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Tarım ve Doğa Dergisi,
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Responses of *Saccharomyces cerevisiae* Cells Grown in Cultures Prepared from Different Tea Infusions to Oxidative Stress

Görkem KIRMIZIKAYA[1], Tuba OKUTAN[2], Oğuz Ayhan KİREÇCİ[3], Ökkeş YILMAZ[4]
[1, 2, 4] Department of Biology, Faculty of Science, Fırat University, Elazığ, Türkiye, [3] Department of Plant and Animal Production, Hizan Vocational School, Bitlis Eren University, Bitlis, Türkiye
[1]https://orcid.org/0000-0001-8516-4933, [2]https://orcid.org/0000-0001-8745-0343, [3]https://orcid.org/0000-0003-2205-4758
[4]https://orcid.org/0000-0002-8276-4498
✉: gkirmizikaya@firat.edu.tr

ABSTRACT

Tea is one of the most consumed beverages. *Saccharomyces cerevisiae*, a model organism in studies on oxidative stress and toxicology, was used to investigate the effects of tea on oxidative stress induced by H_2O_2. *S. cerevisiae* cultures were prepared from black, green and white tea infusions and incubated at 30°C for 72 hours. Glutathione-S-transferase enzyme activity and total protein spectrophotometric, malondialdehyde, glutathione and alpha-tocopherol and ergosterol analyses from cell pellets obtained from cultures were performed by HPLC, and fatty acids were performed by GC device. Although protein level in tea infusion groups was higher ($p<0.001$) compared to control and H_2O_2 groups, malondialdehyde level decreased ($p<0.001$). Glutathione and GST levels were decreased in other tea infusion groups except for black tea infusion and black tea infusion+H_2O_2 groups ($p<0.001$). Ergosterol levels decreased in both tea infusion and H_2O_2+tea infusion groups ($p<0.05$; $p<0.001$). While palmitic acid increased ($p<0.01$) in tea infusions and H_2O_2 groups, palmitoleic acid decreased ($p<0.05$). Stearic and oleic acid levels decreased in tea infusion groups ($p<0.05$). As a result, it has been observed that the water-soluble components of tea have effects on fatty acid biosynthesis, other metabolic products and oxidative stress.

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Glutathione-S-transferase
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Farklı Çay İnfüzyonlarından Hazırlanan Kültürlerde Yetiştirilen *Saccharomyces cerevisiae* Hücrelerinin Oksidatif Strese Tepkileri

ÖZET

Çay, en çok tüketilen içeceklerden biridir. Çayın, H_2O_2 ile oluşturulan oksidatif stres üzerindeki etkilerini araştırmak için *Saccharomyces cerevisiae*, oksidatif stres ve toksikoloji ile ilgili çalışmalarda model organizma, kullanılmıştır. Siyah, yeşil ve beyaz çay infüzyonlarından *S. cerevisiae* kültürleri hazırlandı ve 30°C'de 72 saat inkübe edildi. Kültürlerden elde edilen hücre peletlerinden glutatyon-S-transferaz enzim aktivitesi ve total protein spektrofotometrik, malondialdehit, glutatyon ve alfa tokoferol ve ergosterol analizleri HPLC ile, yağ asitleri ise GC cihazı ile yapıldı. Çay infüzyon gruplarında protein düzeyi kontrol ve H_2O_2 gruplarına göre daha yüksek ($p<0.001$) olmasına rağmen, malondialdehit düzeyi azalmıştır ($p<0.001$). Siyah çay infüzyonu ve siyah çay infüzyonu+H_2O_2 grupları dışındaki diğer çay infüzyon gruplarında glutatyon ve GST seviyeleri azaldı ($p<0.001$). Ergosterol seviyeleri hem çay infüzyonu hem de H_2O_2+çay infüzyonu gruplarında azaldı ($p<0.05$; $p<0.001$). Çay infüzyonlarında ve H_2O_2 gruplarında palmitik asit artarken ($p<0.01$), palmitoleik asit azalmıştır ($p<0.05$). Çay infüzyon gruplarında stearik ve oleik asit seviyeleri azaldı ($p<0.05$). Sonuç olarak, çayın suda çözünen bileşenlerinin, yağ asidi biyosentezi, diğer metabolik ürünlerin ve oksidatif stres üzerinde etkileri olduğu görülmüştür.

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INTRODUCTION

Camelia sinensis L. is a flowering plant species belonging to the Theaceae family, and it has been stated that tea obtained from its leaves and buds has a history dating back to 5000 years and approximately 2/3 of the world's population consumes tea (Elhadad et al. 2020; Takim & Aydemir 2022). Moreover, it has been reported that the antioxidant effect of tea is due to the flavonoids it contains (Jain et al. 2013). White tea is obtained from the buds of the tea plant and subjected to the withering process (Hilal & Engelhardt 2009). Green tea is a fermented tea produced by drying fresh leaves and exposing them to evaporation. It has a complex composition consisting of 15-20% protein by dry weight, 5-7% soluble carbohydrate components, 5% minerals and 1-4% amino acid mixture (Cabrera et al. 2006). Black tea is produced as a fermented tea following the withering of the leaves and becomes palatable by being completely oxidized during processing (Negishi et al. 2004).

The phenolic substances with the highest antioxidant effect in tea leaves are catechins, which constitute 30% of the dry weight (Almajano et al. 2008; Horžić et al. 2009). Theaflavins and thearubigins in black tea and the catechins in green tea are the components responsible for the physiological effects of tea. It has been emphasized that epigallocatechin-3-gallate in green tea is generally the most biologically active compound (Lorenz 2013). Phenolic compounds demonstrate antioxidant effects by binding free radicals, forming chelates with metals, and inactivating the lipoxygenase enzyme (Huang et al. 2005). Nevertheless, the polyphenolic compounds in tea have beneficial antioxidant activity, they also have pro-oxidant properties due to their molecular properties (Babich et al. 2008; Lorenz 2013).

Reactive Oxygen Species (ROS), which are also formed under normal metabolic conditions, can change the cellular redox balance with the increase of oxidative stress caused by the effect of toxic substances (Meng et al. 2017). Hydrogen peroxide (H_2O_2) formed during metabolic activities causes the formation of hydroxyl (OH•), a reactive and harmful free oxygen radical.

S. cerevisiae is preferred as a model organism for toxicological studies and to identify new natural compounds with antioxidant functions to protect against oxidative stress. Thence, due to the metabolic similarity of *S. cerevisiae* cells to humans, the reactive effects of infusions of tea, which is widely consumed by humans, on *S. cerevisiae* were investigated. Since tea varieties are produced by subjecting the leaves to different processes, it is important to know which tea is a potentially more beneficial beverage in terms of antioxidant activity. Thus, it was aimed to investigate the biochemical effects on *S. cerevisiae* cells in in vitro cultures with H_2O_2 added by preparing 0.2% infusions from white, green and black tea species.

MATERIALS and METHODS

Preparation of Tea Infusions and In Vitro Yeast Cell Culture

After brewing 2 g of dry tea leaves in 1 L of distilled water at room temperature for 12 hours, tea infusions were prepared by filtering them through filter papers.

Groups within the experimental study: Control (C), H_2O_2 (H), Black tea (BT), Green tea (GT), White tea (WT), Black tea + H_2O_2 (BTH), Green tea + H_2O_2 (GTH), and White tea + H_2O_2 (WTH) groups were separated.

For each group, 20 g peptone, 20 g yeast extract, 40 g D (+) Glucose were weighed and dissolved in 1000 ml distilled water. Each culture medium was divided into 200 ml cap bottles (n=5) and then sterilized in an autoclave at 121°C for 15 minutes. After the cell medium was cooled, yeast cells were inoculated into the culture medium of all groups under sterile conditions. In addition, 100 µL of 37% H_2O_2 was added to the H, BTH, GTH and WTH groups. All groups were incubated at 30°C for 72 hours. Cell pellets were then collected by centrifugation at 6000 rpm at +4°C for 5 minutes and their wet weights were determined. Cell pellets were washed with isotonic physiological buffer solution and cleared of culture broth residues.

Extraction of Cell Pellets with Tris-ETDA Buffer

Cell pellets were homogenized in 20 mM Tris-EDTA (pH=7.4) buffer at +4°C and then centrifuged. Supernatants were collected after centrifugation for analysis of total protein, GSH, GSSG, MDA, and Glutathione S-transferase (GST). The remaining cell pellet was homogenized with a 3/2, (v/v) n-hexane/isopropanol mixture (Hara & Radin 1978) and the supernatant fraction was used for fatty acid, D2, alpha-tocopherol and ergosterol analysis (Katsanidis & Addis 1999).

Measurement of GSH and GSSG by HPLC

2 ml of supernatant was taken and centrifuged by adding 2 ml of 10% perchloric acid. Then, 1 ml of supernatant was taken and analyzed by HPLC device (Prominence LC-2030 C 3D plus, Shimadzu, Kyoto, Japan). 50 mM $NaClO_4$ was used as mobile phase (acidified with 0.1% H_3PO_4) and ODS-3 column was used as analytical column. Measurements were made at a wavelength of 215 nm (Yilmaz et al. 2009).

MDA Assay by HPLC

2 ml of Tris-EDTA homogenate was taken and 2 ml of 10% perchloric acid was added and centrifuged. Then, 1 ml of supernatant was taken and analyzed by HPLC device (Prominence LC-2030 C 3D Plus, Shimadzu, Kyoto, Japan). A mixture of 30 mmol KH_2PO_4 and methyl alcohol (82.5-17.5%, pH=4) was used as mobile phase and ODS-3 column was used as analytical column. Measurements were made at a wavelength of 254 nm (Karatepe 2004).

Analysis of Fatty Acids

For extraction of lipids, cell pellets were homogenized with a 3/2, (v/v) n-hexane/isopropyl alcohol mixture and centrifuged (Hara & Radin 1978). For fatty acid analysis, 2 ml of supernatant was taken and 2% methanolic H_2SO_4 was added. It was left for 12 hours at 55°C. Fatty acid methyl esters were extracted with n-hexane and analyzed in GC 2010 Plus gas chromatography (Shimadzu, Kyoto Japan). Rxt-2330 GC column (Supelco, Sigma, USA) was used as the column during the analysis. For analysis, the column temperature was 138-218°C, the injection temperature was 240°C, and the detector temperature was 290 was programmed. Helium gas was used as the carrier gas. The results were determined as %. Calculation was done with the LC/GC 5.91 operating program.

Analysis of Lipophilic Molecules

10% KOH was added to the supernatants obtained for analysis and mixed and kept at 85°C for 15 minutes. Unsaponified lipophilic molecules were extracted with 2x5 ml hexane and evaporated under nitrogen gas. The remaining residue was dissolved in 1 ml of mobile phase mixture acetonitrile/methanol (75+25%, v/v). The mobile phase flow rate was 1 ml min^{-1}. The PDA detector was used in the analysis and Nucleodur C18 (Germany) was used as the column. Analyzes were performed at 205 nm wavelength (Katsanidis & Addis 1999; Lopez-Cervantes et al. 2006).

Determination of GST Activity

Glutathione S-transferase activity was performed according to the method of Habig et al. (1974). 1 mM 1-chloro-2,4-dinitrobenzene, 1 mM GSH and 0.1 M phosphate buffer (pH=6.5) were used for this measurement. The reaction mixture was completed to 2.9 ml with phosphate buffer by adding 0.1 ml of GSH and 0.1 ml of CDNB, and the reaction was started by adding 0.1 ml of cell homogenate. Next, the change in absorbance was measured at a wavelength of 340 nm. Results were calculated using the GST standard.

Measurement of Total Protein

The amount of protein in the samples was measured spectrophotometrically according to the method described by Lowry et al. (1951).

Statistical Analysis

SPSS package program was used for statistical analysis of the results. ANOVA and LSD test were used to determine the differences between the groups. Statistical significance was accepted as $p<0.05$. Data are given as mean± Standard error of mean .

RESULTS

Changes in total protein levels in cell pellets treated with tea infusions and hydrogen peroxide are shown in Table 1. Total protein levels in the tea infusion groups were approximately twice that of the C and H groups. The amount of MDA was found to be higher in all infusion groups compared to the control group. However, when the H group added hydrogen peroxide was compared with the BTH and GTH groups, a decrease was observed in both groups ($p<0.05$, $p<0.01$). Although there was a partial decrease in the WTH group, no statistical difference was found.-

Table 1. Variation of total protein, MDA, GSH, GSSG, and GST concentrations in tea infusions and hydrogen peroxide added cell pellets (g cell pellet)

Çizelge 1. Çay infüzyonlarında ve hidrojen peroksit eklenmiş hücre peletlerinde (g hücre peleti) toplam protein, MDA, GSH, GSSG ve GST konsantrasyonlarının değişimi

Groups	Total Protein (mg g^{-1})	MDA (nmol g^{-1})	GSH (µg g^{-1})	GSSG (µg g^{-1})	GST (µg g^{-1})
C	1.81±0.15	13.62±0.46	220.16±3.00	26.99±1.81	19.88±0.59
H	1.63±0.07 ^{a}	28.54±0.98 ^{d}	152.29±4.44 ^{d}	35.42±1.55 ^{d}	23.80±0.60 ^{b}
BT	3.62±0.07 ^{d}	20.09±1.11 ^{d}	310.48±4.89 ^{d}	58.32±1.17 ^{d}	28.00±0.89 ^{d}
BTH	3.52±0.32 ^{d}	24.79±0.76 ^{d}	251.65±6.05 ^{c}	56.77±1.91 ^{d}	23.03±1.67 ^{b}
GT	3.35±0.33 ^{d}	25.99±1.03 ^{d}	98.24±1.26 ^{d}	20.29±0.57 ^{c}	11.43±0.30 ^{c}
GTH	2.62±0.16 ^{c}	22.77±0.89 ^{d}	105.07±3.95 ^{d}	20.03±0.58 ^{c}	9.10±0.24 ^{d}
WT	3.49±0.20 ^{d}	17.26±0.98 ^{b}	181.33±4.87 ^{c}	19.41±0.70 ^{c}	16.63±0.58 ^{b}
WTH	3.77±0.13 ^{d}	27.31±0.64 ^{d}	98.78±4.63 ^{d}	8.10±0.70 ^{d}	14.60±1.36 ^{b}

C: Control; H: H_2O_2; BT: Black Tea; BTH: Black Tea+H_2O_2; GT: Green Tea; GTH: Green Tea+H_2O_2; WT: White Tea; WTH: White Tea+H_2O_2

** The evaluations in the tables were made between the control group and other groups, and the statistical signs are between the control group and other groups.

a: $p>0.05$ is not statistically significant; b: $p<0.05$ is statistically significant; c: $p<0.01$ is more statistically significant; d: $p<0.001$ is most statistically significant.

The GSH level was decreased in the H group compared to the control group. It was higher in the BT and BTH groups than in the control group. In addition, a significant decrease was observed in GSH levels in GT, GTH, WT and WTH groups compared to both C and H groups ($p<0.05$). GSH and GSSG levels were significantly decreased in GT, GTH, WT and WTH groups compared to C and H groups ($p<0.05$).

Glutathione S-Transferase (GST, EC.2.5.1.18) activity were higher in H, BT and BTH groups than in group C ($p<0.05$). However, the activity of GST decreased in GT, GTH, WT and WTH groups (Table 1).

The α-tocopherol level did not change in the BT group compared to C, but decreased in the GT and WT groups and in the tea infusion groups with added H_2O_2 ($p<0.001$). In the tea infusion and tea infusion groups to which H_2O_2 was added, the ergosterol level decreased at different rates compared to the control group ($p<0.05$). Although vitamin D level was higher in the BT group, it decreased in the GT and WT groups ($p<0.05$) (Table 2).

Table 2. Concentrations of lipophilic molecules in cell pallets developed in the medium of tea infusions (μg g^{-1} cell pellet)

Çizelge 2. Çay infüzyonları ortamında geliştirilen hücre paletlerindeki lipofilik moleküllerin konsantrasyonları (μg g^{-1} hücre peleti)

Groups	α-Tocopherol	Vitamin D2	Ergosterol
C	2.64±0.38	1.14±0.12	165.45±7.63
H	3.78±0.34 ^{c}	0.90±0.13 ^{a}	151.21±6.09 ^{b}
BT	2.78±0.40 ^{a}	2.65±0.94 ^{b}	101.94±7.02 ^{c}
BTH	1.00±0.12 ^{d}	1.04±0.41 ^{a}	90.69±5.82 ^{c}
GT	1.16±0.32 ^{d}	0.79±0.15 ^{b}	79.76±11.12 ^{d}
GTH	1.11±0.22 ^{d}	1.04±0.41 ^{a}	73.34±6.93 ^{d}
WT	0.73±0.47 ^{d}	0.73±0.47 ^{b}	116.51±31.68 ^{b}
WTH	1.26±0.27 ^{c}	0.91±0.12 ^{a}	122.82±6.43 ^{b}

C: Control; H: H_2O_2; BT: Black Tea; BTH: Black Tea+H_2O_2; GT: Green Tea; GTH: Green Tea+H_2O_2; WT: White Tea; WTH: White Tea+H_2O_2
** The evaluations in the tables were made between the control group and other groups, and the statistical signs are between the control group and other groups.
a: $p>0.05$ is not statistically significant; b: $p<0.05$ is statistically significant; c: $p<0.01$ is more statistically significant; d: $p<0.001$ is most statistically significant.

The amount of palmitic acid (16:0) was found to be significantly increased in all tea infusion groups compared to the control group ($p<0.05$). In addition, the 16:0 level was found to be high in the tea infusion groups with added hydrogen peroxide. However, palmitoleic acid (16:1, n-7) levels were decreased at different rates in both BT, GT, and WT groups and in tea infusion groups with added hydrogen peroxide compared to the control group ($p<0.05$). The amounts of stearic acid (18:0) and oleic acid (18:1, n-9) in the tea infusion groups decreased at different rates compared to the control group.

It was observed that 18:0 and 18:1 n-9 levels were decreased in the hydrogen peroxide and tea infusion groups compared to the control group ($p<0.05$), while there was no difference at the 18:0 level in the GTH group ($p>0.05$). The amount of 18:1, n-7 was higher in GT, WT, GTH, and WTH groups compared to the control group ($p<0.05$). While the amount of heptadecanoic acid (17:0) decreased in GT and WT groups compared to the control group ($p<0.05$), the amount of cis-heptadecenoic acid (17:1) increased in the tea infusion groups ($p<0.001$, $p<0.05$). The increase in the amount of 17:1 was observed to increase only in the WTH group among the groups to which hydrogen peroxide was added ($p<0.001$). (Table 3-4). The amount of cis-heptadecenoic acid (17:1) was found to be significantly higher in the tea infusion groups and in the WTH group compared to the control group (Table 3-4).

Although the amount of linoleic acid (18:2, n-6) decreased in the tea infusion groups, the amount of linolenic acid (18:3, n-3) was lower in the GT group and higher in the BT and WT groups ($p<0.001$) (Table 3). While the amount of 18:2 decreased in H and WTH groups ($p<0.05$), no difference was found in BTH and GTH groups ($p>0.05$) (Table 4). Lignoceric acid (24:0) was significantly higher in the GT group ($p<0.01$) and partially decreased in the WT group ($p<0.05$). Yet, the amount of 24:0 was slightly higher in the BTH group ($p<0.05$), and no difference was found in the other groups (Table 3-4). Moreover, the total saturated fatty acid ratio increased in the tea infusion groups, BTH and GTH groups compared to the control group, it was determined that the total unsaturated fatty acid ratio decreased (Table 3-4).

DISCUSSION

When the control and tea infusion groups were compared, the protein levels in the infusion groups were found to be higher than the control group (Table 1). In the analyzes made, it was stated that more than 1% of the dry weight of tea contains free amino acids.

It has been determined that these amino acids consist of 20 L-form amino acids that take part in protein synthesis. (Tan et al. 2011). Free amino acids in the structure of tea are active components that affect the taste, aroma and color of tea (Pastoriza et al. 2017). The reason why the amount of protein in the groups of tea infusion is higher than that of the C and H groups may be due to the high content of free amino acids in the infusions of the teas.

Table 3. Comparison of fatty acid ratios with control, hydrogen peroxide, and tea infusions added groups (%)
Çizelge 3. Yağ asidi oranlarının kontrol, hidrojen peroksit ve çay infüzyonları eklenmiş gruplarla karşılaştırılması (%)

Fatty acids	C	BT	GT	WT
10:0	0.84±0.06	1.57±0.10 ^{d}	1.36±0.22 ^{d}	0.33±0.01 ^{d}
12:0	2.97±0.09	4.04±0.24 ^{c}	4.17±0.32 ^{c}	2.49±0.07 ^{a}
14:0	2.74±0.20	4.61±0.40 ^{c}	4.34±0.09 ^{c}	5.32±0.11^{d}
16:0	29.80±0.50	33.53±0.33 ^{c}	33.87±0.74 ^{c}	37.64±0.43 ^{d}
16:1, n-7	26.57±0.65	24.70±0.43 ^{b}	25.59±0.68 ^{b}	15.23±0.32 ^{d}
17,0	0.62±0.03	0.46±0.05 ^{a}	0.35±0.02 ^{c}	0.34±0.05 ^{c}
17:1	0.51±0.05	2.36±0.17 ^{d}	3.01±0.11 ^{d}	0.85±0.85 ^{b}
18:0	10.69±0.40	7.48±0.55 ^{c}	8.99±0.38 ^{b}	8.03±0.17 ^{b}
18:1, n-9	18.11±0.55	13.38±0.66 ^{c}	9.33±0.23 ^{d}	17.86±0.22 ^{a}
18:1, n-7	1.69±0.09	2.11±0.13 ^{a}	4.44±0.93 ^{d}	7.95±0.83 ^{d}
18:2, n-6	3.22±0.32	2.12±0.22 ^{b}	2.13±0.15 ^{b}	1.98±0.10 ^{b}
18:3, n-3	1.31±0.15	2.40±0.21 ^{d}	0.70±0.12^{d}	3.38±1.02 ^{d}
24:0	0.99±0.36	1.23±0.20 ^{a}	1.72±0.50 ^{c}	0.60±0.09 ^{b}
∑ Saturated	48.62±1.59	52.93±1.29 ^{b}	54.80±1.89 ^{c}	53.75±1.11 ^{c}
∑ Unsaturated	51.32±1.72	47.07±1.69 ^{b}	45.20±1.32 ^{c}	46.25±2.09 ^{b}

C: Control; H: H_2O_2; BT: Black Tea; BTH: Black Tea+H_2O_2; GT: Green Tea; GTH: Green Tea+H_2O_2; WT: White Tea; WTH: White Tea+H_2O_2
** The evaluations in the tables were made between the control group and other groups, and the statistical signs are between the control group and other groups.
a: p>0.05 is not statistically significant; b: p<0.05 is statistically significant; c: p<0.01 is more statistically significant; d: p<0.001 is most statistically significant.

Table 4. Comparison of fatty acid ratios with control, hydrogen peroxide, and tea infusions added groups (%)
Çizelge 4. Yağ asidi oranlarının kontrol, hidrojen peroksit ve çay infüzyonları eklenmiş gruplarla karşılaştırılması (%)

Fatty acids	H	BTH	GTH	WTH
10:0	0.84±0.05 ^{a}	1.33±0.03 ^{d}	0.96±0.02 ^{a}	0.31±0.01 ^{d}
12:0	3.38±0.23 ^{a}	3.93±0.23 ^{b}	3.27±0.13 ^{a}	2.35±0.14 ^{a}
14:0	2.91±0.11 ^{a}	2.13±0.13 ^{a}	3.10±0.28 ^{a}	3.06±0.22 ^{a}
16:0	30.46±0.61 ^{a}	34.53±0.78 ^{c}	32.53±0.35 ^{b}	35.56±0.46 ^{c}
16:1,n-7	29.99±0.58 ^{c}	23.08±1.09 ^{b}	22.38±0.14 ^{b}	18.86±0.33 ^{c}
17,0	0.53±0.02 ^{a}	0.46±0.05 ^{a}	0.34±0.09 ^{b}	0.33±0.05 ^{b}
17:1	0.61±0.04 ^{a}	0.59±0.07 ^{a}	0.78±0.06 ^{a}	1.59±0.13 ^{d}
18:0	8.37±0.31 ^{c}	9.17±0.22 ^{a}	11.31±0.47 ^{a}	6.88±0.26 ^{c}
18:1, n-9	18.07±0.49 ^{a}	17.15±0.33 ^{a}	15.41±0.42 ^{b}	16.86±0.60 ^{b}
18:1, n-7	1.40±0.06 ^{a}	2.09±0.20 ^{a}	2.81±0.28 ^{b}	8.29±0.28 ^{d}
18:2, n-6	2.24±0.19 ^{b}	2.81±0.15 ^{a}	3.84±0.18 ^{a}	2.54±0.31 ^{b}
18:3, n-3	0.55±0.19 ^{d}	0.79±0.14 ^{c}	1.90±0.18 ^{b}	2.83±0.44 ^{d}
24:0	0.65±0.04 ^{a}	1.74±0.14 ^{b}	1.37±0.35 ^{a}	0.54±0.17 ^{a}
∑ Saturated	47.14±1.37 ^{a}	53.32±1.58 ^{c}	52.88±1.69 ^{c}	49.03±1.31 ^{a}
∑ Unsaturated	52.86±1.43 ^{a}	46.68 ±1.71 ^{c}	47.12 ±0.98 ^{c}	50.97±1.75 ^{a}

C: Control; H: H_2O_2; BT: Black Tea; BTH: Black Tea+H_2O_2; GT: Green Tea; GTH: Green Tea+H_2O_2; WT: White Tea; WTH: White Tea+H_2O_2
** The evaluations in the tables were made between the control group and other groups, and the statistical signs are between the control group and other groups.
a: p>0.05 is not statistically significant; b: p<0.05 is statistically significant; c: p<0.01 is more statistically significant; d: p<0.001 is most statistically significant.

Lipid peroxidation level (MDA) was higher in the H group and in the tea infusion groups to which H_2O_2 was added. However, the MDA level in the tea infusion groups was lower than in the H group. These results show that the water-soluble components in the tea content reduce the formation of free radicals in cell metabolism. According to Kırmızıkaya et al. (2021), in

their study with tea types, stated that the MDA level of red meats exposed to tea infusion at +4°C for a week decreased compared to the control group.

However, in association with higher MDA levels in the yeast groups developed in tea infusions than in the control group, it can be concluded that these compounds are due to the pro-oxidant effects. Tang et al. (2019) stated that there are components such as polyphenols, alkaloids, pigments and saponins in the extracts of tea plants. Therefore, the presence of more than one component in a mixture and not knowing their amounts may cause pro-oxidant effects and may cause harmful results instead of beneficial effects.

Yen et al. (1997) reported that tea extracts may act as pro-oxidants or antioxidants due to their ability to reduce iron and scavenge oxy-radicals. Tang et al. (2019) suggested in their study that although the extracts of some tea varieties contain very high levels of phytochemicals, their bioavailability is low. On the other hand, Dani et al. (2008) emphasized that the addition of polyphenols to the commonly used cell culture medium can cause significant amounts of hydrogen peroxide formation and OH• radical formation and oxidative stress.

GSH is among the most important molecules of the protective system against free radicals in all cells. GSH level was decreased in group H compared to group C. It was higher in BT and BTH groups than in group C. However, a decrease was observed in other tea groups. GSH is an antioxidant molecule that is mainly effective against hydrogen peroxide. A decrease in GSH can be seen naturally in groups to which hydrogen peroxide is added. Because when there is not enough NADPH molecule in the environment, the GSSG level also rises. We think that this hypothesis is supported by the higher GSSG level compared to group C.

The GSH level was found to be higher in the BT and BTH groups than in the C group. We think that this is due to the high levels of free amino acids such as glutamic acid, cysteine and glycine (Tang et al. 2019) used in GSH synthesis in black tea infusions. Because black tea is prepared by crushing wilted tea leaves, it contains limited amounts of catechin and more abundant theaflavin (Babich et al. 2008). We think that this view can be supported by the decrease in GSH in the BTH group compared to the BT group. Because HO• radicals formed from hydrogen peroxide convert the H_2O_2 of the GSH molecule to water and molecular oxygen to prevent radical formation, while the formation of GSSG molecules in the cell increases.

The high amount of GSSG in the BT and BTH groups may be due to insufficient glutathione reductase enzyme activity or the lack of sufficient NADPH molecules. In addition, high GSH levels in yeast are an important criterion for recovery from acute peroxide stress (Spector et al. 2001). It was observed that both GSH and GSSG molecules decreased significantly in green and white tea infusion groups compared to C and H groups. We think that the reason for the decrease in GSH and GSSG here is partially different from other groups. Tea plants contain different polyphenols (Almajano et al. 2008). Many of these polyphenols can have a pro-oxidant effect on GSH synthesis and lead to a decrease in GSH level.

Although the majority of research on black tea and health focuses on its antioxidant properties, the catechins and theaflavins found in green tea have also been reported to exhibit pro-oxidant behavior (Babich et al. 2008). When the total amount of GSH decreases, the rate of GSSG may decrease. The high MDA level and low GSH and GSSG in the WTH group supports this hypothesis. Another reason is that all physiological events in the cell system work at a normal level and there is no need for high-level synthesis of molecules such as GSH.

Glutathione S-Transferases (GSTs, EC.2.5.1.18) are a superfamily of multifunctional enzymes that detoxify xenobiotic compounds by binding GSH to a hydrophobic substrate. According to results, activity of GST was higher in H, BT and BTH groups compared to the control group. The yeast cell would need to synthesize the GST enzyme under reasonable conditions to protect itself against toxic H_2O_2 in the environment. GST is an antioxidant enzyme that has a purifying effect on both the toxic effect of H2O2 and the cellular damage caused by the hydroxyl radical. This may be due to the high activity of GST in group H. The activity of GST was higher in the BT group. This result is in parallel with the increase in the activity of GSH. Similarly, the decrease in the activity of GST in green and white tea groups is parallel to the decrease in the amount of GSH. Black tea contains limited amounts of catechins and larger amounts of theaflavin derivatives, which are considered biologically important and health-beneficial active ingredients (Leung et al. 2001). The differences between black tea and green and white tea may be due to the further processing of black tea. As is known, some of the compounds with pro-oxidant effect may be lost during processing.

The *S. cerevisiae* cell has the capacity to synthesize all the molecules it needs in the current culture medium. It also synthesizes lipophilic molecules such as ergosterol and α-tocopherol. As shown in Table 2, α-tocopherol level decreased in GT and WT groups. In addition, it decreased in tea groups with H_2O_2 added compared to C and H groups.

Alpha-tocopherol is a molecule with a very high antioxidant potential, tends to interrupt free radical chain reactions and protect polyunsaturated fatty acids and cell membranes (Tufarelli 2014; Izah et al. 2019). We think that the reason for the increase in α-tocopherol levels in the H_2O_2 added groups and the

decrease in the tea added groups is the prooxidant effect of the water-soluble molecules in the tea infusions on the synthesis. Because when the cell encounters abnormal situations, it activates its mechanisms and protects itself. An example of this is the increase in the amount of α-tocopherol in the H group. However, although the MDA level was high in the groups to which tea infusion was added, the lack of high synthesis of molecules such as α-tocopherol that disrupt the free radical reaction supports the above hypothesis.

Ergosterol is one of the most important lipophilic molecules in the membrane structure of yeast cells, and it plays an important role in vitality, membrane fluidity, and permeability (Hata et al. 2010). *S. cerevisiae* contains all genes necessary for the production of ergosterol (Mantzouridou et al. 2009). Ergosterol, one of the most important sterols in cells such as *S. cerevisiae*, is used as a precursor of vitamin D2. Ergosterol levels decreased in the groups in which both tea infusion and tea infusion and H_2O_2 were added compared to the control group.

Under stress conditions, it has also been found that the ability of the yeast cell to tolerate stress is closely related to ergosterol levels. It has been stated that the ergosterol content of yeast resistant to low sugar fermentation conditions is high and *S. cerevisiae*, which is exposed to alcohol effect for a long time, can increase the ergosterol content in the cell membrane to protect the membrane structure. Similar results were obtained in the *S.cerevisiae* erg6 mutant, in which the ergosterol content in the cell membrane decreased and the cells became more susceptible to alcohol stress (Hu et al. 2017).

As shown in Table 3, the amount of palmitic acid was found to be significantly increased in the whole tea infusion and H_2O_2 added tea infusion groups compared to the control group. These results lead us to the conclusion that some water-soluble compounds in tea plants have a positive effect on fatty acid synthetase enzyme activity and increase the transcription of this enzyme. Fatty acid biosynthesis in yeast cells is similar to higher organisms, although there are some differences. Malonyl-CoA molecules synthesized by the Acetyl-CoA Carboxylase enzyme serve as the precursor molecule for palmitic acid synthesis formed by the multifunctional fatty acid synthetases in yeast, consisting of two subunits encoded by FAS1 and FAS2. The end product of fatty acid synthetases is palmitic acid and can be converted to stearic acid in yeast with an extension encoded by ELO1. Both palmitic and stearic acids can be converted to the monounsaturated fatty acids palmitoleic and oleic acids in yeast by the enzyme Steroyl CoA desaturase (SCD) encoded by the OLE1 gene.

S.cerevisiae can de novo form all essential fatty acids and also readily absorbs a wide variety of exogenous long-chain saturated and polyunsaturated fatty acids from the growth medium and rapidly incorporates them into membrane lipids. As seen in Table 3, while palmitic acid level increased in *S.cerevisiae* groups developed in tea groups compared to control group, palmitoleic acid level decreased. It has been reported that the membrane lipids of *S.cerevisiae* may be affected by different physiological and nutritional conditions. It has been suggested that OLE1 gene expression, which provides the synthesis of single-double-bonded unsaturated fatty acids, is affected by factors such as carbon source, nutritive fatty acids, metal ions, and oxygen levels, and may respond differently to these (Martin et al. 2007).

It was observed that both 18:0 and 18:1 in the fatty acid composition decreased at different rates compared to the control group (Table 3). Although 18:0 decreased in all groups developed in hydrogen peroxide and tea infusion, it was determined that it decreased 18:1 only in GTH and WTH groups (Table 4). It can be thought that the fluctuations in the 18:0 and 18:1 levels are due to the changes in the activities of the elongaz and SCD enzymes.

Ding et al. (2015) found that some molecules in tea inhibit the expression of genes that play a key role in lipid biosynthesis and the biosynthesis of the SCD enzyme. In addition, Yuan et al. (2009) stated that SCD reduced mRNA expression in adipose tissue of rats. Ding et al. (2015) found that the amount of 16:0 increased partially and the amount of 18:0 significantly increased in the groups given PTE. They emphasized that the ratio of unsaturated fatty acids such as 18:1 and the ratio of 18:1n-9/18:0 decreased with increasing PTE concentration. Ding et al. (2015) were found to be similar to the results of their study.

Heptadecenoic acid (17:1 n-9) was found over 1% in BT and GT groups. It was also found to be higher in the WT group than in the control group (Table 3). The amount of 17:1 in the WTH group, one of the tea infusion groups to which hydrogen peroxide was added, was determined as 1.59% (Table 4). We think that this increase in the amount of 17:1, which is generally more than 1% in fatty acid composition, may be due to the effect of water-soluble compounds found in aqueous infusions of tea species. It has been reported that when propionic acid is present in the normal culture medium of *S.cerevisiae* cells, this fatty acid facilitates heptadecenoic acid synthesis by increasing the activity of the propionyl-CoA synthetase enzyme (Pronk et al. 1994). The 17:1 level of *S.cerevisiae* cells developed in the white tea infusion was found to be higher than the C and H groups. This may be due to the high amount of organic acids, acetic acid and propionic acid found in white tea. Dias et al. (2013) reported that the main components of tea are proteins, polysaccharides, polyphenols, minerals and trace elements, amino and organic acids, lignins and

methyl xanthines. When the chemical composition of kombucha infusions was examined, significant levels of propionic acid were found (Huang et al. 2016).

CONCLUSION

Due to the metabolic similarity of *S. cerevisiae* cells to humans, the reactive effects of commonly consumed tea on *S. cerevisiae* in the oxidative stress environment created by hydrogen peroxide were investigated. Though it is known that teas inhibit lipid peroxidation, the present study has shown that infusion forms of teas have pro-oxidant properties. However, it has also been found to have effects on fatty acid synthesis, vitamin synthesis, and the synthesis and use of glutathione, the cell's defense molecule. Although this study was conducted on *S. cerevisiae*, the present results showed that widely consumed tea varieties should be consumed more carefully by humans due to their health effects. More comprehensive and molecular studies are needed to define the specific effects of teas on the metabolism of organisms.

Contribution of the Authors as Summary

Authors declares the contribution of the authors is equal.

Statement of Conflict of Interest

Authors have declared no conflict of interest.

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Antioxidant and Antiradical Properties of *Rhabdosciadium anatolyi* Flowers and Contents of Vitamin, Trace Element and Mineral

Ahmet BAKIR[1], Suat EKİN[2], Mehmet FIRAT[3]
[1,2]Department of Chemistry, Division of Biochemistry, Faculty of Science, Van Yuzuncu Yil University, Van, TÜRKİYE. [2]Department of Biology, Faculty of Education, Van Yüzüncü Yıl University, Van, TÜRKİYE.
[1]https://orcid.org/0000-0003-0797-285X, [2]http://orcid.org/0000-0002-6502-5028, [3]https://orcid.org/0000-0001-5814-614X
✉: ahmetbakir@yyu.edu.tr

ABSTRACT

The aim of this study was to investigate the antioxidant and antiradical activity of the *Rhabdosciadium anatolyi* (*R. anatolyi*) flowers, an endemic plant grown in the Eastern Anatolia Region of Turkey, by determining the level of vitamins E and C, mineral (Ca, Na, Mg, P, K), and trace elements (Mn, Zn, Cu, Fe, V, Cr, Mo, Co, Sr, Pb, Ti, Tl, Sn, Cd, As). Within the scope of the study, mineral and trace element analyzes were carried out by ICP-OES and AAS, Vitamin E by HPLC, Vitamin C, total phenolic content, total flavonoid, antioxidant capacity, hydrogen peroxide, DPPH, ABTS, superoxide, hydroxyl and hemolysis of erythrocytes with phenylhydrazine, radical scavenging activity of the *R. anatolyi* flowers methanol extract were determined spectrophotometrically. According to the results, α-tocopherol, ascorbic acid, phenolic content, flavonoid content and total antioxidant activity of the *R. anatolyi* flowers were 3,99 ± 0,35 µmol kg^{-1}, 346.27 ± 6.51 mg 100 g^{-1}, 21.94 ± 0.37 mg gallic acid g^{-1}, 5.60 ± 0.13 mg quercetin g^{-1} ve 29.65 ± 0.26 mM ascorbic acid g^{-1}, respectively. Consequently, the mineral, trace element, vitamin E and vitamin C, total phenol and flavonoid levels in the *R. anatolyi* flowers were high, and the *R. anatolyi* flowers methanol extract effectively inhibited free radicals. From this point of view, it is thought that it can be used in the preventive treatment of many diseases that may be caused by free radical species and that these data will be a reference for other studies.

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Rhabdosciadium anatolyi Çiçeğinin Antioksidan ve Antiradikal Özellikleri ile Vitamin, İz Element ve Mineral İçerikleri

ÖZET

Bu çalışmanın amacı, Türkiyenin Doğu Anadolu Bölgesi'nde yetişen, endemik bir bitki olan *Rhabdosciadium anatolyi (R. anatolyi)* çiçeğinin antioksidan, antiradikal aktivitesi-ile E ve C vitaminleri, mineral (Ca, Na, Mg, P, K) ve iz element (Mn, Zn, Cu, Fe, V, Cr, Mo, Co, Sr, Pb, Ti, Tl, Sn, Cd, As) düzeylerini belirlemektir. Çalışma kapsamında mineral ve iz element analizleri ICP-OES ve AAS ile E vitamini HPLC ile C vitamini, *R. anatolyi* çiçeği metanol ekstraktının toplam fenolik içeriği, toplam flavonoid içeriği ve antioksidan kapasitesi, hidrojen peroksit, DPPH, ABTS, süperoksit, hidroksil ve eritrositlerin fenilhidrazin ile hemoliz oluşumu sonucu *R. anatolyi* çiçeğinin radikal süpürücü aktivitesi spektrofotometrik olarak tespit edildi. *R. anatolyi* çiçeğinin α-tokoferol, askorbik asit, fenolik ve flavonoid içeriği ile toplam antioksidan aktivitesi sırasıyla 3,99 ± 0,35 µmol kg^{-1}, 346.27 ± 6.51 mg 100 g^{-1}, 21.94 ± 0.37 mg gallik asit g^{-1}, 5.60 ± 0.13 mg kuersetin g^{-1} ve 29.65 ± 0.26 mM askorbik asit g^{-1} olarak tespit edilmiştir. Sonuç olarak *R. anatolyi* çiçeğinin içeriğinde mineral, iz element, E ve C vitaminleri, toplam fenol ve flavonoid düzeylerinin yüksek olduğu, *R. anatolyi* çiçeği metanol ekstraktının serbest radikalleri inhibe etmede etkili olduğu

Biyokimya

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Anahtar kelimeler
Antioksidan
İz element
Rhabdosciadium anatolyi
Serbest radikal
Vitamin

belirlendi. Bu açıdan bakıldığında serbest radikal türlerinin neden olabileceği birçok hastalığın koruyucu tedavisinde kullanılabileceği ve ayrıca bu verilerin başka çalışmalar için bir referans olacağı düşünülmektedir.

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INTRODUCTION

With the first civilizations, human beings have taken nature and events in nature under a keen observation. The first observation here is that it has been more or less determined that the assets in the environment would be used as raw and cookable, and that medicinal quality substances can be obtained. When we look at the historical process, the direct and indirect contribution of plants in meeting the basic needs of human beings such as nutrition, shelter, treatment and clothing is an important phenomenon (Küçüker, 2015). In general, it is a fact that plants are the first natural treatment material that people find healing when they get sick, in both in the past and today (Tanker et al., 1998; Mohammed et al.,2020).

Food products are in a critical position to strengthen immune system and live in a way that will increase health level to higher levels. In addition to nutrition, functional foods taken into the organism with nutrients have therapeutic and protective effects from diseases with their special active substance content. In recent years, it is seen that studies on traditional medicine have intensified and the active ingredients in these nutrients have positive effects on the immune system with their secondary compounds (Gürsel, 2014).

Although the treatment of living organisms with plants goes back to ancient times, herbal treatment has gained even more importance with the acceleration of studies on the damage caused by free radicals on living organisms. Because free radicals are a process in which both endogenous and exogenous factors that the living organism is exposed to, emerge at the end of a series of biochemical processes (Dröge, 2002). It is only possible for a living organism to turn this process in its favor with a correct mechanism. If we say that plants are in a critical position in this mechanism, we would not be wrong at all.

600 genera belonging to the Apiaceae family have been recorded so far (Çağın, 2005). The *R. anatolyi* species is a member of the Apiaceae family, belonging to the genus *Rhabdosciadium* Boiss. This plant is an endemic species that spreads only at 2400-2800 m in the Hakkari province region of Turkey. It is known that the Apiaceae family has been used as medicine and food for a long time, as well as having the largest, most cosmopolitan and economic importance (Burnie, 1996; Hançer et al., 2017).

The aim of this study is to investigate the content of *R. anatolyi* flowers, which an endemic plant is growing around Hakkari province in the Eastern Anatolia Region, which has a rich flora, by various methods. For this purpose, total phenol, flavonoid and antioxidant capacity, vitamin E and C content, trace element and mineral levels of flowers methanol extract of *R. anatolyi* flowers were determined. On the other hand, DPPH, ABTS, superoxide (O_2-), hydrogen peroxide (H_2O_2), hydroxyl (HO·) and anti-hemolytic activity methods were used to determine the radical scavenging effect of *R. anatolyi* flowers.

MATERIALS and METHODS

Plant Material and Extraction Processes

The *R. anatolyi* flowers used in the study was collected in Hakkari Province, Yüksekova town, Sat Mountains, Şitazin Gera Mezin (Sat glacial lake/ Big lake) region, at an altitude of 2356 meters and at 37º 22' 41" N - 44º 10' 08" D coordinates. Species identification of the plant was carried out by Research Assistant Mehmet FIRAT at Van Yüzüncü Yıl University. The witness plant sample is stored in the herbarium with the code 34041 (VANF). The plant specimen was dried in a cool shade, not exposed to sunlight. The dried part of the plant was pulverized in the herb grinder. 20 g were weighed on a precision balance and 400 mL of methanol (75%) was added. It was kept in a magnetic stirrer at 24 ºC (room temperature) for 48 hours. It was then filtered on filter paper. In order to remove the added methanol, it was treated in the evaporator and lyophilized at -65°C for 24 hours. The dried extract was stored at +4 ºC to be used in the study.

Determination of Vitamin C

The determination of the vitamin C amount of the *R. anatolyi* flowers was carried out by measuring spectrophotometrically at 521 wavelengths. Stock solutions of vitamin C were prepared in 4000 mg mL^{-1} metaphosphoric acid. After 1 gram of plant was weighed and transferred to the tubes, 2,4 dinitrophenylhydrazine was added and kept in a 90

°C water bath. After the water bath, sulfuric acid solution was added to them. The tubes were brought to room temperature and vortexed. Finally, measurements were made (Shimadzu UV 1800, Japan) and their absorbance was recorded. The absorbic acid concentrations of the samples were calculated using the obtained calibration chart (Brewster, 1984; Golubkina et al., 1989).

Determination of Vitamin E

Standart solutions and calibration

Stock solutions of vitamin E (α-tocopherol) were prepared in 500 µg mL^{-1}. Methanol was used to dilute the standard solutions. Calibration was calculated using linear regression analysis of the peak area of standard solution concentrations.

Extraction process

In the study, vitamin E amounts of *R. anatolyi* flowers were determined by modifying the method used, in accordance with (Sahin et al., 2005; Al-Saleh et al., 2006). From the dried plant samples and ground in the shade, 5 g was weighed and extracted with n-hexane and ethanol. 0.01% BHT was added to them, vortexed, and kept in the dark for 24 hours. Then centrifuged at +4 °C and 4000 rpm for 10 minutes. The supernatant was filtered using whatman filter paper. Then 500 µl of n-hexane was added and evaporated with nitrogen gas (37 °C). After drying, the residue was dissolved in a mixture of 0,2 mL methanol + tetrahydrofuran and made ready for analysis.

Chromatographic conditions

Analyzes, Gl Science C_{18} reverse phase HPLC column (250x4.6 mm ID), methanol + tetrahydrofuran (80:20) mobile phase, at a flow rate of 1500 µL min^{-1}, at a temperature of 25 °C. Thermo Scientific Finnigan Surveyor model in high performance liquid chromatography, using a PDA array detector, applications in a volume of 100 µL in dark-colored vials in tray autosampler (-8 °C) were performed at 290 nm α-tocopherol. Chromatographic analysis concentrated by isocratic elution (40 ºC).

Determination of Total Phenol Content

The Folin-Ciocelteu (FCR) marker was used to determine the total phenol content of the *R. anatolyi* flowers extract, in accordance with (Yi et al., 1997; Gamez-Meza et al., 1999). After adding 0.3 mL of 2% Na_2CO_3 to the flowers extract samples prepared by diluting with methanol, 0.1 mL of foline reagent was added and incubated for 2 hours at room temperature. The absorbances of the samples were read at 765 nm wavelength. Phenolic contents were expressed as gallic acid equivalents per weight (mg GAE g^{-1}) of the prepared extract.

Determination of Total Flavonoid Content

In order to determine the flavonoid content of the *R. anatolyi* flowers extract used in the study, 100 µL of potassium acetate was added to 0.5 mL of the stock solution diluted with methanol, and 0.1 mL of $Al(NO_3)_3$ and 4.6 mL of ethanol were added. At the end of this process, the solutions were vortexed and incubated for 40 minutes at room temperature. Finally, the absorbances of the samples were read against the control sample at a wavelength of 415 nm (Lamasion et al., 1990). The total favonoid amounts of the samples were given as mg g^{-1} as quercetin equivalent.

Determination of Total Antioxidant Capacity

The spectrophotometric method developed according to Prieto et al. (1999) was modified and used for the quantitative determination of the total antioxidant capacity of the *R. anatolyi* flowers extract. The main goal of this method is based on the reduction of acidic Molybdenum (VI) to Molybdenum (V) and the formation of a green colored phosphate/Molybdenum (V) composition at acidic pH. 0.2 mL of the flowers extract of the *R. anatolyi* flowers diluted with methanol in different concentrations was taken, and 2000 µL of reagent solution (0.6 M sulfuric acid, 0.028 M sodium phosphate and 0.004 M ammonium molybdate) was added to them, and then they were kept in a water bath at 95 ºC for 90 minutes. The samples were cooled at room temperature in an ice bath and read against the control sample at a wavelength of 695 nm. Antioxidant capacities of the samples were given as mM ascorbic acid g^{-1}.

DPPH Radical Scavenging Capacity

This method, which is used for the determination of antioxidant activity, is based on the principle of changing the purple color as a result of adding any synthetic antioxidant compound to the DPPH radical solution prepared with methanol (Cuendet et al., 1997; Chen et al., 2009). In case determine the DPPH radical scavenging feature of the *R. anatolyi* flowers extract, 5 mL of 0.004% DPPH solution was added to the solutions of different concentrations prepared by diluting with methanol and incubated for 30 minutes at room temperature. The absorbances of the samples were read against the control sample at a wavelength (517 nm). The % inhibition values obtained were plotted against the concentration and the values (IC_{50}) of *R. anatolyi* flowers extracts that inhibited DPPH radical by 50% were determined. Butylated hydroxy toluene (BHT) was used as a positive control.

$$\text{İnhibisyon (\%)} = \left[\frac{A_{blank} - A_{sample}}{A_{blank}}\right] \text{x } 100$$

ABTS Radical Scavenging Capacity

ABTS radical is based on the principle of decreasing color intensity by scavenging it by a compound obtained by the reaction of ABTS salt and $K_2S_2O_8$, a strong oxidizing agent (Re et al., 1999). The $ABTS^{\cdot+}$ radical scavenging effect of the *R. anatolyi* flowers extract was performed with the prepared 100 mM phosphate buffer with (pH: 7.4). 2.45 mM potassium persulfate solutions were prepared with 2 mM ABTS solution. Then, these two solutions were mixed and mixed with a magnetic stirrer for 10-18 hours at room temperature/dark environment. The prepared solution was calculated by reading its absorbance in the spectrophotometer at 734 nm. The stable free radical scavenging ability of the molecules was done with trolox, a synthetic antioxidant. For the control preparation, the ABTS solution was diluted with buffer (pH: 7.4) so that the absorbance was 0.70 at 734 nm. $ABTS^{\cdot+}$ radical scavenging activity (%) was calculated with the help of the following formula.

$$\text{İnhibisyon (\%)} = [\frac{A_{blank} - A_{sample}}{A_{blank}}] \text{x } 100$$

Determination of Hydrogen Peroxide (H_2O_2) Scavenging Activity

The hydrogen peroxide removal activity of the extract of the flowers part of the *R. anatolyi* flowers prepared with methanol was determined by reading it at a wavelength of 230 nm with a spectrophotometer device. First, 43 mM hydrogen peroxide (H_2O_2) solution was prepared in the prepared phosphate buffer (pH: 7.4). The volume of the *R. anatolyi* flowers extract taken at different concentrations and BHT solutions, which is the standard antioxidant substance used in the study, was completed with a buffer solution up to 0.4 mL. Then 600 µL of hydrogen peroxide solution was added. After 10 minutes of incubation at room temperature, the decreasing amount of hydrogen peroxide was recorded at 230 nm (Ruch, 1989).

Superoxide ($O_2^{\cdot}$) Radical Scavenging Capacity

The scavenging effect of the *R. anatolyi* flowers extract on superoxide anion radicals was determined by spectrophotometric measurement of nitro blue tetrazolium (NBT) at 560 nm (Zhishen et al., 1999). As a result of testing the solutions prepared at different concentrations, the most appropriate 45 µg mL^{-1} was prepared with phosphate buffer of 0.05 M pH: 7.8, so that the concentrations of the standard antioxidant substance BHT solutions to be compared with were the same. Equal proportions of riboflavin, L-methionine and NBT (200 µl) were added to these prepared solutions. The resulting reaction mixture was exposed to fluorescent light for 40 minutes at room temperature. The scavenging effect of the *R. anatolyi* flowers extract on superoxide anion radicals was compared with that of BHT, which has antioxidant properties. Absorbance was recorded at 560 nm against the water blank. The % inhibition value according to the change in the absorption of the control was determined according to the formula below.

$$\text{İnhibisyon (\%)} = [\frac{A_{blank} - A_{sample}}{A_{blank}}] \text{x } 100$$

Hydroxyl ($OH^{\cdot}$) Radical Scavenging Capacity

2.8 mM deoxyribose, 0.001 M $FeCl_3$, EDTA, ascorbic acid and H_2O_2 solutions were added to the flowers part samples of the *R. anatolyi* flowers prepared from different concentrations of the extract in equal proportions and finalized. Volume was made up with 1 mL of 20 mM phosphate buffer (pH: 7.4). After vortexing, the reaction mixture was incubated at 37 °C for 1 hour. Then, 1 mL of TBA and 1 mL of TCA were added and vortexed again, and then boiled at 100 °C for 35 minutes. The absorbance of the colored mixture formed by the released MDA with TBA was read against the control sample at 532 nm (Kunchandy and Rao., 1990). IC_{50} values were determined by plotting the % inhibition values obtained against different concentrations of the plant extract. The results were compared with BHT. The % inhibition value according to the change in the absorption of the control was determined according to the formula below.

$$\text{İnhibisyon (\%)} = [\frac{A_{blank} - A_{sample}}{A_{blank}}] \text{x } 100$$

Hemolysis of Erythrocytes by Phenylhydrazine and Radical Scavenging Activity of *R. anatolyi* Flowers

1 mL of phenylhydrazine, 0.1 mL of 20% PCV, 1,850 mL of buffer were added to the samples prepared from different concentrations of the methanol extract of the flowers part of the *R. anatolyi* flowers. After incubation at 37 °C for 1 hour, it was centrifuged at 4000 rpm for 10 minutes. After the supernatant was transferred to other tubes, its absorbance at 540 nm was read against the control sample. Results were compared with BHT (Valenzuela, 1977).

Trace Element and Mineral Determination

Quantification of the minerals in the flowers of the *R. anatolyi* flowers was carried out using the dry burning method. The flowers part of the plant, which was dried and ground before, was weighed according to this method and placed in porcelain crucibles. 2 mL of ethyl alcohol-sulfuric acid mixture was added to each sample. The prepared samples were burned in a fume hood. Then it was left in the ash furnace set at 250 °C. Porcelain crucibles placed in the muffle furnace were started at 250 °C and the temperature was increased by 50 °C every hour to 550 °C. While

the temperature was at 550 ºC, porcelain crucibles were kept open in the muffle furnace until the next day. Then, 5000 µL of hydrochloric acid (HCl) was added to them. Finally, it was filtered on filter paper and made ready for elemental analysis. Analyzes of Ca, K, Mg, Na and Fe elements were performed using Atomic Absorption Spectrometer (AAS). In addition, P, Zn, Cu, Co, Mn, Cd, Pb, Cr, Ti, Sr, As, Tl, Sn, Mo and V elements were analyzed using Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES).

Statistical Analyses

The means and standard error of the data were expressed as (X ± SEM). Group plots were created by finding mean and standard error values (X ± SEM). Nonlinear regression analysis was used to determine IC_{50} values. Measurements of the samples were performed in triplicate.

RESULTS and DISCUSSION

To determine the antioxidant properties of methanol flowers extract of *R. anatolyi* flowers, total antioxidant capacity, total phenolic and total flavonoid contents, DPPH, ABTS, hydroxyl radical, superoxide radical, hydrogen peroxide and anti-hemolytic activity were measured. In addition, *R. anatolyi*'s vitamin E and C, element (Fe, Zn, Cu, Co, Mn, Cd, Pb, Cr, Ti, Sr, As, Tl, Sn, Mo and V) and mineral (Ca, K, Mg, Na and P) levels were determined and the results are shown in Tables 1 and 2.

Table1. Vitamin E and C, total phenolic and flavonoid content of *R. anatolyi* flowers, total antioxidant capacity, element (Cu, Zn, Fe, Co, Mn, Cd, Pb, Cr, Ti, Sr, As, Tl, Sn, Mo and V) and mineral (Ca, K, Mg, Na and P) levels

Çizelge 1. R. anatolyi çiçeğinin E ve C vitamini, toplam fenolik ve flavonoid içeriği, toplam antioksidan kapasitesi, element (Cu, Zn, Fe, Co, Mn, Cd, Pb, Cr, Ti, Sr, As, Tl, Sn, Mo ve V) ve mineral (Ca, K, Mg, Na ve P) düzeyleri

Parameters	*Rhabdosciadium anatolyi* ($\bar{X}$ ± SEM)
α-tocopherol (µg g^{-1})	3.99 ± 0.35
Vitamin C (mg 100 g^{-1})	346.27 ± 6.51
Total phenolic content (mg GA g^{-1})	21.94 ± 0.37
Total flavonoid content (mg QE g^{-1})	5.60 ± 0.13
Total antioxidant capacity (mM A.A g^{-1})	29.65 ± 0.26
Sn (µmol kg^{-1})	0.22 ± 0.086
V (mmol kg^{-1})	0.017 ± 0.00033
Ti (mmol kg^{-1})	0.066 ± 0.0028
Cr (mmol kg^{-1})	0.012 ± 0.00026
Cu (mmol kg^{-1})	0.079 ± 0.0061
Sr (mmol kg^{-1})	0.088 ± 0.0059
As (µmol kg^{-1})	0.15 ± 0.062
Tl (µmol kg^{-1})	0.35 ± 0.063
Cd (µmol kg^{-1})	0.21 ± 0.044
Co (µmol kg^{-1})	2.56 ± 0.11
Pb (µmol kg^{-1})	1.81 ± 0.021
Mo (µmol kg^{-1})	0.83 ± 0.14
Mg (mmol kg^{-1})	3.64 ± 0.0063
Fe (mmol kg^{-1})	0.064 ± 0.0017
Mn (mmol kg^{-1})	0.43 ± 0.021
P (mmol kg^{-1})	0.081 ± 0.0028
Na (mmol kg^{-1})	2.03 ± 0.027
Ca (mmol kg^{-1})	2.61 ± 0.11
K (mmol kg^{-1})	2.09 ± 0.037
Zn (mmol kg^{-1})	0.38 ± 0.015

Values are expressed as mean ± standard error of mean (X ± SEM). Samples were performed in triplicate.

The graph showing the inhibition percentages and IC_{50} values of BHT, which is the positive control, of hydroxyl (OH^-) and hydrogen peroxide (H_2O_2) radicals of the methanol extract of *R. anatolyi* flowers is shown in Figure 2.

For *R. anatolyi* flowers and BHT, the values showing the hemolysis formation of erythrocytes with phenylhydrazine, the % inhibition of radical scavenging activity of *R. anatolyi* flowers and the change of IC_{50} values, and the percentages of superoxide radical inhibition corresponding to the concentration of the BHT standard (45 µg mL^{-1}) are given in Figure 3.

Figure 1 shows % inhibition and IC_{50} values of $ABTS^{\cdot+}$ radical and BHT and DPPH radical for *R. anatolyi* flowers and trolox.

Recent studies have focused on the term functional nutrients, and studies have been conducted and are still being carried out, showing that plants (phytochemicals) can contribute to health, especially the prevention of diseases such as cardiovascular, cancer and osteoporosis (Hasler, 2002; Çoşkun, 2011).

Table 2. % Inhibition and IC_{50} (µg mL^{-1}) values in the methanol extract of the flowers part of the *R. anatolyi* flowers compared with positive controls.

Çizelge 2. Pozitif kontrollerle karşılaştırıldığında R. anatolyi çiçek kısmına ait metanol ekstraktındaki % İnhibisyon ve IC_{50} (µg mL^{-1}) değerleri.

	Control	% Inhibition ($\bar{X}$ ± SEM)	IC_{50} (µgmL^{-1})($\bar{X}$ ± SEM)
DPPH·		71.27 ± 4.47	95.28 ± 1.61
	BHT	79.64 ± 3.49	71.18 ± 2.28
OH·		79.53 ± 0.17	136.96 ± 1.65
	BHT	79.30 ± 0.98	57.74 ± 8.22
H_2O_2		54.55 ± 0.61	29.81 ± 0.54
	BHT	55.16 ± 0.26	10.85 ± 2.28
ABTS		91.14 ± 0.20	29.44 ± 0.49
	Trolox	90.20 ± 0.34	51.75 ± 3.36
$PhNHNH_2$		53.19 ± 1.03	77.68 ± 2.23
	BHT	52.68 ± 0.17	63.03 ± 6.02

Values are expressed as mean ± standard error of mean (X ± SEM). Samples were performed in triplicate.DPPH: 2,2-difenil-1-pikrilhidrazil, OH: Hidroksil, H_2O_2: Hidrojen peroksit, ABTS: 2,2'-azinobis (3-etilbenzotiazolin-6-sülfanot), $PhNHNH_2$: Fenilhidrazin.

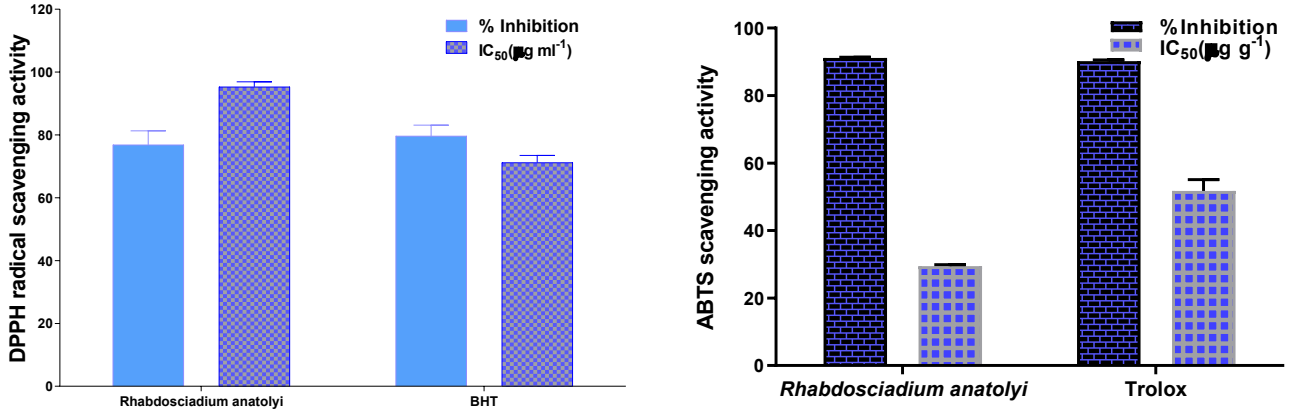

Figure 1. % inhibition of DPPH and ABTS radicals and IC50 values for BHT and trolax of *R. anatolyi* flowers

Şekil 1. R. anatolyi çiçeğinin BHT ve trolaks için DPPH ve ABTS radikallerinin % inhibisyonu ve IC_{50} değerleri.

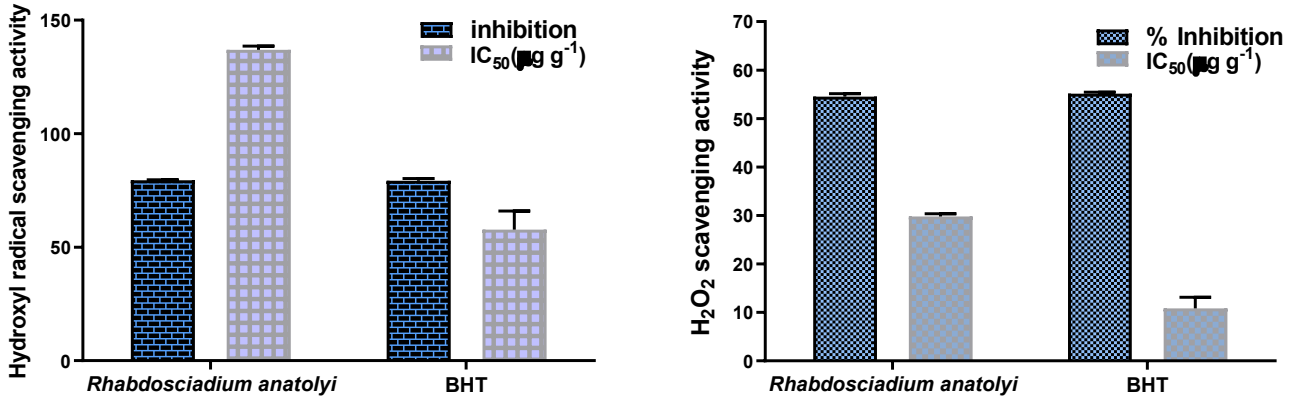

Figure 2. Graph showing the change in hydroxyl radical scavenging activity of *R. anatolyi* flowers and BHT, and % inhibition and IC_{50} values of BHT and hydrogen peroxide radical scavenging activity.

Şekil 2. R. anatolyi çiçeğinin ve BHT'nin hidroksil radikali süpürme aktivitesi ile BHT ve hidrojen peroksit radikali süpürme aktivitesine ait % inhibisyon ve IC_{50} değerlerindeki değişimi gösteren grafik.

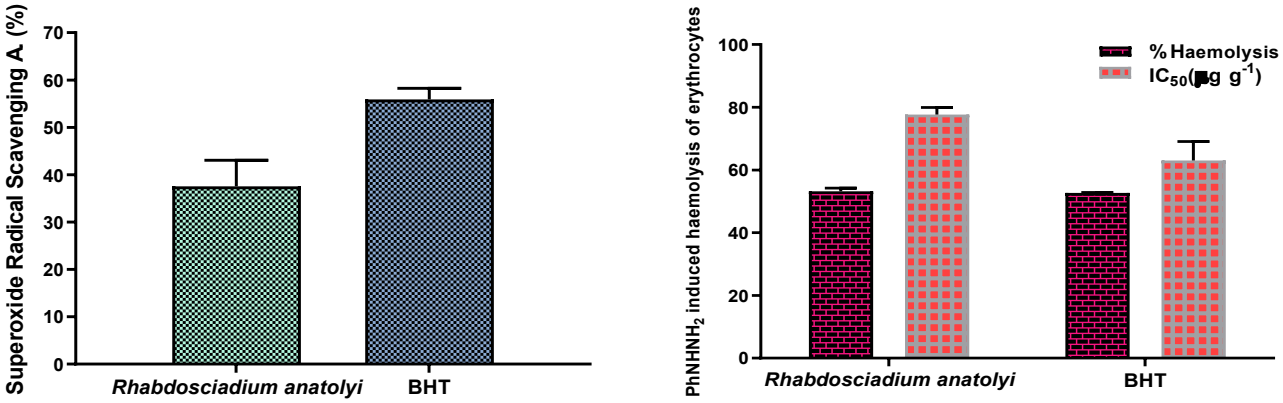

Figure 3. Graph of % inhibition and IC_{50} values showing % inhibition change of *R. anatolyi* flowers superoxide radical and hemolysis of erythrocytes with phenylhydrazine for R. anatolyi plant and BHT.

Şekil 3. R. anatolyi çiçeğinin süperoksit radikalinin % inhibisyon değişimi ve R. anatolyi çiçeği ile BHT için eritrositlerin fenilhidrazin ile hemoliz oluşumu gösteren % inhibisyon ve IC_{50} değerlerine ait grafik.

Carotenoids, flavonoids and polyphenols, which are phytochemicals or phytonutrients, not only protect plants from many diseases and fungi, but also add features such as color, smell and taste to plants (Mosley, 2018). These phytochemical compounds found in plants prevent the oxidation of free radicals, which are produced by lipids, carbohydrates and proteins as waste products as a result of various reactions in the organism (Çoban & Patır, 2010). Phenolic compounds are powerful and natural antioxidants that scavenge these free radicals formed in various conditions. In this study, total phenol, total flavonoid and total antioxidant capacities of *R. anatolyi* flowers were determined. The total amount of phenol was determined using the gallic acid standard graph, the total flavonoid quercetin standard graph, and the total antioxidant was determined using the ascorbic acid standard graph. Total phenol, flavonoid and antioxidant amounts were determined as 21.94 ± 0.37 mg gallic acid g^{-1}, 5.60 ± 0.13 mg quercetin g^{-1} and 29.65 ± 0.26 mM ascorbic acid g^{-1}, respectively.

Apart from proteins, lipids and carbohydrates, which are macronutrients, vitamins are compounds that are essential to perform some special cellular events and are needed at trace levels in the organism (Ferrier, 2019). Vitamin C, which is included in the water-soluble class of vitamins, is important in two respects; The first is that it is a very powerful antioxidant and the other is that it is essential for some vertebrates, including humans (Korkmaz et al., 2012). Apart from the known role of vitamin C in collagen synthesis in connective tissues, studies on its interactions with various chemicals and metal ions have shown that free radicals have important roles in reactions involving electron transport and membrane biochemical strengthening (Hacışevki, 2009). The primary function of vitamin E is to play an antioxidant role in maintaining the non-enzymatic oxidation of cell components. In addition, studies have shown that ascorbic acid regenerates active vitamin E (Ferrier, 2019). The amount of vitamin C in the flowers extract of the *R. anatolyi* flowers was determined as 346.27 ± 6.51 mg 100 g^{-1}, and the value of vitamin E was determined as 3.99 ± 0.35 µg g^{-1}. In the literature review, there is a not study on vitamins E and C related to both *R. anatolyi* flowers and closely related species *R. microcalycinum*, *R. oligocarpum*, *R. urusakii*, *R. alignense*, *R. petiolare*, *R. aucheri*, *R. hizanense* (Fırat & Güzel, 2019) and *R. strauss* species. However, the vitamin C content of the closely related species *Chaerophyllum macropodum* was determined as 20.53 ± 5.08 mg 100 g^{-1} , in accordance with Tunçtürk et al., (2008). As a result, it was determined that the vitamin C content in the *R. anatolyi* flowers was more significant and at a good level compared to the other plant in question. In addition, the value of vitamin E will be a reference for the studies to be done in this plant species.

The living organism generally consists of elements that it needs both roughly in abundance and at trace levels. Carbon, hydrogen, oxygen and nitrogen are the most abundant elements. They constitute 96% of body weight (Wada, 2004; Keha & Küfrevioğlu, 2012). The essential trace elements of the human body include elements such as Zn, Cu, Se, Cr, Co, I, Mn and Mo. Although these elements make up only 0.02% of the total body weight, they play important roles, for example, as active enzyme centers or trace bioactive substances (Wada, 2004). Mg, Fe, Zn, Cu and Cr elements have biochemical functions that have the potential to affect the physical performance of living things. These elements function as structural or catalytic components of enzymes, regulating cellular energy and gas transport, antioxidant defense, membrane receptor functions, second messenger and integration of physiological systems. Thus, mineral elements regulate the use of macronutrients

(Lukaski, 2004). Trace elements, which have an important and critical position in the nutrition of living organisms, have a very complex historical process (Shkolnik, 2012). Because although there are elements whose usefulness to the organism has been defined, it is a fact that there are elements whose functions have not yet been fully explained. Some trace elements such as copper, zinc and selenium cover both humoral and cellular immunity (Berger et al., 1998). These essential elements interact directly with free radical formation and free radical scavengers (Bendich, 1993). In addition, antioxidant enzymes need trace elements such as Cu, Fe, Zn, Se to provide catalytic activity. It is known that glutathione peroxidase enzyme needs Se element, superoxide dismutase enzyme needs Fe, Cu and Zn elements and catalase enzyme needs Fe element.

In the study, the mineral and trace element levels of the *R. anatolyi* flowers were examined. Values found are Ca 2.61 ± 0.11 mmol kg^{-1}, K 2.09 ± 0.037 mmol kg^{-1}, Mg 3.64 ± 0.0063 mmol kg^{-1}, Na 2.03 ± 0.027 mmol kg^{-1}, Fe 0.064 ± 0.0017 mmol kg^{-1}, Mn 0.43 ± 0.021 mmol kg^{-1}, P 0.081 ± 0.0028 mmol kg^{-1}, Zn 0.38 ± 0.015 mmol kg^{-1}, V 0.017 ± 0.00033 mmol kg^{-1}, Ti 0.066 ± 0.0028 mmol kg^{-1}, Cr 0.012 ± 0.00026 mmol kg^{-1}, Cu 0.079 ± 0.0061 mmol kg^{-1}, Sr 0.088 ± 0.0059 mmol kg^{-1}, As 0.15 ± 0.062 µmol kg^{-1}, Tl 0.35 ± 0.063 µmol kg^{-1}, Cd 0.21 ± 0.044 µmol kg^{-1} Co 2.56 ± 0.11 µmol kg^{-1}, Pb 1.81 ± 0.021 µmol kg^{-1}, Mo 0.83 ± 0.14 µmol kg^{-1} and Sn 0.22 ± 0.086 µmol kg^{-1} contents. When these values were compared, it was determined that Mg > Ca > K > Na > Mn > Zn > Sr > P > Cu > Ti > Fe > V > Cr > Co > Pb > Mo > Tl > Sn > Cd > As. As a result, it was determined that the plant flowers contains both macrominerals and trace elements. When the mineral levels of the *R. anatolyi* flowers are compared with the similar species *Echinophora tenuifolia* L., it has been found that it has an important mineral such as Na element (<1 mg $gün^{-1}$). In addition, the presence of trace elements such as Co, Mo, which are needed at ultra-trace levels in living organisms, have been determined.

DPPH is a stable radical. The change in absorbance of the DPPH radical was measured and the absorbance corresponding to the concentration was plotted and determined from the non-linear curve. The sample amount that halves the DPPH concentration is determined in µgmL^{-1} and expressed as the IC_{50} value. IC_{50} antioxidant activity is defined as the effective concentration, which expresses the amount of antioxidant consumed to reduce the initial DPPH concentration by 50%. The lower the IC_{50} value, the higher the radical scavenging activity (Brand-Williams, W., 1995). The capacity of the methanol extract of *R. anatolyi* flowers, which we used within the scope of the study, to scavenge DPPH radical, which is a free radical that can react with compounds that can donate hydrogen atom, was investigated. The highest inhibition percentage was 71.27 ± 4.47 µg mL^{-1} for the plant, and 79.64 ± 3.49 µg mL^{-1} for BHT, the positive control. The IC_{50} value was 95.28 ± 1.61 µg mL^{-1} for the plant, and 71.18 ± 2.28 µg mL^{-1} for BHT, which was the positive control. When these IC_{50} values were compared, it was determined that it was effective close to the standard and the concentration value of the plant extract, which inhibited the DPPH radical by 50%, was low. Compared with other plant species, it has been determined that it has a good scavenging activity and has high and significant values in terms of DPPH radical scavenging activity.

Hydrogen peroxide (H_2O_2) is an oxidizing agent, non-reactive, and its main importance is that it is a source of hydroxyl radicals in the presence of reactive transition metal ions (Kumar, 2011). Hydrogen peroxide can easily pass through cell membranes and directly attack the cellular target. For; example high levels of hydrogen peroxide can inactivate the glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase in mammalian cells. (Halliwell, 1992). The main damage that hydrogen peroxide can cause in the cell results in DNA fragmentation, single strand breakage and formation of DNA protein crosslinking. The hydrogen peroxide (H_2O_2) radical scavenging capacity of *R. anatolyi* flowers was investigated. The highest inhibition percentage of plant flowers methanol extract was determined as 54.55 ± 0.61 µg mL^{-1}, and 55.16 ± 0.26 µg mL^{-1} for BHT. Concentration values inhibiting half of the hydrogen peroxide (H_2O_2) radical concentration were determined as 29.81 ± 0.54 µg mL^{-1} for the plant and 10.85 ± 2.28 µg mL^{-1} for BHT. Peroxisomes, which are in an important position in neutralizing free oxygen radicals, are important in that they contain enzymes that synthesize hydrogen peroxide as well as enzymes that break down (Çoşkun., 2005). From this point of view, it is seen that the scavenging activity of hydrogen peroxide (H_2O_2) radical, which is a free radical, of *R. anatolyi* flowers methanol extract has a lower % inhibition compared to synthetically used BHT, but a higher IC_{50} value.

The superoxide (O_2-) anion radical is the radical that has been shown to form first in living things (Kılınç & Kılınç, 2002). This radical is much less reactive than the hydroxyl radical but can attack a number of biological targets. Therefore, this radical reacts with nitric oxide, a free radical produced by various cell types, particularly phagocytes and vascular endothelial cells, to give peroxynitric (ONOO-) (Halliwell, 1992). Methanol extract of *R. anatolyi* flowers was compared with BHT, a synthetic antioxidant with known antioxidant properties. % inhibition activity of superoxide anion (O_2.-) radical at a concentration of 45 µg mL^{-1} is as *R. anatolyi* > BHT and values were determined as 37.55 ± 5.49 µg mL^{-1}

for the plant and 55.91 ± 2.32 µg mL^{-1} for BHT. When the superoxide anion radical values were examined, it was determined that the methanol extract of the *R. anatolyi* flowers effectively removed the superoxide radical at the concentration in question, compared to the BHT of the same value.

The hydroxyl radical (OH-), which has a very short half-life, is perhaps the most reactive type of ROS identified in biological systems (Kılınç & Kılınç, 2002; Özcan et al., 2015). Where the hydroxyl radical is formed, it causes great damage to many molecules such as thiols and fatty acids, causing the formation of other different radicals. Namely, thiols in the natural antioxidant group, which are the subject of important researches such as anti-cancer, are oxidized in the presence of oxygen and sulfide radicals are released as the final product, as well as reagents such as superoxide radical and hydroxide radical (Kavas, 1994).

The hydroxyl (OH^-) radical scavenging capacity of *R. anatolyi* flowers was investigated. The highest inhibition percentage of the plant flowers methanol extract was determined as 79.53 ± 0.17 µg mL^{-1}, and 79.30 ± 0.98 µg mL^{-1} for BHT. Concentration values inhibiting half of the hydroxyl (OH^-) radical concentration were determined as 136.96 ± 1.65 µg mL^{-1} for the plant and 57.74 ± 8.22 µg mL^{-1} for BHT. It is seen that the scavenging activity of the hydroxyl (OH^-) radical, which is a strong free radical, of the plant methanol extract is slightly higher than the synthetically used BHT, but the IC_{50} value is almost half-lower. As a result, when the IC_{50} values were examined, it was determined that *R. anatolyi* > BHT and the hydroxyl (OH^-) radical scavenging activity of the plant did not have a good scavenging activity compared to BHT, which is a synthetic antioxidant.

ABTS exists in a non-radical and colorless form. However, by oxidation of persulfate (potassium or sodium persulfate) it forms a blue/green $ABTS^{\cdot+}$ radical. This radical is very stable. The generation of $ABTS^{\cdot+}$ radical cation forms the basis of one of the spectrophotometric methods applied to measure the total antioxidant activities of pure solutions (Re et al., 1999). The addition of antioxidants to the preformed radical cation reduces the antioxidant concentration of the $ABTS^{\cdot+}$ radical to some extent and on a time scale, and the duration of the reaction, depending on the antioxidant activity. Thus, the degree of color change as percent inhibition of the $ABTS^{\cdot+}$ radical cation is determined as a function of concentration and time and is calculated based on the reactivity of a synthetic antioxidant to be used as a standard under the same conditions (Re et al., 1999). The lightening of the blue/green color of the $ABTS^{\cdot+}$ cation radical occurs in the presence of antioxidants. $ABTS^{\cdot+}$ radical scavenging activity of trolox antioxidant, which we used as a comparison of *R. anatolyi* flowers methanol extract at the same rate, was investigated. The highest inhibition percentage of plant flowers methanol extract was determined as 91.14 ± 0.20 µg mL^{-1}, and 90.20 ± 0.34 µg mL^{-1} for trolox. The concentration values inhibiting half of $ABTS^{\cdot+}$ cation radical concentration were determined as 29.44 ± 0.49 µg mL^{-1} for the plant and 51.75 ± 3.36 µg mL^{-1} for trolox. These values show that plant methanol extraction has been found to scavenge $ABTS^{\cdot+}$ radical scavenging activity at a better level compared to the comparator trolox.

Phenylhydrazine is a hemolytic agent. Yellow to pale brown crystals or a yellowish oily liquid, phenylhydrazine is sparingly soluble in water and miscible with other organic solvents. Limited data on toxicokinetics indicate that phenylhydrazine is well absorbed by inhalation, oral and dermal routes and readily binds to hemoglobin in red blood cells. Therefore, exposure to phenylhydrazine can damage red blood cells, potentially causing anemia and damage to other tissues such as the spleen and liver (Anonymous, 2000). It was investigated whether R. anatolyi plant flowers has phenylhydrazine radical scavenging activity in terms of antioxidant as a result of hemolysis of erythrocytes with phenylhydrazine. As a comparison, the phenylhydrazine radical scavenging activity of BHT, a synthetic antioxidant, was investigated. Hemolysis formation of erythrocytes with phenylhydrazine of plant flowers methanol extract was determined as 53.19 ± 1.03 µg mL^{-1} for the plant, and 52.68 ± 0.17 µg mL^{-1} for BHT. The IC_{50} concentration values inhibiting half of the phenylhydrazine radical concentration were determined as 77.68 ± 2.23 µg mL^{-1} for the plant and 63.03 ± 6.02 µg mL^{-1} for BHT. These results show that both the % inhibition and IC_{50} value of the plant were higher than BHT and the radical scavenging capacity of the plant flowers methanol extract was found to be lower than BHT, a monohydric phenolic antioxidant.

CONCLUSION

When looking at the general study on *R. anatolyi* flowers, total phenolic, flavonoid and total antioxidant capacity, vitamin E and C content, trace element and mineral levels, and antioxidant scavenging status against some radicals were evaluated. The level of active content of each plant can certainly differ depending on the conditions such as soil structure, geographical location, climate in which it grows, apart from the family it is in. It has been determined that the methanol extract of *R. anatolyi* flowers is rich in vitamin C content, phenolic, flavonoid and total antioxidant capacity is at sufficient level, and trace element and mineral levels have a significant ratio. It is seen that the superoxide radical and $ABTS^{\cdot+}$ radical outperform the synthetic antioxidants used in comparison. On the other hand, when the DPPH,

hydrogen peroxide, hydroxyl and phenylhydrazine radical scavenging activities were compared with the synthetic antioxidants used for comparison, it was determined that the methanol extract of the plant flowers had antiradical activity when evaluated as a whole.

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Author's Contributions

Author 1: Plant collection, data interpretation, evaluation of analysis results, article writing, review and editing. Author 2: Methodology, validation, statistics, data improvement, and auditing. Author 3: Collecting plant material. All authors accept responsibility for all content.

Conflict of Interest Statement

There was no conflict of interest among the authors in this study.

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Green Synthesis, Characterization, and Evaluation of Biocompatible Structures of Gold Nanoparticles in Biomedical Applications (Antibacterial, Antifungal, and Anticancer)

Ayşe BARAN[1], Cumali KESKIN[2]✉
[1]Department of Biology, Graduate Education Institute, Mardin Artuklu University, Mardin, Türkiye, [2]Department of Medical Services and Techniques, Vocational Higher School of Healthcare Studies, Mardin Artuklu University, Mardin, Türkiye
[1]https://orcid.org/0000-0002-2317-0489,[2]https://orcid.org/0000-0003-3758-0654
✉: cumalikeskin@artuklu.edu.tr

ABSTRACT
Gold nanoparticles (AuNPs) stand out due to their low toxicity and high compatibility, and the large and modifiable surface areas they provide. In this study, the leaves of *Celtis tournefortii* Lam. (CT) were used for the green synthesis of gold nanoparticles (AuNPs) first time. The size, shape, surface charge, and functionality of the synthesized AuNPs are described in detail. The suggested mechanisms of action on the tested target cells are highlighted. The biological activities (antibacterial, antifungal, and anticancer) of "green" AuNPs and their further biomedical application possibilities are also discussed. Synthesized AuNPs displayed a spherical appearance, surface plasmon resonance band at 553.67 nm wavelength, and surface charge of -16.53 mV. Particle morphology, size, and surface charge were observed to be affected by the leaf extract used in the reduction reaction. FTIR and TGA-DTA data revealed that functional groups from the CT extract participate in the synthesis and stabilization of AuNPs. AuNPs showed antibacterial and antifungal effects on all the strains and yeast tested by microdilution method (MIC). AuNPs showed dose-dependent cytotoxic activity on cancerous cell lines (SKOV-3, CaCo2, and U118). The obtained results highlight a potentially low-cost green synthesis method using CT leaf extract to synthesize AuNPs showing important biological properties.

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Altın Nanopartiküllerin Yeşil Sentezi, Karakterizasyonu ve Biyouyumlu Yapılarının Biyomedikal Uygulamalarda (Antibakteriyel, Antifungal ve Antikanser) Değerlendirilmesi

ÖZET
Altın nanopartiküller (AuNP'ler), toksisitelerinin düşük olması ve yüksek uyumlulukları ile sağladıkları geniş ve düzenlenebilen yüzey alanlarının olmasından dolayı dikkat çekmektedirler. Bu çalışmada, *Celtis tuernofortii* Lam. (CT) yaprakları altın nanoparçacıkların (AuNP'ler) yeşil sentezi için ilk kez kullanıldı. Sentezlenen AuNP'lerin boyutu, şekli, yüzey yükü ve işlevselliği ayrıntılı olarak tanımlandı. Test edilen hedef hücreler üzerinde önerilen etki mekanizmaları vurgulandı. "Yeşil" AuNP'lerin biyolojik aktiviteleri (antibakteriyel, antifungal ve antikanser) ve bunların diğer biyomedikal uygulama olasılıkları da tartışıldı. Sentezlenen AuNP'ler küresel bir görünüm, 553.67 nm dalga boyunda yüzey plazmon rezonans bandı ve -16.53 mV yüzey yükü sergiledi. Partiküllerin morfolojisi, boyutu ve yüzey yükünün indirgeme reaksiyonunda kullanılan yaprak ekstraktından etkilendiği gözlemlendi. FTIR ve TGA-DTA verileri, CT özünden elde edilen fonksiyonel grupların AuNP'lerin sentezine ve stabilizasyonuna katıldığını ortaya koydu. AuNP'ler, mikrodilüsyon yöntemi (MIC) ile test edilen tüm suşlar ve mayalar üzerinde antibakteriyel ve antifungal etkiler gösterdi. AuNP'ler kanser hücre hatları (SKOV-3, CaCo2 ve U118) üzerinde doza bağlı sitotoksik aktivite gösterdi. Elde edilen sonuçlar, önemli biyolojik özellikler gösteren AuNP'leri sentezlemek için CT yaprak özütü kullanan, potansiyel olarak düşük maliyetli bir yeşil sentez yöntemini vurgulamaktadır.

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Anahtar Kelimeler
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Yeşil sentez
Nanotıp

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INTRODUCTION

Nanotechnology is growing rapidly for the production of new materials in this field, and these products at the nanoscale level have superior features such as large superficial area, magnetic, optical, and conductibility (Velmurugan et al., 2014; Baran 2019a). Among these products, studies with metallic nanoparticles (MNPs) such as gold (Au), silver (Ag), titanium (Ti), iron (Fe), copper (Cu), and zinc (Zn) are the most favored (Asghar et al., 2018; Baran, et al., 2019; Baran, 2019; Pandiyan et al., 2019; Baran, et al., 2021). There are various approaches such as biological, physical, and chemical protocols to produce nanoparticles. Among the biological synthesis protocols, plant-based synthesis processes attract a lot of attention thanks to their advantages such as ease, not requiring special conditions, and excess product (Al-ogaidi et al. 2017, Patil et al. 2018, Rolim et al. 2019). The leaves (Baran, 2019b), roots (Marslin et al. 2015), flowers (Remya et al. 2015), and sometimes the whole plant can be used in the synthesis of NPs. Functional groups of phytochemicals found in plant sources (such as phenolic groups, alcohol groups, and amine groups) are considered responsible for the formation of metallic nanoparticles, the biological reduction of metals, and also in stabilization (Shankar et al. 2016; Some et al. 2019).

Recent studies agree on the diverse advantages of nanogold upon dissimilar nanomaterials, primarily owing to immensely optimized methods for the generation of gold nanoparticles of numerous dimensions and shapes, featuring together exclusive characteristics. The AuNPs are among the important NPs used in diagnosis and treatment in biomedical applications (Rautray and Rajananthini 2020). AuNPs are accepted as medical agents for anticancer, antimicrobial, anti-inflammatory, and antidiabetic applications. Moreover, they are used as theragnostic implementation for various diseases such as cancer, diabetes, Parkinson's, Alzheimer's, AIDS, arthritis, hepatitis, cirrhosis, spinal cord injury, tuberculosis, and circulatory system diseases (Patra et al. 2015, Kumar et al. 2017, Mohammadi et al. 2019, Abu-Dief et al. 2020, Arroyo et al. 2020).

Antibiotic resistance is a serious problem. In the face of resistance developed by microorganisms, the search for antimicrobial agents maintains its importance. Many studies are explaining that AuNPs synthesized by bioderived synthesis methods show effective antimicrobial activity (Donga et al. 2020, Tripathy et al. 2020, Hatipoğlu 2021, Mandhata et al. 2021, Mehravani et al. 2021).

Cancer, which is a difficult disease to treat that many people around the world are trying to fight, constitutes an important field of study where various methods are researched for its treatment, and alternative methods are developed day by day. Effective and positive results have been obtained in studies that foresee the use of AuNPs, which are synthesized by environmentally friendly synthesis methods, as an anticancer agent due to their biocompatible properties (González-Ballesteros et al. 2017, Haddada et al. 2019, Satpathy et al. 2020, Padalia & Chanda 2021).

Celtis tournefortii Lam., which is called by various names such as "Dağdagan", is a member of the *Ulmaceae* family and is a deciduous tree that grows in tropical regions and high temperate regions, with an average height of five meters. It grows in countries such as Turkey, Azerbaijan, Iran, Iraq, Greece, Croatia, and Ukraine. In studies conducted to determine the chemical contents of the genus *Celtis*, it has been stated that it contains phytochemicals such as phenolic acids, coumarins (coumarin, esculetine), tannins (gallic acid, flavan-3-ols (catechins), chlorogenic acid), flavonoids (quercetin, rutin, naringenin), steroids (brassinosteroids), terpenoids (menthol, geraniol), and alkaloids (caffeine, capsaicin) (Keser et al., 2017; Yıldırım et al., 2017; Holopainen et al., 2018; Gecibesler, 2019; Baran et al., 2022).

Therefore, the present study focused to improve the green synthesis and characterization of AuNPs using the aqueous extract from fresh *C. tournefortii* plant leaves and to study the antimicrobial, antifungal, and anticancer activities.

MATERIALS and METHODS

Materials

Leaves were collected from *C. tournefortii* L. trees (oriental hackberry, dağdağan) at the end of August in Mardin Kızıltepe Region (at position 37°17' 07.3" N and 40° 29' 03.0" E) (Figure 1). After pre-washing with tap water, washing was also done with distilled water. It was dried at room condition and prepared for extraction. Then, 100 g of the dried leaves were weighed and mixed with 500 ml of distilled water and allowed to boil on the heater. It was cooled at room conditions, filtered with filter paper, taken at +4 °C, and made ready for synthesis. A solution with a concentration of five millimolar (mM) was prepared from the compound Alpha Aesar Tetrachloroauric (III) acid trihydrate (in Kandel, Germany).

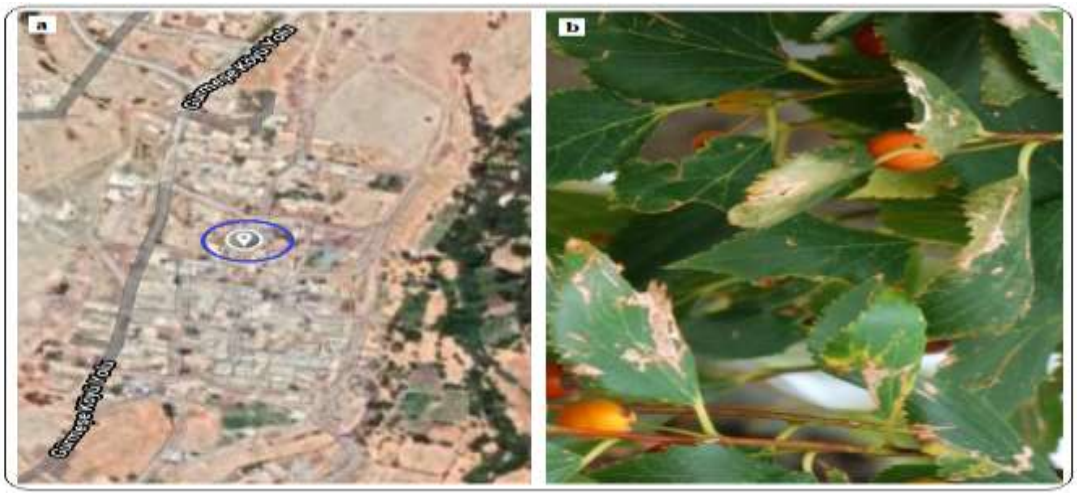


Figure 1. Plant of *C. tournefortii* L. a. location information, b. morphological view of leaves
Şekil 1. Celtis tournefortii Lam. Bitkisinin; a. yetiştiği alana ait konum, b. bitki yapraklarının morfolojik görünümü

Synthesis and Characterization of CT-AuNPs

The extract prepared using C. tournefortii (CT) leaves was mixed with five mM HAuCl4 solution (1/1 ratio). The nanoparticle synthesizing reaction was permitted to progress at room conditions for various periods.

Nanomaterials dried at 100 °C for 12 hours and powdered can be stored in a sterile tube at room temperature for later use. Gold nanoparticles can be stored for a long time in a stable state without oxidation after synthesis.

With the samples taken due to color change, wavelength scans were performed with measurements in the 300-800 nm range by Perkin Elmer One UV-Vis spectrophotometry. The formation and existence of CT-AuNPs were evaluated by examining the maximum absorbance bands.

For the determination of the functional groups of phytochemicals in the CT extract responsible for the reduction reactions of AuNPs, the FTIR spectra of both the plant extract and the liquid fractions formed after the reaction were examined with (Perkin Elmer One) FTIR (spectrum range 4000-400 cm^{-1}). After the synthesis, the crystal patterns and sizes of the CT-AuNPs were defined by the Rigaku Miniflex 600 XRD (range of 20-80 2-theta, Cu∝= 1,5406) (Baran, 2020).

SEM (EVO 40 LEQ), FE-SEM, AFM (Park System XE-100), and TEM (Jeol Jem 1010) micrographs were used to determine the morphological appearance of CT-AuNPs. The elemental profile of the particles obtained after synthesis was revealed by the RadB-DMAX II computer-controlled EDX. Surface charges and size distributions of AuNPs were analyzed using zeta potentials and zeta sizer distributions (Marven) devices. The resistance of CT-AuNPs against heat treatments were examined with the data obtained with the TGA-DTA (Shimadzu TGA-50, heating rate: 10 °C/min in the range of 25-900 °C, flow rate: 20 mL/min, atmosphere: N_2(g)).

Antipathogenic Properties of CT-AuNPs

The antipathogenic effects of AuNPs were evaluated on pathogenic microorganisms by determining the Minimum Inhibition Concentration (MIC), which suppresses their growth using the Micro-Dilution method. Gram (+) *Bacillus subtilis* ATCC 11774 (*B. subtilis*) and *Staphylococcus aureus* ATCC 29213 (*S. aureus*) and gram (-) *Pseudomonas aeruginosa* ATCC27833 (*P. aeruginosa*) and *Escherichia coli* ATCC25922 (*E. coli*), and *Candida albicans* yeast were used test to antipathogenic properties of CT-AuNPs. *S. aureus*, *C. albicans*, and *E. coli* microorganisms were supplied from Inonu University Medical Faculty Hospital Microbiology Laboratory, Malatya, Turkey. *P. aeruginosa* and *B. subtilis* were also obtained from Mardin Artuklu University Microbiology Research Laboratory, Mardin, Turkey.

Microorganisms used in the assay were grown in a suitable medium (Bacteria's: Nutrient Agar, Yeast: Sabora Dextrose Agar) in an incubator at 37 °C overnight. After the growth control was done, solutions were prepared for each microorganism according to the McFarland standard 0.5 turbidity criteria (Emmanuel et al. 2015) by using microorganism colonies on the medium plates. Then, Müller Hinton medium (for bacteria) and Roswell Park Memorial Institute (RPMI) 1640 broth (for *C. albicans*) were prepared and pipetted in appropriate amounts into 96 microplate wells. Some of the microplate wells were used for sterilization and control steps for growth control. Solutions containing CT-AuNPs with different concentrations were prepared, added to the microplate wells, and the distribution of CT-AuNPs in the medium was made with a series of microdilution. The same steps were applied to antibiotics (Colistin: Gram-negative strains, Vancomycin: Gram-positive strains,

Fluconazole: C. albicans yeast), HAuCl4 solution. Microorganism solutions prepared according to Mc Farland standard 0.5 turbidity standard were transferred to microplate wells in the appropriate amount. Afterward, the microplates were incubated in an oven at 37 °C. One day after the interaction, microplates were examined for growth. The concentration of the well before the well where the growth started was determined as the MIC value where the growth was suppressed.

Cytotoxic Activities of CT-AuNPs

The cytotoxic effects of CT-AuNPs on healthy (Human Dermal Fibroblast: HDF) and cancerous (Caco-2: Colorectal adenocarcinoma; U118: Glioblastoma; Sk-ov-3: Human ovarian sarcoma) cell lines (American Type Culture Collection (ATCC) in Dicle University Scientific Research Center, Cell Culture Laboratory, Diyarbakır, Turkey) were determined by the MTT method. CaCo-2, U118, and HDF cell lines were incubated in DMEM (Dulbecco Modified Eagle) and Skov-3 cell line in RPMI (Roswell Park Memorial Institute) medium at 37 °C. The environment for growth was set to 95% air and 5% CO_2 and humidity conditions. After the cell lines were controlled by a hemocytometer and reached 80% confluence, they were re-suspended to different concentrations. Cell lines were then transferred to 96-well microplates and incubated overnight. Then, varying concentrations of AuNPs were added to the cell lines cultured in the microplate wells and left to interact with the nanoparticles for 48 hours, and then MTT solution was added to the wells and incubated for 3 hours. After waiting for another 15 minutes by adding DMSO, the data of the absorbance spectrum of the cells were measured (using Thermo Multi ScanGo) at 540 nm. By using the absorbance values of the cell lines, the concentrations of AuNPs that suppressed viability in the cell lines were calculated by the formula expressed below (Awad et al. 2019).

% viability=U/C*100

U: Absorbance values of cells treated with AgNPs; C: Absorbance values of control (healty) cells (without AgNPs in the medium).

RESULTS and DISCUSSION

Biophysical Characterization of Biogenic CT-AuNPs

UV-vis spectrum data

UV-vis spectroscopy is often used to measure molecules in solution or inorganic ions and complexes. The absorption of electromagnetic radiation by molecules or atoms varies depending on the type of atoms in the molecule. The vibrations that will occur due to the formation of NPs on the plasma surface are the result of the reduction of the charged ions in the aqueous medium. UV-vis with the samples to be taken depending on the time after the color change is observed. The presence and formation of NPs can be detected with the same maximum absorbance data to be obtained in wavelength scans made in the device. In the green synthesis of AuNPs using Dagdagan leaf extract, a color change from yellow to dark pink-red was observed 20 minutes after the extract and five mM $HAuCl_4$ solution were mixed. Depending on the intensity of color formation, samples were taken from the reaction medium, and wavelength scans were made utilizing the UV-vis spectrophotometer. Maximum absorbances were read at 553.67 nm wavelength in the measurements (Figure 2). The characteristic data (pink-red color change) indicating the formation and presence of AuNPs refers to the conversion of the Au^{+4} form to the Au^{0} form by bioreduction. The fact that the maximum absorbance value (553.67 nm) is constant for the samples measured at different time intervals as a result of the color change is proof that the reaction has taken place and is in a stable structure. The data obtained are consistent with the absorbance values of AuNPs reported in previous studies (Jafarizad et al. 2019, Babu et al. 2020, Karim et al. 2020, Rautray & Rajananthini 2020).

X-ray diffraction (XRD) analysis

By using X-ray diffraction analysis, the crystalline size, structure, and phase purity of the synthesized gold nanoparticles were confirmed. The XRD pattern of biosynthesized gold nanoparticles is depicted in Figure 3. The X-ray diffraction pattern of AuNPs produced from an aqueous extract of *Celtis tourneofortii* showed dominant peaks in the 2 range originating from the (111°), (200°), (220°), and (311°) planes with the cubic crystalline structure of nanogold at 38.00, 44.19, 64.33, and 77.26. The mean particle size was calculated as 33.35 nm using the Debye Sherer (CuK∝=1.540) equation. Based upon the consequence of XRD and peak density, it can be concluded that the CT leaf extract during the process of formation of AuNP has a respectable impact on the crystalline structure of the NPs.

FTIR analysis data

FTIR spectroscopy is a potent definition instrument for the classifying of compounds, and materials by examining them in the matchless mode of vibration and rotation. Hence, it is one of the primary techniques for classifying and definition of compounds. After biosynthesis and purifying, the MNPs can at once be reachable for functionality testing. FTIR spectrums were given in Figure 4 a,b. As seen in Figure 4 a, FTIR spectra of the plant extract and the reaction liquids obtained as a result of the synthesis were examined to determine the functional groups of phytochemicals that may be responsible for the reduction of the Au^{+4}

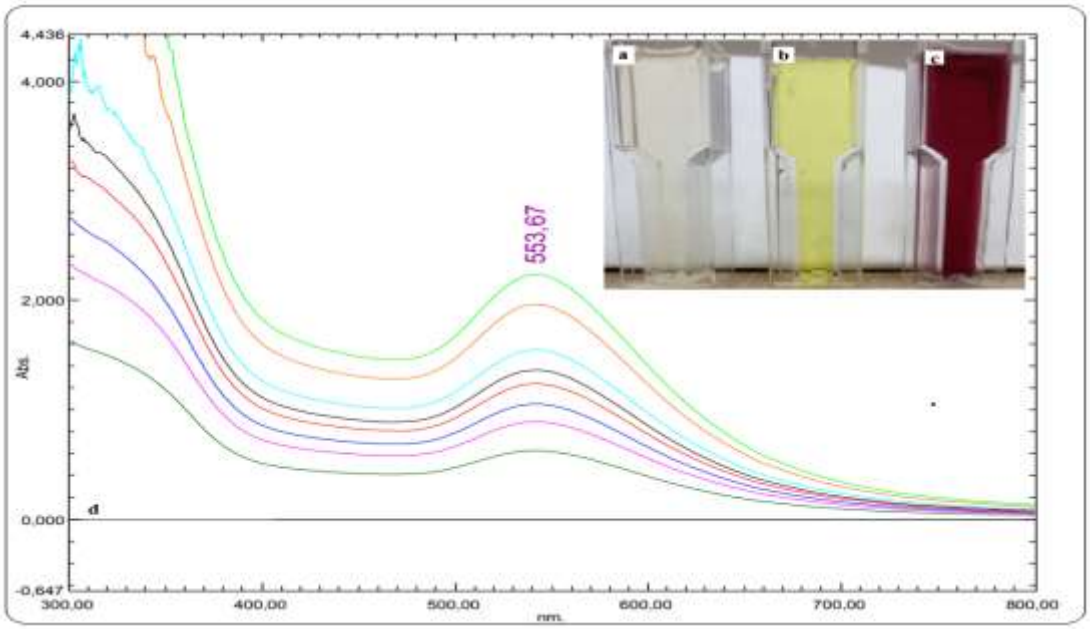


Figure 2. The formation and presence of AuNPs using CT leaf extract; a, b, and c, respectively, the 5 mM of $HAuCl_4$ solution, the plant extract, the color change resulting from the synthesis, and also d. Time-dependent UV-vis spectrum bands

Şekil 2. a. 5 mM $HAuCl_4$ çözeltisi b. *Celtis tournefortii Lam. yaprak özütü, c. Sentez sonucu AuNP'lerin oluşumuna bağlı renk değişimi görünümleri ve d. AuNP'lerin oluştuğunu ve varlığını gösteren UV-vis spektrum dataları*

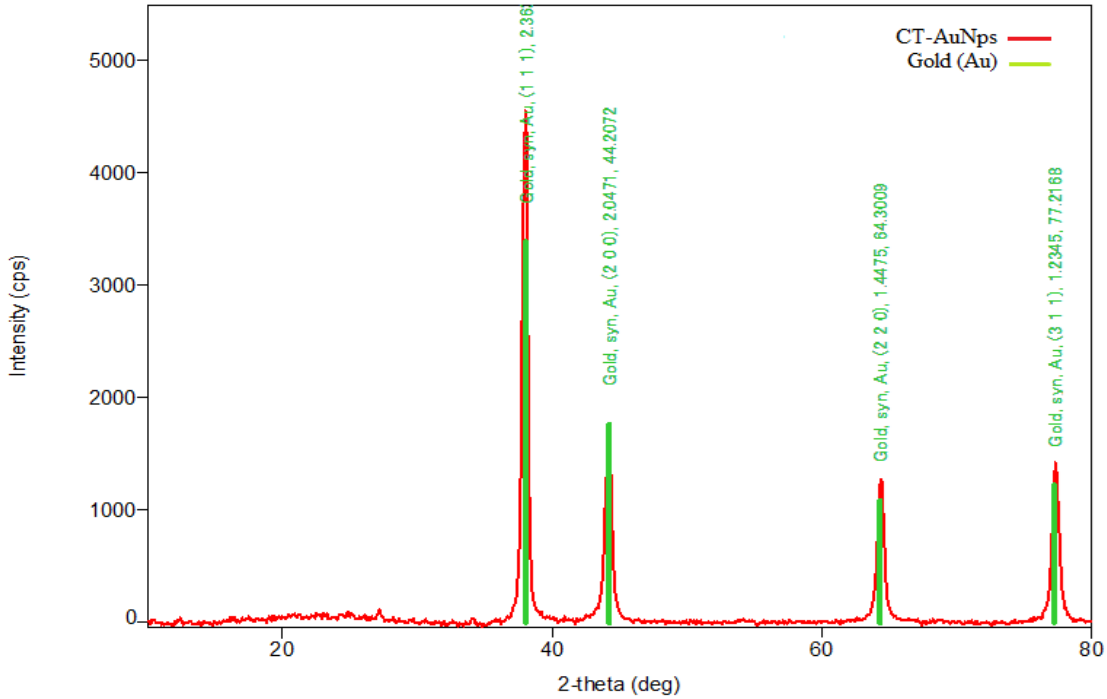


Figure 3. X-ray diffraction pattern of bio-based CT-AuNPs after synthesis

Şekil 3. Biyosentezi yapılan CT-AuNP'lerin kristal desenlerine ait X-ray kırınım deseni

form, which is responsible for bioreduction, to the Au˚ form in CT plant extract. Even though the FT-IR spectra of CT leaf aqueous extract and CT-AuNP appear to be similar, various bands of CT-AuNP are less intense than those of CT extract. CT-AuNP exhibits a slight shift in their various band positions when compared to the CT extract spectrum, indicating the essential role of CT extract in the reduction and stabilization of the formed AuNPs. In Figure 4 b, frequency shifts occur at 3288. 12 cm^{-1}, 2123. 20 cm^{-1} and 1635.27 cm^{-1} imply that alcohols/phenols (-OH; hydroxyl groups), $-CH_3$ (methylene groups), and

proteins (X-C=O; carbonyl groups) were potent in the bioreduction and stability, respectively. In alignment with numerous works, hydroxyl (-OH) and carboxylic acid (-COOH) are the two important functional groups that are related to the formation of NPs. Particularly, in the course of the genesis of NPs, the -COOH group assists in stabilization, meanwhile -OH groups help with the reduction processing (Usman et al. 2019, Padalia and Chanda 2021, Rauf et al. 2021). The carbonyl groups from the amino acid residues and peptides of proteins and the hydroxyl groups of alcohols have a great affinity to bind metals, acting as encapsulating agents to prevent the nanoparticles from aggregating (Lin et al. 2015). As a result, the FT-IR spectrum shows that the treatment of $AuHCl_4$ with the aqueous leaf extract of *C. tournefortii* resulted in the successful production of AuNPs.

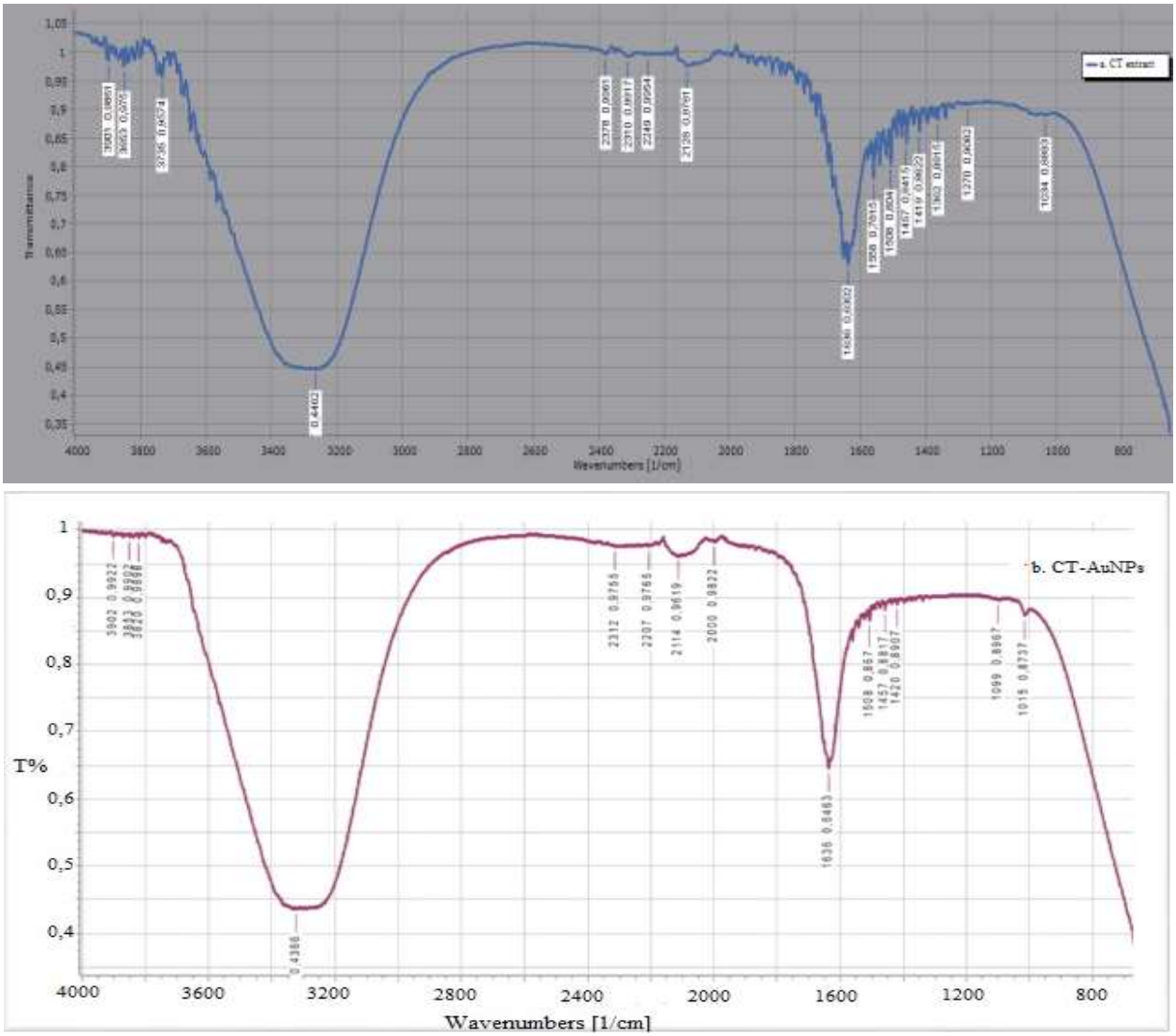


Figure 4. a- CT leaf aqueous extract FTIR spectrum b- CT-AuNPs FTIR spectrum
Şekil 4. a. CT yaprak özütü ve b. CT-AuNP'lerin FTIR spektrumları

FESEM, TEM micrographs and EDX profiles of CT-AuNPs

TEM and FESEM were used to reveal the size and morphology of the synthesized CT-AuNPs (Figure 5). The material appears to be composed of homogeneous AuNPs. The level of contamination is minimal (taking into account the biological source of the reducing agent). Biologic materials are used in the synthesis of AuNPs not only for size and shape control but also to provide superior properties to the AuNPs such as antimicrobial and cytotoxic properties. The TEM images show monodispersed and spherical nanoparticles in each case, indicating that the polyphenols act not only as a reducing agent but also as a capping agent, limiting their growth from 2 to 32 nm (Figure 5). Many of the CT-AuNPs appear to have a spherical morphology, which is often the case when no compounds are employed to favor anisotropic growth (Padalia & Chanda 2021). The dimensions of the CT-AuNPs ranged from 2 nm to 32 nm, with an average diameter of 14±6 nm.

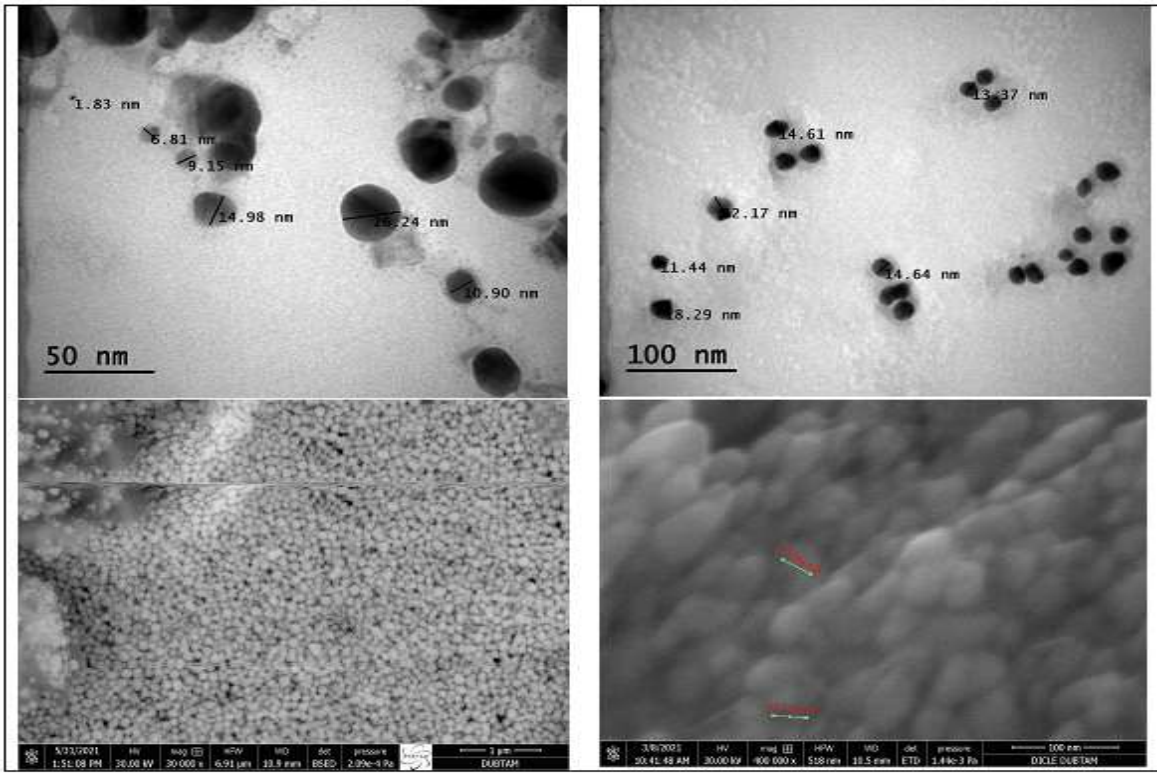
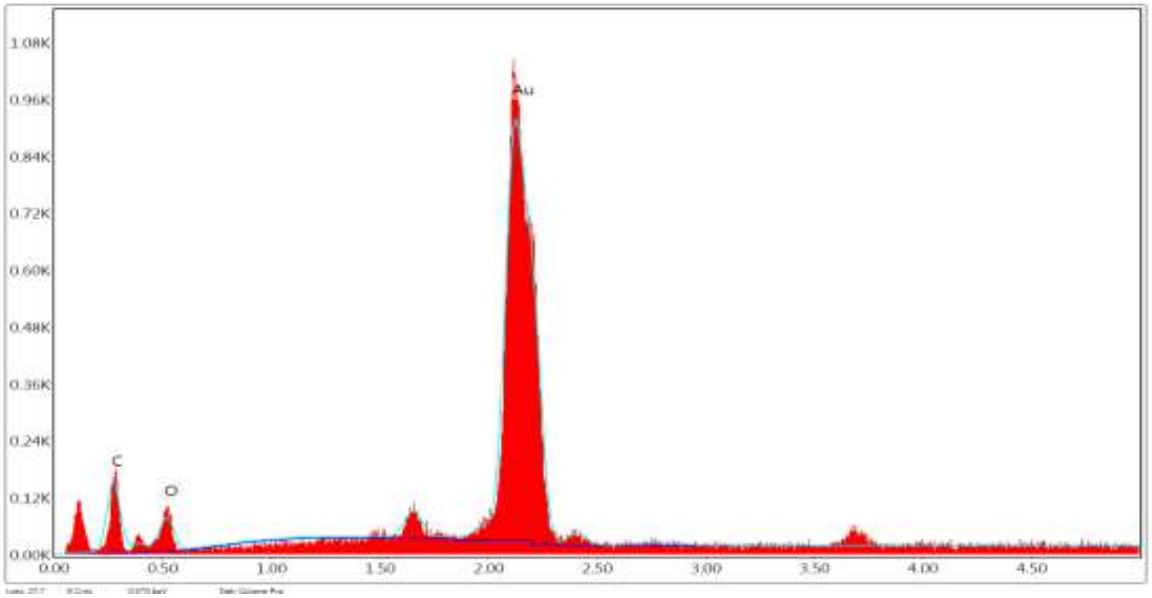


Figure 5. Morphological views of AuNPs synthesized with CT aqueous leaf extract; a and b parts indicate the TEM, c and d parts FESEM micrographs

Şekil 5. CT yaprak özütü ile sentezlenen AuNP'lerin morfolojik görünümleri; a ve b TEM, c ve d FESEM mikrografileri



Element	Wt %	Atomic %	Error %	Net Int.	Net Error %	K Ratio	Z	R	A	F
C K	33.28	69.97	10.45	78.35	3.49	0.1460	1.2166	0.8169	0.3605	1.0000
O K	14.82	23.38	16.40	37.72	7.15	0.0231	1.1795	0.8404	0.1322	1.0000
Au L	51.90	6.65	9.23	245.80	7.12	0.4581	0.7432	1.2002	1.0554	1.1252

Figure 6. Elemental composition of particles formed as a result of biosynthesis using CT leaf aqueous extract

Şekil 6. CT yaprak özütü kullanılarak biyosentez sonrası partiküllerin element kompozisyonu

The elemental composition of CT-AuNPs was examined by EDX analysis. Absorption of metallic gold nanoparticles was determined by strong gold nanocrystal signals (51.90%) at 1.7, 2.2, 2.4, and 3.7 keV. Weak signals such as carbon (33.28%) and oxygen (14.82%) were also recorded (Figure 6). These signals are thought to originate from biomolecules on the surface of NPs and be possibly derived from CT aqueous extract as the starting material involved in stabilizing AuNPs (Doan et al. 2020, Hosny et al. 2021, Mandhata et al. 2021).

TGA-DTA analysis data of CT-AuNPs

As seen in Figure 7, the resistance of AuNPs formed as a result of synthesis to heat treatment was investigated through TGA-DTA data at 25-1000 °C. It was observed that mass losses occurred at three different temperature points. The first mass loss was between 29.25 and 248.66 °C (13.66%), the second mass loss was between 310.33 and 555.34 °C (44.58%), and the third mass loss was formed between 556.18 and 834.74 °C (15.51%) (Figure 7). The first of these mass losses was due to water loss, and the others losses occurred due to inorganic compounds. These changes demonstrated the presence of phytochemicals around the formed nanoparticles; also, these phytochemicals are responsible for the surface charge and associated with stability (Baran, et al., 2019; Doan et al., 2020; Sepahvand et al., 2020 Padalia & Chanda, 2021).

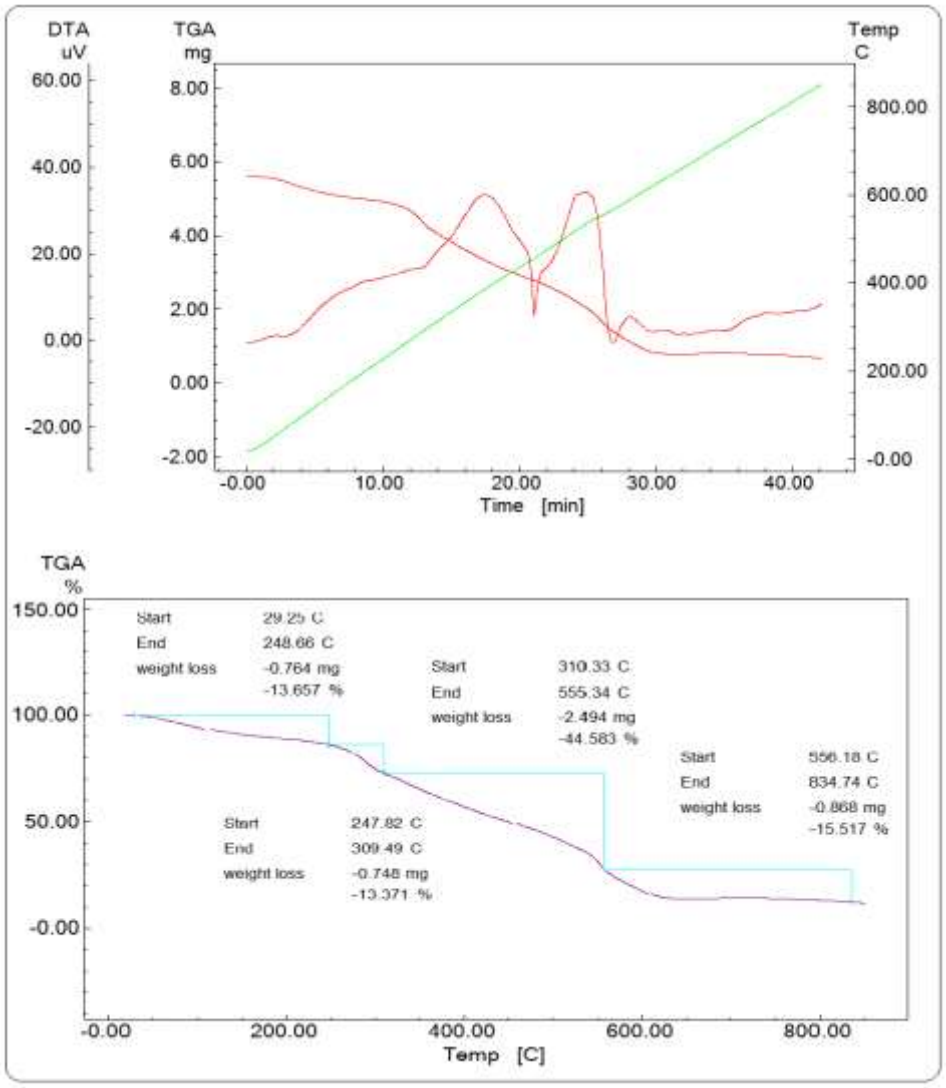


Figure 7. Temperature points at which TGA-DTA mass loss of CT-AuNPs occurs after synthesis
Şekil 7. Sentez sonrası CT-AuNP'lerin TGA-DTA sıcaklık noktalarında kütle kayıpları

Zeta potential and zeta sizer distributions data of CT-AuNPs

The stability of colloidal nanoparticles can be evaluated quantitatively using zeta potential, a measurement of the effective electrical potential on the surface of the particle. The zeta potential of the surface

charge distributions of AuNPs synthesized with CT leaf aqueous extract was determined as -16.53 mV (Figure 8a). In other environmentally friendly synthesis studies, it has been reported that the zeta potential distribution of AuNPs may have different values (Chinnaiyan et al., 2019; Khan et al., 2019; Tripathy et al., 2020).

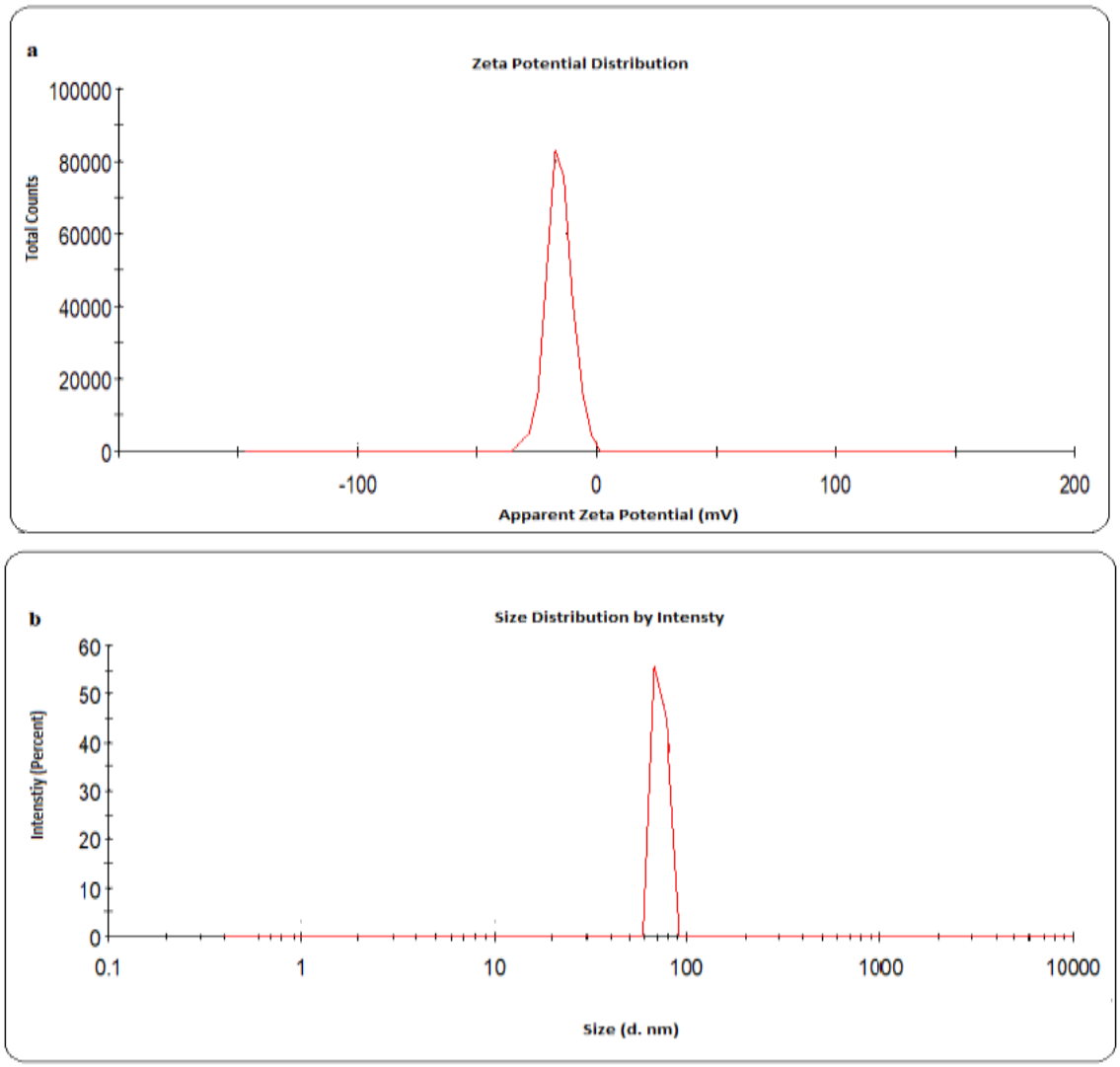


Figure 8. CT-AuNPs of after green synthesis; a. zeta potential, b. density-dependent size distribution graphs
Şekil 8. Yeşil sentez sonrasında CT-AuNP'lerin; a. zeta potansiyeli b. yoğunluğa bağlı boyut dağılımı grafileri

It is very important that AuNPs, as therapeutic agents, especially in drug delivery systems, ensure their stability under challenging conditions such as cell or blood circulation (Giljohann et al. 2010). The negative zeta potential distributions of the synthesized AuNPs provide pH stability, but also prevent the formation of properties that destabilize such as aggregation and fluctuation. Phytochemicals are the factor that causes the surface charge distribution of AuNPs to be negative (Khan et al. 2019, Webster 2020).

It has been reported that gold nanoparticles show different size distributions in environmentally friendly synthesis studies using plant materials (Usman et al., 2019; Perveen et al., 2021).

AFM micrograph of CT-AuNPs

AFM analysis gives us a grip on the topography, and roughness of metallic nanoparticles. The color scale helps determine the average size range on the scale bar. AFM imaging was applied in distinct magnification ranges of 12 and 50 µm (Figure 9). The AFM analysis performed to examine the topographic distribution of the synthesized CT-AuNPs and to evaluate their morphological structure and dimensions revealed that they were monodisperse, spherical in morphology, and less than 50 nm in size, which was consistent with other studies (Francis et al. 2017, Rauf et al. 2021).

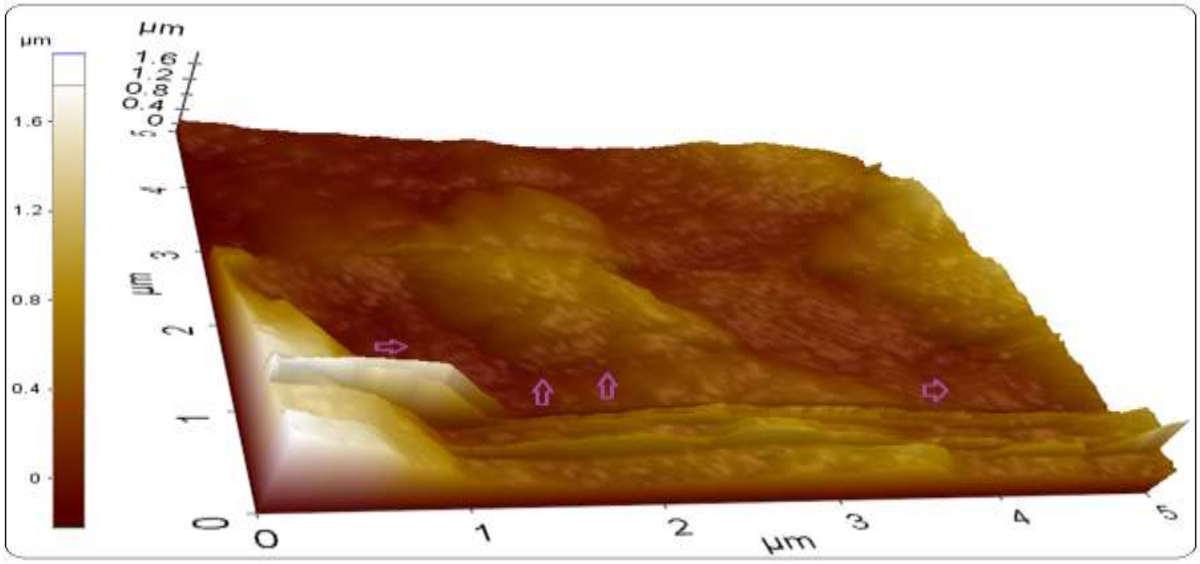


Figure 8. Topographic image of AuNPs synthesized with CT-aqua leaf extract
Şekil 8. CT-sulu yaprak özütüyle sentezlenen AuNP'lerin topografik görüntüsü

Biomedical Applications of CT-AuNPs

Antimicrobial effects of synthesized AuNPs

Regular and insufficient use of antibiotics has improved drug resistance to pathogen bacterial strains owing to genetic mutations. This situation makes the present antibiotics lesser potent for treating chronic illnesses. The improvement of strong and cost-potent drugs is the preference of recent studies. Metallic nanoparticles have superior properties that can easily serve these purposes. AuNPs interact with microorganisms due to electrostatic attraction (Babu et al. 2020). AuNPs induce an increase in Reactive Oxygen Species (ROS) and inhibit important steps of energy metabolism and transcription. After the interaction with nanoparticles, microorganisms reduce the ATP level by changing the membrane potential and inhibiting the ATPase activity, thus slowing down the metabolism. AuNPs also accelerate the biological collapse of the microorganism by suppressing the binding of tRNA to the small subunit of the ribosome (Cui et al. 2012, Jha et al. 2017, Babu et al. 2020, Donga et al. 2020, Webster 2020). The microdilution method was used to determine the MIC value of CT-AuNPs, HAuCL4 solution, and standard antibiotics on pathogen strains. At concentrations ranging from 0.01 to 0.50 g/mL, the synthesized CT-AuNPs were found to have a strong inhibitory effect on the growth of the tested organisms (Table 1).CT-AuNPs exhibited more effectiveness on gram-positive and negative strains at very low concentrations when compared to tested standard antibiotics (Colistin and Vancomycin), and HAuCI4 solution. Moreover, it was observed that the suppressive effect of CT-AuNPs on the C. albicans yeast was four times lower than the standard antibiotic (Fluconazole) and eight times lower concentration than the HAuCI4 solution when compared (Table 1). Researchers reported similar results that support in this results (Baran et al., 2020; Baran et al., 2021).

Table 1. Minimum Inhibition Concentrations (µg mL^{-1}) value of CT-AuNPs, standard antibiotics and Gold (III) chloride solution ($HAuCl_4$)
Çizelge 1.

Pathogen Microorganisms	CT-AuNPs	$HAuCl_4$	Standard Antibiotics*
S. aureus	0.02	1.00	2.00
B. subtilis	0.01	0.50	0.50
E. coli	0.03	2.00	4.00
P. aeruginosa	0.50	1.00	4.00
C. albicans	0.25	2.00	1.00

* Colistin for gram negatives, Vancomycin for gram positives, Fluconazole for yeast.

Cytotoxic effects of CT-AuNPs

Various properties of nanomaterials play a decisive role in their toxic effect. These include the shape of the NPs, but also their properties such as concentration, charge, exposure time, the chemistry of the surface composition, degree of deposition, and size. When the cell size is small, AuNPs easily pass through the cell

membrane and cause changes in the morphological structure of the cell. They cause an increase in ROS and changes in the nuclear structure. They induce apoptosis by activating caspase enzymes, besides, they spread the apoptotic signal by increasing cytochrome c release by affecting mitochondrial permeability, and as a result, they cause cell death (Rolim et al. 2019, Barabadi et al. 2020, Donga et al. 2020, Webster 2020). Various properties of nanoparticles are the characteristics that determine their effectiveness in toxic activity. Among these features of AuNPs; are surface charges, concentrations, interaction times, shapes, degrees of deposition, and sizes are the most important (Remya et al. 2015, Swamy et al. 2015). The cell viability and suppressing concentrations of AuNPs synthesized by CT leaf extract on CaCo-2, Skov-3, and U118 cell lines were investigated using the MTT method (Table 2). 100 µg/mL concentration of CT-AuNPs showed a suppressive effect on viability in all cell lines. Also, the 25 and 200 µg/mL concentrations of CT-AuNPs in healthy cell lines (HDF) caused suppression of proliferation by 74.66% and 50.6%. Moreover, the 25 µg/mL CT-AuNPs concentration exhibited suppressing activity on the proliferation of the CaCo-2 cell line at 88.58% percentage. On the other hand, the 100 µg/mL CT-AuNPs concentration suppressed growing of the U118 and Skov-3 cell lines at 92.50% and 92.46% percent respectively.

Table 2. Cytotoxic effects of CT-AuNPs on cell lines
Çizelge 2.

	Concentrations (µg/mL)			
Cell Lines	**25**	**50**	**100**	**200**
HDF	25.34*	27.55	33.80	49.43
CaCo-2	11.42	11.42	11.45	11.41
U118	68.54	58.89	7.54	10.78
Skov-3	58.79	51.86	7.50	9.49

*% viability rates of cells lines

CONCLUSION

This study demonstrated that AuNPs can be produced without the use of chemicals from Celtis tournefortii leaf extract in an environmentally friendly, toxic residue-free, and cost-effective method. UV-vis spectroscopy, UV-vis, XRD, FTIR, TEM, AFM, FESEM, EDX, TGA-DTA, zeta potential, and zeta sizer techniques were used to characterize CT-AuNPs. Gold nanoparticles prepared with this plant have a strong antipathogenic and cytotoxic effect, with a homogeneous distribution and a spherical appearance with a mean size of 31.30 nm, and the absence of any significant toxicity was evaluated and verified pending the current work. CT-AuNP showed good anticancer properties against CaCo-2, U118, and Skov-3. Hence, Celtis tournefortii leaf aqueous extract stabilized AuNPs be able to effectually as a vigorous means against cancerous cell lines. CT-AuNP exhibited significant antimicrobial activity against varied human pathogenic bacteria and yeast. As a whole, the study's findings highlighted green AuNPs' promising potential for anticancer and antimicrobial activity. As a result, a translation of the methodology is proposed as a simple, commercially viable, and environmentally friendly therapeutic approach in the field of nanomedicine and biomedicine.

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Author's Contributions

The authors declare that they have contributed equally to the article.

Statement of Conflict of Interest

Authors have declared no conflict of interest.

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New Records for the Turkish Freshwater Algal Flora in Twenty Five River Basins of Türkiye, Part I: Bacillariophyta

Tolga COŞKUN[1✉], Abuzer ÇELEKLİ[2], Cüneyt Nadir SOLAK[3], Tuğba ONGUN SEVİNDİK[4], Nilsun DEMİR[5], Elif Neyran SOYLU[6], Haşim SÖMEK[7], Tolga ÇETİN[8], Yakup KARAASLAN[9], Burak ÖTERLER[10], Faruk MARAŞLIOĞLU[11], Elif YILMAZ[12], Hatice TUNCA[13], Uğur GÜZEL[14], Merve KOCA[15], Bengü TEMİZEL[16]

[1, 5, 15]Ankara University, Faculty of Agriculture, Fisheries and Aquaculture Engineering, 06120, Ankara, Türkiye, [2]Gaziantep University, Faculty of Arts and Science, Department of Biology, 27310, Gaziantep, Türkiye, [3, 12]Dumlupınar University, Faculty of Arts and Science, Department of Biology, 43100, Kütahya, Türkiye, [4, 13, 14]Sakarya University, Faculty of Arts and Science, Department of Biology, 54050, Sakarya, Türkiye, [6, 16] Giresun University, Faculty of Arts and Science, Department of Biology, 28200, Giresun, Türkiye, [7]İzmir Katip Çelebi University, Faculty of Fisheries, Department of Inland Water Biology, 35620, İzmir, Türkiye, [8, 9] T.R. Ministry of Agriculture and Forestry, Directorate General of Water Management, 06560, Ankara, Türkiye, [10] Trakya University, Faculty of Science, Department of Biology, 22030, Edirne, Türkiye, [11]Hitit University, Faculty of Arts and Science, Department of Biology, 19040, Çorum, Türkiye

[1]https://orcid.org/0000-0001-5732-7424, [2]https://orcid.org/0000-0002-2448-4957, [3]https://orcid.org/0000-0003-2334-4271
[4]https://orcid.org/0000-0001-7682-0142, [5]https://orcid.org/0000-0002-3895-7655, [6]https://orcid.org/0000-0002-7583-3416
[7]https://orcid.org/0000-0003-4281-9738, [8]https://orcid.org/0000-0002-7817-3222, [9]https://orcid.org/0000-0001-8993-4771
[10]https://orcid.org/0000-0002-9064-1666, [11]https://orcid.org/0000-0002-7784-9243, [12]https://orcid.org/0000-0002-7814-3429
[13]https://orcid.org/0000-0003-3724-5215, [14]https://orcid.org/0000-0003-1358-3519, [15]https://orcid.org/0000-0002-2538-9388
[16]https://orcid.org/0000-0002-5217-3013
✉: tolga.coskun@yahoo.com.tr

ABSTRACT

The present study aimed to contribute to the algal flora of Türkiye by Bacillariophyta (diatom) as new records for the Turkish freshwater. Phytobenthos and phytoplankton were sampled three times (spring, summer, and autumn) a year between 2017 and 2019. Samples of the phytoplankton were collected with a water sampler from three depths, and samples of diatoms were obtained as epiphytic in the littoral zone of the lakes. However, if macrophytes were absent, epilithic or epipelic diatoms were sampled in lakes. Also, samples of phytobenthos were preferred as epilithic in rivers. However, if stones were absent, epiphytic or epipelic diatoms were sampled. During the studied period, a total of 895 diatom taxa were determined as planktonic (378 taxa) and benthic (860 taxa) in lakes and rivers of 25 river basins of Türkiye, and a total of 39 new records were identified. The highest diatom taxa were determined in the Fırat-Dicle, Konya, Antalya, and Büyük Menderes basins with 11, 11, 10, and 6 taxa, respectively. On the other hand, new records were not detected in 10 basins.

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Türkiye'deki 25 Nehir Havzasından Türkiye Tatlı Su Alg Florası İçin Yeni Kayıtlar, Bölüm I: Bacillariophyta

ÖZET

Bu çalışma, Türkiye'nin tatlı su alg florası için Bacillariophyta (diatom) ait yeni kayıt olarak katkıda bulunmayı amaçlanmıştır. Fitobentoz ve fitoplankton örneklemeleri, 2017 ve 2019 yılları arasında yılda üç kez (ilkbahar, yaz ve sonbahar) yapıldı. Göllerdeki Fitoplanktonun örneklemesi, üç derinlikten su örnekleyici ile toplanırken, diatom örnekleri göllerin littoral bölgesinde epifitik olarak elde edildi. Bununla birlikte, makrofitin bulunmadığı göllerde diatom örneklemeleri epilitik ve epipelik olarak yapıldı. Ayrıca, nehirlerdeki fitobentoz örneklemesi epilitik olarak yapılırken, taşın bulunmadığı nehirlerde epifitik veya epipelik olarak örneklenmiştir. Çalışma süresince, Türkiye'nin 25 nehir havzasına ait göl ve nehirlerde planktonik (378 takson) ve bentik (860 takson) olmak üzere toplam 895 belirlenmiş ve toplam 39 yeni kayıt teşhis edilmiştir. En yüksek diatom taksonlarının sırasıyla 11, 11, 10 ve 6 taksonla; Fırat-Dicle, Konya, Antalya ve Büyük Menderes havzalarında belirlenmiştir. Bununla birlikte, 10 havzada yeni kayıta rastlanılmamıştır.

Biyoloji

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Anahtar Kelimeler
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INTRODUCTION

Diatoms (unicellular siliceous algae, Bacillariophyta) are the most diverse photosynthetic eukaryotic microorganisms that are distributed worldwide in nearly all aquatic ecosystems (Round et al., 1990; Taxböck et al., 2020). Their ecological requirements are very different (Round et al., 1990), and their diversity can alter according to the environmental conditions (Ács et al., 2004). They are the important indicators in studies of climate change, acidic precipitation, water pollution, and water quality (Smol and Stoermer, 2010). For most of the diatom species it was thought that they are widespread, and even cosmopolitan in their spatial distribution (Kristiansen, 1996; Finlay and Clarke, 1999). However, in recent studies, a lot of studies support that they exhibit a biogeography (Bouchard et al., 2004; Vyverman et al., 2007). Diatom diversity can be influenced by several factors such as habitat availability, habitat area, the evolutionary history of lineages and regions, geogenic variables, hydrological stability, dispersal limitation, migration, and the cumulative effects of stochastic variation (Hubbell, 2001; Martiny et al., 2006; Cantonati and Lange-Bertalot; 2006, Telford et al., 2006; Cantonati and Spitale, 2009; Karger et al., 2011; Kraft et al., 2011; Lessard et al., 2012; Teittinen et al., 2017). The number of studies should be increased for the better understand of the biogeography of diatoms and the factors affecting their distribution.

In Türkiye, more than 300 taxonomical and ecological studies about diatoms have been reported (Solak et al., 2012; Taşkın et al., 2019). In these studies, planktonic diatoms in lakes have received more attention, while epilithic and epipelic communities were other mostly studied groups especially in rivers (Solak et al., 2012). In recent years, due to the increase of water quality assessment projects, several studies were done using diatom indices (e.g., Sevindik and Kucuk, 2016; Çelekli et al., 2018; Şanal and Demir, 2019; Çetin and Demir, 2019; Tokatlı et al., 2019; Solak et al., 2020; Maraşlıoğlu and Bektaş 2022). Until now, 1848 diatom taxa including brackish water species were recorded in the freshwater algal flora of Türkiye (Taşkın et al., 2019; Maraşlıoğlu and Gönülol, 2021), and 101 new diatom records were given in recent years (Baykal et al., 2009; Sevindik et al., 2011; Solak et al., 2016; Maraşlıoğlu and Soylu, 2018; Şahin and Akar 2018; Varol et al., 2018; Ayça Oğuz et al., 2020; Solak et al., 2021; Şahin 2022).

Due to Türkiye's European Union accession process, several projects have been implemented and funded by Directorate-General for Water Management (DGWM) and the General Directorate of State Hydraulic Works (DSİ) of the Ministry of Agriculture and Forestry specified for biological quality components. This study is one of the outcomes of the "Establishment of Reference Monitoring Network in Türkiye" project, financially and technically supported by DGWM. In this project, 275 lakes and 586 rivers in 25 river basins were studied, and a total of 1363 phytoplankton taxa and 860 phytobenthos taxa were detected. A total of 895 diatoms (Bacillariophyta) taxa were recorded as planktonic (378 taxa) and benthic (860 taxa). The present study aimed to contribute to the algal flora of Türkiye by describing 39 taxa in Bacillariophyta as new records for the Turkish freshwater algal flora. By contributing to the diatom flora in Türkiye together with the data obtained from this study, a database will be established for future diatom biogeography studies. For this reason, the distribution of the newly identified taxa in the Turkish basins was also given.

MATERIALS and METHODS

Study Area

As a consequence of the intersection of different climate types, noticeable altitude differences, and the position of the mountains relative to the Mediterranean, Aegean, and Black Seas, there are 7 different climatic zones in Türkiye (Solak et al., 2012). Therefore, the average annual temperature and precipitation values are very diverse in different regions (Anonymous, 2004). For instance, the average annual precipitation ranges from 200 mm in Central Anatolia to 2500 mm in the north-eastern coastal area of the Black Sea (Şensoy et al., 2008). Thus, water availability varies in the basins depending on the geographical size of the river basins and the average annual precipitation and temperature values (Akın and Akın, 2007). The Fırat-Dicle Basin is the largest single volume of available exploitable freshwater resources (28.5%) (Foreign Relation Office of DSİ 2014).

Türkiye has 25 river basins (Figure 1), and the names of the basins are usually referred to by the name of the greatest river they contain. Türkiye has 107 major rivers (Solak et al., 2012). Most of Türkiye 's rivers are

found in the Black Sea Region. These are Çoruh, Yeşilırmak, Kızılırmak, and Sakarya. All these rivers flow into the Black Sea. The longest river (1,355 km) in Türkiye is Kızılırmak. The most important streams flowing into the Mediterranean Sea are the Asi, Seyhan, Göksu, and Ceyhan rivers. Meriç, Küçük Menderes, and Büyük Menderes are the rivers flowing into the Aegean Sea (Şahin, 2010). River basins in Türkiye consist of 200 natural lakes, 806 reservoirs, and 1000 ponds (Foreign Relation Office of DSİ, 2014). Burdur, Susurluk, Van Lake, and Konya are important basins where the natural lake numbers are high (Hoşgören, 1994). However, in the last century, as a result of climate change and improper usage of groundwaters, decreases have been observed in the area and depth of natural lakes (i.e. Tuz, Akşehir, Beyşehir) (Solak et al., 2012).

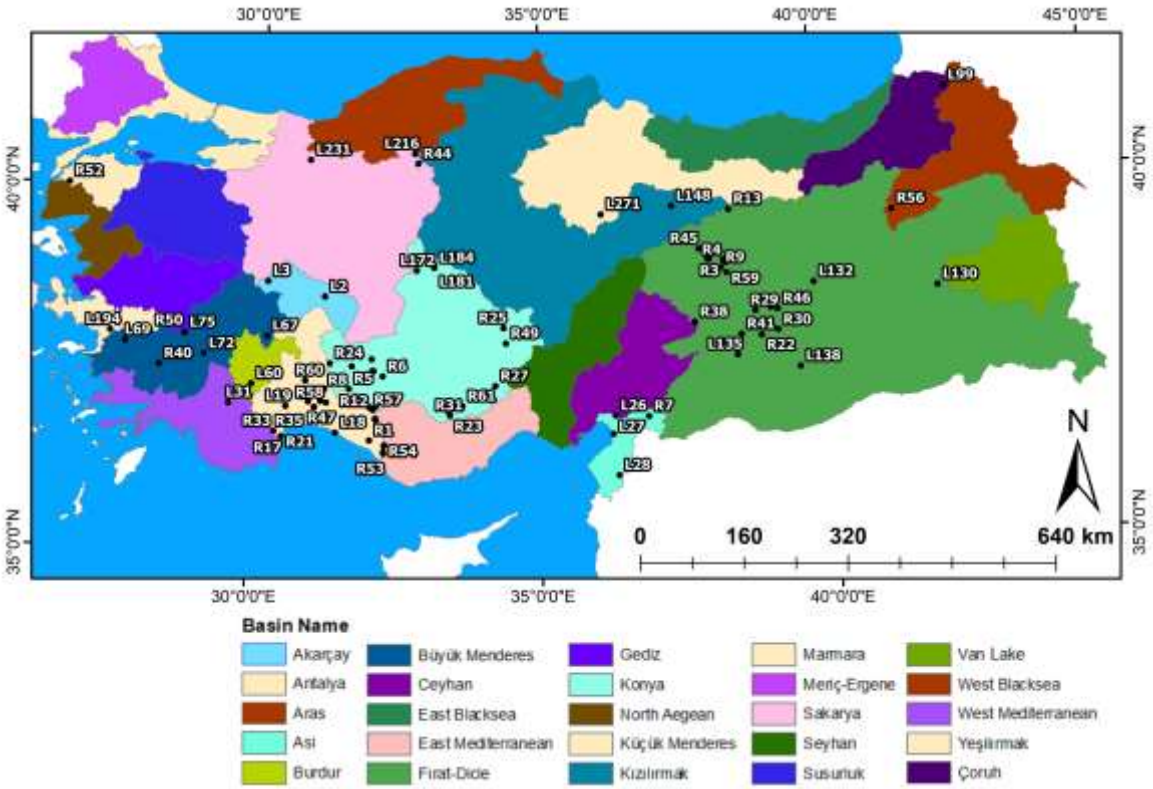

Figure 1. River Basins of Türkiye (L: Lake, R: River) and locations of the newly described taxons
Şekil 1. Türkiye'nin nehir havzaları ve yeni tanımlanan taksonların yerleri (L: Göl, R: Nehir)

Sampling and Identification:

Phytoplankton was sampled three times (spring, summer, and autumn) a year in 2017, 2018, and 2019 at the one, two, or three monitoring stations in each lake. The number of stations to be sampled were determined as one station for lakes that have a surface area smaller than 50 ha, two stations for lakes having a surface area between 50 and 500 ha, and three stations for lakes with a surface area higher than 500 ha (Anonymous, 2015). One of the selected stations was determined at the deepest point of the lake. In 275 lakes, samples of the phytoplankton were collected with a 1 L Hydrobios water sampler from the euphotic zone (Secchi disk depth × 2.5). Plankton net with a pore diameter of 50 µm was also used for collecting quantitavive samples of phytoplankton. All samples were fixed with Lugol's solution.

In each lake, phytobenthos was sampled three times (spring, summer, and autumn) a year in 2017, 2018, and 2019 at the one, two, or three monitoring stations. Samples were obtained in the littoral zone of the lakes, near the stations which were selected for phytoplankton sampling. Epiphytic diatoms were preferred, however, if macrophytes were absent, epilithic or epipelic diatoms were sampled. In each river, phytobenthos was sampled three times (spring, summer, and autumn) a year in 2017, 2018, and 2019. Epilithic diatoms were preferred in rivers, however, if stones were absent, epiphytic or epipelic diatoms were sampled. For epilithic diatoms, at least five stones were randomly selected and the upper surface of the stones was brushed with the bristle brush in 100 mL of distilled water. For epiphytic diatoms, generally, hard-surfaced macrophytes such as reeds were selected, and submerged leaves or stems were brushed in 100 mL of distilled water. For epipelic diatoms, samples were collected from the most superficial layer (~10mm in depth) of the sediment using a core sampler (3 cm in diameter) (EN 15708, 2004). All samples were fixed with Lugol's solution.

Laboratory Analysis:

In the laboratory, diatom samples were cleaned with hydrochloric acid and hot hydrogen peroxide, and permanent slides were mounted with Naphrax according to EN 15708 (2004). The diatom taxa were identified by using different types of compound microscopes (1000 × magnifications) in different laboratories according to the identification books of Krammer and Lange-Bertalot (1986; 1991a; 1991b; 1999) and Krammer (2003). Identified taxa were checked with the checklist of Aysel (2005), Taşkın et al. (2019), and the database of Turkish algae (Maraşlıoğlu and Gönülol, 2021), and then determined as new taxa for Turkish freshwater algal flora. The currently accepted nomenclature and distribution of taxa have been given according to Guiry and Guiry (2021). The new records were photographed with the cameras attached to the microscopes.

RESULTS and DISCUSSION

During the studied period, a total of 895 diatom taxa were recorded as planktonic (378 taxa) and benthic (860 taxa), and 39 of them were found as new records (Table 1). The information about their dimensions, habitat, ecology and locations were given in Table 2. Their images were shown in Figure 2-4.

During the three years, 39 new records in 21 genera have been identified in the 15 basins among 25 river basins in Türkiye. The genera best shown in terms of species richness were *Gomphonema* (6) and *Pinnularia* (4). The majority of the new records were determined to belong to the Cymbellales (13 taxa) and Naviculales (16 taxa) orders, respectively. On the other hand, Cocconeidales and Eunotiales members were represented with 4 and 2 taxa, respectively. Moreover, species belonging to each of the Surirellales, Thalassiosirales, and Thalassiophysales orders have been identified.

In the basins, 19 taxa were only found in lakes, while 10 of them were found only in rivers and 10 of them were distributed in both lakes and rivers. The insufficient diatom research in lakes compare to river studies in **Türkiye** (Solak et al., 2012) is probably the main reason why more new records were found in lakes during in this study.

Rare species constituted 36.4% of 39 new records, while 63.6% of them are the species that are widely distributed in the world. *Achnanthidium rostropyrenaicum*, *Cocconeis pseudolineata*, *Craticula paramolesta*, *Cymbella exigua*, *Encyonema bipartitum*, *Encyonema lapponicum*, *Fallacia gemmifera*, *Gomphonema angustivalva*, *Gomphonema parvuliforme*, *Gomphonema pratense*, *Halamphora paraveneta*, *Navicula supergregaria*, *Pinnularia neohalophila*, *Sellaphora japonica*, *Surirella lacrimula* were identified as rare taxa (Guiry and Guiry, 2021). Rare species were distributed in Antalya, Fırat-Dicle, Konya, Marmara, Burdur, Sakarya, Yeşilırmak, Büyük Menderes, Küçük Menderes and Kızılırmak basins. 10 rare species were found only in one station, while 4 of them were distributed in different stations in one basin. Only *C. pseudolineata* and *G. angustivalva* were reported in different stations of the Antalya, Konya, and Fırat-Dicle basins. Therefore, we can conclude that the distribution area of these two species is wider in Türkiye.

The highest number of new records were found in both river and lake habitats with freshwater features in Fırat-Dicle (11 taxa), Konya (11 taxa), Antalya (10 taxa), and Büyük Menderes (6 taxa) basins. Due to its large geographical area and high-altitude differences (Şahin, 2010), the number of new records was found higher in the Fırat-Dicle basin. Moreover, relatively less studies were done in that region previously (Solak et al., 2012; Taşkın et al., 2019; Maraşlıoğlu and Gönülol, 2021). Although the majority of the species were detected in freshwater habitats, *Fallacia gemmifera*, *Navicula supergregaria*, and *Thalassiosira baltica* were found in brackish lake habitats. These species have been reported in marine and brackish habitats of Europe, Asia, the Arctic, North America, South America, the Atlantic Islands, and Australia (Rumrich et al., 2000; Guiry and Guiry, 2021). *T. baltica* has also been reported as a short life span, fast-growing, high environmentally tolerant, and invasive species (Ricciardi and Rasmussen, 1998; Edlund et al., 2000).

Seven species (*Brachysira neglectissima*, *Cymbopleura kuelbsii*, *Encyonema bipartitum*, *Encyonema lapponicum*, *Navicula supergregaria*, *Neidium densestriatum*, *Pinnularia grunowii*) identified in this study have been reported as sensitive in terms of water quality indicators (Van Dam et al., 1994; Wojtal, 2009; Hofmann et al., 2011). However, the majority of the species that were reported as new records in this study have been indicated as tolerant or resistant to pollution (Van Dam et al., 1994; Wojtal 2009; Hofmann et al., 2011). The distribution of the diatoms in different water quality levels of the lakes or rivers in the basins must be investigated.

CONCLUSION

As a result, 39 new records were reported for the freshwater algal flora of **Türkiye** with this study, and it was observed that these taxa were distributed in different regions in the world. The number of new diatom records for the algal flora of **Türkiye** is expected to increase in the future.

Table 1. Number of studied lakes and rivers in 25 river basins
Çizelge 1. 25 nehir havzasında incelenen göl ve nehir sayısı

	River Basins	Number of Locations		Number of Taxa		New Records			Locations of New Records	
		Lakes	Rivers	Lakes	Rivers	Lakes	Rivers	Number of Taxa*	Lakes**	Rivers***
2017	**Antalya**	9	36	144	236	5	23	10	L12, L15, L17, L18, L19	R1, R8, R14, R15, R16, R17, R18, R19, R21, R32, R33, R34, R35, R37, R39, R43, R44, R48, R54, R55, R59, R61
	Büyük Menderes	13	32	150	170	5	2	6	L67, L68, L69, L72, L75	R51, R40
	Gediz	6	28	63	101	-	-	-	-	-
	Konya	18	39	208	248	7	13	6 (11)	L4, L6, L10, L12, L13, L27, L28, L38	R5, R6, R10, R11, R12, R23, R24, R25, R27, R31, R50, R58, R62
	Küçük Menderes	6	13	98	101	1	-	1	L194	-
	North Aegean	5	19	198	158	-	-	-	-	-
	Susurluk	9	23	63	60	-	-	-	-	-
	Western Mediterranean	13	44	108	132	1	-	1	L31	-
2018	**Akarçay**	10	17	153	157	2	-	2	L2, L3	-
	Burdur	6	11	86	63	1	-	1	L60	-
	East Blacksea	7	18	47	57	-	-	-	-	-
	Kızılırmak	23	23	122	79	1	-	1	L148	-
	Marmara	9	9	113	84	-	1	1	-	R53
	Meriç-Ergene	5	6	96	65	-	-	-	-	-
	Sakarya	23	36	79	76	5	1	3	L19, L39	R45
	Yeşilırmak	14	21	89	41	1	-	1	L271	-
	West Blacksea	14	19	117	97	-	-	-	-	-
2019	**Aras**	3	16	66	71	-	1	- (1)	-	R56
	Asi	8	21	162	149	3	3	1 (2)	L26, L27, L28	R2, R7, R52
	Ceyhan	18	33	104	94	-	-	-	-	-
	Çoruh	8	12	79	51	1	-	- (1)	L99	-
	East Mediterranean	12	32	105	104	-	-	-	-	-
	Fırat-Dicle	17	39	153	174	6	19	5 (11)	L130, L132, L135, L138	R3, R4, R9, R13, R20, R22, R28, R29, R30, R36, R38, R41, R46, R47, R49, R57, R60, R63
	Seyhan	12	33	70	107	-	-	-	-	-
	Van Lake	7	6	89	41	-	-	-	-	-
	Total	**275**	**586**	**895**		**39**	**63**	**39**	**-**	

*has been shown in parentheses, the total number of new records in the basin
** The codes of the lake points are stated in Maraşlıoğlu et al., (2021)
***R1:** Akçay Creek, **R2:** Algana Creek, **R3:** Armutağaç Creek, **R4:** Aşağıdecde Creek, **R5:** Bendboğazı Creek, **R6:** Bıçakçı Creek, **R7:** Bostanlık Creek, **R8:** Boyalıçay Creek, **R9:** Büyük Creek, **R10:** Çamurluiret Creek, **R11:** Çarşamba Creek, **R12:** Çarşamba Creek, **R13:** Çelikler Creek, **R14:** Değirmen Creek, **R15:** Dim Creek, **R16:** Dim Creek, **R17:** Düzlübel Creek, **R18:** Eğreğin Creek, **R19:** Fabrika Creek, **R20:** Gazel Creek, **R21:** Göynük Creek, **R22:** Gurik Creek, **R23:** Güdet Creek, **R24:** Huzur Creek, **R25:** Ilısu River, **R26:** İnasar Creek, **R27:** İvriz Creek, **R28:** Kaldırma Creek, **R29:** Karaçamur Creek, **R30:** Karagedik Creek, **R31:** Karagöz Creek, **R32:** Karagöz Creek, **R33:** Karaman Creek, **R34:** Kargı Creek, **R35:** Karpuz Creek, **R36:** Kartal Creek, **R37:** Kartal Creek, **R38:** Kavurma Creek, **R39:** Kelmen Creek, **R40:** Kızılıçukur Creek, **R41:** Komikan Creek, **R43:** Kuru Creek, **R44:** Kürkgeçit Creek, **R45:** Kütüklü Creek, **R46:** Mağaracık Creek, **R47:** Mutolar Creek, **R48:** Natıflar Creek, **R49:** Norsil Creek, **R50:** Okçu Creek, **R51:** Ovacık Creek, **R52:** Pekmez Creek, **R53:** Menderes Stream, **R54:** Sapadere Creek, **R55:** Sapadere Creek, **R56:** Seyran Creek, **R57:** Simirtas Creek, **R58:** Sülek Creek, **R59:** Utice Creek, **R60:** Yalnızkaz Creek, **R61:** Yazılı Creek, **R62:** Yeşil Creek, **R63:** Yoncalık Creek

Table 2. List of Bacillariophyta taxa identified as new records in 25 basins in Türkiye
Çizelge 2. 25 havzada yeni kayıt olarak tanımlanan Bacillariophyta takson listesi

No	Taxa	Synonym(s)	Dimensions (µm)				Habitat	Type of Life	Water Quality Indication**	Basin(s) in Türkiye***		Distribution in the World*
			L	W	D	S (10 µm)				Basin	Location	
1	*Achnanthidium rostropyrenaicum* Jüttner and Cox	-	18	4.5	-	20	(Fre)-L, R	Epl	T	AN	R16, R35, R53	R
2	*Brachysira neglectissima* Lange-Bertalot	-	18	4.5	-	35	(Fre)-R	Epl	S	AN, FD	R15, R21, R26, R34, R36, R39	W
3	*Caloneis strelnikovae* Levkov and Williams	-	51	14	-	17	(Fre)-L	Epp	S/T	AK	L3	W
4	*Cocconeis euglyptoides* (Geitler) Lange-Bertalot	-	11	6	-	20	(Fre)-L, R	Phy-Epl	T	AN, KO	L12, L15, L19, L169, L181, L185, R11, R12, R15, R16, R23, R25, L26, R27, R31, R33, L38, R49, R53, R54, R58, R60	W
5	*Cocconeis pseudolineata* (Geitler) Lange-Bertalot	*Cocconeis placentula* var. *pseudolineata* Geitler	28	14	-	23	(Fre)-L, R	Epl	S/T	AN, FD, KO	L12, R20, R24, R27, R49, R59	R
6	*Craticula paramolesta* Lange-Bertalot, Cavacini, Tagliaventi and Alfinito	-	12	3.5	-	23	(Fre)-R	Epl	S/T	FD	R3, R9, R29, R62	R
7	*Cymbella affinis var. neoprocera* Silva	*Cymbella excisa* var. *procera* Krammer	31	8.5	-	10	(Fre)-L, R	Epl-Epp	S/T	AS, FD	L26, R2, R7, R22, R30, R41, R52,	W
8	*Cymbella cantonatii* Lange-Bertalot	-	37	10	-	10	(Fre)-L, R	Epl-Epp	S/T	FD	L138, R4, R45, R56	W
9	*Cymbella exigua* Krammer	-	19	8.5	-	15	(Fre)-L	Epp	T	MA	R52	R
10	*Cymbopleura kuelbsii* Krammer	-	30	7.5	-	10	(Fre)-L, R	Epl-Epp	S	AN, FD	L130, L132, L135, R37, R47	W
11	*Encyonema bipartitum* (A. Mayer) Krammer	*Cymbella bipartita* Mayer	17	5	-	11	(Fre)-R	Epl	S	FD	R38	R
12	*Encyonema lapponicum* (A. Cleve) Krammer	*Cymbella aequalis* var. *lapponica* (A. Cleve) Krammer	38	8	-	8	(Fre)-L	Epp	S	BU	L60	R
13	*Encyonopsis krammeri* Reichardt	-	12	3	-	28	(Fre)-L	Epl-Epp	S/T	AN, KO	L12, L169, L182, R1, R17, R18, R39, R43	W
14	*Eunotia circumborealis* Lange-Bertalot and Nörpel	*Eunotia septentrionalis* var. *bidens* Hustedt	45	7	-	13	(Fre)-L	Epp	S/T	SA	L231	W
15	*Eunotia ruzickae* Bílý and Marvan	-	60	6	-	13	(Fre)-L	Epp	S/T	SA	L216	W

Abbreviations; For Basin(s); AK: Akarçay, AN: Antalya, AR: Aras, AS: Asi, WM: Western Mediterranean, EM: Eastern Mediterranean, WB: Western Black Sea, EB: Eastern Black Sea, BU: Burdur, KM: Küçük Menderes, BM: Büyük Menderes, CE: Ceyhan, CO: Çoruh, FD: Fırat Dicle, GE: Gediz, KI: Kızılırmak, KO: Konya, NA: North Aegean, MA: Marmara, ME: Meriç Ergene, SA: Sakarya, SE: Seyhan, SU: Susurluk, VL: Van Lake, YE: Yeşilırmak. **For Habitat;** Fre: Freshwater, Bra: Brackish, R: River, L: Lake. **For Dimensions;** L: Length, W: Weight, D: Diameter, S: Striae number. **For Life Type;** Phy: Phytoplankton, Epl: Epilithic, Epp: Epiphytic. **For Water quality indicator;** S: sensitive, T: tolerant, S/T: indifference. **For Distribution;** R: Rare, W: Widely.

Table 2. Continued
Çizelge 2. Devamı

No	Taxa	Synonym(s)	Dimensions (µm)				Habitat	Type of Life	Water Quality Indication **	Basin(s) in Türkiye***		Distribution in the World*
			L	W	D	S (10 µm)				Basin	Location	
16	*Fallacia gemmifera* (Simonsen) D.G.Mann	*Navicula gemmifera* (Simonsen) D.G.man	15	4	-	20	(Bra)-L	Phy	S/T	KI	L148	R
17	*Gomphonema acutiusculum* (O.Müller) A.Cleve	*Gomphonema lanceolatum var. acutiusculum* O.Müller	43	10	-	9	(Fre)-L	Phy-Epl	S/T	WM	L31	W
18	*Gomphonema angustivalva* Reichardt	-	13	3.5	-	15	(Fre)-L, R	Phy-Epl	T	AN, FD, KO	L18, L19, R8, R10, R13, R14, R16, R32, R42, R57, R58, R61	R
19	*Gomphonema cymbelliclinum* Reichardt and Lange-Bertalot	-	37	6.5	-	10	(Fre)-L, R	Epl	T	AN, KO	R5, R14, R19	W
20	*Gomphonema parvuliforme* Levkov	-	23	8	-	10	(Fre)-L	Epl	T	KM	L194	R
21	*Gomphonema pratense* Lange-Bertalot and Reichardt	-	60	9.5	-	8	(Fre)-R	Epl	S/T	KO	L174	R
22	*Gomphonema procerum* Reichardt and Lange-Bertalot	-	38	6	-	11	(Fre)-L	Epp	S/T	AS	L27, L28	W
23	*Halamphora paraveneta* (Lange-Bertalot, Cavacini, Tagliaventi and Alfnito) Levkov	*Amphora paraveneta* Lange-Bertalot, Cavacini, Tagliaventi & Alfinito	40	20	-	11	(Fre)-L	Epl	T	BM	L75	R
24	*Navicula supergregaria* Rumrich and Lange-Bertalot	-	28	8	-	13	(Bra)-L	Epl	S	BM	L69, L72	R
25	*Navicula vilaplanii* Lange-Bertalot and Sabater	*Navicula longicephala* var. *vilaplanii* Lange-Bertalot & Sabater	15	5	-	19	(Fre)-R	Epl	S/T	BM, KE	R40, R50	W
26	*Neidium densestriatum* (Østrup) Krammer	*Caloneis ladogensis* var. *densestriata* Østrup	24	12	-	26	(Fre)-L, R	Phy-Epl	S	KO	L181, L184	W
27	*Pinnularia grunowii* Krammer	-	38	8	-	11	(Fre)-L	Epl	S	BM	L68	W
28	*Pinnularia neohalophila* Kulikovskiy, Genkal and Mikheeva	*Pinnularia rhombarea* var. *halophila* Krammer	52	11.5	-	10	(Fre)-L	Epl-Epp	S/T	YE	L271	R
29	*Pinnularia sinistra* Krammer	-	17	4	-	11	(Fre)-L, R	Phy-Epl-Epp	T	AN, CO, KO	L17, L99, L174, R33	W
30	*Pinnularia subanglica* Krammer	-	53	8	-	11	(Fre)-L	Epl	S/T	BM	L68	W

Table 2. Continued
Çizelge 2. Devamı

No	Taxa	Synonym(s)	Dimensions (µm)				Habitat	Type of Life	Water Quality Indication**	Basin(s) in Türkiye***		Distribution in the World*
			L	W	D	S (10 µm)				Basin	Location	
31	*Placoneis paraelginensis* Lange-Bertalot	-	11	7	-	12	(Fre)-R	Epl	S/T	AN, AR, FD	L17, R4, R55	W
32	*Planothidium biporomum (Hohn and H*ellerman) Lange-Bertalot	*Achnanthes biporoma* M.H.Hohn & J.Hellerman	10	3.5	-	12	(Fre)-L, R	Epl	T	KO	R10, R24	W
33	*Sellaphora atomoides* (Grunow) Wetzel and Van de Vijver	*Navicula atomoides* Grunow	9.5	3.5	-	30	(Fre)-R	Epl	S/T	FD	R20, R28, R29, R38, R45, R48, R59	W
34	*Sellaphora japonica* Kobayasi	*Stauroneis japonica* H.Kobayasi	20	5.5	-	24	(Fre)-R	Epl	S/T	SA	R44	R
35	*Sellaphora nigri* (De Notaris) Wetzel and Ector	*Navicula nigri* De Notaris	5	3	-	26	(Fre)-R	Epl	S/T	FD	R46	W
36	*Stauroneis amphicephala* Kützing	*Stauroneis anceps* var. *amphicephala* Kützing	35	11	-	19	(Fre)-L, R	Phy-Epl	T	KO	L175, R5	W
37	*Staurophora tackei* (Hustedt) Bahls	*Navicula tackei* Hustedt	20	5.5	-	24	(Fre)-L	Epl	S/T	BM	L67	W
38	Surirella lacrimula English	*Surirella neglecta* E.Reichardt	24	10	-	27	(Fre)-R	Epl-Epp	T	KO	L172, R6, R10, R24, R61	R
39	*Thalassiosira baltica* (Grunow) Ostenfeld	*Coscinodiscus polyacanthus* var. *balticus* Grunow			30 diam		(Bra)-L	Epp	S/T	AK	L2	W

*Distribution has been evaluated according to AlgaeBase (Guiry and Guiry 2021)
**has been given according to Van Dam et al. (1994); Wojtal (2009); Hofmann et al. (2011)
*** The codes of the lake points are stated in Maraşlıoğlu et al. (2021)

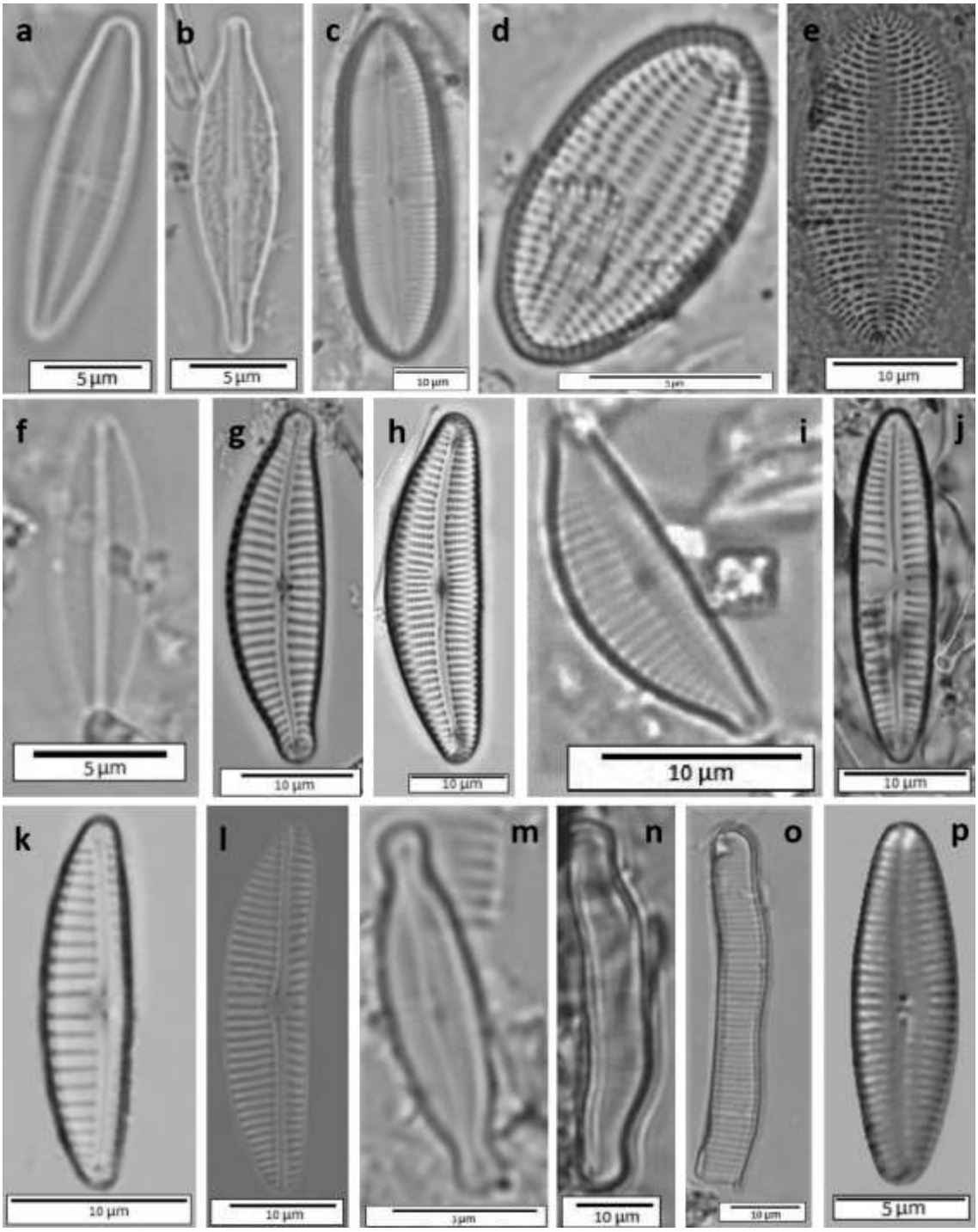

Figure 2. **a)** *Achnanthidium rostropyrenaicum*, **b)** *Brachysira neglectissima*, **c)** *Caloneis strelnikovae*, **d)** *Cocconeis euglyptoides*, **e)** *Cocconeis pseudolineata*, **f)** *Craticula paramolesta*, **g)** *Cymbella affinis* var. *neoprocera*, **h)** *Cymbella cantonatii*, **i)** *Cymbella exigua*, **j)** *Cymbopleura kuelbsii*, **k)** *Encyonema bipartitum*, **l)** *Encyonema lapponicum*, **m)** *Encyonopsis krammeri*, **n)** *Eunotia circumborealis*, **o)** *Eunotia ruzickae*, **p)** *Fallacia gemmifera*

Şekil 2. ***a)*** *Achnanthidium rostropyrenaicum,* ***b)*** *Brachysira neglectissima,* ***c)*** *Caloneis strelnikovae,* ***d)*** *Cocconeis euglyptoides,* ***e)*** *Cocconeis pseudolineata,* ***f)*** *Craticula paramolesta,* ***g)*** *Cymbella affinis var. neoprocera,* ***h)*** *Cymbella cantonatii,* ***i)*** *Cymbella exigua,* ***j)*** *Cymbopleura kuelbsii,* ***k)*** *Encyonema bipartitum,* ***l)*** *Encyonema lapponicum,* ***m)*** *Encyonopsis krammeri,* ***n)*** *Eunotia circumborealis,* ***o)*** *Eunotia ruzickae,* ***p)*** *Fallacia gemmifera*

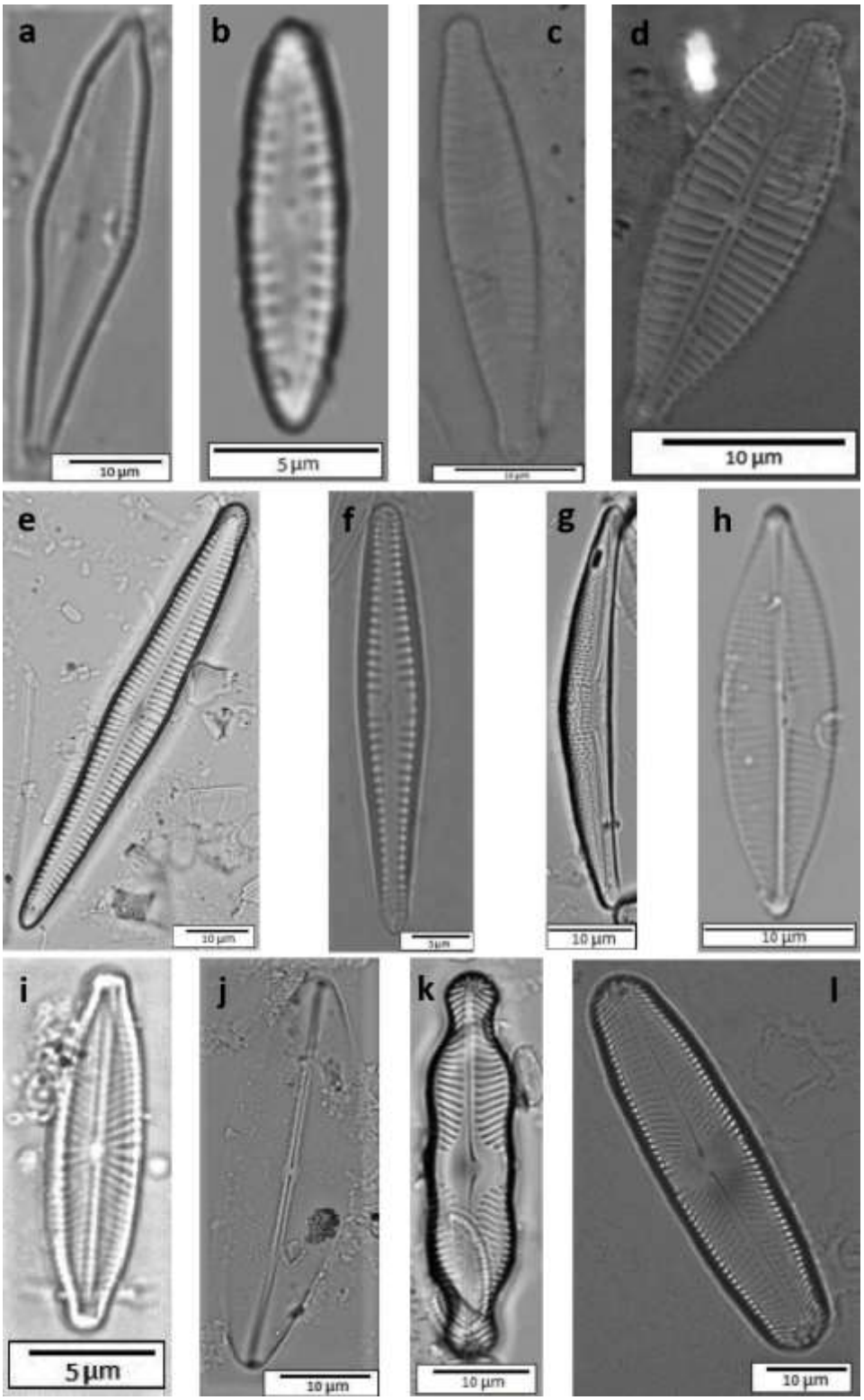

Figure 3. **a)** *Gomphonema acutiusculum*, **b)** *Gomphonema angustivalva*, **c)** *Gomphonema cymbelliclinum*, **d)** *Gomphonema parvuliforme*, **e)** *Gomphonema pratense*, **f)** *Gomphonema procerum*, **g)** *Halamphora paraveneta*, **h)** *Navicula supergregaria*, **i)** *Navicula vilaplanii*, **j)** *Neidium densestriatum*, **k)** *Pinnularia grunowii,* **l)** *Pinnularia neohalophila*

Şekil 3. ***a)*** *Gomphonema acutiusculum,* ***b)*** *Gomphonema angustivalva,* ***c)*** *Gomphonema cymbelliclinum,* ***d)*** *Gomphonema parvuliforme,* ***e)*** *Gomphonema pratense,* ***f)*** *Gomphonema procerum,* ***g)*** *Halamphora paraveneta,* ***h)*** *Navicula supergregaria,* ***i)*** *Navicula vilaplanii,* ***j)*** *Neidium densestriatum,* ***k)*** *Pinnularia grunowii,* ***l)*** *Pinnularia neohalophila*

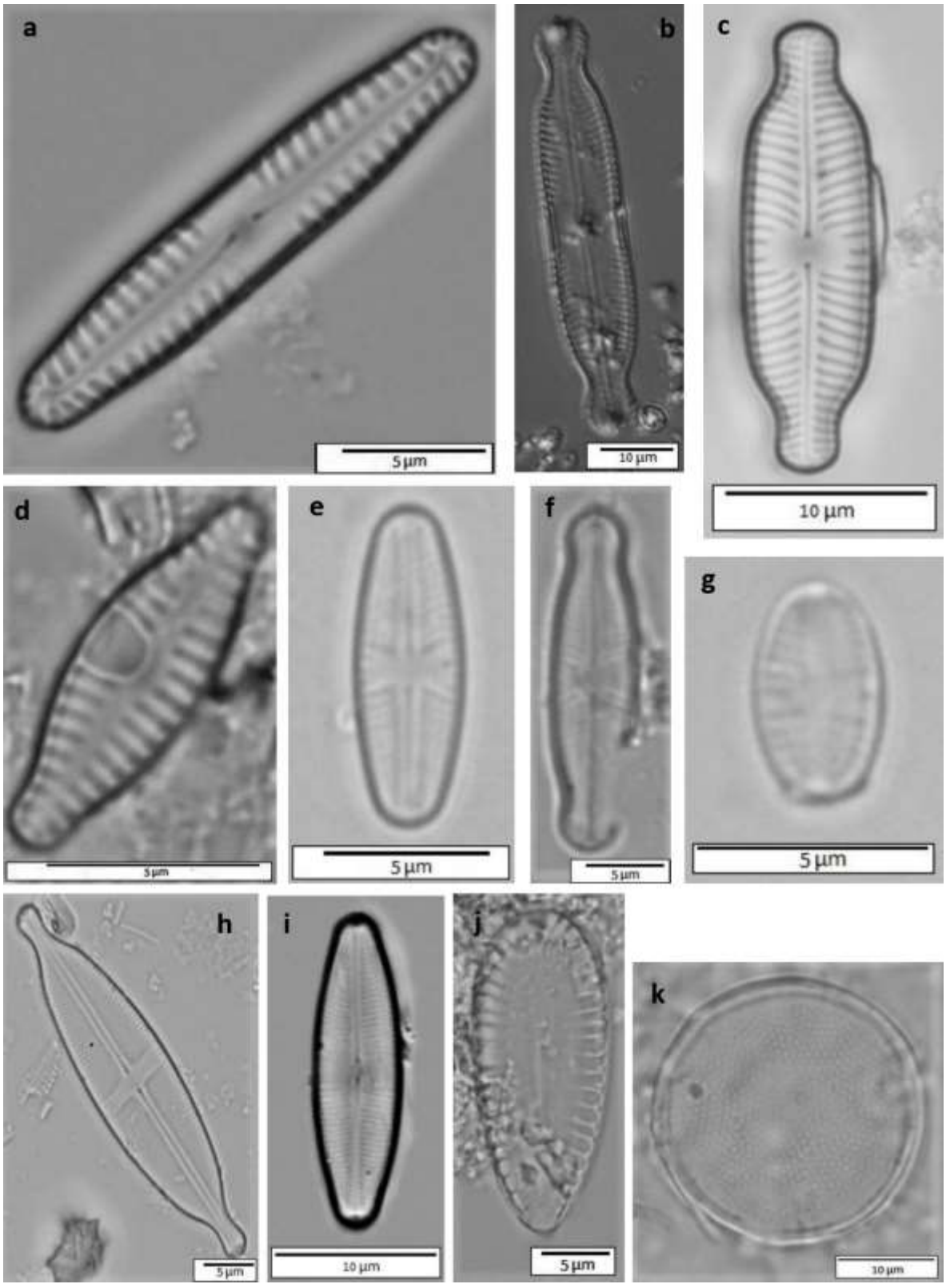

Figure 4. **a)** *Pinnularia sinistra*, **b)** *Pinnularia subanglica*, **c)** *Placoneis paraelginensis*, **d)** *Planothidium biporomum*, **e)** *Sellaphora atomoides*, **f)** *Sellaphora japonica*, **g)** *Sellaphora nigri*, **h)** *Stauroneis amphicephala*, **i)** *Staurophora tackei*, **j)** *Surirella lacrimula*, **k)** *Thalassiosira baltica*

Şekil 4. ***a)*** *Pinnularia sinistra,* ***b)*** *Pinnularia subanglica,* ***c)*** *Placoneis paraelginensis,* ***d)*** *Planothidium biporomum,* ***e)*** *Sellaphora atomoides,* ***f)*** *Sellaphora japonica,* ***g)*** *Sellaphora nigri,* ***h)*** *Stauroneis amphicephala,* ***i)*** *Staurophora tackei,* ***j)*** *Surirella lacrimula,* ***k)*** *Thalassiosira baltica*

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Researchers' Contribution Rate Statement Summary

First Author: Conceptualization, Writing-review, editing, Data Curation, Formal Analysis, Investigation, Methodology, Visualization

Second Author: Data Curation, Formal Analysis, Investigation, Visualization

Third Author: Conceptualization, Data Curation, Formal Analysis, Investigation, Methodology, Visualization

Fourth Author: Conceptualization, Data Curation, Formal Analysis, Investigation, Methodology, Visualization

Fifth Author: Conceptualization, Data Curation, Formal Analysis, Investigation, Visualization

Sixth Author: Data Curation, Formal Analysis, Investigation, Visualization

Seventh Author: Data Curation, Formal Analysis, Investigation, Methodology, Visualization

Eighth Author: Funding Acquisition, Project Administration, Resources

Ninth Author: Funding Acquisition, Project Administration, Resources

Tenth Author: Conceptualization, Data Curation, Formal Analysis, Investigation, Visualization

Eleventh Author: Conceptualization, Data Curation, Formal Analysis, Investigation, Methodology, Visualization

Twelfth Author: Data Curation, Investigation

Thirteenth Author: Data Curation, Investigation

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Conflict of Interest Statement

The article's authors declare that they do not have any conflict of interest.

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Sarıveliler İlçesinden (Karaman-Türkiye) Bir Yeni Miksomiset Kaydı: *Arcyria afroalpina* Rammeelo

Gönül EROĞLU[1]
[1]Selçuk Üniversitesi, Fen Fakültesi, Biyoloji Bölümü, Konya, Türkiye
[1]https://orcid.org/0000-0001-6323-2077
✉: gnleroglu@gmail.com

ÖZET

Arcyria afroalpina Rammeelo, 2016 yılında Karaman ili Sarıveliler ilçesinden toplanan materyallere nem odası tekniği uygulanması sonucunda gelişmiştir. *A. afroalpina*, Türkiye için bir yeni miksomiset kaydı olup, Türkiye miksomisetlerine ilave edilmiştir. Taksonu tanımlayıcı stereomikroskop ve ışık mikroskop görüntüleri makale içinde verilmiştir.

Biyoloji

Araştırma Makalesi

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Anahtar Kelimeler
Myxomycetes
Yeni kayıt
Taksonomi
Morfoloji

A New Myxomycetes Record from Sarıveliler District (Karaman-Turkey): *Arcyria afroalpina* Rammeelo

ABSTRACT

Arcyria afroalpina Rammeelo was developed as a result of applying the moist chamber technique to materials collected from Sarıveliler district of Karaman province in 2016. *A. afroalpina* is a new myxomycete record for Turkey and has been added to Turkish myxomycetes. Taxon descriptive stereomicroscope and light microscope images are given in the article.

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GİRİŞ

Türkiye'de cıvık mantarlar olarak bilinen (Sesli ve ark., 2020) miksomisetler, spor üreten bir sporokarpa sahip olma özelliklerinden dolayı bir protozoan mantar analoğu olarak kabul edilmiş ve amoeboid protistlerin önemli bir taksonomik grubu olan *Amoebozoa*'ya dahil edilmiştir (Lado ve ark., 2016). Morfolojik karakterler temel alınarak yapılan geleneksel sınıflandırmada, *Ceratiomyxomycetidae* (*Ceratiomyxales*), *Myxogastromycetidae* (*Echinosteliales*, *Liceales*, *Trichiales*, *Physarales*) ve *Stemonitomycetidae* (*Stemonitales*) alt sınıfları tanımlanmaktadır (Martin & Alexopoulos, 1969; Poulain ve ark., 2011). Leontyev ve ark. (2019), mevcut sınıflandırmanın grup içindeki evrimsel ilişkileri yeterince yansıtmadığını göz önünde bulundurarak moleküler verilere dayanarak *Cribrariales* (temel grup), *Reticulariales*, *Licelaes* ve *Trichiales*'i *Lucisporomycetidae* (açık renkli sporlular) alt sınıfı ve *Echinosteliales* (temel grup), *Clastodermatales*, *Meridermatales*, *Stemonitidales* ve *Physarales*'i içeren *Columellomycetidae* (koyu renkli sporlular) alt sınıfını önermişlerdir (Sá ve ark., 2022).

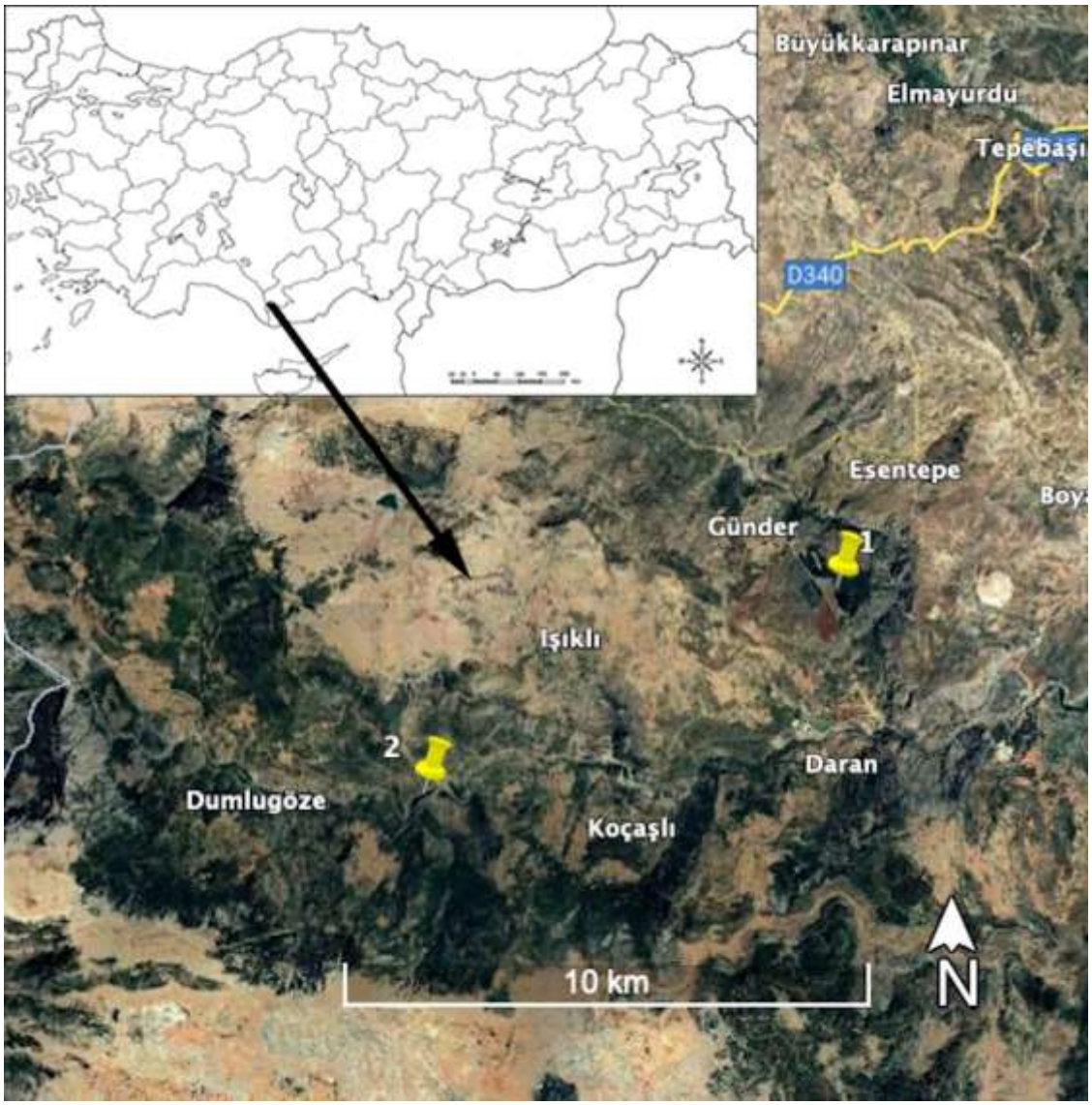
Miksomisetler Dünya çapında karasal habitatlarda yaşayan yaklaşık 1000 amoebozoan türünden oluşmaktadır (Lado, 2022). Türkiye'de ise 311 taksonla temsil edilmektedir (Eroğlu, 2021; Baysal & Eroğlu, 2022; Baba & Sevindik, 2022a; b). Bu türlerin içinde Dünya'dan 55 (Lado, 2022), Türkiye'den ise 20 (Sesli ve ark., 2020; Baba, 2021) *Arcyria* taksonu bildirilmiştir. *Arcyria*'nın sporoforları genellikle yoğun gruplar halinde yani sporokarpiktir. Karakteristik olarak sapın içi spor benzeri hücrelerle doludur.

Kapillitium dişliler, halkalar, yarım halkalar, ağsı süsler, sırtlar, dikenler, spiralller, siğiller veya bunların kombinasyonu ile süslenmiş içi boş filamentlerden (tübüller) oluşmaktadır. Eğer sporofor sapsızsa, spor süslemeleri her zaman ya ağsı ya da siğilli, kapillitium ise düz spiral süslüdür (García-Cunchillos ve ark., 2022).

Literatür araştırmaları sonucunda nem odası tekniğiyle gelişen örneğin, *A. afroalpina* olduğu tespit edilmiştir. Bu takson, Türkiye için yeni bir miksomiset kaydıdır.

MATERYAL ve METOD

Çalışma bölgesi olan Sarıveliler, Karaman ilinin ilçelerinden birisidir. Sarıveliler, İç Anadolu Bölgesinin güneyinde, Orta Torosların güney yamaçlarında Göksu havzasını kapsayan, Taşeli Platosunda yer alır. İlçe doğusunda Ermenek, kuzey doğusunda Başyayla, kuzeyinde Taşkent, güneyinde Gazipaşa, güney batısında Alanya ilçeleri yer alır. İlçenin iklimi karasal iklim olup, yazları oldukça sıcak ve kurak, kışları ise soğuk ve kar yağışlı geçmektedir. İklime bağlı olarak ilçe genelinde geniş orman arazileri bulunmaktadır (Bağcı ve ark., 2016). İlçedeki orman alanları, Karaman ili orman alanlarının %11.78'sini oluşturmaktadır. Bu alanlarda sedir (*Cedrus* sp. Trew.), köknar (*Abies* sp. Mill.), ardıç (*Juniperus* sp. L.), meşe (*Quercus* sp. L.) ve çam ağaçları (*Pinus brutia* Ten. ve *P. nigra* J. F. Arnold) vardır (Anonim, 2022). Yeni kaydın tespit edildiği materyaller Sarıveliler ilçesine bağlı Günder ve Dumlugöze köylerinden toplanmıştır (Şekil 1).



Şekil 1. Çalışma alanının haritası (Google Earth'ten alınmıştır)
Figure1. Map of the study area (adopted from Google Earth)

2016 yılında Sarıveliler ilçesine yapılan arazi çalışmasında toplanan materyallere nem odası tekniği (Gilbert & Martin, 1933) uygulanmıştır. Hazırlanan kültürler ilk ay gün aşırı, sonraki üç ay haftada bir kez

stereomikroskop altında incelenmiştir. Gelişen örnekler substralarıyla beraber kesilerek mukavvalara yapıştırılmış ve laboratuvar ortamında kurumaya bırakılmıştır. Örneklerin bulunduğu mukavvalar aynı boyuttaki kutulara konularak fungaryum materyali haline getirilmiştir. Örneğin makroskobik özellikleri stereomikroskop (Leica S8APO) ve mikroskobik özellikleri ise ışık mikroskobu (Leica DM750) aracılığıyla tespit edilip tanımlayıcı fotoğrafları çekildikten sonra başlıca miksomiset teşhis kaynaklarından (Poulain ve ark., 2011; Stephenson, 2021) faydalanılarak teşhisi yapılmıştır. Ayrıca Rammeloo (1981), Lado ve ark. (2011; 2013)'nın yapmış oldukları çalışmalardan da faydalanılmıştır. Taksonun ismi, yazar ismi ve sinonimi online nomenklatür bilgi sisteminden kontrol edilmiştir (Lado, 2022). Bu çalışmada, Stephenson (2021) tarafından kullanılan sistematik takip edilmiştir. Kutu üzerine etiket yapıştırılarak fungaryum materyali haline getirilen *A. afroalpina*, S. Ü. Mantarcılık Uygulama ve Araştırma Merkezi Fungariumu'nda saklanmaktadır.

BULGULAR ve TARTIŞMA

Arazi ve laboratuvar çalışmaları sonucunda tespit edilen *A. afroalpina*'nın sistematiği, sinonimi, takson tanımı, lokalitesi (il, ilçe, köy, substrat, toplama tarihi, harita üzerindeki lokalite numarası (LN), koordinat, yükseklik, toplama numarası) aşağıda verildiği gibidir.

Eumycetozoa L. S. Owen

Myxomycetes G. Winter

Lucisporimycetidae Leontyev, Schnittler, S. L. Stephenson, Novozhilov & Shchepin

Trichiidia Leontyev, Schnittler, S. L. Stephenson, Novozhilov & Shchepin

Trichiales T. Macbr.

Trichiaceae Chevall.

Arcyria afroalpina Rammeloo Bull. Jard. Bot. Belg. 51(1/2): 229 (1981)

Syn: *Arcyria afroalpina* var. *mexicana* Lizárraga, G. Moreno & Illana, Oesterr. Z. Pilzk. 14:106 (2005)

Sporokarp saplı sporangiumdur. Sporoteka küresel, 0.1-0.4 mm çapında ve toplam yükseklik 0.6-1.2 mm'dir. Sap uzun ve ince, 0.5-1.0 mm uzunluğunda, açık sarı renkli, tabanda daha koyu renklidir (Şekil 2a-b). Sapın içi 8-(12.1)-15.5 µm çapında spor benzeri kistlerle doludur (Şekil 2e). Peridium kalikulus haricinde geçicidir. Kalikulus yassı ve sporotekanın çapından daha dardır. Kalikulus iç yüzeyi ince siğillidir. Siğillerin birleşmesiyle ince ağsı görünümlüdür (Şekil 2d). Kapillitium tübüler, hafif elastik, kalikulusa sıkıca bağlıdır. Kapillitium 2.5-(3.5)-4.5 µm çapında, hafifçe siğiller ile süslenmiş, siğiller küçük tepecikler oluşturmak üzere kaynaşmıştır (Şekil 2c). Işık mikroskobunda sporlar sarımsı, 7.3- (8.3)-9.2 µm çapındadır (Şekil 2f).

Lokalite: Karaman, Sarıveliler, Günder köyü, *Pinus brutia* döküntü dalları, 07.05.2016, LN: 1, 36°35′22″K 32°40′31″D, 1147m, GE213; bilinmeyen devrik kütük odunu, 07.05.2016, LN: 1, 36°35′22″K 32°40′31″D, 1147m, GE197; Dumlugöze köyü, kesik *P. brutia* kütük odunu, 01.07.2016, LN: 2, 37°53′06″K 32°18′36″D, 916 m, GE 249.

A. afroalpina ve *A. marginoundulata* Nann.-Bremek. & Y. Yamam. morfolojik olarak birbirine çok benzerdir. Fakat her iki türün birbirinden farklı özellikleri vardır. Sporlarının büyüklüğü ve spor süsünün dağınık siğilli olması, sporokarpın rengi en belirgin farklılıklarındandır. *A. afroalpina* toprak sarı rengindeyken, *A. marginoundulata* beyaz-açık toprak sarısı renktedir. Kalikulus özelliklerine bakıldığında *A. afroalpina*'nın kalikulusu küçük, düz veya ince siğilli, *A. marginoundulata*'nınki ise sporotekanın 1/5'i kadar, radyal olarak kıvrımlı, kenarda dalgalı eşmerkezli katlar bulunmaktadır (Poulain ve ark., 2011). Diğer taraftan Rammeloo (1981)'nun orijinal tür tanımında sporların daha büyük olduğu, ancak çalışmadaki tür özellikleri Lado ve ark. (2011)'nın yaptığı *A. afroalpina* tür tanımına oldukça fazla benzer olduğu görülmektedir. Ayrıca *A. afroalpina*'nın sapının uzun (Stephenson, 2021) olmasından dolayı *A. globosa* Schwein. taksonundan da farklıdır (Ronikier ve ark., 2013).

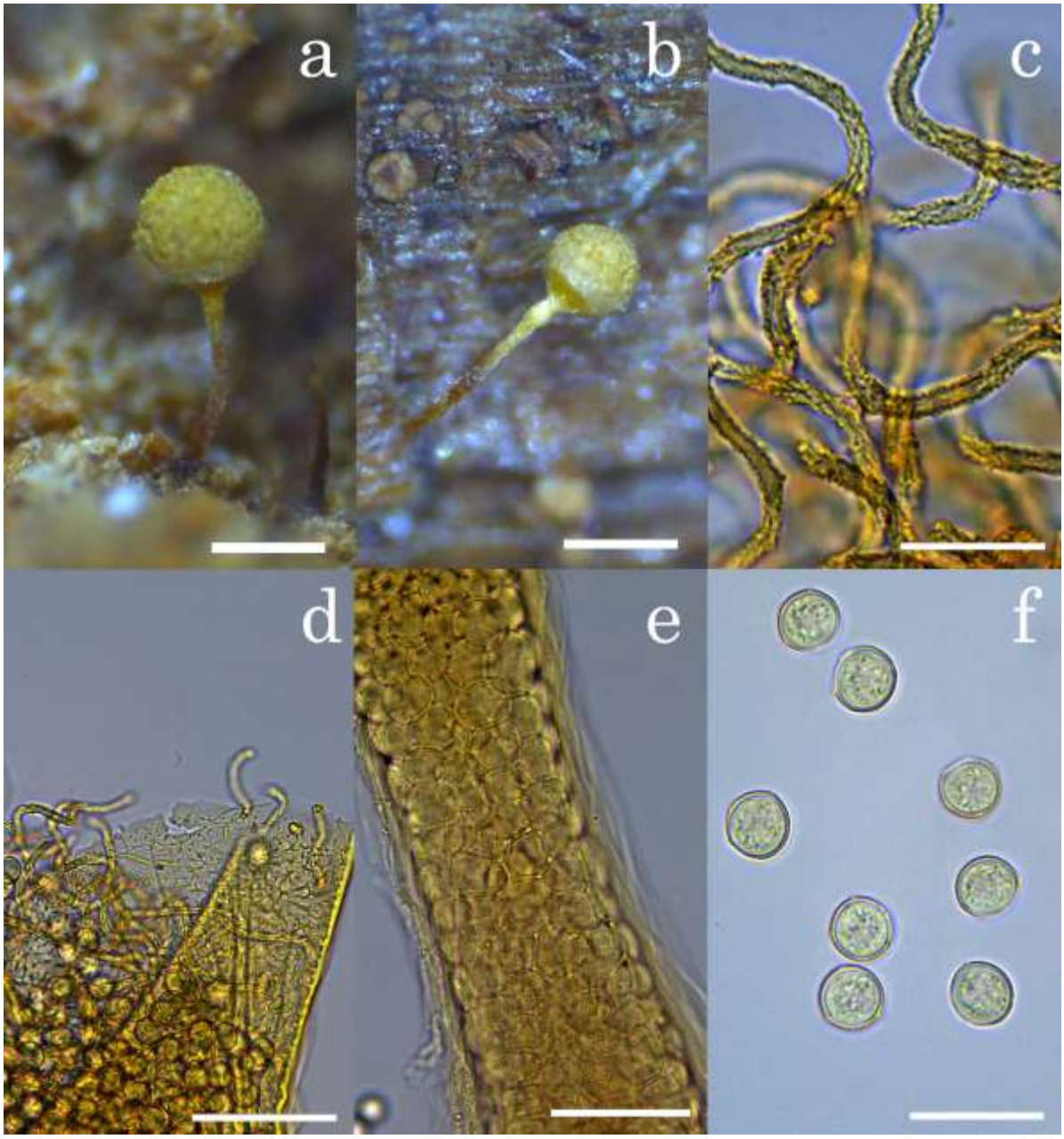
SONUÇ ve ÖNERİLER

Taksonlar arasındaki farklılıklar ve literatür taramaları sonucunda örnek *A. afroalpino* olarak teşhis edilmiştir. Bu takson Türkiye için bir yeni miksomiset kaydı olarak Türkiye miksobiyota listesine ilave edilmiştir. *Arcyria* türlerinin sporokarp ve kapillitial iplik özelliklerinden dolayı teşhisleri, diğer miksomiset örneklerine göre daha kolay teşhis edilebileceği söylenebilir.

Arcyria cinsinin karakteristik özellikleri olmasına rağmen kapillitial iplik, spor, kalikulus gibi mikroskobik özelliklerinin dikkatle incelenmesi önerilebilir. Ayrıca tür özelliklerinin belirlenebilmesi için türün ilk tanımı yapılan çalışma ve tür ile ilgili diğer çalışmalardaki tanımlar ve fotoğraflar dikkatlice incelenip karşılaştırma yapılabilir.

TEŞEKKÜR

Bu çalışma S. Ü. Bilimsel Araştırma Projeleri Koordinatörlüğü (Proje no: 15401116) tarafından maddi yönde desteklenmiştir.



Şekil 2. *Arcyria afroalpina* a-b. Sporokarp (SM), c. Kapillitial iplikler (IM), d. Kalikulus, kapillitial iplikler ve sporlar (IM), e. Sap içindeki spor benzeri kistler (IM), f. Sporlar (IM) (Stereomikroskop=SM, Işık mikroskobu=IM) (Ölçekler: a-b:25 µm, c: 20 µm; d-f: 50 µm)

Figure2. Arcyria afroalpina a-b. Sporocarp (SM), c. Capillitial threads (LM), d. Calyculus, capillitial threads and spores (LM), e. Spore-like cysts (LM) in the stalk, f. Spores (LM) (Stereomicroscope=SM, Light Microscope=LM) *(Scales: a-b: 25 µm, c: 20 µm; d-f: 50 µm)*

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Determination of Metal(loid)s in Mavi Dam Lake Sediment (Ankara) and Evaluation of Health Risks Level

Şeyda FIKIRDEŞICI ERGEN[1], Çağrı TEKATLI[2], Ahmet ALTINDAĞ[3], Gamze KAMIŞLI[4]
Kübra KOCATÜRK DÖNGEL[5], Evren TUNCA[6]
[1-5]Department of Biology, Faculty of Science, Ankara University, Ankara, [6]Fatsa Faculty of Marine Science, Ordu University, Ordu, Türkiye
[1]https://orcid.org/0000-0002-4623-1256, [2]https://orcid.org/0000-0001-5252-3506, [3]https://orcid.org/0000-0002-9900-5914
[4]https://orcid.org/0000-0002-0605-462X ,[5]https://orcid.org/0000-0003-4923-9015, [6]https://orcid.org/0000-0002-2842-2411
✉: fikirdesici@science.ankara.edu.tr

ABSTRACT
This study revealed the current metal(loid) status of the Mavi Dam Lake, which is one of the important wetlands for Ankara, established the accumulation relations between metal(loid)s separately, and seek an answer to the question of whether the current metal(loid)s status poses a risk of public health. The amounts of 13 metal(loid)s were determined. Sediment quality guidelines were calculated to understand the ecological risk of metal(loid)s in the sediment and the results were compared with limit values. It was determined that Ni constitutes 51.28% of the total toxic effects of metals detected in the sediment. Ni and Cr revealed a strong correlation between cluster and correlation analyses and were involved in the same factor in the principal component analysis. Additionally, it was determined that As, Cd, Co, Cr, and Ni may pose carcinogenic risks in terms of public health by contact with the lake or ingestion. In conclusion, it was revealed that the lake being studied should be regularly monitored for all metal(loid)s, especially Ni, and Cr.

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Keywords
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Toxicity
Multivariate statistical analyses

Mavi Baraj Gölü Sedimentinde (Ankara) Metal(loid)lerin Belirlenmesi ve Sağlık Risk Düzeyinin Değerlendirilmesi

ÖZET
Bu çalışma kapsamında Ankara için önemli sulak alanlardan biri olan Mavi Baraj Göl'ün mevcut metal(loid) durumunun ortaya konması, ayrı ayrı metal(loid) arası birikim ilişkilerinin durumu, mevcut metal(loid) durumun canlılar için risk teşkil edip etmediği sorularına cevap aranmıştır. 13 metal(loid) miktarı belirlenmiştir. Sedimentteki metal(loid)lerin ekolojik riskini anlamak için sediment kalite kılavuzları hesaplanmış ve sonuçlar sınır değerlerle karşılaştırılmıştır. Sedimentte araştırılmış metallerin toplam toksik etkilerinin %51.28'ini Ni oluşturduğu tespit edilmiştir. Küme ve korelasyon analizleri ile Ni-Cr arasında güçlü bir ilişki olduğu tespit edilmiş, temel bileşen analizinde de aynı faktörde yer aldığı gözlenmiştir. Ayrıca As, Cd, Co, Cr ve Ni'in göl teması veya yutulması ile halk sağlığı açısından kanserojen risk oluşturabileceği belirlenmiştir. Sonuç olarak, çalışılan gölün başta Ni ve Cr olmak üzere tüm metal(loid)ler için düzenli olarak izlenmesi gerektiği ortaya konmuştur.

Hidrobiyoloji

Araştırma Makalesi

Makale Tarihçesi
Geliş Tarihi : 09.11.2022
Kabul Tarihi : 24.02.2023

Anahtar Kelimeler
Ekotoksikolojik durum
Sediment kalite rehberi
Sağlık risk değerlendirilmesi
Toksisite
Çok yönlü istatistiksel analiz

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INTRODUCTION
Metal(loid)s (Ms) reach wetlands (Jia et al., 2019), lakes (Chen et al., 2019), rivers (Li et al., 2019), and reservoirs (Nguyen et al., 2019) through the entry of untreated wastewater from agricultural, industrial, and domestic sources. Ms that reach aquatic ecosystems via stream flow and atmospheric deposition, as well as runoff, are then deposited in the sediment through adsorption, co-precipitation, and hydrolysis (Guo et al., 2018). They cause serious

concern due to their bioaccumulation, toxicity, and persistency in the environment and food webs in the aquatic ecosystems they reach (Jordanova et al., 2018; Saher et al., 2019).

Sediments are like a sink of Ms in aquatic environments and the metal concentration is always higher than in the water above it (Liu et al., 2018; Levent et al., 2019). However, Ms in the sediment can be released back into the water through a changes in the surrounding chemical (dissolved oxygen, pH, redox potential, etc.) and physical (salinity, degradation, flood, temperature, etc.) factors (Islam et al., 2015). Therefore, it would be appropriate to suggest that the secondary source of Ms in aquatic ecosystems is sediments. For all these reasons, sediments play a vital role in maintaining the ecological conditions of the water bodies (Pal & Mandal, 2019).

Ms can bioaccumulate not only in aquatic ecosystems but also in the tissues of living organisms that benefit from water. Therefore, they have toxic effects on aquatic organisms, terrestrial organisms, and eventually humans (Li et al., 2018). For this reason, it is significant to examine the level of contamination and toxicity of Ms in aquatic ecosystems and sediments for protecting both ecosystems and human health (Fang et al., 2017). Therefore, the Sediment Quality Standards (SQGs) have been developed and used by many researchers to evaluate the current Ms status in sediments (Yoo et al. 2015; Fikirdeşici-Ergen et al., 2021).

The Mavi Dam Lake is one of the important lakes for Ankara. A large part of the area, which is now under protection, is used as a picnic area. Around the lake, there are streams that fill with rain water in winter and dry up in summer. Saray, Bayındır, Yunuslar, and Karanlık streams feed the lake water. Many roads pass through the area, and a highway runs through the middle of the lake (Yeni, 1995).

In this study, 13 heavy Ms (Al, As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Mo, Ni, Pb, and Zn) in the sediment of the Mavi Dam Lake were investigated. The amounts of these Ms were compared with the limit values, such as probable effect level (PEL), threshold effect level (TEL), effects range low (ERL), effects range median (ERM), reported using the Sediment Quality Guidelines (SQGs). The effects of Ms were evaluated with sediment evaluation methods including degree of contamination (Cd), modified degree of contamination (mCd), contamination factor (CF) and enrichment factor (EF), Geoaccumulation index (Igeo), toxic unit (TU), Pollution Load Index (PLI), mean ERM quotients (m-ERM-q), and mean PEL quotients (m-PEL-q). Additionally, risk indices developed and modeled by the USEPA (2004) were used to determine possible carcinogenic risks to public health due to exposure to Ms in the sediment. Some researchers have also used these indices (Kusin et al., 2018; Song et al., 2019; Ustaoğlu & Islam, 2020). The accumulation relations of the Ms were also evaluated statistically.

MATERIAL and METHODS

Sampling and Analyses

In this study, surface sediment and water samples were taken from 17 sampling sites in the Mavi Dam Lake were collected in April 2021 (Figure 1, Table 1). Surface sediment was taken from 1-2 cm using plastic materials (1-2 cm) and transported to the laboratory in polyethylene storage containers. They were stored in a refrigerator at +4 degrees until analysis. The samples were digested and analyzed according to the MA270 method by Bureau Veritas Mineral Laboratoires Canada (ACME LAB.). Concentrations of elements were determined by ICP-MS. Samples were studied and analyzed by Bureau Veritas Mineral Laboratoires Canada (ACME LAB.) according to the AQ270 method.

Table 1. Coordinates of the study area
Çizelge 1. Çalışma alanı koordinatları

Stations	Coordinates (WGS84)	
	X	Y
1.Station	32.989903°	39.910116°
2. Station	32.988849°	39.910343°
3. Station	32.989762°	39.911543°
4. Station	32.988363°	39.913525°
5. Station	32.989764°	39.911542°
6. Station	32.990846°	39.908893°
7. Station	32.993886°	39.911145°
8. Station	32.994752°	39.908854°
9. Station	32.995603°	39.910948°
10. Station	32.998064°	39.911984°
11. Station	33.001869°	39.912092°
12. Station	32.993973°	39.915223°
13. Station	32.995627°	39.914055°
14. Station	32.998089°	39.916232°
15. Station	32.999867°	39.919461°
16. Station	33.000849°	39.918541°
17. Station	33.000675°	39.915639°

Sediment Quality Assessment Methods

In this study, Turekian and Wedepohl data were used as reference data (Turekian & Wedepohl, 1961). These reference data are the most preferred data (PEL (Smith et al., 1996), ERL and ERM (Long & Morgan, 1991).

Contamination factors (C_f^i) (Hakanson, 1980)

$$C_f^i = C^i / C_n^i \quad (1)$$

C^i= Amount of metal

C_n^i= Reference values

Figure 1. Stations sampled in the Mavi Dam Lake
Şekil 1. Mavi Baraj Gölü örnekleme istasyonları

Degree of contamination (C_d) (Hakanson, 1980)

$$C_d = \sum_{i=1}^{n} C_f^i \quad (2)$$

C_f^i= Contamination factors

Modified degree of contamination (mC_d) (Abrahim & Parker, 2008)

$$mC_d = \frac{\sum_{i=1}^{n} C_f^i}{n} \quad (3)$$

C_f^i= Contamination factors

n= Number of metals studied

Pollution load index (PLI) (Tomlinson et al., 1980)

$$PLI = (C_{f1} \times C_{f2} \times C_{f3} \ldots . \times C_{fn})^{1/n} \quad (4)$$

C_{f1}= Contamination factors

n= Number of metals studied

Enrichment factor (EF) (Hasan et al., 2013)

$$EF = \frac{C_n/C_{ref}}{B_n/B_{ref}} \quad (5)$$

C_n= Amount of metal

C_{ref}= Amount of metal in reference sample

B_n= Amount of reference elements in the sample

B_{ref}= Value of reference Ms in reference sample

Al was chosen as the reference Ms.

Geoaccumulation Index (I_{geo}) (Müller, 1969)

$$I_{geo} = \log_2 \frac{C_n}{1.5 \times B_n} \quad (6)$$

C_n= number of metals

B_n= Amount of metal in reference sample

1.5= natural fluctuation coefficient

Mean effect range median quotients (m-ERM-Q) (Long & Morgan, 1991), and mean probable effect-level quotients (m-PEL-Q) (Carr et al., 1996).

$$m-ERM-Q = \frac{\sum_{i=1}^{n} C_i/ERM_i}{n} \quad (7)$$

$$m-PEL-Q = \frac{\sum_{i=1}^{n} C_i/PEL_i}{n} \quad (8)$$

C_i= number of metals

n= Number of metals studied

Total toxic unit (Σ TU) and relative toxic unit

$$\Sigma TUs = \sum_{i=1}^{n} C_i/PEL_{C_i} \quad (9)$$

$$\text{Relative TU} = \frac{C_i/PEL_{C_i}}{\Sigma TUs} X100 \quad (10)$$

Σ TU is the sum of the values obtained with the ratio of the number of metals detected in the samples to the PEL value of these Ms. The relative toxic unit is the percentage ΣTU of the toxic unit value of each Ms.

Potential Public Health Risk Calculation

In this study, dermal and ingestion routes to the body were used as the basis for public health risk calculation. The formula used to calculate the exposure values is as follows (USEPA, 2004; Song et al., 2019; Ustaoğlu & Islam, 2020).

$$Exp_{ing} = \frac{C_{sed} \times IR \times CF \times EF \times ED}{BW \times AT} \quad (11)$$

Exp_{ing} defines the risk of exposure to metals in the sediment through ingestion (mg/kg/day); IR is the amount of daily intake (IR=114 mg/day); The unit

conversion factor is CF (CF = 10^{-6} kg/mg); EF refers to the frequency of exposure from sediment (EF = 350 days/year); The exposure time is expressed in ED. (ED = 30 years), BW used for body weight of an adult (BW = 70 kg); AT means the total number of days in 30 years (AT = 10,950 days) (Iqbal et al. 2013).

$$Exp_{derm} = \frac{C_{sed} \times CF \times SA \times AF \times ABS \times EF \times ED}{BW \times AT} \quad (12)$$

Exp_{derm} defines the risk of dermal exposure to metals in sediment; SA is the exposed skin area (SA = 5700 cm^2); the adhesion index of Ms per unit skin area is defined as AF (AF = 0.07 mg/cm^2); the dermal adsorption rate from the sediment is ABS (ABS = 0.001) (Kusin et al., 2018; Song et al. 2019).

The health risks caused by the metal(loids) in the sediment were evaluated together with the hazardous ratios (HQs) as to the health risk calculation guidelines (USEPA, 2004; Wang et al., 2015).

$$HQ = \frac{Exp_{ing}/Exp_{derm}}{RfD_{ing}/RfD_{derm}} \quad (13)$$

$$HI = \sum(HQ_{ing} + HQ_{derm}) \quad (14)$$

Hazardous ratios below the exposure concentration detected by ingestion or skin contact are HQ. RFD is accepted as the reference value for negative health effects caused by Ms contamination. The reference values for exposure through skin contact and ingestion are considered the same (Iqbal et al., 2013; Wang et al., 2015). If the value is below 1 (HI < 1), no significant risk of non-carcinogenic effects is expected. However, if the HI value is above 1 (HI > 1), non-carcinogenic risk effects may arise, which tend to increase with increasing HI value (USEPA, 2004).

$$CRing = Exp_{ing} \times CSF \quad (15)$$

$$CRderm = Exp_{derm} \times CSF \quad (16)$$

$$\sum LCR = CR_{ing} + CR_{derm} \quad (17)$$

As, Cd, Cr, and Pb are the Ms that can create carcinogenic risks. Lifetime cancer risk (LCR) is used to calculate the public health risk caused by carcinogenic Ms (Ustaoğlu & Islam, 2020). The values for cancer slope factor (CSF) of As, Cd, Cr, and Pb were defined by USEPA (2012) as 1.5, 6.3, 0.5, 0.0085, and mg kg^{-1} day^{-1}, respectively. The range of 1.0×10^{-6}–1.0×10^{-4} was considered the acceptable LCR range, and 1.0×10^{-4} was considered the tolerable threshold for cancer risk (Wang et al., 2015).

RESULTS and DISCUSSION

Distribution and Contamination Status of Ms in the Surface Sediment of the Lake

The findings and limit values of 13 Ms (Al, As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Mo, Ni, Pb, and Zn) obtained from 17 selected stations are presented in Table 2. The amount of Hg, which has a highly toxic effect, was also examined, but it was found below the analytical detection limit (0.05 mg kg-1). Moreover, the same Ms were investigated in water samples and all of them were found below the detection limits.

Table 2. Amount of detected metal(loid) (mg kg^{-1}) and limit values
Çizelge 2. Tespit edilen metal(loid) miktarı (mg kg^{-1}) ve limit değerler

	Al	As	Cd	Co	Cr	Cu	Fe	Mn	Mo	Ni	Pb	Zn
Mean	10285.7	5.0	0.1	10.3	31.1	23.3	13899.9	484.0	0.3	41.3	11.4	56.3
Min	2882	1.34	0.053	3.28	10.3	5.53	4564	134	0.059	12.5	1.73	12.3
Max.	16490	7.75	0.208	14.3	54.9	57.6	23619	1995	0.4450	70.8	30.99	92.0
Std. Dev.	±239.14	±0.095	±0.003	±0.19	±0.59	±0.75	±297.68	±23.83	±0.005	±0.85	±0.37	±1.27
PEL		17.00	3.53		90.00	197.00				36.00	91.30	315.00
ERM		85.00	9.00		145.00	390.00				50.00	110.00	270.00
TEL		5.9	0.60		37.30	35.70				18.00	35.00	123.00
ERL	X	33.00	5.00	X	80.00	70.00	X	X	X	30.00	35.00	120.00
Earth crust	80000	13.00	0.30	19.00	90.00	45.00	47200	850.00	2.6	68.00	20.00	95.00

Al (10285.7±239.14 mg kg^{-1}), Fe (13899.9±297.68 mg kg^{-1}) and Mn (484.0±23.83 mg kg^{-1}) were in the highest amounts among the elements detected in the sediment (Iqbal & Shah, 2014; Diami et al., 2016; Ustaoğlu & Islam, 2020). This was an expected result because most of these metals are abundant in the upper and lower parts of the Earth's crust (Turekian & Wedepohl, 1961) and many studies support this result (Yaroshevsky, 2006; Ravisankar et al., 2015; Fikirdeşici-Ergen et al., 2021). In the study, the concentrations of all Ms did not exceed the average shale values.

When the results of all Ms were analyzed according to the limit values, all of them except Ni were found below the limit values (Table 2). The resulting Ni value was above the PEL, and ERM. The effects of amounts of Ms obtained below the TEL and ERL values on living organisms are rare. The amounts of Ms obtained above the PEL and ERM are likely to have toxic effects on

living organisms. At concentrations below ERL means that it can be affect less than 10% of the population (Hakanson, 1980). At concentrations above ERM, means it can be affect more than 50% of the population (Hakanson, 1980). Therefore, the amount of Ni (41.3±0.85 mg kg^{-1}) in the sediment is likely to have a toxic effect on the living organisms in the environment (Table 2).

Ni is of great importance for today's technology due to its use in petroleum, stainless steel, metal alloys, batteries, coins, and galvanic coatings (Mudd & Jowitti, 2014). Ni, Hg, Pb, and Cd are on the list of priority pollutants (Environmental Quality Standards (EQS) Directive 105/EC 2008). Although in fresh waters, Ni is predominantly in the soluble form of Ni^{2+}, it is more commonly found in complexes with chloride and sulfate (Binet et al., 2018). Ni in anaerobic freshwater sediments can precipitate as Ni sulfide, which reduces the bioavailability of living organisms in the sediment. Furthermore, Mn and Fe oxides can bind to Ni in both aerobic and anaerobic sediments (Schlekat et al., 2016). Ni is considered to be one of the toxic metals for living organisms other than plants (Bocca et al., 2019).

Evaluation of Results Using Sediment Assessment Methods

Contamination factor (CF) gives information about the distribution of Ms. The CF value was calculated below one for all Ms, indicating low contamination. PLI is a comparative and simple empirical index used to determine the level of Ms contamination (Hossain et al., 2014). The PLI result of the concentrations of Ms in the lake sediment was less than 1, indicating that the lake sediment is not polluted. The degree of contamination (C_d) and modified degree of contamination (mCd) values also showed that the pollution level of the lake is at a minimum (Table 3-4).

EF was used to analyze potential Ms sources in the surface sediment of the lake. The EF value helps understand the degree of contamination of the sediment by Ms by comparing it with the background rate. If the EF result is 1, it can be explained that Ms originated in the earth's crust. If the EF result is greater than 1, it can be explained that significant amount of Ms resulting from unnatural-weathering processes and anthropogenic effects (Zhang & Liu, 2002). In this study, EF results were found to be from 2 to 5 for the average Ms in the lake, excluding Mo (Table 4). EF values of Cu, Co, Mn, Ni, Pb, and Zn were remarkable. Especially, Ni and Zn are very close to the level of significant enrichment, indicating anthropogenic activity (Islam et al., 2015).

TU values are based on total concentrations. This value is a pre-indicator of the effects of Ms (Niu et al., 2020). Ni constitute 51.28% of the total toxic effects of metals detected in the sediment. This is followed by Cr constituting 15.45% and As constituting 13.15% of the total toxic effect. Therefore, it was determined that Ni, Cr, and As, which can demonstrate high toxic effects, have a high-risk level. Some studies in the literature support this result (Tunca et al., 2018, Fikirdeşici-Ergen et al., 2021).

When the m-ERM-Q result was evaluated, it was determined that the Ms accumulated in the lake was at the second level (21%) according to the scale. A rate of 21% indicates that the Ms accumulated in the lake has a 21% toxic effect on living organisms The m-PEL-Q result shows that the lake is moderately affected by the tested Ms (Table 3,4).

Potential Public Health Risk Calculation

In the literature, there are many studies including the human health risk assessment methods applied for sediment-induced Ms exposure (Khalil et al., 2011; Kusin et al., 2018; Ustaoğlu & Islam, 2020). Three methods are commonly used to assess human health risk. These are ingestion, skin contact, and inhalation. Ingestion and dermal contact routes were used in this study to assess human health risk.

HQ and HI values greater than 1 indicate adverse health effects due to the presence of Ms in the sediment (Table 5). Because of this study, it was determined that the Hqing, Hqderm, and HI values of As, Cd, Co, Cr, and Ni, which was the Ms tested in the sediment, were above 1. This suggested a carcinogenic health risk and showed that there are risks that may arise with ingestion Ms or skin contact. Moreover, it was determined that the risk of carcinogens caused by the ingestion of Ms is higher than the risk of skin contact. The lifetime cancer risk (LCR) results of As, Cd, Cr, and Pb are given in Table 5. The LCR value of Cr (Cr>As>Cd>Pb) was higher than the values of other Ms. This means that the risk of carcinogens caused by Cr is higher than other Ms. Moreover, the fact that the calculated LCR values for As, Cd, Cr, and Pb were in the range of 1.00E-06 to 1.00E-04, which is suggested by the USEPA, explains the importance of monitoring these Ms in the sediment (USEPA, 2004).

Multivariate Statistical Analyses

To understand the Ms-Ms interactions in the Mavi Dam Lake sediment, various analyses (correlation, cluster, and principal component analyses) were performed. Three factors were found to explain 72.55% of the total variance. Al, Cr, Fe, Co, Ni, and As were associated with 48.19% of the total variance explained by the first factor (F1). The second factor (F2) is 13.87% of the total variance explained in relation to Cu, Zn, Cd and Pb; the third factor (F3) was found to explain 10.48% of the total variance and was associated with Mn and Mo metals.

Table 3. Sediment assessment scale
Çizelge 3. Sediment değerlendirme ölçeği

Contamination factor (Hakanson, 1980)	
Cf<1	low contamination
1≤Cf<3	moderate contamination
3≤Cf<6	considerable contamination
Cf≥6	high contamination
Degree of contamination (C_d)(Hakanson, 1980)	
Cd≤8	low degree of contamination
8≤Cd<16	moderate degree of contamination
16≤Cd<32	considerable degree of contamination
Cd≥32	very high degree of contaminations
Modified degree of contamination (mCd)(Abrahim & Parker, 2008)	
mCd < 1.5	nil to very low degree of contamination
1.5 ≤ mCd < 2	low degree of contamination
2 ≤ mCd < 4	moderate degree of contamination
4 ≤ mCd < 8	high degree of contamination
8 ≤ mCd < 16	very high degree of contamination
16 ≤ mCd < 32	extremely high degree of contamination
mCd ≥ 32	ultra high degree of contamination
Pollution load index (PLI) (Tomlinson et al., 1980)	
PLI <1	no pollution
PLI is >1	deterioration
Enrichment factor (EF) (Hasan et al., 2013)	
<1	no enrichment
1 to 3	minor enrichment
3 to 5	moderate enrichment
5 to 10	moderately severe enrichment
10 to 25	severe enrichment
25 to 50	very severe enrichment
>50 extremely	severe enrichment
Geoaccumulation index (Igeo) (Müller, 1969)	
Igeo≤0	practically uncontaminated
0<Igeo<1	uncontaminated to moderately contaminated
1<Igeo<2	moderately contaminated
2<Igeo<3	moderately to strongly contaminated
3<Igeo<4	strongly contaminated
4<Igeo<5	strongly to extremely contaminated
Igeo≥5	extremely contaminated
Ratio of average effects range median (m-ERM-Q) (Long et al., 2000)	
m-ERM-q<0.1	9%
0.11<m-ERM-q<0.5	21%
0.51 <m-ERM-q <1.5	49%
m-ERM-q>1.50	76% probability of being toxic
Ratio of average probable effect level (m-PEL-Q) (Carr et al., 1996)	
m-PEL-Q<0.1	unimpacted
0.1< m-PEL-Q<1	moderately impacted
m-ERM-Q>1	highly impacted

The correlation between Ni-Cr-Fe-Co-Cu is significant. The highest correlation was observed between Ni-Cr (.860**) and Ni-Fe (.843**) (Table 6). These findings were also supported by PCA and CA results (Table 7–8). According to the PCA, these three are included in component number 1 (Table 7, Figure 2). When the CA analysis results were examined, it was seen that these 3 elements were in the same cluster (Table 8, Figure 3). Furthermore, the closest distance in the proximity matrix, that is, the strongest correlation, was between Ni-Cr (1.61) and Ni-Fe (1.65). The high correlation between Fe and Ni indicates the affinity of Ni with Fe oxide in the sediment (Taghipour et al. 2011, Ghorbani et al. 2015, Paoli et al. 2017). The correlation between Ni-Cr (.860**) and Ni-Co (.840**), which were among those with the highest correlations, can be attributed

to the same geogenic origin from the parental material (Dankoub et al., 2012; Otari & Dabiri, 2015; Salmanpour et al., 2018).

Table 4. Evaluation of mean concentrations of Ms with a sediment assessment guide
Çizelge 4. Sediment değerlendirme kılavuzu ile ortalama Ms konsantrasyonlarının değerlendirilmesi

	Al	As	Cd	Co	Cr	Cu	Fe	Mn	Mo	Ni	Pb	Zn
Cf	0.13	0.38	0.33	0.54	0.35	0.52	0.29	0.57	0.12	0.61	0.57	0.59
Cd						5						
mCd						0.50						
EF	1.00	2.99	2.59	4.22	2.69	4.03	2.29	4.43	0.90	4.72	4.43	4.61
Igeo	-3.54	-1.96	-2.17	-1.47	-2.12	-1.53	-2.35	-1.40	-3.70	-1.30	-1.40	-1.34
PLI						0.302						
m-ERM-Q						0.21						
m-PEL-Q						0.32						
TTU						2.24						
TU		13.15	1.27		15.45	5.29				51.28	5.58	7.99

Table 5. Health risk assessment of Ms in sediment
Çizelge 5. Sedimentte Ms'nin sağlık riski değerlendirmesi

		Exposure assessment		Non-carcinogenic risk			Carcinogenic risk		
	RfD	Exping	Expderm	HQing	HQderm	HI	CRing	CRderm	LCR
Al	1.00E+00	1.33E-02	4.65E-05	1.33E-02	4.65E-05	1.33E-02			
As	0.30E-03	1.77E-02	6.20E-05	5.91E+01	2.07E-01	5.93E+01	4.28E-03	1.50E-05	4.29E-03
Cd	1.00E-03	0.59E+01	2.05E-02	5.86E+03	2.05E+01	5.88E+03	3.59E-04	1.26E-06	3.60E-04
Co	3.00E-04	2.76E-01	9.66E-04	9.20E+02	3.22E+00	9.23E+02			
Cr	3.00E-03	6.50E-03	2.27E-05	2.17E+00	7.58E-03	2.17E+00	8.86E-03	3.1E-05	8.89E-03
Cu	4.00E-02	2.90E-03	9.98 E-06	7.13E-02	2.49E-04	7.15E-02			
Fe	7.00E-01	2.35E-02	8.24E-05	3.36E-02	1.18E-04	3.37E-02			
Mn	1.40E-01	3.21E-02	1.12E-04	2.29E-01	8.02E-04	2.30E-01			
Mo	5.00E-03	2.00E-04	5.99E-07	3.42E-02	1.20E-04	3.43E-02			
Ni	2.00E-02	0.79E+01	2.77 E-02	3.96E+02	1.39E+00	3.98E+02			
Pb	3.00E-03	1.00E-04	1.99E-07	1.90E-02	6.65E-05	1.91E-02	5.52E-05	1.93E-07	5.54E-05
Zn	3.00E-01	5.90E-03	2.05E-05	1.96E-02	6.85E-05	1.96E-02			

Table 6. Ms correlations
Çizelge 6. Ms korelasyonları

	Al	Cr	Mn	Fe	Co	Ni	Cu	Zn	As	Mo	Cd	Pb
Al	1											
Cr	.551*	1										
Mn	.262	.203	1									
Fe	.537*	.721**	.466	1								
Co	.437	.804**	.492*	.619**	1							
Ni	.373	.860**	.336	.843**	.840**	1						
Cu	.465	.813**	.325	.554*	.645**	.690**	1					
Zn	.228	.485*	.309	.495*	.387	.502*	.650**	1				
As	.259	.482	.018	.558*	.357	.535*	.336	.396	1			
Mo	.078	-.076	.230	.110	-.069	-.066	.184	.392	.260	1		
Cd	-.441	-.218	-.228	-.571*	-.287	-.419	-.185	.100	-.085	.103	1	
Pb	.488*	.480	.338	.377	.489*	.424	.525*	.485*	.609**	.426	.142	1

**Correlation is significant at the 0.01 level (2-tailed).
*Correlation is significant at the 0.05 level (2-tailed).

CONCLUSION

The sediment quality was evaluated by investigating the Ms found in the sediment of the Mavi Dam Lake. Additionally, the possible toxic effects of the current accumulation of the sediment on living organisms were also examined. According to the results, it was determined that 51.28% of the total toxic effects of the Ms in the sediment were caused by Ni. When evaluated

according to the sediment quality guideline, the accumulation of Ni was found to be significant. In this study, the possible health risks of the lake sediment to humans were assessed. It was seen that exposure to As, Cd, Co, Cr, and Ni in the sediment through ingestion or skin contact may be carcinogenic. Although it is thought that the sediment contamination will not directly threaten human life, the water-sediment relationship and the sedimentation-release mechanism draw attention to the extent of the danger. In the long term, anthropogenic pressure will also increase this risk. In conclusion, all data show that it would be correct to monitor lake sediment regularly.

Table 7. PCA rotated component matrix
Çizelge 7. PCA döndürülmüş bileşen matrisi

	1	2	3
Al	.763		
Cr	.760	.570	
Mn			.894
Fe	.925		
Co	.800		
Ni	.864		
Cu		.573	
Zn		.671	.554
As	.603		
Mo			.611
Cd		.514	
Pb		.784	

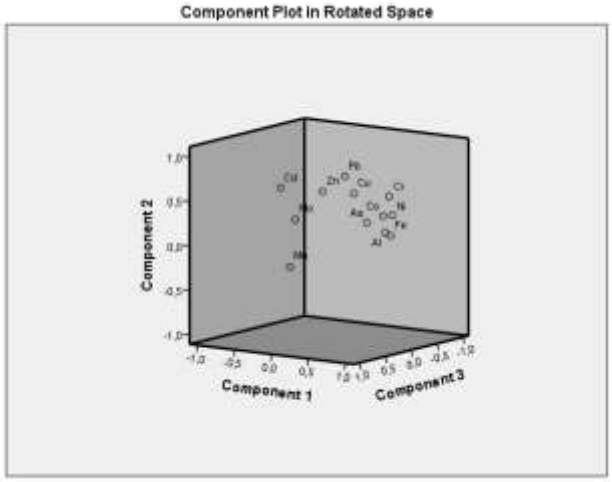

Figure 2. PCA analysis of Ms in the sediment of the Mavi Dam Lake
Şekil 2. Mavi Baraj Gölü sedimentinde Ms'nin PCA analizi

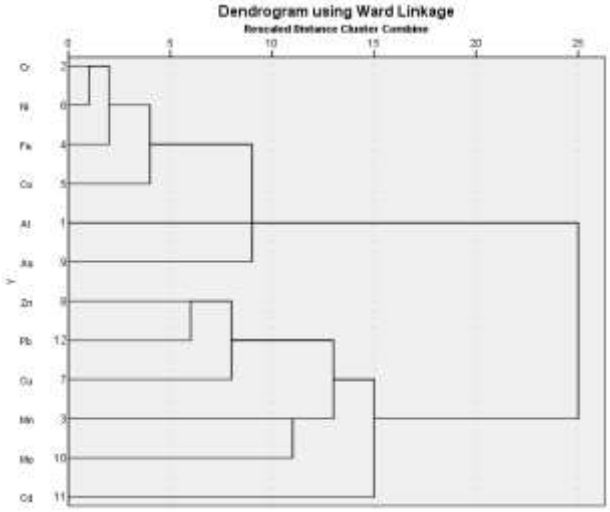

Figure 3. Cluster analysis of Ms in the sediment of the Mavi Dam Lake
Şekil 3. Mavi Baraj Gölü sedimentinde Ms'nin kümeleme analizi

Çizelge 8. CA yakınlık matrisi
Table 8. CA proximity matrix

	Al	Cr	Mn	Fe	Co	Ni	Cu	Zn	As	Mo	Cd	Pb
Al	0.000	3.537	5.806	3.304	3.409	3.558	4.384	4.722	4.396	4.856	6.982	4.295
Cr		0.000	5.860	2.344	2.466	1.610	3.774	3.477	3.679	5.349	6.201	3.759
Mn			0.000	5.182	5.029	5.421	5.624	4.173	5.325	4.974	6.064	5.568
Fe				0.000	2.666	1.655	4.478	3.972	3.426	5.294	7.027	4.462
Co					0.000	1.958	4.222	4.059	3.978	5.449	6.495	3.563
Ni						0.000	4.479	3.826	3.337	5.543	6.518	3.860
Cu							0.000	3.896	5.058	5.322	6.039	4.109
Zn								0.000	4.498	4.352	5.436	3.487
As									0.000	5.167	6.190	4.239
Mo										0.000	5.529	4.633
Cd											0.000	5.457
Pb												0.000

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The authors contributed equally to the article.

Author's Contributions

The authors declare that they have contributed equally to the article.

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Investigation of the Antibiotic Profiles and Phlogenetic Relationships of the *Lactobacillus* Species Isolated From Goat's and Cow's Milk

Suna KIZILYILDIRIM[1] ✉, Fatih KÖKSAL[2]
[1]Süleyman Demirel University, Faculty of Pharmacy, Pharmaceutical Microbiology Depertmant, 32260, Isparta, Türkiye, [2]Cukurova University, Faculty of Medicine, Medical Microbiology Department, 01380, Adana, Türkiye
[1]https://orcid.org/0000-0002-1039-8556, [2]https://orcid.org/0000-0003-0790-1525
✉: sunakizilyildirim@sdu.edu.tr

ABSTRACT

Lactobacillus is naturally abundant in raw milk. Lactobacilli may develop antibiotic resistance as a result of unconscious antibiotic usage in animals. The aim of this study was to identify *Lactobacillus* species from raw goat's and cow's milk, investigate phylogenetic relationships, and examine the antibiotic profiles of these strains. In this study, the milk of 10 cows and 5 goats was obtained from some farms in Adana. The Crystal method was used to phenotypically identify different colonies assumed to be *Lactobacillus* that were cultured in milk samples. The disc diffusion test was used to determine their resistance to antibiotics. By using the PFGE method, the phylogenetic relationships of *Lactobacillus* strains were examined. A total of 18 *Lactobacillus* strains were isolated from 10 different cows' milk and 10 *Lactobacillus* strains were identified from 5 distinct goats' milk. When the antibiotic susceptibility profiles of the *Lactobacillus* strains isolated from cow's milk were examined, it was found that all strains were sensitive to vancomycin and chloramphenicol, and 38.9% of them were resistant to some antibiotics. All the *Lactobacillus* strains isolated from goat's milk were shown to be susceptible to ampicillin, vancomycin, chloramphenicol, and 40% of all strains were found to be resistant to some antibiotics. The PFGE analysis showed that 28 *Lactobacillus* strains were separated into 21 pulsetypes, and the strains in the A-B-C-D-E-F-G pulsetypes were found to be 100% similar. Consequently, the sensitivity of *Lactobacillus* species to antibiotics requires more investigation.

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Keçi ve İnek Sütünden İzole Edilen *Lactobacillus* Türlerinin Antibiyotik Profillerinin ve Filogenetik İlişkilerinin Araştırılması

ÖZET

Lactobacillus, çiğ sütte bol miktarda ve doğal olarak bulunur. Hayvanlarda bilinçsiz antibiyotik kullanımı sonucu, laktobasiller antibiyotik direnci geliştirebilir. Bu çalışmanın amacı çiğ keçi ve inek sütünden elde edilen *Lactobacillus* türlerini belirlemek, filogenetik ilişkileri araştırmak ve bu suşların antibiyotik profillerini incelemektir. Çalışmada Adana'daki bazı çiftliklerden 10 inek ve 5 keçi sütü elde edilmiştir. Kristal yöntemi, süt örneklerinde kültürlenen *Lactobacillus* olduğu varsayılan farklı kolonileri fenotipik olarak tanımlamak için kullanıldı. Antibiyotiklere dirençlerini belirlemek için disk difüzyon testi kullanıldı. PFGE yöntemi kullanılarak *Lactobacillus* suşlarının filogenetik ilişkileri incelendi. 10 farklı inek sütünden 18 *Lactobacillus* suşu izole edildi ve 5 farklı keçi sütünden 10 *Lactobacillus* suşu tanımlandı. İnek sütünden izole edilen *Lactobacillus* suşlarının antibiyotik duyarlılık profilleri incelendiğinde, bakterilerin tamamının vankomisin ve kloramfenikole duyarlı olduğu, %38,9'unun bazı antibiyotiklere dirençli olduğu tespit edildi. Keçi sütünden izole edilen tüm *Lactobacillus* suşlarının ampisilin, vankomisin, kloramfenikol'e duyarlı olduğu gösterilmiş ve bunların %40'ının bazı antibiyotiklere dirençli olduğu saptanmıştır. PFGE analizi 28 *Lactobacillus* suşunun 21 pulsetipine ayrıldığını ve A-B-C-D-E-F-G pulsetiplerindeki suşların

Mikrobiyoloji

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Keçi sütü
Lactobacillus
PFGE

%100 benzer olduğunu göstermiştir. Sonuç olarak, *Lactobacillus* türlerinin antibiyotik duyarlılıkları konusunda daha kapsamlı araştırmaların yapılması gerekmektedir.

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INTRODUCTION

Probiotics are live microorganisms that confer a health benefit on the host, as defined by the Food and Agriculture Organization of the United Nations and the World Health Organization. At the beginning of the 20th century, Ilja Metchnikoff reported that the longevity of Bulgarians is due to consumption of fermented milk products. Probiotic bacteria have been used as a health-promoting factor for a very long time (Zawistowska-Rojek & Tyski, 2018). Probiotics contribute to gastrointestinal and urogenital problems, allergic diseases and more generally, to improve the function of the digestive system and support the immune system (Villavicencio et al., 2018).

Probiotic properties have been seen in many genera of bacteria and fungi, but most used probiotics belong to the species of *Lactobacillus* and *Bifidobacterium*. Also, other bacteria genera, like *Streptococcus*, *Enterococcus*, and *Bacillus*, as well as members of the yeast genus *Saccharomyces* can have probiotic properties. The most common probiotic species contain: *Lactobacillus acidophilus*, *Lactobacillus johnsonii*, *Lactobacillus gasseri*, *Lactobacillus casei*, *Lactobacillus rhamnosus*, *Lactobacillus plantarum*, *Bifidobacterium longum*, *Bifidobacterium breve*, *Bifidobacterium bifidum* and *Bifidobacterium infantis* (Zawistowska-Rojek & Tyski, 2018). *Lactobacillus* strains are important members of the human and animal microbiomes, and are found in a variety of food products (Zhang et al., 2018). *Lactobacillus* contains 51 species among them the species *L. helveticus*, *L. kefiranofaciens*, *L. delbrueckii* and *L. kefiri*, which are commonly found in fermented milk (Georgalaki et al., 2021). Probiotics are widely available in raw's milk and conventional dairy products. Fresh or fermented cow's and goat's milk is consumed in different regions of the world. The presence of high counts of probiotic bacteria in both cow's and goat's milk important a source for public health (Reuben et al., 2020). Probiotic bacteria are becoming more and more resistant to clinically significant antibiotics, and this is linked to their improper usage in farm animals (Jaimee & Halami, 2016). Antibiotic resistance genes that can be passed on to pathogenic bacteria can be transferred by probiotic bacteria (Danielsen & Wind, 2003). It is quite concerning that lactic acid bacteria, a healthy component of the microflora, are developing antibiotic resistance (Jaimee & Halami, 2016). It is very important to determine the antibiotic susceptibility of probiotic bacteria (Danielsen & Wind, 2003).

The aim of the study is to identify different species of *Lactobacillus* in goat's and cow's milk collected from various farms in the province of Adana, as well as to investigate phylogenetic relationships and examine the antibiotic profiles of these strains.

MATERIALS and METHODS

In the study, milk of 10 cow's and 5 goat's, which were offered for daily consumption between 10.01.2022 and 07.02.2022, was taken from some farms in Adana province. Milk samples were taken into sterile capped plastic tubes kept in an ice box and transported to the laboratory. To identify the *Lactobacillus*, 10 mL and 40 mL of each sample were inoculated into de Man, Rogosa, and Sharpe (MRS) broth and incubated at 37°C under anaerobic conditions. All tubes with turbidity were then incubated on MRS agar plates and incubated for 24 to 72 hours at 37°C under anaerobic conditions. All strains were tested for Gram staining, catalase test, and coagulase reaction. The Crystal (BD BBL CRYSTAL ANR) technique was used to phenotypically identify several colonies thought to be *Lactobacillus* (Kızılyıldırım & Köksal, 2021).

Antibiotic Susceptibility

The susceptibility of *Lactobacillus* to antibiotics was evaluated by the disc diffusion test according to the criteria recommended by the National Committee for Clinical Laboratory Standards (Xu et al., 2012). The antibiotic susceptibility of the strains was assessed using antibiotics such as ampicillin (10 µg), vancomycin (30 µg), tetracycline (30 µg), erythromycin (15 µg), gentamicin (10 µg), and chloramphenicol (30 µg).

Phylogenetic Relationship of *Lactobacillus* strains

The phylogenetic relationships of *Lactobacillus* strains were done as previously described. Using the CHEF-DRII system (Bio-Rad Laboratories), DNA fragments were electrophoresed in 0.5 x TBE buffer for 22 hours at 14°C (Brennan et al.,2002; Xu et al., 2012). The GelComparII software program (version 4.0 Applied Maths, Sint-Martens-Latem, Belgium) was used to examine the PFGE data. The relationship between the strains were determined according to the "Dice"

similarity coefficient depending on the bands. The isolates with 100% similarity in band profiles were evaluated in the same cluster (Xu et al., 2012).

RESULTS and DISCUSSION

In the study, a total of 18 *Lactobacillus* strains were recovered from 10 different cow's milk, including seven *L. casei* (38.9%), five *L. rhamnosus* (27.8%), four *L. acidophilus* (22.2%), and two *L. fermentum* (11.1%). One of the milk samples (R1) included a combination of *L. acidophilus*, *L. casei*, and *L. rhamnosus*. Three milk samples (R6-R8-R9) had only one strain of *Lactobacillus*. Six samples of milk (R2-R3-R4-R5-R7-R10) included two different species of *Lactobacillus* (Table 1).

Table 1. *Lactobacillus* species isolated in cow's milk
Çizelge 1. İnek sütünden izole edilen Lactobacillus türleri

Number	Strain no	*Lactobacillus*	Number	Strain no	*Lactobacillus*
1	R1.1	*L. acidophilus*	10	R5.1	*L. acidophilus*
2	R1.2	*L. casei*	11	R5.2	*L. casei*
3	R1.3	*L. rhamnosus*	12	R6	*L. rhamnosus*
4	R2.1	*L. fermentum*	13	R7.1	*L. rhamnosus*
5	R2.2	*L. rhamnosus*	14	R7.2	*L. casei*
6	R3.1	*L. acidophilus*	15	R8	*L. fermentum*
7	R3.2	*L. casei*	16	R9	*L. casei*
8	R4.1	*L. casei*	17	R10.1	*L. casei*
9	R4.2	*L. acidophilus*	18	R10.2	*L. rhamnosus*

When the antibiotic susceptibility profiles of the *Lactobacillus* strains isolated from cow's milk were examined, it was found that seven strains (38.9%) were resistant to antibiotics and 11 strains (61.1%) were sensitive. Of the resistant *Lactobacillus* strains, six were resistant to tetracycline, two to gentamicin, one to ampicillin, and one to erythromycin. Vancomycin and chloramphenicol sensitivity were discovered in all strains. One of the *L. rhamnosus* strains showed resistance to both tetracycline and gentamicin. One strain of *L. casei* showed multiple resistance to ampicillin, tetracycline, and gentamicin. Among the *L. rhamnosus* strains, gentamicin resistance was found in one strain (20%) and tetracycline resistance in four (80%). Only one of the *L. fermentum* strains had tetracycline resistance (50%). In *L. casei* strains, one strain showed resistance to tetracycline (14.2%), gentamicin (14.2%), and ampicillin (14.2%), while one strain showed resistance to erythromycin (14.2%). All *L. acidophilus* strains have been demonstrated to be antibiotic susceptible (Table 2).

Table 2. Antibiotic susceptibility profiles of *Lactobacillus* strains isolated from cow's milk
Çizelge 2. İnek sütünden izole edilen Lactobacillus suşlarının antibiyotik duyarlılık profilleri

No	Strain no	*Lactobacillus*	*Amp	*Van	*Gen	*Ery	*Chl	*Tet
1	R1.1	*L. acidophilus*	S	S	S	S	S	S
2	R1.2	*L. casei*	S	S	S	S	S	S
3	R1.3	*L. rhamnosus*	S	S	S	S	S	R
4	R2.1	*L. fermentum*	S	S	S	S	S	R
5	R2.2	*L. rhamnosus*	S	S	S	S	S	R
6	R3.1	*L. acidophilus*	S	S	S	S	S	S
7	R3.2	*L. casei*	S	S	S	S	S	S
8	R4.1	*L. casei*	S	S	S	S	S	S
9	R4.2	*L. acidophilus*	S	S	S	S	S	S
10	R5.1	*L. acidophilus*	S	S	S	S	S	S
11	R5.2	*L. casei*	S	S	S	S	S	S
12	R6	*L. rhamnosus*	S	S	R	S	S	R
13	R7.1	*L. rhamnosus*	S	S	S	S	S	S
14	R7.2	*L. casei*	S	S	S	S	S	S
15	R8	*L. fermentum*	S	S	S	S	S	S
16	R9	*L. casei*	R	S	R	S	S	R
17	R10.1	*L. casei*	S	S	S	R	S	S
18	R10.2	*L. rhamnosus*	S	S	S	S	S	R

*Amp: Ampicillin, Van: Vancomycin, Gen: Gentamicin, Ery: Erythromycin, Chl: Chloramphenicol, Tet: Tetracycline.

Gad et al. (2014) isolated a total of 152 *Lactobacillus* spp. from 180 pharmaceutical and dairy samples. *Lactobacillus* isolates have shown the highest penicillin resistance. Almost a high percentage of

Lactobacillus isolates showed moderate resistance to cephalexin and a low percentage were resistant to cefoperazone (Gad et al., 2014). Hleba et al. (2021) reported that *Lactobacilli* isolated from milk and dairy products were resistant to erythromycin (21.4%), ampicillin (30.9%), and tetracycline (14.2%), but completely sensitive to gentamicin (Hleba et al., 2012). Bargezar et al. (2021) isolated *L. brevis*, *L. acidophilus*, *L. plantarum*, and *L. casei* species from six different raw milk cheeses. It was reported that 57% of the strains were resistant to kanamycin and 28% were resistant to tetracycline, with no resistance to chloramphenicol or erythromycin found (Barzegar et al., 2021).

The differences in *Lactobacillus* species and numbers in the studies are related to both the number of samples and the methods used in identification. In this study, the phenotypic method was used for the identification of *Lactobacilli*. However, if it was identified by the genotypic method, the number and type of *Lactobacilli* could change. On the other hand, antibiotic profiles in studies may differ depending on the antibiotic groups used and the number of strains.

A total of ten *Lactobacillus* strains were found in five different goat's milk, including five *L. casei* (50%), three *L. rhamnosus* (30%), and two *L. fermentum* (2%) strains. Each goat's milk sample (G1-G2-G3-G4-G5) included two distinct strains of *Lactobacillus* (Table 3).

Table 3. *Lactobacillus* species isolated in goat's milk
Çizelge 3. Keçi sütünden izole edilen Lactobacillus türleri

Number	Strain no	*Lactobacillus*
1	G1.1	*L. casei*
2	G1.2	*L. rhamnosus*
3	G2.1	*L. fermentum*
4	G2.2	*L. casei*
5	G3.1	*L. rhamnosus*
6	G3.2	*L. casei*
7	G4.1	*L. casei*
8	G4.2	*L. fermentum*
9	G5.1	*L. casei*
10	G5.2	*L. rhamnosus*

It was shown that six strains (60%) were sensitive and four strains (40%) were resistant when the antibiotic susceptibility profiles of the *Lactobacillus* strains isolated from goat's milk were examined. Two of the *Lactobacillus* strains had tetracycline resistance, one had gentamicin resistance, and two had erythromycin resistance. All strains were found to be sensitive to ampicillin, vancomycin and chloramphenicol. One of the *L. rhamnosus* strains demonstrated both erythromycin and gentamicin resistance. Two of the *L. casei* isolates showed a 50% tetracycline resistance. Two of the *L. rhamnosus* strains had erythromycin resistance (66.7%), whereas one had gentamicin resistance (33.3%). All strains were found to be sensitive to ampicillin, vancomycin, and chloramphenicol (Table 4).

Table 4. Antibiotic susceptibility profiles of *Lactobacillus* strains isolated from goat's milk
Çizelge 4. Keçi sütünden izole edilen Lactobacillus suşlarının antibiyotik duyarlılık profilleri

No	Strain no	*Lactobacillus*	*Amp	*Van	*Gen	*Ery	*Chl	*Tet
1	G1.1	*L. casei*	S	S	S	S	S	R
2	G1.2	*L. rhamnosus*	S	S	R	R	S	S
3	G2.1	*L. fermentum*	S	S	S	S	S	S
4	G2.2	*L. casei*	S	S	S	S	S	R
5	G3.1	*L. rhamnosus*	S	S	S	R	S	S
6	G3.2	*L. casei*	S	S	S	S	S	S
7	G4.1	*L. casei*	S	S	S	S	S	S
8	G4.2	*L. fermentum*	S	S	S	S	S	S
9	G5.1	*L. casei*	S	S	S	S	S	S
10	G5.2	*L. rhamnosus*	S	S	S	S	S	S

*Amp: Ampicillin, Van: Vancomycin, Gen: Gentamicin, Ery: Erythromycin, Chl: Chloramphenicol, Tet: Tetracycline.

The most prevalent *Lactobacillus* species found in goat's milk are *L. plantarum*, *L. rhamnosus*, *L. casei*, and *L. paracasei*. The high-potential *Lactobacillus* selection derived from goat's milk is industrially significant (Marroki et al., 2011).

Marroki et al. (2014) identified 19 *Lactobacillus* strains from goat's milk, including *L. plantarum* (13), *L. pentosus* (3), *L. rhamnosus* (2), and *L. fermentum*. *Lactobacillus* strains were discovered to be penicillin and erythromycin sensitive. All of the strains were resistant to vancomycin. It has been reported that resistance rates to other antibiotics differ according to *Lactobacillus* strains (Marroki & Bousmaha-Marroki, 2014).

In another investigation, antibiotic susceptibilities of 61 *Lactobacillus* strains (*L. plantarum* (28), *L. pentosus* (22), *L. fermentum* (6) and *L. rhamnosus* (5)) isolated from 14 raw goat's milk samples were tested. Most of the strains were more sensitive to β-lactam group antibiotics (penicillin G (52%), ampicillin (82%) and amoxicillin (80%)). It was also susceptible to

cefotaxime (39%) and imipenem (56%). In addition, high susceptibility to protein synthesis inhibitors such as erythromycin (48%), tetracycline (49%), chloramphenicol (80%), and fusidic acid (26%) were observed. All isolates were resistant to oxacillin, ceftazidine, ceftriaxone, vancomycin, and trimitoprim-sulfamide (Bousmaha-Marroki & Marroki, 2015). Marroki et al. (2011) reported that *Lactobacillus* strains isolated from goa't milk were sensitive to tetracycline, erythromycin and resistant to vancomycin, kanamycin and gentamicin (Marroki et al., 2011). The results of this study are similar to other studies on goat's milk in terms of antibiotic profiles and *Lactobacillus* species. However, further research is needed on the antibiotic profiles of the strains of *Lactobacillus* isolated from goat's milk samples.

It is believed that the primary means of transmission of bacteria resistant to antibiotics across populations of animals and people is through the food chain (Erginkaya et al., 2018). In particular, probiotic organisms are thought to transmit antibiotic resistance genes to pathogenic bacteria. It should be remembered that some *Lactobacillus* species can be resistant to antibiotics and can help other microorganisms acquire antibiotic resistance genes (Wang et al., 2019). In this regard, the antibiotic susceptibilities of probiotic microorganisms in foods should be evaluated, and more extensive research is necessary.

In the evaluation of clonal relationships of *Lactobacillus* strains by the PFGE method, it was observed that 28 strains were divided into 21 pulsetypes. The two-membered A-B-C-D-E-F-G pulsetypes *Lactobacillus* strains were found to be similar (100%). The other 14 strains were separated into unrelated single-membered pulsetypes (Figure 1). It was observed that *L. rha*mnosus isolates in the A pulse type were resistant to tetracycline, *L. rhamnosus* (R6) strains in the E pulsetype were resistant to tetracycline and gentamicin, and *L. rhamnosus* (R10.2) was found to be resistant to tetracycline. Additionally, tetracycline resistance was detected in *L. casei* strains of the G pulsetype, as well as gentamicin and erythromycin resistance in *L. rhamnosus* (G1.2) *and L.* rhamnosus (G3.1) strains of the F pulsetype. Tetracycline resistance was also found in *L. rhamnosus* strains of the F pulsetype.

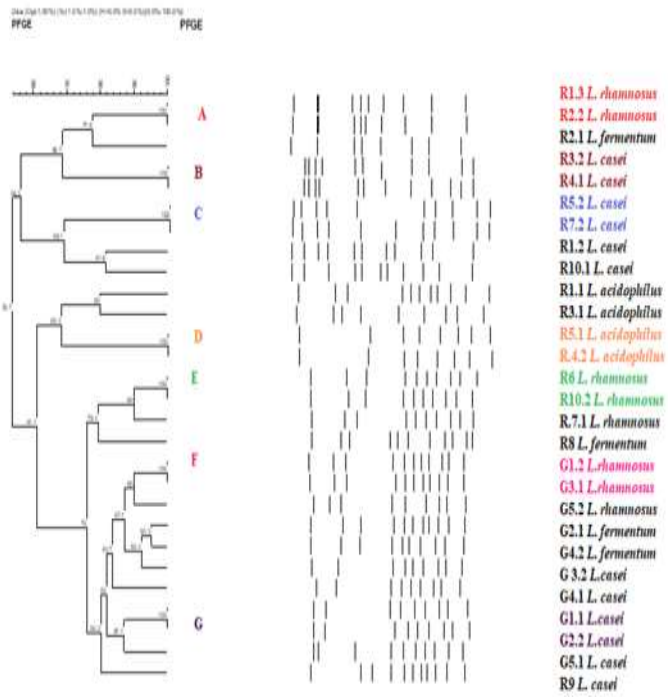

Figure1. Phylogenetic relationship of *Lactobacillus* strains
Şekil 1. Laktobasil suşlarının filogenetik ilişkisi

Similar to this study, Xu et al. (2012) found that the PFGE analysis separated 33 *Lactobacillus* strains into 17 pulsetypes. According to their findings, different *Lactobacillus* strains exhibited the same PFGE patterns and likely descended from a common ancestor of these strains. They found that all antibiotic resistance patterns of each strain were similar in A, C, F, J, K, and M pulsetypes, and some strains with

different pulsetypes exhibited the same antibiotic resistance spectrum (Xu et al., 2012).

CONCLUSION

In conclusion, we strongly believe that studies on the investigation of *Lactobacillus* species and elucidation of their antibiotic susceptibility profiles in foods such as milk and dairy products should continue. It should be encouraged to consciously use antibiotics in animal illnesses in order to prevent antibiotic resistance.

Author's Contributions

The authors declare that they have contributed equally to the article.

Statement of Conflict of Interest

Authors have declared no conflict of interest.

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Optimization of Keratinase Enzyme synthesized by *Micrococcus luteus* using Taguchi DOE Method

Özden CANLI TAŞAR[1] ✉, Gani Erhan TAŞAR[2]
[1] Yüksek Teknoloji Uygulama ve Araştırma Merkezi YÜTAM, Erzurum Teknik Üniversitesi, Erzurum, Türkiye.
[2]Kahta Meslek Yüksek Okulu, Adıyaman Üniversitesi, Adıyaman, Türkiye
[1]https://orcid.org/0000-0002-4313-5373, [2]https://orcid.org/0000-0002-9217-0706
✉: ozden.tasar@erzurum.edu.tr

ABSTRACT
Keratinase is an important enzyme used for degradation of the keratinous wastes, especially slaughterhouse and poultry-derived wastes, that cause environmental pollution. In the current study, optimum conditions for keratinase production by *Micrococcus luteus* Y23-18 strain were investigated using Taguchi DOE L9 orthogonal array. For this purpose, the selected environmental factors were initial pH, incubation temperature and time. The optimal conditions were obtained as pH 9.5, temperature 30˚C and 3 days. The obtained results showed that keratinase activity was enhanced approximately 2.3-folds (34.95 U mL^{-1}) when compared with the unoptimized conditions (15.33 U mL^{-1}). As a result, *M. luteus* Y23-18 is an effective keratinase producer microorganism and Taguchi design of experiment is a useful tool for optimization.

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Micrococcus luteus Tarafından Sentezlenen Keratinaz Enziminin Taguchi DOE Yöntemi Kullanılarak Optimizasyonu

ÖZET
Keratinaz, keratinöz atıkların, özellikle çevresel kirliliğe yol açan mezbaha ve kümes hayvancılığı kökenli atıkların parçalanmasında kullanılan önemli bir enzimdir. Mevcut çalışmada, *Micrococcus luteus* Y23-18 suşu tarafından keratinaz enziminin üretiminin Taguchi DOE L9 ortogonal dizisi kullanılarak optimizasyonu araştırılmıştır. Bu amaçla seçilen çevresel faktörler, başlangıç pH değeri, inkübasyon sıcaklığı ve zamandır. Optimal şartlar 9.5 pH değeri, 30˚C sıcaklık ve 3 gün olarak belirlenmiştir. Elde edilen sonuçlar keratinaz aktivitesinin, optimize edilmeyen durumla (15.33 U mL^{-1}) karşılaştırıldığında yaklaşık olarak 2.3 kat (34.95 U mL^{-1}) arttığını göstermiştir. Sonuç olarak, *M. luteus* Y23-18 etkili bir keratinaz üretici mikroorganizmadır ve Taguchi deney dizaynı optimizasyon için kullanışlı bir araçtır.

Mikrobiyoloji

Araştırma Makalesi

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Anahtar Kelimeler
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Keratinaz
Taguchi deney dizaynı
Optimizasyon

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INTRODUCTION
Keratin is an insoluble fibrillar protein that has high resistance against proteolytic digestion in the skin of birds, reptiles, and mammals. It has two types, the first one is acidic Type I and the other one is neutral Type II (Scott & Untereiner, 2004). Keratinase (EC 3.4.4.25) is an important enzyme that catalyses the hydrolysation of the keratin (Sharma & Kango, 2021; Vidmar & Vodovnik, 2018). An increase in the population of the world causes environmental pollution, hence the elimination of the waste becomes more important for human health. Keratin-originated industrial materials, especially slaughterhouse and poultry wastes, require particular elimination methods depending on their natural structure. In this context, side industrial wastes are critical, like cattle hair and chicken feather, etc. There are approximately 1,5 billion cattle in the world (Murray-Tortarolo & Jaramillo, 2020) and waste cattle hair has not been used for commercial protein source for animal feedstock (Cai et al., 2022). However, cattle hair has valuable bioactive peptides to be recycled (Etemadian

et al., 2021). Chicken meat and chicken-derived products have been consumed continually all over the world TUIK (TUIK Turkey Statistical Institute, 2022) reported the chicken meat consumption to be 582444 tons which was derived from 330211 chicken, during January-March 2022 period in Turkey. The poultry wastes are keratin-rich materials and the chicken feathers contain ß-keratin (38%), α-keratin (41%), and amorphous keratin (21%) (Daroit & Brandelli, 2014). The poultry wastes consist of infertile eggs, feathers, empty shells and dead embryos that have dangerous structure for the environment (Chen & Jiang, 2014; Nowak et al., 2017; Prabakaran & Valavan, 2021; Simpson, 1991). Keratinous wastes are processed various techniques including alkaline extraction, ionic fluids, oxidative reduction, steam flash explosion, microwave radiation, thermal hydrolysis, enzymatic hydrolysis, and microbial fermentation (Martinez et al., 2020); however, keratinase-based degradation of these wastes is more sustainable and environmentally friendly (Zhang et al., 2022).

Keratinases hydrolyse the recalcitrant structural keratins (Gupta et al., 2013). Keratinases are generally used in leather finishing processes, industrial waste water treatments, cosmetic industry, and organic fertilizer. Microbial keratinases are produced by bacteria, commonly, Gram positive bacteria like *Bacillus halotolerans* (Devi et al., 2022), *B. subtilis* (Zhang et al., 2022), *Microbacterium*, and *Micrococcus luteus* (Laba et al., 2015) etc.

Statistical tools are preferred due to their possibility for random experiments when compared with classical one-factor-at- a time (OFAT, namely one- variable- at- a time, OVAT) method. OFAT is a confidential optimization technique for specific requirements of the growth conditions and valuable product formation using addition or depletion of the factors, or causes variations in environmental conditions. However, all the experiments were done respectively. On the contrary, statistical optimization methods usually present randomly-organized experimental runs to gain interaction between the factors. Use of statistical designs offers the opportunity for rapid screening and shows the individual roles of each factor (Canlı Taşar, 2020; Canli Tasar, 2022; Farid et al., 2013) and the Taguchi DOE method increases the robustness of products with high quality and less labour (Kivak, 2014; Rao et al., 2008).

MATERIALS and METHOD

All of the medium components were purchased from Sigma (USA) and Merck (Germany).

Microorganism and medium

The keratinase producer bacteria were isolated from contaminated soil of a local slaughterhouse in Erzurum, Turkey. Among twenty-five keratinase producing-microorganisms, skim milk agar plates were employed for their protease activities (data not shown). The greatest clear zone was produced by a bacterium named HP2, hence it was further used and identified as *Micrococcus luteus* Y23-18 strain using 16S rRNA sequence analysis (Figure 1). ITS1 and ITS4 primers were used for amplification of the ITS gene region for sequencing under in vitro conditions at Macrogen (Netherlands) and pGEM-T Easy Vector Systems (Promega UK) were used for cloning of the PCR products. BioEdit was employed to determine and analysis the results from the database. The nitrogen source was used as powdered ram horn that was obtained from a local slaughterhouse. The ram horns were dried, milled and powdered. The keratinase production medium was designated as following (g L^{-1}): 10 glucose, 3 powdered ram horn, 1 KH_2PO_4, 0,2 $MgSO_4$, 0,1 $CaCl_2$. For inoculum preparation, the same medium was used in 250 mL Erlenmeyer flask containing 100 mL of medium, and one loopful of 24-h-old *M. luteus* Y23-18 strain from a nutrient agar plate was added. The flask was incubated at 30˚C and 200 rpm for 24 h. 0,5 mL of suspension (1.5_{600nm}) was used as the inoculum material for each experiment.

Enzyme Assay

The keratinase enzyme activity was calculated using colorimetric method. The keratin azure (azokeratin) was preferred for assays with a bit of modification (Gonzalo et al., 2020; Letourneau et al., 1998; Suntornsuk & Suntornsuk, 2003). Keratin azure solution was prepared with 0,4 g of keratin azure to 100 mL of 0.01 M (pH 7,5) Tris-HCl buffer. The reaction mixture consisted of 1 mL enzyme source (culture filtrate) and 1 mL of keratin azure solution. Then, the tubes were taken to incubation at 50˚C in a shaking incubator at 200 rpm for an hour. At the end of the incubation time, the reaction mixture was allowed to boil for 5 min, then the mixture was centrifugated at 5000 x g for 20 min. The supernatant was used for spectrophotometrically measurement for the release of the azo dye at 595 nm. On the other hand, the same process was run for the control without incubation. One-unit (U) keratinase enzyme activity was determined as the enzyme amount that caused 0.1 absorbance increase between the sample and the control at 595 nm in an hour under the experimental conditions. All the experiments were done at 200 rpm agitation speed in 50 mL medium-containing flasks. Minitab® 19.1.1 Statistical Software (United States) was used. All the experiments were run three times and the means were taken.

Taguchi methodology

For Taguchi DOE methodology, L9 orthogonal array was preferred for the optimization of keratinase production using initial pH of the medium, temperature and time factors at three levels (Table 1).

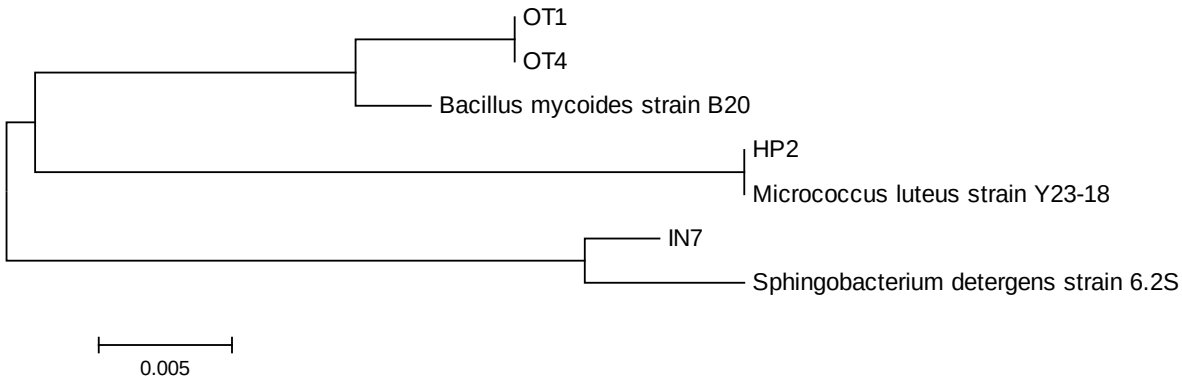

Figure 1. Neighbour joining phylogenetic tree on the basis of 16S rRNA gene sequence data of *Micrococcus luteus* 23-18 strain.

Şekil 1. Micrococcus luteus Y23-18 suşuna ait 16S rRNA gen sekans bilgisine bağlı olarak neighbour joining yöntemi ile oluşturulmuş filogenetik ağaç

Table 1. Optimization parameters and selected levels
Çizelge 1. Optimizasyon parametreleri ve seçilen seviyeler

Serial No	Factors	Level 1	Level 2	Level 3
1	pH	9	9.5	10
2	Temperature (˚C)	30	32	34
3	Time (d)	1	2	3

A full factorial design is useful to investigate all the factors at each level; however, this method is not useful depending on the large number of the factors and levels. Taguchi DOE methodology has three quality characteristics following: the larger- the better, the nominal-the better and the smaller-the better. It was aimed to enhance the keratinase activity, the larger-the better characteristic was utilized and the equation is shown below:

$S/N = -10 \log10 (1/n \sum_{i=1}^{n} \mathbf{1}/\boldsymbol{Yi}^{2})$

where S/N are performance statistics. In the equation, the n determines the number of repetitions and the *Yi* is a performance value of the i*th* experiment. S/N ratio was calculated to find out the selection of the best value by the researcher (Jean & Tzeng, 2003). Taguchi DOE method uses S/N ratio to interpret the results instead of the average values (Tan et al., 2005; Canlı Taşar, 2020).

Analysis of variance

The analysis of variance (ANOVA) was used to find out the optimal levels of the factors. The highest effects obtained from the factors had maximum impact on the keratinase activity. The effects of each parameter were indicated individually. Minitab® 19.1.1 Statistical Software (United States) was employed as the data software.

RESULTS AND DISCUSSION

The obtained results in the current study showed that keratinase production was strongly affected by the environmental conditions (Table 2). Optimal pH values for keratinase production were reported in a pH range between 5 to 10 (Abdel-Fattah et al., 2018; Bockle et al., 1995). In a previous study, maximum keratinase activity was obtained at 6.0 pH and 42˚C by *B. licheniformis* ALW1 using corn steep liquor (Abdel-Fattah et al., 2018). However, the optimal pH and incubation temperature were obtained as 9.5 and 30˚C in the current study, respectively. In another previous study, keratinase production by *M. luteus* the optimum values for pH and temperature were determined as 9.4 and 55˚C, respectively (Laba et al., 2015), which was closer to the current study except temperature. This difference may be resulted from substrates and strains.

In a previous study using classical OFAT method, the optimal conditions were obtained as 9.0 for initial pH, similar to the current study. However, the optimal temperature and time were obtained as 40˚C and 96 hr by *Ochrobacterium intermedium* (Sharma & Kango, 2021). On the other hand, *Streptomyces pactum* DSM 40530 was reported as optimally active at pH range from 7.0 to 10.0 and for temperature from 40 to 75˚C that was not closer to the obtained results (Bockle et al., 1995).

S/N ratio determines the deviation of the quality criterions from the results (Canli et al., 2013; Sharma et al., 2005). The minimisation of the undesirable effects cause enhancement for enzyme production. The minimum keratinase activity (15.33 U mL^{-1}) was obtained from the 1st experimental design (unoptimized conditions), while the maximum activity (30.21 U mL^{-1}) was obtained from the 4th design.

Table 2. Taguchi L9 orthogonal array and keratinase activity and S/N ratios
Çizelge 2. Taguchi L9 ortogonal dizisi ve keratinaz aktivitesi ve S/N oranları

Exp. No.	pH	Temperature	Time	Keratinase (U mL^{-1})	S/N ratios
1	1	1	1	15.33	21.2632
2	1	2	2	22.11	26.8918
3	1	3	3	21.23	26.5390
4	2	1	2	30.21	29.6030
5	2	2	3	27.37	28.7455
6	2	3	1	23.15	27.2910
7	3	1	3	28.39	29.0633
8	3	2	1	20.13	26.0769
9	3	3	2	22.25	26.9466

Response data for S/N ratios and their comparison were given (Table 3). Delta value presents the changes between the maximum and minimum means for the factor. The rank value explains the rank of each Delta. Table 3 demonstrates that initial pH had more relative impact than the incubation time and incubation temperature. The analysis of variance (ANOVA) showed the rank values obtained from the basis of the amplitude of S/N variation (Table 4).

Main effects plot of S/N ratios showed the greatest impacts of the factors at optimal levels (Figure 2). The optimal levels for the factors were pH 9.5, 30˚C and 3 days for the initial pH, incubation temperature and incubation time, respectively. For prediction analyses, Taguchi DOE uses the main effects plot results. At the end of this experiment, keratinase activity was found as 34.95 U mL^{-1} which was closer to the predicted result (35.65 U mL^{-1}).

Table 3. Response table for means.
Çizelge 3. Ortalamalar için yanıt tablosu

Level	pH	Temperature	Time
1	20.56	25.64	20.54
2	26.91	23.20	24.86
3	23.59	22.21	25.66
Delta	6.35	3.43	5.13
Rank	1	3	2

Table 4. Analysis of variance for means.
Çizelge 4. Ortalamalar için varyans analizi

Source	DF	Seq SS	Adj SS	Adj MS	F	P
pH	2	60.588	60.588	30.294	10.92	0.084
Temperature	2	18.728	18.728	9.364	3.38	0.228
Time	2	45.596	45.596	22.798	8.22	0.108
Residual Error	2	5.547	5.547	2.773		
Total	8	130.459				

DF: Degree of freedom; Seq SS: Sequential sum of square; Adj SS: Adjusted sum of square; Adj MS: Adjusted mean of squares; F: F value; P: P value.

The percentage contribution of each factor on the keratinase production was shown in Figure 3. These values were calculated using sequential sum of square of a factor to the total sequential sum of square. pH had the most percentage contribution, the incubation time and temperature had less contribution among the other factors. In a prior study about glucose oxidase production by *Rhodotorula glutinis,* pH had less impact than temperature while the lowest impact was obtained from time (Canli Tasar 2022). This result might be caused by the environmental conditions depending on the target product and medium composition.

CONCLUSION

Keratinase enzyme is a commercially valuable and produced mainly by bacteria. In the current study, *M. luteus* Y23-18 strain was employed for keratinase production as the producer microorganism. Initial pH, incubation temperature and incubation time were selected as the optimization factors and Taguchi DOE was utilized. Optimized factors were determined using Taguchi L9 orthogonal array and the results showed that keratinase production is strongly affected by the environmental conditions. The optimal conditions for keratinase production by *M. luteus* Y23-18 were detected as 9.5 for pH, 30˚C for incubation temperature and 3 days for incubation time. At the end of the optimization, the maximum enzyme activity was obtained as 34.95 U mL^{-1}. Taguchi DOE presented an economical and quickly activity-enhancing way for the enzyme production process. The keratinase production was increased approximately 2.3-folds when compared with the unoptimized conditions. As a conclusion, *M. luteus* has a valuable capacity for keratinase production and Taguchi DOE is a powerful tool for optimization.

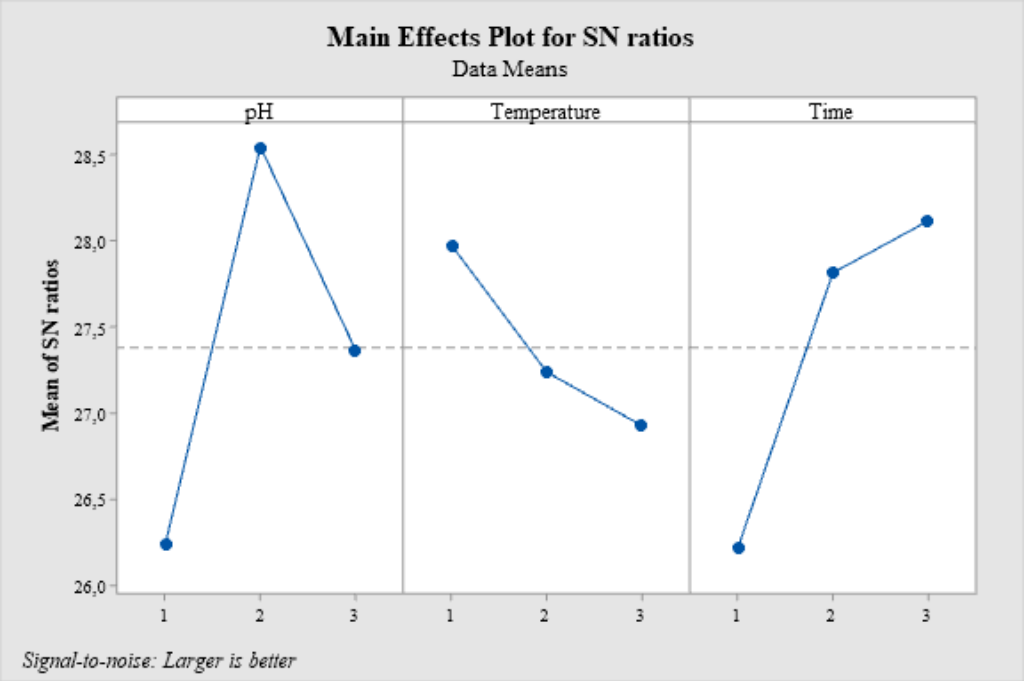

Figure 2. Main effects plots for S/N ratios
Çizelge 2. S/N oranları için asıl etki plotları

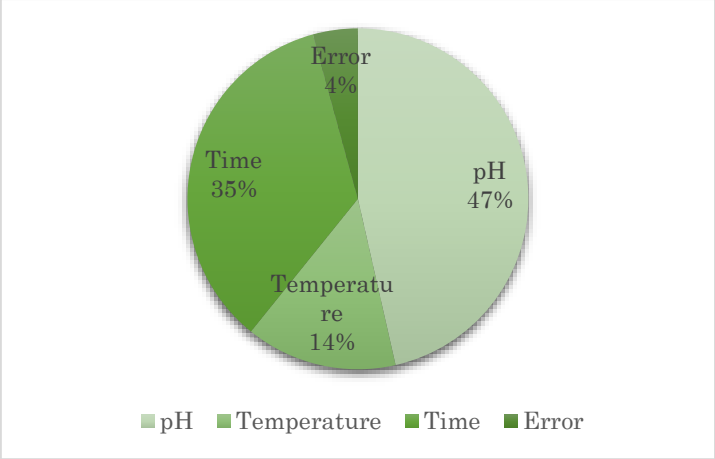

Figure 3. Percentage contribution of each factor on the keratinase production
Çizelge 3. Keratinaz üretimi üzerine etki eden her faktörün yüzde dağılımı

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Author contributions

The authors declare that they have contributed equally to the article.

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Evaluation of In-vitro Anticandidal Activity of 99 Different Commercial Plant Extract, Fixed and Essential Oils against Vaginal *Candida albicans* Isolates

Gülçin ÖZCAN ATEŞ[1✉], Savaş KANBUR[2]
[1,2] Çanakkale Onsekiz Mart University, Vocational School of Health Services, Department of Medical Services and Techniques, Çanakkale, Türkiye
[1]https://orcid.org/0000-0002-8467-2378, [2]https://orcid.org/0000-0002-8770-0194
✉: gulcinozcan@comu.edu.tr

ABSTRACT

Plant extracts (PE), fixed oils (FO) and essential oils (EO) are used in traditional medicine to treat various diseases. This study evaluated the anticandidal activity of 100 different commercially available PEs, FOs, and EOs against 19 *Candida albicans* vaginal isolates and *C. albicans* ATCC 10231. It was determined that 20 EOs and FOs had anticandidal activity. *Piper nigrum* FO, pine turpentine EO, pine tar EO, and *Eugenia caryophyllata* EO showed the highest anticandidal activity. The minimum inhibitory concentrations (MIC) and minimum fungicidal concentrations (MFC) values of these FO and EOs were between 0.125 and 2 µL mL^{-1}. The volatile components of these FOs and EOs were determined by GC-MS analysis. There were six components in *E. caryophyllata* EO, 38 in Pine turpentine EO, 39 in *P. nigrum* FO, and 119 in Pine tar EO. In conclusion, this FOs and EOs can be used to treat vulvovaginal candidiasis.

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99 Farklı Ticari Bitki Ekstrakt, Sabit ve Uçucu Yağın Vajinal *Candida albicans* İzolatlarına Karşı In-vitro Antikandidal Aktivitesinin Değerlendirilmesi

ÖZET

Bitki ekstrakt (PE), sabit yağları (FO) ve esansiyel yağları (EO) çeşitli rahatsızlıkların tedavisinde geleneksel tıpta kullanılmaktadır. Bu nedenle bu çalışmada ticari olarak satışı bulunan 99 adet PE, FO ve EO'nun 19 *Candida albicans* vajinal izolatına ve *C. albicans* ATCC 10231 standart kültürüne karşı antikandidal aktivitesi değerlendirilmiştir. 20 adet PE, FO ve EO'nun antikandidal aktiviteye sahip olduğu belirlendi. En yüksek antikandidal aktiviteyi ise *Piper nigrum* FO, pine turpentine EO, pine tar EO ve *Eugenia caryophyllata* EO gösterdiği belirlenmiştir. Bu FO ve EO'ların minimum inhibitör konsantrasyonu (MİK) ve minimum fungisidal konsantrasyonu (MFC) değerlerinin ise en düşük 0.125 µL mL^{-1} ve en yüksek ise 2 µL mL^{-1} olduğu belirlenmiştir. Bu yağların uçucu bileşenleri ise GC-MS analizi ile belirlenmiştir. Karanfil yağında 6 bileşen, çam terebentin yağında 38 bileşen, karabiber yağında 39 bileşen ve çam katranın da ise 119 bileşen tanımlanmıştır. Sonuç olarak yeni antifungal bileşenlerin belirlenmesine katkı sağlayacak bitki esansiyel yağları belirlenmiştir.

Mikrobiyoloji

Araştırma Makalesi

Makale Tarihçesi
Geliş Tarihi : 30.11.2022
Kabul Tarihi : 23.03.2023

Anahtar Kelimeler
Antikandidal aktivite
Bitki sabit yağ
Esansiyel yağ
Vajinal Candida

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INTRODUCTION

Candida species are commensal members of the human microbiota. They can colonize the mucosal surfaces of the oral cavity, vagina, skin, scalp, and nails. (Gonçalves et al., 2016; Tsega & Mekonnen, 2019; Permana et al., 2021). All women can carry *Candida* in the vagina without causing infection. *Candida* can cause vulvovaginal candidiasis (VVC) in various conditions that impair host immunity (Gonçalves et al., 2016; Ghaddar et al., 2020). VVC is

the most common fungal disease affecting the genital tract of women all over the world and is considered an important public health problem. The clinical symptoms of VVC are not specific. The most common clinical manifestations are vaginal pain, vulvar and vaginal erythema, and fissures with vulvar itching, burning, and irritation leading to dyspareunia and dysuria. The morbidity of VVC is the biggest problem, and it is not associated with mortality. It causes pain and suffering, and changes in self-anxiety and job performance, especially in women. Most importantly, it affects their sexual and emotional relationships and creates mental problems. The treatment of VVC is crucial, considering all these adverse effects. In addition, if not treated, many different complications may occur, such as pelvic inflammatory disease, pelvic abscess, infertility, menstrual disorders, ectopic pregnancy, and spontaneous abortion (Gonçalves et al., 2016, Tsega & Mekonnen, 2019). VVC is more critical, especially during pregnancy, because VVC has been reported to cause preterm birth, infant mortality, and invasive neonatal candidiasis in pregnant women (Tsega & Mekonnen, 2019).

Antimicrobial agents are critical in reducing the global burden of infectious diseases. Today, however, the effectiveness of antibiotics is decreasing due to the development of resistant pathogenic microorganisms. This resistance to antimicrobial agents severely threatens public health and all kinds of antibiotics. The incidence of antimicrobial resistance, including drugs of last resort used in treating infectious diseases, is increasing worldwide. Therefore, alternative antimicrobial strategies are urgently needed, reassessing the therapeutic use of older drugs such as herbs and plant-based products (Mandal & Mandal, 2011; CDC, 2022).

Throughout history, plants have been used in traditional medicine for therapeutic purposes. It was used to treat infectious diseases even when there was no knowledge about microorganisms. Plant extracts (PE) and essential oils (EO) have many impressive properties, including antiviral, antidiabetic, spasmolytic, and antioxidant activity. It has immunomodulatory, psychotropic, and expectorant effects and cancer-suppressive activities. Considering the unpleasant side effects of synthetic drugs used today, such as nephrotoxicity or ototoxicity, the use of plants with lower toxicity in treating diseases makes it even more attractive (Lang & Buchbauer, 2012; Ibišević et al., 2020). Due to resistance to synthetic antimicrobial agents, we must focus on developing alternative treatment protocols, especially with natural sources such as plants (Nalbantbaşi & Gölcü, 2009; Lang & Buchbauer, 2012; Kola-Mustapha et al., 2021).

This study aimed to determine PE, plant fixed oil (FO), and EOs that have activity similar to or higher than fluconazole and amphotericin B antifungal drugs against the vaginal *Candida albicans* ((C. P. Robin), Berkhout 1923) isolates, an opportunistic pathogen.

MATERIAL and METHOD

Culture

Nineteen vaginal *C. albicans* isolates were obtained from the Istanbul Zeynep Kamil Gynecology and Pediatrics Training and Research Hospital Microbiology Laboratory (Turkey) in 2019. 19 vaginal *C. albicans* isolates and *C. albicans* ATCC 10231 standards were checked on HiCrome™ Candida Differential Agar (M1297A, Himedia, India).

Plant Extracts and Essential Oils

Ninety-nine different PEs, FOs, and EOs were obtained in the markets and online shopping in Turkey. Information about the provided PEs, FOs and EOs is given in Table 1.

Method

Inoculum Preparation

To determine the antifungal activity, firstly, the isolates were resuscitated. For this purpose, 19 vaginal *C. albicans* isolates and *C. albicans* (ATCC 10231) cultures were inoculated into Sabouraud Dextrose Agar plates, then plates incubated at 36 ± 2°C for 18-24 hours. Revived isolates were then adjusted to 0.5 McFarland (1 - 5 x 10^6 cells mL^{-1}) cell density with physiological saline (PS, 0.85% NaCl) using a McFarland densitometer.

Determination of Anticandidal Activity by Agar Well Diffusion Method

The antifungal activity of 99 commercial PE, FO, and EOs against *C. albicans* was evaluated using the agar well diffusion method. Amphotericin B and fluconazole antifungal discs were used as positive controls. Within 15 minutes of the inoculum suspension preparation, the suspension was applied with a sterile cotton swab to the dried surface of the Mueller-Hinton Agar + 2% Glucose + 0.5 µg mL^{-1} Methylene Blue Agar (Himedia, India) plate. Afterwards, within 15 minutes, wells with a diameter of 6 mm were drilled with a cork borer set, and 20 µL of PE, FO, and EOs were added to the wells. Zone diameters were measured after incubation at 36 ± 2°C for 24 hours. The study was carried out in 3 parallels. All data are given as mean (X) ± standard deviation (Sx) using the Minitab 17.0 program.

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration

As a result of agar well diffusion, four effective oils were selected. The minimum inhibitory concentration

Table 1. Plant extracts, fixed and essential oils.
Tablo 1. Bitki ekstrakt, sabit ve esansiyel yağlar

No	Ingredients	Type	Produced By	No	Ingredients	Type	Produced By
1	*Urtica sp.*	FO	A	*51*	*Calendula officinalis* flower	FO	T
2	*Apricot kernel*	FO	A	*52*	*Petroselinum crispum* seed	FO	T
3	*Citrus lemon*	EO	A	*53*	*Chamomilla recutita*	FO	T
4	*Vitis vinifera* seed	FO	A	*54*	Black garlic *(Allium sativum)*	FO	T
5	Taurus mint oil (*Mentha pulegium* oil, eucalyptus oil, orange oil)	EO	B	*55*	*Cananga odorata*	EO	U
6	*Aesculus hippocastanum*	FO	L	*56*	*Pinus palustris*	EO	V
7	*Nigella sativa*	FO	C	*57*	Pine tar	FO	J
8	Black garlic oil (*Allium sativum* bulb oil %50 + Sunflower seed oil %50)	FO	M	*58*	*Amygdalus amara*	FO	K
9	*Aesculus hippocastanum*	EO	N	*59*	*Salvia triloba*	EO	K
10	*Carthamus tinctorious*	FO	D	*60*	*Rubus idaeus*	PE	K
11	Pine turpentine	EO	D	*61*	*Aloe vera*	EO	K
12	*Prunus amygdalus dulcis*	FO	D	*62*	*Pimpinella anisum*	FO	K
13	Adiyaman mint (*Mentha pulegium*, orange oil, lemon oil)	EO	O	*63*	*Juniperus communis*	EO	K
14	Anise	FO	F	*64*	*Carthamus tinctorius*	FO	K
15	*Nigella sativa*	FO	E	*65*	*Persea gratissima*	FO	K
16	*Alpinia sp.*	EO	F	*66*	*Calendula officinalis*	EO	K
17	*Daucus carota sativa*	FO	E	*67*	*Citrus bergamia*	EO	K
18	*Cucurbita pepo* seed	FO	E	*68*	*Salvia rosmarinus*	EO	K
19	*Lavandula stoechas*	EO	F	*69*	*Triticum sp.*	FO	K
20	*Prunus armeniaca* kernel	FO	E	*70*	*Juglans sp.*	FO	K
21	*Actinidia deliciosa*	FO	F	*71*	*Pinus sylvestris*	EO	K
22	*Sesamum indicum* seed	FO	E	*72*	*Melaleuca alternifolia*	EO	K
23	*Prunus persica* kernel	FO	E	*73*	*Menthe-chn oil*	EO	K
24	*Persea sp.*	FO	P	*74*	*Nigella sativa*	FO	K
25	*Triticum sp.*	FO	P	*75*	*Primula elatior*	EO	K
26	*Juglans sp.*	FO	P	*76*	*Laurus sp.* (semen)	FO	K
27	*Cucurbita pepo* seed	FO	P	*77*	*Laurus sp.*	EO	K
28	*Prunus armeniaca* kernel	FO	P	*78*	*Ocimum basilicum*	EO	K
29	*Sesamum indicum* seed	FO	P	*79*	*Rosa sp.*	FO	K
30	Chili oil (*Capsicum annuum* oleum, *Helianthus annuus* seed oleum)	FO	G	*80*	*Sinapis sp.*	EO	K
31	Jasmine oil (*Jasminum officinale* flower oil, propylene glycol)	EO	G	*81*	*Daucus carotae*	FO	K
32	Patchouli oil (*Pogostemon cablin* leaf oil, *Helianthus annuus* seed oil)	EO	G	*82*	*Cocos nucifera*	EO	K
33	*Sinapsis alba*	FO	R	*83*	*Elaegnus angustifolia*	EO	K
34	*Carthamus tinctorius*	FO	H	*84*	*Centaurium minus*	EO	K
35	*Calendula officinalis* flower	EO	H	*85*	*Lavandula cariensis*	EO	K
36	*Citrus bergamia*	EO	H	*86*	*Eugenia caryophyllata*	EO	K
37	*Ocimum basilicum*	EO	H	*87*	*Lavandula angustifolia*	EO	K
38	*Cucurbita pepo* seed	FO	H	*89*	*Anemone apennina*	EO	K
39	*Lavandula cariensis*	FO	H	*90*	*Eucalyptus sp.*	EO	K
40	*Piper nigrum*	FO	H	*91*	*Olea europea*	FO	K
41	*Prunus armeniaca* kernel	FO	H	*92*	*Cedrus sp.*	EO	K
42	*Citrus lemon*	EO	H	*93*	*Allium cepa*	FO	K
43	*Punica granatum* seed	FO	H	*94*	*Cinnamomum sp.*	EO	K
44	*Momordica chantia* fruit	PE	H	*95*	*Amygdalus dulcus*	FO	K
45	*Petroselinum sativum*	EO	H	*96*	*Vitis vinifera*	FO	K
46	Balsam	EO	H	*97*	*Jasminum sp.*	EO	K
47	*Cedrus libani*	EO	H	*98*	*Cananga oderata*	EO	K
48	Pine turpentine oil (Turpentine, Tocopheryl acetate)	EO	I	*99*	*Lilium candidum*	EO	K
49	*Ricinus communis* seed, tocopheryl acetate)	EO	I	*100*	*Zingiber officinale*	EO	K
50	*Foeniculum vulga re*	FO	S				

was achieved by microdilution as specified in NCCLS M27-A2. 0.5 McFarland cell suspension was diluted 1:1000 with RPMI 1640 medium with 0.165 M MOPS with 0.2% glucose (Himedia, India) and 3% DMSO, and the final cell density was 0.5 - 2.5 x 10^3 cells mL^{-1}. Six different concentrations (4, 2, 1, 0.5, 0.25, and 0.1 µL mL^{-1}) of PE, FO, and EOs were prepared in RPMI 1640 medium. 100 µL of RPMI 1640 medium containing 2x PE, FO, and EOs was added to each well of the U-bottom microplates. Then, 100 µL of the suspension containing 1:1000 diluted cell suspension was added to the wells containing 2x PE, FO, and EOs. The final volume in each well was ensured to be 200 µL. RPMI 1640 medium containing 4 µL mL^{-1} PE, FO, and EO was used as the negative control, and RPMI 1640 medium containing only culture was used as the positive control. Microplates were evaluated at 660 nm in a microplate reader (Thermo Multiscan FC) after 24 hours and 48 hours of incubation at 37°C. The study was carried out in 3 parallels. The first well without growth was determined as the MIC value.

The minimum fungicidal concentration (MFC) value was determined after incubation at the appropriate temperature and time by planting with the drip planting method from the defined MIC value and the subsequent three wells. The MFC MIC^{-1} ratio was used to interpret the activity of the PE, FO, and EOs (Gatsing et al., 2009; Snoussi et al., 2018; Mseddi et al., 2020).

Determination of Essential Oil Volatile Component Composition

GS-MS determined the volatile components of four oils. Volatile compounds were analyzed using a gas chromatograph 7890 A connected to an MSD 5975 C (Agilent Technologies) series mass spectrometer. CP WAX 52 was determined using a CB capillary column (50 m x 0.25 mm ID, df:0.2 µm). The carrier gas is helium at a 1.2 mL min^{-1} flow rate. The temperature schedule for the GC is 60°C initial temperature, and after 2 minutes, it is increased to 220°C with a temperature increase of 2°C min^{-1}. After reaching 220°C, the temperature was held constant for 20 min. 100 µL of sample is dissolved in 1 ml of hexane, and 1 µL is the injection volume. The injector temperature is 240°C, and the detector temperature is 250°C. The mass spectrometer was operated in electron impact mode at 70 eV. Integrations were made with MSDCHEM software.

RESULTS

Agar Well Diffusion

The data in this study were obtained by sequential application of different techniques to evaluate the anticandidal activities of 99 PE, FO, and EO against 19 vaginal *C. albicans* isolates and *C. albicans* ATCC 10231 standard cultures. The first step is determining whether EOs have antifungal activity against *C. albicans*. For this purpose, the agar well diffusion method was used, and it was determined that 79 PE, FO, and EO did not have anticandidal activity. On the other hand, 20 FOs and EOs were determined to have anticandidal activity. Inhibition zone diameters of PE, FO, and EOs with anticandidal activity are given in Tables 2 and 3.

Tables 2 and 3 show that the inhibition zones of PE, FO, and EOs against the tested isolates vary. It was determined that *P. anisum* FO (62) and *C. bergamia* EO (67) gave inhibition zones of 7 - 10 mm only against the V1c isolate. *L. stoechas* EO (19), *J. officinale* EO (31), and *A. vera* EO (61) did not have antifungal activity against 16 isolates. On the other hand, pine turpentine EO (48), pine tar EO (57), and *E. caryophyllata* EO (86) were determined to have anticandidal activity against all isolates. It was determined that *C. lemon* EO (42) and *Cinnamomum sp.* EO (94) had anticandidal activity against 19 isolates, and *C. bergamia* EO (36) and *O. basilicum* EO (78) had anticandidal activity against 18 isolates. Therefore, *L. stoechas* EO (19), *J. officinale* EO (31), *A. vera* EO (61), *P. anisum* FO (62), and *C. bergamia* EO (67) inhibited the growth of a very limited number of clinical isolates. *C. lemon* EO (3), Taurus mint EO (5), *N. sativa* FO (7), pine turpentine EO (11), *N. sativa* FO (15), *P. nigrum* FO (40), *C. lemon* EO (42), *O. basilicum* EO (78) (excluding V8c isolate), *E. angustifolia* EO (83) and *Lavandula angustifolia* EO (87) yielded a zone diameter of 10 - 20 mm. When these ten EOs were compared with reference antifungal agents, it was determined that they had limited effects on the growth of clinical isolates.

Fluconazole and amphotericin B were used as positive controls. The efficacy of antifungal agents was confirmed by *C. albicans* ATCC 10231. Reference values for clinical *Candida* isolates are given in Table 4. As seen in Tables 2 and 3, it was determined that clinical isolates formed inhibition zones between 15 - 25 mm and 30 - 42 mm against amphotericin B and fluconazole, respectively. In short, it was determined that all tested clinical isolates were susceptible to two antifungal agents. *C. bergamia* EO (36), pine turpentine EO (48), pine tar FO (57), *E. caryophyllata* EO (86), and *Cinnamomum sp.* EO (94) gave a ≥20 mm inhibition zone against tested all isolates. As a result, they formed a zone of inhibition as much as antifungal agents.

Minimum Inhibitory Concentration (MIC) and Minimum Fungicide Concentration (MFC)

In the second step of the study, two FOs and two EOs were selected, which were determined to have high

anticandidal activity by the agar well diffusion method. MIC values of these four selected oils against *C. albicans* isolates were evaluated using the microplate method. The MIC values of the selected two FOs and two EOs are given in Table 5. It was determined that MIC values vary between 0.5 and 2 µL mL^{-1} for pine turpentine EO (48) and *P. nigrum* FO (40). It was determined that the MIC value of *E. caryophyllata* EO (86) was 0.25 µL mL^{-1} for all isolates. The MIC value of pine tar FO was determined to be 0.125 µL mL^{-1} for all isolates.

This study determined the MIC value of the first well without growth. The MFC value was determined after incubation at the appropriate temperature and time by planting with the drip planting method from the well determined as the MIC value and the next three wells. MFC values of the four selected FOs and EOs are given in Table 5. In line with Table 5, it was determined that MFC values vary between 2 µL mL^{-1} for pine turpentine EO (48) and *P. nigrum* FO (40). It was determined that the MFC value of pine turpentine EO (48) and *E. caryophyllata* EO (86) (except for one isolate) was 1 µL mL^{-1} for all *C. albicans* isolates. The MFC value of pine tar FO was determined to be <0.125 µL mL^{-1} for all isolates.

Table 2. Inhibition zone diameters (mm) of PEs, FOs, and EOs against vaginal C. albicans isolates using the agar well diffusion assays.

Tablo 2. Agar kuyusu difüzyon analizi vajinal C. albicans izolatlarına karşı PE'lerin, FO'ların ve EO'ların inhibisyon zon çapları (mm).

No	Isolates									
	V1c	V2b	V3c	V4c	V5c	V6c	V7c	V8c	V9c	V10c
3	6.00 ± 0.01*	12.25 ± 0.62	6.00 ± 0.01	12.67 ± 0.71	11.72 ± 1.56	9.00 ± 0.40	11.28 ± 0.72	12.92 ± 0.59	10.31 ± 0.73	9.30 ± 0.65
5	10.49 ± 0.78	10.85 ± 0.59	9.77 ± 1.18	11.04 ± 0.74	10.87 ± 0.68	10.49 ± 0.77	10.19 ± 0.68	10.35 ± 0.73	9.45 ± 0.69	6.00 ± 0.01
7	10.49 ± 0.33	8.23 ±0.46	7.19 ± 0.22	8.54 ± 0.53	8.08 ± 0.96	10.93 ± 0.74	8.78 ± 0.74	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01
11	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	8.36 ± 0.40	6.00 ± 0.01	11.57 ± 0.31	6.00 ± 0.01
15	13.82 ± 0.66	11.30 ± 0.50	9.39 ± 0.97	6.00 ± 0.01	6.00 ± 0.01	12.26 ± 1.07	6.00 ± 0.01	9.63 ± 0.96	12.22 ± 1.61	9.85 ± 0.83
19	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	8.54 ± 1.02	9.02 ± 0.73	7.93 ± 0.84	6.00 ± 0.01	6.00 ± 0.01
31	6.00 ± 0.01	7.43 ± 0.35	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	7.81 ± 1.40	6.00 ± 0.01	8.73 ± 0.46	6.00 ± 0.01
36	17.69 ± 0.65	18.07 ± 1.78	11.77 ± 0.74	19.65 ±2.28	6.00 ± 0.01	21.24 ± 1.99	13.84 ± 0.92	20.36 ± 2.26	9.34 ± 1.63	12.28 ± 1.29
40	10.02 ± 0.38	11.39 ± 1.75	6.00 ± 0.01	6.00 ± 0.01	8.78 ± 0.76	8.57 ± 0.74	11.86 ± 1.34	10.60 ± 1.07	7.91 ± 0.98	6.00 ± 0.01
42	11.52 ± 1.05	9.94 ± 1.72	10.02 ± 1.66	11.23 ± 0.78	10.72 ± 0.83	11.02 ± 1.07	16.33 ± 2.71	17.57 ± 0.64	14.08 ± 0.91	12.86 ± 1.29
48	26.88 ± 0.70	19.54 ± 0.91	18.14 ± 1.69	22.70 ± 2.44	23.47 ± 0.66	29.01 ± 1.99	35.36 ± 5.15	34.75 ± 2.66	26.02 ± 2.74	16.55 ± 0.84
57	20.97 ± 0.94	15.92 ± 1.60	15.64 ± 1.77	17.27 ± 2.47	14.98 ± 1.13	16.30 ± 3.33	19.45 ± 0.65	25.11 ± 1.26	18.32 ± 0.53	9.70 ± 0.95
61	7.94 ± 0.67	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	9.61 ± 0.75	6.00 ± 0.01	16.99 ± 0.62	6.00 ± 0.01	10.12 ± 1.29
62	7.50 ± 0.77	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01
67	9.11 ± 1.86	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01
78	11.19 ± 1.12	12.78 ± 2.04	11.25 ± 1.02	9.58 ± 1.96	9.84 ± 0.89	6.00 ± 0.01	9.44 ± 0.77	23.21 ± 1.14	14.23 ± 1.92	9.75 ± 0.25
83	10.10 ± 1.50	8.46 ± 0.45	6.00 ± 0.01	7.02 ± 0.87	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	8.18 ± 0.77	6.00 ± 0.01
86	30.68 ± 4.92	24.25 ± 3.40	23.00 ± 1.67	19.78 ± 3.23	21.09 ± 3.59	21.08 ± 1.38	18.32 ± 0.99	27.38 ± 2.02	17.55 ± 2.26	25.08 ± 3.64
87	10.77 ± 1.01	10.24 ±1.20	9.56 ± 1.06	6.00 ± 0.01	9.43 ± 1.28	6.00 ± 0.01	9.27 ± 1.05	8.39 ± 0.69	6.00 ± 0.01	10.36 ± 1.40
94	30.11 ± 1.36	24.00 ± 2.07	15.16 ± 2.19	17.84 ± 1.02	24.18 ± 2.25	21.51 ± 1.89	31.89 ± 1.96	25.98 ± 1.51	20.09 ± 1.32	16.71 ± 0.80
*FLU***	38.70 ± 1.95	35.59 ± 0.15	41.22 ± 0.22	35.20 ± 0.37	34.09 ± 1.80	39.60 ± 2.54	37.15 ± 1.16	26.23 ± 0.56	34.42 ± 0.46	31.74 ± 1.26
*AMP****	20.33 ± 0.43	19.35 ±0.63	22.41 ± 0.12	21.39 ± 0.65	21.98 ± 0.86	21.61 ± 0.91	20.32 ± 0.45	19.61 ± 0.33	18.06 ± 0.25	15.10 ± 0.01

*Results are given as X + Sx **FLU: Flucanozole (25 mcg) (SD232-5CT, Himedia, India), ***AMP: Amphotericin B (20 IU, 21.19 mcg, Bioanalyse, Turkey)

Table 3. Inhibition zone diameters (mm) of PEs, FOs, and EOs against vaginal C. albicans isolates using the agar well diffusion assays.

Tablo 3. Agar kuyusu difüzyon analizi vajinal C. albicans izolatlarına karşı PE'lerin, FO'ların ve EO'ların inhibisyon zon çapları (mm).

No	Isolates									
	V12c	V13c	V14b	V15c	V16b	V17c	V18c	V19c	V20c	10231
3	6.00 ± 0.01*	9.42 ± 0.29	10.33 ± 0.48	10.11 ± 1.22	11.15 ± 2.05	9.67 ± 0.43	6.00 ± 0.01	8.75 ± 0.43	8.67 ± 0.78	6.00 ± 0.01
5	6.00 ± 0.01	9.81 ± 0.68	10.44 ± 1.17	11.18 ± 2.22	10.18 ± 0.53	10.79 ± 1.11	9.86 ± 0.42	6.00 ± 0.01	8.84 ± 0.65	6.00 ± 0.01
7	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	9.84 ± 0.88
11	10.65 ± 0.92	10.69 ± 0.49	9.33 ± 0.71	6.00 ± 0.01	6.00 ± 0.01	9.01 ± 0.54	9.18 ± 0.45	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01
15	6.00 ± 0.01	9.68 ± 0.77	10.62 ± 0.50	8.75 ± 0.59	10.93 ± 0.68	8.62 ± 0.11	9.48 ± 0.31	6.00 ± 0.01	10.38 ± 0.45	11.72 ± 0.99
19	7.23 ± 1.05	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01
31	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01
36	18.98 ± 2.88	10.93 ± 0.93	12.64 ± 2.55	16.87 ± 3.77	19.17 ± 3.42	11.91 ± 0.46	6.00 ± 0.01	13.57 ± 1.08	9.93 ± 0.43	18.03 ± 2.97
40	12.81 ± 0.71	7.56 ± 1.23	6.00 ± 0.01	6.00 ± 0.01	11.09 ± 0.81	9.86 ± 0.59	9.97 ± 0.20	8.02 ± 0.30	10.17 ± 0.63	9.17 ± 0.64
42	18.91 ± 0.95	17.92 ± 4.04	14.70 ± 0.78	19.91 ± 6.22	15.32 ± 0.58	12.81 ± 0.42	15.80 ± 0.72	13.14 ± 0.80	6.00 ± 0.01	11.66 ± 1.08
48	38.49 ± 2.96	34.23 ± 4.31	36.23 ± 2.58	38.23 ± 2.60	38.60 ± 2.22	33.27 ± 0.96	34.16 ± 2.12	16.78 ± 2.45	27.55 ± 0.82	18.79 ± 1.44
57	21.61 ± 1.93	16.88 ± 1.45	19.81 ± 1.65	21.53 ± 0.41	19.98 ± 2.91	16.06 ± 1.57	20.90 ± 1.74	15.30 ± 0.18	23.06 ± 0.91	9.61 ± 1.65
61	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	9.48 ± 0.49	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01
62	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01
67	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01
78	15.68 ± 1.23	16.19 ± 0.97	11.84 ± 0.61	14.60 ± 0.89	16.13 ± 1.09	12.52 ± 0.78	11.14 ± 0.60	10.48 ± 1.47	6.00 ± 0.01	11.94 ± 1.06
83	11.35 ± 1.37	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01
86	23.37 ± 1.85	23.15 ± 3.86	16.99 ± 0.59	18.30 ± 0.70	18.38 ± 1.29	15.43 ± 0.83	18.19 ± 1.51	18.27 ± 1.28	19.66 ± 0.38	13.83 ± 1.71
87	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	6.00 ± 0.01	12.62 ± 1.99
94	32.98 ±1.96	22.41 ± 3.24	21.08 ± 2.34	15.08 ± 1.13	18.00 ± 0.67	13.47 ± 0.93	15.44 ± 1.37	13.82 ± 0.87	6.00 ± 0.01	14.33 ± 1.33
*FLU***	30.03 ± 0.91	42.03 ± 0.69	41.88 ± 0.58	26.65 ± 0.90	39.79 ± 1.21	42.17 ± 1.92	33.67 ± 0.28	38.39 ± 0.76	35.30 ± 0.77	26.83 ± 1.16
*AMP***	22.12 ± 0.56	24.87 ± 1.06	23.73 ± 0.30	22.93 ± 0.39	22.89 ± 0.45	23.47 ± 0.53	22.48 ± 0.25	22.80 ± 0.38	19.72 ± 0.28	16.54 ± 0.26

*Results are given as X + Sx. **FLU: Flucanozole (25 mcg) (SD232-5CT, Himedia, India), ***AMP: Amphotericin B (20 IU, 21.19 mcg, Bioanalyse, Turkey)

Table 4. Inhibition zones in mm against clinical isolates and evaluation of reference antifungal agents

Tablo 4. Klinik izolatlara karşı mm cinsinden inhibisyon bölgeleri ve referans antifungal ajanların değerlendirilmesi

Antifungal agents	*Resistant*	*Susceptible - Dose dependent*	*Sensitive*
Fluconazole 25mcg	14	15-18	19
Amphotericin B 10 mcg	9	10-14	15

The MFC MIC^{-1} ratio used to interpret the activity of the essential oil was determined and given in Table 5. The MFC MIC^{-1} ratio is considered fungistatic when the MFC MIC^{-1} ratio is >4, and fungicidal agents when the MFC MIC^{-1} ratio is ≤4 (Gatsing et al. 2009; Snoussi et al. 2018; Mseddi et al. 2020). Therefore, as can be seen in Table 5, it was determined that four selected essential oils were fungicidal agents.

Volatile Composition of *Piper nigrum* FO (40), Pine Turpentine EO (48), Pine Tar FO (57), and *Eugenia caryophyllata* EO (86)

Volatile composition of *P. nigrum* FO (40), pine turpentine EO (48), pine tar FO (57), and *E.*

caryophyllata EO (86) were identified by GC-MS. Volatile compositions are given in Table 6. *P. nigrum* FO (40) was composed of ten main compounds, and these compounds are 38.376% dehydroabietic acid, 10.152% trans beta-caryophyllene, 9.615% delta-3-carene, 6.772% benzenemethanol, 5.836% limonene, 4.289% beta pinene, 3,578% alpha pinene, 2.9399% propylene glycol, 2.796% delta-elemene and 2.069% 1,2,3,-propanetriol, triacetate. Pine turpentine EO (48) was determined that it consisted of 5 main compounds: 73.261% alpha pinene, 12.111% beta pinene, 3.338% limonene, 3.152% camphene and 3.123% delta-3-carene. Volatile component analysis of pine tar FO (57); the first four main compounds were determined as 9.915% limonene, 7.65% alpha pinene, 5.365% delta-3-carene and 4.269% alpha-terpineol. *E. caryophyllata* EO (86) consists of 3 main compounds: 91.01% eugenol, 5.720% caryophyllene, and 1.814% propylene glycol. These main compounds are responsible for possible anticandidal activity in all EOs and FOs.

Table 5. MIC, MFC value, and MFC MIC-1 ratio (µL mL-1) of four selected FO and EO.
Tablo 5. Seçilen dört FO ve EO'nun MIC, MFC değeri ve MFC MIC-1 oranı (µL mL-1).

Isolates	*Piper nigrum* FO (40)			Pine turpentine EO (48)			Pine tar FO (57)			*Eugenia caryophyllata* EO (86)		
	MIC	MFC	MFC MIC^{-1}	MIC	MFC	MFC MIC^{-1}	MIC	MFC	MFC MIC^{-1}	MIC	MFC	MFC MIC^{-1}
V1c	1	2	2	2	2	1	<0.125	<0.125	<0.125	0.5	1	2
V2b	1	2	2	0.5	1	2	<0.125	<0.125	<0.125	0.5	1	2
V3c	1	2	2	0.5	1	2	<0.125	<0.125	<0.125	0.5	1	2
V4c	1	2	2	1	1	1	<0.125	<0.125	<0.125	0.5	1	2
V5c	1	2	2	0.5	1	2	<0.125	<0.125	<0.125	0.5	1	2
V6c	1	2	2	0.5	1	2	<0.125	<0.125	<0.125	0.5	1	2
V7c	2	2	1	1	1	1	<0.125	<0.125	<0.125	0.5	1	2
V8c	1	2	2	1	1	1	<0.125	<0.125	<0.125	0.5	1	2
V9c	1	2	2	0.5	1	2	<0.125	<0.125	<0.125	0.5	1	2
V10c	1	2	2	0.5	1	2	<0.125	<0.125	<0.125	0.5	1	2
V12b	1	2	2	0.5	1	2	<0.125	<0.125	<0.125	0.5	1	2
V13c	1	2	2	1	1	1	<0.125	<0.125	<0.125	0.5	1	2
V14b	1	2	2	0.5	1	2	<0.125	<0.125	<0.125	0.5	1	2
V15c	1	2	2	0.5	1	2	<0.125	<0.125	<0.125	0.5	1	2
V16b	2	2	1	0.5	1	2	<0.125	<0.125	<0.125	0.5	1	2
V17c	1	2	2	1	1	1	<0.125	<0.125	<0.125	0.5	1	2
V18c	1	2	2	0.5	1	2	<0.125	<0.125	<0.125	0.5	1	2
V19c	1	2	2	0.5	1	2	<0.125	<0.125	<0.125	0.5	1	2
V20c	1	2	2	0.5	1	2	<0.125	<0.125	<0.125	0.5	1	2
10231	2	2	1	0.5	1	2	<0.125	<0.125	<0.125	0.5	1	2

Table 6. Volatile component composition of P. nigrum FO (40), pine turpentine EO (48), pine tar FO (57), and E. caryophyllata EO (86)
Tablo 6. P. nigrum FO (40), çam terebentin EO (48), çam katranı FO (57) ve E. caryophyllata EO'nun (86) uçucu bileşen bileşimi

Compounds	*Piper nigrum FO (40)*		*Pine turpentine EO (48)*		*Pine tar FO (57)*		*Eugenia caryophyllata EO (86)*	
	RT	*Abundance %*	*RT*	*Abundance %*	*RT*	*Abundance %*	*RT*	*Abundance %*
(4aR, 6R, 8aR)-4-acetyl-1,1,3,6-tetramethyl-4a,5,6,7,8,8a-hexahydro-1H-2-benzopyran	-	-	-	-	92.992	0.769	-	-
1,1'-biphenyl, bis(1-methylethyl)-	-	-	-	-	84.819 86.947	2.143 0.414	-	-
1,2,3,-Propanetriol, triacetate	56.221	2.069	-	-	-	-	-	-
1,3,8-p-Menthatiene	-	-	-	-	14.450	0.038	-	-
1,3-Cyclohexadinene, 1,3,5,5-tetramethyl-$$ 1,3,5,5-tetramethy-1,3-cyclohexadiene	-	-	30.227	0.296	9.237	1.866	-	-
1,3-Dioxolane, 4-methy-2-phenyl	44.381	0.189	-	-	-	-	-	-
1,3-Pentadiene, 1,1-diphenyl- (Z)-	84.597	1.924	-	-	-	-	-	-
1,4.a.beta.-dimethyl-7-isopropyl-1,2,3,4,4a,9,10,10a.alpha.-octahdrophenanthrene	-	-	-	-	70.234	2.351	-	-
1,4a.beta-dimethyl-7-isopropyl-2,3,4,4a,9,10-hecahydrophenanthrene	-	-	-	-	72.193	1.866	-	-

-1,7-exo-trimethylenebicyclo[3.2.1]octane	-	-	48.076	0.021	-	-	-	-
1-[2-(trimethylsilyl)ethynyl]-3-phenylcyclohexene	-	-	-	-	73.751 79.166	0.467 0.511	-	-
10,11-deihydrobenzo[k]fluoranthene	-	-	-	-	85.02	1.046	-	-
1-Methoxy-4-(1'-methylethyl)cyclohexa-1,4-diene	-	-	-	-	50.75	0.400	-	-
1-tert-butyl-1-(naphtyl-1)-1-silacyclobutane	-	-	-	-	71.971	0.345	-	-
2-(2'-thienyl)-6-phenylpropenenitrile	72.442	1.360	-	-	-	-	-	-
2-(3-isopropyl-4-methoxyphenyl)-6-methylpyridazin-3(2H)-one	-	-	-	-	66.322 64.575	0.424 0.226	-	-
2,3-dimethyl-2-cyclopenten-1-one	-	-	-	-	28.039	0.180	-	-
2,3-Xylenol	-	-	-	-	56.44	0.377	-	-
2,4,6-octatriene, 2,6-d,methyl-	-	-	-	-	20.237	0.808	-	-
2,5-bis(dimethylchlorosilyl)furan	-	-	-	-	88.657	0.513	-	-
2,5-Dimethyl-4-(2,5-dimethylphenyl)pyridine	-	-	-	-	95.253	0.763	-	-
2-acetylfuran	-	-	-	-	26.287	0.094	-	-
2-butanone, 3,3-dimethyl-	-	-	-	-	27.684	0.095	-	-
2-Cyclopenten-1-one, 3,4-dimethyl-	-	-	-	-	24.829	0.025	-	-
2-Cyclopenten-1-one, 3-ethyl-2-hydroxy	-	-	-	-	47.279	0.175	-	-
2-cyclopenten-1-one, 3-methyl	-	-	-	-	26.919	0.121	-	-
2-Pyridineacetonitrile, .alpha.-(4-methoxyphenyl)-	-	-	-	-	83.175	0.251	-	-
2-trimethylsiloxymethyl-4-trimethylsiloxy-1-penten-3-yne	-	-	-	-	65.183	0.337	-	-
3,5-dimethyl-cyclopentenolone	-	-	-	-	42.149	0.140	-	-
3,6-nonadien-1,9-dicarboxylic acid, 5,5-dimethyl-, dimethylester	-	-	-	-	59.709	0.320	-	-
3-ethyl-2-methyl-2H-naphtho[2,3-b]pyran-5,10-dione	-	-	-	-	74.645	0.487	-	-
4,14-dimethyl[2.2]metacyclophane	68.507	0.939	-	-	-	-	-	-
4b,8-dimethyl-2-isopropylphenanthrene, 4b,5,6,7,8,8a,9,10-octohydro-	-	-	-	-	72.62	3.765	-	-
4-Terpineol	-	-	31.498	0.098	31.555	1.112	-	-
5,8-dihydroxy-3-methyl-1,2-dihydro-9,10-antraquinone	-	-	-	-	71.088	0.873	-	-
5.beta.-Podocarpa-8,11,13-tiren-16-oic acid, methyl ester	-	-	-	-	88.947	0.480	-	-
5-acetyl-4,7-dimethoxy-6-hydroxybenzofuran	-	-	-	-	93.641	0.498	-	-
7H-Cyclohepta[b]maohtalen-7-one, 8,9-dihydro-9,9-dimethyl	74.364	0.520	-	-	-	-	-	-
7-Oxabicyclo[2.2.1]heptane, 1-methyl-4-(1-methylethyl)-	-	-	-	-	10.516	0.072	-	-
7-phenyl-3,3,5-trimethyl-1,2-dihydro-3H-pyrazolo/3,4-d/pyridazin-4(5H)-one	-	-	-	-	65.748	0.691	-	-
Acetic acid	-	-	-	-	23.962	0.258	-	-
Alloocimene	-	-	-	-	6.846	1.041	-	-
Alpha pinene	6.587	3.578	6.766	73.261	6.641	7.650	-	-
Alpha terpinene	-	-	-	-	10.618	0.866	-	-
Alpha terpineol	-	-	36.757	0.231	38.862	4.269	-	-
Alpha terpinolene	-	-	14.667	0.104	14.725	2.621	-	-
Alpha-copaene	-	-	23.434	0.023	25.305	0.028	-	-
Alpha-Cubebene	23.422	0.083	-	-	-	-	-	-
Alpha-Humulene	34.889	0.614	-	-	-	-	34.894	0.462
Alpha-iso-methyl ionone	-	-	-	-	-	-	-	-
Alpha-Longipinene	-	-	-	-	-	-	-	-
Alpha-Muurolene	27.304	0.026	-	-	38.025	0.213	-	-
Alpha-pinene oxide	-	-	19.094	0.203	-	-	-	-
Alpha-Selinene	37.816	0.130	-	-	-	-	-	-
Amorphene	-	-	36.011	0.121	-	-	-	-
Azacyclotridecan-2-one, 1-(3-aminopropyl)	-	-	-	-	76.852	1.115	-	-
Azulene	-	-	-	-	38.772	0.159	-	-
Benzene, 1-(1,1-dimethylethyl)-4-ethyl-	-	-	-	-	20.745	0.047	-	-
Benzene, 1,1'methylenebis[4-methyl-	-	-	-	-	69.351	0.343	-	-
Benzene, 1,3-dimethyl-	-	-	-	-	9.445	1.292	-	-
Benzene, 1-ethenyl-4-methyl	-	-	-	-	18.363	0.398	-	-
Benzene, 1-ethyl-3-methyl	-	-	-	-	13.821	0.033	-	-
Benzene, 1-ethyl-3-methyl-	-	-	-	-	12.332	0.506	-	-
Benzene, 1-ethyl-4-(1-methylethyl)	-	-	-	-	17.132	0.199	-	-
Benzene, 1-methyl-2-(1-methyl-2-propenyl)-	-	-	-	-	24.344	0.150	-	-
Benzene, 1-methyl-3-(1-methylethyl)-	-	-	-	-	14.186	2.110	-	-
Benzene, 1-methyl-4-(1-methylethyl)-	-	-	-	-	25.163	0.045	-	-
Benzene, 1-metyl—2-(1-methyl-2-propenyl)-	-	-	-	-	22.463	0.785	-	-
Benzene, 4-ethyl-1,2-dimethyl-	-	-	-	-	16.701	0.193	-	-
Benzene, diethylmethy	-	-	-	-	20.989	0.086	-	-
Benzene,1,2,3-trimethyl	-	-	-	-	17.29	0.673	-	-
Benzenemethanol	46.351	6.772	-	-	-	-	-	-
Beta ocimene	-	-	-	-	19.11	2.105	-	-
Beta pinene	8.447	4.289	8.519	12.111	8.485	0.899	-	-
Beta terpineol	-	-	-	-	33.159	0.366	-	-
Beta-Bisabolene	38.172	0.067	-	-	-	-	-	-
Beta-citronellol	-	-	-	-	-	-	-	-

Beta-elemene	30.704	0.334	-	-	-	-	-	-
Beta-phellandrene	-	-	-	-	11.645	0.430	-	-
Beta-Selinene	37.519	0.336	-	-	-	-	-	-
Bicyclo[3.1.0]hex-2-ene, 2-methyl-5-(1-methylethyl)-	-	-	-	-	10.128	0.133	-	-
Bicyclo[3.1.1]heptan-3-one, 2,6,6-trimethyl-	-	-	26.612	0.069	-	-	-	-
Bicyclo[5.2.0]nonane, 2-methylene-4,8,8-trimethyl-4-vinyl-	-	-	-	-	24.548	0.026	-	-
Borneol	-	-	36.903	0.349	36.97	2.059	-	-
Bornyl acetate	-	-	-	-	30.198	0.345	-	-
Butane 2,2-dimethyl-	12.667	0.068	-	-	-	-	-	-
Cadinene	-	-	39.738	0.146	-	-	-	-
Calarene	-	-	29.707	0.038	-	-	-	-
Camphene	-	-	7.5 21.763	3.152 0.013	7.51	1.382	-	-
Camphor	-	-	-	-	26.618	0.616	-	-
Carene	-	-	-	-	12.5954	0.106	-	-
Caryophyllene	-	-	-	-	-	-	30.98	5.720
Caryophyllene oxide	51.039	0.423	-	-	-	-	51.036	0.580
Copaene	-	-	25.27	0.081	-	-	-	-
Corylon	-	-	-	-	43.986	0.576	-	-
Creosol	-	-	-	-	50.306	3.670	-	-
Cycloheptane, 4-methylene-1methyl-2-(2-methy-1-propen-1-yl)-1-vinyl-	54.339	0.063	-	-	-	-	-	-
*Cycloheptane, 5-ethyldine*1-methyl-*	-	-	-	-	12.902	0.093	-	-
Cyclohexanecarboxylic acid, 1,3-dimethyl-2-[2-[3-(1-methylethyl)phenyl]ethyl]-, methyl ester, (1.alpha,2.alpha.,3.alpha.)-	-	-	-	-	81.729	0.300	-	-
Cyclohexanemethanol, 4-hydroxy-.alpha.,.alpha.,4-trimethyl	-	-	-	-	57.136	0.204	-	-
Cyclohexene, 5-methyl-3-(1-methylethyl)-, trans	-	-	-	-	13.619	0.098	-	-
Dehydroabietic acid	82.395	38.376	-	-	-	-	-	-
Dehydroabietic aldehyde	78.714	0.309	-	-	-	-	-	-
Dekta-Cadinene	-	-	-	-	39.772	0.536	-	-
DELTA 3-Carene	9.59	9.615	9.613	3.123	9.655	5.364	-	-
Delta-Cadinene	39.726	0.217	-	-	-	-	-	-
Delta-elemene	24.091	2.796	-	-	-	-	-	-
D-Frenchyl alcohol	-	-	-	-	30.561	0.930	-	-
Dimethyl 4-phenyl-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate	80.487	1.522	-	-	-	-	-	-
Ethanone, 1-(1,2,3,5,6,7-hexahydro-1,1,5,5-tetramethyl-s-indacen-4-yl)-	-	-	-	-	82.406	0.506	-	-
Ethyl benzene	-	-	-	-	9.006	0.101	-	-
Ethylphenol	-	-	-	-	56.65	0.830	-	-
Eugenol	-	-	-	-	60.246 64.259	0.354 0.225	60.279	91.010
Fenchone	-	-	20.216	0.034	-	-	-	-
Fenchene	-	-	-	-	7.320	0.254	-	-
Fenchyl alcohol	-	-	30.505	0.079	-	-	-	-
Furfural	-	-	-	-	24.099	0.274	-	-
Gamma Terpinene	13.052	0.140	-	-	13.103	0.300	-	-
Gamma-gurjunene	-	-	-	-	24.68	0.031	-	-
Gamma-Terpineol	-	-	-	-	62.436	0.166	-	-
Germacrene-d	-	-	30.668	0.025	-	-	-	-
Guaiacol	-	-	-	-	45.48	1.397	-	-
Indan, 2-butyl-5-hexyl	-	-	-	-	90.098	0.324	-	-
Inden	-	-	-	-	24.932	0.055	-	-
Isoborneol	-	-	-	-	-	-	-	-
Isoeugenol	-	-	-	-	68.225	1.191	68.301	0.321
Isolongifolene	-	-	29.355	0.419	-	-	-	-
Izal	-	-	-	-	53.053	0.327	-	-
Junipene	-	-	-	-	29.42	1.379	-	-
Limonene	11.247	5.836	11.279	3.338	11.365 8.16	9.915 0.317	-	-
Linalool	28.543	0.055	-	-	-	-	-	-
Longicyclene	-	-	-	-	25.614	0.190	-	-
Longipinene	-	-	23.917	0.059	-	-	-	-
Methanone,(4-nitrophenyl)(4-methylphenyl)	-	-	-	-	72.882	0.358	-	-
Methyl abietate	81.695	0.519	-	-	-	-	-	-
Muurolene	-	-	37.973	0.053	-	-	-	-
Myrtenal	-	-	32.763	0.090	-	-	-	-
Myrtenol	-	-	41.794	0.270	-	-	-	-
Naphtalene, 1-methyl-7-(1-methylethyl)	-	-	-	-	58.224	0.296	-	-
Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1(1-methylethyl)-, (1.alpha.,4a.alpha.,8a.alpha.)-	-	-	-	-	36.045	0.370	-	-
Naphthalene, 6-ethyl-1,2,3,4-tetrahydro-1,1,4,4-tetramethyl-7-(1-methylethyl)-	-	-	-	-	76.274	0.332	-	-
Norbornane, 2-isopropylidene	-	-	-	-	8.013	0.951	-	-
o-Cymene	14.122	0.565	-	-	-	-	-	-
p-Cresol	-	-	-	-	57.034	0.334	-	-

p-Cymene	-	-	14.131	0.298	14.055	0.434	-	-
p-Cymene-8-ol	-	-	44.884	0.086	-	-	-	-
p-Ethylguaiacol	-	-	-	-	53.882	2.507	-	-
Phellandrene	10.077	0.221	-	-	-	-	-	-
Phenanthrene, 3,6-dimethy	76.069	0.847	-	-	-	-	-	-
Phenol, 2,6-dimethyl-	-	-	-	-	48.177	0.153	-	-
Phenol, 2-ethyl-4,5-dimethyl-	-	-	44.656	0.023	-	-	-	-
Phenol, 2-ethyl-5-methyl	-	-	-	-	60.499	0.162	-	-
Phenol, 3,5-dimethyl-	-	-	-	-	60.922	0.537	-	-
Phenol, 3-ethyl-5-methyl	-	-	-	-	46.17	0.165	-	-
Phenyl 3-(2-methylthio)-thienyl sulfide	63.191	1.130	-	-	-	-	-	-
Pinocarveol	-	-	34.355	0.303	-	-	-	-
Podocarp-8-en-15-oic acid, 13.beta.-methyl-13-vinyl-, methyl ester	-	-	-	-	83.381	0.754	-	-
Prehnitol	-	-	-	-	22.35	0.326	-	-
Propylene glycol	31.232	2.939	-	-	-	-	31.224	1.814
Propylguaiacol	-	-	-	-	57.574	0.877	-	-
Sabinene	11.594 8.76	0.443 0.358	11.614	0.433	-	-	-	-
Sclaren	-	-	-	-	84.462	0.521	-	-
Selin-4,7(11)-diene	33.326	0.058	-	-	-	-	-	-
Simonellite	-	-	-	-	79.832	0.762	-	-
Trans Beta Caryophyllene	31.048	10.152	30.970	0.090	-	-	-	-
Trans-(+)-carveol	-	-	44.095	0.044	-	-	-	-
Trans-2-caren-4-ol	-	-	37.768	0.028	-	-	-	-
Trans-Caryophyllene	33.584	0.032	-	-	31.032	0.695	-	-
Trans-Isolimonene	-	-	-	-	13.00	0.055	-	-
Verbenol	-	-	35.683	0.820	-	-	-	-
Ylangene	-	-	41.567	0.022	-	-	-	-
Z-Citral	-	-	26.882	0.027	-	-	-	-

RT: Retentime time (min). -: compounds not detected.

DISCUSSION

Antibiotics saved countless lives around the world with the discovery of penicillin by Sir Alexander Fleming in 1928. However, along with life-saving antibiotics, life-threatening antibiotic resistance has also emerged. Therefore, the current study will offer a clue for plant-based antimicrobial discovery against the global threat of antimicrobial resistance. (Asmerom et al., 2020).

Recently, the rate of systemic fungal infections caused by *Candida* species has increased due to antifungal resistance, especially in immunocompromised hosts (AIDS) and haematological and cancer diseases. Topical and systemic antifungal agents, which are cytotoxic and fungistatic, are used in the treatment of fungal infections. Thus, the antifungal drugs used by the patient to regain his health cause more side effects. Moreover, there is information in the literature about which plant is used for which disease in traditional medicine. For this reason, there is an increasing trend towards herbs and herbal medicines as viable alternatives to synthetic antifungal drugs (Mansourian et al., 2014). Therefore, this study evaluated the anticandidal activity of commercially available plant extracts, fixed and essential oils against *C. albicans* vaginal isolates.

Clove *(Syzygium aromaticum* (L.) Merril. & Perry, syn. *Eugenia aromaticum* or *E. caryophyllata*), a member of the Myrtaceae family, is one of the oldest and most valuable spices of the Orient. It is used in medicine, especially because of its antiseptic and antimicrobial properties. It has also been determined to have antiparasitic, antiviral, antioxidant, antimutagenic, anti-inflammatory and antithrombotic properties. It is also used orally for asthma and various allergic disorders. Sesquiterpenes found in cloves have also been investigated as potential anti-carcinogenic substances (Ranasinghe et al., 2002; Pinto et al., 2009; Rana et al., 2011; Mansourian et al., 2014; Mittal et al., 2014). Clove oil contains high levels of eugenol, and it has been determined that this compound has antifungal activity (Ranasinghe et al., 2002; Mansourian et al., 2014). Various studies have examined clove oils' antifungal activity and volatile component composition (Ranasinghe et al., 2002; Pinto et al., 2009; Mansourian et al., 2014; Mittal et al., 2014). Mansourian et al. (2014) evaluated the antifungal activity of *S. aromaticum* methanol extract against 21 oral *C. albicans* isolates and *C. albicans* ATCC 10231 standard culture isolated from the mouth of patients with prosthetic stomatitis who were referred to the Faculty of Dentistry Tehran University, by agar well diffusion method. They used nystatin as a positive control and methanol as a negative control. They found that the zone of inhibition was 29.62 ± 1.28 mm for the clinical isolate, 29.67 ± 0.58 mm for the standard culture, and 28.48 ± 1.17 and 28.67 ± 0.58 mm for the positive control, respectively. In this study, it was determined that it gave an inhibition zone between 16.99 and 30.68 mm for clinical isolates and 13.83 mm for standard culture. Pinto et al. (2009) were investigated the composition and antifungal activity of EO obtained from *S. aromaticum*. They determined that EO contained 85.3% eugenol by GC-MS. They evaluated antifungal activity against four standard cultures (*C. albicans* ATCC 10231, *C. krusei* ATCC 6258, *C.*

parapsilosis ATCC 90018, and *C. tropicalis* ATCC 13803) and five clinical isolates (*C. albicans* D1, *C. albicans* D5, *C. glabrata* D10R, *C. krusei* D39, and *C. tropicalis* D42). They determined that the MIC value was 64 µL mL^{-1} (v v^{-1}) against all tested isolates except for *C. parapsilosis* ATCC 90018 (0.32-0.64 µL mL^{-1}). They determined that the MFC value was 0.64 µL mL^{-1} for *C. krusei* ATCC 6258, *C. tropicalis* ATCC 13803, *C. albicans* D5, *C. krusei* D39, and *C. tropicalis* D42, and 0.64-1.25 µL mL^{-1} for other yeasts. Yassin et al. (2020) evaluated the anticandidal activity of *S. aromaticum* ethyl acetate extract against *C. albicans, C. glabrata* and *C. tropicalis* vaginal isolates. They determined that the ethyl acetate extract gave 20.9, 14.9 and 30.7 mm inhibition zones for *C. albicans, C. glabrata* and *C. tropicalis*, respectively, and contained 58.88% eugenol. In this study, it was determined that commercial *E. caryophyllata* EO contains 91.01% eugenol, its MIC value is 0.25 µL mL^{-1}, and its MFC value is 0.5 µL mL^{-1}. This study determined the MFC MIC^{-1} value was 2, and they were accepted as fungicidal agents (Gatsing et al., 2009; Snoussi et al., 2018; Mseddi et al., 2020). MIC and MFC values were lower than in other studies due to the high eugenol content of commercial *E. caryophyllata* EO and the fact that it was an effective compound for antifungal activity. It should be noted that clove oil may have toxic or irritating properties; however, despite these harmful properties, it has a wide range of uses (Hammer et al., 1999; Mansourian et al., 2014). As a result, it was determined that the higher the eugenol, the volatile component of *E. caryophyllata* EO, the higher its antifungal activity. *E. caryophyllata* EO with high eugenol content appear as an important therapeutic alternative to antifungal drugs. These results should be determined and grounded, especially by further in vivo studies.

Pinus sp. species belonging to the Pinaceae family is the most dominant conifer tree in Turkey, and there are five species in Turkey: *P. brutia* Ten., *P. nigra* Arn., *P. sylvestris* L., *P. pinea* L. and *P. halepensis* Mill. The term "turpentine essential oil", which refers to the so-called "terpene oil", "turpentine spirits", "pine terpene", "gum turpentine", "turpentine from Bordeaux" or "pine oleoresin", is obtained by hydrodistillation of gum pine. Due to its pleasant smell, turpentine is used in several industries (pharmaceuticals, perfumes, and other chemicals industries such as household cleaning products, paints, pesticides, rubber, varnishes, etc.). Turpentine is used for the traditional treatment of respiratory system and urinary tract diseases, back pain, and stomach and dermatological conditions (Gülçin et al., 2003; Tümen & Reunanen, 2010). Hassan and Amjid (2009) determined the volatile components of the EO of *P. roxburghaii* stems collected from the Lahore region of Punjab province by GC-MS. They identified 17 components in the EO of *P. roxburghaii* stems. The main component in the EO is α-pinene (41.9%), followed by 3-carene (16.3%), caryophyllene (12.3%), p-cymene (1.9%), terpinenol (1.8%), limonene (1.7%), borneol acetate (1.1%), caryophyllene oxide (1.0%), camphene (0.9%), tepinyl acetate (0.8%), ß-phallenderene (0.7%), farnecene (0.6%), o-cymene (0.4%), butanoic acid (0.3%), 3-methyl-, 2-phenylethyl ester (0.3%), 1-terpinene- 4-ol (0.2%), farnesyle acetate (0.2%) and γ-terpinene (0.2%). They determined that *P. roxburghaii* EO has antifungal activity against *Aspergillus flavus, Aspergillus terreus*, and *Trichoderma viride*. Tümen and Reunanen (2010) identified the components obtained by hydrodistillation of turpentine oil from oleoresin samples collected from *P. sylvestris* L. trees in three different locations in Denizli (Acıpayam, Çal, and Çamlıbel), Turkey by GC-MS. They identified fifty-four components from turpentine oil, accounting for about 96.2% to 98.2% of the total. They determined that the main components of turpentine oil are α-pinene, ß-pinene, camphene, longifoline, delta-3-carene, limonene, and ß-caryophyllene. Ghaffari et al. (2019) determined the chemical composition of essential oil obtained from different aerial parts of *P. eldarica* from Tabriz, Iran, in June 2018. They determined that the chemical components of EO obtained from the needles of the tree are D-germacrene (18.17%), caryophyllene (15.42%), and γ-terpinene (12.96%), and ß-pinene (10.62%). They determined that the chemical components of EO obtained from the bark are limonene (16.99%), caryophyllene oxide (13.22%), and drimenol (13.2%). Finally, they stated that the chemical components of EO obtained from pollen are α-pinene (25.64%) and limonene (19.94%). In total, 83 components were characterized in EOs using GC-MS analysis; mainly, they found sesquiterpene hydrocarbons in needle EOs and monoterpene hydrocarbons in pollen and bark EOs. They determined that ß-Pinene, ß-myrcene, limonene, and caryophyllene were present in all three plant parts. They determined that the MIC value of *P. eldarica* bark against *C. albicans* of EO was 125 µg mL^{-1}. Kurti et al. (2019) identified 112 compounds in their chemical profile of total and fragmented EOs of *P. heldreichii, P. peuce, P. mugo, P. nigra*, and *P. sylvestris*. In this study, 38 components of pine turpentine EO (48) and 117 components of pine tar FO (57) were identified. The main components of pine turpentine EO (48) were determined to be α-pinene (73.261%), ß-pinene (12.111%), limonene (3.338%), camphene (3.152%), and delta-3-carene (3.123%). The main components of pine tar FO were found to be limonene (9.915%), α-pinene (7.65%), delta-3-carene (5.364%), and α-terpineol (4.269%). Compared to the literature, the main components in the studies are similar, but their ratios and number of compounds

vary. This is due to the difference in species and the differences in the way the EO is obtained. Therefore, different results were obtained in antifungal activity as the chemical composition changed. This study determined that the MIC value of commercial pine turpentine EO (48) varied between 0.5 - 2 µL mL^{-1}, and the MFC value varied between 1 - 2 µL mL^{-1}. MFC MIC^{-1} value was determined as 1-2, and fungicidal agents were accepted when the MFC MIC^{-1} ratio was ≤ 4. In pine tar FO (57), the MIC value was determined to be below <0.125 µL mL^{-1}. Therefore, further studies are needed.

E. angustifolia L., a member of the Elaeagnaceae family, which includes three genera and about 50 species, can grow in different habitats (such as Eurasia, Australia, North America and Malaysia). The leaves of *E. angustifolia* L. are used as tea, animal feed, and wood pulp, while the fruits are consumed fresh or as jam and beverage. Due to their high therapeutic potential bioactive content, its leaves and flowers have been used to treat various diseases such as asthma, flatulence, jaundice, nausea, stomach upsets and vomiting. Medicines were prepared and applied from *E. angustifolia* L. for treating stomach ailments (such as ulcers and stomach pain) in Turkish folk medicine. The fruit has been used as an analgesic agent to reduce pain in rheumatoid arthritis in Iran. It has been reported recently that the fruit and fruit seeds of *E. angustifolia* have muscle relaxant activity and antibacterial, anti-inflammatory, and antinociceptive effects (Incilay, 2014; Hamidpour et al., 2019). Okmen and Türkcan (2014) investigated the antimicrobial activity of methanol extracts of *E. angustifolia* plant samples collected at Muğla Sıtkı Koçman University Campus in July 2012. They reported that did not give an inhibition zone against *C. albicans* RSKK 02029. Incılay (2014) evaluated the antimicrobial activity of *E. angustifolia* flower, fruit, fruit peel, and leaf collected from the Malatya region. It was determined that the extracts from flower and leaf methanol: water (80:20) had the highest antimicrobial activity against Gram-positive bacteria, and their MIC values ranged from 62.5 to 500 µg mL^{-1}. Monjazeb Marvdashti et al. (2023) determined that the MIC and MFC values of *E. angustifolia* L. whole fruit ethanolic extract against *C. albicans* PTCC 5027 were 2 and 4 mg mL^{-1}, respectively. This study determined that *E. angustifolia* EO gave inhibition zones between 7.02 and 11.35 mm. The chemical composition, MIC, and MFC values of this EO were not determined, since the study was carried out, especially with those that gave high inhibition zones.

CONCLUSION

Considering the current and still growing problem of drug resistance, the antimicrobial properties of plant extract, fixed, and essential oils can be considered valuable resources. While an in-depth analysis of the mechanisms of action may be useful in the search for new therapeutic molecules (i.e., the only active ingredient found in the essential oil), synergistic mechanisms between the components of essential oils are known to be necessary. Consequently, as the present work is preliminary, it will lead to the discovery of bioactive compounds that will fuel the future field of plant-based antimicrobial discovery and development. In line with the results of this study, it can be concluded that important therapeutic agents are as effective as alternatives to anticandidal agents. However, safety and toxicity issues will need to be addressed if these plant extracts are used for medicinal purposes. Therefore, further research on the isolation and characterization of bioactive compounds is necessary. All the results should be evaluated together, and the basis for further in vivo studies should be established.

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Author Contributions

GÖA; conceived and designed the experiments, performed the experiments, analyzed data and SK; contributed vaginal candida isolates. GÖA and SK; writing—review, and editing. All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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Pimpinella major (Apiaceae); a New Record for the Flora of Türkiye and Contributions to Its Taxonomy

Lütfi BEHÇET[1], Hikmet CENGİZ[2]✉
[1]Bingöl University, Faculty of Science and Art, Department of Molecular Biology and Genetics, Bingöl, TÜRKİYE, [2]Bingöl University, Institute of Science, Bingöl, Department of Biology, Bingöl, TÜRKİYE
[1]https://orcid.org/0000-0001-8334-7816, [2]https://orcid.org/0000-0001-6788-0012
✉: cenghikmet@hotmail.com

ABSTRACT
In this study, the *Pimpinella major* (L.) Huds. (Apiaceae), which is distributed in Europe and mostly in the European part of Russia, recorded for the first time in Bingöl (Türkiye). The new record close to *P. saxifraga*, whose distribution is also known in Turkey; it differs from it in that its stem is hollow and angular-deeply furrowed (sulcate) and its ripe dried fruit is distinctly ridged. In addition, the characteristics of *P. major* samples collected from Bingöl were compared with the known diagnostic features of this species and some differences (variations) were emphasized.

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ÖZET
Bu çalışmada, Avrupa'da ve çoğunlukla Rusya'nın Avrupa kısmında yayılış gösteren *Pimpinella major* (L.) Huds. (Apiaceae) türü ilk kez Bingöl (Türkiye)'den kaydedilmiştir. Türkiye'de de dağılımı bilinen *P. saxifraga*'ya yakın olan bu tür; gövdesinin içi boş, köşeli-derin oluklu (sulkat) olması ve olgun kuru meyvelerinin belirgin çıkıntılara sahip olması ile ondan farklıdır. Ayrıca Bingöl'den toplanan *P. major* örneklerinin özellikleri bu türün bilinen tanısal özellikleri ile karşılaştırılmış ve bazı farklılıklar (varyasyonlar) üzerinde durulmuştur.

Botanik

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Anahtar Kelimeler
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INTRODUCTION
Some parts of the world have been studied in detail from a floristic point of view, and the flora of these areas has been revealed in full or with little incompleteness (Mirek et al. 2015). Such well-studied countries are generally not very wide and the land is not very rugged. Türkiye has a very rich flora due to its geographical location, climatic diversity and rugged terrain. However, it cannot be said that this diversity of Turkey has been fully determined today. The publication of many new vascular plant taxa and new records from Türkiye every year is proof of this claim.

Bingöl can be evaluated among the provinces whose flora is well known, with recent floristic studies (Behçet & İlçim 2015; Doğan et al. 2015; Duran et al. 2015; Çinbilgel et al. 2016; İlçim & Behçet 2016; Behçet et al. 2017; Behçet & Yapar 2020, 2021; Hamzaoğlu et al. 2020; Sinan et al. 2021), a new record for Turkey (Pınar et al. 2018) and an interesting distribution of a lost endemic taxon (Behçet & Yapar

2019) have been published. These publications reveal that the floristic richness of Bingöl is known enough well.

During the floristic researches, the second author collected interesting Apiaceae specimens from the surroundings of Güzgülü village (Yedisu, Bingöl/Türkiye). These specimens could not be identified using the Flora of Türkiye account (Matthews 1972; Davis et al. 1988; Güner et al. 2000). Eventually, specimens were identified as *Pimpinella major* (L.) Huds. using the generic account in Flora Europaea volume 4 (Tutin 1968) and Flora of the USSR volume 16 (Shishkin 1973), it was a new record for the Flora of Türkiye.

As a result of the changing ecology, plant species can show some morphological adaptations according to their environment (Table 1). This Apiaceae member, whose distribution was collected from a location far away from previously known regions (in eastern Türkiye) (Figure 1), also shows some variations due to the changing ecology.

Table 1. Comparison of some morphological features of *Pimpinella major* collected from Bingöl with the descriptions of this species in Russian and European floras and *P. saxifraga* features

Çizelge 1. Bingöl'den toplanan Pimpinella major'un bazı morfolojik özelliklerinin Rusya ve Avrupa floralarında yer alan bu türün tanımları ile ve P. saxifraga özellikleri ile karşılaştırılması

Characters	Flora of the USSR (Shishkin 1973)	Flora Europaea (Tutin 1968)	Examples examined in this study	*Pimpinella saxifraga* (Shishkin 1973; Tutin 1968; Matthews 1972)
Stem	40 - 100 cm high, straight, hollow, deeply furrowed, glabrous, with clusters of leaves at base	up to 100 cm, glabrous or rarely puberulent, deeply sulcate (very rarely terete), hollow, branched above	**80 – 140 cm** high, sulcate, hollow, **the lower parts retrorsely puberulent, above parts glabrous**	**15-60 cm, cylindrical, almost or quite solid usually terete, thinly ribbed**
Lower leaves	Lower leaves petioled, simple-pinnate, with **2-4** pairs of ovate or oblong, **2.5-7 x 1-4 cm,** cuneate acute or acuminate, rounded or slightly cordate, unequally acute- or incised-dentate **leaflets, with scabrous margins**	Lower leaves 1(-3)-pinnate with **3-9 segments**; **segments up to 100 mm long**, ovate or oblong, dentate, rarely pinnatisect	**Basal leaves 25 - 60 cm long (including petiol)**, 1-pinnat; **petiole 1,5-3 cm dilated at the base and retrorsely puberulent,11 – 41 cm long, with 7-11 segments; segments up to 65 x 50 mm,** ovate, depply dentate, lobed, rarely pinnatisect, **sparsely hairy on lower surface, glabrous above;** rounded, slightly cordate or cuneate at the base,	with rosette of radical leaves leafy only in lower part, basal leaves 5-20 cm long, almost leafless above
Cauline leaves		Cauline leaves smaller, with inflated, sheath-like petioles with membranous margins	**Lower and median cauline leaves 12-36 cm long,** 1-2 pinnate, with 9-13 segments; segments upto 45x35 mm, pinnatisected, and **lowest segments with 2-4 mm long petiolule; petiole 25- 105 mm long, 20-35 mm long part of the petiole base has a sheath (white scarious edge) structure**	Cauline leaves much reduced, median cauline leaves with cuneate leaflets more deeply cut into narrow lobes at base, nearly pinnate, sessile on sheaths;
Upper leaves				
Uppermost leaves	**Upper leaves sessile, terminal leaflets 3-lobed or 3-partite, median and upper cauline leaves sessile on dilated sheath with white scarious margin,**	Pinnately divided, lobes few	**Upper cauline leaves** sessile on dilated sheath , with narrower , more	upper leaves with simple pinnate or 3-partite small blade with lanceolate or sublinear lobes;

	leaflets narrower, more deeply dissected uppermost leaves small, trifid or obsolete		deeply dissected, trisect **Uppermost leaves** small, trifide, sessile on inflated sheath 10-20 mm long	uppermost leaves with reduced blad
Bracts and bracteoles	Involucre (bracts) and involucels (bracteols) usually absent	Bracts absent; bracteoles usually absent, rarely few, caducous	Bracts – bracteoles absent, or rarely bract 1	
Ray numbers in an umbel, ray length and indumentum, per umbellule flower number	**9—15**, thin, **glabrous**	**10-25**, slender.	**13-31**, 1.5-3.5 cm long, glabrous **or sparsely hairy**, **19-24 flowered per umbellule**	**5-25,** thin,glabrous, **10-20 flowered per umbellule**
Petal	white or **pink,** the outer ca.1.4 mm long	white to **deep pink**	**white**, 1-1.5 x 0.9-1.1 mm, **puberulent or with pointed papillae on back**	white, rarely pink, ca. 1 mm long, bristly-hairy on back,
Fruits	Fruit glabrous, oblong-ovoid, 2.5-3.5 x 1.5-2 mm, dorsal ribs prominent, styles 1.5-2 mm long	Fruit 2.5-3.5 mm long, ovoid-oblong; ridges prominent, whitish	**2.3-3** x 1.5-2.1 mm, **oblong-ovoid**, glabrous, ridges prominent, whitish (Figure 5)**, styles 1.5-1.6 mm long**	2 —2.5 x 1.5-2 mm **brodly ovoid, ridges inconspicuous, styles not exceeding 1 mm**
Stamen	--	--	Anther 1-1.2 mm long, filament 3 mm long	

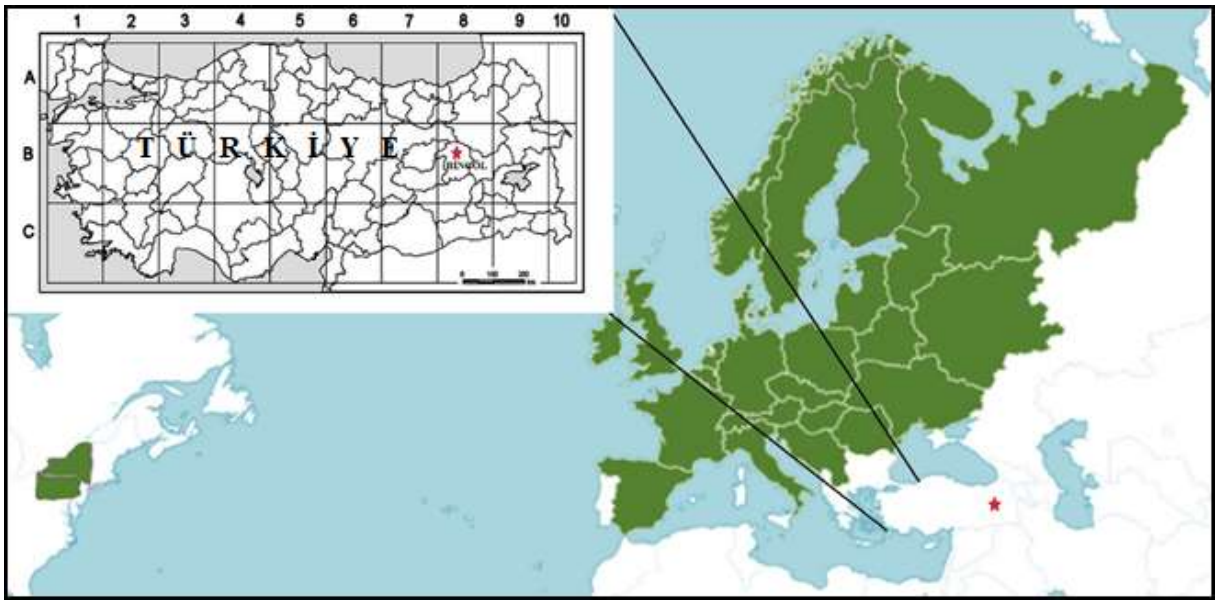

Figure 1. Distribution map of *Pimpinella major* in the World (■) (from POWO 2022) and Türkiye (★)
Şekil 1. Pimpinella major'un Dünya (■) ve Türkiye'deki (★)yayılış haritası (POWO 2022'den)

The genus *Pimpinella* L. (Apiaceae) which contains approximately 150-170 species in the world, has 32 taxa (22 species, 5 subspecies and 5 varieties) distributed in Türkiye and 8 of them are endemic (Pimenov & Leonov 1993; Pu & Watson 2005; Menemen 2012; Fırat 2019). With this new rerecord, the number of *Pimpinella* members has increased to

33. The habitats of the members of the genus are variable; there are members that can develop in different habitats from arid rocky areas to moist-aquatic places (Tutin 1968; Matthews 1972; Pignatti 1982; Hartvig 1986; Velayos 2003; Menemen 2012; Yeşil et al. 2016). In this study, *Pimpinella major* is reported as a newly founded taxon Türkiye. In addition, we provide its description of morphological characters, illustrations and photographs of *P. major* and a key to allied taxa in Türkiye.

MATERIALS and METHODS

Specimens of *Pimpinella major* (Figure 2-5) were collected from Yedisu (Bingöl/Türkiye) (Figure 1) in June 2020 and June 2021. The specimens were identified using keys to the genus *Pimpinella* in volume 4 of Flora Europaea (Tutin 1968) and volume 16 of Flora of the USSR (Shishkin 1973). A detailed morphological study of *P. major* was undertaken based on fresh and dried material. The morphological characteristics of *P. major* with the descriptions given in the floras of Europe (Tutin 1968) and the USSR (Shishkin 1973) were compared with the characteristics of the samples collected from Bingöl and some differences were revealed. Collected materials are deposited at the Herbarium of Bingöl University, Arts and Science Faculty (BIN) and ANK.

RESULTS and DISCUSSION

Pimpinella major (L.) Hudson Fl. Angl. ed. 1 (1762) 110; Mill. Gard. Diet, ed. VIII (1768) No.l; Wolff in Engl. Pflanzenr. IV, 228(1927)289. (Figure 2-5).

Syn: =*Pimpinella saxifraga* var. *major* L. Sp. pi. (1753) 264. =*P. magna* L. Mant. 11(1771)217; Ldb. Fl. Ross. II, 254; Shmal'g., Fl. I, 393. =*P. austriaca* Mill. Gard. Diet. ed. VIII (1768) No. 5. =*P. orientalis* Gouan Illustr. (1773) 21.=*P. media* Weber in Wigg. Prim. Fl. Holsat. (1782) 26.- =*P. angustifolia* Gilib. Fl. lithuan. 11(1782)42. =*P. rubra* Hoppe et Schleich. ex Spreng. in Schult. Syst. Veg. VI (1 820) 384. =*P. tenuifolia* Schwaegr. et Koerte ex Steud. Nomencl. ed. II, 2 (1841) 335. =*P. rugosa* Kunze in Flora, XXIX (1846) 654. ≡*Tragoselinum majus* Lam. Fl. Franc. 111(1778)448. = *T. magnum* Moench, Meth. (1794)99. =*Carum magnum* Baill. Hist. PI. VII (1 880) 178. =*Apium pimpinella* Car. in Pari. Fl. ital. VIII (1 889) 452. - Ic: Rchb. fil. XVII, tab. 27.- Exs.: G.R.F. No. 2634; PI. Finl. exs. No. 830; E. Woloszczak, Fl. polon. exs. No. 728.

Figure 2. *Pimpinella major* **a**- habit, **b**-root, **c**- close-up view of the stem, **d**- appearance of hollow and sulcate structure in the cross section of the stem

Şekil 2. Pimpinella major a- genel görünüm, b-kök, c- gövdenin yakından görünümü, d- gövdenin enine kesitinde içi boş ve oluk yapının görünümü

Figure 3. Basal (A), lower (B) and median cauline (C,D) leaves view of *Pimpinella major* collected from Türkiye
Şekil 3. Türkiyeden toplanan Pimpinella major'un Bazal (A), alt (B) ve orta gövde yapraklarının (C,D) görünümü

Description: Perennial. **Root** fusiform. **Stem** 40–140 cm high, hollow, deeply sulcate, retrorsely puberulent (glabrous at above), branched above, with clusters of leaves at base. **Basal leaves** 25 – 60 cm long (incl. petiole), 1(–3) pinnate; with 3–11 leaflets; leaflets up to 65 x 50 mm, ovate, deeply dentate, lobed, rarely pinnatisect, rounded, slightly cordate or cuneate at the base, sparsely hairy on the lower surface, glabrous above and with scabrous margin; petiole 11 – 41 cm long, 1.5-3 cm dilated at the base and retrorsely puberulent. **Lower cauline leaves** 12–36 cm long (incl. petiole and sheath), 1–2 pinnate, with 9–13 segments; segments up to 45 x 35 mm, pinnatisect, and lowest segments with 2–4 mm long petiolule, the 20–35 mm part of the 25–105 mm long petiole has formed a sheath (with scarious white edges). **Median and upper cauline leaves** sessile on dilated sheath with white scarious margin, leaflets narrower, more deeply dissected. **Uppermost leaves** small, trifid or obsolete, terminal leaflets small, 3-lobed or 3–partite, sessile on the inflated sheath 10–20 mm long. **Umbels** of 9–31 thin glabrous or sparsely hairy rays; rays 1.5-3.5 cm long, subequal, per ray 19-24 flowered, bract and bracteole usually absent, rarely bract one. **Pedicels** scabrid, 4–6 mm long. **Petals** white to deep pink, puberulent or with pointed papillae on back (the specimens in Türkiye are white and puberulent), the outer 1–1.5 x 0.9–1.1 mm. **Anthers** 1–1.2 mm and filament 3 mm long. **Fruit** oblong-ovoid, glabrous, 2.3–3.5 x 1.5–2.1 mm, dorsal ribs protruding, canals 4 under valleculae, 4 toward commissure, styles 1.4–1.6 mm long, stylopodium mamillate-depressed. 2n= 20.

Flowering: June–July, **Fruiting:** June–August

Distribution: Europea and Türkiye (POWO 2022) (Figure 1).

Type: 373.14 (LINN, lectotype, designated here by J.-P. Reduron) (Jonsell & Jarvis 2002).

Specimens examined: Türkiye B8 square, Bingöl: Yedisu town, Güzgülü village, stream sides- damp slopes 1518 m, 39°25'36.50"N, 40°29'15.98"E, 24.06.2020, *Hikmet Cengiz* 2860; ibid, 30.06.2020, *Hikmet Cengiz* 2915; ibid, 22.06.2021, *Hikmet Cengiz* 3719.

Ecology: Specimens of this new record for Türkiye were collected by the second author from the humid slopes 1500-1600 meters around Güzgülü village of Yedisu town of Bingöl. The climax vegetation of Güzgülü village and its surroundings is composed of oak forests (*Quercus petraea* (Matt.) Liebl. subsp. *pinnatiloba* (K.Koch) Menitsky and *Q. libani*

Figure 4. Views of leaf morphology and change in size from the lower part to the tip (1-8) of the stem of *Pimpinella major*

Şekil 4. Pimpinella major gövdesinin alt kısmından yukarıya (1-8) doğru yaprak morfolojisi ve boyut değişiminin görünümleri

G.Olivier taxa are the dominant). Oak species in places; woody shrub members of *Crataegus* L, *Lonicera* L., *Sorbus* L., *Rosa* L. genera are included. The covers of cultivated plants such as *Salix alba* L. and *Populus alba* L. also draw attention along the streams. Taxa such as *Atriplex laevis* Ledeb., *Bidens tripartita* L., *Bunium simplex* (K.Koch) Klyuikov, *Epilobium hirsutum* L., *Eremurus spectabilis* M.Bieb., *Chaerophyllum bulbosum* L., *Cirsium macrobotrys* (K.Koch) Boiss., *Cucubalus baccifer* L., *Gentiana cruciata* L., *Geranium divaricatum* Ehrh., *Inula salicina* L., *Juncus atratus* Krock., *Lepidium latifolium* L., *Lathyrus pratensis* L., *L. rotundifolius* Willd. subsp. *miniatus* (M.Bieb. ex Steven) P.H.Davis, *Lithospermum arvense* L., *Lycopus exaltatus* L. , *Medicago lupulina* L., *Melissa officinalis* L. subsp. o*fficinalis, Pastinaca sativa* L. subsp. *urens* (Req. Ex Gren. & Godr.) Čelak., *Phleum pratense* L., *P. tuberosa* L., *Poa trivialis* L., *Polygonatum orientale* Desf., *Potentilla recta* L., *Senecio mollis* Willd., *Sium sisarum* L. var. *lancifolium* (M.Bieb.) Thell., *Scutellaria galericulata* L. *Tragopogon albinervis* Freyn & Sint., *Silene vulgaris* (Moench) Garcke subsp. *commutata* (Guss.) Hayek, *Stachys setifera* C.A.Mey. subsp. *lycia* (Gand.) R.Bhattacharjee, *S. spectabilis* Choisy ex DC. *Trifolium pratense* L. var. *americanum* Harz., *Verbena officinalis* L. and *Vicia sativa* L. subsp. *nigra* (L.) Ehrh. var. *nigra*, which generally prefer humid-aquatic environments, participate in important species that develop together with the *Pimpinella major*.

Pimpinella major specimens (Figure 2,5) were collected from Bingöl in June 2020-2021. This taxon has a large size compared to other *Pimpinella* taxa of Türkiye. *P. major* morphologically resembles *P. saxifraga.* However, the deeply sulcate structure on the stem of the plant and the hollow feature seen in the stem cross-sections (Fig. 2c and d) and its ripe dried fruit is distinctly ridged (Fig. 5c) confirmed the idea that this plant could not be *P. saxifraga.* Because in the *Pimpinella* keys in the relevant volumes of the European (Tutin 1968) and USSR (Shishkin 1973) floras; the flowers are white (not yellow), the fruits are glabrous and the stems are sulcate and the hollow *Pimpinella* species is *P. major*.

Figure 5. Umbel views of *Pimpinella major* in flower (A) and fruit (B) periods and ridged fruit image(C)

Şekil 5. Pimpinella major'un çiçek (A), *meyve* (B) *dönemlerine ait umbel görünümleri ve damarlı meyve (C) görüntüsü*

When the characteristics of *Pimpinella major* specimens collected from Bingöl and the descriptions given in European and Russian floras are compared; Some variations due to ecology have been detected in plant height, indumentum, leaf characteristics, number of rays and lengths and these are given in the Table 1.

Considering the leaf structures of *P. major* from the basal to the upper part of the stem, it is seen that there is a lot of variability and structural difference. This much variability in the leaf morphology of the plant we collected from Türkiye does not take place sufficiently in European and USSR floras. For example, although the basal leaves of *P. major* are up to 60 cm long (incl. petiol) and the base of the petiole is 2-3.5 cm wide (not in scarious sheath structure with white margin), the leaves on the stem have a different structure (Figure 3, Table 1). However, the caulin leaves of this species are quite different from the basal leaf in terms of both structure and size. The lower cauline leaves are smaller than the basal leaves; They both carry a petiole (non-sheath shape) and a wide scarious sheath with white margins on the lower part. The leaflets in the basal leaf do not have petiolules; they are less dissected (Figure 3).

When the leaf structure features from the lower parts of the plant stem to the top are examined, there is a significant reduction in size from the bottom to the top; there are also quite a lot of variations in structural features (Table 1). While the leaves in the lower and middle parts of the stem carry both sheath and petiole; the leaves above bear sheath only (Figure 3,4). The leaflets on the lower leaves are less dissected than the leaflets on the upper leaves, and the number of segments is larger. In addition, the petiolule structure, which is not seen in the leaflets of basal leaves, is generally evident in cauline leaves.

Although the definition of this taxon prepared according to the samples collected from the distribution areas of *Pimpinella major* outside Türkiye (such as Europe and Russia) and the P*. major* samples collected from Bingöl show some differences (Table 1); In addition to its fruit structure dimensions, it also complies with the aforementioned definitions with its hollow and sulcate stems.

Today, the genus *Pimpinella* is represented in Türkiye by a total of 33 taxa (with the addition of *P. major*), 23 species (8 of which are endemic to Türkiye), 5 subspecies and 5 varieties.

Recommended diagnostic key:

- Stem deeply sulcate, hollow, up to 140 cm long, ridges of fruit prominent, styles 1.5-1.6 mm long…… ***P. major***

- Stem cylindrical, thinly ribbed, almost or quite solid, 15-60 cm long, ridges of fruit inconspicuous, styles not exceeding 1 mm …….. ***P. saxifraga***

Author's Contributions

The contribution of the authors is equal.

Statement of Conflict of Interest

Authors have declared no conflict of interest.

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Mersin İlindeki (Türkiye) Bazı Doğal ve Kültürel Sit Alanlarının Floristik Yapısı Üzerine Bir Çalışma

Ahmet Zafer TEL[1], İshak ORTAÇ[2], Ahmet İLÇİM[3], Ergün ÖZUSLU[4]✉
[1]Iğdır Üniversitesi, Ziraat Fakültesi, Tarımsal Biyoteknoloji Bölümü, Iğdır, Türkiye, [2]Adıyaman Çocuk Evleri Sitesi Müdürlüğü, Adıyaman, Türkiye, [3]Mustafa Kemal Üniversitesi, Fen Edebiyat Fakültesi, Biyoloji Bölümü, Hatay, Türkiye, [4]Gaziantep İslam Bilim ve Teknoloji Üniversitesi, Mühendislik ve Doğa Bilimleri Fakültesi, Gaziantep, Türkiye
[1]https://orcid.org/0000-0002-1204-3839, [2]https://orcid.org/0000-0002-5880-8438, [3]https://orcid.org/0000-0001-8169-2472
[4]https://orcid.org/0000-0002-4480-8365
✉: ergun.ozuslu@gibtu.edu.tr

ÖZET

Bu çalışmada Mersin ili sınırları içerisinde bulunan, doğal ve kültürel sit özelliği taşıyan Aphrodisias Sit Alanı ve Tisan Mevkii, Taşucu Kalesi ve Boğsak Adası, Eğribük Koyu ve Dana Adası, Gilindere Mağarası, Aydıncık Adaları ve Beşparmak Adaları'nın florası üzerine bir envanter çalışması yapmak ve Mersin ilinin floristik ve biyolojik çeşitlilik araştırmalarına katkı sağlamak amaçlanmıştır. Mersin ili sınırlarındaki adalarda daha önce doğrudan flora tespitine yönelik çalışma yapılmamıştır. Araştırma alanları olan Mersin iline ait dört farklı bölgede toplam 26 familya ve 57 cinse ait 66 farklı takson tespit edilmiştir. Tespit edilen taksonların fitocoğrafik bölgelere göre dağılımı şöyledir: Akdeniz (Doğu Akdeniz dahil) 37 takson (%56.07), Geniş yayılışlı 11 takson (%16.66) ve bilinmeyen 18 (%27.27) taksondur. Avrupa - Sibirya ve İran - Turan coğrafik bölge elementi bulunmamaktadır. Araştırma alanında toplam 1 endemik ve 1 nadir bitki taksonu tespit edilmiştir.

Botanik

Araştırma Makalesi

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Anahtar Kelimeler
Ada
Biyolojik Çeşitlilik
Flora
Mersin
Taksonomi

A Study on the Floristic Structure of some Natural and Cultural Sites in Mersin (Türkiye)

ABSTRACT

This study was designed to make an inventory study on the flora of Aphrodisias Protected Area and Tisan Locality, Taşucu Castle and Boğsak Island, Eğribük Bay and Dana Island, Gilindere Cave, Aydıncık Islands and Beşparmak Islands, which are located within the borders of Mersin province and have the characteristics of natural and cultural sites. It was also aimed to contribute to the floristic and biodiversity researches of Mersin province. No studies have been conducted for the direct determination of flora on the islands within the borders of Mersin province before. A total of 66 different taxa belonging to 26 families and 57 genera were identified in four different regions of Mersin province, which was the research area. The distribution of detected taxa according to phytogeographic regions was as follows: Mediterranean (including Eastern Mediterranean) 37 taxa (56.07%), 11 widely distributed taxa (16.66%) and 18 unknown (27.27%) taxa. There was no Europe - Siberia and Iran - Turan geographic region element. A total of 1 endemic and 1 rare plant taxon were identified in the research area.

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Mersin
Taxonomy

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GİRİŞ

Türkiye Florası zengin bir bitki çeşitliliğine sahiptir. Bu çeşitlilik 1700'lü yıllardan beri araştırılmakta olup halen Türkiye Florasına yeni türler eklenmektedir. Türkiye florası üzerine ilk çalışmalar Tournefort ve Boissier tarafından yapılmış (Boissier, 1867–1884; Baytop, 2010). P.H. Davis ise 1965–1988 yılları arasında Türkiye Florası üzerine kapsamlı bir çalışma yaparak on ciltlik eseri yayımlamıştır. İkinci ek cilt

2000 yılında yayımlanmıştır (Davis, 1965-1985; Davis ve ark., 1988; Güner ve ark., 2000). Bugün Resimli Türkiye Florası'nın hazırlanması çalışmaları devam etmekte olup ilk üç cildi yayınlanmıştır (Güner ve ark., 2014; 2018; 2022). Bitki coğrafyası açısından holoarktik flora aleminin Doğu Akdeniz bölümü floristik açıdan zengin bir bölgedir (Avcı, 1993). Akdeniz fitocoğrafik bölgesi, bitki coğrafyası özellikleri, iklim ve edafik faktörler ile zengin bir bitki çeşitliliğine ve endemik bitkilere sahiptir (Güner ve ark., 2012; Balos ve ark., 2022). Doğu Akdeniz bölgesinin Batı ve Orta Toroslar flora bölgesinde yer alan Mersin ili floristik bakımdan oldukça zengin olmasına rağmen yapılan çalışmalar oldukça sınırlıdır.

Doğu Akdeniz Bölgesinde gerçekleştirilen bu araştırmada Mersin ili sınırları içerisinde bulunan, doğal ve kültürel sit özelliği taşıyan Aphrodisias Sit Alanı ve Tisan Mevkii, Taşucu Kalesi ve Boğsak Adası, Eğribük Koyu ve Dana Adası, Gilindere Mağarası çevresi, Aydıncık Adaları ve Beşparmak Adaları'nın florası üzerine bir envanter çalışması yapmak ve Mersin ilinin floristik ve biyolojik çeşitlilik araştırmalarına katkı sağlamak amaçlanmıştır.

Çalışma alanına yakın bölgelerde; Zeren ve İspirgil (2001), Orcan ve ark. (2004), Tel ve Tatlı (2004), Aksay (2006), Dinç (2008), Tel (2009), Yıldızbakan ve ark. (2010), Yıldıztugay ve Küçüködük (2010 a), Şirin (2012), Savran ve ark. (2012), Tel ve Tak (2015), Anonim (2016), Tel ve İlçim (2016), Ortaç (2017), Tel ve ark. (2018), Tel ve Tak (2018), Tel ve ark. (2019), ve Şen (2019), Tel ve ark. (2021) ve Tel ve ark. (2022a; 2022b) tarafından floristik çalışmalar yapılmıştır. Çalışma alanı olan Mersin ili sınırlarındaki adalarda daha önce doğrudan flora tespitine yönelik çalışma yapılmamıştır. Bu bakımdan yapılan bu çalışma adaların bitki örtüsünün belirlenmesi açısından önem arz etmektedir.

MATERYAL ve METOD

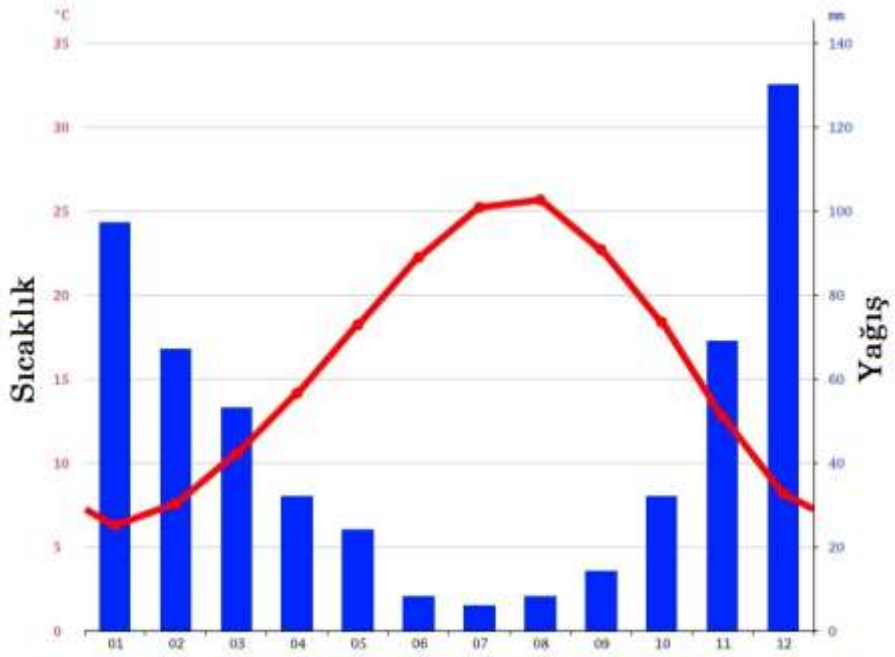
Bu araştırmanın materyalini 2014-2016 yılları arasında Mersin ilindeki doğal sit alanı olan bölgelerinden toplanan bitki örnekleri oluşturmaktadır. Alanda bitkiler gözleme dayalı olarak belirlenmiş, teşhisinde zorluk çekilen bitki taksonları kurutulmuş, herbaryum materyali haline getirilmiştir. Teşhisinde zorluk yaşanan bazı bitkilerin tayinleri arazi çalışmalarının bazılarına katılan Ergün Özuslu tarafından yapılmıştır. Örneklerin teşhis ve tayinlerinde Türkiye Florası kullanılmıştır (Davis, 1965-1985; Davis ve ark., 1988). Toplanan bitki örnekleri Adıyaman Üniversitesi Herbaryumunda bulunmaktadır. Çalışma alanında belirlenen bitkilerin listesi alfabetik olarak Ek-1'de verilmiştir. Bitki isimleri verilirken Türkiye Bitkileri Listesi (Güner ve ark., 2012), Uluslararası Bitki İsimleri İndeksi (Anonim, 2022 a), The Plant List (Anonim, 2022 b) ve www.bizimbitkiler.org (Anonim, 2022 c)'e göre kabul edilenler kullanılmıştır. Endemik ve nadir olan taksonların tehlike sınıfları belirlenmiştir (Ekim ve ark., 2000; Güner ve ark., 2012). Mersin il sınırları içerisinde bulunan çalışma alanlarında tespit edilen bitki taksonlarının toplandığı alanlar numaralandırılarak Şekil 1'de gösterilmiş ve Çizelge 1'de koordinat ve genel özellikleri verilmiştir. Buna göre: [1] Aphrodisias Sit Alanı ve Tisan Mevkii, [2] Taşucu Kalesi ve Boğsak Adası, [3] Eğribük Koyu ve Dana Adası, [4] Gilindere Mağarası, Aydıncık Adaları ve Beşparmak Adasını belirtmektedir.

Şekil 1. Çalışma alanlarının googleeart görüntüsü (Anonim, 2022 d)
Figure 1. Google earth image of workspaces (Anonymous, 2022 d)

Floristik listedeki kısaltmalar şu şekilde verilmiştir; Akd. Elm.: Akdeniz Elementi; D. Akd. Elm. : Doğu Akdeniz Elementi; Ge. Yay.: Geniş Yayılışlı; İr.-Tur. Elm.: İran Turan Elementi; Av.-Sib. Elm.: Avrupa Sibirya Elementi; End.: Endemik; EN: Tehlikede; LC: En az endişe verici; CR: Kritik; VU: Zarar görebilir; DD: Veri yetersiz; NT: Tehdit altına girebilir.

Mersin İlinin İklim Özellikleri

Çalışma alanlarının bulunduğu Mersin'de sıcaklık ortalaması 19.2 °C, yıllık yağış ortalaması 965 mm'dir. Alanda yıllık en yüksek sıcaklık ortalaması (Maksimum) 33.5 °C, en düşük sıcaklık ortalamasının ise 5.8 °C olduğu görülmüştür. Ortalama sıcaklığın en yüksek olduğu ay Ağustos olup sıcaklık değeri 44.2 °C'dir. En düşük ortalama maksimum sıcaklık ocak ayında olup 22.5 °C'dir. Mart ayının sonunda başlayan ve Ekim ayı sonuna kadar devam eden bir kurak devre gözlenmektedir. Mersin ilinin iklim diyagramı Şekil 2'de verilmiştir.



Şekil 2. Mersin ili iklim diyagramı (Anonim, 2022 e)
Figure 2. Climate diagram of Mersin province (Anonymous, 2022 e)

BULGULAR ve TARTIŞMA

Çalışmadaki araştırma alanları, Mersin ilinde bulunan, doğal ve kültürel sit özelliği taşıyan Aphrodisias Sit Alanı ve Tisan Mevkii, Taşucu Kalesi ve Boğsak Adası, Eğribük Koyu ve Dana Adası, Gilindere Mağarası, Aydıncık Adaları ve Beşparmak Adaları olmak üzere dört lokaliteyi kapsamaktadır. Çalışma alanlarının listesi ve çalışma alanlarının genel özellikleri Çizelge 1'de gösterilmiştir.

Çizelge 1. Çalışma alanları ve genel özellikleri
Table 1. Study areas and general characteristics

No *(No)*	Çalışma Alanı *(Study Area)*	Büyüklüğü (hektar) *(Size)*	Koordinatlar *(Coordinates)*
1	Aphrodisias Antik Kenti Köserelik Adası ve Tisan Mevkii	928.59	36.15634643629833; 33.689177950148846
2	Taşucu Kalesi ve Boğsak Adası	28	36.267533065896515; 33.82734384089982
3	Eğribük Koyu ve Dana Adası	260	36.18958106898156; 33.76447897233691
4	Gilindere Mağarası, Aydıncık ve Beşparmak Adaları	641.4	36.12256474590515; 33.5338958531703

Araştırma alanına yakın bölgelerde yapılan çalışmalarda; Mersin Üniversitesi Kampüs Florasının Tespiti adlı çalışmada 29 familya, 67 cins ve 75 tür bulunduğu (Zeren & İspirgil, 2001), Everest ve Rauss (2004) tarafından, Kozlar Yaylasında yapılan çalışmada 272 tür tespit edildiği, Aksay (2006) tarafından, Pusat Dağında yapılan çalışmada 37 familya, 101 cins ve 151 takson bulunduğu, Dinç (2008) tarafından, Cocakdere Vadisinde yapılan çalışmada 73 familyaya ait 282 cins ve 506 takson tespit edildiği, Yıldızbakan ve ark. (2010) tarafından, Cehennemdere Yaban Hayatı Geliştirme Sahasında yapılan çalışmada 105 familya, 1786 adet bitki türü bulunduğu, bu türlerin 428 tanesinin endemik olduğu,

Yıldıztugay ve Küçüködük (2010 a) tarafından, Anamur Antik Kenti ve çevresinde yapılan çalışmada 350 takson, 260 cins ve 74 familyaya ait 510 bitki tespit edildiği, endemizim oranının %3.7 olduğu, Yıldıztugay ve Küçüködük (2010 b) tarafından, Kaş Yaylası ve çevresinde yapılan çalışmada 73 familyaya ait 279 cins ve 470 takson bulunduğu, endemik takson sayısının 62, endemizm oranının %13.2 olduğu, Şirin (2012) tarafından Büyükeğri Dağı ve çevresinde yapılan çalışmada 46 familya, 155 cins ve 255 takson belirlendiği, endemizm oranının %21.1 olduğu, Savran ve Paksoy (2016) tarafından Gülek Boğazında yapılan çalışmada 82 familya, 370 cins ve ait 839 takson bulunduğu ve endemizm oranının %18.8 olduğu, Şen (2019) tarafından Anamur Yaylalarında yapılan çalışmada 109 familya, 390 cins, 611 takson yer aldığı ve endemizm oranının % 13.25 olduğu, Hamzaoğlu ve Koç (2019) tarafından yapılan çalışmada Mersin'den Türkiye için yeni Dianthus kaydı verildiği, Üzgör-Ün ve ark. (2021) tarafından yapılan çalışmada Asteraceae, Fabaceae ve Lamiaceae familyasından toplamda 83 cinse ait 214 takson teşhis edildiği, teşhis edilen taksonların endemizm oranının %11.57 (25 takson) olduğu, Topal ve ark. (2022) tarafından yapılan çalışmada 23 familyada 68 cinse ait 256 farklı geofit taksonu tespit edildiği belirlenmiştir.

Araştırma alanlarının bulunduğu Mersin ilinine ait dört farklı bölgede toplam 26 familya ve 57 cinse ait 66 farklı bitki taksonu tespit edilmiştir (Çizelge 8). Çalışma alanından toplam 121 bitki örneği toplanmıştır. Bu taksonların çalışma alanlarına göre dağılımı; Aphrodisias Sit Alanı ve Tisan Mevkinde 29, Taşucu Kalesi ve Boğsak Adasında 39, Eğribük Koyu ve Dana Adasında 25, Gilindere Mağarası, Aydıncık Adaları ve Beşparmak Adasında 28 takson tespit edilmiştir. Belirlenen bitkilerin bitkicorafyası bölgelerine göre dağılımı şu şekildedir: Akdeniz 37 takson (%56.07), Geniş yayılışlı 11 takson (%16.66) ve bilinmeyen 18 (%27.27) taksondur. Avrupa-Sibirya ve İran-Turan elementi bulunmamaktadır (Çizelge 2).

Çizelge 2. Taksonların fitocoğrafik bölgelere göre dağılımı

Table 2. Distribution of taxa by phytogeographic regions

Fitocoğrafik Bölge *(Phytogeographic Region)*	Takson Sayısı *(Number of Taxa)*	Oran (%) *(Ratio)*
Akdeniz	37	56.07
Avrupa - Sibirya	0	0
İran -Turan	0	0
Geniş Yayılışlı	11	16.66
Bilinmeyen	18	27.27
Toplam	66	100

Alandan toplam 1 endemik ve 1 nadir bitki taksonu tespit edilmiştir (Çizelge 3). Akdeniz elementi olan taksonların çokluğu, araştırma alanlarının tamamının bu bölgede yer almasından kaynaklanmaktadır. Araştırma alanında endemizm oranı %1.51'dir. Araştırma alanında tespit edilen endemik ve nadir takson Doğu Akdeniz fitocoğrafik bölge elementidir. Tespit edilen endemik taksonun EN (Tehlikede) ve nadir taksonun VU (Zarar görebilir) kategorisinde yer aldığı tespit edilmiştir.

Çizelge 3. Çalışma alanlarında tespit edilen familya, takson, endemik takson sayıları

Table 3. Number of families, taxa and endemic taxa detected in the study areas

	Çalışma Alanı *(Study Area)*	Familya *(Family)*	Takson *(Taxon)*	Endemik Takson *(Endemic Taxon)*	Nadir Takson *(Rare Taxon)*
1	Aphrodisias Sit Alanı ve Tisan Mevkii	19	29	-	-
2	Taşucu Kalesi ve Boğsak Adası	18	39	-	-
3	Eğribük Koyu ve Dana Adası	19	25	-	-
4	Gilindere Mağarası, Aydıncık Adaları ve Beşparmak Adasını	14	28	1	1

Çalışma alanları ayrı ayrı değerlendirildiğinde; Aphrodisias Sit Alanı ve Tisan Mevkiinde 19 familya 26 cinse ait toplam 29 takson belirlenmiştir. Taksonların fitocoğrafik bölgelere dağılımı 17 takson Akdeniz, 4 takson Geniş Yayılışlı ve 8 takson bilinmeyendir. Alanın içerdiği takson sayısına göre en büyük familyalar Lamiaceae 4, Fabaceae 3, Liliaceae 3 şeklindedir (Çizelge 4). Çalışma alanında endemik bitki tespit edilmemiştir.

Taşucu Kalesi ve Boğsak Adasında 18 familya 36 cinse ait toplam 39 takson belirlenmiştir (Çizelge 3). Taksonların fitocoğrafik bölgelere dağılımı 25 takson Akdeniz, 4 takson geniş yayılışlı ve 10 takson bilinmeyen şeklindedir. Çalışma alanında, içerdikleri tür ve türaltı takson sayısına göre en büyük üç familya sırasıyla Fabaceae 8, Liliaceae *7*, Asteraceae 5 şeklindedir (Çizelge 4). Çalışma alanında endemik bitki tespit edilmemiştir.

Eğribük Koyu ve Dana Adasında 19 familya 24 cinse ait toplam 25 takson belirlenmiştir. Taksonların fitocoğrafik bölgelere dağılımı şöyledir; 16 takson Akdeniz, 2 takson geniş yayılışlı ve 7 takson bilinmeyendir. Bu bakımdan çalışma alanında en çok takson Akdeniz ve bilinmeyenler şeklinde olduğu görülmektedir. Araştırma alanı Akdeniz Bölgesi içerisinde yer aldığından, toplanan bitki örneklerinde birinci sırada Akdeniz elementi bitkiler yer almaktadır. Bu durumun fitocoğrafya açısından

beklenen bir sonuç olduğu söylenebilir. Çalışma alanında, içerdikleri tür ve türaltı takson sayısına göre en büyük 3 familya Liliaceae 3, Lamiaceae 2, Oleaceae 2 şeklindedir. Araştırma alanında en fazla cins içeren üç familya ve oranları Çizelge 4'te verilmiştir. Çalışma alanında en fazla takson içeren fitocoğrafik bölge Akdeniz fitocoğrafik bölgesi olup, çalışma alanının Doğu Akdeniz bölgesinde bulunmasından dolayı beklenen bir durumdur.

Gilindere Mağarası, Aydıncık Adaları ve Beşparmak Adasınında 14 familya 26 cinse ait toplam 28 takson belirlenmiştir. Taksonların fitocoğrafik bölgelere dağılımı 20 takson Akdeniz, 4 takson Geniş Yayılışlı ve 4 takson bilinmeyen şeklindedir. Çalışma alanında içerdikleri tür ve türaltı takson sayısına göre ilk üç familya Fabaceae 5, Asteraceae 4, Liliaceae 4 şeklindedir (Çizelge 4). Çalışma alanında 1 adet endemik takson bulunmaktadır. Bu takson *Alkanna hispida* Hub.-Mor. olup, Tehlikede (EN) kategorisinde yer almaktadır.

Çizelge 4. Çalışma alanlarının familya, cins ve takson sayılarına göre karşılaştırılması
Table 4. Comparison of the study areas according to the number of families, genus and taxa

		Familya (Takson sayısına göre) *(Family (According to the number of taxa))*			Familya (Cins sayısına göre) *(Family (According to the number of genus))*		
		Familya	Takson	Oranı (%)	Familya	Cins	Oranı (%)
1	Aphrodisias Sit Alanı ve Tisan Mevkii	Lamiaceae	4	13.79	Fabaceae	3	11.53
		Fabaceae	3	10.34	Lamiaceae	3	11.53
		Liliaeae	3	10.34	Liliaceae	3	11.53
		Diğerleri	19	65.51	Diğerleri	17	65.38
2	Taşucu Kalesi ve Boğsak Adası	Fabaceae	8	20.51	Fabaceae	7	29.16
		Liliaceae	7	17.94	Liliaceae	6	25
		Asteraceae	5	12.82	Asteraceae	5	20.83
		Diğerleri	19	74.10	Diğerleri	17	70.83
3	Eğribük Koyu ve Dana Adası	Liliaceae	3	12	Fabaceae	3	8.57
		Lamiaceae	2	8	Liliaceae	3	8.57
		Oleaceae	2	8	Lamiaceae	2	5.71
		Diğerleri	18	72	Diğerleri	16	45.71
4	Gilindere Mağarası, Aydıncık Adaları ve Beşparmak Adası	Fabaceae	5	17.24	Fabaaceae	5	17.85
		Asteraceae	4	13.79	Aasteraceae	4	14.28
		Liliaceae	4	13.79	Liliaceae	4	14.28
		Diğerleri	15	51.72	Diğerleri	15	53.57

Çalışma alanı ve yakın bölgeleri endemizm oranları bakımından bir karşılaştırıldığında, en yüksek endemizm oranının %21.10 ile Büyükeğri Dağı ve Çevresinin Florası çalışmasında (Şirin, 2012), en düşük oranının ise %1.51 ile araştırma alanında olduğu görülmüştür. Büyükeğri Dağı (Mut, İçel) ve Çevresinin yükselti ve habitat çeşitliliğinin fazla olmasından dolayı alanın endemizm oranı da yüksektir (Çizelge 5).

Çizelge 5. Araştırma alanı ve yakın bölgelerinde yapılan çalışmaların endemizm oranları
Table 5. Endemism rates of studies conducted in the research area and its nearby regions

Araştırma Alanı *(Research Area)*	Endemizm Oranı (%) *(Endemism Rate)*
Araştırma Alanı	1.51
Anamur (Mersin) Yaylalarının Florası (Şen, 2019)	13.25
Gülek Boğazı'nın (Mersin-Adana) Florası (Savran & Paksoy, 2016)	18.8
Büyükeğri Dağı ve Çevresinin Florası (Şirin, 2012)	21.10
Anamur Antik Kenti ve çevresinin (Mersin) florası (Yıldıztugay & Küçüködük, 2010a)	3.7
Kaş Yaylası ve çevresinin (Mersin) Florası (Yıldıztugay & Küçüködük, 2010b)	13.2

Araştırma alanları ile yakın bölgelerde yapılan floristik çalışmaların sonuçları, fitocoğrafik bölgelere göre kıyaslandığında alanların tamamında Akdeniz fitocoğrafik bölge elementinin yüksek olduğu görülmüştür (Yıldıztugay & Küçüködük, 2010 a; b; Şirin, 2012; Savran & Paksoy, 2016; Şen, 2019). Bu durum söz konusu alanların Akdeniz bölgesinde bulunmasından kaynaklandığı söylenebilir (Çizelge 6).

Araştırma alanı, yakın bölgelerle en çok takson içeren familyalar bakımından kıyaslandığında Anamur (Mersin) Yaylalarının Florası, Gülek Boğazı'nın (Mersin-Adana) Florası, Büyükeğri Dağı (Mut, İçel) ve Çevresinin Florası, Anamur Antik Kenti ve çevresinin (Mersin) florası adlı çalışmalarda Asteraceae familyasının ilk sırada, araştırma alanı ile Kaş Yaylası ve çevresinin (Anamur - Mersin) Florasında ise Fabaaceae familyasının ilk sırada yer aldığı görülmüştür (Yıldıztugay & Küçüködük, 2010a; b; Şirin, 2012; Savran & Paksoy, 2016; Şen, 2019). Tüm çalışmalarda Asteraceae ve Fabaceae familyalarının ilk iki sırada bulunduğu belirlenmiştir (Çizelge 7).

Çizelge 6. Araştırma alanı ile yakın bölgelerdeki taksonların fitocoğrafik bölgelere göre dağılım oranları (%)
Table 6. Distribution rates of taxa in the study area and nearby regions by phytogeographic regions (%)

Araştırma Alanı *(Research Area)*	Akdeniz Elementi *(Mediterranean Element)*	Avrupa-Sibirya Elementi *(Euro-Siberian Element)*	İran-Turan Elementi *(Irano-Turanian Element)*	Geniş Yayılışlı ve Bilinmeyen *(Widespread and Unknown)*
Araştırma Alanı	56.07	0	0	43.93
Anamur (Mersin) Yaylalarının Florası (Şen, 2019)	51.21	3.60	6.21	54.17
Gülek Boğazı'nın (Mersin-Adana) Florası (Savran & Paksoy, 2016)	19.60	5.60	24.90	49.90
Büyükeğri Dağı (Mut, İçel) ve Çevresinin Florası (Şirin, 2012)	28.20	2.30	15.40	54.10
Anamur Antik Kenti ve çevresinin (Mersin) florası (Yıldıztugay & Küçüködük, 2010 a)	35.10	4.90	3.10	56.90
Kaş Yaylası ve çevresinin (Anamur - Mersin) florası (Yıldıztugay & Küçüködük, 2010 b)	33.60	4.30	10.20	51.90

Çizelge 7. Araştırma alanı ve yakın bölgelerinde yapılan çalışmalarda en çok takson içeren familyalar
Table 7. Families containing the most taxa in the studies conducted in the research area and nearby regions

Araştırma Alanı *(Research Area)*	Asteraceae	Fabaceae	Lamiaceae	Brassicaceae
Araştırma Alanı	9	12	6	7
Anamur (Mersin) Yaylalarının Florası (Şen, 2019)	54	42	47	37
Gülek Boğazı'nın (Mersin-Adana) Florası (Savran & Paksoy, 2016)	89	88	61	69
Büyükeğri Dağı (Mut, İçel) ve Çevresinin Florası (Şirin, 2012)	33	26	19	23
Anamur Antik Kenti ve çevresinin (Mersin) florası (Yıldıztugay & Küçüködük, 2010 a)	40	35	15	17
Kaş Yaylası ve çevresinin (Anamur - Mersin) Florası (Yıldıztugay & Küçüködük, 2010 b)	58	59	31	31

SONUÇ ve ÖNERİLER

Aphrodisias Antik Kenti, Köserelik Adası ve Tisan Mevkii Mersin İlinin Silifke ilçesinde bulunmaktadır. Alan, I. Derece Doğal sit olup Kızılçam ile kaplıdır. Denize doğru dik yamaçları içermektedir. Kıyı şeridinde orman ve çalı formasyonu göze çarpmaktadır. Alanın kıyı şeridi haricinde düzlüklerde yazlık yerleşim yerleri ve tarımsal faaliyetler görülmektedir. Araştırma alanı 928.59 ha alan kaplamakta olup, 6.05 ha ada ve adacıklar, 451.87 ha alan fundalık, 450.15 ha orman ve 20.51 ha alan turistik alandır. Çalışma alanı yaklaşık 50-200 metre yüksekliğe sahip alüvyal malzeme ve kireçtaşlarından oluşmuş olup kıyı kesimleri ve yarım adadan oluşan bir jeomorfoloji göstermektedir. Doğal Sit Alanında toplam 30 flora taksonu belirlenmiştir. Doğal Sit Alanında tespit edilen 30 taksondan "kritik tür" olarak değerlendirilen herhangi bir taksona rastlanmamıştır.

Taşucu Kalesi ve Boğsak Adası Mersin İlinin Silifke ilçesinde bulunmaktadır. Taşucu Kalesi I. derece; Boğsak Adası ise I. ve II. derece Doğal Sit alanıdır. Alan, yaklaşık 28 hektar büyüklüğe sahip olup askeri alan içerisinde yer almaktadır. Çalışma alanı ve çevresi yaklaşık 30 metre yükseltiye sahip tipik ada, yarım ada ve kıyı oluşumlarına sahiptir. Alanda Akdeniz maki vejetasyonu bitkileri gözlenmektedir. Bu bitkiler *Phillyrea latifolia* L., *Ceratonia siliqua* L., *Pistacia terebinthus* L. subsp. *palaestina, Olea europaea* L. var. *Sylvestris, Calicotome villosa* (Poiret) Link, gibi türlerden oluşmaktadır. Doğal sit alanında toplam 40 flora taksonu belirlenmiştir. Tespit edilen 40 taksondan "kritik tür" olarak değerlendirilen takson yoktur.

Eğribük Koyu ve Dana Adası Mersin ilinin Silifke ilçesinde bulunmaktadır. Alan, I. Derece Doğal Sit alanı olup yaklaşık 260 hektar büyüklüğe sahiptir. Alan kıyı şeridini ve Dana Adası'nı kapsamaktadır. Kıyı şeridinde orman ve çalı formasyonu göze çarpmaktadır. *Pinus brutia* formasyonu hâkim durumdadır. Mersin Akdere sahilinin güneybatısında, çok dik ve sarp bir arazi yapısına sahip olan alan doğal bir peyzaj yapısına sahiptir. Eğribük Koyu'na 2.5 km uzaklıkta olan Dana Adası da 1. Derece Sit Alanıdır (Şekil 3). Akdeniz, doğal bitki örtüsü öne çıkmakta ve fundalık vasfı taşımaktadır.

Doğal Sit Alanında toplam 25 flora taksonu belirlenmiştir. Bu 25 takson içerisinde "kritik tür" olarak değerlendirilen takson yoktur. Akdeniz kıyısından 2 km uzaklıkta olan Dana adasının vejetasyon yapısı anakara ile benzer özellikler göstermektedir. Adada çalı olarak *Rhus coriaria, Capparis spinosa, Paliurus spina-christii* gibi türler göze çarpar.

Gilindere Mağarası, Aydıncık ve Beşparmak Adaları Mersin ilinin Aydıncık ve Gülnar ilçelerinde bulunmaktadır. Alan, I. Derece Doğal Sit alanıdır.

Çizelge 8. Alanda tespit edilen bitki listesi
Table 8. Plant list detected in the area

No *(No)*	Familya Adı *(Family Name)*	Takson Adı *(Taxon Name)*	Fitocoğrafik Bölge *(Phytogeographic Region)*	Tehlike Kategorisi *(Threataned Category)*	Bulunduğu Sit Alanı *(Sit Area)*	Toplama Tarihi *(Collection Date)*	Toplayıcı Numarası *(Collector Number)*
1	Cupresaceae	*Cupressus sempervirens* L.	-	-	1, 3	03.04.2016	1004
2	Pinaceae	*Pinus brutia* Ten.	*D.Akd. Elm.*	-	1, 2, 3, 4	07.04.2016	1011
3	Anacardiaceae	*Pistacia terebinthus* L. supsp. *palaestina* (Boiss.) Engler	*D.Akd. Elm.*	-	1, 2, 3, 4	07.04.2016	1017
4	Anacardiaceae	*Pistacia terebinthus* L. subsp. *terebinthus*	*Akd. Elm.*		2	07.05.2016	1018
5	Anacardiaceae	*Pistacia leutiscus* L.	*Akd. Elm.*		2	07.05.2016	1016
6	Anacardiaceae	*Rhus coriaria* L.	-	-	2	07.05.2016	1019
7	Asteraceae	*Artemisia absinthium* L.	-	-	2	03.04.2016	1034
8	Araceae	*Arum dioscoridis* Sm. var. *liepoldtii* (Schott) Engler	*D.Akd. Elm.*	-	2	03.04.2016	1425
9	Asteraceae	*Carthamus lanatus* L.	Gen. Yay.	-	2	07.05.2016	1038
10	Asteraceae	*Cichorium intybus* L.	Gen. Yay.	-	4	03.04.2016	1046
11	Asteraceae	*Crupina crupinastrum* (Moris) Vis.	Gen. Yay.	-	2	04.05.2016	1050
12	Asteraceae	*Inula graveolens* (L.) Desf.	*Akd. Elm.*	-	1, 3	22.03.2016	1056
13	Asteraceae	*Inula viscosa* (L.) Aiton	*Akd. Elm.*	-	4	07.04.2016	1058
14	Asteraceae	*Phagnalon graecum* Boiss.	*D.Akd. Elm.*	-	2, 4	22.07.2016	1066
15	Asteraceae	*Senecio vernalis* Waldst. & Kit.	Gen. Yay.	-	4	07.04.2016	1070
16	Asteraceae	*Staehelina lobelii* DC.	*D.Akd. Elm.*	-	2	07.05.2016	1075
17	Boraginaaceae	*Alkanna hispida Hub.-Mor.*	*D.Akd. Elm.*	End./EN	4	22.07.2016	1084
18	Boraginaaceae	*Echium angustifolium* Miller	*D.Akd. Elm.*	-	2	07.04.2016	1092
19	Boraginaceae	*Heliotropium europaeum* L.	*Akd. Elm.*	-	1, 3, 4	08.05.2016	1094
20	Capparaceae	*Capparis spinosa* L. var. *spinosa*	-	-	1, 3, 4	08.05.2016	1129
21	Caryophyllaceae	*Dianthus polycladus* Boiss.	*D.Akd. Elm.*	*VU*	4	08.05.2016	1132
22	Caryophyllaceae	*Silene vulgaris* (Moench) Garcke *var. commutata* (Guss.) Coode & Cullen	-	-	2	23.07.2016	1140
23	Chenopodiacee	Halimione *portulacoides* (L.) Aellen	-	-	1, 3	08.05.2016	1145
24	Cistaceae	*Cistus creticus* L.	*Akd. Elm.*	-	1, 3, 4	08.05.2016	1152
25	Cistaceae	*Cistus salviifolius* L.	Gen. Yay.	-	1	08.05.2016	1153
26	Cistaceae	*Halimium umbellatum* (L.) Spach	*Akd. Elm.*	-	2	23.07.2016	1154
27	Ephedraceae	*Ephedra campylopoda* C. A. Meyer	-	-	2	03.04.2016	1008
28	Ericaceae	*Arbutus andrachne* L.	-	-	1, 3	22.07.2016	1171
29	Ericaceae	*Arbutus unedo* L.	-	-	1	22.03.2016	1172
30	Fabaceae	*Anagyris foetida* L.	*Akd. Elm.*	-	2	04.05.2016	1183
31	Fabaceae	*Anthyllis vulneraria* L. subsp. *boissieri* (Sag.) Bornm.	Gen. Yay.	-	4	22.07.2016	1184
32	Fabaceae	*Calicotome villosa* (Poiret) Link	*Akd. Elm.*	-	1,2,3,4	22.07.2016	1189
33	Fabaceae	*Ceratonia siliqua* L.	*Akd. Elm.*	-	1,2,3,4	04.05.2016	1190
34	Fabaceae	*Genista acanthoclada* DC.	*D.Akd. Elm.*	-	4	25.10.2016	1200
35	Fabaceae	*Lupinus varius* L.	*Akd. Elm.*	-	2	23.07.2016	1216
36	Fabaceae	*Medicago marina* L.	-	-	4	08.05.2016	1217
37	Fabaceae	*Pisum sativum* L. subsp. *elatius* (Bieb.) Aschers. & Graebn var. e*latius*	*Akd. Elm.*	-	2	04.04.2016	1227
38	Fabaceae	*Trifolium campestre* Schreb.	Gen. Yay.	-	2	22.07.2016	1232
39	Fabaceae	*Trifolium stellatum* L. var. *stellatum*	-	-	2	04.05.2016	1236

40	Fabaceae	*Trifolium repens* L. var. *giganteum* Lag-Foss	-	-	1,3	04.05.2016	1234
41	Fabaceae	*Vicia cracca* L. subsp. *stenophylla* Vel.	Gen. Yay.	-	2	23.07.2016	1239
42	Fagaceae	*Quercus coccifera L.*	*Akd. Elm.*	-	1,3,4	22.07.2016	1246
43	Lamiaceae	*Ajuga chamaepitys* (L.) Schreber subsp. *chia* (Schreber) *Arcangeli var. chia*	*Gen. Yay.*	-	1	22.03.2016	1264
44	Lamiaceae	*Lavandula stoechas* L. subsp. *stoechas*	*D.Akd. Elm.*	-	1	22.03.2016	1273
45	Lamiaceae	*Micromeria myrtifolia* Boiss. & Hohen.	*D.Akd. Elm.*	-	2,4	22.07.2016	1277
46	Lamiaceae	*Salvia viridis* L.	*Akd. Elm.*	-	2	07.04.2016	1297
47	Lamiaceae	*Teucrium creticum* L.	*D.Akd. Elm.*	-	1,3,4	22.03.2016	1309
48	Lamiaceae	*Teucrium polium* L.	*Ge. Yay.*	-	1,3	22.03.2016	1310
49	Liliaceae	*Allium ampeloprasum* L.	*Akd. Elm.*	-	2	23.07.2016	1438
50	Liliaceae	*Allium neapolitanum* Cyr.	*Akd. Elm.*	-	2	03.04.2016	1439
51	Liliaceae	*Asparagus acutifolius* L.	*Akd. Elm.*	-	1,2,3,4	24.10.2016	1441
52	Liliaceae	*Asphodelus aestivus* Brot.	*Akd. Elm.*	-	2,4	22.07.2016	1443
53	Liliaceae	*Muscari comosum* (L.) Miller	*Akd. Elm.*	-	2	23.07.2016	1448
54	Liliaceae	*Smilax aspera* L.	-	-	1,2,3,4	22.07.2016	1454
55	Liliaceae	*Urginea maritima* (L.) Baker	*Akd. Elm.*	-	1,2,3,4	23.10.2016	1456
56	Oleaceae	*Olea europaea* L. var. *sylvestris* (Miller) Lehr	*Akd. Elm.*	-	1,2,3,4	23.10.2016	1329
57	Oleaceae	*Phillyrea latifolia* L.	*Akd. Elm.*	-	1,2,3,4	22.07.2016	1330
58	Papaveraceae	*Papaver rhoeas* L.	*Ge. Yay.*	-	1,3,4	26.10.2016	1336
59	Plantaginaceae	*Plantago afra* L.	-	-	2	23.07.2016	1338
60	Plumbaginaceae	*Limonium angustifolium* (Tausch) Turrill	*Akd. Elm.*	-	1,3	22.07.2016	1344
61	Poaceae	*Avena sterilis* L. subsp. *ludoviciana* (Durieu)	-	-	2	23.07.2016	1463
62	Poligonaceae	*Polygonum maritimum* L.	-	-	2	22.07.2016	1346
63	Rhamnaceae	*Paliurus spina- christi* Miller	-	-	4	22.07.2016	1369
64	Rosaceae	*Sarcopoterium spinosum* (L.) Spach Gillet et Magne	*D.Akd. Elm.*	-	1,2,3,4	22.07.2016	1383
65	Rutaceae	*Ruta chalepensis* L.	-	-	1,2,3	24.10.2016	1386
66	Santalaceae	*Osyris alba* L.	*Akd. Elm.*	-	1,3	22.07.2016	1391

Şekil 3. Dana adasının denizden fotoğrafı
Figure 3. Photograph of Dana Island from the sea

Şekil 4. *Alkanna hispida* Hub.-Mor. (Kıllı havacıva)
Figure 4. *Alkanna hispida* Hub.-Mor. (Kıllı havacıva)

Aynı zamanda Orman ve Su İşleri Bakanlığı tarafından "Gilindire Mağarası Tabiat Anıtı" olarak ilan edilmiştir. Alan, yaklaşık 641.4 hektar büyüklüğe sahiptir. Kıyı şeridinde orman ve çalı formasyonu göze çarpmaktadır. *Pinus brutia* formasyonu hakim durumdadır. Alan, 12 km. kıyı şeridine sahiptir. Deniz

ve kıyı çizgisinin girintili çıkıntılı olduğu alanda irili ufaklı burunlar ve koylar yer almaktadır. Kalker kayalıklardan oluşan alanda ağaç ve çalı formunda *Teucrium creticum* L., *Sarcopoterium spinosum* (L.) Spach, *Calicotome villosa* (Poiret) Link, *Olea europaea* L. var. *sylvestris* (Miller) Lehr, *Genista acanthoclada* DC, *Pinus brutia* Ten., türleri ve otsu *Urginea maritima* (L.) Baker, *Asphodelus aestivus* Brot, *Anthyllis vulneraria* L. subsp. *boissieri* (Sag) Bornm *Papaver rhoeas* L. *Senecio vernalis* Waldst. & Kit. ve endemik *Alkanna hispida* türleri göze çarpmaktadır. Alanda kaya ve maki vejetasyonu bulunmaktadır. Doğal Sit Alanında toplam 28 flora taksonu belirlenmiştir. Belirlenen taksonlardan 1 taksonun endemik (*Alkanna hispida* Hub.-Mor.) ve 1 taksonunda nadir (*Dianthus polycladus* Boiss.) olduğu tespit edilmiştir. Doğal Sit Alanında bulunan ve "kritik tür" olarak değerlendirilen *Alkanna hispida* Hub.-Mor. (Kıllı havacıva) (Şekil 4) Tehlikede (EN) ve nadir tür *Dianthus polycladus* Boiss. (Belen karanfili) ise Zarar Görebilir (VU) kategorisinde yer almaktadır.

TEŞEKKÜR

Bu çalışmanın bir kısmı İshak ORTAÇ'ın yüksek lisans çalışmasından, Adıyaman Üniversitesine ait FEFYL-2016-0010 nolu BAP projesi sonuç raporundan ve T.C. Çevre ve Şehircilik Bakanlığının 2014/151078 nolu Ekolojik Temelli Bilimsel Araştırma Projesinden üretilmiştir. Maddi destekte bulunan her iki kuruma teşekkür ederiz.

Araştırmacıların Katkı Oranı Beyan Özeti

Yazarlar makaleye eşit oranda katkı sağlamış olduklarını beyan eder.

Çıkar Çatışması Beyanı

Makale yazarları aralarında herhangi bir çıkar çatışması olmadığını beyan ederler.

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Detoxification Efficiency of Micropropagated *Alternanthera reineckii* Briq. against Zinc Oxide Nanoparticles in Human Keratinocyte Cells

Bugrahan EMSEN[1], Ibrahim CINAR[2], Muhammet DOGAN[3]
[1]Department of Biology, Kamil Özdağ Faculty of Science, Karamanoğlu Mehmetbey University, Karaman, Türkiye, [2]Department of Medical Services and Techniques, Karamanoğlu Mehmetbey University, Karaman, Türkiye, [3]Department of Nutrition and Dietetics, Faculty of Health Sciences, Karamanoğlu Mehmetbey University, Karaman, Türkiye
[1]https://orcid.org/0000-0002-9636-2596, [2]https://orcid.org/0000-0002-0509-913X, [3]https://orcid.org/0000-0003-3138-5903
✉: bugrahanemsen@gmail.com

ABSTRACT

Considering the rapid developments in nanotechnology, scientific research in the field of nanotoxicology is required in order to prevent the dangers of nanotechnology on human health. For this purpose, we tested the cytotoxic effect of ZnO nanoparticle (NP), which is included in many cosmetic products, on human keratinocyte cells (HaCaT). In addition, we evaluated to potentially inhibit this cytotoxic effect with an aquatic plant, *Alternanthera reineckii* Briq. produced by tissue culture method. The nodal explants of *A. reineckii* were cultured in Murashige & Skoog basal medium (MS) including the combinations of 0.25-1.25 mg/L Thidiazuron (TDZ) and 0.25 mg/L indole-3-butyric acid (IBA). Maximum number of shoots per explant (22.50 shoots/explant) was obtained in the culture medium with 0.75 mg/L TDZ+0.25 mg/L IBA. The highest shoot length (1.77 cm) was determined in MS medium with 0.25 mg/L TDZ+0.25 mg/L IBA. Acetone and water extracts were obtained from *A. reineckii* through Soxhlet extraction. The cytotoxic effect of ZnO alone on HaCaT was inhibited by acetone and water extracts. The cell viability, which decreased to 26.04% with the effect of ZnO, increased up to 67.83% with the application of acetone extract. Overall, our results revealed the protective potential of this plant against nanotoxicity induced by ZnO and shed light on future studies.

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Mikroçoğaltılan *Alternanthera reineckii* Briq.'nin İnsan Keratinosit Hücrelerinde Çinko Oksit Nanopartiküllerine Karşı Detoksifikasyon Etkinliği

ÖZET

Nanoteknolojideki hızlı gelişmeler göz önüne alındığında, nanoteknolojinin insan sağlığı üzerindeki tehlikelerinin önüne geçebilmek için nanotoksikoloji alanında bilimsel araştırmalara ihtiyaç duyulmaktadır. Bu amaçla birçok kozmetik üründe yer alan ZnO nanopartikülünün (NP) insan keratinosit hücreleri (HaCaT) üzerindeki sitotoksik etkisini test ettik. Ek olarak, doku kültür yöntemi ile üretilen bir su bitkisi olan *Alternanthera reineckii* Briq. ile bu sitotoksik etkiyi inhibe etme potansiyelini değerlendirdik. *A. reineckii*'nin nodal eksplantları, 0,25-1,25 mg/L Thidiazuron (TDZ) ve 0,25 mg/L indol-3-bütirik asit (IBA) kombinasyonlarını içeren Murashige & Skoog bazal ortamında (MS) kültüre edildi. Eksplant başına maksimum sürgün sayısı (22,50 sürgün/eksplant) 0,75 mg/L TDZ+0,25 mg/L IBA içeren kültür ortamında elde edilmiştir. En yüksek sürgün uzunluğu (1,77 cm) 0,25 mg/L TDZ+0,25 mg/L IBA içeren MS ortamında belirlenmiştir. *A. reineckii*'den Soxhlet ekstraksiyonu yoluyla aseton ve su özütleri elde edilmiştir. Tek başına ZnO'nun HaCaT üzerindeki sitotoksik etkisi, aseton ve su özütleri ile inhibe edilmiştir. ZnO'nun etkisiyle %26,04'e düşen hücre canlılığı, aseton özütü uygulamasıyla %67,83'e kadar yükselmiştir. Genel olarak, sonuçlarımız bu bitkinin ZnO tarafından indüklenen nanotoksisiteye karşı koruyucu potansiyelini ortaya koymuş ve gelecekteki çalışmalara ışık tutmuştur.

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INTRODUCTION

In recent years, nanotechnology has appeared in many areas. Thanks to nanotechnology, analysis of nanometer-sized structures, determination of physical properties of nanometer-sized structures, and superior material production can be made (Das et al. 2019; Mohajerani et al. 2019; Jahan & Isildak, 2021). Nano-sized metal and metal oxide particles are indispensable raw materials of advanced technology and their application areas are spread over many different sectors. Metal and metal oxide nanoparticles (NPs) have high catalytic, magnetic, chemical and optical characteristics. These properties vary according to the surface properties, shapes and sizes of the NPs (Kavitha et al. 2022). Zinc oxide (ZnO) is also an important NP with the aforementioned properties (Sagadevan et al. 2018). ZnO NPs can be used in sensor, surface dyes, textile products, fabrics and materials such as plastics (Uma et al. 2019; Abdullah et al. 2020; Agustina et al. 2020). Moreover, as ZnO particles become transparent when they are reduced to nano-size, they are widely preferred in personal care products, especially sun creams (Gollavilli et al. 2020).

The use of NPs in many areas has also caused their side effects. Due to the very small size of nano materials and their large surface area, they show very high chemical and biological activity. If these particles enter the body and pass for a certain time, diseases such as inflammation, wheezing and coughing may occur (Monsé et al. 2019). Ways of exposure to NPs are by inhalation, digestion, injection into the skin or body (Braakhuis et al. 2015). Especially the use of nanocosmetics has increased in recent years. However, there are risks that may occur with their increased penetration through the skin. Risk of insoluble NPs in sunscreen preparations exists (Lee et al. 2020).

It is important to include herbal extracts in the product, especially in order to reduce the risk of nanomaterials found in cosmetic products. Thus, the possibility of reducing the toxic effect of the nanomaterial will increase. *Alternanthera reineckii* Briq. was used as plant material in this study. *A. reineckii* is an aquatic plant originating from South America. This plant is in a form adapted to living in and out of water thanks to the imbalance in the current at the banks of the Amazon river (Anderson et al. 2015). *A. reineckii* was propagated by tissue culture techniques. Plant tissue culture is the production of plants or plant products from the whole plant or various parts of the plant under sterile conditions in an artificial nutrient medium (El-Sherif 2019; Celik et al. 2020; Ozelci & Yigit, 2022). This method is mainly based on the totipotency property of plant cells. The ability to divide while forming the complete genome of the cell is called totipotency. Apart from the totipotency feature in plants, their growth and metabolism developments are also important (Rani & Kumar 2017). Recently, tissue culture technique has been widely used in many plant-based studies such as antioxidant activity (Dilkalal et al. 2021), stress physiology (Hosseini Tafreshi et al. 2021), and secondary metabolite production (Jirakiattikul et al. 2021). We have benefited from this technique because of its advantages such as preventing the collection of plants from nature and providing rapid and multiple plant production under *in vitro* conditions.

To the best of our knowledge, we found that the protective effect of *A. reineckii* has not been tested against ZnO-induced nanotoxicity. Therefore, in the present study, we propagated *A. reineckii in vitro* in the desired amount and examined its protective property against cytotoxic damage induced by ZnO NPs on the human keratinocyte cells.

MATERIALS and METHODS

ZnO NPs and Their Characterization

For ZnO NPs, nano powder sample, white powder in the nanoscale range <200 nm with a high purity of 99.9+% and CAS number 1314-13-2, purchased from US research Nanomaterials Inc, Houstan, TX, USA, is used in this study. Characterization of ZnO NP is performed with X-ray diffraction (XRD) and scanning electron microscope (SEM) analysis to identify the crystal structure, the average crystallite size and morphology of NP.

In Vitro Regeneration

A. reineckii plants were taken as sterile stock plants. Murashige and Skoog (MS) basal medium with vitamins were used as nutrient media in the culture studies. The nodal explants were placed in MS medium including 30 g/L sucrose, 7 g/L agar and the combinations of 0.25-1.25 mg/L Thidiazuron (TDZ) and 0.25 mg/L indole-3-butyric acid (IBA). It was sterilized by keeping it under 1.2 atmospheres pressure at 121°C for 20 minutes. Then, the nutrient media were sterilized by keeping them under 1.2 atmospheres pressure at 121°C for 20 minutes. The culture experiment was terminated at the end of eight weeks. In the activity studies, *A. reineckii* in the MS nutrient medium containing 0.75 mg/L TDZ + 0.25 mg L IBA,

where the best results were obtained, were used.

Extraction

Plants (10 g) left to dry at room conditions were subjected to extraction (Soxhlet extraction). The filtered extracts were then concentrated by means of a rotary evaporator. The stock extracts obtained were dissolved with 0.5% dimethyl sulfoxide (DMSO) before the experiments.

Culture of the Cell Lines

Dulbecco's Modified Eagle Medium (DMEM) was used for culturing the human keratinocyte cell line (HaCaT). DMEM in high glucose was supplemented with 1% penicillin-streptomycin, 1% L-glutamine and 10% heat-inactivated fetal bovine serum (FBS). The cell cultures were maintained at 37°C in a humidified atmosphere with 5% CO_2.

Antiproliferative Activities

Cells were seeded at 1×10^4 cells/well in 96-well flat-bottomed microtiter plates. After 24 h incubation, ZnO NPs (50 mg/L) and extracts with different concentrations of *A. reineckii* were added to the wells alone and in combination and kept in a CO_2 incubator at 37°C for 48 h. Final extract concentrations in the wells were numbered between 1-10 and showed in Table 1. Negative control (NC) cultures received 0.5% DMSO alone. MTT procedure was use for cytotoxic activity (Emsen et al. 2018).

Table 1. Different extract experiments obtained from *A. reineckii*
Çizelge 1. A. reineckii'den elde edilen farklı özüt deneyleri

Extracts at different concentrations *(Farklı konsantrasyonlarda özütler)*	Abbreviation of the extract treatments *(Özüt uygulamalarının kısaltması)*
Acetone/water extract at 1.95 mg/L concentration	AE1/WE1
Acetone/water extract at 3.91 mg/L concentration	AE2/WE2
Acetone/water extract at 7.81 mg/L concentration	AE3/WE3
Acetone/water extract at 15.63 mg/L concentration	AE4/WE4
Acetone/water extract at 31.25 mg/L concentration	AE5/WE5
Acetone/water extract at 62.5 mg/L concentration	AE6/WE6
Acetone/water extract at 125 mg/L concentration	AE7/WE7
Acetone/water extract at 250 mg/L concentration	AE8/WE8
Acetone/water extract at 500 mg/L concentration	AE9/WE9
Acetone/water extract at 1000 mg/L concentration	AE10/WE10

Statistical Analyses

Differences between effects of different TDZ-IBA doses on shoot regeneration of *A. reineckii* from nodal explant were tested on Duncan post hoc work, an ANOVA test ($p < 0.05$). Hierarchical clustering and heatmap analyses were used to measure the distances between viabilities in HaCaT cells treated with ZnO NPs alone or combined with acetone and water extracts of *A. reineckii*. SPSS 21.0 was preferred to perform the analyses.

RESULTS

Analysis of SEM Image and XRD Spectrum of ZnO NPs

XRD patterns of resulting material in the range of 2θ = 25–75^0 was obtained (Figure 1). The well-defined sharp Bragg peaks represented extremely crystalline nature of the material with the hexagonal crystal structure (known as zincite). The particle size was changing from 37.3 nm to few hundred nm. The phase identification was made using JCPDS database and the ZnO NPs were well organized and fitted the standard of ZnO (JCPDS no: 36-1451). SEM image of ZnO NP was given in Figure 2. ZnO NPs were distributed in random as in the SEM image.

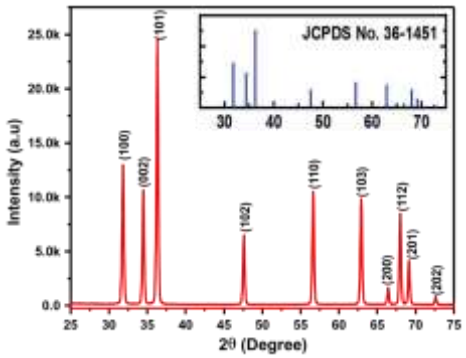

Figure 1. Characterization of ZnO NPs XRD pattern. Inset shows the X-ray diffraction pattern of ZnO nano powder. Standard pattern of ZnO (JCPDS 36-1451)

Şekil 1. ZnO NP'lerin XRD modelinin karakterizasyonu. Ekli küçük resim ZnO nano tozunun X-ışını kırınım modelini gösterir. ZnO'nun standart modeli (JCPDS 36-1451)

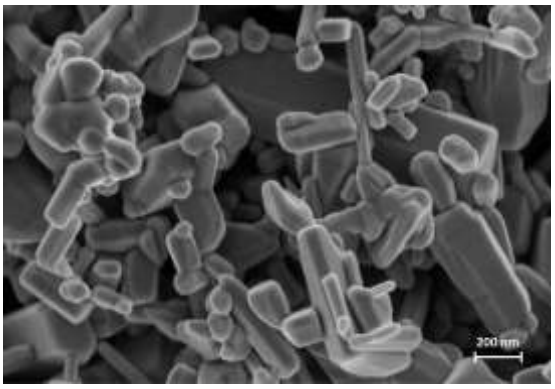

Figure 2. SEM image of ZnO NPs
Şekil 2. ZnO NP'lerin SEM görüntüsü

In Vitro Shoot Regeneration of *A. reineckii*

The nodal explants of *A. reineckii* were cultured for *in vitro* shoot regeneration in MS nutrient solution containing the combinations of 0.25-1.25 mg/L TDZ and 0.25 mg/L IBA. The first shoot formation was observed on the 12th day in the culture media fortified with 0.75 mg/L TDZ + 0.25 mg/L IBA. At the end of six weeks, the experiment was terminated and multiple shoot formation from the nodal explants was recorded (Table 2).

Shoot regeneration percentage was ranked between 66.66% and 100% (Table 2). The lowest shoot regeneration frequency (66.66%) was determined in the explants without growth regulators (control group). Mean shoot lengths ranked between 1.10-1.77 cm. The longest shoot (1.77 cm) was obtained in cultures with 0.25 mg/L TDZ + 0.25 mg/L IBA, while the shortest shoot (1.10 cm) was determined in cultures with 1.25 mg/L TDZ + 0.25 mg /L IBA.

Cytotoxicity Activities

Viability rates of HaCaT cells treated with ZnO NPs and *A. reineckii* extracts were tested. The application that decreased the cell viability (26.04±1.09%) the most was ZnO NPs alone. AE and WE experiments alone showed a certain amount of cytotoxic effect on cells at different concentrations. While AE10 reduced the cell viability rate to 74.61±1.42%, this rate was 86.40±2.06% for WE10 application. The experimental groups with the highest cell viability among the combined AE+ZnO NPs and WE+ZnO NPs applications were AE6+ZnO (67.83±1.84%) and WE2+ZnO (65.85±2.69%), respectively (Figure 4, 5).

Heatmap and HCA analyses ranked experiments according to cell viability data and included extract and ZnO NPs experiments into different clusters. Accordingly, the experiments tested for HaCaT cells were divided into 3 clusters. ZnO NPs experiment was located under cluster 3 alone and was separated from other clusters. Applications under cluster 1 were NC and AE, WE extract trials. The combined applications of extract+ZnO NPs were placed under cluster 2 (Figure 6a).

The colour gradient appearing on the heatmap also coincided with the cluster analysis. According to heatmap analysis, ZnO NPs trial differed from other extract applications with blue color. The other extract trials, except AE9 and AE10, had red color intensity. The viability rates of cells exposed to AE9 and AE10 treatments were 78.83±1.55% and 74.61±1.42%, respectively. Combined applications, except AE8, AE9, AE10 + ZnO NPs, were in black intensity with a medium shade (Figure 6b).

Figure 3. *In vitro* shoot regeneration of *A. reineckii.* Multiple shoot regeneration from nodal explants in MS medium including 0.75 mg/L TDZ + 0.25 mg L IBA after four weeks (a) and (b) eight weeks of culture
Şekil 3. *A. reineckii*'nin *in vitro* sürgün rejenerasyonu. Dört hafta (a) ve (b) sekiz haftalık kültürden sonra 0,75 mg/L TDZ + 0,25 mg L IBA içeren MS ortamında nodal eksplantlardan çoklu sürgün rejenerasyonu

DISCUSSION

While exploring potential application areas of nanotechnology, the risks and uncertainties that NPs may pose on living things and the environment should not be ignored. The size and density of NPs play an important role in the toxic effects of NPs (Braakhuis et al. 2014). Studies on the toxic effects of nanoparticles have been investigated in many different organisms and have produced similar results for different NPs. In a study with ZnO and titanium dioxide (TiO_2) NPs, their ecotoxic effects on the green algae *Pseudokirchneriella subcapitata* were evaluated. The researchers exhibited that algae growth was inhibited by the increase in NP concentration, and ZnO NP caused the cell membrane to become unstable (Lee & An 2013). It is also known

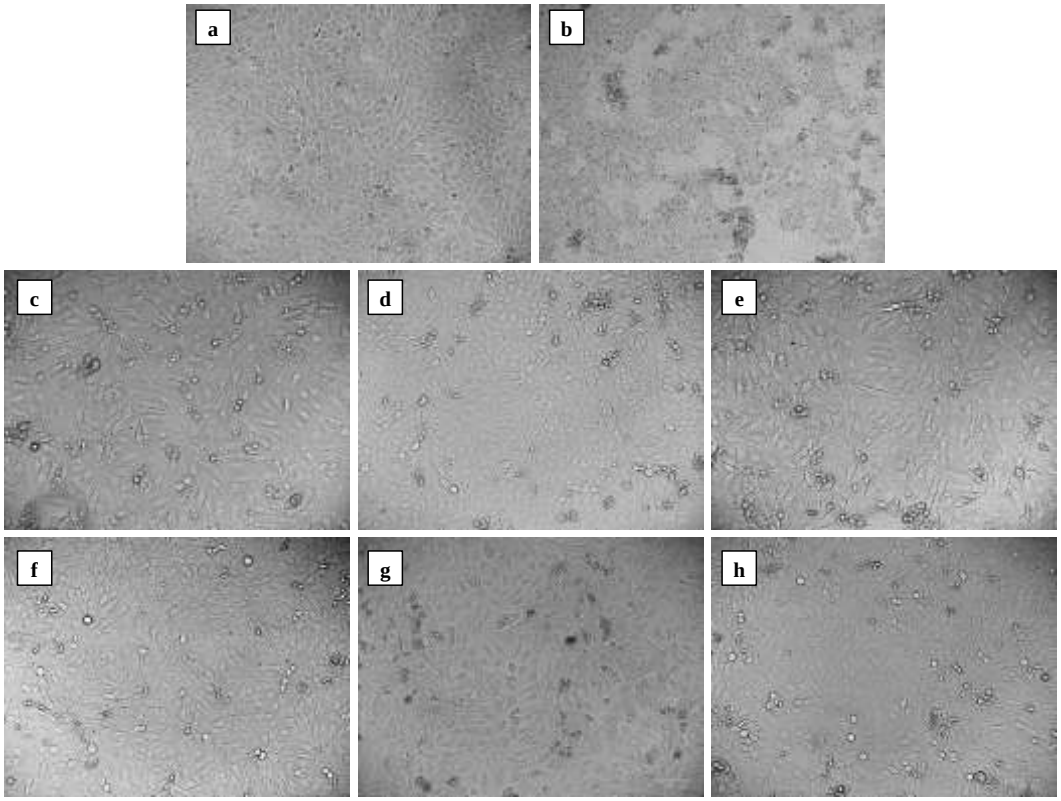

Figure 4. Effect of ZnO NPs alone (b), AE1+ZnO NPs (c), AE5+ZnO NPs (d), AE10+ZnO NPs (e), WE1+ZnO NPs (f), WE5+ZnO NPs (g), WE10+ZnO NPs (h) on cell viability in HaCaT cells observed under the fluorescent cell imager-bright field channel (magnification 175×). Control-treated cells were regarded as 100% viable (a).

Şekil 4. Tek başına ZnO NP'lerin (b), AE1+ZnO NP'lerin (c), AE5+ZnO NP'lerin (d), AE10+ZnO NP'lerin (e), WE1+ZnO NP'lerin (f), WE5+ZnO NP'lerin (g), WE10+ZnO NP'lerin (h) floresan hücre görüntüleyici-parlak alan kanalı (175× büyütme) altında gözlenen HaCaT hücrelerinde hücre canlılığı üzerindeki etkileri. Kontrol ile muamele edilen hücreler %100 canlı olarak kabul edilmiştir (a).

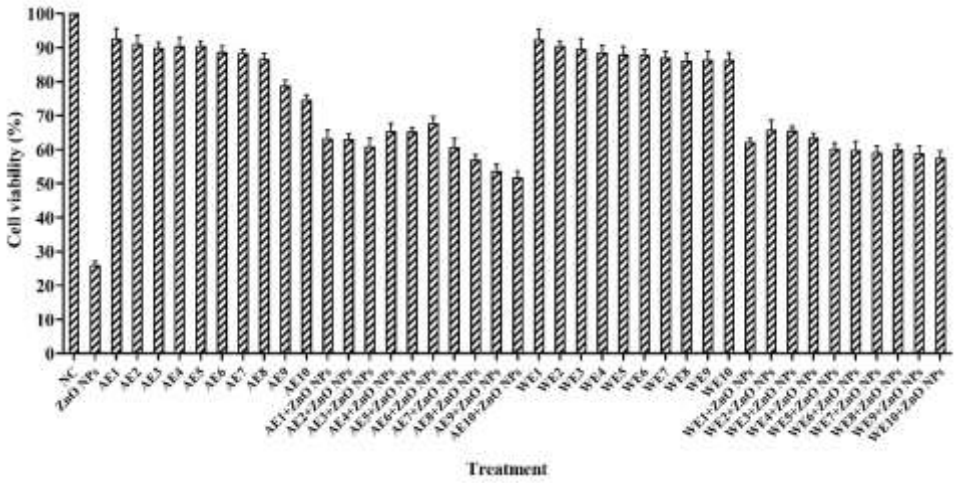

Figure 5. Viability rates obtained by MTT analysis in HaCaT cells treated with ZnO NPs alone or combined with acetone and water extracts of *A. reineckii*. Data represent mean ± standard deviation from 3 independent experiments.

Şekil 5. Tek başına ZnO NP'lerle veya A. reineckii'nin aseton ve su özütleri ile kombine edilmiş HaCaT hücrelerinde MTT analizi ile elde edilen canlılık oranları. Veriler, 3 bağımsız deneyden elde edilen ortalama ± standart sapmayı temsil etmektedir.

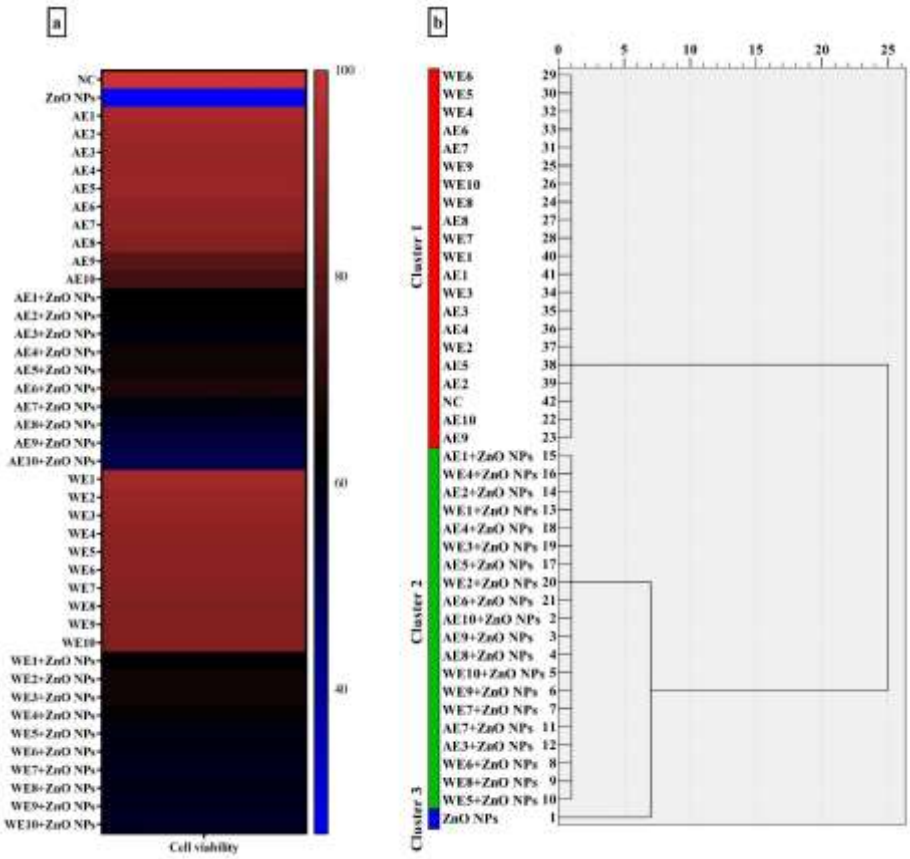

Figure 6. Heatmap of ZnO NPs alone or combined with acetone and water extracts of *A. reineckii* in HaCaT cells. High and low viabilities were represented by red and blue color, respectively. The scale of color intensity was positively correlated to cell viability (a). Dendrogram built from cell viability rates in HaCaT cells (b)

Şekil 6. HaCaT hücrelerinde A. reineckii'nin tek başına veya aseton ve su özütleri ile birlikte ZnO NP'lerinin ısı haritası. Yüksek ve düşük canlılık, sırasıyla kırmızı ve mavi renkle temsil edilmiştir. Renk yoğunluğu ölçeği, hücre canlılığı ile pozitif olarak ilişkiliydi (a). HaCaT hücrelerinde hücre canlılığı oranlarından oluşturulan dendrogram (b)

Table 2. Effects of different TDZ-IBA doses on shoot regeneration of *A. reineckii* from nodal explant
Çizelge 2. Farklı TDZ-IBA dozlarının nodal eksplanttan A. reineckii'nin sürgün rejenerasyonu üzerindeki etkileri

Growth regulators (mg/L) *(Büyüme düzenleyiciler)*				
TDZ	IBA	Shoot regeneration *(Sürgün rejenerasyonu)* (%)	Shoots per explants *(Eksplant başına sürgün)*	Shoot length *(Sürgün uzunluğu)*(cm)
0	0	66.66b	1.67c	1.15b
0.25	0.25	88.89ab	12.33b	1.77a
0.50	0.25	100.00a	17.67ab	1.59a
0.75	0.25	100.00a	22.50a	1.27b
1.00	0.25	88.89ab	21.67a	1.25b
1.25	0.25	83.33ab	16.00b	1.10b

Means followed by different small letters within same column were significantly different ($p < 0.05$)

that ZnO NPs causes genetic damage. Akbaba & Türkez (2018) tested the genetic damage level of ZnO NPs on lymphocytes by means of chromosome aberration and micronucleus analyses and reported that 500 ppm and higher concentrations showed genotoxic effects.

In the current study, the cytotoxic potential of ZnO NPs on HaCaT cells was evaluated and it was determined that the viability of the cells was greatly reduced. Similarly, there are different studies showing that ZnO NPs have different toxic mechanisms on HaCaT cells. In one study, the mechanism of skin toxicity caused by ZnO has been investigated. The researchers revealed that ZnO NPs could induce the inflammatory response in HaCaT cells. This induction was enhanced by reactive oxygen species (ROS)-extracellular signaling (Jeong et al. 2013). Another study showed that ZnO NPs could cause a potential

ROS generation. In this study, the data demonstrated a significant decrease of glutathione level in HaCaT cells exposed to ZnO NPs and it was suggested that there might be a link between the nanotoxicity mechanism and oxidative stress, active oxygen production, antioxidant defense mechanisms, and apoptosis (Lee et al. 2012). Studies have focused on the inhibition of nanotoxicity in cells. Resveratrol was preferred in some of the studies within this scope. Giordo et al. (2020) pronounced that resveratrol, an important antioxidant, inhibited mitochondrial dysfunction and oxidative stress caused by ZnO NPs in Zebrafish. In another similar study, genetic damage, oxidative stress and cytotoxicity in human pulmonary alveolar epithelial cells induced by ZnO NPs were tolerable by using resveratrol. The researchers' opinion is that the high antioxidant capacity of the compounds used inhibits the resulting nanotoxicity (Emsen & Turkez 2017).

The use of herbal products to inhibit nanotoxicity will reduce the level of side effects. Medicinal and aromatic plants used in many fields such as food, medicine and cosmetics are important in this regard (Giannenas et al. 2019). Tissue culture techniques, a current and biotechnological method, have made a significant contribution to the mass production of the medicinal and aromatic plants (Máthé et al. 2015). In order to obtain extract or active substance, the plants collected from nature can also disrupt the ecological balance. For this reason, the production of plants *in vitro* with tissue culture techniques makes a great contribution to such studies. In our study, we produced *A. reineckii* by tissue culture techniques and inhibited the nanotoxicity caused by ZnO through different extracts obtained from *A. reineckii*.

When shoot regeneration frequencies were examined, very high and very low hormone combinations had a negative effect on the shoot regeneration of the explants. Similarly, shoot tip explants of *Ceratophyllum demersum* L. were cultured and a decrease in shoot regeneration frequencies with high and low hormone application was reported (Emsen & Dogan 2018).

When shoot lengths were examined, the highest length value was obtained in the lowest concentrations of TDZ (0.25 mg/L). The increase in the ratio of TDZ in the culture medium caused the shoots to remain short. Similarly, negative effects of TDZ on shoot lengths were previously reported by Dewir et al. (2018) and Novikova & Zaytseva (2018). It has been reported that transferring *in vitro* cultures to nutrient media supplemented with low concentrations of TDZ (0.01 to 1.0 µM) would be a correct solution to avoid TDZ-induced adverse events such as short shoots (Novikova & Zaytseva 2018; Novikova et al. 2020). Another effective approach was to transfer the shoots in TDZ culture medium to hormone-free nutrient medium or to medium containing a plant growth regulator such as zeatin, 6-benzylaminopurine or GA_3 (Sujatha et al. 2008; Dhavala & Rathore 2010). In our current study, the shoot lengths were sufficient for us, as we conducted an activity study.

CONCLUSIONS

In general, the present study demonstrated that *in vitro* propagated *A. reineckii* showed protective role against ZnO-induced nanotoxicity. Especially the combined application of acetone extract obtained from this plant and ZnO highly inhibited the ZnO-induced cytotoxic effect. This result presented the idea that the extracts of *A. reineckii* can be added in certain proportions to products containing ZnO used in the cosmetic field.

Author's Contributions

BE designed the experiments and carried out extraction, cell culture, cytotoxicity experiments, IC carried out ZnO nanoparticle characterization, MD carried out *in vitro* regeneration process and all authors analysed the data and wrote the manuscript.

Statement of Conflict of Interest

Authors have declared no conflict of interest.

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Melissa officinalis: Antibacterial and Antioxidant Potential, Phenolic Profile and Enzyme Activities

Canan ÜLGEN[1], Arzu BİRİNCİ YILDIRIM[2], Arzu UÇAR TÜRKER[3]
[1]Şırnak University, Technology and Research Center, Şırnak, Türkiye, [2]Bolu Abant Izzet Baysal University, Department of Field Crops, Faculty of Agriculture, Bolu, Türkiye, [3]Bolu Abant Izzet Baysal University, Department of Biology, Faculty of Science and Art, Bolu, Türkiye
[1]https://orcid.org/0000-0002-8272-3370, [2]https://orcid.org/0000-0001-7712-2813, [3]https://orcid.org/0000-0001-9617-6673
✉: turker_a@ibu.edu.tr

ABSTRACT
Popularly referred to as lemon balm, *Melissa officinalis* L., has been used as a cure for gastrointestinal disorders, respiratory and cardiovascular diseases, mental and central nervous system problems, various cancers, headache, nervousness, and rheumatism. In this study, the phenolic profile, antioxidant potential, antibacterial activity, and enzyme activity of lemon balm grown in nature in Bolu, Turkey were determined. Furthermore, comparisons were made with plants grown *in vitro*. Individual phenolic analysis with HPLC-DAD showed that the most prevalent phenol was rosmarinic acid in both extracts and naturally-grown plants had higher amount than *in vitro*-grown ones. Similarly, naturally-grown plants had considerably greater levels of total phenol-flavonoid, scavenging activity for free radicals (DPPH), and phenol synthesis related enzyme (PAL). As for the enzymatic antioxidant activity (SOD and CAT), naturally-grown plants were found to have higher CAT activity and lower SOD activity. As a remarkable result, although plants grown *in vitro* showed moderate antibacterial activity, no effect was observed in naturally-grown plants. In general, these results showed that the *M. officinalis* grown in nature is exposed to more biotic and abiotic stress and increases their phenolic content remarkably and consequently antioxidant capacity.

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ÖZET
Halk arasında oğul otu olarak bilinen *Melissa officinalis* L., mide bağırsak rahatsızlıkları, solunum ve kalp damar hastalıkları, zihinsel ve merkezi sinir sistemi problemleri, çeşitli kanserler, baş ağrısı, sinirlilik ve romatizma için şifa olarak kullanılmaktadır. Bu çalışmada, Bolu, Türkiye'de doğada yetişen oğul otu bitkisinin fenolik profili, antioksidan potansiyeli, antibakteriyel etkisi ve enzim aktivitesi belirlenmiştir. Ayrıca *in vitro* yetiştirilen bitkilerle de karşılaştırılma yapılmıştır. HPLC-DAD ile yapılan bireysel fenolik analiz her iki özütde en yaygın fenolün rosmarinik asit olduğunu ve doğal olarak yetişen bitkilerde *in vitro* yetiştirilenlerden daha yüksek miktarda rosmarinik asit bulunduğunu göstermiştir. Benzer şekilde, doğal olarak yetişen bitkilerin önemli ölçüde daha yüksek toplam fenol-flavonoid seviyelerine, serbest radikalleri temizleme aktivitesine (DPPH) ve fenol sentezi ile ilgili enzime (PAL) sahip olduğu görülmüştür. Enzimatik antioksidan aktivitesi olarak (SOD ve CAT), doğal olarak yetişen bitkilerin daha yüksek CAT aktivitesine ve daha düşük SOD aktivitesine sahip olduğu bulunmuştur. Dikkat çekici bir sonuç olarak *in vitro* yetiştirilen bitkiler orta derecede antibakteriyel aktivite göstermesine rağmen doğal olarak yetişen bitkilerde etki görülmemiştir. Genel olarak bu sonuçlar, doğada yetişen *M. officinalis' in* daha fazla biyotik ve abiyotik strese maruz kaldığını ve fenolik içeriğini ve dolayısıyla antioksidan kapasitesini

Bitki Fizyolojisi

Araştırma Makalesi

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Anahtar Kelimeler
Antibakteriyel
Antioksidan
Oğul otu
Melissa officinalis
Fenol

önemli ölçüde arttırdığını göstermiştir.

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INTRODUCTION

The Lamiaceae plant *Melissa officinalis* L., commonly known as bee balm or lemon balm, is indigenous to the Mediterranean basin and southern Europe (Davis, 1978). It has been used as a medicinal plant in the remedy of headache, mental and central nervous system problems, respiratory and cardiovascular diseases, various cancers, gastrointestinal disorders, nervousness, and rheumatism (Adinee et al., 2008; Shakeri et al., 2016; Petrisor et al., 2022). It has a worldwide medical reputation, especially as an antioxidant, anxiolytic, antidepressant, antipyretic, carminative, anti-inflammatory, spasmolytic expectorant, antimicrobial, sedative, digestive, antitumor, hypoglycemic, antinociceptive, hypolipidemic, antihypertensive, hepatoprotective, and memory enhancer (Gbolade & Lockwood, 1989; Barros et al., 2013; Shakeri et al., 2016; Ulgen et al., 2020; Petrisor et al., 2022). According to phytochemical studies, the principal active components of *M. officinalis* include volatile substances, triterpenes, phenolic acids, and flavonoids (apigenin and luteolin derivatives) (Barros et al., 2013). According to WHO monographs on several medicinal plants, hydroxycinnamic acids, often known as rosmarinic acid, are among the recognized phytochemicals and are indicators of quality control (Shakeri et al., 2016). Essential oil, flavonoids, and phenolic acids, including rosmarinic and caffeic acids, have all been linked to these biological effects (Mencherini et al., 2007). Recently, the aqueous extracts and decoctions of lemon balm's aerial portions have attracted significant interest as potential food ingredients due to their antimicrobial and antioxidant properties. They give extra health advantages as well as good protection in a number of dietary compositions (Carocho et al., 2016; Caleja et al., 2018). It is also widely used for seasoning and flavoring (Gbolade & Lockwood, 1989; Carocho et al., 2015).

Phenolic compounds are the most significant secondary metabolites that accumulate spontaneously in plants and play a crucial role in the ability of plants to withstand stressful situations as non-enzymatic antioxidants (Selmar & Kleinwächter, 2013). Additionally, plants elevate antioxidant enzyme expression like SOD (superoxide dismutase), CAT (catalase) and ascorbate peroxidase to combat oxidative stress brought on by biotic and abitoc stresses, which generates reactive oxygen species (ROS) like hydrogen peroxide, hydroxyl radicals, singlet oxygen and superoxide (Sharma et al., 2012; Ighodaro et al., 2018). The primary enzyme in the manufacture of phenol is PAL (phenylalanine ammonia lyase), which serves in higher plants as a secondary metabolic route. The defensive mechanism PAL, which is responsible for the formation of phenolic constituents in plants, is the principle stimulating reaction in plants to numerous biotic and abiotic factors (MacDonald & D'Cunha, 2007).

M. officinalis attract particular attention due to their medicinal and health benefit properties. They have been used in food, nutraceutical, pharmaceutical, and cosmetic industries (Carović-Stanko et al., 2016). The purpose of this study is to indicate the phenolic profiles, antibacterial potential and antioxidant capacities (enzymatic and non-enzymatic) of *M. officinalis* making a comparison between naturally-grown and *in vitro*-grown shoots.

MATERIAL and METHOD

Plant Materials and Extraction

M. officinalis L. subsp. *officinalis* L. was collected from Bolu, Turkey and identified utilizing "Flora of Turkey and the East Aegean Islands" (Davis, 1978) keeping voucher specimens-AUT-2008 in Department of Biology (BAIBU). Field-grown shoots were obtained from the plant. *In vitro*-grown shoots were regenerated according to our prior research (Ulgen et al., 2020; Ulgen et al., 2021). Field-grown and *in vitro*-grown lemon balm shoots were collected, and the shoots were then lyophilized and crushed into a powder. Methanolic extract was obtained at 40 °C for 18 h and then vacuum-vaporized at 40 °C. The residual part was then lyophilized after being dissolved in distilled water.

Phenolic Constituents

Individual Phenolic Compounds

Two methanolic extracts obtained from naturally-grown and *in vitro*-grown *M. officinalis* were quantitatively evaluated for rosmarinic acid, quercetin, rutin hydrate, gallic acid monohydrate, myricetin and caffeic acid (Sigma®) using high performance liquid chromatography (HPLC) combined with a diode array detector (DAD) (VWR-Hitachi LaChrom Elite®). Operational parameters were

configured as previously reported in our study (Turker et al., 2021).

Total Phenol and Flavonoid Content

Colorimetric methods (Folin-Ciocalteu and aluminum chloride) were used respectively for the determination of phenolic and flavonoid content in methanol extracts according to our previous report (Turker et al., 2021). For total phenol content, they were given as mg g^{-1} gallic acid equivalent (GAE) g^{-1} dry extract, and for total flavonoid content, as mg g^{-1} catechol equivalent (CE) g^{-1} dry extract.

Antioxidant Activity

Scavenging activity of free radicals in *M. officinalis* methanol extracts was appraised using the DPPH (1,1-diphenyl-2-picrylhydrazyl) test according to our previous report (Turker et al., 2021). The method relies on the elimination of DPPH by substances at 517 nm using a UV-vis Spectrophotometer (Hitachi U-1900®). Ascorbic acid was utilized as an antioxidant reference.

Antibacterial Activity

Using 3 Gram-positive bacteria [*Staphylococcus epidermidis* (ATCC 12228), *Staphylococcus aureus* (ATCC 25923) and *Streptococcus pyogenes* (ATCC 19615)] as well as 7 Gram-negative bacteria [*Serratia marcescens* (ATCC 8100), *Escherichia coli* (ATCC 25922), *Salmonella typhimurium* (ATCC 14028), *Pseudomonas aeruginosa* (ATCC 27853), *Proteus vulgaris* (ATCC 13315), *Escherichia coli* (ATCC 25922), *Enterobacter cloacae* (ATCC 23355) and *Klebsiella pneumoniae* (ATCC 13883)], the disc diffusion assay was utilized to determine two distinct methanolic extracts of *M. officinalis* according to our previous report (Turker et al., 2021). Concentration of each bacterial culture was adjusted to the 0.5 McFarland with saline (0.9% NaCl) using densitometer McFarland Densitometer (Biosan®) and then cotton swabs were employed to inoculate Mueller Hinton agar plates. Filter paper discs containing 13 µl of the extracts (sterilized with 0.22 µm filter) were placed into inoculated petri plates. Positive controls include erythtomycin, tetracycline, and ampicillin (Bioanalyse®). Water and methanol were used as negative controls. Inhibitory zones after 16–18 hours of incubation at 37°C in petri dishes were determined.

Enzymatic Antioxidant Activity

Extraction of Enzymes and Protein Designation

To assess SOD and CAT enzyme activity, *M. officinalis* shoots obtained from 2 distinct sources were first processed for the extraction of enzymes and protein measurement. Lowry technique (Lowry et al., 1951) was used to calculate the protein content of the shoots employing bovine serum albumin as a reference protein. It was performed in regard to our preceding report (Ulgen et al., 2021).

Superoxide Dismutase (SOD) Enzyme Activity

The method mentioned in Ulgen et al. (2021) was used to monitor SOD activity. Absorbance was measured at 560 nm. One SOD unit was defined as the quantity of protein required to reduce nitroblue tetrazolium (NBT) by 50% in the reaction, and enzyme activity was reported as Unit mg^{-1} protein.

Catalase (CAT) Enzyme Activity

The method mentioned in Ulgen et al. (2021) was used to monitor CAT activity. CAT activity was evaluated by measuring the reduction in absorbance at 240 nm induced by catalase enzyme breakdown of H_2O_2. The H_2O_2 extinction coefficient of 0.0392 mM cm^{-1}, which is utilized to indicate activity, is given as mmol H_2O_2 mg^{-1} protein.

Phenylalanine Ammonium Lyase (PAL) Activity

The method mentioned in Ulgen et al. (2021) was used to monitor PAL activity. PAL activity was measured at 270 nm.

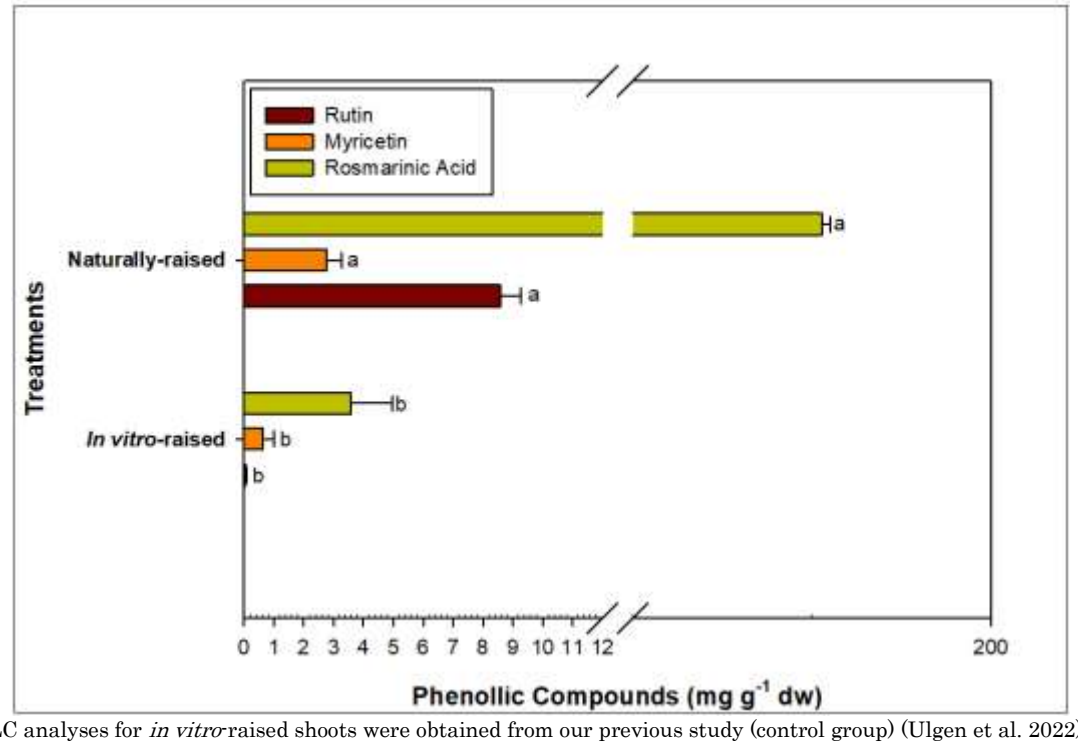
Data Analysis

All experiments had a totally randomized design. Data were analyzed using ANOVA as well as Duncan's multiple range tests in SPSS version 26 (SPSS Inc, Chicago, IL, USA). All of the data in the tables was provided as a mean value with a SE (standard error).

RESULTS and DISCUSSION

Six individual phenolic constituents included in methanol extracts of naturally-grown shoot materials were analyzed with HPLC-DAD system (Figure 1). HPLC analysis results for *in vitro*-raised shoots were collected from our previous work (control group) (Ulgen et al., 2021) to compare with naturally-raised ones. Rosmarinic acid was found to be the most prevalent phenol in both extracts and naturally-grown *M. officinalis* shoots contained higher amount of phenols than *in vitro*-grown shoots (Figure 1). In parallel to our results, the most prevalent phenolic in this species, according to several earlier studies was rosmarinic acid (Caniova & Brandsteterova, 2001; Fecka & Turek, 2007; Lee, 2010; Ziaková & Brandsteterová, 2003; Draginic et al., 2022; Petrisor et al., 2022; Abdellatif et al., 2023; Silva et. al., 2023). Naturally-grown and *in vitro*-grown plants involved rosmarinic acid (190.56±0.5 mg g^{-1} and 3.58±1.4 mg g^{-1}), rutin (8.58±0.7 mg g^{-1} and 0.07±0.01 mg g^{-1}) and myricetin (2.76±0.5 mg g^{-1} and 0.64±0.36 mg g^{-1}), respectively. Caffeic acid, quercetin and gallic acid were not detected in both extracts. Although the second most abundant phenol in natural plants was rutin, it was myricetin in *in*

vitro-grown plants. On the other hand, in another study, HPLC-DAD evaluation of an *M. officinalis* extract revealed the presence of rosmarinic acid (principal), caffeic acid, and m-coumaric acid (the least prevalent) (Dastmalchi et al., 2008). Pereira et al. (2014) listed caffeic acid, gallic acid, chlorogenic acid and ellagic acid in *M. officinalis* ethanol extract. Moreno et al. (2006) also detected rosmarinic acid, carnosic acid and carnosol in the leaves and flowers of *M. officinalis*, and they reported 5.5 g rosmarinic acid 100 g extract^{-1} in flowering plants. Barros et al. (2013) evaluated the phenolic constituents of garden grown and *in vitro*-cultured *M. officinalis* infusions. In parallel to our study, they found rosmarinic acid as the abundant phenolic being the lowest in *in vitro*-grown materials (15.46 mg g^{-1}) that is higher than our result (3.58 mg g^{-1}). On the contrary, naturally-grown sample in our study contained higher rosmarinic acid (190.56 mg g^{-1}) than garden-grown materials (20.96 mg g^{-1}) in the study of Barros et al. (2013). Spiridon et al. (2011) revealed the presence of caffeic acid (5.78%), p-coumaric acid (6.78%), rosmarinic acid (11.9%), quercetin glucoside (16.46%) and kaempherol diglucoside (59.09%) with HPLC-MS technique.



*Results of HPLC analyses for *in vitro*-raised shoots were obtained from our previous study (control group) (Ulgen et al. 2022) to make a comparison with naturally-raised shoots.

Figure 1. HPLC-DAD analysis of phenolic constituents in naturally-raised and *in vitro*-raised *M. officinalis*.

Şekil 1. Doğal olarak yetişen ve in vitro olarak yetiştirilen M. officinalis' in fenolik bileşenlerin HPLC-DAD analizi.

Total phenolic and flavonoid contents (Figure 3) and antioxidant capacity (Figure 4) of naturally-grown extracts were determined. Furthermore, to compare naturally-raised shoots to *in vitro*-raised shoots, we took the control group's total phenol-flavonoid and antioxidant results from our earlier work (Ulgen et al., 2021). It was observed that naturally-raised sample comprised higher total phenol (374.35±1.3 mg GAE g^{-1} dry extract) and flavonoid (327.61±1.6 mg CE g^{-1} dry extract) content than *in vitro*-raised extract (31.66±0.7 mg GAE g^{-1} and 32.50±0.6 mg CE g^{-1} dry extract, respectively). Significantly, the naturally-grown shoots had 12 and 6 times higher total phenol and flavonoid content, respectively, compared to *in vitro*-grown ones (Figure 3). The result obtained from natural habitat plant in our study was better than the findings of other previous studies. For example, Moreno et al. (2006) reported total phenolics of water, methanol and acetone extracts of *M. officinalis* as 3 g, 12 g and 19 g of GAE 100 g of extract^{-1}, respectively.

Atanassova et al. (2011) exhibited total phenol and flavonoid content in 80% aqueous methanol extract of *M. officinalis* as 48.86 mg GAE 100 g DW^{-1} and 45.06 mg CE 100 g DW^{-1}, respectively. Mabrouki et al. (2018) demonstrated total phenol and flavonoid content in ethanol extract of *M. officinalis* as 63.00 mg GAE g^{-1} and 3.61 mg QE g^{-1} dry extract. Draginic et al. (2022) found out total phenol and flavonoid level in ethanolic macerate as 73.39 mg GAE g^{-1} and 6.23 mg QE g^{-1} dry extract, respectively. Furthermore, Spiridon et al. (2011) indicated *M. officinalis* total phenol and flavonoid content as 54.9 mg GA g^{-1} and 25.8 mg R g^{-1}, respectively.

DPPH free radical scavenging activity of both extracts showed that naturally-grown plants were more

effective (Figure 4) and strong antioxidant capacity was observed with naturally-raised plants (IC_{50}=34.86 µg mL^{-1}). *In vitro* propagated plants exhibited very low antioxidant capacity (IC_{50}>200 µg mL^{-1}) comparing to naturally growing plants. The presence of phenolic acids, notably hydroxycinnamic acid derivatives like rosmarinic acid, is thought to be the cause of the antioxidant action of *M. officinalis* extracts (Caniova & Brandsteterova, 2001). Our result obtained from naturally-grown plants was in good agreement with some previous studies. For example, free radical scavenging activities (IC_{50}) of ethanolic macerate of *M. officinalis* was reported as 9.95 µg mL^{-1} by Draginic et al. (2022). Moreno et al. (2006) exhibited IC_{50} of methanol and acetone extracts of *M. officinalis* as 18 and 25.6 µg mL^{-1}, respectively. IC_{50} value of *M. officinalis* ethanol extract was determined as 18.16 µg mL^{-1} by Mabrouki et al. (2018). Atanassova et al. (2011) recorded IC_{50} value of 80 % aqueous methanol as 10.87 µg mL^{-1}. Moreover, Mencherini et al. (2007) reported considerable free radical scavenging action in a concentration-dependent manner for aqueous ethanol extract and rosmarinic acid showing IC_{50} values as 18.5 and 3.1 µg mL^{-1}, respectively. On the other hand, some studies reported the antioxidant capacity that is lower than our findings. Dastmalchi et al. (2008) reported IC_{50} value of naturally-grown *M. officinalis* leaves as 134.16 µg mL^{-1} and Spiridon et al. (2011) showed the effectiveness of *M. officinalis* for antioxidants capacity as IC_{50} = 87.28 µg mL^{-1}. *M. officinalis*'s therapeutic effects in the avoidance and treatment of oxidative stress-related illnesses, such as cardiovascular and neurological disorders, can be ascribed to its antioxidant power.

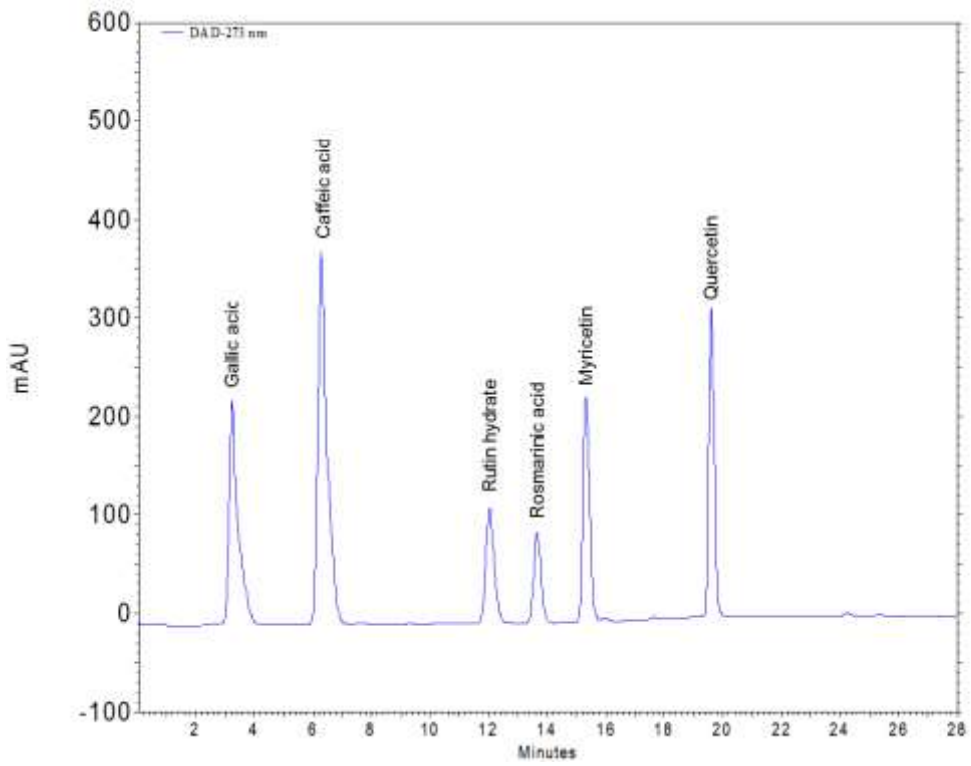
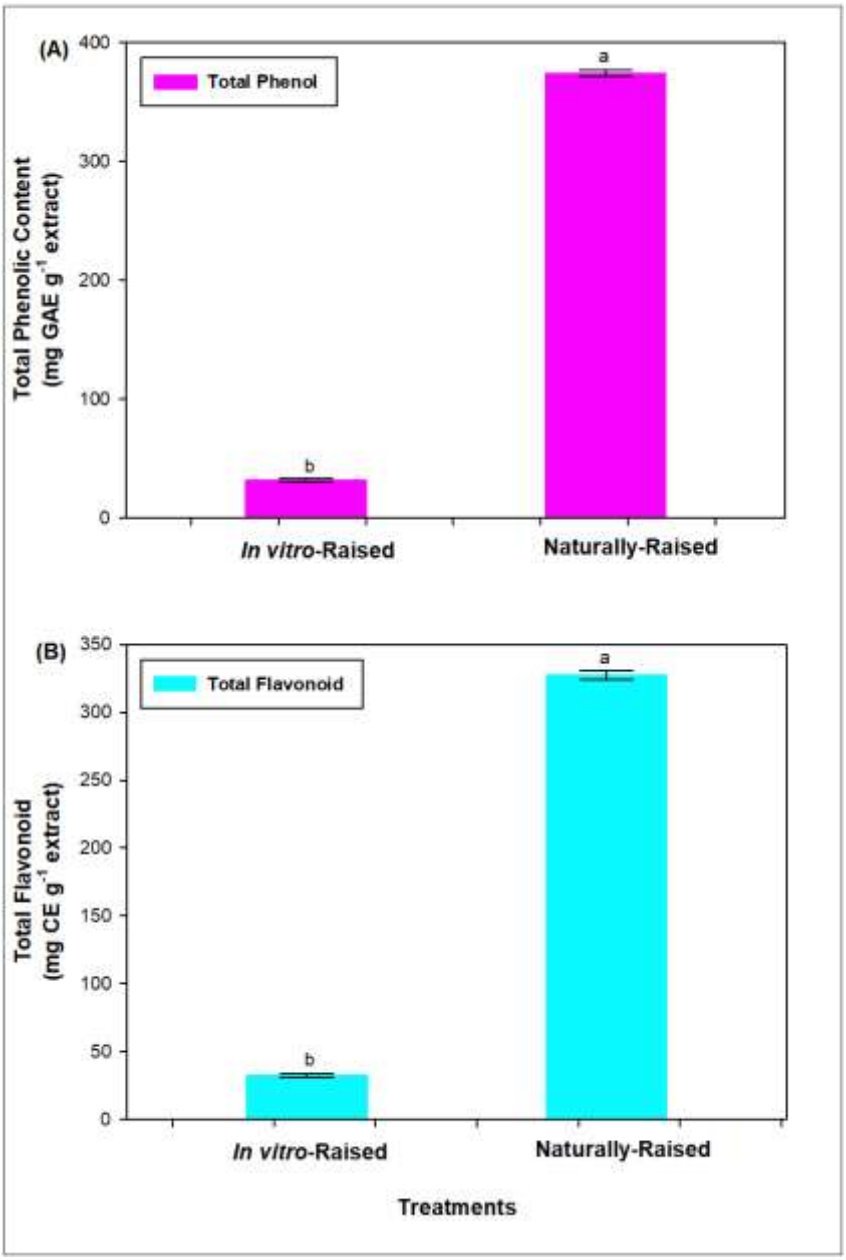


Figure 2. HPLC chromatogram of the phenolic standards. Retention times: 1. Gallic acid-3.2 min, 2. Caffeic acid-6.2 min, 3. Rutin hydrate-12.0 min, 4. Rosmarinic acid-13.6 min, 5. Myricetin-15.3 mi n, 6. Quercetin-19.6 min

Şekil 2. Fenolik standartların HPLC kromatogramı. Retensiyon zamanları: 1. Gallik asit-3.2 dk, 2. Kafeik asit-6.2 dk, 3. Rutin hidrat-12.0 dk, 4. Rosmarinik asit-13.6 dk, 5. Myricetin-15.3 dk, 6. Quercetin-19.6 dk.

Antibacterial activity of shoots obtained from nature and *in vitro*-propagation was presented in Table 1. Antibacterial assay showed the opposite result from antioxidant activity. It is quite intriguing because only the *in vitro*-grown *M. officinalis* showed antibacterial action against 8 pathogens and naturally-grown plants were not effective against tested bacteria (Table 1). Antibacterial activity of *in vitro*-grown *M. officinalis* may be related to the essential oil components involved in the plant. Because, it was observed that *in vitro* propagated plants were more aromatic than the shoots that were collected from nature. Rosmarinic acid is not thought to have a determinant effect on antibacterial activity of *M. officinalis* in our study because this phenolic acid is much less in *in vitro*-grown plants than in wild-collected ones. Similarly, Moreno et al. (2006) reported no antibacterial activity of rosmarinic acid at low concentrations (5–250 µg mL^{-1}). Mencherini et al.

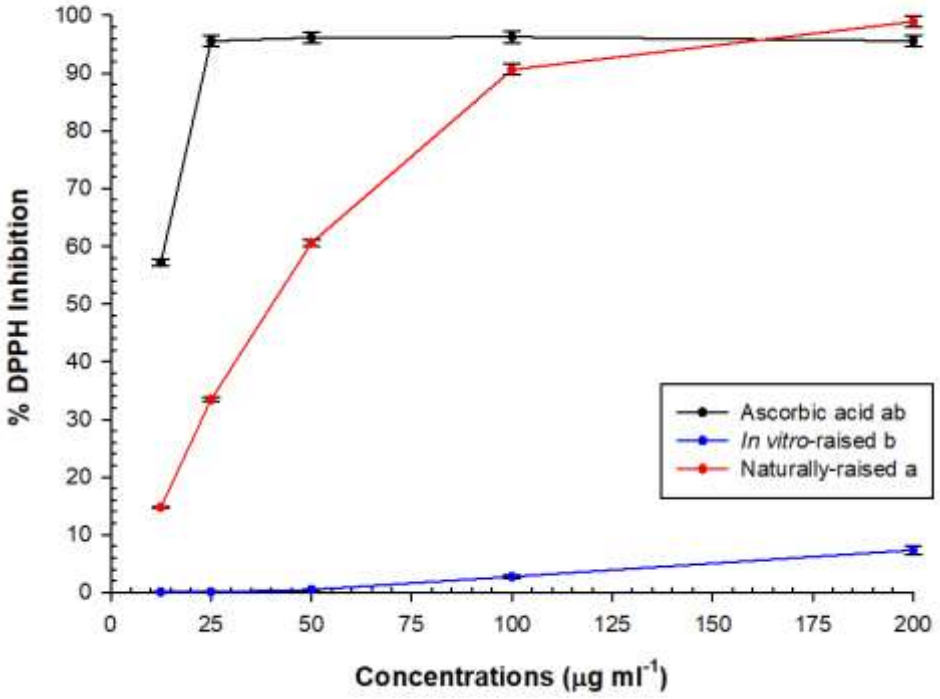
(2007) indicated the bacteriostatic effects of rosmarinic acid at higher concentrations like 0.12 mg mL µg mL^{-1} against Gram positive bacteria (*S. epidermidis* and *S. aureus*) and 2.0 mg mL µg mL^{-1} against Gram negative bacteria (*E. coli* and *P. aeruginosa*). According to prior studies, the antibacterial effect of rosmarinic acid was observed at very high concentrations. In our study, since the rosmarinic acid concentration in the crude extract was lower than its pure form, its antibacterial activity was probably not sufficient. Ivanov et al. (2022) reported that rosmarinic acid exhibited weak potency against *S. aureus* and moderate activity against *S. pyogenes* and *E. cloacae*. Mabrouki et al. (2018) indicated antibacterial activity of *M. officinalis* ethanol extract against *S. aureus* (25 mm inhibition zone), *P. aeruginosa* (17.5 mm) and *E. coli* (18.1 mm). Generally main components of *M. officinalis* essential oil were ß-caryophyllene, citral, geranial, neral, germacrene D, thymol and C-citronellal and they demonstrated antibacterial efficacy against the studied microorganisms (Ehsani et al. 2017; Çelebi et al., 2023; Petrisor et al., 2022).



*Results of total phenol and flavonoid content for *in vitro*-raised shoots were obtained from our previous study (control group) (Ulgen et al. 2022) to make a comparison with naturally-raised shoots.

Figure 3. Total phenol (A) and flavonoid (B) content of naturally-raised and *in vitro*-raised *M. officinalis*. GAE: Gallic acid equivalent, CE: Catechol equivalent.

Şekil 3. Doğal olarak yetişen ve in vitro olarak yetiştirilen M. officinalis' in toplam fenol (A) ve flavonoit (B) içeriği. GAE: Gallik asit eşdeğeri, CE: Katekol eşdeğeri.



*Results of % DPPH inhibition for *in vitro*-raised shoots were obtained from our previous study (control group) (Ulgen et al. 2022) to make a comparison with naturally-raised shoots.

Figure 4. DPPH free radical scavenging activity of naturally-raised and *in vitro*-raised *M. officinalis*. IC_{50} values: Ascorbic acid: <200 µg/ml, *In vitro*-raised: >200 µg/ml and Naturally-raised: 34.86 µg/ml.

Şekil 4. Doğal olarak yetişen ve in vitro olarak yetiştirilen M. officinalis' in DPPH serbest radikal süpürücü aktivitesi. IC_{50} değerleri: Askorbik asit: <200 µg/ml, in vitro yetiştirilen: >200 µg/ml and doğal olarak yetişen: 34.86 µg/ml.

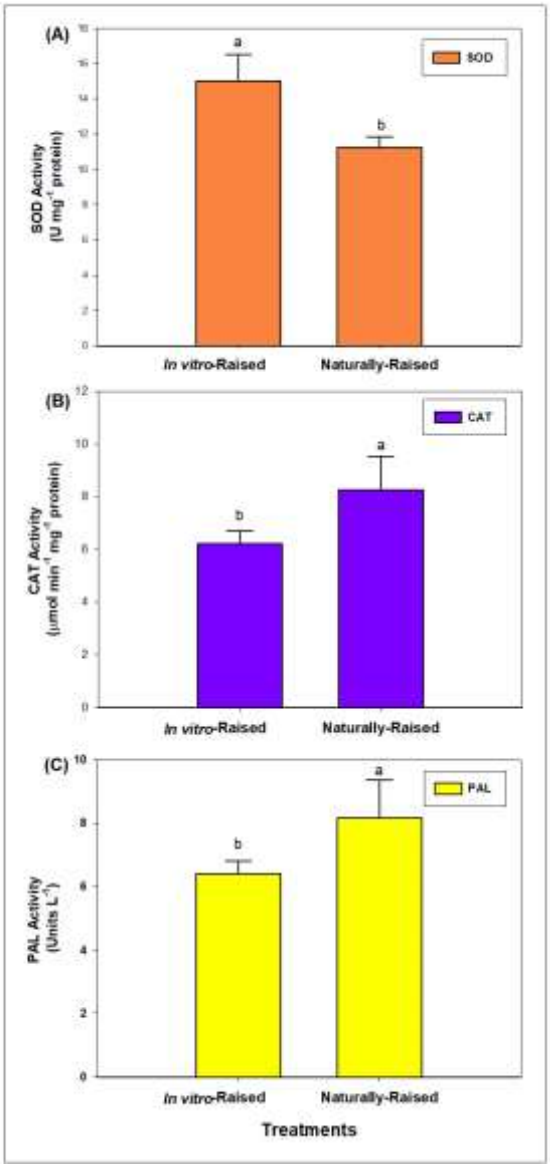
Table1. Antibacterial properties of naturally-raised and *in vitro*-raised *M. officinalis*. Different letters in each column indicate a significant difference ($P<0.05$).

Çizelge 1. Doğal olarak yetişen ve in vitro olarak yetiştirilen M. officinalis' in antibakteriyel özellikleri. Her sütundaki farklı harfler önemli bir fark gösterir (P <0.05).

Tested Bacteria	*In vitro*-raised	Naturally-raised	Ampicillin	Tetracycline	Erythtomycin
S.aureus	11±0.5^{b}	-	29±0.3^{b}	27±0.3^{b}	25±0.3^{c}
S.epidermidis	10±0.3^{c}	-	-	-	30±0.6^{b}
S.pyogenes	15±0.6^{a}	-	54±0.3^{a}	42±0.3^{a}	50±0.3^{a}
S.marcences	-	-	-	20±0.3^{e}	-
S.typhimurium	8±0.3^{d}	-	24±0.3^{c}	22±0.6^{d}	-
P.aeruginosa	8±0.3^{d}	-	-	11±0.3^{f}	-
P.vulgaris	10±0.1^{bc}	-	13±0.3^{f}	23±0.3^{d}	10±0.3^{d}
K.pneumonia	-	-	-	20±0.1^{e}	8±0.3^{e}
E.cloacae	9±0.3^{c}	-	21±0.3^{d}	25±0.1^{c}	-
E.coli	9±0.3^{c}	-	16±0.3^{e}	25±0.3^{c}	-

S. marcences and *K. pneumonia* were not susceptible to tested extracts. The best antibacterial activity was observed against *S. pyogenes* with 15 mm inhibiton zone. Generally, natural lemon balm inhibited Gram positive bacteria (*S. pyogenes*, *S. aureus* and *S. epidermidis*) more than Gram negative in our study. It might be due to the fact that gram-positive bacteria have single-layered cell walls, which differ from those of other bacteria. Gram-negative bacteria, on the other hand, have multi-layered cell walls, which may provide them resistance to plant extracts (Turker et al., 2021). Reviewed literature has demonstrated that *M. officinalis* extracts have greater antibacterial property against Gram-positive bacteria (Mencherini et al., 2007; Canadanović-Brunet et al., 2008; Stefanović and Comic, 2012; Abdellatif et al., 2023; Silva et. al., 2023). Some inhibitory potentials of lemon balm were also recorded against certain Gram-negative strains

(Carocho et al., 2015; Caleja et al., 2018; Abdellatif et al., 2023; Silva et. al., 2023). The majority of the tested microorganisms were susceptible to the reference antibiotic discs (ampicillin, tetracycline and erythtomycin) (Table 1). Methanol was employed as a negative control to alter the final concentrations of all extracts. Negative controls (methanol or water) had no inhibitory effect. Abdel-Naime et al. (2019) demonstrated antibacterial efficacy of *M. officinalis* crude extract and its derived fractions against a variety of human pathogenic microorganisms, which is consistent with the findings of our investigation. Their samples displayed varying degrees of inhibitory effectiveness against *P. aeruginosa* and *S. aureus*, with MICs ranging from 1.65 to 191.40 g ml^{-1}, whereas no antibacterial effects were noticed against *K. pneumoniae* and *E. coli*.



*Results of SOD, CAT and PAL activities for *in vitro*-raised shoots were obtained from our previous study (control group) (Ulgen et al. 2022) to make a comparison with naturally-raised shoots.

Figure 5. SOD, CAT and PAL activities of naturally-raised and *in vitro*-raised *M. officinalis*.

Şekil 5. Doğal olarak yetişen ve in vitro olarak yetiştirilen M. officinalis' in SOD, CAT ve PAL activiteleri.

SOD and CAT are two antioxidant enzymes that are not only essential but also crucial to the antioxidant defense of biological systems against free radical damage. SOD and CAT activities provide a first-line antioxidant defense system that plays a critical and basic role in overall defense mechanisms and tactics in biological systems (Ighodaro et al., 2018). Superoxide dismutase (SOD) is the cell's initial detoxifying enzyme and the most effective antioxidant. It catalyzes the dismutation of two molecules of superoxide anion (O_2) to hydrogen peroxide (H_2O_2) and molecular oxygen

(O_2), making the potentially hazardous superoxide anion less dangerous. CAT enzyme catalyzes the breakdown or reduction from hydrogen peroxide (H_2O_2) to water and molecular oxygen, providing the detoxification process beginning by SOD (Sharma et al., 2012). SOD and CAT activity of naturally-grown plants were detected and compared with *in vitro*-grown plants (Ulgen et al., 2021). We used enzyme activity values of SOD, CAT and PAL for *in vitro*-grown shoots from our previous study (Ulgen et al., 2021) to make a comparison between naturally-raised and *in vitro*-raised shoots. Although naturally-grown plants had higher CAT activity, *in vitro*-propagated plants exhibited higher SOD activity (Figure 5). Wild-grown plants are exposed to more biotic and abiotic stress so increased CAT activity was observed comparing to *in vitro*-propagated ones. SOD levels decrease with aging, although free radical production rises in biological systems (Ighodaro et al. 2018). The reason of lower SOD activity of the plant collected from nature may be related to the age of the wild plant that is relatively older than *in vitro*-grown ones. Many studies demonstrated increased SOD and CAT activity in *M. officinalis* that is exposed to different stress conditions like heat (Pistelli et al., 2019), salt (Ghasemian et al., 2021) and magnetic field (Ulgen et al., 2021).

An essential biosynthetic enzyme called phenylalanine ammonium lyase (PAL) catalyzes the initial step in the synthesis of phenolics, which are comprised in the body's defense system against a variety of biotic and abiotic stressors (Ghanati et al., 2007). Wild-raised shoots had higher PAL activity as expected comparing to *in vitro*-grown ones (Figure 5). It has been proven that the high phenol content of plants collected from nature is due to high PAL activity. Elevated PAL activity was exhibited with some stress factors like salt (Safari et al., 2020) and magnetic field (Ulgen et al., 2021).

It is obvious that different results have been obtained in different studies conducted in different parts of the world in terms of phenolic content and antioxidant activity of *M. officinalis*. The reason of this is the production and concentration of accumulated phenolics that are affected by a variety of internal and external variables, including plant physiology, harvesting time, geographical variation, age, developmental stage, soil composition, climate, elevation and pathogen attack type (Mabrouki et al., 2018; Pratyusha, 2022). Plants respond to environmental stress by increasing phenolic components (Turker & Yildirim, 2018; Turker et al., 2018; Thakur et al., 2019). Since plants grown *in vitro* are in a more controlled environment, they are relatively less exposed to stress than those collected from nature so *in vitro*-grown plants may not require a lot of phenol synthesis to survive.

CONCLUSION

Although many studies have been conducted on the antioxidant potency and phenolic constituents of *M. officinalis*, this study firstly ascertained the phenolic profile and antioxidant capacity of *M. officinalis* grown in Bolu, Turkey and antibacterial activity of *in vitro*-grown material was demonstrated for the first time. It has been determined that naturally-grown *M. officinalis* has strong antioxidant potency due to its high phenolic content, especially rosmarinic acid. High phenolic content of wild growing plants that are exposed to more stressful environmental conditions was verified with high enzyme activities of PAL and CAT. Essential oil of *in vitro*-grown materials that was more aromatic than plants collected from nature should be investigated in the next studies. Furthermore, subsequent studies should perform on enhancing the quantity of phenolics like rosmarinic acid in *in vitro*-grown plants by exerting various stress types.

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Author's Contributions

The contribution of the authors is equal.

Statement of Conflict of Interest

Authors have declared no conflict of interest.

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Some Physiological and Biochemical Effects of *Bacillus thuringiensis* LU3 Biopriming in Common Wheat (*Triticum aestivum* L.) under Salt Stress

Müge TEKER YILDIZ[1], Okan ACAR[2] ✉, Furkan ÖZTÜRK[3], Nurcihan HACIOĞLU DOĞRU[4]
[1,3] Çanakkale Onsekiz Mart University, School of Graduate Studies, Biology Department, Çanakkale, Türkiye, [2,4]Çanakkale Onsekiz Mart University, Faculty of Science, Biology Department, Çanakkale, Türkiye
[1]https://orcid.org/0000-0001-7657-9811, [2]https://orcid.org/0000-0002-9818-8827, [3]https://orcid.org/0000-0002-9752-058
[4]https://orcid.org/0000-0002-5812-9398
✉: oacar@comu.edu.tr

ABSTRACT
Salt stress is one of the main abiotic stresses limiting sustainable crop production in the world. Biopriming is the technique involving the use of beneficial and environmentally friendly biological agents to improve the physiological functioning of seeds. Plant growth-promoting rhizobacteria (PGPR) are found in the rhizosphere of plants and have the potential to cope with salinity stress. In this study, the effects of *Bacillus thuringiensis* LU3 (Bt LU3) biopriming application on two common wheat (*Triticum aestivum* L.) varieties (Sultan-95 and Tosunbey) under salt stress (0, 100 and 200 mM NaCl) on physiological (root and shoot length, biomass, dry weight, specific leaf area (SLA)), and biochemical parameters (pigment content, total protein content, hydrogen peroxide content (H_2O_2), lipid peroxidation content (TBARS) and antioxidant enzyme activities (peroxidase activity (POX), glutathione reductase activity (GR))) were investigated. As a result, it was determined that salt-sensitive Sultan-95 had better growth with Bt LU3 biopriming compared to salt-tolerant Tosunbey.

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Tuz Stresi Altındaki Ekmeklik Buğdayda *Bacillus thuringiensis* LU3 ile Biyopriming'in Bazı Fizyolojik ve Biyokimyasal Etkileri

ÖZET
Tuz stresi, dünyada sürdürülebilir tarımsal üretimi sınırlayan başlıca abiyotik streslerden biridir. Biyopriming, tohumların fizyolojik işleyişini iyileştirmek için faydalı ve çevre dostu biyolojik ajanların kullanımını içeren bir tekniktir. Bitki büyümesini teşvik eden rizobakteriler (PGPR), bitkilerin rizosferinde bulunur ve tuzluluk stresi ile başa çıkma potansiyeline sahiptir. Bu çalışmada, tuz stresi altında (0, 100 ve 200 mM NaCl) iki ekmeklik buğday (*Triticum aestivum* L.) çeşidine (Sultan-95 ve Tosunbey) *Bacillus thuringiensis* LU3 (Bt LU3) biyopriming uygulamasının fizyolojik (kök ve gövde uzunluğu, biyokütle, kuru ağırlık, spesifik yaprak alanı (SLA)) ve biyokimyasal parametreler (pigment içeriği, toplam protein içeriği, hidrojen peroksit içeriği (H_2O_2), lipid peroksidasyon içeriği (TBARS) ve antioksidan enzim aktiviteleri (peroksidaz aktivitesi (POX), glutatyon redüktaz aktivitesi (GR))) araştırılarak tuza duyarlı Sultan-95'in Bt LU3 biyopriming ile tuza dayanıklı Tosunbey'e göre daha iyi büyüme ve performans gösterdiği belirlenmiştir.

Bitki Fizyolojisi

Araştırma Makalesi

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Anahtar Kelimeler
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Tuz stresi
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INTRODUCTION
Common wheat (*Triticum aestivum* L.) is a strategic food source, with an estimated area of more than 200 million hectares in 2020. The area of cultivation of wheat is expected to remain fairly constant in 2030 (Erenstein et al., 2021). Each year, approximately 200

million tons of wheat are produced (FAO, 2021). Abiotic and biotic stresses are the major factors limiting crop production in the changing global climate (Islam et al., 2016; Hossain et al., 2021). Salt stress is one of the most prominent abiotic stress factors limiting plant yield, it constitutes a threat to global food security by negatively affecting plant growth and development. Nevertheless, osmotic stress occurs when salt accumulated in the soil and the plant restricts the water uptake from the roots, restricting the nutrient uptake, and causing the nutrient balance and membrane properties to be damaged (Munns & Tester, 2008; Rahnama et al., 2010).

Reactive oxygen species (ROS) are produced naturally in plant cells such as photosynthesis and respiration. When the balance between the increase in the content of ROS in cells and detoxification is disturbed, oxidative stress occurs, and the antioxidant defense system plays an important role in the prevention of oxidative damage. Antioxidant defense systems constitute of enzymatic antioxidants (such as Superoxide Dismutase (SOD); Catalase (CAT), Ascorbate Peroxidase (APX), Glutathione Reductase (GR), etc.) or non-enzymatic antioxidants (Ascorbic Acid (ASH), Glutathione (GSH), etc.) (Gill & Tuteja, 2010).

Because of the complexity of basic salt tolerance mechanisms have limited success in the responses of plants to salinity during their growth stages of them (Taie et al., 2013). On the other hand, the seeds are exposed to pre-treatment with different agents via priming (Ali et al., 2017; Subramanyam et al., 2019), thereby improving the metabolic activity, germination, and seedling formation of the seed under adverse conditions. Biopriming is used as an alternative new seed priming method by combining physiological (seed hydration) and biological (seed inoculation with beneficial organisms) mechanisms (Migahid et al., 2019 Rhaman et al., 2020).

It has been reported that plant growth-promoting rhizobacteria (PGPR) and biopriming applications provide an advantage in reducing the negative effects of chemical fertilizers and pesticides on the environment based on sustainable agriculture (Kloepper & Schroth, 1978). In salt conditions, PGPR has been reported to contribute to plant growth in sorghum, chickpeas, maize, wheat, and rice (Sarig et al., 1992; Jacoud et al., 1998; Alam et al., 2001; Hamaoui et al., 2001; Saubidet et al., 2002). Paul & Nair (2008) reported that PGPR bacteria *Pseudomonas flourescens* isolated from coastal agricultural soils, produces proteins that alleviate the effect of salt stress and may be a suitable inoculant for plant growth in saline soils. Similarly, it was determined that wheat seedlings inoculated with indole 3-acetic acid (IAA) producing *Pseudomonas* isolates increased root and shoot growth by 40% and 52%, respectively, under salt stress (100 mmol L^{-1} NaCI) (Egamberdieva, 2009).

Although nickel (Ni) is a heavy metal, it is an essential microelement for plants (Ain et al., 2016). It has been determined that 2% of 35 heterotrophic bacterial isolates obtained from the root rhizosphere of the nickelophilic *Alyssum pinifolium* in Ezine (Çanakkale-Türkiye), which is distributed (Esen, 2016) in soils containing very high Ni (1702 mg g^{-1}) but not saline (0.33 dS m^{-1}), have high salt tolerance (Öztürk & Hacıoğlu Doğru, 2020). It is well known that the content of Na^+ increased with salt stress negatively affects the membrane integrity and the water potential in the root zone. Nevertheless, positive results are obtained with seed pre-treatment in reducing the negative effects of salt stress, which reduces plant growth and yield, or in improving the damage.

In this study, we focused on effects of *Bacillus thuringiensis* LU3 biopriming applications on physiological (root and shoot length, biomass, dry weight, specific leaf area (SLA)) and biochemical parameters (pigment content, total protein content, hydrogen peroxide content (H_2O_2), lipid peroxidation content (TBARS) and antioxidant enzyme activities (peroxidase activity (POX), glutathione reductase activity (GR))) in two wheat varieties (Sultan-95 and Tosunbey) under salt stress (0, 100 and 200 mM NaCI).

MATERIALS and METHODS

Bacterial Strain

The *Bacillus thuringiensis* LU3 (Bt LU3) strain was used in this study (Öztürk & Hacıoğlu Doğru, 2020). It was previously isolated from *A. pinifolium* rhizospheric soil in the Ezine region of Çanakkale (Türkiye) and showed its ability to synthesize IAA and inorganic phosphate (Karakaş et al., 2022).

Experimental Material and Seed Biopriming

In his study, two common cultivars (*T. aestivum* L.) were used. Salt-tolerant Tosunbey variety (Önay, 2019) was obtained from Ankara Field Crops Central Research Institute (Türkiye) and the susceptible salt-sensitive Sultan-95 variety (Önay, 2019) was obtained from Transitional Zone Agricultural Research Institute (Eskisehir-Türkiye). Surface sterilization of seeds was carried out with a 5% sodium hypochlorite solution (Abdul-Baki et al., 1979). Bt LU3 strain was cultured in nutrient broth at 28°C for 4 days. The bacterial cells were harvested by centrifugation for 4 min at 2000g. The pellets were re-suspended in sterile distilled water (dH_2O). The bacterial culture was adjusted to 10^8 colony-forming units (CFU) mL^{-1}. This bacterial suspension of Bt LU3 was used to biopriming wheat seed for 24 h. Then the seeds were washed and dried with blotting paper. The study was carried out according to the completely randomized block design with three replicates. Plants were grown in triplicate

and each group contained at least 3 petri dishes (Figure 1). Plants were grown in an *in vitro* plant growth chamber at 24±2°C in a photoperiod (light/dark cycle of 16/8 h). Additionally, the seedlings were irrigated with Hoagland nutrient solution (100%) (Hoagland & Arnon, 1950). Following the growth, the tissues were stored in a deep freeze (-20°C) by sampling the leaf tissues of 7d-old seedlings.

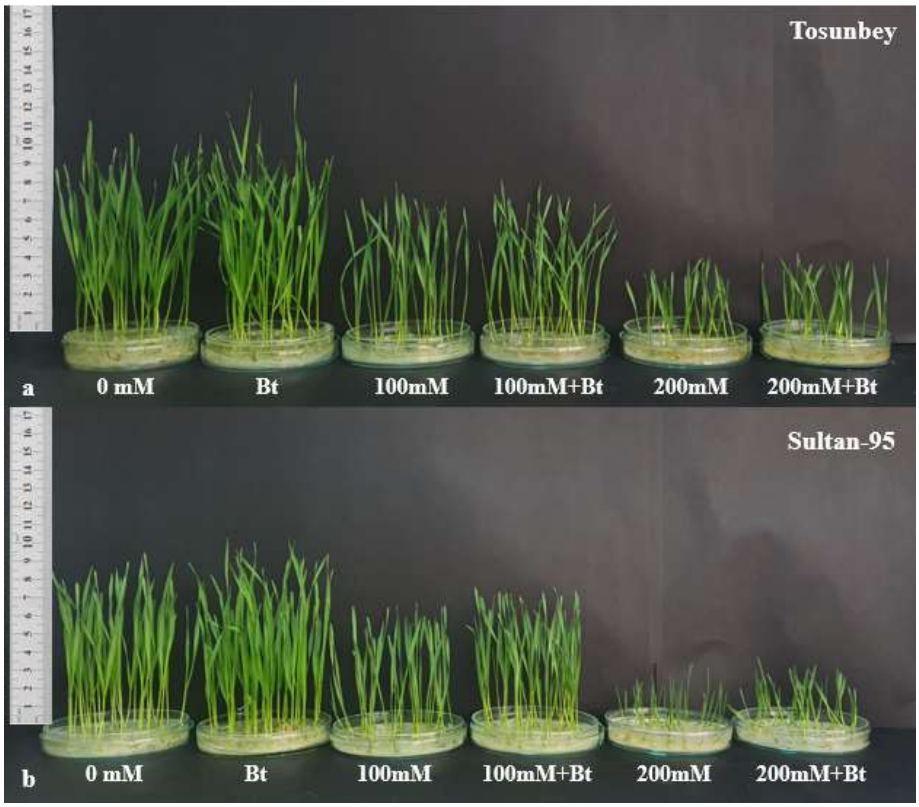

Figure 1. Wheat seedlings inoculated and uninoculated with Bt LU3 at different salt concentrations (Control; 0 mM, Bt; Bt LU3, Salt stress; 100 and 200 mM NaCl, Bt LU3 +salt stress; 100 mM+Bt, 200 mM+Bt) (a: Salt-tolerant variety; Tosunbey, b: Salt-sensitive variety; Sultan-95).

Şekil 1. Farklı tuz konsantrasyonlarında Bt LU3 aşılı ve aşısız buğday fideleri (Kontrol; 0 mM, Bt; Bt LU3, Tuz stresi; 100 ve 200 mM NaCl, Bt LU3+tuz stresi; 100 mM+ Bt, 200 mM+Bt) (a: Tuz stresine dayanıklı çeşit; Tosunbey, b: Tuz stresine duyarlı çeşit; Sultan-95).

Root and Shoot Length (cm)

The green part up to the root was determined as the shoot length (cm) and the root part as the root length (cm) of the wheat seedlings in all groups with the help of a ruler.

Biomass and Dry Weight (g plant^{-1})

Biomass the weight of three seedlings from each group was determined by weighing on the precision scale (g plant^{-1}). For dry weight, three seedlings from each group were dried in an oven at 80°C for 24 h (g plant^{-1}).

Specific Leaf Area (SLA) (cm^2 mg^{-1})

After the wheat seedlings were visualized in the Image J program, they were dried in an oven at 70°C for 24 h and weighed on a precision balance. It was calculated according to Wilson (1999) using the following formula 1:

$$SLA = \text{Area (cm}^2\text{) / Dry weight (mg)} \quad (1)$$

Pigment Content (mg g^{-1})

Determination of pigment content, was made on 0.1 g tissues taken from the leaves of the plants, were homogenized in 80% acetone. The absorbance values determined spectrophotometrically from the homogenate at 663, 645, and 480 nm were calculated according to Arnon (1949) using the following formula 2:

Chlorophyll a (Chla) = (A663 x 12.70) – (A645 x 2.69) x 10/mg (2)

Chlorophyll b (Chlb) = (A645 x 22.90) – (A663 x 4.68) x 10/mg

Total chlorophyll (Chlt) = (20.2 x A645) + (8.02 x A663) x 10/mg

Carotenoid (Car) = ((A480 + (A663 x 0.114) – (A645 x 0.638))/112.5 x 10/mg

Total Protein Content (mg mL^{-1})

Leaf tissue samples were homogenized with 50 mM NaP buffer (pH 7.8, 1 mM EDTA) and then centrifuged for protein analysis. 0.1 g of Coomassie Brilliant Blue G 250 was mixed in a tube with a protein reagent containing ethanol (50 mL) and ortho-phosphoric acid (100 mL). The absorbance values determined at 595 nm in the spectrophotometer were used to calculate the total protein content (mg g^{-1}) on the standard graph (Bradford, 1976).

Hydrogen Peroxide Content (H_2O_2) (µg mL^{-1})

The H_2O_2 content, a mixture of plant tissue (0.1 g), 3 mL of H_2SO_4, and cold acetone were homogenized with homogenization buffer and centrifuged. Supernatants were determined at 550-800 nm (µg mL^{-1}) spectrophotometric with reading buffer containing H_2SO_4, purified water, ferrous ammonium sulfate, xylenol orange, sorbitol, and ethanol (e-FOX) (Cheeseman, 2006).

Lipid Peroxidation Content (TBARS) (nmol g^{-1})

The thiobarbituric acid reactive substance (TBARS) levels in leaf tissues were measured and analyzed according to the method of Madhava Rao & Sresty (2000) (nmol g fresh $weight^{-1}$, €=155 mM^{-1} cm^{-1}). Leaf samples (0.1 g) were homogenized in 2.5 mL trichloroacetic acid (TCA 0.1%). The supernatant was mixed with 4 mL trichloroacetic acid (20% TCA) containing thiobarbituric acid (0.5% TBA). The mixture was then exposed to 95°C temperature for 30 min. Following cooling, the absorbance values were recorded at 532 nm and 600 nm.

Peroxidase Activity (POX; EC 1.11.1.7)

The POX activity was quantified by homogenizing tissue samples with 2 mL of 0.05 M (pH 6.5) sodium acetate buffer and using 0.05 M (pH 6.5) sodium acetate buffer, 0.1M pyrogallol, and 0.09 M H_2O_2 solutions. It was determined by a spectrophotometer at 300 nm (Kanner & Kinsella, 1983).

Glutathione Reductase Activity (GR; EC 1.6.4.2)

The GR activity, as the decrease in the content of oxidized glutathione in the presence of NADPH, was calculated by the absorbance value by spectrophotometer at 340 nm (€=6.2 mM^{-1} cm^{-1}). The reaction mixture contained 0.025 mM sodium phosphate buffer (pH 7.8), 0.5 mM GSSG, and 0.12 mM $NADPH.Na_4$ and 0.1 mL. 1 enzyme unit was determined as the content of oxidized glutathione (µmol mL^{-1}) per minute (Foyer & Halliwell, 1976).

Statistical Analysis

The results were given as means ± standard error of five replicates. The compiled data were subject to an ANOVA (ONE-WAY) and the differences between the means were compared by the Tukey test to assess the effect of biopriming with Bt LU3 on physiological parameters and biochemical analysis in *T. aestivum* during salt stress. Those comparisons with $P \leq 0.05$ were taken as significantly different. The data were analyzed by using Statistical Package for the Social Sciences (SPSS 27.0) statistical software.

RESULTS

Root and Shoot Length

Bt LU3 application increased the root length by 5% in Sultan-95 compared to the control and no changed it in the Tosunbey variety. While 100 mM NaCl decreased root length by 26-28% in both varieties compared to the control, Bt LU3 application improved this reduction by 11% in Sultan-95 and 3% in Tosunbey. Similarly, 200 mM NaCl reduced root length by 58-63% in both varieties, while Bt LU3 application improved this reduction by 7% in Sultan-95 and 5% in Tosunbey (Figure 2). 100 mM NaCl reduced the shoot length of Sultan-95 by 28%, while Bt LU3 application improved it by 11%. However, this improvement was limited in 200 mM NaCl application. In Tosunbey, both salt concentrations reduced the shoot length by 25-57%. Bt applications did not cause a statistically significant change in these reductions. Our results showed that Bt LU3 biopriming was more effective in Sultan-95 compared to Tosunbey in reducing root and shoot length inhibition caused by salt stress (Figure 2).

Biomass and Dry Weight (g $plant^{-1}$)

Bt LU3 biopriming increased biomass by 9% in Sultan-95 and decreased by 14% in Tosunbey compared to control. Compared to the control with 100 mM NaCl, the biomass decreased by 43% in Sultan-95 and by 55% in Tosunbey, while it increased by 3% in both varieties with Bt LU3 biopriming. Biomass, which decreased 65% in Sultan-95 and 61% in Tosunbey with 200 mM NaCl, increased by 6% in Sultan-95 and 3% in Tosunbey with Bt LU3 biopriming (Figure 3). However, with Bt LU3 biopriming, only 33% of dry weight was recovered in Sultan-95 under 200 mM NaCl stress (Figure 4). Our results indicate that Bt LU3 biopriming has a better effect on the reduction in biomass and dry weight in Sultan-95 under high salt stress (Figure 3, Figure 4).

Specific Leaf Area (SLA) (cm^2 g^{-1})

Biopriming of Bt LU3 in both varieties increased SLA by 3% compared to the control. While 100 mM NaCl decreased SLA by 22% in both varieties, Bt LU3 application improved it by 11% only in Sultan-95.

While 200 mM NaCl stress reduced SLA by 61% in Sultan-95 and 50% in Tosunbey, Bt LU3 biopriming improved this reduction by 29% in Sultan-95 and 18% in Tosunbey (Figure 5). The results show that the reduction in SLA with severe salt stress is significantly improved in both varieties with Bt LU3 (Figure 5).

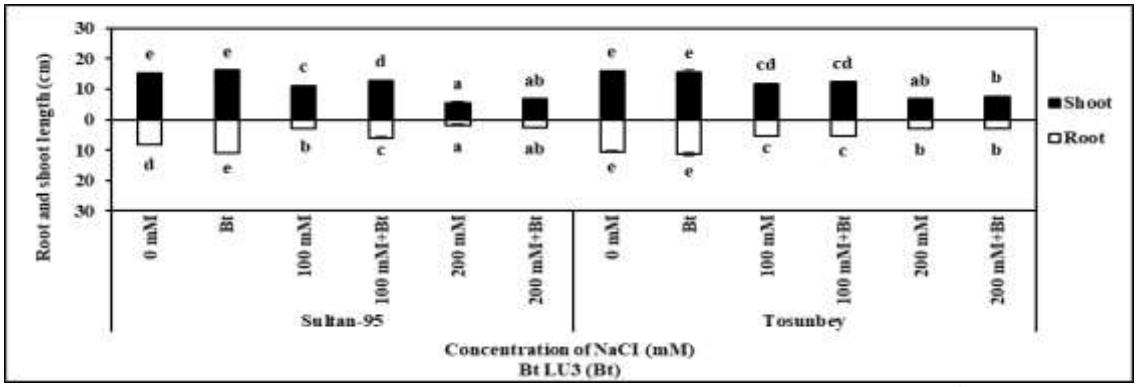

Figure 2. The effects of Bt LU3 biopriming on root and shoot length of two *T. aestivum* L. (Sultan-95 and Tosunbey) varieties under salt stress (Control; 0 mM, Bt; Bt LU3, Salt stress; 100 and 200 mM NaCl, Bt LU3+salt stress; 100 mM+Bt, 200 mM+Bt). (P <0.05).

Şekil 2. Tuz stresi altında iki T. aestivum L. (Sultan-95 ve Tosunbey) çeşidinin kök ve gövde uzunluğu üzerine Bt LU3 biyopriming'in etkileri (Kontrol; 0 mM, Bt; Bt LU3, Tuz stresi; 100 ve 200 mM NaCl, Bt LU3+tuz stresi; 100 mM+ Bt, 200 mM+Bt) (P <0.05).

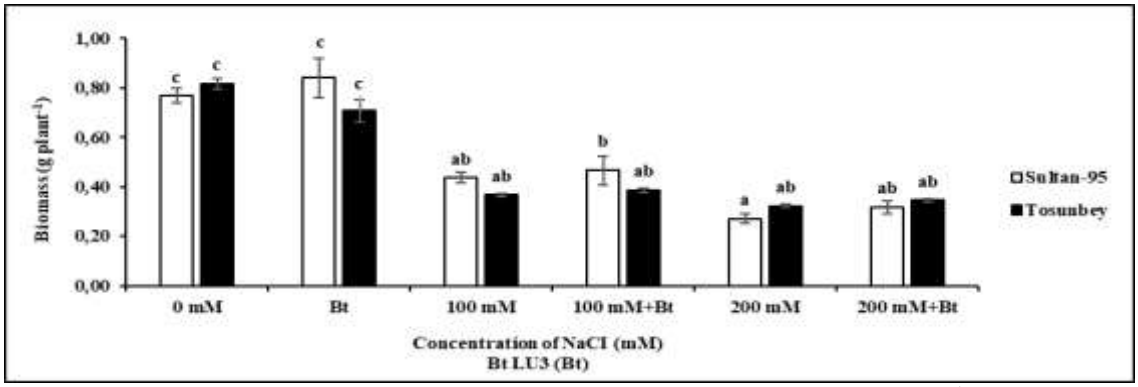

Figure 3. The effects of Bt LU3 biopriming on the biomass of two *T. aestivum* L. varieties (Sultan-95 and Tosunbey) under salt (Control; 0 mM, Bt; Bt LU3, Salt stress; 100 and 200 mM NaCl, Bt LU3+salt stress; 100 mM+Bt, 200 mM+Bt) (P <0.05).

Şekil 3. Tuz stresi altında iki T. aestivum L. çeşidinin (Sultan-95 ve Tosunbey) biyokütlesi üzerine Bt LU3 biyopriming'in etkileri (Kontrol; 0 mM, Bt; Bt LU3, Tuz stresi; 100 ve 200 mM NaCl, Bt LU3+tuz stresi; 100 mM+ Bt, 200 mM+Bt) (P <0.05).

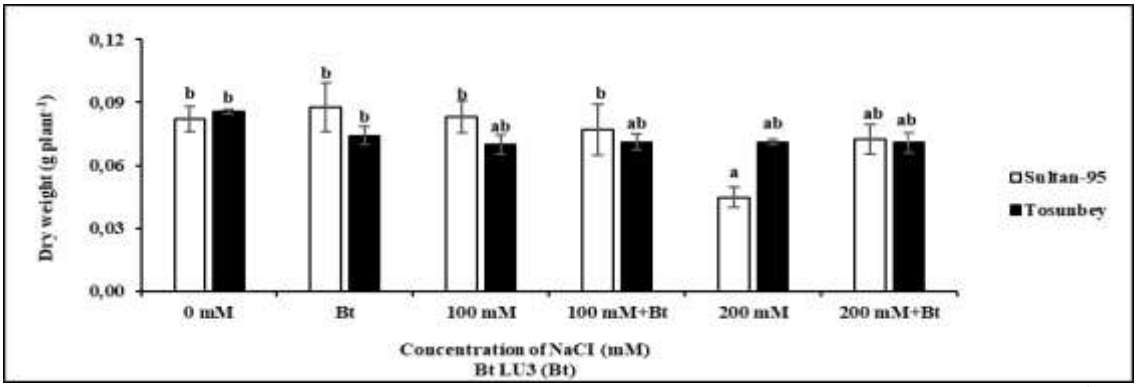

Figure 4. The effects of Bt LU3 biopriming on the dry weight of two *T. aestivum* L. varieties (Sultan-95 and Tosunbey) under salt stress (Control; 0 mM, Bt; Bt LU3, Salt stress; 100 and 200 mM NaCl, Bt LU3+salt stress; 100 mM+Bt, 200 mM+Bt) (P <0.05).

Şekil 4. Tuz stresi altında iki T. aestivum L. çeşidinin (Sultan-95 ve Tosunbey) kuru ağırlığı üzerine Bt LU3 biyopriming'in etkileri (Kontrol; 0 mM, Bt; Bt LU3, Tuz stresi; 100 ve 200 mM NaCl, Bt LU3+tuz stresi; 100 mM+ Bt, 200 mM+Bt) (P <0.05).

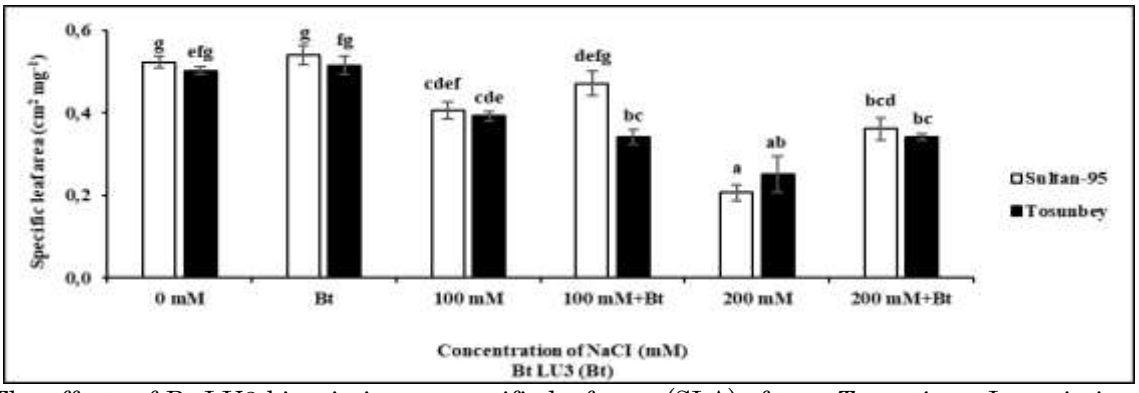

Figure 5. The effects of Bt LU3 biopriming on specific leaf area (SLA) of two *T. aestivum* L. varieties (Sultan-95 and Tosunbey) under salt stress (Control; 0 mM, Bt; Bt LU3, Salt stress; 100 and 200 mM NaCl, Bt LU3+salt stress; 100 mM+Bt, 200 mM+Bt) (P <0.05).

Şekil 5. Tuz stresi altında iki T. aestivum L. çeşidinin (Sultan-95 ve Tosunbey) spesifik yaprak alanı (SLA) üzerine Bt LU3 biyopriming'in etkileri (Kontrol; 0 mM, Bt; Bt LU3, Tuz stresi; 100 ve 200 mM NaCl, Bt LU3+tuz stresi; 100 mM+ Bt, 200 mM+Bt) (P <0.05).

Pigment content (mg g^{-1})

Firstly, the content of Chla in Sultan-95 decreased by 28% with 100 mM NaCl and by 36% with 200 mM NaCl compared to the control. It was determined that Bt LU3 biopriming increased the decreased Chla content by 28% and 59%, respectively, with 100 and 200 mM NaCl (Figure 6 a). Secondly, Chlb contents increased by 21% and 22%, respectively, with Bt LU3 application in Sultan-95 under 100 and 200 mM NaCl stress. These increases were determined as 55% and 41% for the Tosunbey variety, respectively (Figure 6 b). Thirdly, it was determined that the number of Car increased by 12% with Bt LU3 biopriming with only 200 mM NaCl application in the Sultan-95 variety, and 24% and 33%, respectively, compared to control with 100 and 200 mM NaCl in Tosunbey variety (Figure 6 c). As a result, the Chlt increased by 22% and 17%, respectively, in the Sultan-95 variety with 100 mM+Bt and 200 mM+Bt applications, while it increased by 44% and 46%, respectively, in the Tosunbey variety (Figure 6 d).

Our pigment results indicate that the content of Chla, Chlb, Car, and Chlt levels increased by 20%, 66%, 45%, and 80%, respectively, in the Tosunbey compared to mild salt stress treatment with Bt LU3 biopriming. However, with Bt LU3 biopriming, Chla, Chlb, and Chlt content increased by 28%, 90%, and 37%, respectively, in the Sultan-95 compared to mild salt stress application. Chla, Car and Chlt contents increased by 28%, 17%, and 90%, respectively, with Bt LU3 biopriming compared to 200 mM NaCl application in the Tosunbey variety. In Sultan-95, these increases were determined as 59%, 24% and 45%, respectively (Figure 6). Accordingly, Bt LU3 biopriming reduced salt stress-induced chlorosis in both varieties compared to control and even increased it dramatically in the Tosunbey.

Total Protein Content

The protein content of the Sultan-95 variety increased with all treatments compared to the control. The protein content increased by 2% compared to the control with only 200 mM NaCl+Bt application in the Tosunbey variety (Figure 7).

Hydrogen Peroxide Content (H_2O_2) (µg mL^{-1})

The H_2O_2 content decreased by 21-27% with Bt LU3 biopriming compared to control in both varieties. In contrast, it increased 31-33% with 100 mM NaCl and 51-58% with 200 mM NaCl. Bt LU3 application decreased H_2O_2 production by 26% in Sultan-95, 8% in Tosunbey under 100 mM NaCl stress, 63% in Sultan-95 under 200 mM NaCl stress, and 39% in Tosunbey (Figure 8).

Our results show that the content of H_2O_2, which increased with salt stress, decreased in both varieties with Bt LU3 biopriming, and remained below the control level in 200 mM+Bt application, especially in Sultan-95 (Figure 8).

Lipid Peroxidation Content (TBARS) (nmol g^{-1})

Lipid peroxidation levels increased 1.3 times and 2.1 times in Sultan-95 with 100 mM and 200 mM NaCl, respectively. These increases were determined as 49% and 87% in the Tosunbey, respectively. Bt LU3 application alone caused a non-significant increase in both varieties. However, TBARS levels in Sultan-95 plants under 100 mM and 200 mM NaCl stress were decreased by Bt LU3 by 59% and 106%, respectively. Similarly, Bt LU3 application in the Tosunbey variety decreased TBARS levels by 11% and 40%, respectively (Figure 9). Our results indicate that lipid peroxidation developed by salt stress decreased in both varieties with Bt LU3 application, and the most effective reduction occurred in the salt-sensitive Sultan-95 (Figure 9).

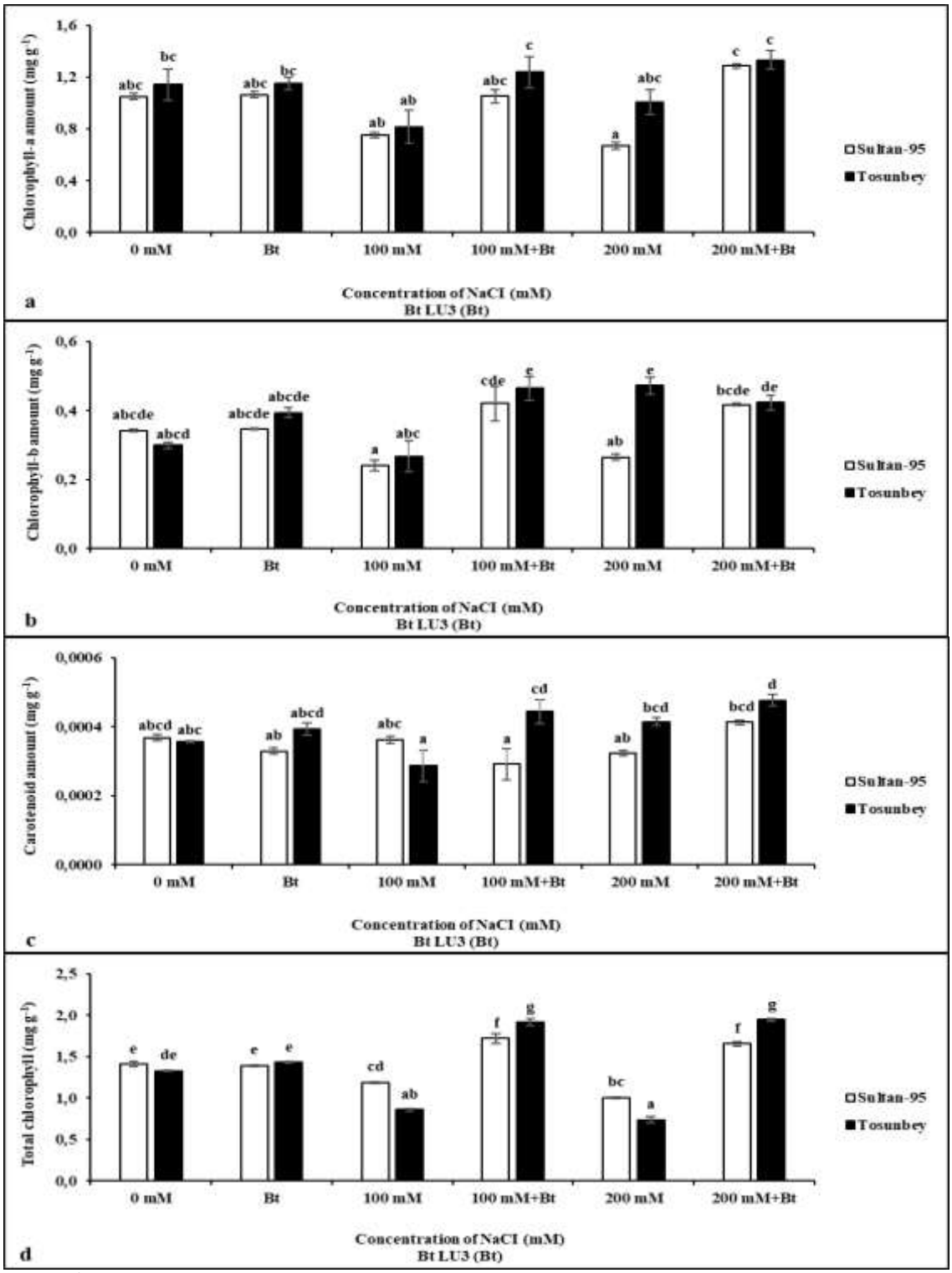

Figure 6 (a, b, c, d). The effects of Bt LU3 biopriming on pigment content of two *T. aestivum* L. varieties (Sultan-95, Tosunbey) under salt stress (Control; 0 mM, Bt LU3; Bt, Salt stress; 100 and 200 mM NaCl, Bt LU3+salt stress; 100 mM+Bt, 200 mM+Bt). (a; Chlorophyll a content (Chla), b; Chlorophyll b content (Chlb), c; Carotenoid (Car), d; Total chlorophyll content (Chlt)) ($P < 0.05$).

Şekil 6 (a, b, c, d). Tuz stresi altında Bt LU3 biyopriming'in iki T. aestivum L. çeşidinin (Sultan-95, Tosunbey) pigment içeriği üzerine etkileri (Kontrol; 0 mM, Bt LU3 grubu; Bt, Tuz stresi; 100 ve 200 mM NaCl, Bt LU3+tuz stresi; 100 mM+ Bt, 200 mM+Bt). (a; Klorofil a içeriği (Chla), b; Klorofil b içeriği (Chlb), c; Karotenoid (Car), d; Toplam klorofil içeriği (Chlt)) (P <0.05).

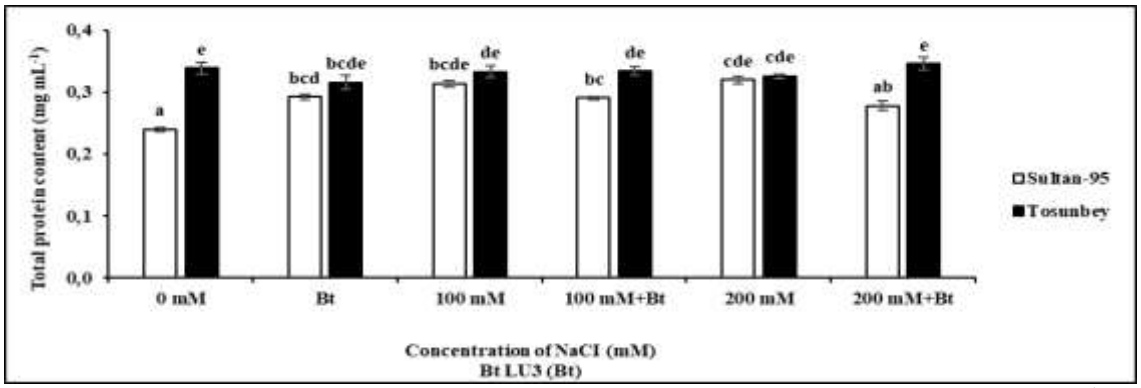

Figure 7. The effects of Bt LU3 biopriming on total protein content of two *T. aestivum* L. varieties (Sultan-95 and Tosunbey) under salt stress (Control; 0 mM, Bt; Bt LU3, Salt stress; 100 and 200 mM NaCl, Bt LU3+salt stress; 100 mM+Bt, 200 mM+Bt) (P <0.05).

Şekil 7. Tuz stresi altında iki T. aestivum L. çeşidinin (Sultan-95 ve Tosunbey) toplam protein içeriği üzerine Bt LU3 biyopriming'in etkileri (Kontrol; 0 mM, Bt; Bt LU3, Tuz stresi; 100 ve 200 mM NaCl, Bt LU3+tuz stresi; 100 mM+ Bt, 200 mM+Bt) (P <0.05).

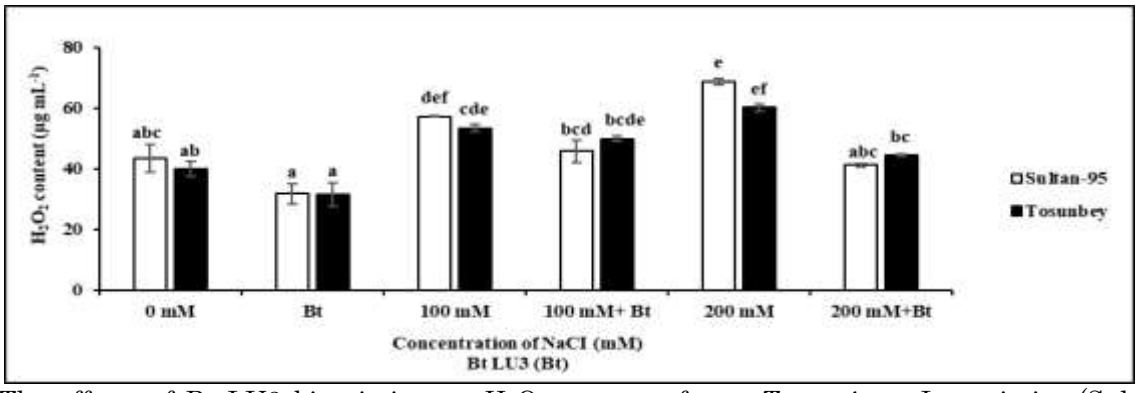

Figure 8. The effects of Bt LU3 biopriming on H_2O_2 content of two *T. aestivum* L. varieties (Sultan-95 and Tosunbey) under salt stress (Control; 0 mM, Bt; Bt LU3, Salt stress; 100 and 200 mM NaCl, Bt LU3+salt stress; 100 mM+Bt, 200 mM+Bt) (P <0.05).

Şekil 8. Tuz stresi altında Bt LU3 biyopriming'in iki T. aestivum L. çeşidinin (Sultan-95 ve Tosunbey) H_2O_2 içeriği üzerindeki etkileri (Kontrol; 0 mM, Bt; Bt LU3, Tuz stresi; 100 ve 200 mM NaCl, Bt LU3+tuz stresi; 100 mM+ Bt, 200 mM+Bt) (P <0.05).

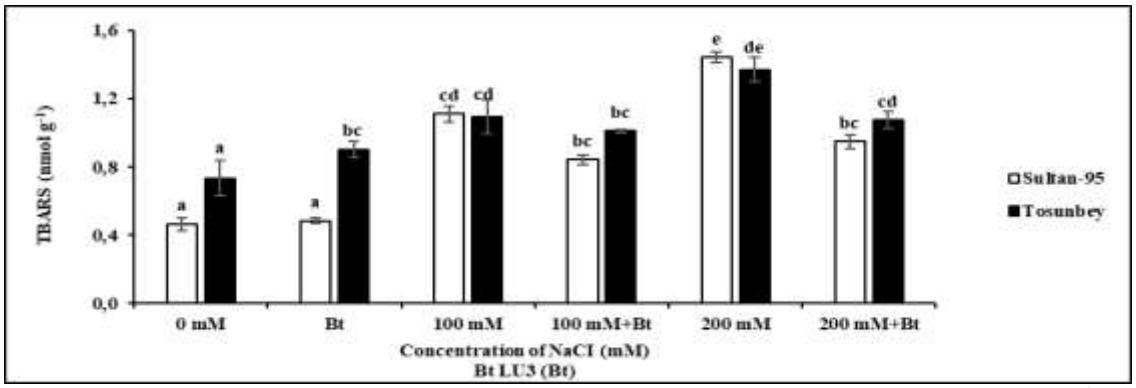

Figure 9. The effects of Bt LU3 biopriming on TBARS content of two *T. aestivum* L. varieties (Sultan-95 and Tosunbey) under salt stress (Control; 0 mM, Bt; Bt LU3, Salt stress; 100 and 200 mM NaCl, Bt LU3+salt stress; 100 mM+Bt, 200 mM+Bt) (P <0.05).

Şekil 9. Tuz stresi altında iki T. aestivum L. çeşidinin (Sultan-95 ve Tosunbey) TBARS içeriği üzerine Bt LU3 biyopriming'in etkileri (Kontrol; 0 mM, Bt; Bt LU3, Tuz stresi; 100 ve 200 mM NaCl, Bt LU3+tuz stresi; 100 mM+ Bt, 200 mM+Bt) (P <0.05).

Peroxidase Activity

POX activity increased by 43% and 65% in Sultan-95 with 100 mM and 200 mM NaCl, respectively. These increases were determined as 9% and 19% in Tosunbey, respectively. In both varieties, the Bt LU3 application alone increased POX activities by 56-59%. However, the application of 100 mM and 200 mM NaCl+Bt increased the POX activities in Sultan-95 by 32% and 94%, respectively. Similarly, Bt LU3 application in the Tosunbey variety increased the POX activities by 11% and 24%, respectively (Figure 10). Our results showed that Bt LU3 application increased salt stress-induced POX increases in both varieties and the most effective increase occurred in the salt-sensitive Sultan-95 (Figure 10).

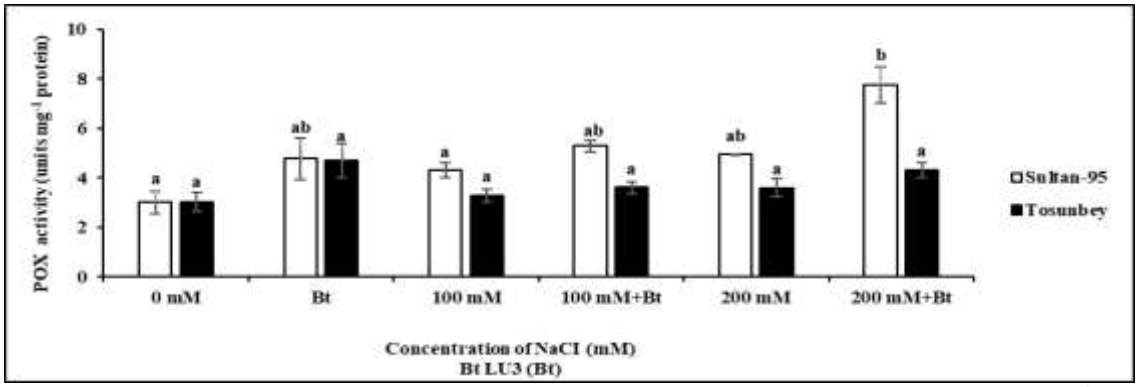

Figure 10. The effects of Bt LU3 biopriming on POX activity of two *T. aestivum* L. varieties (Sultan-95 and Tosunbey) under salt stress (Control; 0 mM, Bt; Bt LU3, Salt stress; 100 and 200 mM NaCl, Bt LU3+salt stress; 100 mM+Bt, 200 mM+Bt) (P <0.05).

Şekil 10. Bt LU3 biyopriming'in iki T. aestivum L. çeşidinin (Sultan-95 ve Tosunbey) tuz stresi altındaki POX aktivitesi üzerindeki etkileri (Kontrol; 0 mM, Bt; Bt LU3, Tuz stresi; 100 ve 200 mM NaCl, Bt LU3+tuz stresi; 100 mM+ Bt, 200 mM+Bt) (P <0.05).

Glutathione Reductase Activity

GR activity increased by 23% and 34%, respectively, with 100 mM and 200 mM NaCl applications in Sultan-95. Similarly, these increases were determined as 36% and 61% in the Tosunbey, respectively. The Bt LU3 application in both varieties increased GR activities by 7% in Sultan-95 and by 41% in Tosunbey. However, 100 mM and 200 mM NaCl+Bt application increased GR activities by 49% in Sultan-95. Similarly, the Bt LU3 application in the Tosunbey variety increased GR activities by 26% and 81%, respectively (Figure 11). Our results showed that the Bt LU3 application increased salt stress-induced GR increases in both varieties and the most effective increase occurred in the salt-tolerant Tosunbey (Figure 11).

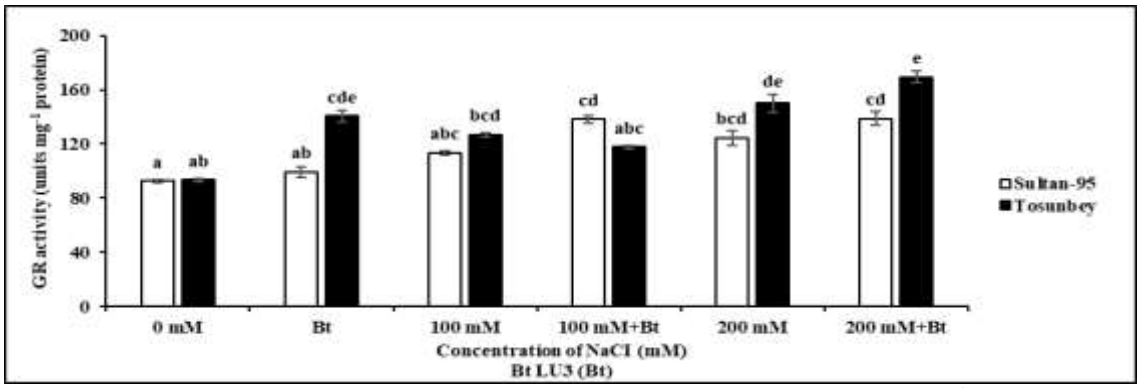

Figure 11. The effects of Bt LU3 biopriming on GR activity of two *T. aestivum* L. varieties (Sultan-95 and Tosunbey) under salt stress (Control; 0 mM, Bt LU3; Bt, Salt stress; 100 and 200 mM NaCl, Bt LU3+salt stress; 100 mM+Bt, 200 mM+Bt) (P <0.05).

Şekil 11. Tuz stresi altındaki iki T. aestivum L. çeşidinin (Sultan-95 ve Tosunbey) GR aktivitesi üzerine Bt LU3 biyopriming'in etkileri (Kontrol; 0 mM, Bt LU3 grubu; Bt, Tuz stresi; 100 ve 200 mM NaCl, Bt LU3+tuz stresi; 100 mM+ Bt, 200 mM+Bt) (P <0.05).

DISCUSSION and CONCLUSION

PGPR promotes plant growth and is known to increase tolerance to environmental stresses (Stefan et al., 2013; Pandey et al., 2018). Salt stress limits root and shoot length in wheat. In our study, Bt LU3 biopriming increased root and shoot lengths in salt-sensitive Sultan-95 under 100 mM NaCl but not in salt-tolerant Tosunbey varieties under all salt stress. Inoculation with *Bacillus halodenitrificans* in soils under high salt stress has been reported to improve growth parameters in wheat (Ramadoss et al., 2013). On the other hand, it is known that root length increases dramatically with *Bacillus thuringiensis* AZP2 biopriming in wheat under drought stress (Timmusk et al., 2014; Raheem et al., 2018).

Besides, salt stress causes a decrease in the biomass and dry weight of the common wheat (*T. aestivum* L.) plant. Our obtained results showed that decreased biomass (g plant^{-1}) and dry weight (g plant^{-1}) with high salt stress had a better healing effect on salt-sensitive Sultan-95. Similarly, it was reported that the dry weight increased by 40-52% in 120-days wheat seedlings inoculated with *Bacillus* sp. compared to the control (Hajiabadi et al., 2021). In addition, it has been reported that *Bacillus thuringiensis* AZP2 biopriming significantly increased the dry weight (g plant^{-1}) of wheat under drought stress (Timmusk et al., 2014).

Our results indicate that the Bt LU3 biopriming application, which decreased with all salt stress applications, increased significantly in Sultan-95, while it was effective only at 200 mM NaCl in Tosunbey. Similarly, our biomass results are consistent with root and shoot length of Sultan-95 under salt stress results. Salt-tolerant plants have unchanged or increased chlorophyll levels under salt stress, while chlorophyll content is reduced in salt-tolerant plants (Rahneshan et al., 2018). In our study, it was shown that while the pigment contents (Chla, Chlb and Chlt) of both varieties dramatically decreased with all salt stress applications. Despite of salt stresses, Bt LU3 biopriming increases all pigment amounts to control levels. Interestingly, the increases in pigment contents of the salt-sensitive variety with Bt LU3 biopriming increased to the salt-tolerant variety. But Bt LU3 biopriming dramatically increased the pigment contents, especially in the salt-tolerant variety. Inoculation with *Bacillus* sp. has been reported to induce chlorophyll biosynthesis in soybean (*Glycine max*) limiting the reductions in chlorophyll content caused by salt stress and reducing the adverse effect (El-Esawi et al., 2018). On the other hand, Chla and Chlb contents decrease as a result of insufficient absorption of elements such as K^+, Mg^{+2}, Fe and P, increase in Na^+ content, decrease in 5-aminolevulinic acid accumulation or deterioration of some enzymes (such as chlorophyllase, peroxidase) due to phenolic compounds in common wheat under the salt stress (Hajiabadi et al., 2021). The use of Bt LU3 biopriming and PGPR increased the pigment content of both varieties in our study. It has been stated that this may be related to the production of siderophores in plants (Hajiabadi et al., 2021). During the growth of plants, intermediate products of oxygen are constantly produced in the processes of photosynthesis and respiration. Under normal conditions, electron transfer to oxygen and associated intermediate toxic radical molecules (OH, H_2O_2, O_2^{-} and O_2, etc.) are formed (Parida & Das, 2005). As a result of salt stress, the water potential decreases and causes osmotic stress with ion accumulation in plants. Water scarcity causes oxidative stress by increasing the production of ROS such as OH, H_2O_2, O_2^{-} and O_2 (Desingh & Kanagaraj, 2007). Plants form an antioxidant defense system against ROS resulting from salt stress with the activity of certain antioxidant enzymes such as CAT, POX and SOD (Desingh & Kanagaraj, 2007). ROS production in wheat is triggered by unsuitable conditions such as salt stress. Our results showed that POX activities increased in both varieties for ROS detoxification, especially in the Sultan-95 variety. However, GR activity in the ascorbate-glutathione pathway was effectively increased in the Tosunbey variety. Besides, Bt LU3 biopriming significantly increased GR activity in Sultan-95 under moderate salt stress. According to these results, it can be said that the Bt LU3 biopriming application induced an increase in antioxidant activity in the salt-sensitive variety.

On the other hand, our results indicate that salt stress and the increase in antioxidant enzyme activities in both varieties are associated with decreases in the content of TBARS and H_2O_2. All data showed that Sultan-95 had a more effective antioxidant capacity with Bt LU3 biopriming compared to Tosunbey. Our results are consistent with the findings of El-Esawi et al. (2018). PGPR-promoted antioxidant enzyme production is known to reduce excessive ROS production and maintain membrane stability (Afridi et al., 2019). In addition, *in vitro* application of the *B. amyloliquefaciens* SQR9 strain on maize (*Zea mays*) seedlings has been reported to increase POX and GR activities (Wang et al., 2016). Moreover, the increase in antioxidant enzyme activities (APX, SOD, CAT, POX) has been shown to play a role in reducing the harmful effects of high salinity on soybean (*Glycine max*) growth (El-Esawi et al., 2018). On the other hand, it is stated that the activation of antioxidant enzymes increases plant growth and performance through the up-regulation of genes that mediate tolerance under salt stress conditions (El-Esawi et al., 2018).

Table 1. Correlations between physiological and biochemical parameters in wheat seedlings.
Çizelge 1. Buğday fidelerinde fizyolojik ve biyokimyasal parametreler arasındaki korelasyonlar.

Variable	Root	Shoot	Biomass	DW	SLA	Chla	Chlb	Car	Chlt	Protein	H_2O_2	TBARS	POX	GR
Root	1													
Shoot	,887**	1												
Biomass	,819**	,868**	1											
DW	,527**	,417*	,632**	1										
SLA	,809**	,802**	,832**	,635**	1									
Chla	0,065	0,146	0,035	0,225	0,143	1								
Chlb	-0,127	-0,057	-0,229	0,083	-0,062	,583**	1							
Car	0,172	0,081	-0,005	0,128	0,182	,407**	0,112	1						
Chlt	-0,175	-0,111	-0,263	-0,081	-0,302	,677**	,629**	0,055	1					
Protein	-,250*	-0,187	-,364*	-0,155	-,413*	-0,007	0,104	-0,041	0,154	1				
H_2O_2	-,596**	-,735**	-,697**	-,555**	-,761**	-,354*	-0,261	-,432**	0,031	0,202	1			
TBARS	-,639**	-,640**	-,799**	-,557**	-,731**	-0,148	0,049	-,276*	0,094	,356**	,712**	1		
POX	-0,225	-0,15	-0,162	-0,058	0,007	0,052	0,096	0,208	-0,007	-0,159	-0,171	0,137	1	
GR	-,514**	-,428**	-,540**	-,339*	-,380*	0,212	,549**	0,09	,430**	,283*	0,048	,486**	,291*	1

Root: Root length, Shoot: Shoot length, DW: Dry weight, SLA: Specific leaf area, Chla: Chlorophyll a content, Chlb: Chlorophyll b content, Car: Carotenoid, Chlt: Total chlorophyll content, Protein: Total protein content, H_2O_2: Hydrogen peroxide content, TBARS: Lipid peroxidation content, POX: Peroxidase activity, GR: Glutathione reductase activity.
Kök: Kök uzunluğu, Gövde: Gövde uzunluğu, DW: Kuru ağırlık, SLA: Spesifik yaprak alanı, Chla: Klorofil a içeriği, Chlb: Klorofil b içeriği, Car: Karotenoid, Chlt: Toplam klorofil içeriği, Protein: Toplam protein içeriği, H_2O_2 : Hidrojen peroksit içeriği, TBARS: Lipit peroksidasyon içeriği, POX: Peroksidaz aktivitesi, GR: Glutatyon redüktaz aktivitesi
**: Significant correlations P<0.01, and *: P<0.05

Triple interaction (Variety x NaCl x Bt LU3) was statistically significant for all parameters except all pigment results and POX activities (Table 1). This result indicated that there was not any effect of Bt biopriming on pigment contents and POX levels. The correlation between the physiological parameters showed that there was a positive relationship between Root, Shoot, SLA, Biomass and DW. The correlation between also the biochemical parameters showed that there was a positive relationship between GR, TBARS, Protein, H_2O_2. According to these results physiological parameters shown strictly correlation together via Bt biopriming. On the other hand, GR activity more induced than the POX activity against salt stress (Table 1). Our results, a strictly correlation was determined with Bt biopriming application in physiological parameters. Additionally, the positive correlation between salt stress-induced H_2O_2 amount, and GR activity indicates that GR works more effectively than POX in antioxidant defense with Bt biopriming (Table 1).

Consequently, it was determined that the salt-sensitive Sultan-95 cultivar had better growth parameters with Bt LU3 biopriming application compared to the salt-tolerant Tosunbey variety. It can be said that the responses in growth are related to the induction of the antioxidant capacity of the tolerant variety and promoted by the Bt LU3 biopriming application. As a result, more comprehensive studies on biopriming applications of rhizobacteria are required in the context of plant-bacteria-soil relations research for the development of sustainable agricultural practices.

Author Contribution Rates

The authors declare that they contribute equally to the article.

Conflict of interest/Competing interests

The authors declare that there is no conflict of interest.

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Evaluation of the Histopathological Changes Accompanied for the Toxic Effects of Diazinon on the Spleen of Mice

Esra AKAT ÇÖMDEN[1], Özlem ÇAKICI[2]
Ege University, Science Faculty, Biology Department, Zoology Section 35100 Bornova-Izmir, Türkiye
[1]https://orcid.org/0000-0001-7080-3106, [2]https://orcid.org/0000-0003-3589-1440
✉: ozlem.cakici@ege.edu.tr

ABSTRACT

This study was carried out to determine the histopathological changes caused by diazinon in the spleen of Swiss albino mice. Experimental groups containing low dose (30 mg/kg), medium dose (60 mg/kg) and high dose (120 mg/kg) were exposed to diazinon through oral administration for 30 consecutive days. Separation and hemorrhage in the capsule, congestion, enlarged white pulp, amyloid formation, and karyolysis in some megakaryocytes were determined in the splenic parenchyma of the low dose group. An increase in the number of enlarged white pulps, hemorrhage within splenic parenchyma, accumulation of cells into dilated sinusoids and amyloid formation were examined in the medium dose group. Some cells passing from the splenic parenchyma into dilated sinusoids were also observed. Intensive congestion, necrotic areas within spleen tissue, an increase in the number of karyolytic megakaryocytes, fibrosis and some cells passing from the splenic parenchyma into enlarged sinusoids were prominent histological lesions in the high dose group. These results showed that diazinon caused severe dose-related histopathological damages and had the capacity to disrupt functions of the spleen.

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Diazinonun Farelerin Dalağı Üzerindeki Toksik Etkilerine Eşlik Eden Histopatolojik Değişikliklerin Değerlendirilmesi

ÖZET

Bu çalışma, diazinonun İsviçre albino farelerinin dalağında oluşturduğu histopatolojik değişiklikleri belirlemek amacıyla yapıldı. Düşük doz (30 mg/kg), orta doz (60 mg/kg) ve yüksek doz (120 mg/kg) içeren deney grupları, ardışık 30 gün boyunca oral yoldan diazinona maruz bırakıldı. Düşük doz grubunun dalak parankiminde kapsülde ayrılma ve kanama, tıkanıklık, beyaz pulpa büyümesi, amiloid oluşumu ve bazı megakaryositlerde karyoliz tespit edildi. Orta doz grubunda genişlemiş beyaz pulpa sayısında artış, dalak parankiminde kanama, hücrelerin genişlemiş sinüzoidlerde birikmesi ve amiloid oluşumu incelendi. Dalak parankiminden dilate sinüzoidlere geçen bazı hücreler de gözlendi. Yoğun konjesyon, dalak dokusunda nekrotik alanlar, karyolitik megakaryosit sayısında artış, fibrozis ve dalak parankiminden büyümüş sinüzoidlere geçen bazı hücreler yüksek doz grubundaki belirgin histolojik lezyonlardı. Bu sonuçlar diazinonun doza bağlı ciddi histopatolojik hasarlara neden olduğunu ve dalağın fonksiyonlarını bozma kapasitesine sahip olduğunu gösterdi.

Zooloji

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INTRODUCTION

Environmental pollution caused by pesticide residues as a result of their intensive use in agriculture and industry is a major concern. Overuse of pesticides has led to an increased risk of environmental pollution, detrimental effects on food security, biodiversity, and water sources (Carvalho, 2006; Queyrel et al., 2016). Organophosphorus pesticides have been extensively used all over the world. These pesticides have adverse effects on non-target organisms including humans (Pundir & Malik, 2019). They were reported to cause harmful impacts on mammalian organs such as liver and kidney (Çakıcı & Akat, 2013) and heart (Öğütçü, 2006). Moreover, deaths of over 100.000 birds worldwide were attributed to organophosphate insecticide monocrotophos (Hooper et al., 2002). Organophosphorus pesticides cause toxic effects by irreversible inhibition of acetylcholinesterase at the cholinergic synapses in the nervous system (Timbrell, 2001), leading to accumulation of acetylcholine at the nerve terminals and neuromuscular junctions which resulted in respiratory failure and death (Krieger, 2010).

Diazinon is one of the most widely used organophosphorus insecticides to control insects in crops, lawns, fruit, and vegetables (Marete et al., 2020). It was determined in groundwater, drinking water wells, soils, fruits, crops, and even human sera (Aggarwal, 2013; Jafari et al., 2018). Diazinon causes the inhibition of acetylcholinesterase in the nervous system of humans (Zhang et al., 2010). In addition to its neurotoxic effect, diazinon induces vascular toxicity (Razavi, 2013), oxidative stress (Hernández-Moreno et al., 2018) and cardiotoxicity (Danaei et al., 2019). It also disrupts cytochrome P450 enzymes in the human liver and can bind to human serum albumin through the mainly hydrophobic interactions, and hydrogen bond (Sams et al., 2004; Jafari et al., 2018). A report from the U.S. Environmental Protection Agency noted the existence of diazinon metabolites in the urine of children (Egeghy et al., 2011). Furthermore, diazinon induces DNA or chromosomal damage in rodent and human cells in vitro (Guyton et al., 2015). Although diazinon has low persistence in the environment, it is a nonspecific insecticide and highly toxic to animals and humans. Its major degradation products are hydroxy diazinon, diazoxon, hydroxy diazoxon and 2-isopropyl-6-methyl-4- pyrimidinol, which may cause acute toxic effects to workers employed in the manufacture and application of this pesticide. Burgess et al. (2008) reported that different physiological symptoms such as headache, abdominal cramps, difficulty in breathing, and even death can result from acute diazinon exposure in human (Kouloumbos et al., 2003; Čolović et al., 2010).

The spleen is the biggest and most important secondary lymphoid organ closely associated with the circulatory system. It is responsible for initiating immune response, filtering the foreign substances from blood, removing old or damaged erythrocytes and reutilization of iron of hemoglobin (Mebius & Kraal, 2005; Mescher, 2016). The spleen has a prominent function in hematopoiesis particularly throughout fetal life in humans. However, it actively contributes to blood cell production during the lifespan of mice (Sieff & Williams, 1995). While the marrow compartment alone is the major lifelong blood-forming organ in humans, both the spleen and bone marrow are the primary site of blood cell production throughout the postnatal life in mice (Palis & Yoder, 2001). Neishabouri et al. (2007) reported toxic effects of diazinon on some internal organs, including spleen of mice. Zeinali et al. (2022) investigated the potential protective impact of chyrsin against immunotoxicity induced by diazinon. In general, these studies mentioned limited histopathologic effects of diazinon. To that end, we aimed to study detailed histopathological impacts of diazinon on the spleen of Swiss albino mice. Therefore, the current study will provide a better understanding of the possible side effects of diazinon on other nontarget organisms including humans.

MATERIAL and METHOD

Experimental Animals and Design

The mean weight range of the mice was 25-30 g. Body weight was measured weekly. After 15 days of acclimatization, the mice were randomly classified into a control group or diazinon-treated group, each containing 10 mice which were maintained with same sex in cages. Experimental groups contained low dose (30 mg/kg), medium dose (60 mg/kg), and high dose (120 mg/kg). Diazinon (purity 99%) was supplied by AgroBest Grup (Izmir, Turkey). All experimental groups were kept under standard laboratory circumstances at 12-h dark/light cycles, 45±5% relative humidity, and 22±3°C temperature. The control group received normal laboratory chow. Experimental mice were fed daily with laboratory chow mixed with diazinon for 30 consecutive days. Animals were given standard laboratory chow and tap water ad libitum. There was no death in any diazinon-treated group during these experiments. All animal care and experimental procedures were approved by Ege University Animal Experiments Ethics Committee (permit no: 2011-047). The animals were treated humanely and with regard for alleviation of suffering.

Histopathological Analyses

After 30 days following exposure to diazinon, the mice

were euthanized under ether anesthesia. Spleens of the experimental mice were quickly removed and fixed in Bouin's solution for 24 hours. They were then transferred in ethanol (70%, 96%, 100%) for dehydration and put into xylol for transparency. Thereafter, the samples were embedded in paraffin following standard histological methods. Processing was manual and 5µm-thickness deparaffinized sections were stained with Harris hematoxylin-eosin (H&E, Sigma Aldrich, St Louis, Mo, USA) to demonstrate the general morphology of the tissue. After the stain with Periodic acid Schiff (PAS, Sigma Aldrich, St Louis, Mo, USA), counterstaining was carried out with H&E. PAS staining showed severe fibrosis in the splenic parenchyma. They were examined and photographed with a Zeiss Axioscope light microscope connected to an AxioCam Erc5S digital camera (Carl Zeiss, Oberkochen, Germany).

RESULTS

The present study was designed to determine harmful effects of diazinon on the splenic parenchyma of mice. Based on our results, there was no difference between male and female mice. Light microscopic observations showed that diazinon caused dose-related destructive changes in the spleen such as congestion, enlarged white pulp, amyloid formation, karyolysis in megakaryocytes, sinusoidal dilation, accumulation of cells in enlarged sinusoids, necrotic areas, and fibrosis.

Control Group

The spleen is mainly composed of two functional parts called the white and red pulps. The organ was mainly composed of the red pulp which was clearly distinguishable from the white pulp. In the splenic parenchyma, the splenic artery was divided into small arterioles that constituted the central arterioles. The central arteriole was enveloped by lymphoid tissue forming the splenic white pulp. Lymphocytes were predominant in the white pulp surrounded by the red pulp. Numerous sinusoids were observed in the splenic tissue (Fig. 1a). Erythrocytes, macrophages, and megakaryocytes were also present in the red pulp (Fig. 1b).

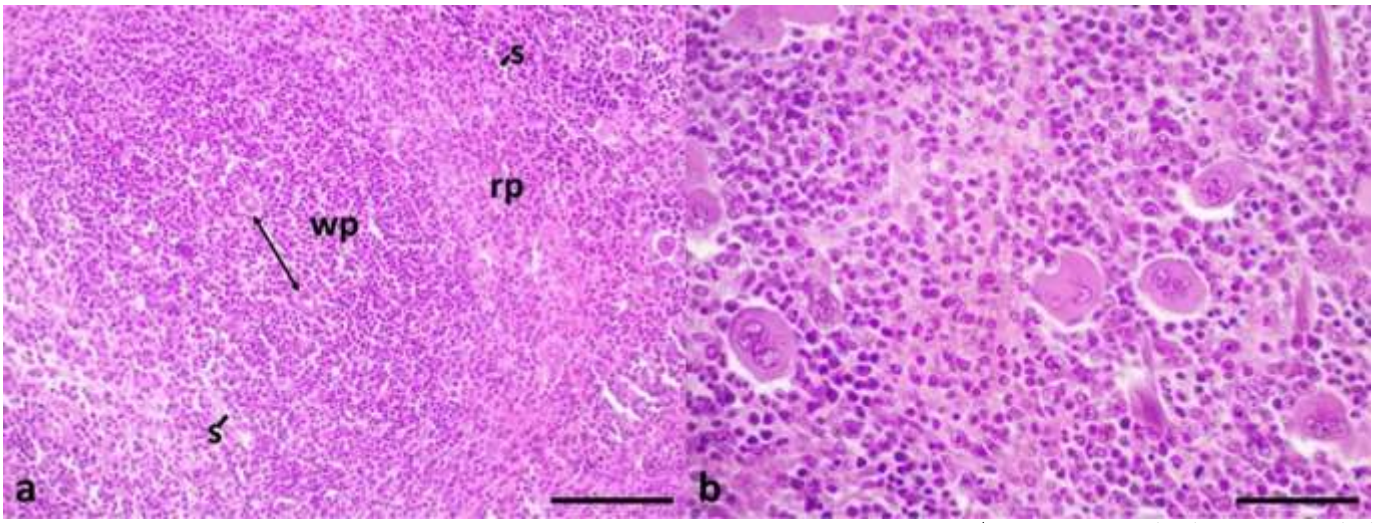

Figure 1. Light microscopic view of spleen in the control group after 30 days, **a)** White pulp (wp), red pulp (rp), central arterioles (arrows) in white pulp, sinusoids (s), scale bar: 100 µm, **b)** Detailed appearance of megakaryocytes, scale bar: 50 µm, H&E: Hematoxylin and Eosin

Şekil 1. *30 gün sonra kontrol grubundaki dalağın ışık mikroskobu görünümü,* ***a)*** *Beyaz pulpa (wp), kırmızı pulpa (rp), beyaz pulpada merkezi arteriyoller (oklar), sinüzoidler (s), ölçek: 100 µm,* ***b)*** *Megakaryositlerin ayrıntılı görünümü, ölçek: 50 µm, H&E: Hematoxylin ve Eosin*

Low Dose: 30 mg/kg diazinon exposed group

Separation and hemorrhage in the splenic capsule were determined in the low dose group. Congestion within the splenic parenchyma was also detected when compared with the control group (Fig. 2a). Enlarged white pulps (Fig. 2b) and amyloid formation in dilated sinusoids were important findings (Fig. 2c). Additionally, karyolysis in some megakaryocytes was examined (Fig. 2d).

Medium dose: 60 mg/kg diazinon exposed group

In the medium dose group, an increase in the number of enlarged white pulps was observed (Fig. 3a). Hemorrhage within the splenic parenchyma and accumulation cells in dilated sinusoids were also detected (Fig. 3b). Amyloid formation and some cells passing from the splenic parenchyma into dilated sinusoids were also determined (Fig. 3c).

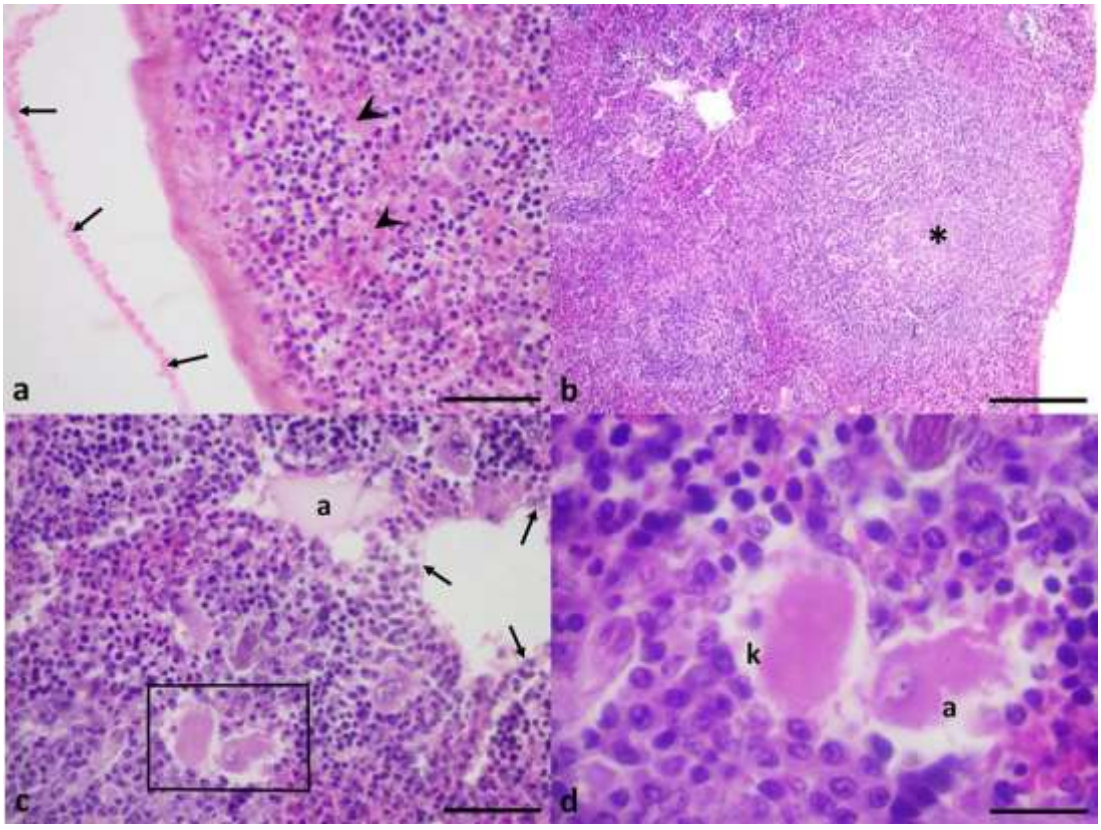

Figure 2. Light microscopic view of spleen in the low dose group after 30 days of diazinon exposure, **a)** Hemorrhage (arrow) in separated capsule. Congestion (arrowheads) within the splenic parenchyma, scale bar: 50 µm, **b)** Enlarged white pulp (asterisk), scale bar: 200 µm, **c)** Amyloid formation in dilated sinusoid (a) and enlarged sinusoid (arrow), scale bar: 50 µm **d)** Karyolysis (k) and amorphous (a) in megakaryocytes, scale bar: 20 µm, H&E: Hematoxylin and Eosin

Şekil 2. *30 günlük diazinon maruziyetinden sonra düşük doz grubunda dalağın ışık mikroskobu görünümü,* ***a)*** *Ayrılmış kapsülde kanama (ok). Dalak parankiminde konjesyon (ok başları), ölçek: 50 µm,* ***b)*** *Genişlemiş beyaz pulpa (yıldız), ölçek: 200 µm,* ***c)*** *Genişlemiş sinüzoidde amiloid (a) oluşumu ve genişlemiş sinüzoid (ok), ölçek: 50 µm,* ***d)*** *Karyoliz (k) ve megakaryositlerde şekilsizlik (a), ölçek: 20 µm, H&E: Hematoxylin ve Eosin*

High dose: 120 mg/kg diazinon exposed group

In the high dose group, intensive congestion (Fig. 4a) and necrotic areas within the splenic parenchyma (Fig. 4b) were detected. An increase in the number of karyolytic megakaryocytes was clearly examined (Fig. 4c). Moreover, fibrosis and some cells passing from spleen tissue into enlarged sinusoids were observed (Fig. 4d).

All histopathological changes were presented in Table 1. Evaluation of histopathological defects were made in a blinded manner by two persons.

Table 1. Histological alterations on the spleen of mice after exposure to diazinon (30, 60 and 120 mg/kg)
Çizelge 1. *Diazinona maruziyet sonrasında farelerin dalağındaki histolojik değişiklikler (30, 60 and 120 mg/kg)*

Tissue	Histopathological changes	Control	30 mg/kg	60 mg/kg	120 mg/kg
Spleen	Congestion	0	++	++	+++
	Separation of capsule	0	+++	0	0
	Dilated sinusoids	0	++	+++	++
	Enlarged white pulp	0	++	+++	0
	Karyolysis in megakaryocytes	0	+	+	+++
	Amyloid	0	++	++	0
	Cells in dilated sinusoids	0	0	+++	+
	Fibrosis	0	0	0	+++
	Necrotic areas	0	0	0	++

Note: Histopathological defects were presented based on their severity (None=0; Mild=+; Moderate=++; Severe=+++)
Not: *Histopatolojik hasarlar ciddiyetlerine göre belirtildi (Hiç=0; Zayıf=+; Orta=++; Şiddetli=+++)*

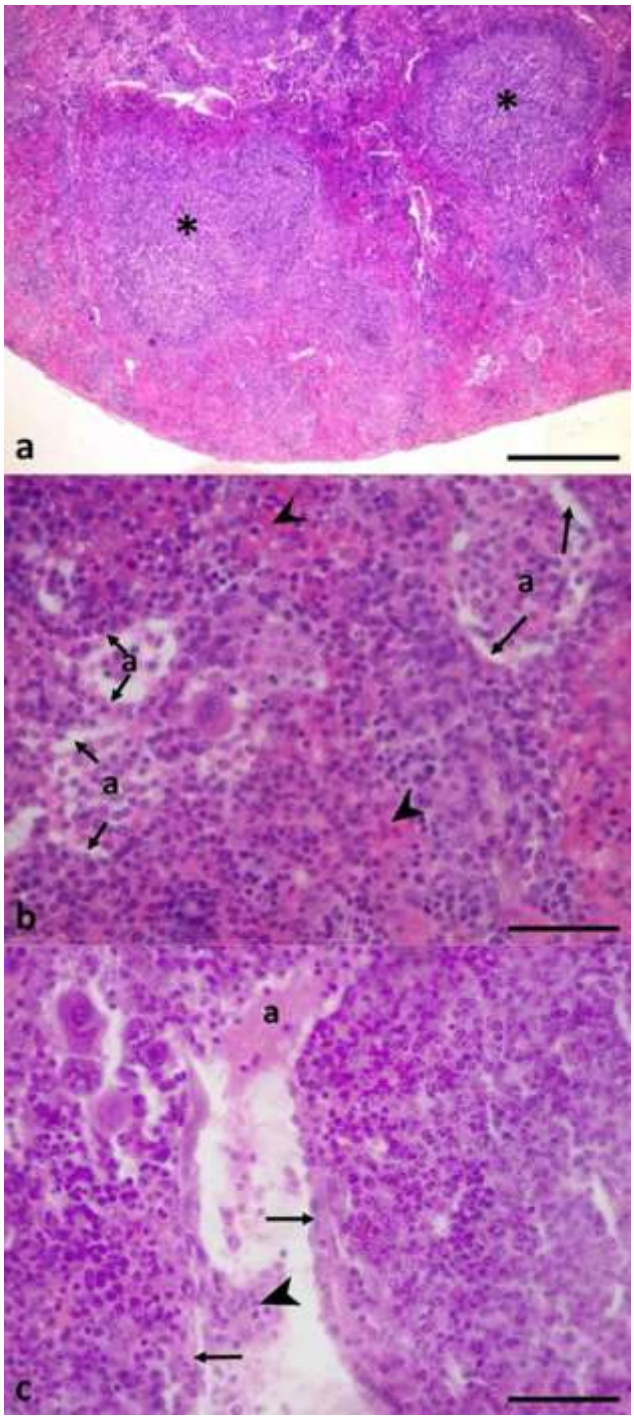

Figure 3. Light microscopic view of spleen in the medium dose group after 30 days of diazinon exposure, **a)** Enlarged white pulp (asterisk), scale bar: 400 µm, **b)** Congestion within splenic parenchyma (arrowheads) and accumulation cells (a) in dilated sinusoids (arrows), scale bar: 50 µm, **c)** Amyloid formation (a) and some cells (arrowhead) passing from spleen parenchyma into dilated sinusoids (arrows); scale bar: 50 µm, H&E: Hematoxylin and Eosin

Şekil 3. *30 günlük diazinon maruziyetinden sonra orta doz grubunda dalağın ışık mikroskobu görünümü,* ***a)*** *Genişlemiş beyaz pulpa (yıldız), ölçek: 400 µm,* ***b)*** *Dalak parankimasında konjesyon (ok başları) ve genişlemiş sinüzoidlerde hücrelerin birikimi (a), ölçek: 50 µm,* ***c)*** *Amiloid oluşumu (a) ve dalak parankimasından genişlemiş sinüzoidlere (oklar) geçen bazı hücreler (ok başı); ölçek: 50 µm, H&E: Hematoxylin ve Eosin*

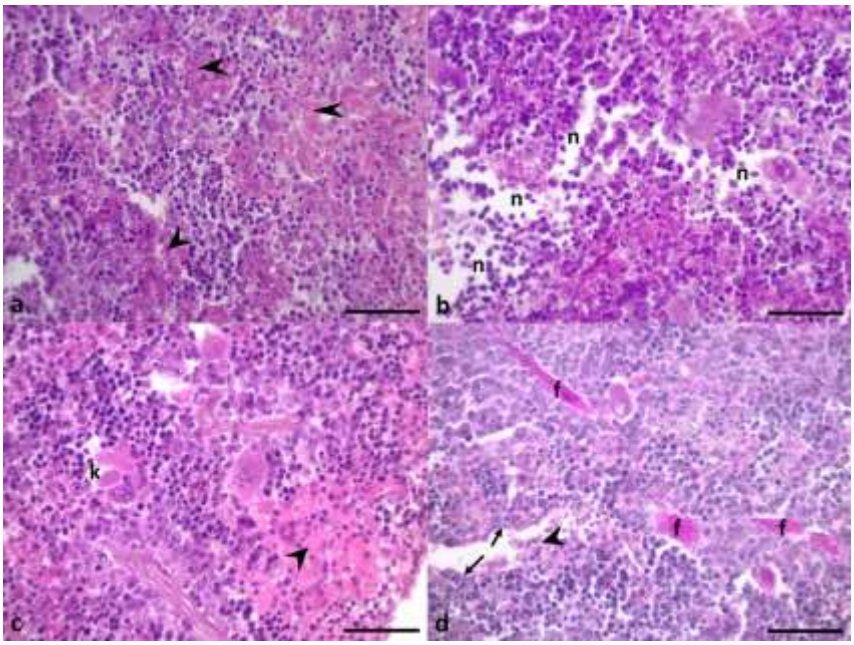

Figure 4. Light microscopic view of spleen in high dose group after 30 days of diazinon exposure, **a)** Intensive congestion within the splenic parenchyma (arrowheads), **b)** Wide necrotic areas (n), **c)** Karyolytic (k) megakaryocytes and congestion (arrowhead) in splenic parenchyma; H&E: Hematoxylin and Eosin, **d)** Fibrosis (f) and some cells (arrowhead) passing from spleen parenchyma into enlarged sinusoids (arrows); scale bars: 50 µm, PAS: Periodic Acid Schiff

Şekil 4. *30 günlük diazinon maruziyetinden sonra orta doz grubunda dalağın ışık mikroskobu görünümü,* ***a)*** *Dalak parankiminde yoğun konjesyon (ok başları),* ***b)*** *Geniş nekrotik alanlar (n),* ***c)*** *Karyolitik (k) megakaryositler ve dalak parankimasında konjesyon (ok başı); H&E: Hematoxylin ve Eosin,* ***d)*** *Fibrözis (f) ve dalak parankimasından genişlemiş sinüzoidlere (oklar) geçen bazı hücreler (ok başları); ölçekler: 50 µm, PAS: Periodic Acid Schiff*

DISCUSSION

The spleen, which is the largest lymphoid organ in the body, acts a prominent role in the defense mechanisms of organisms. Additionally, it serves as a blood cell reservoir and performs the destruction of old or damaged erythrocytes. It also acts to eliminate abnormal neutrophils and platelets, and it is a phagocytic filter to remove bacteria. Abnormality in this role, e.g. hypersplenism, results in an increase in the number of abnormal neutrophils, erythrocytes, platelets or any composition of these blood factors (Podder et al., 2010).

In the spleen there are two main functional parts, the red and white pulps. The localization of white pulp is around central arterioles, and it consists of the periarteriolar lymphoid sheath (PALS), primary or secondary follicles and marginal zone. PALS is known as the T-cell region. The localization of splenic B cells are mainly primary or secondary follicles adjacent to the PALS. The adjacent follicles and PALS are surrounded by a marginal zone that contains a mixture of cell types, and the junction between the marginal zone and the red pulp is not always apparent (Elmore, 2006). The spleen acts a vital role in hematopoiesis throughout the life of mice (Sieff & Williams, 1995). Therefore, many megakaryocytes were observed in the histological section of the spleen in both control and treatment groups in this study. Megakaryocytes are giant polyploid cells predominantly scattered in the bone marrow typically near sinusoidal capillaries. Their polyploid nuclei are large and irregularly lobulated with coarse chromatin. Myeloid stem cells give rise to megakaryocytes. Thrombopoietin, a humoral factor formed by the liver, is known to arrange megakaryocytes and the production of platelets (Mescher, 2016). Hematopoiesis is the dynamic process whereby all formed elements of the blood arise from the multipotent precursor cells produced by hematopoietic stem cells. While hematopoiesis in the adult human occurs primarily within the red bone marrow, several other tissues including yolk sac, liver, and spleen serve as primary sites of hematopoiesis during mammalian embryogenesis (Tavassoli, 1991; Zon, 1995). Both the spleen and bone marrow serve as the primary sites of blood cell production throughout the life of the postnatal mouse. On the other hand, bone marrow is the major lifelong blood-forming organ in humans (Sieff & Williams, 1995).

Due to fact that reports related to the negative impacts of pesticides on mammalian splenic tissue

are limited and the spleen is the site of direct and indirect toxicity, the current work was performed to elucidate the harmful impacts of diazinon on the splenic parenchyma of Swiss albino mice. Therefore, the results of this study can only be discussed with a few other searches. Separation and hemorrhage in the capsule and congestion within the splenic parenchyma were determined in the low dose group. After exposure to the aromatic amine 3,4-dichloroaniline (herbicide), a dose-related enlargement of splenic red pulp was described with prominent vascular congestion and increased red pulp cellularity in a fish species, *Pomatoschistus microps* (Monteiro et al., 2006). In our study, enlarged white pulp and amyloid formation in dilated sinusoids were important findings in the low dose group. Lovat et al. (2015) reported that mouse models of Non-Hodgkin Lymphomas showed splenomegaly with enlargement of the white pulp secondary to expansion/accumulation of B lymphocytes. Neishabouri et al. (2007) investigated some toxic effects of diazinon on mice internal organs. Authors found that the ratio of spleen red pulp to white pulp demonstrated an important increase. In addition, diazinon caused white pulp atrophy with capsular and trabecular damages in the spleen.

In the medium dose group, congestion within the splenic parenchyma of Swiss albino mice was observed. Similar observations were noticed by Mohany et al. (2011) who reported that imidacloprid insecticide caused congestion in the red pulp of the spleen of male albino rats. We also examined accumulation of cells in dilated sinusoids, amyloid formation, and some passing cells from splenic cords into dilated sinusoids, an increase in the number of enlarged white pulps in the medium dose group. Korani et al. (2011) noted hypertrophic white pulps after exposure to $AgNO_3$. The white pulp of mice also revealed hypertrophy after being exposed to zinc oxide, cyclodextrin and cefepime (Oprea et al., 2016). Dkhil (2009) reported that there was an apparent enlargement of the white pulp because of cellular proliferation. Handy et al. (2002) mentioned the diazinon effects on some organs of mice. However, they did not present detailed findings on these organs including spleen. They reported the hyperplasia of white and red pulps and hemorrhage after exposure to diazinon. In this study, intensive congestion and some cells passing from spleen tissue into enlarged sinusoids were observed in the high dose group. An increase in the number of karyolytic megakaryocytes were also examined. Studies of diazinon's mutagenicity showed that it can damage DNA in human blood cells and cells from laboratory animals (Grover et al., 2003). Moreover, disintegration of the splenic tissue and fibrosis were observed. Similar lesions were determined in the spleen of rats exposed to Bisphenol A (Ahmed et al., 2015) and Levantine frog, *Pelophylax bedriagae* exposed to Carbaryl (Çakıcı, 2018). All these histopathological changes may be attributed to functional disruption of the spleen. Due to the primary role of megakaryocytes in blood cell production, an increase in the number of karyolytic megakaryocytes showed that diazinon has the capacity to hinder blood cell formation. According to Handy et al. (2002), chronic diazinon exposure resulted in necrotic degeneration of the trabeculae in both spleen and thymus of mice. El-Bendary et al. (2014) showed that profenofos and chlorpyrifos caused hepatic and splenic lesions including congestion and hemorrhage in male mice. Due to the splenic necrose, it exhibited a reduction in the number of lymphocytes in the white pulp of the high dose group in this study. On the other hand, Dkhil (2009) reported that it was difficult to differentiate the limit between white and red pulps in the spleen of malaria-infected mice. Similar finding was observed in the high dose group of our study.

CONCLUSION

Diazinon induced considerable histological defects in the splenic tissue of Swiss albino mice. Since there is little research related to the impacts of pesticides on the spleen of mammals, the current research will be useful for other mammalian toxicologic investigations.

Researchers' Contribution Rate Statement

The authors declare that they have contributed equally to the article.

Conflict of Interest Statement

Authors declare no conflict of interest.

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Bolkar Dağları Üzerindeki Bazı Yüksek İrtifa Gölleri Bentik Makroomurgasız Faunası

Selvi İLHAN[1], Selda ÖZTÜRK[2]✉,Sevil SUNGUR[3], Burak SEÇER[4], Erdoğan ÇİÇEK[5]
[1,4,5]Nevşehir Hacı Bektaş Veli Üniversitesi, Fen Edebiyat Fakültesi, Biyoloji Bölümü, 50300 Nevşehir, [2]Kapadokya Üniversitesi, Sağlık Bilimleri Yüksekokulu, 50400 Nevşehir, [3]Nevşehir Hacı Bektaş Veli Üniversitesi, Sağlık Hizmetleri MYO, 50300 Nevşehir
[1]https://orcid.org/0000-0002-3947-1470, [2]https://orcid.org/0000-0002-5639-7962, [3]https://orcid.org/0000-0003-4018-6375
[4]https://orcid.org/0000-0002-8763-131X, [5]https://orcid.org/0000-0002-5334-5737
✉: seldaozturkk50@gmail.com

ÖZET

Bu çalışma, Bolkar Dağları üzerinde yer alan bazı yüksek irtifa göllerine ait (Yazıgöl, Yarıkgöl, Otlugöl, İsimsiz Göl, Eğrigöl ve Alagöl) bentik makroomurgasız faunasının belirlenmesi amacıyla Temmuz ve Ağustos 2019 tarihlerinde gerçekleştirilmiştir. Elde edilen makrobentik faunanın sistematik açıdan incelenmesi sonucunda sekiz takım (Diptera, Coleoptera, Trichoptera, Rhynchobdellida, Haplotaksida, Sphaeriida, Hygrophila ve Hemiptera) içerisinde sekiz familya ve 22 cinse mensup 23 tür tespit edilmiştir. Göllere ait tür çeşitliliği ve türler arasındaki yoğunluk ilişkilerinin belirlenebilmesi için Shannon-Wiener çeşitlilik (H) ve Shannon-Evenness yoğunluk (EH) indeksleri uygulanmış olup en yüksek çeşitlilik (H) 2.17 değeri ile Yarıkgöl'de gözlenirken bunu 1.94; 1.55; 1.08; 0.40 ve 0.38 değerleri ile sırasıyla Alagöl, Otlugöl, İsimsiz Göl, Yazıgöl ve Eğrigöl takip etmiştir. En dengeli dağılım (EH) ise Yarıkgöl ve İsimsiz Göl (0.73) olarak hesaplanırken bunu 0.59; 0.47; 0.25 ve 0.24 değerleri ile sırasıyla Otlugöl, Alagöl, Yazıgöl ve Eğrigöl izlemiştir. Belirlenen taksanın dağılımına göre en yüksek benzerlikler Otlugöl ve Yarıkgöl ile Eğrigöl ve İsimsiz Göl arasında tespit edilmiştir. En düşük benzerlikler ise İsimsiz Göl ve Yarıkgöl arasında hesaplanmıştır. Bu çalışma bölgedeki göller üzerinde yapılmış ilk çalışma niteliğinde olup göllerin makroomurgasız faunası ilk kez ortaya çıkarılmıştır.

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Bolkar dağları

Benthic Macroinvertebrate Fauna of Some High Altitude Lakes on the Bolkar Mountains

ABSTRACT

This study was carried out in July and August 2019 to determine the benthic macroinvertebrate fauna of some high altitude lakes (Yazıgöl, Yarıkgöl, Otlugöl, İsimsiz Lake, Eğrigöl, and Alagöl) located on the Bolkar Mountains. As a result of the systematic examination of the obtained macrobenthic fauna, 23 species belonging to eight families and 22 genera were identified in eight orders (Diptera, Coleoptera, Trichoptera, Rhynchobdellida, Haplotaxida, Sphaeriida, Hygrophila and Hemiptera). Shannon-Wiener diversity (H) and Shannon-Evenness density (EH) indices were applied, respectively, in order to determine the species diversity of the lakes and the density relationships among the species. Accordingly, while the highest diversity (H) was observed in Yarıkgöl with a value of 2.17, ıt was followed by Alagöl, Otlugöl, İsimsiz Göl, Yazıgöl and Eğrigöl with values of 1.94; 1.55; 1.08; 0.40 and 0.38, respectively. While the most balanced distribution (EH) was calculated as Yarikgöl and İsimsiz Lakes, which have the same value (0.73), it was followed by Otlugöl, Alagöl, Yazıgöl and Eğrigöl with the values of 0.59; 0.47; 0.25 and 0.24, respectively. Cluster analysis based on the Bray-Curtis similarity index was applied to determine the similarities between the lakes according to the distribution of the determined taxa. Accordingly, the highest similarities were found between Otlugöl and Yarıkgöl, Eğrigöl and İsimsiz Lakes, which has the same similarity rate. The lowest similarities were calculated between İsimsiz Lake and Yarıkgöl. This

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study is the first study on these lakes in the region, and the macroinvertebrate fauna of the lakes was listed for the first time.

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GİRİŞ

Yüksek dağlar ve sıradağlar zoocoğrafik bağlamda canlıların yayılış sınırlarını etkileyen önemli doğal bariyerlerdir. Ekstrem iklim özelliklerine sahip olan yüksek irtifa bölgeleri bu koşullara adapte olmuş endemik türleri barındırmaları bakımından önem arz ederler. Bu bölgeler üzerinde mevsimsel veya daimi su kütleleri özelliğinde yüksek irtifa gölleri bulunmaktadır (Ustaoğlu ve ark., 2008). Bu göllerin yer aldığı dağlık bölgelerdeki iklimsel farklılıklar gerek karasal gerekse sucul ekosistemlerdeki canlı popülasyonlarının dağılımlarını sınırlandırmaktadır (Dullinger ve ark., 2012). Bu nedenle bu ekosistemler diğer sucul habitatlara nazaran daha düşük çeşitlilik ve ekstrem çevre şartlarına uyum sağlamış canlılar tarafından temsil edilirler (Galas, 2004; Krno ve ark., 2006; Sömek & Ustaoğlu, 2016).

Ova gölleri ile kıyaslandığında, nispeten küçük ekosistemler olan yüksek irtifa gölleri, bulundukları konum itibari ile bakir alanlar olup antropojenik kaynaklı tarımsal veya evsel kirleticilerden neredeyse hiç etkilenmezler. Bu sebeple çevresel bozulmaların ekosistem üzerindeki etkilerinin test edilmesi bakımından referans alan özelliği taşırlar (Galas, 2004; Taşdemir & Ustaoğlu, 2016). Fakat günümüzdeki artan küresel iklim değişimi sonucu gelişen atmosferik kirleticilerin birikimi bu sucul sistemler üzerinde bir tehdit oluşturmaktadır. Buna bağlı olarak gerek su kalitesinde gerekse canlı kompozisyonunda birtakım olumsuz değişimler ortaya çıkabilmektedir. Diğer yandan sahip oldukları benzersiz doğal güzellikleri sebebiyle rekreasyon, kamp, dağcılık, doğa sporları vb. gibi faaliyetlere açık olan bu ekosistemlerdeki faunanın tespiti yeterli düzeyde tamamlanamadan kaybedilme tehlikesi ile karşı karşıyadır (Bayrak, 2015; Kıymaz, 2018; Baydar, 2020). Son zamanlarda antropojenik etkiler ve küresel iklim değişiminin 6. tür yok oluşunu tetiklediğini ve tahmin edilenden daha hızlı ve etkili şekilde kendini göstereceği ileri sürülmektedir. Her ne kadar tür yok oluşlarında omurgalı canlılara ve mercan resiflerine dikkat çekilse de omurgasızların daha fazla etkileneceği ve maalesef bu konuda insan oğlunun elinde veri eksikliği olduğu itiraf edilmektedir (Ceballos ve ark., 2020; Cowie ve ark., 2022).

Sucul ekosistemlerin önemli bileşenlerinden olan bentik makroomurgasızlar, sahip oldukları farklı beslenme düzeyleri ile geniş bir niş işgal ederler. Bu canlı grupları besin piramidinin alt basamaklarından üst basamaklarına doğru madde ve enerjinin aktarılmasında kilit bir role sahiptirler (Şahin, 1991; Kıymaz, 2018). Ayrıca, çevresel etkilere cevapları farklılık gösteren birçok canlı grubunu bünyelerinde barındırmaları ile sucul sistemlerin genel ekolojik yapısı ve su kalitesi hakkında yargıya varılmasına olanak sağlamaktadırlar (Dügel & Kazancı, 2004). Bu nedenle makroomurgasızlar gerek lentik gerekse lotik sistemlerin sağlığının belirlenmesinde en elverişli ve yaygın olarak kullanılan önemli biyoindikatörler olarak bilinirler (Akbaba & Boyacı, 2015; Dügel, 2016). Dolayısıyla sucul ekosistemlerdeki makrobentik faunanın dağılımı ile bu dağılım üzerinde rol oynayan çevre faktörlerinin belirlenmesi, gerek biyoçeşitlilik gerekse ekosistem izleme ve indeks çalışmaları kapsamında önem taşımaktadırlar (Bayrak, 2015; Kıymaz, 2018; Baydar, 2020).

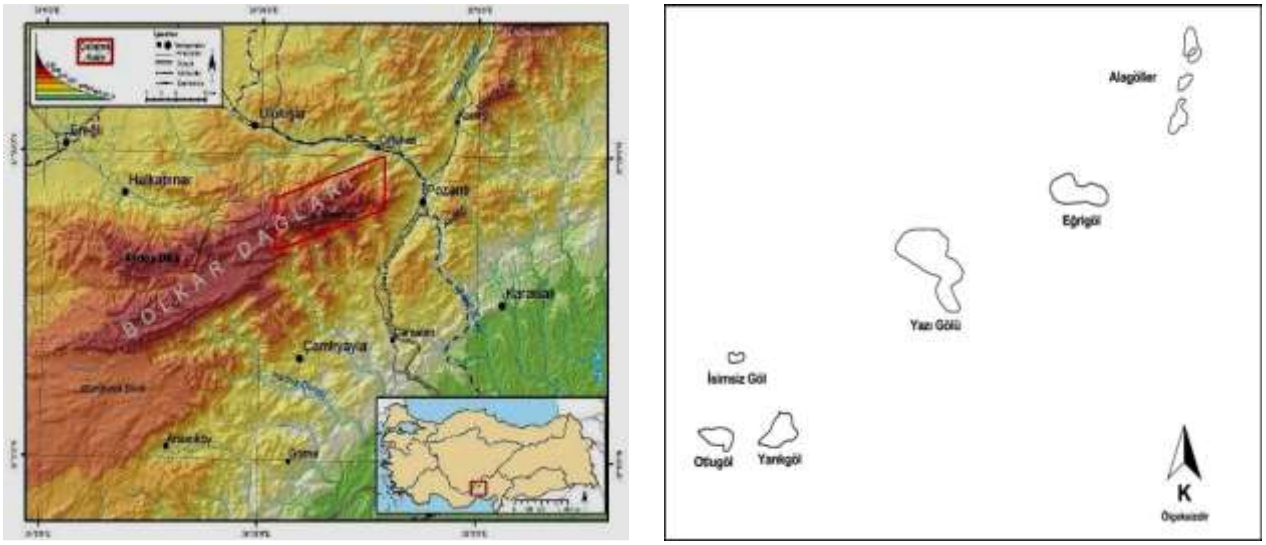
Avrupa, Akdeniz ve İran bölgeleri ile Sahara Arabian alt bölgeleri arasında konumlanmış olan Bolkar Dağları, Güney Anadolu bölgesi Orta Toroslar kısmında yer almaktadır. Aladağlardan sonra ikinci en yüksek dağ sırası olarak bilinen bu dağlar, yer aldığı coğrafik konum itibariyle Akdeniz ile İç Anadolu bölgeleri arasında sınır ve bariyer oluşturmaktadırlar (Uçak, 2013). Değişken bir iklim yapısına sahip olan Bolkar Dağları'nın güney yamaçları genel olarak Akdeniz iklim özelliğine sahipken, kuzey yamaçları İç Anadolu Bölgesinde hüküm süren tipik karasal iklim özelliği sergilemektedir (Gemici, 1993). Bolkar Dağları'nın orta bölümünde yer alan Alpin kuşak, Temmuz ayına kadar karlarla örtülü olup bir kısmı mevsimsel bir kısmı ise daimi olan irili ufaklı pek çok yüksek irtifa gölünü barındırmaktadır. Diğer yandan, dağ silsilesinin güney yamaçlarındaki nemli mikro iklim özelliği sergileyen derin vadiler Avrupa-Sibirya floristik elemanlarının yanında bazı relikt formları barındırmaları ile de elzem ekosistemlerdir (Gemici, 1993). Bu nedenle Bolkarlar, çeşitlilik açısından gerek Türkiye'nin gerekse Avrupa'nın en önemli ve zengin dağlarından birisi olarak kabul edilmektedir (Gürses ve ark., 1996). Bu öncül çalışma ile Bolkar Dağları üzerinde yer alan bazı yüksek irtifa göllerine ait bentik makroomurgasız faunasının belirlenmesi amaçlanmıştır.

MATERYAL VE METOT

Çalışma kapsamında Temmuz ve Ağustos 2019

tarihlerinde Bolkar Dağlarında bulunan bazı yüksek irtifa göllerinde arazi çalışmaları yapılmıştır. Örnekleme işlemleri her göle ait üç lokaliteden bir defaya mahsus olmak üzere yürütülmüştür. İncelenen göllerin isimleri yöre halkına sorularak kaydedilmiş olup geçici bir göl için isim kullanılmadığı için "İsimsiz göl" olarak adlandırılmıştır.

Bu çalışmada altı gölde (Şekil 1 ve Çizelge 1) farklı habitatlardan (kum, çakıl, vejetasyon vb.) bireylerin yakalanmasına dikkat edilecek şekilde multihabitat örnekleme yöntemi sürdürülerek ve 500 µm göz açıklığına sahip dip kepçesi kullanılarak bentik makroomurgasız örneklemesi yapılmıştır. Toplanan bentoz, çamurlarından arındırıldıktan sonra %4'lük formaldehit çözeltisi ihtiva eden plastik kaplar içerisinde fikse edilerek Nevşehir Hacı Bektaş Veli Üniversitesi Hidrobiyoloji Araştırma Laboratuvarına taşınmıştır. Laboratuvara taşınan bentik faunanın teşhis işlemleri Leica EZ-4D marka stereo mikroskop ve Leica DM-500 marka ışık mikroskobu altında gerçekleştirilmiştir.



Şekil 1. Bolkar Dağları yer bulduru haritası ve örnekleme istasyonlarının konumu
Figure 1. Location map of the Bolkar Mountains and the location of the sampling stations

Çizelge 1. Örnekleme istasyonları bilgileri
Table 1. Sampling stations information

No	Göl (Lake)	Rakım (m) (Altitude)	Koordinat (Coordinate)	Özellik (Characteristic)
1	Yazıgöl	2778	37°19'09.18"K-34°24'40.95"D	Mevsimsel-dip yapısı çamurlu
2	Yarıkgöl	2740	37°15'25.97"K-34°20'43.79"D	Daimi-dip yapısı çamurlu
3	Otlugöl	2731	37°15'37.38"K-34°21'25.17"D	Daimi-dip yapısı çamurlu su bitkileri mevcut
4	İsimsiz Göl	2692	37°16'44.28"K-34°20'37.47"D	Mevsimsel-dip yapısı çamurlu ve çakıllı
5	Eğrigöl	2715	37°21'09.93"K-34°27'58.90"D	Daimi-dip yapısı çamurlu
6	Alagöller	2905	37°23'17.49"K-34°30'37.65"D	Daimi-dip yapısı çamurlu ve çakıllı

Haplotaksida ve Diptera larvalarına ait teşhislerde diseksiyon işlemi yapılarak geçici ve daimi preparatlar hazırlanmış, diseksiyona ihtiyaç duyulmayan diğer grupların ise morfolojileri üzerinden değerlendirmeye gidilmiştir. Taksonomik kategorizasyon ve tür tayinlerinde; Coleoptera için Illies (1955), Brauer (1909); Trichoptera için Ulmer (1961), Jansson ve Vuoristo (1979), Brohmer (1979), Wallace ve ark. (1990); Sphaeriida için Parenzon (1974) ve Nordsieck (1968); Heteroptera için Stichel (1960,1961), Wagner (1965), Stobbe (1985); Hygrophila için Glöer ve ark. (1985); Rhynchobdellida için Elliot ve Mann (1979); Diptera için Şahin (1987, 1991), Pennak (1991); Haplotaksida için Brinkhurst ve Jamieson (1971), Brinkhurst ve Gelder (1991), Milligan (1997), Timm (1999) ve Wetzel ve ark., (2000)'dan yararlanılmıştır.

Göllerde tespit edilen makrobentik faunaya ait komünite yapısının incelenebilmesi için baskınlık analizi (Kocataş, 1997), türlerin dağılımlarına bağlı olarak istasyonlar arasındaki ayrımların belirlenebilmesi için Bray-Curtis benzerlik indeksine dayalı Cluster kümeleme analizi (Bray & Curtis, 1957) uygulanmıştır. Ayrıca göl istasyonları arasındaki ayrımlar n-MDS (non-metric multidimensional scaling) analizi (Clarke, 1993) ile görselleştirilmiştir. Tür çeşitliliği ve türler arasındaki yoğunluk ilişkilerinin belirlenebilmesi için ise sırasıyla Shannon-Wiener çeşitlilik ve Shannon-Evenness yoğunluk indeksleri (Shannon & Wiener, 1949) uygulanmıştır. Analizler PAST 3,0 ve CAP 4,0 paket

programları üzerinden gerçekleştirilmiştir.

BULGULAR ve TARTIŞMA

Örnekleme çalışmaları sonucunda toplamda 4971 birey incelenmiş ve sekiz takım (Diptera, Coleoptera, Trichoptera, Rhynchobdellida, Haplotaksida, Sphaeriida, Hygrophila ve Hemiptera) içerisinde sekiz familyaya mensup 22 cins ve 23 tür belirlenmiştir (Çizelge 2 ve Şekil 2).

Çizelge 2. Göllere ait tespit edilen taksanın dağılımı (*: Cins düzeyindeki tolerans seviyesi, YG: Yazıgöl, YRG: Yarıkgöl, OG: Otlugöl, EG: Eğrigöl, AG: Alagöl)

Table 2. Distribution of taxa detected in lakes (: Tolerance level evaluated at genus level, YG: Yazıgöl, YRG: Yarıkgöl, OG: Otlugöl, EG: Eğrigöl, AG: Alagöl)*

Sistematik Kategori (Takım/Familya/Tür) Systematic Category (Order/Family/Species)	Göller (Lakes)						Tolerans (Tolerance)	Referans (Reference)
	YG	YRG	OG	İG	EG	AG		
Diptera								
Chironomidae								
Procladius sp.	+	+	+			+	9	Bode ve ark., 1996
Psectroladius limbatellus (Brundin, 1949)	+	+	+			+		
Cladotanytarsus mancus (Walker, 1856)	+						5*	Bode ve ark., 1996
Virgatanytarsus arduennensis (Goetghebuer, 1922)	+					+		
Chironomus tentans (Fabricius, 1805)			+	+	+	+		
Micropsectra paraecox (Wiedemann, 1818)					+	+	7*	Bode ve ark., 2002
Macropelopia nebulosa (Meigen, 1804)					+	+		
Chironomus riparius (Meigen, 1804)		+	+		+	+	10*	Bode ve ark., 1996
Coleoptera								
Dytiscidae								
Porhydrus lineatus (Fabricius, 1775)	+					+		
Agabus biguttatus (Olivier, 1975)						+	5*	Bode ve ark., 1996
Dytiscus marginalis (Linnaeus, 1758)		+						
Graptodytes veterator (Zimmermann, 1918)		+						
Trichoptera								
Limnephilidae								
Limnephilidae Gen. sp.	+			+	+	+	4	Hauer & Lamberti, 1996
Limnephilus bipunctatus (Curtis, 1834)						+	3*	Bode ve ark., 1996
Rhynchobdellida								
Glossiphoniidae								
Helobdella stagnalis (Linnaeus, 1758)		+	+			+	8	Bode ve ark., 2002
Haplotaksida								
Naididae								
Tubifex tubifex (Müller, 1774)		+		+	+	+	10	Bode ve ark., 1996
Potamothrix hammoniensis (Michaelsen, 1901)		+						
Bothrioneurum vejdovskyanum (Stolc, 1886)			+				7	Bode ve ark., 2002
Ilyodrilus templetoni (Southern, 1909)				+			10	Bode ve ark., 1996
Sphaeriida								
Sphaeriidae								
Sphaerium rivicola (Lamarck, 1818)		+				+	6*	Bode ve ark., 1996
Pisidium sp.		+				+	6	Bode ve ark., 1996
Hygrophila								
Planorbidae								
Planorbis carinatus (Müller, 1774)		+	+					
Hemiptera								
Corixidae								
Micronecta sp.		+	+					
TOPLAM	356	102	77	26	3151	1294	4971	

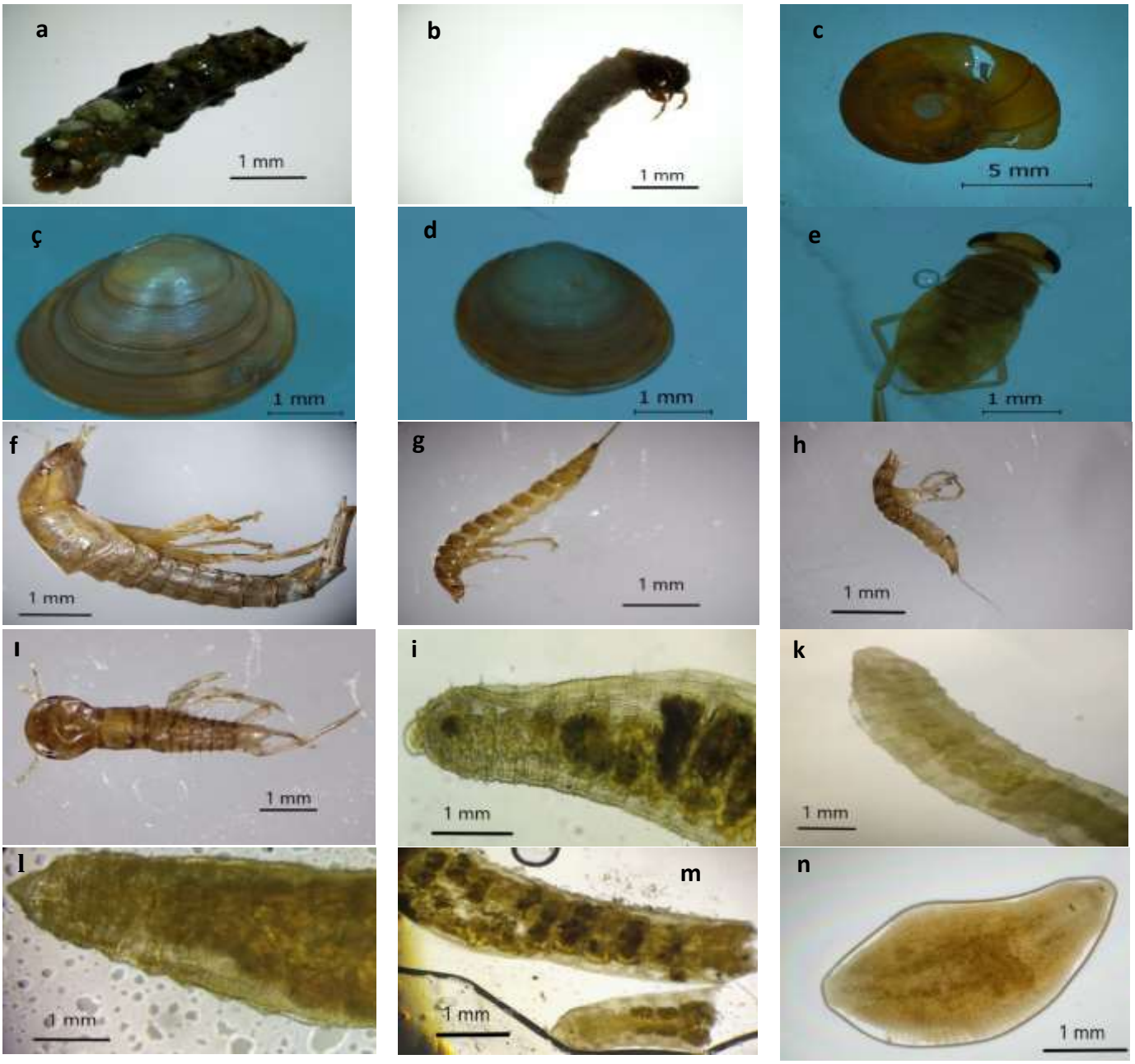
Tespit edilmiş olan sekiz takıma göre, incelenen 4971 birey içerisinde en yüksek baskınlık (%69.26) 3443 birey ile Haplotaksida takımında (Naididae) gözlenirken bunu 888 birey (%17.86) ile Diptera takımı (Chironomidae), 311 birey (%6.26) ile de Sphaeriida takımı (Sphaeriidae) izlemiştir. En düşük baskınlık ise 3 birey (%0.06) ile Hemiptera takımında (Corixidae) gözlemlenmiştir (Şekil 3).

Tür kategorisinde yapılan değerlendirmeye göre en baskın türün 3414 birey (%68.68) ile *T. tubifex*'e ait olduğu belirlenirken bunu 474 birey (%9.54) ile *Procladius* sp.'nin izlediği görülmüştür. Baskınlığın en düşük gözlendiği taksonlar ise birer birey (%0,02) ile *A. biguttatus* ve *B. vejdovskyanum* olarak tespit edilmiştir.

Her bir göle ait makrobentik faunanın dağılımları değerlendirilecek olursa; Yazıgöl'de üç takım içerisinde (Diptera, Coleoptera ve Trichoptera), üç familyaya (Chironomidae, Dytiscidae ve Limnephilidae) mensup altı tür belirlenmiştir. İncelenen 356 birey içerisinde en yüksek baskınlık 326 birey (%91.57) ile *Procladius* sp. türünde gözlenirken,

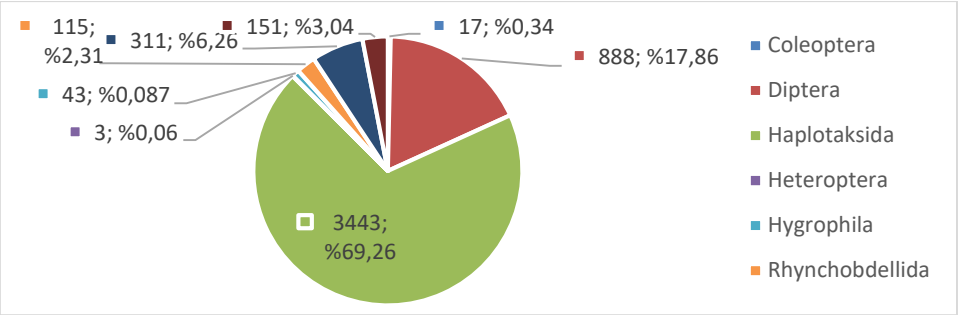
en düşük baskınlık ise ikişer birey (%0.56) tespit edilmiş olan *V. arduennensis* ve *P. lineatus* türlerinde gözlenmiştir.

Yarıkgöl'de 102 birey incelenmiş ve yedi takım içerisinde (Diptera, Coleoptera, Rhynchobdellida, Haplotaksida, Sphaeriida, Hygrophila ve Hemiptera) yedi familyaya mensup (Chironomidae, Dytiscidae, Glossiphoniidae, Naididae, Sphaeriidae, Planorbidae ve Corixidae) 12 tür tespit edilmiştir. Bu gölde en yüksek baskınlık (20 birey; %19.61) *Procladius* sp. türünde tespit edilirken, en düşük baskınlık ikişer birey (%1.96) ile *G. veterator* ve *Micronecta* sp. türlerinde gözlenmiştir.



Şekil 2. Tespit edilen bazı türlere ait genel vücut görüntüleri (a: Limnephilidae Gen. sp, b: *Limnephilus bipunctatus*, c: *Planorbis carinatus*, ç: *Pisidium* sp., d: *Spharium rivicola*, e: *Micronecta* sp., f: *Dytiscus marginalis*, g: *Graptodytes veterator*, h: *Porhydrus lineatus*, ı: *Agabus biguttatus*, i: *Potamothrix hammoniensis*, k: *Bathrioneurum vejdovskyanum*, l: *Tubifex tubifex*, m: *Ilyodrilus templetoni*, n: *Helobdella stagnalis*)

Figure 2. General body images of some detected species (a: Limnephilidae Gen. sp, b: Limnephilus bipunctatus, c: Planorbis carinatus, ç: Pisidium sp., d: Spharium rivicola, e: Micronecta sp., f: Dytiscus marginalis, g: Graptodytes veterator, h: Porhydrus lineatus, ı: Agabus biguttatus, i: Potamothrix hammoniensis, k: Bathrioneurum vejdovskyanum, l: Tubifex tubifex, m: Ilyodrilus templetoni, n: Helobdella stagnalis)



Şekil 3. Tespit edilen takımlara ait % baskınlıklar
Figure 3. % dominance of the teams detected

Su bitkilerinin bulunduğu Otlugöl'de beş takım (Diptera, Rhynchobdellida, Haplotaksida, Hygrophila ve Hemiptera) ve beş familyaya mensup (Chironomidae, Glossiphoniidae, Naididae, Planorbidae, Corixidae) sekiz tür tespit edilmiştir. İncelenen bireyler içerisinde en yüksek baskınlık 26 birey (%33.77) ile *P. carinatus* türünde gözlenirken bunu 20 birey (%25.97) ile *C. tentans* izlemiştir. *Procladius* sp., *P. limbatellus*, *C. riparius* ve *Micronecta* sp. türlerinden ise birer birey (%1.3) tespit edilmiştir.

En fazla bireyin (3151) örneklendiği Eğrigöl'de ise *T. tubifex* 'in %92.03'lük oran ile baskın tür olduğu görülmüştür. Bu gölde üç takım içerisinde (Diptera, Trichoptera ve Haplotaksida) üç familyaya mensup (Chironomidae, Limnephilidae ve Naididae) altı tür tespit edilmiştir.

Geçici bir göl olan İsimsiz Göl'de en baskın tür olarak (15 birey ve %57.69) Limnephilidae Gen. sp.'ye rastlanmıştır.

En yüksek tür çeşitliliğine sahip olan Alagöl'de altı takım içerisinde (Diptera, Coleoptera, Trichoptera, Rhynchobdellida, Haplotaksida ve Sphaeriida), altı familyaya mensup (Chironomidae, Dytiscidae, Limnephilidae, Glossiphoniidae, Naididae ve Sphaeriidae) 15 tür tespit edilmiştir. İncelenen 1294 birey içerisinde %38.95'lik oran ile *T. tubifex* türünün en baskın olduğu belirlenirken *C. riparius* ve *A. biguttatus* türlerinden ise birer bireye rastlanmıştır.

Çalışma kapsamında birey sayısı olarak en baskın grubu %69.26'lık oran ile Haplotaksida (Nadidae) oluşturmaktadır. Bu takımın üyeleri genellikle kozmopolit türlerdir ve Türkiye'deki bazı yüksek dağ göllerinden kayıtları bildirilmiştir (Brinkhurst, 1969; Geldiay & Tareen, 1972; Milbrink, 1980; Ustaoğlu, 1980; Taşdemir ve ark., 2004; Yıldız ve ark., 2007). Nadidae familyasına mensup türler yüksek ekolojik toleranslarıyla bilinmektedir (Brinkhurst ve Jamieson, 1971). Çalışmamızda bu familyaya mensup tespit edilen *T. tubifex* türü 4 istasyonda (Yarıkgöl, İsimsiz Göl, Eğrigöl ve Alagöl) gözlenmiştir. Bu türün (tolerans seviyesi=10) organik madde girdisinin yüksek olduğu ötrofik sucul sistemlerde bol miktarda bulunduğu bilinmektedir (Bode ve ark., 1996; Yıldız, 2003). Eğrigöl ve Alagölde en baskın tür olarak *T. tubifex*'e rastlanmış olması göller etrafında yoğun bir şekilde koyun otlatılmasından ve koyun sürülerinin su ihtiyaçlarını karşılayabilmek için her gün bu göllere uğramasından kaynaklanmaktadır. Yarıkgöl'de tespit edilmiş olan *P. hammoniensis* türü de ötrofik karakterli göllerde bulunan ve organik kirliliğin indikatörü olarak bilinen kozmopolit bir türdür (Ustaoğlu ve ark., 2008). Otlugölde tespit edilen *B. vejdovskyanum* taksonunun genellikle kumlu ve çakıllı habitatlarda dağılım gösterdiği ve ötrofikasyona karşı toleranslı bir tür (tolerans seviyesi=7) olduğu bildirilmiştir (Bode ve ark., 1996; Kathman & Brinkhurst, 1998; Odabaşı ve ark., 2017).

Birey sayısı bakımından ikinci baskın grup olan Diptera (Chironomidae) takımı içerisinde tespit edilen *C. tentans* ve *C. riparius* türleri (tolerans seviyeleri=10) genellikle ötrof göllerde dağılım gösteren toleranslı türlerdir (Hilsenhoff, 1987; Bode ve ark., 1996). *Chironomus* cinsine ait türler kirlilik araştırmalarında biyolojik yöntemlerde kullanılan yaygın bir gruptur (Kazancı ve ark., 1997; Ayık, 2006). Toleranslı olarak bilinen *M. praecox* (tolerans seviyesi=7) ve *M. nebulosa* türleri (Bode ve ark., 2002; Kökçü, 2016) Eğrigöl ve Alagöl'de tespit edilmiştir. Diğer bir toleranslı canlı olan *Procladius* (*Holotanypus*) sp. (tolerans seviyesi=9) taksonu (Bode ve ark., 1996; Kökçü, 2016) Yazıgöl, Yarıkgöl, Otlugöl ve Alagöl'de tespit edilmiştir. Bu durum antropojenik etkilerden uzak olan bir gölde çok düşük düzeydeki organik madde girdisinin dahi canlı topluluğunun varlığı ve baskınlığı üzerinde nasıl hızlı bir etki yaptığını ortaya koymaktadır. Diğer yandan Yazıgölde tespit edilen *C. mancus* (tolerans seviyesi=5) türünün ise serin ve oksijence zengin suları tercih ettiği bildirilmiştir (Bode ve ark., 1996; Akyıldız, 2013).

Ryhnchobdellida takımı içerisinde Hirudinae alt sınıfına mensup *H. stagnalis* (tolerans seviyesi=8) (Bode ve ark., 2002) türüne Yarıkgöl, Otlugöl ve Alagöl'de rastlanmıştır. Ektoparazit olarak kabul edilen *H. stagnalis*'in bentik omurgasızların vücut sıvıları ile beslendikleri bilinmektedir (Elliot & Mann, 1979). Nitekim bu türün yüksek tür çeşitliliğine sahip yoğun vejetasyonun bulunduğu göllerde dağılım gösterdiği tespit edilmiştir.

Köklü bitkilerin var olduğu sığ sularda yayılış gösteren *P. carinatus* (Planorbidae) türüne (Baker, 1945; Zhadin, 1952) Yarıkgöl ve Otlugöl'de rastlanmıştır.

Sphaeriidae familyasına mensup türler, sucul sistemlerdeki çözünmüş ve partikül halindeki kirletici maddeleri süzerek bünyelerinde biriktirdiklerinden biyoindikatörler olarak bilinirler (Viarengo & Canesi, 1991; Viarengo ve ark., 2007). Bu familya üyelerinden *Pisidium* sp. ve *S. rivicola* türleri (tolerans seviyeleri=6) (Bode ve ark., 1996) Yarıkgöl ve Alagöl'de tespit edilmiştir.

Tatlı su habitatlarında yüksek popülasyon oranları ve organik kirliliğe karşı düşük toleranslarıyla bilinen Trichoptera larvaları su kalitesi belirleme çalışmalarında kullanılan önemli biyolojik indikatörlerdendir (Wiggins & Mackay, 1978; Bouchard, 2004). Limnephilidae familyasına mensup Limnephilidae Gen. sp. (tolerans seviyesi=4) (Hauer & Lamberti, 1996) türüne Yazıgöl, İsimsiz Göl, Eğrigöl ve Alagöl'de rastlanırken, *L. bipunctatus* türü (tolerans seviyesi=3) (Bode ve ark., 1996) yalnızca Alagöl'de tespit edilmiştir.

Coleoptera takımından Dytiscidae familyası üyeleri olan *P. lineatus, A. biguttatus* (tolerans seviyesi=5) (Bode ve ark., 1996), *D. marginalis* ve *G. veterator* türleri Yazıgöl, Yarıkgöl ve Alagöl'de tespit edilmiştir. Bu familyanın üyeleri genellikle bütün sucul habitatlara adapte olmuş canlılar olarak bilinirler (Borror ve ark., 1981; Nilsson, 1996).

Yoğun vejetasyon ve çamur substratlı alanlarda bulunan Corixidae (Tully ve ark., 2004) üyelerinden *Micronecta* sp. türü Yarıkgöl ve Otlugöl'de tespit edilmiştir.

Göllerde kozmopolit ve toleransı yüksek olan türlerin yanı sıra biyoindikatör olarak değerlendirilebilecek türlere de rastlanmıştır. Tolerans seviyesi yüksek olan türlerin çok düşük düzeydeki bir organik kirlilik yükü karşısında çok hızlı bir şekilde baskın hale geldikleri gözlemlenmiştir. Bu durum göllerin kirliliğe karşı ne kadar kırılgan olduğunu da ortaya koymaktadır.

Göllerin İndeks Değerleri

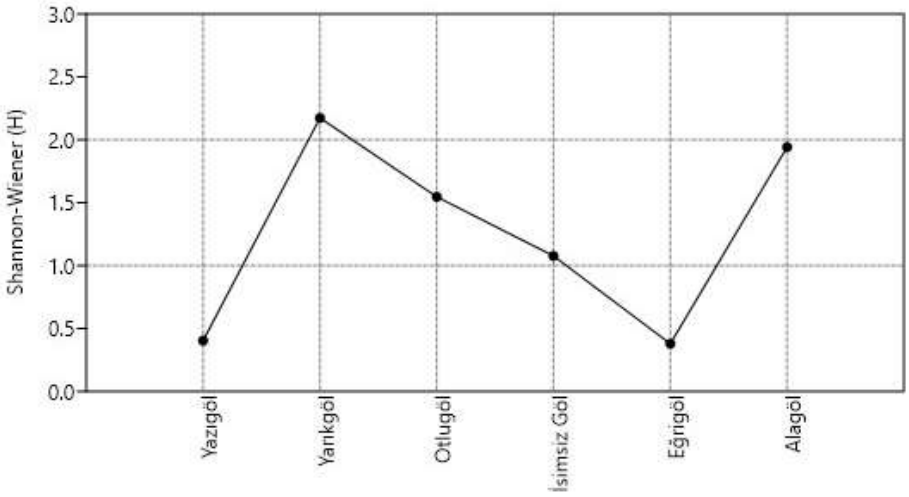
Tespit edilen makrobentik faunaya ait bolluk değerleri kullanılarak hesaplanan Shannon-Wiener çeşitlilik indeksi (H) sonuçlarına göre çeşitliliğin en yüksek hesaplandığı istasyon (2.17) Yarıkgöl olurken bunu 1.94; 1.55; 1.08; 0.40 ve 0.38 H değerleri ile sırasıyla Alagöl, Otlugöl, İsimsiz Göl, Yazıgöl ve Eğrigöl takip etmiştir. Türlerin dağılımları üzerinden dengelilik ve eşitliğin hesaplandığı Shannon Evenness yoğunluk indeksi (EH) sonuçlarına göre ise; en dengeli dağılım gösteren istasyonlar aynı değere sahip olan (0.73) Yarıkgöl ve İsimsiz Göl olarak hesaplanırken bunu 0.59 EH değeri ile Otlugöl izlemiştir. En düşük dengeli dağılım sergileyen istasyonlar ise 0.24; 0.25 ve 0.47 EH değerleri ile sırasıyla Eğrigöl, Yazıgöl ve Alagöl olarak tespit edilmiştir (Çizelge 3, Şekil 4-5).

Tür zenginlikleri bakımından aynı olan istasyonlara ait hesaplanan çeşitlilik değerlerindeki farklılıklar orada bulunan türlerin dağılım özelliklerindeki farklılıklarından kaynaklanmaktadır. Aynı şekilde daha düşük tür içermesine rağmen çeşitliliği ifade eden H değeri bakımından daha yüksek orana sahip olan istasyonlar da türlere ait dağılım özellikleri ile ilişkilidir. Dolayısıyla Yarıkgöl'e ait tür zenginliğinin Alagöl'e göre az sayıda olmasına rağmen hesaplanan çeşitlilik değerinin (H) daha yüksek hesaplanması, orada bulunan popülasyonların daha dengeli (homojen) dağılım sergilemesi ile açıklanmaktadır. Nitekim göl ekosistemlerine ait türlerin dengelilik-eşitlik özelliklerinin hesaplanması için uygulanan Shannon Evenness indeks (EH) sonuçlarına göre de Yarıkgöl'e ait hesaplanan EH değeri 0.73 iken, Alagöl'de bu değer 0.47 olarak hesaplanmıştır. Diğer yandan 0.73 EH değeri ile İsimsiz Göl ve Yarıkgöl aynı dağılım özelliği sergilerken, 0.24 EH değeri ile Eğrigöl en az dengeli dağılımın gözlendiği istasyon olarak tespit edilmiştir. Eğrigöl ve Alagöl'de EH değerinin düşük çıkması bu göllerde *T. tubifex*' in birey sayısı bakımından diğer istasyonlara göre çok fazla sayıda tespit edilmesi sebebiyle dağılım özelliği bakımından homojenliği düşürmesinden kaynaklanmaktadır.

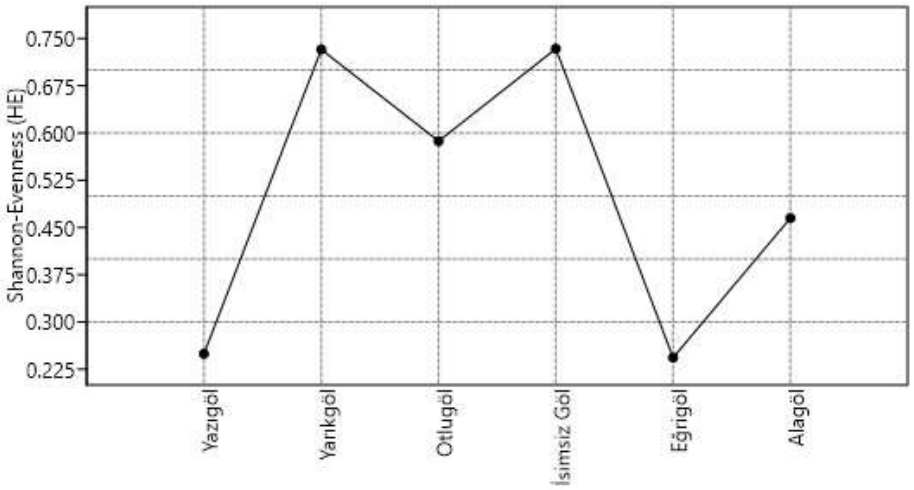
Çizelge 3. Göllere ait tür sayısı, birey sayısı ve hesaplanan indeks değerleri
Table 3. Number of species, number of individuals and calculated index values of the lakes

	Yazıgöl	Yarıkgöl	Otlugöl	İsimsiz Göl	Eğrigöl	Alagöl
Tür Sayısı	6	12	8	4	6	15
Birey Sayısı	356	102	77	26	3151	1294
Hesaplanan İndeksler						
Shannon-Wiener (H) Çeşitlilik	0.40	2.17	1.55	1.08	0.38	1.94
Shannon Evenness (EH) Yoğunluk	0.25	0.73	0.59	0.73	0.24	0.47

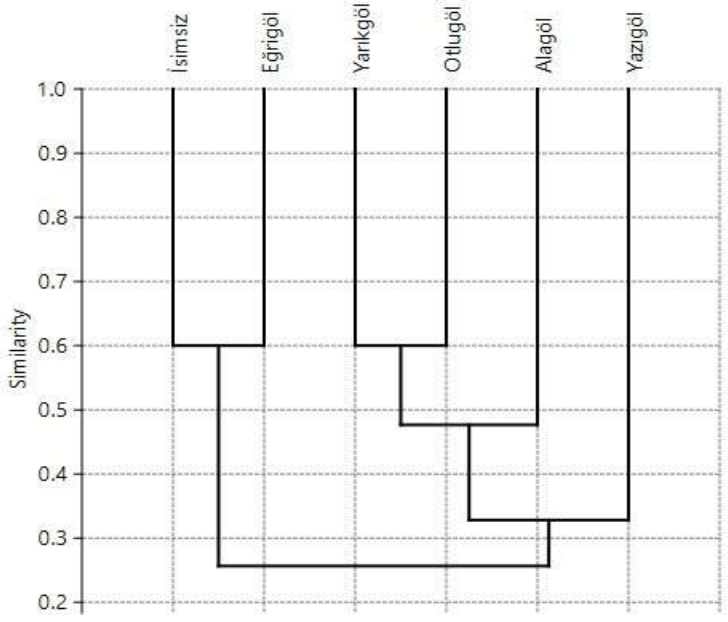
Göller arasındaki en yüksek benzerlikler Otlugöl ile Yarıkgöl ve Eğrigöl ile İsimsiz Göl arasında tespit edilmiştir (Çizelge 4, Şekil 6 ve Şekil 7). Alagöl ile Eğrigöl arasındaki benzerlik oranı ise %0.57 olarak belirlenmiştir. En düşük benzerlikler (%0.13) İsimsiz Göl ile Yarıkgöl arasında hesaplanmıştır. Göl istasyonlarına ait hesaplanan benzerlik oranları ve bu oranlara göre belirlenen Cluster kümeleme dendogramı ve n-MDS grafiği sırasıyla Çizelge 4, Şekil 6 ve Şekil 7'de sunulmuştur.



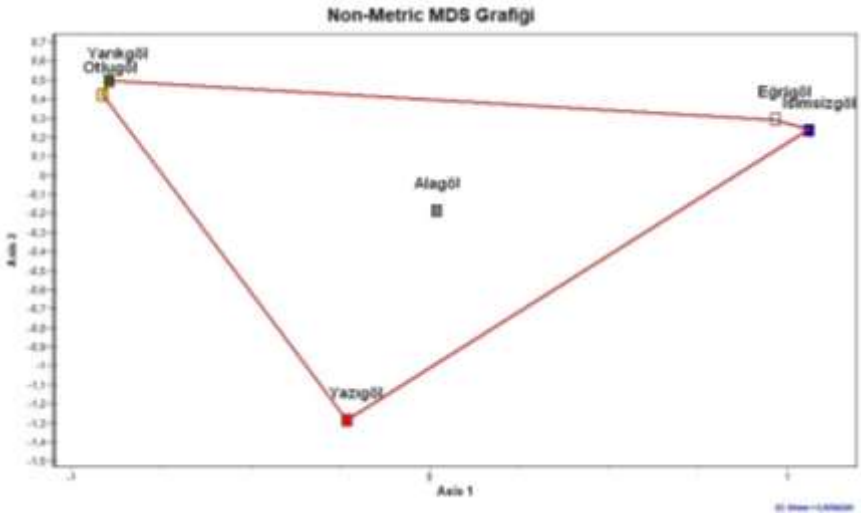
Şekil 4. Shannon-Wiener çeşitlilik değerleri arasındaki ilişki
Figure 4. Relation between Shannon-Wiener diversity values



Şekil 5. Shannon-Evenness yoğunluk değerleri arasındaki ilişki
Figure 5. Relation between Shannon-Evenness density values



Şekil 6. Göller arasındaki benzerliklere bağlı Cluster kümeleme dendogramı
Figure 6. Cluster dendogram based on similarities between lakes



Şekil 7. Göller arasındaki ayrımların n-MDS analizi ile gösterimi
Figure 7. Representation of the distinctions between lakes by n-MDS analysis

Çizelge 4. İstasyonlar arasındaki benzerlik oranları (Bray-Curtis)
Table 4. Similarity rates between stations (Bray-Curtis)

	Yazıgöl	Yarıkgöl	Otlugöl	İsimsiz Göl	Eğrigöl	Alagöl
Yazıgöl	1					
Yarıkgöl	0.22	1				
Otlugöl	0.29	0.60	1			
İsimsiz Göl	0.20	0.13	0.17	1		
Eğrigöl	0.17	0.22	0.29	0.60	1	
Alagöl	0.48	0.52	0.43	0.32	0.57	1

SONUÇ

İzole ekosistemler olan yüksek rakımlı dağ gölleri ve buzul gölleri bu ortamlara adapte olan birçok nadir türü bünyesinde barındırırlar. Bu ekosistemlerde yaşayan canlıların çoğu geniş bir ekolojik toleransa sahiptirler (Ustaoğlu ve ark., 2008). Bu nedenle bu alanlarda dağılım gösteren taksonların tespit edilerek baskınlık ve çeşitlilik değerlerinin hesaplanması, gerek indikatör olabilecek taksonların belirlenmesinde gerekse hassas alanların korunmasına yönelik çalışmaların başlatılmasında önem taşımaktadır. Fakat Türkiye'de yapılan limnolojik çalışmalar genellikle ulaşım açısından kolaylık sağlayan ova gölleri üzerinde yürütülmüştür. Buna karşın yüksek irtifada yer alan buzul veya diğer farklı orjinli göller üzerinde ise çok az sayıda çalışma gerçekleştirilmiştir (Balık ve ark., 2003; Ustaoğlu ve ark., 2004; Yıldız ve ark., 2005, 2007; Topkara ve ark., 2009, 2011; Taşdemir ve ark., 2011; Öztürk ve ark., 2022).

Bu çalışma ile Bolkar Dağları üzerinde bulunan ve daha önce üzerinde hiçbir çalışmanın yapılmadığı altı önemli yüksek irtifa gölünün makrobentik faunası ilk kez ortaya konularak kayıt altına alınmıştır. Arazi çalışmaları sırasında bazı geçici göllerin iklime bağlı olarak Ağustos ayının ortasından sonra tamamen kuruduğu ve bazı göllerin ise kurumaya yüz tuttuğu gözlemlenmiştir. Elde edilen sonuçlar, göllerdeki biyoçeşitliliğin tespitinde bir ön çalışma niteliğindedir. Bu nedenle Bolkar Dağları üzerindeki tüm yüksek irtifa göllerinin de dahil edildiği ve daha uzun bir örnekleme periyoduna sahip bir çalışma ile bu göllerin makroomurgasız faunanın tam olarak ortaya konulması büyük önem taşımaktadır. Bunun yanı sıra bu çalışmada örneklenmiş olan göllerden bir tanesinin henüz isminin bile bulunmadığı düşünüldüğünde bölgedeki göllerin coğrafik ve biyoekolojik özelliklerini ortaya koyacak keşif niteliği taşıyan bir çalışmanın gerçekleştirilmesinin önemli olduğu düşünülmektedir.

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Evaluation of Oxidative Stress And Growth Alterations on Arthrospira Platensis Gomont and Chlorella Vulgaris Beijerinck (Beijerinck) by Cambio

Şükrüye ER[1], Hatice TUNCA[2], Ali DOĞRU[3], Tuğba ONGUN SEVİNDİK[4]
[1]Adapazarı ENKA Schools, Sakarya, Turkiye [2,3,4] Sakarya Unıversity Science Faculty Biology Department, Sakarya, Turkiye
[1]https://orcid.org/ 0000-0002-0815-8430, [2]https://orcid.org/ 0000-0003-3724-5215, [3]https://orcid.org/ 0000-0003-0060-4691
[4]https://orcid.org/ 0000-0001-7682-0142
✉: esukriye@gmail.com

ABSTRACT

This study aims to evaluate the toxicity effects of different concentrations of Cambio on *Chlorella vulgaris* (0-500 µg mL^{-1}) and *Arthrospira platensis* (0-50 µg mL^{-1}) algae by determining the changes in chlorophyll-*a* amount, OD 750 (biomass) and antioxidant parameters (the activities of Superoxide dismutase (SOD), Ascorbate peroxidase (APX), Glutathione reductase (GR) and the contents of malondialdehyde (MDA), hydrogen peroxide (H_2O_2), proline). *A. platensis* is being a cyanobacterium used commercially because of its high nutrient content. *C. vulgaris* used for medical and commercial purposes due to the capability of bioremediation, the structure of drug raw material, and nutrient compound. Ecotoxicological studies on these cosmopolitan algae are important for determining the harmful effects of chemicals on freshwater ecosystems. Cambio was toxic to *A. platensis* cells at the highest concentration, however, it stimulated the growth of *C. vulgaris*. For *A. platensis* application, the activity of Superoxide dismutase significantly decreased at moderate concentrations ($p<0.05$), while the activity of Ascorbate peroxidase decreased at the highest concentration ($p<0.05$). Moreover, the activity of Glutathione reductase rose at 20 µg mL^{-1} concentration. Malondialdehyde and H_2O_2 did not show significant changes, but the proline content showed significant increases in all Cambio concentrations compared to the control ($p<0.05$). However, for *C. vulgaris* application the antioxidant parameters did not show any alterations. These results are indicated that the effects of Cambio on *A. platensis* are more destructive than *C. vulgaris*.

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Cambio'nun Arthrospira Platensis Gomont ve Chlorella Vulgaris Beijerinck (Beijerinck) Üzerinde Oluşturduğu Oksidatif Stresin Ve Büyüme Değişimlerinin Değerlendirilmesi

ÖZET

Bu çalışma, Cambio'nun farklı konsantrasyonlarının *Chlorella vulgaris* (0-500 µg mL^{-1}) ve *Arthrospira platensis* (0-50 µg mL^{-1}) alglerinde oluşturduğu biyokütle (klorofil-*a* miktarı, OD 750) ve antioksidan parametrelerindeki (Süperoksit dismutaz (SOD), Askorbat peroksidaz (APX), glutatyon peroksidaz (GR) enzim aktiviteleri ve malondialdehit (MDA), hidrojen peroksit (H_20_2) ve prolin içerikleri) değişimleri belirlemeyi amaçlamaktadır. *A. platensis*, yüksek besin içeriği nedeniyle ticari olarak kullanılan bir siyanobakteridir. *C. vulgaris*, biyoremediasyon kabiliyeti, ilaç hammaddesinin yapısı ve besin bileşimi nedeniyle tıbbi ve ticari amaçlar için kullanılmaktadır. Cambio, en yüksek konsantrasyonda *A. platensis* hücreleri için toksiktir, ancak *C. vulgaris*' in büyümesini uyarmıştır. Kozmopolit olan bu iki algin üzerinde yapılan ekotoksikolojik çalışmalar kimyasalların tatlı su ekosistemlerinde meydana getirdiği zararlı etkilerin belirlenmesi adına önemlidir. *A. platensis* uygulamasında, süperoksit dismutaz aktivitesi orta konsantrasyonlarda; Askorbat peroksidaz aktivitesi ise en yüksek konsantrasyonda anlamlı olarak azalmıştır ($p<0.05$). Ayrıca,

Ekotoksikoloji

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Anahtar Kelimeler
Herbisit
Stres
Antioksidan
Büyüme parametreleri
Alg ekotoksikolojisi

Glutatyon redüktazın aktivitesi, 20 µg mL^{-1} konsantrasyonunda artış göstermiştir. Malondialdehit ve H_2O_2 miktarında önemli değişiklikler görülmemiştir. Ancak prolin içeriği, kontrole kıyasla tüm Cambio konsantrasyonlarında önemli artışlar göstermiştir ($p<0.05$). Ancak *C. vulgaris* uygulaması için antioksidan parametrelerde herhangi bir değişiklik gözlenmemiştir. Bu sonuçlar Cambio'nun *A. platensis* üzerindeki etkilerinin *C. vulgaris* üzerindeki etkilerine göre daha yıkıcı olduğunu göstermektedir.

Atıf İçin: Cambio'nun *Arthrospira platensis* Gomont ve *Chlorella vulgarıs* Beıjerınck (Beıjerınck) Üzerinde Oluşturduğu Oksidatif Stresin ve Büyüme Değişimlerinin Değerlendirilmesi (2023). *KSÜ Tarım ve Doğa Derg 26* (5), 1120-1134. https://doi.org/10.18016/ksutarimdoga.Vi.1174954.

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INTRODUCTION

Cambio, which is widely used as a pesticide in corn fields in Turkey, belongs to the chemical family of the sulfonylurea group and its active ingredient is nicosulfuron. It is a selective herbicide used after the emergence against annual and perennial grasses and some broad-leaved weeds that are problematic in corn fields. This herbicide inhibits the synthesis of leucine, isoleucine, and valine amino acids by inhibiting the plant's acetolactate (ALS) enzyme. It halts the production of other plant components by blocking these essential amino acids (Rey-Caballero et al. 2016; Wu et al. 2022). In this way, it stops the development of target weeds by preventing cell division and consequently causes the death of the plants (PMRA-ARLA, 1996; Serim et al., 2017).

Cellular homeostasis is characterized by a baseline level of ROS that depends on the organism developmental stage, circadian clock, and environmental and physiological conditions. Different biotic and abiotic stresses such as temperature, heavy metals, pesticides, light intensity and nutrition restrictions can disrupt this homeostasis, uncouple metabolic pathways and lead to the accumulation of ROS in different cell compartments (Bhatnagar-Mathur et al., 2008; Hasanuzzaman et al., 2012; Mittler et al., 2022). The increase of free radicals in the cell is an important reason for cell damage. Especially, cell membranes may be affected by free radicals and may lose their fluidity and permeability through lipid peroxidation (Tunca et al., 2022). When oxidative stress progress, living things try to get rid of this situation by using antioxidant defense mechanisms (Xu et al., 2022; Özyurt et al., 2021). Superoxide dismutase (SOD) is one of the most important antioxidant enzymes that acts as the first step of free radical detoxification. While catalyzing the formation of molecular oxygen (O_2) and hydrogen peroxide (H_2O_2) from superoxide radicals, it can make the superoxide anions less harmful (Valentine et al., 1998; Ighodaro et al., 2017). Ascorbate peroxidase (APX) is an enzymatic antioxidant that defends the cell from ROS in many organisms such as high plants, algae, and flagella. It uses an ascorbic electron donor and catalyzes the reduction of H_2O_2 to water. The task is very similar to catalase but has a higher affinity to H_2O_2. Glutathione reductase (GR) is an enzyme that is highly conserved in bacteria, yeasts, algae, plants, animals, and humans. It sustains the cell against ROS by forming a GSH pool by oxidation of NADPH (Anjum, 2010). MDA is generated by lipid peroxidation as an aldehyde metabolite, (Cao et al. 2022; Tang et al., 2022).

In recent years, increasing usage of herbicides in agricultural activities has indirectly affected aquatic ecosystems (Relyea, 2005; Schmitt-Jansen & Altenburger, 2005; Schuler & Rand, 2008; Vervliet-Scheebaum et al., 2010). Unfortunately, the toxicity and environmental destruction of these compounds acting on non-target organisms are ignored by laws and policies (Niedobova et al., 2022). Microalgae are the non-target organism for herbicides but these creatures are the most sensitive due to being primary producers of food-chain in aquatic ecosystems (Cedergreen & Streibig, 2005, Yang et al., 2022, Walsh, 1978). These features cause to transport of the xenobiotics to higher trophic levels. Primary producers being indicators provide us with important information about pollution in these systems (Tunca et al., 2022). *Arthrospira platensis* is an alkaliphilic cyanobacterium used commercially because of its high nutrient content. *Arthrospira* sp. has been recorded in Turkish algal flora (Aysel, 2005). The studies about the biology and physiology of this algae are important, due to being cosmopolitan. The fact that this alga exhibits its metabolic responses to environmental stimuli in the natural environment makes it a reliable microorganism in ecotoxicological studies (Sili et al. 2012). *C. vulgaris* also used for medical and commercial purposes due to the capability of bioremediation, the structure of drug raw material, and nutrient compound. *C. vulgaris* is unicellular and coccoid (Kookal et al. 2023; Tamil Selvan et al. 2023).

This study aims to determine the effects of Cambio on the development of *C. vulgaris* and *A. platensis* algae and to evaluate the changes in antioxidant and growth

parameters.

MATERIAL and METHOD

Algae culture and treatment

A. platensis M2 (Culture collection No: SLSP01) and *C.* vulgaris were obtained from Soley Microalgae Institute California (USA) and Çukurova University (Turkey), respectively. Spirulina Medium (Aiba & Ogawa, 1977) was used in the culture of *A. platensis* and BG11 Medium (Rippka et al., 1979) was used in the culture of *C. vulgaris*. The 50 mL of cultures were grown under the conditions of 93 µmol photons m^{-2} s^{-1}, in 12:12 h circadian rhythm at 30±1 ºC and 25±1 ºC for *A. platensis* and *C. vulgaris,* respectively. Various concentrations of Cambio compound (40 g/L, nicosulfuron EC, İstanbul, Turkey) for *C. vulgaris* (100, 200, 300, 400, 500 µg mL^{-1}) and *A. platensis* (10, 20, 30, 40, 50 µg mL^{-1}) were added to the culture medium. The concentration ranges were determined according to preliminary laboratory experiments.

Cell growth assay

Optic density (OD) of the algae was measured for 7 days under control and stressed conditions by using a spectrophotometer taking absorbance at 750 nm. Cultures were diluted at a 1/10 ratio with BG11 Medium for *C. vulgaris* and Spirulina Medium for *A. platensis*. At the same time, BG11 Medium and Spirulina Medium were used as curves, and each measurement was conducted every 24 h for 7 days. To determine the Chlorophyll-*a* contents, methanol extractions were performed and the samples were measured at the absorbance of 665 nm wavelength for 7 days (MacKinney, 1941).

Antioxidant enzyme activities

On the 7th day, the centrifugation was applied to 2 mL culture solutions of the control and each Cambio-exposed algae medium, at 15.000 rpm for 20 min at 4 °C. Pellets resulting from centrifugation were stored at -20 °C until the enzyme assays. The Bradford (1976) method was performed to determine the protein concentrations of algal cell extracts, using bovine serum albumin (BSA) as a standard. The modified method of Beyer and Fridovich (1987) was performed to determine the SOD (EC 1.15.1.1.) activity, while the Sgherri et al. (1994) method was modified to determine the GR (EC 1.6.4.2) activity and the modified method of Wang et al. (1991) was used to determine the APX (EC 1.11.1.11) activity, by estimating the decreasing rate of ascorbate oxidation at 290 nm. Detailed information about methods was given in Kılıç et al. (2019), Günsel et al. (2018) and, Önem et al. (2018).

Nonenzymatic parameters

The modified method of Heath and Packer was performed to determine the MDA content. For determination of the H_2O_2 content, 0.5 mL of supernatant was mixed with 1 mL of 1 M KI and 0.5 mL of 0.1 M Tris–HCl (pH 7.6) solutions. After 90 min, the absorbance of the samples was measured at 390 nm according to explained in Tunca et al., (2020). The method of Weimberg (1987) was followed for the determination of proline content. Detailed information about methods was given in Tunca et al. (2020).

Statistical analysis

The one-way ANOVA was used to analyze the differences between the control and treated samples, according to LSD. The confidence interval was selected as 95%. Three biological replicate cultures were used for each treatment. The mean values and standard errors (SE) of each application were given in the Figures.

RESULTS

Biomass and chlorophyll-a content

OD750 measurements and chlorophyll-*a* content of *C. vulgaris* and *A. platensis* cultures in Cambio application are given in Figures 1 and 2, respectively. In *A. platensis* cultures, the application caused a significant increase in biomass accumulation in a dose-dependent manner during the first 2 days ($p < 0.05$). However, at the end of 4th day, the continuous decrease was observed in biomass accumulation in a dose-dependent manner ($p < 0.05$). Generally, significant decrease was seen in chlorophyll-*a* content of *A. platensis* cells in a dose-dependent manner at the end of 2nd day ($p < 0.05$). In *C. vulgaris* cultures, Cambio added to the culture medium for 7 days caused a significant increase in biomass accumulation (OD750 absorbance) in a dose-dependent manner at the beginning of first day ($p < 0.05$). However, during progressive days, there was a significant increase in the amount of chlorophyll-*a* in a dose-dependent manner after the 4th day ($p < 0.05$).

Antioxidant enzyme activities

The activity of SOD significantly increased at 20 and 50 µg mL^{-1} concentrations in *A. platensis* cultures, while it did not change significantly at all Cambio concentrations in *C. vulgaris* cultures ($p < 0.05$) (Figure 3). SOD, APX, and GR activities of *C. vulgaris* cultures did not show significantly changes compared to control (Figures 4a and 5a). APX activity of *A. platensis* significant decreased at 50 µg mL^{-1} concentration ($p < 0.05$) (Fig. 4b), while GR activity significantly increased at 20 µg mL^{-1} concentration ($p < 0.05$) (Fig. 5b).

Nonenzymatic parameters

The MDA amounts of *A. platensis* and *C. vulgaris*

cultures exposed to different Cambio concentrations did not show significant changes compared to the control ($p > 0.05$) (Figures 6 and 7). H_2O_2 amount did not alter in either organism. On the other hand, the proline content displayed increases at 20, 30, 40, and 50 µg mL^{-1} concentrations in *A. platensis* application but it did not change in *C. vulgaris* at all Cambio concentrations compared to the control ($p< 0.05$) (Figure 8).

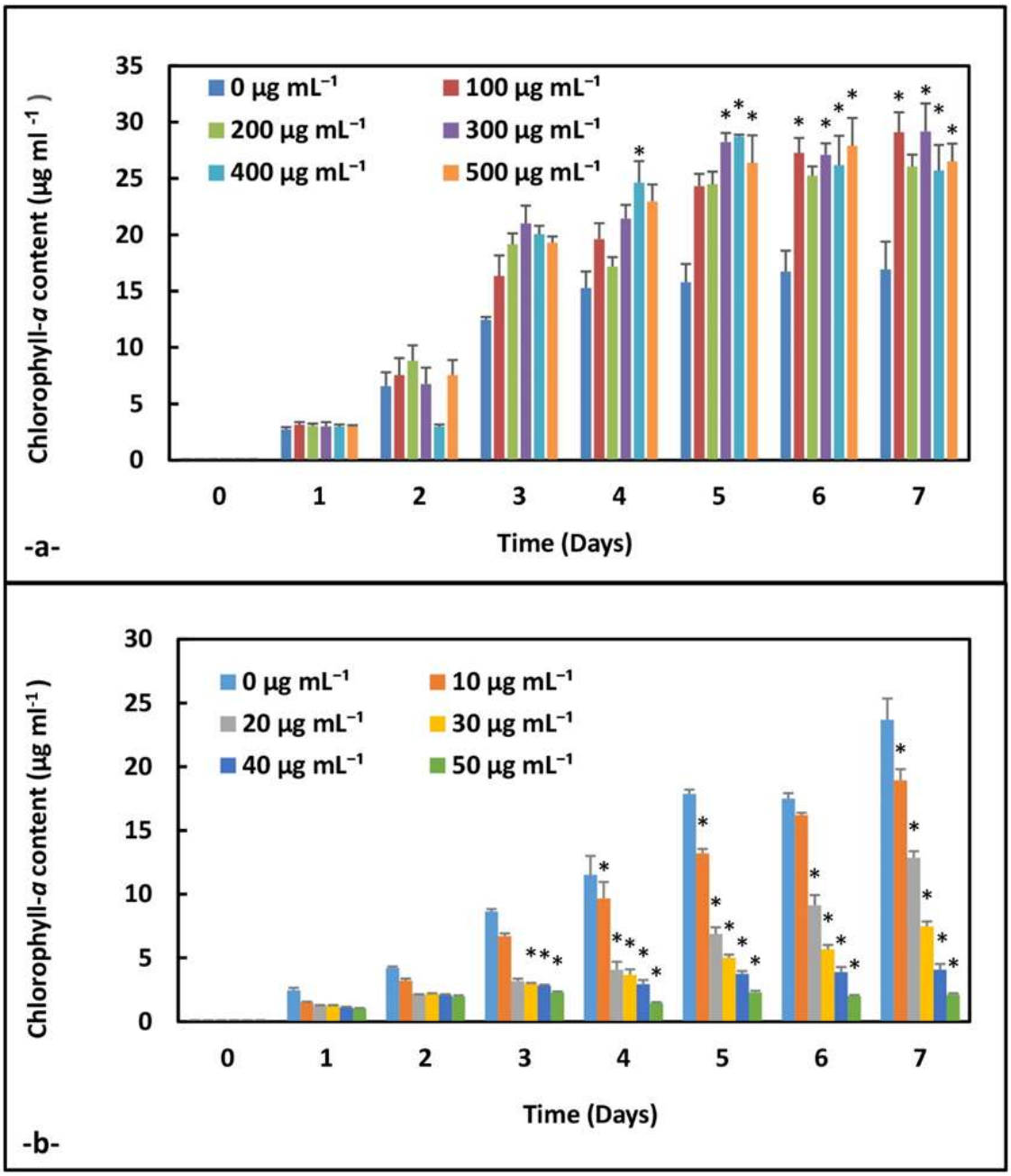

Figure 1. Alterations of chlorophyll-*a* content in (a) *C. vulgaris* and (b) *A. platensis* by Cambio treatment for 7 days. The asterisks show statistical differences at the 95% confidence interval. Experiments were carried out in triplicate. SE is shown as bars on the chart.

Şekil 1. *(a) C. vulgaris ve (b) A. platensis alglerinde 7 gün boyunca Cambio uygulaması ile klorofil-a içeriğindeki değişiklikler. Yıldız işaretleri, %95 güven aralığında istatistiksel farklılıkları göstermektedir. Deneyler üç tekrar halinde gerçekleştirilmiştir. SE, grafikte çubuklar olarak gösterilmektedir.*

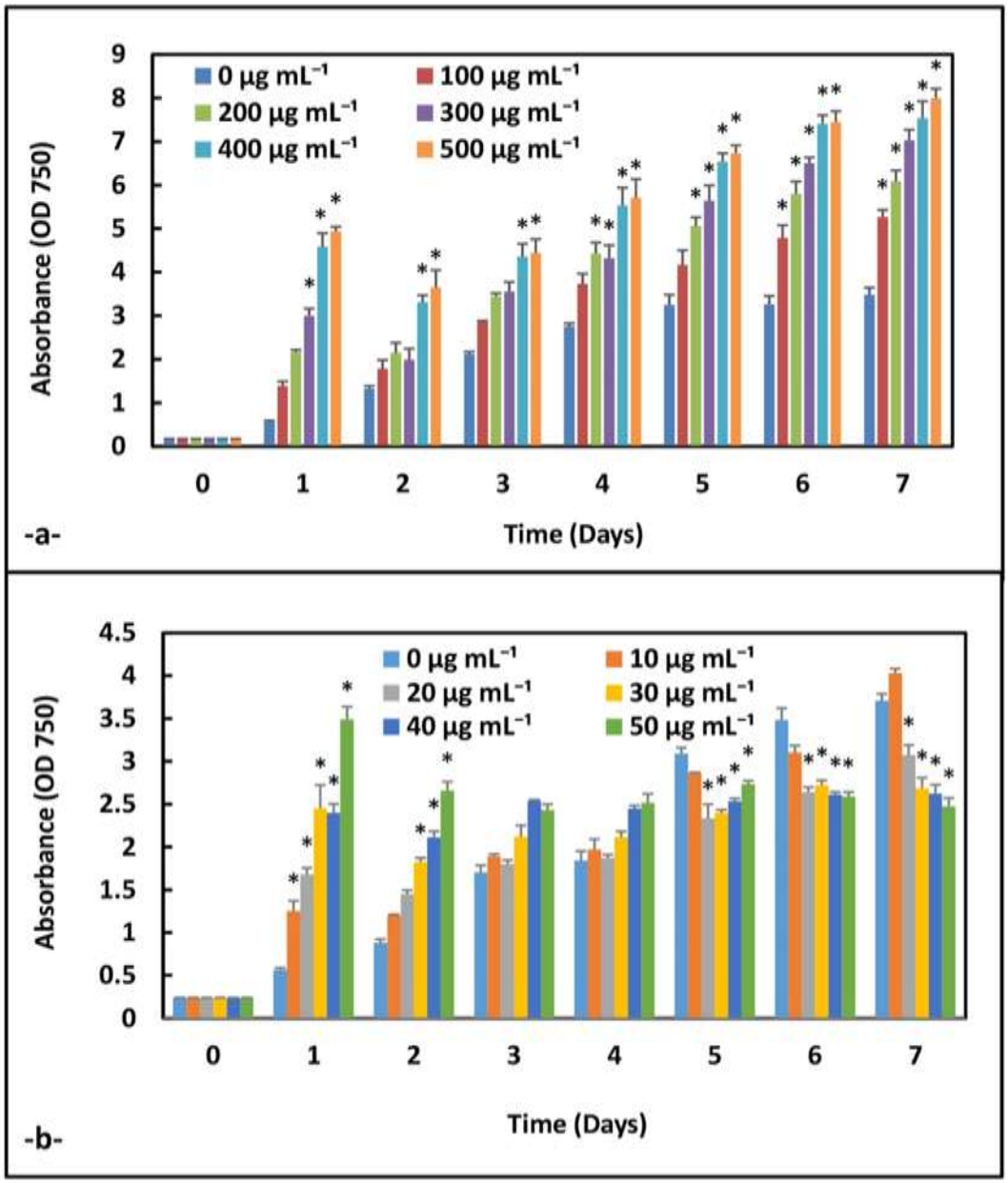

Figure 2. Alterations of OD 750 absorbances in (a) *C. vulgaris* and (b) *A. platensis* by Cambio treatment for 7 days. The asterisks show statistical differences at a 95% confidence interval. Experiments were carried out in triplicate. SE is shown as bars on the chart.

Şekil 2. *(a) C. vulgaris ve (b) A. platensis alglerinde 7 gün boyunca Cambio uygulaması ile büyüme eğrilerindeki değişiklikler. Yıldız işaretleri, %95 güven aralığında istatistiksel farklılıkları göstermektedir. Deneyler üç tekrar halinde gerçekleştirilmiştir. SE, grafikte çubuklar olarak gösterilmektedir.*

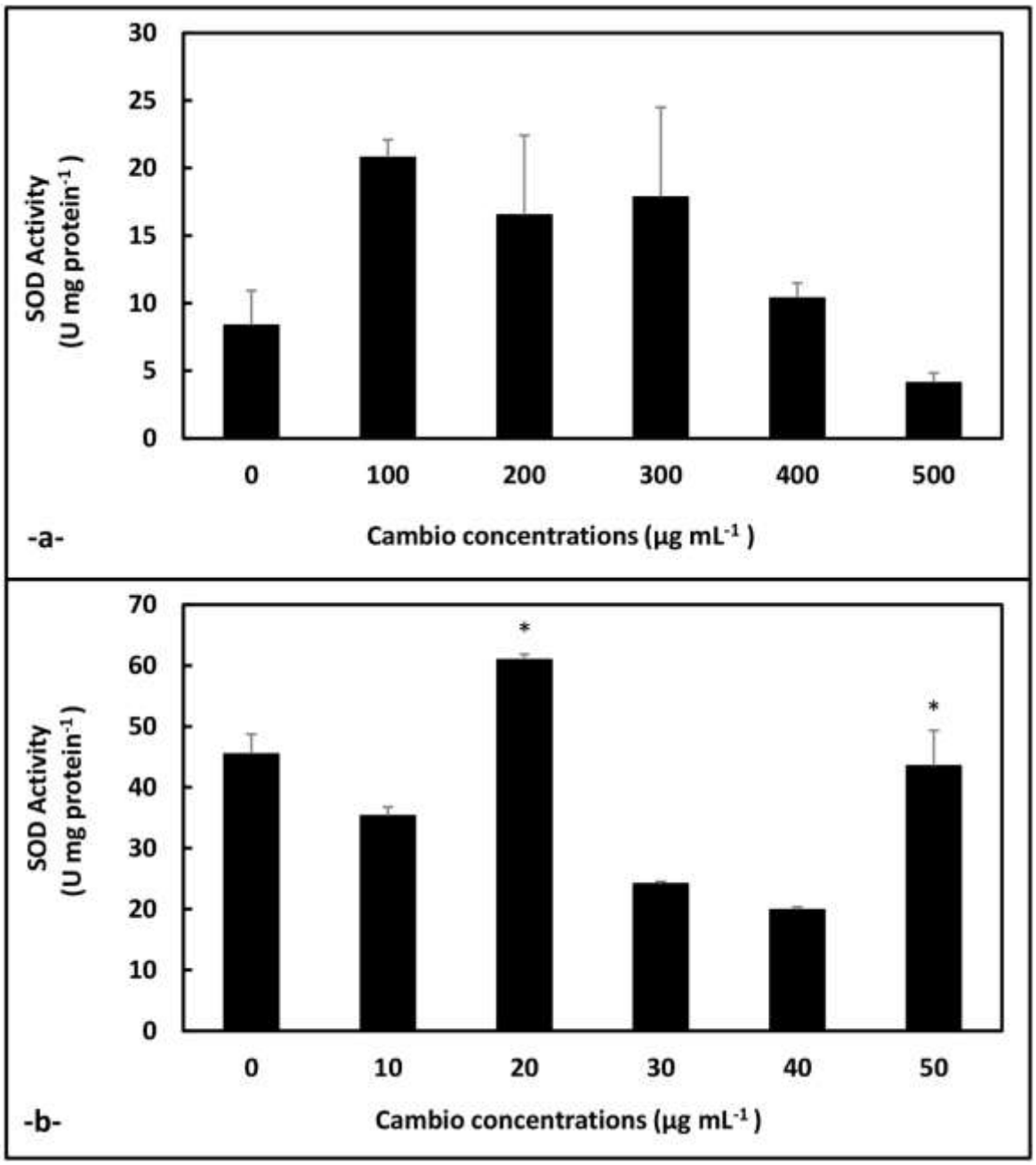

Figure 3. Alterations of SOD activities of (a) *C. vulgaris* and (b) *A. platensis* by Cambio treatment. The asterisks show statistical differences at a 95% confidence interval. Experiments were carried out in triplicate. SE is shown as bars on the chart.

Şekil 3. *(a) C. vulgaris ve (b) A. platensis'in Cambio uygulamasıyla SOD aktivitelerinde meydana gelen değişiklikler. Yıldız işaretleri, %95 güven aralığında istatistiksel farklılıkları göstermektedir. Deneyler üç tekrar halinde gerçekleştirilmiştir. SE, grafikte çubuklar olarak gösterilmektedir.*

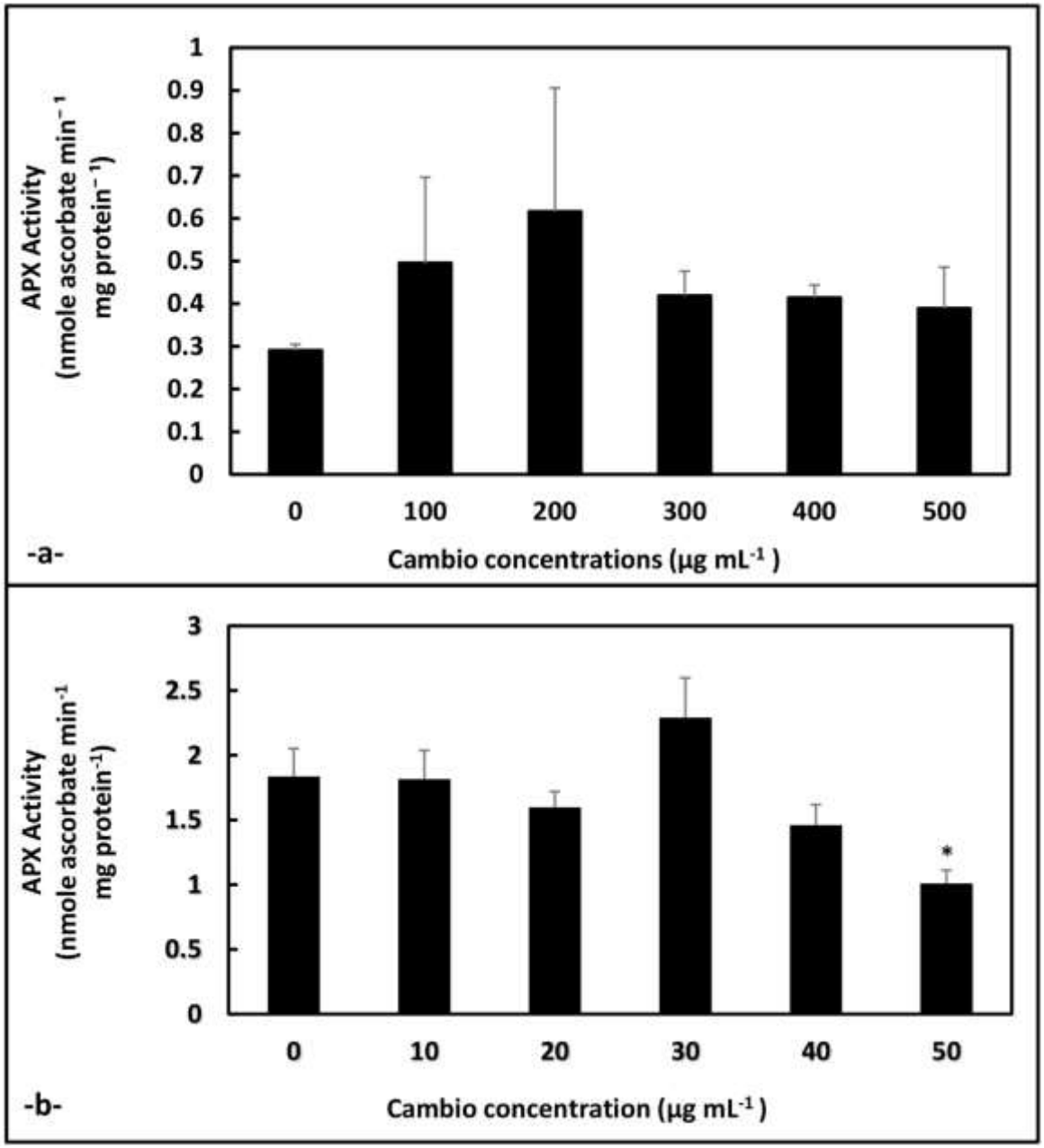

Figure 4. Alterations of APX activities of (a) *C. vulgaris* and (b) *A. platensis* by Cambio treatment. The asterisks show statistical differences at the 95% confidence interval. Experiments were carried out in triplicate. SE is shown as bars on the chart.

Şekil 4. *(a) C. vulgaris ve (b) A. platensis'in APX aktivitelerinde Cambio uygulaması ile meydana gelen değişiklikler. Yıldız işaretleri, %95 güven aralığında istatistiksel farklılıkları göstermektedir. Deneyler üç tekrar halinde gerçekleştirilmiştir. SE, grafikte çubuklar olarak göstermektedir.*

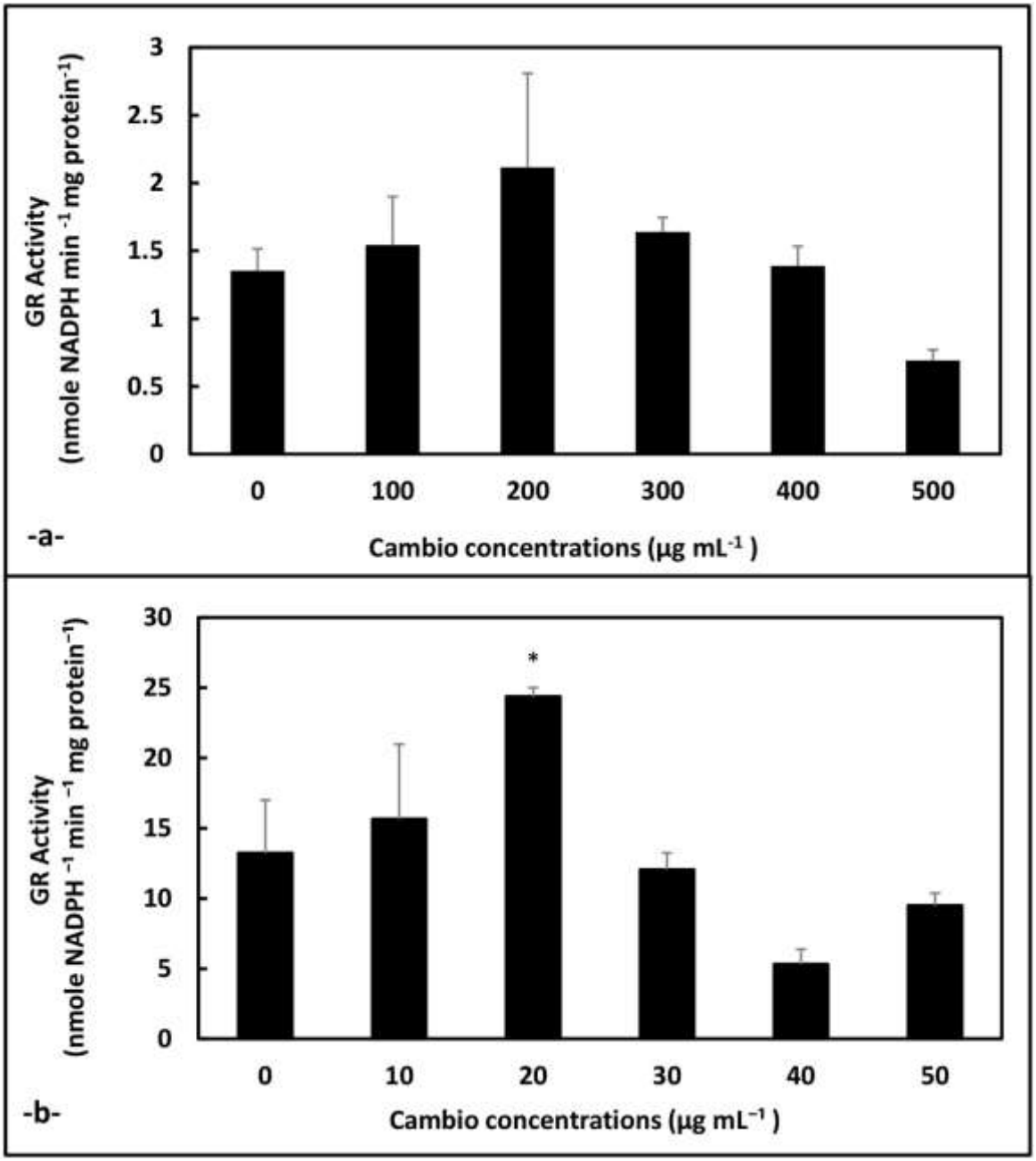

Figure 5. Alteration of GR activities of (a) *C. vulgaris* and (b) *A. platensis* Cambio treatment. The asterisks show statistical differences at the 95% confidence interval. Experiments were carried out in triplicate. SE is shown as bars on the chart.

Şekil 5. *(a) C. vulgaris ve (b) A. platensis Cambio uygulaması ile GR aktivitelerinde meydana gelen değişiklikler. Yıldız işaretleri, %95 güven aralığında istatistiksel farklılıkları göstermektedir. Deneyler üç tekrar halinde gerçekleştirilmiştir. SE, grafikte çubuklar olarak gösterilmektedir.*

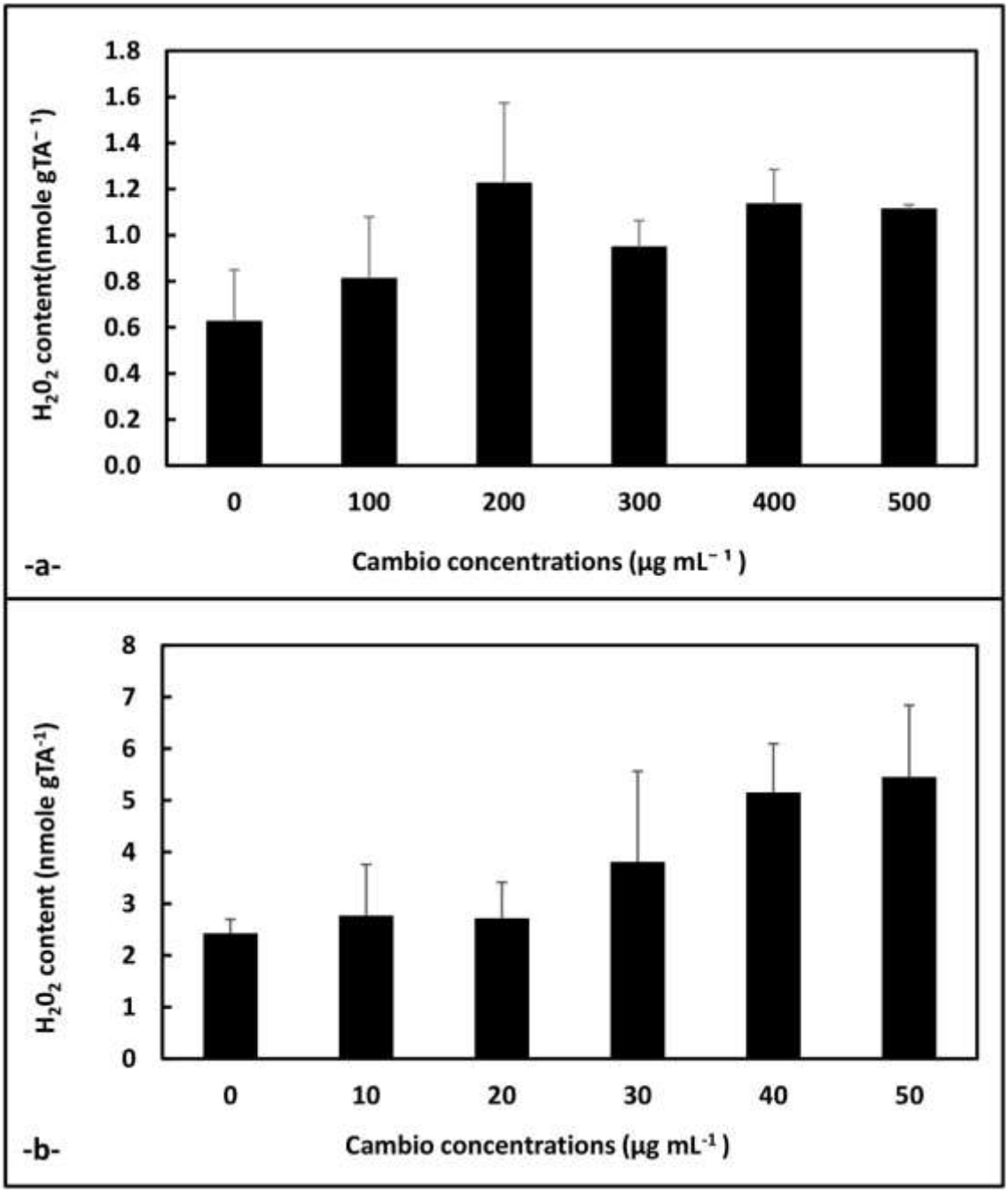

Figure 6. Alterations of H_2O_2 content in (a) *C. vulgaris* and (b) *A. platensis* by Cambio treatment. The asterisks show statistical differences at the 95% confidence interval. Experiments were carried out in triplicate. SE is shown as bars on the chart.

Şekil 6. *Cambio uygulamasıyla (a) C. vulgaris ve (b) A. platensis alglerindeki H_2O_2 içeriğinde meydana gelen değişiklikler. Yıldız işaretleri, %95 güven aralığında istatistiksel farklılıkları göstermektedir. Deneyler üç tekrar halinde gerçekleştirilmiştir. SE, grafikte çubuklar olarak gösterilmektedir.*

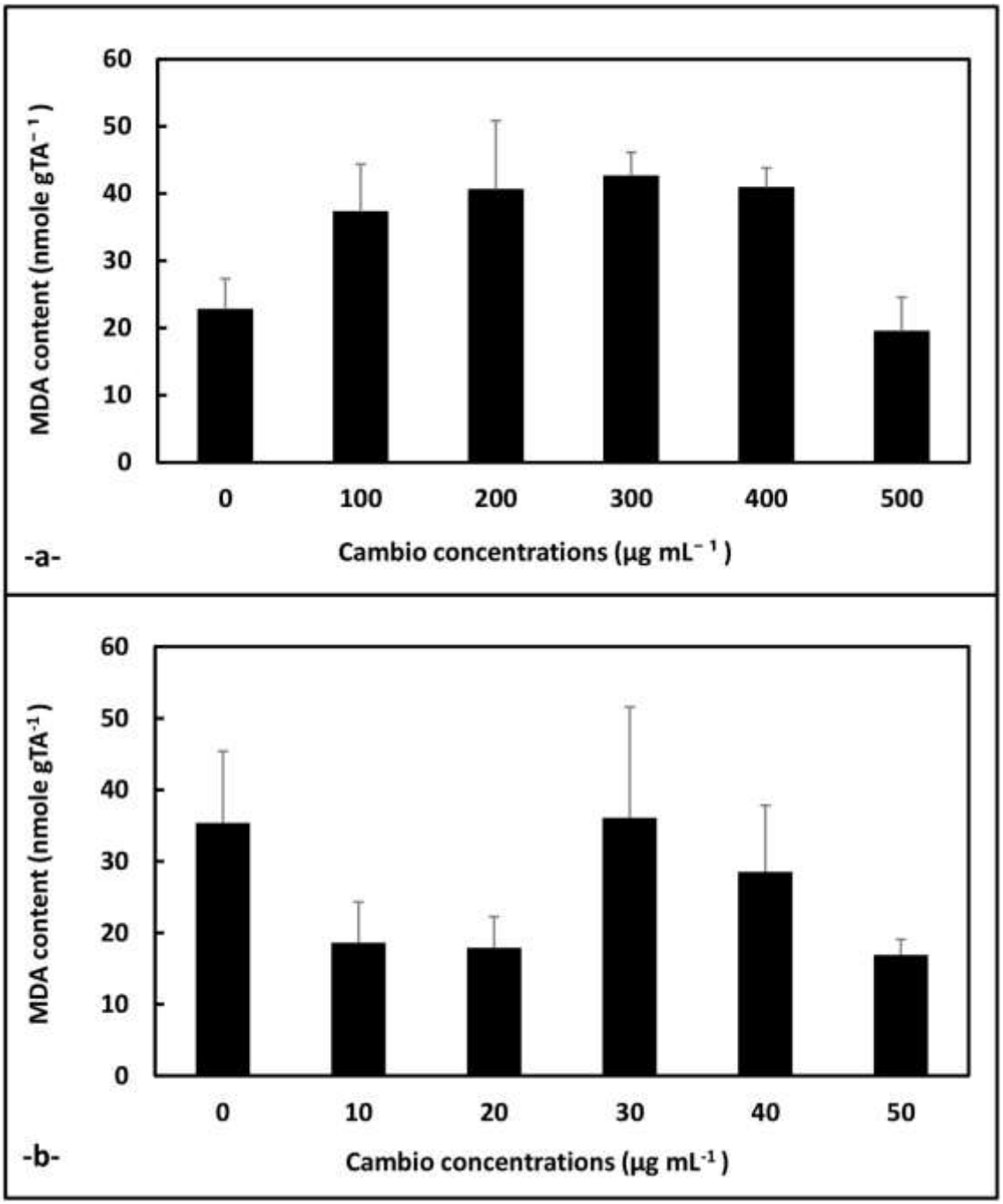

Figure 7. Alterations of MDA content in (a) *C. vulgaris* and (b) *A. platensis* by Cambio treatment. The asterisks show statistical differences at the 95% confidence interval. Experiments were carried out in triplicate. SE is shown as bars on the chart.

Şekil 7. *(a) C. vulgaris ve (b) A. platensis'te Cambio işlemi ile MDA içeriğinde meydana gelen değişiklikler. Yıldız işaretleri, %95 güven aralığında istatistiksel farklılıkları göstermektedir. Deneyler üç tekrar halinde gerçekleştirilmiştir. SE, grafikte çubuklar olarak gösterilmektedir.*

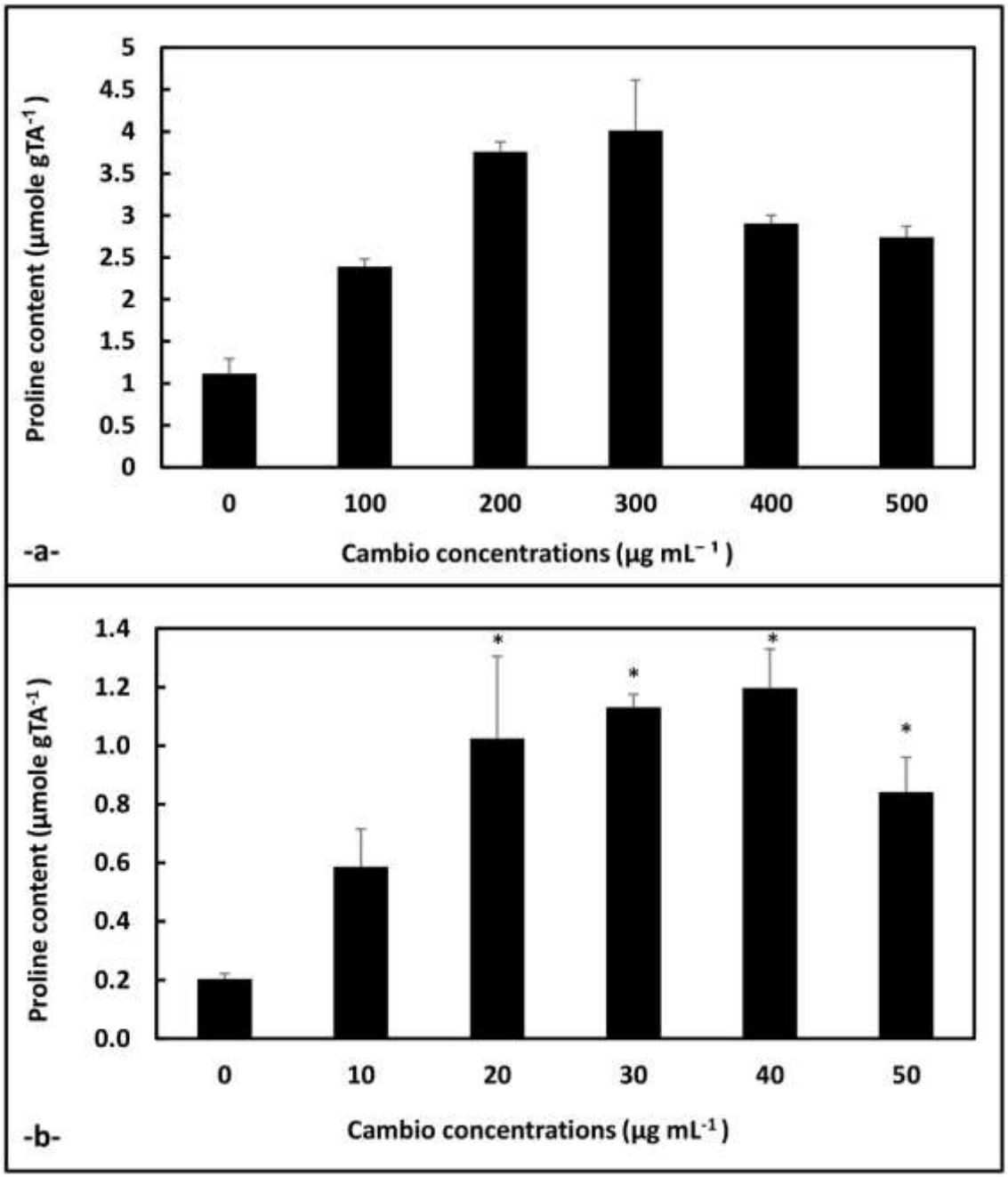

Figure 8. Alterations of Proline content in (a) *C. vulgaris* and (b) *A. platensis* Cambio treatment. The asterisks show statistical differences at the 95% confidence interval. Experiments were carried out in triplicate. SE is shown as bars on the chart.

Şekil 8. *(a) C. vulgaris ve (b) A. platensis Cambio tedavisinde Prolin içeriğinde meydana gelen değişiklikler. Yıldız işaretleri, %95 güven aralığında istatistiksel farklılıkları göstermektedir. Deneyler üç tekrar halinde gerçekleştirilmiştir. SE, grafikte çubuklar olarak gösterilmektedir.*

DISCUSSION

Until environmental toxicology studies scientifically contradict, Cambio has been considered a herbicide that can be used in agriculture (PMRA-ARLA, 1996; EPA, 2004). In the literature, there is no study on the effect of this herbicide on aquatic system organisms. Therefore, the effect of Cambio on two different algae (one is eukaryotic and the other is prokaryotic) was investigated in this study. The effective concentrations of Cambio for *C. vulgaris* were around 10 times higher than for *A. platensis*. It is found that higher concentrations (> 20 µg mL^{-1}) of Cambio have growth inhibitory effects on *A. platensis* cyanobacteria according to OD750 nm absorbance and chlorophyll-a content. These different effect of two application may arise from the detoxification capability of the organisms having different cell types (Lynch and Marinov, 2018).

Bozic et al. (2016) observed that chlorophyll production increased due to herbicide stress when they used

nicosulfuron in sunflower breeding. They have attributed this to the inhibition of acetolactate synthase (ALS) and different defense responses of plants to nicosulfuron. Wang et al. (2022) found that nicosulfuron led to the disruption in the function of PSII in sugar beet leaf. It is obvious that nicosulfuron caused to the decrease in photosynthetic parameters in lower by significant photoinhibition. Leboulanger et al., (2001) have examined the effect of Atrazine and Nicosulfuron herbicides on the growth rate of *Pseudokirchneriella subcapitata*, *C. vulgaris*, *Navicula accomoda*, and *Oscillatoria limnetica* algae. They have observed that EC50s for Atrazine arranged between 40 and 100 µg L^{-1}, whereas overall, nicosulfuron inhibited only the growth of *O. limnetica.* Similarly, Seguin et al. (2001) have reported that the EC50 of Atrazine range between 4 and 400 µg L^{-1} concentrations on different microalgae and nicosulfuron have been found less toxic atrazine. Nicosulfuron have been grouped in sulfonylurea herbicides, and in some studies, these herbicide group has been found less toxic to algae (Soares et al., 2022). Cambio is also a sulfonylurea herbicide, however, its toxicity was very effective on *A. platensis.* On the other hand, it stimulated the growth of *C. vulgaris* in the study. This can be attributed to either the perception of the herbicide by *C. vulgaris* as a stress factor or the use of the herbicide as a substrate. Nyström et al., (1999) investigated that the sulfonylurea sensitivity displayed differences according to algae species. Since these pesticides are capable of blocking the synthesis of essential amino acids (Seguin et al., 2001; Ma et al., 2002), Neilson and Larsson (1980) have suggested that the sulfonylurea sensitivity in the algae arises from the different properties of the incorporation of amino acids.

SOD enzyme activity in *A. plantensis* cultures firstly showed a tendency to increase at 20 and 50 µg mL^{-1} concentrations but it did not change at 30 and 40 µg mL^{-1} concentrations. It may have been occurred due to running out of the cellular concentrations of the enzyme at these concentrations but it may have triggered the gene expression of the enzyme at 50 µg mL^{-1} concentration. Qian et al. (2008) have exposed different concentrations of glufosinate to *C. vulgaris* cultures and they have observed a similar increase in SOD activity. They explained this situation that glufosinate produced superoxide radicals ($\cdot O^{2-}$) in the cells. To explain the enhancement of SOD activity, it can be suggested that the herbicide may be effect directly the SOD gene or, it may increase the $\cdot O_2$ radicals in the medium (Wang and Ki, 2020). Moreover, Cypermethrin insecticide has been tested on *Scenedesmus obliquus* algae, and it has been found that this insecticide has enhancing effect at low concentrations on SOD activity (Wang et al., 2012). This sensitivity could be affected by the blocking of a specific enzyme which plays a role in the production essential amino acids. In this way, the production of chlorophyll may decrease. And also, SOD activity reductions may arise from a deficiency of photosynthetic metabolism (Önem et al., 2018). The activity of SOD significantly decreased at moderate concentrations and Cambio caused decreases in chlorophyll-*a* content of *A. platensis*.

In *A. platensis* cultures exposed to Cambio concentrations, only 20 µg mL^{-1} showed a significant increase in GR activity compared to control. Bajguz (2010) observed that when heavy metals such as cadmium, copper, and lead were applied to *C. vulgaris*, GR activity increased. Geoffroy et al. (2002) suggested that when they applied the different concentrations of diuron on *S. obliquus*, they obtained poor GR stimulation results. The antioxidant defense system and also production of ROS were decreased due to the phenylurea (diuron).

In the study, it was observed that *A. platensis* cultures exposed to different concentrations of Cambio did not change the MDA and H_2O_2 amounts compared the control. Lipid peroxidation end- product known as MDA, which is the indicator of oxidative stress in the cells (Freire et al. 2023). H_2O_2 is a weak reducing compound and causes the formation of hydroxyl radical, and thus cell damage (lipid peroxidation) (Özcan et al., 2015). The absence of significant changes in the amount of H_2O_2 in the cell suggests that it may be due to the effectiveness of enzymes that consume H_2O_2 content, such as the APX (Mallick and Mohn, 2000). In the study, SOD and APX enzyme activities conduct to the H_2O_2 and MDA levels at the same concentration. Likewise, the MDA content was balanced with protective effect of the proline content. In other words, these antioxidant defense systems were related with the levels of oxidant biomarkers. The free proline amount of *A. platensis* cultures displayed increases between 20-50 µg mL^{-1} concentrations with the Cambio application. It has been reported that ROS, which occurs due to the toxicity of endosulfan during photosynthesis, causes proline accumulation due to membrane peroxidation and oxidation problems (Kumar et al., 2008). In addition, studies with *Spirulina* and *Anabaena* species suggest that proline, which has a metal chelating feature due to metal ion stress, increases with metal toxicity and consequently, it increases the amount of protein in the cells (Sultan & Fatma 1999; Kumar et al., 2004; Choudhary et al., 2007). It is known proline prevents the formation of ROS in stress conditions (Phetchuay et al., 2019), and works as a function and structure stabilizer by interacting with macromolecules such as protein and cell membrane as an OH-ion trap (Rezayian et al. 2019).

The activities of SOD, APX, GR enzymes, and the amount of MDA, H_2O_2, and proline did not show a significant change in *C. vulgaris*. In the study on *C.*

vulgaris, the results indicated that Cambio did not cause oxidative stress to increase at all concentrations. Moreover, it was seen that this herbicide promotes growth in this algae, and thus lipid peroxidation and oxidative damage prevents.

CONCLUSION

The study indicated that the effects of Cambio on *A. platensis* are more destructive than *C. vulgaris.* This situation can be explained by the different cell structures of these organisms. Since that the advanced detoxification mechanisms of *C. vulgaris* can tolerate higher levels than the concentrations in the *A. platensis* application. Moreover, it should be noted that one freshwater ecosystem is not exposed to a uniform herbicide/pesticide, and any pesticide entering the system can also degrade and forms different products. Therefore, different combinations of pesticides on microorganisms should be studied (Delorenzo et al., 2001). Because, in sites polluted with solvents, two or more organic compounds are often found together in the environment (Wu et al., 2014). The use of these small single-celled organisms as quick and reliable indicators in the detection of environmental contamination compared to the other plants will be a pioneer for further studies.

Declaration of Contribution

The authors declare that they have contributed equally to the article.

Conflict of Interest

The authors of the article declare that there is no conflict of interest between them.

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Mikrodalga ile Kurutulmuş Kereviz Yapraklarının (Apium graveolens L.) Kuruma Hızının ve Bazı Kalite Parametrelerinin Belirlenmesi

Esra KÖMÜŞ[1], Ferda SARI[2]✉
[1]Sivas İl Millî Eğitim Müdürlüğü, SİVAS, [2]Sivas Cumhuriyet Üniversitesi Teknik Bilimler Meslek Yüksekokulu, Bitkisel ve Hayvansal Üretim Bölümü, 58140, Sivas
[1]https://orcid.org/0000-0001-8573-2777, [2]https://orcid.org/0000-0002-2249-3489
✉:fesari@cumhuriyet.edu.tr

ÖZET

Bu çalışmada kereviz yaprakları (Apium graveolens L.) mikrodalga tekniği ile kurutulmuştur. Mikrodalgada kurutma işlemi 180, 360, 600 ve 900 W mikrodalga çıkış gücünde yapılmıştır. Uygulanan farklı mikrodalga çıkış gücünün kereviz yapraklarının kuruma kinetiği, toplam fenolik madde içeriği, antioksidan kapasite, klorofil a, b ve toplam klorofil miktarı üzerine etkileri incelenmiştir. Kereviz yapraklarının kuruma davranışlarını açıklamak için Page, Newton ve Weibull olmak üzere 3 farklı model test edilmiştir. Uygulanan tüm koşullarda Page modelinin kereviz yapraklarının farklı mikrodalga çıkış gücünde kurutulmasında kuruma kinetiğini açıklayan en iyi model olduğu görülmüştür. Kereviz yapraklarının kurutulması azalan hız periyodunda gerçekleşmiş olup uygulanan mikrodalga çıkış gücünün artmasıyla kuruma oranı artmış ve kuruma süresi azalmıştır. Kurutulan kereviz yapraklarının toplam fenolik madde içeriği ve antioksidan kapasitesi 180, 360 ve 600 W uygulamasında taze örneğe göre azalırken 900 W'da artmıştır. Uygulanan tüm mikrodalga çıkış güçleri kereviz yapraklarının klorofil a, b ve toplam klorofil içeriğinde taze yapraklara göre azalmaya neden olmuştur. Bu azalma en yüksek 180 W'da en düşük ise 900 W'da tespit edilmiştir.

Gıda Bilimi

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Anahtar Kelimeler
Mikrodalga
Kurutma
Kereviz (*Apium graveolens* L.) yaprağı

Determination of Drying Rate and Same Quality Parameters of Celery Leaves (Apiumgraveolens L.) Dried by Microwave

ABSTRACT

In this study celery leaves (*Apiumgraveolens* L.) were dried by microwave technique. The microwave drying process was carried out at 180, 360, 600, and 900 W microwave outputpowers. The effects of different microwave output powers applied on drying kinetics, total phenolic content, antioxidant capacity, chlorophyll a, b, and total chlorophyll contents of celery leaves were investigated. In order to explain the drying behavior of celery leaves, three different drying models, namely, Page, Newton, and Weibull were tested. In all conditions applied, it was found that the Page model was the best to describe the drying kinetics in drying of celery leaves at different microwave output powers. It was observed that the drying of celery leaves took place in the falling rate period throughout the drying process. The drying rate increased,so drying time decreased as microwave output power increased. The total phenolic content and antioxidant capacity of the dried celery leaves decreased at 180, 360, 600 W, but increased at 900 W compared to the fresh sample. All the and microwave output powerscaused a decrease in chlorophyll a, b, and total chlorophyll contents of celery leaves compared to fresh ones. This reduction was determined to be the highest at 180 W and the lowest at 900 W.

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GİRİŞ

Kurutma, gıdalarda mevcut suyun büyük bir kısmının uzaklaştırılarak, su aktivitesinin mikroorganizma faaliyetini önleyecek, enzimatik ve kimyasal reaksiyonları yavaşlatacak veya durduracak seviyeye düşürülmesi işlemidir (Doymaz, 2010; Inchuen ve ark., 2010). Mikrodalga ile kurutma hızlı kurutma sağlaması, enerji tüketiminin az olması ve besin içeriğinin korunması gibi nedenlerden dolayı son yıllarda yaygın olarak kullanılan kurutma yöntemlerinden biri haline gelmiştir (Maskan, 2000; Soysal, 2004; Alibaş, 2007). Kurutma proseslerinde mikrodalga kullanımı ısı transfer hızını artırdığından diğer kurutma yöntemlerinden daha etkilidir.Mikrodalga ile kurutmada ısı yüzeyden transfer olmaz, üretilen elektromanyetik enerji materyal tarafından absorblanır. Materyal bünyesindeki polar moleküller (su) hedef alındığından, elektromanyetik alan doğrudan seçici bir ısıtma yapmaktadır. Mikrodalgalar gıda maddeleri tarafından absorbe edildiği zaman ısı doğrudan materyal içerisinde oluşmakta ve gıdanın içindeki su bu ısı sayesinde kısa sürede buharlaşmaktadır. Bu nedenlerden dolayı mikrodalga teknolojisi ile kurutma işlemi son yıllarda diğer yöntemlere alternatif olarak kullanılmaya başlanmıştır (Zhang ve ark., 2006; Inchuen ve ark., 2010; Bejar ve ark., 2011).

Kereviz çok eskiden beri kültüre alınmış *Apiales* takımının *Apiaceae* familyasından, taze olarak tüketildiğinde tek yıllık, tohumu için üretildiğinde ise iki yıllık bir bitkidir (Bayraktar, 1981; Vural ve ark., 2000). Kerevizin besin maddesi olarak kullanılmasından çok önceleri halk arasında sinir sistemi üzerine yatıştırıcı olarak, eklem iltihabı, ses kısıklığı ve böbrek ağrısı gibi birçok hastalığa karşı ilaç olarak kullanıldığına dair bilgilere rastlanmıştır (Bayraktar, 1981). Bunların yanı sıra astım, bronşit, dalak ve karaciğer hastalıklarını tedavi etmek için kullanılan kerevizin günümüzde de kök, gövde ve yaprakları tıbbi ve aromatik bitki olarak kullanılmaktadır (Popović ve ark., 2006; Choochoteve ark., 2004). Yapılan araştırmalarda kerevizin kan basıncını düşürdüğü, kalp fonksiyonlarını düzenlemenin yanı sıra pankreası uyararak insulin salgılamasını ve dolayısıyla kan şekerini düzenlediği belirlenmiştir (Popovićve ark.,2006, Nagella ve ark., 2012). Ayrıca kerevizin yüksek tansiyonu düzenlediği, karaciğer ve mideyi kuvvetlendirdiği ve baş ağrılarına iyi geldiği bildirilmektedir (Kızıldağ ve ark.,2016). Kereviz; antioksidan aktivitesi (Ninfali ve Bacchiocca 2003), antikanserojenik etkisi (Sultana ve ark., 2005), antihiperlipidemik etkisi (Tsi ve Tan 2000; Iyer ve Patil 2011) vb. özellikleri nedeniyle oldukça ilgi çeken bir bitki haline gelmiştir (He ve ark., 2016).Kerevizinyaprakları ise gıda üretim sektöründe taze veya kurutularak, garnitür olarak, et, çorba veya soslu yemeklerde dünya genelinde yaygın olarak kullanılan bir baharattır (Kaiserve ark.,2013).

Yapılan literatür çalışmalarında kereviz yaprağının kurutulması ve kurutma kinetiği üzerine herhangi bir çalışmaya rastlanmamıştır. O nedenle buçalışmada mikrodalga ile kereviz yapraklarının (*Apiumgraveolens* L.) kurutulması ve uygulanan farklı mikrodalga çıkış güçlerinin kereviz yapraklarının kuruma kinetiği, toplam fenolik madde içeriği, antioksidan kapasitesi ve klorofil a, b ve toplam klorofil miktarı üzerine etkileri incelenmiştir.

MATERYAL ve METOD

Materyal

Çalışmada kullanılan kereviz yaprakları Sivas'ta bulunan yerel bir marketten temin edilmiştir. Kereviz yaprakları denemelerde kullanılana kadar 4±1°C'de muhafaza edilmiş ve 24 saat içerisinde denemeye alınmıştır. Kullanılan tüm kimyasallar analitik saflıktadır.

Yöntem

Örnekhazırlama

Kereviz yapraklarının deneme öncesinde buzdolabısıcaklığından oda sıcaklığına gelmesi sağlanmıştır. Daha sonra sapları ayrılmış ve musluk suyu ile yıkanarak yaprakların yüzeyindeki fazla su havlu kağıt ile uzaklaştırılmıştır.

Kurutma

Kurutma işlemi mikrodalga fırında (HMT84M651, Bosch, Stuttgart, Almanya) 180, 360, 600 ve 900 W mikrodalga çıkış güçlerinde yapılmıştır. Kereviz yapraklarının başlangıç nem içeriği kızılötesi nem tayin cihazında (Shimadzu, MOC63u) belirlenmiştir. Sağlıklı ve zedelenmemiş yaklaşık 5 g yaprak alınarak cam petri kabı içerisine tek sıra olacak şekilde dizilerek, mikrodalga fırına yerleştirilmiştir. Kurutma kinetiğinin belirlenmesi için her 15 saniyede bir kurutma durdurularak tartım yapılmıştır. Tartımda 0.01 g hassasiyete sahip dijital terazi (AND GX 4000) kullanılmıştır. Her bir ağırlık ölçümünün süresi 10 saniyeyi geçmeyecek şekilde deneme üç tekrarlı olarak gerçekleştirilmiştir.

Nemiçeriğinin ve kurumahızınınbelirlenmesi

Kereviz yapraklarının mikrodalga ile kurutulması sırasında, herhangi bir t süresindeki nem içeriği aşağıdaki gibi hesaplanmıştır;

$$Mt = \frac{m-KM}{m} \quad (1)$$

Mt: Herhangi bir t süresindeki nem içeriği (g su g^{-1} KM)
m : Numunenin kütlesi(g)
KM : Numunenin içerdiği kuru madde miktarı (g)

Kuruma hızı (g su g^{-1} KM sn), aşağıdaki eşitlik kullanılarak, nem içeriğine karşılık kuruma süresi eğrilerinin türevlerinin alınması ile bulunmuştur.

$$\text{Kuruma Hızı} = -\frac{M_{t+dt}-M_t}{dt} \quad (2)$$

M_{t+dt} : t + dt süresindeki nem içeriği (g su g^{-1} KM)
dt : Kuruma süresi (saniye, sn)

Nemoranınınhesaplanması

Örneklerin nem oranı ağırlık değişimleri üzerinden hesaplanmış ve kurutma kinetiği ile ilgili modellemelerde nem oranı değerleri kullanılmıştır. Nem oranı (MR) aşağıdaki eşitlik yardımıyla hesaplanmıştır.

$$MR = \frac{M_t-M_e}{M_o-M_e} \quad (3)$$

MR: Nem oranı (birimsiz)
M_t: Herhangi bir süredeki nem miktarı(g su g^{-1} KM),
M_o: Başlangıç nem miktarı (g su g^{-1} KM),
M_e: Denge nem miktarı (g su g^{-1} KM)

Gıdaların mikrodalga ile kurutulması işleminde daha önce yapılan çalışmalarda (Maskan, 2000) belirtildiği gibi Me değeri diğer ifadelerden çok daha küçük olduğu için sıfır kabul edilmiştir.

Kurutmaprosesininmodellenmesi

Deneysel veriden yararlanarak nem oranı ve kuruma süresi arasındaki ilişkiyi göstermek amacıyla Çizelge 1'de verilen Newton, Page ve Weibull olmak üzere 3 farklı model test edilmiş ve istatistiksel olarak kıyaslanmıştır. Matematiksel modellerin deneysel verilere uyumu MINITAB (16) istatistik programında doğrusal olmayan regresyon yapılarak belirlenmiştir. En uygun modelin belirlenmesinde validasyon çalışması yapılmıştır. Modeller ile nem oranları tahmin edilirken iki tekerrür sonucunun ortalaması kullanılmış ve model uygunluğunun validasyonu 3. tekerrür sonucu ile yapılmıştır.
Validasyon için modelin kuruma eğrilerine uyumunu belirleyen parametrelerden regresyon katsayısı (R^2), yanlılığı (bias) ölçmek için ortalama eğilim hatası (MBE), doğruluğu (accuracy) ölçmek için ortalama karesel hatanın karekökü (RMSE) ve Ki-Kare (χ^2) hesaplanmıştır. RMSE, MBE ve χ^2 değerleri aşağıdaki eşitlikler kullanılarak saptanmıştır(Walther ve Moore 2005).

$$RMSE = \left[\frac{1}{N}\sum_{i=1}^{N}\left(MR_{tah,i} - MR_{den,i}\right)^2\right]^{1/2} \quad (4)$$

$$MBE = \frac{1}{N}\sum_{i=1}^{N}\left(MR_{tah,i} - MR_{den,i}\right) \quad (5)$$

$$\chi^2 = \frac{\sum_{i=1}^{N}(MR_{tah,i}-MR_{den,i})^2}{N-z} \quad (6)$$

N : Gözlem sayısı
z : Kullanılan modeldeki katsayı sayısı
$MR_{den,i}$: Deneysel olarak gözlenen i. düzey
$MR_{tah,i}$: Modellerden tahmin edilen i. düzey

Analizler

Örneklerinekstraksiyonu

Kurutulmuş kereviz yaprakları kahve öğütücüde (Sinbo) öğütüldükten sonra gözenek aralığı 300 µm ve 150 µm olan eleklerden (RETSCH) geçirilmiş ve analizlerde bu aralıktaki örnekler kullanılmıştır. Örneklerin ekstraksiyonu için 0.2 g örnek üzerine 10 mL 50 °C'deki saf su konularak, karşım yine 50°C'ye ayarlanmış çalkalamalı su banyosunda 30 dakika tutulmuştur. Süre sonunda örnekler 1 dakika vortekste karıştırılmış ve kaba filtre kağıdından süzülmüştür.Bu ekstraktlar örneklerin toplam fenolik madde içeriği ile antioksidan kapasitelerinin belirlenmesinde kullanılmıştır. Ekstraktlar analiz edilinceye kadar -18°C'de muhafaza edilmiştir.

Toplamfenolikmaddeanalizi

Örneklerin toplam fenolik madde içeriği Obanda ve Owuor (1997) tarafından belirtilen Folin-Ciocalteu spektrofotmetrik yönteminin modifiye edilmesiyle, 3 tekrarlı olarak gerçekleştirilmiştir. 0.5 ml ekstrakt, saf su ile 3 kez seyreltilmiş 0.5 mL Folin-Ciocalteu reaktifi ile karıştırılmış, karışıma 5 dakika sonra 1 ml sodyum karbonat çözeltisi (%35) ilave edilip tüp içeriği hafifçe çalkalanmıştır. Son olarak karışıma 1 mLsaf su ilave edilerek iyice karıştırılmıştır. Elde edilen karışım 30 dakika karanlıkta bekletildikten sonra oluşan mavi rengin absorbansı spektrofotometrede (OPTİMA SP3000) 700 nm dalga boyunda örnek yerine saf su kullanılarak hazırlanan köre karşı okunmuştur. Sonuçlar farklı konsantrasyonlarda (0-50 ppm; R^2=0.999) hazırlanmış gallik asit çözeltileri ile elde edilmiş olan kalibrasyon eğrisinin regresyon eşitliğinden hesaplanmış ve sonuçlar mg gallik asit eşdeğeri (GAE) g^{-1} kuru madde (KM) olarak ifade edilmiştir.

Antioksidankapasitetayini

Örneklerin antioksidan kapasitesi, DPPH yöntemi (Türkmen ve ark. 2009) kullanılarak belirlenmiştir. Bu amaçla, 50 µL ekstrakt(2mg mL^{-1}), metanolde hazırlanmış1950 µL DPPH radikali ($6x10^{-5}$ M) ile karıştırılmıştır. Kontrol örneğinde ekstrakt yerine saf su kullanılmıştır. Reaksiyon karışımı vorteks karıştırıcıda karıştırılıp oda sıcaklığında 60 dakika süreyle karanlıkta bekletilmiştir. Sürenin bitimindekarışımın absorbansı spektrofotometrede 517 nm'de metanole karşı okunmuştur. Antioksidan kapasite (%AK), aşağıdaki eşitlikten yararlanılarak hesaplanmıştır.

$$AK(\%) = \frac{Abs_{Kontrol}-Abs_{Örnek}}{Abs_{Kontrol}} x100 \quad -(7)$$

Abs kontrol : Örnek içermeyen DPPH çözeltisinin absorbansı,
Abs örnek : Örnek içeren DPPH çözeltisinin absorbansı

Klorofilekstraksiyonu ve analizi

Klorofil ekstraksiyonu %80 konsantrasyonundaki soğuk aseton ile 3 tekrarlı yapılmıştır (Shivanna ve Subban 2014; Sun ve Li 2017). Havana 0.05 g örnek alınmış ve üzerine feofitinizasyon oluşumunu en aza indirmek için 0.01 g $MgSO_4$ ilave edilmiştir. Bu karışım üzerine 3 mL% 80'lik aseton eklenerek 30 sn ezilmiştir. Süre sonunda süpernatant falkon tüpüne aktarılmış ve havandaki kalıntı üzerine tekrar 3 mLaseton ilave edilerek ekstraksiyona devam edilmiştir. Bu işlem toplam 18 mL % 80'lik aseton ile 6 aşamada tamamlanmıştır. En son ilave edilen 3 mL % 80'lik asetonla, ezme işleminden sonra havandaki kalıntının tümü kazınarak falkon tüpüne aktarılmıştır. Falkon tüplerine toplanan ekstraktlar 1 dakika vortekste karıştırılmış ve filtre kâğıdından süzülmüştür.Elde edilen klorofil ekstraktlarının UV spektrofotometrede 645 nm ve 663 nm'de absorbansları ölçülmüştür. Örneklerin klorofil a, klorofil b ve toplam klorofil içerikleri aşağıda belirtilen eşitlikler kullanılarakmg klorofil g^{-1} KM cinsinden hesaplanmıştır.

$$Klorofil\ a\ (mg\ L^{-1}) = 12.72 * A_{663} - 2.59 * A_{645} \quad (8)$$
$$Klorofil\ b\ (mg\ L^{-1}) = 22.88 * A_{645} - 4.67 * A_{663} (9)$$
$$Toplam\ klorofil\ a\ (mg\ L^{-1}) = 20.29 * A_{645} - 8.05 * A_{663} (10)$$

İstatistikselanaliz

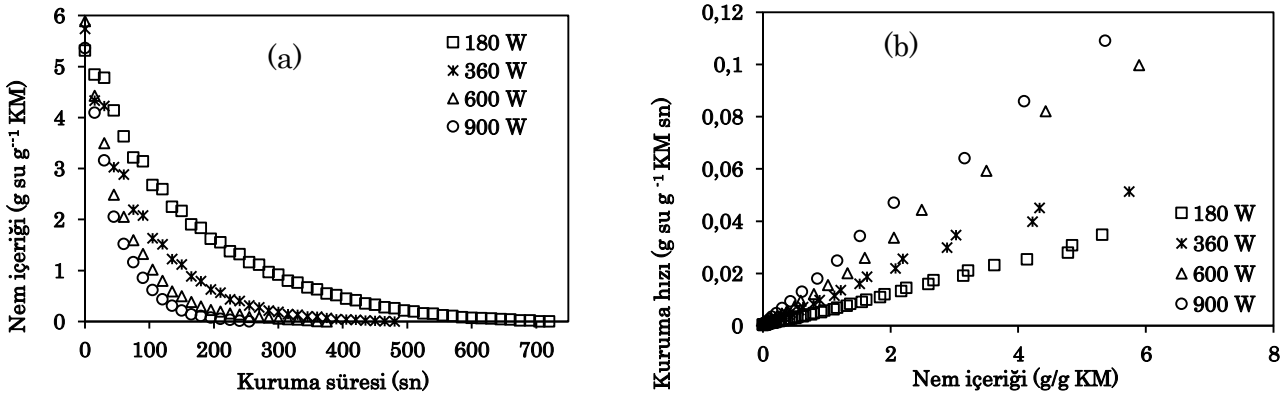
Çalışma sonucunda elde edilen verilere Statistica paket program (1995) kullanılarak varyans analizi yapılmıştır. Varyans analiz sonuçlarının önemli bulunması durumunda hangi gruplar arasında fark olduğu çoklu karşılaştırma testlerinden en küçük önemli fark (LSD) testi uygulanarak belirlenmiştir ($p<0.05$).

BULGULAR ve TARTIŞMA

MikrodalgaÇıkışGücününKerevizYapraklarınınKurutmaKinetiğiÜzerineEtkisi

Nemiçeriğideğişimi

Çalışmada kullanılan kereviz yapraklarının ortalama nem miktarları 5.38 g g^{-1} KM olarak bulunmuştur. Gıdaların mikrodalga enerjisi ile kuruma sonrası içerebilecekleri minimum nem içeriği değerinin 0.1 g su g^{-1} KM olduğu kabul edilmektedir (Maskan, 2000). Bu nedenle test edilen her bir mikrodalga çıkış gücü, numune nem içeriği yaklaşık 0.1 g g^{-1} KM seviyeye düşene kadar uygulanmıştır.Kereviz yapraklarının farklı mikrodalga çıkış güçlerinde (180, 360, 600 ve 900 W)kuruma süreleri sırasıyla 720, 480, 375 ve 255 snolarak gerçekleşmiştir. Kuruma süresi en düşük 900W, en yüksek ise 180W çıkış gücünde tespit edilmiştir. Mikrodalga çıkış gücünün 180 W'tan 360, 600 ve 900 W'a çıkarılmasıyla kuruma sürelerinde sırasıyla % 33.33, % 47.92, ve % 64.58'lik bir azalma görülmüştür. Yapılan diğer çalışmalarda da benzer durum tespit edilmiştir (Funebo ve Ohlsson 1998; Soysal, 2004; Özkan ve ark., 2007; Karaaslan ve Tunçer, 2009).Kereviz yapraklarının farklı çıkış güçlerinde kuruması sırasında nem içeriklerinin zamana bağlı olarak değişimi Şekil 1 (a)'da verilmiştir. Şekilden de görüldüğü gibi, kuruma süresi mikrodalga çıkış gücünden etkilenmekte, çıkış gücünün artmasına bağlı olarak nem kaybı hızlanmakta ve kuruma süresi kısalmaktadır.Yapılan diğer çalışmalarda da mikrodalga çıkış gücünün artmasıyla kurutma süresinin azaldığı belirtilmiştir (Sharma ve Prasad, 2001; Özkan ve ark., 2007; Alibaş, 2012).



Şekil 1Farklı mikrodalga çıkış güçlerinde kurutulmuş kereviz yapraklarının nem içeriği (a) ve kuruma hızı (b)
Figure 1. Moisture content (a) and drying rate (b) of celery leaves dried at different microwave output powers.

Örneklerin nem içeriğindeki azalmanın başlangıçta hızlı, sonlara doğru ise yavaş seyrettiği görülmektedir. Kurutmanın başlangıcında en hızlı kuruma 900 W'da gerçekleşmiştir. Kuruma eğrilerinde görülen hızlı düşüşler örneklerdeki su kaybının yüksek olduğunu göstermektedir. Aynı durum maydanozun (Soysal ve

ark., 2006), mango, guava ve aonlanın (Kumar ve Sagar, 2014) ve asma yaprağının (Alibaş, 2012) kurutulması sırasında da görülmüştür. Mikrodalga çıkış gücünün artması daha yüksek ısı emilimine neden olarak ürün sıcaklığının artmasına, dolayısıyla nem transferinin hızlanmasına neden olmaktadır. Bu durumda daha hızlı kuruma oranı ve daha kısa kuruma süresi görülmektedir. Daha önce yapılan çalışmalarda da benzer durum tespit edilmiştir (Soysal ve ark., 2006; Alibaş, 2012; Kumar ve Sagar, 2014; Doymaz ve ark., 2015; Hihat ve ark., 2017).

Kurumahızıdeğişimi

Farklı mikrodalga çıkış güçlerinde kurutulan kereviz yapraklarının kuruma hızı değerleri Şekil 1 (b)'de verilmiştir. Kereviz yapraklarının kuruma hızları 180 W'da 0.0349 g su g^{-1} KM sn, 360 W'da 0.0423 g su g^{-1} KM sn, 600 W'da 0.0958 g su g^{-1} KM snve 900 W'da0.1091 g su g^{-1} KM snolarak bulunmuştur. Mikrodalga çıkış gücü arttıkça kuruma hızının da arttığı görülmektedir. Mikrodalga güç seviyesinin kuruma hızı üzerine etkili olduğu yapılan diğer çalışmalarda da belirtilmiştir (Funebo ve Ohlsson,1998; Maskan, 2000; Sharma ve Prasad, 2001; Soysal, 2004; Özkan ve ark., 2007). Ayrıca kuruma hızı sabit olmayıp, kuruma azalan hız peryodunda gerçekleşmiştir. Kurutmanın ilk aşamalarında örneklerin nem içeriğinin yüksek olması nedeniyle daha fazla mikrodalga emilimi olmaktadır. Dolayısıyla daha fazla nem difüzyonu ile daha yüksek kuruma hızları gözlenmiştir. Kurutma ilerledikçe üründeki nem azaldığından mikrodalga emilimi azalarak kuruma hızında düşüşe neden olmuştur.

Kurumaeğrilerininmatematikselmodellereuygunluğu

Tarımsal ürünlerin kuruma eğrilerinin matematiksel olarak ifade edilmesinde birçok eşitlik kullanılmaktadır. Bu çalışmada kereviz yapraklarının farklı mikrodalga çıkış güçlerinde kurutulması sırasında elde edilen verilerden yararlanarak lineer olmayan regresyon analiz yöntemi yardımıyla kuruma süreleri ile nem oranı değişimi eğrilerinin matematiksel modellenmesi 3 farklı modele göre yapılmış (Çizelge 1) ve bu modellere ilişkin kinetik parametreler hesaplanmıştır. Uygulanan modellere ilişkin denklemler, model katsayıları, bu denklemlerin uygulanmasıyla elde edilen R^2, MBE, RMSEve χ^2 hesaplanmış vesonuçlar Çizelge 2'de verilmiştir. Bir modelin deneysel verilere uygunluğunun tespitinde R^2 değerinin yüksek olması, MBE, RMSE ve χ^2değerlerinin mümkün olduğunca düşük olması beklenmektedir (Sarsavadia ve ark., 1999, Soysal ve ark., 2006; Özkan ve ark., 2007). Modellere ilişkin R^2 dikkate alınarak modeller karşılaştırıldığında test edilen üç model için hesaplanan katsayıların oldukça yüksek ve birbirine yakın olduğu görülmektedir.

Çizelge 1. Kereviz yapraklarının kuruma eğrilerinin modellemesinde kullanılan eşitlikler
Table 1. Equations used for modeling of drying curves of celery leaves

Model *(Model)*	Eşitlik *(Equation)*	Kaynaklar *(References)*
Newton	$MR = \exp(-k.t)$	Ayensu (1997), Roberts ve ark., (2008)
Page	$MR = \exp(-k.t^n)$	Soysal (2004)
Weibull	$MR = \exp\left(-t/\alpha\right)^{\beta}$	Babalis ve ark., (2006)

k: Kinetik sabit (sn^{-1}) t: Kuruma süresi (sn) n: Page modele ait katsayı, α ve β:sırasıyla Weibull model skala parametresi (s) ve şekil parametresi

Ortalama eğilim hatası başka bir ifadeyle ortalama yanlılık hatası (MBE) tahmin edilenin deneysel olandan ortalama sapmasını ifade eder ve ideal koşullarda MBE değeri sıfıra eşit veya yakın olmalıdır. MBE değerinin pozitif bir değer olması modelin verileri yüksek tahmin ettiğini, negatif bir değer olması ise modelin verileri daha düşük tahmin ettiğini ifade eder (Walther ve Moore 2005; Kingsly ve Singh, 2007). Çizelge 2 incelendiğinde 180 W ve 360 W uygulaması dışındaki tüm mikrodalga çıkış güçlerinde en düşük MBE değeri Page ve Weibull modellerinde elde edilmiştir. RMSE modelden elde edilen tahmini değerler ile deneysel değerler arasındaki gerçek sapmayı göstermektedir ve daima pozitiftir. Bu değerin sıfıra eşit veya yakın olması modelin verileri doğru tahmin ettiğini ifade eder (Walther ve Moore 2005; Kingsly ve Singh, 2007). Modellere ilişkin RMSE değerleri karşılaştırıldığında 900 W dışındaki diğer tüm mikrodalga çıkış güçlerinde en düşük RMSE değeri Page ve Weibull modellerinde tespit edilmiştir. Modelin gözlenen değerlere uygunluğunun iyiliğini ifade eden χ^2 değerinin düşük olması uygunluğun arttığını göstermektedir. Modellere ilişkin χ^2 değerleri karşılaştırıldığında 900 W dışındaki diğer tüm mikrodalga çıkış güçlerinde en düşük χ^2 değeri Page ve Weibull modellerinde tespit edilmiştir. Nitekim her üç model için R^2, MBE, RMSE ve χ^2 değerleri dikkate alındığında Page ve Weibull modellerinin kereviz yapraklarının kuruma davranışlarını açıklamak için Newton modelinden daha uygun olacağı sonucuna varılmıştır. Ancak Page modeli Weibull'dan daha basit olduğundan kolay karşılaştırma sağlamakta ve tarımsal ürünlerin kurutulmasında yaygın olarak kullanılmaktadır. Bu nedenle kereviz örneklerinin kuruma davranışını açıklayan en uygun modelin Page modeli olduğuna karar verilmiştir. Bu model nar (Calín-Sanchez ve ark., 2014), kabak dilimleri (Alibaş, 2007), elma (Doymaz, 2010) ve ıspanağın (Özkan ve

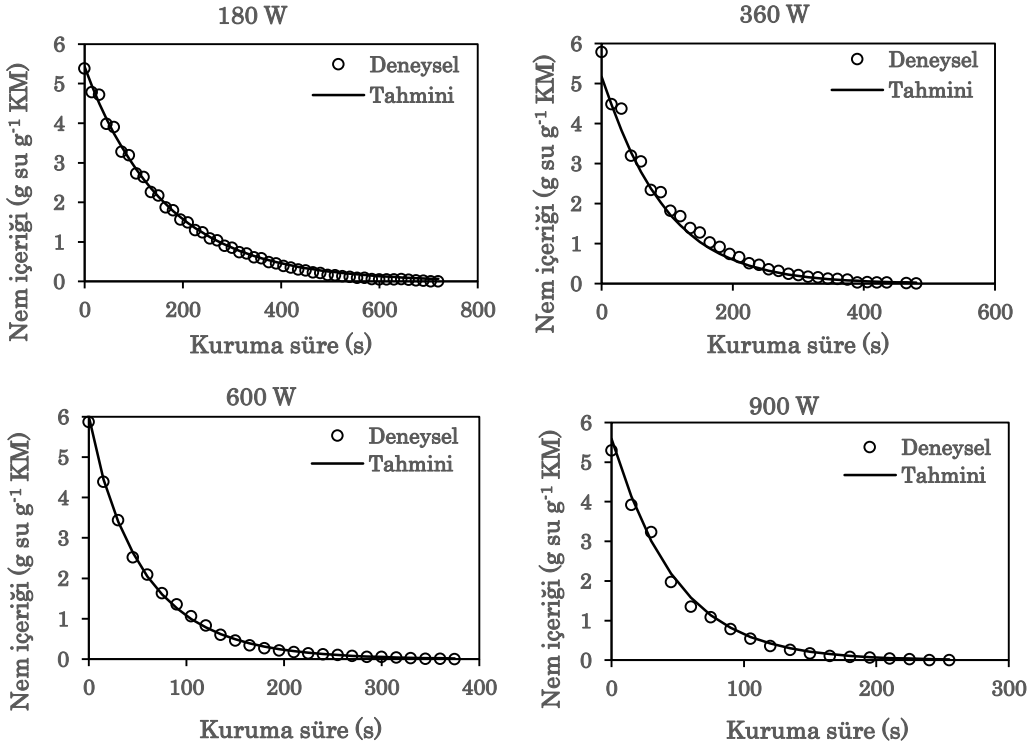
ark., 2007) kuruma davranışını tahmin etmek için de bazı çalışmalarda kullanılmıştır. Page model parametrelerinden k, 0.00541097-0.0246278 sn^{-1} olarak, n ise 0.921739-1.07986 arasında bulunmuştur. Uygulanan mikrodalga çıkış gücü artıkça k değerlerinde artış gözlemlenmiştir. Bu durum yüksek mikrodalga uygulamasının neden olduğu yüksek sıcaklıktan dolayı kurumanın kısa sürede gerçekleştiğini göstermektedir. Daha önce yapılan çalışmalarda da benzer bulgular tespit edilmiştir (Soysal, 2004; Wang ve ark., 2007; Özkan ve ark., 2007).

Table 2. Parameters related to the models applied in the drying of celery leaves at different microwave output powers and data of compatibility with the models.

Çizelge 2.Farklı mikrodalga çıkış güçlerinde kereviz yapraklarının kurutulmasında uygulanan modellere ilişkin parametreler ve modellerle uyumluluk verileri.

Çıkış gücü (W) *Out Power (W)*	Model *Model*	Katsayılar *Coefficients*	R^2	RMSE	MBE	χ^2
180	Page	k:0.00541097; n:1.02165	0.9982	0.014675	0.008961	0.000225
	Newton	k:0.00607298	0.9982	0.015588	0.009437	0.000248
	Weibull	α:165.462; ß:1.02165	0.9981	0.014677	0.008965	0.000225
360	Page	k:0.0169299; n:0.921739	0.9947	0.01897	0.00501	0.000383
	Newton	k:0.0117154	0.9939	0.02147	0.00276	0.000476
	Weibull	α:83.5109; ß:0.921739	0.9947	0.01897	0.00501	0.000383
600	Page	k:0.0225074; n:0.939195	0.999	0.00692	0.000029	0.000051
	Newton	k:0.0173376	0.9987	0.01054	0.00197	0.000116
	Weibull	α:56.7998; ß:0.939195	0.999	0.00692	0.000029	0.000051
900	Page	k:0.0246278; n:1.07986	0.9981	0.01296	0.00144	0.000189
	Newton	k:0.0203153	0.9984	0.01212	0.00370	0.000156
	Weibull	α:50.0181; ß:1.07986	0.9981	0.01296	0.00144	0.000189

k: s^{-1}, α:s



Şekil 2. Farklı mikrodalga çıkış güçlerinde kurutulan kereviz yapraklarının deneysel ve tahmini nem içerikleri
Figure 2.Experimental and predicted moisture contents of celery leaves dried at different microwave output powers

Farklı mikrodalga çıkış güçlerinde kurutulan kereviz yapraklarına ait deneysel değerler ile Page denklemi ile tahmin edilen değerlerin karşılaştırılması Şekil 2'de verilmiştir. Buna göre deneysel değerler ile Page modeli tahmini değerleri arasında oldukça yüksek bir uyum olduğu görülmektedir. Benzer sonuçlar

maydanoz (Soysal, 2004), soğan (Arslan ve Özcan, 2010), şeftali (Zhu ve Shen, 2014) ve sarımsak (Demiray ve Tülek, 2014) gibi gıdaların kuruma davranışını inceleyen çalışmalarda da belirtilmiştir.

Farklımikrodalgaçıkışgüçlerindekurutmanınkerevizyapraklarınınkaliteparametreleriüzerineetkisi

Farklı mikrodalga çıkış güçlerindekurutmanın kereviz yapraklarında toplam fenolik madde miktarı, antioksidan kapasite, klorofil a, b ve toplam klorofil miktarı üzerine etkisi incelenmiştir. Mikrodalga çıkış güçlerinintest edilen bağımlı değişkenler üzerine etkisine ilişkin Varyans analiz sonuçları Çizelge 3'te verilmiştir.

Toplamfenolikmadde ve antioksidankapasiteüzerineetkisi

Yapılan varyans analizine göre (Çizelge 3) mikrodalga çıkış gücünün, toplam fenolik madde miktarı ve antioksidan kapasite üzerine etkisi istatistiki olarak önemli bulunmuştur (sırasıyla $p<0.001$ ve $p=0.048$). Taze kereviz yapraklarının toplam fenolik madde miktarı 16.47 mg GA/g KM olarak bulunmuştur (Çizelge 4). He ve ark. (2016) kereviz yapraklarının toplam fenolik madde miktarını oldukça geniş bir aralıkta, 8.7-25.1 mg GAE g^{-1} KM olarak, tespit etmişlerdir. Bu çalışmada kullanılan taze kereviz yapraklarının toplam fenolik madde içeriği bu değerler arasındadır.

Çizelge 3. Farklı mikrodalga çıkış güçlerinde kurutmanın kereviz yapraklarının kalite parametreleri üzerine etkisine ilişkin varyans analizi

Table 3. Analysis of variance on the effect of drying at different microwave out powers on the quality parameters of celery leaves

Faktör *Factor*	Bağımlı değişken *Dependent variable*	SD[1]	Kareler Ortalaması *Mean of squares*	F değeri *F value*	p değeri *p value*
Çıkış gücü	Toplam fenolik madde	4	7.84	26.05	<0.001
	Antioksidan kapasite		14.44	3.52	0.048
	Klorofil a		59.68	326.66	<0.001
	Klorofil b		7.01	211.86	<0.001
	Toplam Klorofil		109.03	361.20	<0.001

[1] Serbestlik Derecesi

Çizelge 4. Farklı mikrodalga çıkış güçlerinde kurutulan kereviz yapraklarının toplam fenolik madde miktarları (mg GA g^{-1} KM) ve antioksidan kapasiteleri (% inhibisyon)

Tablo 4. Total phenolic content (mg GA g^{-1}DM) and antioxidant capacity (inhibition %) of celery leaves dried at different microwave output powers

Çıkış gücü (W) *Out power (W)*	Toplam fenolik madde *Total phenolics*	Antioksidan kapasite *Antioxidant capacity*
Taze	16.47 ± 0.21^{b}	73.60 ± 0.87^{a}
180	13.50 ± 0.33^{d}	68.94 ± 4.14^{b}
360	15.43 ± 0.64^{c}	70.94 ± 0.70^{ab}
600	16.42 ± 0.84^{bc}	71.72 ± 1.11^{ab}
900	17.87 ± 0.48^{a}	74.48 ± 0.94^{a}

*: Aynı sütundaki farklı harfler istatistiksel olarak farklılık olduğunu göstermektedir ($p<0.05$).

Kurutulmuş kereviz yapraklarında en düşük toplam fenolik madde içeriği çıkış gücü 180 W uygulandığındaelde edilmiştir (Çizelge 4). Bu düzey taze örnekte ve diğer uygulanan çıkış güçlerinden önemli düzeyde farklılaşmıştır ($p<0.05$). En yüksek toplam fenolik madde içeriği ise 900 W uygulamasında saptanmıştır. Mikrodalga çıkış gücü 900 W'ın altına düşürüldüğünde ise kuru örneklerin toplam fenolik madde içeriklerinin taze örneklerinkinden daha düşük olduğu belirlenmiştir ($p<0.05$). 180, 360 ve 600 W mikrodalga uygulaması toplam fenolik madde miktarında sırasıyla % 18, % 6.31, % 0.30 azalmaya neden olurken; 900 W uygulaması % 8.5'lik bir artışa neden olmuştur.Görülen azalma düşük mikrodalga çıkış gücünde kurutma işleminin uzun sürmesi nedeniyle fenolik bileşiklerin zarar gördüğü, artış ise yüksek mikrodalga çıkış gücünde kurutmada lifli yapının gevşeyerek fenolik maddelerin ekstraksiyon çözeltisine geçişinin kolay olması ile açıklanabilir (İzli ve ark., 2017; Bejar ve ark., 2011; Akbaş ve ark., 2018).Düşük mikrodalga çıkış güçleri fenolik bileşikleri parçalayan oksidatif enzimlerin inaktivasyonu için yeterli olmadığından ve kurutmanın uzun sürmesine neden olmasından dolayı örneklerin daha fazla ısıya maruz kalmasına ve fenolik bileşiklerde kayba neden olmaktadır (Ancos ve ark., 1999; Al Juhaimi ve ark., 2016). Çalışmada kullanılan 180 ve 360 W uygulamasının hücre duvarının parçalanmasına ve enzim inaktivasyonuna yetersiz geldiği, mikrodalga çıkış gücünün 600 W ve özellikle 900 W olması durumda ise hücre yapısının bozulması ve enzim inaktivasyonu için kısmen yeterli olduğu söylenebilir.

Farklı mikrodalga çıkış güçlerinde kurutulan kereviz

yapraklarının antioksidan kapasiteleri taze örnekte % 73.60 olarak bulunmuştur (Çizelge 4). He vd.'nin (2016) yaptıkları çalışmada farklı ekstraksiyon koşullarında kereviz yapraklarının antioksidan kapasitesini % 48-82 arasında bulmuşlardır.Çalışmada kullanılan taze kereviz yapraklarının antioksidan kapasitesi bu değerler arasındadır.Kurutulmuş kereviz yapraklarında ise antioksidan kapasite %68.94-%74.48 arasında saptanmıştır (Çizelge 4). En düşük antioksidan kapasite 180 W uygulamasında elde edilmiştir. Bu güç seviyesi taze ve diğer uygulanan çıkış güçlerinden önemli düzeyde farklılaşmıştır ($p<0.05$). Taze örneklerin antioksidan kapasitesi 180, 360 ve 600 W mikrodalga uygulamasıyla azalmıştır. Mikrodalga çıkış gücü 900 W olduğunda ise antioksidan kapasitede artış tespit edilmiştir. Ancak 360, 600 ve 900 W uygulamaları ile taze örnekler arasında antioksidan kapasite yönünden saptanan farklılık istatistiki olarak önemli bulunmamıştır ($p>0.05$). Benzer bulgular Akbaş ve ark.,(2018), Inchuen ve ark., (2010) ve İzli ve ark., (2017) tarafından da tespit edilmiştir.Daha önce yapılan çalışmalarda bitkisel materyallerin toplam fenolik madde içeriği ile antioksidan kapasiteleri arasında ilişki olduğu belirtilmiştir (Velioğlu ve ark.,1998; Inchuen ve ark., 2010). Örneklerin antioksidan kapasitesindeki değişim fenolik bileşiklerde görülen eğilimle aynı olmuştur.

Klorofil a, klorofil b ve toplamklorofilüzerineetkisi

Farklı mikrodalga çıkış güçlerinde kurutmanın kereviz yapraklarının, klorofil a, b ve toplam klorofil miktarları üzerine etkisi istatistiki olarak önemli bulunmuştur ($p<0.05$) (Çizelge 3.). Taze kereviz yapraklarının klorofil a, klorofil b ve toplam klorofil içerikleri sırasıyla 17.49, 5.57, 23.05 mg g^{-1} KM olarak belirlenmiştir (Çizelge 5). Beklenildiği gibi ve bu konuda diğer birçok materyalde yapılan birçok araştırma sonuçları (Shivanna ve Subban, 2014; Kumar ve ark., 2015; Rubinskiene ve ark., 2015;Jinasena ve ark., 2016) ile de uyumlu olarak klorofil a miktarı klorofil b'den fazla bulunmuştur. Literatürde kereviz yapraklarının klorofil miktarına ilişkin herhangi bir çalışmaya rastlanmamıştır.
Mikrodalga uygulaması klorofil bileşiklerinde azalmaya neden olmuştur. Benzer bulgular hibiskus yapraklarının (Kumar ve ark., 2015), nane yapraklarının (Rubinskiene ve ark., 2015) ve köri yapraklarının (Shivanna ve Subban 2014) kurutulmasında da tespit edilmiştir.

Çizelge 5.Farklı mikrodalga çıkış güçlerinde kurutulan kereviz yapraklarının klorofil a, klorofil b ve toplam klorofil miktarları (mg g^{-1} KM)

Table 5. Chlorophyll a, chlorophyll b and total chlorophyll contents (mg g^{-1}DM)of celery leaves dried at different microwave output powers

Çıkış gücü (W) *Out power (W)*	Klorofil a *Chlorophyll a*	Klorofil b *Chlorophyll b*	Toplam klorofil *Total chlorophyll*
Taze	17.49 ± 0.87^{a}	5.57± 0.25^{a}	23.05 ± 1.11^{a}
180	7.14± 0.21^{c}	1.96 ± 0.09^{c}	9.10 ± 0.30^{c}
360	7.43 ± 0.23^{bc}	2.06 ± 0.11^{c}	9.49 ± 0.34^{bc}
600	7.51 ± 0.14^{bc}	2.23 ± 0.08^{bc}	9.62 ± 0.06^{bc}
900	8.09 ± 0.15^{b}	2.43 ± 0.28^{b}	10.18 ± 0.22^{b}

*: Aynı sütundaki farklı harfler istatistiksel olarak farklılık olduğunu göstermektedir ($p<0.05$).

Kereviz yapraklarına uygulanan farklı çıkış güçlerinin klorofil a, b ve toplam klorofil içeriğine etkisi karşılaştırıldığında ise genel olarak çıkış gücü arttıkça klorofil miktarında istatistiki olarak önemli olmamakla beraber, daha düşük bir azalma olduğu görülmektedir. 900 W'da diğer güç uygulamalarından daha yüksek miktarda klorofil saptanmıştır(Çizelge 5). Kuru yaprakta en düşük klorofil a, b ve toplam klorofil içeriği 180 W çıkış gücünde tespit edilmiştir. Ancos ve ark., (1999) 285, 570 ve 850 W gibi farklı mikrodalga çıkış güçleri uyguladıkları kivilerin klorofil a ve b miktarlarını taze örneklerden düşük bulurken,bu çalışmadan elde edilen sonuçlara benzer şekilde çıkış gücünün artmasıyla daha fazla klorofil a ve b tespit etmişlerdir.Kereviz yapraklarının klorofil içeriğinin 180 W uygulamasında düşük olmasının, kuruma süresinin uzun olmasından dolayı klorofillerin daha uzun süre ısıya maruz kalarak degradasyona uğramasından kaynaklandığı düşünülmektedir.Ayrıca çıkış gücü arttıkça klorofilde daha az kaybın meydana gelmesinin yüksek çıkış güçlerinde hücre duvarında daha fazla zararın oluşması nedeniyle klorofillerin serbest kalması ve dolayısıyla ekstraksiyon çözeltisine geçişinin kolaylaşmasından kaynaklanabileceği düşünülmektedir.

SONUÇ ve ÖNERİLER

Bu çalışmada mikrodalga fırında 180, 360, 600 ve 900 W çıkış güçlerinde kurutulan, kereviz yapraklarının kuruma kinetiği, toplam fenolik madde içeriği, antioksidan kapasite ve klorofil a, b ve toplam klorofil miktarı üzerine mikrodalga çıkış güçlerinin etkisi incelenmiştir. Elde edilen sonuçlara göre, kuruma eğrilerinin kinetiğini açıklayan en iyi modelin Page model olduğu ortaya konmuştur. Kereviz

yapraklarının incelenen kalite parametreleri,uygulanan mikrodalga çıkış gücüne bağlı olarak azalma göstermiştir. Bu azalma 900 W uygulamasında daha az olmuştur. Ancak denemelerin yapılması sırasında 900 W uygulamasında kısa sürede yüksek sıcaklıkların oluşması kurutmanın kontrolünü zorlaştırmış ve üründe yanmalara neden olabileceği tespit edilmiştir. Kalite parametrelerine bakıldığında 600 W uygulamasında da 900 W'a yakın sonuçlar tespit edilmiştir. Bu nedenle kereviz yapraklarının mikrodalga fırında kurutulmasında 600 W uygulaması önerilmektedir.

Teşekkür

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Evaluation of Pomological Characteristics and Bioactive Compounds of Wild Sea Buckthorn (*Hippophae Rhamnoides* L.) and Hawthorn (*Crataegus songarica*) from Walnut-Fruit Forest Kyzyl-Unkur, Kyrgyzstan

Janyl ISKAKOVA[1] ✉, Zhyldyz OSKONBAEVA[2], Jamila SMANALIEVA[3,4]

[1]Kyrgyz-Turkish Manas University, Environmental Engineering Department, Engineering Faculty, pr. Aytmatov 56, 720044 Bishkek, Kyrgyzstan, [2]Rhine-Waal University of Applied Sciences, Faculty of Life Sciences, Marie-Curie-Strasse 1, 47533 Kleve, Germany, [3]Kyrgyz State Technical University named after I. Razzakov, Department of Food Production Technology, Faculty of Technology, pr. Aytmatov 66, 720044 Bishkek, Kyrgyzstan, [4]Max Rubner-Institut, Federal Research Institute of Nutrition and Food, Department of Nutritional Behaviour, Department of Nutritional Behaviour, Haid-und-Neu-Straße 9, 76131 Karlsruhe, Germany

[1]https://orcid.org/0000-0002-1614-3984, [2]https://orcid.org/0000-0003-3835-4165, [3]https://orcid.org/0000-0002-3929-4291

✉: janyl.iskakova@manas.edu.kg

ABSTRACT

There are different berries and fruits naturally growing in the walnut-fruit forests of Kyrgyzstan, however, their composition and bioactive compounds have not been studied. This study aims to contribute to the limited literature on dietary fibres, ash, bioactive compounds such as vitamin C, polyphenols, antioxidant activity, and physical parameters of wild sea buckthorn (*Hippophae rhamnoides* L.) and hawthorn (*Crataegus songarica*) from walnut-fruit forests of Kyrgyzstan. The standard food analysis techniques and DPPH assay were used to determine the nutritional composition and antioxidant activity of the samples, respectively. The total amount of polyphenols in the extracts was determined by the Folin-Ciocalteau micro method. The content of vitamin C in fresh sea buckthorn was higher than in hawthorn, but hawthorn has advantages in terms of the amount of phenolic compounds and antioxidant activity. Both studied species have high nutritional values and are recommended to be used in the diet to improve the food security of the local population.

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Kırgızistan Kyzyl-Unkur Ceviz-Meyve Ormanından Yabani İğde (*Hippophae Rhamnoides* L.) ve Alıcın (*Crataegus songarica*) Pomolojik Özelliklerinin ve Biyoaktif Bileşiklerinin Değerlendirilmesi

ÖZET

Kırgızistan'ın ceviz-meyve ormanlarında doğal olarak yetişen farklı meyveler vardır, ancak bunların bileşimi ve biyoaktif bileşenleri araştırılmamıştır. Bu çalışma, ceviz-meyve ormanındaki yabani iğdesi (*Hippophae rhamnoides* L.) ve alıcın (*Crataegus songarica*) C vitamini, polifenoller, antioksidan aktivite gibi biyoaktif bileşenleri, diyet lifleri, kül ve fiziksel parametreleri hakkında sınırlı literatüre katkıda bulunmayı amaçlamaktadır. Standart gıda analiz yöntemleri yabani bitkilerin bileşimini ve antioksidan aktiviteyi belirlemek için DPPH analizi kullanılmıştır. Ekstraktlardaki toplam polifenol miktarı Folin-Ciocalteau mikro yöntemi ile belirlenmiştir. Taze deniz iğdesindeki C vitamini içeriği alıçtan daha yüksektir, ancak alıç, fenolik bileşik miktarı ve antioksidan aktivite açısından avantajlıdır. İncelenen her iki türün de yerel nüfusun gıda güvenliğini artırmak için beslenmede kullanılması önerilmektedir.

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INTRODUCTION

Wild berries are natural food sources with high nutritional value and can help for enhancing food security (Powell et al., 2015). The natural and climatic conditions of Kyrgyzstan allow the growth of numerous wild fruit trees and shrubs in forests and high mountains.

Sea buckthorn (*Hippophae rhamnoides* L.) is a thorny shrub that grows widely on the sea and river coasts of Kyrgyzstan. All parts of sea buckthorn contain about 200 bioactive components, including flavonoids, phenolic compounds, tocopherols, fatty and organic acids, fats, vitamins (A, E, K, C, B_1, and B_2), amino acids, terpenes, tannins, and microelements (Kumar et al., 2011). Sea buckthorn is recommended for the prevention and treatment of cardiovascular diseases due to its high polyphenol content (Cheng et al., 2003; Skalski et al., 2019). In addition, sea buckthorn seed oil extracts are known to have significant anti-atherogenic and cardioprotective effects, as well as positive effect on atherosclerosis (Basu et al., 2007), acute alcohol intoxication (Wen et al., 2016), burn wound (Ito et al., 2014; Koskovac et al., 2017), depression (Tian et al., 2015) etc. In Kyrgyz folk medicine, sea buckthorn is used to treat diseases of gastrointestinal tract (Pawera et al., 2016). In recent years, in the food industry of Kyrgyzstan, sea buckthorn has been used as a functional ingredient in the production of cereal drink "Bozo" (Smanalieva et al., 2022) and for the production of jams and juices. In addition, dried sea buckthorn berries are used as an additive to tea and cereal products.

Crataegus spp. – is a genus of approximately 300 species of shrubs and trees, and grows easily in shallow, sandy or stony soil. The Asian hawthorn species *Crataegus songarica* grows in the walnut-fruit forests of Kyrgyzstan with yellow, red (Figure 1) and black fruits. The plant is a tree or small shrub with thorns up to 15 mm in length. According to researchers, antioxidant phytochemicals of the genus *Crataegus* are procyanidins, flavonoids, flavonols, glycosylated flavanones, and triterpene pentacyclic acids (Liu et al., 2010; Yang & Liu 2012; Sytařová et al., 2020). Since ancient times, *Crataegus* spp. has traditionally been used in folk medicine to treat heart problems such as hypertension, arrhythmia, and congestive heart failure (Yang & Liu, 2012). In Europe and China, hawthorn fruit is also eaten and used to make commercial products such as wine, jam and candy (Edwards et al., 2012).

Unfortunately, the scientific literature lacks information on bioactive compounds and antioxidant activity, and there is very limited old data on the nutritional composition of wild sea buckthorn and hawthorn berries from the walnut-fruit forest of Kyrgyzstan. Therefore, this study aimed to contribute to the limited literature on chemical composition (dry matter, sugars, pH, titratable acidity, ash, fibre, vitamin C) and the radical scavenging activity, total polyphenols of sea buckthorn and hawthorn berries, which are required for documentation in the national food composition database.

MATERIAL and METHODS

Biological Material

Sea buckthorn and hawthorn samples were harvested (2 kg) at full maturity in 2017 and 2018 in the walnut-fruit forests in Kyzyl-Unkur village, Kyrgyzstan (Figure 1). The samples were transported to the laboratory in air-conditioned vehicles in a portable refrigerator at a temperature of 4–6 °C (within 9–12 h). All samples were stored in plastic bags under two different conditions: at 4–6 °C for physical properties analysis and at -23 °C for chemical composition analysis.

Analysis of Nutritional Value and Pomological Characteristics

The AOAC methods were used (AOAC, 1990) to determine moisture content (No. 930.15), titratable acidity (No. 949.08) and ash content (No. 942.05). The concentration of reducing sugars (glucose and fructose) was determined by the iodometric method according to AOAC methods 939.03. Ascorbic acid was determined using the AOAC 967.21 titration method with a solution of 2,6-dichloroindophenol (AOAC, 2019). The crude fibre content was determined by filtre bag technique (No. 962.09). All chemical analyses were performed in triplicate, mean values and standard deviations (SD) were determined.

The physical properties of fruits (fruit weight, size, sphericity, average radius, aspect ratio, volume, fruit and bulk density) included the examination of randomly selected 25 fruits in three replications at a natural moisture content of 66.03% for sea buckthorn and 71.48% for hawthorn by weight basis (w.b.) according to Mohsenin (2019):

The sphericity of fruits φ (–) was defined by formula(1):

$$\varphi = \frac{(D_{max} \cdot D_i \cdot D_{min})^{\frac{1}{3}}}{D_{max}} \quad (1)$$

Sphericity expresses the characteristic shape of a solid object in relation to the shape of a sphere of the same volume.

The surface area A (mm^2) was calculated by the formula (2) of the surface area of a sphere and multiplied by sphericity, where r_a is the average fruit radius (mm):

$$A = \left(\pi \cdot r_a^2\right) \cdot \varphi \quad (2)$$

The aspect ratio R_a (–) was calculated by formula (3):

$$R_a = \frac{D_{max}}{D_{min}} \quad (3)$$

Fruit volume V (mm^3) and solid density ρ_s ($kg\ m^{-3}$) were determined experimentally from liquid displacement. Briefly, 3 g of fruit samples were placed into a 100 mL beaker filled with 50 mL of distilled water. The volume of water displaced by fruits was calculated as follows (4):

$$V_s = \frac{M_{bws} - M_{bw}}{\rho_w} \quad (4)$$

Where M_{bws} is the weight of the beaker, water and fruits; M_{bw} is the weight of the beaker and water. The measurement was repeated three times.

Figure 1. The appearance of the fresh sea buckthorn and hawthorn
Şekil 1. Taze iğde ve alıç görünümü

The bulk density ρ_b ($kg\ m^{-3}$) was determined by dividing the fruit weight M_f by the beaker (1000 mL) volume V_b (5). The procedure was repeated three times:

$$\rho_{b=} \frac{M_f}{V_b} \quad (5)$$

Total Phenolic Compounds Measurement

Sample extracts for polyphenol analysis were prepared according to Kalt et al. (1999). The total amount of polyphenols in the extracts was determined by the Folin-Ciocalteau micro method using a UV-VIS spectrophotometer (Specord 50, Analytik Jena, Germany) at a wavelength of 765 nm. The total amount of polyphenols was expressed as mg gallic acid equivalents (GAE) per 100 g fresh sample.

Analysis of Total Antioxidant Capacity

Both extracts and analysis of total antioxidants were performed according to Hangun-Balkir & McKenney (2012) using a solution of 80% ethanol. Individual solutions of antioxidants were prepared at five concentrations (1, 2.5, 5, 7.5 and 10 µg mL^{-1}) in 80% ethanol. A radical solution of 0.01% DPPH (2,2 diphenyl-1-picrylhydrazyl) in 80% ethanol solution was used. The absorbance of the control and sample solutions was measured at 517 nm by using a UV-VIS

spectrophotometer (Specord 50, Analytik Jena, Germany). Values are expressed as inhibition concentration (IC_{50}), the concentration of the samples that causes 50% scavenging of the DPPH radical.

Statistical Analyses

All chemical analyses were carried out in triplicate and physical properties - in 20 replicates. Results were expressed as mean values with SD calculated using the SPSS software version 13 for statistical evaluation (SPSS Inc., Chicago, IL, USA).

RESULTS and DISCUSSION

Evaluation of Physical Attributes of Sea Buckthorn and Hawthorn

Measuring the physical attributes of agricultural products is an important tool in the design of sorting, grading, conveying, processing, and packaging equipment (Sahin & Sumnu, 2006). This is also important for the determination of pomological characteristics. Table 1 compares the physical parameters of sea buckthorn (*Hippophae salicifolia)* from the trans-Himalayan region (Yadav et al., 2006; Jaiswal et al., 2017) and Turkey (Sezen et al., 2015). Sea buckthorn from the walnut-fruit forests of Kyrgyzstan has close physical parameters with the genotypes of sea buckthorn from the Himalayas. Fresh sea buckthorn fruits have an average length (D_{max}) of 6.36 mm, an average width (D_{in}) of 4.7 mm, and a thickness (D_{min}) of 4.6 mm. The calculated sphericity was 0.81, which corresponds to an oval shape (ovoid). The fruit surface area was 17.33 mm^2. The solid density (ρ_s) and bulk density (ρ_b) of the fresh samples were 1.05 and 0.53 g cm^{-3}, respectively. The weight of 100 berries was 10.44 g, which is significantly lower than the results of Jaiswal et al. (2017) 36.27 ÷ 91.2 g.

The investigated physical attributes of hawthorn (*Crataegus songarica)* at a natural moisture content of 71.48% by fresh weight basis are given in Table 1. Hawthorn fruits are purple-red in colour with 2-4 seeds, 9-11 mm in diameter, and with a sphericity of 0.89 (Figure 1). According to Turkish authors, D_{max} and D_i were found to be 19.34 mm, and 14.39 mm for *Crataegus* spp. (Özcan et al., 2005), 12.25 mm and 11.37 mm for *Crataegus Monogyna* Jacq. var. *Monogyna* (Yalçın-Dokumacı et al., 2021), and within the range of 2.21 ÷ 17.68 mm, and 1.67 ÷ 15.72 mm for 11 hawthorn species (Gundogdu et al., 2014), respectively. The sphericity of hawthorn berries from Turkey was high at 0.95 ÷ 1.22 (Özcan et al., 2005; Yalçın-Dokumacı et al., 2021). It should be noted that sphericity values around 1 indicate that the fruit is round, while values above 1 indicate an oval shape. The aspect ratio and surface area of the studied hawthorn were determined to be 1.22 and 68.05 mm^2, respectively. The solid and bulk densities of the fruits were 0.88 g cm^{-3} and 591.67 kg m^{-3}, respectively. The average weight of hawthorn berries was 0.49 g per berry and the volume of one hawthorn fruit was 0.56 cm^3. This is significantly lower compared to reports from Turkey, for example, Yalçın-Dokumacı et al. (2021) have measured the average weight of one hawthorn as 0.93 g, Özcan et al. (2005) - 3.03 g, and Gundogdu et al. (2014) - 0.58 ÷ 3.48 g per one berry. The volume of the fruit studied by Özcan et al. (2005) was also significantly higher and equaled 3.08 cm^3. In terms of weight, volume, surface area and length of hawthorn berries harvested in the walnut-fruit forests of Kyrgyzstan are much smaller than berries grown in Turkey. This can be explained by the huge difference in hawthorn cultivars (Gundogdu et al., 2014) and climatic conditions of the locations. Meisen et al. (2020) stated higher variability in the physical properties of walnuts between walnut trees within the same site than between different sites.

Evaluation of Nutritional Composition of Sea Buckthorn and Hawthorn

The moisture content, inverted sugars, organic acid, fibre, and ash content of sea buckthorn and hawthorn are given in Table 2. Fresh sea buckthorn features 66.03 g per 100 g of moisture content, 1.00 ÷ 1.13 g per 100 g of inverted sugars, 1.78 ÷ 2.12 g per 100 g of organic acids, 6.13 g per 100 g of fibres, and 1.75 g per 100 g of ash content. The moisture content of the investigated sea buckthorn was close to fresh berries of *H. tibetana* and *H. rhamnoides* from the Himalayas (67.2 ÷ 76.9%) (Ranjith et al., 2006). Compared to berries from the Czech Republic, the moisture content (84.2 ÷ 87.4%) is significantly lower than reported by Sytařová et al. (2020). According to Bal et al. (2001), the differences in moisture content are associated with differences in origin and climate. Other values of sea buckthorn are consistent with sea buckthorn data from other countries (Ranjith et al., 2006; Ercisli et al., 2007; Bal et al., 2011). For comparison, sea buckthorn grown in Poland contained similar value of sugars 1.34 ÷ 2.87 g per 100 g and organic acids from 2.48 to 2.79 g per 100 g fresh weight (Tkacz et al., 2019). The crude fibre content was 6.13 g per 100 g, which is significantly higher than indicated by Jaroszewska & Biel (2017). In our study, the ash content was determined with seeds, therefore, the ash content of sea buckthorn was higher compared to the data obtained for sea buckthorn berries grown in the Himalayas. According to Ranjith et al. (2006), the ash contents in the seedless berries *H. rhamnoides* and *H. salicifolia* were 1.05% and 0.26%, respectively, which is lower than our results. The sugar/acid ratio of fruit is considered an important indicator of flavor quality (Albertini et al., 2006). The sugar/acid ratio of sea buckthorn was 0.51 and was consistent with the data of Tkacz et al. (2019) who reported that the sugar/organic acid ratio ranged from 0.40 to 2.99.

Table 1. Physical attributes (mean ± SD, n = 20) of fresh sea buckthorn and hawthorn
Çizelge 1. Taze iğde ve alıçların fiziksel özellikleri (ortalama ± SD, n = 20)

Physical characteristic Fiziksel özellik	Kyrgyzstan Kyzyl-Unkur *Hippophae rhamnoides* L. Kırgızistan Kızıl-Ünkür *Hippophae rhamnoides* L.	Literature data for *Hippophae rhamnoides* L. *Hippophae rhamnoides* L için literatür verileri	Kyrgyzstan Kyzyl-Unkur *Crataegus songarica* Kırgızistan Kızıl-Ünkür *Crataegus songarica*	Literature data *Crataegus songarica* *Crataegus songarica* için literatür verileri
Altitude, m	1466	3500 (Jaiswal et al., 2017); 2000-3000 (Yadav et al., 2006); 1025 (Sezen et al., 2015)	1466	
Average length, D_{max} (mm)	6.35 ± 0.63	6.5 ÷ 7.5 (Jaiswal et al., 2017); 5.78 ÷ 7.92 (Yadav et al., 2006); 6.46 ÷ 9.14 (Sezen et al., 2015)	11.06 ± 0.67	19.34 ± 0.18 (Özcan et al., 2005); 12.25 ± 0.064 (Yalçın-Dokumacı et al., 2021); 2.21 ÷ 17.68 (Gundogdu et al., 2014)
Average width, D_i (mm)	4.70± 0.03	5.49 ÷ 6.99 (Jaiswal et al., 2017); 5.51 ÷ 7.24 (Yadav et al., 2006); 5.48 ÷ 7.18 (Sezen et al., 2015)	9.38 ± 0.96	14.39 ± 0.12 (Özcan et al., 2005); 11.37 ± 0.082 (Yalçın-Dokumacı et al., 2021); 1.67 ÷ 15.72 (Gundogdu et al., 2014)
Thickness, D_{min} (mm)	4.60 ± 0.38	4.74 ÷ 6.28 (Jaiswal et al., 2017)	9.08 ± 0.87	
Sphericity, φ (–)	0.81	0.66 ÷ 0.91 (Jaiswal et al., 2017)	0.89 ± 0.04	1.22 ± 0.01 (Özcan et al., 2005); 0.95 ± 0.036 (Yalçın-Dokumacı et al., 2021)
Aspect ratio, R_a (–)	1.37		1.22 ± 0.08	
Surface area, A (mm^2)	17.33 ± 2.22	76.8 ÷ 154.7 (Jaiswal et al., 2017)	68.05 ± 12.90	
Weight, m (g)	10.44 ± 0.69 of 100 berries	3.63 ÷ 9.12 g of 100 berries (Jaiswal et al., 2017); 11.53 ÷18.87 g of 100 berries (Yadav et al., 2006); 15 ÷ 26 g of 100 berries (Sezen et al., 2015)	0.49 ± 0.08 g of 1 berry	3.03 (Özcan et al., 2005); 0.93 (Yalçın-Dokumacı et al., 2021); 0.58 ÷ 3.48 g of 1 berry (Gundogdu et al., 2014)
Volume, V (cm^3)	0.11 ± 0.02		0.56 ± 0.04	3083.3 ± 261.41 mm^3 (Özcan et al., 2005)
Bulk density, ρ_b (kg m^{-3})	0.53 ± 0.02 g cm^{-3}		591.67 ± 1.75	466.06 ± 3.39 (Özcan et al., 2005)
Fruit density, ρ_s (g cm^{-3})	1.05 ± 0.19	0.65 ÷ 1.4 (Jaiswal et al., 2017)	0.88 ± 0.14	1065.98 ± 28.18 kg m^{-3} (Özcan et al., 2005)

Table 2. Chemical composition (mean ± SD, n = 3) of fresh sea buckthorn and hawthorn
Çizelge 2. Taze iğde ve alıçların kimyasal bileşimi (ortalama ± SD, n = 3)

Index Gösterge	Kyrgyzstan Kyzyl-Unkur *Hippophae rhamnoides* L. Kırgızistan Kızıl-Ünkür *Hippophae rhamnoides* L.	Literature data for *Hippophae rhamnoides* L. *Hippophae rhamnoides L* için literatür verileri	Kyrgyzstan Kyzyl-Unkur *Crataegus songarica* Kırgızistan Kızıl-Ünkür *Crataegus songarica*	Literature data for *Crataegus songarica* *Crataegus songarica* için literatür verileri
Moisture content, g per 100 g	66.03 ± 0.15	67.2 ÷ 76.9 (Ranjith et al., 2006); 88.22 ÷ 86.92 (Tkacz et al., 2019); 84.2 ÷ 87.4 (Sytařová et al., 2020)	71.48 ± 0.24	80 ÷ 97.65 (Gundogdu et al., 2014); 68 ÷ 70.13 (Mironeasa et al., 2016)
Invert sugars, g per 100 g	1.03 ± 0.05	1.34 ÷ 2.87 (Tkacz et al., 2019); 0.35 ÷ 1.56 (Criste et al., 2020)	2.69 ± 0.26	15.66 ÷ 32.27 (Gundogdu et al., 2014)
pH	3.23 ± 0.00	2.7 ÷ 2.9 (Tkacz et al., 2019); 2.63 ÷ 2.98 (Ercisli et al., 2007)	4.74 ± 0.02	4.29 ÷ 5.99 (Gundogdu et al., 2014); 3.12 ÷ 4.09 (Türkoğlu et al., 2005)
Titratable acidity, g malic acid per 100 g	1.95 ± 0.11	2.0 ÷ 3.7 (Tkacz et al., 2019); 2.64 ÷ 4.54 (Ercisli et al., 2007)	0.75 ± 0.01	0.22 ÷ 2.40 (Gundogdu et al., 2014); 0.49 ÷ 0.96 mg $100g^{-1}$ (Türkoğlu et al., 2005)
Total crude fibre, g per 100 g	6.13 ± 0.66	6.2-7.3 (Ranjith et al., 2006)	2.25 ± 0.19	4.67 (Özcan et al., 2005)
Ash content, g per 100 g	1.75 ± 0.04	0.26 ÷ 1.05 (Ranjith et al., 2006); 0.31 ÷ 0.43 (Tkacz et al. (2019)	0.66 ± 0.06	2.28 (Özcan et al., 2005); 2.77 (Yalçın-Dokumacı et al., 2021)
Sugar/acid ratio	0.52	0.40 ÷ 2.99 (Tkacz et al., 2019); 0.40 ÷ 1.90 (Tiitinen et al., 2005)	3.36	7.75 (Lou et al., 2020)

The moisture content of hawthorn berries was close to the values obtained by Mironeasa et al. (2016) (68.0 ÷ 70.13%) and slightly lower than those reported by Gundoğdu et al. (2014) (80 ÷ 97.6%) in Turkish hawthorn berries. The titratable acidity and pH of fresh hawthorn berries were 0.08 and 4.74, respectively, which were in the same range as Gundoğdu et al. (2014) (0.22 and 4.26), but higher than those reported by Türkoğlu et al. (2005) (0.49 and 3.12). The ash content of hawthorn berries was lower (0.66 g per 100 g) compared to Turkish hawthorn (2.28 g per 100 g) (Özcan et al., 2005). Inverted sugars in

studied hawthorn berries were found to be very low compared to hawthorn from Turkey (15.66 g per 100 g) (Gundogdu et al., 2014), and from China (12.32 g per 100 g) (Liu et al., 2010). In this study, the total crude fibre content of hawthorn was determined to average 2.25 g per 100 g, whereas the crude cellulose determined by Özcan et al. (2005) averaged 4.67 g per 100 g. The determined ash content of Kyrgyz hawthorn berries was found to be low compared to the values reported by Mironeasa et al. (2016) and Yalçın-Dokumacı et al. (2021). The sugar/acid ratio of hawthorn berries was found to be 3.36, which is significantly lower than the sugar/acid ratio from China, which was 7.75 for frozen hawthorn (Lou et al., 2020). Cultivated genotypes from Russia and Finland have a sugar/acid ratio of 0.4 ÷ 1.9 (Tiitinen et al., 2005).

Antioxidant Compounds and Antioxidant Activity of Sea Buckthorn and Hawthorn

The measured content of vitamin C, total phenolics, and antioxidant activity of sea buckthorn and hawthorn are given in Table 3. Fresh sea buckthorn contained on average 181.9 mg per 100 g of vitamin C and complied with the findings of other researchers. For example, Tkacz et al. (2019) measured vitamin C content in the range of 61.02 ÷ 158.81 mg per 100 g of fresh weight (f.w.). According to Araya-Farias et al. (2011), fresh sea buckthorn fruits harvested in Quebec (Canada) contain 184.63 mg per 100 g of vitamin C, also from Himalaya was in the same range of 168.3 ÷ 184.0 mg per100 g (Ranjith et al., 2006). In addition, the investigated samples of sea buckthorn contain high amounts of total phenolic compounds (TPC) 386.23 GAE mg per 100 g of f.w. For comparison, sea buckthorn from Canada contains TPC of 175.25 mg per 100 g f.w. (Araya-Farias et al., 2011), berries from Poland feature 55.13 ÷ 115.70 mg of phenolic compounds in 100 g f.w. (Tkacz et al., 2019). According to Sytařová et al. (2020), the total polyphenol content in sea buckthorn ranged from 70 ÷ 362 mg GAE per 100 g (in berries) and 188 ÷ 372 mg GAE per 100 g (in leaves) of fresh matter.

Table 3. Content of vitamin C, total phenolics and antioxidant activity (mean ± SD, n = 3) of sea buckthorn and hawthorn (f. w.)
Çizelge 3. İğde ve alıç (t. a.) C vitamini içeriği, toplam fenolikler ve antioksidan aktivite (ortalama ± SD, n = 3)

Sample Numune	Vitamin C, mg per 100 g C vitamin, mg 100 g'da	Total phenolic content, mg GAE per 100 g Toplam polifenol içeriği, mg GAE 100 g'da	Antioxidant activity by DPPH, IC_{50} µg mL^{-1} DPPH, IC_{50} µg mL^{-1} ile antioksidan aktivite
Hippophae rhamnoides L.	181.88 ± 5.00	386.23 ± 5.00	3.8 ± 0.30
Literature data	61.02 ÷ 158.81 (Tkacz et al., 2019); 184.63 mg per 100 g f. w. (Araya-Farias et al., 2011)	175.25 mg per 100 g f. w. (Araya-Farias et al., 2011); 10.12 ÷ 18.66 mg g^{-1} (Criste et al., 2020)	0.11 ÷ 2.27 (Chaman et al., 2011)
Crataegus songarica	43.34 ± 0.30	669.57 ± 5.00	2.5 ± 0.05
Literature data	20 ÷ 90 mg per 100 g f. w. (García-Mateos et al., 2013) 1.55 ÷ 9.42 mg per 100 g f. w. (Gundogdu et al., 2014)	52 ÷ 558 mg per 100 g f. w. (García-Mateos et al., 2013); 184 ÷248 mg per 100 g f. w. (Edwards et al., 2012)	0.08 ÷ 0.35 µg mL^{-1} (García-Mateos et al., 2013)

In hawthorn, vitamin C content was 43.34 mg 100 g of f.w. For comparison, hawthorn from Mexico has a vitamin C content of 20 ÷ 90 mg per 100 g f.w. (García-Mateos et al., 2013), while hawthorn from Turkey has a significantly lower content of vitamin C 1.55 ÷ 9.42 mg per 100 g f. w. (Gundogdu et al., 2014). The TPC of the hawthorn was 669.57 GAE mg per 100 g, which was higher than that of hawthorn from Mexico 52 ÷ 558 mg 100 g f.w. (García-Mateos et al., 2013). However, Edwards et al. (2012) reported TPC of hawthorn in the range of 184 ÷ 248.18 mg per 100 g f.w. The TPC of dried hawthorn was 3450 mg GAE per 100 g (Tadić et al., 2008), due to the concentration of solids during drying. In hawthorn fruits in this study, the TPC was significantly higher than that of sea buckthorn ($p<0.01$).

A higher content of total polyphenols was also found in barberry 891 mg per 100 g f.w. and rosehip 813 mg per

100 g f.w. from the Kyzyl-Unkur walnut-fruit forest (Smanalieva et al., 2020).

The radical scavenging concentration IC_{50} is the antioxidant concentration at which 50% inhibition of free radical activity is observed. If this concentration is low, the antioxidant capacity or activity is considered to be high. The measured IC_{50} of the investigated sea buckthorn in ethanol extract was 3.8 µg mL^{-1}, and hawthorn was 2.5 µg mL^{-1}. For comparison, the free radical scavenging concentration IC_{50} of sea buckthorn from Pakistan was 0.11 and 2.27 µg mL^{-1} in methanolic and aqueous extracts, respectively (Chaman et al., 2011). In the study of Varshneya et al. (2012), the antioxidant activity in methanol extracts of sea buckthorn pomace from the Himalayas was 179.77 µg mL^{-1}. The IC_{50} of hawthorn (2.5 µg mL^{-1}) was significantly lower than that of sea buckthorn, but higher than that of barberry (1.7 µg mL^{-1}) and rosehip (1.3 µg mL^{-1}) (Smanalieva et al., 2020). García-Mateos et al. (2013) found an inhibitory concentration IC_{50} of 20 hawthorn genotypes in methanolic extracts in the range of 0.08 ÷ 0.35 µg mL^{-1}. It should be noted that the antioxidant extract in methanol exhibits the maximum activity in all antioxidant methods (Chaman et al., 2011).

CONCLUSION

Physical characteristics and chemical composition, as well as bioactive components, such as vitamin C and phenolic compounds of wild sea buckthorn (*Hippophae rhamnoides* L.) and hawthorn (*Crataegus songarica)* of the walnut-fruit forests of Kyrgyzstan, were determined for the first time and compared with all available data from other researchers documented in the scientific literature. The results showed that the moisture content of sea buckthorn is significantly lower than in berries from Europe. In terms of physical characteristics, sea buckthorn and hawthorn can be placed in the medium range for size, weight, and other physical attributes. Hawthorn berries harvested in the walnut-fruit forests of Kyrgyzstan are significantly smaller than berries grown in Turkey. The contents of crude fibre and ash in hawthorn were below other recorded values. This can be explained by the huge difference in the varieties of hawthorn and the climatic conditions of the locations. High vitamin C values were measured in sea buckthorn, however, the total phenolic content and antioxidant activity were higher in hawthorn. Therefore, wild berries are recommended to be consumed in daily nutrition, which requires further research on the development of new recipes and processing technologies. An integrated approach to agroforestry is needed to grow more productive hawthorn and sea buckthorn genotypes.

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Author's Contribution

The plant material belonging to this study was collected by Zh.O. Designing the study and deciding on the appropriate experimental methods were carried out by J.I. and J.S. Experimental analyses of the study were performed by J.I. and Zh.O. Formal and statistical analyzes were performed by J.S. Article draft text was written by J.I., Zh.O., and J.S. The manuscript was finalised with the critical feedback on the study, analysis and article provided by J.I., Zh.O., and J.S.

Conflict of Interest

The authors have no conflicts of interest to declare that they are relevant to the content of this article.

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Çukurova Koşullarında Iyi ve Konvansiyonel Tarım Uygulamalarının Yapıldığı Turunçgil Bahçelerinin Mineral Beslenme Yönünden Karşılaştırılması

Ayfer ALKAN TORUN[1] ✉, Funda PEŞMEN[2]
[1,2] Çukurova Üniversitesi, Ziraat Fakültesi, Toprak Bilimi ve Bitki Besleme Bölümü, Adana, Türkiye
[1]https://orcid.org/0000-0001-5353-8224, [2]https://orcid.org/0000-0001-9857-3613
✉: atorun@cu.edu.tr

ÖZET

Yoğun ve bilinçsiz gübre kullanımından kaynaklanan çevre kirliliğini azaltmak, sürdürebilirliğin sağlanması ve bunun insanlar üzerindeki etkisi güncel konuların başında gelmektedir. Dolayısıyla verim ve kalitede yaşanan düşüşleri önlemek ve üretimin ekonomik olarak sağlanması için, son yıllarda dengeli gübreleme/beslenme programlarını amaçlayan ve konvansiyonel tarıma alternatif olan iyi tarım uygulamaları öne çıkmaktadır. Bu araştırma Çukurova bölgesinde konvansiyonel ve iyi tarım uygulamaları yapılan turunçgil (portakal, limon ve mandarin) bahçelerinin beslenme durumunu ortaya koymak ve karşılaştırmak amacı ile yapılmıştır. Bu amaçla, Adana'nın Karataş ilçesinde iyi tarım uygulamaları (İTU) ve konvansiyonel tarım uygulamalarının (KTU) yapıldığı 80 farklı turunçgil bahçesinden alınan yaprak örneklerinde besin elementi analizleri gerçekleştirilerek mineral beslenme durumları karşılaştırılmıştır. Sonuçlar genel olarak değerlendirildiğinde ağaçların K beslenmesi bakımından büyük oranda yetersiz olduğu buna karşılık N, P, Ca ve Mg açısından önemli bir beslenme probleminin olmadığı görülmüştür. Ayrıca, yaprak örneklerindeki element konsantrasyonlarının KTU yapılan alanlarda kısmen daha yüksek olduğu belirlenmiştir. Çalışmaya konu olan alanlardan alınan yaprak örneklerindeki Fe ve Cu konsantrasyonlarının kritik konsantrasyon sınır değerlerine göre %100'ünün yeterli düzeyde ve üzerinde olduğu buna karşılık Zn konsantrasyonunun iki uygulamada da %85 oranında ve Mn konsantrasyonunun İTU yapılan bahçelerde %20 ve KTU yapılan bahçelerde %25 oranında kritik konsantrasyon sınır değerlerinden düşük olduğu tespit edilmiştir. Sonuç olarak, İTU yapılan alanlarda çiftçilerin gübreleme alışkanlıklarının değişmediği, bitkinin besin ihtiyacının toprak ve bitki analizlerine dayalı olarak belirlenerek uygulamaların buna göre yapılması gerektiği ve bunu teşvik eden uygulamaların sürdürülebilirliğinin önemli olduğu anlaşılmıştır.

Toprak Bilimi

Araştırma Makalesi

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Anahtar Kelimeler
Turunçgil
İyi tarım uygulamaları
Konvansiyonel tarım
Besin elementi
Karşılaştırma

Comparison of Citrus Orchards with Good and Conventional Agricultural Practices in Terms of Mineral Nutrition in Çukurova Conditions

ABSTRACT

Reducing environmental pollution caused by the intensive and unconscious use of fertilizers, ensuring sustainability and its impact on people are among the current issues. In recent years, good agricultural practices aimed balanced nutrition programs and were an alternative to conventional agriculture have become prominent to reduce environmental pollution caused by the intensive and unconscious use of fertilizers, to prevent decreases in yield and quality, and to ensure production economically. This study was carried out to reveal and compare the nutritional status of citrus (orange, lemon, and mandarin) orchards in Çukurova region where conventional and good agricultural practices are applied. For this purpose, leaf samples were taken from 80 different citrus orchards in Karataş district of Adana where good agricultural practices (GAP) and conventional agricultural practices (CFP) were carried out, nutrient analysis was carried out and mineral

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nutrition status was determined comparatively. In general, it was seen that trees were largely insufficient in terms of K, but there was no significant nutritional problem in terms of N, P, Ca, and Mg. In addition, element concentrations in leaf samples were partially higher in the areas where GAP was applied. According to the critical concentration limit values, Fe and Cu concentrations in leaf samples taken from study areas were at a sufficient level and above 100% whereas Zn concentration was 85% in both applications and Mn concentrations were lower than the critical concentration limit values by 20% in orchards with GAP and 25% in orchards with CFP. As a result, it was understood that fertilization habits of the farmers did not change in areas where GAP was applied, nutrient needs of plants should be determined based on soil and plant analyzes and applications should be made accordingly, and sustainability of practices that encourage this is important.

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GİRİŞ

Birleşmiş Milletler tarafından yayımlanan küresel beslenme raporunda 2020 yılında yaklaşık 811 milyon insanın yetersiz beslendiği belirtilmiştir (UN, 2021). Bu durumda nüfus ile besin üretim dengesinin sağlanabilmesi için üretim alanlarını artırma çabası yerine ekilmekte olan alanlardan elde edilen ürün miktarının artırılması amaçlanmalıdır (Çetiner & Tuzla 2005). Bitkilerin büyüme ve gelişmesini teşvik etmek ve birim alandan alınacak verim miktarını arttırmak amacıyla tüm dünyada aşırı bir şekilde kimyasal gübre kullanılmaktadır. Modern tarımda gübreleme yapılırken çoğu zaman bitkiden alınacak maksimum verim hedeflenmekte, ancak bu kimyasalların toprak ve çevreye vereceği zarar göz ardı edilmektedir. Oysa bazen aşırı miktarlarda kullanılan bu kimyasal gübreler her ne kadar bitkilerde verim ve kaliteyi arttırsa da toprak yapısında bozulmalara, toprakta bulunan mikroorganizmaların faaliyetlerinin azalmasına ve biyolojik dengenin bozulmasına neden olmaktadır (Topbaş ve ark., 1998; Vessey, 2003; Sönmez ve ark., 2008). Ekolojik sistemde hatalı uygulamalar sonucu bozulan bu doğal dengeyi yeniden kurmak için, insana ve çevreye dost üretim sistemlerini içeren, esas olarak sentetik kimyasal tarım ilaçları ve gübrelerin kullanımını en aza indirgeyen metotların tarımsal üretimde kullanılmasına ihtiyaç vardır. Bu nedenlerle birçok ülkede konvansiyonel tarımdan çevre dostu üretim tekniklerine geçilmeye başlanmıştır (Zengin, 2007). Çevre dostu üretim tekniklerinde kullanılan alternatiflerden birisi de iyi tarım uygulamalarıdır.

Günümüzde Konvansiyonel Tarım Uygulamaları (KTU)'na alternatif İyi Tarım Uygulamaları (İTU)'nın da hayata geçirilmesiyle beraber özellikle çok yıllık bitkilerin kalite, ekonomi ve çevre ilişkilerinin toplamı değerlendirildiğinde; doğru bitki besleme uygulamaları, üzerinde daha önemle durulması gereken konuların başında gelmektedir. Bitkilerin verim ve kalite açısından gelişiminin en önemli faktörlerinden biri tartışmasız olarak besin elementlerinin dengeli gübreleme ile bitkiye verilmesidir. Obreza ve Morgan (2011), turunçgil gübrelemesinde toprağa uygulanan fosfor (P)'un (P_2O_5 olarak) azot (N)'un yarısı kadar verilmesini, toprağın P'ce çok fakir olması durumunda bu oranın biraz daha artırılabileceğini belirtmiştir. Potasyumun (K_2O olarak) N'un yarısı veya aynı oranda, Mg ise N'un %20'si kadar uygulanmasının yeterli olacağını bildirmişlerdir. Aynı çalışmada, pH>6.5 olan topraklarda Ca uygulamasına ihtiyaç olmadığı, Mn, Cu ve B gübrelemesi için sırasıyla N'un %5; % 2.5 ve % 0.33'ü kadar, Zn, Fe ve molibden (Mo)'in ise bitkide noksanlık görülmesine bağlı olarak uygulanması önerilmiştir. Turunçgillerde N, P ve K uygulama dozlarının meyve verimine, yaşına, turunçgilin türü/çeşidine, dikim sıklığına, toprak tekstürüne ve lokasyona bağlı olarak değişkenlik göstermektedir (Haifa. 2016). Bitkinin sağlıklı gelişim gösterebilmesi için etkili olan diğer faktörler ise; bitkinin türü, çeşidi, toprak özellikleri ve yapılan kültürel uygulamalardır (Jifon ve ark., 2009; Roccuzzo ve ark., 2012; El-Jendoubi ve ark., 2013). Çukurova bölgesindeki tarımsal üretimde önem arz eden ürünler grubu arasında turunçgillerin yer aldığı bilinmektedir. Adana ili Karataş ilçesi % 28'lik turunçgil üretimi ile bölgede üretim deseninde önemli paya sahiptir (Anonim, 2021). Turunçgillerde dengeli ve yeterli beslenmeye engel olan etmenleri belirlemek ve sağlıklı bir gübre programı uygulayabilmek için toprak, yaprak, meyve ve biyokimyasal analizleri kapsayan bütüncül bir yaklaşımla doğru bilgiye ulaşılacağı düşünülmektedir (Robinson, 1980; Gallasch ve ark., 1984; Swietlik, 1996; Srivastava ve ark., 2000;

Srivastava & Singh, 2001).

Literatür çalışmaları genel olarak değerlendirildiğinde dünya genelinde artan nüfusun gıda ihtiyaçlarının karşılanması için yapılan yoğun tarım üretimi beraberinde çevresel sorunları da gündeme getirmiştir. Üretim yapılan alanların doğru kullanılması ve çevreyle etkileşimi gibi konuların ışığında tarım alanlarının daha verimli kullanılması ve tahribatların en aza indirilmesi için çeşitli çalışmalara ihtiyaç duyulmaktadır. Besin elementlerinin yoğun kullanımından kaynaklanan çevre kirliliğinin azaltılması, verim ve en önemlisi de kalitede yaşanan düşüşleri önlemek ve üretimin ekonomik olarak sağlanması ancak dengeli gübreleme programlarının uygulanması ile mümkün olduğu görülmektedir. Türkiye'de önemli turunçgil üretim alanlarına sahip Adana iline bağlı Karataş ilçesinde yukarıda belirtilen çevresel ve ekonomik kaygılar doğrultusunda turunçgil yetiştiriciliğinde İTU ve KTU yapılan alanlarda besin elementi durumu ile ilgili çalışmaların yetersiz olduğu görülmüştür. Bu çalışma, Çukurova bölgesinde konvansiyonel ve iyi tarım uygulamaları yapılan turunçgil bahçelerinin beslenme durumunu ortaya koymak ve karşılaştırmak amacı ile yapılmıştır. Adana'nın Karataş ilçesinde İTU ve KTU yapılan 80 farklı turunçgil (portakal, limon ve mandarin) bahçesinden alınan yaprak örnekleriyle bitki besin elementi konsantrasyonları mikro ve makro elementler olarak karşılaştırılmıştır.

MATERYAL ve METOD

Bu çalışma, Adana ili Karataş ilçesinde "İyi Tarım Uygulamaları" (İTU) ile "Konvansiyonel Tarım Uygulamalarının" (KTU) yapıldığı alanları temsilen seçilen turunçgil üretim bahçelerinden bitki örneklemesi yapılması şeklinde yürütülmüştür. Çalışmada farklı turunçgil türlerinden portakal, limon ve mandarin yetiştiriciliği yapılan 80 adet bahçeden örneklemeler gerçekleştirilmiştir. Söz konusu bahçelerdeki ağaçların yaş aralığı 4 ile 13 yıl arasında değişmektedir. Çalışma alanında belirlenmiş olan en yaygın toprak serileri Oymaklı, Çanakçı, Arıklı, Arpacı, Mürsel, Helvacı ve Gemisure serileridir (Dinç ve ark, 1995). Çalışma alanı örnekleme noktaları da Şekil 1'de görülmektedir.

Şekil 1. Çalışma alanı örnekleme noktaları
Figure 1. Sample area usage points

Örnekleme Yöntemi

Yaprak örnekleri, ağaçların mineral beslenme düzeyini saptamak amacıyla Karataş ilçesinde İTU ve KTU yapıldığı alanları temsilen seçilen bahçelerde yer alan ağaçlardan, portakal, limon ve mandarin türlerinin çeşitliliği sebebiyle olgunlaşma ve hasat süreleri Eylül ve Ocak ayları aralığında hasat olgunluk döneminde, herbir bahçeyi temsil edecek miktarda, ağacın meyvesiz dallarından sürgün uçlarından itibaren üstten 4. ile 6. yaprak olacak şekilde ve ağaçların dört bir tarafından alınarak

gerçekleştirilmiştir (Ertarğın, 2014).

Laboratuvar analizleri

Belirlenen dönemlerde alınan bitki örnekleri polietilen torbalarda laboratuvara getirilerek yıkanmış ve yıkanan bitki örnekleri 48 saat boyunca 70 °C'ye ayarlanmış etüvde kurutulmuştur. Kurutulan örnekler daha sonra agat değirmeninde öğütülmüş ve analize hazır hale gelen örnekler aşağıda belirtilen yöntemlere göre analiz edilmiştir.

Azot (N): Kjeldahl metoduna göre belirlenmiştir (Kacar ve İnal, 2010).

Potasyum (K), Kalsiyum (Ca), Magnezyum (Mg), Fosfor (P), Bakır (Cu), Mangan (Mn), Demir (Fe) ve Çinko (Zn): Kuru yakma yöntemine göre belirlenmiştir. Örnekler 0.2 g tartılarak 550 ˚C'de kül fırınında porselen krozelerde yakılmış, yanan örneklerin üzerine 2 ml 1/3'lük HCl ve 18 ml saf su eklenerek son hacim 20 ml' ye tamamlanıp mavi bant filtre kağıdından süzülmüştür (Kacar ve İnal, 2010). Elde edilen süzüklerde K, Ca, Mg, Cu, Mn, Fe ve Zn konsantrasyonu atomik absorpsiyon spektrofotometre (Varian FS 220) ve P konsantrasyonu ise spektorofotometre cihazında (Shimadzu UV-1800) okunarak belirlenmiştir (Kacar ve İnal, 2010).

BULGULAR ve TARTIŞMA

Adana ili Karataş ilçesi farklı tarımsal üretim (iyi tarım ve konvansiyonel tarım) uygulamalarının yapıldığı turunçgil üretim alanlarının beslenme durumunu ortaya koymak için yürütülen bu çalışmada söz konusu alanlardan hasat döneminde alınan yaprak örneklerine ait minimum, maksimum ve ortalama makro ve mikro besin elementi konsantrasyonları Çizelge 1.'de verilmiştir.

Çalışma alanından alınan bitki örneklerinin N konsantrasyonu İTU yapılan bahçelerde %2.09-%3.13 ve ortalama %2.64 olarak, KTÜ yapılan bahçelerde aynı değerler sırasıyla, % 2.08-% 3.26 ve % 2.66 olduğu görülmüştür. Çalışmaya konu olan örneklerin N konsantrasyonları değerlendirildiğinde; İTU yapılan bahçe örneklerinde %87 oranında, KTU yapılan bahçelerde ise %80 oranının yeterli düzeyin üzerinde olduğu tespit edilmiştir. Azot konsantrasyonu bakımından genel olarak her iki uygulamada ciddi bir beslenme probleminin olmadığı hatta aşırı uygulama yapıldığı görülmüştür (Çizelge 2). Torun ve ark. (2005), turunçgillerde mineral beslenme bozukluklarının (N ve Ca fazlalığı, Mn ve Zn noksanlığı gibi) Akdeniz bölgesinde özellikle Çukurova'da yapılan çalışmalarda belirlendiğini bildirmiştir. Aynı çalışmada, Çukurova bölgesinde turunçgil üretim alanlarında ciddi düzeyde N fazlalığının olduğu bildirilmiştir (Torun ve ark., 2005).

Çizelge 1. Iyi ve Konvansiyonel Tarım Uygulamalarının Yapıldığı Turunçgil Bahçelerinden alınan yaprak örneklerinin makro ve mikro besin elementi ve bunların minimum, maksimum ve ortalama değerleri

Table 1. Macro and micro nutrient elements and their minimum, maximum and average values of leaf samples taken from Citrus Orchards with Good and Conventional Agricultural Practices.

Besin Elementleri	İyi Tarım Uygulaması *Good Agricultural Practice*			Konvansiyonel Tarım Uygulaması *Conventional Agriculture Practice*		
Nutrient Elements	Min.	Max.	Ort.	Min.	Max.	Ort.
N (%)	2.09	3.13	2.64	2.08	3.26	2.66
P (%)	0.12	1.19	0.31	0.14	0.69	0.29
K (%)	0.24	1.76	0.89	0.23	3.32	0.90
Ca (%)	2.48	12.65	6.91	2.88	15.77	6.81
Mg (%)	0.29	1.12	0.66	0.39	1.01	0.64
Cu (mg kg^{-1})	5.7	20.9	10.9	5.8	41.5	10.8
Zn (mg kg^{-1})	7.2	54.4	17.5	7.6	48.7	15.2
Fe (mg kg^{-1})	72.0	170.0	112.0	80.0	194.0	115.0
Mn (mg kg^{-1})	16.0	108.0	40.0	12.0	111.0	39.0

Bitki örneklerinin K konsantrasyonunun İTU yapılan bahçelerde %0.24-%1.76 ve ortalama %0.89, KTÜ yapılan bakçelerde aynı değerler sırasıyla, % 0.23-% 3.32 ve % 0.90 olduğu görülmüştür. Turunçgillerin örnekleme döneminde İTU ve KTU yapılan bahçelerden alınan yaprak örneklerinden elde edilen K konsantrasyonunun sonuçlara göre İTU yapılan bahçelerden alınan örneklerde %75 oranında, KTU yapılan bahçelerden alınan örneklerde ise yaklaşık %80 oranında K bakımından yetersiz olduğu belirlenmiştir (Çizelge 2). Sönmez ve ark. (2014) Batı Akdeniz bölgelerini kapsayan çalışmada; toprakların K konsantrasyonu yeter düzeydeyken, bahçelerin büyük bir kısmının yaprak örneklerinin ise düşük düzeyde K içerdiklerini tespit ettiklerini ve bu durumun bahçelerde K beslenmesi ile ilgili bir problem olduğunu bildirmişlerdir. Yürütülen çalışmada belirlenen K sonuçlarının Torun ve ark. (2005) tarafından yürütülen çalışmayla da uyumlu olduğu görülmüştür. Potasyum turunçgillerde meyve

veriminde, meyve iriliğinde ve kalitesinde oldukça önemli bir elementtir. Potasyum noksanlığının turunçgillerde meyve veriminde düşüşe neden olması beklenen bir sonuçtur. Nitekim Bhargava ve ark. (1993) ağaç başına 0, 200 ve 400 gr K_2O uygulamalarında elde edilen verimlerin sırasıyla 31.9, 36.2 ve 37.5 kg ağaç$^{-1}$ olduğunu saptamışlardır. Aynı çalışmada K 'un verim yanında meyve iriliğinde de artışa yol açtığı belirlenmiştir.

Çalışmaya konu olan örneklerin P konsantrasyonunun İTU yapılan bahçelerde %0.12 ve %1.19 ve ortalama %0.31 olarak, KTÜ yapılan bahçelerde aynı değerler sırasıyla, % 0.14-% 0.69 ve % 0.29 olduğu görülmüştür. Yaprak örneklerinde belirlenen P konsantrasyonuna göre, İTU ve KTU yapılan bahçe örneklerinde noksanlığa rastlanmamıştır. İyi tarım uygulamaları ve KTU yapılan bahçe topraklarında P konsantrasyonu açısından dikkate değer bir fark olmadığı ve genellikle noksanlık olmadığı da tespit edilmiştir. Son yıllarda yapılan çalışmalarda, özellikle topraklarda P'un yetersiz olduğu alanlarda toprağa silisyum (Si) uygulaması ve/veya ortamda Si'un varlığı bitkilerde P alımını arttırdığı bildirilmiştir (Neu ve ark., 2017; Bakır ve ark., 2018). Bu durumun yani Si'un hem bitkiler tarafından P alımını ve hem de bitkiye alınan P'un hareketliliğini/taşınımını arttırması şeklinde ifade edilmektedir. Literatür çalışmalarına bakıldığında birçok bitki deseninde P noksanlığı altında Si uygulamasının ve/veya varlığının bitki gelişimini iyileştirdiği birçok araştırmacı tarafından ortaya konmuştur. Örneğin; domates (Zhang ve ark., 2019), mısır (Owino-Gerroh & Gascho, 2005), çeltik (Pati ve ark., 2016; Hu ve ark., 2018), buğday (Kostic ve ark., 2017; Neu ve ark., 2017) ve patates (Soltani ve ark., 2017; Soratto ve ark., 2019) bitkilerinde görülmüştür.

Örneklenen alanlardan alınan bitkilerin Ca ve Mg konsantrasyonları incelendiğinde; İTU ve KTU yapılan bahçelerdeki örneklerin birbirine yakın oranda Ca ve Mg konsantrasyonu içerdiği ve iki uygulama da bitki beslenmesi bakımından genelde noksanlık olmadığı görülmüştür. Yapılan çalışmalarda turunçgiller için önemli bir besin elementi olan Ca noksanlığına pek rastlanmadığı bildirilmiştir (Chapman, 1968). Embleton ve ark. (1973) yapraklardaki optimum Ca değerinin %3.0-5.5 arasında olduğunu vurgulamıştır. Çolakoğlu (1971), İzmir çevresindeki Satsuma mandarin bahçelerinden alınan yaprak örneklerinin % 87'sinde Ca içeriğinin %3-6 arasında değiştiğini belirtmiştir. Çakmak ve ark. (2003), Çukurova bölgesinde yürüttükleri çalışmada aldıkları 1119 yaprak örneklerinin %58.2'sinin yeter düzeyde %41.5 oranında ise yüksek ve aşırı yüksek düzeyde Ca içerdiğini, P açısından %70.3 oranında yeter konsantrasyona sahipken K'un %52.7 oranda yeter düzeyde olduğunu belirlemişlerdir.

Sonuçlar makro besin elementleri açısından genel olarak değerlendirildiğinde, İTU yapılan bahçelerden alınan yaprak örneklerinde ortalama N konsantrasyonunun %2.64 ve KTU yapılan bahçelerden alınan yaprak örneklerinde %2.66 olarak belirlenmiştir. Söz konusu değerler İTU ve KTU' na göre P, K, Ca ve Mg için sırasıyla ortalama %0.31 ve %0.29, %0.89 ve %0.90, %6.91 ve %6.81 ve %0.66 ve %0.64 olarak belirlenmiştir (Çizelge 1).

Çalışmaya konu olan alanlardan alınan yaprak örneklerindeki mikro element konsantrasyonları Fe ve Cu değerlerinin kritik konsantrasyon sınır değerlerine göre genelde yeterli düzeyde olduğu buna karşılık Zn ve Mn konsantrasyonunun kritik konsantrasyon sınır değerlerinden belirgin düzeyde daha düşük olduğu tespit edilmiştir (Çizelge 2). Turunçgillerin örnekleme döneminde İTU ve KTU yapılan bahçelerden alınan yaprak örneklerinden elde edilen sonuçlara göre İTU yapılan bahçelerden ve KTU yapılan bahçelerden alınan örneklerin tamamının Fe ve Cu konstanrasyonunun yeterli ve fazla olduğu, iki uygulama arasında fark olmadığı görülmüştür (Çizelge 2).

Sönmez ve ark. (2014)'nın toprakların mikro element içeriklerini inceledikleri bir araştırmada Fe, Zn, Mn ve Cu yönünden yeterli durumda olduklarını belirtmişlerdir. Yüksek toprak pH'ı ve kireç dikkate alındığında bitkilerin mikro element beslenmesi açısından problem yaşaması muhtemel görüldüğünü ve bitki analiz sonuçları incelendiğinde, Fe ve Cu'ın yeterli gözükmesine rağmen Mn ve Zn noksanlığı gösteren bahçelerin oldukça fazla olduğunu belirtmişlerdir. Bu durumda yüksek pH, yüksek kireç, düşük organik madde vb. toprak özelliklerinin bitkilerin besin elementi alımında olumsuz etki yaratmasının muhtemel olduğu ve bu faktörlerin iyileştirilmesi ile de başarılı sonuçların elde edilebileceği düşünülmektedir (Turan ve Horuz, 2012).

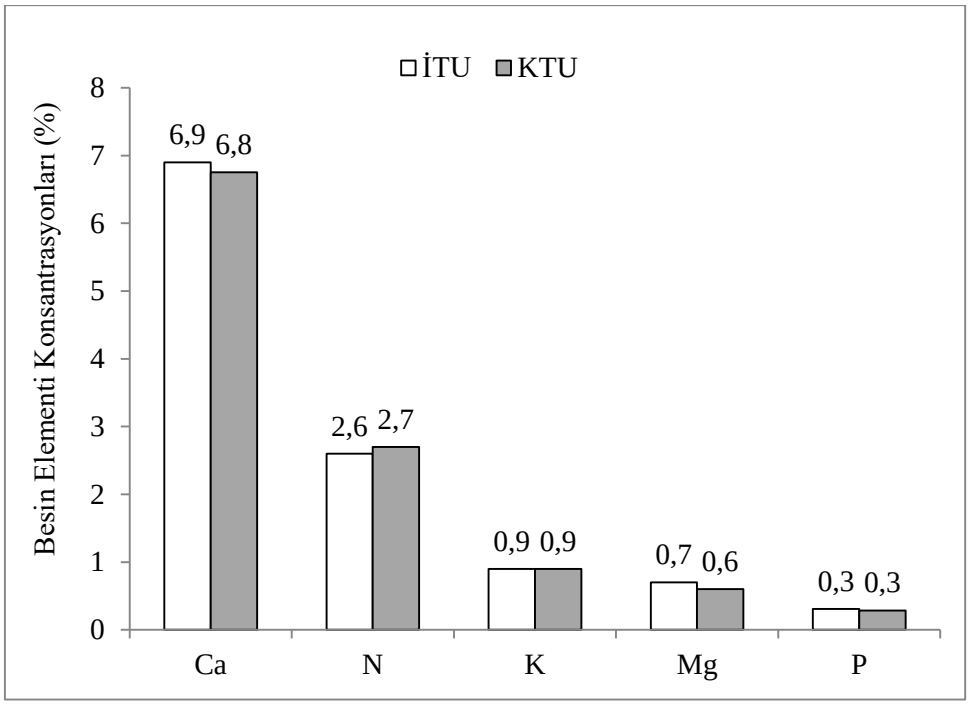
Çalışma alanlarından alınan yaprak örneklerindeki besin elementi konsantrasyonları karşılaştırmalı olarak değerlendirildiğinde hem İTU hemde KTU yapılan alanlardan alınan yaprak örneklernin makro elementlerden miktar olarak en fazla Ca içerdiği bu elementi sırasıyla N, K, Mg ve P'un izlediği belirlenmiştir (Şekil 2). Elementler genel olarak değerlendirildiğinde KTU yapılan alanlardan alınan yaprak örneklerindeki element konsantrasyonlarının İTU yapılan alanlardan alınan örneklerdeki konsantrasyon değerlerine göre kısmen daha yüksek olduğu belirlenmiştir. Söz konusun farkın Ca için %1.5 oranında olduğu görülmektedir (Şekil 2).

Çalışma alanlarından alınan yaprak örneklerindeki mikro elementler değerlendirildiğinde ise bitkilerin miktar olarak en fazla Fe içerdiği bunu sırasıyla Mn, Zn ve Cu'ın takip ettiği görülmüştür. Turunçgil bahçelerinin beslenme durumlarının belirlenmesi için yapılan bir çalışmada, bahçelerden alınan yaprak örneklerinin % 83'ünün yeterli düzeyde Fe içerdiği

Çizelge 2. Iyi ve Konvansiyonel Tarım Uygulamalarının Yapıldığı Turunçgil Bahçelerinden alınan yaprak örneklerine ait bazı besin elementi analiz sonuçlarının sınır değerlerine göre sınıflandırılması

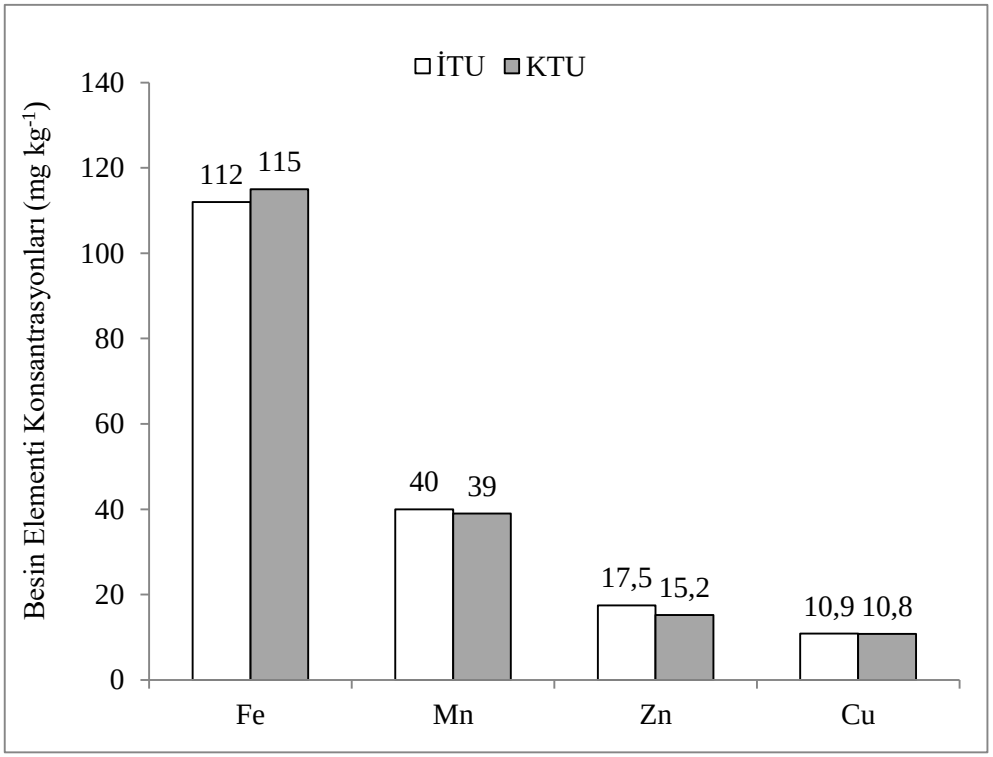
Table 2. Classification of leaf samples taken from Citrus Orchards with Good and Conventional Agricultural Practices according to the limit values of some nutrient analysis results

Besin Elementi	Sınır Değerleri	Değerlendirme	İyi Tarım		Konvensiyonel Tarım		Genel Toplam	
			Good Agriculture		*Conventional agriculture*		Total	
Nutrion Element	*Reference Values*	*Evaluation*	Örnek Sayısı / *Number of Samples*	%	Örnek Sayısı / *Number of Samples*	%	Örnek Sayısı / *Number of Samples*	%
N (%)	<2.20	Çok az	3	7.5	2	5.0	5	6.3
	2.20-2.40	Az	2	5.0	6	15.0	8	10.0
	2.50-2.70	Yeterli	18	45.0	14	35.0	32	40.0
	2.80-3.00	Fazla	13	32.5	14	35.0	27	33.8
	>3.00	Çok fazla	4	10.0	4	10.0	8	10.0
P (%)	<0.09	Çok az	-	-	-	-		-
	0.09-0.11	Az	-	-	-	-	-	-
	0.12-0.16	Yeterli	4	10.0	7	17.5	11	13.7
	0.17-0.30	Fazla	23	57.5	19	47.5	42	52.5
	>0.30	Çok fazla	13	32.5	14	35.0	27	33.7
K (%)	< 0.70	Çok az	13	32.5	15	37.5	28	35.0
	0.70-1.10	Az	17	42.5	17	42.5	34	42.5
	1.20-1.70	Yeterli	8	20.0	7	17.5	15	18.8
	1.80-2.40	Fazla	2	5.0	-	-	2	2.5
	>2.40	Çok fazla	-	-	1	-	1	1.3
Ca (%)	<1.50	Çok az	-	-	-	-	-	-
	1.50-2.90	Az	1	2.5	2	5.0	3	3.8
	3.00-4.90	Yeterli	5	12.5	5	12.5	10	12.5
	5.00-7.00	Fazla	15	37.5	16	40.0	31	38.8
	>7.00	Çok fazla	19	47.5	17	42.5	36	45.0
Mg (%)	<0.20	Çok az	-	-	-	-	-	-
	0.20-0.29	Az	1	2.5	-	-	1	1.3
	0.30-0.49	Yeterli	5	12.5	6	15.0	11	13.8
	0.50-0.70	Fazla	18	45.0	21	52.5	39	48.8
	>0.70	Çok fazla	16	40.0	13	32.5	29	36.3
Fe (mg kg^{-1})	<35	Çok az	-	-	-	-	-	-
	35-59	Az	-	-	-	-	-	-
	60-120	Yeterli	25	62.5	25	62.5	50	62.5
	121-200	Fazla	15	37.5	15	37.5	30	37.5
	>200	Çok fazla	-	-	-	-	-	-
Cu (mg kg^{-1})	<3	Çok az	-	-	-	-	-	-
	3-4	Az	-	-	-	-	-	-
	5-16	Yeterli	36	90.0	37	92.5	73	91.3
	17-20	Fazla	3	7.5	2	5.0	5	6.3
	>20	Çok fazla	1	2.5	1	2.5	2	2.5
Zn (mg kg^{-1})	<18	Çok az	29	72.5	31	77.5	60	75.0
	18-24	Az	5	12.5	3	7.5	8	10.0
	25-100	Yeterli	6	15.0	6	15.0	12	15.0
	101-300	Fazla	-	-	-	-	-	-
	>300	Çok fazla	-	-	-	-	-	-
Mn (mg kg^{-1})	<18	Çok az	1	2.5	2	5.0	3	3.8
	18-24	Az	7	17.5	8	20.0	15	18.8
	25-100	Yeterli	31	77.5	27	67.5	58	72.5
	101-300	Fazla	1	2.5	3	7.5	4	5.0
	>300	Çok fazla	-	-	-	-	-	-



Şekil 2. Iyi ve Konvansiyonel Tarım Uygulamalarının Yapıldığı Turunçgil Bahçelerinden alınan yaprak örneklerinin makro element konsantrasyonları

Figure1. Macro element concentrations of leaf samples taken from Citrus Orchards with Good and Conventional Agricultural Practices



Şekil 3. Iyi ve Konvansiyonel Tarım Uygulamalarının Yapıldığı Turunçgil Bahçelerinden alınan yaprak örneklerinin mikro element konsantrasyonları

Figure2. Micro element concentrations of leaf samples taken from Citrus Orchards with Good and Conventional Agricultural Practices

belirlenmiştir (Pınar & Arslan, 2007). Söz konusu sonuçlar Sönmez ve ark. (2014) tarafından yürütülen çalışmada yaprak örneklerinin Fe analiz sonuçlarının turunçgil bahçelerinin % 1.6'sının düşük, % 79.7'sinin

yeterli ve % 18.7'sinin yüksek düzeyde Fe konsantrasyon sonucuyla da uyumludur.

Örneklenen alanlardan alınan bitkilerdeki mikro besin elementleri uygulamalar bazında karşılaştırmalı olarak değerlendirildiğinde İTU ve KTU yapılan bahçelerden elde edilen ortalama mikro besin elementi konsantrasyonlarının birbirine benzer olduğu görülmüştür (Şekil 3).

SONUÇ ve ÖNERİLER

Karataş-Adana bölgesinde İyi tarım ve Geleneksel tarım uygulamaları yapılan alanlardaki bitkilerin yeşil aksam besin elementi konsantrasyonları açısından önemli bir değişim olmadığı saptanmıştır. Bu bağlamda bahçelerden alınan yaprak örneklerinden elde edilen sonuçlara göre ağaçların K bakımından her iki uygulamada da büyük oranda yetersiz olduğu buna karşılık N, P, Ca ve Mg açısından ciddi bir beslenme probleminin olmadığı görülmüştür. Ayrıca her iki uygulamada bitkide eksikliği tespit edilen Zn ve Mn gibi besin elementlerinin alımını artırmak için topraktan veya yapraktan uygulama yapılabileceği gibi, toprakta besin elementi alımını muhtemelen olumsuz etkileyecek (yüksek pH, yüksek kireç, düşük organik madde vb.) faktörlerin düzeltilmesi ile başarılı sonuçların elde edilebileceği düşünülmektedir. Turunçgillerde dengeli ve yeterli beslenmeye yol açan etmenleri belirlemek ve sağlıklı bir gübre programı uygulayabilmek için toprak, yaprak, meyve ve biyokimyasal analizleri kapsayan bir yaklaşım gerektirmektedir. Dolayısıyla yaprak örneklerinin yanı sıra insan beslenme zincirine giren meyvede besin kalitesinin de araştırılması bütüncül bir değerlendirme için önemli olacaktır. Besin element girdisi ve çıktısının takibini öngören İTU gibi tarımsal üretim sistemlerinde doğru uygulama ve bu üretim sisteminin içinde yer alan kontrol mekanizmalarının doğru çalışması/işletilmesinin önemli olduğu ortaya çıkmıştır.

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Yazarlar makaleye eşit oranda katkı sağlamış olduklarını beyan eder.

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Influence of Basic Drying Techniques on Color, Protein and Mineral Composition of Coriander Leaves

İlknur ALİBAŞ[1] ✉, Aslıhan YILMAZ[1]
[1]Bursa Uludağ Üniversitesi, Ziraat Fakültesi, Biyosistem Mühendisliği Bölümü, 16059, Nilüfer / Bursa
[1]https://orcid.org/0000-0002-1898-8390, [2]https://orcid.org/0000-0002-4913-905X
✉: ialibas@uludag.edu.tr

ABSTRACT
Coriander leaves were weighed at 20 ± 0.02 g and dried with natural drying at shade, convective drying at 50°C and 1 m s^{-1} air velocity, and microwave drying at 200 and 800 W. The drying periods were led 4680, 630, 85, and 16.50 minutes for natural, 50°C, 200 W, and 800 W, respectively. Whereas energy consumption was not recorded in natural drying, energy consumption at 50°C, 200 W, and 800 W was recorded as 10.290, 0.283, and 0.220 kWh, respectively. The closest results to fresh leaves in terms of color parameters were measured at 800 W, followed by 200 W. Similarly, at 800 and 200 W, the most successful results were obtained with regard to calcium, magnesium, and iron. Also, it was analyzed that the chlorophyll content, protein, phosphorus, potassium, and zinc were preserved at the maximum level in the 800 W microwave drying method. Although all drying methods cause similar reductions for sodium, it was determined that manganese was well preserved at 200 W. Among the dried samples, the highest copper level was analyzed in natural drying and microwave drying at 800 W. To sum up, the most convenient drying technique for coriander leaves was 800 W in terms of drying and quality parameters.

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Temel Kurutma Tekniklerinin Kişniş Yaprağının Renk, Protein ve Mineral İçeriği Üzerine Etkisi

ÖZET
Kişniş yaprakları 20 ± 0.02 g tartılmış ve gölgede doğal kurutma, 50°C ve 1 m s^{-1} hava hızında konvektif kurutma ve 200 ve 800 W'da mikrodalga kurutma yöntemleri ile kurutulmuştur. Kurutma süreleri doğal, 50°C, 200 W ve 800 W için sırasıyla 4680, 630, 85 ve 16.50 dakika olarak ölçülmüştür. Doğal kurutmada herhangi bir enerji tüketimi kaydedilmezken, 50°C, 200 W ve 800 W'ta enerji tüketimi sırasıyla 10.290, 0.283 ve 0.220 kWh olarak hesaplanmıştır. Renk parametreleri bakımından taze örneğe en yakın sonuçlar 800 W'ta, ardından 200 W'ta kurutulan örneklerde ölçülmüştür. Benzer şekilde 800 ve 200 W'ta kalsiyum, magnezyum ve demir açısından en başarılı sonuçlar elde edilmiştir. Ayrıca, 800 W mikrodalga kurutma yönteminde kurutulan örneklerde klorofil içeriği, protein, fosfor, potasyum ve çinkonun maksimum düzeyde korunduğu tespit edilmiştir. Tüm kurutma yöntemleri sodyum için benzer azalmalara neden olsa da, manganın 200W'da en yüksek düzeyde korunduğu belirlenmiştir. Kurutulan örnekler arasında en yüksek bakır seviyesi doğal ve 800 W mikrodalga kurutma yönteminde kurutulan örneklerde analiz edilmiştir. Özetle, kişniş yaprakları için kurutma ve kalite parametreleri açısından en uygun kurutma tekniği 800 W olmuştur.

Makale Konusu
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INTRODUCTION

Coriander is an aromatic plant and annual herbaceous species from the Umbelliferae family (Sarimeseli, 2011). The high amount of fiber, protein, vitamins A and C, iron, zinc, calcium, and phosphorus in coriander is significant for human nutrition (Yilmaz & Alibas, 2017).

One of the most effective ways of four-season consuming coriander, which has an important place and benefit in human nutrition, is drying (Hihat et al., 2017). With drying, the water, which is in high proportions in the material, is significantly removed; thus, transportation, storage, and packaging become easier (Alibas et al., 2021; Boyar et al., 2022).

The most common and elderly drying method known for spice production is the natural drying method, which is done by laying in the shade (Yilmaz & Alibas, 2017). Although it seems like an important advantage that there is no energy consumption in the natural drying method, the method has also drawbacks such as long drying period, large drying area, high labor requirement, mold and aflatoxin growth in the product due to high moisture content in closed areas, and being vulnerable to rodents and insects of products. Therefore, more hygienic and technological methods, such as convective and microwave drying, are being developed today (Hihat et al., 2017).

On the other hand, it is an indisputable fact that convective drying, which is the most broadly way for drying almost all kinds of agricultural products, causes excessive loss of color, odor, and minerals in spices due to the long drying time (Yilmaz & Alibas, 2017; Yilmaz et al., 2021). Therefore, microwave drying is a drying method used as an alternative to the natural drying method for drying aromatic plants because of many advantages such as short drying times, low energy requirement, uniform drying, better preservation of vitamins, protein, and nutrients, and preventing color and odor losses (Hihat et al., 2017; Yilmaz & Alibas, 2017).

Some studies have been found in the literature about drying coriander leaves. However, it has been observed that there is no study cooperatively examining the energy consumption, thermal properties, color parameters, chlorophyll content, protein, and nutritional elements of dried coriander leaves. The main focus of the study is this deficiency in the literature.

The objectives of the study were i) drying coriander with convective, microwave, and natural dehydration techniques, ii) comparing of energy consumption of different drying methods, iii) detecting the color parameters, chlorophyll content, protein, and minerals of raw and dried coriander leaves, iv) determining the most appropriable dehydration technique regarding both drying and quality parameters.

MATERIAL and METHOD

Drying Material

Coriander (*Coriandrum sativum* L.) was harvested from the garden of a producer in Nilüfer, Bursa, Türkiye. Right after the harvesting process, the products were brought to the laboratory without losing time, and they, surrounded by wet pillows, were stored in a humidity-controlled cooler at +4°C until the drying process so that they did not lose their initial moisture content. All drying processes, except natural drying, were completed within 24 hours. Also, 20 ± 0.02 g samples were used in each drying process, and the leaves were selected from healthy and representative of the average.

Drying Systems

Convective and microwave drying processes were carried out using a combined dryer (Electrolux, EVY7800AAX, U.S.A.) whose operating circumstances were 3000 W, 50 Hz, and 230 ± 10 V. While the convective drying mode of the dryer, whose drying area was 410 x 329 mm, operates with a sensitivity of 5°C between 30 and 300°C, the microwave mode runs at ten output powers between 100 and 1000 W.

Natural drying was fulfilled in a shaded room without sunlight at 25°C and 60% ±5 relative humidity. The weight reduction was measured manually every three hours during drying.

In the study, 50°C, broadly used to dehydrate fruits and vegetables, especially spices, was used to represent convective drying. Also, an air velocity of 1 m s^{-1} in convective drying ensured hot air circulation in the dryer. On the other hand, the microwave-drying process was symbolized by low output power at 200 W and high output power at 800 W to preserve the nutrients, especially color, in spices and green leafy products. The time-dependent weight loss data were collected instantly with a data logger located under the drying area of the dryer for both drying techniques.

In both drying methods, the dryer's energy consumption was measured with the help of a mono-phase electric counter, and the difference between the initial and final readings on the electric counter was calculated by the total energy consumption.

Mathematical Modeling of Drying Data

Coriander leaves were kept in a dry air sterilizer at 105°C for 24 hours to determine the initial moisture content. Based on the initial and final weights, the moisture content of the material with respect to the dry basis was determined with the following equation (Eq 1).

$$MC_{db} = (W_{total} - W_{dry\ matter}) / (W_{dry\ matter}) = (W_{water}) / (W_{dry\ matter}) \quad (1)$$

where: MC_{db} is moisture content on the dry basis (kgwater $kg^{-1}_{dry\ matter}$), W_{total} is the initial weight (g), W_{water} is the water weight (g), and W_{dry} matter is the dry weight of the leaves (g).

The drying rate of the leaves was detected by considering the next formulation (Eq 2):

$$DR = (M_{t+dt} - M_t) / d_t \quad (2)$$

where: DR is the drying rate ($kg_{water}\ kg^{-1}_{dry\ matter}\ min^{-1}$), $Mt+dt$ is the moisture content at $t+dt$ time ($kg_{water}\ kg^{-1}_{dry\ matter}$), and dt is the drying time at t time (min).

The time-dependent moisture ratios of coriander leaves were found with the help of the following equation (Eq 3):

$$MR = (M - M_e) / (M_o - M_e) \quad (3)$$

where: MR is the moisture ratio, M is the moisture content at any time ($kg_{water}\ kg^{-1}_{dry\ matter}$), M_o is the initial moisture content ($kg_{water}\ kg^{-1}_{dry\ matter}$), and M_e is the equilibrium moisture content ($kg_{water}\ kg^{-1}_{dry\ matter}$). Since all drying processes were carried out under controlled conditions where the relative humidity did not change, this value was accepted as zero (Yilmaz & Alibas, 2017).

Experimental moisture ratio data were converted into prediction ones by means of the NLREG 6.2 statistical program using five different thin-layer drying equations in Table 1. The coefficient of determination (R^2), standard errors (SEE), root mean square errors ($RMSE$), and chi-square (χ^2) of the estimation were calculated by the same program. The R^2 was used as the main criterion in the selection of the model closest to the experimental data. Also, SEE, $RMSE$, and χ^2 values were evaluated as secondary criteria in the selection of equations with the same R^2 value, respectively (Alibas et al., 2020).

Thermal Properties

Thermal properties such as specific heat, thermal conductivity, thermal diffusivity, and thermal effusivity of coriander leaves as a function of moisture content regarding the dry base were detected by calculating. The specific heat and thermal conductivity of different drying techniques were calculated by Equations 4 and 5, respectively (Alibas & Yilmaz, 2022).

$$Cp = 837 + 3348\ (M/(1+M)) \quad (4)$$

$$k = 0.49 - 0.44 \exp(-0.206\ M) \quad (5)$$

where: Cp is the specific heat of the leaves ($J\ kg^{-1}\ K^{-1}$) and k is the thermal conductivity ($Wm^{-1}\ K^{-1}$).

The thermal diffusivity and thermal effusivity of the drying processes were calculated using the densities of the leaves. Density, thermal diffusivity, and thermal diffusivity values were determined using the following equations (Eq 6, 7, and 8), respectively (Alibas & Yilmaz, 2022).

$$\rho = 147.95\ (M / M_0) + 691.46 \quad (6)$$

$$\alpha = k / (\rho\ Cp) \quad (7)$$

$$e = \sqrt{(k\ \rho\ Cp)} \quad (8)$$

where: ρ is the density of the leaves ($kg\ m^{-3}$), α is the thermal diffusivity ($m^2\ s^{-1}$), and e is the thermal effusivity ($W\ m^{0.5}\ m^{-2}\ K^{-1}$).

Color Parameters

The color parameters of coriander leaves were determined with the help of a colorimeter (Konica, Minolta CR10, Japan) running according to the CIELAB principle. According to the method, L^*, a^* b^*, C, and $a°$ are represented the brightness, greenness, yellowness, chroma, and hue angles, respectively (Alibas & Yilmaz, 2022).

On the other hand, some indicators, known as total color difference (ΔE), browning index (BI), and whitening index (WI), are used to express the color changes that occur in the leaves during drying. Accordingly, ΔE, x, BI, and WI were determined using Equations 9, 10, 11, and 12, respectively.

$$\Delta E = \sqrt{(L_f - L_d)^2 + (a_f - a_d)^2 + (b_f - b_d)^2} \quad (9)$$

$$x = (a + (1.75\ L)) / [(5.645\ L) + (a - (3.012\ b))] \quad (10)$$

$$BI = [100\ (x - 0.31)] / 0.17 \quad (11)$$

$$WI = 100 - \sqrt{((100 - L)^2 + a^2 + b^2)} \quad (12)$$

Total Protein and Nutrients

Total protein analysis was carried out as to the Kjeldahl method (Alibas et al., 2021). The Kjeldahl method takes place in three stages: combustion, distillation, and titration. In the first step of the combustion process, 5 g of combustion salt and 15 ml of H_2SO_4 with 99% purity were added to the 0.2 - 0.5 g thoroughly crushed sample taken into the combustion tube. The combustion tubes, which were taken to the combustion block by adding boiling stone, were first burned at 200 - 250°C for 30 minutes and then at 350 - 380°C for 60 minutes. During the burning process, the samples were checked, and the burning process was continued until the sample colors turned blue-green. During the combustion stage, the organic components in the samples reacted with the oxygen in the chemical mixtures, and the organic components were decomposed. At the end of the combustion, 100 ml of distilled water was added to the samples in the combustion tubes and allowed to cool.

In the second stage, the distillation process, the cooled solutions in the combustion tubes were placed in the distillation device, and 4-5 ml of 40% sodium hydroxide

was added to the solution automatically. The distillation process was continued until a total of 100 - 150 ml of distillate (ammonium borate) was collected. After the distillation process was completed, the tip of the reflux cooler was washed with distilled water and cleaned before each experiment.

In the titration step, which is the last step, the ammonium borate solution was neutralized by titration with 0.1 M H_2SO_4 solution using an automatic burette. The total nitrogen (*N*) amount was calculated from the amount of acid consumed for the titrated solution. The total protein content was determined by multiplying the calculated total nitrogen amount with the protein conversion coefficient.

Mineral contents of coriander leaves were also determined by the Kacar method. In the analysis, a nitric-perchloric acid mixture was used and all macro and micronutrients except boron were measured. A solution was prepared in an Erlenmeyer by adding a nitric-perchloric acid mixture (4:1 v/v) to the samples with a mass of 0.2 - 0.5 g. The mixture was homogenized by shaking the erlenmeyer slightly. The upper surface of the Erlenmeyer was closed with a watch glass and left to stand in the fume hood for 20-30 minutes. After waiting, the samples were rested in a water bath at 30-40°C for 24 hours. At the end of this period, the samples were taken to the heater tray in the fume hood, and the temperature of the heater tray was gradually increased up to 200°C. Thus, most of the nitric acid in the solution was removed, and the solution got a light yellow color. The heating process gradually increased until a light yellow color appeared. After the intensive white smoke of perchloric acid completely covered the Erlenmeyer, the wet burning process was continued for at least 30 minutes. When the intensive smoke disappeared and the 1 ml colorless liquid remaining in the Erlenmeyer became clear, the solution was taken from the heating plate and left to cool. After the solution was cooled to room temperature, it was washed with distilled water and taken into a 100 ml flask. The solution was made ready for measuring by completing the volume of the flask with distilled water and shaking it well. Phosphorus contents of the samples were measured with a UV-VIS spectrophotometer (PG Instrument, T60 Split Beam UV/VIS, UK); however, a flame photometer (Eppendorf, Elex 6361, Germany) was used for sodium, potassium, and calcium. Also, iron, copper, zinc, and manganese were analyzed with an ICP-OES (Perkin Elmer, OPTIMA 2100 DV, USA) (Alibas et al., 2020).

Data Analysis

The averages, standard errors of estimated, and LSD analyses of the data obtained in the study were analyzed with the JMP Pro 14 statistical program. All drying and quality parameters were performed in triplicate except for the color measurements performed in 20 replications. The time-dependent moisture content obtained from the experimental drying data was modeled through the NLREG 6.2 statistical program using five thin-layer drying models presented in Table 1. Drying constants and coefficients in these thin-layer drying models were determined with the help of the same statistical program.

Table 1. Thin-layer drying equations used in modeling experimental data (Alibas & Yilmaz, 2022)
Çizelge 1. Deneysel verilerin modellenmesinde kullanılan ince tabaka kurutma denklemleri (Alibas & Yilmaz, 2022)

Model Number	Equation number	Equation
1	Page	$MR = \exp(-kt^n)$
2	Logaritmic	$MR = a\exp(-kt) + c$
3	Two-term exponential	$MR = a\exp(-kt) + (1-a)\exp(-kat)$
4	Midilli et al.	$MR = a\exp(-kt^n) + bt$
5	Alibas	$MR = a\exp\big((-kt^n) + (bt)\big) + g$

MR, moisture ratio; *a, b, g, n,* drying coefficients; *t*, drying period, min; *k,* drying constant, min^{-1}.

RESULTS and DISCUSSION

Drying Kinetics of Coriander Leaves

Figure 1 shows the time-dependent moisture content of coriander leaves dried by natural, convective, and microwave drying methods. According to the figure, the longest drying time was recorded in natural drying (4680 minutes), while the shortest drying period was found at 800 W (16.50 minutes). Microwave drying technique at 800 W, determined as the shortest drying period among all drying techniques, took 283.64, 38.18, and 5.15 times shorter than natural drying, convective drying at 50°C, and microwave drying methods at 200 W, respectively. Also, the drying period decreased with the increase in microwave output power. Sarimeseli (2011) dried coriander leaves via microwave drying at 180, 360, 540, 720, and 900 W. They noted that 900 W took 3.5, 2.63, 1.75, and 1.25 times shorter than 180, 360, 540, and 720 W, respectively. Hihat et al. (2017) dried coriander leaves by convective drying at 40, 60, 80, 100, and 120°C and microwave drying at 100, 300, 500, 700, and 900 W. Also, they determined that the drying time decreased with increasing both the drying temperature and the microwave output power. Silva et al. (2008) dried coriander leaves in a convective drying technique at 50°C. They obtained findings parallel to our study in terms of drying time. In research in which coriander leaves were dried at 55°C by convective drying method, Venkanna et al. (2019) highlighted

that the drying time was 2.63 times shorter than the finding we recorded at 50°C. Yilmaz & Alibas (2017) dried coriander leaves at 50°C and noted that the drying period was 3.71 times shorter than our findings.

Figure 2 includes the drying rates of coriander leaves dried using natural, convective at 50°C, and microwave drying at 200 and 800 W. According to the figure, the lowest average drying rate was determined in the natural drying method with 0.00034 kg_{water} kg_{DM}^{-1} min^{-1}, but the highest one in the microwave drying method at 800 W with 0.24933 kg_{water} kg_{DM}^{-1} min^{-1}. The highest average drying rate at 800 W, determined as the shortest drying time, was 40.15 and 5.97 times higher than convective drying at 50°C and microwave drying at 200 W, respectively. Also, the drying rate determined in the natural drying method was 18.26 and 122.84 times lower than 50°C and 200 W. The average drying rate increased with the increase in microwave output power. Similar findings were detected by some researchers (Sarimeseli, 2011; Yilmaz & Alibas, 2017; Mouhoubi et al., 2022).

Modeling of Drying Data

Figure 3 gives the time-dependent experimental and estimated moisture contents of coriander leaves dried by natural, convective, and microwave drying methods. Accordingly, we observed that 78% of the separable moisture ratio evaporated away from the product in the 420th minute of the drying period at 50°C. However, we determined that the separable moisture ratio removed from the coriander leaves at the same time in the natural drying trials was only 5%. Similarly, 23.10% of the separable moisture was removed from the product in the tenth minute of the drying time at 200 W. Strikingly, in the same period, 76.97% of the separable moisture evaporated from the product at 800 W. Similar results were found in similar studies in the literature (Lutovska et al. 2016; Yilmaz & Alibas, 2017; Alibas et al. 2021).

Table 2 shows the statistical parameters, namely the coefficient of determination (R^2), the standard errors of the estimated (*SEE*), the root mean square error (*RMSE*), and the chi-square (χ^2), as well as drying constants and coefficients between the experimental drying data and the predicted ones obtained through five different thin-layer drying equations. Accordingly, the closest estimation results to the experimental ones in natural drying, microwave drying at 800 W, and convective drying at 50°C were obtained with the Midilli et al. equation. On the other hand, the closest estimated results to the experimental ones at 200 W were determined by the Alibas' equation. Similarly, in a study in which coriander leaves were dried at 50°C by convective drying method, Silva et al. (2008) obtained the closest approximation to the experimental results with the Midilli et al. equation. In an investigation in which coriander leaves were dried at 100 W, Yilmaz & Alibas (2017) determined the closest estimate to the experimental data with the Alibas' model. Similarly, Aral & Beşe (2016) stated that the closest estimation data to the experimental results of hawthorn fruit dried at 50°C were obtained with the Midilli et al. equation. In a study in which amaranth leaves were dried at 200 W, Mujaffar & Loy (2016) found the closest approximation to experimental MR with the Alibas' model.

With the increase of microwave output power, the drying constant (k) of the most successful model also increased. The lowest drying constant was obtained in natural drying and microwave drying at 200 W.

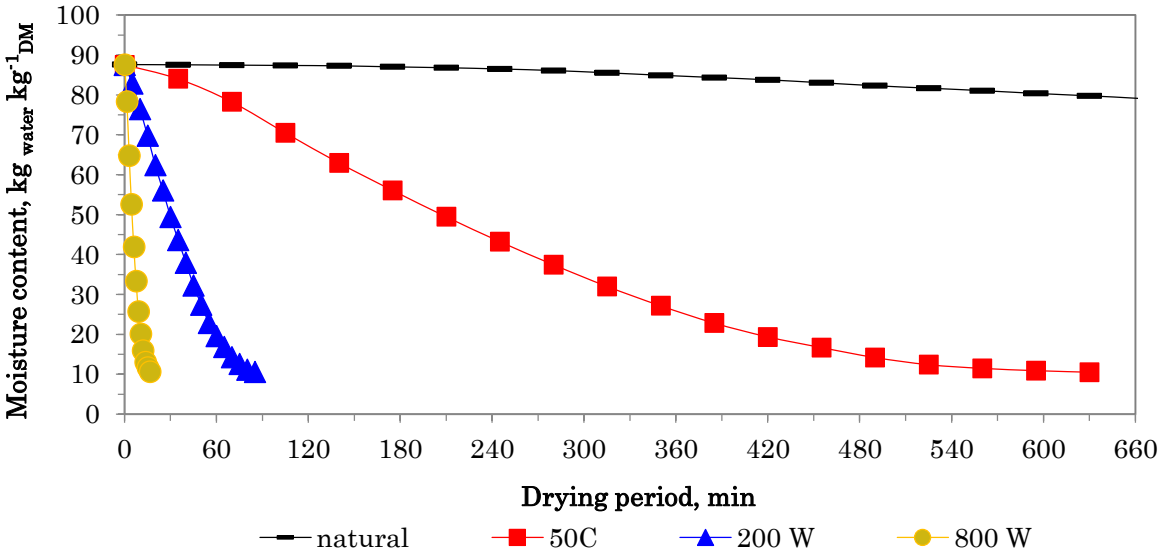

Figure 1. The time-dependent moisture content of coriander leaves dried by natural, convective, and microwave drying methods

Şekil 1. Doğal, konvektif ve mikrodalga kurutma yöntemleriyle kurutulan kişniş yapraklarının zamana bağlı nem içeriği

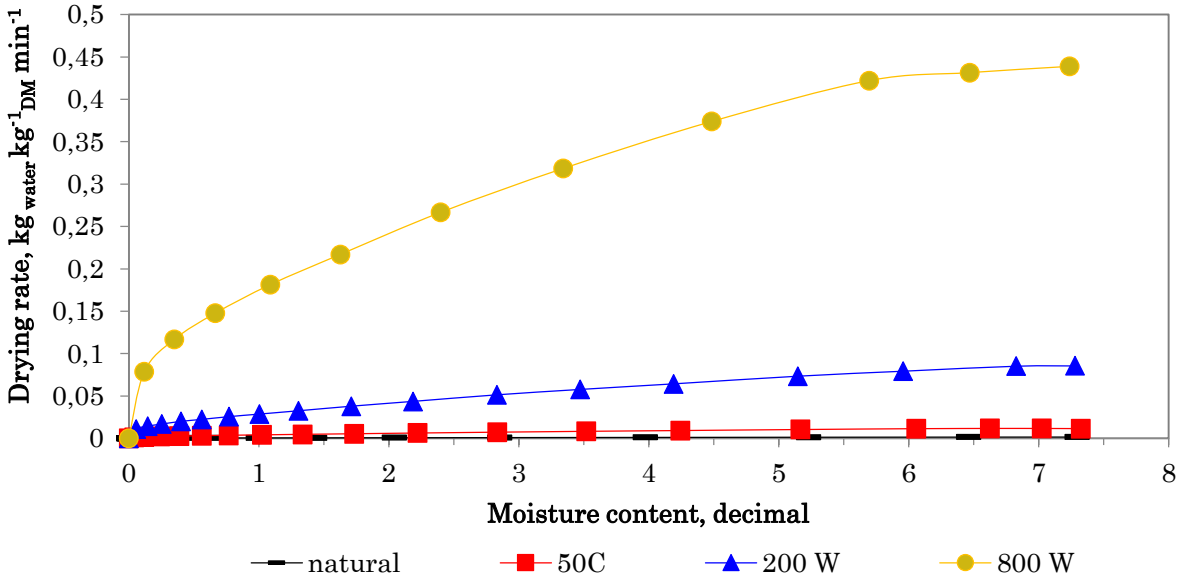

Figure 2. The drying rates of coriander leaves dried by natural, convective, and microwave drying methods
Şekil 2. Doğal, konvektif ve mikrodalga kurutma yöntemleriyle kurutulan kişniş yapraklarının kuruma oranları

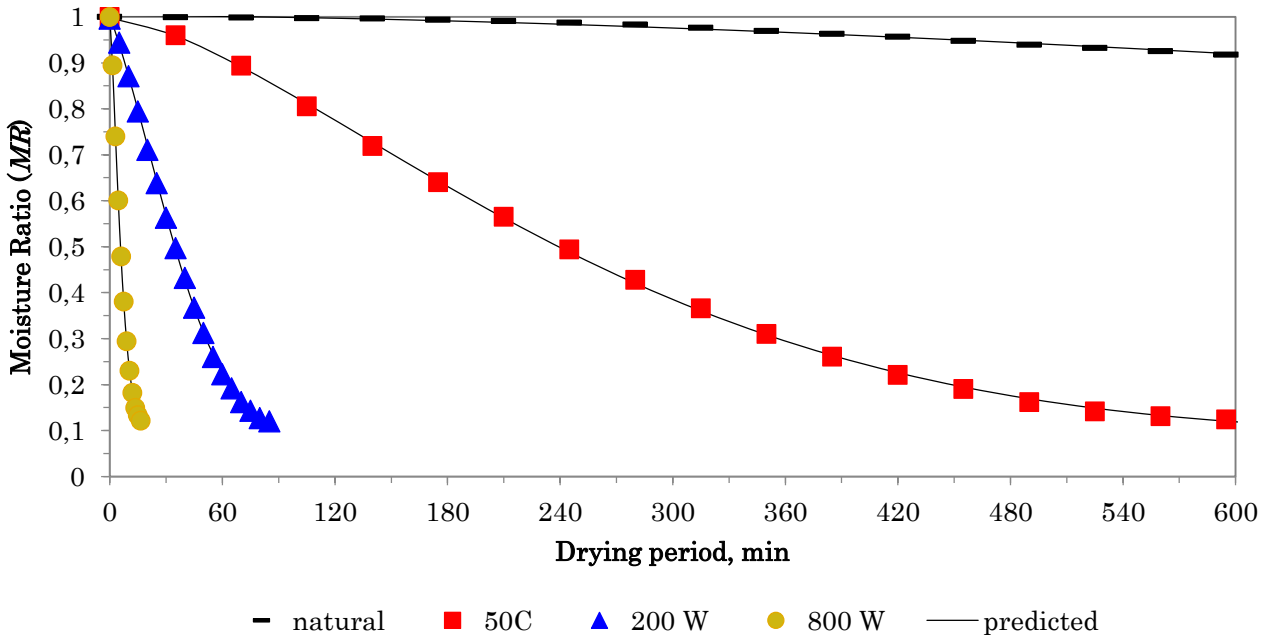

Figure 3. The moisture ratios of coriander leaves dried by natural, convective, and microwave drying methods
Şekil 3. Doğal, konvektif ve mikrodalga kurutma yöntemleriyle kurutulan kişniş yapraklarının nem oranları

Total Energy Consumption

Table 3 reflects the total energy consumption of natural, convective, and microwave drying methods. We noted that convective drying causes considerably higher energy consumption than microwave drying. Accordingly, we found that the energy consumption recorded in the convective drying method at 50°C was 36.36 and 46.77 times higher than at 200 and 800 W, respectively. Also, the total energy consumption decreased with the increase in microwave output power. Wang et al. (2004) dried potato slices with different microwave power densities and found that energy consumption decreased with increasing microwave power density. In an investigation in which tomato slices were dried with different microwave output powers, Çelen & Kahveci (2013) reported that the total energy consumption increased with the decrease in microwave output power. In a study on drying basil leaves, Alibas et al. (2021) emphasized that the energy consumption recorded in the convective drying method at 50°C was quite high

compared to the microwave drying method. Alibas & Yilmaz (2022) dried orange slices with microwave and convection drying methods and found that the total energy consumption decreased with the increase in microwave output power. Also, they determined that the energy consumption was at the maximum level of 50°C, the lowest temperature applied in the study.

Table 2. Statistical parameters, drying constant and coefficients related to thin layer drying equations used in modeling coriander leaves dried by natural, convective and microwave drying methods

Çizelge 2. Doğal, konvektif ve mikrodalga kurutma yöntemleriyle kurutulan kişniş yapraklarının modellenmesinde kullanılan ince tabaka kurutma denklemlerine ilişkin istatistiksel parametreler, kuruma sabiti ve katsayılar

Model	Natural Drying at 25°C and 60% relative humidity in a shade room								
	R^2	SEE	RMSE	χ^2	k	n	a	b	g
1	0.9995	0.0073	$1.4005\ 10^{-2}$	$3.6921\ 10^{-4}$	0.0088	1.6014			
2	0.9917	0.0293	$1.8051\ 10^{-13}$	$6.5165\ 10^{-26}$	0.0006		2.1585		-1.1033
3	0.9991	0.0093	$8.4764\ 10^{-3}$	$1.3525\ 10^{-4}$	0.0005		2.0282		
4	0.9998	0.0044	$5.3166\ 10^{-5}$	$6.0301\ 10^{-9}$	0.0002	1.6302	1.0024	$8.0254\ 10^{-6}$	
5	0.9994	0.0085	$1.4055\ 10^{-10}$	$4.5153\ 10^{-20}$	1.0999	1.0001	1.0439	1.2805	
Model	Convective Drying at 50°C								
	R^2	SEE	RMSE	χ^2	k	n	a	b	g
1	0.9980	0.0141	$1.2031\ 10^{-2}$	$1.3750\ 10^{-4}$	0.0075	1.3400			
2	0.9918	0.0295	$1.3922\ 10^{-10}$	$1.9382\ 10^{-20}$	0.0094		1.2409		-0.1803
3	0.9988	0.0109	$6.4355\ 10^{-3}$	$3.9344\ 10^{-5}$	0.0011		1.9168		
4	0.9997	0.0057	$1.3509\ 10^{-4}$	$1.9262\ 10^{-8}$	0.0019	1.4738	0.9980	0.0001	
5	0.9997	0.0059	$3.8086\ 10^{-10}$	$1.6212\ 10^{-19}$	0.9999	1.7802	0.9153	-0.0011	
Model	Microwave Drying at 200 W								
	R^2	SEE	RMSE	χ^2	k	n	a	b	g
1	0.9993	0.0081	$2.1177\ 10^{-3}$	$5.0451\ 10^{-6}$	0.0062	0.0001			
2	0.9946	0.0234	$8.4615\ 10^{-14}$	$8.5916\ 10^{-27}$	0.0001		1.3120		-0.2688
3	0.9994	0.0075	$1.2980\ 10^{-3}$	$1.8953\ 10^{-6}$	0.0153		0.0325		
4	0.9995	0.0065	$9.1239\ 10^{-5}$	$1.0703\ 10^{-8}$	0.0009	1.4112	0.9930	0.0004	
5	0.9998	0.0043	$9.0998\ 10^{-10}$	$1.1465\ 10^{-18}$	0.0002	2.1200	0.9185	-0.0131	
Model	Microwave Drying at 800 W								
	R^2	SEE	RMSE	χ^2	k	n	a	b	g
1	0.9975	0.0164	$1.0763\ 10^{-2}$	$1.1584\ 10^{-4}$	0.0001	1.2023			
2	0.9937	0.0271	$1.4370\ 10^{-2}$	$2.2527\ 10^{-4}$	0.1167		1.1120		0.0011
3	0.9976	0.0159	$1.0743\ 10^{-5}$	$1.1541\ 10^{-9}$	0.0099		1.7735		
4	0.9998	0.0047	$6.9568\ 10^{-11}$	$5.8077\ 10^{-21}$	0.0899	1.3458	1.0005	0.0045	
5	0.9998	0.0055	$1.5195\ 10^{-9}$	$3.0787\ 10^{-18}$	0.0499	1.4728	0.9103	-0.0260	

Thermal Properties

Tables 4 and 5 include the average thermal and average thermal properties per unit time of coriander leaves dried with different drying methods. Accordingly, the highest average thermal properties were obtained in the natural drying method. On the other hand, the lowest average thermal properties in the unit of time were obtained by the same method. However, the lowest average thermal properties and the highest average thermal properties per unit time were determined at 800 W. Also, the average thermal properties per unit time increased with the increase of microwave output power, but the average thermal properties decreased. Esmaiili et al. (2007) detected the thermal diffusivity of seedless grapes dried at 50°C as 9.60 x 10^{-8} $m^2\ s^{-1}$. Yu et al. (2015) calculated the thermal conductivity, specific heat, and thermal diffusivity of canola seed dried at 50°C as 0.22 W m^{-1} K^{-1}, 2766 J kg^{-1} K^{-1}, and 7.3 x 10-8 $m^2\ s^{-1}$, respectively. Olaoye & Ogunleye (2018) detected the specific heat and thermal diffusivity of ginger slices dried at 45°C as 1568 J kg^{-1} K^{-1} and 3.149 $m^2\ s^{-1}$. Lemus-Mondaca et al. (2021) stated that the density, specific heat, thermal diffusivity, thermal conductivity, and thermal effusivity of stevia leaves dried at 40°C were 116.20 kg m^{-3}, 3340 J kg^{-1} K^{-1}, 4.36 $m^2\ s^{-1}$, 0.169 W m^{-1} K^{-1}, and 225.98 W $s^{0.5}$ m^{-2} K^{-1}, respectively.

Color Parameters and Total Chlorophyll Concentration

Table 6 shows the color parameters, total color difference, browning index, whitening index, and total chlorophyll concentration of coriander leaves dried with different drying methods. According to the table, brightness (L^*), greenness (a^*), yellowness (b^*), chroma (C), and hue angle (a°) results closest to the fresh product were obtained in samples dried at 800 W. However, the lowest color parameters were detected in the convective drying method at 50°C. While the maximum total color difference was measured in the

samples dried in the convective drying method at 50°C, the minimum ones occurred in the samples dried at 800 W. The total color difference in the samples dried at 800 W was 19.28%, 88.04%, and 50.41% lower than the microwave drying at 200 W, convective drying at 50°C, and natural drying methods, respectively. In the convective drying method at 50°C, the forced airflow, which was effective during the long drying period, caused an increase in oxidation and so significant losses in color parameters.

Table 3. The operating parameters of coriander leaves dried by different methods
Çizelge 3. Farklı yöntemlerle kurutulan kişniş yapraklarının kuruma parametreleri

Methods	*DRP* **	*ADR* **	*EC* **
	min	kg $_{water}$ kg$^{-1}_{DM}$ min^{-1}	kWh
Natural	4680.00 ± 75.50^{a}	0.00034 ± 0.00001^{c}	0.000 ± 0.000^{c}
50°C	630.00 ± 8.66^{b}	0.00621 ± 0.00009^{c}	10.290 ± 0.141^{a}
200 W	85.00 ± 2.89^{c}	0.04177 ± 0.00142^{b}	0.283 ± 0.010^{b}
800 W	16.50 ± 0.29^{c}	0.24933 ± 0.00436^{a}	0.220 ± 0.004^{bc}

**P<0.01, Column mean values with different superscripts are significantly different. ±SEE
DRT: drying period (min^{-1}), *ADR*: the average drying rate (kg $_{water}$ kg$^{-1}_{DM}$ min^{-1}), and *EC*: energy consumption (kWh)

Table 4. The average thermal properties of coriander leaves dried by different methods
Çizelge 4. Farklı yöntemlerle kurutulan kişniş yapraklarının ortalama termal özellikleri

Methods	A_{Cp} **	A_k **	A_ρ ^{ns}	A_a **	A_e **
	J kg^{-1} K^{-1}	W m^{-1} K^{-1}	kg m^{-3}	m^2 s^{-1}	W s$^{0.5}$ m^{-2} K^{-1}
Natural	3526.75 ± 41.15^{a}	0.3269 ± 0.0079^{a}	804.14 ± 27.25	1.12 x 10^{-07} ± 1.91 x 10$^{-09\ a}$	963.69 ± 27.37^{a}
50°C	3147.46 ± 46.67^{b}	0.2402 ± 0.0041^{b}	757.92 ± 23.77	9.59 x 10^{-08} ± 1.01 x 10$^{-09\ b}$	756.93 ± 8.86^{b}
200 W	3188.08 ± 20.29^{b}	0.2463 ± 0.0008^{b}	760.37 ± 8.85	9.72 x 10^{-08} ± 3.02 x 10$^{-10\ b}$	772.70 ± 3.67^{b}
800 W	3127.04 ± 10.83^{b}	0.2357 ± 0.0013^{b}	755.66 ± 3.89	9.50 x10^{-08} ± 2.24 x 10$^{-10\ b}$	746.15 ± 4.91^{b}

**P<0.01, ^{ns} not significant, Column mean values with different superscripts are significantly different. ±SEE A_{Cp}: the average specific heat (J kg^{-1} K^{-1}), A_k: the average thermal conductivity (W m^{-1} K^{-1}), A_ρ: the average density (kg m^{-3}), A_a: the average thermal diffusivity (m^2 s^{-1}), and A_e: the average thermal effusivity (W s$^{0.5}$ m^{-2} K^{-1}).

Table 5. The average thermal properties per unit time of coriander leaves dried by different methods
Çizelge 5. Farklı yöntemlerle kurutulan kişniş yapraklarının birim zamanda ortalama termal özellikleri

Methods	δ_{Cp} **	δ_k **	δ_ρ **	δ_a **	δ_e **
	J kg^{-1} K^{-1} min^{-1}	W m^{-1} K^{-1} min^{-1}	kg m^{-3} min^{-1}	m^2 s^{-1} min^{-1}	W s$^{0.5}$ m^{-2} K^{-1} min^{-1}
Natural	0.754 ± 0.009^{d}	6.99 x 10^{-05} ± 1.68 x10^{-06d}	0.172 ± 0.006^{d}	2.39 x 10^{-11} ± 4.07 x10^{-13d}	0.206 ± 0.006^{d}
50°C	4.996 ± 0.074^{c}	3.81 x 10^{-04} ± 6.56 x10^{-06c}	1.203 ± 0.038^{c}	1.52 x 10^{-10} ± 1.60 x10^{-12c}	1.201 ± 0.014^{c}
200 W	37.507 ± 0.239^{b}	2.90 x 10^{-03} ± 9.90 x10^{-06b}	8.946 ± 0.104^{b}	1.14 x 10^{-09} ± 3.55 x10^{-12b}	9.091 ± 0.043^{b}
800 W	189.517 ± 0.656^{a}	1.43 x 10^{-02} ± 7.59 x10^{-05a}	45.797 ± 0.236^{a}	5.76 x 10^{-09} ± 1.36 x10^{-11a}	45.221 ± 0.298^{a}

**P<0.01, Column mean values with different superscripts are significantly different. ±SEE δ_{Cp}: the average specific heat per unit time (J kg^{-1} K^{-1} min^{-1}), δ_k: the average thermal conductivity per unit time (W m^{-1} K^{-1} min^{-1}), δ_ρ: the average density per unit time (kg m^{-3} min^{-1}), δ_a: the average thermal diffusivity per unit time (m^2 s^{-1} min^{-1}), and δ_e: the average thermal effusivity per unit time (W s$^{0.5}$ m^{-2} K^{-1} min^{-1}).

The highest results in terms of the browning index were recorded in samples dried in the convective drying method at 50°C. While the browning index closest to the fresh product was measured in the samples dried in the microwave drying method at 200 W, the lowest ones were obtained in the samples dried by the natural drying method. The browning index decreased with the increase of microwave output power; that is, the color of the samples was lightened. The closest whitening index to fresh samples was obtained in samples dried at 800 W, followed by samples dried in microwave drying at 200 W and natural drying methods, respectively. The drying method with the lowest whitening index, that is, a high rate of darkening in the samples, was determined as 50°C. The whitening index of the samples dried by the convective drying method is 36.13% lower than the fresh samples.

Sarimeseli (2011) determined that the yellowness, chroma, and hue angles of coriander leaves dried at 720 W were higher than microwave drying at 180, 360, 540, and 900 W. Yilmaz & Alibas (2017) stated that the brightness of coriander leaves dried with the natural drying method is 1.26 times higher than our findings. Also, the brightness of coriander leaves dried at 50°C was parallel to our study. On the other hand, similar to our study, the lowest results for greenness, yellowness, chroma, and hue angle were determined in the convective drying method at 50°C. In a study in which mint leaves were dried using natural, convective, and microwave drying methods, Kripanand et al. (2015) obtained the closest brightness and greenness to the fresh product in the samples dried at 900 W, but the lowest ones in the samples dried at 65°C. Also, they emphasized that with the increase of microwave output power, color parameters closer to the fresh product are obtained. Raja et al. (2019) underlined that the maximum total color change in *Carica papaya* L. leaves was measured in samples dried by convective drying method at 50°C. In a study in which thyme leaves were dried by natural, convective, and microwave drying methods, Yilmaz et

al. (2021) determined that the highest losses in terms of brightness, greenness, yellowness, chroma, hue angle, and total color difference were measured in samples dried by convective drying technique at 50°C. The lowest browning index was obtained in the samples dried in the natural drying method; however, the lowest whitening index was also found in the samples dried by the convective drying method at 50°C. In an investigation in which basil leaves were dried in the shade using convective and microwave drying techniques, Alibas et al. (2021) emphasized that the greenness, yellowness, chroma, and hue angle closest to the fresh product were obtained at fresh 700 W.

The highest total chlorophyll concentration was measured in samples dried at 800 W, followed by the lowest ones found in fresh samples and samples dried by convective drying method at 50°C, respectively. The results obtained at 800 W, where the highest chlorophyll content was recorded, were 1.08, 1.33, and 1.17 times higher than the samples dried by natural drying, convective drying at 50°C, and microwave drying at 200 W, respectively. In a study in which green tea leaves were dried by natural drying, sun drying, convective drying at 60, 80, and 100°C, and microwave drying at 800 W, Roshanak et al. (2016) emphasized that the chlorophyll content closest to the fresh product was obtained in the samples dried by microwave drying method at 800 W. In an investigation in which coriander leaves were dried via convective at 50°C and microwave drying methods at 100, 500, and 1000 W, Yilmaz & Alibas (2017) measured the lowest chlorophyll concentration were dried samples at 50°C, followed by fresh samples. Yilmaz et al. (2021) dried thyme leaves by natural drying in the shade, convective drying at 50°C, and microwave drying at 200, 600, and 1000 W. They measured the highest chlorophyll concentration in samples dried by natural drying and microwave drying at 1000 W but the lowest ones at 50°C. Alibas et al. (2021) dehydrated the basil leaves through natural drying, convective drying at 50°C, and microwave drying at 100, 300, 500, 700, and 900 W. They determined that the highest concentration of chlorophyll was achieved at 700 W.

Table 6. Color parameters and chlorophyll content of coriander leaves dried via different techniques
Çizelge 6. Farklı tekniklerle kurutulan kişniş yapraklarının renk parametreleri ve klorofil içeriği

Method	*L****	*a****	*b****	*C***	*a°**	*ΔE***	*BI***	*WI***	*TCC*** *nmol cm^{-2}*
Fresh	45.43 ± 0.29^{a}	-6.67 ± 0.11^{a}	26.10 ± 0.89^{a}	26.94 ± 0.86^{a}	104.40 ± 0.51^{a}	0.00 ± 0.00^{a}	68.05 ± 3.24^{ab}	39.11 ± 0.16^{a}	231.17 ± 6.87^{e}
Natural	35.00 ± 0.32^{d}	-4.60 ± 0.26^{c}	18.73 ± 0.47^{c}	19.30 ± 0.46^{c}	103.83 ± 0.87^{a}	12.95 ± 0.66^{d}	61.68 ± 2.00^{b}	32.19 ± 0.23^{c}	331.26 ± 8.77^{b}
50°C	31.33 ± 0.50^{e}	-3.87 ± 0.10^{d}	18.67 ± 0.28^{c}	19.06 ± 0.29^{c}	101.69 ± 0.11^{b}	16.19 ± 0.91^{e}	74.56 ± 2.02^{a}	28.73 ± 0.46^{d}	268.70 ± 4.05^{d}
200 W	37.10 ± 0.57^{c}	-4.90 ± 0.11^{bc}	20.97 ± 0.53^{b}	21.54 ± 0.50^{b}	103.22 ± 0.57^{ab}	10.27 ± 0.38^{c}	68.08 ± 4.30^{ab}	33.51 ± 0.68^{c}	306.77 ± 4.73^{c}
800 W	38.70 ± 0.75^{b}	-5.27 ± 0.08^{b}	21.07 ± 0.37^{b}	21.72 ± 0.38^{b}	104.04 ± 0.15^{a}	8.61 ± 0.29^{b}	63.01 ± 0.24^{b}	34.95 ± 0.58^{b}	358.44 ± 7.16^{a}

** P<0.01, *P<0.005, Column mean values with different superscripts are significantly different. ±SEE
*L**, Brightness; *a**, greenness; *b**, yellowness; *C*, chroma; *a°*, hue angle (°), *ΔE*, total color difference; *BI*, browning index; *WI*, whitening index; *TCC*, total chlorophyll content (nmol cm^{-2}).

Total Protein and Nutrients

Table 7 shows the macro and micronutrients, as well as the total protein content of fresh and dried coriander leaves. The total protein content closest to the fresh product was measured in samples dried at 800 W but the lowest ones at 50°C. In terms of total protein content, a decrease of 28.24% was recorded in samples dried at 800W compared to fresh products, while a decrease of 55.25% in samples dried at 50°C. On the other hand, the total protein content of coriander leaves dried by the natural drying method was found to be 44.81% lower than the fresh samples. However, we noted that the total protein content also increased with the increase in microwave output power. Danso-Boateng (2013) dried basil leaves with natural, convective, and microwave drying methods and emphasized that the highest total protein content was obtained by the microwave drying method.

Phosphorus (*P*) and potassium (*K*) contents closest to the fresh product were measured in samples dried at 800 W. While the lowest *P* was determined at 50°C, the highest *K* loss was determined in the samples dried by natural drying and convective drying methods at 50°C. While the highest calcium (*Ca*), magnesium (*Mg*), and iron (*Fe*) contents were recorded in the samples dried by microwave drying method at 200 and 800 W, the maximum losses for these minerals were obtained by natural drying and convective drying method at 50°C. The copper (*Cu*) content closest to fresh coriander leaves was measured in samples dried by microwave drying at 800 W and natural drying methods, and the *Cu* content of samples dried by these methods was 1.51 and 1.49 times lower than fresh samples, respectively. The closest manganese (*Mn*) content to fresh produce was recorded at 200 W, but the lowest ones were in natural drying and convective drying at 50°C. Accordingly, compared to the fresh samples, the *Mn* content in the samples dried at 200 W was 1.43 times lower than in the fresh samples but 1.97 and 2.01 times lower than samples dried using natural drying and convective drying at 50°C, respectively. The closest zinc (*Zn*) content to the fresh product was measured at 800 W. The zinc content of products dried by this method was 1.5 times lower than fresh leaves. On the other hand, the lowest *Zn* concentration was recorded at 50°C. The *Zn* content in the products dried at 50°C

was 1.75 times lower than the fresh coriander leaves. Strikingly, we noted that the drying methods had no effect on the sodium (*Na*) content. In a study in which rosemary leaves were dried with different drying methods, Arslan & Özcan (2008) detected that the copper content was better preserved in the samples dried by the microwave drying method compared to convective drying. Aljuhaimi & Özcan (2018) dried the germinated peanut kernels using convective and microwave drying methods. They underlined that the potassium, phosphorus, calcium, iron, and zinc concentrations in the samples dried by the microwave drying method were higher than the convective ones. In a study in which thyme leaves were dried by natural, convective, and microwave drying methods, Yilmaz et al. (2021) noted that the potassium and zinc in the leaves dried at 1000 W, the highest microwave output power used, were preserved at the maximum level. Alibas et al. (2021) measured the maximum magnesium, sodium, potassium, and iron of basil leaves at 700 W after fresh coriander leaves. Also, they achieved the highest copper concentration in natural drying.

Table 7. Total protein and mineral composition of coriander leaves dried by different techniques
Çizelge 7. Farklı tekniklerle kurutulan kişniş yapraklarının toplam protein ve mineral içeriği

Method	*TP* ** *mg g-1*	*P* ** *mg g-1*	*K* ** *mg g-1*	*Ca* ** *mg g-1*	*Mg* ** *mg g-1*	*Na* ** *mg g-1*	*Fe* ** *mg g-1*	*Cu* ** *µg g-1*	*Mn* ** *µg g-1*	*Zn* ** *µg g-1*
Fresh	345.93 ± 3.12^{a}	5.38 ± 0.02^{a}	59.71 ± 0.48^{a}	17.82 ± 0.26^{a}	6.76 ± 0.24^{a}	4.29 ± 0.04^{a}	2.36 ± 0.08^{a}	18.34 ± 0.46^{a}	83.76 ± 0.82^{a}	41.31 ± 0.75^{a}
Natural	238.88 ± 2.23^{d}	4.13 ± 0.06^{c}	30.42 ± 0.24^{d}	9.77 ± 1.02^{c}	3.62 ± 0.11^{c}	1.93 ± 0.04^{b}	0.99 ± 0.05^{c}	12.25 ± 0.39^{b}	42.50 ± 1.43^{d}	24.27 ± 0.21^{cd}
50°C	222.44 ± 2.11^{e}	3.88 ± 0.05^{d}	29.96 ± 0.42^{d}	9.58 ± 0.16^{c}	3.50 ± 0.07^{c}	1.86 ± 0.03^{b}	0.89 ± 0.04^{c}	11.13 ± 0.40^{c}	41.62 ± 0.76^{d}	23.58 ± 0.65^{d}
200 W	249.90 ± 0.53^{c}	4.18 ± 0.05^{c}	37.97 ± 0.11^{c}	11.46 ± 0.83^{b}	4.78 ± 0.09^{b}	2.29 ± 0.04^{b}	1.40 ± 0.04^{b}	11.40 ± 0.38^{c}	58.46 ± 1.46^{b}	25.15 ± 0.40^{c}
800 W	269.76 ± 2.49^{b}	4.45 ± 0.06^{b}	45.80 ± 1.60^{b}	12.19 ± 0.69^{b}	4.89 ± 0.12^{b}	2.32 ± 0.07^{b}	1.46 ± 0.08^{b}	12.12 ± 0.23^{b}	56.21 ± 1.09^{c}	27.54 ± 0.29^{b}

** P<0.01, Column mean values with different superscripts are significantly different. ±SEE
TP, total protein content (mg g^{-1}); *P*, phosphorus (mg g^{-1}); *K*, potassium (mg g^{-1}); *Ca*, calcium (mg g^{-1}); *Mg*, magnesium (mg g^{-1}); *Na*, sodium (mg g^{-1}); *Fe*, iron (mg g^{-1}); *Cu*, copper (µg g^{-1}); *Mn*, manganese (µg g^{-1}); *Zn*, zinc (µg g^{-1}).

The linear correlations of quality parameters

Table 8 highlights the linear correlations among fresh and dried coriander leaf quality parameters. Although we found many significant negative and positive relationships in the study, we discussed relationships above 90%. Accordingly, we detected positive correlations between the brightness and whitening index, total protein, phosphorus, potassium, magnesium, iron, manganese, or zinc at the level of 98.70%, 93.81%, 92.68%, 94.66%, 91.56%, 92.30%, 91.61%, and 90.45%, respectively. On the other hand, we observed that the brightness had negative associations with greenness and total color difference at the rate of 92.81% and 96.23%. Also, we found negative relationships between greenness and whitening index, total protein, phosphorus, potassium, magnesium, or iron at the level of 90.79%, 91.52%, 91.58%, 90.75%, 90.02%, and 91.23%, respectively. On the other hand, we determined that yellowness had positive correlations with chroma and manganese at the level of 99.90% and 91.71%. Chroma had strong positive associations with phosphorus, manganese, or zinc at the level of 90.08%, 92.54%, and 90.17%, respectively. We found powerful negative correlations between total color change and whitening index, total protein, phosphorus, potassium, calcium, magnesium, iron, manganese, or zinc, all over 90%. However, the whitening index had positive correlations with total protein and potassium at the level of 90.46% and 91.85%, respectively.

We found positive associations between total protein content and phosphorus, potassium, calcium, magnesium, iron, copper, manganese, or zinc at the rates of 98.13%, 96.41%, 95.59%, 96.34%, 97.63%, 94.09%, 95.84%, and 97.46%, respectively. Similarly, phosphorus had robust positive relations with potassium, calcium, magnesium, iron, copper, manganese, or zinc at the level of 94.62%, 90.81%, 92.71%, 94.10%, 92.53%, 93.83%, and 96.66%, respectively. On the other hand, we found strong positive correlations of over 90% between potassium and calcium, magnesium, iron, manganese, or zinc. Similarly, calcium had over 90% positive correlations with magnesium, iron, copper, manganese, or zinc. Also, we observed positive relationships between magnesium and iron, manganese, or zinc at the level of 98.99%, 98.08%, and 91.61%, respectively. Although iron had positive correlations with manganese and zinc at the level of 98.02% and 94.03%, there was a positive relationship between copper and zinc at the rate of 95.96%. Also, manganese had a strong positive correlation with zinc at a rate of 93.04%.

Alibas et al. (2020) determined that phosphorus had strong positive relationships with potassium and magnesium. They also emphasized highly positive associations between potassium and magnesium or copper. Alibas et al. (2021) stated a strong positive correlation between total protein and zinc, or phosphorus had positively correlated with calcium, magnesium, copper, and zinc. However, they found that calcium had significantly associated with magnesium, copper, zinc, and manganese. Also, they underlined a strong positive correlation between yellowness and chroma. Yilmaz & Alibas (2021) found a negative relationship between the whitening index and the total color difference. Also, they noted that

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Table 8. Linear correlations among measured quality parameters during drying of parsley leaves by different technique.
Çizelge 8. Maydanoz yapraklarının farklı tekniklerle kurutulması sırasında ölçülen kalite parametreleri arasındaki doğrusal korelasyonlar

*L**	*a**	*b**	*C*	*a°*	*ΔE*	*BI*	*WI*	*TCC*	*TP*	*P*	*K*	*Ca*	*Mg*	*Na*	*Fe*	*Cu*	*Mn*	*Zn*	
1.0000	-0.9281	0.8800	0.8946	0.4890	-0.9623	-0.2098	0.9870	-0.3242	0.9381	0.9268	0.9466	0.8639	0.9156	0.3947	0.9230	0.8319	0.9161	0.9045	*L**
	1.0000	-0.8292	-0.8534	-0.6886	0.8897	0.2073	-0.9079	0.3162	-0.9152	-0.9158	-0.9075	-0.8454	-0.9002	-0.4194	-0.9123	-0.8461	-0.8884	-0.8805	*a**
		1.0000	0.9990	0.1717	-0.8851	0.2729	0.7948	-0.4533	0.8725	0.8877	0.8804	0.8624	0.8726	0.1981	0.8667	0.8302	0.9171	0.8919	*b**
			1.0000	0.2154	-0.8953	0.2386	0.8133	-0.4485	0.8857	0.9008	0.8927	0.8691	0.8847	0.2174	0.8801	0.8412	0.9254	0.9017	*C*
				1.0000	-0.4037	-0.7031	0.5610	0.0923	0.4580	0.4511	0.4390	0.3477	0.4311	0.4176	0.4588	0.3802	0.3656	0.3712	*a°*
					1.0000	0.1211	-0.9371	0.3956	-0.9660	-0.9356	-0.9514	-0.9276	-0.9558	-0.4606	-0.9556	-0.8833	-0.9440	-0.9267	*ΔE*
						1.0000	-0.3540	-0.3261	-0.1020	-0.0557	-0.0905	0.0326	-0.0499	-0.3249	-0.0821	0.0091	0.0403	0.0035	*BI*
							1.0000	-0.2488	0.9046	0.8843	0.9185	0.8142	0.8822	0.4392	0.8897	0.7756	0.8668	0.8493	*WI*
								1.0000	-0.4831	-0.4548	-0.3946	-0.5377	-0.4600	-0.5027	-0.4909	-0.6121	-0.5054	-0.5872	*TCC*
									1.0000	0.9813	0.9641	0.9559	0.9634	0.5368	0.9763	0.9409	0.9584	0.9746	*TP*
										1.0000	0.9462	0.9081	0.9271	0.4916	0.9410	0.9253	0.9383	0.9666	*P*
											1.0000	0.9162	0.9601	0.4774	0.9550	0.8448	0.9480	0.9270	*K*
												1.0000	0.9417	0.4856	0.9552	0.9083	0.9518	0.9353	*Ca*
													1.0000	0.5068	0.9899	0.8730	0.9808	0.9161	*Mg*
														1.0000	0.5043	0.5213	0.4496	0.4620	*Na*
															1.0000	0.8950	0.9802	0.9403	*Fe*
																1.0000	0.8696	0.9596	*Cu*
																	1.0000	0.9304	*Mn*
																		1.0000	*Zn*

*L**, Brightness; *a**, greenness; *b**, yellowness; *C*, chroma; *a°*, hue angle (°), *ΔE*, total color difference; *BI*, browning index; *WI*, whitening index; *TCC*, total chlorophyll content (nmol cm^{-2}); *TP*, total protein content (mg g^{-1}); *P*, phosphorus (mg g^{-1}); *K*, potassium (mg g^{-1}); *Ca*, calcium (mg g^{-1}); *Mg*, magnesium (mg g^{-1}); *Na*, sodium (mg g^{-1}); *Fe*, iron (mg g^{-1}); *Cu*, copper (µg g^{-1}); *Mn*, manganese (µg g^{-1}); *Zn*, zinc (µg g^{-1}).

total protein had strong positive correlations with phosphorus, potassium, magnesium, copper, and manganese. However, they found that sodium had strongly associated with copper and manganese. Yilmaz et al. (2021) underlined a strong negative correlation between the whitening index and the total color difference, but the total color difference had a high positive correlation with zinc. However, they also reported a strong positive association between chroma and yellowness. They noted that phosphorus had robust positive associations with potassium, calcium, magnesium, copper, and manganese.

CONCLUSION

Coriander leaves, whose moisture content was 87.56% ± 0.04 and weight was 25 ± 0.02 g, were dried using natural drying, convective drying at 50°C, and microwave drying at 200 and 800 W until the final moisture content was 10.57 ± 0.07 in 4680, 630, 85, and 16.50 minutes, respectively. Experimental data were modeled using five different thin-layer drying equations. For all drying methods, except 200 W, Midilli et al. equation was determined as the most suitable model, but the Alibas equation obtained the best estimation at 200 W. While no energy consumption was required in the natural drying method, the energy consumption at 50°C, 200 W and 800 W was determined as 10.290, 0.283, and 0.220 kWh, respectively.While no energy consumption was required in the natural drying method, the energy consumption at 50°C, 200 W and 800 W was determined as 10.290, 0.283, and 0.220 kWh, respectively. The highest average thermal properties were obtained in the natural drying method but the lowest ones were at 800 W. Conversely, the lowest average thermal properties per unit time were detected in the natural drying method, but the highest results were at 800 W.

The closest color parameters to the fresh coriander leaves were determined at 800 W, followed by 200 W. Whereas the closest browning index to fresh samples was reached in samples dried at 200 W, the closest whitening index to fresh leaves was determined at 800 W. However, the highest chlorophyll content was measured in the microwave drying method at 800 W, and the lowest ones were obtained in the convective drying method at 50°C.

The closest results to fresh samples in terms of total protein, phosphorus, potassium, and zinc were found in samples dried at 800 W. However, the calcium, magnesium, and iron contents closest to fresh leaves were measured in samples dried at 800 and 200 W. After fresh samples, the highest copper concentration was obtained by natural drying and microwave drying at 800 W. Although the manganese results closest to the fresh samples were measured at 200 W, similar results were obtained in sodium content for all drying methods. In the study, the highest results in terms of both drying and quality parameters were recorded in the microwave drying method at 800 W.

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Author's Contributions

The contribution of the authors is equal.

Statement of Conflict of Interest

Authors have declared no conflict of interest.

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Kırşehir İlinde Seralarda Kullanılan Sulama Sularının Kalite Parametrelerinin Belirlenmesi

Sedat BOYACI[1], Ömer ERTUĞRUL[2], Gülden ÖZGÜNALTAY ERTUĞRUL[3], Derya DURAN GÖKALP[4]
[1,2,3]Kırşehir Ahi Evran Üniversitesi, Ziraat Fakültesi, Biyosistem Mühendisliği Bölümü, Kırşehir, Türkiye. [4]Kaman İlçe Tarım ve Orman Müdürlüğü, Kırşehir, Türkiye.
[1]https://orcid.org/0000-0001-9536-1736, [2]https://orcid.org/0000-0003-0774-1728, [3]https://orcid.org/0000-0002-8433-1872
[4]https://orcid.org/0000-0002-1211-5556
✉: sedat.boyaci@ahievran.edu.tr

ÖZET

Çalışma, Kırşehir ilinde sera işletmelerinde kullanılan sulama sularının kalitelerinin belirlenmesi amacıyla yürütülmüştür. Bu amaçla, ilde yer alan 12 sera işletmesinden sulama suyu örnekleri alınmıştır. Ayrıca, 0-30 ve 30-60 cm derinliklerinden üretim dönemi başlangıcı ve sonunda toprak örnekleri alınmıştır. Sulama suyu örneklerinde belirlenen pH değerleri 5.47-8.61, elektriksel iletkenlik (EC) ise 35-1720 dS m^{-1} arasında değişim göstermektedir. İşletmelerde kullanılan sulama sularının kalsiyum, magnezyum ve potasyum konsantrasyonları bakımından bir sorun oluşturmazken, işletmelerin %75'inin sulama suları yüksek düzeyde sodyum içermektedir. Sulama suyu örneklerinde karbonat iyonu bulunmazken, bikarbonat konsantrasyonları açısından örneklerin 10 tanesi orta sınıfında, 2 tanesinde ise önemsiz bulunmuştur. Klor sınıflamasına göre işletmelerin %91.67'si 1.sınıf ve %8.33'ü ise 3. sınıfa dahil olmuştur. Sülfat konsantrasyonları bakımından %90'ı 1. sınıfa, %10'u 2. sınıfa girmektedir. Sodyum Adsorbsiyon Oranı değerleri bakımından %91.67'si 1. sınıfa, %8.33'ü 2. sınıfta yer almıştır. Kalıcı Sodyum Karbonat değerleri bakımından ise sorun bulunmadığı belirlenmiştir. %Na değerlerine göre sınıflandırıldığında örneklerin %58.33'ü 1. sınıf, %33.33'ü 2. sınıf ve %8.33'ü 4. sınıf olarak belirlenmiştir. Bor konsantrasyonlarının bakımından %83.33'i 1.sınıf, %8.33'u 2. sınıf ve %8.33'ü 4. sınıfta yer almıştır. Yetiştirilen bitkiler dikkate alındığında toprak reaksiyonlarının sebze yetiştiriciliği açısından uygun, EC değerlerine bakıldığında, bazı sera topraklarının hafif ve orta tuzluluk gösterdiği belirlenmiştir. Çalışma sonucunda, sera işletmelerinde sulama suyu analizlerinin düzenli olarak yapılması ve topraktaki tuzluluk düzeyinin takip edilmesi yetiştiricilik açısından önemli görülmüştür.

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The Evaluation of the Quality Parameters Irrigation Waters Used in Greenhouses of Kırşehir Province

ABSTRACT

The study aimed to determine the quality of irrigation water used in greenhouse enterprises in Kırşehir, Türkiye. For this purpose, irrigation water samples were taken from 12 greenhouses located in the province. In addition, soil samples were taken at the beginning and end of the production period from 0-30 and 30-60 cm depths. The pH values determined in the irrigation water samples varied between 5.47 to 8.61, and the electrical conductivity (EC) varied between 35 to 1720 dS m^{-1}. While the calcium, magnesium and potassium concentrations of the irrigation water used in the enterprises did not pose a problem 75% of the enterprises contain high levels of sodium in their irrigation waters. Besides, despite the fact that there were no carbonate ions in the irrigation water samples, 10 of the samples were in the moderate range and 2 of them were in lower range in terms of bicarbonate concentrations,. According to the chlorine classification, 91.67% of the enterprises were in the 1st class and 8.33% were in the 3rd class. As for sulphate concentrations, 90% were in the 1st class and 10% were in the 2nd class. In terms of Sodium Adsorption Rate values, 91.67% of them were in the 1st class and 8.33% of them were

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in the 2nd class. It was determined that there was no problem in terms of permanent sodium carbonate values. When classified according to %Na values, 58.33% of the samples were determined as the 1st class, 33.33% as the 2nd class and 8.33% as the 4th class. In terms of boron concentrations, 83.33% were in the 1st class, 8.33% in the 2nd class and 8.33% in the 4th class. Considering the plants grown, it was determined that the soil reactions were suitable for vegetable cultivation, and when the electrical conductivity values were considered, some greenhouse soils showed low and medium salinity. As a result of the study, regular analyses of irrigation water in greenhouse enterprises and monitoring the salinity level in the soil were considered important in terms of aquaculture.

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GİRİŞ

Tarımsal üretimde ürün miktarının arttırılması, ancak bitki gelişimini etkileyen faktörlerin sağlanmasıyla yapılabilmektedir. Sulama, bitki gelişimini etkileyen en önemli faktörlerden birisidir. Doğal koşullarda yağışlar, bitkilerin su ihtiyaçlarının tamamını karşılayamadığı için sulama bitki gelişimi için büyük bir önem teşkil etmektedir. Bu nedenle sulamada kullanılacak sulama suyunun kaliteli olması oldukça önemlidir (Altın & Sönmez, 2020). Sulama ile toprak, su ve bitki arasında iyi ve doğru bir dengenin oluşturulması temel amaçtır. Bu nedenle sulama, bitki gelişmesi için yeterli su koşulunu sağlayan bir işlem olarakta tanımlanır. Eğer, toprakta gereğinden fazla miktarda su varsa sulama ile ürün miktarında bir azalma ve daha önemliside, toprakta tuzluluk, alkalilik ve taban suyu gibi sorunlar meydana gelmektedir (Altan ve ark., 2003). Sulama suyunun kalitesi bitki gelişiminde önemli bir rol oynar. Suyun kalitesi içerdiği tuz ve toksik element miktarına bağlıdır. Tuz içeriği yüksek olan sulama suyu, toprak profilinin çözünebilir tuz içeriğinde ve drenaj sularının tuz yükünde bir artışa sebep olur. Drenaj suyuna ulaşamayan tuzlar toprakta birikir. Tüm bitkiler tuz içeren iyonların en uygun miktarlarına gereksinim duyarlar. Artan tuz miktarı yetiştiriciliği yapılan bitkinin zarar görmesine yol açar (Grismer, 1990). Toprakta tuz birikimi, uygulanan su miktarı ile ilişkilidir ve bitkinin olgunluk evrelerine doğru en yüksek tuz konsantrasyonlarına ulaşır (Tekin ve ark., 2014). Tuzlu sulama sularıyla toprağa iletilen tuzların, çok az miktarını bitkilerin yapılarına almaları nedeniyle zaman içerisinde toprakta tuz birikmektedir. Kış aylarında düşen yağışlarla fazla veya düzenli yıkamaların yapılmadığı topraklar verimliliklerini kaybetmekte ve ekonomik boyutu gittikçe artan iyileştirme uygulamalarının yapılması gerekmektedir (Yurtsever & Güngör, 1990). Tekin (2018a) kış yağışlarının yetersiz olduğu veya yıkama gereksiniminin karşılanmadığı durumda, sulama suyu ile profile giren tuzun zamanla birikeceği, sulama suyu tuzluluğunun artması ile de toprak tuzluluğunun artacağını bildirmiştir. Seralarda sulama suyu kalitesi ile ilgili çalışmalarda; Sönmez & Kaplan (1996) Kumluca ve Finike yörelerindeki seralarda kullanılan suların kalitelerini belirlemek amacıyla yapmış oldukları çalışmada, sulama sularının genelde orta veya fazla tuzlu olduğunu ve örneklerin büyük çoğunluğunun SAR (Sodyum Adsorbsiyon Oranı) ve %Na bakımından 1. sınıf olup sorun oluşturmadığını, klor içerikleri bakımından değişkenlik göstermekle birlikte genellikle 1. ve 2. sınıfta yer aldığını, bor ve sülfat içerikleri bakımından 1. ve 2. sınıf sulama suyu kalitesine sahip olduklarını belirlemişlerdir. Sera sulama sularının büyük bir kısmı tuzluluk hariç diğer özellikleri açısından önemli düzeyde sorun olmadığı az sayıdaki sorunlu suların genellikle yörelerin denize en yakın kuyu suları olduğunu bildirmişlerdir. Ayrancı (2006) Muğla-Ortaca yöresindeki seralarda kullanılan yeraltı sulama suyu örneklerinin %76'sının C_2S_1, %24'ünün ise C_3S_1 sınıfına girdiğini bildirmiştir. İncelenen sulama sularının tamamı SAR ve %Na bakımından sorun teşkil etmeyen 1. sınıf sulama sularıdır. Sulama sularında karşılaşılan en önemli sorun kaynağının klorür olduğu saptanmıştır. Ayrıca analiz sonucunda suların 19'unda (%76) sülfat tespit edilmemiştir. Öktüren Asri ve ark. (2010) Antalya-Serik yöresini temsilen seralardan aldıkları sulama suyu örneklerinin %68'inin C_2, %32'sinin C_3 tuzluluk sınıfında olduğu, SAR ve %Na bakımından ise incelenen tüm örneklerin 1. sınıfta yer aldığı ve genel olarak yörede faaliyet gösteren seraların sulama sularında önemli düzeyde sorun olmadığı belirlenmiştir. Sönmez ve Kaplan (2004) Demre yöresindeki sera topraklarının 0-20 ve 20-40 cm derinliklerde genel olarak orta ve fazla tuzlu, sulama sularının ise genel olarak orta tuzlu (C_2) ve fazla tuzlu (C_3) sınıfına girdiğini bildirmektedir. Toprakların tuz içeriklerinde dönemsel farklılıklar olsada sera topraklarının ve sulama sularının büyük bir kısmının

tuzluluk açısından sorun oluşturacak nitelikte olduğu ortaya konulmuştur. Yörede bulunan işletmelerde yetiştiricilikte tuzluluğa dayanıklı bitki çeşitlerinin tercih edilmesi gereklilik olarak görülmekte ve bunun yanısıra toprak tuzluluğunun izlenerek gerekli önlemlerin alınması gerekmektedir. Öktüren Asri ve Sönmez (2009) Antalyanın, Merkez ve Serik ilçelerinde topraksız kültürde domates yetiştirilen seralardaki bitkilerin beslenme durumlarının ve sulama suyu kalitelerinin belirlenmesi amacıyla yürüttükleri çalışmada, sulama suyu örneklerinin pH'ları genel olarak nötr ve hafif alkali karakterde olup, tuzluluk bakımından I. ve II sınıf (C_1 ve C_2), sodiklik açısından I. sınıf (S_1), CI- ve SO_4^{-2} içerikleri bakımından I. sınıf, B içerikleri yönünden de I. ve II. sınıf sulama suları olduğu ve topraksız kültürde yetiştiricilik yapan seraların sulama suyu kalitesi bakımından sorun teşkil etmeyen bölgelerde kurulduğu belirlenmiştir. Sulama suyunun kimyasal bileşenleri, bitki büyümesini doğrudan toksisite veya eksiklik yoluyla yada besinlerin bitki kullanılabilirliğini değiştirerek dolaylı olarak etkileyebilmektedir. Sulama suyunun kalitesini değerlendirmek amacıyla, bitki büyümesi için önemli olan faktörleri ve bu faktörlerin kabul edilebilir seviyelerini veya konsantrasyonlarının belirlenmesi gerekir. Sulama suyunun bir laboratuvar tarafından test edilmesi bu süreçteki ilk adımdır. Sonuçların doğru şekilde yorumlanması, su kalitesi sorunlarını düzeltmemize ve/veya ürün kaybını önlemek için gübre ve sulama tekniklerini seçmemize olanak sağlar (Will & Faust, 1999). Bu çalışma, Kırşehir ilindeki seralardan alınan sulama suyu örneklerinin analiz edilerek, ilde sera işletmelerindeki su kalitesinin belirlenmesi amacıyla yürütülmüştür. Elde edilen sonuçlara göre, işletmelerde kullanılan sulama sularının kimyasal özellikleri ve sulama suyu kaynaklı sorunlar belirlenerek sorunların çözümüne yönelik öneriler ortaya konulmuştur.

MATERYAL ve METOD

Çalışmada, Kırşehir ilinde seralarda kullanılan sulama sularının kalitelerini belirlemek amacıyla yöreyi temsil eden 10 adet topraklı, 2 adet topraksız olmak üzere toplam 12 işletmeden toprak ve sulama suyu örnekleri alınmıştır. Alınan toprak örnekleri (10 adet) işletmelerden üretim döneminin başlangıcı ve sonunda birer kez, su örnekleri (12 adet) ise üretim döneminde bir kez alınmıştır. İncelenen sera işletmelerine ait bazı genel bilgiler Çizelge 1'de verilmiştir.

Çizelge 1. İncelenen sera işletmelerine ait bazı genel bilgiler
Table 1. Some general information about the greenhouses enterprises

Sera no	Üretim şekli	Sera alanı (m^2)	Su kaynağı
Sera 1	Topraklı	1000	Kuyu
Sera 2	Topraklı	1000	Gölet
Sera 3	Topraklı	1000	Kuyu
Sera 4	Topraklı	1000	Kuyu
Sera 5	Topraklı	1000	Baraj gölü
Sera 6	Topraklı	1000	Kuyu
Sera 7	Topraklı	1000	Gölet
Sera 8	Topraklı	2000	Kuyu
Sera 9	Topraklı	800	Kuyu
Sera 10	Topraklı	1008	Kuyu
Sera 11	Topraksız	126000	Kuyu
Sera 12	Topraksız	100000	Kuyu

Çalışma alanında topraklı tarım yapan işletmelerde genel olarak yetiştiricilik dönemleri Mart-Temmuz, Ağustos-Kasım arasında olmak üzere 2 dönem şeklinde gerçekleştirilmektedir. Seralarda domates, hıyar ve biber üretimi yapılmakla birlikte topraklı tarım yapılan seralarda dekara ortalama domateste 3000, hıyarda 3300 ve biberde 4000 adet fide dikilmektedir. Topraksız tarım yapılan seralarda jeotermal enerji kaynağı kullanıldığı için üretim yıl boyunca yapılmakta ve dekara 2700-3600 adet domates fidesi dikilmektedir. İşletmelerde su, baraj gölü, gölet ve kuyulardan olmak üzere farklı kaynaklardan temin edilmektedir. İncelenen işletmelerde kuyu derinlikleri 20-600 metre arasında değişim göstermekte olup su kaynaklarına uzaklık ise 5-7500 m arasında değişmektedir. Topraklı tarım yapan işletmelerin tamamında sulama suyu damla sulama lateralleri ile verilirken, topraksız tarım yapan işletmelerde spagetti borular kullanılmaktadır. Topraklı tarım yapılan işletmelerde drenaj sistemi mevcut olmayıp tıkanan lateralleri açmak için 2 işletmede fosforik asit uygulaması yapılırken, topraksız tarım işletmelerinde ise fosforik asit, hidrojen peroksit, sodyum hidroksit gibi uygulamalar yapılmaktadır. Ayrıca, topraklı tarım yapılan işletmelerde su ve toprak analizleri yapılmadığı, topraksız tarım yapılan seralarda ise sulama suyu analizlerinin düzenli olarak yapıldığı belirlenmiştir.

İşletmelerden alınıp laboratuvara getirilen 0-30 ve 30-60 cm'lik toprak derinliklerden alınan toprak

örnekleri, hava kuru hale getirildikten sonra 2 mm'lik elekten geçirilerek analiz için hazırlanmıştır. Toprakların pH ve elektriksel iletkenlik (EC) değerleri 1:1 toprak/su çözeltilerinde pH ve EC metre ile ölçülmüştür (Rhoades ve ark., 1999). Çalışmada işletmelerden alınan 12 adet sulama suyu örneği Kırşehir Ahi Evran Üniversitesi Merkez laboratuvarında analiz edilmiştir. Analiz edilen örneklerin pH ve elektriksel iletkenlik (EC)değerleri (Eutech PC 700) ile, Mg^{2+}, Na^{+}, K^{+}: AAS direkt okuma (Agilent 240 AA Atomik Absorpsiyon Spektrometresi), Ca^{2+}: Flame fotometre direkt okuma (JENWAY / PFP7 Flame Photometer), (CO_3^{-}, HCO_3^{-}), Sülfürik asit titrasyonu (USSL, 1954), Cl: Mohr metodu (Gümüş nitrat titrasyonu) (USSL, 1954), $SO4^{2-}$: Baryum klorür metodu (Eltan, 1998) (Spektrofotometrik) (Thermo Scientific™ GENESYS™ 10S UV-Vis Spectrophotometer), B: Azomethine-H yöntemiyle (Thermo Scientific™ GENESYS™ 10S UV-Vis Spectrophotometer) belirlenmiştir. Elde edilen verilerden %Na (Christiansen ve ark., 1977), kalıcı sodyum karbonat değeri (RSC) (Eaton, 1950), sodyum adsorpsiyon oranı (SAR) (USSL, 1954) ve geçirgenlik göstergesi (GG) (Doneen & Wenderson, 1960) değerleri hesaplanmıştır.

BULGULAR ve TARTIŞMA

Sera Topraklarının pH ve EC değerleri

Çalışmada, Kırşehir ilinde belirlenen 10 adet topraklı tarım serasından 0-30 ve 30-60 cm toprak derinliklerinden alınan toprak örneklerinin pH ve EC değerleri Çizelge 2'de verilmiştir.

Çizelge 2. İncelenen sera topraklarının pH ve EC değerleri
Table 2. Soil pH and EC values of the greenhouses

Sera no	Derinlik (cm)	Üretim dönemi başlangıcı		Üretim dönemi sonu	
		EC (dS m^{-1})	pH	EC (dS m^{-1})	pH
Sera 1	0-30	1.53	7.39	1.17	7.43
	30-60	2.34	7.30	0.43	7.80
Sera 2	0-30	1.03	7.85	1.26	7.69
	30-60	0.87	7.97	1.28	7.70
Sera 3	0-30	2.90	7.65	1.30	7.73
	30-60	3.19	7.61	5.44	7.47
Sera 4	0-30	1.24	7.95	2.94	7.48
	30-60	1.22	7.94	1.98	7.73
Sera 5	0-30	4.88	7.56	3.70	7.53
	30-60	4.89	7.51	4.56	7.49
Sera 6	0-30	1.22	8.00	1.93	7.49
	30-60	1.67	7.77	1.79	7.60
Sera 7	0-30	1.66	7.96	1.58	7.88
	30-60	2.07	7.91	1.24	7.95
Sera 8	0-30	1.86	7.77	0.82	7.94
	30-60	1.11	7.90	1.95	7.65
Sera 9	0-30	0.94	7.56	1.21	7.66
	30-60	1.47	7.96	0.93	7.75
Sera 10	0-30	0.93	8.05	1.30	7.76
	30-60	0.96	8.20	0.82	8.09

Toprakların üretim dönemi başlangıcı toprakların 0-30 ve 30-60 cm derinlikleri için pH değerleri sırasıyla 7.39-8.05 ile 7.30-8.20 aralığında değişim göstermiştir. Topraklar sınıflandırıldığında üretim dönemi başlangıcında her iki toprak derinliği için seraların %90'ının hafif alkalin, %10'unun nötr reaksiyon gösterdiği belirlenmiştir. Üretim dönemi sonu bu dağılım, 0-30 ve 30-60 cm toprak derinlikleri için sırasıyla 7.43-7.94 ve 7.47-8.09 arasında değişim göstermiştir. Buna göre, toprakların 0-30 cm derinliği için %70'inin hafif alkalin, %30'unun nötr reaksiyon, 30-60 cm için %80'inin hafif alkalin, %20'sinin nötr reaksiyon gösterdiği belirlenmiştir. Boyacı ve ark. (2021) Kırşehir ili seralarında yapmış oldukları çalışmalarında toprakların pH değerlerinin 7.51-8.32 arasında değişim gösterdiğini ve sınır değerleri ile karşılaştırıldığında seraların tamamının hafif alkalin grubunda olduğunu bildirmiştir. Domates bitkisi hafif asit ve nötr toprak reaksiyonlarında gelişebilirken, hıyar bitkisi nötr veya hafif alkalin reaksiyonlu toprakları tercih eder (Sevgican, 1989) ve yetiştiricilik açısından en uygun toprak pH değerleri 5.5-6.8 arasındadır (Kütevin & Türkeş, 1985). Biber bitkisi ise yüksek pH'lara toleranslıdır (Şeniz, 1992). Çalışmada, incelenen sera topraklarının pH değerlerinin yetiştiriciliği yapılacak bitki türüne göre değerlendirilmesi gerektiği belirlenmiştir. Ayrıca asit reaksiyonlu topraklara gübrelemeden önce kireç, alkali reaksiyonlu topraklara ise uygun miktarlarda

kükürt veya asit karakterli gübreler verilerek toprak reaksiyonlarının düzenlenmesi bitki yetiştiriciliği açısından oldukça önemlidir.

Üretim dönemi başlangıcında 0-30 ve 30-60 cm derinlikleri için toprakların elektriksel iletkenlik değerlerine bakıldığında, toprakların %80'inin tuzluluk göstermediği, %10'unun ise hafif tuzluluk ve %10'unun orta tuzluluk gösterdiği belirlenmiştir. Üretim dönemi sonu 0-30 cm toprak derinliği için toprakların elektriksel iletkenlik değerlerine bakıldığında, toprakların %80'inin tuzluluk göstermediği, %20'sinin ise hafif tuzluluk gösterdiği belirlenmiştir. Üretim dönemi sonu toprakların 30-60 cm derinliği için %80'inin tuzluluk göstermediği, %20'sinin orta tuzluluk gösterdiği belirlenmiştir. Boyacı ve ark. (2021) Kırşehir ili seralarında yapmış oldukları çalışmalarında 0-20 cm sera topraklarda EC'nin 0.17-1.98 dS m^{-1} arasında değişim gösterdiği ve sınır değerleri ile karşılaştırıldığında sera topraklarının tamamının tuzsuz olduğunu belirlemiştir. Şeniz (1992) biber bitkisi tuzluluğa karşı hassas, hıyar bitkisiyse tuza orta derecede toleranslıdır. Domates bitkisinin tuzluluğa karşı toleranslıdır (Campos ve ark., 2006; Sönmez & Kaplan, 2007). Akay & Kaplan (1995) yapmış oldukları çalışmalarında hıyar ve domates yetiştiriciliği yapılan sera işletmelerinin topraklarındaki tuz konsantrasyonlarının dönemsel olarak arttığını ve bu artışın nedeni olarak ta gübre uygulamaları gösterilmiştir. Tura ve Tolossa (2020) düşük kaliteli sulama suları, bitki verimliliğini olumsuz etkileyebilir. Tuz miktarının aşırı artması durumunda ise verimde kayıplar meydana gelir. Verim kaybını önlemek için topraktaki tuzların, bitki verimini etkileyebilecek olan miktarın altında bir konsantrasyonda tutulması gerekmektedir. Yapılan çalışmada, 5 işletmenin topraklarının 0-30 ve 30-60 cm derinliklerinde EC değerlerinin artış gösterdiği belirlenmiştir (Çizelge 2). Toprakların farklı derinliklerinde genel olarak bir tuzluluk problemi olmasa da hafif tuzluluk ve orta tuzluluk gösteren seralarda yıkamaların yapılmaması nedeniyle ilerleyen yıllarda tuzluluk sorunlarıyla karşılaşılabileceğinden önlem alınması gerekmektedir. Seralarda toprakların 0-30 ve 30-60 cm'lik derinliklerde tuzluluk artışlarının, üreticilerin toprağa ilave ettiği yapay veya doğal gübrelerden kaynaklandığı, azalışların ise fazla miktarda sulama suyu uygulanması neticesinde toprakların yıkanmasıyla açıklanabilir.

Sera Sulama Suyu Örneklerinin Kimyasal Özellikleri

Çalışmada Kırşehir ilinde işletmelerden alınan toplam 12 adet sulama suyu örneğine ait kimyasal analiz sonuçları Çizelge 3'te, sulama suyu örneklerinin kalite sınıflarına göre değerlendirilmesi Çizelge 4'te verilmiştir.

Bitki yetiştiriciliği açısından sulama sularının niteliklerini önemli ölçüde belirleyen faktörlerin başında sulama suyunun tuzluluğu ile anyon ve katyonların konsantrasyonları gelmektedir (Altın & Sönmez, 2020). Araştırmada sulama suyu örneklerinde ölçülen pH değerleri, 5.47-8.61 arasında değişim göstermiştir (Çizelge 3). Ortalama olarak ölçülen pH değeri ise 7.47 olarak ölçülmüştür. Sulama sularında 6.5-8.4 pH aralığı değerleri problem oluşturmamaktadır (Ayers & Wescot, 1989). İncelenen suların 1 tanesi dışında (Sera 5) pH değerlerinin genel olarak uygun değerler arasında olduğu ve sorun oluşturmadığı belirlenmiştir. Sulama sularında pH değerlerinin önerilen sınır değerlerden farklı olması yetiştirilen bitkilerde dengesiz beslenme veya toksik

Çizelge 3. İncelenen sera sulama sularının kimyasal analiz değerleri
Table 3. Results of the chemical analyses of greenhouse irrigation waters

Seralar	pH	EC (µmhos cm^{-1})	Katyonlar (meq L^{-1})				Anyonlar (meq L^{-1})				SAR	RSC	%Na	B (ppm)	Sulama sınıfı
			Ca	Mg	K	Na	CO_3	HCO_3	Cl	SO_4					
Sera 1	7.57	384	3.10	1.76	0.05	1.52	-	5.34	0.62	0.46	0.97	0.48	23.63	0.28	C_2S_1
Sera 2	8.21	480	2.23	2.48	0.05	2.43	-	5.36	1.00	0.84	1.59	0.65	33.84	0.30	C_2S_1
Sera 3	8.04	572	2.91	1.93	0.02	2.80	-	4.85	1.15	1.66	1.80	0.01	36.54	0.50	C_2S_1
Sera 4	7.65	550	2.87	1.25	0.02	2.90	-	4.55	1.62	0.86	2.02	0.44	41.21	0.34	C_2S_1
Sera 5	8.61	1720	3.62	2.97	0.25	9.02	-	1.95	8.51	5.42	4.97	-4.65	56.86	0.22	C_3S_1
Sera 6	7.25	947	3.96	1.86	0.02	2.95	-	6.93	1.79	0.07	1.73	1.11	33.58	0.42	C_3S_1
Sera 7	7.44	536	3.82	2.95	0.02	2.94	-	7.18	1.86	0.69	1.60	0.41	30.23	0.28	C_2S_1
Sera 8	7.44	950	3.26	3.15	0.08	3.25	-	6.19	2.56	0.99	1.82	-0.21	33.40	0.40	C_3S_1
Sera 9	7.35	996	3.51	2.15	0.12	1.56	-	6.56	0.52	0.25	0.93	0.90	21.28	2.37	C_3S_1
Sera 10	7.97	420	1.88	2.15	0.12	3.17	-	4.96	1.49	0.89	2.23	0.92	43.24	0.67	C_2S_1
Sera 11	6.68	350	0.16	0.82	0.01	0.81	-	0.87	0.94	0.003	1.16	-0.11	44.82	0.10	C_2S_1
Sera 12	5.47	35	0.0012	0.55	0.0002	6.54	-	0.45	0.08	0.07	12.49	-0.10	92.27	0.18	C_1S_2
Min.	5.47	35	0.0012	0.55	0.0002	0.81	-	0.45	0.08	0.003	0.93	-4.65	21.28	0.10	
Ort.	7.47	661.67	2.61	2.00	0.06	3.32	-	4.60	1.84	1.02	2.78	-0.01	40.91	0.51	
Maks.	8.61	1720	3.96	3.15	0.25	9.02	-	7.18	8.51	5.42	12.49	1.11	92.27	2.37	

Çizelge 4. Sera sulama sularının kalite sınıflarına göre değerlendirilmesi
Table 4. Evaluation of greenhouse irrigation waters according to quality classes

Ölçülen değerler	Sınıflar	Değerlendirme	Örnek Sayısı	%
EC, (µmhos cm^{-1}) (USSL, 1954)	C1	<250	1	8.33
	C2	250-750	7	58.33
	C3	750-2250	4	33.33
	C4	>2250	-	-
SAR (Ayers and Westcot, 1989)	S1	0-10	11	91.67
	S2	10-18	1	8.33
	S3	18-26	-	-
	S4	>26	-	-
%Na (Christiansen ve ark., 1977)	1	0-40	7	58.33
	2	40-60	4	33.33
	3	60-70	-	-
	4	70-80	1	8.33
	5	80-90	-	-
Cl (me L^{-1}) (Christiansen ve ark., 1977)	1	0-3	11	91.67
	2	3-6	-	-
	3	6-10	1	8.33
	4	10-15	-	-
	5	15-20	-	-
	6	>20	-	-
B (ppm) (Christiansen ve ark., 1977)	1	0-0.5	10	83.33
	2	0.5-1.0	1	8.33
	3	1.0-2.0	-	-
	4	2.0-3.0	1	8.33
	5	3.0-4.0	-	-
	6	>4.0	-	-
SO_4 (me L^{-1}) (Christiansen ve ark., 1977)	1	0-3	11	91.67
	2	3-6	1	8.33
	3	6-9	-	-
	4	9-12	-	-
	5	12-15	-	-
	6	>15	-	-
RSC (Eaton, 1950)	1	<1.25	12	100.00
	2	1.25-2.5	-	-
	3	>2.5	-	-

maddelerin birikmesine neden olmaktadır (Kanber ve ark., 2003). Gübreleme ile verilen bitki besin elementlerinden en yüksek düzeyde yarar sağlamak amacıyla fertigasyon değerlerinin asidik olması önerilmektedir (Maltaş & Kaplan, 2018). Buna göre sulama suyunda pH değerlerinin yüksek olması durumunda üretim yapılırken, asit kullanımı ile pH değerinin azaltılması, optimum bitki beslenmesi bakımından uygulanabilir bir yöntem olarak önerilebilir.

Sulama suyu örneklerinde belirlenen elektriksel iletkenlik değerleri, 35-1720 µmhos cm^{-1} arasında ortalama 661.67 µmhos cm^{-1} olarak belirlenmiştir (Çizelge 3). İncelenen örnekler USSL (1954)'e göre sınıflandırıldığında, örneklerin %8.33'ünün 1. sınıf, %58.33'ünün 2. sınıf ve %33.33'ünün 3. sınıfına girdiği görülmektedir (Çizelge 4). Sulama, yüksek tarımsal üretimi güven altına alan son derece gerekli ve önemli bir uygulamadır (Kaman ve ark., 2022). Ancak nispeten yüksek konsantrasyonda tuz içeren su ile sulama, bitkisel üretim ve toprak üzerinde olumsuz etkilere sahiptir (Yasuor ve ark., 2020). Yüksek tuz miktarı, bitki köklerinin su ve bitki besin elementlerinin alımını zorlaştırması nedeniyle su ve besin elementi eksiklikleri ortaya çıkmaktadır. Toplam çözünebilir tuzları sulama suyundan uzaklaştırmak için ters osmoz ve deiyonizasyon uygulamalarının yapılması iyi bir çözüme ulaşmak için fayda sağlayacaktır (Will & Faust, 1999). Seralarda 2. ve 3. sınıf sulama suları tuza orta veya yüksek oranda dayanıklı bitkilerin yetiştiriciliği için kullanılabilir. Aynı zamanda bu alanlarda yıkama gereksinimi ortaya çıkmakta ve drenaj sistemlerine gereksinim duyulmaktadır. İşletmelerde yetiştirilen bitkiler domates, hıyar ve biber tuza orta derecede dayanıklı olduklarından (Maas, 1986) bu alanlarda yetiştirilebilse de işletmelerde yıkama yapılmadığından ve bir drenaj sistemi olmadığından ileriki yetiştirme dönemlerinde toprakta sorunlarının

ortaya çıkması muhtemeldir. Topraksız tarım işletmeleri olan Sera11 ve Sera 12'de gelen sulama suları ters osmozdan geçirilerek kullanılmaktadır. Sera 12'de gelen sulama suyunun EC değeri 2800 µmhos cm^{-1} iken ters osmoz uygulaması ile birlikte bu değer 35 µmhos cm^{-1}'e kadar düşürülmüştür. EC değeri yüksek olan sulama sularına sahip işletmelerde bu ve benzeri uygulamalar ile elektriksel iletkenlik değerini düşürmek amacıyla uygulanması önemli görülmüştür.

İncelenen sulama sularında kalsiyum (Ca) konsantrasyonları 0.0012-3.96 me L^{-1} arasında değişim göstermektedir (Çizelge 3). Kalsiyum, bitki gelişimi için gerekli elementlerden olup sulama sularındaki uygun miktarı 40-100 ppm (2-5 me L^{-1}) arasındadır (Will & Faust, 1999). Buna göre örneklerin kalsiyum düzeylerine bakıldığında %25'inin düşük, %75'ininse yeterli olduğu belirlenmiştir. Magnezyum (Mg) konsantrasyonlarına bakıldığında ise 0.55-3.15 me L^{-1} arasında değişmektedir (Çizelge 3). Will ve Faust (1999) tarafından bildirilen 30-50 ppm (2.5-4.2 me L^{-1}) yeterlilik sınır değerlerine göre incelenen örneklerin %75'inin düşük, %25'inin yeterli düzeyde magnezyum içerdiği belirlenmiştir. Örneklerin potasyum (K) konsantrasyonlarının 0.0002-0.25 me L^{-1} arasında değiştiği belirlenmiştir (Çizelge 3). Sularda çok düşük düzeylerde bulunan potasyumun birkaç ppm'den fazla olması gübreler veya diğer kaynaklar ile sulama sularına iletildiği anlamı taşımaktadır (Ayrancı, 2006). Kalsiyum (Ca) ve magnezyum (Mg) yetiştirilen ürünler için temel elementlerdir ve sodyumun olumsuz etkilerini azaltarak toprağın katyon dengesinde temel bir rol oynarlar. Genellikle suda nitratlar, klorürler, sülfatlar, karbonatlar ve bikarbonatlar gibi tuzların ayrışmasıyla üretilen iyonlar şeklinde bulunurlar. Sudaki kalsiyum ve magnezyum tuzu içeriğinin kapsamı sertlik ile temsil edilir (De Pascale ve ark., 2013). Sulama sularında kalsiyum, magnezyum ve potasyumun fazla bulunması toprağın fiziksel ve kimyasal özelliklerine etki ederek toprağı kolay işlenebilir hale getirerek infiltrasyon kapasitesini arttırır (Kanber & Ünlü, 2010). Yapılan çalışmada, araştırmacıların önerileri doğrultusunda elementlerin sodyum zararını azaltması, bitki gelişmesi ve toprak özellikleri açısından önemli olduğu yetiştirme döneminde toprak ve sulama suyu analizleri ile izlemenin yapılmasının önemli olduğu belirlenmiştir.

Sera sulama suları için uygun sodyum (Na) düzeyinin 50 ppm (2.2 me L^{-1}) olduğu belirtilmiştir (Will & Faust, 1999). Buna göre Na konsantrasyonları 0.81-9.02 me L^{-1} arasında değişmekte olup örneklerin %25'inin optimum değerden düşük, %75'inin ise yüksek düzeyde Na içerdiği belirlenmiştir (Çizelge 3). Yüksek sodyum (Na), tuzluluk sorunlarına katkıda bulunabileceği, ortamdaki magnezyum ve kalsiyum mevcudiyetini engelleyebileceği ve yaprak yanıklarına neden olabileceği için yetiştiriciler için sorun teşkil eder (De Pascale ve ark., 2013). Sulama suları ve yağışlar aracılığıyla toprağa ulaşan sodyum iyonları toprak kolloidlerinin disperse olmasına neden olarak toprağın strüktürel yapısının bozulmasına yol açar. Toprak gözenekliliğinin bozulması nedeniyle de toprağın hava ve su geçirgenliği azalır. Bunun yanı sıra toprak çözeltisinin pH değerleri kültür bitkilerinin yetiştirilemeyeceği düzeylere çıkabilir (Ayyıldız, 1990). Toprağa fazla miktarda Na iyonu içeren sulama suyu uygulandığında, toprağa sızması güç olur ve toprak yüzeyinde gölcükler meydana gelir. Toprak kuruduğunda yüzeyde çatlamalar oluşup, sürüldüğünde çok sert kesekler ortaya çıkar. Öte yandan, toprağın çözünebilir ve değişebilir Na iyonu miktarı fazla ise böyle topraklara uygulanan yüksek Na içerikli sular, toprak yüzeyinde çok uzun zaman kalır, toprak içine sızamaz ve yüzeyden buharlaşıp kaybolur (Kanber & Ünlü, 2010). Yapılan çalışmada, Na düzeyinin bazı işletmelerde sınır değerlerden düşük ve bazılarında yüksek olduğu belirlenmiştir. Sulama sularının Na düzeyinin düşük olması durumunda potasyum alımını ve kullanımını etkileyeceği, fazla olması durumunda ise bitkilerde toksik etki ortaya çıkaracaktır. Bu etkinin belirlenmesi amacıyla toprak, su ve bitki doku analizlerinin yapılması etkinin belirlenmesi bakımından önemlidir. Ayrıca, sulama suyunun uzun süre kullanılması durumunda toprak geçirgenliğinde ortaya çıkacak değişim Doneen (1966)'ya göre değerlendirildiğinde, seralarda toprakların geçirgenliğinin düşük olması durumunda (2 cm h^{-1}'ten düşük), Sera 1-2-3-4-7 ve 10'da ilerleyen dönemlerde toprakların geçirgenliğinde %35'lik azalmanın meydana gelmesi beklenebilir. Toprak geçirgenliğinin orta geçirgenlikte olması durumunda (2-12 cm h^{-1}) Sera 1-2-9 ve 10'un geçirgenliğinde %25'lik bir azalmanın beklenebileceği, toprakların geçirgenliğinin yüksek olması durumunda da sera topraklarının geçirgenliğinin etkilenmeyeceği belirlenmiştir.

İncelenen sulama suyu örneklerinde karbonat (CO_3) iyonu saptanmamıştır (Çizelge 3). Sulama sularında müsaade edilen bikarbonat (HCO_3) değeri 1.5-8.5 me L^{-1} arasındadır (Ayers & Wescot, 1989). Buna göre incelenen sulama suyu örneklerinde bikarbonat konsantrasyonları 0.45-7.18 me L^{-1} arasında (Çizelge 3) değişmekte olup örneklerin 10 tanesi orta sınıfta, 2 tanesinde ise önemsiz bulunmuştur. Sulama sularının karbonat ve bikarbonat içerikleri, nötralize edilebilen bileşiklerin konsantrasyonunu ifade etmektedir (Ayrancı, 2006). Sulama suyundaki yüksek CO_3 ve HCO_3 içeriği, özellikle mikro sulamada (damla sulama, mikro yağmurlama) kullanılan su dağıtım ekipmanlarını tıkayabilir. Karbonat seviyesi sınıflandırmada belirtilen limiti aşarsa, suyun fiziksel veya kimyasal olarak arıtılması ihtiyacı değerlendirilmelidir (De Pascale ve ark., 2013). Damla sulama sistemlerinin en önemli kısıtlarından biri

damlatıcıların zamanla tıkanması ve bunun sonucunda sistem performansında önemli bir düşüş yaşanmasıdır. Damlatıcıların kısmen veya tamamen tıkanması, su dağıtım homojenliğini ve sulama verimini düşürerek üretim ve kalite kayıplarına neden olabilir (Tekin ve ark., 2016; Tekin, 2018b). Yapılan çalışmada seralarda sulamaların damlama ve spagetti borular ile yapıldığı belirlenmiştir. Sulama sularında orta sınıfta olan işletmelerde sulama ekipmanlarında ortaya çıkacak tıkanıklık probleminin takip edilmesi gerektiği, mümkünse sulama suyunun filtrasyon işleminden sonra bitkiye ulaştırılması önemli görülmüştür. İşletmelerde uniformite testleri yapılarak damlatıcılardaki tıkanıklıklar için fosforik asit veya benzeri uygulamaların yapılmasına dikkat edilmesi gerekmektedir.

İncelenen örnekler klor (Cl) elementi bakımından 0.08-8.51 me L^{-1} arasında değişim göstermiştir (Çizelge 3). Christiansen ve ark. (1977)'ye göre değerlendirildiğinde incelenen örneklerin %91.67'sinin 1.sınıf ve %8.33'ünün ise 3. sınıfta olduğu görülmüştür (Çizelge 4). Sulama sularında Cl en sorunlu anyon olarak kabul edilmektedir. 5 me L^{-1}'nin altındaki Cl konsantrasyonu içeren sularla duyarlı bitkilerin, 5-10 me L^{-1} arasında değerlere sahip sular ile orta hassas bitkilerin, 10 me L^{-1} üzerinde değerlere sahip sular ile dayanıklı bitkilerin sulanmasında sakınca olmadığı belirtilmiştir (Maas, 1990). Sudaki klorür, suda bulunan klorür tuzlarının ayrışmasından ve arıtılmış atık suyun klorlanmasından kaynaklanır. Yüksek klorür genellikle yüksek sodyum konsantrasyonu ile ilişkilidir. Klorür toprak tarafından emilmez, ancak yapraklarda birikerek kökler tarafından emildiği dolaşımdaki çözelti içinde kolayca hareket eder. Yüksek konsantrasyonlarda, nitratların emilimini ve organik asitlerin hücreler içinde ve arasında taşınmasını engelleyebilir. Klorürden kaynaklanan toksisite belirtileri, yaprak dokusunun yanması ve kuruması (uçlardan başlayıp kenarlar boyunca devam ederek), esmerleşme, erken sararma ve yaprak düşmesi olarak ortaya çıkar (De Pascale ve ark., 2013). Sera 5'in Cl sınıfının 3. sınıfta olduğu belirlenmiştir. İşletmede Nisan-Ağustos aylarında domates, Ekim-Mart dönemlerinde ise marul yetiştiriciliği yapılmaktadır. Domates bitkisinde verim kaybı oluşmaması için gerekli maksimum Cl düzeyi 875 ppm ve marulda ise 350 ppm olarak belirlenmiştir (De Pascale ve ark., 2013). Buna göre domates bitkisinin yetiştiriciliği için herhangi problem ortaya çıkmayacağı ancak sonraki dönemde yetiştirilen marul bitkisi için Cl'nin sorun oluşturabileceği söylenebilir. Buna göre işletmelerde verim kayıpları yaşanmaması için bitki özelliklerinin dikkate alınması yetiştiricilikte verim kayıplarının yaşanmaması bakımından son derece önemlidir.

Sülfat (SO_4) konsantrasyonları bakımından incelenen sulama suyu örneklerinde sülfat miktarı 0.003-5.42 me L^{-1} arasında değişim göstermiştir (Çizelge 3). Christiansen ve ark. (1977)'e göre sınıflandırıldığında sulama sularının %91.67'si 1. sınıfa, %8.33'ü ise 2. sınıfa girmektedir (Çizelge 4). Sülfür (S) ve klor (Cl), bitki büyümesi için gerekli elementlerdir. Bazı ürünler önemli miktarlarda kükürt giderir. Ancak, sulama suyunda bu elementin fazla miktarları mevcutsa, doğrudan toksisitenin bir sonucu olarak ürünlere zarar verebilir. Kükürt genellikle suda sülfat (SO_4) şeklinde bulunur. Bununla birlikte, indirgeyici ortamlarda sülfatlar, daha yüksek fitotoksik etkiye sahip olan sülfitlere (SO_3^-) dönüştürülebilir ve sülfürler demirin çökelmesine neden olarak bitkilerde toksisite semptomlarına yol açar (De Pascale ve ark., 2013). İncelenen işletmelerde genel olarak sülfat problemi olmadığı Sera 5'te ise kullanıma dikkat edilmesi gerektiği belirlenmiştir.

Sodyum Adsorpsiyon Oranı (SAR) değerleri (USSL, 1954)'e göre sınıflandırılmış olup miktarları 0.93-12.49 arasında (Çizelge 3) değişen örneklerin SAR açısından %91.67'si 1. sınıfta, %8.33'ü ise 2. sınıfta (Çizelge 4) yer almıştır. Suyun topraklarda oluşturacağı sodyum zararı, katyon derişimine bağlıdır. Sodyum içeriği yüksek ise sodyum zararı fazladır. Buna karşı, sulama suyunda kalsiyum ve magnezyumun uygun oranlarda bulunması, topraklarda istenen özelliklerin oluşmasını sağlar (Kanber & Ünlü, 2010). Sodyum (Na) topraktaki kolloidler tarafından emilir ve geçirgenlik üzerinde önemli etkileri ortaya çıkar. Ancak, suyun içerdiği sodyumun toprak tarafından etkin bir şekilde emilme riski, kalsiyum (Ca) ve magnezyum (Mg) varlığı ile azaltılır (De Pascale ve ark., 2013). Yapılan analizlerde SAR değerinin genel olarak 1. sınıfta yer aldığı (%91.67) ve herhangi bir sorun bulunmadığı ancak Sera 12'de dikkat edilmesi gerektiği belirlenmiştir.

İncelenen sulama sularının Artık Bikarbonat (RSC) değerleri -4.65 ile 1.11 me L^{-1} arasında değişmiştir (Çizelge 3). İncelenen örneklerin RSC değeri bakımından Eaton (1950)'in bildirdiği 1.25 me L^{-1} değerinden daha düşük düzeydedir. Sulama sularının RSC içeriği, toprakların fiziksel özelliklerini etkilemekte ve siyah alkali olarak tanımlanan sodyumlu toprakların oluşmasına neden olmaktadır (Kanber & Ünlü, 2010). Sulama sularında yüksek konsantrasyonda bikarbonat bulunursa, toprak çözeltisinin daha konsantre duruma gelmesi halinde kalsiyum ve magnezyum karbonat olarak çökelmeye başlar. Bu koşullarda, toprak çözeltisinin kalsiyum ve magnezyum konsantrasyonu azalır. Bu durumda sodyumun nispi oranı artar ve dominant hale geçerek sodyum zararına neden olur (Ayyıldız, 1990). Çalışmada işletmelerde artık bikarbonat değerlerine bakıldığında herhangi bir sorun bulunmadığı belirlenmiştir.

Sulama suyu örneklerinin %Na değerleri 21.28-92.27 me L^{-1} arasında (Çizelge 3) değişmekte olup,

Christiansen ve ark. (1977)'e göre örneklerin %58.33'ünün 1. sınıf, %33.33'ünün 2. sınıf ve %8.33'ünün 4. sınıfa dahil olduğu belirlenmiştir (Çizelge 4). Sulama suyunun kalitesini belirleyen sodyum ve buna bağlı olarak alkalilik oluşturma tehlikesi, sodyum katyonunun mutlak konsantrasyonu yanında, sodyumun diğer katyonların toplam konsantrasyonuna göre oransal miktarının yüksek olmasına da bağlıdır (Sönmez & Kaplan, 1996). Buna göre, sulama suyundaki sodyum miktarı düşük olsa da sodyumun diğer katyonların toplamına oranı yüksek bir değer oluşturuyorsa yine sodyum zararı ortaya çıkabilir. Bu nedenle sulama suyunda %Na değerlerinin, belirlenen sınır değerlerin üzerine çıkmaması gerekmektedir (Ayrancı, 2006). Sodyum fiziko-kimyasal yapısı nedeniyle topraklarda belirli bir değerin üzerine çıkması durumunda toprak yapısında dispersiyona neden olur. Değişebilir Na yüzdesi yüksek olan topraklar disperse (ayrışma) hale geçerek balçıklaşır ve geçirgenlikleri azalır (Kanber ve ark., 1992). Topraklı tarım serası olan Sera 4, Sera 5 ve Sera 10 da sulama suyu kaynaklı %Na zararının toprak ve bitki analizleri ile birlikte değerlendirilmesinin önemli olduğu belirlenmiştir.

Bor (B) konsantrasyonları bakımından incelenen sulama suyu örneklerinde bor değerleri 0.10-2.37 ppm arasında değişim göstermiştir (Çizelge 3). Christiansen ve ark. (1977)'ye göre incelenen örneklerin %83.33'ü 1.sınıf, %8.33'ü 2. sınıf ve %8.33'ü 4. sınıfta yer almaktadır (Çizelge 4). Bor elementi, bütün bitkilerin gereksinim duyduğu önemli bir mikro besin elementi olup çok küçük miktarlarda alınmaktadır. Yetersiz miktarda bor ile beslenme bitkinin hayati fonksiyonlarını azaltırken, normalin üzerinde bor ile beslenmesi durumunda toksik etkiye neden olabilmektedir. Bu nedenle bor elementinin 0.50 ppm'den yüksek konsantrasyonları, duyarlı bitkilerde önemli zararlar meydana getirirken, 1.0 ppm'den fazla bor içeriğine sahip suların sulama suyu olarak kullanılması durumunda bitki ve toprakta önemli zararlara neden olmaktadır (Uygan & Çetin, 2004). Bor (B) bitki yaşamı için gerekli bir elementtir, ancak çok düşük konsantrasyonlarda bile toksik olabilir. Genel olarak, toksik bor konsantrasyonları neredeyse sadece kurak bölgelerdeki topraklarda jeotermal ve volkanik bölgelerdeki kuyu ve kaynak sularında bulunurken, çoğu yüzey suyu kabul edilebilir seviyelerde bor içerir. Bu element ev deterjanlarında sodyum perborat şeklinde bulunduğundan, konut arıtma tesislerinden çıkan sulardan dolayı sulama suyunda önemli miktarlarda bor bulunabilir. Sulama suyunda 0.2-0.5 mg L^{-1} seviyeleri normal kabul edilir. Bununla birlikte, 0.3 mg L^{-1} değerinin üzerindeki seviyeler hassas bitkiler için zararlı olabilmektedir. Bor içeriği 4.0 mg L^{-1} değerinin üzerindeki sulama suyu hemen hemen tüm bitkiler için uygun değildir. Bitkiler, iki uç değer arasında değişen farklı tolerans seviyelerine sahiptir. Bor'un toksik etkileri ilk olarak yaşlı yapraklarda sararma, klorotik lekeler veya yaprağın uç ve kenarlarında kurumuş doku şeklinde kendini gösterir. Bitki yaşı ayrıca duyarlılığı veya sorunun boyutunu etkiler. Fideler genellikle aynı türden olgun bitkilerden daha hassastır. Su kaynağında yüksek düzeylerde olduğunda bor problemlerini en aza indirmeye yönelik yönetim stratejileri, gübre kaynaklarından bor elementini ortadan kaldırmayı, ortam pH'ını arttırmayı ve kalsiyum seviyesini arttırmayı içerir (De Pascale ve ark., 2013). Yapılan çalışmada Sera 9 da borun 4. sınıfta yer aldığı görülmüştür. İşletmede kuyu derinliği 120 m'dir. Sera işletmesinin bulunduğu yerin jeotermal yeraltı su kaynaklarının bulunduğu bölge üzerine kurulması nedeniyle bor konsantrasyonunun yüksek düzeyde olabileceği belirlenmiştir. Yüksek bor düzeyinin giderilmesi için ters ozmoz işleminin yapılması ve ters ozmozun verimliliğini arttırmak içinse suyun pH'sının hafif alkali olarak ayarlanması (pH 7.5) gereklidir (Will & Faust, 1999).

SONUÇ ve ÖNERİLER

Kırşehir ilinde sera işletmelerinde kullanılan sulama sularının kalitelerinin belirlenmesi amacıyla yürütülen çalışmada ilde yer alan 10 adet topraklı ve 2 adet topraksız tarım serasında olmak üzere 10 seranın toprak pH ve EC değerleri, 12 serada ise sulama suyu analizleri yapılmıştır.

İncelenen sera topraklarının genel olarak hafif alkalin topraklar olduğu ve pH değerlerinin yetiştiriciliği yapılacak bitki türüne göre değerlendirilmesi gerektiği belirlenmiştir. Toprakların elektriksel iletkenlik değerlerine bakıldığında, toprakların büyük bir kısmının tuzluluk göstermediği hafif ve orta tuzluluk gösteren seralarda sulama suyu tuzluluğu ve gübre kullanımına dikkat edilmesi gerektiği belirlenmiştir.

Çalışmada incelenen 1 işletmede sulama sularının C_1 (1. sınıf), 7 işletmede C_2 (2. sınıf) ve 4 işletmede C_3 (3. sınıf) sınıfında olduğu belirlenmiştir. SAR sınıflarına bakıldığında 11 işletmede S_1 (1. sınıf), 1 işletmede S_2 (2. sınıf) olduğu belirlenmiştir. Buna göre C_2S1 ve C_3S_1 sınıfına giren sulama suları, tuza orta veya yüksek oranda dayanıklı bitkiler için kullanılabilir. Özellikle C_3 sınıfına giren sulama sularına sahip işletmelerde drenaj sisteminde olamadığı düşünüldüğünde yıkama gereksiniminin ortaya çıkmasına neden olmaktadır. Topraksız tarım yapan seralarda bu sorun ters ozmoz uygulaması ile çözülsede sistemin maliyetli olması nedeniyle topraklı tarım yapan küçük işletmelerde bunun yerine yıkama yapılması ve mümkünse drenaj sistemlerinin tesis edilmesi ileriki yıllarda toprakta oluşacak tuzluluk probleminin önlenmesi açısından önemlidir. Ayrıca araştırma yapılan ilde jeotermal kaynakların bulunması nedeniyle ortaya çıkacak yüksek bor düzeylerine dikkat edilmesi önerilmektedir.

TEŞEKKÜR

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Araştırmacıların Katkı Oranı Beyan Özeti

Yazarlar makaleye eşit oranda katkı sağlamış olduklarını beyan eder.

Çıkar Çatışması Beyanı

Makale yazarları aralarında herhangi bir çıkar çatışması olmadığını beyan ederler.

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Gaziantep, Kahramanmaraş ve Adıyaman İllerinde Bademde Zararlı *Eurytoma amygdali* Enderlin (Hymenoptera: Eurytomidae)'nin Popülasyon Gelişimi

Hakan USANMAZ[1], Mahmut Murat ASLAN[2]

[1]Antepfıstığı Araştırma Enstitüsü, Gaziantep; [2]Kahramanmaraş Sütçü İmam Üniversitesi, Ziraat Fakültesi, Bitki Koruma Bölümü, Kahramanmaraş
[1]https://orcid.org/0000-0001-8187-3169, [2]https://orcid.org/0000-0002-4586-1301
✉: hakan.usanmaz@tarimorman.gov.tr

ÖZET

Bu çalışmada 2016-2017 yılları arasında Gaziantep, Kahramanmaraş ve Adıyaman illeri badem bahçelerinde ana zararlı durumunda bulunan badem iç kurdu *Eurytoma amygdali* End. (Hymenoptera: Eurytomidae)'nin popülasyon gelişiminin belirlenmesiergin çıkış seyrinin belirlenmesi amaçlanmıştır. Popülasyon takibi çalışmaları, Gaziantep (Oğuzeli, Şahinbey, Şehitkamil) Kahramanmaraş (Pazarcık) ve Adıyaman (Besni) illerinde iki yıl boyunca yürütülmüştür. Her ilçede seçilen bahçelere popülasyon takibi amacıyla şifon kafesler yerleştirilmiş olup, içerisine geçen yıldan kalan ve zararlı ile bulaşık olduğu tahmin edilen en az 200'er meyve bırakılmıştır. Şifon kafeslerin kontrolü mart ayının ortasından itibaren çalışma süresince haftalık olarak yapılmıştır. İlk erginlerin görülmesiyle birlikte, zararlının çıkış yapan erginleri ağız aspiratörü yardımıyla kafeslerden uzaklaştırılmış ve kayıt altına alınmıştır. Gaziantep'te zararlının ilk çıkışlarının nisan ayının ilk 3 haftası içerinde başladığı; Kahramanmaraş ili Pazarcık ilçesinde mart ayı sonu ile nisan ayının son haftası arasında gerçekleştiği ve Adıyaman ili Besni ilçesinde ise mart ayı sonu ile nisan ayının ilk haftasında zararlının erginlerinin doğada görülmeye başladığı belirlenmiştir. Ayrıca *E.amygali*'nin bölgede tek döl verdiği, popülasyonun ise en yüksek seviyesine ilk ergin çıkışından itibaren yaklaşık bir hafta içinde ulaştığı da bu çalışma ile tespit edilmiştir.

Entomoloji

Araştırma Makalesi

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Anahtar Kelimeler
Badem
Eurytoma amygdali
Badem iç kurdu
Popülasyon
Çıkış zamanı

Population Development of *Eurytoma amygdali* Enderlin (Hymenoptera: Eurytomidae) Harmful in Almonds in Gaziantep, Kahramanmaraş and Adıyaman Provinces

ABSTRACT

In this study, it was aimed to determine the population development of the almond seed wasp *Eurytoma amygdali* End. (Hymenoptera: Eurytomidae). Population follow-up studies were carried out regularly for two years in the provinces of Gaziantep (Oğuzeli, Şahinbey, Şehitkamil), Kahramanmaraş (Pazarcık) and Adıyaman (Besni). Chiffon cages have been placed in the selected orchards in each district for the purpose of monitoring the population, and at least 200 fruits from last year, which are estimated to be infested with pests, are left inside. Chiffon cages were checked weekly during the study, starting from mid-March. After the first adults were seen, the emerging adults of the pest were removed from the cages with the help of a mouth aspirator and recorded. In Gaziantep, the first emergence of the pest started in the first 3 weeks of April; It has been determined that it occurs between the end of March and the last week of April in Pazarcık district of Kahramanmaraş province, and the adults of the pest begin to appear in nature at the end of March and the first week of April in Besni district of Adıyaman province. In addition, it was determined that *E.amygali* gave only one offspring in the region, and the population reached its highest level in about a week from the first adult emergence.

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GİRİŞ

Türkiye sahip olduğu iklim koşulları ve toprak yapısı bakımından meyve yetiştiriciliği açısından önemli bir konuma sahiptir. Birçok meyve türünde olduğu gibi sert kabuklu meyve çeşitlerinin üretimi açısından oldukça büyük bir potansiyeli olan Türkiye badem, antepfıstığı, fındık ve ceviz gibi türlerin anavatanı durumundadır. Rosaceae familyasına bağlı Badem (Prunus dulcis Mill.), önemli sert kabuklu meyve türleri arasındadır. Bademin anavatanı Orta Asya ve Batı Asya'dır (Küden ve ark., 2000). FAO'nun son 10 yıllık verilerine göre dünyadaki badem üretim alanı, 2010 yılında 1.739.078 hektar iken, 2020 yılında bu rakam 2.162.263 hektara yükselmiştir. Toplam badem üretimi ise 2010 yılında 2.575.821 tondan 4.140.043 tona çıkmıştır. Dünya'da en fazla badem üretiminin yapıldığı ülkeler sıralamasında, 2020 yılında Türkiye 52.370 hektar alanda 159.187 ton badem üretimiyle beşinci sırada yer almaktadır (FAO, 2020).

Badem bahçelerinin sürekli artmasıyla birlikte bitki koruma sorunları da yıldan yıla artmaktadır. Badem üretiminde verim ve kaliteyi etkileyen birçok faktör vardır. Bu faktörlerin başında zararlılar -hastalıklar ile mücadele ve diğer kültürel işlemlerin doğru ve zamanında yapılmaması gelmektedir. Tüm kültürü yapılan bitki türlerinde olduğu gibi badem alanlarında da verim kayıplarına sebep olan pek çok böcek türü bulunmaktadır. Kaplan (2022), badem bahçelerinde ekonomik anlamda zararlı olan Tropinota hirta (Poda,) (Coleoptera: Cetoniidae)' nın farklı şekildeki tuzaklarda yakalanma durumunu belirlenmek amacıyla Mardin ilinde 2018 yılında bir çalışma yürütmüştür. Bolu ve ark. (2005), 2002-2004 yıllarında yürüttükleri çalışmada Güneydoğu ve Doğu Anadolu badem bahçelerinde bulunan böcek faunasını ortaya çıkartmak amacıyla bir çalışma yürütmüşlerdir. Elazığ, Diyarbakır ve Mardin illerinde bulunan badem bahçelerine mart-kasım ayları arasında sörveyler düzenlenmiştir. Yapılan bu sörveyler sonunda 11 takım, 56 familyaya ait toplam 205 böcek türü tespit etmişlerdir. Bu böceklerin erginleri ya da ergin öncesi dönemleri direk meyvede zarar oluşturduğu gibi ağacın kök, gövde gibi kısımlarında da zarar vererek önemli verim kayıplarına sebep olmanın yanında ağaçları tamamen kurutabilmektedir. Bu zararlı böcek türlerinin başında badem meyveleri içinde beslenerek direk meyve kayıplarına neden olan Badem içkurdu (*Eurytoma amygdali*) gelmektedir. *E. amygdali*' bademin yanısıra kayısı ve erik meyvelerinde de zarar yaptığı tespit edilmiştir (Baspinar ve ark., 2018). Zararlının erginlerinin badem meyvesi üzerinde açtıkları çıkış deliklerinden doğaya mart ayının sonu, nisan ayının başı gibi çıktığı tespit edilmiştir (Kouloussis ve Katsoyannis, 1993). Çıkış yapan ergin dişilerin çiftleştikten sonra yumurtalarını gelişmekte olan badem meyvelerini içine bıraktıkları ve yumurtadan çıkan larvaların badem meyvesi içinde embriyo kısmı ile beslenmek sureti ile zarar oluşturmaktadır (Tsourgianni,1989; Tzanakakis et al., 1991; Kouloussis, 2008; Meister, 2010).

E. amygdali'nin Dünyada badem üretiminin yapıldığı birçok ülkede verim kayıplarına sebep olduğu tespit edilmiştir. (Plaut, 1971; Talhouk, 1977; Zerova & Fursov, 1991; Kouloussis & Katsoyannos, 1995; Tenranifar et al., 2002). *Eurytoma amygdali* Dünya'da olduğu gibi Türkiye'de bulunan badem bahçelerinde ciddi verim kayıplarına neden olmakta ve badem üretimini mücadele yapılmadığı takdirde sınırlandırmaktadır.(Nizamlıoglu, 1962; Ekici & Günaydın, 1969; Doganlar ve ark. 2006; Barut, 2007; Yeşilyaprak, 2015). Mentjelos & Atjemis (1970) tarafından Yunanistan'da yürütülen çalışmada zararlının bulaşma oranının %35-79 arasında değiştiğini; Baktır & Aker (2015), Kıbrıs'ta zararlı yüzünden meydana gelen meyve kaybının % 40'ların üzerinde olduğunu; Cakar (1980) ise, Makedonya'da badem meyvelerinde oluşan zararı % 71 olarak tespit etmişlerdir. Bolu & Özgen (2007), Güneydoğu Anadolu ve Doğu Anadolu bölgelerinde bulunan Mardin, Elazığ ve Diyarbakır illerinde *E. amygdali*'nin bulaşma oranının belirlenmesi için bir çalışma yürütmüşlerdir ve zararlının genel bulaşma oranının %30 ile %60 arasında değiştiğini belirlemişlerdir. Yiğit ve ark. (2020); *Eurytoma amygdali* Enderlein ile Malatya ili badem bahçelerinde yapmış olduğu çalışmada; Ferragnes çeşidinde 81%, Cristomorto çeşidinde 85%, Nonpareil çeşidinde ise %97 oranında bulaşıklık tespit etmişlerdir. Bu ilde, zararlının Nisan ayının sonunda sıcaklığın 25 °C'ye çıktığında çıkış yaptığını belirlemişlerdir.

Zararlının neden olduğu zarar oranının en aza indirilmesi ve zararlı ile bulaşık meyvelerin bahçeden uzaklaştırılması zamanında kimyasal mücadele ile mümkün olmaktadır. Bu sebeple yürütülen bu çalışmada bademin önemli zararlılarından biri olan *E. amygdali*'nin mücadele zamanının tespit edilmesi amacıyla; Gaziantep, Kahramanmaraş (Pazarcık) ve Adıyaman (Besni) illerinde bulunan yetişkin badem bahçelerindeki çıkış zamanları ve popülasyon gelişimleri 2 yıl boyunca takip edilmiştir. Böylece; ilgili lokasyonlarda kimyasal mücadelenin yapılması gereken en doğru zaman ortaya konmuştur.

MATERYAL ve METOT

Çalışmanın ana materyalini; Gaziantep (Oğuzeli, Şahinbey, Şehitkamil ilçeleri) Kahramanmaraş (Pazarcık) ve Adıyaman (Besni) illerinde bulunan badem bahçeleri, bu bahçelerden elde edilen *E. amygali*'nin ergin dönemlerine ait örnekler, ağız aspiratörü, öldürme şişeleri, fotoğraf makinesi, budama makası, çerçevesi tahta kenarları tül olan kültür kafesleri (80X150 cm) ile iklim veri kayıt cihazı (Hobo marka) materyal olarak kullanılmış olup günlük maksimum sıcaklık, günlük minimum sıcaklık ve günlük ortalama nem gibi verilerden yararlanılmıştır.

Gaziantep ilinin Şahinbey, Şehitkamil ve Oğuzeli ilçeleri ile Kahramanmaraş ilinin Pazarcık ilçesinde ve Adıyaman ilinin Besni ilçesinde bulunan badem bahçelerinde 2016-2017 yılları arasında *E. amygdali*'nin ilk çıkış zamanını ve popülasyon gelişimini takip etmek için her il için seçilen 3'er bahçede çalışmalar yürütülmüştür. Bahçelerin mümkün olduğu kadar farklı lokasyonlardan seçilmesine özellikle dikkat edilmiştir. Gaziantep ilinde seçilen bahçeler Ferragnes-Ferraduel çeşitlerini içeren 15-20 yaşında ağaçlardan oluşan ve sulama imkanına sahip bahçelerken; Kahramanmaraş Pazarcık ilçesindeki bahçeler 10-15 yaşlarında Teksas, Nonpareil, Ferragnes, Ferraduel çeşitlerine sahip ve sulama imkanı olmayan bahçelerdir. Besni ilçesinde ise bahçelerden ikisi 10-20 yaşlarında Nonpareil, Ferragnes, Ferraduel çeşitleriyle kurulmuş ve sulama imkanı olmayan bahçelerken; diğeri ise tescilli bir çeşit ile kurulmamış ve sulama imkanı olmayan, 14 yaşında bir bahçedir.

E. amygdali'nin ilk ergin çıkışını ve ergin çıkış seyrinin belirlenmesi amacıyla bahçe içerisine ağaçın güneydoğu yönüne 40 x 50 x 50 cm boyutlarında alt tarafı sunta, etrafı tül ile kaplı şifon kafesler kullanılmıştır. Bu kafeslere zararlı ile bulaşık olduğu tahmin edilen en az 200'er adet kararmış meyve konularak mart ayının ortasından itibaren haftada bir kez kontrol edilmiştir. İlk ergin çıkışı tespit edildikten sonra çıkışlar düzenli olarak kaydedilmiştir. Çıkış yapan zararlının erginleri haftalık olarak ağız aspiratörü yardımı ile kafes içinden uzaklaştırılmıştır. Çalışma başlangıcında her bahçeye birer adet iklim kaydedici hobo yerleştirilerek sıcaklık ve orantılı nem değerlerinin kaydedilmesi sağlanmıştır.

BULGULAR ve TARTIŞMA

Eurytoma amygdali'nin Gaziantep İlindeki Popülasyon Değişimi

Gaziantep ilinde Oğuzeli, Şehitkamil, Şahinbey ve ilçelerinde bölgeyi en iyi şekilde temsil edecek 1'er bahçede popülasyon takibi çalışmaları yürütülmüştür. 2016 yılında Oğuzeli ilçesinde ilk ergin *E. amygdali* çıkışı 30 mart tarihinde gerçekleşmiştir. Bu dönem badem meyvelerin mercimek tanesi kadar olduğu döneme denk gelmiştir. Maksimum ergin çıkışları nisan ayının ilk haftasında gerçekleşmiş ve ergin çıkışlar nisan ayının son haftasına kadar devam etmiştir. İlk ergin çıkışının başladığı günlerde maksimum sıcaklıklar 26-27 °C arasında gerçekleşirken, minimum sıcaklıklar 6-11°C arasında, ortalama nispi nem ise %32-34 arasında değişmektedir. Bir sonraki yıl ise ilk ergin *E.amygdali* çıkışı 17 nisan tarihinde başlamıştır. Maksimum ergin çıkışları 37 adet ergin ile 24 nisan tarihinde gerçekleşmiş ve ergin çıkışları mayıs ayının ortasına kadar devam etmiştir. Zararlın popülasyon takibinde tek tepe noktası oluşturduğu ve zararlının tek döl verdiği görülmüştür. İlk ergin uçuşlarının başladığı günlerde maksimum sıcaklıklar 21-22 °C arasında değişmekte olup popülasyonun maksimum seviyeye ulaştığı günlerde ise 28-30 C° arasında değişmektedir (Şekil 1). İki yıl arasında ergin çıkış tarihleri arasında yaklaşık 2 haftalık bir fark görülmektedir. Bu fark yıllar arasındaki iklim değişikliğinden kaynaklanmaktadır.

Şahinbey ilçesine ait 2 yıllık popülasyon gelişimi Şekil 2'de verilmiştir. Çalışmanın ilk yılında erginlerin nisan ayının ilk haftasında doğada görülmeye başladığı tespit edilmiştir. Popülasyon tepe noktasına 11 nisan tarihinde ulaşmış olup, ergin çıkışları mayıs ayının başında son bulmuştur. Ergin çıkışının başladığı günlerde maksimum sıcaklıklar 26-27 °C seviyelerinde, minimum sıcaklıklar ise 6-10 °C arasında değişmektedir. Çalışmanın ikinci yılında ise ilk ergin çıkışı 17 nisan tarihinde kayıt altına alınmış olup, 1 hafta sonra ergin çıkışları en yüksek seviyeye ulaşmıştır. 15 Mayıs tarihinde ise çıkışlar sona ermiştir. Ergin çıkışının başladığı günlerde maksimum sıcaklıklar 21-26 °C arasında değişmekte, minimum sıcaklıklar 8-10°C arasında ve ortalama nispi nem ise % 60 değerlerindedir.

Şehitkamil ilçesinde 2016 yılında ilk ergin çıkışı nisan ayının başında gerçekleşmiştir. Zararlının popülasyonu maksimum seviyeye 8 nisan tarihinde ulaşmış ve çıkışlar nisan ayının sonuna kadar devam etmiştir (Şekil 3). Ergin çıkışlarının başladığı günlerde günlük maksimum sıcaklıklar 26-27 °C arasında değişmiştir. Bir sonraki yıl ise ilk ergin çıkışı 19 nisan tarihinde görülmüş, popülasyon tepe noktasına 26 nisan tarihinde ulaşmıştır. Zararlı mayıs ayının ortalarına kadar doğada görülmeye devam etmiştir (Şekil 3). Ergin çıkılarının başladığı günlerde günlük maksimum sıcaklıklar 22-23 °C arasında değişmiştir.

Gaziantep ilinde *E.amygdali*'nin 2016 ve 2017 yıllarına ait popülasyon gelişiminde zararlının ilk çıkışlarının mart ayının sonu ile nisan ayının ikinci haftası arasında gerçekleştdiği ortaya konmuştur. Ancak 2016 yılında erginlerin doğada görülme zamanı ile 2017 yılında doğada görülmeye başlama zamanı arasında yaklaşık 1-2 haftalık bir fark söz konusu olmuştur. Bu durumumun iki yıl arasında gerçekleşen

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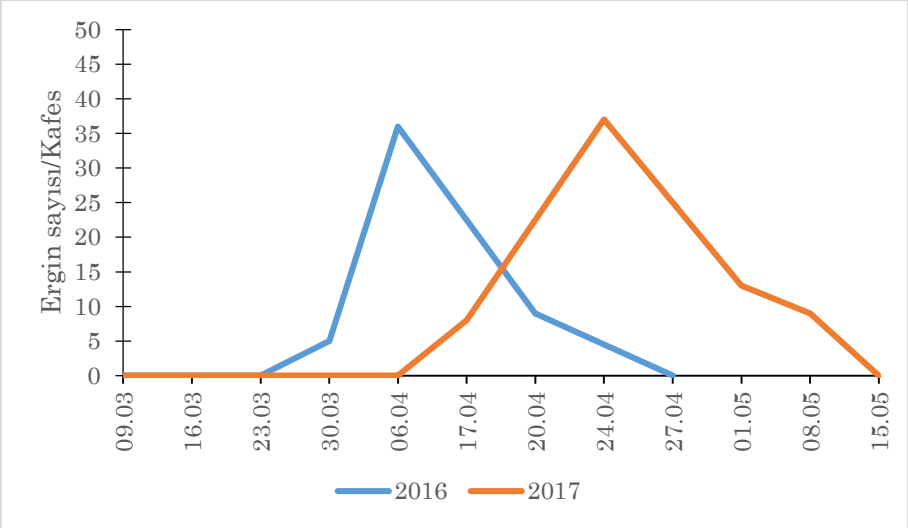
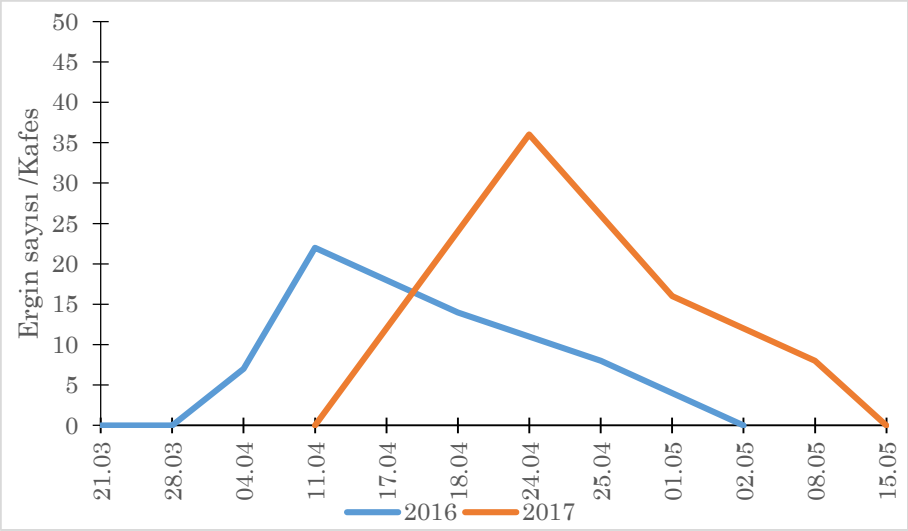
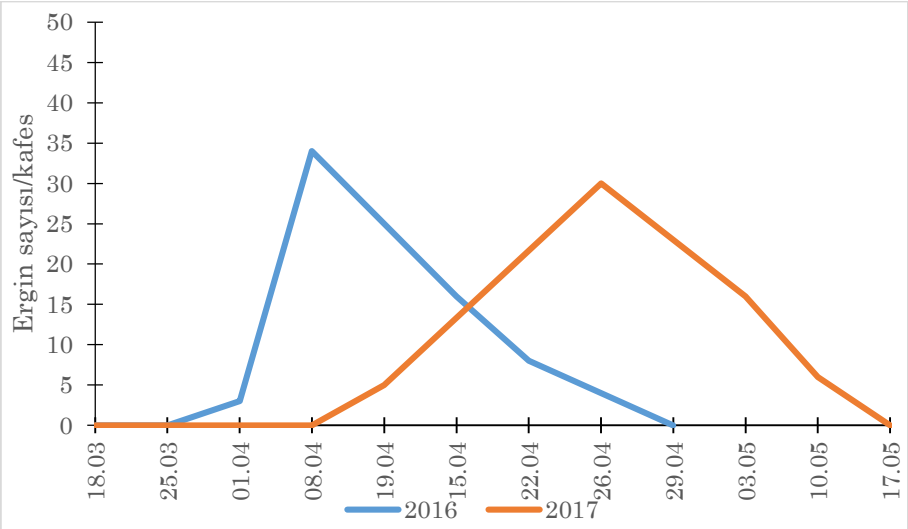


Şekil 1. *Eurytoma amygdali*'nin Gaziantep ilinin Oğuzeli ilçesindeki ergin çıkış seyri (2016-2017)
Figure 1. Adult emergence of *Eurytoma amygdali* in Oğuzeli district of Gaziantep province (2016-2017)



Şekil 2. *Eurytoma amygdali*'nin Gaziantep ilinin Şahinbey ilçesindeki ergin çıkış seyri (2016-2017)
Figure 2. Adult emergence of Eurytoma amygdali in Şahinbey district of Gaziantep province (2016-2017)



Şekil 3. *Eurytoma amygdali*'nin Gaziantep ilinin Şehitkamil ilçesindeki ergin çıkış seyri (2016-2017)
Figure 3. Adult emergence of *Eurytoma amygdali* in Şehitkamil district of Gaziantep province (2016-2017)

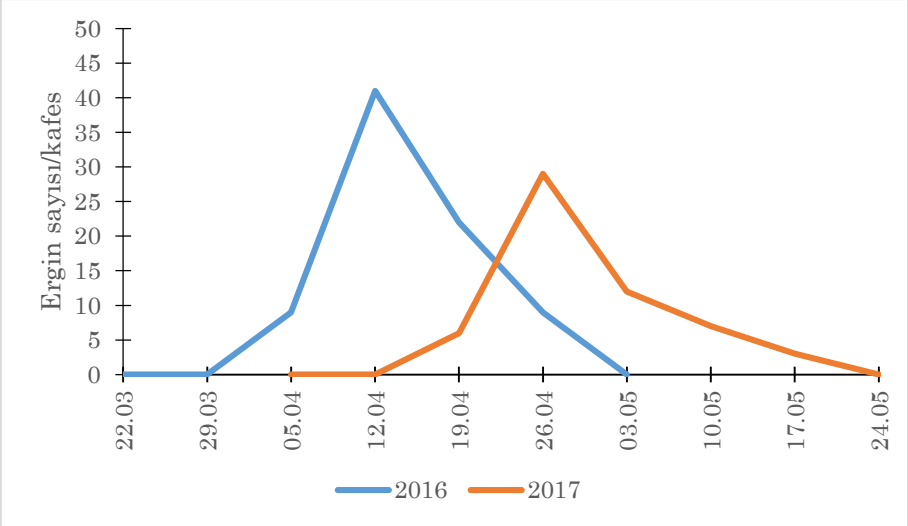
ekolojik farklılıklardan kaynaklandığı düşünülmektedir. Çalışmanın yürütüldüğü 3 bahçede de ilk çıkış tarihinden itibaren ortalama 1 hafta içinde popülasyonun maksimum seviyeye ulaştığı belirlenmiştir. Ayrıca zararlının yılda 1 döl verdiği popülasyon takibi çalışmaları sonucu tespit edilmiştir

Tolga (2019), yürüttüğü çalışmada Muğla iline bağlı Datça ilçesinde *E.amygdali*'nin ilk uçuşlarına 2014 yılında 14 mart tarihinde başladığını; 2015 yılında ise 18 mart tarihinde başladığını bildirmiştir. Ayrıca zararlının popülasyonunun 21.03.2014 ve 03.04.2015 tarihlerinde en yüksek seviyeye ulaştığını belirterek, maksimum ergin çıkışlarının ilk ergin çıkışından yaklaşık bir hafta içinde gerçekleştiğini tespit etmiştir. Datça ilçesi ile bu çalışmanın yapıldığı bölge arasında görülen ergin çıkış tarihleri arasındaki yaklaşık 1-2 haftalık farkın ekolojik faktörlerden kaynaklandığı sanılmaktadır. Dünyada ise Katsoyannos et al. (1992), 1986-1989 tarihleri arasındaki 4 yıl süresince Yunanistan'ın Selanik bölgesinde yürüttükleri çalışmada, Retsou badem çeşidindeki zarar görmüş bademlerden ergin çıkışını takip etmişlerdir. Çalışmanın başlangıcından itibaren yakalanan erginlerin ilk olarak 13 – 24 Nisan tarihleri arasında ortaya çıktığı ve maksimum seviyeye 17 Nisan - 11 Mayıs tarihleri arasında ulaştığını belirlemişlerdir. Bu iki çalışma ile elde edilen sonuçların örtüştüğü görülmektedir.

Eurytoma amygdali'nin Kahramanmaraş İlindeki Popülasyon Değişimi

Kahramanmaraş ilinde Pazarcık ilçesinde Yumaklıcerit, Bölükçam ve İncirli yörelerinde bölgeyi en iyi şekilde temsil edecek 1'er bahçede çalışmalar yürütülmüştür.

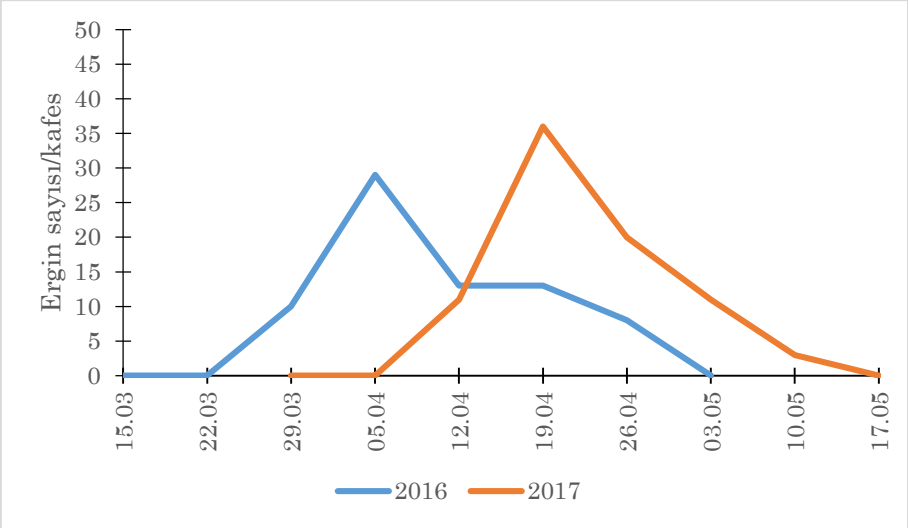
Pazarcık/Yumaklıcerit yöresinde 2016 yılında ilk ergin çıkışı 5 nisan tarihinde gerçekleşmiştir. Popülasyon maksimum seviyesine 12 nisan tarihinde ulaşmış ve çıkışlar 3 hafta boyunca devam etmiştir (Şekil 4). İlk ergin çıkışının gerçekleştiği günlerde maksimum sıcaklıklar 25-30 °C arasında değişmektedir. Bir sonraki yıl ise ilk erginler doğada nisan ayının 3. haftası görülmeye başlamış olup, maksimum ergin çıkışları 26 nisan tarihinde gerçekleşmiştir. Ergin çıkışları 4 hafta boyunca devam etmiştir (Şekil 4). İlk ergin çıkışının gerçekleştiği günlerde maksimum sıcaklıklar 23-29 °C arasında değişmekte ve çıkışların maksimum seviyeye ulaştığı günlerde ise 30°C civarlarındadır. Zararlının çıkışları 2016 yılında 3 hafta sürerken; 2017 yılında ise 4 hafta sürmüştür.



Şekil 4. *Eurytoma amygdali*'nin Kahramanmaraş ilinin Pazarcık/Yumaklıcerit yöresindeki ergin çıkış seyri (2016-2017)

Figure 4. Adult emergence of Eurytoma amygdali in Pazarcık/Yumaklıcerit district of Kahramanmaraş province (2016-2017)

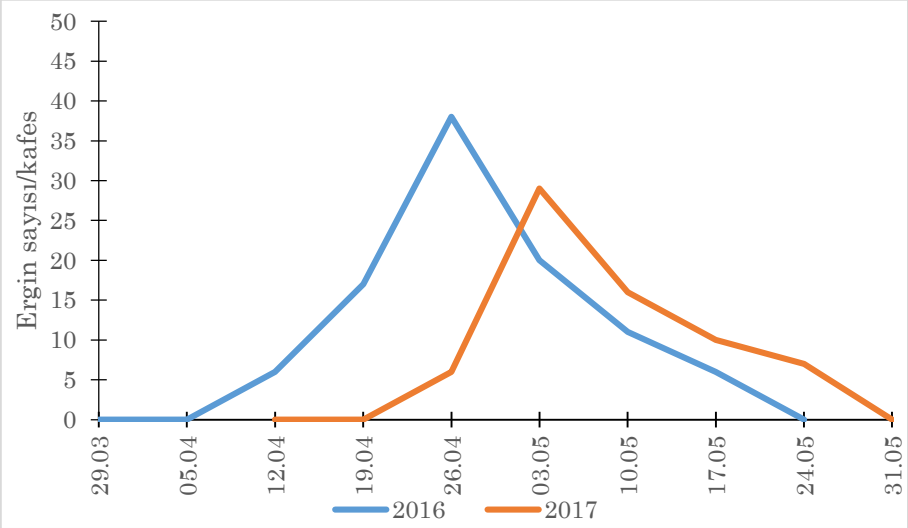
Pazarcık/Bölükçam yöresinde 2016 yılında ilk erginler mart ayının son haftasında görülmüştür. Popülasyon en yüksek seviyesine 5 nisan tarihinde ulaşmış olup, çıkışlar mayıs ayının ilk haftasına kadar devam etmiştir (Şekil 5). İlk ergin uçuşlarının başladığı günlerde maksimum sıcaklık değerleri 28-30°C arasında, minimum sıcaklık değerleri 3-6 °C arasında, ortalama nispi nem ise % 49-54 arasında değişmektedir. Sonraki yılda ise ilk ergin çıkışı 12 nisan tarihinde gerçekleşmiştir. Maksimum ergin çıkışları nisan ayının 3. haftasında gerçekleşmiş olup; mayıs ayının ortasına kadar zararlının erginleri görülmeye devam etmiştir. İlk ergin çıkışının başladığı günlerde maksimum sıcaklık değerleri 24-27 °C arasında değişmektedir. Her iki yılda da ergin çıkışları maksimum seviyeye yaklaşık 1 hafta içinde ulaşmıştır.



Şekil 5. *Eurytoma amygdali*'nin Kahramanmaraş ilinin Pazarcık/Bölükçam yöresindeki ergin çıkış seyri (2016-2017)

Figure 5. Adult emergence of Eurytoma amygdali in Pazarcık/Bölükçam district of Kahramanmaraş province (2016-2017)

Pazarcık/İncirli yöresinde 2016 yılında ilk ergin çıkışı 12 nisan tarihinde gerçekleşmiştir. Ergin çıkışları maksimum değerine nisan ayının son haftasında ulaşmış ve çıkışlar 24 mayıs tarihinde son bulmuştur (Şekil 6). İlk ergin çıkışının gerçekleştiği günlerde günlük maksimum sıcaklıklar 28-30 °C'ler arasında değişmektedir. Sonraki yıl ise İncirli'de ilk erginlere nisan ayının son haftasında rastlanılmıştır. Ergin çıkışları maksimum değerine 3 mayıs tarihinde ulaşmış ve çıkışlar mayıs sonuna kadar devam etmiştir. İlk ergin çıkışının gerçekleştiği günlerde günlük maksimum sıcaklıklar 22-28 °C'ler arasında değişmektedir.



Şekil 6. *Eurytoma amygdali*'nin Kahramanmaraş ilinin Pazarcık/İncirli yöresindeki ergin çıkış seyri (2016-2017)

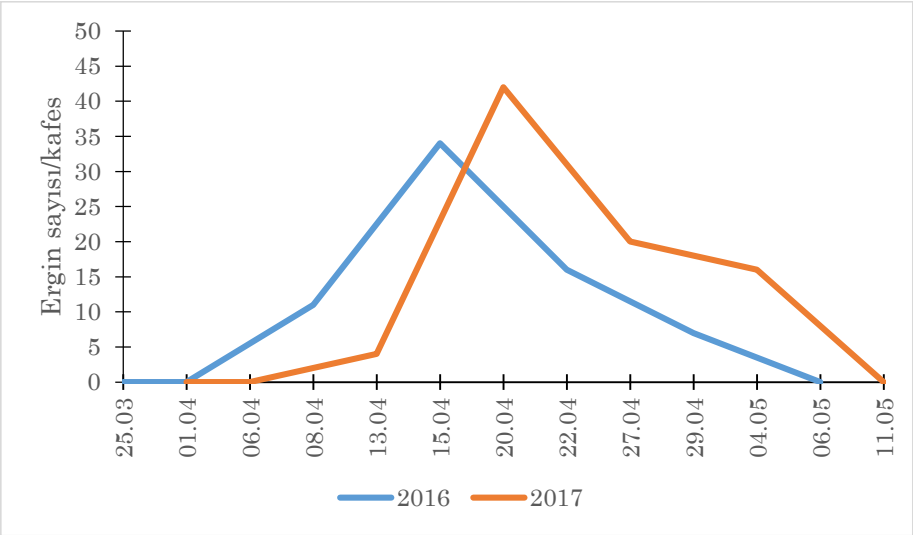
Figure 6. Adult emergence of Eurytoma amygdali in Pazarcık/İncirli district of Kahramanmaraş province (2016-2017)

Pazarcık ilçesinde 3 farklı yörede 2016-2017 yıllarında yürütülen popülasyon takibi çalışmalarna göre *E. amygdali*'nin ilk ergin çıkışları Mart ayı sonu ile Nisan ayı sonu arasındaki sürede gerçekleşmiştir. Çalışmanın yürütüldüğü bölgede ergin çıkışları her iki yılda da İncirli yöresindeki bahçede diğer bahçelere göre daha geç görülmüştür. Bu durumun İncirli'deki popülasyon takibinin yapıldığı bahçenin rakımının yüksek olması ve dağlık bir alanda yer almasının rol oynadığı düşünülmektedir. Barut (2007), Kahramanmaraş (Merkez)'da *E. amygdali*'nin 2007 yılında ilk ergin çıkışını 15.04.2007 tarihinde gerçekleştirdiğini ve çıkışların 19.05.2007 tarihine kadar devam ettiğini tespit etmiştir. Popülasyonun

maksimum seviyeye ise 7 gün içinde ulaştığında ayrıca ortaya koymuştur. Arambourg ve ark. (1985), ise *E. amygdali*'nin Doğu Avrupa ve Orta Doğuda ilk kez Fransa'da Bouches - du – Rhane eyaletinde badem üzerinde kaydetmişlerdir. Ayrıca zararlının yılda 1 döl verdiğini, populasyonun şubat–mart aylarında oluştuğunu ve erginlerin nisan ayında çıktığını tespit etmişlerdir. Söz konusu çalışmalar ile bu bölgede elde edilen sonuçlar birbiriyle paralellik göstermektedir.

Eurytoma amygdali'nin Adıyaman İlindeki Popülasyon Değişimi

Adıyaman ili Besni ilçesinde Köseceli beldesi, Tekağaç ve Konuklu köylerinde bölgeyi en iyi şekilde temsil edecek şekilde 1'er bahçede çalışmalar yürütülmüştür. Besni/Köseceli beldesinde 2016 yılında ilk ergin çıkışları 8 nisan tarihinde gerçekleşmiştir. Popülasyon yoğunluğu en yüksek seviyesine yaklaşık bir hafta sonra ulaşmış; zararlının erginleri mayıs ayının ilk haftasına kadar uçuşlarına devam etmiştir (Şekil 7). İlk ergin çıkışlarının başladığı günlerde maksimum sıcaklık değerleri 26-28 °C arasında, minimum sıcaklıklar 6-11 °C arasında ve ortalama nispi nem ise % 48-70 arasında değişmektedir. Popülasyonun maksimum seviyeye ulaştığı günlerde ise maksimum sıcaklık değeri 30 °C, minimum sıcaklık değeri 11°C ve ortalama nispi nem ise %30 civarlarındadır. Çalışmanın ikinci yılı olan 2017 yılında ilk ergin çıkışı nisan ayının 2. haftasının sonlarında gerçekleşmiştir. Ergin çıkışları maksimum değerine 20 nisan tarihinde ulaşmış olup, ergin çıkışları 11 mayıs tarihine kadar devam etmiştir. İlk ergin çıkışlarını başladığı günlerde maksimum sıcaklık değerleri 26-28 °C arasında, minimum sıcaklık 6-10 °C arasında ve ortalama nisbi nem değerleri ise % 45-60 arasında değişmektedir.



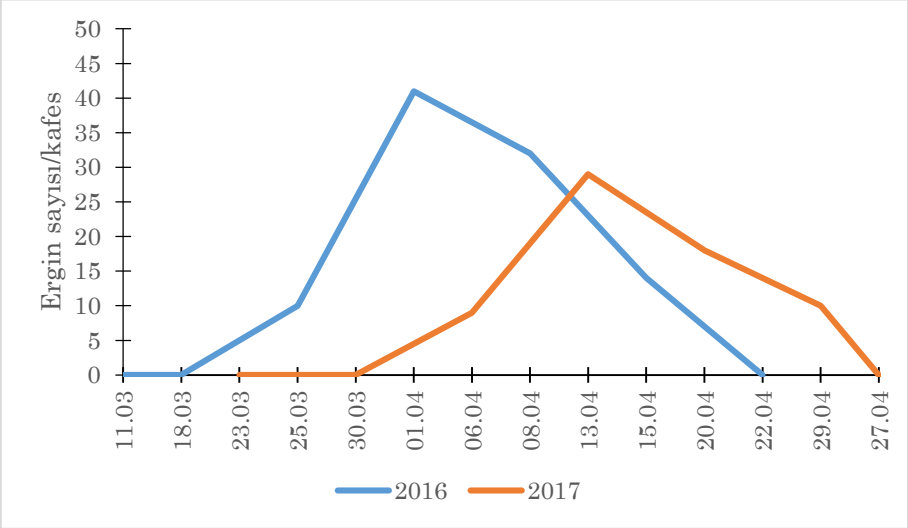
Şekil 7. *Eurytoma amygdali*'nin Adıyaman ilinin Besni/Köseceli yöresindeki ergin çıkış seyri (2016-2017)
Figure 7. Adult emergence of Eurytoma amygdali in Besni/Köseceli district of Adıyaman province (2016-2017)

Besni ilçesine bağlı Konuklu köyünde 2016 yılında ilk ergin uçuşlarının 25 mart tarihinde gerçekleştiği; maksimum ergin çıkışlarının ise nisan ayının ilk günlerinde görüldüğü tespit edilmiştir. Ayrıca ergin çıkışları 22 nisan tarihine kadar devam etmiştir (Şekil 8). İlk ergin çıkışlarının başladığı günlerde maksimum sıcaklıklar 27-29 °C arasında, minimum sıcaklıklar ise 9-11°C arasında değişmektedir. Popülasyon takibinin 2. yılında ise ilk ergin uçuşları nisan ayının ilk haftasında başlarken, maksimum ergin çıkışları bir hafta içinde gerçekleşmiştir. Ergin çıkışları mayıs ayının başına kadar devam etmiştir. İlk ergin çıkışlarının başladığı günlerde maksimum sıcaklıklar 24-28 °C arasında değişmektedir.

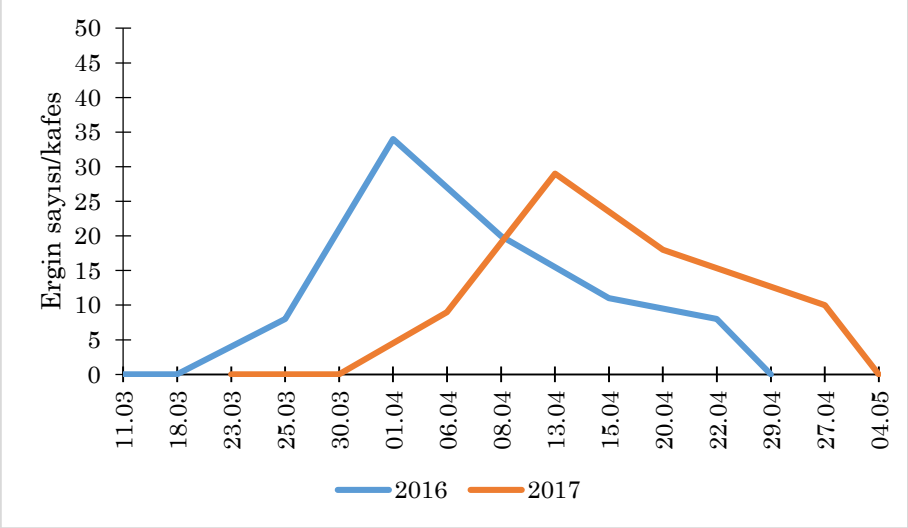
Besni ilçesi Tekağaç köyünde 2016 yılında ilk ergin çıkışı mart ayının son haftası görülmüştür. Popülasyon en yüksek seviyesine nisan ayının başında ulaşmış, ergin çıkışları ise 29 nisan tarihinde kadar sürmüştür (Şekil 9). İlk ergin çıkışlarının başladığı günlerde maksimum sıcaklık değerleri 26-25 °C arasında değişmektedir. Bir sonraki yıl ise ergin çıkışı 06 nisan tarihinde gerçekleşmiştir. Maksimum ergin çıkışları ise nisan ayının 2. haftasında görülmüş olup; zararlının ergin çıkışları mayıs ayının ilk haftasına kadar sürmüştür. İlk ergin çıkışlarının başladığı günlerde maksimum sıcaklık değerleri 26-25 °C arasında değişmektedir.

Besni ilçesinde elde edilen sonuçlara göre Konuklu ve Tekağaç köylerinde *E. amygdali*'nin ergin çıkışlarının aynı dönemde başladığı, Köseceli beldesinde ise bu bölgelerden yaklaşık bir hafta sonra ilk ergin uçuşlarının gerçekleştiği belirlenmiştir. Besni'de ilk ergin çıkışlarının Mart ayı sonu ile Nisan ayının ilk haftası içinde başladığı tespit edilmiştir. Yeşilyaprak (2015), Şanlıurfa ilinde badem bahçelerinde yürüttüğü çalışmada Bozova ve Karaköprü ilçelerinde *E. amygdali*'nin ilk ergin çıkışlarının nisan ayının ikinci haftasında görüldüğünü ve en yüksek ergin çıkışının

bundan 1 hafta sonra gerçekleştiğini belirlemiştir. Ayrıca Duval & Milan (2010), Fransa'da *E. amygdali*'nin ergin çıkışının mart ayının ortasında başlayıp mayıs ayı sonlarına kadar sürdüğünü belirlemişlerdir. Yürütülmüş olan bu çalışmalar arasındaki farkın ekolojik farklılıklar sebebiyle meydana geldiği düşünülmektedir.



Şekil 8. *Eurytoma amygdali*'nin Adıyaman ilinin Besni/Konuklu yöresindeki ergin çıkış seyri (2016-2017)
Figure 8. Adult emergence of *Eurytoma amygdali* in Besni/Konuklu district of Adıyaman province (2016-2017)



Şekil 9. *Eurytoma amygdali*'nin Adıyaman ilinin Besni/Tekağaç köyünde ergin çıkış seyri (2016-2017)
Figure 9. Adult emergence of *Eurytoma amygdali* in Besni/Tekağaç district of Adıyaman province (2016-2017)

SONUÇ ve ÖNERİLER

Gaziantep, Kahramanmaraş ve Adıyaman illerinde yürütülen bu çalışmada badem bahçelerinde önemli verim kayıplarına neden olan *E. amygdali*'nin doğada Nisan ayı başından itibaren görülmeye başladığı ve çıkışlarının mayıs ayı sonlarına kadar devam edebildiği gözlemlenmiştir. Popülasyonun en yüksek seviyesine iklim koşullarına bağlı olarak 1 hafta içinde ulaştığı tespit edilmiştir. Ancak zararlının badem meyvelerinin meyve iç kabuğu sertleştikten sonra yumurtasını meyve içine bırakamadığı belirlenmiştir. İlk ergin çıkışından itibaren 7-10 gün sonra çıkışların maksimum seviyeye ulaştığı ve çıkışların 3-4 hafta devam edebildiği de saptanmıştır. Üreticilerin badem ağaçları çiçeklerini döktükten sonra zararlıya karşı yoğun bir ilaçlama yapmaya başladığı ve meyve kabuğunun sertleştiği dönemden sonra bile ilaçlamalarına devam ederek çevreye ciddi zararlar verdikleri de görülmüştür. Zararlı ile mücadele zamanının ilaçlama başarısında önemli bir yeri olduğu için bahçelerde yapılacak popülasyon takibine bağlı olarak mücadele zamanının tayin edilmesi tavsiye edilmektedir. Tüm bunların yanında zararlının bahçedeki popülasyonunu azaltmak için badem ağaçları üzerinde bir önceki yıldan kalan ve zararlının kışladığı kararmış badem meyvelerinin bahçelerden uzaklaştırılması verim kayıplarının azaltılması açısından fayda sağlayacaktır.

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Araştırmacıların Katkı Oranı Beyan Özeti

Yazarlar makaleye eşit oranda katkı sağlamış olduklarını beyan eder.

Çıkar Çatışması Beyanı

Makale yazarları aralarında herhangi bir çıkar çatışması olmadığını beyan ederler.

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Laktik Asit Bakteri İnokulasyonu Uygulanan Kuşkonmaz Bitkisinden Silo Yemi Olarak Yararlanma Olanakları

Fatma AKBAY[1], Tuğba GÜNAYDIN[2], Seda ARIKAN[3], Mustafa KIZILŞİMŞEK[4]✉
[1,2,3,4] Kahramanmaraş Sütçü İmam Üniversitesi, Ziraat Fakültesi, Tarla Bitkileri Bölümü, Kahramanmaraş
[1]https://orcid.org/0000-0002-0156-9974, [2] https://orcid.org/0000-0002-4458-1287, [3]https://orcid.org/0000-0002-7545-8660
[4]https://orcid.org/0000-0002-0295-0603
✉: mkizil@ksu.edu.tr

ÖZET

Kuşkonmaz (*Asparagus officinalis* L.) bitkisi, *Asparagaceae* familyasına giren ve içerisinde yaklaşık 300 tür barındıran *Asparagus* cinsine ait bir tür olup, kültürü yapılan ve ekonomik değeri yüksek olan bir sebzedir. Kuşkonmaz bitkisinin hayvan yemi olarak kullanımı ile ilgili çalışmalar oldukça kısıtlıdır. Kuşkonmaz bitkisi ilkbaharda taze sebze olarak hasadı yapıldıktan sonra gelişmeye bırakılır ve bitkilerin kış dinlenmesine girmesinden önce gelişen vejetatif aksamı hasat edilir. Bu çalışmada, söz konusu vejetatif aksamın silaj yapımı olanakları araştırılmıştır. Çalışmada, kuşkonmaz bitkisinin gelişme dönemi sonunda biçilen sürgünleri kullanılıp, bu sürgünler teorik olarak 2-3 cm ebadında parçalanmış daha önceden bir TUBİTAK projesi sonucu elde edilen 5 adet laktik asit bakterisi (LAB) ile aşılanarak silolanmıştır. Araştırmadan elde edilen sonuçlara göre; 879.92 kg da^{-1} yeşil ot verimi ve 324.60 kg da^{-1} kuru ot verimi ile alternatif bir kaba yem kaynağı olabileceği, potansiyel beslenme değerinin ruminant hayvanlar için uygun olduğu belirlenmiştir. Diğer yandan, LAB katkısı kontrol grubuna göre silajların pH, asetik asit, propiyonik asit içeriklerini ve kuru madde kaybını düşürürken, laktik asit içeriklerini ise önemli düzeyde arttırmıştır. Özellikle silaj kalitesini iyileştirmede, LS-65-2-1 kod numaralı *L. bifermentas* izolatının ön plana çıktığı belirlenmiştir

Tarla Bitkileri

Araştırma Makalesi

Makale Tarihçesi
Geliş Tarihi : 28.12.2002
Kabul Tarihi : 07.03.2023

Anahtar Kelimeler
Alternatif yem
Fermantasyon ürünleri
Inokulant
Silaj mikrobiyolojisi

Usage Opportunity of *Asparagus officinalis* L. Inoculated with Lactic Acid Bacteria as Silage Feed

ABSTRACT

Asparagus (*Asparagus officinalis* L.) plant is a species belonging to the Asparagus genus, and contains about 300 species It is a cultivated vegetable with high economic value. Studies on its use as animal feed are very limited. After the asparagus plant is harvested as a fresh vegetable in the spring, it is left to develop and the vegetative parts of the plants are harvested before they go into winter dormancy. In this study, the silage making possibilities of the vegetative part were investigated. The vegetative parts of the asparagus plant, which were cut as forage at the end of the growing period, were used and the forage were inoculated with 5 lactic acid bacteria (LAB), obtained from a TUBITAK project, for making silage. It has been determined that Asparagus can be an alternative roughage source with 879.92 kg da-1 green forage yield and 324.60 kg da^{-1} dry matter yield. Its potential nutritional value is determined as suitable for ruminant feeding. Moreover, LABs inoculation decreased pH, acetic acid, propionic acid content, dry matter loss, whereas increased lactic acid content significantly compared to control group. It has been determined that *L. bifermentas* isolate code number LS-65-2-1 come to forefront in terms of improving silage quality.

Field Crops

Research Article

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GİRİŞ

Silaj yapımı, diğer birçok fermente edilmiş ürünler gibi, uzun yıllardan bu yana hayvansal üretimde yararlanılan bir yeşil yem saklama metodudur. Günümüzde, dünyada uygulanan fermantasyon prosesi içerisinde en büyük payın silaj yapımı olduğu, sadece AB'de yılda yaklaşık 300 milyon ton silaj yapıldığı bildirilmiştir (Jatkauskas & Vrotniakiene, 2009). Silaj yapımı, en yaygın kullanılan şekli ile "zengin yeşil yemlerin anaerobik şartlar altında meydana gelen laktik asit fermantasyonu sayesinde, uzun süre bozulmadan saklama metodu" şeklinde tanımlanmaktadır (Basmacıoğlu & Ergül, 2002; Kızılşimşek ve ark., 2016). Silaj, günümüzde birçok ülkede ruminant diyetlerinin temel bileşeni konumuna gelmiş olup, Türkiye'deki üretimi gün geçtikçe artmaktadır. Silajın temel kalite kriterlerinin başında, besleme değeri, yem tüketimi ve sindirilebilirliği gelmektedir.

Yüksek protein içerikli yemler genel olarak suda çözünen karbonhidratlar (SÇK) bakımından yetersizdir. Yonca, fiğ gibi baklagil bitkileri yüksek protein içeriğine sahip olup, en kıymetli yem bitkilerinden sayılmakla beraber, SÇK bakımından fakir olduklarından (<%1.5), istenilen kalitede bir fermantasyon oluşmaz ve silaj kaliteleri düşük olur. (Dordevic ve ark. 2016). Yonca ile yapılan silajlarda yeterli laktik asit (LA) üretimi olmamakta ve bunun yerine asetik asit (AA), etanol ve CO_2 üretimi gerçekleşmektedir. Bu durum, yonca üzerindeki epifitik mikroflora kompozisyonunda yeterli sayıda laktik asit bakterisi (LAB) bulunmamasından, var olan LAB türlerinin etkin olmamasından, yetersiz SÇK içeriğinden ve yüksek tamponlama kapasitesinden kaynaklanmaktadır (Liu ve ark., 2016). Tamponlama kapasitesi, temel olarak silaj içerisindeki asitlik derecesinin ölçüsü olan pH seviyesini bir birim düşürmek için gerekli olan asit miktarı olarak tanımlanmaktadır (Wilson, 1935).

Kuşkonmaz bitkisi sürgünleri, bir kaynağa göre %32.69 (Aberoumand, 2009), başka bir kaynağa göre de >%30 (Lopez ve ark. 1996) gibi yoncadan bile oldukça yüksek protein (Guo ve ark., 2020) içeriğine sahip bir tür olması nedeni ile, hayvan yemi olarak dikkate değer bir özellik taşımaktadır. Bununla birlikte, içerdiği çok yüksek SÇK (gövdede %17.4, yapraklarda %5.5) değerleri (Guo ve ark., 2020), mısır bitkisinden bile daha fazladır. Bu özelliğinin silaj fermantasyon kalitesine çok önemli ve olumlu bir katkı sağlaması, ayrıca yonca ve fiğ silajında sıkça görülen tamponlama kapasitesini de önemli ölçüde azaltması beklenmektedir.

Kuşkonmaz (*Asparagus officinalis* L.) bitkisinin anavatanı içerisinde, Akdeniz ve Ege Bölgelerinin kıyı şeritleri de yer almakla beraber, Türkiye'de fazla tanınmayan ve kültürü fazla yapılmayan, çok az miktarda konserve yapımı için üretilen bir bitkidir. Daha çok doğadan toplanarak taze olarak tüketilmektedir (Korkmaz ve ark., 2020). Taze sürgün hasadı ilkbahar başından itibaren belli aralıklarla yapılmakta ve lokasyona göre değişmekle birlikte yaklaşık yaz başına kadar devam etmektedir. Hasat sona erdikten sonra oluşan sürgünler hasat edilmeden bırakılmakta, bitkinin bir sonraki sezon için besin maddesi birikimi yapması hedeflenmektedir. Bırakılan sürgünler, kış soğukları bastırmadan önce veya ilk don tarihinden 4-6 hafta önce biçilerek ya toplanmakta ya da toprak yüzeyine bırakılmaktadır. Bu sürgünler biçildikleri sırada %30-40 civarında kuru madde içermekte ve hayvan beslenmesinde kullanılma potansiyelleri bulunmaktadır (Bhowmik & Matsui, 2003).

Dünya literatürü incelendiğinde, kuşkonmaz bitkisi ile ilgili çalışmaların neredeyse tamamı, taze sebze olarak kullanımı, içerdiği besin elementleri, depolama ve pazarlama konuları ve kök bölgesindeki besin madde birikimi gibi konularda olduğu ve bu konulardaki araştırmaların oldukça yüksek sayılara ulaştığı görülmektedir (Nindo ve ark., 2003; Villanueva ve ark., 2005; Köklü ve ark., 2020; Ye ve ark., 2022). Bununla birlikte, *Asparagus officinalis* türünün hayvan yemi veya silo yemi olarak kullanımı ile ilgili yapılmış çalışmalar son derece kısıtlı ve yok denecek kadar az olduğu görülmüştür. Bitkinin silo yemi olarak değerlendirilme olanakları ile ilgili olarak literatürde çok önemli bir eksiklik olduğu belirlenmiştir. Kültürü yapılan kuşkonmaz bitkisinin sap ve yapraklarından silaj yapımı ile ilgili ulaşılabilen tek literatür Guo ve ark., (2016)'nın yaptıkları bir çalışmadır. Araştırmacılar kuşkonmaz bitkisinin sürgün ve yapraklarından yapılan silajlara LAB, LAB+selüloz, LAB+pirinç kepeği ve LAB+selülüz+pirinç kepeği ilavesi ile yaptıkları çalışma sonucunda, pirinç kepeği ve seloloz ilavelerinin silaj kalitesini kötüleştirdiğini, buna karşılık LAB ilavesinin asetik asit içeriğini artırdığını, aerobik bozulmayı azalttığını ve silaj kalitesine çok önemli ve olumlu katkılarda bulunduğunu tespit etmişlerdir. Bununla birlikte kuşkonmaz sürgün ve yapraklarından yüksek kaliteli silaj elde edilmesinin ancak LAB ilavesi ile mümkün olabileceğini bildirmişlerdir.

Bu çalışmada, yüksek SÇK içeriğine de sahip olan kuşkonmaz bitkisi son sürgünleri ve yapraklarının hasat edilerek silaj yapılması olanakları araştırılmıştır. Silaj yapımı sırasında daha önceden bir 110O694 no'lu projesi kapsamında izole edilmiş ve seçilmiş 5 adet LAB izolatı inokule edilerek, fermantasyon kalitesinin iyileştirilmesi hedeflenmiştir.

MATERYAL ve METOD

Çalışmada kullanılacak kültürü yapılan kuşkonmaz bitkileri (*Asparagus officinalis* L.), Kahramanmaraş Sütçü İmam Üniversitesi, Ziraat Fakültesi, Bahçe Bitkileri Bölümü yetiştirme ve uygulama arazisinde, çeşitli araştırmalarda kullanılan 7 yaşındaki bitkilerdir. Söz konusu bitkilerden, normal yetiştirme teknikleri ile yetiştirilmiş, herhangi bir özel uygulama yapılmamış olan bitkilerden alınan örneklerle bu çalışma yürütülmüştür. Bitkilerin mevsim sonu sürgünleri, Eylül-Ekim 2021 tarihinde, yaklaşık %30-40 kuru madde (KM) içeriğinde iken biçilmiş, doğrama makinesi ile teorik olarak 2-3 cm boyunda parçalanıp, daha sonra LAB inokulasyonları yapılarak silolanmıştır.

LAB materyali olarak, daha önce bir TÜBİTAK 110O694 no'lu projesi kapsamında izole edilmiş ve belli özelliklerine göre seçilmiş 5 adet LAB izolatı kullanılmıştır. Çizelge 1'de verilen bu izolatlar, yüksek LA üretme yeteneğinde olup, anaerobik şartlar altında agresif bir gelişme gösteren izolatlardır. LAB türlerinin tamamı *Lactobacillus* genusuna ait olup, birer adet *brevis, gasseri, plantarum, buchneri* ve *bifermentans* türlerini içermektedir. Bunlardan *L. buchneri* ve *L. brevis* türleri fizyolojik karakter bakımından heterofermentatif olup, diğerleri homofermentatif niteliktedir.

Çizelge 1. Araştırmada kullanılan LAB türleri ve özellikleri
Table 1. LAB types and properties used in the research

İzolat Adı (*Isolate name*)	Tür Adı (Type Name)	Laktik Asit Üretimi (mmol L^{-1}) (*Lactic acid production*)	Laktik Asit/Toplam Fermente Ürün (%) (*Lactic acid/total fermented product*)	Fizyolojik Karakter (*Physiological character*)
LS-55-2-2	*Lactobacillus brevis*	70.02	81.79	Heterofermentatif
LS-51-2-1	*Lactobacillus gasseri*	53.85	94.24	Homofermentatif
LS-71-2-3	*Lactobacillus plantarum*	52.39	96.93	Homofermentatif
LS-31-1-4	*Lactobacillus buchneri*	59.08	85.12	Heterofermentatif
LS-65-2-1	*Lactobacillus bifermentans*	56.65	94.66	Homofermentatif

Örnekleme Yöntemi

Denemede kullanılacak kuşkonmaz parseli 120 cm sıra arası ve 50 cm sıra üzerine dikilmiş olan toplam 7 sıradan oluşan ve her bir sıranın 50 m uzunluğunda olduğu bir parselde bulunmaktadır. Bitkiler 7 yaşında oldukları için gelişme göstermişler ve sıra arası ve sıra üzeri mesafeleri bir miktar kapatmışlardır. Bu nedenle morfolojik özelliklerin seçiminde tek bitki üzerinden gidilmemiştir, belli işaretlenmiş alanlar üzerinden ölçüm ve gözlemler yapılmıştır. Bu amaçla, 7 sıranın kenarlardan birer sırası kenar tesiri olarak bırakılmış, ortada kalan 5 sıra içerisinde, 3 m eninde ve 5 m uzunluğunda 3 adet parsel oluşturulmuştur. Her parselden rastgele seçilen 10 sürgünün toprak yüzeyinden en üst noktasına kadar olan mesafe cm cinsinden ölçülerek bitki boy ortalaması alınmıştır. Sap çapı (mm) ise her parselden rastgele seçilen 10 bitkinin toprak yüzeyinden 25 cm yukarısındaki kısmından kumpas kullanılarak mm cinsinden ölçülmüştür ve ortalaması alınarak belirlenmiştir. Rastgele seçilen 10 sürgün, toprak yüzeyinden biçilecek, sapları ve yaprakları ayrılmış ve tartılarak toplam ağırlığa oranlanmıştır. Her parsel (2.4x5 m=12 m^2) tamamen biçilip tartılmış ve dekara yeşil ot verimi hesaplanmıştır. Parselden alınan 2 kg ağırlığındaki yeşil aksam, ağırlığı sabit oluncaya kadar gölgede kurutulmuş, tartılarak kuru ağırlığı belirlenmiştir ve yeşil ağırlığa oranlanarak kuru madde oranı hesaplanmıştır. Bu değer kuru ot verimi hesabında dikkate alınmıştır (Ertekin ve ark., 2020).

Silaj materyali ve silajların hazırlanması

Her parselden yaklaşık 10±1 kg yeşil ot olacak şekilde hasat edilen bitkiler alınmıştır ve bitki parçalayıcıda teorik olarak 2-3 cm uzunluğunda doğranmıştır. Her bir parsel doğrandıktan sonra, parçalayıcı önce fırça ile, temizlenip, ardından alkol ile silinerek diğer örneklere mikrobiyel bulaşma engellenmiştir. Silaj yapımı için, bir naylon sergi üzerine doğranan yeşil ot, iyice karıştırıldıktan sonra, yaklaşık 1.5 kg ağırlığında 6 muamele grubuna bölünmüştür. Muamele grupları 1.kontrol, 2.*Lactobacillus buchneri,* 3.*Lactobacillus bifermentans*, 4.*Lactobacillus plantarum*, 5.*Lactobacillus brevis ve 6.Lactobacillus gasseri* şeklinde düzenlenmiştir. Silajlara katkı maddesi 10^5 kob g^{-1} ilavesinden sonra silajlar her muamele grubunda 3'er tekerrür olmak üzere plastik torbalara vakumlanarak doldurulmuş ve 60. gün süre ile (22±2 ^oC) laboratuvar ortamında fermantasyona bırakılmıştır.

Kimyasal ve mikrobiyolojik analizler

Silaj kuru örneklerinde Nötr deterjan lif (NDF), Asit deterjan lif (ADF) ve asit deterjan lignin (ADL) analizleri Ankom Fiber Analiz cihazından (Fiber Analyser, ANKOM marka, A220 model) yararlanılarak yapılmıştır (Van Soest ve ark. 1991). Silajların azot (N) içeriği Kjeldahl metodu kullanılarak saptanmıştır. Ham protein (HP) ise N x 6.25 formülü ile hesaplanmıştır (AOAC, 1990). Suda çözünen karbonhidrat (SÇK) içerikleri Deriaz (1961)'in

bildirdiği metoda göre Somogyi-Nelson ajanları kullanılarak ve spektrofotometrede 550 nm dalga boyunda ölçülerek yapılmıştır. Açılan silajların organik asit kompozisyonu (Laktik asit, asetik asit, butrik asit ve propiyonik asit) ve etanol içeriği (%) HPLC cihazında belirlenmiştir (Quiros ve ark. 2009). Hazırlanan örnekler, örnek temizleme prosedüründen sonra HPLC'de 42 °C'de 0,6 ml dk^{-1} akış hızında ve RID dedektör kullanılarak tespit edilmiştir.

Fermantasyonun 60. gününde açılan silajlardan 25 g örnek alınarak 225 ml Ringer solusyonu içerisinde el mikseri ile yüksek devirde 1 dk karıştırıldıktan sonra Whatman 54 filtre kağıdından süzülmüş ve pH değeri belirlenmiştir. Elde edilen süzüğün, 10 kat seyreltme serileri hazırlanıp ve uygun besi yerlerine ekilmiştir. LAB için MRS besi yeri, küfler ve mayalar için MEA besi yeri ve enterobakteriler için VRBG besi yeri kullanılmıştır. MRS besi yerinde anaerob şartların sağlanması için örnek ekiminden sonra bir kat daha dökülmüştür. MRS ve MEA 37 °C'de 48 saat, VRBG besi yeri ise 32 °C'de 18 saat inkübe edildikten sonra sayılmıştır (Seale ve ark. 1990).

İstatistik Analizler

Elde edilen verilerden tarlada ölçülen veya tarla denemelerine ait agronomik özelliklere ilişkin veriler, sadece bilgi edinmek amacıyla yapılacağından, herhangi bir istatistik analize tabi tutulmamıştır. Zaten agronomik özellikleri değiştirecek bir uygulama bu araştırmada mevcut değildir. Buna karşın, laboratuvarda yapılan silaj ile ilgili veriler ise tesadüf parselleri deneme düzenine göre varyans analizine tabi tutulmuş, uygulamalar arasındaki farklılıklar LSD testi ile belirlenmiştir. Verilerin değerlendirilmesinde JMP istatistik paket programı kullanılmıştır (JMP, 2007).

BULGULAR ve TARTIŞMA

Kuşkonmaz bitkisine ait agronomik özellikler Çizelge 2'de verilmiştir. Elde edilen ortalama değerlere göre yapılan hasat zamanında kuşkonmaz bitki boyunun 161.77 cm, sap çapının 10.44 cm, sap oranının %50.17 ve yaprak oranının %49.88 olduğu belirlenmiştir. Toplam yeşil ot veriminin 879.92 kg da^{-1} ve kuru ot veriminin 324.60 kg da^{-1} olduğu belirlenmiştir.

Çizelge 2. Kuşkonmaz bitkisine ait agronomik özellikler
Table 2. Agronomic characteristics of asparagus plant

Agronomik Özellikler (Agronomic Feature)	***Ortalama Değerler (Mean Values)***
Bitki Boyu (cm) *(Plant Height)*	161.77
Sap Çapı (cm) *(Stem diameter)*	10.44
Sap Oranı (%) *(Stem Ratio)*	50.17
Yaprak Oranı (%) *(Leaf Ratio)*	49.88
Yeşil Ot Verimi (kg da^{-1}) *(Green Forage Yield)*	879.92
Kuru Ot Verimi (kg da^{-1}) *(Hay Yield)*	324.60

Başlangıç materyale ait KM değerleri arasında oluşan farkın istatistiki olarak önemli olduğu ($P<0.01$), KM içeriklerinin %36.11-37.83 arasında değiştiği, en yüksek KM değerinin (LS-71-2-3) *L. plantarum* izolatından elde edildiği Çizelge 3'de görülmektedir. Bu değeri (LS-65-2-1) *L. bifermentans* izolatının izlediği, en düşük KM içeriğinin ise istatistiki olarak aynı grupta yer alan kontrol ve (LS-31-1-4) *L. buchneri* izolantında tespit edilmiştir. Olgunlaşmış silajların (T_{60}) KM içeriklerinin %34.55-36.90 arasında değiştiği ($P<0.05$), en yüksek KM değerinin (LS-71-2-3) *L. plantarum* izolantından elde edildiği, bu değeri %36.13 ile *L.brevis* izolantının izlediği, en düşük KM içeriğinin ise aşılanmamış (kontrol) kuşkonmaz silajından elde edildiği belirlenmiştir. Çalışmada LAB inokulasyonlarının kuşkonmaz silajlarının kuru madde içeriğini arttırdığı açıkça söylenebilir. Nitekim, birçok araştırmacı mikrobiyal aşıyıcıların KM içeriğinin olumlu etkilediğini bildirmiştir (Henderson, 1993; Driehuis ve ark., 1997; Kızılşimşek ve ark., 2020).

Yüksek KM içeriğinin düşük pH elde edilmesinde önemli katkı sağladığı, bununla birlikte, yüksek KM ile silaj yapıldığında kuru madde korunumunun daha yüksek olduğu bilinmektedir (Kızılşimşek ve ark., 2020). İnokulasyon yapılmamış (kontrol) kuşkonmaz silajlarında KM kaybı %4.32 iken, *L. plantarum* inokulasyonu ile bu kaybın %2.46'lara kadar düştüğü ortaya çıkmıştır. Öte yandan, taze materyaldeki KM (T_0) değerlerine bakıldığında, *L.buncheri* izolatı ile kontrolün aynı grupta yer aldığı, fakat silaj açımında (T_{60}) *L. buncheri* izolatının kontrole göre KM kaybının daha az olduğu belirlenmiştir. Diğer bir ifadeyle, hem yüksek KM içeriğinin hem de LAB inokulasyonun kuru madde kaybını azalttığı açıkça söylenebilir (Weinberg & Muck, 1996). Çalışmada yüksek KM içeriği yönünden *L. plantarum* izolantının diğer izolatlara göre daha başarılı olduğu da belirlenmiştir.

Silaj başlangıç dönemine ait pH değerlerinin 5.80-5.95 arasında değiştiği ($P<0.05$), en düşük pH değerlerinin kontrol ve *L. bifermentans* izolantından elde edildiği, en yüksek değerin ise *L. plantarum* izolatından elde edildiği Çizelge 3'de görülmektedir. Genellikle, kaliteli silaj için pH değerinin 3.8 ile 4.2 arasında olması istenilmektedir. Çalışmada, fermantasyonun 60. gününde açılan silajların (T_{60}) pH'sının 3.95-4.60

arasında değiştiği (P<0.01), en yüksek pH değerinin kontrol silajından elde edildiği belirlenmiştir. Dolayısıyla, kuşkonmaz bitkisine hasat öncesi dönemde mikrobiyal katkıların, kontrol grubu silajlara oranla daha iyi bir fermantasyon sağlayabileceğini göstermektedir. Benzer şekilde bazı araştırmacılar inokulasyon uygulamanın pH düşürmede etkili olduğunu bildirmişlerdir (Meryy ve ark., 1995; Weinberg ve ark., 1998; Filya, 2003, Filya ve ark., 2007). Diğer yandan, bazı araştırmacılar pH düşürmede LAB türününde önemli olduğu belirlemişerdir (Ertekin & Kızılşimşek 2020; Günaydın ve ark., 2023). Nitekim çalışmada, *L. buchneri, L. bifermentans, L. plantarum, L. gasseri* izolatlarının *L. brevis* izolantına göre daha başarılı olduğu söylenebilir.

Fermantasyonun 60. gününde açılan kuşkonmaz silajlarının mikrobiyolojik analiz sonuçları Çizelge 4'te sunulmuştur. LAB inokulasyonun laktik asit bakteri sayısı, enterobakteri sayısını istatistiki olarak etkilerken, maya sayısını etkilemediği belirlenmiştir. Araştırmada başlangıç ve 60 günlük fermantasyon dönemi sonrasında muamele gruplarında küf tespit edilmemiştir.

Çizelge 3. LAB ile aşılanmış kuşkonmaz bitkisinin KM ve pH değerleri
Table 3. KM and pH values of asparagus plant inoculated with LAB

Bakteri İnokulantları **Bacteria Inoculant**		**KM (T_0) DM(T_0)**	**KM (T_{60}) DM(T_{60})**	**pH (T_0)**	**pH (T_{60})**
Kontrol	Kontrol *(Control)*	36.11^{c}	34.55^{c}	5.80^{c}	4.60^{a}
LS-31-1-4	*Lactobacillus buchneri*	36.23^{c}	35.93^{abc}	5.84^{bc}	3.95^{c}
LS-65-2-1	*Lactobacillus bifermentans*	37.16^{ab}	35.85^{abc}	5.80^{c}	3.98^{c}
LS-71-2-3	*Lactobacillus plantarum*	37.83^{a}	36.90^{a}	5.95^{a}	3.97^{c}
LS-55-2-2	*Lactobacillus brevis*	37.01^{b}	36.13^{ab}	5.91^{ab}	4.15^{b}
LS-51-2-1	*Lactobacillus gasseri*	37.01^{b}	35.50^{bc}	5.85^{abc}	3.99^{c}
Ortalama *(Mean)*		36.89	35.81	5.86	4.11
LSD		0.67**	1.40*	0.10*	0.07**
CV (%)		1.00	2.14	0.95	0.96

^{a,b,c} Farklı simgelere sahip ortalama değerler arasında istatistiki olarak önemli farklılık vardır, **P<0.01*P<0.05 istatistiki düzeyde önemli,
^{a,b,c} Values within a row with different superscripts differ significantly at **P<0.01, *P<0.05
CV (%): Varyasyon Katsayısı, LSD: Asgari önem farkı, KM: Kuru madde oranı, T_0: Taze materyal, T_{60}: Silolamanın 60.günü
LSD: Least significance difference, C.V: Coefficient variation, DM: Dry matter ratio, T_0: Fresh material, T_{60}: 60 days of silage

Çizelge 4. Kuşkonmaz silajlarına ait mikrobiyolojik analiz sonuçları
Table 4. Microbiological analysis results of asparagus silages

Bakteri İnokulantları *(Bacteria Inoculant)*		**Laktik Asit Bakteri (T_{60})** *(Lactic acid bacteria) (T_{60})*	**Enterobakteri (T_{60})** (*Enterobacteria*) (T_{60})	**Maya (T_{60})** (*Yeast*) (T_{60})
Kontrol	Kontrol *(Control)*	5.63^{a}	3.00^{a}	4.14
LS-31-1-4	*Lactobacillus buchneri*	3.67^{c}	2.11^{ab}	4.25
LS-65-2-1	*Lactobacillus bifermentans*	4.93^{ab}	2.65^{a}	4.45
LS-71-2-3	*Lactobacillus plantarum*	4.29^{bc}	1.56^{b}	3.71
LS-55-2-2	*Lactobacillus brevis*	4.29^{bc}	1.85^{b}	4.12
LS-51-2-1	*Lactobacillus gasseri*	4.41^{bc}	1.70^{b}	4.18
Ortalama *(Mean)*		4.53	2.10	4.14
LSD		0.82**	0.79**	öd *(ns)*
CV (%)		9.96	6.51	20.68

^{a,b,c} Farklı simgelere sahip ortalama değerler arasında istatistiki olarak önemli farklılık vardır, **P<0.01 istatistiki düzeyde önemli,
^{a,b,c} Values within a row with different superscripts differ significantly at **P<0.01
öd: Önemli Değil, CV (%): Varyasyon Katsayısı, LSD: Asgari önem farkı, T_0: Taze materyal, T_{60}: Silolamanın 60.günü
ns:non significant, LSD: Least significance difference, C.V: Coefficient variation

Çalışmada laktik asit bakteri sayısının 3.67-5.63 $\log_{10}$ kob TM^{-1} arasında değiştiği görülmektedir (P<0.01). En yüksek laktik asit bakteri sayısı kontrol uygulamasında (5.63 $\log_{10}$ kob TM^{-1}) kaydedilmiştir. Bu değeri LS-65-2-1 kod numaralı *L. bifermentans* izolatının izlediği, en düşük değerin ise LS-31-1-4 kod nuramalı *L. buchneri* izolatında belirlenmiştir. Kontrolde yüksek miktarda tespit edilen LAB sayısı çalışmada en düşük pH değerinin kontrol silajından elde edilmesini sağlamalıydı. Dolayısıyla, bu durum Ertekin & Kızılşimşek (2020)'ın belirttiği gibi, laktik asit üretiminin kontrol silajlarında az olması ile açıklanabilir. Öte yandan, başarılı bir silajda fermantasyon sonucunda enterobakteri sayısının az olması istenilen bir sonuçtur. Diğer bir ifadeyle, silajlarda istenilmeyen enterobakterileri ve mayalar mevcut SÇK'lar için LAB ile bir rekabet halindedir ve bu durum pH'ın düşmesini de olumsuz etkilemektedir. Fermantasyon sonucunda silajların enterobakteri sayılarının 1.70-3.00 $\log_{10}$ kob TM^{-1} arasında değiştiği

(P<0.01), en yüksek enterobakteri sayısının inokulasyon yapılmamış (kontrol) silajlardan elde edildiği, en düşük enterobakteri sayısının ise *L. plantarum, L. brevis* ve *L. gasseri* izolatlarından elde edilmiştir. Dolayısıyla, LAB izolatlarının enterobakteri sayısını azalttığı açıkça söylenebilir. Olgunlaşmış silajların maya sayıları arasında istatistiki olarak önemi bir farklılık oluşmadığı görülmektedir. Değerler incelendiğinde, özellikle, kontrole kıyasla *L. plantarum, L. brevis* ve *L. gasseri* izolatlarından daha düşük maya sayısı elde edilmiştir. Driehuis ve ark. (1999), LAB aşılama ile silaj içerisinde zamanla maya varlığının azaldığını bildirmişlerdir.

Kuşkonmaz silajlarının SÇK içerikleri arasında istatistiki olarak bir farklılık oluşmadığı Çizelge 5'de görülmektedir. Aynı çizelgeden taze materyale ait SÇK içeriklerinin %3.12-5.05 arasında değiştiği, değerler incelendiğinde *L. brevis* (LS-55-2-2) izolatının SÇK içeriğinin kontrole ve diğer izolatlara kıyasla daha yüksek olduğu belirlenmiştir. Bununla birlikte, fermantasyon sonucunda *L. plantarum* izolatı ve kontrolün diğer izolatlara kıyasla SÇK içeriklerinin daha yüksek olduğu görülmektedir. Dolayısıyla, kontrol uygulamasında birçok izolata göre yüksek olan SÇK miktarının, fermantasyon sırasında kaybı daha yüksek olduğu ifade edilebilir. Diğer yandan, LAB inokulasyonu ile, fermantasyonun sonunda bile yeteri kadar SÇK bulunması, pH'nın yeteri kadar düştüğü, mikroorganizma faaliyetlerinin de durduğuna dikkat çekmektedir. Hızla düşen pH, SÇK korur ve uzun süreli fermantasyonu önleyerek proteoliz oluşumu ve deaminasyonu azaltmaktadır (Muck, 1993). Bu bağlamda, mikrobiyal aşıyıcıların besin kaybını azaltmada ve SÇK içeriğini arttırmada etkili bir yol olduğu söylenebilir (Weinberg ve ark., 1995). Buna karşılık, çalışmadan elde edilen sonuçlara benzer şekilde, Taylor ve ark. (2002), LAB katkısının silajın SÇK içeriğini istatistiki olarak etkilemediğini bildirmişlerdir.

Çizelge 5. LAB ile aşılanmış kuşkonmaz silajının SÇK değerleri
Table 5. WSC values of asparagus silage inoculated with LAB

Bakteri İnokulantları *(Bacteria Inoculant)*		**SÇK (T_0)** *(WSC) (T_0)*	**SÇK (T_{60})** *(WSC) (T_{60})*
Kontrol	Kontrol *(Control)*	4.82	0.99
LS-31-1-4	*Lactobacillus buchneri*	4.76	1.52
LS-65-2-1	*Lactobacillus bifermentans*	4.73	1.23
LS-71-2-3	*Lactobacillus plantarum*	3.12	0.90
LS-55-2-2	*Lactobacillus brevis*	5.05	1.56
LS-51-2-1	*Lactobacillus gasseri*	4.46	1.20
Ortalama *(Mean)*		4.49	1.23
LSD		öd *(ns)*	öd *(ns)*
CV (%)		19.22	21.13

Öd:Önemli değil, CV (%): Varyasyon katsayısı, LSD: Asgari önem farkı, SÇK: Suda çözünen karbonhidrat, T_0: Taze materyal, T_{60}: Silolamanın 60.günü
öd:non significant, LSD: Least significance difference, WSC: Water soluble carbohydrates CV: Coefficient variation, T_0: Fresh material, T_{60}: 60 days of silage

Kuşkonmaz bitkisine farklı laktik asit bakteri inokulasyonu yapılan ve inokulasyon yapılmamış (kontrol) silajların HP, NDF, ADF ve ADL değerleri ve istatistiki oluşan gruplar Çizelge 6'da verilmiştir. Fermantasyonun 60. gününde açılan silajlarının HP içerikleri arasında oluşan farkın istatistiki olarak önemli olduğu (P<0.01), HP içeriklerinin %6.87-8.00 arasında değiştiği, *L. brevis* (LS-55-2-2) bakteri izolatıyla yüksek HP içeriğinin elde edildiği belirlenmiştir.

Çalışmada NDF ve ADL içerikleri arasında oluşan farkın istatistiki olarak önemli olmadığı, buna karşılık bakteri izolatlarının ADF içeriklerini önemli derecede etkilediği belirlenmiştir. Aynı çizelgeden NDF içeriklerinin %50.38-56.97 gibi dar bir aralıkta değiştiği, bu nedenle farkın istatistiki olarak önemli olmadığı, değerler incelendiğinde *L. bifermentans* (LS-65-2-1) izolatından düşük NDF içeriğinin elde edildiği görülmektedir. Ek olarak, ADF içeriklerinin %32.99-36.04 arasında değiştiği (P<0.01), en düşük ADF içeriğinin *L. bifermentans* (LS-65-2-1) izolatından elde edildiği belirlenmiştir. Öte yandan, LAB inokulantları arasında en yüksek NDF ve ADF içeriği *L. gasseri* (LS-51-2-1) izolatından elde edildiği görülmektedir. Çalışmada ADL içeriklerinin %10.61-11.52 gibi çok dar bir aralıkta değiştiği, farkın istatistiki olarak önemli olmadığı, fakat kontrol grubundan daha düşük ADL içeriğinin elde edildiği görülmektedir.

Literatür incelendiğinde, genellikle aşıyıcıların beslenme değeri üzerindeki etkileri tutarsızlık göstermektedir. Ertekin & Kızılşimşek (2020), farklı LAB uyguladıkları yonca silajında, NDF ve ADF içeriklerinin LAB inokulasyonundan etkilenmediğini, fakat en düşük NDF ve ADF içeriğinin *L. bifermentans* izolatından elde edildiğini bildirmişlerdir. Tabacco ve ark. (2011), LAB inokulantlarının ADF, NDF ve ADL değerlerini etkilemediğini bildirmişlerdir. Acosta Aragon ve ark. (2012), LAB aşıyıcıların NDF

içeriklerini istatistiki olarak etkilemediği, buna karşılık ADF içeriklerini azaltığını ve HP içeriğini arttırdığını bildirmişlerdir. İflazoğlu Mutlu ve ark. (2015), fermantasyonun 60. günde LAB şuşlarının NDF ve HP içeriğini etkilemediği, ADF içeriğini etkilediği ve ADL içeriğini arttırdığını bildirmişler. Kızılşimşek ve ark. (2020), LAB aşıyıcıların NDF ve ADF içeriğini etkilemediği, HP içeriğini ise arttırdığını bildirmişlerdir. Besin içeriklerindeki bu farklılık, çalışmalarda kullanılan bitki çeşitlerinin, KM içeriklerinin, LAB izolantlarının farklı olmasından kaynaklanabileceği gibi, çalışmalarda kullanılan LAB yoğunluğunun farklı olmasından da kaynaklanabilir.

Çizelge 6. LAB ile aşılanmış kuşkonmaz silajının HP, NDF, ADF ve ADL değerleri (T_{60})
Table 6. HP, NDF, ADF and ADL values of asparagus silage inoculated with LAB (T_{60})

Bakteri İnokulantları *(Bacteria Inoculant)*		**HP** *(CP)*	**NDF** *(NDF)*	**ADF** *(ADF)*	**ADL** *(ADL)*
Kontrol	Kontrol *(Control)*	7.92^{ab}	52.55	34.34^{bc}	10.61
LS-31-1-4	*Lactobacillus buchneri*	7.61^{abc}	54.26	34.37^{bc}	11.14
LS-65-2-1	*Lactobacillus bifermentans*	7.39^{bcd}	50.38	32.99^{c}	11.31
LS-71-2-3	*Lactobacillus plantarum*	6.87^{d}	53.76	35.74^{ab}	11.52
LS-55-2-2	*Lactobacillus brevis*	8.00^{a}	54.09	34.42^{bc}	11.19
LS-51-2-1	*Lactobacillus gasseri*	7.26^{cd}	56.97	36.04^{a}	11.32
Ortalama *(Mean)*		7.51	53.67	34.65	11.18
LSD		0.57**	öd *(ns)*	2.39**	öd *(ns)*
CV (%)		4.15	4.26	1.51	7.43

^{a,b,c} Farklı simgelere sahip ortalama değerler arasında istatistiki olarak önemli farklılık vardır,**P<0.01*P<0.05 istatistiki düzeyde önemli,
^{a,b,c} Values within a row with different superscripts differ significantly at **P<0.01, *P<0.05
CV (%): Varyasyon Katsayısı, LSD: Asgari önem farkı, öd: önemli değil, HP: Ham Protein, ADF: Asit Deterjan Lif, NDF: Nötr Deterjan Lif, ADL: Asit Deterjan Lignin, T_{60}: Silolamanın 60.günü
LSD: Least significance difference, CV: Coefficient variation, ns: non-significant, CP: Crude protein, ADF: Acid detergent fiber, NDF: Nötr detergent fiber, ADL: Acid detergent ligninT$_{60}$: 60 days of silage

Fermantasyon sonrasında, laktik asit değerinin %0.60-1.25 arasında değiştiği, farklılığın istatistiki olarak önemli olmadığı belirlenmiştir (Çizelge 7). Öte yandan, fermantasyon sonucunda (LS-65-2-1) *L. bifermentans* ve (LS-51-2-1) *L. gasseri* izolantlarının hem pH'ı istenilen seviyeyi düşürmede başarılı olduğu hem de diğer izolantlara ve kontrole kıyasla fermantasyon sonucunda laktik asit düzeyinin yüksek olduğu söylenebilir. Diğer bir ifadeyle, fermantasyon sonucunda homofermentatif LAB aşıyıcıların heterofermentatif LAB aşıyıcılara göre laktik asit üretim etkinliğinin daha yüksek olduğu söylenebilir. Baytok ve ark. (2005), tarafından rapor edilen bulgular ile uyumludur.

Çizelge 7. LAB inokulantı uygulanan kuşkonmaz silajlarının fermantayon ürünlerine ait ortalama değerler (T_{60})
Table 7. Average values of fermentation products of asparagus silage applied LAB inoculant (T_{60})

Bakteri İnokulantları *(Bacteria Inoculant)*		**Laktik Asit (%)** *(Lactic Acid) (%)*	**Asetik Asit (%)** *(Acetic Acid) (%)*	**Propiyonik Asit (%)** *(Propionic Acid)* **(%)**
Kontrol	Kontrol *(Control)*	0.99	1.28^{a}	0.26^{bc}
LS-31-1-4	*Lactobacillus buchneri*	0.64	1.31^{a}	0.33^{b}
LS-65-2-1	*Lactobacillus bifermentans*	1.14	0.50^{b}	0.18^{bc}
LS-71-2-3	*Lactobacillus plantarum*	0.80	1.12^{a}	0.50^{a}
LS-55-2-2	*Lactobacillus brevis*	0.60	1.35^{a}	0.12^{c}
LS-51-2-1	*Lactobacillus gasseri*	1.25	0.36^{b}	0.16^{bc}
Ortalama *(Mean)*		0.90	0.99	0.24
LSD		öd *(ns)*	0.61**	0.17**
CV (%)		36.67	33.32	35.26

^{a,b,c} Farklı simgelere sahip ortalama değerler arasında istatistiki olarak önemli farklılık vardır, **P<0.01 istatistiki düzeyde önemli,
^{a,b,c} Values within a row with different superscripts differ significantly at **P<0.01
öd: Önemli Değil, CV (%): Varyasyon Katsayısı, LSD: Asgari önem farkı, T_{60}: Silolamanın 60.günü
ns:non significant, LSD: Least significance difference, CV: Coefficient variation, T_{60}: 60 days of silage

Çalışmada bütirik asit ve etanol içeriği silajların hiçbirinde tespit edilmemiştir. Bu nedenle çizelgede yer verilmemiştir. Kılıç (1986), iyi kalite özelliğine sahip bir silaj yeminde bütirik asit oluşumunun hiç istenmediğini bildirmiştir. Bu doğrultuda kaliteli bir silaj elde edildiği söylenebilir. Alçiçek & Özkan (1997) ise asetik asit içeriğinin %0.8'in üzerinde olmaması gerektiğini bildirmişlerdir. Çalışmada asetik asit içerikleri arasında oluşan farkın istatistiki olarak önemli olduğu (P<0.01), (LS-65-2-1) *L. bifermentans* (%0.50) ve (LS-51-2-1) *L. gasseri* (%0.36) izolantlarının asetik asit düzeylerinin tatminkâr bir seviyede olduğu

belirlenmiştir. Silaj için istemenmeyen fermantasyon ürünlerinden biri olan propiyonik asitinin bakteri izolantlarından istatistiki olarak önemli derecede etkilediği (P<0.01), (LS-55-2-2) *L. brevis* izolantından ise en düşük değerin elde edildiği belirlenmiştir. Çalışmada (LS-65-2-1) *L. bifermentans* ve (LS-51-2-1) *L. gasseri* izolantlarının kontrole kıyasla sırasıyla laktik asit üretimini sırasıyla %15 ve %20 oranlarında arttırdığı, buna karşılık silajda istenmeyen asetik asit %61 ve %72 ve propiyonik asit oluşumunu ise %31 ve %38 oranlarında düşürdüğü tespit edilmiştir. Birçok araştırmacı silaj katkı maddesi olarak değerlendirilen laktik asit bakteri izolantlarının laktik asit üretimini arttırmada ve istenmeyen fermantasyon ürünleri düşürmede başarılı olduğunu bildirmişlerdir (Weinberg ve ark., 1993; Filya ve ark., 2000; Kızılşimşek ve ark., 2007; Ertekin & Kızılşimşek 2020).

SONUÇ ve ÖNERİLER

Kuşkonmaz bitkisi yaşına bağlı olarak 3-8 hafta hasat edildikten sonra, hasat mevsimi sonunda oluşan sürgünler hasat edilmeden bırakılmakta ve bitkinin bir sonraki sezon için köklerinde besin maddesi depolaması beklenmektedir. Daha sonra da bu sürgünler biçilerek atılmakta ve oldukça önemli bir ekonomik kayıp oluşturmaktadır. Dolayısıyla bu durum tarımın sürdürülebilirliği ilkesi ile de çatışmaktadır. Nitekim araştırma sonucunda 879.92 kg da^{-1} yeşil ot verimi ile alternatif bir kaba yem kaynağı olabileceği, özellikle kuru madde içeriğinin ve kuru ot veriminin yüksek olduğu belirlenmiştir.

Farklı laktik asit bakteri ile yapılan silajlarında ise LAB inokulasyonlarının kuşkonmaz silajlarının kuru madde içeriğini arttırdığı, özellikle (LS-65-2-1) *L. bifermentans,* (LS-71-2-3) *L. plantarum* ve (LS-55-2-2) *L. brevis* türünün aşılanmamış (kontrole) göre daha iyi performans gösterdiği belirlenmiştir. KM kaybı yönünden (LS-71-2-3) *L. plantarum* izolantının diğer izolatlara göre daha başarılı olduğu da ortaya çıkan sonuçlar arasındadır. Kontrol grubundan elde edilen yüksek pH değeri, mikrobiyal aşıyıcılar ile düşürülmüştür. Yüksek laktik asit bakteri sayısı bakımından LS-65-2-1 kod numaralı *L. bifermentans* izolatı, düşük enterobakteri ve maya sayısı ile (LS-71-2-3) *L. plantarum,* (LS-55-2-2) *L. brevis* ve (LS-51-2-1) *L. gasseri* izolatları ön plana çıkmıştır. Çalışmada (LS-65-2-1) *L. bifermentans* ve (LS-51-2-1) *L. gasseri* izolantlarının kontrole kıyasla sırasıyla laktik asit üretimini arttırdığı, buna karşılık silajda istenmeyen asetik asit ve propiyonik asit oluşumunu ise düşürdüğü tespit edilmiştir. Silaj yem kalitelerine göre en yüksek HP içeriği (LS-55-2-2) *L. brevis,* (LS-31-1-4) *L. buncheri* ve (LS-65-2-1) *L.bifermentans,* düşük ADF içeriği (LS-65-2-1) *L. bifermentans* izolatında saptanmıştır. Çalışmada NDF, SÇK ve ADL değerleri arasında istatistiki olarak bir farklılık oluşmamıştır.

Tüm değerler incelendiğinde kuşkonmaz bitkisinin alternatif kaba yem kaynağı olabileceği ve potansiyel beslenme değerinin ruminant beslemesi için uygun olduğu belirlenmiştir. Kuşkonmaz silajına uygulanan laktik asit bakteri izolatları ile de silaj kalitesinin iyileştirildiği ve birçok özellik bakımından (LS-65-2-1) *L. bifermentans* izolatının ön plana çıktığı belirlenmiştir. Araştırma sonucunda; kuşkonmaz ve bu özelliklere sahip bitkiler için (LS-65-2-1) *L. bifermentans* izolatının kullanılması önerilmektedir. Dahası, yüksek SÇK içeriğine sahip kuşkonmaz bitkisinin baklagil gibi zor silolanan bitkilerin içerisine katkı maddesi olarak da kullanılabileceği ve bu nedenle kuşkonmaz bitkisinin kullanım alanının artabileceği düşünülmektedir.

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Yazarlar makaleye eşit oranda katkı sağlamış olduklarını beyan eder.

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Ekmeklik Buğday Çeşitlerinde Başaklanma Döneminde Uzaklaştırılan Bazı Fotosentez Organlarının Tarımsal Özellikler Üzerine Etkisinin Belirlenmesi

İlke Aybüke BÜYÜKYATIKÇI[1], Köksal YAĞDI[2]✉
[1] Bursa Uludağ Üniversitesi Ziraat Fakültesi Tarla Bitkileri Bölümü Görükle/Bursa, [2] Bursa Uludağ Üniversitesi Ziraat Fakültesi Tarla Bitkileri Bölümü Görükle/Bursa
[1]https://orcid.org/0000-0003-4330-631X, [2]https://orcid.org/0000-0003-1567-9397
✉: kyagdi@uludag.edu.tr

ÖZET

Bursa Uludağ Üniversitesi Ziraat Fakültesi Uygulama ve Araştırma Merkezinde 2018-2019 yıllarında yürütülen bu çalışmada, ekmeklik buğday çeşitlerinde başaklanma döneminde bazı fotosentez organlarının uzaklaştırılmasının verim ve kalite özellikleri üzerine etkisinin belirlenmesi amaçlanmıştır. Çalışmada iki ekmeklik buğday çeşidi (Pehlivan ve Golia) materyal olarak kullanılmış olup araştırma tesadüf bloklarında bölünmüş parseller deneme desenine göre üç tekerrürlü olarak yürütülmüştür. Çeşitlerin başaklanma dönemlerinde kesilerek uzaklaştırılan fotosentez organlarının elde edilen sonuçlara göre bitki boyu, başak boyu, başakta başakçık sayısı, başakta tane sayısı, başakta tane ağırlığı, bin tane ağırlığı, hektolitre ağırlığı, sedimantasyon değeri, gluten oranı ve hasat indeksi değerlerini önemli ölçüde azalttığı belirlenmiştir. Buna karşılık protein oranında artış gözlemlenmiştir. Kılçıklı bir çeşit olan Golia çeşidi uygulamalardan kılçıksız bir çeşit olan Pehlivan çeşidine göre daha fazla etkilenmiştir.

Tarla Bitkileri

Araştırma Makalesi

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Anahtar Kelimeler
Ekmeklik buğday
Fotosentez organları
Verim özellikleri
Kalite özellikleri

Determination of The Effects of Some Photosynthesis Organs Removed at Heading Stage on Agricultural Traits in Bread Wheat Varieties

ABSTRACT

This study conducted in 2018-2019 at Bursa Uludağ University Faculty of Agriculture Application and Research Center, and aimed to determine the effect of removing some photosynthesis organs on the yield and quality characteristics of bread wheat varieties during the heading stage. In the study, 2 bread wheat varieties (Pehlivan and Golia) were used as material, and the research was carried out in randomized split blocks design with three replications. According to the results obtained, the photosynthesis organs removed during the spiking period of the varieties significantly reduced the plant height, spike height, number of spikes per spike, grain number per spike, grain weight per spike, thousand-grain weight, hectolitre weight, sedimentation value, gluten ratio, and harvest index values. On the other hand, an increase in protein ratio was observed. The Golia cv. which is an awned cultivar, has been more affected by the applications than the Pehlivan cv., which is an awnless cultivar.

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GİRİŞ

Buğday gerek dünyadaki diğer ülkelerde gerekse Türkiye'de insan beslenmesindeki en temel besinlerin hammaddesi olması itibariyle diğer tarımsal ürünlere göre daha fazla önem arz etmektedir. Yurdumuzda tarım yapılabilir alan içerisinde %41'lik payı tahıllar oluştururken toplam tahıl alanı içerisinde % 49'luk payı buğday oluşturmaktadır (Anonim,2018a). Türkiye buğday ekim alanı 2018/19 üretim sezonu itibarıyla dünya buğday ekim alanının %3,5'ini oluşturmaktadır (Anonymous,2018). Bu alan aynı zamanda Türkiye'de toplam işlenen tarım alanın

%20'sini teşkil etmektedir. Türkiye'de buğday ekim alanı 2019 yılı itibarıyla 6,8 milyon ha olup bu alandan elde edilen üretim 19 milyon tondur. Buna göre yurdumuz buğday verimi ortalama 278 kgda^{-1}'dır (Anonymous,2019).

Tahıllarda tane verimi esas olarak fotosenteze bağlıdır. Fotosentez, yeşil bitkilerde yaşam için gerekli olan organik maddelerin biriktirilmesini sağlar (Balkan & Gençtan, 2009). Başaklanma öncesi dönemde, fotosentez sonucu oluşan besin maddeleri, yaprak, kardeş, sap, kök ve başak organlarının gelişimi için kullanılmakta; başaklanmadan sonraki besin maddeleri ise tanelere taşınmaktadır. Tane doldurma dönemi boyunca fotosentez ürünlerinin büyük bir kısmı, buğday bitkisinin üst bölümündeki tanelere yakın fotosentez organlarından karşılanmaktadır (Austin & Jones, 1975).

Buğdayda, sap, yapraklar, gövde, başak ve kılçıklar fotosentetik organlardır (Birsin, 2005). Bitki gelişimi için yapraklar çok önemlidir. Bitkideki fotosentez bölgelerinden temelde yapraklar sorumludurlar. Buğdayda bayrak yaprağı, gövde ve kılçıklar fotosentez yoluyla dane doldurmaya katkıda bulunurlar. Özellikle bayrak yaprağının dane verimi üzerine katkısı fazladır (Blum, 1985; Merah et al., 2018). Bayrak yaprakları ve kılçıklar fotosentezde önemli bir etkiye sahiptir. Buğdayda, yapraklar, özellikle bayrak yaprakları, yüksek verime katkıda bulunan organlar olarak kabul edilirken, kılçıklar ise yan organlar olarak kabul edilmiştir. Kılçıkların özellikle dane doldurma aşamalarında kılçıklı buğday çeşitlerinde dane verimine katkısının fazla olduğu bildirilmektedir (Li et al., 2006).

Bu çalışma, ekmeklik buğday çeşitlerinde başaklanma döneminde uzaklaştırılan bazı fotosentez organlarının tarımsal özellikler üzerine etkisinin belirlenmesi amacıyla yürütülmüştür.

MATERYAL ve METOD

Bu çalışma, Bursa Uludağ Üniversitesi Ziraat Fakültesi Uygulama ve Araştırma Merkezinde 2018-2019 sezonunda yürütülmüştür. Tesadüf bloklarında bölünmüş parseller deneme desenine göre üç tekerrürlü olarak yürütülen deneme, ana parselleri çeşitler, alt parselleri ise uygulamalar olacak şekilde (toplam 42 parsel) kurulmuştur. Çalışmada her parsel 5 m uzunluğunda ve 1,2 m genişliğinde 6 m²'den oluşmaktadır. Çalışmada bitki materyali olarak Pehlivan ve Golia çeşitleri kullanılmıştır. Denemenin ekimi 14 Kasım 2018 tarihinde deneme mibzeri ile yapılmıştır. Nisan ayının son haftası içerisinde (2019) çeşitlerin tam başaklanma döneminde bazı fotosentez organlarının uzaklaştırılması işlemi uygulanmıştır. Bitkilerin hasadı ise 2019 Temmuz ayının ilk haftasında yapılmıştır.

Çeşitlerin başaklanma dönemlerinde (Zadoks 59. Dönem) (Zadoks et al.,1974) deneme parsellerinden her tekerrürden rastgele belirlenen 30 bitkinin ana sapı üzerinde aşağıdaki uygulamalar yapılmıştır:

1. Uygulama (UYG1): Kontrol

2. Uygulama (UYG2): Bitkideki tüm yapraklar uzaklaştırılmıştır

3. Uygulama (UYG3): Kılçıklar uzaklaştırılmıştır

4. Uygulama (UYG4): Bayrak yaprak hariç tüm yapraklar uzaklaştırılmıştır

5. Uygulama (UYG5): Bayrak yaprak uzaklaştırılmıştır

6. Uygulama (UYG6): Bayrak yaprak ve kılçıklar uzaklaştırılmıştır

7. Uygulama (UYG7): Bayrak yaprak ve altındaki birinci yaprak uzaklaştırılmıştır

Çalışmada bitki materyali olarak kullanılan iki ekmeklik buğday çeşidinde; bitki boyu, başak boyu, başakta başakçık sayısı, başakta tane sayısı, başakta tane ağırlığı, bin tane ağırlığı, hektolitre ağırlığı, protein oranı, sedimantasyon değeri, gluten oranı ve hasat indeksi özellikleri incelenmiştir. Bu değerlerin tespitinde, her parsel için Uluöz, (1965); Kırtok, (1982); Akkaya ve Akten (1988); Dinçer, (1991) ve Çölkesen ve ark., (1994)' in uygulamış oldukları yöntemler esas alınarak gözlem, ölçüm ve analizler aşağıdaki şekilde yapılmıştır:

-Bitki Boyu (cm): Her parselden tesadüfi olarak seçilen 10 örnek bitkide, kök boğazından başakçığın en üst ucuna kadar (kılçık hariç) olan kısmı cm olarak ölçülmüştür.

-Başak Boyu (cm): Her parselden alınan 10 adet örnek bitkide başak eksenin çıktığı boğum ile en üst başakçığın ucuna kadar olan kısım kılçık hariç ölçülerek cm cinsinden belirlenmiştir.

-Başakta Başakçık Sayısı (adet): Başak boyları ölçülen 10 başaktaki başakçıklar sayılmıştır.

-Başakta Dane Sayısı (adet): Her parselden rastgele alınan 10 adet başağın tek başak harman makinesinde harmanlanması ile elde edilen danelerin sayılmasıyla bulunmuştur.

-Başakta Dane Ağırlığı (g): Her parselden rastgele alınan 10 adet başağın tek tek harmanlanması ile elde edilen daneler 0,01 g duyarlılıktaki terazi ile tartılarak dane ağırlığı değerleri belirlenmiştir.

-Bin Dane Ağırlığı (g): Her parselden alınan materyallerden 3 defa 100 buğday danesi sayılıp, 0,01 g duyarlılığa sahip terazide ölçülmesi sonucunda çıkan değerlerin ortalaması alınarak 10 ile çarpılarak 1000 dane ağırlığı hesaplanmıştır.

-Hasat İndeksi (%): Her uygulamaya ait dane verimi, o uygulamaya ait biyolojik verim değerine oranlanmış ve yüzde (%) olarak hasat indeksi hesaplanmıştır.

-Hektolitre Ağırlığı (kg/100lt): Denemelerden elde edilen daneler hektolitre aleti ile ölçülerek 100 lt'nin

ağırlığına oranlanmıştır.

-Protein oranı (%): Elde edilen dane örnekleri öğütülerek Kjeldahl metoduna göre iki paralel halinde azot miktarı tespit edilerek hesaplanmıştır.

-Sedimantasyon Değeri (ml): Bir tüpün içine 50 ml brom fenol konulduktan sonra 3,2 g un numunesi tüpün içine aktarılmış ve el ile 10 defa sert bir şekilde çalkalanmıştır. Sonrasında sedimantasyon makinesinde 5 dk salınıma bırakılmıştır ve salınım bittikten sonra 25 ml laktik asit çözeltisi üzerine eklenmiştir. Tüpler tekrar 5 dk salınıma bırakılmış ve süre sonunda tüp alınıp sabit bir yerde 5 dk bekletildikten sonra okuma yapılmıştır.

-Gluten Oranı (%): Öğütülme sonrası elde edilen un örneklerinden 10 g un alınarak gluten makinesine konulmuştur, % 2'lik tuzlu su içerisinde 5 dk yıkandıktan sonra makineden alınmıştır. Alınan örnek gluten indeks makinesi bantlarına yerleştirilmesinden sonra örnek 600 dk/devir dönerek sağlam ve sağlam olmayan kısım birbirinden ayrılmıştır. Her ikisinin toplamı yaş gluten olarak belirlenmiştir.

Çalışmanın yürütüldüğü Bursa Uludağ Üniversitesi Araştırma ve Uygulama Merkezi topraklarının genellikle ağır bünyeli ve tuzluluk gruplandırılmasında tamamının tuzluluk yönünden bir problemi olmadığı belirlenmiştir. pH gruplandırılmasında ise %50'sinden fazlasının orta alkali grubunda olduğu anlaşılmıştır. Deneme yerinin toprakları organik madde açısından sınıflandırıldığında humusça fakir olduğu ve sürekli tarım yapıldığından dolayı azalan organik maddenin topraklarda artırılmasının gerekli olduğu belirlenmiştir. Araştırma topraklarının büyük bir kısmı kireççe fakir olup değişebilir potasyum, kalsiyum, magnezyum kapsamları oldukça yüksektir (Deveciler, 2005).

Denemenin yürütüldüğü Bursa ili Akdeniz ve Karadeniz iklimleri arasında geçiş göstermektedir. İlde kışlar çok sert geçmezken yaz dönemlerinde de fazla kuraklık görülmez (Anonim, 2018b). Bursa ili denemenin yürütüldüğü 2018-2019 yılı yetiştirme dönemi içinde toplam yağış miktarı 442,6 ml olarak belirlenmiştir. Kasım – Şubat aylarında ortalama yağış 69,4 ml olarak düşerken, sapa kalkma ve erme dönemi olan Mart – Mayıs aylarında ortalama yağış 37,27 ml ve fizyolojik erme ve sonraki dönemleri kapsayan Haziran ayında 31,2 ml yağış düşmüştür. Denemenin yapıldığı 2018-2019 yılı yetiştirme sezonunda toplam sıcaklık 123,3 °C, ortalama sıcaklık 13,7 °C olarak ölçülmüştür. Kasım – Şubat aylarında ortalama sıcaklık 7,83 °C olarak ölçülmüşken, sapa kalkma ve erme dönemi olan Mart – Mayıs aylarında ortalama sıcaklık 14,23 °C ve fizyolojik erme ve sonraki dönemleri kapsayan Haziran ayında 24,5 °C olarak ölçülmüştür (Anonim, 2018c).

Araştırmada elde edilen değerler, "JMP 7" istatistik analiz programı kullanılarak tesadüf bloklarında bölünmüş parseller deneme desenine göre varyans analizi gerçekleştirilmiştir. Ortalamaların karşılaştırılması için LSD testi uygulanmıştır. Önemlilik durumuna göre elde edilen bulgular her özellik için ayrı ayrı gruplandırılmıştır (Peterson, 1994).

BULGULAR ve TARTIŞMA

Çalışmada saptanan bitki boyu, başak boyu, başakçık sayısı, başakta tane sayısı ve ağırlığı ile hasat indeksi özelliklerine ait varyans analizi sonuçları Çizelge 1'de, bin tane ağırlığı, protein oranı, sedimantasyon değeri, glüten oranı ve hektolitre ağırlığı değerlerine ait varyans analizi sonuçları da Çizelge 2'de verilmiştir. Buna göre; bitki boyu, başakta tane sayısı ve ağırlığı ile hasat indeksi için çeşitler, uygulamalar ve çeşit x uygulama interaksiyonu, başak boyu için uygulamalar, başakçık sayısı için uygulamalar ve çeşit x uygulama interaksiyonu etkisi %1 olasılık düzeyinde istatistiki olarak önemli bulunmuştur (Çizelge1). Bin tane ağırlığı, protein oranı ve gluten oranı özellikleri için çeşitlerin; yine bu üç özellik ve hektolitre ağırlığı için uygulamaların ve bin tane ağırlığı, protein oranı, gluten oranı, sedimantasyon değeri ve hektolitre ağırlığı özellikleri için ise çeşit x uygulama
interaksiyonlarının etkisi istatistiki olarak önemli bulunmuştur (Çizelge 2).

Çalışmada saptanan ortalama bitki boyu değerleri Çizelge 3'de verilmiştir. Buna göre; çeşitlerin ortalama bitki boyu değerleri Golia çeşidi için 65.6 cm Pehlivan çeşidi için ise 100.8 cm olarak saptanmıştır. Farklı uygulamalara ait bitki boyu ortalamaları 80.81-87.18 cm arasında değişmiştir. En uzun bitki boyu kontrol uygulamasında, en kısa bitki boyu ise kılçıkların uzaklaştırıldığı UYG3 uygulamasından elde edilmiştir. Bitki boyunu en fazla etkileyen uygulama kılçıkların uzaklaştırılması olmuştur ve %8 oranında bir boy azalması meydana gelmiştir. En az etkileyen uygulama ise %1.60 azalma ile bayrak yaprağının uzaklaştırılması (UYG5) olmuştur. Çeşit x uygulama interaksiyonlarında ise ortalama bitki boyu değerleri 61.53-103.96 cm arasında geniş bir değişim göstermiştir. Pehlivan çeşidini en fazla etkileyen uygulama bayrak yaprak ve altındaki birinci yaprağın uzaklaştırıldığı UYG7'de olmuştur. Bu uygulama ile 96.00 cm bitki boyu saptanmış ve %7.65 oranında bir azalma söz konusu olmuştur. Pehlivan çeşidinde bayrak yaprak hariç tüm yaprakların uzaklaştırıldığı UYG4 ve bayrak yaprağının uzaklaştırıldığı UYG5 uygulamalarından sırasıyla 102.26 cm ve 103.86 cm ile en yüksek bitki boyunun saptandığı kontrol ile aynı gruba giren sonuçlar elde edilmiştir. Golia çeşidini en fazla etkileyen uygulama ise 61.53 cm ile en yüksek değerin saptandığı kontrol uygulamasına oranla % 12.59'luk azalma ile kılçıkların

uzaklaştırıldığı UYG3 uygulamasında bulunmuştur. Bu sonuçlar; buğdayda bayrak yaprağının uzaklaştırılmasının bitki boyunun kısalmasına sebep olduğunu açıklayan Mahmood et al., (1991) ve Chowdhry et al., (1999) bulgularını destekler niteliktedir. Buna karşılık kılçıkların kesilmesinin bitki boyu üzerine fazla etki göstermediğini açıklayan Saghir et al.,(1968) sonuçları ile de uyumsuzluk söz konusu olmuştur.

Çizelge 1. İki ekmeklik buğday çeşidi ve yedi farklı uygulamanın bitki boyu, başak boyu, başakçık sayısı, başakta tane sayısı başakta tane ağırlığı ve hasat indeksi özelliklerine ait varyans analizi sonuçları (kareler ortalaması değerleri)

Table 1. Variance analysis results (mean square values) of plant height, spike length, number of spikelets, number of grains per spike, grain weight per spike and harvest index of two bread wheat varieties and seven different treatments

Varyasyon Kaynağı *Sources of Variations*	SD *DF*	Bitki Boyu *Plant Height*	Başak Boyu *Spike Length*	Başakçık Sayısı *Number of Spikelet per spike*	Başakta Tane Sayısı *Number of Grains per Spike*	Başakta Tane Ağırlığı *Grain Weight per Spike*	Hasat İndeksi *Harvest Index*
Çeşitler *Cuyltivars*	1	13069.82**	0.77	2.38	5201.49**	0.581**	740.12**
Uygulama *Treatment*	6	34.2**	0.35**	2.77**	14.31**	0.053**	9.18**
Ç X U C X T	6	18.98**	0.13	2.43**	10.39**	0.031**	7.11**
Bloklar *Replications*	2	3.68	0.01	0.21	0.51	0.006	1.01
Ana Parsel Hatası *Main Parcel Error*	2	3.20	0.10	1.07	2.49	0.003	0.97
Hata *(Error)*	24	1.61	0.05	0.22	0.97	0.001	0.68

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

Çizelge 2. İki ekmeklik buğday çeşidi ve yedi farklı uygulamanın bin tane ağırlığı, protein oranı, sedimantasyon değeri, gluten oranı ve hektolitre ağırlığı özelliklerine ait varyans analizi sonuçları (kareler ortalaması değerleri)

Table 2. Variance analysis results (mean square values) of 1000 grain weight, protein content, sedimentation value, gluten content and hectoliter weight of two bread wheat varieties and seven different treatments

Varyasyon Kaynağı *Sources of Variations*	SD *RF*	Bin Tane Ağırlığı *1000 Kernel Weight*	Protein Oranı *Protein Ratio*	Sedimantasyon Değeri *Sedimentation*	Gluten Oranı *Gluten Ratio*	Hektolitre Ağırlığı *Hectolitre Weight*
Çeşitler (Ç) *Cuyltivars*	1	579.13*	11.02**	21.42	62.90*	1.31
Uygulama(U) *Treatment*	6	55.07**	2.37**	1.96	70.92**	31.99**
Ç X U C X T	6	9,.39**	1.19**	2.98 *	34.90**	3.94*
Bloklar *Replications*	2	7.09	0.83	3.42	1.72	6.41
Ana Parsel Hatası *Main Parcel Error*	2	1.49	0.19	4.57	0.24	0.76
Hata *(Error)*	24	1.10	0.20	0.66	1.65	1.30

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

Başak boyu özelliğine ait ortalama değerler incelendiğinde, çeşitlerin başak boyu değerlerinin ortalama olarak 7.47-7.74 cm arasında değiştiği görülmektedir. Buna göre; çeşitlerin ortalama en uzun başak boyu 7.74 cm ile Golia çeşidinde, ortalama en kısa başak boyu ise 7.47 cm ile Pehlivan çeşidinde saptanmıştır. Farklı uygulamalara ait başak boyu ortalamaları ise 7.35 cm ile 8.03 cm arasında değişmektedir. En uzun başak boyu bayrak yaprağının uzaklaştırıldığı UYG5 uygulamasından, en kısa başak

boyları ise kılçıkların uzaklaştırıldığı UYG3 ve bayrak hariç tüm yaprakların uzaklaştırıldığı UYG4 uygulamalarından elde edilmiştir. Bayrak yaprağının uzaklaştırılması uygulaması (UYG5) ortalama başak boyunu kontrol uygulamasına oranla %5.51 oranında artırmıştır. Bayrak yaprak hariç diğer tüm yapraklar uzaklaştırılması (UYG4) ise ortalama başak boyunu %3.41 ve kılçıkların uzaklaştırılması (UYG3) da %3.28 oranında bir boy azalmasına neden olmuştur. Çeşit x uygulama interaksiyonları incelendiğinde başak boyu değerlerinin 7.23 cm ile 8.16 cm arasında değiştiği tespit edilmiştir. En uzun başak boyu değeri Pehlivan çeşidinde 8.16 cm ile bayrak yaprağının uzaklaştırıldığı UYG5 uygulamasında, Golia çeşidinde ise 8.10 cm ile bayrak yaprak ve altındaki birinci yaprağın uzaklaştırıldığı UYG7 uygulamasından elde edilmiştir. Bayrak yaprak hariç diğer yaprakların uzaklaştırıldığı UYG4 ve bayrak yaprak ve kılçıkların birlikte uzaklaştırıldığı UYG6 uygulamalarından Pehlivan çeşidinde 7.23 cm ile en kısa başak boyu değerleri elde edilmiştir. Elde edilen sonuçlar, başak boyunda kılçıkların kesilmesinin %2.04 oranında azalmaya sebep olduğu ve kılçıkların ve bayrak yaprağının aynı anda kesilmesinin %6.82 oranında başak boyunda azalma meydana getirdiğini açıklayan Khaliq et al., (2008) bulguları ile uyum içerisindedir. Bayrak yaprak kesilerek gelişen buğdayda başak uzunluğunun azaldığını gözlemleyen Mahmood et al., (1991) ile bayrak yaprağının kesilmesinin başak boyu üzerinde %5.09 oranında azalma olduğunu bildiren Khaliq et al., (2008)'in bulgularıyla ise uyumsuzluk gösterdiği tespit edilmiştir (Çizelge3).

Başakta başakçık sayısı özelliğine ait çeşitlerin ortalama başakta başakçık sayısı incelendiğinde değerlerin 16.72-17.20 adet arasında değiştiği görülmüştür (Çizelge3). Herhangi bir uygulamanın yapılmadığı UYG1 kontrol uygulamasında başakta başakçık sayıları Pehlivan çeşidinde 17.26 adet, Golia çeşidinde ise 18.23 adet olarak tespit edilmiştir. Farklı uygulamalara ait başaktaki başakçık sayısı ortalamaları 16.05-17.83 adet arasında değişmiştir. En fazla başakta başakçık sayısının saptandığı bayrak yaprağının uzaklaştırıldığı UYG5 uygulamasında kontrol uygulaması ile aynı gruba giren sonuçlar elde edilmiştir. En az başakta başakçık sayısı ise kılçıkların uzaklaştırıldığı UYG3 uygulamasından elde edilmiştir. Başakta başakçık sayısını en fazla etkileyen uygulama kılçıkların uzaklaştırıldığı bu uygulama olmuştur ve %9.57 oranında bir azalma meydana gelmiştir. Çeşit x uygulama interaksiyonları incelendiğinde ise ortalama başakta başakçık sayısı değerlerinin 15.33-18.93 adet aralığında değişim gösterdiği görülmektedir. Kontrol uygulaması ile karşılaştırıldığında kılçıkların uzaklaştırıldığı UYG3 uygulaması Pehlivan çeşidinde %2.89'luk bir azalmaya neden olurken, Golia çeşidinde ise %15.90 oranında azalma söz konusu olmuştur. Pehlivan çeşidinde bayrak yaprağının uzaklaştırılması %9.67 oranında başakta başakçık sayısında artışa neden olurken aynı uygulama Golia çeşidinde ise %8.22'lik bir azalmaya neden olmuştur. Araştırmada çeşitlere göre değişen ve istatistiki olarak önemli olumlu ya da olumsuz değişimlere sebep olduğu saptanan değerler ile bayrak yaprak kesilmesinin başakçık sayısına olumlu veya olumsuz bir etki yapmadığını bildiren Mahmood et al., (1991), Chowdhry et al., (1999), Birsin (2005) ve Balkan ve Gençtan (2009)'ın bulgularıyla farklılıklar saptanmıştır.

Çeşitlerin ortalama başakta tane sayısı değerleri Pehlivan çeşidi için 30.55 adet, Golia çeşidi için 52.81 adet olarak saptanmıştır Bu özellik için farklı uygulamalar sonucu elde edilen 39.45-43.68 adet arasında değişmiştir. En yüksek değer kontrol uygulamasından elde edilmiştir (Çizelge 3). En düşük ortalama başakta tane sayısı ise 39.45 adet ile bayrak yaprak ve kılçıkların birlikte uzaklaştırıldığı UYG6 uygulamasından elde edilmiştir. Kontrol uygulamasına oranla UYG6 uygulamasında %9.68 oranında bir azalma meydana gelmiştir. Başakta tane sayısını en az etkileyen uygulama ise %1.67 azalma ile bayrak yaprak ve altındaki birinci yaprağın uzaklaştırılmasında (UYG7) belirlenmiştir. Diğer uygulamalar ise bu değerlerin arasında yer almışlardır. Çeşit x uygulama interaksiyonlarında ise ortalama başakta tane sayısı 28.80 adet ile 56.23 adet arasında değişim göstermiştir. Pehlivan çeşidini en fazla etkileyen uygulama bayrak yaprak ve kılçıkların birlikte uzaklaştırıldığı UYG6 olmuştur. Bu uygulama ile kontrol uygulamasına göre başakta tane sayısında %7.48'lik azalma meydana gelmiştir. Golia çeşidinde en fazla değişim bayrak yaprak hariç tüm yaprakların uzaklaştırıldığı UYG4 ile bayrak yaprak ve kılçıkların birlikte uzaklaştırıldığı UYG6 da olmuştur ve bu uygulamalar sırasıyla %12.14 ile %10.96 oranlarında azalmaya neden olmuştur. Başakta tane sayısında bayrak yaprağının uzaklaştırılmasına ait çalışmaların sonuçları incelendiğinde; Chowdhry et al., (1999) bayrak yaprağının kesilmesinin başaktaki tane sayısını azalttığını ifade ederken bu uygulamanın Ali et al., (2010) %3.66, Alam et al., (2008) %9.9, Birsin (2005) %12,8, Mahmood et al., (1991) %12.9, ve Duwayri (1984) %11.1'lik bir azalma meydana getirdiğini bildirmişlerdir. Buldukları değerler çalışmadaki değerler ile uyum içerisindedir. Balkan ve Gençtan (2009), kılçıkların uzaklaştırılmasının başakta tane sayısının birinci yıl sonuçlarında %3.54 ve Ali et al., (2010) da aynı uygulamanın başakta tane sayısında %8.32'lik önemli düzeyde azalmaya neden olduğunu bildirmektedirler. Çalışmada ise kılçıkların uzaklaştırılması başakta tane sayısı bakımından Pehlivan çeşidinde bir etki yapmazken, Golia çeşidinde önemli derecede bir azalmaya sebep olmuştur.

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Çizelge 3. Çalışmada incelenen özelliklere ait ortalama değerler ve önemlilik grupları
Table3. Mean values and significance groups of the investigated traits

	Bitki Boyu *Plant Height*			Başak Boyu *Spike Lenght*			Başakçık Sayısı *Number of Spikelet*			Başakta Tane Sayısı *Number of Grains per Spike*		
	P*	G**	Uyg. Ort.	P	G	Uyg. Ort.	P	G	Uyg. Ort.	P	G	Uyg. Ort.
UYG1	103.96±1.27a	70.40±.1.22e	87.17±18.41A	7.46±0.15	7.76±0.15	7.61±0.21B-D	17.26±0.11cd	18.23±0.40ab	17.75±0.59A	31.13±0.95ef	56.23±1.81a	43.68±13.81A
UYG2	99.93±0.09c	62.43±1.07h1	81.18±20.54C	7.53±0.15	7.86±0.16	7.70±0.23BC	16.96±0.57d	16.50±0.61de	16.73±0.58B	30.73±0.71ef	54.26±1.00b	42.50±12.91B
UYG3	100.10±1.55c	61.53±1.55ı	80.81±21.17C	7.30±0.26	7.43±0.50	7.36±0.37D	16.76±0.64de	15.33±0.95f	16.05±1.07C	31.83±0.64e	52.26±0.35c	42.05±11.20BC
UYG4	102.26±2.85ab	64.23±0.06gh	83.25±20.90B	7.23±0.15	7.46±0.31	7.35±0.25D	17.06±0.55d	16.13±0.25e	16.60±0.64BC	30.93±1.00ef	49.23±1.78d	40.08±10.09DE
UYG5	103.86±1.10a	67.70±1.11f	85.78±19.83A	8.16±0.12	7.90±0.10	8.03±0.18A	18.93±0.06a	16.73±0.55de	17.83±1.26A	30.33±0.87e-g	51.83±1.15c	41.08±11.81CD
UYG6	100.13±1.97bc	65.30±0.36g	82.71±19.12B	7.23±0.06	7.70±0.26	7.46±0.31CD	16.80±0.92de	16.13±0.25e	16.46±0.70BC	28.80±0.10g	50.10±0.30d	39.45±11.67E
UYG7	96.00±0.78d	67.70±1.65f	81.85±15.54BC	7.40±0.20	8.10±0.35	7.75±0.46B	16.63±0.35de	18.03±0.06bc	17.33±0.79A	30.13±1.55fg	55.76±0.59ab	42.95±14.08AB
Çeşit Ort.	100.8 ±8.73A	65.6 ±9.09B		7.47±0.34	7.74±0.33		17.20±0.87	16.72±1.09		30.55±1.16B	52.81±2.74A	
LSD(%5)	: Ç.0,83; U.1,55; Ç x U = 2,20			Ç. 0,15; U. 1,28			Ç: 0,33; U: 0,63; Ç x U: 0,89			Ç: 0,66; U: 1,23; Ç x U: 1,75		

	Başakta Tane Ağırlığı *Grain Weight per Spike*			Hasat İndeksi *Harvest Index*			Bin Tane Ağırlığı *1000 Kernel Weight*			Protein Oranı *Protein Ratio*		
	P	G	Uyg. Ort.	P	G	Uyg. Ort.	P	G	Uyg. Ort.	P	G	Uyg. Ort.
UYG1	1.26±0.05fg	1.69±0.04a	1.48±0.24A	33.00±0.78e	41.44±0.24a	37.22±4.65A	42.25±1.15a	34.47±0.94d	38.36±4.36A	8.04±0.11e	10.19±0.18b-d	9.12±1.19D
UYG2	0.98±0.03ı	1.34±0.03c-e	1.16±0.20C	28.01±0.77h	39.50±0.26bc	33.75±6.32E	32.37±1.69e	27.80±0.79f	30.08±2.77E	9.96±0.71cd	10.81±0.36ab	10.39±0.69B
UYG3	1.23±0.02fg	1.40±0.03bc	1.32±0.09B	32.44±1.31ef	38.331.14±c	38.38±3.41BC	42.59±0.62a	34.97±0.40d	38.78±4.20A	10.90±0.23ab	11.3,6±0.82a	11.13±0.59A
UYG4	1.10±0.04h	1.44±0.04b	1.27±0.19B	30.97±1.45g	40.530.46±ab	35.75±5.32B	36.90±1.49c	32.33±1.43e	34.61±2.83C	10.15±0.49b-d	10.27±0.46bc	10.21±0.42BC
UYG5	1.23±0.07fg	1.36±0.02cd	1.30±0.09B	29.04±0.68h	38.750.05±c	33.90±5.34DE	38.00±0.56bc	31.70±2.05e	34.85±3.73C	9.65±0.37cd	11.55±0.34a	10.60±1.08AB
UYG6	1.27±0.07ef	1.30±0.01d-f	1.28±0.05B	31.50±1.23fg	36.81±0.33d	34.15±3.02DE	41.83±1.33a	31.53±2.02e	36.68±5.85B	9.84±0.66cd	9.82±0.25cd	9.83±0.45C
UYG7	1.19±0.09g	1.39±0.07bc	1.29±0.13B	30.58±0.98g	38.94±0.73c	34.76±4.64CD	38.75±0.50b	27.92±0.90f	33.33±5.96D	9.44±0.28d	11.17±0.92a	10.30±1.12BC
Çeşit Ort.	1.18±0.11B	1.14±0.12A		30.76±1.91B	39.18±1.41A		38.95±3.61A	31.53±2.92B		9.71±0.91B	10.74±0.77A	
LSD(%5) :	Ç:0,02; U: 0,05; Ç x U: 0,07			Ç: 0,53; U: 0,99; Ç x U: 1,40			Ç:0,67; U: 1,26; Ç x U: 1,78			Ç:0,28; U: 0,53; Ç x U: 0,76		

	Sedimantasyon Değeri *Sedimantation*			Gluten Oranı *Gluten Ratio*			Hektolitre Ağırlığı *Hectoliter Weight*		
	P	G	Uyg. Ort.	P	G	Uyg. Ort.	P	G	Uyg. Ort.
UYG1	26.66±1.15ab	26.66±1.15ab	26.66±1.03	27.83±0.59bc	29.90±1.15ab	28.86±1.40A	76.99±0.81ab	77.13±0.53ab	77.06±0.62AB
UYG2	26.00±0.0ab	24.00±0.0cd	25.00±1.09	20.66±0.96gh	17.90±1.49ı	19.28±1.44D	70.62±1.18f	71.42±0.98ef	71.02±1.06D
UYG3	26.00±0.0ab	26.00±2.0ab	26.00±1.26	31.63±0.29a	24.66±0.75de	28.15±3.85A	78.13±1.44a	77.04±1.35ab	77.58±1.39A
UYG4	26.00±0.0ab	25.33±1.25bc	25.66±0.82	22.20±1.70fg	26.70±0.82cd	24.45±2.74B	76.38±1.46ab	77.11±1.06ab	76.74±0.92AB
UYG5	27.33±1.15a	23.33±1.15d	25.33±2.42	23.73±1.23ef	23.73±0.06ef	23.73±0.78B	73.14±0.76de	74.36±0.33cd	73.75±0.85C
UYG6	26.66±1.15ab	23.33±1.16d	26.00±1.27	26.26±1.75cd	18.00±0.78ı	22.13±4.68C	76.24±0.63a-c	75.46±0.64bc	75.85±0.71B
UYG7	27.33±1.15a	25.33±1.15bc	26.33±1.51	24.83±2.75de	19.13±0.21h1	21.98±3.58C	76.24±1.41a-c	72.74±3.22de	74.49±2.93C
Çeşit Ort.	26.57±0.93	25.14±1.49		25.30±3.72A	22.86±4.48B		75.39±2.60	75.04±3.19	
LSD(%5) :	Ç:0,62; Ç x U: 1,64			Ç: 0,78; U: 1,47; Ç x U: 2,08			U: 1,32; Ç x U: 1,87		

*P: Pehlivan ** G: Golia

Başakta tane ağırlığına ait çeşitlerin ortalama değerleri incelendiğinde, değerlerin 1.18-1.42 g arasında değiştiği görülmektedir. Başakta tane ağırlığı açısından en yüksek değerler Golia çeşidinden elde edilmiştir. Farklı uygulamalara ait ortalama başakta tane ağırlığı değerlerinde ise en yüksek başaktaki tane ağırlığı 1.48 g ile kontrol uygulamasından elde edilmiştir. Ortalama en düşük değerler tüm yaprakların uzaklaştırıldığı UYG2 uygulamasında saptanmıştır. Diğer uygulamalardan ise 1.27-1.32 g arasında ve aynı istatistiki gruba giren değerler elde edilmiştir. Çeşit x uygulama interaksiyonları incelendiğinde, en yüksek başakta tane ağırlığı 1.69 g ile Golia çeşidinin kontrol uygulamasından (UYG1) elde edilmiştir. Pehlivan çeşidinde başakta tane ağırlığında en büyük etkiyi tüm yaprakların uzaklaştırıldığı UYG2 uygulaması yapmıştır. Bu uygulama ile 0.98 g başakta tane ağılığı saptanmış ve kontrole göre %20.32'lik azalma olmuştur. Bayrak yaprak hariç diğer tüm yaprakların uzaklaştırıldığı UYG4 uygulaması ile %10.56 oranında bir azalma söz konusu olmuştur. Golia çeşidinde ise en fazla değişim bayrak yaprak ve kılçıkların birlikte uzaklaştırıldığı UYG6, tüm yaprakların uzaklaştırıldığı UYG2 ve bayrak yaprağının uzaklaştırıldığı UYG5 uygulamalarından sırasıyla %23.07, %20.71 ve %19.52 oranlarında azalma belirlenmiştir (Çizelge 3). Bayrak yaprağının kesilmesinin başakta tane ağırlığında Mahmood et al., (1991) %11.2 oranında, Ali et al., (2010) %14.14 oranında, Birsin (2005) %34 oranında ve Balkan ve Gençtan (2009) 2005 yılında %21.12 ve 2006 yılında %16.33 oranında azalma meydana getirdiğini bildirmektedirler. Kılçıkların uzaklaştırılmasının ise başakta tane ağırlığında Ali et al., (2010) %18.10, Birsin (2005) %13.1, Balkan ve Gençtan (2009) 2005 yılında %7.26 ve 2006 yılında %4.78 oranlarında azalma meydana getirdiğini ifade etmişlerdir. Araştırmada çeşitlere göre değişen oranlarda da olsa genel olarak uygulamaların azalan yöndeki etkisi bu sonuçlar ile paralellik göstermektedir.

Çalışmada Pehlivan çeşidi için ortalama hasat indeksi değeri %30.76, Golia çeşidi için ise %39.18 olarak saptanmıştır. Golia çeşidi kısa boylu olması nedeniyle daha yüksek hasat indeksi değerine sahip olmuştur. Farklı uygulamalara ait ortalama hasat indeksi değerleri %33.75 ile %37.22 arasında değişmiştir. En yüksek hasat indeksi değerleri kontrol uygulamasından (%37.22) elde edilmiştir. Hasat indeksi değerlerinde UYG2 uygulaması ile %9.32 ve UYG5 uygulaması ile %8.91'lik bir azalma meydana geldiği belirlenmiştir.Çeşit x uygulama interaksiyonlarına ait hasat indeksi değerleri %28.01 ile %41.44 arasında değişen değerler almıştır. Pehlivan çeşidinde bayrak yaprağının uzaklaştırıldığı UYG5 uygulaması %11.99 ve tüm yaprakların uzaklaştırıldığı UYG2 uygulaması ise %15.2 oranında bir azalmaya neden olmuştur. Golia çeşidinde bayrak yaprak hariç tüm yaprakların uzaklaştırıldığı UYG4 uygulaması %40.53 ile en yüksek hasat indeksi değerinin saptandığı kontrol uygulaması ile aynı grupta yer almıştır. Bayrak yaprak uzaklaştırılması Golia çeşidinin hasat indeksi değerinde (%6.49) Pehlivan çeşidine oranla daha az bir azalmaya sebep olmuştur. Hasat indeksi bakımından Golia çeşidinde en fazla azaltıcı etkiyi %11.17 ile bayrak yaprak ve kılçıkların birlikte uzaklaştırıldığı UYG6 uygulaması yapmıştır. Kılçıkların uzaklaştırıldığı UYG3 uygulaması ile Pehlivan ve Golia çeşitlerinin hasat indeksi değerlerinde sırasıyla %1.69 ve %7.50 oranlarında azalmaya neden olmuştur (Çizelge 3). Serin iklim tahıllarında hasat indeksinin yüksek olması, tane veriminin de yüksek olması anlamına gelmesinin yanı sıra, bitki boyunun kısa olması anlamına da gelmektedir (Budak ve Yıldırım, 1995). Ayrıca hasat indeksinin yüksek veya düşük olması çevresel faktörlerden de kaynaklanabilmektedir. Farklı bölgelerde yapılan çalışmalarda araştırıcılar ekmeklik buğday hasat indeksinin % 22.6-42.6 arasında değiştiğini belirlemişlerdir (Turan, 2008; Ayter, 2010; Özen, 2014). Çalışmada da doğal olarak kısa boylu Golia çeşidinden uzun boylu Pehlivan çeşidine göre daha yüksek hasat indeksi değerleri elde edilmiştir.

Çalışmada çeşitlerin ortalama bin tane ağırlığı değerleri Golia çeşidi için 31.53 g, Pehlivan çeşidi için ise 38.95 g olarak belirlenmiştir. En yüksek bin tane ağırlığı değeri 38.36 g ile kontrol uygulaması UYG1 ve kontrol uygulaması ile aynı gruba giren kılçıkların uzaklaştırıldığı UYG3 uygulamasından (38.78 g) elde edilmiştir. En düşük değerler ise tüm yaprakların uzaklaştırıldığı UYG2 uygulamasında belirlenmiş ve bu uygulama ile kontrole göre %21.8 oranında bir azalma meydana gelmiştir. Çeşit x uygulama interaksiyonlarına ait bin tane ağırlığı değerleri 27.80-42.59 g arasında değişmiştir. Pehlivan çeşidinde de Golia çeşidinde de bin tane ağırlığını en fazla etkileyen uygulama tüm yaprakların kesildiği UYG2'de olmuştur. Bu uygulama ile Pehlivan çeşidinde 32.37 g ve Golia çeşidinde 27.80 g bin tane ağırlığı saptanmıştır. Ayrıca bu uygulama, kontrol uygulamasına kıyasla Pehlivan çeşidinde %23.38 ve Golia çeşidinde ise %19.35 oranında düşüş meydana getirmiştir. Pehlivan çeşidinde kılçıkların uzaklaştırıldığı UYG3 ve bayrak yaprak ve kılçıkların birlikte uzaklaştırıldığı UYG6 uygulamalarından sırasıyla 42.59 g ve 41.83 g ile kontrol uygulamasıyla aynı gruba giren sonuçlar elde edilmiştir. Bayrak yaprağının uzaklaştırıldığı UYG5 uygulaması ile Pehlivan çeşidinde 38.00 g ve Golia çeşidinde 31.70 g bin tane ağırlığı belirlenmiştir ve sırasıyla %10.05 ve %8.03'lük azalma gözlemlenmiştir (Çizelge3). Bayrak

yaprağının uzaklaştırılması ile bin tane ağırlığında Khaliq et al., (2008) göre %8.86, Alam et al., (2008) göre %7.65, Balkan ve Gençtan (2009) göre %9.32, Ali et al., (2010) göre ise %11.86 oranında azalma meydana geldiğini tespit etmişlerdir. Asghar and Ingram (1993) ile Alam et al., (2008) buğdayda tüm yaprakların kesilmesinin bin tane ağırlığında %13.2 oranında azalmaya sebep olduğunu ifade etmişlerdir. Bu değerler araştırmadaki değerlerle paralellik göstermektedir. Sonuçlar, bayrak yaprak uzaklaştırılmasının bin tane ağırlığını olumsuz yönde etkilediğini ifade eden Chhabra ve Sethi (1989), Mahmood et al.,(1991), Chowdhry et al., (1999)'nın bulgularını destekler niteliktedir.

Çalışmada çeşitlerin ortalama protein oranı değerleri Pehlivan çeşidinde %9.71, Golia çeşidinde %10.74 olarak belirlenmiş olup en yüksek değer Golia çeşidinden elde edilmiştir. Farklı uygulamalara ait ortalama protein değerleri ise %9.12 ile %11.13 arasında değişmiştir. En düşük protein oranı kontrol uygulamasında belirlenmiştir. Kontrol uygulamasına kıyasla diğer uygulamalarda protein oranı değerleri daha yüksek gözlemlenmiştir. Kılçıkların uzaklaştırıldığı UYG3 uygulamasında protein oranında %22.03 ve bayrak yaprağının uzaklaştırıldığı UYG5 uygulamasında da %16.22 oranında artış olmuştur. Çeşit x uygulama interaksiyonlarına ait protein oranı değerleri %8.04 ile %11.55 arasında değişen değerler almıştır. Kontrol uygulaması ile karşılaştırıldığında Golia çeşidinde UYG6 hariç her uygulamada artış gözlemlenmiştir. Pehlivan çeşidinde en fazla artış kılçıkların uzaklaştırıldığı UYG3 ve bayrak yaprak hariç tüm yaprakların uzaklaştırıldığı UYG4 uygulamalarından sırasıyla %35.57 ile %26.24'lük artış kaydedilmiştir. Golia çeşidinde ise en fazla artış kılçıkların uzaklaştırıldığı UYG3 ve bayrak yaprağının uzaklaştırıldığı UYG5 uygulamalarından elde edilmiştir. Bu uygulamalarda sırasıyla %11.48 ve %13.34 oranında artış gözlenmiştir. Sonuçlar, bayrak yaprağının uzaklaştırılması ile protein oranında artış meydana geldiğini açıklayan Mahmood et al.,(1991)'in sonuçları ile paralellik göstermektedir. Benzer şekilde Birsin,(2005)'in bayrak yaprağının uzaklaştırılmasının protein oranını %2.8 oranında arttırdığı sonucu da, araştırmada elde edilen genel olarak uygulamaların protein oranını arttırdığı sonucu ile paralellik göstermektedir. Aynı lokasyonda buğdayda protein oranı ile ilgili yürütülen çalışmalarda ise, Yağdı, (2004) %11.85- 13.44; Sözen ve Yağdı, (2005) %2.27-10.90; Kurt, (2012) %9.70-11.80; Metin, (2019) %12.17-14.98; Yıldırım,(2019) %12.15 protein oranı değerleri saptamışlardır.

Çalışmada ortalama sedimantasyon değerleri 25.14 ml ile 26.57 ml değerleri arasında değişim göstermiştir. En yüksek değerler Pehlivan çeşidinde belirlenmiş olup ortalama sedimantasyon değerleri 25.00 ml ile 26.66 ml arasında değişmiştir. Çeşit x uygulama interaksiyonlarına ait sedimantasyon değerleri incelendiğinde, en yüksek değerlerin 27.33 ml ile Pehlivan çeşidinden bayrak yaprağının uzaklaştırıldığı UYG5 ve bayrak yaprak ile altındaki birinci yaprağın birlikte uzaklaştırıldığı UYG7 uygulamasından elde edildiği saptanmıştır. En düşük değerler ise Golia çeşidinin bitkideki tüm yaprakların uzaklaştırıldığı UYG2 ve bayrak yaprağının uzaklaştırıldığı UYG5 uygulanmalarından elde edilmiştir. Bu uygulamalar sedimantasyon değerinde sırasıyla %9.97 ve %12.49 oranında azalmaya neden olmuştur. Buna karşılık yapılan uygulamaların Pehlivan çeşidi üzerindeki etkisi belirgin olmamış ve kontrol ile uygulamalardan aynı istatistik gruba giren sonuçlar elde edilmiştir (Çizelge 3). Sedimantasyon değeri buğdayların gluten kalitesi hakkında bilgi veren bir özelliktir. Çağlayan ve Elgün, (1999), sedimantasyon değerinin çeşit, çevre ve yetiştirme tekniği yanında süne ve kımıl zararına bağlı olarak değişebileceğini ifade etmişlerdir. Buğdayda 36 ml ve üzeri çok iyi, 25 ml – 36 ml arası iyi, 15 ml – 24 ml orta, 15 ml ve altı zayıf sedimantasyon değeri olarak nitelendirilmektedir (Özkaya & Kahveci,1990). Buna göre çalışmada saptanan Golia çeşidindeki UYG2 ve UYG5 uygulamaları orta, diğer uygulamalar ise iyi olarak değerlendirilmektedir. Bursa koşullarında yapılmış olan sedimantasyon değeri ile ilgili çalışmalarda, Sözen ve Yağdı,(2005) 19.5-31.34 ml; Kınabaş, (2011) 17.89-27.37 ml; Kurt, (2012) 32.06-34,68 ml; Metin, (2019) 11.0-19.33 ml arasında değişen değerler tespit etmişlerdir.

Çeşitlerin ortalama gluten oranı değerleri Golia çeşidi için %22.86, Pehlivan çeşidi için ise %25.30 olarak saptanmıştır. Farklı uygulamalara ait gluten oranı ortalamaları %19.28 ile %28.86 arasında değişmiştir (Çizelge 3). En yüksek gluten oranı kontrol uygulaması (UYG1) ile kılçıkların uzaklaştırıldığı UYG3 uygulamasından elde edilirken, en düşük ortalama gluten oranı değerleri tüm yaprakların uzaklaştırıldığı UYG2 uygulamasından elde edilmiştir. Kontrol uygulamasına kıyasla UYG2 uygulaması gluten oranında %33.19'luk azalmaya neden olmuştur. Çeşit x uygulama interaksiyonları incelendiğinde, gluten oranı değerlerinin %17.90-31.63 arasında geniş bir değişim göstermiştir. Pehlivan çeşidini en fazla etkileyen tüm yaprakların uzaklaştırıldığı UYG2 uygulaması olmuştur. Bu uygulamada %20.66 gluten oranı belirlenmiştir ve bu uygulama kontrole kıyasla gluten oranında %25.76'lık azalmaya neden olmuştur. Golia çeşidini de en fazla etkileyen uygulamalar ise gluten oranında %40.13'lük azalmaya neden olan tüm yaprakların uzaklaştırıldığı UYG2 uygulaması ile %39.79'luk azalmaya neden olan bayrak yaprak ve kılçıkların birlikte uzaklaştırıldığı UYG6 uygulaması olmuştur. Ünal (2002), gluten özelliği bakımından, unda %20'den az değerlerin düşük, %20-27 arası orta, %28-35 arasının iyi ve %35

üzeri değerlerinin ise yüksek gluten miktarı olduğunu belirlemiştir. Buna göre çalışmada yer alan uygulama ortalamalarına göre UYG1 ve UYG3 uygulamaları iyi, UYG4, UYG5, UYG6, UYG7 uygulamaları orta ve UYG2 uygulaması ise düşük gluten değerine sahip olduğu görülmüştür. Gluten oranı ile ilgili yürütülen diğer çalışmalarda, Kınabaş, (2011) %16,99-24,99; Kurt, (2012) %25,05-36,30; Metin, (2019) %24,51-51,95; Yıldırım, (2019) %34,6-50,6 arasında değişen değerler saptamışlardır.

Çalışmada Golia çeşidinde 75,04 kg, Pehlivan çeşidinde ise 75,39 kg hektolitre ağırlığı değeri ölçülmüştür. Farklı uygulamaların ortalama hektolitre ağırlığı değerleri incelendiğinde, en yüksek hektolitre ağırlığının 77.58 kg ile kılçıkların uzaklaştırıldığı UYG3 uygulamasından elde edildiği anlaşılmaktadır. Bu uygulamayı aynı istatistiki grupta yer alan kontrol uygulaması (77.06 kg) ve UYG4 uygulaması (76.74 kg) izlemişlerdir. En düşük hektolitre ağırlığı ise tüm yaprakların uzaklaştırıldığı UYG2 uygulamasından elde edilmiştir ve bu uygulama hektolitre ağırlığında %7.83 oranında azalmaya neden olmuştur (Çizelge 3). Çeşit x uygulama interaksiyonunda en yüksek hektolitre ağırlığı 78.13 kg ile Pehlivan çeşidinin kılçıkların uzaklaştırıldığı UYG3 uygulamasında saptanmıştır. Kontrol uygulaması ile bayrak yaprak hariç tüm yaprakların uzaklaştırıldığı UYG4 uygulaması da iki çeşitte en yüksek değerin saptandığı UYG3 uygulaması ile aynı gruba giren sonuçlar elde edilmiştir. En düşük değerler ise tüm yaprakların uzaklaştırıldığı UYG2 uygulamasında saptanmıştır. Bu uygulama ile Pehlivan çeşidinde 70.62 kg ve Golia çeşidinde ise 71.42 kg hektolitre ağırlığı saptanmış olup, sırasıyla kontrol uygulamasına göre %8.27 ve %7.40 oranında bir azalma söz konusu olmuştur (Çizelge 3). Hektolitre ağırlığı buğdayın un randımanını etkileyen önemli bir kriterdir ve çeşit, çevre şartları, kültürel uygulamalar, yatma, hastalık ve zararlı gibi faktörlere bağlı olarak değişmektedir (Şener ve ark.,1997; Atlı, 1999). Genel olarak buğdaylarda hektolitre ağırlığı 65-84 kg arasında değişmektedir. Ekmeklik buğdaylarda 76 kg'ın üstünde bir hektolitre ağırlığı istenen bir durumdur (Yürür,1998). UYG1, UYG3, UYG4 ve UYG6'da saptanan araştırma sonuçları bu sınırlar içerisindeyken, tüm yaprakların uzaklaştırıldığı UYG2, bayrak yaprağının uzaklaştırıldığı UYG5 ve bayrak yaprak ile altındaki birinci yaprak uzaklaştırıldığı UYG7 uygulamalarının sonuçları ise arzu edilen hektolitre ağırlığı değerinin altında sonuçlar vermişlerdir. Bursa koşullarında yapılmış olan bazı çalışmalarda hektolitre ağırlığı değerleri, Yağdı, (2004) 79.00-80.93 kg; Sözen ve Yağdı (2005) 80.30-82.0 kg; Kurt, (2012) 73.88-77.53 kg; Yıldırım, (2019) 77.65 kg olarak bulunmuştur.

SONUÇ ve ÖNERİLER

Bursa ekolojik koşullarında gerçekleştirilen bu çalışmada ekmeklik buğday çeşitlerinde başaklanma döneminde uzaklaştırılan bazı fotosentez organlarının tarımsal özellikler üzerine etkisinin belirlenmesi amacıyla yürütülmüştür.

Araştırma sonucunda çeşitlerin başaklanma dönemlerinde kesilerek uzaklaştırılan fotosentez organlarının bitki boyu, başak boyu, başakta başakçık sayısı, başakta tane sayısı, başakta tane ağırlığı, bin tane ağırlığı, hektolitre ağırlığı, sedimantasyon değeri, gluten oranı ve hasat indeksi değerlerini önemli ölçüde azalttığı belirlenmiştir. Protein oranında ise artış gözlemlenmiştir. Bu özellikler arasında glüten oranı her iki çeşitte de tüm yaprakların uzaklaştırılması uygulaması (UYG2) sonucunda en fazla düşüşün (%25,76-40,13) saptandığı özellik olmuştur. Buna karşılık her iki çeşit için de kılçıkların uzaklaştırıldığı (UYG3) uygulaması başta olmak üzere genel olarak fotosentez organlarının uzaklaştırılmasının protein oranını arttırdığı saptanmıştır.

Başaklanmadan sonra kesilerek uzaklaştırılan fotosentez organlarının başakta tane sayısı, başakta tane ağırlığı ve bin tane ağırlığı üzerine etkileri çeşitlere göre farklı olmuştur. Araştırmada kılçıklı olan Golia çeşidi uygulamalardan kılçıksız Pehlivan çeşidine göre daha fazla etkilenmiştir.

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Yazarlar makaleye eşit oranda katkı sağlamış olduklarını beyan eder.

Çıkar Çatışması Beyanı

Makale yazarları aralarında herhangi bir çıkar çatışması olmadığını beyan ederler.

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