in Table 2 shows that null-hypothesis was rejected in all food groups. Hence, both rural and urban consumers had a monetary illusion to purchase these ten kinds of food especially vegetable oil, instead of considering the real incomes and prices. Moreover, as the results of Wald test are shown separately for rural and urban households in Table 3, the symmetry nature of coefficients in the system was rejected, implying that the price coefficient of the jth commodity in the equation relating to the share of the ith commodity was not equal to the ith commodity price factor in the equation relating to the share of jth commodity.

After performing the required tests and approving the estimation method as systematically, the results of the demand system estimation using the Iterative Seemingly Unrelated Regressions and the Eviews 10 software package are presented in Table 4 and Table 5. Since the problem statement in this study is the analysis of vegetable oil demand for urban and rural households, therefore, we focused more on this part of the equation system. According to Table 4, in the expenditure share of rural vegetable oil equation, all variables except the price of Vegetables and Legumes food group are significant. The coefficients of the intended equation show that increasing the price of food groups of Cereals, Meat, Vegetable Oils, Fruits and Nuts, Sugary and Spices have a negative and inverse effect on the expenditure share of Vegetable Oils, and rising prices for Dairy, Animal Oils and Drinks increase the expenditure share of vegetable oil. With respect to the vegetable oil expenditure-share equation in Table 4, all variables except for the variable of Drink price were also significant. Thus, increasing the prices of the food groups of Cereals, Meat, Fruits and Nuts, Vegetables, Sugary and Drinks decrease the expenditure share of Vegetable Oil in urban household's food basket and the rise in prices for Dairy groups, Animal Oil, Vegetable Oils and Spices have had an adverse effect on the share of vegetable oil expenditure.

To evaluate the goodness-of-fit and also, the existence of autocorrelation in the estimated equations, the R^2 and DW values related to AIDS estimation are shown in Tables 4 and 5 by the food groups, separately. The R^2 values for the rural and urban households' vegetable oil expenditure share were estimated to be 0.84 and 0.89, respectively. it can therefore be concluded that the model explains well the vegetable oil expenditure shares in the rural and urban household's expenditures. The DW statistic values were close to 2, indicating that there is no autocorrelation in the estimated equations.

Table 2. Homogeneity test of demand system (Wald test)

Households	Commodity group	statistics	Prob.	Hypothesis
Rural	Cereal	82.54	0.000	rejected
	Meat	87.46	0.000	rejected
	Dairy	102.52	0.000	rejected
	Butter and animal oil	197.45	0.000	rejected
	Vegetable oil	92.19	0.000	rejected
	Fruit and Nuts	365.19	0.000	rejected
	Vegetables and legumes	32.55	0.000	rejected
	Sugary	8.95	0.002	rejected
	Spices	2.77	0.090	rejected
	Drinks	134.07	0.000	rejected
Urban	Cereal	110.69	0.000	rejected
	Meat	137.85	0.000	rejected
	Dairy	125.78	0.000	rejected
	Butter and animal oil	20.72	0.000	rejected
	Vegetable oil	6.31	0.012	rejected
	Fruit and Nuts	287.23	0.000	rejected
	Vegetables and legumes	5.81	0.015	rejected
	Sugary	26.02	0.000	rejected
	Spices	14.37	0.001	rejected
	Drinks	141.64	0.000	rejected

Table 3. The hypothesis test of symmetry (Wald test)

Household	Commodity group	Statistics	Prob.	Hypothesis
Rural	All	3049.54	0.000	rejected
Urban	All	2294.61	0.000	rejected

Since in an AIDS approach, the dependent variable is the expenditure share of the food group and the independent variable is the logarithm of food groups' price and income, it is necessary to calculate the elasticities to measure the changes in the demand quantity relative to the food groups' price and income changes.

According to the values of estimated parameters in Tables 4 and 5, also by employing the elasticity equations regarding the Almost Ideal Demand System, the elasticity values were determined for each food group. The results of different elasticities for rural and urban households are given in Tables 6, 7, 8 and 9. From the expenditure elasticity view, the expenditure elasticity shows the demand changes of specific food groups in the face of income changes. Based on the expenditure elasticities, commodities are divided into three groups. The commodity with expenditure elasticity greater than 1 are luxury goods, the commodity with an elasticity between 0 and 1 are normal goods and finally for those with elasticity lower than 0 are inferior goods. As can be seen in Tables 6 and 8, the rural and urban expenditure elasticity for vegetable oil was found to be 0.872 and 0.889, respectively. It is concluded that vegetable oil is a normal good for both rural and urban households, and also the value of the aforementioned elasticity is nearly equal. For rural and urban households, assuming all the other variables constant, the mean value of expenditure elasticity indicates that an average increase of 1% in households' income would cause a 0.872% and 0.889% increase in the quantity demanded for vegetable oil, respectively.

To know how consumer's respond to price changes, both compensated (Hicks) and uncompensated (Marshall) price elasticities matrix were calculated. For rural households as it can be seen in Table 6, the mean values of uncompensated (Marshal) own-price elasticities are negative, which is consistent with the demand theory. The value of own-price elasticity for vegetable oil was 1.073. This implies that the rural consumers demand for vegetable oil is elastic demand, and a 1% increase in price will demand to decrease by 1.073%. For urban households, the uncompensated (Marshal) own-price elasticity of vegetable oil was detected not to be demand elastic, unlike the rural households (Table 8). This implies that the mean value is less than unity and a 1% increase of price leads to demand decrease by 0.280%.

The demand reaction of a special food-group to a change in the price of another food-group was also measured by cross-price elasticity. The complementary and substitution relationship between various food-groups determined by the

negative and positive signs of cross-price elasticity, respectively. The results of uncompensated cross-price elasticity are shown in Tables 6 and 8. For rural areas, according to the results in table 6, the food-groups of cereal, meat, fruit and nuts, sugar and spices are complementary; the groups of dairy, butter and animal oils, vegetables and legumes, and drinks are substitute goods for the group of vegetable oil. In comparison, for urban households, in the group of vegetable oils, on the other hand, cereals, meat, fruits and nuts, sugar, spices, vegetable and legumes are complementary; dairy, butter and animal oils, and drinks are substitute goods (Table 8).

The interpretation of compensated (Hicks) own and cross-price elasticities for both rural and urban areas are the identical for uncompensated, with this difference that in this type of elasticities, the effect of a change in real income is adjusted due to a change in the price, and changes in demand are only due to price changes, while uncompensated elasticities inclusive the both effects of the income and price of price changes (Table 7 and 9).

Finally, in order to evaluate the correctness of compensated, uncompensated and income elasticities, the relationship between elasticities was investigated based on the demand rules of microeconomic (Henderson & Quandt, 1971):

a- The weighted (the budget share of good i) sum of the income elasticities is equal to the unit

b- The weighted sum of uncompensated own and cross-price elasticities is equal to the negative weight of the commodity whose price has changed

c- The weighted sum of the compensated own and cross-price elasticities is zero

$$\sum_{i=1}^{n} w_i \xi_{ij} = 0$$

d- Hicks decomposition process of a demand change

$$e_{ij} = \xi_{ij} + w_i \pi_i$$

As can be seen in Tables 6, 7, 8 and, 9 the relationship between the several elasticities is established.

Finally, in this section, we investigated the urban and rural consumers' welfare by increasing the vegetable oil prices. For this purpose, vegetable oil price over the five scenarios of 20%, 40%, 60%, 80% and, 100% has been increased. The Compensating Variation (CV) to determine the welfare change was applied. The total and per household's CV are presented in Table 10. The CV results showed that with the increase in the prices under the 20%, 40%, 60%, 80% and 100% scenarios, the welfare of both urban and rural consumers will decrease. For instance, in 100% scenario, in order to reach the initial level of utility, the government compensation payments should be 21539.3 and 15510.3 billion Rials in urban and rural regions, respectively. As well as, the results of per household CV showed that to offset the effects of price increases, the rural households (2471700.8 Rials) need to pay more than urban households (1105237.3 Rials).

Table 4. ITSUR Parameter Estimates from the LA-AIDs Models (Rural households)

model	lpc	lpm	lpd	lpba	lpv	lpfn	lpvl	lps	lps	lpd	lm		DW
Cereal	0.095	-0.047	0.013	0.024	-0.05	-0.02	-0.05	-0.02	-0.003	-0.003	0.017	0.85	1.47
	(49.9)	(-24.9)	(7.67)	(7.17)	(-11.1)	(-14.2)	(-18.2)	(-11.0)	(-2.37)	(-0.95)	(12.02)		
Meat	-0.022	0.107	-0.01	0.003	0.004	0.005	-0.01	-0.001	0.004	-0.005	0.002	0.76	1.59
	(-11.4)	(53.9)	(-5.01)	(0.99)	(0.81)	(3.37)	(-3.15)	(-0.48)	(2.63)	(-1.52)	(1.77)		
Dairy	-0.017	-0.010	-0.021	0.008	0.010	-0.010	-0.007	0.002	0.003	0.00	-0.014	0.89	1.45
	(-16.8)	(-10.0)	(-22.2)	(4.55)	(4.16)	(-13.3)	(-4.75)	(2.10)	(4.68)	(-0.04)	(-18.0)		
Butter and animal oil	0.001	0.004	-0.001	-0.038	0.001	-0.001	0.002	0.002	0.0001	0.004	-0.002	0.45	1.80
	(3.07)	(9.22)	(-3.93)	(-44.6)	(0.18)	(-4.86)	(3.29)	(4.22)	(0.46)	(5.03)	(-7.68)		
Vegetable oil	-0.004	-0.012	0.004	0.005	-0.003	-0.003	0.001	-0.007	-0.003	0.004	-0.005	0.84	1.88
	(-8.6)	(-22.4)	(9.09)	(5.19)	(-2.67)	(-8.96)	(0.23)	(-13.7)	(-8.00)	(4.26)	(-13.5)		
Fruit and Nuts	-0.010	0.002	-0.000	-0.002	0.034	0.045	-0.016	0.023	0.008	0.0005	0.015	0.93	1.34
	(-9.5)	(2.13)	(-0.22)	(-1.4)	(12.8)	(56.0)	(-9.7)	(20.4)	(9.6)	(0.30)	18.5		
Vegetables and legumes	-0.032	-0.029	0.014	0.003	0.016	-0.011	0.075	0.001	-0.004	-0.009	-0.003	0.82	1.33
	(-30.9)	(-28.7)	(14.5)	(1.95)	(6.5)	(-14.7)	(45.5)	(1.72)	(-6.1)	(-5.2)	(-4.7)		
Sugary	-0.003	-0.002	0.003	-0.004	-0.001	-0.000	0.002	0.011	-0.002	0.006	-0.0004	0.69	1.63
	(-5.5)	(-3.1)	(4.9)	(-4.05)	(-0.79)	(-0.95)	(2.03)	(16.8)	(-5.08)	(5.2)	(-0.75)		
Spices	-0.002	-0.007	0.004	-0.000	0.003	-0.000	0.008	-0.001	0.001	-0.008	-0.0006	0.71	1.53
	(-6.04)	(-18.1)	(12.2)	(-0.4)	(3.07)	(-2.9)	(12.3)	(-2.7)	(5.2)	(-11.1)	(-1.9)		
Drinks	-0.003	-0.004	-0.007	0.0007	-0.011	-0.001	0.001	-0.008	-0.003	0.011	-0.0081	-	-
	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)		

Note: values in parenthesis are t-values. Drinks are calculated from adding-up conditions for that reason t-values are not available

Table 5. ITSUR Parameter Estimates from the LA-AIDs Models (Urban householders)

Model	lpc	lpm	lpd	lpba	lpv	lpfn	lpvl	lps	lps	lpd	lm		DW
Cereal	0.136	-0.071	-0.0001	0.024	-0.039	-0.023	-0.047	-0.020	-0.009	-0.013	-0.002	0.82	1.51
	(74.24)	(-37.5)	(-0.001)	(7.96)	(-14.4)	(-17.4)	(-15.5)	(-14.4)	(-6.82)	(-4.30)	(-1.66)		
Meat	-0.039	0.139	-0.003	-0.002	-0.003	-0.001	-0.015	-0.008	0.002	0.004	-0.001	0.86	1.57
	(-22.3)	(75.4)	(-1.51)	(-0.93)	(-1.43)	(-1.14)	(-5.15)	(-6.15)	(1.84)	(1.52)	(-0.90)		
Dairy	-0.015	-0.017	-0.007	-0.0001	0.001	-0.006	-0.005	0.0042	0.0040	0.006	-0.020	0.75	1.61
	(-15.9)	(-17.5)	(-7.09)	(-0.55)	(1.00)	(-8.46)	(-3.30)	(5.68)	(5.44)	(3.61)	(-24.9)		
Butter and animal oil	0.002	0.003	-0.005	-0.017	0.0014	-0.001	0.003	0.002	0.0005	0.002	-0.003	0.65	1.79
	(4.17)	(7.53)	(-9.55)	(-20.3)	(1.91)	(-3.71)	(3.90)	(5.95)	(1.45)	(3.16)	(-8.24)		
Vegetable oil	-0.009	-0.011	0.002	0.002	0.028	-0.005	-0.002	-0.005	-0.003	0.0006	-0.004	0.89	1.76
	(-18.4)	(-22.3)	(3.45)	(2.58)	(38.1)	(-13.4)	(-3.40)	(-14.9)	(-8.68)	(0.71)	(-10.49)		
Fruit and Nuts	-0.019	0.006	0.0001	-0.0001	0.013	0.052	-0.019	0.019	0.009	0.006	0.026	0.53	1.43
	(-16.9)	(5.04)	(0.09)	(-0.06)	(7.72)	(59.1)	(-9.82)	(21.62)	(10.36)	(3.08)	(26.35)		
Vegetables and legumes	-0.014	-0.033	0.012	0.0004	0.0006	-0.010	0.076	-0.001	-0.004	-0.006	0.008	0.64	1.54
	(-42.5)	(-33.1)	(10.9)	(0.24)	(0.46)	(-14.4)	(46.9)	(-1.97)	(-6.01)	(-3.59)	(9.72)		
Sugar	-0.007	-0.002	0.001	-0.005	0.001	-0.001	0.003	0.017	-0.0006	0.0059	0.003	0.62	1.69
	(-11.3)	(-4.2)	(1.86)	(-4.70)	(1.14)	(-1.51)	(2.69)	(33.5)	(-1.15)	(5.09)	(6.35)		
Spices	-0.004	-0.006	0.006	-0.0003	0.0003	-0.001	0.006	-0.001	0.003	-0.007	(0.002	0.67	1.61
	(-10.9)	(-15.3)	(12.6)	(-0.46)	(0.59)	(-3.60)	(8.52)	(-4.44)	(11.03)	(-10.0)	(6.21)		
Drinks	-0.0007	-0.0052	-0.0056	-0.0006	-0.003	-0.001	0.0016	-0.005	-0.002	0.001	-0.008	-	-
		-	-	-	-	-	-	-	-	-	-		

Note: values in parenthesis are t-values. Drinks are calculated from adding-up conditions for that reason t-values are not available

Table 6. Mean Values of Expenditure and uncompensated demand price Elasticity (Marshall Elasticity's of AIDS) for rural

Group elasticity	Expenditure	Price									
		Cereal	Meat	Dairy	Butter And animal oil	Vegetable oil	Fruit and Nuts	Vegetables and legumes	Sugar	spices	drinks
Cereal	1.066	-0.661	-0.191	0.044	0.092	-0.198	-0.081	-0.216	-0.085	-0.015	-0.014
Meat	1.013	-0.114	-0.484	-0.046	0.017	0.018	0.022	-0.050	-0.005	0.019	-0.026
Dairy	0.867	-0.123	-0.067	-1.183	0.079	0.103	-0.080	-0.052	0.027	0.038	0.003
Butter And animal oil	0.677	0.252	0.563	-0.163	-5.370	0.039	-0.162	0.329	0.253	0.029	0.488
Vegetable oil	0.872	-0.070	-0.244	0.117	0.115	-1.073	-0.067	0.022	-0.165	-0.070	0.094
Fruit and Nuts	1.162	-0.151	-0.009	-0.020	-0.030	0.350	-0.548	-0.198	0.231	0.078	0.0007
Vegetables and legumes	0.973	-0.216	-0.201	0.101	0.025	0.116	-0.075	-0.473	0.014	-0.033	-0.065
Sugar	0.992	-0.068	-0.038	0.060	-0.093	-0.024	-0.008	0.042	-0.776	-0.049	0.117
Spices	0.981	-0.075	-0.237	0.156	-0.010	0.101	-0.026	0.265	-0.037	-0.945	-0.260
Drinks	0.753	-0.026	-0.073	-0.199	-0.024	-0.347	-0.019	0.089	-0.258	-0.094	-0.647
$e_{ij} = \xi_{ij} + w_i\pi_i$		-0.070	-0.244	0.117	0.115	-1.073	-0.067	0.022	-0.165	-0.070	0.094
$\sum_{i=1}^{n} w_i\xi_{ij} = 0$		-0.269	-0.207	-0.110	-0.008	-0.045	-0.096	-0.144	-0.052	-0.031	-0.032

$\sum_{i=1}^{n} w_i\pi_i=1$

Table 7. Mean Values of compensated demand price Elasticity (Hicks Elasticity's of AIDS) for rural

Group elasticity	Price									
	Cereal	Meat	Dairy	Butter nd animal oil	Vegetable oil	Fruit and Nuts	Vegetables and legumes	Sugar	Spices	Drinks
Cereal	-0.375	0.029	0.162	0.101	-0.150	0.021	-0.062	-0.029	0.018	0.020
Meat	0.158	-0.273	0.065	0.026	0.065	0.120	0.096	0.047	0.051	0.006
Dairy	0.110	0.112	-1.089	0.087	0.142	0.003	0.072	0.073	0.065	0.032
Butter And animal oil	0.435	0.704	-0.088	-5.364	0.070	-0.097	0.427	0.289	0.051	0.510
Vegetable oil	0.164	-0.062	0.214	0.123	-1.034	0.016	0.148	-0.120	-0.043	0.123
Fruit and Nuts	0.161	0.231	0.108	-0.020	0.404	-0.436	-0.030	0.292	0.115	0.038
Vegetables and legumes	0.045	0.0004	0.209	0.043	0.161	0.018	-0.322	0.065	-0.002	-0.033
Sugar	0.198	0.167	0.170	-0.084	0.020	0.087	0.185	-0.724	-0.018	0.149
Spices	0.188	-0.033	0.264	-0.001	0.146	0.067	0.406	0.013	-0.914	-0.227
Drinks	0.176	0.082	-0.116	0.031	-0.313	0.053	0.198	-0.218	-0.070	-0.622
$\sum_{i=1}^{n} w_i \xi_{ij} = 0$	0	0	0	0	0	0	0	0	0	0

Table 8 Mean Values of Expenditure and uncompensated demand price Elasticity (Marshall Elasticity's of AIDS) for urban

Group elasticity	expenditure	Price									
		Cereal	Meat	Dairy	Butter and animal oil	Vegetable oil	Fruit and Nuts	Vegetables and legumes	Sugar	Spices	Drinks
Cereal	0.990	-0.475	-0.272	0.001	0.095	-0.152	-0.090	-0.181	-0.078	-0.374	-0.052
Meat	0.993	-0.180	-0.358	-0.013	-0.012	-0.017	-0.007	-0.069	-0.038	0.011	0.021
Dairy	0.812	-0.091	-0.012	-1.051	-0.006	0.020	-0.033	-0.022	0.047	0.043	0.060
Butter And animal oil	0.679	0.273	0.427	-0.466	-2.576	0.144	-0.089	0.341	0.227	0.062	0.261
Vegetable oil	0.889	-0.199	-0.254	0.061	0.055	-0.280	-0.112	-0.055	-0.139	-0.081	0.018
Fruit and Nuts	1.226	-0.230	0.003	-0.023	-0.003	0.108	-0.578	-0.196	0.159	0.075	0.048
Vegetables and legumes	1.058	-0.312	-0.253	0.081	0.002	0.002	-0.082	-0.460	-0.013	-0.034	-0.045
Sugar	1.074	-0.175	-0.077	0.021	-0.111	0.020	-0.024	0.051	-0.644	-0.014	0.120
Spices	1.075	-0.175	-0.243	0.198	-0.012	0.009	-0.471	0.191	-0.052	-0.878	-0.252
Drinks	0.699	0.051	-0.123	-0.172	-0.020	-0.127	-0.022	0.099	-0.198	-0.066	-0.952
$e_{ij} = \xi_{ij} + w_i \pi_i$		-0.199	-0.254	0.061	0.055	-0.280	-0.112	-0.055	-0.139	-0.081	0.018
$\sum_{i=1}^{n} w_i e_{ij} = -w_j$		-0.261	-0.217	-0.108	-0.010	-0.040	-0.116	-0.139	-0.048	-0.030	-0.027
$\sum_{i=1}^{n} w_i \pi_i = 1$											

Table 9. Mean Values of compensated demand price Elasticity (Hicks Elasticity's of AIDS) for urban

Group elasticity	Price									
	Cereal	Meat	Dairy	Butter and animal oil	Vegetable oil	Fruit and Nuts	Vegetables and legumes	Sugar	Spices	Drinks
Cereal	-0.217	-0.057	0.108	0.106	-0.112	0.024	-0.043	-0.030	-0.007	-0.025
Meat	0.079	-0.142	0.094	-0.002	0.022	0.108	0.069	0.009	0.041	0.049
Dairy	0.121	0.057	-0.962	0.002	0.053	0.061	0.090	0.087	0.067	0.082
Butter And animal oil	0.451	0.573	-0.392	-2.569	0.171	-0.010	0.436	0.260	0.082	0.280
Vegetable oil	0.032	-0.070	0.157	0.065	-0.244	-0.008	0.068	-0.096	-0.054	0.043
Fruit and Nuts	0.090	0.270	0.109	0.009	0.157	-0.435	-0.026	0.218	0.112	0.082
Vegetables and legumes	-0.036	-0.023	0.196	0.013	0.045	0.041	-0.312	0.037	-0.002	-0.016
Sugar	0.105	0.155	0.138	-0.099	0.064	0.101	0.201	-0.592	0.017	0.150
Spices	0.105	-0.009	0.315	-0.0003	0.053	0.078	0.341	-0.001	-0.872	-0.222
Drinks	0.233	0.028	-0.096	-0.012	-0.099	0.059	0.197	-0.164	-0.045	-0.933
$\sum_{i=1}^{n} w_i \xi_{ij} = 0$	0	0	0	0	0	0	0	0	0	0

Table 10. Compensated variation of vegetable oil price changes

	Compensating variation (CV)				
	20%	40%	60%	80%	100%
Total urban CV (10^9 Rials)	7996.7	14149.1	18456.9	20920.4	21539.3
Per urban household CV (Rials)	410333.6	726024.2	947071.7	1073476.1	1105237.3
Total rural CV (10^9 Rials)	3446.6	6721.3	9823.4	12753.1	15510.3
Per rural household CV (Rials)	549491.8	1071107.8	1565448.1	2032312.3	2471700.8

Conclusions and policy implications

This study examined the vegetable oil demand in Iranian's rural and urban households, separately. The COICOP data structure was used for the classification of food items in the household's food basket. Food items were classified into ten food groups i.e. Cereal, Meat, Dairy, Butter, Vegetable Oil, Fruits and Nuts, Vegetables and Legumes, Sugary, Spices, and Drinks. To estimate the vegetable oil demand equations and elasticities, the LAIDS model and ISUR technique were employed. The results revealed that vegetable oil was an elastic (-1.073) food groups in the rural household food basket, unlike it was an inelastic (-0.280) food item in urban consumers. The vegetable oil was also found to be a necessity (normal) food item for both rural (0.872) and urban (0.889) consumers. According to the results, the following suggestions are provided for policymakers:

1. The rural consumers of vegetable oils are more responsive to price changes than urban consumers because of its near substitution goods like butter and animal oil (0.115). Thus, the vegetable oil market in urban areas is stable than in rural areas.

2. Consumption of vegetable oil in the urban area has a low demand own-price elasticity, implying the importance of vegetable oil in the urban diet. The urban consumers will therefore incur a large part of the cost of rising prices due to the absence of protection policies.

3. Given that the per capita consumption of vegetable oil in rural (14.5 kg) and urban (13.29 kg) households are higher than the global average (12.5 kg), being inelastic in the urban area leads to inefficient price policy while policy reforms in marketing and trade are likely to impact the consumer's behaviors and consumption pattern reforms in the rural area.

4. Concerning vegetable oil income elasticity, as Iranian household income grows, demand for vegetable oil will continue to increase with a lower ratio.

5. In all cases, the absolute value of uncompensated own-price elasticities is greater than the absolute compensated elasticities. This indicates that consumers' response to commodity price changes is higher when income is not compensated.

6. Given the negative welfare effects associated with price reform, policymakers should mitigate these effects by design the different compensation payments for both rural and urban regions.

Compliance with Ethical Standards

Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

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