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Determination of in Vitro Antioxidant, Antimicrobial Properties and COX-1/COX-2 Enzyme Inhibition Activity of *Capparis Sicula*

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ABSTRACT

Since synthetic drugs cause many side effects and have a high cost, there has been increasing interest in the development of herbal-based drugs that have fewer side effects and are relatively inexpensive. *Capparis sicula* is traditionally used in the treatment of some diseases among people. For this purpose, the antioxidant and antimicrobial properties of the methanol extract of the *Capparis sicula* plant and its inhibitory effects on COX-1 and COX-2 enzymes were investigated. In the study, the antioxidant properties of the *Capparis sicula* plant were determined by DPPH and CUPRAC methods, while its antimicrobial properties were determined by the disk diffusion method. The effect of *Capparis sicula* on COX-1 and COX-2 enzymes was determined colorimetrically using commercial kits. The results showed that *Capparis sicula* had a significant antioxidant effect, but did not have any antimicrobial effect on standard strains of *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli* and *Candida albicans*. In addition, the inhibitory effect on the COX-1 enzyme was 4.23% for the first time, and the inhibition effect on the COX-2 enzyme was determined as 23.21%. As a result, the pharmaceutical, food and cosmetic industries can use *Capparis sicula* as an important source of natural raw materials.

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Capparis sicula'nın in vitro Antioksidan, Antimikrobiyal Özellikleri ve COX-1/COX-2 Enzim Inhibisyon Aktivitesinin Belirlenmesi

ÖZET

Sentetik ilaçlar birçok yan etkiye ve yüksek maliyete neden olduğundan daha az yan etkisi olan ve nispeten daha ucuz olan bitkisel bazlı ilaçların geliştirilmesi artan bir ilgi görmüştür. *Capparis sicula* halk arasında bazı hastalıkların tedavisinde geleneksel olarak kullanılmaktadır. Bu amaçla sunulan çalışmada *Capparis sicula* bitkisinin metanol ekstraktının antioksidan ve antimikrobiyal özellikleri ile COX-1 ve COX-2 enzimleri üzerindeki inhibisyon etkileri araştırılmıştır. Çalışmada *Capparis sicula* bitkisinin antioksidan özellikleri DPPH ve CUPRAC yöntemleri ile belirlenirken, antimikrobiyal özellikleri disk difüzyon yöntemi ile belirlenmiştir. *Capparis sicula*'nın COX-1 ve COX-2 enzimleri üzerindeki etkisi ise, ticari kitler kullanılarak kolorimetrik olarak belirlendi. Sonuçlar, *Capparis sicula*'nın önemli bir antioksidan etkiye sahip olduğunu, ancak standart *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli* ve *Candida albicans* suşları üzerinde herhangi bir antimikrobiyal etkiye sahip olmadığını gösterdi. Ayrıca COX-1 enzimi üzerindeki inhibisyon etkisi ilk kez

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%4.23, COX-2 enzimi üzerindeki inhibisyon etkisi ise %23.21 olarak tespit edilmiştir. Sonuç olarak, ilaç, gıda ve kozmetik endüstrileri, *Capparis sicula*'yı önemli bir doğal hammadde kaynağı olarak kullanabilir.

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INTRODUCTION

Capparis is a plant belonging to the Capparidaceae family, that can survive for many years and has been used by people for centuries to heal disease and its symptoms. The *Capparis* plant is also known as bubu, gebre, kapari, kebere, and pickle herb in different parts of Turkey (Duman & Özcan 2014). Numerous studies have reported that *Capparis* is effective in pain relief, anti-diarrhoea, treatments for allergies, infections, diuretic and diabetes-related symptoms (Singh et al. 2011; Tlili et al. 2010; Tlili et al. 2011; Argentieri et al. 2012; Arslan & Bektaş 2010; Bektaş et al. 2012a; Boga et al. 2011; Husseini et al. 2013).

Capparis sicula (C. sicula) is a shrub that is widely distributed in Mediterranean countries. Since antiquity, people in Greece have been using the tips of the *C. sicula* to make a sauce. People living in Mediterranean countries have used it as a condiment for many years (Inocencio et al. 2000). *C. sicula* is widely used in Turkey. People in and around Adana, a large city in southern Turkey, use its buds in particular as an analgesic. In this region, people crush its flower and bud parts and wrap them around their kneecaps to alleviate joint pain. In addition, it is used to treat rheumatic diseases and inflammation of the lung, and *C. sicula* has a significant enzyme inhibitory property (Dafni et al. 1984; Mahasneh et al. 1996; Abbas et al. 1992; Marrelli et al. 2014). For this reason, it is important to determine the inhibitory effect of *C. sicula* on COX-1 and COX-2 enzymes.

Cyclooxygenase enzymes (COXs) catalyze two reactions. One combines arachidonic acid (AA) with the oxygen molecule to form prostaglandin G_2 (PGG_2), and the other is the conversion of PGG_2 to prostaglandin H_2 (PGH_2). COXs perform an important initial reaction in the AA metabolic pathway, resulting in the generation of proinflammatory prostaglandins, thromboxanes, and prostacyclins. Prostaglandins regulate the contraction of smooth muscles in metabolism, blood pressure, and platelet aggregation. In addition, their overexpression causes pain and fever (Lee et al. 2003). Three isoforms of the COX enzyme are known, namely COX-1, COX-2, and COX-3. Of these, COX-1, as a constitutive enzyme, is responsible for protecting the gastric mucosa, regulating platelet aggregation, and synthesizing prostaglandins responsible for renal blood flow, while COX-2 has been reported to be prominent in oncogenesis, pain, and inflammation. Since NSAIDs used in the treatment of inflammation inhibit COX-1, and COX-2, they have been reported to cause gastrointestinal ulceration, kidney damage, and hepatic side effects in long-term use (Abdu-Allah et al.2020; Khoshneviszadeh et al. 2016)

Inflammation is part of the body's defense mechanisms, where metabolism is initiated as a protective response against pathogens, foreign bodies, or injury (Raikar & Shingade, 2018). It can also be a symptom of infections that can be detected after their symptoms have been observed (Panda et al. 2020). NSAIDs are known to reduce inflammation. They are used to heal some diseases caused by inflammation, such as rheumatoid arthritis, and fever, and to relieve daily pain (Bindu et al. 2020). There are many drugs on the market to treat inflammatory diseases. But very few of them are non-toxic. Gastrointestinal problems that develop upon the use of anti-inflammatory drugs are a dilemma for the medical world, today. In this context, comprehensive studies conducted using ethnobotanical plants with anti-inflammatory and analgesic properties are critical for opening new horizons in the treatment of inflammatory diseases (Igbe et al. 2010).

Therefore, investigating plant extracts' ability to inhibit enzymes can help us discover compounds that could effectively treat various diseases (Liu et al. 2018; Orhan et al. 2017). It is a very old practice to use herbs and plant extracts to prevent infections; however, the effects of most of them have not been scientifically proven (Ellof 1998). In addition, currently, there is little information available [to us] on how and which parts of these plants are good for treating various diseases. For this reason, it is recommended to conduct further scientific studies to support traditionally used plants and develop new natural products against the harms of synthetic products (Özcan 2020). From the literature review, it has been seen that the number of studies on the biological properties and traditional use of *C. sicula* is limited.

The present study was conducted to investigate the antioxidant and antimicrobial properties of *C. sicula* and its effect on COX-1 and COX-2 enzymes.

MATERIALS and METHODS

Plant Samples and Preparation of Plant Extracts

The plant samples were collected from the pine groves of the region known as Kemal Hill, between Karaömerli and Kılbaş Villages in Adana Province, between May and July 2020. The scientific diagnosis of *C. sicula* was made by Mehmet FIRAT, a lecturer at Yüzüncü Yil University, Faculty of Education, Department of Biology. Flora of Turkey and the East Aegean Islands was the primary source for scientific plant identification (Güner et al. 2000).

Preparation of Plant Extracts

After the plants were dried without exposure to sunlight, they were broken into small particles with the help of a hand blender. Then, a methanol extract was prepared from its flower buds (10 g 100 mL^{-1}). The solvent was removed from the medium with the help of an evaporator. The ready-to-use extracts were used fresh.

Antioxidant Assays

Free Radical Scavenging Activity (DPPH)

The free radical scavenging activity of DPPH (2,2-diphenyl-1-picrylhydrazyl, free radical) was performed with minor modification according to the method specified by Brand-Williams et al. (1995). As a free radical, 10^{-4} M DPPH was prepared in methanol. 0.02, 0.04, 0.06, 0.08, 0.10 g mL^{-1} and 3.9 ml of DPPH solution were added into the test tubes, respectively, and the mixtures were incubated for 30 minutes in a dark environment at room temperature. At the end of the incubation, their absorbance at 517 nm against the blank (methanol) was read using the spectrophotometer. The amount of DPPH removed from the reaction medium was calculated with the following formula. Experiments were carried out three times.

% Inhibition = $[(A_0 - A_1)/A_0] \times 100$.

A_0 refers to control absorbance, A_1 refers to the absorbance of the sample.

Determination of Total Antioxidant Capacity (TAC) by CUPRAC Method

In a glass tube, 1 mL of copper (II) solution, neocuproine solution and ammonium acetate buffer were added sequentially. 10 µL of solutions prepared at different concentrations of *C. sicula* extract (0.02-0.10 g mL^{-1}) as well as distilled water were added to the same tube. The total volume was made up of 4 mL. The resulting solution was kept closed for 30 minutes at room temperature. The absorbance value was measured at 450 nm (Apak et al. 2004). Ascorbic acid (AsA) was used as a standard and was prepared as 4.4 $x10^{-4}$ M. Total antioxidant capacity was calculated based on AsA. The experiments were carried out three times.

Determination of Antimicrobial Properties

Antimicrobial activity was investigated according to the disk diffusion method (NCCLS 1997). *Staphylococcus aureus* (ATCC 33862), *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 35218), *Candida albicans* (ATCC 90028) standard strains were activated by inoculating Mueller Hinton Broth (OXOID) and fungal strains in Sabouraud Dextrose (SD) Broth (DIFCO) and incubated for 24 hours at 35±2°C. Their concentrations were adjusted according to MCFarland 0.5 (108 CFU mL^{-1}) (Barry & Thornsberry 1985). Bacteria Mueller Hinton Agar (OXOID) and yeast fungus Sabouraud Dextrose (SD) Agar (OXOID) were left on the media for 15 minutes before being applied with a glass baguette. 25 µl of plant extracts were absorbed into 6 mm diameter sterile standard discs and left in the culture medium (Barry & Thornsberry 1985). Afterwards, the samples were incubated for 24 hours at 37 ºC and their inhibition diameters were determined. The extract with the highest concentration was used to determine the antimicrobial effect. The standard strains used in the study were obtained from the Public Health Institution of Turkey.

Detection of COX-1 and COX-2 Enzyme Inhibition

The inhibition capacity of COX enzymes was determined by using commercial kits (COX ovine/human, Inhibitor Screening Assay Kit item No. 560131). Results were read at 415 nm on a Micro Elisa plate reader (Bio-Tek). Calculations were made according to the Kit procedure. In the COX enzyme inhibition experiments, the extract prepared at a concentration of 0.10 g mL^{-1} was used by diluting 10 times.

RESULTS and DISCUSSION

The World Health Organization (WHO) reports that 80% of the world's population benefits from medicinal plants for their health needs. In addition, the active substance in 20% of commercial drugs is of plant origin (Gurib-Fakim 2006). Extraction and analysis of plants are vital for discovering new drugs and modernising existing formulations (Arulmozhi et al. 2019a). Alternatively, there is an increasing interest in research on natural antioxidants because of the toxic effects of existing synthetic antioxidants on the liver and their carcinogenicity (Grice 1986; Wichi 1988).

For this purpose, in the presented study, the antioxidant properties of methanol extracts (0.02-0.10 mg mL^{-1}) of the *C. sicula* plant prepared at different concentrations were determined by DPPH and CUPRAC tests. In the DPPH test, it was determined that the antioxidant property increased due to the increase in concentration compared to the control group (Figure 1).

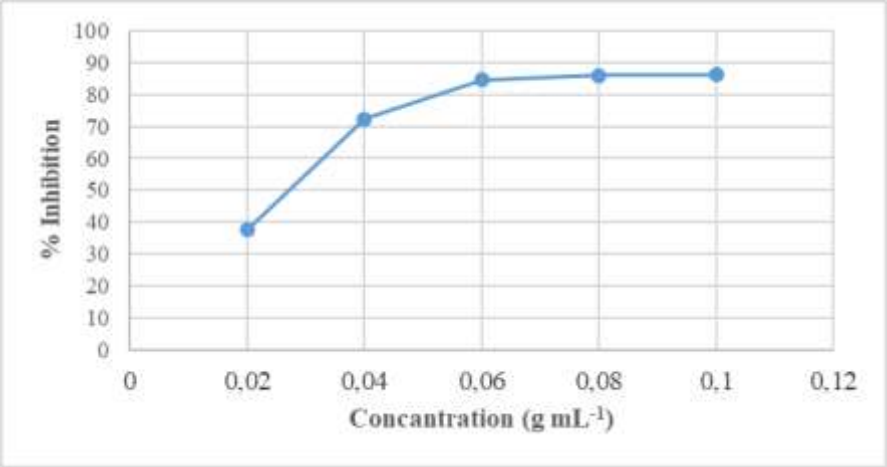

Figure 1 DPPH scavenging activity of *C. sicula* plant extract at different concentrations. The average of three measurements for each concentration was taken

Şekil 1. Farklı konsantrasyonlarda C. situla akstraktlarının DPPH temizleme aktivitesi. Her konsantrasyon için üç ölçümün ortalaması alındı.

AsA was used as a standard in the analyses performed by the CUPRAC method. A single concentration of AsA was used, and the TAC value was found to be 0.206 ± 0.062 (mmol AsA g^{-1}). It was determined that the *C. sicula* plant reached higher values than AsA at the concentrations of 0.02 and 0.04 g mL^{-1}. These results show that *C. sicula* has an important antioxidant capacity (Table 1).

Table 1 CUPRAC test antioxidant activity results of *C. sicula* extract and AsA. Each value is shown as X±SD (n=3).

Çizelge 1. C. sicula ve AsA CUPRAC test antioksidan aktivite sonuçları. Her değer X±SD olarak gösterilmiştir (n=3).

C. sicula (g mL^{-1})	**PLUG (mmol AsA /g- *C. sicula*)**	**A(M)**	**PLUG (mmol AsA /g-AsA)**
0.02	0.216 ± 0.107		
0.04	0.210 ± 0.096		
0.06	0.142 ± 0.107	$4.44x10^{-4}$	0.206 ± 0.062
0.08	0.175 ± 0.154		
0.10	0.185 ± 0.181		

In parallel with the results obtained from the present study, Subramanian & Ramani (2020) examined the antioxidant activities of 4 different extractions of the *Capparis brevispina* DC plant, and they determined that the IC_{50} value of the ethanol extract was 37.23 µg mL^{-1}, the IC_{50} value of the water extract was 41.78 µg mL^{-1}, the IC_{50} level of the chloroform extract was 42.44 µg mL^{-1}, and hexane extract was 56.34 µg mL^{-1}. Preetha et al. (2020) investigated the antioxidant effect of ethanolic and hydroethanolic extracts of *Capparis decidua* fruits in their study and showed that plant fruits exhibited a very good antioxidant activity compared to the control group (Preetha et al., 2020).

In a study examining the antioxidant activities of ethanol and water extracts of *C. spinosa* subsp. *spinosa* var. *spinosa*, *C. aegyptia*, *C. zoharyi*, *C. ovata* subsp. *ovata*, *C. sicula* subsp. *sicula* and *C. orientalis* plants by DPPH and ABTS tests, it was reported that the plants showed an antioxidant activity at varying rates. In the same study, the IC_{50} value of the leaves of the *C. sicula* subsp *sicula* grown in Tunisia was found to be 74.78 g mL^{-1} by the DPPH test. After the *C. sicula* subsp *sicula* plant was examined with the ABTS method, the IC_{50} value of its ethanolic extract was found to be 62.63 g mL^{-1} (Aichi-Yousfi et al. 2016). In addition, lyophilized and methanolic extracts of *C. spinosa* have been reported to have significant antioxidant effects (Bonina et al. 2002).

Assadi et al. (2021) reported that *C. spinosa* fruit

extracts showed antioxidative and antidiabetic effects in experimentally induced type 2 diabetes in rats administered with high fat and low dose streptozotocin. In the study conducted by Tlili et al. (2017) on rats treated with CCl_4, they noted that methanol extracts of *C. spinosa* leaves decreased the level of malondialdehyde (MDA), and their results supported the use of this herb, which is traditionally used in the prevention of kidney and liver diseases.

Plant-based medicines have been used by humans in the treatment of diseases for a long time. Plants have always been the source of new medicines (Ganapathy 2017). In the last twenty years, many scientists have discovered new antimicrobial agents from different natural sources (Vidya et al. 2012). In recent years, pathogenic microorganisms have become more resistant to current antibiotics due to their overuse. Therefore, further studies are needed to discover more economical antimicrobial agents with less toxicity (Arulmozhi et al. 2018).

In the presented study, methanol extracts of *C. sicula* flower buds did not show any antibiotic effect on microorganisms at the studied concentrations (Table 2.).

Table 2. Results of antimicrobial effect of *C. sicula*
Çizelge 2. C. situla'nın antimikrobiyal sonuçları

C. sicula (g mL^{-1})	**Microorganism**	**Antimicrobial Effect**
0.10 g mL^{-1}	*Staphylococcus aureus* (ATCC 33862)	-
	Pseudomonas aeruginosa (ATCC 27853)	-
	Escherichia coli (ATCC 35218)	-
	Candida albicans (ATCC 90028)	-

Unlike the obtained data, Arulmozhi et al. (2019) showed the antimicrobial effect of leaf extracts of the *C. zeylanica* plant on six pathogenic organisms, including *Staphylococcus epidermidis, Enterococcus faecalis, Salmonella paratyphi, Shigella dysen-teriae, Mycobacterium tuberculosis,* and *Candida albicans*. They reported that the plant had an antimicrobial effect on all strains in the study.

Preetha et al, (2020) reported in their study investigating the antimicrobial effect of ethanolic and hydroethanolic extracts of *Capparis decidua* fruits that plant fruits showed very good antimicrobial effects on strains of *Enterococcus faecalis, Klebsiella pneumonia, Pseudomonas aeruginosa, Streptococcus mutans* and *Escherichia coli.* Mazarei et al. (2017) reported that *Capparis spinosa* L. leaf polysaccharides showed antioxidant activity in the DPPH test. In addition, they showed that the antimicrobial effect of the polysaccharides on gram-negative bacteria consisting of *Escherichia coli*, *Shigella dysenteriae* and *Salmonella typhi* was higher than the effect on gram-positive bacteria of *Bacillus panis* and *Staphylococcus aureus*. In their antimicrobial study, Subramanian & Ramani (2020) showed that different *C. brevispina* extracts had minimal inhibition against gram-negative bacteria (*Escherichia coli* MTCC 739 and *Pseudomonas aeruginosa* MTCC 2453) and selected fungi (*Aspergillus niger* MTCC 5889 and *Aspergillus flavus* MTCC 9390). But in the same study, they reported the presence of resistance to gram-positive bacteria (*Bacillus subtilis* MTCC 2423; *Staphylococcus aureus* MTCC 2940). In their study, Anjuma et al. (2020) investigated the antibacterial effect of the methanolic extract of *Capparis decidua* and found that the floral particles of the plant had an antimicrobial effect on *E. cloacae* (MIC 250 lg mL^{-1}), *K. pneumonia* (MIC 250 lg mL^{-1}), *S. paratyphi* (MIC 1000 lg mL^{-1}), *S. typhi* (MIC 500 lg mL^{-1}) and *S. marcescens* (MIC 250 lg mL^{-1}). Moreover, in the same study, it was determined that the aerial parts of plant had an antimicrobial effect on pathogenic microorganisms including *A. junii* (MIC 250 lg mL^{-1}), *E. cloacae* (MIC 250 lg mL^{-1}), *E. coli* (MIC 250 lg mL^{-1}), *M. luteus* ATCC-4617 (MIC 250 lg mL^{-1}), *P. vulgaris* (MIC 250 lg mL^{-1}), *P. aeruginosa* (MIC 250 lg mL^{-1}), *S. paratyphi* (MIC 500 l g mL^{-1}), *S. typhimurium* (MIC 250 lg/mL), *S. dysenteriae* (MIC 250 lg mL^{-1}), and *S. aureus* (MIC 500 lg mL^{-1}). The difference in the results of the present study may be due to the concentration used, as well as the soil and climatic conditions in the region where the plant grows.

NSAIDs are drugs that are frequently used in the treatment of pain, inflammation, and fever, and they show an important therapeutic effect in curing inflammatory diseases (Vonkeman et al. 2010). It is thought that the action mechanism of these drugs depends on the inhibition of COX enzymes (Ulbrich et al. 2002). There are two main isoforms of the COX enzyme. COX-1 is a structural enzyme found in normal tissues. COX-2, on the other hand, is an inducible enzyme that is rarely expressed under physiological conditions and is increasingly expressed in inflammation and tumorigenesis. Arachidonic acid is synthesised by COX-2 to prostaglandin E_2 and a number of inflammatory mediators are

synthesised. Therefore, COX-2 is an important enzyme in inflammatory events (Zhang et al. 2013). In addition, it has been reported that expensive and selective COX-2 inhibitors have side effects (Emery et al. 1999). Alternatively, safer and cheaper drugs can be developed from medicinal plants (Gautam & Jachak, 2009).

In the present study, the inhibitory effect of methanol extracts of *C. sicula* flower buds on COX-1 and COX-2 enzymes was determined for the first time. While the *C. sicula* methanol extract inhibited the COX-1 enzyme by 4.23%, it inhibited COX-2 by 23.21% (Table 3). The inhibitory effect of methanol extract on COX-2 was 5.48 times greater than its effect on COX-1.

Table 3. COX-1 and COX-2 % inhibition values of methanol extract of *C. sicula* plant
Çizelge 3. C. sicula bitkisinin metanol ekstraktının COX-1 ve COX-2 % inhibisyon değerleri.

Enzyme	inhibitor	% Inhibition Value
COX-1	*C. sicula*	4.23%
COX-2	*C. sicula*	23.21%

Likewise, a study conducted on mice reported that ethanol, and the water extracts of the leaves of *Capparis zeylanica* showed dose-dependent analgesic effects and water extract was more effective than the ethanol extract (Ghule et al. 2007). Tekulu et al. (2020) reported that the extracts obtained from the roots of the plant *Capparis tomentosa* Lam showed anti-inflammatory effects. Bektaş et al. (2012a) investigated the anti-inflammatory effect of the *Capparis ovata* plant and found that the fruits of the plant and methanol extracts of its flower buds showed a significant anti-inflammatory effect. In their study, they found that both extracts showed a significant inhibitory effect in the prostaglandin E_2 inflammation model (Bektaş et al. 2012b). In a study on mice, it was reported that natural products obtained from *Capparis ecuadorica* HH (Capparaceae) had great anti-inflammatory potential as they blocked the inflammatory response in (LPS)-indued RAW 264.7 cells (Song et al. 2020). In their study, Rahimi et al. (2020) reported that *C. spinosa* reduced brain inflammation. In another study, it was reported that alcoholic extracts of *Capparis spinosa* exhibited a strong anti-inflammatory effect in rats. In the study, it was stated that this anti-inflammatory activity of alcoholic extracts was associated with the presence of polyprenols such as cappaprenol-12, cappaprenol-13, and cappaprenol-14 containing 12, 13, and 14 isoprenoid units, respectively (Al-Said et al. 1988). Various studies have stated that aqueous and chloroform extracts of *C. spinosa* indicated an anti-inflammatory effect on rats (Zhou et al. 2010; Ageel et al. 1986). In addition, it has been reported that the leaves, stems and roots of *Capparis erythrocarpos* plant have analgesic effects (Twumasia et al. 2019).

CONCLUSION

Consequently, it was determined that the methanol extract obtained from the flower buds of the *C. sicula* plant showed a high antioxidant effect but did not show any antimicrobial effect on the bacterial and fungal strains used in the present study. In addition, the inhibitory effect on COX-1 and COX-2 enzymes was detected *in vitro* for the first time. With this study, the high antioxidant capacity of *C. sicula* and its inhibitory effect on COX enzymes were clearly demonstrated. *C. sicula* may be a good alternative for use in the pharmaceutical, cosmetic, and food industries. However, further studies are needed for the safe and effective use of *C. sicula*.

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Contribution Rate Statement Summary of Researchers

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

Conflict of Interest Statement

The authors of the article declare that there is no conflict of interest between them.

REFERENCES

Abbas, J.A., El-Oglah, A.A. & Mahasneh, A.M. (1992). Herbal plants in the traditionalmedicine of Bahrain. *Econ Bot*, *46*, 158-163.

Abdu-Allah, H.H., Abdelmoez, A.A., Tarazi, H., El-Shorbagi, ANA. & El-Awady, R. (2020). Conjugation of 4-aminosalicylate with thiazolinones afforded non-cytotoxic potent in vitro and in vivo anti-inflammatory hybrids. *Bioorg Chem, 94*, 103378

Ageel, A.M., Parmar, N.S., Mossa, J.S., Yahya, M.A.A., Said, M.S.A. & Tariq, M. (1986). Anti-inflammatory activity of some Saudi Arabian medicinal plants. *Agents Action, 17*, 383-384.

Aichi-Yousfi, H., Meddeb, E., Rouissi, W., Hamrouni, L., Rouz, S., Rejeb, M.N. & Ghrabi-Gammar, Z. (2016). Phenolic composition and antioxidant activity of aqueous andethanolic leaf extracts of six Tunisian species of genus Capparis –Capparaceae. *Indus Crop Prod*, *92*, 218–226.

Al-Said, M.S., Abdelsattar, E.A., Khalifa, S.I. & Al-Feraly, F.S. (1988). Isolation and identification of an anti-inflammatory principle from *Capparis spinosa*. *Die Pharmazie*, *43*, 640-641.

Anjuma, S., Asif, M., Zia, K., Jahan, B., Ashraf, M., Hussainb, S., Hayat, M.Q., Shahd, M.R. & Tahir, M.N. (2020). Biological and phytochemical studies on Capparis decidua (Forssk) Edgew from Cholistan desert. *Nat Prod Res, 34*(16), 2315–2318.

Apak, R., Güçlü, K., Özyürek, M. & Karademir, S.E. (2004). Novel total antioxidant capacity index for dietary polyphenols and vitamin C and E, using their cupricion reducing capability in the precence of neocuproine: CUPRAC *Method. J of Agr and Food Chem, 52*, 7970-7981.

Argentieri, M., Macchia, F., Papadia, P., Fanizzi, F.P. & Avato, P. (2012). Bioactive compounds from *Capparis spinosa* subsp rupestris. *Ind Crop Prod, 36*(1), 65-69.

Arslan, R. & Bektas, N. (2010). Antinociceptive effect of methanol extract of *Capparis ovata* in mice. *Pharma Biol, 48*(10), 1185- 1190.

Arulmozhi, P., Vijayakumar, S., Praseetha, P.K. & Jayanthi, S. (2019a). Extraction methods and computational approaches for evaluation of antimicrobial compounds from *Capparis zeylanica* L*. Anal Biochem, 572*, 33-44.

Arulmozhi, P., Vijayakumar, S. & Kumar, T. (2018). Phytochemical analysis and antimicrobial activity of some medicinal plants against selected pathogenic microorganisms. *Microbe Pathog, 123*, 219-226.

Assadi, S., Shafiee, S.M., Erfani, M. & Akmali, M. (2021). Antioxidative and antidiabetic effects of *Capparis spinosa* fruit extract on high-fat diet and low-dose streptozotocin-induced type 2 diabetic rats. *Biomed Pharmacother, 138*, 111391.

Barry, A.L. & Thornsberry, C. (1985). Susceptibility Tests: Diffusion Test Procedures. In: Lennette EH, Balows A, Hausler WJ Jr, Shadomy HJ (ed.) Manual of clinical microbiology, 4th edn, American Society for Microbiology, Washington, D.C. 978-987.

Bektas, N., Arslan, R., Goger, F., Kirimer, N. & Ozturk, Y. (2012a). Investigation for anti-inflammatory and anti-thrombotic activitiesof methanol extract of *Capparis ovata* buds and fruits. *J of Ethnopharm, 142*, 48–52.

Bindu, S., Mazumder, S. & Bandyopadhyay, U. (2020). Non-steroidal anti-inflammatory drugs (NSAIDs) and organ damage: a current perspective. *Biochem Pharmacol 180*, 114147

Boga, C., Forlani, L., Calienni, R., Hindley, T., Hochkoeppler, A. & Tozzi, S. (2011). On the antibacterial activity of roots of *Capparis spinosa L. Nat Prod Res, 25*(4), 417-421.

Bonina, F., Puglia, C., Ventura, D., Equino, R., Tortora, S. & Sacchi, A. (2002). In vitro antioxidant and in vivo photoprotective effects of a liophilized extract of *Capparis spinosa* L. buds. *J. Cosmet Sci, 53*, 321–335.

Brand-Williams, W., Cuvelier, M.E. & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *Lebensm Wiss Technol, 28*(1), 25- 30.

Dafni, A., Yaniv, Z. & Palevitch, D. (1984). Ethnobotanical survey of medicinal plants in northern Israel. *J Ethnopharmacol, 10*(3), 295-310.

Duman, E. & Özcan, M.M. (2014). Physicochemical properties of seeds of Capparis species growing wild in Turkey. *Environ Monit Assess, 186*(4), 2393-2398.

Ellof, J.N. (1998). Which extractant should be used for the screening and isolation of antimicrobial components from plants? *J Ethnopharmacol, 60*, 1–6.

Emery, P.H., Zeidler, T.K., Kvien, M., Guslandi, M. & Naudin, R. (1999). Celecoxib versus diclofenac in long-term management of rheumatoid arthritis: Randomized double-blind comparison. *Lancet, 354*, 2106-2111.

Ganapathy, S. (2017). Antibacterial and molecular docking studies of bioactive component from leaves of *Stachytarpheta cayennensis* (rich.). *Vahl Res J Phytochem, 11*(1), 28–34.

Gautam, R. & Jachak, S.M. (2009). Recent developments in anti-inflammatory natural products. *Med Pic Rev, 29*, 767-820.

Ghule, B.V., Murugananthan, G. Yeole, P.G. (2007). Analgesic and antipyretic effects of *Capparis zeylanica* leaves. *Phytoterapia, 78*, 365–369.

Grice, H.C. (1986). Safety evaluation of butylated hydroxy toluene (BHT) in the liver, lung and the gastrointestinal tract. *Food Chem Toxicol, 24*, 1127–1130

Güner, A., Özhatay, N., Ekim, T. & Başer, K.H.C. (2000). Flora of Turkey and the East Aegean Islands, Edinburgh Univ. Pres. Edinburgh

Gurib-Fakim, A. (2006). Medicinal plants: traditions of yesterday and drugs of tomorrow. *Mole Aspect Med, 27*(1), 1-9

Husseini, H.F., Hasani-Rnjbar, S., Nayebi, N., Heshmat, R., Sigaroodi, F.K. & Ahvazi, M. (2001). *Capparis spinosa* L. (Caper) fruit extract in treatment of type 2 diabetic patients: A randomized double-blind placebo-controlled clinical trial. *Complement Ther Med, 21*(5), 447-452.

Igbe, I., Ching, F.P. & Eromon, A. (2010). Anti inflammatory activity of aqueous fruit pulp extract of *Hunteria umbellata* K. Schum in acute and chronic inflammation. *Acta Pol Pharm, 67*, 81-85.

Inocencio, C., Rivera, D., Alcaraz, F. & Tomas-Barberan, F.A. (2000). Flavonoid content of commercial capers (*Capparis spinosa*, *C. sicula* and *C. orientalis*) produced in Mediterranean

countries. *Eur Food Res Technol, 212*, 70-74.

Khoshneviszadeh, M., Shahraki, O., Khoshneviszadeh, M., Foroumadi, A., Firuzi, O., Edraki, N., Nadri, H., Moradi, A., Shafiee, A. & Miri, R. (2016). Structure-based design, synthesis, molecular docking study and biological evaluation of 1, 2, 4- triazine derivatives acting as COX/15-LOX inhibitors with anti-oxidant activities. *J Enzym Inhib Med Chem, 31*, 1602-1611.

Lee, J.L., Mukhtar, H., Bickers, D.R., Kopelovich, L. Athar, M. (2003). Cyclooxygenases in the skin: pharmacological and toxicological implications. *Toxicol Appl Pharmacol, 192*, 294-306.

Liu, H., Zhu, Y., Wang, T., Qi, J. & Liu, X. (2018). Enzyme-site blocking combined with optimization of molecular docking for efficient discovery of potential tyrosinase specific inhibitors from *Puerariae lobatae* radix. *Molecules, 23*(10), 2612.

Mahasneh, A.M., Abbas, J.A. & El-Oqlah, A.A. (1996). Antimicrobial activity of extracts of herbal plants used inthe traditional medicine of Bahrain. *Phytot her Res 10*, 251–253.

Marrelli, M., Loizzo, M.R., Nicoletti, M., Menichini, F. & Conforti, F. (2014). In vitro investigation of the potential health benefits of wild Mediterranean dietary plants as anti-obesity agents with α amylase and pancreatic lipase inhibitory activities. *Sci Food Agric, 94*, 2217-2224.

Mazarei, F., Jooyandeh, H., Noshad, M. & Hojjati, M. (2017). Polysaccharide of caper (*Capparis spinosa* L.) Leaf: Extraction optimization, antioxidant potential and antimicrobial activity. *Int J Biol Macromol, 95*, 224-231.

Orhan, I.E., Senol, F.S., Haznedaroglu, M.Z., Koyu, H., Erdem, S.A., Yılmaz, G., Cicek, M., Yaprak, A.E., Ari, E. & Kucukboyaci, N. (2017). Neurobiological evaluation of thirty-one medicinal plant extracts using microtiter enzyme assays. *Clin. Phytosci, 2*(9). https://doi.org/10.1186/s40816-016-0023-6.

Özcan, K. (2020). Antibacterial, antioxidant and enzyme inhibition activity capacities of Doronicum macrolepis (FREYN&SINT): An endemic plant from Turkey. *Saudi Pharm, 28*, 95–100.

Panda, S.S., Girgis, A.S., Thomas, S.J., Capito, J.E., George, R.F., Salman, A., El-Manawaty, M.A. & Samir, A. (2020). Synthesis, pharmacological profile and 2D-QSAR studies of curcumin amino acid conjugates as potential drug candidates. *Eur J Med Chem, 196*, 112293

Preetha, S., Roy, A. & Rajeshkumar, S. (2020a). In-vitro free radical scavenging, antibacterial, cytotoxicity of ethanolic/hydroethanolic extract of *Capparis decidua*. *Int J Pharm Res*, 2912-2921.

Rahimi, V.R., Rajabian, A., Rajabi, H., Vosough, E.M., Mirkarimi, H.R., Hasanpour, M., Iranshahi, M., Rakhshandeh, H. & Askari, V.R. (2020). The effects of hydro-ethanolic extract of *Capparis spinosa* (*C. spinosa*) on lipopolysaccharide (LPS)-induced inflammation and cognitive impairment: Evidence from in vivo and in vitro studies. *J of Ethnopharm, 256*, 112706.

Raikar, P. & Shingade, S. (2018). Synthesis, characterization and screening of pyrazoline derivatives for anti-inflammatory activity. *IJPSR, 9*, 2446 – 2450.

Singh, P., Mishra, G., Sangeeta Srivastava, S., Jha, K.K. & Khosa, R.L. 2011. Traditional uses, phytochemistry and pharmacological properties of *Capparis decidua*: An Overview. *Der Pharm Lett, 3*(2), 71-82.

Song, B.R., Lee, S.J., Kim, J.E., Choi, H.J., Bae, S.J., Choi, Y.J., Gong, J.E., Noh, J.K., Kim, H.S., Kang, H.G., Hong, J.T. & Hwang, D.Y. (2020). Anti-inflammatory effects of *Capparis ecuadorica* extract in phthalic-anhydride -induced atopic dermatitis of IL-4/Luc/CNS-1 transgenic mice. *Pharm Biol, 58*(1), 1263-1276.

Subramanian, S.K. & Ramani, P. (2020a). Antioxidant and cytotoxic activities of Indian caper (*Capparis brevispina* DC (Capparaceae) leaf extracts. *Eur J Integr Med, 33*, 101038.

Tekulu, G.H., Hiluf, T., Brhanu, H., Araya, E.M., Bitew, H. & Haile, T. (2020). Anti-inflammatory and anti-nociceptive property of *Capparis tomentosa* Lam. root extracts. *J Ethnopharm, 253*, 1126542.

Tlili, N., Elfalleh, W., Saadaoui, E., Khaldi, A., Triki, S. & Nasri, N. (2011). The caper (Capparis L.): Ethnopharmacology, phytochemical and pharmacological properties. *Fitoterapia, 82*(2), 93-101.

Tlili, N., Feriani, A., Saadoui, E., Nasri, N. & Khaldi, A. (2017). *Capparis spinosa* leaves extract: Source of bioantioxidants with nephroprotective and hepatoprotective effects. *Biomed Pharmacother, 87*,171-179.

Tlili, N., Khaldi, A., Triki, S. & Munne-Bosch, S. (2010). Phenolic Compounds and Vitamin Antioxidants of Caper (*Capparis spinosa*). *Plant Food Hum Nutr, 65*(3), 260-265.

Twumasia, M.A., Tandohb, A., Manteb, P.K., Ekuadzia, E., Boakye-Gyasic, M.E., Bennehd, C.K., Kumadohe, D. & Woodeb, E. (2019). Leaves and stems of Capparis erythrocarpos, more sustainable than roots, show antiarthritic effects. *J of Ethnopharm, 238*, 111890.

Ulbrich, H., Fiebich, B. & Dannhardt, G. (2002). Cyclooxygenase-1/2 (Cox-1/Cox-2) and 5-lipoxygenase (5-LOX) inhibitors of the 6,7-diaryl-2,3-1H-dihydropyrrolizine type. *Eur J Med Chem, 37*, 953–959.

Vidya, S.M., Krishna, V., Manjunatha, B.K., Rajesh, K.P.G., Bharath, B.R. & Manjunatha, H. (2012). Antibacterial and molecular docking studies of

entagenic acid, a bioactive principle from seed kernel of Entada pursaetha *DC. Med Chem Res, 21*, 1016–1022.

Vonkeman, H.E. & Van de Laar, M.A.F.J. (2010). Nonsteroidal anti-inflammatory drugs: adverse effects and their prevention. *Semin Arthritis Rheum, 39*, 294–312.

Wichi, H.P. (1988). Enhanced tumor development by butylated hydroxyanisole (BHA) from the prospective of effect on forestomach and oesophageal squamous epithelium. *Food Chem Toxicol, 26*, 717–723.

Zhang, Y., Shi, S., Zhao, M., Chai, X., Tu, P. & Coreosides, A.D. (2013). C14-polyacetylene glycosides from the capitula of *Coreopsis tinctoria* and its anti-inflammatory activity against COX-2. *Phytoterapia, 87*, 93–97.

Zhou, H., Jian, R., Kang, J., Huang, X. & Li, Y. (2010). Anti-inflammatory effects of caper (*Capparis spinosa* L.) fruit aqueous extract and the isolation of main phytochemicals. *J Agric Food Chem, 85*, 12717-12721.

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Farklı Bölgelerde Yetişen Mersin (*Myrtus communis* L.) Meyvelerinin Bazı Fitokimyasal Özelliklerinin Karakterizasyonu

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ÖZET

Türkiye'de Akdeniz florasında yaygın olarak bulunan, tıbbi ve aromatik bir bitki olan *Myrtus communis* L. (Mersin) günümüzde çeşitli fitoterapötik uygulamalarda sıklıkla karşılaşılmaktadır. Bu çalışmada, *Myrtus communis* L. bitkisinin meyvelerinden ekstraksiyon yöntemiyle elde edilen sabit yağların verimleri hesaplanmış ve kimyasal kompozisyonu gaz kromotografisi-kütle spektroskopisi (GC-MS) ile tespit edilmiştir. En yüksek sabit yağ verimi %5.43 olarak Bursa iline ait örneklerden elde edilmiştir. Analiz sonuçlarında yağ kompozisyonu içerisinde 11 farklı bileşen tespit edilmiştir. Tüm bölgelerde en fazla bulunan yağ asitleri sırasıyla %73.97-68.96 linoleik asit (C18:2), %16.60-12.04 oleik asit (C18:1) ve %8.86-8.51 palmitik asittir (C16:0). Bu çalışmanın sonuçları *Myrtus communis* L. meyvesinin zengin fitokimyasal içeriği ve yüksek besleyici özelliği sayesinde gıda, tıp ve birçok farklı alanda kullanılabilirliğini ortaya koymuştur.

Biyokimya

Araştırma Makalesi

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Anahtar Kelimeler
Myrtus communis L.
Mersin bitkisi
Fitokimyasal karakterizasyon
Sabit yağ
Yağ asitleri

Characterization of Some Phytochemical Properties of Myrtle (*Myrtus communis* L.) Fruits Grown in Different Regions

ABSTRACT

Myrtus communis L. is a medicinal and aromatic plant and common in the Mediterranean flora in Türkiye. Today, it is being more prominent thanks to its various phytotherapeutic applications. In this study, the yields of fixed oils obtained by extraction process from the fruits of *Myrtus communis* L. were calculated and their chemical composition was determined by gas chromatography-mass spectroscopy (GC-MS). The highest fixed oil yield was obtained from Bursa region's samples as 5.43%. Eleven different phytochemical components were determined in the fixed oil composition. The most abundant fatty acids in all regions were found as linoleic acid (C18:2) (73.97-68.96%), oleic acid (C18:1) (16.60-12.04%), and palmitic acid (C16:0) (8.86-8.51%), respectively. The results revealed that *Myrtus communis* L. fruit can be used in food, medicine, and many different fields because of its rich phytochemical content and high nutritional properties.

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GİRİŞ

Tıbbi aromatik bitkiler; ilaç, gıda, kozmetik gibi birçok farklı alanda kullanılmaktadır. Tıbbi aromatik bitkilerin en dikkat çekici özelliği olan tedavi amaçlı kullanımı birçok araştırma konularında da yer almaktadır. Bitkilerle iyileştirme, tamamlayıcı tedavi, geleneksel tedavi ve doğal tedavi gibi farklı isimlerle kullanılmaktadır (Demirezer, 2010). "Tıbbi bitkilerle tedavi" anlamına gelen "Fitoterapi" terimi ise ilk defa Fransız hekim olan Henri Lecrerc (1870-1955) tarafından kullanılmıştır (Hernández-Ceruelos ve ark, 2017).

Türkiye iklim ve bitki çeşitliliği, geniş yüz ölçümü ve tarımsal potansiyeli sayesinde tıbbi ve aromatik bitkiler ticaretinde önde gelen ülkelerden biri konumundadır. Türkiye'de tıbbi olarak kullanılan

bitkilerin sayısı kesin olarak bilinmemekle beraber, 500 civarında olduğu tahmin edilmektedir. Bu bitkilerin yaklaşık 200 tıbbi ve aromatik bitkinin ihraç potansiyelinin olduğu bilinmektedir. Bu bilgiler doğrultusunda, Türkiye'nin bu alanda büyük bir çalışma potansiyeline sahip olduğu görülmektedir (Faydaoğlu & Sürücüoğlu, 2011).

Türkiye'de Akdeniz florasında yaygın olarak bulunan Myrtaceae familyasında yer alan *Myrtus communis* L. (Mersin) bitkisi tıbbi olarak kullanımı ile öne çıkmaktadır. Bu familyanın 3000 türü olup, yaygın olarak bulunan ve en çok bilinenler arasında okaliptüs (Eucalyptus), karanfil (Syzygium) ve yenibahar (Pimenta) taksonları yer almaktadır. *Myrtus communis* L. bitkisi her daim yeşil, kısa boylu (1-3 m arasında boylanabilen) ve çalı formunda olan çok yıllık bir bitki olarak bilinmektedir. Doğal olarak yetişme alanları arasında Akdeniz ülkeleri, Avustralya'nın tropikal bölgeleri, Orta Doğu ülkeleri ve Kuzey Amerika'nın ılıman bölgeleri yer almaktadır. Türkiye, Fransa gibi ülkelerde ise yabani olarak yetişmektedir. Türkiye'de yaygın olarak "Mersin" ismiyle bilinmesine karşılık bilhassa Güney sahillerinde "Hambeles", "Murt" ya da "Adi Mersin" isimleriyle de yer almaktadır. *Myrtus communis* L. bitkisinin meyveleri üzüm yapısına benzer ve çoğunlukla morumsu siyah renktedir (Şekil 1). Ekşimsi bir lezzete sahip olan meyveleri fazla miktarda sert ve küçük tohumlara sahiptir. *Myrtus communis* L. bitkisinin ekstraktları yaygın olarak hipoglisemik madde, antiseptik ve dezenfektan ilaç yapımında kullanıldığından önemli aromatik ve tıbbi bitkiler arasında yer almaktadır (Şahin ve ark., 2020).

Son zamanlarda fenolik bileşik içeriği fazla olan bitki türleri, çoklu doymamış yağ asitleri ve esansiyel yağlar, sağlığı iyileştirici potansiyel etkileri (antioksidan, antikanser ve antienflamatuar vb.) sebebiyle büyük ilgi görmektedir (Messaoud & Boussaid, 2011). Son yıllarda yapılan çalışmalarda, insanların daha sağlıklı olmalarında diette bulunanan yağlar ve içeriğindeki yağ asitlerinin kimyasal yapılarının ve miktarlarının ilişkisini gösteren bulgular elde edilmiştir (Lauritzen ve ark., 2000). Bu anlamda Türkiye'de yaygın olarak bulunan doğal antioksidan kaynaklarından biri olan *Myrtus communis* L. bitkisinin kullanımı ve araştırılması giderek önem kazanmaktadır.

Çaylı ve Akyüz (2019) ısı perdelerinin kullanımı ile ısı kayıplarının azaltılacağını ve ısı tasarrufu sağlanacağını bildirmişlerdir.

Şekil 1. Bursa-Gemlik bölgesinden toplanan *Myrtus communis* L. meyveleri
Figure 1. Fruits of Myrtus communis L. collected from Bursa-Gemlik region

Myrtus communis L. bitkisinin yaprağından elde edilen esansiyel yağların antioksidan aktivite ve anti-mutajenik aktivite içerdiği bilinmektedir. Ancak, *Myrtus communis* L. bitkisi yalnızca yapraklarında bulunan uçucu yağlar nedeniyle değil, aynı zamanda meyvelerinde bulunan yağ asitleri sebebiyle de önemli bir bitki olarak tanımlanmaktadır. *Myrtus communis* L. meyveleri üzerine yapılan çalışmalarda çoğunlukla, uçucu bileşenleri ve fenolikleri üzerine odaklanılmıştır. Bununla beraber, *Myrtus communis* L. meyveleri ve tohumlarının yağ asidi içerikleri üzerine çok az çalışma yapıldığı görülmektedir. Kıvrak (2018), yaptığı çalışmada *Myrtus communis* L. yapraklarından elde edilen uçucu yağ ve tohumlarından soğuk pres yöntemiyle elde edilen yağın kimyasal analizini gaz kromotografisi – kütle spektroskopisi (GC-MS) yardımıyla gerçekleştirmiştir. Analiz sonuçlarına göre uçucu yağın en önemli ana bileşenleri; 1,8-cineol (%21.68), alfa-pinen (%18.02), linalol (%14.12) olarak tespit edilirken sabit yağda ana yağ asitleri linoleik asit (%77.59) ve palmitik asit (%10.36) olarak tespit edilmiştir (Kıvrak, 2018). Maxia

ve arkadaşları (2011) yaptığı çalışmada *Myrtus communis* L. esansiyel yağının topikal anti-inflamatuar etkisini bildirmiştir (Maxia ve ark., 2011). Mansouri ve ark. (2001) yaptığı çalışmada, *Myrtus communis* L. bitkisinin yapraklarının metanol ekstraktının 6 gram pozitif ve 4 gram negatif bakteriden oluşan toplam 10 mikroorganizma karşısında güçlü bir antibakteriyel etki gösterdiği rapor edilmiştir (Mansouri ve ark., 2001).Yapılan başka bir çalışmada ise Türkiye'den seçilen dört genotipin meyvelerinden hidrodistilasyon ile izole uçucu yağların kimyasal bileşenleri GC-FID (gaz kromotografi alev iyonlaşmalı dedektör) ve GC-MS analizleri ile karakterize edilerek *Myrtus communis* L. türlerinde bulunan esansiyel yağlarının ana bileşenleri; 1,8-Cineole (%29.20-31.40), linalool (%15.67-19.13), alfa-terpineol (%8.40-18.43), alfa-pinen (%6.04-20.71), ve geranil asetat (%3.98-7.54) şeklinde tespit edilmiştir (Kordali ve ark., 2016). Tümen ve arkadaşları, *Myrtus communis* L.'nin meyve ve yapraklarından elde edilen uçucu yağların yara iyileştirme aktivitelerini eksizyon ve insizyon yara modellerini kullanarak değerlendirdiği bir çalışma gerçekleştirmiştir (Tümen ve ark., 2017).

Bu çalışmada, üç farklı bölgede doğal olarak yetişen *Myrtus communis* L. meyvelerinde bulunan yağ asitlerinin tespit edilmesi ve meyvelerinin fiziksel özelliklerinin sabit yağ verimi üzerinde olası bir etkisi olup olmadığının belirlenmesi amaçlanmıştır. İzmir, Bursa ve Isparta bölgelerinden toplanan *Myrtus communis* L. bitkisinin meyvelerinden hekzan ekstraksiyonu ile elde edilen sabit yağlar GC-MS ile karakterize edilmiştir. Türkiye tıbbi ve aromatik bitki zenginliği içerisinde yer alan farklı yetişme alanlarından toplanmış *Myrtus communis* L. bitkisinin meyvelerinin fitokimyasal içeriğinin belirlenmesiyle bitkinin sağlık alanında etkin bir şekilde kullanılmasına katkı sağlayacağı öngörülmektedir.

MATERYAL ve METOD

Materyal

Bu çalışma kapsamında materyal olarak İzmir-Urla, Bursa-Gemlik ve Isparta-Sütçüler olmak üzere üç farklı şehir ve bölgeden toplanan *Myrtus communis* L. bitkisinin olgunlaşmış meyveleri kullanılmıştır. Numunelerin toplandığı koordinatlar ve toplanma zamanı Çizelge 1'de verilmiştir. Meyvelerin teşhisi Bursa Teknik Üniversitesi (BTÜ) Orman Fakültesi Laboratuvarlarında yapılmıştır.

Meyveler teşhis edildikten sonra oda şartlarında hava kurusu hale gelene kadar belirli periyotlarda karıştırılarak bekletilmiş ve sonra kilitli poşetlerde muhafaza edilmiştir. Ekstraksiyon işleminde kullanılan n-hekzan VWR Chemical Inc.'den %98.5 saflıkta temin edilmiştir. GC-MS analizinde ve ön işlemlerde kullanılan metil alkol ve sodyum hidroksit (NaOH) analiz saflığında Sigma-Aldrich'ten satın alınmıştır.

Çizelge 1. Numune alanları ve toplanma zamanları
Table 1. Sample collection areas and collection times

İl-İlçe	Koordinat (X,Y)	Toplanma zamanı
İzmir-Urla	38.3704234 - 26.5191641	Aralık 2022
Bursa-Gemlik	40.4715964 - 29.0113266	Aralık 2022
Isparta-Sütçüler	37.4347142 - 30.8976957	Aralık 2022

Sabit Yağ Eldesi

Olgunlaşan meyveler laboratuvar ortamında hava kurusu hale getirildikten sonra havanda ezilmiştir. Ezilirken havanda kalan yağ n-hekzan ile yıkanarak numune balonuna alınmıştır. Ezilmiş numuneler sabit tartıma gelene kadar yaklaşık 2 saat süreyle 50 °C sıcaklıkta vakumlu etüvde kurutulduktan sonra, her bir örnekten yaklaşık 30 gram tartılarak selüloz kartuşa yerleştirilmiştir. Yerleştirilen örnekler soxhlet cihazında (Behrotest) n-hekzan ile 6 saat süreyle ekstrakte edilmiştir. Soxhlet ekstraksiyonu sonunda balonda toplanan ekstraktın içindeki hekzanı uzaklaştırabilmek için su soğutmalı vakumlu evaporator (Heidolph) kullanılmıştır. Su banyosunda 50 °C sıcaklık ve 250 mmbar vakum altında hekzan tamamen uçurulmuş ve geriye kalan sabit yağın ağırlığı tartılarak verim hesaplanmıştır. Sabit yağın verimi (w/w) Eşitlik 1 yardımıyla hesaplanmıştır.

$$\%\ Sabit\ yağ\ verimi = \frac{m_{SY}}{m_0} \times 100 \text{ (Eşitlik 1)}$$

Formülde; m_{sy} = Elde edilen sabit yağın ağırlığı (g), M_0 = Meyvenin kuru ağırlığını (g) ifade etmektedir.

Elde edilen sabit yağlar cam viallere alınmış ve analizlerde kullanılmak üzere 4 °C'de buzdolabında muhafaza edilmiştir.

Gaz Kromatografisi – Kütle Spektrometresi (GC-MS) Analizi

Sabit yağların kimyasal içerikleri BTÜ Merkez Laboratuvarında bulunan Agilent marka gaz kromatografisi/kütle spketroskopisi (GC/MS) cihazı ile analiz edilmiştir. Sabit yağ numuneleri cihaza verilmeden önce içeriğindeki serbest yağ asitleri metillendirilmek suretiyle metil esterlerine dönüştürülmüştür. Bunun için bir falkon tüpü içerisine sabit yağ örneği konulmuş ve üzerine metanol ile hazırlanan 0.2 N NaOH çözeltisi eklenmiş ve iyice

karıştırılmıştır. Daha sonra karışımın üzerine hekzan eklenerek seyreltilmiştir. Nihai karışım 10 dakika santrifüjlenmiş ve esterleşmiş hekzan fazı viale alınarak analize tabi tutulmuştur. Bileşenlerin tayini için uygulanan metotta taşıyıcı gaz olarak Helyum (He) gazı, sabit faz olarak ise Agilent HP-5MS (%5 fenil metil siloksan çapraz bağlı; 0.25 µm; 30 m x 0.250 mm) kapiler kolonu kullanılmıştır. Sıcaklık programı 60 °C'de 10 dk bekletilip 4 °C dk^{-1} artış hızı ile sıcaklığın 220 °C'ye yükseltilmesi, 220 °C'de 10 dk bekletilip yine 1 °C artış hızı ile 300 °C'ye yükseltilmesi şeklinde uygulanmıştır. Çalışmada, enjektör sıcaklığı 250 °C, iyonizasyon enerjisi 70 eV olarak belirlenmiştir.

İstatistik Analizler

Her bir bölgeden rastgele alınan 40 adet meyvenin çap ve ağırlık ölçümleri alınmıştır. Üzerinde durulan özelliklerden sürekli değişkenler için tanımlayıcı istatistikler; Ortalama, Standart Sapma, Minimum ve Maksimum değerler olarak ifade edilirken, Kategorik değişkenler için sayı ve yüzde olarak ifade edilmiştir. Her bölge için ayrı ayrı olmak üzere çap ile ağırlık arasındaki ilişkiyi belirlemek ve ağırlıktan yararlanarak çapı tahmin etmek üzere, Regresyon analizi yapılmıştır. Hesaplamalarda istatistik anlamlılık düzeyi %5 olarak alınmış ve hesaplamalar için SPSS (ver. 2.0) istatistik paket programı kullanılmıştır.

BULGULAR ve TARTIŞMA

Sabit Yağ Verimi

Üç farklı ilden toplanan *Myrtus communis* L. bitkisinin sabit yağ verimleri Çizelge 2'de verilmiştir.

Çizelge 2. Farklı illere ait sabit yağ verimleri
Table 2. The fixed oil yields of different regions

İl-İlçe	Verim (%)
İzmir-Urla	3.26 ± 1.02
Bursa-Gemlik	5.43 ± 0.81
Isparta-Sütçüler	4.04 ± 0.94

Myrtus communis L. meyvelerinin sabit yağ miktarı en yüksek verim %5.43 ile Bursa bölgesinden elde edilmiştir. İzmir bölgesinden toplanan bitkinin meyvelerinin yağ verimi %3.26 oranı ile diğer bölgelere göre daha düşük bulunmuştur. Bu çalışmada meyvelerdeki mezokarp ve tohum yağ içeriği birlikte değerlendirilmiştir. Daha önce Muğla bölgesi *Myrtus communis* L. meyvelerinin kullanıldığı çalışmada yağ verimi mezokarp ve tohum için sırasıyla %0.38 ve %4.87 bildirilmiştir (Çakır, 2004).

Sabit yağ içeren meyve ve tohumların yağ verimleri ve içeriklerinin, genetik varyasyon, coğrafik lokasyon, iklim, yağış miktarı, toprak özellikleri gibi farklı koşullardan etkilendiği ve değişiklik gösterdiği bilinmektedir (Karaca & Aytaç, 2007). Bu gibi farklılıkların biyoaktivitelerinde de değişikliğe yol açıp açmaması ve ilişkili özellikleri yeni araştırma konuları olarak ilgi uyandırmaktadır.

Sabit Yağın Kimyasal Karakterizasyonu

Farklı illere ait *Myrtus communis* L. meyve sabit yağ örneklerinin GC-MS analizleri sonucunda 11 farklı bileşen tespit edilmiş ve analiz sonuçları Çizelge 3, Çizelge 4 ve Çizelge 5'te verilmiştir.

Sabit yağın ana etken maddesi olarak linoleik asit (C18:2) %73.97 oranla en yüksek Bursa örneğinde tespit edilmiş olup diğer bölgelerde ise 68.96-71.16 arasında bulunmuştur. İkinci etken madde olarak tekli doymamış yağ asidi olan oleik asit (C18:1) %16.60 oranla en yüksek Isparta örneğinde tespit edilmiştir. İzmir-Urla örneğinde %15.58 ve Bursa-Gemlik örneğinde ise %13.51 oleik asit tespit edilmiştir. Doymuş yağ asitlerinden Palmitik asit %8.86 ve Stearik asit ise %3.26 oranla en fazla İzmir örneğinde tespit edilmiştir.

Meyvede bulunan ve GC-MS ile karakterize edilen perikarp ve tohum yağlarının bileşenlerinin sınıflandırılması Çizelge 6'da verilmiştir. Doymamış yağ asitlerinin Bursa, Isparta ve İzmir örneklerinde sırasıyla %87.57, %86.74 ve %85.66 oranlarında ana fraksiyonu oluşturduğu tespit edilmiştir. Üç bölge için toplam doymuş yağ asitlerinin bulunma oranı ortalama %11.72 olup %12.12 oranı ile İzmir örneğinde en yüksek tespit edilmiştir. Ayrıca 1,8-cineole ve alfa-pinene başta olmak üzere örneklerin meyve yağında %0.43-%2.88 arasında terpen sınıfını bileşenlere rastlanmıştır.

Çizelge 3. *Myrtus communis* L. meyvesi İzmir örneğinin sabit yağ GC-MS analiz sonuçları
Table 3. Fixed oil GC-MS analysis results of Myrtus communis L. *fruits from İzmir*

Alıkonma zamanı (dk)	Bileşen adı	Bulunma (%)	CAS- numarası
6.74	α-Pinene	0.14	000080-56-8
10.62	D-Limonene	0.08	005989-27-5
10.74	1,8-Cineole	0.42	000470-82-6
25.39	α-Terpinene	0.12	000099-86-5
47.00	Methyl palmitate (C16:0)	8.86	000112-39-0
52.22	Methyl linoleate (C18:2)	71.16	000112-63-0
52.40	Methyl oleate (C18:1)	15.58	000112-62-9
53.21	Methyl stearate (C18:0)	3.26	000112-61-8
	Toplam Karakterizasyon Yüzdesi	**%99.62**	

Çizelge 4. *Myrtus communis* L. meyvesi Bursa örneğinin sabit yağ GC-MS analiz sonuçları
Table 4. Fixed oil GC-MS analysis results of Myrtus communis L. *fruits from Bursa*

Alıkonma zamanı (dk)	Bileşen adı	Bulunma (%)	CAS-numarası
6.73	α-Pinene	0.06	000080-56-8
10.62	D-Limonene	0.03	000138-86-3
10.73	1,8-Cineole	0.26	000470-82-6
21.29	ß-Ocimene	0.02	013877-91-3
25.39	α-Terpinene	0.04	998044-86-7
29.70	ß-Selinene	0.02	017066-67-0
47.01	Methyl palmitate (C16:0)	8.68	000112-39-0
52.28	Methyl linoleate (C18:2)	73.97	000112-63-0
52.42	Methyl oleate (C18:1)	13.51	000112-62-9
53.21	Methyl stearate (C18:0)	3.12	000112-61-8
58.91	Methyl eicosanoate (C20:1)	0.09	001120-28-1
	Toplam Karakterizasyon Yüzdesi	**%99.8**	

Çizelge 5. *Myrtus communis* L. meyvesi Isparta örneğinin sabit yağ GC-MS analiz sonuçları
Table 5. Fixed oil GC-MS analysis results of Myrtus communis L. *fruits from Isparta*

Alıkonma zamanı (dk)	Bileşen adı	Bulunma (%)	CAS- numarası
6.74	α-Pinene	0.83	000080-56-8
8.39	ß-Pinene	0.21	018172-67-3
9.80	3-Carene	0.24	013466-78-9
10.62	D-Limonene	0.41	005989-27-5
10.73	1,8-Cineole	0.99	000470-82-6
28.29	Caryophyllene	0.19	000087-44-5
47.00	Methyl palmitate (C16:0)	8.51	000112-39-0
52.22	Methyl linoleate (C18:2)	68.96	000112-63-0
52.40	Methyl oleate (C18:1)	16.60	000112-62-9
53.21	Methyl stearate (C18:0)	2.74	000112-61-8
58.90	Methyl eicosanoate (C20:1)	0.11	001120-28-1
	Toplam Karakterizasyon Yüzdesi	**%99.79**	

Çizelge 6. *Myrtus communis* L. meyve yağları bileşenlerinin sınıflandırılması
Table 6. Classification of Myrtus communis L. *fruit fixed oil components*

Bileşen sınıfı	İzmir-Urla (%)	Bursa-Gemlik (%)	Isparta-Sütçüler (%)
Terpen bileşenler	0.75	0.43	2.88
Doymuş yağ asitleri	12.12	11.80	11.24
Doymamış tekli yağ asitleri	15.58	13.60	16.70
Doymamış çoklu yağ asitleri	71.16	73.97	68.96
Toplam doymamış yağ asitleri	86.74	87.57	85.66
Toplam	99.62	99.81	99.79

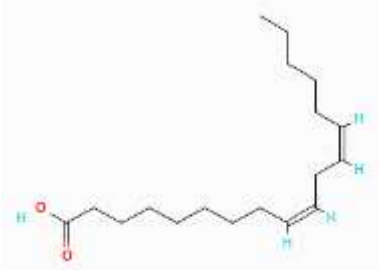
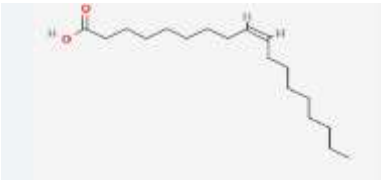
Genetik özellikler, iklim, sıcaklık, coğrafik konum, toprak özellikleri gibi farklı koşulların bitki içindeki yağ asit kompozisyonunda değişikliğe yol açtığı bilinmektedir (Baydar & Turgut, 1999). Çalışmaya dahil olan üç bölgede bitkinin fizyolojik büyüme ve gelişme farklılıkları ve yağ kompozisyonunun içeriği bu sebepler doğrultusunda farklılık gösterdiği düşünülmektedir.

Myrtus communis L. Meyve yağında tespit edilen ana bileşen yağ asitlerinin fonksiyonları Çizelge 7'de gösterilmiştir.

Linoleik asit içeriği ile zengin çoklu doymamış yağ asit profili her üç bölgede de karakteristik olarak saptanmıştır. Linoleik asit ve oleik asit önceki çalışmalarda *Myrtus communis* L. meyve yağ kompozisyonunda majör yağ asit bileşenleri olarak belirtilmiştir (Çakır, 2004). Linoleik asit ve oleik asitin enflamatuar fazı hızlandırarak gerçekleşen yara iyileştirici özelliği bildirilmiştir (Rodrigues ve ark., 2012). İnsan organizmasında sentezlenemeyen ve canlılık için elzem olan Linoleik asit, sağlık için kilit rol oynayan Omega-6 yağ asidi sınıfında değerlendirilmektedir. 18 karbon atomu içeren Linoleik asit, yapısındaki 2 çift bağın ilki, metil grubuna en yakın 6. karbonda bulunmaktadır. Bu sebeple Omega-6 olarak adlandırılır (Aydın, 2004). Omega yağ asitlerinin kalp damar, kanser oluşumu, santral sinir sistemi ve beyin sağlığı üzerine etkilerini araştıran çalışmalar, diyette birlikte bulunma oranlarının önemini ortaya koymaktadır (Gogus & Smith, 2010). Omega-6/omega-3 oranının

organizmanın fizyolojisi ile uyumlu olarak sağlanması antioksidan özellik etkiler için önemlidir; diyet içeriğindeki omega-6/omega-3 oranı 2-3:1 arası olduğunda inflamasyonu önlediği ve oranın 5:1 düzeyini geçmemesi gerektiği ifade edilmiştir (Simopoulos, 2002; Salar & Ayşe, 2021). Sonuç olarak, temel yağ asitleri vücut tarafından sentezlenemediğinden gıdalarla belli oranlarda alınması gerekir.

Çizelge 7. *Myrtus communis* L. meyvesinin ana bileşen yağ asitlerinin fonksiyonları
Table 7. Functions of the main fatty acids of Myrtus communis L. *fruits*

Bileşen	Kimyasal formül	Fonksiyon
Linoleik Asit (C18:2) 	$C_{18}H_{32}O_2$	▪ Kardiyoprotektif ▪ Ateroskleroz önleyici ▪ Beyin ve sinir sistemi gelişimi ▪ Yüksek antioksidan ▪ Anti mutajenik aktivite ▪ Cilt bariyerini güçlendirici
Oleik Asit (C18:1) 	$C_{18}H_{34}O_2$	▪Santral sinir sistemini onarıcı ▪Antitrombotik ▪Antienflamatuar
Palmitik Asit (C16:0) 	$C_{16}H_{32}O_2$	▪Cilt ve mukoza onarıcı ▪Lipofilik biyoaktiviteyi arttırma ▪UV ışınlarına karşı koruma

Kolesterol düzeyi gibi bazı metabolik değerlerin, arteriyel kan basıncı düzeylerinin uygun seviyelerde tutulması, kas ve bağ dokusunun güçlenmesi, cilt ve mukoza sağlığı açısından yağlar ve yağ asitleri önemlidir (Lauritzen ve ark., 2000).

Beslenmede bazı yağların kullanımı ise linoleik asidin ve metabolik öncülüğünü yaptığı araşidonik asitin (C20:4) tüketiminde artışa sebep olmaktadır. Bilindiği gibi Araşidonik asit proinflamatuvar etki gösteren eikosanoid metabolitleri, (TXA2, PGE2, PGI2 vb.) ve lökotrienlerin sentezinde rol almaktadır (Imig, 2020).

Linoleik asidin kardiyovasküler sistem üzerinde vaskuler dokuda inflamasyonu önleyici anti-inflamatuar etkisi bulunmaktadır (Jandacek, 2017). Düşük dansiteli kolesterol düzeylerini düşürerek ve hızlı kan pıhtılaşmasını inhibe ederek kalp krizi riskini azaltır. Ayrıca immun sistemde olumlu yönde etki gösterir (Özcan, 2019).

Meyvelerin ortalama, minimum ve maksimum çap ve ağırlık değerleri Çizelge 8'de özetlenmiştir. Meyve çapları incelendiğinde, üç il arasında istatistik olarak anlamlı fark bulunmuştur ($p=0.00$). Aralarında anlamlı fak bulunan meyve çaplarının istatistik LSD karşılaştırması Çizelge 9'da verilmiştir.

Çizelge 8. *Myrtus communis* L. meyvelerin çap ve ağırlık değerleri
Table 8. Diameter and weight values of Myrtus communis L. *fruits*

	Ortalama		Minimum		Maksimum	
İl	Çap (mm)	Ağırlık (g)	Çap (mm)	Ağırlık (g)	Çap (mm)	Ağırlık (g)
İzmir	6.16±0.15	0.142±0.053	4.70	0.073	9.20	0.280
Bursa	5.18±0.09	0.123±0.033	4.04	0.078	6.67	0.231
Isparta	5.89±0.09	0.124±0.026	4.56	0.074	7.01	0.189

Buna göre, Bursa ili meyve ortalama çap değerinin diğer illerden faklı olduğu görülmüştür. İzmir ve Isparta illeri meyve çap ortalama değerleri birbirine yakındır. Bursa'dan toplanan örnekler ise ortalama 5.18±0.09 mm çap değeri ile iller arasında en küçük çap değerine sahiptir. Buna karşın en yüksek verim değeri (%5.43) Bursa örneklerinden elde edilmiştir.

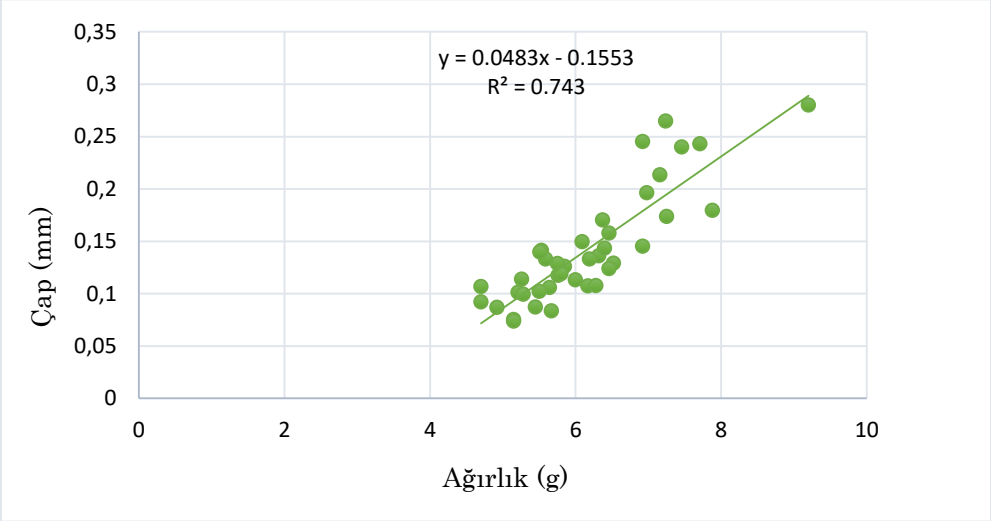
Diğer taraftan, üç bölgedeki meyve ağırlıkları ortalaması arasındaki farkların istatistik olarak

önemli olmadığı görülmüştür (p=0.06). Meyvelerin çap ve ağırlıkları arasındaki bölgesel ilişki Şekil 2, 3 ve 4'te gösterilmiştir. Analizler sonucunda verilerin normal dağılım gösterdiği belirlenmiştir. Meyve numuneleri, bölgeler arasında kıyaslandığında meyve çapları arasında anlamlı bir fark bulunurken, ağırlıkları arasında anlamlı bir farklılık yoktur. Bu sonuca göre; meyve ağırlığının sabit yağ verimi üzerinde tek başına doğrudan bir etkisi olmadığı düşünülmektedir.

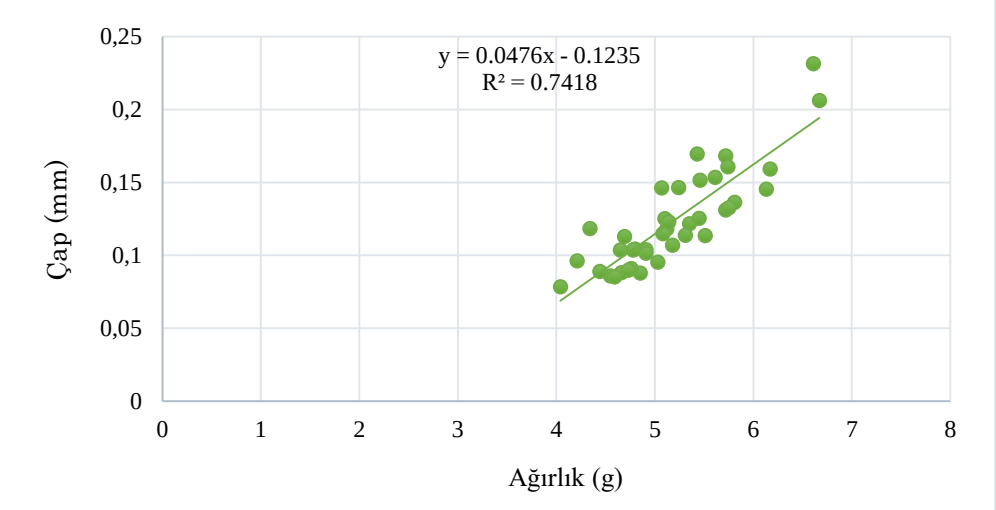
Çizelge 9. *Myrtus communis* L. meyvesine ait çap değerlerinin LSD istatistik karşılaştırılması
Table 9. LSD statistical comparison of diameter values of Myrtus communis L. *fruits*

	N1:N2:N3	İl		p
Çap	40:40:40	İzmir (a)	Bursa (b)	0.000*
			Isparta (a)	0.112
		Bursa (b)	İzmir (a)	0.000*
			Isparta (a)	0.000*
		Isparta (a)	İzmir (a)	0.112
			Bursa (b)	0.000*

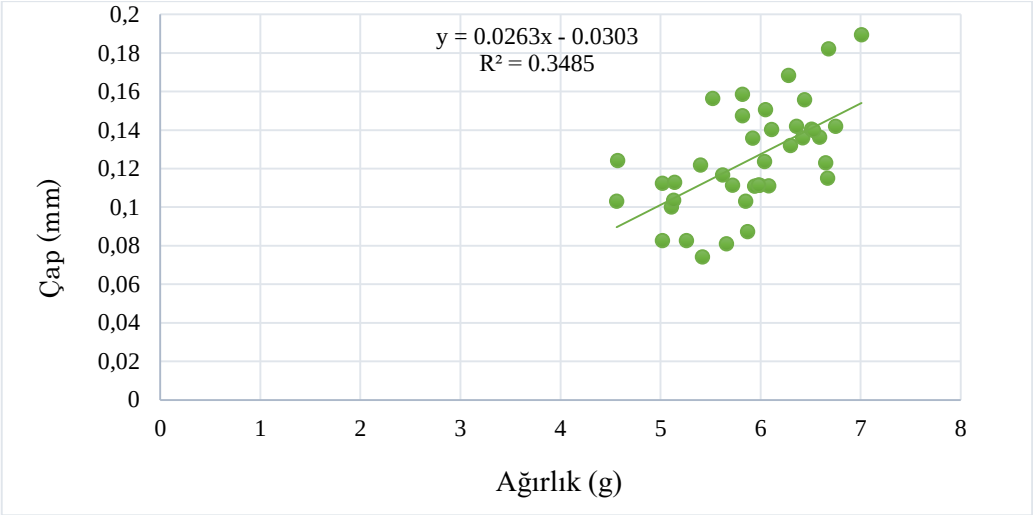
* Ortalama farklar 0.05 düzeyinde anlamlıdır.



Şekil 2. *Myrtus communis* L. İzmir meyve örneklerinin çap ve ağırlık ilişkisi
Figure 2. The relationship between diameter and weight of Myrtus communis L. *fruit from İzmir*



Şekil 3. *Myrtus communis* L. Bursa meyve örneklerinin çap ve ağırlık ilişkisi
Figure 3. The relationship between diameter and weight of Myrtus communis L. *fruit from Bursa*



Şekil 4. *Myrtus communis* L. Isparta meyve örneklerinin çap ve ağırlık ilişkisi
Figure 4. *The relationship between diameter and weight of Myrtus communis* L. *fruit from Isparta*

Tüm bulgular birlikte değerlendirildiğinde; meyve çapının sabit yağ verimi üzerinde etkili olduğu ve çap azaldıkça yağ veriminin arttığı gözlemlenmiştir. Öte yandan; optimum yağ verimi için minimum çap değerine karşılık maksimum ağırlığa sahip meyve örnekleriyle çalışmanın endüstriyel anlamda üretim için daha uygun olacağı düşünülmektedir.

SONUÇ ve ÖNERİLER

Türkiye'de Akdeniz florasında yaygın olarak bulunan Myrtaceae familyasında yer alan *Myrtus communis* L. bitkisi günümüzde çeşitli kozmetik ve tıbbi uygulamalarda sıklıkla kullanılmaktadır. Genellikle uçucu yağı, ekstraktı ve hidrolatı ön plana çıksa da mersin bitkisinin meyvelerinden elde edilen sabit yağda da oldukça önemli fitokimyasallar bulunmaktadır. İzmir, Isparta ve Bursa illerinden toplanan *Myrtus communis* L. meyvelerinden sabit yağ elde edilmiştir. En yüksek sabit yağ verimi %5.43 olarak Bursa örneklerinden elde edilmiştir, bunu sırasıyla Isparta ve İzmir takip etmiştir. Sabit yağların fitokimyasal profilleri incelendiğinde; GC-MS analizleri sonucunda sabit yağ kompozisyonu içerisinde 11 farklı bileşen tespit edilmiştir. En yüksek oranda bulunan bileşenler linoleik asit (%73.97) ve oleik asit (%16.60) olarak tespit edilmiştir. Bölgeler arasındaki bu farklılığın genetik özellikler, iklim, sıcaklık, coğrafik konum, toprak özellikleri gibi farklı koşullardan kaynaklandığı düşünülmektedir. Öte yandan meyvenin çap ve ağırlık gibi fiziksel özelliklerinin yağ verimi üzerinde etkisi olup olmadığını anlamak amacıyla yapılan istatistiksel değerlendirme sonucunda; meyve ağırlığının verim üzerinde tek başına bir etkisi bulunmadığı ancak çap ile birlikte değerlendirilmesi gerektiği sonucuna varılmıştır. Bu çalışma *Myrtus communis* L. meyvesinin sabit yağının endüstriyel ve fitoterapi alanında kullanılabileceğini göstermiştir.

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Çıkar Çatışması Beyanı

Makale yazarları aralarında herhangi bir çıkar çatışması olmadığını beyan ederler.

KAYNAKLAR

Aydın, A. (2004). Sağlığımız ve Omega-3 Yağ Asitleri. *Cerrahpaşa Tıp Fakültesi Sürekli Tıp Eğitimi Etkinlikleri Sempozyum Dizisi*, 41, 181-189.

Baydar, H., & Turgut, İ. (1999). Variations of fatty acid composition according to some morphological and physiological properties and ecological regions in oilseed plants. *Turkish Journal of Agriculture and Forestry*, *23*(7), 81–86. https://journals.tubitak.gov.tr/agriculture/vol23/iss7/10

Çakır, A. (2004). Essential oil and fatty acid composition of the fruits of Hippophae rhamnoides L.(Sea Buckthorn) and Myrtus communis L. from Turkey. *Biochemical Systematics and Ecology*, *32*(9), 809–816. https://doi.org/10.1016/j.bse. 2003.11.010

Demirezer, L. Ö. (2010). Bitkilerin tıpta kullanılması konusundaki sorumluluklarımız. Bitkilerle Tedavi Sempozyumu, İstanbul, Türkiye, 5-6 Haziran 2010 5(6), 87–88.

Faydaoğlu, E., & Sürücüoğlu, M. S. (2011). Geçmişten günümüze tıbbi ve aromatik bitkilerin kullanılması ve ekonomik önemi. *Kastamonu University Journal*

of Forestry Faculty, *11*(1), 52–67. https://dergipark.org.tr/ en/pub/kastorman/issue/17236/180072

Gogus, U., & Smith, C. (2010). n-3 Omega fatty acids: a review of current knowledge. *International Journal of Food Science & Technology, 45*(3), 417–436. https://doi.org/10.1111/j.1365-2621.2009.02151.x

Hernández-Ceruelos, A., Muñoz-Juarez, S., & Vázquez-Alvarado, P. (2017). Phytoparmaceuticals and Its Applications in Therapy. In Recent Advances in Drug Delivery Technology (pp. 202-228). IGI Global.

Imig, J. D. (2020). Eicosanoid blood vessel regulation in physiological and pathological states. *Clinical Science, 134*(20), 2707–2727. https://doi.org/10.1042/CS20191209

Jandacek, R. J. (2017). Linoleic acid: a nutritional quandary. *Healthcare, 5*(2), 25. https://doi.org/10.3390/healthcare5020025

Karaca, E., & Aytaç, S. (2007). Yağ bitkilerinde yağ asitleri kompozisyonu üzerine etki eden faktörler. *Anadolu Tarım Bilimleri Dergisi, 22*(1), 123–131. https://dergipark.org.tr/en/pub/omuanajas/issue/20226/214331

Kıvrak, Ş. (2018). Myrtus communis L.: Characterisation of Essential Oil of Leaves and Fatty Acids of Seeds Using Gas Chromatography-Mass Spectrometry (GC/MSD). *Süleyman Demirel Üniversitesi Fen Bilimleri Enstitüsü Dergisi, 22*(2), 488. https://doi.org/10.19113/sdufbed.93157

Kordali, S., Usanmaz, A., Cakir, A., Komaki, A., & Ercisli, S. (2016). Antifungal and herbicidal effects of fruit essential oils of four Myrtus communis genotypes. *Chemistry & Biodiversity, 13*(1), 77–84. https://doi.org/10.1002/cbdv.201500018

Lauritzen, I., Blondeau, N., Heurteaux, C., Widmann, C., Romey, G., & Lazdunski, M. (2000). Polyunsaturated fatty acids are potent neuroprotectors. *The EMBO Journal, 19*(8), 1784–1793. https://doi.org/10.1093/emboj/19.8.1784

Mansouri, S., Foroumadi, A., Ghaneie, T., & Najar, A. G. (2001). Antibacterial activity of the crude extracts and fractionated constituents of Myrtus communis. *Pharmaceutical Biology, 39*(5), 399–401. https://doi.org/10.1076/phbi.39.5.399.5889.

Maxia, A., Frau, M. A., Falconieri, D., Karchuli, M. S., & Kasture, S. (2011). Essential oil of Myrtus communis inhibits inflammation in rats by reducing serum IL-6 and TNF-α. *Natural Product Communications, 6*(10), 1934578X1100601034. https://doi.org/10.1177/1934578X1100601034

Messaoud, C., & Boussaid, M. (2011). Myrtus communis berry color morphs: A comparative analysis of essential oils, fatty acids, phenolic compounds, and antioxidant activities. *Chemistry & Biodiversity, 8*(2), 300–310. https://doi.org/10.1002/cbdv.201000088

Özcan, GS. (2019). Yüksek Yağlı Diyetle Beslenen Sıçanlarda Myrtus Communis L.'nin Pankreas Üzerine Etkilerinin Histolojik Ve Biyokimyasal Yöntemlerle Değerlendirilmesi [Yüksek Lisans Tezi, Marmara Üniversitesi Sağlık Bilimleri Enstitüsü Histoloji ve Embriyoloji Anabilim Dalı]

Rodrigues, H. G., Vinolo, M. A. R., Magdalon, J., Vitzel, K., Nachbar, R. T., Pessoa, A. F. M., dos Santos, M. F., Hatanaka, E., Calder, P. C., & Curi, R. (2012). Oral administration of oleic or linoleic acid accelerates the inflammatory phase of wound healing. *Journal of Investigative Dermatology, 132*(1), 208–215. https://doi.org/10.1038/jid.2011.265

Salar, B., & Ayşe, U. Z. (2021). Omega Yağ Asitleri: Biyolojik Etkileri ve Bitkisel Kaynakları. Hacettepe University *Journal of the Faculty of Pharmacy, 41*(3), 194–209. https://doi.org/10.52794/hujpharm.920079

Simopoulos, A. P. (2002). The importance of the ratio of omega-6/omega-3 essential fatty acids. *Biomedicine & Pharmacotherapy, 56*(8), 365–379. https://doi.org/1016/S0753-3322(02)00253-6

Şahin, G., Altuntaş, E., & Polatcı, H. (2020). Mersin (Myrtuscommunis L.) mevyesinin fiziksel, mekanik, renk ve kimyasal özellikleri. *Kahramanmaraş Sütçü İmam Üniversitesi Tarım ve Doğa Dergisi, 23*(1), 59–68. https://doi.org/10.18016/ksutarimdoga.vi.588652.

Tümen, I., Süntar, I., & Kurtça, M. (2017). Evaluation Of The Wound Healing and Anti-Inflammatory Activities and Phytochemical Analysis of Myrtus Communis L. *Fresenius Envir Bull*, *26*(7), 4420-4428. https://www.researchgate.net/publication/320311123.

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Evaluation of the Effect of Biomarker Levels Associated with Disease Severity on Mortality in COVID-19 Patients in the Gaziantep Region of Turkey

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ABSTRACT
This study aimed to investigate the effect of clinical and some specific laboratory parameters on the prognosis and mortality of critically ill COVID-19 patients who need to be followed in the intensive care unit (ICU). This is a retrospective cohort study. A total of 180 patients treated in the ICU were included in the study. The data of clinical and levels of D-dimer, cardiac troponin I (cTnI), Ferritin, and CK-MB were researched. The multivariate and univariate logistic regression models were employed to investigate the risk factors affiliated with in-hospital death. There was a significant difference in mortality between women and men (p=0.002). Hypertension was the most common comorbid disease, mortality was detected to be significantly greater in patients over 65 years of age. The serum D-dimer, cTnI, CK-MB, and ferritin levels were found to be higher in patients at risk. In the multivariate logistic regression model, we detected that ferritin above 300 µg/l (p=0.05) alongside cancer was associated with mortality. This study showed that advanced age is an important risk factor as well as the mortality of patients with cancer –especially those with a ferritin value above 300 µg/l and patients with a high cTnI value.

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Türkiye'nin Gaziantep Bölgesinde COVID-19 Hastalarında Hastalık Şiddetiyle İlişkili Biyobelirteç Düzeylerinin Mortalite Üzerine Etkisinin Değerlendirilmesi

ÖZET
Bu çalışma, yoğun bakım ünitesinde (YBÜ) takip edilmesi gereken kritik COVID-19 hastalarının prognoz ve mortalitesi üzerine klinik ve bazı özellikli laboratuvar parametrelerinin etkisini araştırmayı amaçlamıştır. Bu retrospektif bir kohort çalışmasıdır. Yoğun bakımda tedavi edilen toplam 180 hasta çalışmaya dahil edildi. D-dimer, kardiyak troponin I (cTnI), Ferritin ve CK-MB klinik verileri ve düzeyleri araştırıldı. Hastane içi ölümle ilişkili risk faktörlerini araştırmak için çok değişkenli ve tek değişkenli lojistik regresyon modelleri kullanıldı. Kadınlar ve erkekler arasında mortalite açısından anlamlı fark vardı (p=0,002). Hipertansiyon en sık görülen komorbid hastalıktı, mortalite 65 yaş üstü hastalarda anlamlı olarak daha yüksek saptandı. Risk altındaki hastalarda serum D-dimer, cTnI, CK-MB ve ferritin düzeyleri daha yüksek bulundu. Çok değişkenli lojistik regresyon modelinde, kanserle birlikte 300 µg/l'nin (p=0.05) üzerindeki ferritinin mortalite ile ilişkili olduğunu saptandı. Bu çalışma kanserli hastalarda, özellikle ferritin değeri 300 µg/l'nin üzerinde olanlarda ve cTnI değeri yüksek olan hastalarda, ileri yaşın mortalitesi kadar önemli bir risk faktörü olduğunu göstermiştir. Serum D-dimer, cTnI, CK-MB ve ferritin düzeyleri yüksek olan hastalarda mortalite anlamlı olarak daha yüksekti.

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Araştırma Makalesi

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Anahtar Kelimeler
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Kardiyak troponin-I

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INTRODUCTION

COVID-19(Coronavirus disease 2019), a member of the family Coronaviridae, was first observed in December 2019 in Wuhan, China. Severe acute respiratory syndrome, which soon turned into a worldwide spreading COVID-19 pandemic, is an infection brought about by Coronavirus-2 (SARS-CoV-2) (Doruk et al. 2021).

In SARS-CoV-2 disease, the virus mainly binds to the receptor of the angiotensin-converting enzyme 2 (ACE2), which belongs to the cell membrane and is expressed in the lung. The circulatory system also becomes a target of the virus because it expresses ACE2. Interstitial pneumonia in COVID-19, which often causes pneumonia in the lungs, is affiliated with a poor prognosis (Bayrakçı 2022).

The level of cardiac troponin was found to be significantly greater in severely infected patients hospitalized in the intensive care unit (ICU) or who died. Myocardial damage recognized by an increased level of troponin occurs in COVID-19 patients due to non-ischemic myocardial processes, including severe acute respiratory syndrome, particularly with sepsis, hypoxia, pulmonary thrombosis, systemic inflammation, and embolism (Imazio et al. 2020).

It is noted that hypertension, diabetes, cerebrovascular disease, and ischemic heart disease are significantly more frequent in people requiring intensive care or dying from COVID-19. Myocardial damage and dysfunction caused by SARS-CoV-2 are common in COVID-19 patients followed up in ICU, evidenced by the frequent occurrence of troponin elevation and electrocardiographic abnormalities (Salazar et al. 2020).

The clinical signs of COVID-19 are aggravated by the propagation of disseminated intravascular coagulation (DIC), thrombosis, and cytokine storms. Coagulation/fibrinolytic abnormality, which is very often noted in COVID-19, is associated with an increase in D-dimer and plays a significant role in the prognosis. However, assessing the D-dimer value by a physician is important in supporting examination findings (Asakura et al. 2021).

An increase in the levels of cardiac biomarkers is observed in COVID-19 patients, including high-sensitive cardiac troponin I (cTnI), NT-proB-type Natriuretic Peptide (Pro-BNP), myoglobin (MB), creatinine kinase-MB (CK-MB), as well as D-dimer. In particular, the incidence of COVID-19 increases in cancer patients (Huang et al. 2021).

This study was designed to investigate the effect of D-dimer, cTnI, Ferritin, and CK-MB levels on the mortality of critically ill COVID-19 patients who need to be followed up in the ICU.

MATERIALS and METHODS

The study retrospectively analyzed the hospital archives of COVID-19 patients who were admitted to the ICU of Gaziantep Ersin Arslan Educational Research Hospital between May 1 and July 1, 2021. The study included 18-year-old and older patients who were admitted to intensive care with a diagnosis of COVID-19, whose cTnI, D-dimer, and CK-MB were examined within the first day in the ICU.

Demographic data, medical history, clinical data, chronic medication, laboratory findings, comorbidities, intubation status and duration, complications, mortality, and data on mortality and low-dose computed tomography of the lung (LDCT) findings were present in the patient's electronic medical record. The following-up period ended with the patient's discharge or death. The data obtained by the study team during the application hospital stay were collected retrospectively.

cTnI levels were determined by the Abbott cTnI ADV microparticle enzyme immunoassay on the Architect i2000SR Immunoassay Analyzer (Abbott Diagnostics, Chicago, Ill., USA). The normal cTnI level was accepted as ≤34.2 ng/L in men and ≤15.6 ng/L in women according to the laboratory reference data of our hospital. High values than these were considered to be the abnormal cTnI level.

The measurement of CK-MB activity was performed using the Abbott Architect c 8000 devices using the immune inhibition method (Abbott Diagnostics, Chicago, Ill., USA). A 25 U/L was accepted as the upper limit of the normal.

The D-dimer activity was measured in the plasmas of patients by using the immunoturbidimetric method on the STA Compact Max device (Stago, France). The threshold value we used to detect the negative D-dimer's negative and positive predictive values was 00 mg/dl.

Ferritin measurements were made from the serum obtained via centrifuging the blood samples at 4000 rpm for 10 minutes, using the enzyme immune assay method (Beckman Coulter DXI 800 device of SRT company's original kit). The threshold value we used to determine the negative and positive predictive values of ferritin levels was 360 µg/L.

The serums were analyzed with the Enzyme Immune Assay (EIA) method in the CBC laboratory using the kit of Bio Meriux Company (Ferritin EIA).

Statistical analysis was conducted employing the SPSS 15.0 software. Also, continuous and categorical variables were indicated as median, mean, and n (%), respectively. The suitability of the variables for normal distribution was analyzed employing the figures (probability and histogram graphs) and analytical methods (Shapiro-Wilk tests/Kolmogorov-Smirnov).

According to the Kolmogorov-Smirnov test, the data where the p-value was above 0.05 was considered to show normal distribution. The differentiation between the groups was compared using the Chi-square, Fisher, and the comparison of numerical data that was not normally distributed was done with the Mann-Whitney U test. The multivariate and univariate logistic regression models were employed to investigate the risk factors affiliated with in-hospital death. A p-value of 0.05 ($p \leq 0.05$) was regarded to be statistically significant.

To be able to conduct the study, necessary permission was taken from the Ethics Committee for Non-Interventional Clinical Research of Gaziantep University (No: 2022/75).

RESULTS and DISCUSSION

A total of 180 patients, 81 (45.3%) of whom were female and 99 (54.7%) of whom were male, were enrolled in the study. The patient's median age was 64 years. The average age was 63.9±14.7 years (Range 20-94). The difference between men and women in terms of age was compared by the Mann-Whitney U test since age did not show a normal distribution. There was a significant differentiation between women and men (p=0.002). The median age was 61 years for males and the average median age was 60.8±15 years (Range 20-91). The median age was 67 years for women and the average median age was 68.4±12.7 years (Range 43-94) (Table 1).

Table 1. Demographic data of the study
Çizelge 1. Çalışmanın demografik verileri

Parameters		n	Std Dev	CI 95%
Gender		180		
	Male	99 (54.7%)		
	Female	81 (45.3%)		
Age		63.9±14.7 (20-94)	14.7	2.16
	<65 years	94	0.22	0.07
	>65 years	86	0.49	0.03
Hospitalized stay		8.9±6.8 (1-37)	7.05	1.03
Mortality		96		

The diagnosis result of COVID-19 was verified by PCR in 149 (82.8%) patients and by clinical and radiological findings in 31 (17.2%) patients. We did find no differentiation between men and women in terms of PCR positivity (p=0.412).

During diagnosis, a comorbid disease was present in 135 (75%) of the patients. Hypertension was the most common comorbid disease in 35 (19.4%) patients. This was followed by the diagnosis of type 2 diabetes mellitus, which was detected in 28 (15.6%) patients, and cancer, which was detected in 10 (5%) patients. (Table 2).

The median intensive care hospitalization time of the patients was 8.9 days and the average hospitalization time was 8.9±6.8 (Range 1-37). Of the patients who were followed up, 94 (53.3%) died, and 76 (44.7%) patients were discharged. There was no difference in gender between patients who died and survived (p=0.170). Assessed for comorbid diseases, no statistical difference was detected in the presence of comorbid diseases such as type 2 DM and hypertension (p=0.089, p=0.182, and p=0.532, respectively). However, the presence of cancer made a significant difference (p=0.036). Of the 10 patients with cancer, only one was able to be discharged, while all the other patients died.

Advanced age is an important cause of mortality between the mortality reasons of patients. When patients were categorized as over and below 65 years of age, mortality was detected to be significantly greater in patients over 65 years of age. Also, 48 (63.15%) of the 76 patients over the age of 65 died, and 46 (46.15%)of the 104 patients under the age of 65 died. The age differences between those discharged from the hospital and dead patients were statistically significant (p=0.003).

This study shows that 48.33% of COVID-19 patients have an elevated cTnI level on the first day of hospitalization. According to our study, 60 out of 87 (68.96%) of the patients with a high cTnI level (1.49) died.

In this study, founded that the CK-MB values of 22 of the patients in the ICU due to COVID-19 were above the normal value. Of these 22 patients, 19 (86.36%) cases resulted in mortality.

In this study, the D dimer level of 129 patients was above 1.0 and the average D dimer was 5.01. Of these 129 patients, 76 ended up with mortality. D-dimer concentrations were significantly higher in the

decedent patients (5.41µg/mL) than in discharged patients (1.86µg/mL). D-dimer concentrations were above 15µg/mL in 14 of the 96 dead patients (14.58%) and in 3 of the 84 recovered patients (3.5%).

Mortality was significantly higher in 72 (60%) of 120 patients with serum ferritin above 300 µg/L, while 22 (44%) of 50 patients with ferritin under 300 µg/L died (p=0.041).

Table 2. Statistical analysis of the study
Çizelge 2. Çalışmanın istatistiksel analizi

Parameters	n	Mean	Hospital Stay	p<	Mortality %	p<
Gender						
Male	99	55%			57.5%	
Female	81	45%			48.1%	
Ages		63.9				
<65 years	94	52.2%			47.8%	
>65 years	88	47.8%	>20 days	0.004	57.9%	0.006
Comorbidity						
Yes	135	75%			56.2%	0.08
Hypertension	33	18.3%			51.5%	0.06
Diabetes Mellitus	19	10.5%			63.1%	
Hypertension and Diabetes Mellitus	29	16.2%	>25 days	0.005	55.1%	
Malignity	11	6.1%			72.7%	
Other	43	23.9%			53.4%	0.04
No	45	25%	<10 days	0.03	44.4%	
Data from the laboratory						
CKMB (ng/ml)		3.95±2.15				0.07
Troponin (ng/L)		0.82±0.37				0.5
D-Dimer (µg/L)		3.48±1.01				0.05
Ferritin (µg/L)		784±50.8				0.08

*Mann Whitney U test was used with a 95% Confidence Interval (CI) for statistical analysis

In the multivariate logistic regression model, we found that ferritin above 300 µg/L (p=0.05) alongside cancer was associated with mortality

This research is a retrospective cohort research that demonstrates the strength of predicting the clinical progress and prognosis of patients with elevated levels of cardiac cTnI and D-dimer detected on the first day of patients admitted to ICU due to COVID-19.

In this study, 96 of the 180 patients died during follow-up and treatment.

This study shows that 48.33% of COVID-19 patients have an elevated cTnI level on the first day of hospitalization. Patients with elevated troponin-I levels were older and had greater rates of congestive heart disease and cardiovascular risk factors (dyslipidemia, hypertension, diabetes, peripheral vascular disease, chronic kidney disease, cerebrovascular disease). Cardiac troponins are used in routine practice as sensitive and specific biomarkers to diagnose myocardial damage in diseases such as acute coronary syndromes or acute myocarditis (Thygesen et al. 2010).

Elevation of cardiac troponins is seen as a prognostic marker for predicting the negative consequences of heart failure, even without coronary artery stenosis (Kociol et al. 2010). In addition, cardiac troponin uprising has been revealed in many non-myocardial ischemic conditions such as sepsis syndrome, pulmonary embolism, subarachnoid hemorrhage, and renal failure, and has been considered prognostic biomarkers with several clinical outcomes (Freda et al. 2002). In this study, cTnI elevation was strongly associated with poor in-hospital prognosis, including clinical course of patients, need for ICU, and all-cause mortality. Cordeanu et al. revealed that 34% of their patients had a high level of Troponin-I and had a four-fold enhanced risk of death in these patients in comparison with patients with a normal level of troponin-I (Cordeanu et al. 2020).

According to this study, 60 out of 87 (68.96%) of the patients with a high cTnI level (1.49) died. Patients hospitalized in ICU due to COVID-19 and with troponin levels above 1.00 had a high mortality rate.

Majure et al. (2021) conducted a study where troponin was an estimator of death, involving 6247 patients infected with COVID-19, and reported significantly increased mortality rates in the group of patients with high troponin quantity in comparison with patients with normal troponin levels.

Shi et al. (2020) conducted a study on 671 patients with verified COVID-19, the myocardial injury prevalence determined by hs-troponin I above the 99th percentile

was found to be 15.8%. Both cardiac troponin I>0.026 ng/ml (hazard ratio, 4.56, p = .02) and CK-MB >2.2 ng/ml (hazard ratio, 6.62, p <.001) was separately associated with an increase in in-hospital mortality.

We found that the CK-MB value of 22 of the patients in the ICU due to COVID-19 was above the normal value. Of these 22 patients, 19 (86.36%) cases resulted in mortality. This shows that the elevation of cTn and CK-MB quantities in COVID-19 intensive care patients is associated with mortality.

COVID-19 mortality rates vary according to age and gender all over the world. Even if the majority of infections in China and Germany affect young age groups, it particularly strains the elderly population. The mortality rate in China is reported as 2.3%. The proportion of people over 80 is only 3%. The elderly people in Italy are even more seriously affected. Of them, 37.6% are aged 70 and over, and the mortality rate of 7.2% is significantly higher than in China. Mortality in this group increases significantly with age. Also, 12.5% of the 70-79 age group die, 19.7% of the 80-89 age group die, and 22.9% of the very old (≥90 years) group die (4, 5, 6). In the study conducted by Li et al. on 425 patients, there were no patients under the age of 15. Fifty-six percent of the patients were male and the majority of them were 45 years of age or older. In later studies, it was also observed that the disease was more severe in older age and male patients (Bornstein et al. 2020).

A total of 180 patients who received inpatient treatment in intensive care between 1 May 2021 and 1 July 2021 in this hospital were assessed. Firstly, a demographic analysis of these patients was done. When the age of the patients is evaluated, a statistically significant relationship of age is observed between the deceased patients and the recovered patients ($p<0.05$). Mortality in this group increases significantly with age. Also, 12.5% died in the 70-79 age group, 19.7% died in the 80-89 age group, and 22.9% died in the very old (≥90 years) age group. Seventy-six of the deceased patients were over the age of 65 years and the average age was 80.71 years. The number of recovered patients was 84, and the average age was 60.22 years. Advanced age is an important cause of mortality when considering the mortality of patients. Mortality is significantly greater in patients over 65 years old when patients are categorized as over and below 65 years of age. Of the 76 patients over the age of 65, 48 patients (63.15%) died, while 46 (46.15%) of the 104 patients under the age of 65 died.

According to this study, 96 patients out of 180 died and 57 (59.37%) of the deceased patients were male. This shows us that high age and male gender were found to be associated with mortality and the need for intensive care in patients with COVID-19.

Diabetes and hypertension are the most common comorbidities for people with COVID-19 who require hospitalization in ICU. In a study comprising 5,700 patients, conducted by Richardson S. et al., the median age was reported as 63-year-old, and the most frequent comorbidities were diabetes (1808; 33.8%), obesity (1737%; 41.7%), and hypertension (3026%; 56.6%) (Richardson et al. 2020). Recent studies show that COVID-19 patients with hypertension have a greater risk of mortality in general compared to non-hypertensive patients (Zuin et al. 2020).

Retrospective cohort research comprised 126 COVID-19 patients with pre-existing hypertension, as well as 125 COVID-19 patients of similar age and gender without hypertension, and reported that myocardial damage after hypertension is a worse prognosis factor and is straightforwardly linked with higher mortality in COVID-19 (Yang et al. 2020).

In this study, the comorbid disease was present in 135 (75%) of the patients in the course of diagnosis. Hypertension was the most common comorbid disease in 67 (49.52%) patients. This was followed by the diagnosis of type 2 diabetes mellitus, which was detected in 38 (28.14%) patients, and cancer, which was detected in 10 (7.40%) patients. Seventy-six (56.29%) of the 135 patients who were followed up due to COVID-19 died, also only 15 (33.33%) of the 45 patients who did not have additional disease died. Comorbidity significantly increases mortality in patients in intensive care due to COVID-19.

The elevation of the D-dimer indicates a state of hypercoagulation in patients with COVID-19. D-dimer, a fibrin degradation product, is observed to be high in the blood serum of patients with COVID-19. The elevation of D-dimer in COVID-19 is the most sensitive marker among coagulation parameters and indicates a higher risk of developing thrombosis.

Zhang et al. (2020) emphasized that D-dimer levels (>2mg/dl) are an important indicator in determining mortality in COVID-19 patients with pneumonia.

Guan et al. (2020) reported the results of a large retrospective study that for the first time demonstrated the correlation between abnormal D-dimer levels and disease severity in COVID-19 patients. They set a cut-off point at a D-dimer level of more than 0.5 mg/L and revealed that a significantly bigger ratio of individuals with the novel coronavirus with severe symptoms exhibits abnormally high D-dimer levels than the patients with moderate or mild symptoms ($p=0.002$).

In addition, Tang et al. (2020) revealed that patients with COVID-19 at severe disease levels showed nearly 3.5 times higher D-dimer quantities than those with moderate or mild disease.

According to this study, D-dimer levels of patients in intensive care were significantly higher than normal. The reason for this is immobilization, comorbidities,

and intense viral inflammation, and therefore we think that vascular occlusions and microthrombi are more common in patients who are followed up in intensive care.

Also, in-hospital mortality was linked with elevated D-dimer levels, demonstrating that the test could be used as a single helpful biomarker for clinical outcomes in COVID-19 patients.

When COVID-19 infection causes an "inflammatory storm", not only the associated cytokines are greatly elevated, but also some inflammatory biological markers including serum ferritin, CRP, PCT, and SAA are increased. CRP, Ferritin, PCT, and SAA levels increased significantly in the severe and critical COVID-19 cases than in moderate cases (Qin et al. 2020).

Zhou et al. (2020) reported that mortality increases in COVID-19 patients with higher serum ferritin levels.

We found that patients with serum ferritin above 300 µg/L had significantly higher mortality, 72 (60%) of 120 patients with high ferritin died, while 22 (44%) of 50 patients with ferritin under 300 µg/L died (p=0.041).

The outcomes of this study can make significant contributions to clinical practice. Assessing the level of ferritin can contribute to the early identification of patients who need to be remedied in a more screened environment due to the high risk of a bad result. In addition, the assessment of ferritin could allow making treatment decisions to prevent complications and/or death.

In this study, we aimed to determine the determinants of biochemical parameters that may have an impact on the need for treatment, as well as mortality in the ICU, of patients receiving inpatient treatment with a COVID-19 diagnosis.

The study was conducted under some limitations. Although a sufficient number of patients were involved in the research, it was still a retrospective study, the data were collected from a single center, and the processes of comorbid diseases could not be detailed, which constituted the limitations of the study.

CONCLUSION

Consequently, COVID-19 is a disease whose activity is still very high and continues to spread rapidly. Scientists are continuing to look for markers that can help in the early detection of this disease and determining the prognosis.

We believe that the biochemical parameters mentioned in this study are important in determining the prognosis of the disease and may be useful in terms of the need for patients to be referred to the intensive care unit after the initial examination in the emergency department.

As a result, given the numerous potential causes of abnormal cTnI levels, normal troponin levels may be more valuable for clinicians. In this research, we found out that the normal troponin quantity on the first day of hospitalization had a high negative predictive accuracy (99.3-91.7%) in the male and female gender for mortality. Such a powerful biomarker can be used during the admission of patients to the hospital to evaluate and conduct further treatment in high-risk people.

As a consequence, hematological and biochemical parameters can be used as predictive markers during the treatment process, both during the application for diagnostic purposes and in determining the severity and prognosis of the disease, as well as during the follow-up of treatment.

Advanced age and comorbidities in COVID-19 patients are linked with a poor prognosis in critical patients with COVID-19. Also, a greater number of comorbidities are affiliated with the higher disease severity of COVID-19. It can help determine the patients at high mortality risk in the COVID-19 pandemic as a model that summarizes the sum of CCI, age, and comorbidities.

Author's Contribution

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

Statement of Conflict of Interest

Authors have no conflict of interest to declare.

REFERENCES

Asakura, H., & Ogawa, H. (2021). COVID-19-associated coagulopathy and disseminated intravascular coagulation. *International Journal of Hematology*, *113*(1), 45–57.

Bayrakçı, O. (2022). Prognosis of Covid-19 according to pneumonia types. *Asian J Res Inf Dis*, *9*(1), 8–12.

Bornstein, S. R., Dalan, R., Hopkins, D., Mingrone, G., & Boehm, B. O. (2020). Endocrine and metabolic link to coronavirus infection. *Nature Reviews. Endocrinology*, *16*(6), 297–298.

Cordeanu, E.-M., Duthil, N., Severac, F., Lambach, H., Tousch, J., Jambert, L., … Stephan, D. (2020). Prognostic value of troponin elevation in COVID-19 hospitalized patients. *Journal of Clinical Medicine*, *9*(12), 4078.

Doruk, Ö.G., Örmen, M., Tuncel, E.(2021) Biochemical and hematological parameters in Covid-19. *J DEU Med* (Special Issue 1): S71-S80.

Freda, B. J., Tang, W. H. W., Van Lente, F., Peacock, W. F., & Francis, G. S. (2002). Cardiac troponins in renal insufficiency: review and clinical implications. *Journal of the American College of Cardiology*, *40*(12), 2065–2071.

Guan, W.-J., Ni, Z.-Y., Hu, Y., Liang, W.-H., Ou, C.-Q., He, J.-X., … China Medical Treatment Expert Group for Covid-19. (2020). Clinical characteristics of Coronavirus disease 2019 in China. *The New England Journal of Medicine*, *382*(18), 1708–1720.

Huang, Y., Hu, Z., Hu, D., Quan, Z., Zhou, X., Fan, G., … Hu, D. (2021). Clinical characteristics, risk factors, and cardiac manifestations of cancer patients with COVID-19. *Journal of Applied Physiology (Bethesda, Md.: 1985)*, *131*(3), 966–976.

Imazio, M., Klingel, K., Kindermann, I., Brucato, A., De Rosa, F. G., Adler, Y., & De Ferrari, G. M. (2020). COVID-19 pandemic and troponin: indirect myocardial injury, myocardial inflammation, or myocarditis? *Heart (British Cardiac Society)*, *106*(15), 1127–1131.

Kociol, R. D., Pang, P. S., Gheorghiade, M., Fonarow, G. C., O'Connor, C. M., & Felker, G. M. (2010). Troponin elevation in heart failure prevalence, mechanisms, and clinical implications. *Journal of the American College of Cardiology*, *56*(14), 1071–1078.

Majure, D. T., Gruberg, L., Saba, S. G., Kvasnovsky, C., & Hirsch, J. S. (2021). Jauhar R; Northwell Health COVID-19 Research Consortium. The usefulness of elevated troponin to predict death in patients with COVID-19 and myocardial injury. *Am J Cardiol*, *138*, 100–106.

Qin, C., Zhou, L., Hu, Z., Zhang, S., Yang, S., Tao, Y., … Tian, D.-S. (2020). Dysregulation of immune response in patients with Coronavirus 2019 (COVID-19) in Wuhan, China. *Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America*, *71*(15), 762–768.

Richardson, S., Hirsch, J. S., Narasimhan, M., Crawford, J. M., McGinn, T., Davidson, K. W., … Zanos, T. P. (2020). Presenting characteristics, comorbidities, and outcomes among 5700 patients hospitalized with COVID-19 in the New York City area. *JAMA: The Journal of the American Medical Association*, *323*(20), 2052–2059.

Salazar, M., Barochiner, J., Espeche, W., & Ennis, I. (2020). COVID-19, hipertensión y enfermedad cardiovascular. *Hipertension y riesgo vascular*, *37*(4), 176–180.

Shi, S., Qin, M., Cai, Y., Liu, T., Shen, B., Yang, F., … Huang, C. (2020). Characteristics and clinical significance of myocardial injury in patients with severe coronavirus disease 2019. *European Heart Journal*, *41*(22), 2070–2079.

Tang, N., Li, D., Wang, X., & Sun, Z. (2020). Abnormal coagulation parameters are associated with poor prognosis in patients with novel coronavirus pneumonia. *Journal of Thrombosis and Hemostasis: JTH*, *18*(4), 844–847.

Thygesen, K., Mair, J., Katus, H., Plebani, M., Venge, P., Collinson, P., … Study Group on Biomarkers in Cardiology of the ESC Working Group on Acute Cardiac Care. (2010). Recommendations for the use of cardiac troponin measurement in acute cardiac care. *European Heart Journal*, *31*(18), 2197–2204.

Yang, G., Tan, Z., Zhou, L., Yang, M., Peng, L., Liu, J., Cai, J., Yang, R., Han, J., Huang, Y., & He, S. (2020). Effects of Angiotensin II Receptor Blockers and ACE (Angiotensin-Converting Enzyme) Inhibitors on Virus Infection, Inflammatory Status, and Clinical Outcomes in Patients With COVID-19 and Hypertension: A Single-Center Retrospective Study. *Hypertension (Dallas, Tex. : 1979)*, *76*(1), 51–58.

Zuin, M., Rigatelli, G., Zuliani, G., Rigatelli, A., Mazza, A., & Roncon, L. (2020). Arterial hypertension and risk of death in patients with COVID-19 infection: Systematic review and meta-analysis. *The Journal of Infection*, *81*(1), e84–e86.

Zhang, L., Yan, X., Fan, Q., Liu, H., Liu, X., Liu, Z., & Zhang, Z. (2020). D-dimer levels on admission to predict in-hospital mortality in patients with Covid-19. *Journal of thrombosis and hemostasis: JTH*, *18*(6), 1324–1329.

Zhou, F., Yu, T., Du, R., Fan, G., Liu, Y., Liu, Z., Xiang, J., Wang, Y., Song, B., Gu, X., Guan, L., Wei, Y., Li, H., Wu, X., Xu, J., Tu, S., Zhang, Y., Chen, H., & Cao, B. (2020). Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet (London, England)*, *395*(10229), 1054–1062.

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Morphological, Physiological and Biochemical Responses of Safflower (*Carthamus tinctorius* L.) Exposed to Salinity Stress

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ABSTRACT

The morphological, physiological (biomass, water content-WC) and biochemical (proline, membrane damage-malondialdehyde-MDA, H_2O_2 content) responses of safflower to NaCI salt stress in different concentrations (0, 50, 75, 150, and 300 mM) were investigated for the first time in *in vitro* conditions in this study. At the end of the 3-week period, it was determined that NaCl had a negative effect on germination percentages. The percentage of germination was 100% in the control group, while it decreased to 30% in 150 mM NaCl and 5% in 300 mM. In general, morphological development of seedlings was significantly slowed down and seedling growth was not observed at 300 mM concentration. It was determined that the WC, fresh weights, shoot and root length decreased in all NaCl concentrations but there was no significant decrease in dry weights. MDA, proline and H_2O_2 contents increased in safflower seedlings in parallel with the intensity of salt treatments. While the highest MDA and proline content was found in 150 mM NaCl treatment, the highest H_2O_2 content was found in 75 mM NaCl treatment. In the light of these data, it has been proven that the Balcı safflower variety is sensitive by showing a negative effect on the applied salt concentrations.

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Tuz Stresine Maruz Bırakılan Aspir (*Carthamus tinctorius* L.) Bitkisinin Morfolojik, Fizyolojik ve Biyokimyasal Tepkileri

ÖZET

Aspir tohumlarının farklı konsantrasyonlarda (0, 50, 75, 150, 300 mM) NaCI stresine karşı morfolojik, fizyolojik (biyokütle, su içeriği-WC) ve biyokimyasal (proline, membran hasarı-malondialdehit-MDA, H_2O_2 içeriği) tepkileri *in vitro* koşullarda ilk defa incelenmiştir. Üç haftalık kültür sürenin sonunda NaCl tuz stresinin çimlenme yüzdelerini olumsuz etkilediği belirlenmiştir. Çimlenme yüzdesi kontrol grubunda % 100 iken 150 mM NaCl'de % 30'a, 300 mM'de % 5'e düşmüştür. Genel olarak, fidelerin morfolojik gelişimi önemli ölçüde yavaşlamış ve 300 mM konsantrasyonda ise fide büyümesi gözlenmemiştir. Tüm NaCl konsantrasyonlarında su içeriği (WC), taze ağırlık, sürgün ve kök uzunluğunun azaldığı ancak kuru ağırlıklarda önemli bir azalma olmadığı belirlenmiştir. Aspir fidelerinde tuz uygulamalarının yoğunluğuna paralel olarak MDA, proline ve H_2O_2 içerikleri de artmıştır. En yüksek MDA ve proline içeriği 150 mM NaCl uygulamasında ve en yüksek H_2O_2 içeriği 75 mM NaCl uygulamasında bulunmuştur. Bu veriler ışığında, Balcı aspir çeşidinin uygulanan tuz konsantrasyonlarında negatif etki göstererek hassas olduğu kanıtlanmıştır.

Bitki Fizyolojisi

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INTRODUCTION

Safflower (*Carthamus tinctorius* L.) is an annual bushy dicotyledonous plant with its yellow, orange, red, cream-colored and white flowers. Firstly used as an ornamental and dyeing plant purposes, safflower has today become an invaluable plant with a strategic importance in the production of two main products, oil and biodiesel, and it will be even more valuable in the future. Also, it has been reported that safflower seed oil is healthier than canola and olive oils (Dajue & Mündel, 1996). In addition to its use as an alternative oil plant, other areas of use of safflower are animal feed, fuel, paint industries and medical (cardiovascular diseases, analgesic, antipyretic, osteoporosis) (İşler, 2014; Birecikli Hamidi & Akbaş, 2018; Nadas et al., 2023).

In ever-changing world, the environmental conditions in which plants grow may change as a result of various abiotic and biotic stress factors. Salinity, one of the abiotic factors, has a significant impact on every stage of the life cycle of plants (Çulha, 2011). Increasing salinity in the soil as a result of higher NaCl negatively affects plant growth because this situation not only restricts the water uptake from the soil but also inhibits nutrient intake (Pessarakli & Szabolcs, 1999).

Revealing the stress behaviors of plants provides great convenience in breeding varieties with high adaptability to stress conditions. Therefore, plant breeding programs are becoming increasingly important (Özen & Onay, 2013). Safflower is a plant that is not very selective in climatic and soil demands and has high adaptability (Kaya, 2017). It is highly important to conduct breeding activities to produce new varieties with high productivity, quality and resistance to stress in order to improve the cultivation of safflower plants which can easily adapt to different climatic conditions.

Biotechnological methods are increasingly used to overcome the problems that cannot be solved using traditional methods in breeding activities and is an alternative method used in salt stress factor research, tolerance determination and variety selection (Hamidi Birecikli & Akbaş, 2018; Kaya, 2017). However, there are a few studies about the effect of salinity stress factor on the safflower plant (*Carthamus tinctorius* L.) in *in vitro* (Hosseini et al., 2010; Çulha, 2011). This study is the first that was conducted in vitro on the effect of salt stress on the Balcı safflower cultivar. From this point of view, the morphological, physiological and biochemical responses given against NaCl stress of safflower (*Carthamus tinctorius* L.) under *in vitro* culture conditions investigates in this study.

MATERIAL and METHOD

In this study, the seeds of the registered Balcı safflower (*Carthamus tinctorius* L.) were used as plant materials. After the seeds were soaked in 70% ethanol for 30 seconds, optimum surface sterilization was performed by soaking them in %5 NaOCl for 60 minutes. Aseptic safflower seeds were cultured in Magenda GA-7 containing 1/4 MS medium (Murashige & Skoog, 1962) supplemented with 0, 50, 75, 150, and 300 mM NaCl and were left to germinate in the growth chamber. All nutrient media were supported with 30 g sucrose and 5.458 g agar. For each application group, 4 seeds were planted in 20 Magenda GA-7 and a total of 80 seeds were used. All salt stress applications on the germination of safflower seeds were carried out in the growth room where optimum conditions were provided. The growth chamber was provided with mercury fluorescent lamps (400 W, MBFR/U, Thorn) with a light intensity of 30-60 $\mu m/m^2s^1$ and a temperature control system that keeps the ambient temperature constant at 25±2ºC. In addition, the light period of the growth room was adjusted to be 16 hours of light and 8 hours of dark (3000-5000 lux).

After a period of 3 weeks, the germinated ones were determined among the seeds (n: 20) incubated with NaCl applications. Seeds with a radicle outflow of about 2.0 mm were considered germinated. At the end of the 3-week culture period, the aerial/root parts of the safflower seedlings were harvested separately and then kept in deep- freeze (-42 °C) until the analysis was performed. Shoot/root length and fresh/dry weight were measured immediately after harvesting to determine salinity effects on the growth of seedlings. Water contents (WC) of every sample were calculated in % according to the formula below:

$$WC\ \% = (FW - DW) / FW \times 100$$ (DW=Dry weight, FW=Fresh weight). (1)

Malondialdehyde (MDA) content was determined according to Ohkawa et al. (1979). Proline content was calculated spectrophotometrically by using acidic ninhydrin method (Bates et al., 1973; Ghoulam et al., 2002). H_2O_2 content was determined according to Velikova et al. (2000).

Statistical Analysis

A completely randomized design was used to analyze the data. Means were compared by DMRT (Duncan Multiple Range Test) using SPSS 20.0 for Windows (SPSS Inc., USA) to evaluate the presence of any significant difference between the means. Significance refers to the statistics at 0.05 probability level.

RESULTS and DISCUSSION

Salinity stress is known to lead insufficient water uptake, ion toxicity, restrains in metabolic activity, enzymatic inhibition and imbalances in plant growth and thus prevents seed germination to a great extent (Leblebici & Işık, 2018). It has been also reported by a

number of researchers that increasing salt concentrations decrease the percentage of germination in the safflower plant (Çulha & Çakırlar, 2011; Çulha, 2011; Bina & Bostani, 2017). As compatible with the literature, this study conducted on the Balcı safflower variety revealed that NaCl treatments had a negative effect on the percentages of germination when compared with the control group ($p \leq 0.05$). The seeds were germinated 100% in the control group while they were germinated 75% in 50 mM and 75 mM NaCl treatment groups. It was detected that the germination percentage decreased to 30% in 150 mM NaCl treatment and decreased to 5% in 300 mM which is the highest salt treatment (Figure 1). Supporting the findings of this study, Echi et al. (2013) reported that the salinity conditions led to a significant reduction in the germination of safflower seeds and the germination decreased significantly at high salt concentrations (210 mg L^{-1} NaCl) compared to the control group.

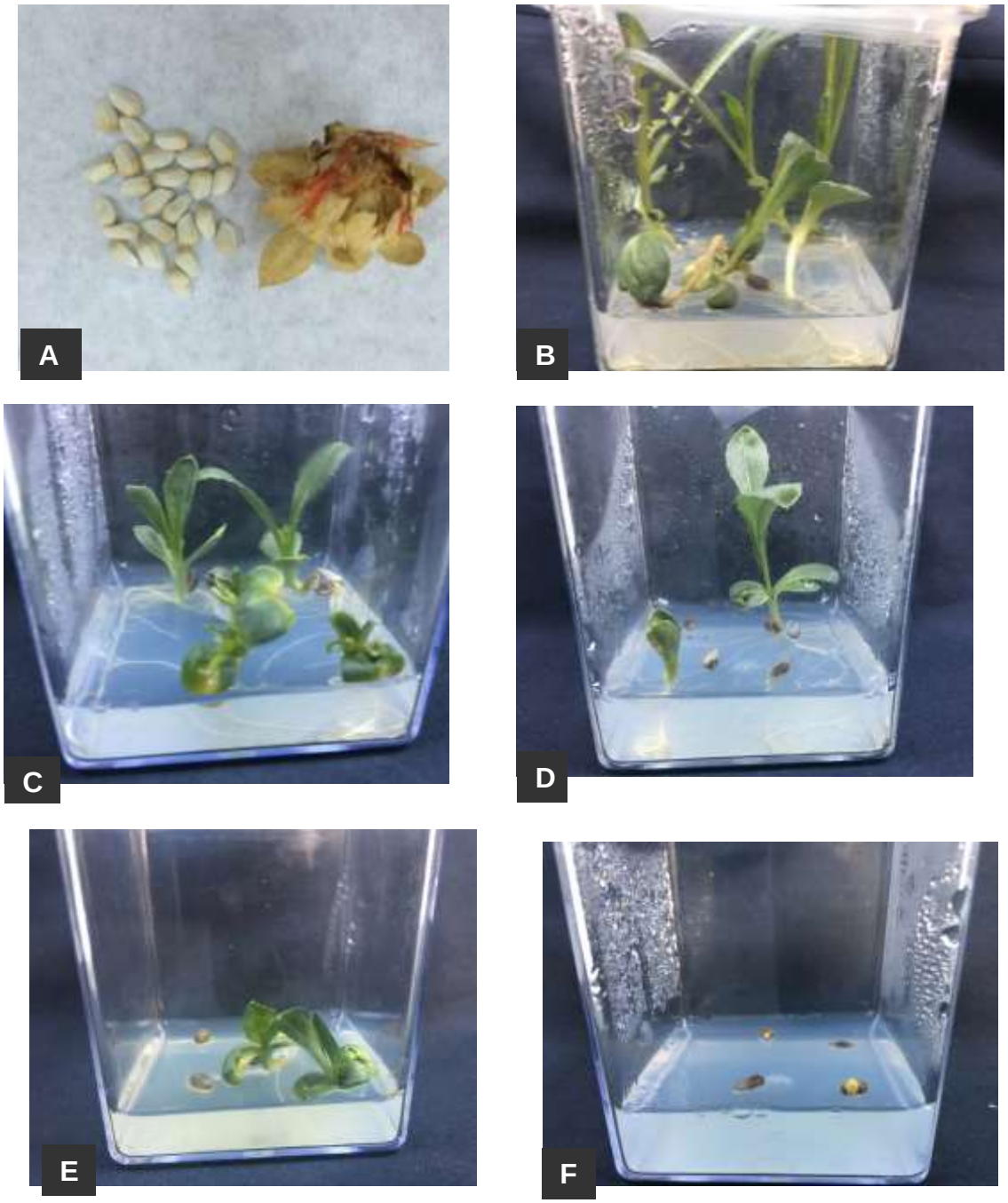

Figure 1. A) A general view of mature seeds of Balcı safflower variety **B)** Control, General views of safflower seeds cultured in **C)** 50 mM NaCl **D)** 75 mM NaCl **E)** 150 mM NaCl **F)** 300 mM NaCl groups for 3 weeks

Şekil 1. A) *Balcı aspir çeşidinin olgun tohumlarının genel görünüşü* ***B)*** *Kontrol grubundaki* ***C)*** *50 mM NaCl* ***D)*** *75 mM NaCl* ***E)*** *150 mM NaCl* ***F)*** *300 mM NaCl grubundaki aspir tohumlarının 3 haftalık genel görünümleri*

The impacts of salt stress on plants may impair the metabolism of plants and result in a reduction in growth. In the present study, a significant decrease was observed in the shoots length of Balcı safflower seedlings compared to the control group depending on increasing NaCl concentrations (Table 1).

Table 1 Effect of salinity stress on shoot and root length (mean ± sd)
Çizelge 1. Tuz stresinin sürgün ve kök boyu üzerindeki etkisi

Treatments	Germination Percentage (%)	Shoot length (cm)	Root length (cm)
Control	100	5.22 ± 2.05^{a}	1.95 ± 1.97^{a*}
50 mM NaCI	75	2.38 ± 1.89^{b}	0.51 ± 0.71^{b}
75 mM NaCI	75	1.79± 0.95^{cb}	0.54 ± 1.11^{b}
150 mM NaCI	30	1.20 ± 0.31^{c}	0.49 ± 0.63^{b}
300 mM NaCI	5	-	-

Numbers given are the mean of 20 materials. Different letters in each column indicate significant difference at $p \leq 0.05$, based on Duncan's multiple range test.
Rakamlar 20 materyalin ortalamasını göstermektedir. Duncan's multiple range testine dayalı olarak, her sütunda farklı harfi alan ortalamalar arasındaki farklılık $p \leq 0,05$ seviyesinde önemlidir.

It was also found that all NaCl salt treatments had a negative effect on the roots length of safflower seedlings and caused a significant reduction compared to control group (approximately 74%). The longest root was obtained from the control group with 1.95 cm and the shortest root was obtained from the 150 mM NaCl treatment with 0.49 cm. Parallel to this study, there are numerous studies reporting that salinity stress causes a reduction in plant length, root length and all growth and productivity parameters (Çulha & Çakırlar, 2011; Echi et al., 2013; Erdal & Çakırlar, 2014; Zhang et al., 2015; Toprak & Tunçtürk, 2018). It is thought that the decrease in plant length and root length may be a result of osmotic pressure differences, Na^{+} accumulation in leaves and inhibition in cell growth (Alasvandyari & Mahdavi, 2017).

In this study, a significant decrease was detected in fresh weights of the green parts of growing seedlings in all NaCl treatments compared to the control group and such decrease was statistically significant ($p \leq 0.05$). As shown in Table 2, when the effects of salt treatments on the dry weights of green parts were analyzed, it was detected that there was no significant decrease when compared to the control group and to each other and the differences were statistically insignificant. In some studies on safflower, decreased fresh and dry weights of shoots in association with the increasing NaCl content were reported (Çulha & Çakırlar, 2011; Zhang et al., 2015; Toprak & Tunçtürk, 2018). It is considered that salinity stress causes a decrease in water content, chlorophyll and carotenoid content in tissues, inhibition of photosynthesis activity and, as a result, weight loss in the plant (Sairam et al., 2002).

Table 2: Effect of salinity stress on water content and fresh/dry weights (mean ± sd)
Çizelge 2. Tuz stresinin taze/kuru ağırlık ve su içeriği üzerindeki etkisi

Treatments	Fresh Weight (g/plant)	Dry Weight (g/plant)	WC (%)
Control	0.22±0.02^{a}	0.02±0.004^{a}	89.31±1.005^{a}
50 mM NaCl	0.16±0.01^{b}	0.02±0.003^{a}	87.23±1.038^{a}
75 mM NaCl	0.15±0.03^{b}	0.01±0.003^{a}	87.32±0.902^{a}
150 mM NaCl	0.16±0.01^{b}	0.02±0.004^{a}	83.95±1.916^{b}
300 mM NaCl	-	-	-

Different letters in each column indicate significant difference at $p \leq 0.05$, based on Duncan's multiple range test.
Duncan's multiple range testine dayalı olarak, her sütunda farklı harfi alan ortalamalar arasındaki farklılık $p \leq 0,05$ seviyesinde önemlidir.

It is reported that the first damage caused by salinity stress in plants indicated itself with water deficiency. The increase in salt content in culture medium reduces the osmotic potential of water and makes it difficult for the root to take up water, causing water deficiency in the plant (Sairam & Srivastava, 2002). As a result of this event which is described as physiological drought, various metabolic irregularities and reduced growth rate occur (Levitt, 1980). In this study, a decrease was detected in water content (WC) compared to the control group as a result of increased in all NaCl concentrations. However, it was found that the decreases observed in 50 and 75 mM NaCl treatment groups were statistically insignificant ($p \leq 0.05$) while the decrease in 150 mM NaCl group was statistically significant compared to the control group (Table 2). Among all the tested applications, the lowest WC ratio was determined in the cultivated safflower seedlings in the 150 mM NaCl application group with 83.95%. In the light of all data, it was determined that high NaCl concentrations caused a decrease in WC ratio in Balcı safflower cultivars and low salt concentrations were not very effective (Table 2). Similarly, Siddiqi and Ashraf (2008) as well as Alasvandyari & Mahdavi

(2017) reported in their studies conducted with safflower varieties that salinity stress decreased water-related parameters (such as relative water content, water potential, and osmotic potential). Çulha (2011) stated in a study conducted with 3 different safflower varieties that water content (relative and real) decreased as a result of increased salinity stress.

Under salt stress, the active oxygen species react with polyunsaturated fatty acids and cause lipid peroxyl radicals to form. These radicals cause the disruption of membrane organization and integrity (Radić et al., 2006). As a result of the peroxidation of lipids in the cell membrane, MDA is formed as a reaction product (Ohkawa et al., 1979). The damage caused by salt stress on the cell membranes of Balcı safflower variety was determined by analyzing the MDA content calculated with thiobarbituric acid test (Table 3). It was observed that NaCl applications led to an increase in MDA content and that it continued rising in parallel with the increased concentration compared to the control group. In the 150 mM NaCl application group, it was detected that MDA content was maximum (4.58 µmol g^{-1}) and this value was statistically significant difference from the values in both NaCl applications and control group. In studies conducted with different safflower varieties, it was reported that salinity stress caused an increase in MDA content as compatible with this findings (Çulha, 2011; Erdal & Çakırlar, 2014; Alasvandyari & Mahdavi, 2017; Kazemeini et al., 2017). In support of the findings of this study, it has been reported that high salt concentrations in different plant species cause an increase in the amount of MDA [rice (Orcan et al., 2019), and wheat (Zhang et al., 2013).

Table 3: Changes in MDA, Proline and H_2O_2 Contents Following Salinity Stress (mean ± sd)
Çizelge 3. Tuz stresinde MDA, prolin ve H_2O_2 içeriğindeki değişimler

Treatments	MDA (µmol g^{-1} TA)	Proline (mmol g^{-1} TA)	H_2O_2 (µmol g^{-1} TA)
Control	2.76±0.04^{d}	0.69±0.01^{d}	14.55±0.69^{d}
50 mM NaCl	3.02±0.02^{c}	0.94±0.04^{c}	17.81±1.04^{c}
75 mM NaCl	3.68±0.05^{b}	1.33±0.03^{b}	34.03±0.87^{a}
150 mM NaCl	4.58±0.05^{a}	2.18±0.03^{a}	25.66±1.10^{b}
300 mM NaCl	-	-	-

Differences between means marked with different letters are significant ($p \leq 0.05$)
Her sütunda farklı harfi alan ortalamalar arasındaki farklılık önemlidir ($p \leq 0.05$)

Plants accumulate large quantities of low molecular weight osmolytes in order to resist the salinity stress factor. There are a number of studies reporting that the content of proline, which is one of these osmolytes, increases in plants under stress (Ashraf & Orooj, 2006; Eyidogan & Öz, 2007). Similarly, in the present study conducted with the Balcı safflower variety, it was determined that the osmotic stress resulting from salinity stress caused an increase in proline content (Table 3). It was observed that proline content increased in proportionally to the NaCl concentration and such increase was statistically significant. The increase in proline content (0.94 mmol g^{-1}) at 50 mM concentration, which was the lowest NaCl application, indicated a significant difference ($p \leq 0.05$) compared to the control. 2.18 mmol g^{-1} proline content which was quite high compared to the control group was detected in the 150 mM NaCl treatment group. Supporting these findings, a number of studies were reported in different safflower varieties that proline content increased as the severity of stress increased (Erdal & Çakırlar, 2014; Alasvandyari & Mahdavi, 2017; Kazemeini et al., 2017).

It is expressed that increased H_2O_2 in plants under salinity stress causes lipid peroxidation and disrupts the membrane structure (Mandhania et al., 2006). In this study, the changes in H_2O_2 contents in all of NaCl salinity stress treatments were analyzed and a significant ($p \leq 0.05$) increase in H_2O_2 content was detected generally in all groups (50, 75, 150 and 300 mM NaCI) compared to the control group (Table 3). Among the salt applications, the highest H_2O_2 content was obtained in safflower seedlings developed at 75 mM NaCI concentration with 34.03 µmol g^{-1}, while the lowest H_2O_2 content was found at 50 mM NaCI application with 17.81 µmol g^{-1}, and these values were found to be much higher than the control group. Çulha (2011) reported in his study conducted with 3 different safflower varieties that H_2O_2 content increased as a result of the induction of oxidative stress as the NaCI content increased. The researcher reported that increased NaCI stress escalated not only the lipid peroxidation but also the ion leakage and, in this case, H_2O_2 acted as a toxic molecule, not as a signal molecule. Chaparzadeh et al. (2004) detected an increase in the H_2O_2 content of the leaves of *Calendula officinalis* plant under salinity stress compared to the control group. The researchers reported that H_2O_2 content had a significant role in the regulation of growth in plants and their development of tolerance to salinity stress as a result of interaction with different enzyme activities.

CONCLUSION

Salinity which is one of the most important environmental stress factors affects the growth and development of plants negatively. However, responses

to salinity stress may vary depending on the growth stages of plants. During seed germination that known the first stage of growth in plants, analyzing the stress effects and responses is regarded as an important step in determining the tolerance degree of plants to salinity. Therefore, we analyzed and assessed in this study the effect of various NaCl concentrations in the germination stage of the Balcı safflower variety. As a result, it was detected that NaCl salt had a negative impact on germination percentage, shoot/root length, fresh weight of green parts but had no effect on dry weight. In addition, significant increases were found in MDA content, proline and H_2O_2 content of seedlings, particularly in high concentrations.

In order for plants to adapt to changing adverse environmental conditions, it is important to determine tolerant plant species and varieties that can cope with abiotic stress factors such as salinity. In this study, for the first time, a scientific data that can be a reference in the selection of possible agricultural areas by determining the sensitivity level of the Balcı safflower variety to salinity stress is presented. Our results showed that Balcı safflower cultivar is sensitive to salinity. Planning future studies to increase the resistance of the plant against salt stress through elicitors will be beneficial in the cultivation of safflower, which is an alternative oil plant.

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Statement of Conflict of Interest

Authors have declared no conflict of interest.

Author's Contributions

The contribution of the authors is equal.

REFERENCES

Alasvandyari, F., & Mahdavi, B. (2017). Effect of glycinebetaine on growth and antioxidant enzymes of safflower under salinity stress condition. *Agriculture & Forestry, 63*(3), 85-95.

Ashraf, M., & Orooj, A. (2006). Salt stress effects on growth, ion accumulation and seed oil concentration in an arid zone traditional medicinal plant ajwain (*Trachyspermum ammi* [L.] Sprague). *Journal of Arid Environments, 64*, 209-220.

Bates, L. S., Waldern, R.P., & Teave, I. D. (1973). Rapid determination of free proline for water stress studies. *Plant and Soil, 39*, 205-207.

Bina, F., & Bostani, A. (2017). Effect of Salinity (NaCl) stress on germination and early seedling growth of three medicinal plant species. *Adv. Life Sci., 4*(3), 77-83.

Birecikli Hamidi, A., & Akbaş, F. (2018). "Balcı" Aspir (*Carthamus tinctorius* L.) Çeşidinin *in vitro* Sürgün Uçlarının Mikroçoğaltımı ve Köklendirilmesi Üzerine Oksin ve Sitokininlerin Etkisi. *Yyü Tar. Bil. Derg. (Yyu J Agr Sci)*, *28*(4), 438-443.

Chaparzadeh, N., D"amico, M. L., Khavari-Nejad, R. A., Izzo, R., & Navari-Izzo, F. (2004). Antioxidative responses of *Calendula officinalis* under salinity conditions. *Plant Physiology and Biochemistry, 42,* 695-701.

Çulha, Ş. (2011). Tuz Stresinin Aspir (*Carthamus tinctorius* L.) Çeşitlerindeki Bazı Fizyolojik ve Biyokimyasal Parametreler Üzerine Etkisinin İncelenmesi (Tez No. 299523) [Yüksek Lisans Tezi, Hacettepe Üniversitesi], Ankara.

Çulha, Ş., & Çakırlar, H. (2011). Tuzluluğun bitkiler üzerine etkileri ve tuz tolerans mekanizmaları. *AKÜ Fen ve Mühendislik Bilimleri Dergisi*, *11*(2), 11-34.

Dajue, L., & Mündel, H. H. (1996). *Safflower Carthamus tinctorius L., Promoting the conservation and use of underutilized and neglected crops. 7*, Institute of Plant Genetics and Crop Plant Research, Gatersleben/International Plant Genetic Resources Institute, Rome, Italy.

Echi, R. M., Aslı, D. E., Vajedi, S. J., & Kashani, Z. F. (2013). The effect of seed pretreatment by salicylhydroxamic acid on germination indices of safflower under salinity stress. *International Journal of Biosciences (IJB)*, *3*(6), 181-189.

Erdal, Ş. Ç., & Çakırlar, H. (2014). Impact of salt stress on photosystem II efficiency and antioxidant enzyme activities of safflower (*Carthamus tinctorius* L.) cultivars. *Turk J. Biol., 38,* 549-560.

Eyidogan, F., & Öz, M. T. (2007). Effect of salinity on antioxidant responses of chickpea seedlings. *Acta Physiol. Plant., 29*, 485-493.

Ghoulam, C., Foursy, A., & Fares, K. (2002). Effects of salt stress on growth, inorganic ions and proline accumulation in relation to osmotic adjustment in five sugar beet cultivars. *Environ. Exp. Bot., 47*, 39-50.

Hosseini, T., Shekari, F., & Ghorbanli, M. (2010). Effect of salt stress on ion content, proline and antioxidative enzymes of two safflower cultivars (*Carthamus tinctorius* L.). *Journal of Food, Agriculture & Environment, 8*(2), 1080-1086.

İşler, N. (2014). Aspir Tarımı [PowerPoint slides]. Hatay@MKÜ. http://www.mku.edu.tr/files/898-7f299b9f-6e6e-4418-aa25-7dd9fbd65874.pdf

Kaya, Ş. (2017). Aspir (*Carthamus tinctorius* L.) Bitkisi Balcı Çeşidinin Doku Kültürüyle *In Vıtro* Çoğaltımı (Tez No. 478572) [Yüksek Lisan Tezi, Yıldız Teknik Üniversitesi], İstanbul.

Kazemeini, S. B., Zamani, A., Motazedian, A., & Shakeri, E. (2017). Evaluation of salinity tolerance in two safflower cultivars using ions relations and

biochemical traits. *Iranian Journal of Field Crop Science, 48* (4), 1211-1225.

Leblebici, S., & Işık, G. (2018). Farklı konsantrasyonlarda kalsiyum karbonat (CaCO3) uygulamasının *Carthamus tinctorius* l.'a (asteraceae) ait farklı varyetelerin tohum çimlenmesi üzerine etkileri. *Anadolu Üniversitesi Bilim ve Teknoloji Dergisi C- Yaşam Bilimleri ve Biyoteknoloji, 7* (1), 63-67.

Levitt, J. (1980). *Responses of Plants to Environmental Stresses II. Water, Radiation, Salt and Other Stresses* (2nd ed.). Academic Press, New York.

Mandhania, S., Madan, S., & Sawhney, V. (2006). Antioxidant defense mechanism under salt stress in wheat seedlings. *Biologia Plantarum, 50* (2), 227-231.

Murashige, T., & Skoog, F. 1962. A revised medium for rapid growth and bioassays with tabacco tissue cultures. *Physiol. Plantarum, 15*, 473-497.

Nakas, D. B. , Avcı, S. & Kaya, M. D. (2023). Response of Different Safflower Genotypes to Anther Culture . Kahramanmaraş Sütçü İmam Üniversitesi Tarım ve Doğa Dergisi , 26 (2) , 293-298 . DOI: 10.18016/ksutarimdoga.vi.1084134

Ohkawa, H., Ohishi, N., & Yagi, Y. (1979). Assay of lipid peroxides in animal tissue by thiobarbituric acid reaction. *Analytical Biochemistry, 95*, 351-358.

Orcan, P., Işıkalan, Ç., & Akbaş, F. (2019). Evaluation of salinity tolerance in rice (*Oryza sativa* L.) using water potential, biomass, membran damage and osmoprotective compound. *Fresenius Bulletin, 28* (4A), 3313-3323

Özen, H. Ç., & Onay, A. (2013). *Bitki Fizyolojisi.* Nobel Akedemik Yayıncılık, Ankara.

Pessarakli, M., & Szabolcs, I. (1999). Soil salinity and sodicity as particular plant/crop stress factors (M. Pessarakli, Ed.). Handbook of Plant Crop Stress. Marcel Deker Press Inc., New York.

Radic´, S., Radic´-Stojkovic´, M., & Pevalek-Kozlina, B. (2006). Influence of NaCl and mannitol on peroxidase activity and lipid peroxidation in *Centaurea ragusina* L. roots and shoots. *Journal of Plant Physiology, 163,* 1284-1292.

Sairam, R. K.,, Rao, K. V., & Srivastava, G. C. 2002. Differantial Response of Wheat Genotypes to Long Termalinity Stres in Relation to Oxsidative Stres, Antioxidant Activity and Osmolyte Concentration. *Plant Science, 163*,1037–1046.

Sairam, R. K., & Srivastava, G. C. (2002). Changes in antioxidant activity in sub-cellular fractions of tolerant and susceptible wheat genotypes in response to long term salt stress. *Plant Science, 162,* 897-904.

Siddiqi, E. H., & Ashraf , M. (2008). Can leaf water relation parameters be used as selection criteria for salt tolerance in safflower (*Carthamus tinctorius* L.). *Pak. J. Bot., 40* (1), 221-228.

Toprak, T., & Tunçtürk, R. (2018). Farklı Aspir (*Carthamus tinctorius* L.) Çeşitlerinin Gelişim Performansları Üzerine Tuz Stresinin Etkisi. *Doğu Fen Bilimleri Dergisi, 1* (1), 44-50.

Velikova, V., Yordanov, I., & Edrava, A. (2000). Oxidative stress and some antioxidant systems in acid rain-treated bean plants: Protective role of exogenous polyamines. *Plant Science, 151*, 59- 66.

Zhang, W., Yang, X., Liu, F., Pei, Y., Yuan, J., & Nie, J. (2015). Effects of saline alkali stress on seed germination of *Carthamus tinctorius* L. *Medicinal Plant, 6* (11-12), 1-6.

Zhang, J., Duan, X., Ding, F., Ma, H., Zhang, T., & Yang, Y. (2013). Salinity induced the changes of root growth and antioxidative responses in two wheat cultivars. *Protoplasma, 10,* 709-715.

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On the Presence and Distribution of *Lycopus exaltatus* L.f. (Lamiaceae) in Türkiye

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ABSTRACT
This study is about confirming the distribution of Lycopus exaltatus (Lamiaceae) in Türkiye, for which there is no reliable information about its existence in Türkiye. In addition, the description of L. exalatus, besides the ecology information in the area where it spreads; differences with Lycopus europaeus, whose distribution is known in Türkiye, are also emphasized.

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ÖZET
Bu çalışma; Türkiye'de varlığına dair güvenilir bilgi bulunmayan *Lycopus exaltatus*'un (Lamiaceae) Türkiye'deki yayılışının doğrulanması ile ilgilidir. Ayrıca *L. exalatus*'un tanımı, yayıldığı alandaki ekoloji bilgilerinin yanı sıra; Türkiye'de yayılışı bilinen *Lycopus europaeus* ile farklılıklar da vurgulanmıştır.

Botanik

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INTRODUCTION

Lamiaceae is one of the biggest families of flowering plants with about 245 genera and 7.886 species (Stevens, 2017). In addition to *Clinopodium debile* (Bunge) Kuntze (Behçet & Altınsoy, 2023), the distribution of which has been newly determined in our country; Lamiaceae is represented by 787 taxa(608 species, 179 subspecies and varieties) belonging to 48 genera in Türkiye, together with recently published new taxa (Celep et al., 2022; Güner Ö, 2022; Aytaç et al., 2022; Duman et al., 2023). Members of the genus *Lycopus* L., which consists of about 15 species in the world, most commonly found in Europe, Asia, and North America. In the 7th volume of Flora of Turkey, the distribution of only *L. europaeus* (Figure 4) among the *Lycopus* members is given and there is no information about the distribution of any other member of this genus in Türkiye.

Although the existence of *Lycopus exaltatus* L.f. in Türkiye is mentioned in the study titled Türkiye Bitkiler Listesi (Damarlı Bitkiler) (Dirmenci 2012); no locality information was given for this species.

There is also a note in the same source that confirmation of the existence of this species in Türkiye is required.

In addition, in Henderson's work on the genus *Lycopus* (1962), there is an explanation as follows: *L. exaltatus* ranges across Eastern Europe from Germany, Austria,

Hungary, and Italy through Russia and Türkiye to the Himalayan Region of India and into Siberia. Although Hendersen (1962) and Dirmenci (2012) mentioned the distribution of *L. exaltatus* in Türkiye; Dirmenci's note that "its presence in Turkey needs to be confirmed" regarding this species has led to doubts about the existence of this species in Türkiye. From this note, understood that there is no definite opinion about the distribution of *L. exaltatus* in Türkiye. Although there is very limited information about the distribution of the species in Türkiye in the mentioned source, the absence of any locality information, the absence of any numbered herbarium specimens, requires confirmation of its existence in Türkiye.

Lycopus is a perennial herb. Occurs mostly in low wetland areas and distinguished by several gross morphological characteristics such as dentate or pinnatifid leaves, flowers in compact, sessile verticillasters in the leaf axils, and a dry, tetrahedral, one-seeded nutlet with corky crests (Henderson, 1962; Moon and Hong, 2006).

An interesting *Lycopus* (very different from *Lycopus europaeus* L., which is very well known in Türkiye) population found in the field study carried out in the summer months (June/July) around the Yedisu district of Bingöl province. With the help of the literature, we reviewed (Henderson, 1962; Mill, 1982; Ball, 1972; Volkova, 1977), It was understood that the population belonged to *Lycopus exaltatus*. However, there is not enough data on the existence of this species in Türkiye. With this study, doubts regarding the distribution of *L. exaltatus* eliminated in Türkiye (Figure 1,3).

MATERIALS and METHODS

Interesting *Lycopus* specimens were collected from Güzgülü village (Yedisu, Bingöl/Türkiye) during botanical trips. These specimens could not be identified according to Flora of Turkey. (Mill, 1982). Eventually, specimens identified as *Lycopus exaltatus* L.f. using the generic account in Flora Europaea volume 3 (Ball, 1972) and Flora of the USSR volume 21 (Volkova, 1977). The examples mentioned are; it was also compared and checked with images of *L. exaltatus* specimens (Figure 1) in Moscow University Herbarium (MW) (Serengin 2023). Photographs of specimens were taken in the field and morphological observations were made using an Olympus SZ51 stereo microscope. Specimens deposited in BIN (the Herbarium of Bingöl University, Arts and Science Faculty).

RESULTS and DISCUSSION

Lycopus exaltatus L.f. Suppl. Pl. 87 (1781) (Figure 1,3)

Homotypic Synonyms

• *Lycopus europaeus* var. *exaltatus* (L.f.) Lej., Rev. Fl. Spa: 7 (1825)

Heterotypic Synonyms

• *Lycopus europaeus* var. *laciniatus* Nyman, Consp. Fl. Eur., Suppl. 2: 259 (1890)

• *Lycopus exaltatus* var. *ovatus* Benth., A.P.De Candolle, Prodr. 12: 180 (1848)

• *Lycopus italicus* L. ex B.D.Jacks., Index Linn. Herb.: 100 (1912), not validly publ.

• *Lycopus laciniatus* Rouy, Bull. Soc. Bot. France 20(Rev. Bibliogr.): 32 (1883), nom. illeg.

• *Lycopus pinnatifidus* Pall., Reise Russ. Reich. 3: 655 (1776), nom. nud.

Turkish name: az kurtayağı (Dirmenci, 2012)

Description: Perennial; rhizome thickened, oblique, sometimes with long creeping stolons; stem 50-100 cm long, 4-angled, erect, simple, rarely branched, sparsely covered in upper part with subappressed hairs and scattered small glands; leaves oblong-ovate, short-petioled or subsessile, deeply pinnatisect (sometimes nearly to midvein), with lanceolate or oblong-lanceolate, mucronate, entire or few-toothed segments, 5-10 cm long, 2-7 cm broad, punctate-glandular, glabrous or short-haired above, the veins (especially the midvein) covered with rather long hairs; flowers numerous, in compact, 15-20- flowered whorls; bracts 6-9 mm; especially the outer, mostly exceeding the whorls, lance-subulate, rigid, hispid; calyx campanulate, 5-toothed, 3.5-4 mm long, glandular, the teeth (1.3-)1.5(-2.0) mm, 3-nerved, the middle tooth conspicuous, aristate; corolla white, 3.5 (4) mm long, 4-lobed, cut to one-third into unequal lobes, the upper lobe slightly emarginate, the lower purple-speckled; stamens exserted one-third their length; nutlets 1 mm long, glandular above, with thickened margins.

Flowering time: June-August

Habitat: Sand and pebble shallows and shores of river and lakes, wood margins, riverside thickets, inundated forests and canals.

Distribution: *Lycopus exaltatus* ranges across Albania, Altay, Austria, Belarus, Bulgaria, Buryatiya, Central European Rus, Czechoslovakia, East European Russia, Germany, Greece, Hungary, Irkutsk, Italy, Kazakhstan, Kirgizstan, Krym, North Caucasus, Northwest European R, Poland, Romania, South European Russi, Transcaucasus, Ukraine, West Himalaya, West Siberia, Xinjiang, Yugoslavia (POWO, 2022) (Figure 2).

Specimens examined: Türkiye (B8 square), Bingöl: Yedisu town; Güzgülü village, wet places, 22.06.2021, 1518 m, 39°25'36.50"K, 40°29'15.98"D, *H.CENGIZ* 3706.

Ecology: The first author collected specimens of *Lycopus exaltatus* from the humid slopes 1500-1600 meters above sea level 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* G.Olivier taxa are the dominant cover). 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*

Figure 1. General view of *Lycopus exaltatus* from Seregin 2023.
Şekil 1. Lycopus exaltatus'un genel görünümü (Seregin 2023'den)

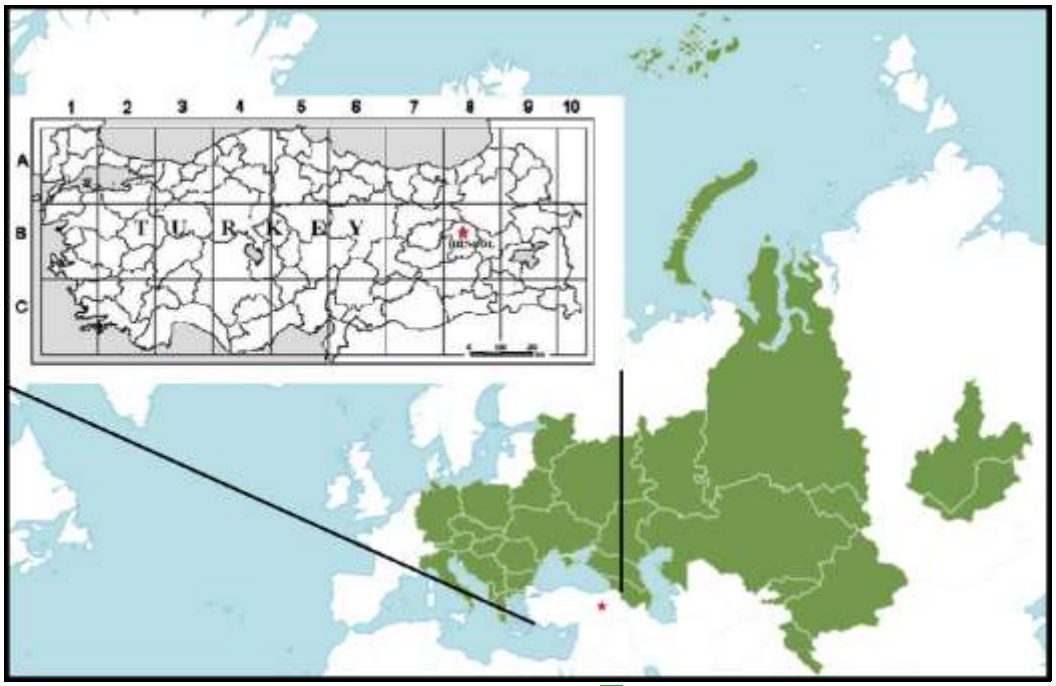

Figure 2. Distribution map of *Lycopus exaltatus* in the World (■) (from POWO 2022) and Türkiye (★)
Şekil 2. Lycopus exaltatus'un Dünya (■) ve Türkiye'deki (★) yayılış haritası (POWO 2022'den)

Figure 3. *Lycopus exaltatus* **A**- habit (Photo by Hikmet CENGİZ), **B**- close-up view of the flowers, **C**- Lower (1), median cauline (2,3,4) to upper cauline (5) leaves
Şekil 3. Lycopus exaltatus A- genel görünüm (Fotoğraf Hikmet CENGİZ tarafından), B- çiçeklerin yakından görünümü, C- en alt (1), orta (2,3,4) ve en üste (5) doğru gövde yaprakları

L., *Lathyrus pratensis* L., *L. rotundifolius* Willd. subsp. *miniatus* (M.Bieb. ex Steven) P.H.Davis, *Lithospermum arvense* L., *Medicago lupulina* L., *Melissa officinalis* L. subsp. o*fficinalis, Pastinaca sativa* L. subsp. *urens* (Req. Ex Gren. & Godr.) Čelak., *Phleum pratense* L., *Phlomis 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 *Lycopus exaltatus*.

There is no mention of a member of the *Lycopus exaltatus* species in Türkiye in 11 volumes of 'Flora of Turkey'. However, in the book '*Türkiye Bitkileri Listesi (Damarlı Bitkiler)*' published by Güner et al., 2012, it is mentioned that there are two species of the genus *Lycopus*, which are *Lycopus exaltatus* and *Lycopus europaeus.* Although one of these species is *Lycopus exaltatus*, but the author of this genus made a note under this species, 'confirmation of its existence in Turkey is required.' There is not enough information about this species in the sources related to the Flora of Turkey. The native range of this species is Europe to South Siberia and West Himalaya. In some studies; although it is mentioned that there is a spread of this species in Türkiye, no details are given. Therefore, it was necessary to confirm its distribution in the Flora of Turkey. As a result of the literature review we made, it was determined that the samples we collected belonged to *Lycopus exaltatus* by looking at the flora of Europe and Russia. With this study, data on the distribution, locality, ecology and habitat of this species in the Flora of Turkey were revealed and the presence of this species in the Flora of Turkey was confirmed.

Although *Lycopus exaltatus is similar to L. europaeus;* it is better developed, robust and its leaves are pinnatifid or pinnatisect from base to apex*;* bracts 6-9 mm; calyx-teeth (1.3-)1.5(-2.0) mm; *Lycopus europaeus* leaves pinnatifid or pinnatisect at base, tooted or shallowly lobed at apex (Figure 4); bracts 3-5 mm; calyx-teeth c. 2 mm.

Figure 4. General view of *Lycopus europaeus* (Photo by Lütfi Behçet)
Şekil 4. Lycopus europaeus'un genel görünümü (Fotoğraf Lütfi Behçet tarafından çekilmiştir)

Identification key for the genus Lycopus distributed in Türkiye:

Lower cauline leaves evenly pinnatifid with forward-angled, linear, acute lobes.

- Leaves pinnatifid or pinnatisect to the apex (not just the leaves at the base and lower part of the stem); bracts 6-9 mm .....*L. exaltatus*
- Leaves pinnatifid or pinnatisect at base, tooted or shallowly lobed at apex; bracts 3-5 mm.....*L. europaeus*

As a result of these studies, it was concluded that the *Lycopus* specimens collected from the Yedisu district of Bingöl province belonged to *Lycopus exaltatus*, and the presence of *L. exaltatus* in Türkiye was revealed and its distribution area was specified. With the determination of the habitat of *L. exaltatus* from Bingöl, the number of taxa of the genus *Lycopus* in Türkiye increased to 2 and numbered specimens of *L. exaltatus* collected from Türkiye are kept in the herbarium of BIN. We hope that it will be useful for the Illustrated Flora of Turkey studies to be written and for those who are interested in the subject.

Author's Contributions

The contribution of the authors is equal.

Statement of Conflict of Interest

Authors have declared no conflict of interest.

REFERENCES

Aytaç, Z., İğci, B. K., Ertuğrul, T., Pınar, N. M., Uluer, D. A., & Özüdoğru, B. (2022). *Marrubium zeydanlii* (Lamiaceae), a new species from Turkey. *Turkish Journal of Botany*, *46*(6), 636-644.

Ball, P. W. (1972). *Lycopus* L. -In: Tutin TG, Heywood VH, Burges NA, Moore DM, Valentine DH, Walters SM, Webb DA (eds.), Flora Europaea Vol. 2: Cambridge Univ. Press, Cambridge, pp.183.

Behçet L. & Altınsoy, İ. (2023). *Clinopodium debile* (Bunge) Kuntze (Lamiaceae), A New Record for the Flora of Turkey. *Kahramanmaraş Sütçü Üniversitesi Tarım ve Doğa Dergisi 26*(3), 504-510.

Celep, F. Karaer, F. & Drew, B. (2022). *Lamium cappadocicum*, a new species from Central Anatolia, Turkey: evidence from molecular and morphological studies, *Turkish Journal of Botany, 46*(6), 614-623.

Dirmenci, T. (2012). *Lycopus* L. In: Güner, A., Aslan, S., Ekim, T., Vural, M. & Babaç, M.T., (eds). Türkiye Bitkileri Listesi (Damarlı Bitkiler). - Nezahat Gökyiğit Botanik Bahçesi ve Flora Araştırmaları Derneği Yayını, İstanbul. pp.559.

Duman, H. (2023). A new annual *Satureja* (Lamiaceae) species from Turkey with molecular evidence, and lectotypification of two species. *Turkish Journal of Botany, 47*(1), 61-72.

Güner, Ö. (2022). *Stachys istanbulensis* (Lamiaceae) a new species from Turkey: evidence from morphological, micromorphological and molecular analysis. *Turkish Journal of Botany, 46*(6), 624-635.

Henderson, N. C. (1962). A taxonomic revision of the genus *Lycopus* (Labiatae). *American Midland Naturalist, 68*, 95-138.

Kahraman, A. & Dogan, M., (2010). Comparative study of *Salvia limbata* C.A. and *S. palaestina* Bentham (sect. Aethiopis Bentham, Labiatae) from East Anatolia, Turkey. *Acta Botanica Croatica, 69*(1), 47–64.

Mill, R. R. (1982). *Lycopus* L. In: Davis, P. H. (ed.), Flora of Turkey and the East Aegean Islands Vol. 7: Edinburgh Univ. Press, Edinburgh. 394–395.

Moon, H-K. & Hong, S.P. (2006). Nutlet morphology and anatomy of the genus *Lycopus* L. (Lamiaceae: Mentheae). *Journal of Plant Research, 119*, 633-644.

POWO, (2022). Plants of the World Online. Facilitated by the Royal Botanic Gardens, Kew. Published on the Internet: http://www.plantsoftheworldonline.org/ (Access: 22.10.2022).

Seregin, A. (2023). Moscow University Herbarium (MW). Version 1.263. Lomonosov Moscow State University. Occurrence dataset https://doi.org/10.15468/cpnhcc accessed via GBIF.org on 2023-01-17. https://www.gbif.org/ occurrence/1697646490

Stevens, P. F. (2017). Angiosperm Phylogeny Website (version 14). Retrieved Feb. 2018, http://www.mobot.org/MOBOT/research/APweb/.

Volkova, E.V. (1977). *Lycopus* L. In: Komarov VL (ed). Flora of the USSR. Vol. XVI Moscow: Bishen Singh Mahendra Pal Singh and Koeltz Scientific Books (English translation): pp. 423-427.

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Pistacia atlantica Desf. Türünün Marmara, Ege ve Akdeniz Bölgelerinde Yayılış Alanları ve Taksonomik Özelliklerinin Belirlenmesi

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ÖZET
Pistacia L. türleri Türkiye florası içerisinde doğal yayılış göstermektedir ve *P. atlantica* Desf. bunlardan biridir. Bu çalışmada *P. atlantica*'nın Marmara, Ege ve Akdeniz bölgelerindeki yayılış alanları, türün morfolojik karakterlerindeki farklılıklar ve varyasyonlar incelenerek taksonomik durumu ve yayılış alanlarının belirlenmesi amaçlanmıştır. Bu araştırmada 62 *Pistacia* örneği incelenmiş olup incelenen örneklerin 25'inin *P. atlantica* türü olduğu saptanmıştır. Yaprak ölçümlerinden elde edilen veriler dikkate alındığında toplanan 25 örneğin yaprak uzunluklarının 88 mm ile 171 mm arasında olduğu, yaprak genişliklerinin ise 68 mm ile 137 mm arasında değişiklik gösterdiği belirlenmiştir. Örneklere ait yaprakların incelenmesi sonucunda terminal yaprakçık uzunluklarının 2.5 ile 7.9 cm arasında, genişliklerinin ise 1.2 ile 2.5 cm arasında değiştiği, yaprak çifti sayısının ise 2 ile 6 arasında olduğu belirlenmiştir. Bu çalışmada *P. atlantica*'nın Türkiye'de Akdeniz bölgesinde Mersin; Ege bölgesinde İzmir, Aydın ve Manisa; Marmara bölgesinde Balıkesir ilinde doğal olarak yayılış gösterdiği tespit edilmiştir.

Botanik

Araştırma Makalesi

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Anahtar Kelimeler
Pistacia
P. atlantica
Taksonomi
Yayılış alanı

Determination of Distribution Areas and Taxonomic Properties of *Pistacia Atlantica* Desf. in Marmara, Aegean and Mediterranean Regions

ABSTRACT
Pistacia L. species show natural spread in the flora of Turkey and *P. atlantica* Desf. is one of them. This study aims to determine the taxonomic status and spreading areas by examining the differences and variations of the spreading areas of *P. atlantica* in the Marmara, Aegean, and Mediterranean regions and the morphological characters of the species. In this study, 62 *Pistacia* samples were examined. Twenty-five of these samples were found to be *P. atlantica.* When we considered the data obtained from leaf measurements, it was determined that the leaf lengths were between 88-171 mm, and the leaf widths varied between 68-137 mm. As a result of the examination of the leaves of the samples, the terminal leaflet lengths ranged between 2.5-7.9 cm and their width ranged between 1.2-2.5 cm and the number of leaf pairs was between 2-6. In this study, it was determined that P. atlantica naturally spreads in Mersin, İzmir, Aydın, Manisa, and Balıkesir provinces in Turkey

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GİRİŞ

Atlas Sakızı olarak bilinen *Pistacia atlantica* Desf. ülkemizde Akdeniz, Ege, Marmara, Karadeniz ve İç Anadolu Bölgelerinde taşlık ve kayalık habitatlarda yayılış gösteren ve *P. eurycarpa* Yalt.'a taksonomik bakımdan çok yakın olan Anacardiaceae familyasına ait bir türdür (Kafkas ve ark., 2001; Kafkas ve ark., 2002; Al-Saghir & Porter, 2012; Amara ve ark., 2020;

Oğuz & Oğuz, 2022). *P. atlantica*, kserofit bir tür olup dünyada yaygın olarak, Yunanistan, Ege Adaları, Türkiye, Kıbrıs, Filistin, Ürdün, Suriye, İran, Irak, Lübnan, Mısır, Tunus, Cezayir, Fas, Filistin, İspanya, Ukrayna, Gürcistan, Azerbaycan, Afganistan ve Pakistan'da yayılış göstermektedir (Zohary, 1996; Al-Saghir & Porter, 2012). Bu türün meyveleri %60 oranında yağ içerdiğinden tohumları yenilmekte, kahve olarak içilmekte, gıda ve ilaç sanayisinde, antepfıstığı anaç ıslahında ve erozyon kontrolünde kullanılmaktadır (Atlı ve ark., 1999; Kafkas & Perl-Tereves, 2001; Atlı ve ark., 2001; Kafkas ve ark., 2002; Barazani & Golan-Goldrish, 2004; Pourreza ve ark., 2008; Amara ve ark., 2020).

Pistacia L. cinsinin kapsamlı ilk sınıflandırılması Zohary (1952) tarafından yapılmıştır. Zohary, bu çalışmasında cinsin tür sayısını 11 olarak belirlemiş ve dört seksiyona ayırmıştır. *Pistacia* cinsinin günümüzde dünyada 13 türü tanımlanmıştır (Engler, 1883; Zohary, 1952; Al-Saghir & Porter, 2012). Al-Saghir ve Porter (2012) yaptığı çalışmada *Pistacia* cinsini 9 tür ve 5 alttür olarak sınıflandırılmıştır. Türkiye florasında *Pistacia* cinsi 6 tür ve 2 alttür ile temsil edilmektedir (Yaltırık, 1967 a; Yaltırık, 1967 b).

Türkiye'de *Pistacia* cinsinin ve *P. atlantica* türünün taksonomisinde karışıklıklar bulunmaktadır (Al-Saghir & Porter, 2012). *P. atlantica*, birçok ülke florasında farklı isimle tanımlanmış olmasının yanı sıra morfolojik karakterler bakımından da farklılıklar bulunmaktadır (Yaltırık, 1967 a; Yaltırık, 1967 b). Türkiye'deki *Pistacia* türlerinin sınıflandırmasına yönelik yapılan çalışmada, Zohary (1952)'nin *P. atlantica* var. *kurdica* olarak tanımladığı bitkiler Yaltırık tarafından *P. eurycarpa* adında yeni bir tür olarak tanımlanmış ve *P. eurycarpa* türünün *P. atlantica* türünden esas olarak yaprak ve meyve özellikleri bakımından farklı olduğunu ileri sürmüştür (Yaltırık,1967 a; Yaltırık,1967 b).

Bu çalışma ile *P. atlantica*'nın Marmara, Ege ve Akdeniz bölgelerindeki yayılış alanları ile türün morfolojik karakterlerindeki farklılıklar ve varyasyonlar incelenerek taksonomik durumu ve yayılış alanlarının belirlenmesi amaçlanmıştır.

MATERYAL ve METOD

Örnekleme Yöntemi

Çalışmanın materyalini Türkiye'nin Marmara, Ege ve Akdeniz bölgelerinde yer alan Mersin, İzmir, Aydın, Denizli, Manisa, Çanakkale ve Balıkesir illerinden 2014 yılında toplanan toplam 62 yabani *Pistacia* örneği oluşturmaktadır. Toplanan örneklerin 58'inin teşhisi yapılmıştır. 4'ü yetersiz numune olduğundan teşhisleri yapılamamıştır.

Laboratuvar analizleri

Toplanan bitki örnekleri herbaryum tekniğine uygun olarak preslenerek kurutulmuş ve herbaryum materyali haline getirilmiştir (Şeçmen ve ark., 2008). Toplanan bitki örnekleri Antepfıstığı Araştırma Enstitüsü Müdürlüğünde muhafaza edilmektedir. Bitki örnekleri Yaltırık (1967 a), Yaltırık (1967 b), Anonim (1998) ve Al-Saghir ve Porter (2012)'den yararlanılarak teşhis edilmiştir. Türlerin ülkemizdeki yayılışının daha kolay izlenebilmesi için Davis (1972)'in önerdiği grid sistemi kullanılmıştır. Bitki isimleri verilirken Türkiye Bitkileri Listesi Damarlı Bitkiler (Güner ve ark., 2012), Uluslararası Bitki İsimleri İndeksi (Anonim, 2022 a), Anonim (2022 b) ve Anonim (2022 c)'ye göre kabul edilenler kullanılmıştır. Çalışmada toplanan bitki örneklerinin yaprak uzunluğu, yaprak genişliği, yaprakçık çifti sayısı, yaprakçık uzunluğu, genişliği, yaprak ucu, yaprak kanat durumu ve meyve ölçüleri cetvel ve kumpas ile ölçülmüştür.

BULGULAR ve TARTIŞMA

Pistacia atlantica nemli, yarı kurak veya kurak bölgelerde 100-2000 m rakımlar arasında yetişebilen yaprak döken bir ağaç türüdür. İran-Turan fitocoğrafik bölge elemanı olmasına rağmen çoğunlukla Akdeniz bölgesinde yayılış göstermektedir (Zohary, 1952). Gri renkte gövde kabuğuna sahip olup 10 m yüksekliğe kadar boylanabilmektedir (Kafkas ve ark., 2001). Geniş küremsi ve dağınık taç şekline sahiptir. Yetişkin ağaçların gövdesinin çapı 1 metreyi geçebilmektedir (Pourreza ve ark., 2008). *Pistacia* türlerinin taksonomisinde yaprak ana damar bağlantıları, yaprakçık büyüklük ve şekli, yaprakçık çifti sayısı, terminal yaprakçığının olması veya olmaması, yaprakçık ucu şekli, meyve büyüklüğü ve şekli gibi morfolojik karakterler kullanılmaktadır (Zohary, 1952; Yaltırık, 1967 a; Yaltırık, 1967 b; Zohary, 1987; Kafkas & Perl-Treves, 2001).

Bu çalışmada Akdeniz, Ege ve Marmara bölgelerinde yapılan incelemeler sırasında 58 bitki örneğinden yaprak ve meyve örnekleri alınmış ve tür içindeki benzerlik ve farklılıklar belirlenmiştir. Bu örnekler içerisinde toplam 25 örneğin *P. atlantica* olduğu belirlenmiştir. *P. atlantica* olarak tanımlanan örneklerin toplandığı iller ve belirlenen tür sayıları Çizelge 1'de verilmiştir.

Yaprak ölçümlerinde elde edilen veriler dikkate alındığında toplanan 25 örneğin yaprak uzunluklarının 88 ile 171 mm arasında, yaprak genişliklerinin ise 68 ile 137 mm arasında değişiklik gösterdiği belirlenmiştir (Çizelge 2). Örneklere ait yapraklarda terminal yaprakçık uzunluklarının 2.5 ile 7.9 cm arasında, genişliklerinin ise 1.2 ile 2.5 cm arasında değiştiği, yaprak çifti sayısının ise 2 ile 6 arasında olduğu belirlenmiştir (Çizelge 2).

Çizelge 1. Araştırmada toplanan örnek sayısı ve *P.atlantica*'nın illere göre dağılımı

Table 1. Number of samples collected in the study and the distribution of P. atlantica by provinces

İl	Toplanan Örnek Sayısı	Belirlenen Tür Sayısı
Mersin	16	6
Denizli	5	4
Aydın	14	2
İzmir	11	6
Manisa	6	5
Çanakkale	3	0
Balıkesir	3	2
Toplam	**58**	**25**

Yapılan incelemelerde *P. atlantica* örneklerinin hepsinin yapraklarında terminal yaprakçığın olduğu belirlenmiştir (Şekil 1; Şekil 2). Yapraklardaki terminal yaprakçıkların büyüklüğü 23, 44 ve 51 örneklerde yan yaprakçıklardan küçük, 18, 19, 35 ve 59 nolu örneklerde yan yaprakçıklar kadar, 1 nolu örnekte ise yan yaprakçıklardan büyük olduğu belirlenmiştir. Uç yaprakçığın şekli 1, 18, 23, 35, 51 ve 59 nolu örneklerde (Mersin, İzmir, Aydın, Manisa, Denizli, Manisa) dar eliptik, 19 ve 44 nolu örneklerde (Manisa, İzmir) elips şeklinde olduğu tespit edilmiştir. Örneklerin hepsinin yapraklarının tüysüz, yaprak kenar şeklinin düz olduğu ve yaprak kenarlarında tüyün olmadığı belirlenmiştir. Yaprakların reçine kokusu açısından değerlendirilmesi sonucunda 1 ve 59 nolu örneklerin zayıf, 18, 19, 23 ve 35 nolu örneklerin orta, 44 ve 51 nolu örneklerin ise güçlü reçine kokusuna sahip oldukları belirlenmiştir.

Çizelge 2. *P. atlantica* olarak belirlenen örneklere ait yaprak ölçüm değerleri.

Table 2. Leaf measurement values of the samples determined as P. atlantica

Örneğin Toplandığı İl	Örnek No	Yaprak Uzunluk (mm)	Yaprak Genişlik (mm)	Terminal Yaprakçık Uzunluk (cm)	Terminal Yaprakçık Genişlik (cm)	Bileşik Yaprak Çifti Sayısı (Adet)	Meyve Uzunluğu (mm)	Meyve Genişliği (mm)
Mersin	1	125	79	5.00	1.90	3	6.65	6.09
Mersin	6	108	90	5.20	1.60	3	4.98	4.56
Manisa	9	152	105	4.50	1.50	3	6.90	7.00
İzmir	15	100	82	5.30	1.30	2	6.92	6.66
İzmir	16	105	82	5.00	1.70	3	7.00	6.00
İzmir	18	116	88	6.50	2.50	4	6.26	5.97
Manisa	19	138	116	5.00	2.00	3	6.70	6.06
Aydın	23	88	113	5.00	2.20	4	7.22	6.58
Aydın	27	120	90	5.50	1.20	4	6.90	7.00
Manisa	28	109	95	5.60	1.70	3	6.90	7.00
İzmir	29	112	98	5.40	2.30	3	7.00	6.90
Mersin	30	135	109	5.10	1.80	3	7.60	6.80
İzmir	32	161	119	6.50	1.90	3	7.10	7.00
Manisa	35	124	82	4.00	1.00	4	6.75	6.09
Mersin	38	107	80	4.00	1.60	3	6.,90	6.20
Denizli	39	167	128	7.90	1.90	3	6.33	6.45
Mersin	43	127	93	5.30	1.40	3	7.10	6.20
İzmir	44	152	68	2.50	1.50	4	7.19	6.91
Denizli	45	133	83	5.20	2.50	3	7.10	6.10
Mersin	48	158	137	6.20	2.10	4	7.72	7.34
Denizli	49	145	105	4.70	1.60	3	5.10	6.10
Denizli	51	171	93	4.00	1.50	6	7.07	6.89
Balıkesir	55	99	70	3.90	1.40	3	7.00	6.10
Balıkesir	56	120	88	5.50	2.00	3	6.00	6.10
Manisa	59	104	74	4.00	1.50	4	7.44	5.94

Meyve ölçümlerinde elde edilen veriler dikkate alındığında toplanan örneklerin meyve uzunluklarının 6.26 ile 7.60 mm arasında olduğu ve meyve genişliğinin ise 5.94 ile 7.00 mm arasında değişiklik gösterdiği belirlenmiştir (Çizelge 2). Örneklere ait meyvelerde yapılan incelemede bütün örneklere ait meyvelerin dış kabuğunun kıvamlılığının sulu olduğu, dış kabuk pürüzlülüğünün ise ağsı şeklinde olduğu belirlenmiştir. Örneklere ait meyve şeklinin dört örnekte küremsi, beş örnekte yumurtamsı, bir örnekte ise yumurtamsı-küremsi olduğu tespit edilmiştir (Şekil 3). Başlangıçta sarı-pembe olan meyvelerin olgunlaşınca petrol yeşili ve koyu yeşil renge döndüğü belirlenmiştir. *P. atlantica* türünün yaygın olarak gelişim gösterdiği bölgeler içerinde ağaç gelişimi açısından bireylerin orta ve kuvvetli gelişim gösterdiği belirlenmiştir (Şekil 4; Şekil 5).

Al-Saghir ve Porter (2012) yaptıkları çalışmada *P. atlantica*'nın yaprak ölçülerini 8-17.6x5.2-14 cm, Bilgin ve ark. (2020) 10.8-17.6x7.0-12.6 cm, Kafkas ve ark. (2002) 13.2x9.1 cm, El Zerey-Belaskri & Benhassaini (2016) 1.4-24.5x1.6x21.9 cm olarak

vermiştir. Yaltırık (1967 a) yaprak ölçülerini vermemiştir. Bu çalışmada ise yaprak ölçüleri 8.8-17.1x6.8-13.7 cm olarak ölçülmüştür.

Şekil 1. *P. atlantica* yaprağı
Figure1. P. atlantica leaf

Şekil 2. *P. atlantica'nın* yaprak ve meyvesi
Figure 2. Leaf and fruits of P. atlantica

Şekil 3. *P. atlan*tica meyvesi
Figure 3. P. atlantica fruit

Şekil 4. *Pistacia atlantica* (Çandarlı, Dikili, İzmir)
Figure 4. Pistacia atlantica (Çandarlı, Dikili, İzmir)

Şekil 5. *Pistacia atlantica* (Haytabey Köyü, Denizli)
Figure 5. Pistacia atlantica (Haytabey village, Denizli)

Al-Saghir ve Porter (2012) yaptıkları çalışmada terminal yaprakçık uzunluğu ve genişliğini 2.7-7x0.5-2 cm, Bilgin ve ark. (2020) ise 3.6-6.6x1.2-2.4 cm, Yaltırık (1967 a) 2.5-8x0.8-2.2 cm, Kafkas ve ark. (2002) 4.8x1.8 cm olarak belirtmiştir. Bu çalışmada ise terminal yaprakçığın uzunluk ve genişliği (2.5-)4-6(-8)x1.2-2(-2.5) cm olarak belirlenmiştir.

Yaprakçık çifti sayısını Yaltırık (1967 a) 2-5 çift, Al-Saghir ve Porter (2012) 3-5 çift, Kafkas ve ark. (2002) 2-5 çift, El Zerey-Belaskri & Benhassaini (2016) 2-8 çift, Bilgin ve ark. (2020) ise 2-5 çift olarak vermiştir. Bu çalışmada ise 2-5(-6) olarak tespit edilmiştir. Meyve ölçülerine bakıldığında Yaltırık (1967 a) meyve ölçümlerini 5-8x5-6 mm, Kafkas ve ark. (2002) 5.2-7.9x5.6-7.5 mm, Bilgin ve ark. (2020) 5.2x7.9x5.6-7.5 mm olarak belirtmiştir. Al-Saghir ve Porter (2012) ise meyve ölçümlerini vermemiştir. Bu çalışmada elde

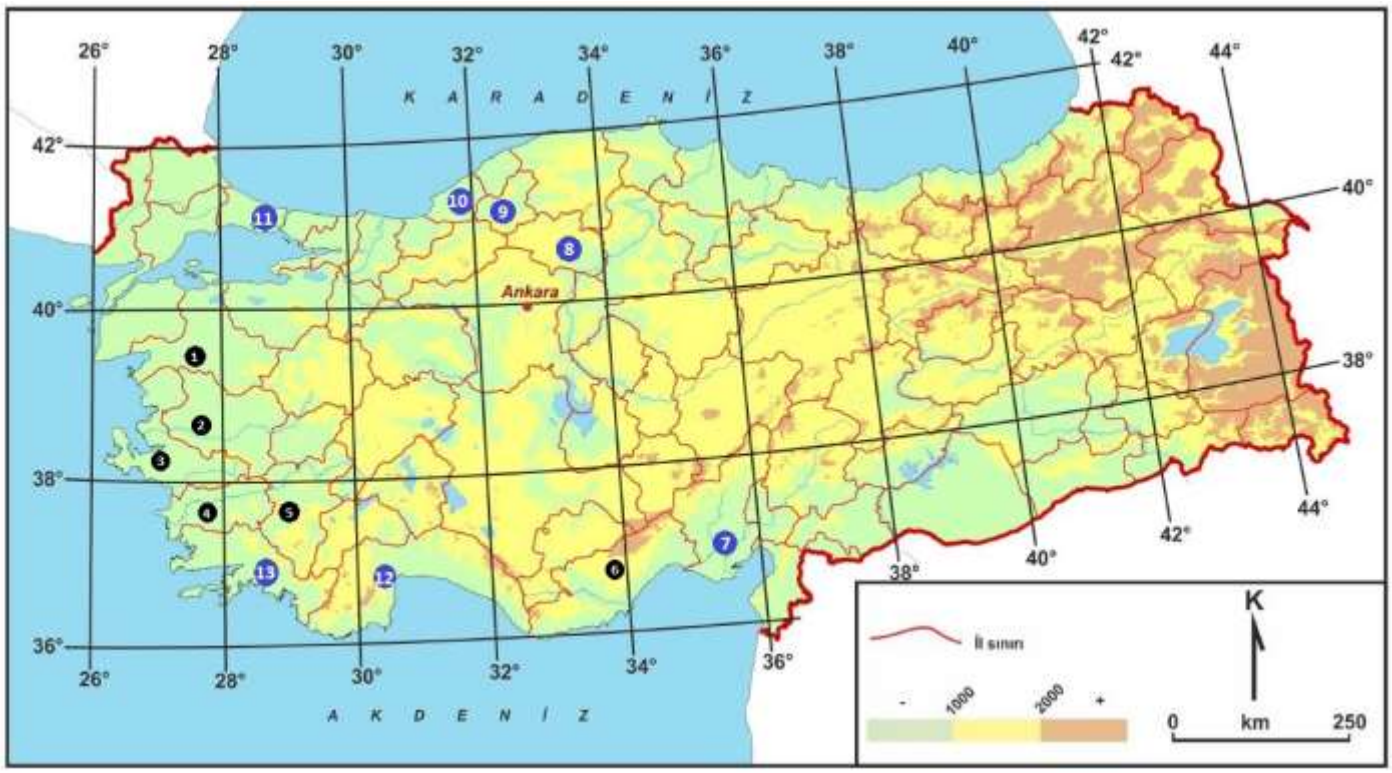
edilen meyve uzunluk ve genişliğine ilişkin değerler ise (5-)6-7(-7.6) x (5-)6-7 mm olarak belirlenmiştir.

P. atlantica'nın taksonomisinde kullanılan yaprak, yaprakçık ve meyvelerine ait morfolojik karakter ölçüleri Yaltırık (1967 a), Kafkas ve ark. (2002), Al-Saghir ve Porter (2012) ve Bilgin ve ark. (2020)'nin çalışmaları ile karşılaştırıldığında bu dört çalışmanın değerleri birbirinden faklı olduğundan türün yeni bir betiminin yapılması gerektiği sonucuna varılmıştır.

Pistacia türleri arasında genetik bariyer bulunmadığından cinsin taksonomik akrabalık ilişkileri oldukça tartışmalıdır (Al-Saghir & Porter, 2012). Dünya'da geniş bir alana yayılmış olan *P. atlantica* ekolojik koşullara uyum sağladığından morfolojik karakterleri çok değişkendir. Bu da *Pistacia* cinsinde olduğu gibi *P. atlantica* türünün taksonomisinde de karışıklığa sebep olmaktadır (Kafkas ve ark., 2001; Kafkas ve ark., 2002; Al-Saghir & Porter, 2012; Amara ve ark., 2020). *P. atlantica,* birçok ülke florasında *Lentiscus atlantica* (Desf.) Kuntze, *Lentiscus mutica* (Fisch. & C.A. Mey.) Kuntze, *Pistacia chia* Desf., *Pistacia atlantica* var. *latifolia* DC., *Pistacia atlantica* subsp. *mutica* (Fisch. & C.A. Mey.) Rech., *Pistacia atlantica* subsp. *cabulica* (Stocks) Rech, *P. mutica* Fisch. & C.A. Mey., *Pistacia mutica* subsp. *cabulica* (Stocks) Engl., *P. cabulica* Stocks, *Pistacia choulettei* Gand., *Pistacia mutica* f. *multijuga* Engl., *Pistacia atlantica* var. *cypricola* H. Lindb., *Pistacia mutica* var. *cypricola* (H. Lindb.) H. Lindb., *Pistacia atlantica* f. *oxycarpa* Zohary olarak isimlendirilmiş ve bu isimler günümüzde sinonim olmuştur (Yaltırık, 1967 a; Yaltırık, 1967 b; Al-Saghir & Porter, 2012).

Atlı ve ark. (2001) ile Tekin ve ark. (2020) Türkiye'de *Pistacia mutica* türünün bulunduğunu belirtmiştir. Ancak, bu tür Türkiye Florasında *P. atlantica*'nın sinonimi olarak belirtilmektedir (Yaltırık, 1967 a; Al-Saghir & Porter, 2012). Bu kayıtlar türün taksonomisinde karışıklık olduğu düşüncemizi desteklemektedir.

P. atlantica'nın Akdeniz, Ege, Marmara ve Karadeniz bölgelerinde yayılış gösterdiği bilinmekte olup, Türkiye Florasında sadece İstanbul, Zonguldak, Karabük, Çankırı, Muğla ve Antalya'dan kayıtlar verilmiştir (Yaltırık, 1967 a). Bu çalışmada *P. atlantica*'nın Türkiye'de Akdeniz bölgesinde Mersin; Ege bölgesinde İzmir, Aydın ve Manisa; Marmara bölgesinde Balıkesir ilinde doğal olarak yayılış gösterdiği tespit edilmiştir (Şekil 6). Çalışmada *P. atlantica* türünün en fazla Ege bölgesinde yayılış gösterdiği, Akdeniz bölgesinde ikinci ve Marmara bölgesinde üçüncü sırada yer aldığı görülmüştür (Çizelge 3).



Şekil 6. P. *atlantica*'nın belirlendiği alanlar (siyah renk: bu çalışmada belirlenen yayılış alanları; mavi renk: daha önceki çalışmalarda belirlenen yayılış alanları) 1-Balıkesir, 2- Manisa, 3- İzmir, 4- Aydın, 5- Denizli, 6- Mersin, 7- Adana, 8- Çankırı, 9- Karabük, 10- Zonguldak, 11- İstanbul, 12- Antalya, 13- Muğla.

Figure 6. Areas where Pistacia atlantica has been identified (black colour: distribution areas determined in this study; blue color: Distribution areas determined in previous studies) 1-Balıkesir, 2- Manisa, 3- İzmir, 4- Aydın, 5- Denizli, 6- Mersin, 7- Adana, 8- Çankırı, 9- Karabük, 10- Zonguldak, 11- İstanbul, 12- Antalya, 13- Muğla.

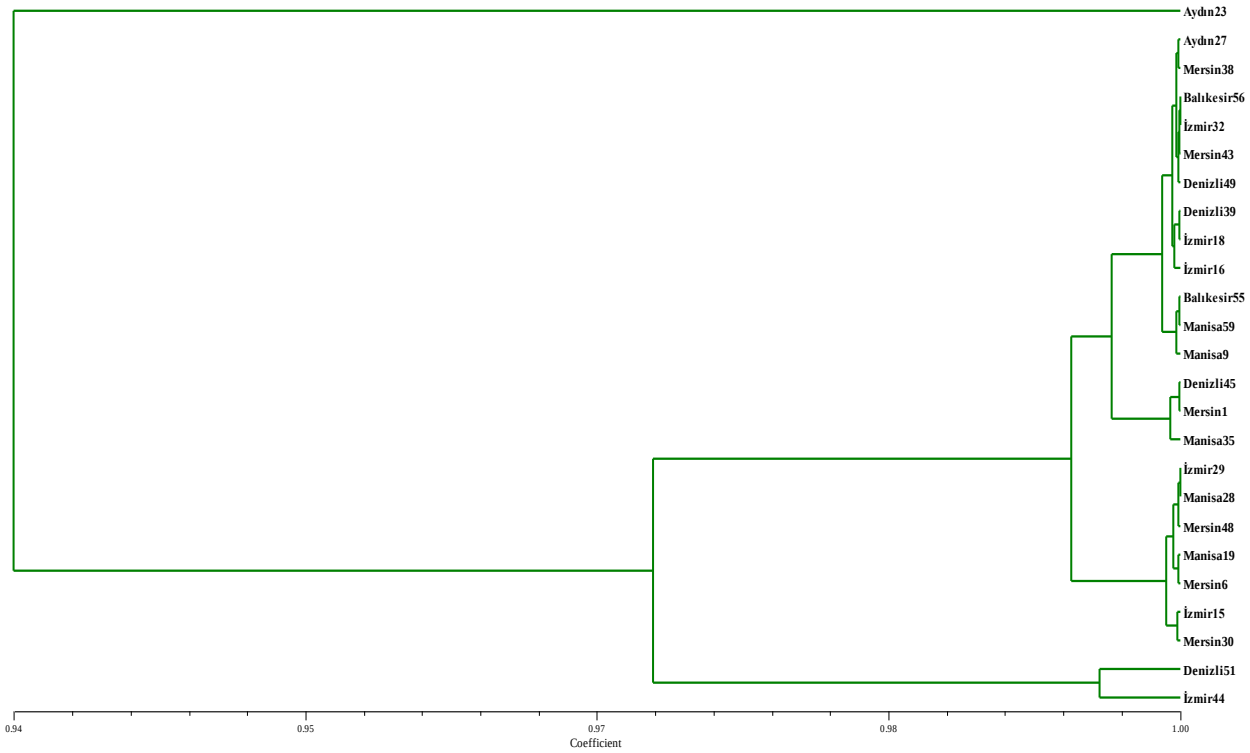
Çizelge 3. *P. atlantica* örnekleri listesi
Table 3. P. atlantica examples list

1	*Pistacia atlantica*, Hamamköy, Mut, Mersin, 142 m., 01.09.2014, K363836,7 D332158,3, A. Yılmaz, K. Sarpkaya, A. Aktan, 1001.
2	*Pistacia atlantica*, Barbanlı Mezarlığı, Mut, Mersin, 233 m., 01.09.2014, K363920 D332434,4, A. Yılmaz, K. Sarpkaya, A. Aktan, 1038.
3	*Pistacia atlantica*, Gezende Barajı, Mut, Mersin, 272 m., 01.09.2014, K36345,6 D331656,8, A. Yılmaz, K. Sarpkaya, A. Aktan, 1043.
4	*Pistacia atlantica*, Barbanlı Mezarlığı, Mut, Mersin, 233 m., 01.09.2014, K363920 D332434,4, A. Yılmaz, K. Sarpkaya, A. Aktan, 1030.
5	*Pistacia atlantica*, Değirmendere, Silifke, Mersin, 263 m., K362538,2 D344235,3, A. Yılmaz, K. Sarpkaya, A. Aktan, 1006.
6	*Pistacia atlantica*, Evkaf Çiftliği, Yeni su yol çatı, Köprü Başı, Mut, Mersin, 267 m., K36289,7 D333757,2, A. Yılmaz, K. Sarpkaya, A. Aktan, 1048.
7	*Pistacia atlantica*, Aliağa Uzunhasanlar Köyü Mezarlığı, İzmir, 42 m., 04.09.2014, K384631,1 D27 454,8, A. Yılmaz, K. Sarpkaya, A. Aktan, 1018.
8	*Pistacia atlantica*, Çandarlı, İzmir, 33 m., 04.09.2014, K385742,3 D265843, A. Yılmaz, K. Sarpkaya, A. Aktan, 1032.
9	*Pistacia atlantica*, Karakuzu Mezarlığı, Aliağa, İzmir, 196 m., K384538,9 D2970, 04.09.2014, A. Yılmaz, K. Sarpkaya, A. Aktan, 1016.
10	*Pistacia atlantica*, Kyrme Yolçatı, Aliağa, İzmir, 8 m., K384529,3 D265633, 04.09.2014, A. Yılmaz, K. Sarpkaya, A. Aktan, 1029.
11	*Pistacia atlantica*, Kyrme Yolçatı, Aliağa, İzmir, 8 m., 04.09.2014, K384529,3 D265633, A. Yılmaz, K. Sarpkaya, A. Aktan, 1044.
12	*Pistacia atlantica*, Çandarlı, Dikili, İzmir, 31 m., 04.09.2014, K385738,8 D2659, A. Yılmaz, K. Sarpkaya, A. Aktan, 1015.
13	*Pistacia atlantica*, Yuntdağı Küçük Belenli, Yunus Emre, Manisa, 517 m., 4.09.2014, K384652,5 D271758,9, A. Yılmaz, K. Sarpkaya, A. Aktan, 1019.
14	*Pistacia atlantica*, Yuntdağı Çamlıca, Muradiye, Manisa, 268, 4.09.2014, K384450,6 D271835,8, A. Yılmaz, K. Sarpkaya, A. Aktan, 1028.
15	*Pistacia atlantica*, Yuntdağı Çamlıca, Muradiye, Manisa, 268 m., 4.09.2014, K384450,6 D271835,8, A. Yılmaz, K. Sarpkaya, A. Aktan, 1009.
16	*Pistacia atlantica*, Yuntdağı Türkmen köyünden sonra Karavelilier Mevki, Yunus Emre, Manisa, 320 m., 4.09.2014, K384438,1 D271237,6, A. Yılmaz, K. Sarpkaya, A. Aktan, 1035.
17	*Pistacia atlantica*, Selendi-Yağcı, Manisa, 16.09.2014, 694 m., K385110,2 D284722,6, A. Yılmaz, K. Sarpkaya, A. Aktan, 1059.
18	*Pistacia atlantica*, Haytabey Köyü, Denizli, 675 m., 02.09.2014, K375958,8 D29653,1, A. Yılmaz, K. Sarpkaya, A. Aktan, 1045.
19	*Pistacia atlantica*, Haytabey Köyü, Denizli, 695 m., 02.09.2014, K3804,2 D29659,1, A. Yılmaz, K. Sarpkaya, A. Aktan, 1049.
20	*Pistacia atlantica*, Haytabey Köyü, Pamukkale, Denizli, 676 m., 02.09.2014, K 37 59 58,8 D 29 6 53,1, A. Yılmaz, K. Sarpkaya, A. Aktan, 1051.
21	*Pistacia atlantica*, Eskihisar Mezarlığı, Denizli, 65 m., 02.09.2014, K375227,6 D2881, A. Yılmaz, K. Sarpkaya, A. Aktan, 1039.
22	*Pistacia atlantica*, Deliktaş Köyü, Balıkesir/Merkez, 403 m., 15.10.2014, K393958,3 D274911,2, A. Yılmaz, K. Sarpkaya, A. Aktan, 1055.
23	*Pistacia atlantica*, Deliktaş Köyü, Balıkesir/Merkez, 399 m., 15.10.2014, K39405,2 D274922,2, A. Yılmaz, K. Sarpkaya, A. Aktan, 1056.
24	*Pistacia atlantica*, 02.09.2014, Sultanhisar, Yağdere Girişi, Aydın, 121 m., K375354 D281237,7, A. Yılmaz, K. Sarpkaya, A. Aktan, 1023.
25	*Pistacia atlantica*, Shisar Eskihisar Mezarlığı, Aydın, 65 m., 03.09.2014, K375227,6 D2881, A. Yılmaz, K. Sarpkaya, A. Aktan, 1027.

Atlı ve ark. (2001) yaptıkları çalışmada *P. atlantica, P. mut*ica, *P. terebinthus, P. palaestina* ve P. *lentiscus*'un Akdeniz ve Güneydoğu Ege bölgesinde yayılış gösterdiğini, Tekin ve ark. (2020) ise yaptıkları çalışmada *P. atlantica, P. mutica, P. terebinthus, P. palaestina, P. vera* and *P. lentiscus*'un Akdeniz ve Ege bölgelerinde, Akdeniz ile İç Anadolu geçiş bölgelerinde yayılış gösterdiğini bildirmiştir. Bilgin ve ark. (2020)

ise *P. atlantica*'nın Ege, İç Anadolu ve Akdeniz ile Ege arasında geçiş bölgelerinde yayılış gösterdiğini belirtmiştir. Bu verilerin bu çalışma sonuçlarıyla örtüştüğü görülmektedir.

Cluster analizi sonucunda elde edilen kdendogram Şekil 7'de verilmiştir. Dendograma göre, incelenen Pistacia atlantica populasyonunun genel anlamda birbirine oldukça benzer bireylerden oluştuğu anlaşılmaktadır. Bununla birlikte Pistacia atlantica genotipleri kendi aralarında iki ana gruba ayrılmaktadır. Birinci grupta Aydın 23 genotipi tek başına yer alırken populasyonun geri kalan bireyleri ikinci grubu meydana getirmişlerdir. İkinci grupta kendi arasında iki alt gruba ayrılırken, Denizli 51 ve İzmir 44 genotipleri diğer genotiplerden nispeten az da olsa farklı olarak bir alt grubu oluşturmuşlardır. Çalışmaya konu olan genotiplerin tamamı da ikinci grubun ikinci alt grubu içinde bir araya gelmişlerdir. İkinci alt grubun üyeleri olan bu genotipler incelenen özellikler ışığında, neredeyse %100 oranında birbirlerinin aynısıdır. Analiz sonucunda belirleenen bu durum taksonomik olarak beklenen bir durumdur. Çünkü *Pistacia atlantica* türü coğrafik olarak geniş bir alanda yayılış göstermektedir. Ayrıca, *Pistacia atlantica* benzer iklim koşullarında bulunmasının doğal sonucudur.



Şekil 7. İncelenen özellikler doğrultusunda oluşturulan benzerlik dendogramı (r=0.83).
Figure 7. Similarity dendogram created in line with the examined features (r=0.83).

SONUÇ ve ÖNERİLER

Bu araştırmada 62 *Pistacia* örneği incelenmiş olup incelenen örneklerin 25'inin *P. atlantica* türü olduğu saptanmıştır. Yaprak ölçümlerinden elde edilen veriler dikkate alındığında toplanan 25 örneğin yaprak uzunluklarının 88 ile 171 mm arasında olduğu, yaprak genişliklerinin 68 ile 137 mm arasında değişiklik gösterdiği belirlenmiştir. Örneklere ait yaprakların incelenmesi sonucunda terminal yaprakçık uzunluklarının 2.5 ile 7.9 cm arasında, genişliklerinin ise 1.2 ile 2.5 cm arasında değiştiği, yaprak çifti sayısının ise 2 ile 6 arasında olduğu belirlenmiştir. *P. atlantica*'nın Akdeniz bölgesinde Mersin; Ege bölgesinde İzmir, Aydın ve Manisa; Marmara bölgesinde Balıkesir ilinde doğal olarak yayılış gösterdiği tespit edilmiştir. *P. atlantica* türünün en fazla Ege bölgesinde yayılış gösterdiği, Akdeniz bölgesinde ikinci ve Marmara bölgesinde üçüncü sırada yer aldığı görülmüştür. İncelenen *P. atlantica* genotiplerine uygulanan cluster analizi neticesinde, gözlemlenen popülasyon her ne kadar birbirine benzerlik gösterse de Aydın 23 genotipi bu genotipler arasında belli oranda farklılık ortaya koymuştur.

Sonuç olarak bu çalışma ile *P. atlantica* türünün ülkemizdeki yeni yayılış alanları belirlenmiştir. *P. atlantica* türünün taksonomisine yönelik yapılan çalışmalarda türün taksonomisinde rol oynayan temel morfolojik karakterlerde farklılık bulunduğu belirlenmiş olup türün yeni bir betiminin yapılması gerektiği sonucuna varılmıştır.

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KAYNAKLAR

Al-Saghir, M.G. & Porter, D.M. (2012). Taxonomic revision of the genus *Pistacia* L. (Anacardiaceae). *American Journal of plant sciences*, *3*, 12-32. http://dx.doi.org/10.4236/ajps.2012.31002.

Amara, M., Bouazza, M., Errouane, K.,Harche, M.K., Nafil, D., Pascalau, R., Smuleac, L. & Al-Saghir, M.G. (2020). Systematic position of *Pistacia atlantica* Desf. subsp. atlantica in north-west Algeria: anatomy and biometry of leaves. *Advanced Research in Life Sciences*, *4*, 48-53. https://doi.org/10.2478/arls-2020-0018.

Anonim, (1998). *Descriptors for pistacia spp. (excluding Pistacia vera L.). Ipgri International Plant Genetic Resources Institute*, Rome.

Anonim, (2022a). Uluslararası Bitki İsimleri İndeksi. https://www.ipni.org. (AlınmaTarihi:01.11.2022).

Anonim, (2022b). Geçerli Bitki İsimleri. https://www. bizimbitkiler.org. (Alınma Tarihi: 02.11.2022)

Anonim, (2022c). The Plant List. https://www. theplantlist.org. (AlınmaTarihi:01.112022).

Atlı, H. S., Arpacı, S., & Ayanoğlu, H. (1999). *Comparison of seedling characteristics of some pistacia species*. In Ak B.E. (ed.), *XI. Grempa seminar on pistachios and almonds*. International Centre for Advanced Mediterranean Agronomic Studies, Zaragoza.

Atlı, H. S., Arpacı, S., Kaşka, N., & Ayanoğlu, H. (2001). *Willd pistacia species in Turkey.* In S. Padulosi, & A. Hadj-Hassan (Eds.), *Towards a comprehensive documentation and use of pistacia genetic diveristy in central & west asia, north africa and mediterranean europe.* International Plant Genetic Resources Institute, Irbid, Jordan.

Barazani O. & Golan-Goldhirsh, A. (2004). Germplasm collection of pistacia at the jacop blaustein research, phenotypic traits and molecular markers. *Information Bulletin of the Research Network on Nuts 12*, 31-32.

Bilgin, Ö.F., Güney, M. & Karcı, H. (2021). Pistacia atlantica'nın morfolojisi ve Türkiyedeki yayılışı (Sözlü bildiri). 4. Uluslararası Mersin Sempozyumu, Mersin, Türkiye, 22-24 Ekim 2020, ss. 365-374.

Davis, P.H. (1972). *Flora of Turkey and Aegean Islands volume 2. Edinburgh University Press*, Edinburgh.

El Zerey-Belaskri, A. & Benhassaini, H. (2016). Morphological leaf variability in natural populations of *Pistacia atlantica* Desf. subsp. *atlantica* along climatic gradient: new features to update *Pistacia atlantica* subsp. atlantica key. *International Journal of Biometeorology*, *60,* 577-589.

Engler, A. (1883). *Pistacia*. (*Monographiae phanerogamarum volume 4*). Paris: Ed. De Condole AC, 284-293.

Güner, A., Aslan, S., Ekim, T., Vural, M., & Babaç, M.T. (2012). *Türkiye bitkileri listesi (damarlı bitkiler).* Nezahat Gökyiğit Botanik Bahçesi ve Flora Araştırmaları Derneği Yayını, İstanbul.

Kafkas, S. & Perl-Treves, R. (2001). Morphological and molecular phylogeny of pistacia species in Turkey. *Theoretical and Applied Genetics 102*, 908-915.

Kafkas, S., Çetiner, M.S. & Perl-Treves, R. (2001). *Molecular variation between and within wild pistacia species in Turkey.* In Ak B.E. (ed.), *XI. Grempa seminar on pistachios and almonds*. International Centre for Advanced Mediterranean Agronomic Studies, Zaragoza.

Kafkas, S., Kafkas, E., & Perl-Treves, R. (2002). Morphological Diversity and a Germplasm Survey of Three Wild Pistacia Species in Turkey. *Genetic Resources and Crop Evolation*, *49*, 261-270.

Oğuz, H.İ. & Oğuz, İ. (2022). *Antepfıstığında kullanılan anaçlar*. Iksad publications, Ankara: Ed. Gündeşli MA, 43-48.

Pourreza, M., Shaw, J.D., & Zangeneh, H. (2008). Sustainability of Wild Pistachio (Pistacia atlantica Desf.) in Zagros Forests, Iran. *Forest Ecology Management, 255*, 3667–3671.

Seçmen, Ö., Gemici, Y., Görk, G., Bekat, L., & Leblebici, E. (2008). *Tohumlu bitkiler sistematiği*. Ege Üniversitesi Fen Fakültesi Kitaplar Serisi No:116, İzmir.

Tekin, H., Karagöz, D., Kılıç, İ.H., Simitçioğlu, B., & Yayla, F. (2020). Morphological-Pomological Characterıstics and Ecologıcal Requırements of Pistachio (*Pistacia vera* L.). *Zeugma Biological Science 1*(2), 15-27.

Yaltırık, F. (1967a). *Pistacia. (Flora of Turkey and the east aegean islands volume 2).* Edinbrugh University Press, Edinburgh: Ed. Davis PH, 544-548.

Yaltırık, F. (1967b). Contributions to the taxonomy of woody plants in Turkey. *Notes from the royal botanic garden*, *28*(1), 11-12.

Zohary, M. (1952). A monographical study of the genus pistacia. *Palestine journal of botany*, *5*(1), 187-228.

Zohary, M. (1987). *Pistacia. (Flora palaestina part 2)*. Israel Academy of Sciences and Humanites, Jerusalem: Ed. Zohary M, 296-300.

Zohary, D. (1996). *The genus pistacia. (Taxonomy, distribution, conservation and uses of pistacia genetic resources).* International Plant Genetic Resources Institute, Palermo, Italy: Eds. Padulosii S, Caruso T, & Barone E, 1-11.

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Farklı Yıllarda Toplanan Benli Şalba (*Salvia pisidica* Boiss. & Heldr. ex Bentham) Türünün Sitotoksik Etkisinin Araştırılması

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ÖZET

Kanser, genomdaki birçok mutasyonun birikimi ile ortaya çıkan sistemik bir hastalıktır. Kanser tedavilerinde kullanılan farklı tedavi yöntemleri nedeniyle zamanla hücrelerde yüksek toksisite ve ilaç direncine neden olması sebebiyle tedavilerdeki başarıyı engellemektedir. Bu nedenle kanser tedavisi için doğal ürünler ve bunların sentetik türevleri büyük bir potansiyel taşımaktadır. Benli Şalba (*Salvia pisidica* Boiss.&Heldr. Ex Bentham) antibakteriyel ve antioksidatif etki başta olmak üzere birçok biyolojik aktiviteye sahiptir. Bu çalışmada farklı yıllarda toplanan bitkileren elde edilen su ekstraktlarının meme kanser hücrelerinde sergilediği sitotoksik etkiler incelenmiştir. Çalışmada 24, 48 ve 72 saatlik inkübasyonlar MCF-7, MDA-MB-231 ve MCF10-A hücre hatlarında yapılmıştır. 2017, 2018 ve 2019 yıllarında toplanan S.pisidica bitkisinin su ekstraktları 1-1000 ng/mL dozlarda hücrelere uygulanmıştır. WST-8 hücre canlılığı belirleme kiti ile sitotoksisite testi inkübasyon süreleri sonunda yapılmıştır. Çalışmalar sonucunda MCF-7 hücrelerinde 2017 yılına ait ekstraktın 48 saatlik inkübasyonda IC50 değeri 1.95 ng/mL, 2019 yılına ait ekstraktın 48 saatlik inkübasyonda IC40 değeri 3.9 ve 1.95 ng/mL olduğu hesaplanmıştır. Daha agresif olan MDA hücrelerinde 2019 yılına ait *S.pisidica* su ekstraktının 48 saatlik inkübasyondaki IC50 değeri ise 7.8 ng/mL'dir. Elde edilen sonuçlara bakıldığında fenolik içeriklerin yıllara bağlı olarak farklılık gösterdiği ve 2017 örneğinde düşük 2019 örneğinde ise yüksek değerlerde olduğu bulunmuştur.

Ekoloji

Araştırma Makalesi

Makale Tarihçesi
Geliş Tarihi : 16.11.2022
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Anahtar Kelimeler
Lamiaceae
Salvia pisidica
Kanser
Sitotoksisite
Ekoloji

Investigation of the Cytotoxic Effect of Benli Şalba (*Salvia pisidica* Boiss. & Heldr. ex Bentham) Species Collected in Different Years

ABSTRACT

Cancer is a systemic disease that occurs with the accumulation of many mutations in the genome. Due to the different treatment methods used in cancer treatments, it prevents the success of the treatments due to the high toxicity and drug resistance in the cells over time. Therefore, natural products and their synthetic derivatives have great potential for cancer treatment. Benli Şalba (*Salvia pisidica* Boiss.&Heldr. ex Bentham) has many biological activities, especially antibacterial and antioxidative effects. In this study, the cytotoxic effects of water extracts of plants collected in different years against breast cancer cells were investigated. In this study, 24, 48 and 72 hour incubations were made in MCF-7, MDA-MB-231, and MCF10-A cell lines. The water extracts of *S.pisidica* plant collected in 2017, 2018 and 2019 were applied to the cells at doses of 1-1000 µg/mL. At the end of the incubation period, cytotoxicity test was performed with the WST-8 cell viability determination kit. In the results of the experiment, it was calculated that the IC50 value of the extract of 2017 was 1.95 ng/mL in 48 hours of incubation, and the IC40 value of the extract of 2019 was 3.9 and 1.95 µg/mL in 48 hours of incubation in MCF-7 cells. In the more aggressive MDA cells, the IC50 value of the 2019 *S.pisidica* water extract in 48 hours of incubation is 7.8 µg/mL. When the

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results obtained were examined, it was found that the phenolic contents differed depending on the year and were low in the samples of 2017 and high in the samples of 2019.

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GİRİŞ

Sağlıklı yaşam bitkilerde bulunan antioksidan etkili maddelerin ve fenolik bileşiklerin etkilerine önem vermektedir. Bitkiler doğal antioksidan bileşiklerin başlıca kaynaklarıdır. Doğal antioksidanların en önemli grupları ise fenolik maddelerdir. Bunlar polifenolik komponentler olup tüm bitki kısımlarında görülürler. Yaygın olan bitkisel fenolik antioksidanlar flavonoidler başta olmak üzere kumarinler, sinnamik asit türevleri, fenolik asitler ve tokoferollerdir (Deveci ve ark., 2016). Araştırmalar fenolik bileşiklerin sağlık üzerinde antidiyabetik, antienflamentuar, antialerjik, antiviral, antimikrobiyal, antitrombotik ve antipatojenik olmak gibi bir çok yararının olduğunu göstermiştir (Atak & Uslu, 2018).

Salvia türleri, halk hekimliğinde yara iyileşmesi, mide, karaciğer ve romatizma ağrılarının hafifletilmesinde ve dünyanın çeşitli yerlerinde soğuk algınlığı tedavisinde infüzyon ve kaynatma şeklinde kullanılmaktadır. Bunlara ek olarak *Salvia*'dan elde edilen uçucu yağlar, gıda tatlandırıcılarında, farmasötiklerde ve parfümeride kullanılmaktadır. *Salvia* türleri esas olarak uçucu yağ ve fenolik asit, flavonoid içeriğinin yüksek olduğu bilinmektedir. Yapılan bir çalışmada toplam fenolik, flavonol içerikleri sırasıyla 54.57mg gallik asit, 16.70 mg kateşin ve 18.19 mg rutin bulunmuştur (Özkan ve ark., 2010). Farmakolojik çalışmalar, Lamiaceae familyasının bitki türlerindeki uçucu yağların antikanser veya antimitojenik/antiproliferatif aktivitelere sahip olduğunu göstermiştir (Lampronti, 2006).

Lamiaceae (Labiatae) familyası 250 cins ve 6500 adet tür içeren çok geniş bir familyadır. Familya çoğu tıbbi ve aromatik özellikte olan adaçayı (*Salvia* sp.), kekik (*Thymus* sp.), nane (*Mentha* sp.), mercanköşk (*Origanum* sp.), biberiye (*Rosmarinus* sp.), lavanta (*Lavandula* sp.), reyhan (*Ocimum* sp.) gibi pek çok bitkiyi kapsamaktadır (Taştan ve ark., 2022). *Salvia* L. (Lamiaceae) cinsinin Türkiye'de yaklaşık 90 türü bulunmaktadır. Bunlardan biri olan *Salvia* türleri temelde uçucu yağ ve fenolik içerik bulundurmaktadır (Özkan ve ark., 2010). Çalışmada kullanılan bitki, Benli Şalba (*Salvia pisidica* Boiss.&Heldr. ex Bentham), Lamiaceae familyasına ait endemik bir bitkidir (Behçet, 2020). Bu tür, menekşe-mavi çiçekleri olan 14-35 cm uzunluğunda çok yıllık bir yarı çalımsı bir bitkidir. Deniz seviyesinden 950 ila 1750 m yükseklikte kuru kireçtaşı yamaçları ve tarla kenarlarını tercih eder. Sadece Burdur, Uşak, Antalya ve Afyon ilinde yetiştiği Anadolu'ya özgüdür ve Antalya'da "ada çayı" adı verilen bir bitki çayı türü olarak yaygın olarak kullanılmaktadır (Hedge, 1982).

Kadınlarda en yaygın görülen ve ölüme neden olan kanserler 2020 dünya kanser istatistiklerine göre sırasıyla; akciğer, serviks ve meme kanserleridir (Ferlay ve ark., 2021). Bunlardan meme kanseri, kadınlarda %15 oranında ölüme neden olmakta ve tanı konulan kanser tiplerinin %30'unu kapsamaktadır (Siegel ve ark., 2019). Bu nedenle meme kanseri 2020 yılında dünyada en çok tanı konulan kanser tipi olarak belirlenmiştir (Ferlay ve ark., 2021).

Kanser tedavilerinde; kemoterapi, radyasyon ve kombinasyon terapileri gibi birçok tedavi stratejisi olmasına rağmen, meydana gelen yan etkiler giderek artmaktadır (Greenwell ve ark., 2015).

Bilinen en eski tedavi şekli bitkisel kaynaklarla tedavidir (Çelik ve ark., 2007). Bitkiler insanlık tarihi boyunca yaşamın vazgeçilmez temel kaynaklarından biridir. Bunun temel nedeni ise insanların bitkileri sadece beslenme için değil çeşitli hastalıkların tedavisi içinde kullanmalarıdır (Deveci ve ark., 2016). İlaçların yan etkilerinin giderek artması ve direnç oluşması da düşünüldüğünde bitkilerden elde edilen doğal ürünler ve bunların sentetik türevleri, kanser tedavileri için umut vaat etmektedir (Zhong ve ark., 2015).

Salvia türlerinden, *S.miltiorrhiza*'da meme kanserine karşı etkili olan ana bileşenler, dihidrotanshinon I (DHT), tanshinone I, tanshinone IIA ve kriptotanshinon dahil olmak üzere yağda çözünür tanshinonlar ve salvianolik asit A, salvianolik asit B, salvianolik asit C ve rosmarinik asit tarafından temsil edilen suda çözünür fenolik asitlerdir. S.miltiorrhiza'nın etkili bileşenlerinden birisi olan dihidrotanshinon-I, antikanser, antiinflamatuar, kardiyoprotektif ve diğer farmakolojik aktiviteleri nedeniyle günümüze kadar kapsamlı olarak incelenmiştir (Chen ve ark., 2019). DHT'nin meme adenokarsinomunda apoptozu ve G1 fazlı hücre döngüsü durmasını indüklediği rapor edilmiştir (Tsai ve ark., 2007). Salvianolik asitler, S. miltiorrhiza'da suda çözünür bileşenlerdir. Esas olarak Sal A, Sal B, Sal C ve RA'yı içermektedir (Wu ve ark., 2020). Sal A, MCF-7 hücrelerinde proliferasyonu ve indüklenen

apoptozu inhibe etmektedir (Cai ve ark., 2014). Sal A, Bcl-2 ve p-Akt ekspresyonunu inhibe etmekte ve PTEN ve Bax ekspresyonunu desteklemektedir. Ayrıca Kaspaz-3, Kaspaz-9 ve PARP bölünmesini indükleyerek MCF-7 hücrelerinde apoptoza yol açmaktadır (Cai ve ark., 2014). Sal B, Caspase-3, Caspase-9 ve Bax ekspresyonunu artırmakta ve MCF-7 hücrelerinde apoptozu teşvik etmek için Bcl-2 ekspresyonunu azaltmaktadır. Sal B, Bcl-xl, Survivin ve p-ERK ekspresyonunu inhibe etmekte ve MCF-7 ve MDA-MB-231 hücrelerinde Caspase-3 ve Caspase-8 aktivasyonunu desteklemektedir (Quan ve ark., 2019).

Kanser çalışmalarında MDAMB-231 hücreleri, MCF7 hücre hattından sonra en yaygın kullanılan hücre hattıdır. Östrojen ekspresyonuyla hormon duyarlılığının yüksek olmasından dolayı MCF7 hücreleri hormon bağımlı çalışmalarda tercih edilen model olarak kabul edilmektedir. Aynı zamanda MCF7 hücreleri meme hücrelerinin luminal epitelyal fenotipinin belirteçlerini protein seviyesinde ifade etmektedir (Levenson & Jordan, 1997). MCF7'nin ifade ettiği belirteçleri MDAMB-231 hücreleri ifade etmez. Yüksek düzeyde vimentin içeren MDAMB-231 hücreleri mezenkimal fenotipin bir belirteci olarak, üçlü negatif meme kanserleri için sıklıkla kullanılan bir modeldir (Mladkova ve ark., 2010). Bu üçlü negatif meme kanserleri beyin ve akciğerler başta olmak üzere diğer organlara metastaz yapabilmektedir. Bundan dolayı, tekrarlama olasılığı diğer meme kanserlerine göre daha yüksektir (Foulkes ve ark., 2010).

Çalışmada farklı yıllarda toplanan *S. pisidica*'nın sitotoksik etkisinin araştırılması amaçlanmıştır.

MATERYAL ve METOD

Bitki Ekstraktlarının Elde Edilmesi

S. pisidica çiçeklenme döneminde Antalya, Korkuteli ilçesinden, yaklaşık 950 m yükseklikten kuru kalker yamaçlardan Mayıs 2017, Mayıs 2018 ve Mayıs 2019 yıllarında toplanmıştır. Toplanan bitki örnekleri oda sıcaklığında, gölgede laboratuvar koşullarında kurutulmuştur. Kurutulan bitki materyali gölgede bir öğütücü ile toz haline getirilmiştir. Ekstraksiyon için 7.5 gr bitki materyali 100 mL su içinde 24 saat aralıklı çalkalanarak ıslatılmıştır. Ekstraksiyon sonunda 1 No'lu Whatman filtre kâğıdı ile süzülmüştür. Süzüntü, 40 ^{o}C'de bir döner buharlaştırıcı ile indirgenmiş basınç altında kuruyana kadar konsantre edilmiştir ve bu işlem her numune için üç kez tekrarlanmıştır ve ardından liyofilize edilmiştir. Kurutulan ekstraktlar kullanılıncaya kadar buzdolabında (4 ^{o}C) saklanılmıştır (Özkan ve ark., 2010).

Fenolik İçerik Analizi (High Performance Liquid Chromatography, HPLC)

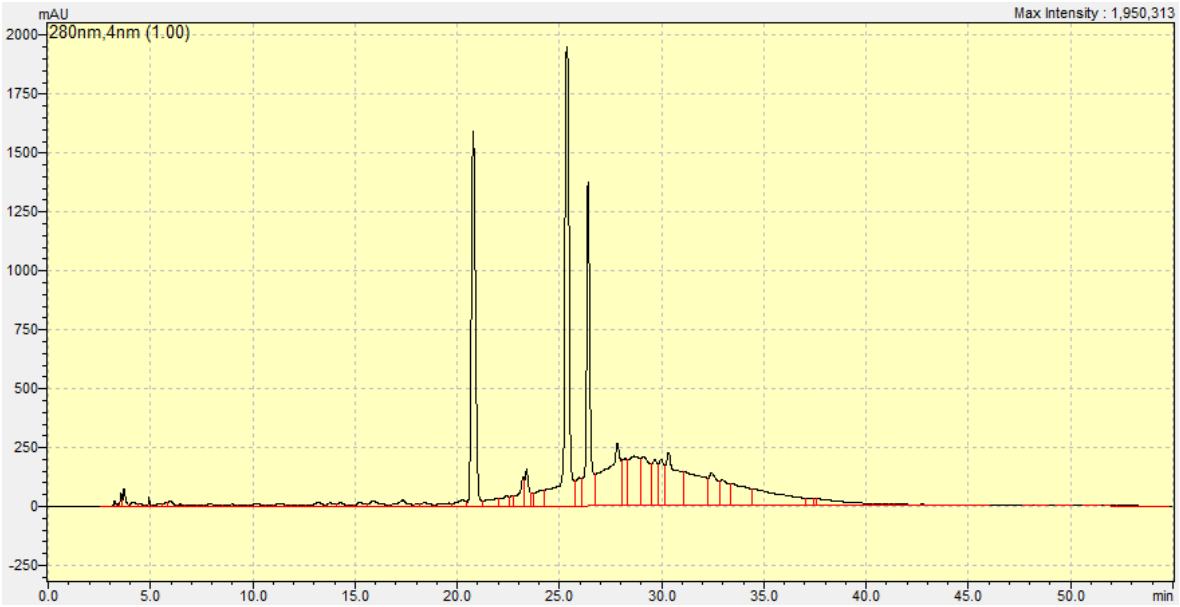
Örneklerin fenolik bileşiminin analizi yüksek performanslı sıvı kromatografisi (HPLC) ile gerçekleştirilmiştir. Kromatografik analiz için örnekler 0.45 µm'lik membran filtreden süzülüp, HPLC (SIL-20A Prominence, Shimadzu, Japonya)'ye 10 µL enjekte edilmiştir. Bileşenlerin ayrımında C18 kolon (LiChroCART® 250-4 250 mm × 4 mm 5 µm Nucleosil® 100) ve DAD dedektör (SPD-M20A Diode Array Detector Shimadzu, Japan) kullanılmıştır (Torun ve ark., 2014). Mobil faz olarak su/asetik asit/metanol (88/2/10, v/v/v) (mobil faz A) ve metanol/asetik asit/su (90/2/8, v/v/v) (mobil faz B) karışımı kullanılmış olup mobil faz akışı 0.9 mL/dk olarak ayarlanmıştır. Mobil faz akış programı başlangıçta A:B 100:0 olup 15. dakikada A:B 85:15, 25. dakikada A:B 50:50, 35. dakikada A:B 30:70, 50. dakikada A:B 25:75 ve 55. dakikada A:B 100:0 olarak uygulanmıştır. Bileşenler maksimum absorbansı verdiği dalga boyunda [vanilik asit (260nm), gallik asit, (+)-kateşin, (-)-epikateşin) (280 nm), ferulik asit, kafeik asit, klorojenik asit ve p-kumarik asit (320 nm)] ölçülmüştür (Şekil 1). Örneklerin fenolik madde bileşenlerinin belirlenmesinde standartlar örneklerin yürütüldüğü koşullarda dört farklı konsantrasyonda tutulma zamanları göz önüne alınarak analiz edilmiş ve standartlardan hazırlanan kalibrasyon eğrileri ile hesaplamalar yapılmıştır.

Hücreler ve Kültür Koşulları

MDAMB-231 (ATCC® HTB-26), MCF-7 (ATCC® HTB-22™), ve MCF10A (ATTC CRL-10317) hücreleri ATCC'den elde edilmiştir. MDAMB-231 ve MCF-7 hücreleri %10 fetal sığır serumu (FBS), 2 mM L-glutamin, 1 mM sodyum piruvat ve 0.02 mM esansiyel olmayan amino asitlerle desteklenmiş RPMI 1640 ortamında çoğaltılmıştır. MCF10A hücreleri, MEMB (Kat no: CC-3151) besiyerine MEGM singleQuats (Kat no: CC-4136) büyüme faktörleri ilave edilerek çoğaltılmıştır.

Hücre Canlılığı Testi

96 kuyucuklu steril plaklara hücreler 1x10^{4} hücre/kuyucuk olacak şekilde ekilmiştir. Besi yerler 24 saatlik inkübasyon sonunda uzaklaştırılmıştır. Her üç ekstrakt %1 FBS içeren besi yeri içinde, 1 µg/ml olan en yüksek dozdan seri sulandırmalar ile dozlar (1.95-1000 ng/ml) hazırlanmıştır. Kuyucuklara ekstraktlar 200 µl şeklinde eklenmiş ve 37°C'lik etüvde %5 CO_2'li atmosferde 24, 48 ve 72 saat inkübe edilmiştir. Her bir inkübasyon süresinin ardından WST-1 hücre proliferasyon kiti (Roche, Cat. No. 11644807001) kullanılarak ekstraktların sitotoksik etkileri belirlenmiştir (Yeh ve ark., 2018). İnkübasyon süresi sonunda plakların absorbans değerleri 450 nm dalga boyunda mikroplaka okuyucuda (Thermo Scientific Multiskan Go), ölçülerek kaydedilmiştir.



Şekil 1. HPLC'de elde edilen örnek kromatogram (1: Vanilik asit, 2: Gallik asit, 3: Klorojenik asit, 4: Kafeik asit, 5: Ferulik asit, 6: p-kumarik asit, 7: Rutin, 8: (-)-epikateşin)

Figure 1. Example chromatogram obtained in HPLC (1: Vanillic acid, 2: Gallic acid, 3: Chlorogenic acid, 4: Caffeic acid, 5: Ferulic acid, 6: p-coumaric acid, 7: Rutin, 8: (-)-epicatechin)

Tripan Mavisi Testi

Hücre canlılığını doğrudan mikroskopta gözlemleyebilmek için kullanılmıştır. Ayrıca bu test elde edilen sitotoksisite test sonuçlarını desteklemek içinde kullanılmıştır. Hücre canlılığı hücre içerisine Tripan mavisinin alınıp alınmamasına göre değerlendirilmiştir. Membran hasarlı hücrelerin içine boya girdiği için bu hücreler mavi renktedir. Canlı hücrelerin içine boya girişi olmadığı için hücreler şeffaf renklidir.

İstatistiksel Analiz

Sitotoksisite testlerinden elde edilen verileri değerlendirmek için Graph-pad InStat istatistik programında Tek Yönlü Anova ve ardından Dunnet çoklu karşılaştırma testi kullanılmıştır. Elde edilen veriler Sigma Plot 10.0 programı ile Ortalama ± SEM değerleri şeklinde grafik haline getirilmiştir. Ayrıca MDA ve MCF-7 hücrelerinde yıllara göre değişen içeriklerin 1.95-1000 µg/mL dozlardaki etkilerinin Pearson Korelasyon Analizi ve Isı Haritası yapılmıştır.

BULGULAR ve TARTIŞMA

Fenolik İçerik: Çalışmada kullanılan *S.pisidica* ekstraktların fenolik içeriklerinin analizi sonuçları Çizelge 1'de verilmiştir. Sonuçlara bakıldığında fenolik içeriklerin yıllara bağlı olarak farklılık gösterdiği ve 2017 örneğinde düşük 2019 örneğinde ise yüksek değerlerde olduğu bulunmuştur.

Çizelge 1. *S.pisidica* Ekstraktlarında Tespit Edilen Fenolik Bileşikler

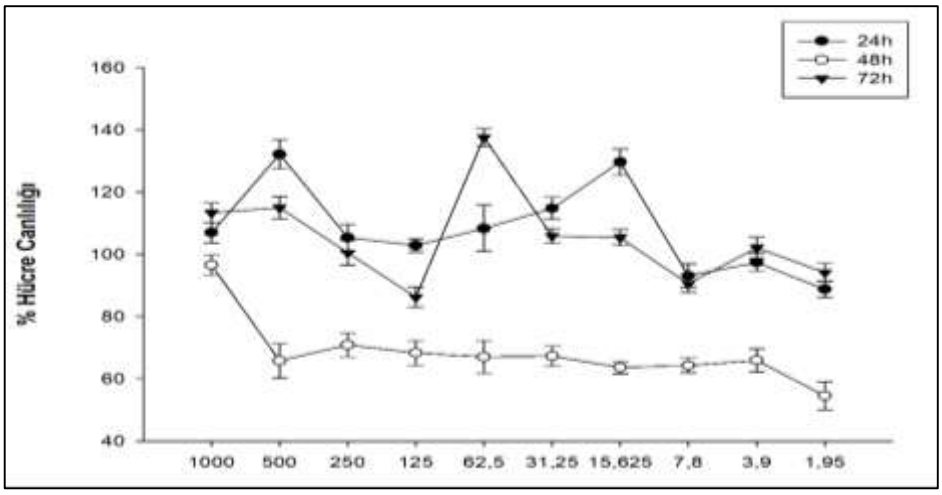
Table 1. Phenolic Compounds Detected in S.pisidica Extracts

Fenolikler (mg/mL) *(Phenolics)*	**2017**	**2018**	**2019**
Vanillic	$0.116^{c}\pm0.00$	$0.148^{b}\pm0.00$	$0.213^{a}\pm0.03$
Gallic	$0.038^{b}\pm0.00$	$0.043^{a}\pm0.00$	$0.031^{c}\pm0.00$
Chlorogenic	$0.655^{c}\pm0.00$	$0.694^{b}\pm0.00$	$1.914^{a}\pm0.04$
Caffeic	$0.008^{c}\pm0.00$	$0.012^{b}\pm0.01$	$0.028^{a}\pm0.01$
Ferulic	$0.128^{b}\pm0.00$	$0.109^{c}\pm0.00$	$0.248^{a}\pm0.01$
p-Coumaric	$2.273^{c}\pm0.02$	$2.378^{b}\pm0.07$	$3.797^{a}\pm0.05$
Rutin	$0.517^{b}\pm0.00$	$0.359^{c}\pm0.02$	$0.594^{a}\pm0.01$
(-)-Epicatechin	$0.505^{c}\pm0.04$	$0.565^{b}\pm0.01$	$0.858^{a}\pm0.02$
(+)-Catechin	Nd	nd	nd
3-hydroxycinnamic acid	Nd	nd	nd
o-Coumaric	Nd	nd	nd

Sonuçlar ortalama ± standart hata, farklı üst simge harfleri olan bir satırdaki değerler önemli ölçüde ($p < 0.05$) farklıdır
nd: not determined (Tespit edilemedi).
Results mean ± standard error, values in a row with different superscripts differ significantly ($p < 0.05$)
nd: not determined.

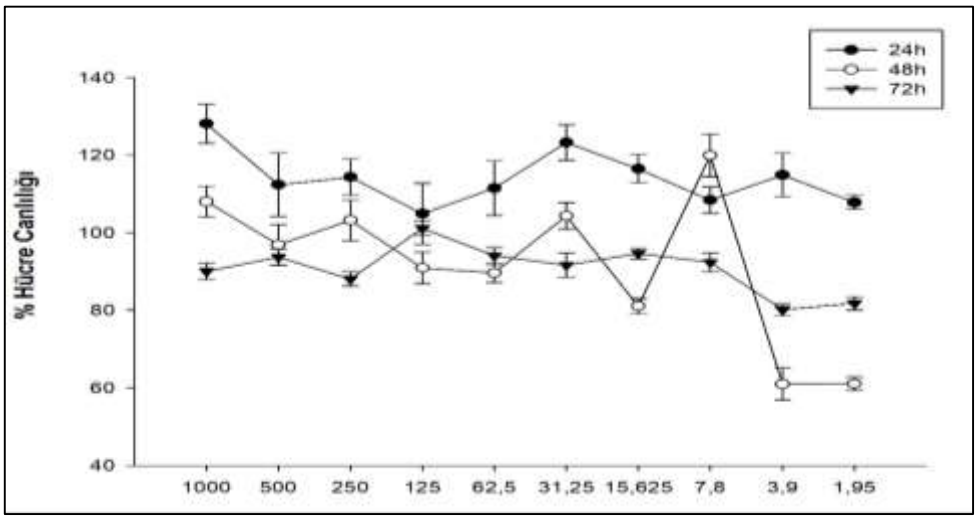
Sitotoksik Etkiler

S.pisidica bitkisinin 2017, 2018 ve 2019 yıllarına ait su ekstraktlarının 24, 48 ve 72 saatlik inkübasyonlarda meme kanseri hücreleri üzerindeki sitotoksik etkileri incelendiğinde, MCF-7 hücrelerinde 2017 yılına ait ekstraktın 48 saatlik inkübasyonda IC50 değeri 1.95 ng/mL olarak belirlenmiştir (Şekil 2). 2019 yılına ait ekstraktın 48 saatlik inkübasyonda IC40 değeri 3,9 ve 1.95 ng/mL olduğu hesaplanmıştır (Şekil 3). Daha agresif olan MDA hücrelerinde 2019 yılına ait *S.pisidica* su ekstraktının 48 saatlik inkübasyondaki IC50 değeri ise 7.8 ng/mL'dir (Şekil 4).



Şekil 2. MCF-7 Hücrelerinde 1.95-1000 µg/mL Aralığındaki Dozlarda 2017 Yılına Ait *S.pisidica* Su Ekstraktı İle Muamelesi Sonucu Kontrole Kıyasla Hücre Canlılığı Yüzdesi.

Figure 2. Percentage of Cell Viability in MCF-7 Cells After Treatment with 2017 S.pisidica Water Extract at Doses in the Range of 1.95-1000 µg/mL Compared to Control.

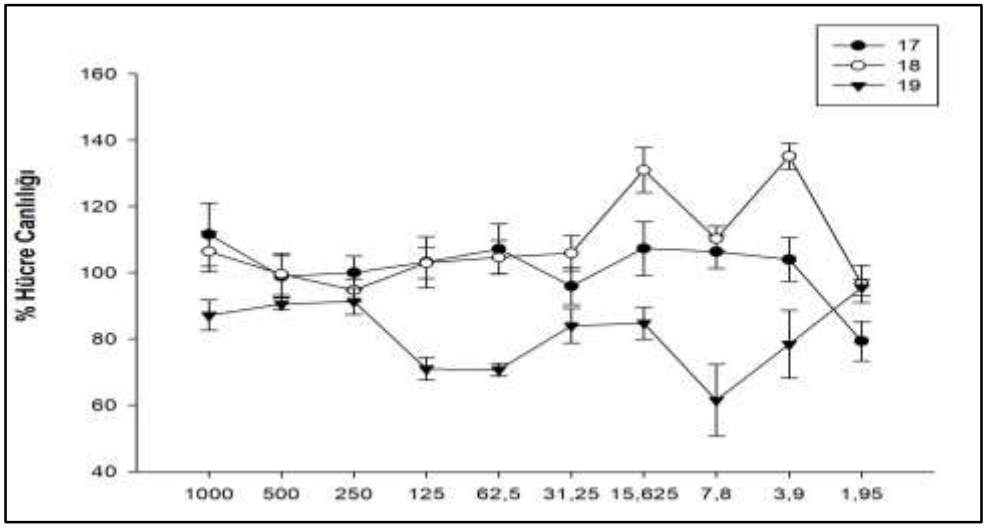


Şekil 3. MCF-7 Hücrelerinde 1.95-1000 µg/mL Aralığındaki Dozlarda 2019 Yılına Ait *S.pisidica* Su Ekstraktı İle Muamelesi Sonucu Kontrole Kıyasla Hücre Canlılığı Yüzdesi.

Figure 3. Percentage of Cell Viability in MCF-7 Cells After Treatment with 2019 S.pisidica Water Extract at Doses in the Range of 1.95-1000 µg/mL Compared to Control.

Çalışmada, fenolik içerikler incelendiğinde yıllara bağlı olarak farklılıklar görülmüştür (Çizelge 1). Fenolik bileşiklerin içeriklerinin farklılık göstermesinin nedenleri arasında toplanma zamanı ve çevresel faktörler etkilidir (Atak & Uslu, 2018). Çalışılan 2017 yılı örneklerinde içerik daha az 2019 örneklerinde içerik daha fazla bulunmuştur. Bunun nedenlerinden biri örneğin bekletilmesi nedeniyle fenolik içeriğinin azalması olabileceği düşünülmektedir. Bir diğer neden olarak bitkinin yetiştiği ortamın iklimsel faktörleri özellikle sıcaklığın etkili olacağı düşünülmüştür. Antalya Devlet Meteoroloji Müdürlüğü'nden alınan Korkuteli'ne ait 2017, 2018 ve 2019 iklimsel verileri (Çizelge 2) incelendiğinde yıllara bağlı olarak aylık sıcaklık ortalamalarında bir farklılık olduğu ve bir artış olduğu görülmektedir. Fialová ve ark. (2015)'in yapmış olduğu çalışmada Lamiaceae familyasına ait *Mentha* L.

Türlerinde yıllara bağlı olarak fenolik içeriklerinin değiştiğini bulmuşlardır. Benzer şekilde Başyiğit & Baydar (2017)'de yapmış olduğu çalışmada Lamiaceae familyasına ait *Salvia officinalis* L. farklı zamanlarındaki hasatlarında fenolik bileşiklerin miktarının değiştiğini rapor etmişlerdir.



Şekil 4. MDA Hücrelerinde 1.95-1000 µg/mL Aralığındaki Dozlarda 2019 Yılına Ait *S.pisidica* Su Ekstraktı İle Muamelesi Sonucu Kontrole Kıyasla Hücre Canlılığı Yüzdesi.

Figure 4. Percentage of Cell Viability in MDA Cells After Treatment with 2019 S.pisidica Water Extract at Doses in the Range of 1.95-1000 µg/mL Compared to Control.

Çizelge 2. Yıllara Bağlı Korkuteli İlçesine Ait Sıcaklık Değerleri (°C)

Table 2. Temperature Values of Korkuteli District in Years (°C)

Yıllar *(Years)*	Aylar *(Monts)*												
	1	2	3	4	5	6	7	8	9	10	11	12	Ortalama *(Average)*
2017	0.2	4	7	10.9	15.7	21.5	25.7	23.5	21.5	13.8	7.6	5.4	13.06
2018	4.4	6.5	9.4	14.8	17.8	20.4	24.9	24	20.8	14.5	9.3	4.3	14.25
2019	2.5	5.2	7.9	10.2	16.7	20.8	23.7	24.6	20.2	16	9.9	5	13.55

S.pisidica ile ilgili yapılmış olan bir çalışmada (Özkan ve ark., 2010) toplam fenolik, flavanol ve flavonol içerikleri, antioksidan aktiviteleri ve antimikrobiyal aktiviteleri incelenmiştir. Çalışmada HPLC ile bir fenolik profili belirlenmemiştir. Çalışmada HPLC ile bir fenolik profili belirlenmiş ve p-Coumaric, chlorogenic ve rutin içeriklerinin *S.pisidica* su ekstraktının içerisinde en yüksek miktarda bulunduğu belirlenmiştir.

Salvia türleri ile yapılan literatürde birçok çalışma yer almaktadır. *S.tebesana* türünün petrol eteri ekstraktı A2780 (yumurtalık), MCF-7 (meme) ve DU145 (prostat) kanseri hücreleri üzerinde sitotoksik etki göstermektedir (Eghbaliferiz 2019). *S.dominica* ve *S.triloba*'dan elde edilen etanol ekstrelerinin T47D ve MCF-7 hücreleri üzerindeki sitotoksik etkileri incelenmiş ve sitotoksik etki sergilediği belirtilmiştir (Abu-Dahab 2014). Bir diğer *Salvia* türü olan *S.suffruticosa* ekstraktının T-47D, MDA-MB-231 ve MCF-7 olmak üzere birçok meme kanseri hücre hatlarına yönelik güçlü inhibitör etki sergilediği göstermektedir (Rustaie, 2018). *S.verticillata*'nın kloroform ve petrol eteri kök ekstrelerinin ise sitotoksik, antioksidan ve antimikrobiyal aktivitelerini analiz edilmiş ve MDAMB-231 hücreleri için IC50 değeri 30.90 ug/ml olarak hesaplanmıştır (Barjaktarevic, 2021).

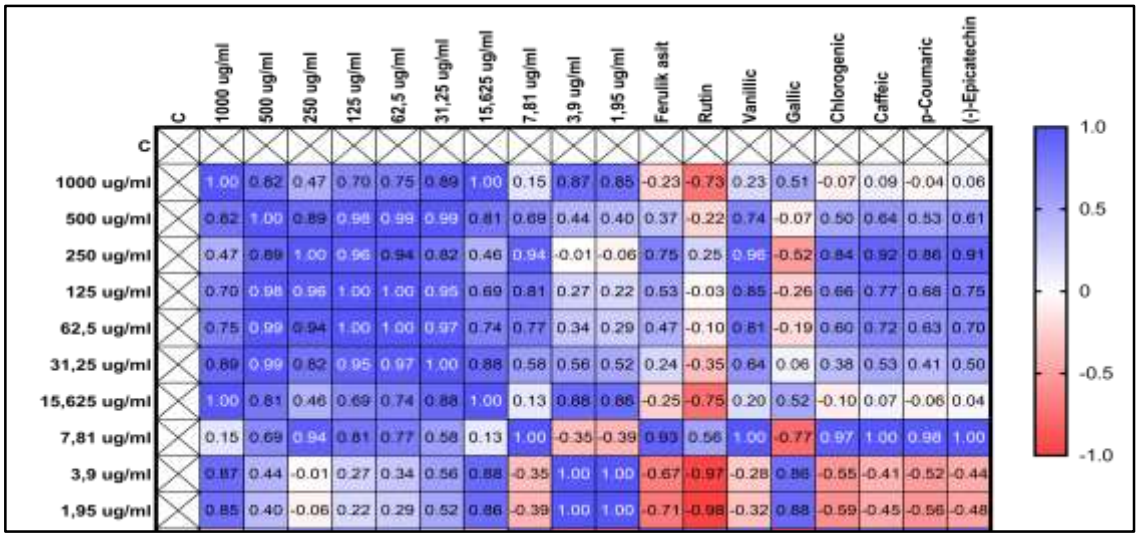
Çalışma kapsamında MCF-7 ve MDA-MB-231 hücreleri ile gerçekleştirilen sitotoksisite denemeleri sonucunda *S.pisidica* su ekstraktının yıllara bağlı olarak farklı sitotoksik etkiler sergilediği belirlenmiştir. MCF-7 hücrelerinde 2017 yılına ait ekstraktın 48 saatlik inkübasyonda IC50 değeri 1.95 µg/mL olarak belirlenmiştir. 2019 yılına ait ekstraktın 48 saatlik inkübasyonda ise IC40 değeri 3.9 ve 1.95 µg/mL olduğu hesaplanmıştır. Daha agresif olan MDA hücrelerinde 2019 yılına ait *S.pisidica* su ekstraktının 48 saatlik inkübasyondaki IC50 değeri ise 7.8 µg/mL'dir.

Rutin, antikarsinojenik, sitoprotektif, antiinflamatuar, antimutajenik etkiler dahil olmak üzere çoklu farmakolojik aktivitelere bir flavonoldür (Saleh, 2019). Yang ve ark. (2017)'de yapmış olduğu çalışmada Rutin, MCF-7 hücreleri için 45.6 µM IC50 değerleriyle, doza bağlı bir şekilde MCF-7 kanser hücrelerinin çoğalmasını inhibe ettiği gösterilmiştir. ElKhazendar ve ark. (2019)'de yapmış olduğu çalışmada ise ferulik asit, 48 saatte sırasıyla 75.4

µg/mL konsantrasyonda MCF-7 hücreleri üzerinde sitotoksik etkiler gösterdiği belirtilmiştir.

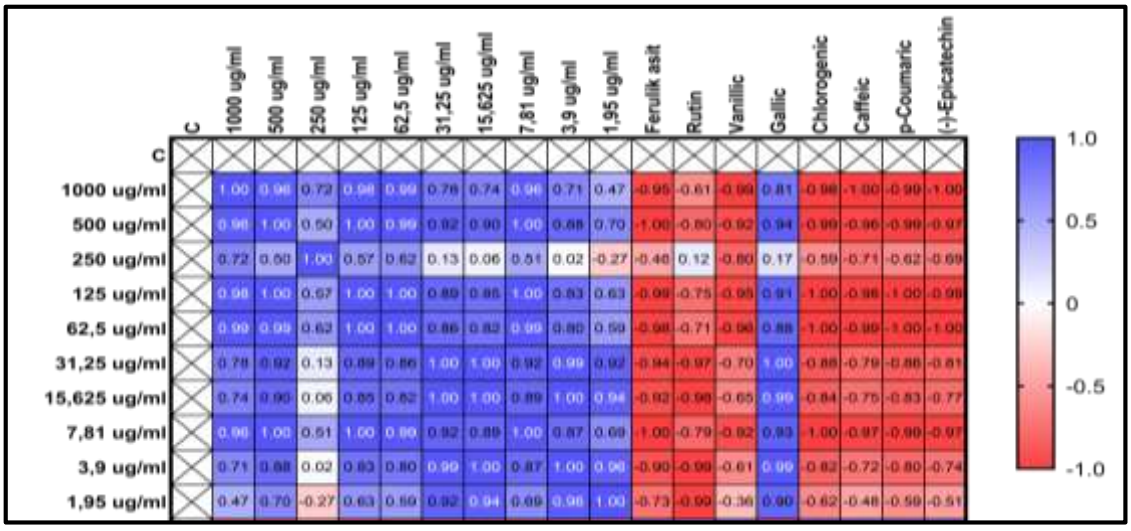
SONUÇ ve ÖNERİLER

Sonuç olarak elde edilen bu veriler ile ekstraktın içerikleri incelendiğinde ferulik asit ve rutin miktarlarındaki değişimlerin sitotoksisitede istatistiksel olarak anlamlı bir farklılık yarattığı görülmektedir. Yıllara göre ferulik asit ve rutin etken maddelerinin miktarları incelendiğinde 2017 yılındaki ekstraktlarda sırasıyla; 0.128 mg/ml ve 0.517 mg/ml; 2018 yılındaki ekstraktlarda 0.109 ve 0.359 mg/ml; 2019 yılındaki ekstraktlarda ise 0.248 ve 0.594 mg/ml olarak belirlenmiştir. Elde edilen sonuçlarda ferulik asit ve rutin miktarındaki değişiklikler ile meme kanserindeki hücre canlılığı arasında negatif yönde güçlü korelasyon olduğu görülmektedir (Şekil 5 ve 6, ferulik asit için r=-0.71; rutin için r=-0.98).



Şekil 5. MCF-7 Hücrelerinde Yıllara göre değişen içeriklerin 1.95-1000 µg/mL Dozlardaki etkilerinin Pearson korelasyon analizi ve ısı haritası.

Figure 5. Pearson correlation analysis and heat map of the effects of year-varying contents in MCF-7 Cells at Doses of 1.95-1000 µg/mL.



Şekil 6. MDA Hücrelerinde Yıllara göre değişen içeriklerin 1.95-1000 µg/mL Dozlardaki etkilerinin Pearson korelasyon analizi ve ısı haritası.

Figure 6. Pearson correlation analysis and heat map of the effects of year-varying contents in MDA Cells at Doses of 1.95-1000 µg/mL.

Tüm sonuçlar değerlendirildiğinde, içeriklerin yıllara bağlı olarak içeriklerinde farklılıklar oluşmakta ve bu farklılıklar sonucu hücre canlılığındaki etkiler de değişmektedir. Tüm içerikler incelendiğinde ferulik asit ve rutin miktarlarının hücre canlılığında azalmaya sebep olabileceği düşünülmektedir.

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.

KAYNAKLAR

Abu-Dahab, R., Abdallah, M.R., Kasabri, V., Mhaidat, N.M.,& Afifi, F.U. (2014). Mechanistic studies of antiproliferative effects of *Salvia triloba* and *Salvia dominica* (Lamiaceae) on breast cancer cell lines (MCF7 and T47D). *Zeitschrift für Naturforschung C, 69*(11-12), 443-451.

Atak, E., & Uslu, M.E. (2018). Fenolik Bileşikler, Ekstraksiyon Metotları ve Analiz Yöntemleri. *MCBÜ Soma Meslek Yüksekokulu Teknik Bilimler Dergisi, 27*(3), 39-48.

Barjaktarevic, A., Ćirović, N., Arsenijevic, N., Volarevic, V., Markovic, B., Mitić, V., Jovanic, V.S., & Cupara, S. (2021). Antioxidant, Antimicrobial and Cytotoxic Activities of *Salvia verticillata* L. Extracts. Indian Journal of *Pharmaceutical Sciences. 83*(6), 1280-1287.

Başyiğit, M., & Baydar, M. (2017). Tıbbi Adaçayı (*Salvia officinalis* L.)'nda Farklı Hasat Zamanlarının Uçucu Yağ ve Fenolik Bileşikler ile Antioksidan Aktivite Üzerine Etkisi. *Süleyman Demirel University Journal of Natural and Applied Sciences. 21*(1), 131-137.

Behçet, L., & Yapar, Y. (2020). Türkiye'den Bazı Kareler İçin Yeni Floristik Kayıtlar. *Bitlis Eren Üniversitesi Fen Bilimleri Dergisi. 9*(4):1482-1495.

Cai, J., Chen, S., Zhang, W., Zheng, X., Hu, S., Pang, C., Lu, J., Xing, J., & Dong, Y. (2014). Salvianolic acid A reverses paclitaxel resistance in human breast cancer MCF-7 cells via targeting the expression of transgelin 2 and attenuating PI3 K/Akt pathway. *Phytomedicine. 21*(12), 1725-1732.

Chen, X., Yu, J., Zhong, B., Lu, J., Lu, J.J., Li, S., & Lu, Y. (2019). Pharmacological activities of dihydrotanshinone I, a natural product from *Salvia miltiorrhiza* Bunge. *Pharmacological Research. 145*, 104254-104254.

Çelik, S., Konkan, R., Erkme, & H. (2007). Tabo A, Erkıran M. Bitkisel ilaçlar ve psikiyatride kullanımı. *Düşünen Adam. 20*(4), 186-195.

Deveci, H.A., Gökhan, N., Kırpık, M.A., Harmankaya, A., & Yıldız, Y. (2016). Fenolik Bileşik İçeren Bitkisel Antioksidanlar. *Kafkas Üniversitesi Fen Bil. Enst. Derg. 9*(1), 26-32.

Eghbaliferiz, S., Soheili, V., Tayarani-Najaran, Z., Asili, J. (2019). Antimicrobial and cytotoxic activity of extracts from *Salvia tebesana* Bunge and *Salvia sclareopsis* Bornm cultivated in Iran. *Physiology and Molecular Biology of Plants. 25*(4), 1083-1089.

ElKhazendar, M., Chalak, J., El-Huneidi, W., Vinod, A., Abdel-Rahman, W.M., & Abu-Gharbieh, E. (2019). Antiproliferative and proapoptotic activities of ferulic acid in breast and liver cancer cell lines. *Tropical Journal of Pharmaceutical Research. 18*(12), 2571-2576.

Ferlay, J., Colombet, M., Soerjomataram, I., Parkin, D.M., Piñeros, M., Znaor, A., & Bray, F. (2021). Cancer statistics for the year 2020: An overview. *International Journal of Cancer. 149*(4), 778–789. https://doi.org/10.1002/IJC.33588

Fialová, S., Tekeľová, D., Rendeková, K., Klinčok, J., Kolárik, M., Kurucová, K., & Grančai, D. (2015). Phenolic compounds variation in *Mentha* L. species in the course of a four-years period Kolísanie fenolových látok v rôznych druhoch *Mentha* L. počas 4 ročného obdobia. *Acta Fac. Pharm. Univ. Comen. LXII*, (Suppl IX), 2-7. ISSN 1338-6786 (online) and ISSN 0301-2298 (print version), DOI: 10.1515/afpuc-2015-0013.

Foulkes, W.D., Smith, I.E., & Reis, J.S. (2010). "Triple-Negative Breast Cancer." *New England Journal of Medicine. 363*(20), 1938-1948.

Greenwell, M., & Rahman, P.K.S.M. (2015). Medicinal Plants: Their Use in Anticancer Treatment. *Int J Pharm Sci Res. 6*(10), 4103–4112. doi: 10.13040/IJPSR.0975-8232.6(10).4103-12.

Hedge, I.C. (1982). *Salvia* P.H. Davis (Ed.), Flora of Turkey and the East Aegean islands, *Edinburgh University Press, Edinburgh. 7*, 400-461

Lampronti, İ.,& Saab, A.M., Gambari, R. (2006). Magnoliophyta Bölümüne Ait Bitkilerden Elde Edilen Uçucu Yağların Antiproliferatif Aktivitesi. *Int. J. Oncol. 29*, 989-995.

Levenson, A.S., & Jordan, V.C. (1997). "MCF-7: the first hormoneresponsive breast cancer cell line." *Cancer Res. 57,* 3071–3078.

Mladkova, J., Sanda, M., Matouskova, E., & Selicharova, I. (2010). "Phenotyping breast cancer cell lines EM-G3, HCC1937, MCF7 and MDA-MB-231 using 2-D electrophoresis and affinity chromatography for glutathione-binding proteins." *BMC Cancer 10*, 449. https://doi.org/10.1186/1471-2407-10-449.

Özkan, G., Sağdıç, O., Göktürk, R.S., Ünal, O., Albayrak, S. (2010). Study on chemical composition and biological activities of essential oil and extract from *Salvia pisidica*. *LWT-Food Scıence And Technology. 43*(1), 186-190.

Quan, G., Chen, W., Wen, Y., Yan, Y., Gu, M., & Pan, Y. (2019). Chemoprotective efficacy of salvianolic acid B via triggering apoptosis in MCF-7 human breast cancer cells. *International Journal of Pharmacology. 15*(1), 110-115.

Rustaie, A., Hadjiakhoondi, A., Akbarzadeh, T., Safavi, M., Samadi, N., & Sabourian, R. (2018). Phytochemical constituents and biological activities of *Salvia suffruticosa*. *Research Journal of Pharmacognosy. 5*(2), 25-32.

Saleh, A., ElFayoumi, H.M., Youns, M., & Barakat, W. (2019). Rutin and orlistat produce antitumor effects

via antioxidant and apoptotic actions. Naunyn-Schmiedeberg's *Archives of Pharmacology. 392*(2), 165-175.

Siegel, R.L., Miller, K.D., & Jemal, A. (2019). Cancer statistics, CA: A Cancer. *Journal for Clinicians. 69*(1), 7-34.

Taştan, P., Birim, D., Fafal, T., Armağan, G., & Kıvçak, B. (2022). Bazı Lamiaceae Bitkilerinin Yağ Asidi Bileşiminin Belirlenmesi. *KSÜ Tarım ve Doğa Derg 25* (6), 1199-1204. https://doi.org/10.18016/ ksu tarimdoga.vi.984818.

Torun, M., Rácz, G., Fogarassy, E., Vatai, G., Dinçer, C., Topuz, A., & Özdemir, O. (2014). Concentration of sage (*Salvia fruticosa* Miller) extract by using integrated membrane process. *Separation and Purification Technology. 132*, 244-251.

Tsai, S.L., Suk, F.M., Wang, C.I., Liu, D.Z., Hou, W.C., Lin, P.J., Hung, L.F., & Liang, Y.C. (2007). Anti-tumor potential of 15, 16-dihydrotanshinone I against breast adenocarcinoma through inducing G1 arrest and apoptosis. *Biochemical Pharmacology. 74*(11), 1575-1586.

Wu, Y., Xu, S., & Tian, X.Y. (2020). The effect of salvianolic acid on vascular protection and possible mechanisms. Oxidative *Medicine and Cellular Longevity*. 5472096. doi: 10.1155/2020/5472096.

Yang, S., Zhang, H., Yang, X., Zhao, H., & Zhu, Y. (2017). Evaluation of antiproliferative activities of rutin on human colon cancer lovo cells and breast cancer MCF-7 cells. *Analytical and Quantitative Cytopathology and Histopathology. 39*(2),99-107.

Yeh, C.N., Wu, R.C., Cheng, C.T., Tsai, C.Y., Chang, Y.R., Yeh, T.S., Wu, T.H., Lee, W.C., Chiang, K.C. (2018). HO-1 Is a Favorable Prognostic Factor for HBV-HCC Patients Who Underwent Hepatectomy. *Cancer Manag Res, 10*, 6049–6059, doi:10.2147/CMAR.S186931.

Zhong, J., Reece, E.A., Yang, P. (2015). Punicalagin exerts protective effect against high glucose-induced cellular stress and neural tube defects. *Biochemical and biophysical research communications. 467*(2), 179-184.

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Investigation of Antimicrobial Activities and 16S rRNA Sequences of Actinomycetes Isolated from Karst Caves in the Eastern Black Sea Region of Türkiye

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ABSTRACT

Considering that most antibiotics originate from actinomycete group bacteria, especially the *Streptomyces* genus, it is predicted that novel actinomycetes isolated from extreme environments such as caves may bring novel antibiotics to the medical world. The study aimed to screen the antimicrobial activity of actinomycetes isolated from the three karst caves in Türkiye and to identify selected isolates with antimicrobial activity by molecular methods. One hundred seventy-nine actinomycetes isolated from Akçakale, Kırklar (Altıntaş), and Köprübaşı Caves in Gümüşhane province in the Eastern Black Sea Region of Türkiye were included in the study. The antimicrobial activity of isolates was investigated using the modified cross-streak agar method against seven Gram-negative bacteria, three Gram-positive bacteria, and one yeast strain. Fifty-three isolates (29.6%) had antimicrobial activity against at least one of the tested microorganisms. The rate of isolates exhibiting antimicrobial activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Enterococcus faecalis*, *Chromobacterium violaceum*, *Klebsiella pneumoniae*, *Salmonella* Typhimurium, *Escherichia coli*, *Acinetobacter haemolyticus*, *Pseudomonas aeruginosa*, *Enterobacter aerogenes*, and *Candida albicans* was 21.2%, 20.0%, 16.8%, 12.8%, 3.4%, 2.8%, 2.2%, 1.1%, 0.6%, 0.6%, and 0.6%, respectively. An actinomycete isolate, TRMS 124, showed antimicrobial activity against ten test microorganisms. The 16S ribosomal RNA (16S rRNA) sequencing was performed for the identification and phylogenetic analysis of 26 isolates randomly selected among actinomycetes that exhibited antimicrobial activity against at least three test microorganisms. As a result, it was determined that 24 isolates showed homology with various *Streptomyces* species and two isolates with *Embleya scabrispora* and *Couchioplanes caeruleus*, respectively. These results showed that karst caves could be good sources for isolating actinomycetes with the potential to produce antimicrobial compounds.

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Doğu Karadeniz Bölgesindeki Karstik Mağaralardan İzole Edilen Aktinomisetlerin Antimikrobiyal Aktivitelerinin ve 16S rRNA Dizilerinin Araştırılması

ÖZET

Günümüzde kullanılan antibiyotiklerin büyük çoğunluğunun başta *Streptomyces* cinsi olmak üzere çeşitli aktinomiset grubu bakterilerden orijin aldığı düşünüldüğünde, mağara gibi ekstrem ortamlardan izole edilecek yeni aktinomisetlerin tıp dünyasına yeni antibiyotikler kazandırabileceği öngörülmektedir. Bu çalışmada, üç farklı karstik mağaradan izole edilen aktinomiset izolatlarının antimikrobiyal aktivitelerinin araştırılması ve etkili izolatların

Mikrobiyoloji

Araştırma Makalesi

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moleküler yöntemlerle tanımlanması amaçlanmıştır. Çalışmaya Türkiye'nin Doğu Karadeniz Bölgesinde bulunan Gümüşhane ilindeki Akçakale, Kırklar (Altıntaş) ve Köprübaşı mağaralarından izole edilen 179 aktinomiset izolatı dahil edilmiştir. İzolatların antimikrobiyal aktiviteleri yedi Gram-negatif, üç Gram-pozitif ve bir maya suşuna karşı çapraz çizgi yöntemi ile araştırılmıştır. Elli üç izolatın (%29,6) test edilen mikroorganizmalardan en az birine karşı antimikrobiyal aktiviteye sahip olduğu bulunmuştur. İzolatların *Staphylococcus aureus*, *Bacillus subtilis*, *Enterococcus faecalis*, *Chromobacterium violaceum*, *Klebsiella pneumoniae*, *Salmonella* Typhimurium, *Escherichia coli*, *Acinetobacter haemolyticus*, *Pseudomonas aeruginosa*, *Enterobacter aerogenes* ve *Candida albicans*'a karşı antimikrobiyal aktivite sergileme oranı sırasıyla %21.2 %20.0, %16.8, %12.8, %3.4, %2.8, %2.2, %1.1, %0.6, %0.6, %0.6 şeklinde bulunmuştur. TRMS 124 olarak adlandırılan bir aktinomiset izolatı, 10 test mikroorganizmasına karşı antimikrobiyal aktivite sergilemiştir. En az üç test mikroorganizmasına karşı antimikrobiyal aktivite sergileyen aktinomisetler arasından randomize olarak seçilen 26 izolatın tanımlanması ve filogenetik analizi için 16S ribozomal RNA (16S rRNA) dizi analizi yapılmıştır. Buna göre 24 izolatın çeşitli *Streptomyces* türleri ile iki izolatın ise sırasıyla *Embleya scabrispora* ve *Couchioplanes caeruleus* ile homoloji gösterdiği tespit edilmiştir. Bu çalışmadan elde edilen bulgular karstik mağaraların antimikrobiyal madde üretme potansiyeline sahip aktinomisetlerin izolasyonu için doğal kaynaklar olabileceğini göstermiştir.

Anahtar Kelimeler
16S ribosomal RNA
Aktinomisetler
Antimikrobiyal aktivite
Mağara
Streptomyces

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INTRODUCTION

Actinomycete is a common name given to members of the order *Actinomycetales* (Prudence et al., 2020). Their members are characterized by having high G+C content of DNA, a Gram-positive cell wall structure, and commonly filamentous morphology (Farda et al., 2022). They are generally found in terrestrial and aquatic environments and play an essential role in maintaining the ecological balance by producing enzymes that decompose organic materials (Devanshi et al., 2021).

Actinomycetes produce a variety of bioactive compounds with antimicrobial activity as a result of their secondary metabolism (Selim et al., 2021). In particular, the number of antibiotics brought to medical use by actinomycetes is considerable. So much so that almost two-thirds of the natural antibiotics introduced to the medical world originated from various actinomycetes, especially of the members of the *Streptomyces* genus (Procópio et al., 2012). Considering that actinomycetes are an antibiotic factory, finding a novel actinomycetes species may mean the discovery of a novel antibiotic. It is estimated that many novel actinomycetes are waiting to be discovered in habitats that have not yet been adequately researched worldwide, such as cave environments (Cheeptham et al., 2013).

Cave environments are aphotic and oligotrophic habitats with high humidity (Kováč, 2018). In such specialized environments, most microorganisms can produce various biomolecules for nutrient competition. Therefore, microorganisms adapted to the cave conditions have the potential to be a source of novel bioactive products, including antimicrobial metabolites (Cheeptham et al., 2013). Actinomycetes are the dominant members of the microbial flora in the cave ecosystem. Therefore, it is highly probable that cave actinomycetes are significant sources for the discovery of novel antibiotics (Rangseekaew & Pathom-Aree, 2019). The best example of this is cervimycin, an aromatic polyketide derivative. Cervimycin was obtained from an actinomycete isolate identified as *Streptomyces tendae*, isolated from the Grotta dei Cervi (Italy) cave (Herold et al., 2005).

Recently, there has been an increase in research on the isolation of actinomycetes from various caves and their potential to produce antimicrobial agents (Belyagoubi et al., 2018; Long et al., 2019; Hamedi et al., 2019;

Syiemiong & Jha, 2019; Jaroszewicz et al., 2021). There are also studies on the isolation and identification of bacteria that have the potential to produce antimicrobial substances from caves in Türkiye. For instance, Yücel & Yamaç (2010) stated that 290 *Streptomyces* spp. isolates were obtained from 19 different karst caves in western Türkiye, and 180 of these isolates were found to exhibit antimicrobial activity against at least one of the tested microorganisms. Yamaç et al. (2011) investigated the potential of these isolates to become novel *Streptomyces* species. For this reason, the biochemical, physiological, nutritional, and morphological characters of the isolates were evaluated, and most of them formed different clusters from the reference *Streptomyces* strains. Consequently, the researchers stated that caves are potential sources for the isolation of novel *Streptomyces* species. Doğruöz-Güngör et al. (2020) investigated the antimicrobial activity of bacteria isolated from Kadıini Cave in Antalya, Türkiye. The researchers reported that the isolates of *Brevibacterium* spp., *Bacillus* spp., and *Pseudomonas* spp. showed antibacterial activity against tested Gram-positive bacteria. In another study, the antimicrobial activity of actinomycetes isolated from various habitats, including cave water, was investigated in Burdur province, Türkiye. As a result, an isolate identified as *Microbiospora* spp. exhibited antimicrobial activity against the tested microorganisms (Bedel, 2020). However, we think that research on the antimicrobial activity of cave microorganisms should increase in Türkiye, which is believed to have more than 40,000 caves and is defined as a "cave paradise country" by many researchers. This study scoped actinomycetes isolated from three different karst caves that have not been opened to tourism and have not been investigated in terms of microflora before, located in the province of Gümüşhane in the Eastern Black Sea Region of Türkiye. The antimicrobial activity of actinomycetes isolates was screened against some laboratory microorganisms, and phylogenetic analyses of selected isolates were determined using the 16S ribosomal RNA (rRNA) sequencing.

MATERIALS and METHODS

Sampling Area and Sample Collection

Between 14-16 October 2016, a total of 71 samples (soil, sand, rock soil, mud, guano, lichen, and water) were collected from the entrance, twilight, and dark zones of Akçakale (40º26'03"N; 39º31'54"E, 1585 m altitude, 290 m length), Kırklar (Altıntaş) (40°18'16.5"N; 39°47'32.09"E, 2160 m altitude, 295 m length), and Köprübaşı Caves (40°31'14.67"N; 39°24'55.96"E, 1050 m altitude, 249 m length) in Gümüşhane province, Türkiye (Figure 1). Of 71 samples, 31 were collected from Akçakale Cave (Figure 2), 32 from Kırklar (Altıntaş) Cave (Figure 3), and eight from Köprübaşı Cave (Figure 4). The samples were taken aseptically and randomly from different regions along the visitable parts of the caves. About 50-100 g samples were transferred into sterile containers using sterile spatulas and forceps (for solid samples) or sterile serological pipettes and injectors (for liquid samples), and transferred to the laboratory in a cooler with ice packs and stored at 4 ºC until processing.

Figure 1. Location of the Köprübaşı Cave (A), Akçakale Cave (B), and Kırklar Cave (C)
Şekil 1. Köprübaşı mağarası (A), Akçakale mağarası (B) ve Kırklar (Altıntaş) Mağarasının (C) konumu

Actinomycetes Isolation

For solid samples, a 5 g sample was weighed under aseptic conditions and mixed in 50 mL sterile phosphate buffer solution (PBS). For liquid samples, about 50 mL sample was centrifuged at room temperature at 10,000 rpm for 5 min. The pellet was then resuspended with 1 mL PBS. The mixtures were then vigorously vortexed at room temperature. Then, serial dilutions of up to 10^{-4} were prepared for each sample, and 100 µL of each dilution was spread on Actinomycetes Isolation agar (AIA, Sigma-Aldrich Co., St. Louis, MO, USA) with supplemented nystatin (40 µg mL^{-1}) as an antifungal agent. The cultures were incubated in the dark at 28 °C for 28 days and examined daily. Subcultures of isolates resembling colony formation of actinomycetes were prepared, and Gram reactions were examined. Actinomycete colonies classically have well-developed radial mycelium, and cells are Gram-positive and filamentous morphology. All actinomycetes with different colony formations

were coded as TRMS [Türkiye-Mağara (cave)-*Streptomyces*] with a number and stored at -80 ºC in nutrient broth, including 20% glycerol.

Screening of Antimicrobial Activity

The antimicrobial activity of actinomycetes isolates was investigated against three Gram-positive bacteria (*Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Bacillus subtilis* ATCC 6633), seven Gram-negative bacteria (*Pseudomonas aeruginosa* ATCC 27853, *Acinetobacter haemolyticus* ATCC 19002, *Chromobacterium violaceum* ATCC 12472, *Escherichia coli* ATCC 25922, *Enterobacter aerogenes* ATCC 13048, *Klebsiella pneumoniae* ATCC 13883, and *Salmonella* Typhimurium ATCC 14028), and one yeast (*Candida albicans* ATCC 10231) strain as representing pathogens. All strains were American Type Culture Collection (ATCC) standard microorganisms and were obtained from the culture collection of the Department of Medical Microbiology, Medicine Faculty, Karadeniz Technical University.

The antimicrobial activity was screened using the modified cross-streak agar method as described previously (Velho-Pereira & Kamat, 2011). This method is commonly used to investigate the antagonism between microorganisms. Briefly, fresh cultures of actinomycetes were inoculated in a straight line on Tryptic Soy agar (TSA, Lab M, Lancashire, UK) plates and incubated at 28 ºC for one week. After the incubation, test microorganisms were adjusted to 0.5 McFarland turbidity standards and inoculated in duplicate perpendicular to the actinomycetes. The plates were incubated at 37 ºC for 24 hours. It was investigated whether the test microorganisms' growth was inhibited on the actinomycetes-facing side.

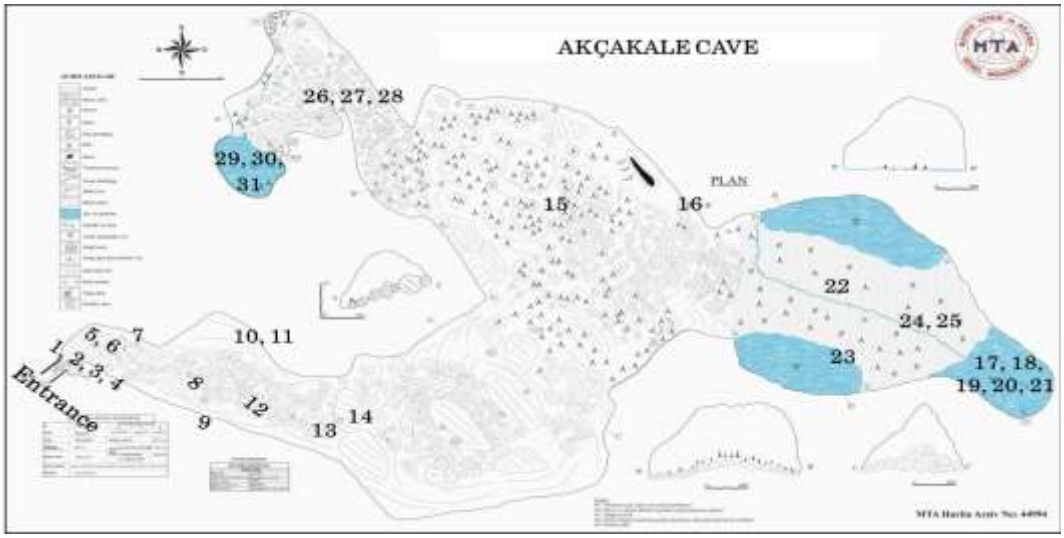

Figure 2. Sampling sites of Akçakale Cave
Şekil 2. Akçakale mağarası örnekleme alanları

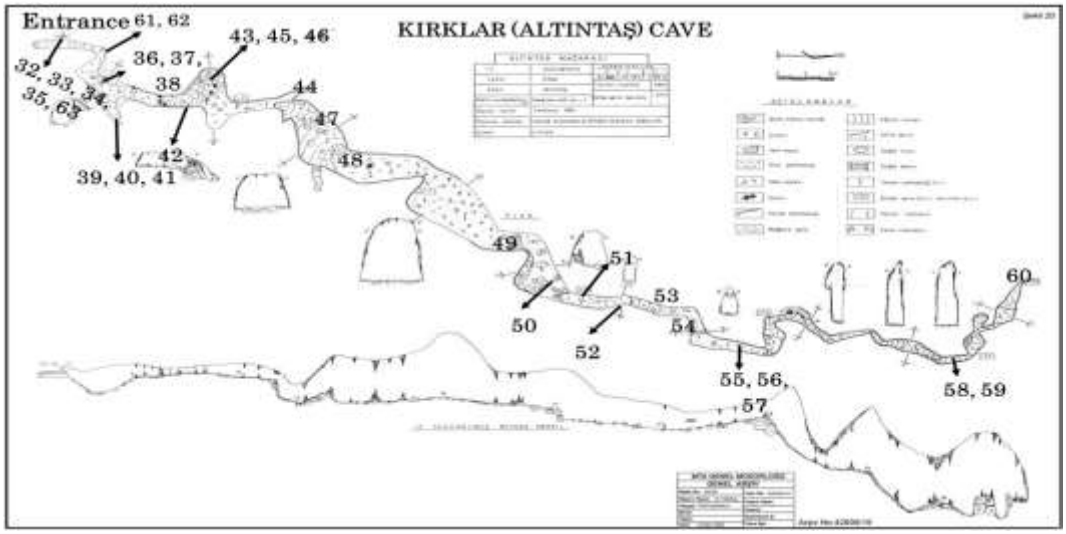

Figure 3. Sampling sites of Kırklar (Altıntaş) Cave
Şekil 3. Kırklar (Altıntaş) mağarası örnekleme alanları

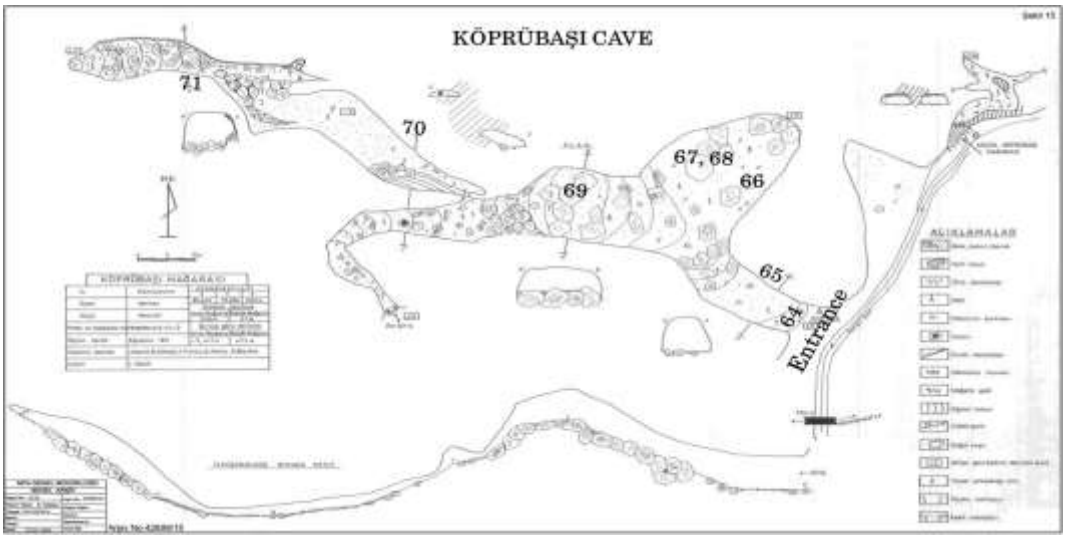

Figure 4. Sampling sites of Köprübaşı Cave
Şekil 4. Köprübaşı mağarası örnekleme alanları

16S rRNA Gene Sequencing and Phylogenetic Analysis

The 16S rRNA sequence analysis of 26 isolates among the actinomycetes exhibiting antimicrobial activity against at least three test microorganisms was performed. Firstly, genomic DNA isolation of the isolates was performed as described by Chen & Kuo (1993). In addition, lysozyme (1 µg mL^{-1}) was added to the lysis buffer of the mentioned protocol and the cells were bead-beating using 1 mm diameter glass beads (Marienfeld, Lauda-Koenigshofen, Germany).

The 16S rRNA gene was amplified by PCR method using the conserved primers as 27F (5'-AGAGTTTGGATCMTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3'). PCR reactions were prepared to contain the following ingredients; 2 µL DNA template, 0.8 µL each primer (10 pmol $µL^{-1}$), 8 µL 5x FIREPol® Master Mix (SolisBioDyne, Tartu, Estonia), and ultra-pure water up to 40 µL. The thermocycler conditions were as follows: initial denaturation at 94 °C for 2 min; 35 cycles of 94 °C for 45 s, 55 °C for 60 s, 72 °C for 60 s; final extension at 72 °C for 10 min (Tufekci et al., 2019). The amplicons (about 1500 bp) were purified before sequencing using the PureLinkTM Quick PCR Purification Kit (Invitrogen, Carlsbad, CA, USA) based on the manufacturer's instructions.

The purified amplicons were sequenced using BigDye terminator chemistry (Applied Biosystems, Foster City, CA, USA) on the ABI 3130 capillary DNA sequencer (Applied Biosystems). The sequencing was performed using primers 27F and 1492R. The results were compared for similarity with sequences in the National Center of Biotechnology Information (NCBI) rRNA/ITS databases using the Basic Alignment Search Tool (BLAST). The partial 16S rRNA sequences were submitted to GenBank with accession numbers OP781986-OP7820011.

A phylogenetic tree was constructed based on the partial 16S rRNA sequences using the Neighbor-Joining (NJ) method (Saitou & Nei, 1987). The evolutionary distances were calculated using the Kimura 2-parameter method (Kimura, 1980). The robustness of the phylogenetic tree was determined with 1.000 bootstrap replicates, using the Molecular Evolutionary Genetic Analysis (MEGA) 11.0 program package (http://www.megasoftware.net) (Tamura et al., 2021). The bootstrap values above 50% were indicated at the nodes of the phylogenetic tree. *Escherichia coli* was used as the outgroup in the phylogenetic tree.

Statistical Analysis

The data were analyzed using the Pearson chi-square test in SPSS 23.0 for Windows (IBM Inc., Armonk, NY, USA) and the statistical significance was taken as $p<0.05$.

RESULTS

A total of 179 actinomycetes were isolated from three caves based on the morphological appearance of the colonies (glabrous or chalky, heaped, folded), and the colors of the aerial and substrate micelles (gray, brown, white, yellow, beige, and orange) (Figure 5). Of them, 31 were from Akçakale Cave, 61 were from Köprübaşı Cave, and 87 were from Kırklar Cave.

The antimicrobial activity of all isolates was screened. Of these, 53 isolates (29.6%) showed antimicrobial activity against at least one of the tested microorganisms. The results are shown in Table 1.

Actinomycetes mainly exhibited antimicrobial activity against Gram-positive bacteria. For example, 21.2% (n=38) of the isolates showed antimicrobial activity against *S. aureus*, 20.0% (n=35) against *B. subtilis*, and 16.8% (n=30) against *E. faecalis*. However, there was no significant difference ($p>0.05$) in susceptibility

among Gram-positive bacteria. On the other hand, 12.8% (n=23) of the isolates had antimicrobial activity against *C. violaceum*, 3.4% (n=6) against *K. pneumoniae*, 2.8% (n=5) against *S.* Typhimurium, 2.2% (n=4) against *E. coli*, 1.1% (n=2) against *A. haemolyticus*, and 0.6% (n=1) against *P. aeruginosa* and *E. aerogenes*. *C. violaceum* was the most susceptible Gram-negative bacteria to actinomycetes ($p<0.05$).

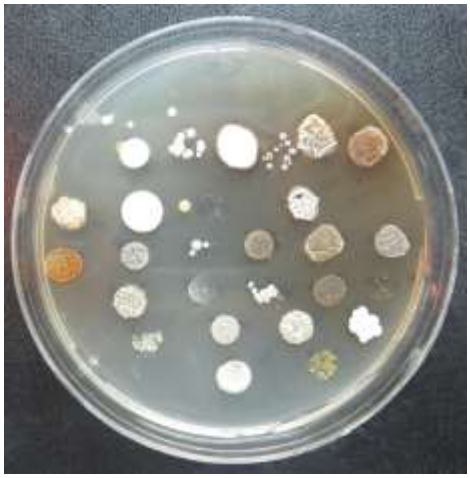

Figure 5. The representative image of subcultured actinomycete isolates

Şekil 5. Alt kültürleri yapılmış aktinomiset izolatlarının temsili görüntüsü

One isolate, designated TRMS 124, exhibited antimicrobial activity against *C. albicans*. TRMS 124 was also the most potent isolate by showing antimicrobial activity against ten microorganisms (*S. aureus*, *E. faecalis*, *B. subtilis*, *E. coli*, *P. aeruginosa*, *E. aerogenes*, *K. pneumoniae*, *S.* Typhimurium, *C. violaceum*, and *C. albicans*). The representative images of the modified cross-streak agar test result are represented in Figure 6.

The 16S rRNA genes (1222 to 1412 bp long) of 26 isolates were sequenced and compared with the sequences deposited in the NCBI rRNA/ITS databases. Twenty-four of the 26 isolates belonged to the *Streptomyces* genus. Moreover, 22 different *Streptomyces* species were identified as closely related species of the isolates. The others were closely related to *Embleya scabrispora* and *Couchioplanes caeruleus* species. The similarity of the isolates to their closest strains was more than 99% (Table 2).

Based on the tree topology, we grouped 24 *Streptomyces* isolates into two main clusters (Figure 7). Cluster I was the largest cluster in the phylogenetic tree and consisted of the TRMS 88, TRMS 117, TRMS 124, TRMS 539, TRMS 543, TRMS 609, TRMS 673, TRMS 713, TRMS 714, TRMS 3120, TRMS 5515, TRMS 5517, TRMS 5814, TRMS 6015, TRMS 6027, TRMS 6124, and TRMS 6127 isolates related to 20 different *Streptomyces* species. Cluster II consisted of seven isolates closely related to *Streptomyces zagrosensis* and *Streptomyces niveus*: TRMS 6025, TRMS 6330, TRMS 6344, TRMS 6712, TRMS 6726, TRMS 6734, and TRMS 6736.

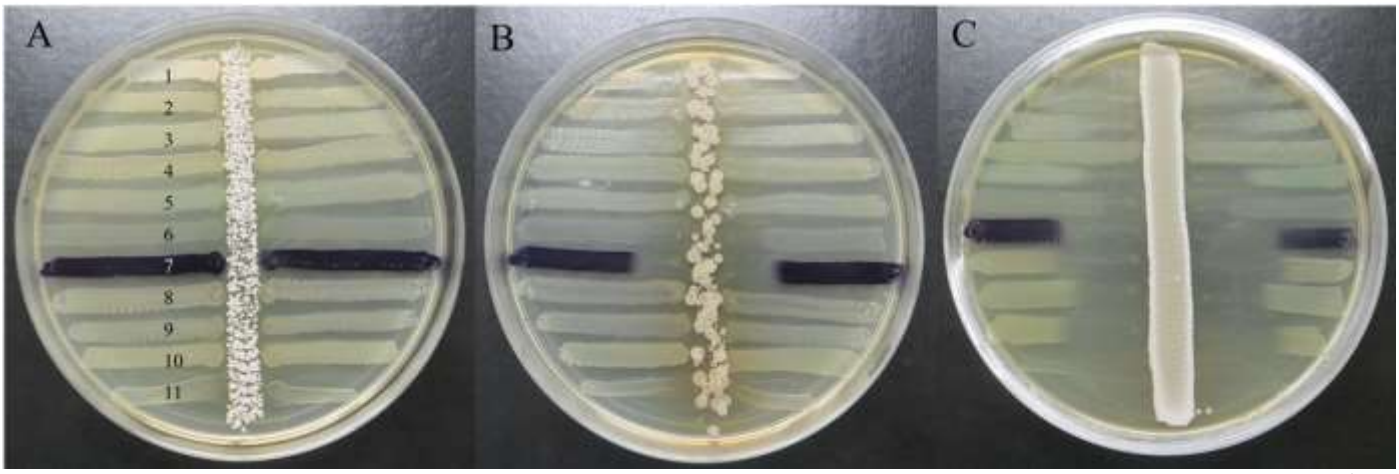

Figure 6. The representative images of the modified cross-streak agar test result. A, TRMS 6615 (no antimicrobial activity); B, TRMS 3120; C, TRMS 124 (1: *S. aureus*, 2: *E. coli*, 3: *B. subtilis*, 4: *A. haemolyticus*, 5: *P. aeruginosa*, 6: *E. faecalis*, 7: *C. violaceum*, 8: *K. pneumoniae*, 9: *S.* Typhimurium, 10: *E. aerogenes*, 11: *C. albicans*)

Şekil 6. Çapraz çizgi yöntemi test sonucunun temsili görüntüleri. A, TRMS 6615 (antimikrobiyal aktivite mevcut değil); B, TRMS 3120; C, TRMS 124 (1: S. aureus, 2: E. coli, 3: B. subtilis, 4: A. haemolyticus, 5: P. aeruginosa, 6: E. faecalis, 7: C. violaceum, 8: K. pneumoniae, 9: S. Typhimurium, 10: E. aerogenes, 11: C. albicans)

Table 1. The antimicrobial activity of the actinomycetes isolates against test microorganisms (+, positive; -, negative)
Çizelge 1. Aktinomiset izolatlarının test mikroorganizmalarına karşı antimikrobiyal aktivitesi (+, pozitif; -, negatif)

Isolate name	Cave	Isolation Source	Test microorganisms										
			S. aureus[1]	*E. faecalis*[1]	*B. subtilis*[1]	*A. haemolyticus*[2]	*E. coli*[2]	*P. aeruginosa*[2]	*E. aerogene*[2]	*K. pneumoniae*[2]	*S.* Typhimurium[2]	*C. violaceum*[2]	*C. albicar*[3]
TRMS 59	Akcakale	Aqueous sample (Sampling site 5)	-	-	-	-	-	-	-	-	-	+	-
TRMS 88*	Akçakale	Rock soil (Sampling site 8)	+	+	+	-	-	-	-	-	-	-	-
TRMS 117*	Akçakale	Rock soil (Sampling site 1)	+	+	+	-	-	-	-	-	-	-	-
TRMS 124*	Akçakale	Rock soil (Sampling site 1)	+	+	+	-	+	+	+	+	+	+	+
TRMS 185	Akçakale	Guano (Sampling site 18)	-	-	-	-	-	-	-	-	-	+	-
TRMS 512	Akçakale	Aqueous sample (Sampling site 5)	-	-	-	-	-	-	-	-	-	+	-
TRMS 515	Akçakale	Aqueous sample (Sampling site 5)	-	-	-	-	-	-	-	-	-	+	-
TRMS 539*	Kırklar	Mud (Sampling site 53)	+	+	+	-	-	-	-	-	-	-	-
TRMS 543*	Kırklar	Mud (Sampling site 54)	+	+	+	-	-	-	-	-	-	-	-
TRMS 553	Kırklar	Soil (Sampling site 55)	+	+	+	-	-	-	-	-	-	-	-
TRMS 609*	Kırklar	Sand (Sampling site 60)	+	+	+	-	-	-	-	-	-	+	-
TRMS 636	Kırklar	Lichen (Sampling site 63)	+	-	-	-	-	-	-	-	-	-	-
TRMS 639	Kırklar	Lichen (Sampling site 63)	+	-	+	-	-	-	-	-	-	-	-
TRMS 673*	Köprübaşı	Soil (Sampling site 67)	+	+	+	-	-	-	-	-	-	+	-
TRMS 713*	Akçakale	Rock soil (Sampling site 7)	+	+	+	+	+	-	-	+	+	+	-
TRMS 714*	Köprübaşı	Soil (Sampling site 71)	+	+	+	+	+	-	-	+	+	+	-
TRMS 814	Akçakale	Rock soil (Sampling site 8)	-	+	-	-	-	-	-	-	-	-	-
TRMS 2224	Akçakale	Rock soil (Sampling site 22)	-	-	-	-	-	-	-	-	-	+	-
TRME 2510*	Akçakale	Rock soil (Sampling site 25)	+	+	+	-	-	-	-	-	-	-	-
TRMS 2514	Akçakale	Rock soil (Sampling site 25)	-	-	-	-	-	-	-	-	-	+	-
TRMS 3120*	Akçakale	Water (Sampling site 31)	+	+	-	-	-	-	-	-	-	+	-
TRMC 3225*	Kırklar	Lichen (Sampling site 32)	+	-	-	-	-	-	-	+	-	+	-
TRMS 4012	Kırklar	Mud (Sampling site 40)	-	-	-	-	-	-	-	-	-	+	-
TRMS 5515*	Kırklar	Soil (Sampling site 55)	+	+	+	-	-	-	-	-	-	-	-
TRMS 5517*	Kırklar	Soil (Sampling site 55)	+	+	+	-	-	-	-	-	-	-	-
TRMS 5611	Kırklar	Soil (Sampling site 56)	+	-	-	-	-	-	-	-	-	-	-
TRMS 5814*	Kırklar	Mud (Sampling site 58)	-	+	+	-	-	-	-	-	-	+	-
TRMS 5818	Kırklar	Mud (Sampling site 58)	+	-	+	-	-	-	-	-	-	-	-
TRMS 6015*	Kırklar	Sand (Sampling site 60)	+	+	+	-	-	-	-	+	-	+	-
TRMS 6025*	Kırklar	Sand (Sampling site 60)	+	+	+	-	-	-	-	-	+	+	-
TRMS 6027*	Kırklar	Sand (Sampling site 60)	+	+	+	-	+	-	-	-	-	+	-
TRMS 6124*	Kırklar	Soil (Sampling site 61)	+	+	+	-	-	-	-	-	-	-	-
TRMS 6127*	Kırklar	Soil (Sampling site 61)	+	+	+	-	-	-	-	-	-	-	-
TRMS 6131	Kırklar	Soil (Sampling site 61)	+	-	-	-	-	-	-	-	-	-	-
TRMS 6133	Kırklar	Soil (Sampling site 61)	+	-	+	-	-	-	-	-	-	-	-
TRMS 6135	Kırklar	Soil (Sampling site 61)	-	-	-	-	-	-	-	-	-	+	-
TRMS 6316	Kırklar	Lichen (Sampling site 63)	-	-	-	-	-	-	-	+	+	+	-
TRMS 6323	Kırklar	Rock soil (Sampling site 63)	+	-	-	-	-	-	-	-	-	-	-
TRMS 6326	Kırklar	Rock soil (Sampling site 63)	+	-	-	-	-	-	-	-	-	-	-
TRMS 6327	Kırklar	Rock soil (Sampling site 63)	-	-	+	-	-	-	-	-	-	+	-
TRMS 6330*	Kırklar	Rock soil (Sampling site 63)	+	+	+	-	-	-	-	-	-	-	-
TRMS 6342	Kırklar	Rock soil (Sampling site 63)	+	-	+	-	-	-	-	-	-	-	-
TRMS 6344*	Kırklar	Rock soil (Sampling site 63)	+	+	+	-	-	-	-	-	-	-	-
TRMS 6712*	Köprübaşı	Soil (Sampling site 67)	+	+	+	-	-	-	-	-	-	-	-
TRMS 6721	Köprübaşı	Soil (Sampling site 67)	-	-	+	-	-	-	-	-	-	-	-
TRMS 6726*	Köprübaşı	Soil (Sampling site 67)	+	+	+	-	-	-	-	-	-	-	-
TRMS 6732	Köprübaşı	Soil (Sampling site 67)	+	+	+	-	-	-	-	-	-	-	-
TRMS 6734*	Köprübaşı	Soil (Sampling site 67)	+	+	+	-	-	-	-	-	-	-	-
TRMS 6735	Köprübaşı	Soil (Sampling site 67)	-	-	+	-	-	-	-	-	-	-	-
TRMS 6736*	Köprübaşı	Soil (Sampling site 67)	+	+	+	-	-	-	-	-	-	-	-
TRMS 6738	Köprübaşı	Soil (Sampling site 67)	+	+	+	-	-	-	-	-	-	-	-
TRMS 6739	Köprübaşı	Soil (Sampling site 67)	+	+	+	-	-	-	-	-	-	+	-
TRMS 6913	Köprübaşı	Soil (Sampling site 69)	-	-	-	-	-	-	-	-	-	+	-

*The 16S rRNA sequence analysis was performed, [1]Gram-positive bacteria, [2]Gram-negative bacteria, [3]Yeast

Table 2. The results of 16S rRNA sequencing analysis
Çizelge 2. 16S rRNA dizi analizinin sonuçları

Isolate		**Closest Strain(s) in Gene Bank**			
Code	**Accession Number**	**Strain**	**Accession Number**	**Identity (%)**	**Query Cover (%)**
TRMS 88	OP781989	*Streptomyces spororaveus* *Streptomyces nojiriensis* *Streptomyces cirratus* *Streptomyces subrutilus* *Streptomyces xanthophaeus* *Streptomyces avidinii* *Streptomyces vinaceus*	NR_112469 NR_112414 NR_112388 NR_112385 NR_112294 NR_041132 NR_041131	99.63	99
TRMS 117	OP781986	*Streptomyces cyaneofuscatus*	NR_115383	100	100
TRMS 124	OP781987	*Streptomyces anulatus* *Streptomyces baarnensis* *Streptomyces praecox* *Streptomyces fimicarius* *Streptomyces caviscabies* *Streptomyces pratensis*	NR_112527 NR_112440 NR_112358 NR_112347 NR_114493 NR_125619	99.93	94
TRMS 539	OP781993	*Streptomyces spororaveus* *Streptomyces nojiriensis* *Streptomyces cirratus* *Streptomyces subrutilus* *Streptomyces xanthophaeus* *Streptomyces avidinii*	NR_112469 NR_112414 NR_112388 NR_112385 NR_112294 NR_041132	99.70	100
TRMS 543	OP781994	*Streptomyces vinaceus* *Streptomyces cirratus*	NR_041131 NR_112388	99.49	100
TRMS 609	OP781998	*Streptomyces microflavus* *Streptomyces alboviridis* *Streptomyces griseus* *Streptomyces erumpens*	NR_103947 NR_112340 NR_112475 NR_112455	99.42	100
TRMS 673	OP782006	*Streptomyces exfoliatus*	NR_041117	99.85	100
TRMS 713	OP781988	*Streptomyces anulatus* *Streptomyces baarnensis* *Streptomyces praecox* *Streptomyces fimicarius* *Streptomyces caviscabies* *Streptomyces pratensis*	NR_112527 NR_112440 NR_112358 NR_112347 NR_114493 NR_125619	99.86	100
TRMS 714	OP782011	*Streptomyces anulatus* *Streptomyces praecox*	NR_112527 NR_112358	100	99
TRME 2510	OP781990	*Embleya scabrispora*	NR_112597	100	100
TRMS 3120	OP781991	*Streptomyces vinaceus* *Streptomyces cirratus*	NR_041131 NR_112388	99.54	100
TRMC 3225	OP781992	*Couchioplanes caeruleus*	NR_037054	99.35	100
TRMS 5515	OP781995	*Streptomyces cirratus* *Streptomyces vinaceus*	NR_112388 NR_041131	99.36	100
TRMS 5517	OP781996	*Streptomyces vinaceus* *Streptomyces cirratus*	NR_041131 NR_112388	99.77	100
TRMS 5814	OP781997	*Streptomyces cyaneofuscatus*	NR_115383	99.85	100
TRMS 6015	OP781999	*Streptomyces microflavus* *Streptomyces alboviridis* *Streptomyces griseus* *Streptomyces erumpens*	NR_103947 NR_112340 NR_112475 NR_112455	99.64	99
TRMS 6025	OP782000	*Streptomyces zagrosensis*	NR_134202	99.85	100
TRMS 6027	OP782001	*Streptomyces lunaelactis*	NR_134822	99.09	100
TRMS 6124	OP782002	*Streptomyces spororaveus* *Streptomyces nojiriensis* *Streptomyces cirratus* *Streptomyces subrutilus*	NR_112469 NR_112414 NR_112388 NR_112385	99.69	100

		Streptomyces xanthophaeus	NR_112294		
		Streptomyces avidinii	NR_041132		
		Streptomyces vinaceus	NR_041131		
		Streptomyces spororaveus	NR_112469		
		Streptomyces nojiriensis	NR_112414		
		Streptomyces cirratus	NR_112388		
TRMS 6127	OP782003	*Streptomyces subrutilus*	NR_112385	99.70	100
		Streptomyces xanthophaeus	NR_112294		
		Streptomyces avidinii	NR_041132		
		Streptomyces vinaceus	NR_041131		
TRMS 6330	OP782004	*Streptomyces niveus*	NR_115784	99.54	100
TRMS 6344	OP782005	*Streptomyces niveus*	NR_115784	99.62	100
TRMS 6712	OP782007	*Streptomyces zagrosensis*	NR_134202	99.76	100
TRMS 6726	OP782008	*Streptomyces zagrosensis*	NR_134202	99.69	100
TRMS 6734	OP782009	*Streptomyces zagrosensis*	NR_134202	99.69	100
TRMS 6736	OP782010	*Streptomyces zagrosensis*	NR_134202	99.92	100

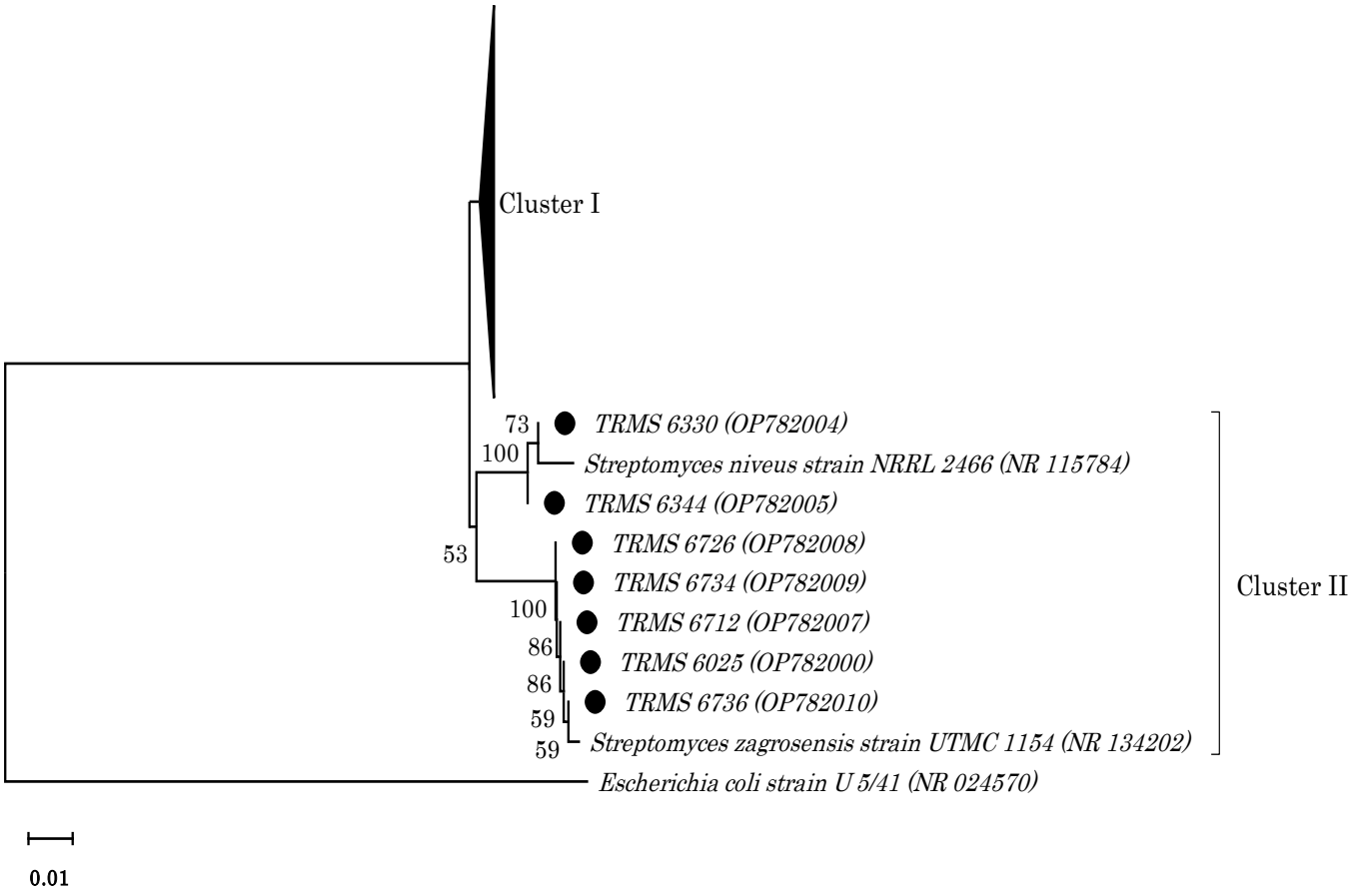

Figure 7. The phylogenetic tree based on the partial 16S rRNA gene sequences of the *Streptomyces* isolates and related species. The phylogenetic tree was constructed using the Neighbor-Joining method with 1.000 bootstrap replicates in the MEGA 11.0 program. The isolates were highlighted with a sircular sign. The accession numbers were demonstrated in parentheses to the right of the isolate name. *Escherichia coli* was used as an outgroup. The scale bar represents 0.01 substitutions per nucleotide position

Şekil 7. Streptomyces türleri ile yakın akrabalık gösteren izolatların ve ilgili türlerin kısmi 16S rRNA gen dizilerine dayanan filogenetik ağaç görüntüsü. Filogenetik ağaç, MEGA 11.0 programında 1.000 bootstrap tekrarı ile Neighbor-Joining yöntemi kullanılarak oluşturulmuştur. İzolatlar siyah daire ile işaretlenmiştir. Erişim numaraları, izolat adının sağında parantez içerisinde gösterilmiştir. Escherichia coli grup dışı kontrol olarak kullanılmıştır. Ölçek çubuğu, nükleotid pozisyonu başına 0.01 baz ikameyi temsil etmektedir

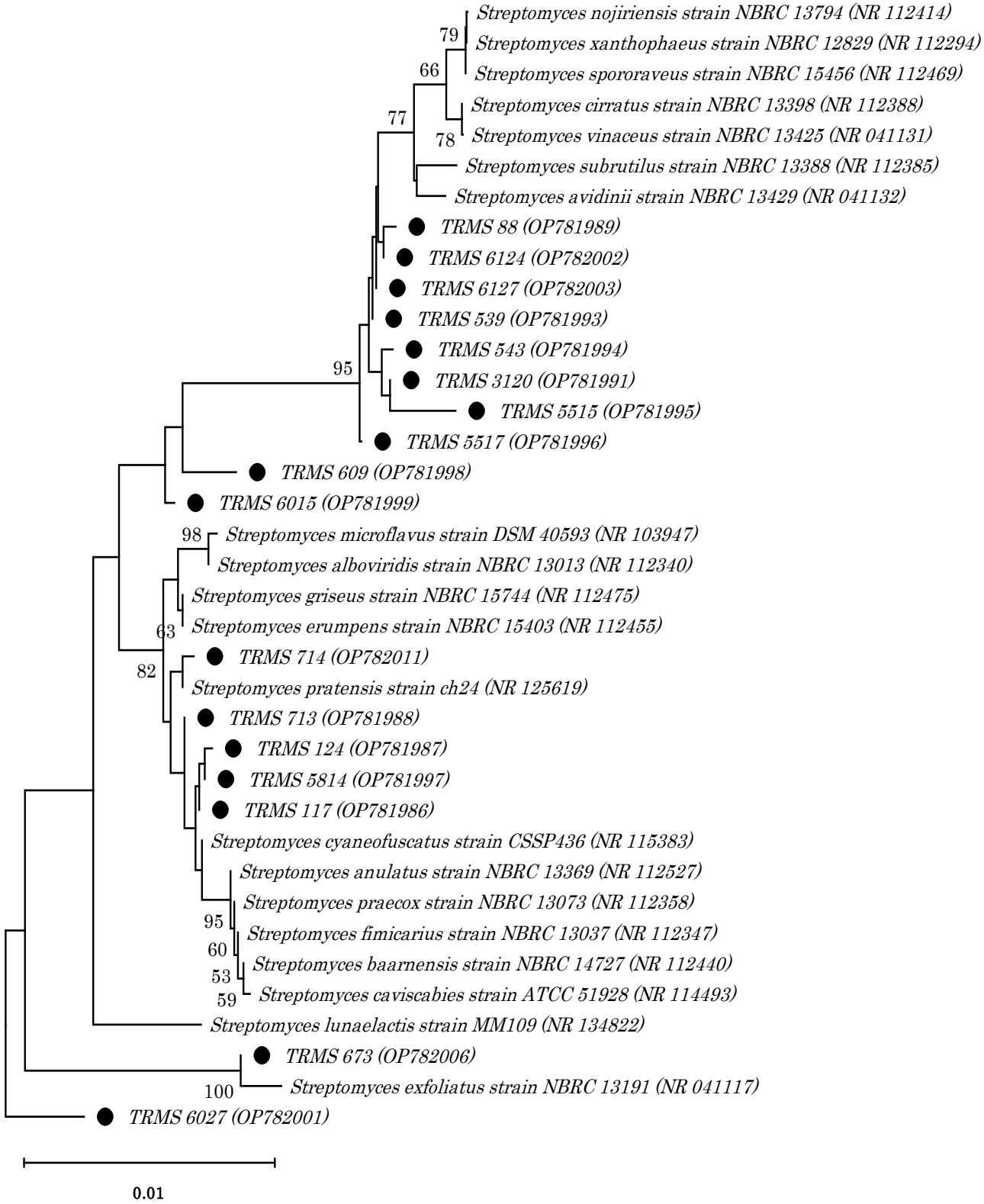

Figure 7 (Continued). Cluster I
Şekil 7 (Devam). Küme I

DISCUSSION and CONCLUSION

The rapid spread of antibiotic resistance among bacteria causes severe problems in the treatment of infectious diseases, leading to economic losses and increased death rates. Therefore, there is a need for the discovery or development of novel antimicrobial agents (Miethke et al., 2021). Considering that two-thirds of the natural antibiotics used today originate from

various actinomycetes members, novel actinomycete strains emerge as a strategic method for finding novel antibiotics (Cheeptham et al., 2013).

Caves have harsh living conditions with a dark and low-nutrient environment. Low nutrient levels can encourage microorganisms to produce various antimicrobial compounds to survive and grow (Hibbing et al., 2010). Therefore, it is predicted that caves may be the natural sources of microorganisms synthesizing effective and new bioactive compounds. Studies on the microbial diversity of caves have reported that the microbial flora of each cave is unique and diverse. Actinomycetes, especially the *Streptomyces* genus, were nevertheless stated as predominant members of the cave flora (Jaroszewicz et al., 2021). In the present study, only the isolation of actinomycetes in the culture of cave samples was focused on, and total aerobic mesophilic microorganism counts were dismissed. As a result, the study continued with 179 actinomycetes isolates thought to have different colony morphology.

There are several studies investigating the antimicrobial activities of actinomycetes isolated from volcanic (Cheeptham et al., 2013) and karst caves (Yücel & Yamaç, 2010; Nimaichand et al., 2015; Maciejewska et al., 2016; Belyagoubi et al., 2018; Hamedi et al., 2019; Syiemiong & Jha, 2019; Jaroszewics et al., 2021; Pradana et al., 2022) in the literature. Furthermore, Yücel & Yamaç (2010), Cheeptham et al. (2013), and Jaroszewics et al. (2021) reported that *Streptomyces* strains isolated from the cave could exhibit antibacterial activity even against resistant strains such as methicillin-resistant *S. aureus*, vancomycin-resistant enterococci, and extended-spectrum beta-lactamases producing *E. coli*. In this study, 53 actinomycetes isolates showed antimicrobial activity against at least one of the tested standard microorganism strains. Besides, the antimicrobial activity of TRMS 124, TRMS 713, and TRMS 714 isolates against at least eight microorganisms suggest that these isolates may be potential sources of broad-spectrum antibiotics. Our findings support that cave actinomycetes can be potential sources of effective antimicrobial agents in line with the literature.

In the current study, the number of isolates exhibiting antimicrobial activity against Gram-positive bacteria was higher. Some reports have also stated that actinomycetes exhibit more antimicrobial activity against Gram-positive bacteria, as in this study (Maciejewska et al., 2016; Belyagoubi et al., 2018). This might be due to the difference in the cell wall structures of Gram-positive and Gram-negative bacteria. Because the cell wall of Gram-negative bacteria contains a more complex structure than Gram-positive bacteria. Lipopolysaccharides (LPS) are the main components found in the outer membrane of Gram-negative bacteria. Moreover, LPS could protect bacterial cells against harmful molecules such as antimicrobial compounds or toxins by providing a structurally effective permeability barrier (Farhana & Khan, 2022). On the other hand, actinomycetes had the most antimicrobial activity against *C. violaceum* among Gram-negative bacteria. Because *C. violaceum* is one of the dominant members of the soil flora and the competition between bacteria, it can be expected that most actinomycetes have an antagonistic effect against *C. violaceum* (Alisjahbana et al., 2021). Also, the isolates other than TRMS 124 had no antimicrobial activity against *C. albicans*.

The 16S rRNA sequence analysis of 26 randomly selected isolates among the actinomycetes exhibiting antimicrobial activity against at least three test microorganisms was performed, and 24 isolates were identified as *Streptomyces* spp. However, previous studies reported that 16S rRNA sequencing alone could be insufficient to distinguish closely related species of the *Streptomyces* genus. Thus, based on the 16S rRNA sequence analysis, some isolates were found to be closely related to more than one *Streptomyces* species at the same score and percentages in this study. Therefore, more research is needed to identify these isolates at the species level. The researchers recommend performing multilocus sequence analysis (MLSA), including housekeeping genes (*atpD*, *gyrB*, *recA*, *rpoB*, *trpB*) for species-level identification of *Streptomyces* spp. (Guo et al., 2008; Labeda, 2011; Rong & Huang, 2012).

The isolates found in cluster II exhibited antimicrobial activity against Gram-positive bacteria, while TRMS 6025 additionally showed antimicrobial activity against *S.* Typhimurium and *C. violaceum*. TRMS 6025, TRMS 6712, TRMS 6726, TRMS 6734, and TRMS 6736 were closely related to *S. zagrosensis*. In addition, since the antimicrobial activity spectrum of these isolates (except TRMS 6025) against the tested microorganisms was the same, we might name TRMS 6712, TRMS 6726, TRMS 6734, and TRMS 6736 as different strains of the same species. Moreover, the fact that TRMS 6025 exhibited antimicrobial activity against more test microorganisms than other isolates with which it is closely related might suggest that TRMS 6025 might be a different species. On the other hand, TRMS 6330 and TRMS 6344 were closely related to *S. niveus*, and these two isolates are likely to be *S. niveus* strains. In addition, *S. niveus* is the source of the novobiocin antibiotic which is an inhibitor of bacterial DNA gyrase (Procópio et al., 2012). Cluster I was a more heterogeneous group in terms of the antimicrobial activity spectrum. This cluster also harbored isolates with the broadest antimicrobial activity spectrum against test microorganisms such as TRMS 124, TRMS 713, and TRMS 714. Furthermore, cluster I contained *S. subrutilus*, *S. vinaceus*, *S. nojiriensis*, *S. xhantophaeus*, *S. anulatus*, and *S.*

griseus which are the producers of antibacterials such as hydroxystreptomycin, viomycin, nojirimycin, geomycin, actinomycin, and streptomycin, respectively (Selim et al., 2021). On the other hand, the antimicrobial effects observed in this study may be attributed to bioactive compounds such as cervimycin A-D, undecylprodigiosin, xiakemycin A, chaxalactin B, as well as the antibiotics mentioned above. Because these bioactive compounds have been purified from some *Streptomyces* spp. isolates with antimicrobial activity isolated from various caves until now and have been held responsible for the antimicrobial effect (Rangseekaew & Pathom-Aree, 2019).

Several considerations limit this study. For example, biochemical tests were not performed for the characterization of actinomycetes. Antimicrobial activity was determined using the modified cross-streak test. Therefore, the results were presented only as the presence (+) or absence (-) of antimicrobial activity. Any quantitative data on antimicrobial activity is not available. Also, antibiotic-resistant strains were not used as the test microorganism.

This study provides preliminary data that actinomycetes isolated from Akçakale, Kırklar, and Köprübaşı Caves may be species with the potential to produce novel antimicrobial compounds. The results supported that caves could be the source of actinomycetes that produce bioactive compounds, as described previously. The antimicrobial activities of these isolates will be further investigated in a follow-up study using the disk diffusion and broth microdilution methods. In addition, the secondary metabolites of these isolates should be determined in detail.

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Author's Contributions

Authors declare the contribution of the authors is equal.

Statement of Conflict of Interest

The authors have declared no conflict of interest.

REFERENCES

Alisjahbana, B., Debora, J., Susandi, E., & Darmawan, G. (2021). *Chromobacterium violaceum*: A review of an unexpected scourge. *International Journal of General Medicine*, *14*, 3259-3270. https://doi.org/10.2147/IJGM.S272193

Bedel, H. (2020). *Farklı Habitatlardan Toplanan Toprak ve Su Kaynaklarındaki Aktinomisetlerin İzolasyonu, Tanımlanması ve Antibakteriyel Aktivitenin Belirlenmesi (Tez No 663288)*. [Yüksek Lisans Tezi, Burdur Mehmet Akif Ersoy Üniversitesi Fen Bilimleri Enstitüsü Gıda Mühendisliği Ana Bilim Dalı]. Yükseköğretim Kurulu Ulusal Tez Merkezi.

Belyagoubi, L., Belyagoubi-Benhammou, N., Jurado, V., Dupont, J., Lacoste, S., Djebbah, F., Ounadjela, F.Z., Benaissa, S., Habi, S., Abdelouahi, D.E., & Saiz-Jimenez, C. (2018). Antimicrobial activities of culturable microorganisms (actinomycetes and fungi) isolated from Chaabe Cave, Algeria. *International Journal of Speleology*, *47*(2), 189-199. https://doi.org/10.5038/1827-806X.47.2.2148

Cheeptham, N., Sadoway, T., Rule, D., Watson, K., Moote, P., Soliman, L.C., Azad, N., Donkor, K.K., & Horne, D. (2013). Cure from the cave: volcanic cave actinomycetes and their potential in drug discovery. *International Journal of Speleology*, *42*(1), 35-47. http://dx.doi.org/10.5038/1827-806X.42.1.5

Chen, W.P. & Kuo, T.T. (1993). A simple and rapid method for the preparation of gram-negative bacterial genomic DNA. *Nucleic acids research*, *21*(9), 2260. https://doi.org/10.1093/nar/21.9.2260

Devanshi, S., Shah, K.R., Arora, S. & Saxena, S. (2021). *Actinomycetes as an environmental scrubber*. In Abdel-Raouf, M.E., & El-Keshawy, M.H. (Eds.), *Crude oil - new technologies and recent approaches*. IntechOpen, London.

Doğruöz-Güngör, N., Çandıroğlu, B., & Altuğ, G. (2020). Enzyme profiles and antimicrobial activities of bacteria isolated from the Kadiini Cave, Alanya, Turkey. *Journal of Cave and Karst Studies*, *82*(2), 106-115. https://doi.org/10.4311/2019MB0107

Farda, B., Djebaili, R., Vaccarelli, I., Del Gallo, M., & Pellegrini, M. (2022). Actinomycetes from caves: An overview of their diversity, biotechnological properties, and insights for their use in soil environments. *Microorganisms*, *10*(2), 453. https://doi.org/10.3390/microorganisms10020453

Farhana, A. & Khan, Y.S (2022). *Biochemistry, Lipopolysaccharide*. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing. Available from: https://www.ncbi.nlm.nih.gov/books/NBK554414/ (Accessed on 1 May 2023).

Guo, Y., Zheng, W., Rong, X., & Huang, Y. (2008). A multilocus phylogeny of the *Streptomyces griseus* 16S rRNA gene clade: Use of multilocus sequence analysis for streptomycete systematics. *International Journal of Systematic and Evolutionary Microbiology*, *58*(1), 149-159. https://doi.org/10.1099/ijs.0.65224-0

Hamedi, J., Kafshnouchi, M., & Ranjbaran, M. (2019). A study on actinobacterial diversity of Hampoeil cave and screening of their biological activities. *Saudi Journal of Biological Sciences*, *26*(7), 1587-

1595. https://doi.org/10.1016/j.sjbs.2018.10.010

Herold, K., Gollmick, F.A., Groth, I., Roth, M., Menzel, K.D., Möllmann, U., Gräfe, U., & Hertweck, C. (2005). Cervimycin A-D: a polyketide glycoside complex from a cave bacterium can defeat vancomycin resistance. *Chemistry (Weinheim an der Bergstrasse, Germany)*, *11*(19), 5523-5530. https://doi.org/10.1002/chem.200500320

Hibbing, M.E., Fuqua, C., Parsek, M.R., & Peterson, S.B. (2010). Bacterial competition: surviving and thriving in the microbial jungle. *Nature Reviews Microbiology*, *8*(1), 15-25. https://doi.org/10.1038/nrmicro2259

Jaroszewicz, W., Bielańska, P., Lubomska, D., Kosznik-Kwaśnicka, K., Golec, P., Grabowski, Ł., Wieczerzak, E., Dróżdż, W., Gaffke, L., Pierzynowska, K., Węgrzyn, G., & Węgrzyn, A. (2021). Antibacterial, antifungal and anticancer activities of compounds produced by newly isolated *Streptomyces* strains from the Szczelina Chochołowska Cave (Tatra Mountains, Poland). *Antibiotics (Basel, Switzerland)*, *10*(10): 1212. https://doi.org/10.3390/antibiotics10101212

Kimura, M. (1980). A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution*, *16*(2), 111-120. https://doi.org/10.1007/BF01731581

Kováč, L. (2018). *Caves as oligotrophic ecosystems*. In: Moldovan, O., Kováč, L. & Halse, S. (Eds.), *Cave ecology*. Springer: Cham, Switzerland.

Labeda, D.P. (2011). Multilocus sequence analysis of phytopathogenic species of the genus *Streptomyces*. *International Journal of Systematic and Evolutionary Microbiology*, *61*(10), 2525-2531. https://doi.org/10.1099/ijs.0.028514-0

Long, Y., Jiang, J., Hu, X., Zhou, J., Hu, J., & Zhou, S. (2019). Actinobacterial community in Shuanghe Cave using culture-dependent and -independent approaches. *World Journal of Microbiology & Biotechnology*, *35*(10), 153. https://doi.org/ 10.1007/s11274-019-2713-y

Maciejewska, M., Adam, D., Martinet, L., Naômé, A., Całusińska, M., Delfosse, P., Carnol, M., Barton, H. A., Hayette, M. P., Smargiasso, N., De Pauw, E., Hanikenne, M., Baurain, D., & Rigali, S. (2016). A phenotypic and genotypic analysis of the antimicrobial potential of cultivable *Streptomyces* isolated from cave moonmilk deposits. *Frontiers in Microbiology*, *7*,1455. https://doi.org/10.3389/fmicb.2016.01455

Miethke, M., Pieroni, M., Weber, T., Brönstrup, M., Hammann, P., Halby, L., Arimondo, P.B., Glaser, P., Aigle, B., Bode, H.B., Moreira, R., Li, Y., Luzhetskyy, A., Medema, M.H., Pernodet, J.L., Stadler, M., Tormo, J.R., Genilloud, O., Truman, A.W., … Müller, R.(2021). Towards the sustainable discovery and development of new antibiotics. *Nature Reviews Chemistry*, *5*(10), 726-749. https://doi.org/10.1038/s41570-021-00313-1

Nimaichand, S., Devi, A.M., Tamreihao, K., Ningthoujam, D.S., & Li, W.J. (2015). Actinobacterial diversity in limestone deposit sites in Hundung, Manipur (India) and their antimicrobial activities. *Frontiers in Microbiology*, *6*, 413. https://doi.org/10.3389/fmicb.2015.00413

Pradana, A.D., Sekarini, D.N., Suma, A.A., & Maliza, R. (2022). Study on the antibacterial effect from moonmilk Pindul Cave, Indonesia. *Iranian Journal of Medical Microbiology*, *16*(2), 165-172. https://doi.org/10.30699/ijmm.16.2.165

Procópio, R.E., Silva, I.R., Martins, M.K., Azevedo, J.L., & Araújo, J.M. (2012). Antibiotics produced by *Streptomyces*. *The Brazilian journal of infectious diseases: an official publication of the Brazilian Society of Infectious Diseases*, *16*(5), 466-471. https://dx.doi.org/10.1016/j.bjid.2012.08.014

Prudence, S.M.M., Addington, E., Castaño-Espriu, L., Mark, D.R., Pintor-Escobar, L., Russell, A.H., & McLean, T.C. (2020). Advances in actinomycete research: An ActinoBase review of 2019. *Microbiology (Reading, England)*, *166*(8), 683-694. https://dx.doi.org/10.1099/mic.0.000944

Rangseekaew, P. & Pathom-Aree, W. (2019). Cave actinobacteria as producers of bioactive metabolites. *Frontiers in Microbiology*, *10*, 387. https://dx.doi.org/10.3389/fmicb.2019.00387

Rong, X. & Huang, Y. (2012). Taxonomic evaluation of the *Streptomyces hygroscopicus* clade using multilocus sequence analysis and DNA-DNA hybridization, validating the MLSA scheme for systematics of the whole genus. *Systematic and Applied Microbiology*, *35*(1), 7-18. https://dx.doi.org/10.1016/j.syapm.2011.10.004

Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution*, *4*(4), 406-425. https://dx.doi.org/10.1093/oxfordjournals.molbev.a040454

Selim, M.S.M., Abdelhamid, S.A., & Mohamed, S.S. (2021). Secondary metabolites and biodiversity of actinomycetes. *Journal, Genetic Engineering & Biotechnology*, *19*(1), 72. https://doi.org/10.1186/s43141-021-00156-9

SPSS, (2015). IBM SPSS Statistics 23.0 for Windows. Armonk, NY.

Syiemiong, D. & Jha, D.K. (2019). Antibacterial potential of actinobacteria from a limestone mining site in Meghalaya, India. *Journal of Pure and Applied Microbiology*, *13*(2), 789-802. https://dx.doi.org/10.22207/JPAM.13.2.14

Tamura, K., Stecher, G., & Kumar, S. (2021). MEGA 11: Molecular evolutionary genetics analysis version 11. *Molecular Biology and Evolution*, *38*(7), 3022-3027. https://dx.doi.org/10.1093/molbev/msab120

Tufekci, E.F., Akcay, S., Kayipmaz, S., & Kiliç, A.O. (2019). Impact of quorum sensing inhibitors on population dynamics of oral bacteria. *Journal of Biology, Agriculture and Healthcare*, *9*(18), 10-19. https://dx.doi.org/10.7176/JBAH

Velho-Pereira, S. & Kamat, N.M. (2011). Antimicrobial screening of actinobacteria using a modified cross-streak method. *Indian Journal of Pharmaceutical Sciences*, *73*(2), 223-228. https://dx.doi.org/10.4103/0250-474x.91566

Yamaç, M., Işık, K., & Şahin, N. (2011). Numerical classifification of *Streptomycetes* isolated from karstic caves in Turkey. *Turkish Journal of Biology*, *35*(4), 473-484. https://doi.org/10.3906/biy-0911-185.

Yücel, S. & Yamaç, M. (2010). Selection of *Streptomyces* isolates from Turkish karstic caves against antibiotic resistant microorganisms. *Pakistan Journal of Pharmaceutical Sciences*, *23*(1), 1–6.

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Mentha pulegium Extracts Showed Strong Antimicrobial And Cytotoxic Effects in Vitro.

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ABSTRACT

Mentha pulegium is a medicinally important and well-known plant and used for various purposes such as medicinal, nutritional and spice. We are analyzed to observe the antimicrobial, antioxidant and cytotoxic properties of *M. pulegium* extracts in this study. The antimicrobial activity of *M. pulegium* was tested using the agar well method. MIC, MBC and antimicrobial activity were tested on *Staphylococcus aureus* (ATCC 25923), *Klebsiella pneumoniae* (ATCC 700603), *Escherichia coli* (ATCC 25322), *Bacillus megaterium* (DSM32) and *Candida albicans* (FMC17) microorganisms. Clindamycin (2 µg) and Amoxicillin (30 µg) were used as positive control and Dimethylsulfoxide (DMSO) as negative control. Cytotoxic activity of extracts at different concentrations obtained from solvents such as acetone, chloroform and methanol using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) test method. Cell death patterns after exposure to different concentrations of the extracts on human breast cancer (MDA-MB-231), human pancreatic cancer (PANC1), human ovarian cancer (OVCAR3) and human lung cancer (A549) cell lines were determined accordingly. As a result, it was determined that *M. pulegium* extract has a strong antimicrobial activity spectrum and cytotoxic effect.

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Mentha pulegium Ekstraktının Güçlü Antimikrobiyal ve Sitotoksik Etkisinin Invitro Olarak Gösterilmesi

ÖZET

Mentha pulegium tıbbi açıdan önemli ve iyi bilinen bir bitkidir ve tıbbi, beslenme ve baharat gibi çeşitli amaçlar için kullanılır. Bu çalışmada *M. pulegium* ekstraktının antimikrobiyal, antioksidan ve sitotoksik özelliklerini görmek için analiz edildi. *M. pulegium*'un antimikrobiyal aktivitesi agar kuyucuk yöntemi kullanılarak test edildi. MIC, MBC ve antimikrobiyal aktivite *Staphylococcus aureus* (ATCC 25923), *Klebsiella pneumoniae* (ATCC 700603), *Escherichia coli* (ATCC 25322), *Bacillus megaterium* (DSM32) ve *Candida albicans* (FMC17) mikroorganizmaları üzerinde test edildi. Pozitif kontrol olarak Klindamisin (2 µg) ve Amoksisilin (30 µg), negatif kontrol olarak Dimetilsülfoksit (DMSO) kullanıldı. 3-(4,5-dimetiltiyazol-2-il) -2,5-difenil tetrazolyum bromür (MTT) test yöntemi kullanılarak aseton, kloroform ve metanol gibi çözücülerden elde edilen farklı konsantrasyonlarda ekstraktların sitotoksik aktivitesi; ekstraktların farklı konsantrasyonlarına maruz kaldıktan sonra µg/ml sitotoksik aktivite ve hücre ölüm şekilleri; Aseton, kloroform ve metanol gibi çözücülerden 3-(4,5-dimetiltiyazol-2-il) -2,5-difenil tetrazolyum bromür (MTT) test yöntemi kullanılarak aseton, kloroform ve metanol insan meme kanseri (MDA-MB-231), insan pankreas kanseri (PANC1), insan yumurtalık kanseri (OVCAR3) ve insan akciğer kanseri (A549) hücre hatları buna göre belirlendi. Sonuç olarak M. pulegium ekstraktının güçlü bir antimikrobiyal aktivite spektrumuna ve sitotoksik etkiye sahip olduğu belirlenmiştir.

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INTRODUCTION

Since ancient times, drugs of natural origin have formed the basis of traditional medicine in different cultures, and today medicinal plants occupy the most important place as a source of new drug molecules (Hassan and Ullah, 2019; Sofowora et al., 2013). It has been reported that approximately 400,000 plant taxa have been identified to date, and approximately 30,000 have been used in traditional medicine for the treatment of many diseases (Top et al., 2019; Erarslan et al., 2020).

Cancer is a complex disease that occurs as a result of disorders in the mechanisms regulating the basic functions of the cell and seriously affects the human population (Şekerli et al., 2017; Greenwell and Rahman, 2015). There are many treatment methods for this life-threatening disease. These are: radiotherapy, surgery, chemotherapy-hormone therapy, immunotherapy, gene therapy, check-point inhibitors and angiogenesis inhibitors (Hasham et al., 2018). Due to the fact that these treatment methods have some side effects (such as chemotherapy) and treatments such as immunotherapy and gene therapy are expensive, interest in researches such as the development of new plant-derived drug candidate molecules and the discovery of new bioactive molecules and the development of new treatment methods is increasing and investments are made in this subject (Kurt et al., 2013; Pucci et al., 2019).

The lack of easy access to primary health care and veterinary services in many rural areas of the world has increased the need for traditional medicine to treat both humans and animals and has helped maintain the use of medicinal plants (McGaw and Eloff, 2008). The need for new, effective and affordable drugs to treat microbial infections is increasing, especially in developing countries of the world, where up to half of deaths are caused by infectious diseases, which is seen as a major challenge in global healthcare (Awouafack et al., 2013).

The development of antibiotic-resistant bacterial strains is due to a number of factors, such as the widespread and inappropriate use of antibiotics and the increase in antibiotic-resistant pathogenic bacteria (Lowy, 2003). According to the data obtained, the problem of antibiotic resistance in humans and animals will continue for a long time (Andersson and Hughes, 2011). However, the development of alternative drug classes is necessary to treat such infectious diseases. Communicable diseases represent an important cause of death, especially in developing countries. For this reason, pharmaceutical companies have focused on developing new antimicrobial drugs in recent years, especially due to the continuous emergence of microorganisms resistant to antimicrobials. A synergy between traditional medicines and products derived from medicinal plants has also been reported (Nascimento et al, 2000). As bacteria resistant to conventional drugs are becoming more common, medicinal plants represent an alternative to antimicrobial action and infection treatment (Sakagami and Kajimura, 2002).

According to the researches of the World Health Organization (WHO), due to the great biological and structural diversity of medicinal plant components used for therapeutic purposes, it constitutes an effective resource for the discovery of antibacterial, antifungal and antiparasitic compounds (Faydaoğlu and Sürücüoğlu, 2013; Ugboko et al., 2020). Plant extracts showing antimicrobial activity are used as preservatives, medicinal purposes, analgesic, anti-inflammatory properties in foods and show a good effect against pathogens (Swamy et al., 2016; Winska et al., 2019). Nowadays, people resort to medicinal plants because of the unavoidable proliferation of diseases and the inadequate treatments (Erdoğan et al., 2013). The genus *Mentha* is from the Labiatae family and is used as an aromatic and medicinal plant (Gonzalez-Tejero et al., 2008). *M. pulegium* species is also called pennyroyal, watermelon and filiskin . It is a perennial, pungent-smelling plant that grows in Western, Southern and Central Europe, Asia, Iran, and Arab countries and grows in a wide area in Turkey (Çöteli et al., 2013). It is also used as a preservative in the food industry (Ahmed et al., 2018). In addition, it is known that it is loved and consumed among the people as a natural flavor and because it is thought to be medicinal.

The Labiatae family is one of the large plant families used as a basis for assessing the formation of some secondary metabolites (Wink, 2003). Plants of the genus *Mentha* are a source of essential oils found in terpenes, various phenolic compounds, tannins, terpenoids, quinones, coumarins, flavonoids, alkaloids, sterols, and saponins, especially in the epidermal glands of leaves, stems, and reproductive structures (Dorman et al., 2003). *M. pulegium* is rich in essential oils in its structure and its essential oil yield varies between 1.90% and 6.20% according to dry weight. The majority of essential oils are composed of a substance called Pulegon (Benlarbi et al., 2014; Gülçin et al., 2020). At the same time, *M. pulegium* has traditionally

been used in medicine for digestive problems, colds, sinusitis, cholera, bronchitis, tuberculosis, carminative, expectorant (Mamadalieva et al., 2020). It is also used topically to kill germs, repel insects and treat skin diseases, gout, venomous bites and mouth sores (Salem et al., 2017).

Considering the problems mentioned in this study, cytotoxic activity of *M. pulegium* species from Labiatae family was determined on human breast cancer (MDA-MB-231), human pancreatic cancer (PANC1), human ovarian cancer (OVCAR3) and human lung cancer (A549) cell lines. intended to be examined. In addition, it was aimed to determine the apoptotic/necrotic activity by double staining (Hoechst 33342 and Propidium Iodide) method. Although the blooming aerial part of *M. pulegium* is widely used for its antiseptic properties, studies on the antimicrobial activity of this plant have been reported so far. (Chalchat et al., 2000; Mamadalieva et al., 2020) It was aimed to investigate the antioxidant activity of the extract obtained from the aerial part of the *M. pulegium* plant and antimicrobial activity experiments with the agar-well method using five different microorganisms and the MIC and MBC values of the applied extract in addition to these.

MATERIAL and METHODS

In this study; The plant *M. pulegium* was obtained naturally. Gram positive (+) in the study *Staphylococcus aureus* ATCC 25923, *Bacillus megaterium* DSM32 and gram negative (-) *Escherichia coli* ATCC 25322, *Klebsiella pneumoniae* ATCC 700603 bacteria and *Candida albicans* FMC17 as fungus were used. Microorganism cultures were obtained from the culture collection of Fırat University, Faculty of Science, Department of Biology, Microbiology Laboratory.

Preparation of Extract and Test of Antimicrobial Effect

The *M. pulegium* plant grown in Diyarbakır and its surroundings was collected in April-May. Above-ground parts of *M. pulegium* were pounded in a porcelain mortar and powdered, then weighed on a precision scale and 1 g of dried *M. pulegium* was taken and dissolved with 10 ml of acetone, 10 methanol and 10 ml of chloroform solvents. After keeping it at room temperature for 72 hours, it was passed through a 0.45 µm minipore filter (Oxoid). Then, the main stock was prepared by performing the extraction process at 55-60˚C in a rotary evaporator for 4-6 hours and dissolving the obtained extract in DMSO (Dimethyl sulfoxide). The extracts were stored at +4 ˚C (Salem, et al., 2017; Dalkılıç et al., 2020). % efficiency calculation was made for the extract obtained (Chalchat et al., 2020). Polar solvents are very effective in separating polyphenols from plant tissues. For this reason, acetone, methanol and chloroform solvents, which are polar solvents, were preferred for the extraction of *M. pulegium* (Wink, 2003; Darman et al., 2003).

Formula 1.

% Efficiency calculation:

$$\%\,Yield = \frac{\text{Amount remaining after extraction (g)}}{\text{Dry amount before extraction (g)}}\ \text{X}\ 100$$

All the materials used were sterilized in an autoclave and the necessary labels were made. Before the experimental study, bacteria were grown in Nutrient Broth (Difco) and yeast were grown in malt extract broth (Difco). The agar well method was used to test the antimicrobial activity of *M. pulegium* plant, acetone, methanol and chloroform extracts. Mueller Hinton Agar (Oxoid) was prepared for the bacteria used in the study and Sabouraud Dextrose Agar was prepared for the fungus and after the sterilization process was completed in the autoclave (15 min at 121°C), bacteria and fungi were inoculated with the help of the loop and according to Mc Farland 0.5 (10^8 microorganism/ml). After it was adjusted and shaken well, 25 ml was placed in sterile petri dishes of 9 cm diameter and the medium was homogeneously distributed. Petri dishes were left to solidify for 5-15 minutes at room temperature. Solidified Müeller Hinton Agar and Sabouraud Dextrose Agar were sterilized and each petri dish was well drilled with cork borer except for the negative and positive controls. Extract at different concentrations (5 mg/ml, 25 mg/ml, 50 mg/ml and 100 mg /ml) was inoculated under aseptic conditions, as 100 µl for each well. After the inoculation process, the petri dishes inoculated with bacteria were incubated at 37±0.1°C for 24 hours, and the petri dishes inoculated with fungi for 48 hours at 25±0.1°C. Standard discs (Amoxicillin 30 µg/disc, Clindamycin 2 µg/disc) were used for positive control and DMSO was used as negative control. The diameters of the inhibition zones formed at the end of the incubation period were evaluated and recorded. This study was carried out in 3 replications.

Minimal Inhibition Concentration

The minimum inhibitory concentration (MIC) is the lowest concentration of a herbal extract or chemical that markedly inhibits the growth of bacteria. Serial dilutions of the *M. pulegium* were prepared at concentrations ranging from 4 to 10,000 µglmI. Nutrient broth (100 µl) was added to all wells of the 96-well microtitration plate. 40 µl of *M. pulegium* was added to the A1-H1 wells and serial dilution was made by taking 100 µl. Each well was inoculated (4 µl) of tested bacteria and the 96-well microtiter plate was then incubated (37°C for 24 hours) so far as the growth requirement of each organism (Hemeg et al., 2020).

Minimal Bactericidal Concentration

Mueller Hinton Agar was prepared and autoclaved (121°C for 15 minutes) and then poured into petri dishes. Each petri dish was divided into four sections and each section was divided into 12 wells. 3µl of each well of the samples in the 96-well microtiter plate, in which the MIC was made, was taken from each well and added to the medium containing Mueller Hinton Agar to its own number. Then incubated in an oven at 37°C for 24 hours. The lowest medium concentration without growth in the subculture on the agar was accepted as MBC. (Dalkılıç et al., 2020).

Statistical Analysis

It was checked whether the results showed a normal distribution, and a paired t-test was applied on the results. For all values, $p<0.05$ was considered statistically significant. All statistical analyzes were done with SPSS version 22.

Cytotoxic Activity

MTT Assay assay: MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) Assay is a method used to measure cell viability, proliferation and cytotoxicity. MTT method; It is based on the principle of reducing MTT, a tetrazolium salt that can pass through the cell membrane, by the active mitochondria in living cells by gaining electrons inside the cell and transforming it into purple colored water-insoluble formazan crystals. Then, formazan crystals are dissolved with suitable solvents and the resulting color change is measured by spectrophotometric methods and the number of viable cells is determined (Talbaoui et al., 2016; Stockert et al., 2012).

Application of MTT Assay

After the cells grown in 25cm² flasks were 90% confluent, the medium in the flask was removed and washed with 5 ml of sterile PBS (Phosphate-bufferedsaline) solution. 1 ml of Trypsin-EDTA was added to the flasks and incubated for 2 minutes at 37˚C in an oven with 5% CO_2. After the cells were separated from the surface, Trypsin-EDTA was inactivated with 5 ml of medium. The cells were taken out of the flask and centrifuged at 1500 rpm for 5 minutes, then the supernatant was removed, the cell pellet was dissolved with 1000 µl of DMEM and the cell count was made with the Countess II automatic cell counting device. After the calculations were made, cell dilution was prepared with standard DMEM and 100 µL DMEM was seeded on 96-well plates with $5x10^3$ cells in each well. Only DMEM was used as a blank in the first row, 2.5 µg/ml Doxorubicin was used as positive control and only medium was used as negative control. Then it was incubated for 24 hours in an oven with 5% CO_2 at 37˚C. After incubation, the medium in the wells was removed and methanol, acetone and chloroform extracts of *M. pulegium* prepared in DMEM were added to the cells in 4 different concentrations of 1000, 500, 250,125 µg/ml 6 repetitions and 5% CO_2 at 37˚C for 72 hours. It was left to incubate in an oven. After the incubation period, 20 µl of MTT solution (5 mg/ml) was added to the wells containing the cells and incubated for 4 hours at 37˚C in a dark environment containing 5% CO_2. After incubation, the medium was removed and formazan crystals were dissolved with 100 µl of DMSO (dimethylsulfoxide). The color change expected to occur afterwards was measured with the ELISA micro-plate reader device at a wavelength of 570 nm. Since no significant effect was observed when the measurement was made, the concentrations were changed. The study was repeated by changing the concentrations to 1000, 500, 250, 125 µg/ml, taking into account the molecular weight of the plant we used in our study and the compounds in its content, and significant results were observed.

Antioxidant Activity

2,2-Diphenyl-1-Picrylhydrazil (DPPH) Radical Scavenging Capacity Method

Antioxidant activity of 100, 50, 25 and 12.5 mg/ml concentrations of *M. pulegium* methanol extract 2,2-diphenyl-1-picrylhydrazil (DPPH) was determined according to the radical scavenging capacity method. The solution was prepared in methanol at concentrations of 100, 50, 25 and 12.5 mg/ml from the lyophilized drug. 100 µl of the prepared solution was taken and 100 µl of DPPH solution was added. 100% Ascorbic acid was used as positive control and 100% methanol was used as negative control. Each concentration was studied in 3 repetitions, then mixed until a homogeneous mixture was obtained and 30 minutes after the mouth was closed. The same procedure was repeated for all concentrations. At the end of this period, the absorbances of each mixture were read in the spectrophotometer at 570, 540 and 492 nm, and the % inhibition values were calculated.

Apoptotic/Necrotic Activity

Double staining (Hoechst 33258 and Propidium Iodide) method

This step; apoptotic and necrotic activity was tested using dual staining (Hoechst 33258 and Propidium Iodide) technique.

The basis of this method is; It is based on the ability of fluorescent dyes to bind to DNA, making the chromatin and thus the nucleus of the cell visible. Hoechst 33258 (HO) dye is a dye that can bind to DNA and thus penetrate through the cell membrane. It is used to stain the nuclei of living and dead cells. Propidium iodide (PI) stain can only be taken up by cells with impaired membrane integrity, thus allowing detection

of late apoptotic / necrotic cells. For apoptotic cells, features such as smaller (pycnotic) and/or fragmented nuclei are sought compared to normal cells, while for necrotic cells, the nucleus is slightly larger than normal cells and has less staining. Thus, in the absence of pycnotic and/or fragmented nuclei: cells with HO (+) / PI (-) observed; Alive. In the presence of pycnotic and/or fragmented nuclei: cells with HO (+) / PI (-) observed; early apoptotic. In the presence of pycnotic and/or fragmented nuclei: cells with HO (+) / PI (+) observed; late apoptotic or secondary necrotic. In the absence of pycnotic and/or fragmented nuclei: cells with HO (+) / PI (+) observed; It was evaluated as necrotic (HO dye blue, PI dye red) and apoptotic/necrotic activity was determined in this context (Cevatemre, 2012).

Application of the double staining (Hoechst 33258 and Propidium Iodide) method

A549 cell line, $10x10^3$ cells in 2 ml DMEM (1% L-Glutamine, 1% Penicillin-Streptomycin and 10% FBS (Fetal Bovine Serum)) in a 6-well plate planted. Then, it was incubated for 24 hours at 37˚C under 5% CO_2 atmospheric conditions. After the incubation, the medium in the wells was emptied and 2 ml of medium containing 1000 µg/ml *M.pulegium* extract was added and left to incubate for 48 hours. After incubation, the medium was removed from the wells and the cells were washed with PBS, then 2 ml of the solution prepared with 5-10 µg/ml HO and 1 µg/ml PI dyes (the dye solution was prepared in 1x PBS (without Ca/Mg)) was added. The plate was then incubated at 37 ˚C in the dark with 5% CO_2 5 for 30 minutes. After incubation, cell morphologies were compared with control groups (negative control: no drug-treated cells, positive control: 2.5 µg/ml Doxorubicin-treated cells) and evaluated under a fluorescent microscope.

RESULTS

Since the blooming aerial part of *M. pulegium* is widely used due to its antiseptic properties, this study was carried out to use methanol, acetone and chloroform extracts of the aerial parts of *M. pulegium* as antioxidant, antibacterial, investigated its antifungal and anticancer properties.

Antimicrobial activity

The antimicrobial activity of *M. pulegium* extract is shown in tables 1, 2 and 3.

In the acetone extract of *M. pulegium*, the highest inhibition zone was observed in *K. pneumoniae* with 13±0.44 mm at 100 mg/ml concentration and in *C. albicans* as 13±0.55. The lowest inhibition zone of *C. albicans* was measured as 10 mm at a concentration of 10±0.32 mg/ml (Table 1).

Table 1. Antimicrobial effect of *M. pulegium* acetone extract (zone diameters mm)
Çizelge 1. *M. pulegium* aseton ekstraktının antimikrobiyal etkisi (zon çapları mm)

Microorganisms	100 mg/ml	50 mg/ml	25 mg/ml	5 mg/ml	Clindamycin	Amoxicillin
E. coli	9±0.13	11±0.11	-	-	22 ± 1.65	-
K. pneumoniae	13±0.44	-	-	-	21 ± 1.24	11 ± 1.21
S. aureus	11±0.16	-	-	-	23 ± 1.88	-
B. megaterium	-	-	-	-	20 ± 1.63	-
C. albicans	13±0.55	10±0.32	-	-	-	-

In the chloroform extract of *M. pulegium*, the highest inhibition zone was seen in *C. albicans* as 11±0.13 mm at 100 mg/ml concentration, and the lowest inhibition zone was 9±0.08 mm in *E. coli* (Table 2).

It was observed that the methanol extract of *M. pulegium* formed effective zone diameters in all microorganisms at 4 different concentrations. While the highest inhibition zone was observed as 25±1.75 mm at 100 mg/ml concentration in *B. megaterium*, the lowest inhibition zone was measured as 13±0.17 mm at 5 mg/ml concentration (Table 3).

Table 2. Antimicrobial effect of *M. pulegium* chloroform extract (zone diameters mm)
Çizelge 2. *M. pulegium* kloroform ekstraktının antimikrobiyal etkisi (zon çapları mm)

Microorganisms	100 mg/ml	50 mg/ml	25 mg/ml	5 mg/ml	Klindamisin	Amoksisilin
E. coli	9±0.08	-	-	-	22 ± 1.65	-
K. pneumoniae	-	-	-	-	21 ± 1.24	11 ± 1.21
S. aureus	-	-	-	-	23 ± 1.88	-
B. megaterium	-	-	-	-	20 ± 1.63	-
C. albicans	11±0.13	10±0.11	-	-	-	-

It has been observed that *M. pulegium* methanol extract has an effective antimicrobial activity on the tested microorganisms and at certain concentrations. Among the positive controls, clindamycin; While it created a zone of 22±1.65 mm diameter against *E.coli*, amoxicillin did not show any effect against the same

bacteria. Clindamycin; formed a zone of 21 ± 1.24 mm against *K. pneumoniae* and amoxicillin with a diameter of 11 ± 1.21 mm against the same bacterium (Figure 1).

Table 3. Antimicrobial effect of M. pulegium methanol extract (zone diameters mm)
Çizelge 3. M. pulegium metanol ekstraktının antimikrobiyal etkisi (zon çapları mm)

Microorganisms	100 mg/ml	50 mg/ml	25 mg/ml	5 mg/ml	Klindamisin	Amoksisilin
E. coli	24±0.57	21±1.15	20±1.32	10±0.09	22 ± 1.65	-
K. pneumoniae	22±0.57	20±0.81	19±1.17	8±0.13	21 ± 1.24	11 ± 1.21
S. aureus	23±1.75	20±1.17	15±0.14	9±0.15	23 ± 1.88	-
B. megaterium	25±1.75	17±0.11	15±0.17	13±0.17	20 ± 1.63	-
C. albicans	19±0.47	16±0.10	16±0.67	12±0.14	-	-

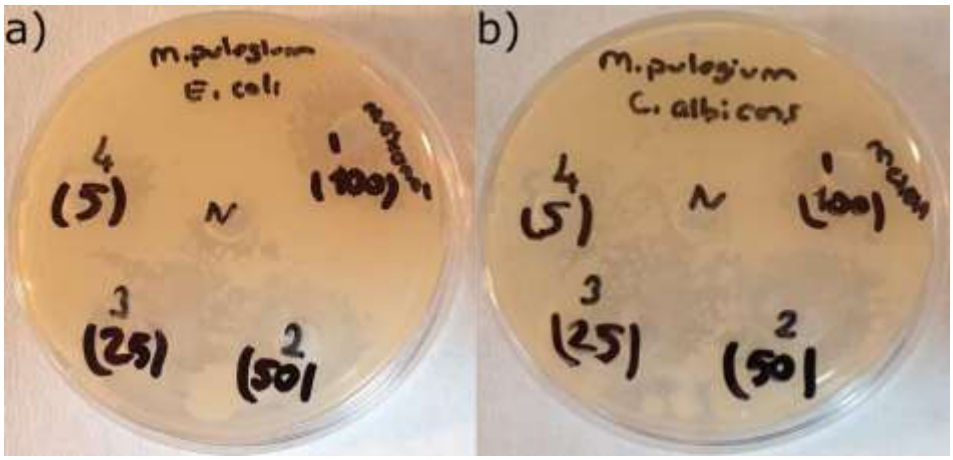

Figure 1. (a) Zones of inhibition of *M. pulegium* extract of *E.coli* in methanol solvent (b) Zones of inhibition of *M. pulegium* extract of *C. albicans* in methanol solvent.
Şekil 1. a) *M. pulegium* ekstraktının *E.coli*'nin metanol çözücüsündeki inhibisyon zonları (b) *M. pulegium* ekstraktının *C. albicans*'ın metanol çözücüsündeki inhibisyon zonları.

MIC and MBC activity

The most significant MIC value (512 µg/ml) in *M.pulegium* extract was observed on *E.coli with acetone and chloroform extracts*. The best MIC value (512 µg/ml) in the extract with methanol was seen in *K. pneumonia*. The MIC value (512 µg/ml) in the chloroform extract had a great effect on inhibiting the growth of *B. megaterium*. As a result of the antimicrobial study of *M. pulegium*, it was observed that different extracts gave an effective result even at low concentrations (Table 4).

Table 4. Minimum inhibition concentration (MIC) and minimum bactericidal concentration (MBC) values of *M. pulegium* extracts (µg/ml)
Çizelge 4. *M. pulegium* ekstraktlarının minimum inhibisyon konsantrasyonu (MIC) ve minimum bakterisidal konsantrasyon (MBC) değerleri (µg/ml)

Microorganism	*E.coli*		*K.pneumonia*		*S.aureus*		*B.megaterium*		*C.albicans*	
	MIC	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**
Acetone extract	512	1024	256	1024	128	256	256	512	512	1024
Chloroform extract	512	512	256	512	256	512	512	1024	512	2048
Methanol extract	64	512	512	512	32	256	32	64	256	1024

Antioxidant activity

The antioxidant activity of *M. pulegium* reduced DPPH by approximately 15% at 570 and 540 nm and 62% at 492 nm at a concentration of 100 mg/ml. It reduced DPPH by approximately 33% at 50 mg/ml concentration, and by approximately 20-34% at 25 and 12.5 mg/ml concentrations, that is, it showed antioxidant activity (Figure 2).

Cytotoxic activity

Medicinal plants are important sources of chemotherapeutic drugs and are used in the prevention and treatment of cancer. (Top et al., 2019) For this reason, the cytotoxic activity of *M.pulegium* species from the Labiatae family was investigated on human breast cancer (MDA-MB-231), human pancreatic cancer (PANC1), human ovarian cancer (OVCAR3) and human lung cancer (A549) cell lines.

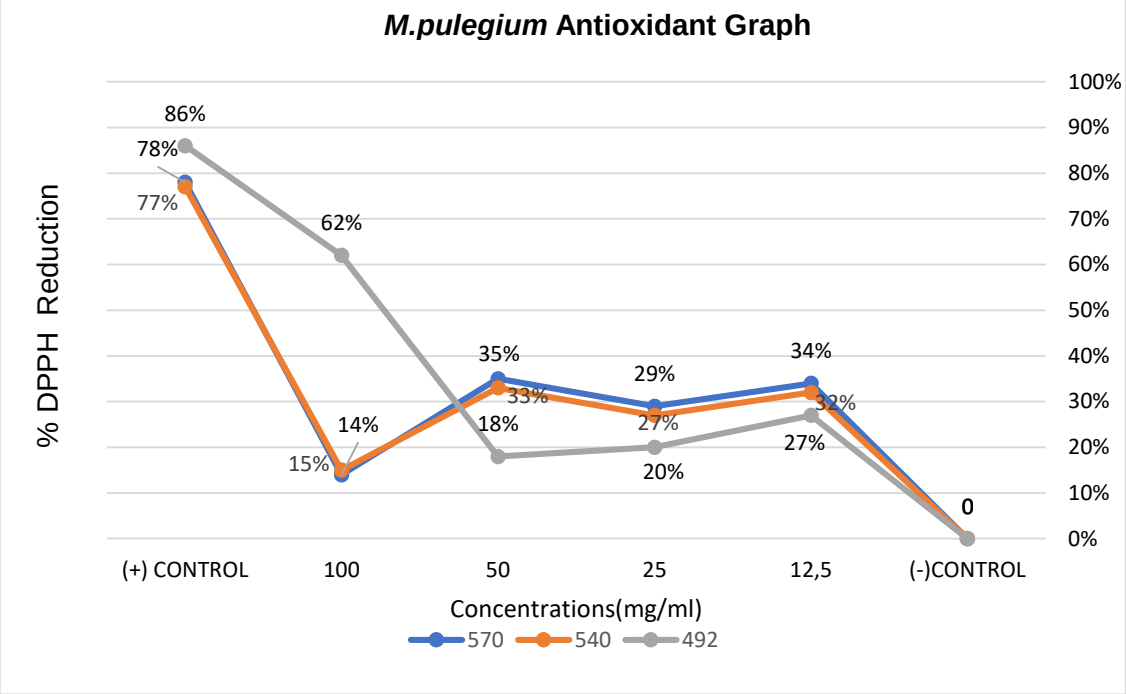

Figure 2. The result of DPPH analysis with *M. pulegium* methanol solvent measured at 3 different nm. (+ control: Ascorbic acid, - control methanol)

Şekil 2. *M. Pulegium* metanol çözücüsü ile 3 farklı nm' de ölçülmüş DPPH analizi sonucu. (+ control: Askorbik asit, -control:metanol)

The results showed that *M. pulegium* extract has a cytotoxic effect against breast cancer cell line. The most effective cytotoxicity was determined as 29.5% total viable cell percentage at 1000 µg/ml concentration prepared with chloroform. The lowest viable cell rate was observed as 110.7% at 250 µg/ml concentration. In the extract prepared with acetone, 50.9% cell viability was determined at 250 µg/ml. The best result of *M. pulegium* methanol extract on MDA-MB 231 cell viability was 51.8% at 1000 µg/ml concentration (Figure 3).

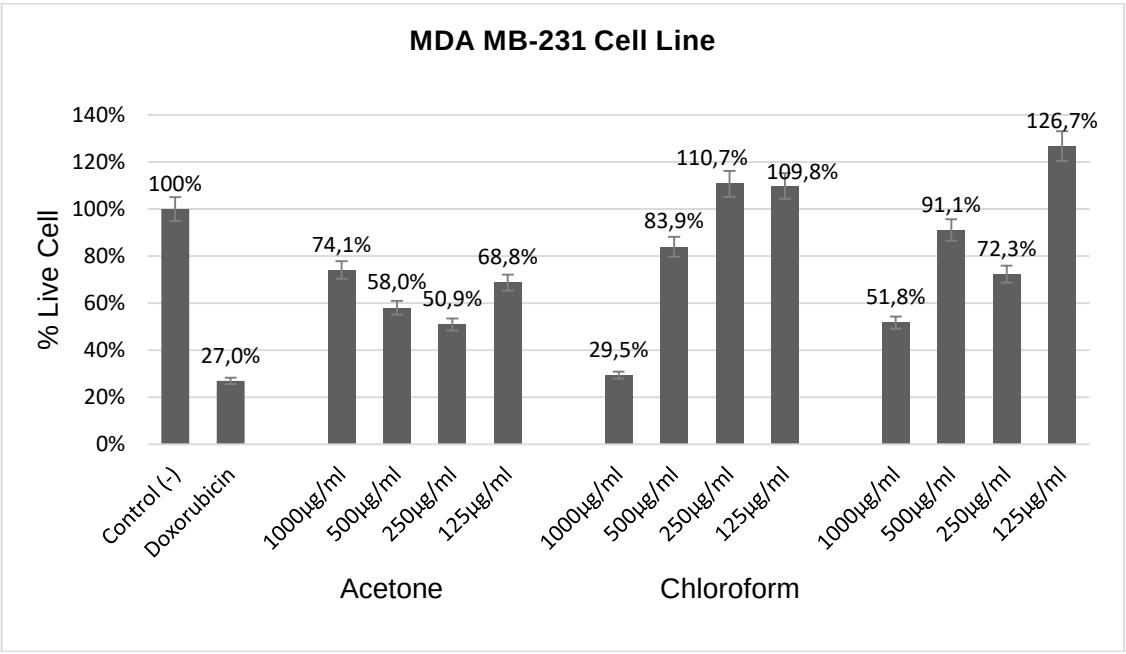

Figure 3. Cytotoxic activity of *M. pulegium* on MDA MB-231 cell line

Şekil 3. MDA MB-231 hücre hattı üzerinde *M. pulegium*'un sitotoksik aktivitesi.

The results showed that *M. pulegium* extract has cytotoxic effect against OVCAR3 cell line. The best cell viability rate in the extract prepared with acetone solvent was determined as 9.9% total viable cell percentage at 250 µg/ml concentration. In chloroform extract, the best cell viability rate was determined as 4.4% total viable cell percentage at 1000 µg/ml concentration. In the extract of *M. pulegium* prepared with methanol solvent, cell viability was observed depending on the dose. The percentage of total cell viability was determined as 9.4%, 24.3%, 53.6% and 78.5% at 1000, 500, 250 and 125 µg/ml concentrations, respectively (Figure 4).

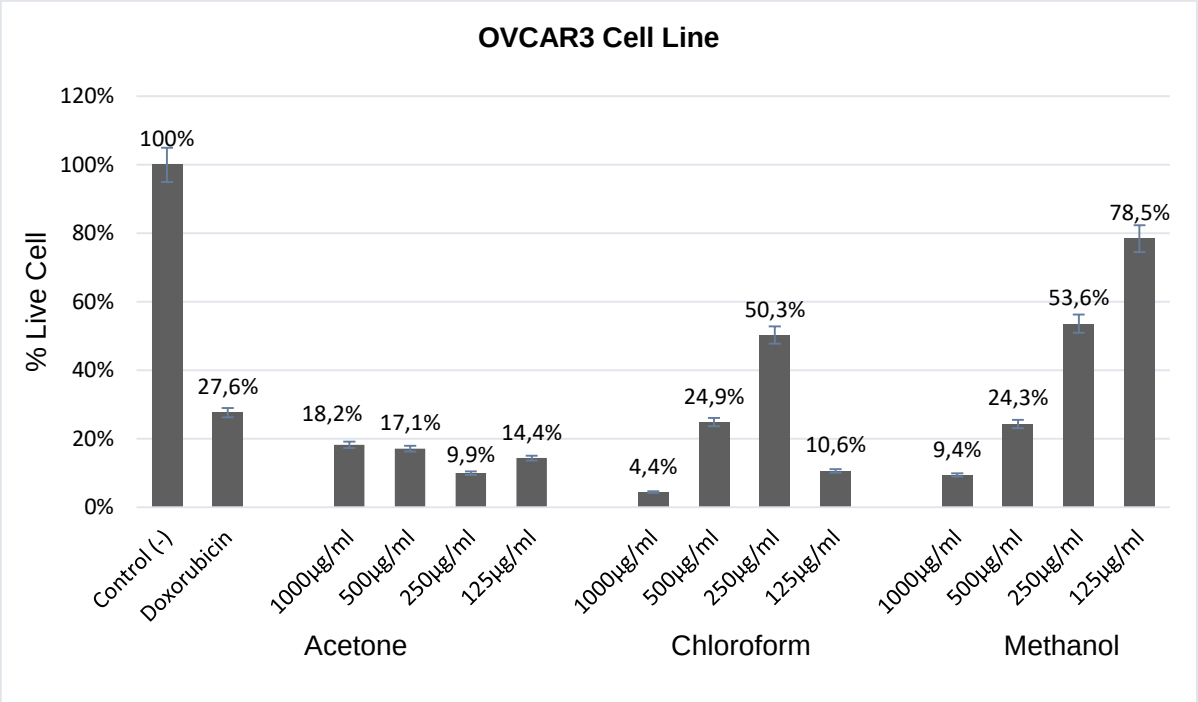

Figure 4. Cytotoxic activity of *M. pulegium* on the OVCAR3 cell line
Şekil 4. *M. pulegium*'un OVCAR3 hücre hattı üzerindeki sitotoksik aktivitesi

In the extract of *M. pulegium* prepared with chloroform solvent, dose-dependent cell viability was observed against the PANC1 cell line. While 43.0% cell viability was observed at 1000 µg/ml concentration of chloroform, it was observed as 97.3% total viable cell percentage at 125 µg/ml concentration. In the extract prepared with acetone, approximately 42% of total cell viability was observed. The best cytotoxic activity in the methanol extract was observed at a concentration of 1000 µg/ml with a total viable cell ratio of 46.2% (Figure 5).

The best cytotoxic activity in acetone solvent against A549 cell line of *M. pulegium* was seen as 20.4% total viable cell percentage at 500 µg/ml concentration. The best cell viability percentage in chloroform was seen as 5.7% at 250 µg/ml concentration. In the methanol extract, on the other hand, the best cytotoxic effect was observed at 500 µg/ml concentration as a percentage of 16.1% total viable cells (Figure 6).

The best cytotoxic activity in acetone solvent against A549 cell line of *M. pulegium* was seen as 20.4% total viable cell percentage at 500 µg/ml concentration. The best cell viability percentage in chloroform was seen as 5.7% at 250 µg/ml concentration. In the methanol extract, on the other hand, the best cytotoxic effect was observed at 500 µg/ml concentration as a percentage of 16.1% total viable cells (Figure 6).

Apoptotic/Necrotic Activity Results

The result of the double staining experiment showed parallelism with the MTT test and a decrease in cells was observed. The results of this test performed to determine the mechanism of this decrease in cells are given in Figure 7.

In line with these results; In the A549 cell line, (-) control cells remained healthy and their number increased. HO stain (-), a dye that can pass through all membranes and stain the nucleus, stained the cells in the control group, but the PI dye, which can stain cells with impaired membrane integrity and necrosis, could not stain this group. For this reason, the cells remained viable and reproduced as expected. Likewise, the cells in the (+) control group, that is, the cells treated with doxorubicin drug, were clearly decreased in number and density and stained with PI dye, which gave us the result that doxorubicin killed the cells by necrosis.

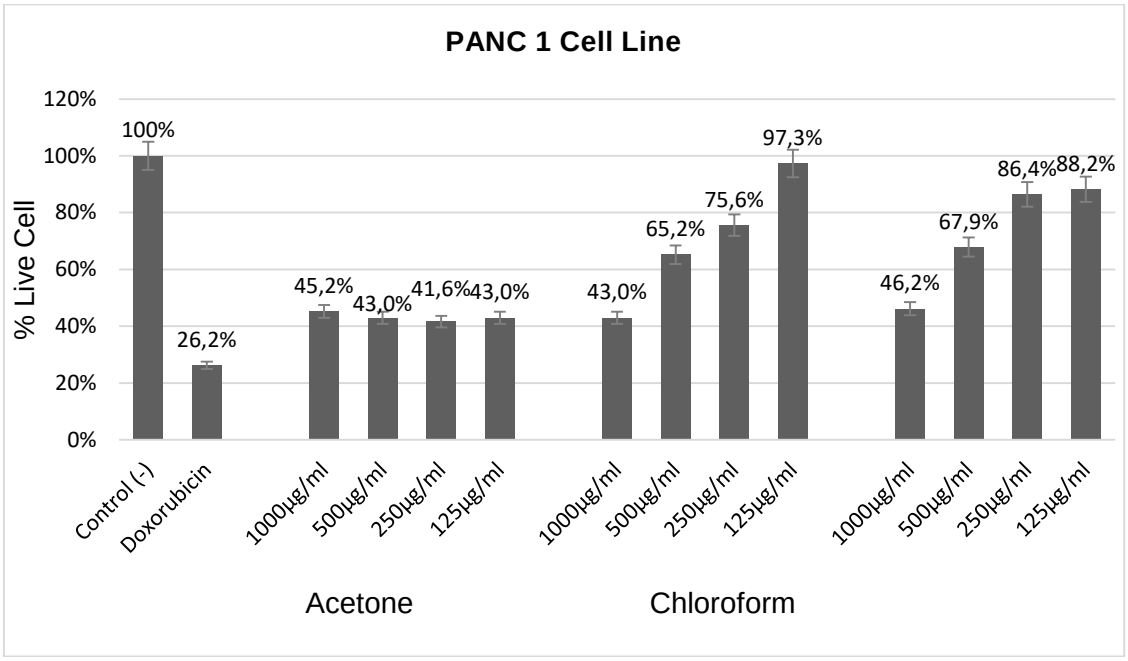

Figure 5. Cytotoxic activity of *M. pulegium* on PANC 1 cell line
Şekil 5. *M. pulegium*'un PANC 1 hücre hattı üzerindeki sitotoksik aktivitesi

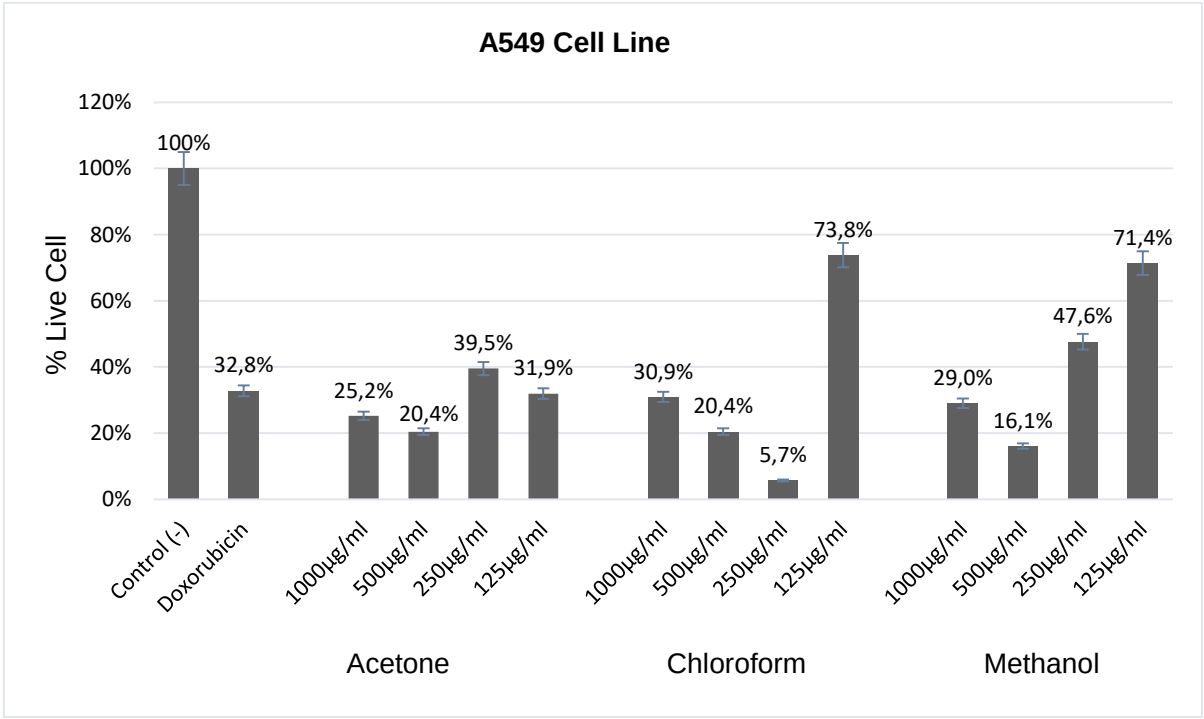

Figure 6. Cytotoxic activity of *M. pulegium* on A549 cell line
Şekil 6. *M. pulegium*'un A549 hücre hattı üzerindeki sitotoksik aktivitesi

Table 5. MDA-MB 231 cell IC50 results
Çizelge 5. MDA-MB 231 hücresi IC50 sonuçları

	MDA-MB 231 cells		
	Mentha pulegium		
	Aseton ekstrat	**Kloroform ekstrat**	**Metanol ekstrat**
IC_{50}	452 µg/ml	125 µg/ml	235µg/ml

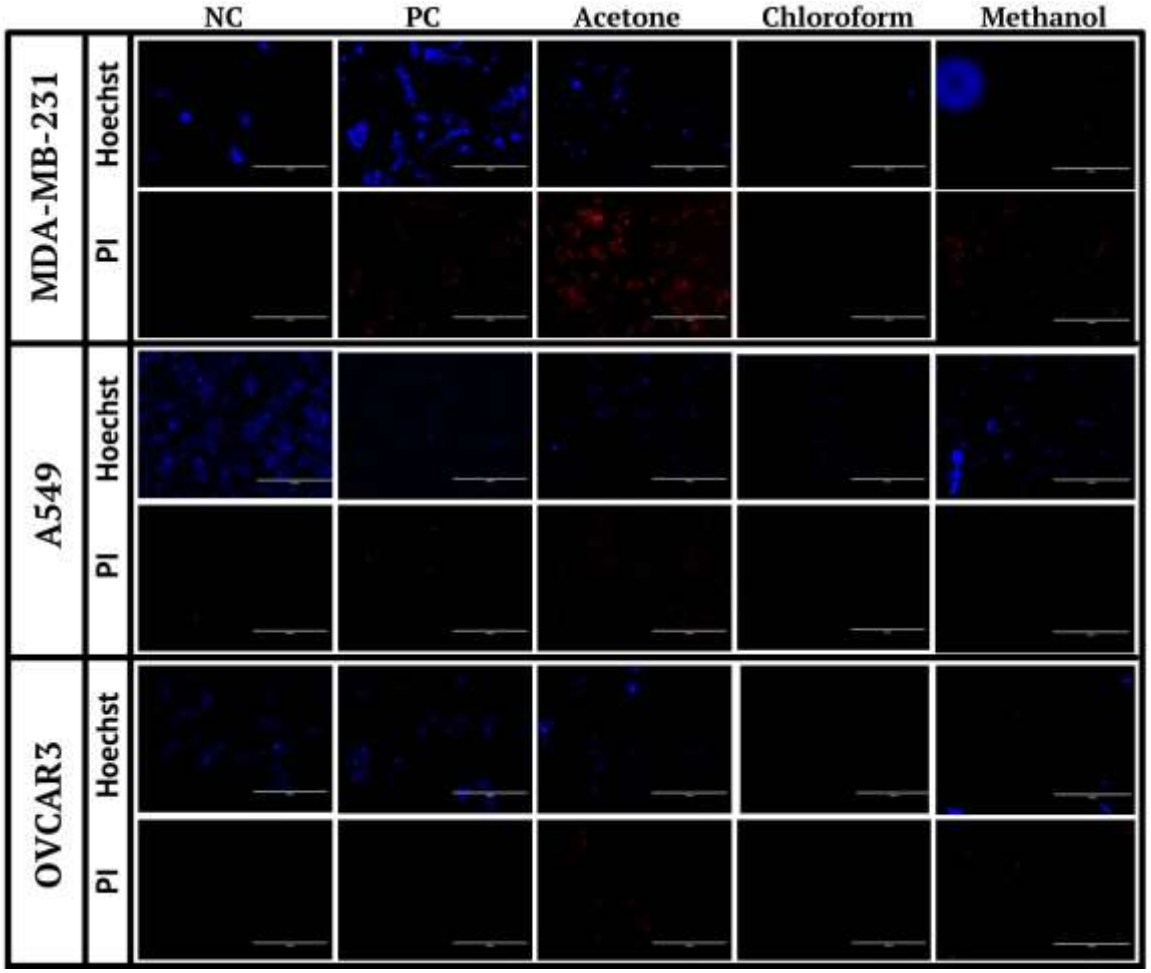

Figure 7. Dual staining apoptic/necrotic activity assay images of *M. pulegium* on MDA-MB 231, A549 and OVCAR3 cell lines

Şekil 7. MDA-MB 231, A549 ve OVCAR3 hücre hatları üzerinde M. pulegium'un ikili boyama apoptik/nekrotik aktivite deneyi görüntüleri

Table 6. OVCAR3 cell IC50 results

Çizelge 6. OVCAR3 hücresi IC50 sonuçları

	OVCAR3 cells		
	Mentha pulegium		
	Aseton ekstrat	**Kloroform ekstrat**	**Metanol ekstrat**
IC_{50}	156 µg/ml	482 µg/ml	483 µg/ml

Table 7. IC50 results for cell PANC1

Çizelge 7. PANC1 hücresi için IC50 sonuçları

	PANC1 cells		
	Mentha pulegium		
	Aseton ekstrat	**Kloroform ekstrat**	**Metanol ekstrat**
IC_{50}	129 µg/ml	500 µg/ml	272 µg/ml

Table 8. IC50 results for cell A549

Çizelge 8. A549 hücresi için IC50 sonuçları

	A549 cells		
	Mentha pulegium		
	Aseton ekstrat	**Kloroform ekstrat**	**Metanol ekstrat**
IC_{50}	225 µg/ml	303 µg/ml	488 µg/ml

In *M. pulegium* extracts applied to cells, the most prominent result was seen in methanol extract. The vesicle shapes and dispersed membrane seen in the cells represent apoptotic morphology. Likewise, not staining with PI dye showed that these cells died by apoptosis. In addition, the cells stained with HO dye in the acetone extract did not give apoptotic morphology and were not stained with PI dye, indicating that the cells remained viable or were early apoptotic cells. On the other hand, the cells to which chloroform extract was applied decreased in number but were not fully stained with either dye, indicating that the cells could be secondary necrotic or viable cells.

IC50 values (50% inhibition of cell growth) were calculated based on the MTT assay results as shown in table 5, table 6, table 7 and table 8. The lowest IC50 value in MDA-MB231 cell line was observed in chloroform extract (Table 5.), while the lowest IC50 value in OVAR3, PANC1 and A549 cell lines was given by acetone extract (Table 6., Table 7., Table 8.).

DISCUSSION and CONCLUSION

Plant species were used as traditional medicines for the treatment of many diseases, especially infectious diseases (Gonzalez-Tejero et al., 2008). Considering the biological potential of plants as a source of antimicrobial drugs, the antimicrobial activity of *M. pulegium* was tested with acetone, chloroform and methanol solvents in this study. In this study, antimicrobial effects of acetone, chloroform and methanol extracts of *M. pulegium* against *K. pneumoniae*, *E. coli*, *S. aureus*, *B. megaterium* and *C. albicans* were determined with agar wells. In addition to antimicrobial activity, minimal inhibition concentration and minimal bactericidal concentration levels were also examined.

Of the five Lamiaceae species; It has been reported that *Mentha piperita, Mentha pulegium, Lavandula angustifolia, Satureja montana* and *Salvia lavandulifolia* have significant antimicrobial effects against some bacteria (*Streptococcus mutans, Streptococcus sanguis, Streptococcus salivarius, Streptococcus pyogenes, Pseudomonas aeruginosa, Lactobacillus acidophilus,* and *Enterecoccus feacalis*) (Nikolic et al., 2014).

In another study, Marzouk et al. (2008) defined the chemical composition of fresh and dried *M. pulegium L.* essential oils. All oils were found to be rich in oxygen monoterpene hydrocarbons. In *M. pulegium* applied to microorganisms, gram-positive species are more sensitive to essential oils from fresh leaves collected in the vegetative state than dried ones (Morzouk et al., 2008). Marzouk et al. found the maximum inhibition zones of fungal species sensitive to the essential oil of *M. longifolia* and *M. pulegium* in the range of 9-15 and 8-25 mm, respectively.

Some researchers investigating the antimicrobial effect of plant extracts have used a wide variety of solvents for different parts of different plants (Cowan, 1999). The main solvents among these are solvents such as acetone, ethanol, n-hexane, methanol, chloroform and methylene chloride (Caceres et al., 1990). It is not possible to make a preference order for all plants and plant parts among these solvents. However, considering the studies in the literature, acetone, chloroform and methanol were preferred as solvents in this study, which is generally used extensively and considering the positive results. In this study, *E. coli* formed an inhibition zone of 24, 21, 20 and 10 mm in length in the methanol extract of *M. pulegium* at concentrations of 100, 50, 25 and 5 mg/ml, respectively. The highest inhibition zone was found to be 25 mm in *B. megaterium*. Based on these results, it is possible to conclude that the essential oil has a stronger antimicrobial activity spectrum than the acetone and chloroform extract compared to the methanol extract.

In many studies, DPPH has been widely used to determine antiradical/antioxidant capacities (Soylu and Çebi, 2017; Mensor et al., 2001; Sharma and Bhat, 2009). In the present study, the antioxidant activity of *M. pulegium* essential oil and methanol extract was determined by three different test systems: DPPH, ascorbic acid and reducing power. It was concluded that *M. pulegium* plant has high bioactive components and depending on these values, it can be used as a potential antioxidant source in the pharmaceutical and food industries. It is important to carry out more studies on the use of this plant in the prevention and treatment of diseases, rich in bioactive components, which have important effects on health, and also to ensure the continuity of the researches towards industrial applications by determining the contributions of this plant in food compositions.

Since cancer is a disease for which there is no cure yet, the development of therapeutic drugs is considered in a special status by international health organizations. Medicinal plants have long been a fruitful resource for the treatment of cancer, which is predicted to be the main cause of death this century (Shoeb, 2006; Tariq et al., 2017). In the study, the cytotoxic activity of three extracts (acetone, chloroform, methanol) obtained from the above-ground part of *M. pulegium* was determined by MTT test in MDA MB-231, PANC-1, OVCAR3 and A549 cell lines. It was determined that *M. pulegium* extract had a cytotoxic effect against MDA-MB-231, PANC1, OVCAR3 and A549 cells at applied concentrations. It was determined that especially *M. pulegium* methanol extracts had a significant cytotoxic effect on the OVCAR3 cell line. It was observed that the solvent that showed the most important effect

among the solvents was the chloroform solvent.

Ovarian cancer (OC) is responsible for the highest tumor-related mortality among gynecological malignancies (Woopen and Shrouli, 2009). In most OC patients diagnosed with advanced stages (III and IV), this creates panic and provokes an emergency to explore a new therapeutic strategy. Plants with medicinal properties attract attention because they are enriched with various chemical compounds that have the potential to treat various diseases. Providing innovative and important clues to a range of pharmacological targets for a human disease management system is a long process (Ebona et al., 2018; Dutta et al., 2018). Although the development of a new drug has faced challenges and difficulties, the emergence of combinatorial chemistry provides a new glimmer of hope, and the effort to discover the drug and a chemical compound has been quite successful (Liu et al., 2001). By incorporating new cytotoxic agents, the standard platinum-based treatment for ovarian cancer Numerous studies have been conducted to increase its effectiveness (Buys et al., 2005). The combination of natural compounds with standard chemotherapeutic drugs may exert additive or synergistic effects in killing cancer cells, thus achieving better therapeutic effect or allowing lower and safer drug doses to be administered (Coşkun, 2017).

As a result of the study, it was observed that *M. pulegium* extract may have active substances that can be used in the development of new drugs and that it has a cytotoxic effect. Obtained IC50 values are 452 µg/ml, 125 µg/ml and 235 µg/ml in MDA-MB 231 cells, 225 µg/ml, 303 µg/ml and 488 µg/ml in A549 cells, 129 µg/ml , 500 µg in PANC1 cells /ml and 272 µg/m were determined as 165 µg/ml, 482 µg/ml and 272 µg/ml in OVCAR3 cells, showing that *M. pulegium* has a promising growth inhibitory effect on cancer cells.

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Contribution Rate Statement Summary of Researchers

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

Conflict of Interest Statement

The authors of the article declare that there is no conflict of interest between them.

REFERENCES

Awouafack, M. D., McGaw, L. J., Gottfried, S., Mbouangouere, R., Tane, P., Spiteller, M., & Eloff, J. N. (2013). Antimicrobial activity and cytotoxicity of the ethanol extract, fractions and eight compounds isolated from Eriosema robustum (Fabaceae). *BMC Complementary and Alternative Medicine*, *13*, 1-9. https://doi.org/10.1186/1472-6882-13-289

Ahmed, A., Ayoub, K., Chaima, A. J., Hanaa, L., & Abdelaziz, C. (2018). Effect of drying methods on yield, chemical composition and bioactivities of essential oil obtained from Moroccan Mentha pulegium L. Biocatalysis and Agricultural Biotechnology, 16, 638-643. https://doi.org/ 10.1016/ j.bcab.2018.10.016

Andersson, D. I., & Hughes, D. (2011). Persistence of antibiotic resistance in bacterial populations. *FEMS microbiology reviews*, *35*(5), 901-911. https://doi.org/10.1111/j.1574-6976.2011. 00289.x

Benlarbi, K. H., Elmtili, N., Macías, F. A., & Galindo, J. C. G. (2014). Influence of in vitro growth conditions in the production of defence compounds in Mentha pulegium L. *Phytochemistry Letters*, *8*, 233-244. https://doi.org/10.1016/j.phytol. 2014.03. 007

Buys, S. S., Partridge, E., Greene, M. H., Prorok, P. C., Reding, D., Riley, T. L., ... & PLCO Project Team. (2005). Ovarian cancer screening in the Prostate, Lung, Colorectal and Ovarian (PLCO) cancer screening trial: findings from the initial screen of a randomized trial. *American journal of obstetrics and gynecology*, *193*(5), 1630-1639. https://doi.org/ 10.1016/j.ajog.2005.05.005

Chalchat, J. C., Gorunovic, M. S., Maksimovic, Z. A., & Petrovic, S. D. (2000). Essential oil of wild growing Mentha pulegium L. from Yugoslavia. *Journal of Essential Oil Research*, *12*(5), 598-600. https://doi.org/10.1080/10412905.2000.9712166

Cevatemre, B. (2012). *Fenretinid ve indol-3-karbinol kombinasyonunun meme kanseri hücre soyları üzerindeki sitotoksik/apoptotik etkilerinin araştırılması* (Doctoral dissertation, Bursa Uludag University (Turkey)).

Cowan, M. M. (1999). Plant products as antimicrobial agents. *Clinical microbiology reviews*, *12*(4), 564-582. https://doi.org/10.1128/CMR.12.4.564

Coşkun, M. D. (2017). *NF-κB ve oksidatif stresin inhibisyonu ile peripheral nöropatinin iyileştirilmesi ve kanser kemoterapi sonuçlarinin geliştirilmesi* (Doctoral dissertation, Bursa Uludag University (Turkey)).

Caceres, A., Cano, O., Samayoa, B., & Aguilar, L. (1990). Plants used in Guatemala for the treatment of gastrointestinal disorders. 1. Screening of 84 plants against enterobacteria. *Journal of Ethnopharmacology*, *30*(1), 55-73. https://doi.org/

10.1016/0378-8741(90)90017-n

Çöteli, E., Erden, Y., & Karataş, F. (2013). Yarpuz (Mentha pulegium L.) bitkisindeki malondialdehit, glutatyon ve vitamin miktarları ile total antioksidan kapasitesinin araştırılması. *Süleyman Demirel Üniversitesi Fen Bilimleri Enstitüsü Dergisi*, *17*(2), 4-10. https://dergipark.org.tr/en/pub/sdufenbed/issue/20801/222003

Dorman, H. D., Koşar, M., Kahlos, K., Holm, Y., & Hiltunen, R. (2003). Antioxidant properties and composition of aqueous extracts from Mentha species, hybrids, varieties, and cultivars. *Journal of agricultural and food chemistry*, *51*(16), 4563-4569. https://doi.org/10.1021/jf034108k

Dalkılıç, S., Dalkılıç, L. K., & Korkmaz, İ. (2020). Geleneksel Kahvaltılık Zahterin Antimikrobiyal Etkisi. *Gümüşhane Üniversitesi Fen Bilimleri Dergisi*, *10*(1), 128-133. https://doi.org/10.17714/gumusfenbil.579489

Dutta, D., Chakraborty, A., Mukherjee, B., & Gupta, S. (2018). Aptamer-conjugated apigenin nanoparticles to target colorectal carcinoma: a promising safe alternative of colorectal cancer chemotherapy. *ACS Applied Bio Materials*, *1*(5), 1538-1556. https://doi.org/10.1021/acsabm.8b00441

Erarslan, Z. B., Ecevit-Genç, G., & Kültür, Ş. (2020). Medicinal plants traditionally used to treat skin diseases in Turkey–eczema, psoriasis, vitiligo. *Journal of Faculty of Pharmacy of Ankara University*, *44*(1), 137-166. https://doi.org/10.33483/jfpau.586114

Erdoğan, E. A., Everest, A., & Kaplan, E. (2013). Antimicrobial activities of aqueous extracts and essential oils of two endemic species from Turkey. *Indian Journal of Traditional Knowledge*, *12*(2), 221-224.

Ebana, R. U. B., Etim, O. E., Edet, U. O., John, R. S., & Osom, A. O. Comparative Evaluation of Phytochemicals and Antibacterial Activity of the Roots and Leaves of Anthocleista vogelii on Some Clinical Isolates.

Faydaoğlu, E., & Sürücüoğlu, M. (2013). Tibbi ve aromatik bitkilerin antimikrobiyal, antioksidan aktiviteleri ve kullanim olanaklari. *Erzincan University Journal of Science and Technology*, *6*(2), 233-265.

Greenwell, M., & Rahman, P. K. S. M. (2015). Medicinal plants: their use in anticancer treatment. *International journal of pharmaceutical sciences and research*, *6*(10), 4103. https://dx.doi.org/10.13040%2FIJPSR.0975-8232.6(10).4103-12

González-Tejero, M. R., Casares-Porcel, M., Sánchez-Rojas, C. P., Ramiro-Gutiérrez, J. M., Molero-Mesa, J., Pieroni, A., ... & ElJohrig, S. (2008). Medicinal plants in the Mediterranean area: synthesis of the results of the project Rubia. *Journal of Ethnopharmacology*, *116*(2), 341-357. https://doi.org/ 10.1016/j.jep.2007.11.045

Gülçin, İ., Gören, A. C., Taslimi, P., Alwasel, S. H., Kılıc, O., & Bursal, E. (2020). Anticholinergic, antidiabetic and antioxidant activities of Anatolian pennyroyal (Mentha pulegium)-analysis of its polyphenol contents by LC-MS/MS. *Biocatalysis and agricultural biotechnology*, *23*, 101441. https://doi.org/10.1016/j.bcab.2019.101441

Hassan, A., & Ullah, H. (2019). Antibacterial and antifungal activities of the medicinal plant veronica biloba. *Journal of chemistry*, *2019*, 1-7. https://doi.org/10.1155/2019/5264943

Hesham, H. M., Lasheen, D. S., & Abouzid, K. A. (2018). Chimeric HDAC inhibitors: Comprehensive review on the HDAC-based strategies developed to combat cancer. *Medicinal Research Reviews*, *38*(6), 2058-2109. https://doi.org/10.1002/med.21505

Hemeg, H. A., Moussa, I. M., Ibrahim, S., Dawoud, T. M., Alhaji, J. H., Mubarak, A. S., ... & Marouf, S. A. (2020). Antimicrobial effect of different herbal plant extracts against different microbial population. *Saudi Journal of Biological Sciences*, *27*(12), 3221-3227. https://doi.org/10.1016/j.sjbs.2020.08.015

Kurt, H., Keşkek, Ş. Ö., Çil, T., & Canataroğlu, A. (2013). Meme kanserli hastalarda tamamlayıcı/alternatif tedavi kullanımı. *Türk Onkoloji Dergisi*, *28*(1), 10-15. https://doi.org/10.5505/tjoncol.2013.827

Lowy, F. D. (2003). Antimicrobial resistance: the example of Staphylococcus aureus. *The Journal of clinical investigation*, *111*(9), 1265-1273. https://doi.org/10.1172/JCI18535

Liu, J., Chen, H., Miller, D. S., Saavedra, J. E., Keefer, L. K., Johnson, D. R., ... & Waalkes, M. P. (2001). Overexpression of glutathione S-transferase II and multidrug resistance transport proteins is associated with acquired tolerance to inorganic arsenic. *Molecular pharmacology*, *60*(2), 302-309. https://doi.org/10.1124/mol.60.2.302

McGaw, L. J., & Eloff, J. N. (2008). Ethnoveterinary use of southern African plants and scientific evaluation of their medicinal properties. *Journal of Ethnopharmacology*, *119*(3), 559-574. https://doi.org/10.1016/j.jep.2008.06.013

Mamadalieva, N. Z., Hussain, H., & Xiao, J. (2020). Recent advances in genus Mentha: Phytochemistry, antimicrobial effects, and food applications. *Food Frontiers*, *1*(4), 435-458. https://doi.org/10.1002/fft2.53

Marzouk, B., Fredj, M. B. H., Chraief, I., Mastouri, M., Boukef, K., & Marzouk, Z. (2008). Chemical composition and antimicrobial activity of essential oils from Tunisian Mentha pulegium L. *Journal of Food Agriculture and Environment*, *6*(1), 78.4

Mensor, L. L., Menezes, F. S., Leitão, G. G., Reis, A. S., Santos, T. C. D., Coube, C. S., & Leitão, S. G. (2001). Screening of Brazilian plant extracts for

antioxidant activity by the use of DPPH free radical method. *Phytotherapy research*, *15*(2), 127-130. https://doi.org/10.1002/ptr.687

Nascimento, G. G., Locatelli, J., Freitas, P. C., & Silva, G. L. (2000). Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria. *Brazilian journal of microbiology*, *31*, 247-256. https://doi.org/10.1590/S1517-83822000000400003

Nikolić, M., Jovanović, K. K., Marković, T., Marković, D., Gligorijević, N., Radulović, S., & Soković, M. (2014). Chemical composition, antimicrobial, and cytotoxic properties of five Lamiaceae essential oils. *Industrial Crops and Products*, *61*, 225-232.

Pucci, C., Martinelli, C., & Ciofani, G. (2019). Innovative approaches for cancer treatment: Current perspectives and new challenges. *Ecancermedicalscience*, *13*. https:// dx.doi.org/ 10.3332%2Fecancer.2019.961

Sofowora, A., Ogunbodede, E., & Onayade, A. (2013). The role and place of medicinal plants in the strategies for disease prevention. *African journal of traditional, complementary and alternative medicines*, *10*(5), 210-229. https://doi.org/ 10.4314/ajtcam.v10i5.2

Sakagami, Y., & Kajimura, K. (2002). Bactericidal activities of disinfectants against vancomycin-resistant enterococci. *Journal of Hospital Infection*, *50*(2), 140-144. https://doi.org/10.1053/ jhin.2001.1150

Swamy, M. K., Akhtar, M. S., & Sinniah, U. R. (2016). Antimicrobial properties of plant essential oils against human pathogens and their mode of action: an updated review. *Evidence-based complementary and alternative medicine*, *2016*. https://doi.org/ 10.1155/2016/3012462

Salem, N., Bachrouch, O., Sriti, J., Msaada, K., Khammassi, S., Hammami, M., ... & Mediouni Ben Jemaa, J. (2017). Fumigant and repellent potentials of Ricinus communis and Mentha pulegium essential oils against Tribolium castaneum and Lasioderma serricorne. *International journal of food properties*, *20*(sup3), S2899-S2913. https:// doi.org/10.1080/10942912.2017.1382508

Stockert, J. C., Blázquez-Castro, A., Cañete, M., Horobin, R. W., & Villanueva, Á. (2012). MTT assay for cell viability: Intracellular localization of the formazan product is in lipid droplets. *Acta histochemica*, *114*(8), 785-796. https://doi.org/ 10.1016/j.acthis.2012.01.006

Soylu, E. N., & Çebi, A., (2017). Scenedesmus sp.'nin DPPH (1, 1-difenil-2-pikrilhidrazil) Serbest Radikal Süpürücü Aktivitesi. *Karadeniz Fen Bilimleri Dergisi*, *7*(2), 146-151. https://doi.org/10.31466/ kfbd.353777

Sharma, O. P., & Bhat, T. K. (2009). DPPH antioxidant assay revisited. *Food chemistry*, *113*(4), 1202-1205. https://doi.org/10.1016/j.foodchem.2008.08.008

Shoeb, M. (2006). Anti-cancer agents from medicinal plants. *Bangladesh journal of Pharmacology*, *1*(2), 35-41. https://doi.org/10.3329/bjp.v1i2.486

Şekerli, M., KILIÇ, N., & DUMAN, D. C. (2017). Liken metabolitlerinin antikanser aktivite etkisinin moleküler düzeyde mekanizmaları. *Türk Hijyen ve Deneysel Biyoloji Dergisi*, *74*(1), 95-102. https://dx.doi.org/10.5505/TurkHijyen.2016.24650

Top, R. (2018). *Bazı önemli tıbbi bitkilerin antioksidan, antimikrobiyal ve antikanser etkilerinin araştırılması* (Master's thesis, Bartın Üniversitesi, Fen Bilimleri Enstitüsü). https:// doi.org/10.17798/bitlisfen.485413

Tariq, A., Sadia, S., Pan, K., Ullah, I., Mussarat, S., Sun, F., ... & Adnan, M. (2017). A systematic review on ethnomedicines of anti-cancer plants. *Phytotherapy Research*, *31*(2), 202-264. https://doi.org/10.1002/ptr.5751

Ugboko, H. U., Nwinyi, O. C., Oranusi, S. U., Fatoki, T. H., & Omonhinmin, C. A. (2020). Antimicrobial importance of medicinal plants in Nigeria. *The Scientific World Journal*, *2020*. https://doi.org/ 10.1155/2020/7059323

Wińska, K., Mączka, W., Łyczko, J., Grabarczyk, M., Czubaszek, A., & Szumny, A. (2019). Essential oils as antimicrobial agents—myth or real alternative?. *Molecules*, *24*(11), 2130. https:// doi.org/10.3390/molecules24112130

Wink, M. (2003). Evolution of secondary metabolites from an ecological and molecular phylogenetic perspective. *Phytochemistry*, *64*(1), 3-19. https:// doi.org/10.1016/s0031-9422(03)00300-5

Woopen, H., & Sehouli, J. (2009). Current and future options in the treatment of malignant ascites in ovarian cancer. *Anticancer research*, *29*(8), 3353-3359.

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Contributions to Curculionidae (Coleoptera) Fauna of Southeastern Anatolia Region of Türkiye with a new Record

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ABSTRACT
In this study, individuals belonging to the Curculionidae family collected from the Southeastern Anatolia Region were examined. Samples were collected by namely the knocking and sweep net method between 1993-2019 dates. The collected specimens were identified by the second author after they were turned into standard museum material. As a result of the study, 11 genera belonging to 5 subfamilies of the Curculionidae (Coleoptera) family and 18 species belonging to these genera were determined. Among these species, 1 species (*Coniocleonus hollbergii* Fåhraeus, 1842) has been reported as a new record for the fauna of Türkiye. In addition, 9 species were reported as new records from the region. The distribution of the species in the world and Türkiye, as well as their regional distribution and hosts, are given.

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Türkiye'nin Güneydoğu Anadolu Bölgesi Curculionidae (Coleoptera) Faunasına Katkılar ve Yeni Bir Kayıt

ÖZET
Bu çalışmada, Güneydoğu Anadolu Bölgesi'nden toplanan Curculionidae familyasına ait bireyler incelenmiştir. Örnekler 1993-2019 tarihleri arasında atrap ve darbe yöntemiyle toplanmıştır. Toplanılan örnekler standart müze materyali haline getirildikten sonra 2. yazar tarafından teşhis edilmiştir. Çalışma neticesinde Curculionidae (Coleoptera) familyasından 5 altfamilyaya ait 11 cins ve bu cinslere ait 18 tür tespit edilmiştir. Bu türler içerisinde 1 tür (*Coniocleonus hollbergii* Fåhraeus, 1842) Türkiye faunası için yeni kayıt olarak bildirilmiştir. Ayrıca 9 tür ise bölgeden yeni kayıt olarak bildirilmiştir. Türlerin dünya ve Türkiye dağılışları ile birlikte bölge yayılışları ve konukçuları verilmiştir.

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INTRODUCTION
Curculionidae is the largest insect family in the superfamily Curculionoidea. The members of this family are most commonly found. Curculionidae shows considerable variation in size and shape. The rostrum is well developed in most species, with capitate antennae appearing in the middle. The Curculionidae species present a complete metamorphosis (egg, larva, pupa, and adult) (Fuentes et al., 2017). All members are phytophagous and the group is important economically. There are many important agricultural and forest pests within the family. They can attack specific parts of the plant, from the roots to the aerial parts; usually, the larvae feed within plant tissues and adults make holes in the fruits, nuts, and other parts (Hoffmann, 1950; Mihajlova, 1978; Domínguez, 2006), except for myrmecophilous, saprophagous and predatory species (Muñiz, 1970).

The fauna of Curculionidae (Coleoptera) of Türkiye is richer, because of different climatic conditions. Both geographic position and climatic differentiations have some effects on fauna (Lodos et al., 2003). The Curculionidae fauna of Türkiye has been studied by many scientists (Lodos, 1960; Lodos et al., 1978, 2003; Sert, 1990, 1995; Gözüaçık and Özgen, 2005; Keskin, 2005; Pehlivan et al., 2005a, b; Gültekin, 2006; Bolu and Legalov, 2008; Bolu and Özgen, 2009; Erbey, 2010; Avgın and Colonnelli, 2011; Erbey and Gürler, 2014; Erbey, 2015). The current study aimed to review the collection material and to extend our knowledge on the fauna on Curculionidae (Coleoptera) in the Southeastern Anatolia Region of Türkiye.

MATERIAL and METHOD

This study was carried out between 1993-2019 in the provinces of Diyarbakır and Şanlıurfa, located in the Southeastern Anatolia Region of Türkiye. The collection of samples in this study; namely knocking, sweeping net and the method of culturing by collecting together with the plants on which Curculionidae larvae feed were used. The samples were prepared as standard museum material. Specimens were collected and identified by the authors. While making diagnoses (Caldara, 1990; Alonso-Zarazaga, 1999; Colonnelli, 2004; Velázquez de Castro et al., 2010; Erbey, 2010; Skuhrovec et al., 2014; Alonso-Zarazaga et al., 2017; Arzanov and Grebennikov, 2017, etc.) resources such as in addition, diagnostic museum materials in the collection of the second author were taken into account.

RESULTS

Family: Curculionidae Latreille, 1802 Subfamily: Ceutorhynchinae Gistel, 1848

Genus: *Coeliodes* Schönherr, 1837

***Coeliodes ruber* Marsham, 1802**

Material examined: 2♀♀4♂♂, 08.IV.1993, 5♀♀2♂♂, 15.IV.1993, 3♀♀5♂♂, 22.IV.1993, 4♀♀6♂♂, 29.IV.1993, 5♀♀5♂♂, 06.V.1993 Şanlıurfa (Multiple samples).

Host plant: *Pistacia vera* L.

Record Host plants: *Quercus* spp. (Colonnelli, 2004), *Rosa* (Lodos et al., 2003).

Distribution in World: Armenia, Austria, Belarus, Belgium, Bulgaria, Czech Republic, France, Georgia, Germany, Hungary, Italy, Moldova, Netherlands, Poland, Romania, Russia (Europe), Slovakia, Spain, Switzerland, Türkiye, Ukraine (Colonnelli, 2004).

Distribution in Southeastern Anatolia Region of Türkiye: *Coeliodes ruber* is the first record for the fauna of Şanlıurfa province. Moreover; this species is also the first record for the fauna of the Southeastern Anatolia Region of Türkiye.

Genus: *Mogulones* Reitter, 1916

***Mogulones crucifer* (Pallas, 1771)**

Material examined: 1♀1♂, 01.V.2002, Diyarbakır (Dicle University Campus).

Host plant: Unknown

Record Host plants: *Cynoglossum* and *Solenanthus* (Colonnelli, 2004).

Distribution in World: Austria, Belarus, Belgium, Bulgaria, Canada, Denmark, Estonia, France, Georgia, Germany, Greece, Hungary, Italy, Netherlands, Spain, Sweden, Switzerland, Kazakhstan, Kyrgyzstan, Lithuania, Moldova, Norway, Poland, Portugal, Romania, Russia (European), Serbia, Siberia (western and central), Slovakia, Slovenia, Türkiye, Ukraine, (Colonnelli, 2004).

Distribution in the Southeastern Anatolia Region of Türkiye: This species is a first record for the fauna of Diyarbakır province. Moreover; this species is also the first record for the fauna of the Southeastern Anatolia Region of Türkiye.

Genus: *Stenocarus* Thomson, 1865

***Stenocarus ruficornis* (Stephens, 1831)**

Material examined: 1♂, 01.V.2007, Diyarbakır (Dicle University Campus).

Host plant: Unknown

Record Host plants: *Papaver*, *Glaucium* (Colonnelli, 2004).

Distribution in World: Austria, Belarus, Belgium, Bulgaria, Czech Republic, Denmark, England, France, Georgia, Germany, Ireland, Kazakhstan, Kyrgyzstan, Macedonia, Moldova, Netherlands, Poland, Portugal, Romania, Russia (Europe), Siberia (western and central), Slovakia, Slovenia, Spain, Sweden, Switzerland, Tunisia, Türkiye, Ukraine (Colonnelli, 2004).

Distribution in the Southeastern Anatolia Region of Türkiye: This species is a first record for the fauna of Diyarbakır province. Moreover; this species is also the first record for the fauna of the Southeastern Anatolia Region of Türkiye.

Subfamily: Curculioninae Latreille, 1802

Genus: *Rhinusa* Stephens, 1829

***Rhinusa asellus* (Gravenhorst, 1807)**

Material examined: 5♀♀ 5♂♂, Diyarbakır (This species has been obtained from laboratory larval culture) (Larvae were collected from Ergani district on 1, 16 and 17 May 2019, and from Yenişehir district on 8 May 2019).

Host plants: *Verbascum* spp.

Record Host plants: *Althaea*, *Prunus*, *Verbascum*

(Lodos et al., 2003).

Distribution in the World: Austria, Bulgaria, Czech Republic, East Palaearctic, French mainland, Germany, Italian mainland, Near East, Poland, Sardinia, Sicily, Slovakia, Switzerland (Anonymous, 2019).

Distribution in Türkiye: Kayseri, Kırşehir, Konya, Yozgat (Sert, 1995); Adana, Mersin, Niğde, Tarsus (Erbey, 2010).

Distribution in the Southeastern Anatolia Region of Türkiye: *Rhinusa asellus* is a first record for the fauna of Diyarbakır province. Moreover; this species is also the first record for the fauna of the Southeastern Anatolia Region of Türkiye.

***Rhinusa tetra* (Fabricius, 1792)**

Material examined: 5♀♀ 5♂♂, Diyarbakır (This species has been obtained from laboratory larval culture) (Larvae were collected from Ergani district on 1, 16, and 17 May 2019, and from Yenişehir district on 8 May 2019).

Host plants: *Verbascum* spp.

Record Host plants: *Pinus*, *Prunus*, *Quercus*, *Sinapis*, *Styrax*, *Triticum*, *Vicia*, *Verbascum* (Lodos et al., 2003).

Distribution in the World: Albania, Austria, Belarus, Bosnia and Herzegovina, Bulgaria, Central European Russia, Corsica, Croatia, Czech Republic, East European Russia, East Palaearctic, European Türkiye, French mainland, Germany, Greek mainland, Hungary, Italian mainland, Macedonia, the former Yugoslav Republic of, Near East, Nearctic region, North Africa, Poland, Portuguese mainland, Romania, Sardinia, Sicily, Slovakia, Slovenia, South European Russia, Spanish mainland, The Netherlands, Ukraine, Yugoslavia (Anonymous, 2019).

Distribution in Türkiye: Adana, Elazığ, Mersin, Niğde, Tarsus (Erbey, 2010; Özgen et. al., 2016)

Distribution in the Southeastern Anatolia Region of Türkiye: *Rhinusa tetra* is the first record for the fauna of Diyarbakır province. Moreover; this species is also the first record for the fauna of the Southeastern Anatolia Region of Türkiye.

Genus: *Tychius* Germar, 1817

***Tychius aureolus* Kieswetter, 1851**

Material examined: 2♀♀1♂, 21.X.1998 Şanlıurfa (Tülmen Village).

Host plant: *Pistacia vera* L.

Record Host plants: *Amygdalus communis* L., *Avena sativa* L., *Medicago sativa* L., *Onobrychis sativa* Lam., *Vicia sativa* L. (Bingöl, 1978; Akkaya, 1995).

Distribution in the World: Central Asia, Europe, Türkiye (Hoffmann, 1954; Lodos et al., 1978).

Distribution in the Southeastern Anatolia Region of Türkiye: This species has been recorded for the provinces of Adıyaman, Batman, Diyarbakır, Gaziantep, Mardin, Siirt, Şanlıurfa and Şırnak in previous studies (Bingöl, 1978; Akkaya, 1995).

Subfamily: Entiminae Schoenherr, 1823

Genus: *Polydrusus* Germar, 1817

***Polydrusus corruscus* Germar, 1824**

Material examined: 8♀♀5♂♂, 15.IV.1993, Şanlıurfa.

Host plant: *Pistacia vera* L.

Record Host plants: *Salix*, *Populus* (Lodos et el., 2003).

Distribution in the World: Caucasus, Corsica, France, Russia (West), Switzerland, Türkiye, Yugoslavia (Dalla Torre et al., 1931 to 1939; Hoffmann, 1950).

Distribution in the Southeastern Anatolia Region of Türkiye: *Polydrusus corruscus* is a first record for the fauna of Şanlıurfa province. Moreover; this species is also the first record for the fauna of the Southeastern Anatolia Region of Türkiye.

Genus: *Sitona* Germar, 1824

***Sitona crinitus* (Herbst, 1795)**

Material examined: 2♀♀2 ♂♂, 22.IV.1993, Şanlıurfa.

Host plant: *Pistacia vera* L.

Record Host plants: *Salix*, *Astragalus*, *Malus*, *Medicago*, *Pyrus*, *Prunus*, *Vicia* (Lodos et al., 2003); *Avena sativa* L., *Lens culinaris* L., *Medicago sativa* L., *Onobrychis sativa* Lam., *Pistacia vera* L., *Vicia sativa* L. (Lodos et al., 1984; Bolu et al., 2005)

Distribution in the World: North Africa, North America, West and Central Asia, Türkiye (Dalla Torre et al., 1931-1939; Lodos et al., 1978; Dieckmann, 1980).

Distribution in the Southeastern Anatolia Region of Türkiye: This species has been recorded for the provinces of the Southeastern Anatolia Region of Türkiye (Adıyaman, Diyarbakır, Gaziantep, Mardin, Siirt, Şanlıurfa) (Lodos et al., 1984; Bolu et al., 2005).

Genus: *Tanymecus* Germar, 1817

***Tanymecus dilaticollis* Gyllenhal, 1834**

Material examined: 2♀♀2♂♂, 08.V.2007, Diyarbakır (Dicle University Campus).

Host plant: Unknown

Record Host plants: *Medicago*, *Pinus*, *Verbascum* (Lodos et al., 2003).

Distribution in the World: Armenia, Austria, Avusturya, Bulgaria, Cyprus, Georgia, Greece, Iran, Iraq, Moldovia, Romania, Serbia, Türkiye, Ukraine (Alonso-Zarazaga et al., 2017).

Distribution in the Southeastern Anatolia Region of Türkiye: This species has been recorded for the provinces of the Southeastern Anatolia Region of

Türkiye (Adıyaman) (Bingöl, 1978; Lodos et al., 2003).

Subfamily: Lixinae Schoenherr, 1823

Genus: *Larinus* Germar, 1824

***Larinus latus* Herbst, 1874**

Material examined: 1♂, 08.V.2007, Diyarbakır (Dicle University Campus).

Host plant: Unknown

Record Host plants: *Gundelia tournefortii* L. (Karaat et al., 1986), *Onopordum* (Hoffmann, 1950; Lodos et al., 2003).

Distribution in the World: Caucasus, Crimea, Iran, Syria, Türkiye, Ukraine, (Ter-Minassian, 1978).

Distribution in the Southeastern Anatolia Region of Türkiye: This species has been recorded for the province of Diyarbakir in previous studies (Karaat et al., 1986).

***Larinus onopordi* Fabricius, 1787**

Material examined: 1♀, 08.V.2007, Diyarbakır (Dicle University Campus).

Host plant: Unknown

Record Host plants: *Centaurea*, *Circium*, *Onopordum*, *Pinus* (Lodos et al., 2003), *Echinops* (Ter-Minassian, 1967).

Distribution in the World: Caucasus, Central Europe, Greece, Hungary, Iran, Kazakhstan, North Africa, Russia, Southern Europe, Syria, Tajikistan, Türkiye, Turkmenistan, Ukraine, (Hoffmann, 1954; Ter-Minasyan, 1978).

Distribution in the Southeastern Anatolia Region of Türkiye: *Larinus onopordi* has been recorded for the province of Gaziantep in previous studies (Lodos et al., 2003). This species is the first record for the fauna of Diyarbakır province.

***Larinus sturnus* Schaller, 1873**

Material examined: 2♀♀3♂♂, 22.V.2014 Diyarbakır (Dicle University Campus); 1♀1♂, 21.IV.2007 Diyarbakır (Köprüköy Village).

Host plant: Unknown

Record Host plants: *Onopordum* (Lodos et al., 2003), *Carduus*, *Centaurea*, *Cirsium* (Compositae) (Balalaikins and Bukejs, 2011).

Distribution in the World: Algeria, Caucasus, Central Asia, Europe, Former Soviet Union (European part), Iran, Türkiye (Hoffmann, 1954; Lodos et al., 1978; Ter-Minasyan, 1978).

Distribution in the Southeastern Anatolia Region of Türkiye: This species is first record for the fauna of Diyarbakır province. Moreover; this species is also the first record for the fauna of the Southeastern Anatolia Region of Türkiye.

Genus: *Lixus* Fabricius, 1801

***Lixus cardui* Olivier, 1808**

Material examined: 2♀♀ 12.III.2007, 1♂ 26.III.2007, 1♀ 20.IV.2007, 1♂ 02.5.2007 Diyarbakır (Dicle University Campus).

Host plant: Unknown

Record Host plants: *Ammiaceae* (Dieckmann, 1983), *Centaurea*, *Onopordum* (Lodos et al., 2003). *Amygdalus communis* L., *Onopordum* sp., *Prunus avium* L. (Bolu et al., 2005; Çınar et al., 2005; Bolu & Legalov, 2007).

Distribution in the World: Caucasus, Central Europe, Hungary, Iran, Italy, Mediterranean Coast, Soviet Union (middle and south of the European part), Türkiye (Hoffmann, 1954; Ter-Minasyan, 1978; Abazzi and Osella, 1992).

Distribution in the Southeastern Anatolia Region of Türkiye: This species has been recorded for the provinces of Diyarbakır and Mardin (Bolu et al., 2005; Çınar et al., 2005; Bolu & Legalov, 2007).

***Lixus elongatus* Goeze, 1777**

Material examined: 1♀ 2♂, 21.IV.2007 Diyarbakır (Köprüköy Village).

Host plant: Unknown

Record Host plants: *Centaurea*, *Cirsium*, *Onopordum*, *Tamarix*, *Verbascum* (Lodos et al., 2003); *Amygdalus communis* L. (Bolu et al., 2005; Bolu & Legalov, 2007).

Distribution in the World: Algeria, Caucasus, Central Asia, Europe, Soviet Union (the southern part of Europe), Türkiye (Hoffmann, 1954; Ter-Minasyan, 1978; Abazzi and Osella, 1992).

Distribution in the Southeastern Anatolia Region of Türkiye: This species has been recorded for Diyarbakır and Mardin provinces of the Southeastern Anatolia Region of Türkiye (Bolu et al., 2005; Bolu & Legalov, 2007).

***Lixus recurvus* Olivier, 1807**

Material examined: 1♀, 10.IV.2007, Diyarbakır (Dicle University Campus).

Host plant: Unknown

Record Host plants: *Heracleum*, *Angelica*, *Falcaria* (Apiaceae) (Gültekin, 2006).

Distribution in the World: Armenia, Azerbaijan, Georgia, Iran, Russia, and Türkiye (Alonso-Zarazaga et al., 2017).

Distribution in the Southeastern Anatolia Region of Türkiye: This species is first record for the fauna of Diyarbakır province. Moreover; this species is also the first record for the fauna of the Southeastern Anatolia Region of Türkiye.

***Lixus scolopax* Bohemann, 1836**

Material examined: 1♀, 21.V.2006, 1♀, 15.V.2007, 1♂, 22.V.2007 Diyarbakır (Dicle University Campus); 2♂♂, 21.IV.2007 Diyarbakır (Köprüköy Village).

Host plant: Unknown

Record Host plants: *Centaurea*, *Onopordum* (Lodos et al., 2003).

Distribution in the World: Caucasus, Mediterranean Coast, Southern Europe, Soviet Union (the southern part of Europe), Türkiye (Ter-Minassian, 1978; Lodos et al., 1978).

Distribution in the Southeastern Anatolia Region of Türkiye: This species has been recorded for the province of Gaziantep in previous studies (Lodos et al., 2003). *Lixus scolopax* is the first record for the fauna of Diyarbakır

Genus: *Coniocleonus* Motschulsky, 1860

***Coniocleonus* (*Augustecleonus*) *hollbergii* (Fåhraeus, 1842)**

Material examined: 1♂, 10.IV.2007, Diyarbakır (Dicle University Campus)

Host plant: Unknown

Record Host plants: *Rumex acetosella* (Polygonaceae), *Calluna* (Ericaceae) (Balalaikins and Bukejs, 2011).

Distribution in the World: Austria, Belarus, Czech Republic, France, Germany, Hungary, Italy, Latvia, Poland, Russia, Sweden, Siberia (Russia) (Alonso-Zarazaga et al., 2017).

Distribution in Türkiye: This species is the first record for insect fauna of Türkiye.

Subfamily: Otiorrhynchinae

Genus: *Otiorhynchus* Germar, 1822

***Otiorhynchus sulcatus* (Fabricius, 1775)**

Material examined: 9♀♀, 26.I.2009 (This species has been obtained from laboratory larval culture).

Host plant: *Vitis vinifera* L.

Record Host plants: *Laurus*, *Myrtus*, *Rosa*, *Smilax* (Lodos et al., 2003).

Distribution in the World: Albania, Austria, Belarus, Belgium, Bulgaria, Croatia, Czech Republic, Denmark, Estonia, Finland, France, Germany, Great Britain, Hungary, Ireland, Italy, Latvia, Lithuania, Luxemburg, Netherlands, Norway, Poland, Portugal, Romania, Russia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Türkiye, Ukraine (Lodos et al., 2003; Alonso-Zarazaga et al., 2017).

Distribution in the Southeastern Anatolia Region of Türkiye: This species is first record for the fauna of Diyarbakır province. Moreover; this species is also the first record for the fauna of the Southeastern Anatolia Region of Türkiye.

DISCUSSION

When the literature is examined, there are not many studies in the Southeastern Anatolia region in Türkiye. Therefore, the fauna in this region is not very well known. For this reason, the faunistic studies carried out here gain importance. In this study, one species, *Stephanocleonus hollbergii*, was recorded as a new record for Türkiye. In addition, 9 species were determined as new records for the region. As it is known, most of the members of the Curculionidae family are harmful because they are phytophagous. They cause significant damage to many agricultural and agricultural products (Hoffmann, 1950; Mihajlova, 1978. There are very large agricultural areas in the Southeastern Anatolia region. Therefore, the determination of the fauna in this region is important in terms of control agricultural pests. In this study, the Curculionidae family, which is distributed in the Southeastern Anatolia Region, was tried to be determined and the contribution of the obtained results to the fauna was evaluated.

CONCLUSION

In this study, 11 genera belonging to 5 subfamilies of Curculionidae (Coleoptera) family and 18 species belonging to these genera were determined. Among these species, 1 species (*Coniocleonus hollbergii* Fåhraeus, 1842) was determined as a new record for the fauna of Türkiye. In addition, 9 species were determined as new records from the region. These species are; *Coeliodes ruber* Marsham, 1802, *Mogulones crucifer* (Pallas, 1771), *Stenocarus ruficornis* (Stephens, 1831), *Rhinusa asellus* (Gravenhorst, 1807), *R. tetra* (Fabricius, 1792), *Polydrusus corruscus* Germar, 1824, *Larinus sturnus* Schaller, *Lixus recurvus* Olivier, 1807 and *Otiorhynchus sulcatus* (Fabricius, 1775).

Author's Contribution

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

Statement of Conflict of Interest

Authors have no conflict of interest to declare.

Statement of Research and Publication Ethics

The authors declare that this study has been performed in accordance with research and publication ethics.

REFERENCES

Abazzi, P. & Osella, G. (1992). Elenco sistematico-faunistico delgi Anthribidae, Rhinomaceridae, Attelabidae, Apionidae, Brentidae, Curculionidae Italiana. *Redia 25* (2), 267-414.

Akkaya, A. (1995). Güneydoğu Anadolu Bölgesinde Baklagil Yem Bitkilerinde Entomolojik Sorunlar ve Çözüm Önerileri (Sözlü bildiri). GAP Bölgesindeki Bitki Koruma Sorunları ve Çözüm Önerileri Sempozyumu, Şanlıurfa, Türkiye, 27-29

Nisan 1995, ss. 304-316.

Alonso-Zarazaga, M. & Lyal, C.H.C. (1999). A world catalogue of families and genera of Curculionoidea (Insecta: Coleoptera) (excepting Scolytidae and Platypodidae). *Entomopraxis*, 1-315.

Alonso-Zarazaga, M., Barrios, H., Borovec, R., Bouchard, P., Caldara, R., Colonnelli, E., Gültekin, L., Hlavac, H., Korotyaev, B., Lyal, C.H.C., Machado, A., Meregalli, M., Pierotti, E., Ren, L., Sanchez-Ruiz, M., Sforzi, A., Silfverberg, H., Skuhrovec, J., Trýzna, M., Velázquez de Castro, A.J. & Yunakov, N.N. (2017). Cooperative Catalogue of Palaearctic Coleoptera Curculionoidea, *Sociedad Entomológica Aragonesa S.E.A., Zaragoza (Spain) November 2*, 1-547.

Anonymous, (2019). Fauna Europaea. https://fauna-eu.org (Update: 20.05.2022).

Arzanov, Y.G. & Grebennikov, G.G. (2017). Cleonini (Coleoptera: Curculionidae: Lixinae) are monophyletic and flightless: tribe overview, rampant adult homoplasy and illustrated global diversity. *Zootaxa 1*, 1-63.

Avgın, S.S. & Colonnelli, E. (2011). Curculionoidea (Coleoptera) from southern Türkiye. *African Journal of Biotechnology 10*(62), 13555-13597.

Bingöl, M.C. (1978). Güneydoğu Anadolu Bölgesinde Kültür Yem Bitkilerinde (Yonca: *Medicaga sativa* L., Korunga: *Onobrychs sativa* Lam., Fiğ: *Vicia sativa* L.) Zarar Yapan Miridae ve Curculionidae Familyalarına Ait Böcek Türleri, Tanınmaları ve Zararları Üzerinde Araştırmalar. Diyarbakır Bölge Zirai Mücadele Araştırma Enstitüsü (Basılmamış uzmanlık Tezi) ss. 80.

Bolu, H., Özgen, I. & Çınar, M. (2005). Dominancy of Insect Families and Species Recorded in Almond Orchards of Türkiye. *Acta Phytopathologica et Entomologica Hungarica 40* (1-2), 145-157.

Bolu, H. & Legalov, A.A. (2008). On the Curculionoidea (Coleoptera) fauna of Almond (*Amygdalus communis* L.) Orchards in South-eastern and Eastern Anatolia in Türkiye. *Baltic Journal of Coleopterology 8* (1), 75-85.

Bolu, H. & Özgen, I. (2009). Diyarbakır, Elazığ ve Mardin İlleri Badem Ağaçlarında Zararlı *Polydrosus roseiceps* Pes. (Coleoptera: Curculionidae)'nin Populasyon Değişiminin Belirlenmesi. *HR.Ü.Z.F. Dergisi 13* (2), 43-47.

Caldara, R. (1990). Revision taxonomica della specie paleartiche del genere *Tychius* Germar (Coleoptera: Curculionidae). *Memorial Society of Italy 25* (3), 45-217.

Colonnelli, E. (2004). Catalogue of Ceutorhynchinae of the world, with a key to genera (Insecta: Coleoptera: Curculionidae). *Argnia, Barcelona*, 3-124.

Çınar, M., Çimen, İ. & Bolu, H. (2004). Elazığ ve Mardin İlleri Kiraz Ağaçlarında Zararlı Olan Türler, Doğal Düşmanları ve Önemlileri Üzerinde Gözlemler. *Türkiye Entomoloji Dergisi 28* (3), 213-220.

Dalla Torre, K.W., Emden, M. Van & Emden, F. Van. (1939). Curculionidae: Brachyderinae I. in: schenkling, S. (Ed.): *Coleopterum catalogus auspiicis et auxilio w. junk 147*, 1-132.

Dieckmann, L. (1980). Beitrage zur insektenfauna der DDR: Coleoptera-Curculionidae (Brachycerinae, Otiorrhynchinae, Brachyderinae). *Beitrage Entomolgy Berlin 30* (1), 145-310.

Erbey, M. (2010). *Bolkar Dağlarının Curculionidae (Coleoptera) familyası üzerinde taksonomik ve morfolojik araştırmalar (Tez no 362311).* [Doktora Tezi, Gazi Üniversitesi Fen Bilimleri Enstitüsü, Biyoloji Anabilim Dalı] Yükseköğretim Kurulu Ulusal Tez Merkezi.

Erbey, M. & Gürler, Y. (2014). A new species of the genus *Aspidiotes* Schoenherr, 1847 (Coleoptera: Curculionidae: Tanymecini) from Middle Anatolia (Türkiye). *Zootaxa 3847* (1), 133-138.

Erbey, M. (2015). A new species of weevil of the genus Rhytideres (Coleoptera: Entiminae: Alophini) from Inner Anatolia (Türkiye). *Zoology in the Middle East 63*(1), 241-245.

Gözüaçık, C. & Özgen, I. (2005). Türkiye faunası için yeni kayıt: *Ceutorhynchus peyerimhoffi* Hustache, 1916 (Coleoptera:Curculionidae). *Türk. Entomol. Derg. 29* (3), 237-239.

Gültekin, L. (2006). Host plant range and biology of *Lixus nordmanni* Hochhuth (Coleoptera, Curculionidae) on hogweed Heracleum L. in eastern Türkiye. *Journal of Pest Science 79*, 23-25.

Özgen, I., Arzanov, Y. & Tanyıldızı, M.Ş. (2016). New Faunistic Records of Weevils Curculionoidea (Coleoptera) Elazığ Provinces. *Journal of Entomology and Zoology Studies 4* (5), 846-850.

Hoffmann, A. (1950). *Faune de France, Coleopteres, Curculionides, premiere partie 52, Paris, 1-486.*

Hoffmann, A. (1954). *Fauna de France, Coleopteres, Curculionides, deuxieme partie 59, Paris, 487-1208.*

Karaat, Ş., Göven, M.A. & Mart, C. (1986). Güneydoğu Anadolu Bölgesinde Bazı Yabancı Otların Zararına Yaşayan Böcek Türleri Üzerinde İlk İncelemeler (Sözlü bildiri). Türkiye I. Biyolojik Mücadele Kongresi Bildirileri, Adana, Türkiye,12-14 Şubat, ss. 186.

Keskin, B. (2005). *İzmir ilinde bulunan Otiorhynchus Germar, 1822 (Coleoptera, Cuculionidae) cinsine bağlı türler üzerinde sistematik araştırmalar (Tez no 169732).* [Doktora Tezi Ege Üniversitesi, Fen Bilimleri Enstitüsü, Biyoloji Ana Bilim Dalı]. Yükseköğretim Kurulu Ulusal Tez Merkezi.

Lodos, N. (1960). *Orta Anadolu'da meyve ağaçlarında zarar yapan Curculionidae (Hortumlu Böcekler) türleri üzerindeki sistematik araştırmalar.* Ege Üniversitesi Matbaası, İzmir.

Lodos, N., Önder, F., Pehlivan, E. & Atalay, R. (1978). *Ege ve Marmara Bölgesi'nin zararlı böcek faunasının tespiti üzerine çalışmalar.* Zirai Mücadele Zirai Karantina Genel Müdürlüğü, Ankara.

Lodos, N., Önder, F., Pehlivan, E., Atalay, R., Erkin, E., Karsavuran, Y., Tezcan, S. & Aksoy, S. (2003). *Faunistic studies on Curculionidae (Coleoptera) of western black sea, central anatolia and mediterranean regions of Türkiye*. Meta Basım Matbaacılık Hizmetleri, İzmir, 1-83.

Mihajlova, B. (1978). Contrubition to the study of fauna of snout beetles (Coleoptera: Curculionidae) of Macedonia. *Fragmenta Balkanica 10* (14), 1-234.

Pehlivan, E., Karsavuran, Y. & Tezcan, S. (2005a). Contributions to the knowledge of the Curculioninae and Phytonominae (Coleoptera: Curculionidae) from Türkiye. *Türk Entomoloji Dergisi 29* (3), 173-182.

Pehlivan, E., Karsavuran, Y. & Tezcan, S. (2005b). Contributions to the knowledge of the Lixinae (Coleoptera: Curculionidae) from Türkiye. *Türk Entomoloji Dergisi 29* (4), 259-272.

Sert, O. (1990). *Anakara ili ve ilçeleri Curculionidae (Coleoptera) familyası üzerinde taksonomik çalışmalar (Tez no 12584)*. [Yüksek Lisans Tezi, Hacettepe Üniversitesi Fen Bilimleri Enstitüsü, Biyoloji Ana Bilim Dalı]. Yükseköğretim Kurulu Ulusal Tez Merkezi.

Sert, O. (1995*). İç Anadolu Bölgesi Curculionidae (Coleoptera) famliyası üzerinde taksonomik çalışmalar (Tez no 47147)*. [Doktora Tezi, Hacettepe Üniversitesi Fen Bilimleri Enstitüsü, Biyoloji Ana Bilim Dalı]. Yükseköğretim Kurulu Ulusal Tez Merkezi.

Ter-Minasyan, M.E. (1978). Weevils of the subfamily Cleoninae in the fauna of the USSR. tribe Lixini, *Zoological Institute, Academy of Sciences of the USSR* 1-166.

Velázquez de Castro, A.L., Friedman, A.L.L. & Borovec, R. (2010). Sitonini of Israel (Coleoptera: Curculionidae: Entiminae). *Israel Journal of Entomology 40*, 71-108.

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First Report of Root-Knot Nematode *Meloidogyne hapla* (Chitwood, 1949) (Nematoda: Meloidogynidae) on *Petroselinum crispum* (Mill.) Nym. ex A.W. Hill) in Türkiye

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ABSTRACT
Parsley (*Petroselinum crispum*) is an important culinary herb originated from the Mediterranean basin, where it still can be found in wild forms, and is taxonomically positioned in the Apiaceae family. In Turkey, it is cultivated extensively in the Mediterranean and Aegean Regions, and in the Marmara Region. There are several diseases and pests affecting the yield and quality in the parsley cultivated areas. Root-knot nematodes are among the most important pests in winter crop production with the ability of a wide host plant range. Root-knot nematodes weaken the parsley plant, causing significant quality losses, and showing signs of stunting and yellowing of the leaves. In this study, parsley plants with typical symptoms of root-knot nematodes were detected in surveys conducted in Çanakkale province, Türkiye. Pure cultures of the root-knot nematode obtained from the roots of parsley were identified up to the species level. Species identification and diagnosis were made by morphological and morphometrical measurements from second-stage juveniles and female individuals obtained from pure cultures. As a result, *Meloidogyne hapla* was detected first time on parsley plants in Türkiye.

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Türkiye'de *Meloidogyne hapla* (Chitwood, 1949) (Nematoda: Meloidogynidae)'nın *Petroselinum crispum* (Mill.) Nym. ex A.W. Hill)'da İlk Konukçu Kaydı

ÖZET
Maydanoz (*Petroselinum crispum*), Akdeniz havzası orijinli, halen yabani formda bulunabilen ve taksonomik olarak Apiaceae familyasında yer alan önemli bir mutfak bitkisidir. Ülkemizde de ticari olarak Akdeniz ve Ege Bölgelerinde, geniş çaplı olarak Marmara Bölgesinde üreticiliği yapılmaktadır. Maydanoz ekim alanlarında verim ve kaliteyi etkileyen çeşitli hastalık ve zararlı bulunmaktadır. Kök-ur nematodları, geniş bir konukçu bitki yelpazesine sahip olma yetenekleri ile kışlık mahsul üretiminde en önemli zararlılar arasında yer almaktadır. Kök-ur nematodları maydanoz bitkisini zayıflatarak önemli kalite kayıplarına neden olup, bitkide bodurlaşma ve yapraklarda sararma belirtileri göstermektedir. Bu çalışmada Türkiye'nin Çanakkale ilinde yapılan surveylerde Kök-ur nematodlarının tipik simptomlarının gözlemlendiği maydanoz bitkileri tespit edilmiştir. Maydanoz köklerinde elde edilen kök-ur nematodunun saf kültürleri tür düzeyine kadar teşhis edilmiştir. Saf kültürlerden elde edilen 2. dönem larvalar ve dişi bireylerden morfometrik ölçümler ve morfolojik yöntemler kullanılarak tür teşhisi yapılmıştır. Çalışma sonucunda *Meloidogyne hapla* Türkiye'de ilk defa maydanoz bitkilerinde tespit edilmiştir.

Bitki Koruma

Araştırma Makalesi

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Anahtar Kelimeler
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Teşhis
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INTRODUCTION

Parsley (*Petroselinum crispum* (Mill.) Nym. ex A.W. Hill), a popular leaf-consumed vegetable plant in the family Apiaceae, is commonly found in subtropical and temperate zones of the Mediterranean Climate zone. This family is the largest and most cosmopolitan family among angiosperms (Pimenov & Leonov, 1993; Hickey & King, 1997; Hançer & Uruşak, 2017). Parsley is a bright, green, pile-rooted plant with fragrant and segmented leaves, the length of leaves varies between 30-100 cm, the seeds are 2.5-3 mm long and with a special odour. It is produced and consumed as a cultural vegetable in all countries of the world (Vural et al., 2000). There are approximately 300 genera and 2500-3000 species of parsley, among which there can be found 97 genera and 400 species in Turkey, and many species are used as food and spicery, on the other hand, it is also used in the pharmaceutical and perfume industries (Seçmen et al., 1986). Parsley has an important place among the vegetables whose leaves are consumed in terms of health, nutrition and economy, and its production is increasing day by day (Ben-Amotz & Fishier, 1998).

There are many pests and diseases that negatively affect the yield and quality of the plant. Some of the pests can be counted as Carrot fly (*Psila rosae* F.), Carrot louse (*Cavariella aegopodii* Scop.), Gray worms (*Agrotis* spp.) and Root-knot nematodes (*Meloidogyne* spp.). Root-knot nematodes have symptoms such as weakening parsley, stunting and yellowing of leaves (Sikora & Fernández, 2005; Mennan et al., 2011). The damage caused by root-knot nematodes on the leaves of the consumed part of parsley is economically important and it is an undesirable situation for the growers. In the previous studies conducted around the world, it has been reported that parsley is a suitable host for Root-knot nematodes (*M. arenaria*, *M. enterolobii*, *M. floridensis*, *M. incognita*, *M. hapla*, *M. hispanica* and *M. javanica*) (Doucet & Pinochet 1992; Sikora & Fernández, 2005; Mennan et al., 2011; Quénéhervé et al., 2011; Maleita et al., 2012; Sasanelli et al., 2015). However, this is the first report of a parsley plant as a host for *M. hapla* species in Turkey.

MATERIAL and METHOD

A field survey was conducted in the areas where parsley is grown in Çanakkale in the winter period of 2021-2022. The roots of plant specimens with typical symptoms of root-knot nematodes were brought to the laboratory and examined under a binocular microscope (Figure 1). After the infected plants were detected, the female egg masses were taken from the root of each sample with forceps and alive second-stage infective juveniles were obtained after 48 hours on a modified Baermann funnel, (8 cm high, 10-12 cm wide Petri dishes were used with a sieve inside) for morphometric measurements. After that, pure cultures were formed by infecting tomato plants with one egg mass for each plant which are susceptible to Root-knot nematodes. After about 60 days, second-stage juveniles (J2s) were obtained from female individuals eggmasses reproduced on pure cultures for diagnostic studies.

Figure 1. Parsley roots infected with *Meloidogyne spp.* a: Sample 13; b: Sample 94
Şekil 1. Meloidogyne hapla ile infekteli maydanoz kökleri a: Örnek 13, b: Örnek 94

The obtained J2s were fixed in TAF solution (Courtney et al., 1955) and then taken into pure glycerin according to the Seinhorst (1959) method, fixed on the slide, and made ready for species identification. The standard measurements used in the morphological diagnosis of second-stage juveniles were made by Jepson (1987) and the species-level diagnosis was made using the Leica DM1000 light microscope according to Chitwood (1949) and Cliff & Hirschmann, (1985). For each infected plant population, 25 root-knot nematode second instar larvae were measured.

Obtained female individuals were cut after being kept in 45% lactic acid and their preparations were made between slide and coverslip in pure glycerin (Hooper, 1986). Morphological identifications of female individuals were made according to Jepson (1987) and Karssen (2002).

RESULTS and DISCUSSION

The results of the diagnosis performed with morphological and morphometric measurement methods showed that the species infecting the roots of the parsley plant was *M. hapla* (Table 1, Figure 2; 3).

In Turkey, as yet, *M. hapla* species host reports were detected on potato (Özarslandan et al., 2005), sugar beet (Alkan, 1962), pepper (Söğüt & Elekçioğlu, 2000), strawberry (Özarslandan et al., 2021) and kiwi (Akyazı et al., 2017).

Previous studies on the host plant of root-knot nematodes that infect parsley in different parts of the world were reported as susceptible to *M. arenaria* and *M. incognita* species (Ibrahim et al., 1983; Doucet & Pinochet 1992; Walker, 2002). In Turkey, so far only *M. arenaria* species were known on Parsley (Mennan et al. 2011).

Table 1. Morphometric measurements of Meloidogyne hapla J2s on Petroselinum crispum
Çizelge 1. Petroselinum crispum'dan elde edilen Meloidogyne hapla'ya ait J2s'lerin morfometrik ölçümleri

Diagnostic Characters	*This Study Sample 13*	*This Study Sample 94*	*Chitwood, 1949*	*Cliff & Hirschmann, 1985*
Body lenght	433.06±30.76 (382.52-496.92)	381.22 ±11.61 (362.17-403.40)	357-467	391.6-605.2
Greatest body width	15.43±1.16 (13.14-17.79)	15.56±0.28 (15.06-16.12)	-	12.8-17.8
Body width at stylet base	9.57±0.57 (8.44-10.95)	9.92±0.35 (9.19-10.40)	-	-
Body width at anus	11,46±1.09 (9.08-13.14)	9.87±0.28 (9.43-10.44)	-	-
Stylet length	15.23±1.01 (14.07-18.81)	14.74±0.44 (14.25-15.89)	10-12	10.1-11.9
DGO	3.64±0.37 (2.98-4.24)	2.70±0.34 (2.16-3.28)	3-4	2.7-4.7
Tail length	52.77±4.68 (42.04-64.36)	45.84±1.61 (42.81-48.33)	46-58	43.6-69.4
Excretory pore to head end	90.65±6.75 (80.88-106.36)	75.18±1.83 (72.14-78.15)	-	75-105.2
Body width at excretory pore	13.76±0.93 (12.09-16.18)	14.41±0.46 (13.10-14.99)	-	-
a	28.15±2.20 (24.27-35.07)	24.50±0.67 (23.16-25.56)	-	-
b	3.69±0.39 (3.00-4.42)	4.25±0.22 (3.77-4.66)	-	-
c	8.24±0.63 (7.25-9.57)	8.32±0.34 (7.49-8.97)	-	-
c'	4.62±0.45 (3.65-5.34)	4.64±0.18 (4.30-4.95)	-	-

Note: All mesurements are in µm (a: Body lenght/Body width, b: Body lenght/Intestine to head end, c: Body lenght/Tail lenght, c': Tail lenght/Body width at anus) and in form: mean ± s.d. (range)

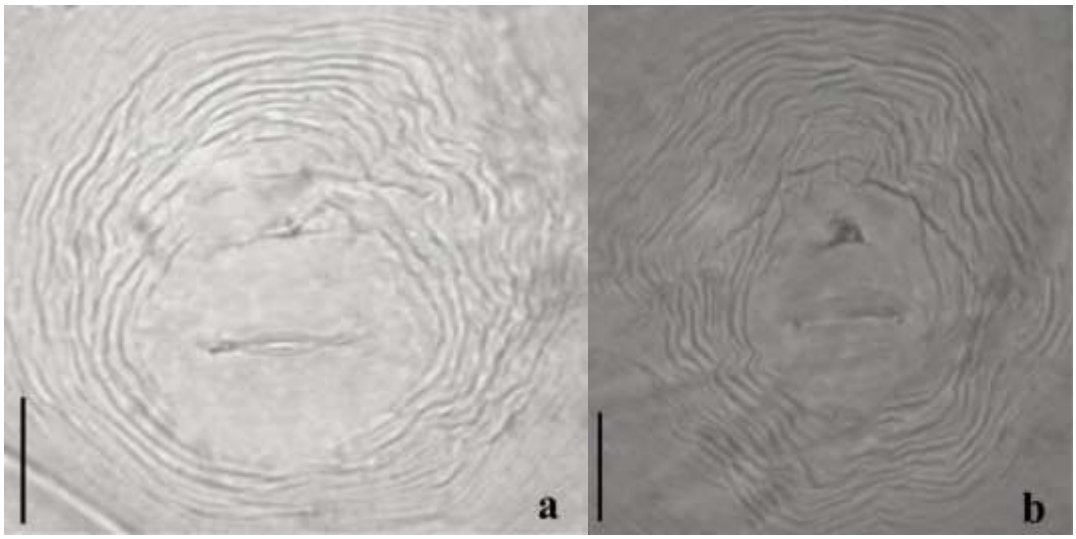

Figure 2. Perineal pattern of Meloidogyne hapla from Petroselinum crispum a: Sample 13; b: Sample 94 (Scale Bar: 20 µm)

Şekil 2. Petroselinum crispum'dan elde edilen Meloidogyne hapla'ya ait perineal pattern a: Örnek 13, b: Örnek 94 (Ölçek Çubuğu: 20 µm)

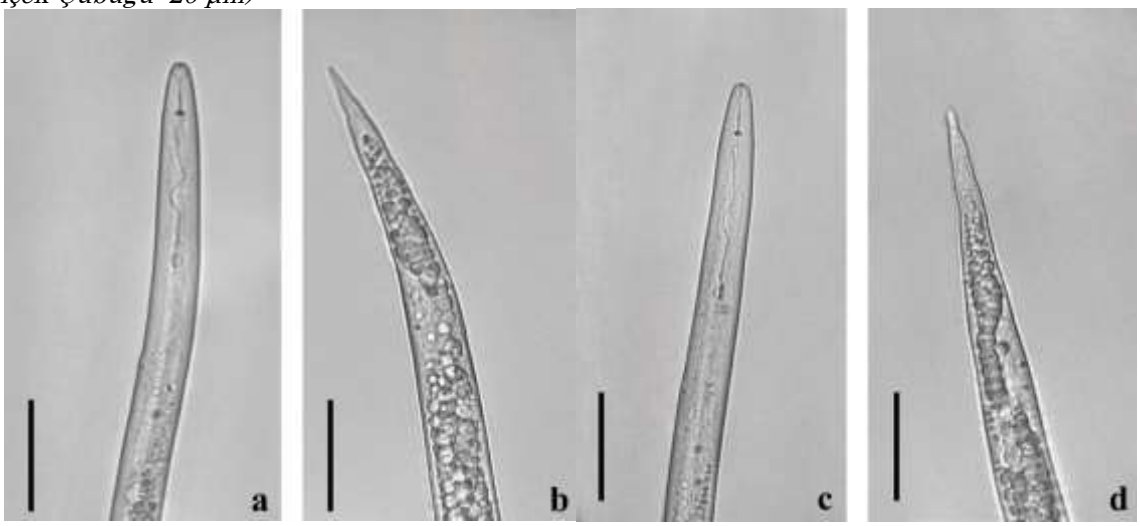

Figure 3. J2s of Meloidogyne hapla from Petroselinum crispum a: Anterior region of Sample 13; b: posterior region of Sample 13; c: anterior region of Sample 94; d: posterior region of Sample 94 (Scale Bar: 20 µm)

Şekil 3. Petroselinum crispum'dan elde edilen Meloidogyne hapla'ya ait J2s a: Örnek 13 anterior bölgesi, b: Örnek 13 posterior bölgesi, c: Örnek 94 anterior bölgesi, d: Örnek 94 posterior bölgesi (Ölçek Çubuğu: 20 µm)

From previous studies, it has been reported that *M. hapla* is rarely detected in Turkey (Yüksel, 1974; Elekçioğlu & Uygun, 1994; Mennan & Ecevit, 1996; Söğüt & Elekçioğlu, 2000), so far *M. hapla* was diagnosed on potato (Özarslandan et al. ., 2005), sugar beet (Alkan, 1962), pepper (Söğüt & Elekçioğlu, 2000; Akyazı et al., 2012), strawberry (Özarslandan et al., 2021), kiwi (Akyazı et al., 2017) plants.

It can be concluded that this study is the first record in Türkiye as a host for *M. hapla* on a parsley plant since the *M. hapla* that infects parsley has not been detected by previous studies. In the future it is recommended to carry out extensive survey studies on parsley growing areas and investigations on control strategies for this plant parasitic nematode using crop rotation, resistant varieties and solarization applications.

Author's Contributions

The contribution of the authors is equal.

Statement of Conflict of Interest

The authors declare no conflict of interest.

REFERENCES

Akyazi, F., Han, H., Cetintas, R., & Felek, A. F. (2012). First report of root-knot nematodes, *Meloidogyne arenaria* and *M. hapla* (Nemata: Meloidogynidae) from pepino in Turkey. *Nematologia Mediterranea*.

Akyazı, F., Joseph, S., Felek, A. F., & Mekete, T. (2017). Mitochondrial haplotype-based identification of root-knot nematodes, *Meloidogyne arenaria* and *Meloidogyne hapla*, infecting kiwifruit in Turkey. *Nematropica*, *47*(1), 34-48.

Alkan, B. (1962). Türkiye'nin zararlı nematod faunası üzerinde ilk incelemeler. *Bitki Koruma Bülteni, 2*(12), 17-25.

Ben-Amotz, A., & Fishier, R. (1998). Analysis of carotenoids with emphasis on 9-cis ß-carotene in vegetables and fruits commonly consumed in Israel. *Food Chemistry*, *62*(4), 515-520.

Chitwood, B. G. (1949). 'Root-knot nematodes'. Part 1. A revision of the genus *Meloidogyne* Goeldi, 1887. *Proceedings of the Helminthological society of Washington, 16*(2), 90-114.

Cliff, G. M., & Hirschmann, H. (1985). Evaluation of morphological variability in *Meloidogyne arenaria*. *Journal of Nematology*, *17*(4), 445.

Courtney, W. D., Polley, D. O. R. O. T. H. Y., & Miller, V. L. (1955). TAF, an improved fixative in nematode technique. *Plant Disease Reporter*, *39*(7), 570-571.

Doucet, M. E., & Pinochet, J. (1992). Occurrence of *Meloidogyne* spp. in Argentina. *Journal of Nematology*, *24*(4S), 765.

Elekçioğlu, İ. H., & Uygun, N. (1994). Occurrence and distribution of plant parasitic nematodes in cash crop in Eastern Mediterranean region of Turkey. In Proceedings of 9th Congress of the Mediterranean Phytopathological Union, Kuşadası-Aydın-Türkiye, 409-410.

Hançer, Ç. K., & Uruşak, E. A. (2017). Apiaceae familyası meyve anatomisindeki "Vitta" terimi ve yerleşimleri. *Avrasya Terim Dergisi*, *5*(2), 19-24.

Hickey, M., & King, C. (1997). *Common families of flowering plants.* Cambridge Univ. Pres., England, 119-127.

Hooper, D. J. (1986). *Handling, fixing, staining and mounting nematodes. In: Laboratory Methods for Work with Plant on Soil Nematodes, (Ed. J.F. Southey)*. Her Majesty's Stationery Office, London, 59-80.

Ibrahim, I. K. A., Rezk, M. A., & Khalil, H. A. A. (1983). Resistance of some plant cultivars to root-knot nematodes, *Meloidogyne* spp. *Nematologia Mediterranea*, 11, 189-192.

Jepson, S. B. 1987. *Identification of root-knot nematodes Meloidogyne species*. CABI Wallingford, Oxfordshire.

Karssen, G. (2002). *The plant parasitic nematode genus Meloidogyne Goeldi, 1892 (Tylenchida) in Europe*. Brill.

Maleita, C. M. N., Curtis, R. H. C., Powers, S. J., & Abrantes, I. (2012). Host status of cultivated plants to *Meloidogyne hispanica*. *European Journal of Plant Pathology*, *133*(2), 449-460.

Mennan, S., Aydinli, G., & Kati, T. (2011). First report of root-knot nematode (*Meloidogyne arenaria*) infecting parsley in Turkey. *Journal of Phytopathology*, *159*(10), 694-696.

Mennan, S., & Ecevit, O. (1996). Bafra ve Çarşamba Ovaları yazlık sebze ekim alanlarındaki kök-ur nematodları (*Meloidogyne* spp.)'nın biyolojisi, yayılışı ve bulaşıklık oranları üzerine araştırmalar. Türkiye III. Entomoloji Kongresi, *24*(28), 700-705.

Özarslandan, A., Dinçer, D., Yavuz, Ş., & Aslan, A. (2021). First report of northern root-knot nematode (Chitwood, 1949) on strawberry in Turkey. *Journal of Nematology*, *53*(1), 1-4.

Özarslandan, A., Söğüt, M. A., & Elekçioğlu, İ. H. (2005). Türkiye'de *Meloidogyne hapla*'nın yeni bir konukçusu. *Harran Üniversitesi Ziraat Fakültesi Dergisi*, *9*(3), 63-64.

Pimenov, M. G., & Leonov, M. V. E. (1993). *The genera of the Umbelliferae: a nomenclator.* Royal Botanic Gardens, Kew.

Quénéhervé, P., Godefroıd, M., Mège, P., & Marıe-Luce, S. (2011): Diversity of *Meloidogyne* spp. parasitizing plants in Martinique Island, French West Indies. *Nematropica*, 41, 191-199.

Sasanelli, N., Vovlas, N., Cantalapiedra-Navarrete, C., Lucarelli, G., Palomares-Rius, J. E., & Castillo, P. (2015). Parasitism and pathogenicity of curly-leaf parsley with the root-knot nematode *Meloidogyne javanica* in Southern Italy. *Helminthologia*, *52*(4), 348-354.

Seçmen, Ö., Gemici, Y., Leblebici, E. & Bakat, L. (1986). Ege Üniversitesi Fen Fakültesi Kitaplar Serisi, 1106, Bornova, İzmir.

Seinhorst, J. W. (1959). A rapid method for the transfer of nematodes from fixative to anhydrous glycerin. *Nematologica*, *4*(1), 67-69.

Sikora, R. A., & Fernandez, E. (2005). *Nematode parasites of vegetables. In Plant parasitic nematodes in subtropical and tropical agriculture*.Wallingford UK: CABI publishing, 319-392.

Söğüt, M. A., & Elekçioğlu, İ. H. (2000). Determination of *Meloidogyne* Goeldi, 1892 (Nemata: Heteroderidae) species races found in vegetable growing areas of the Mediterranean region of Turkey. *Turkish Journal of Entomology*, *24*(1), 33-40.

Walker, J. T. (2002). Susceptibility of eight herbs to common root-knot nematodes. *Journal of Environmental Horticulture*, *20*(2), 101-103.

Yüksel H., 1974. The distribution of root-knot nematodes (*Meloidogyne* spp.) in Turkey. *Ataturk University, Journal of the Faculty of Agriculture*, *5*: 83-105.

Vural, H., Eşiyok, D., & Duman, İ. (2000). Kültür Sebzeleri (Sebze Yetiştirme). *Ege Üniversitesi, Ziraat Fakültesi Yayınları*. İzmir, 440s.

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Interaction of *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 Race 3 with *Meloidogyne javanica* (Treub, 1885) Chitwood, 1949 Race 3 in Tomato and Pepper

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ABSTRACT
More than one species of root-knot nematodes can attack field vegetables and they can interact with each other. In this study, the interaction of *Meloidogyne incognita* race 3 and *M. javanica* race 3 on susceptible host plants (Falkon and Sena) were investigated in a growth chamber under controlled conditions. Experiments were arranged as a randomized plots design with four replications. The incidence of two *Meloidogyne* species in tomato and pepper hosts were determined by esterase phenotypes. No interaction was observed between the species after the mixed inoculation of 1000 J2 *M. incognita* race 3 and 1000 J2 *M. javanica* race 3 on the susceptible tomato plant (P≤0.05). Among mixed inoculation of the two species in the susceptible pepper, only the incidence of *M. incognita* race 3 was increased, while *M. javanica* race 3 was not detected in host plants.

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Domates ve Biber Bitkisinde *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 Irk 3'ün *Meloidogyne javanica* (Treub, 1885) Chitwood, 1949 Irk 3 ile Etkileşimi

ÖZET
Sebze alanlarında bir bitki köküne birden fazla kök-ur nematodu türü birlikte saldırabilir ve birbirleriyle etkileşim halinde olabilirler. Bu çalışmada, *Meloidogyne incognita* ırk 3 ve *Meloidogyne javanica* ırk 3'ün hassas domates (Falkon) ve hassas biber (Sena) bitkileri üzerindeki karşılıklı etkileşimi kontrollü iklim odası koşullarında incelenmiştir. Tesadüf parselleri deneme desenine göre denemeler 4 tekerrürlü olarak kurulmuştur. İki *Meloidogyne* türünün domates ve biber bitkilerinde bulunma durumu esteraz fenotiplerine göre belirlenmiştir. Hassas domates bitkisine 1000 adet *Meloidogyne incognita* ırk 3 ve 1000 adet *M. javanica* ırk 3 ikinci dönem larvasının karışık inokulasyonu sonrasında, türler arasında herhangi bir etkileşim görülmemiştir (P≤0.05). Hassas biber bitkisinde iki türün karışık inokulasyonu sonrasında yalnızca *M. incognita* ırk 3 oranı artarken, *M. javanica* ırk 3 tespit edilmemiştir.

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INTRODUCTION
Root-knot nematodes which cause severe economic losses in vegetable crops, are known to be parasitic in a fairly high number of host plants (Mitkowski & Abawi 2003; Moens et al. 2009; Mukhtar et al. 2013). It has been reported that root-knot nematodes are in close contact with their hosts and can respond directly to their signals (Curtis 2008) and cause significant yield losses particularly in vegetables. Giant cells formed by these groups of nematodes in the roots are close to the xylem and phloem tissues, consequently, the uptake of essential nutrients and water from the soil by the plant roots is prevented (Abad et al. 2003; Siddiqui et al. 2014).

Vegetable varieties are being infected by more than one *Meloidogyne* species at once (Barros et al. 2018) causing damage on plant together. Because the feeding sites are similar, two or more coexisting *Meloidogyne* species may interact and probably pressure the population density of other species. It has been stated that nematodes compete each other for present feeding sites and cause similar histopathological and physiological changes in the host (Khan & Haider 1991). It has been reported that interactions can occur between *Meloidogyne-Meloidogyne*, *Heterodera-Meloidogyne*, *Rotylenchulus reniformis-Meloidogyne*, *R. reniformis-Tylenchulus semipenetrans* nematode species (Eisenback 1985). The relationships between nematode species may be beneficial for one or two species, may have no effect (neutral), or there may be serious competition between species with similar feeding habits (Eisenback 1985; Eisenback and Griffin 1987). Species diversity in the field increases with widespread dispersal and competition of nematodes with weak species (Oostenbrink 1966). Competition between species can occur when the reproductive capacity of one species is greater than that of other species (Brewer 1978). It has been stated that host damage and nematode reproduction depend on factors such as initial population density (Ferris 1974), nematode species (Barker et al. 1976), plant variety (Barker 1978), and environmental conditions (Lucas 1975). It has been reported that one of the critical environmental conditions is temperature. The interactions between *Meloidogyne incognita* and *M. hapla* are greatly affected by temperature (Johnson & Nusbaum 1970). As the temperature increases, *Meloidogyne hapla* and *M. javanica* are suppressed by *M. incognita*, and as the temperature decreases, *M. incognita* and *M. hapla* are suppressed by *M. javanica*. Factors other than temperature may also be important in the dominance of a particular *Meloidogyne* species. Even though *Meloidogyne hapla* generally prefers low temperatures, *M. hapla* suppressed by *M. javanica* at 20 °C. It is also known that the intensity of competition between species is important for the dominance of *M. hapla* (Kinloch & Allen 1972).

Many studies on nematodes have examined the effects of a single nematode species on a particular host plant, but plant parasitic nematodes are usually seen in polyspecific populations rather than being seen in a single-species community (Ostenbrink 1966; Eisenback 1985). These nematode communities are dynamic and nematode individuals are in consistent interaction with each other as well as with plants, the environment, and other organisms (Eisenback 1985). Therefore, in this study, in order to determine the interaction between root-knot nematode species that can be found in a vegetable field and can enter vegetable roots together, the interaction of *Meloidogyne incognita* race 3 and *Meloidogyne javanica* race 3 on susceptible tomato and pepper plants was investigated.

MATERIAL and METHOD

Material

In this study, the second-stage juveniles of race 3 of both *Meloidogyne incognita* and *M. javanica* species were used. As host plants, susceptible tomato, *Solanum lycopersicum* var. Falcon and susceptible pepper *Capsicum annuum* var. Sena was grown from seeds and were used as 3-4 leaf stage seedlings. Polyacrylamide gel electrophoresis was used as the separation method by using *M. javanica* species as markers.

Method

Growing susceptible tomato and pepper seedlings

The experiment was conducted in a fully controlled climate chamber with 16/8 hours of light and darkness, 60±10% relative humidity, 25±2 ºC temperature. The seeds of susceptible tomato (Falcon) and susceptible (Sena) pepper were sown in plastic seed viol trays containing 80% peat and 20% perlite. Due to fast growth rate of tomato plant, tomato seeds were sown 2 weeks after pepper plant. When the tomato and pepper seedlings in the trays reached the 3-4 leaf stage, they were transplanted into 1.5 L volume pots containing 80% autoclaved (at 120 ºC) sand and 20% peat. Trials were assembled as a randomized plots design with 4 replications. Aphicide (Platin chemistry-Effore/Acetamiprid) was applied once for aphid management. Plants fertilization and irrigation were done as necessary.

Extraction of the second-stage juvenile from *M. incognita* race 3 and *M. javanica* race 3

Egg masses were collected from susceptible tomato roots under a stereo microscope (2x) (Nikon SMZ-2B) to assemble the second-stage juveniles of *Meloidogyne incognita* race 3 and *M. javanica* race 3, which were obtained and reproduced from pure culture. Obtained egg masses were incubated for two days at 28 ºC based on the modified Baermann-funnel method (Hooper 1986). At the end of incubation, the second-stage juveniles (J2 s) from hatched eggs were collected in the water (distilled) under the binocular microscope and the number of J2 s ml^{-1} was determined.

Inoculation of *M. incognita* race 3 and *M. javanica* race 3

By the time of susceptible tomato and pepper seedlings reached approximately 14-15 cm in height, four holes with 2 cm in depth were formed and a mixture of 1000 *Meloidogyne incognita* J2 race 3 and 1000 *M. javanica* J2 race 3 combined were inoculated into each pot. After inoculation, the holes were closed, and 50 ml of

distilled water was added to soil to provide the nematodes homogeneously. In order to determine the inoculum viability of *Meloidogyne incognita race* 3 and *M. javanica* race 3, 1000 second-stage juvenile from each species were inoculated into control plants. The irrigation, fertilization and other maintenance of the plants were provided as needed.

Determining of *Meloidogyne* species

During the study, 65 days following of plant inoculation by a mixture of two different root-knot nematodes, all plants were removed from the soil and the roots were carefully washed. The egg masses and galls in the roots of tomato and pepper plants were counted and evaluated according to the index of Hartman & Sasser (Hartman & Sasser 1985). In addition, the roots were dissected, and (milky white color) and transparent females were counted under a binocular microscope. Because the protein content is low in transparent females, only females with high protein content were used for the diagnosis. Single female was placed in an Eppendorf tube containing 5 µl of distilled water. Females were kept at -20 °C for further PAGE (Polyacrylamide Gel Electrophoresis) studies.

Species identification of mixed population in the plant by PAGE

Rad mini-PROTEIN II (Bio-Rad, Philadelphia, PA) electrophoresis unit was used. Before electrophoresis, the females were thawed and homogenized individually in a micro hematocrit plastic tube in 10 µl of extraction buffer. Each sample was loaded into each well of 10 wells containing gels. The standard *Meloidogyne javanica* female extract was placed into wells number 1 and 10. The remaining 8 wells were loaded with the protein extract of test sample females. Electrophoresis was carried out in a discontinuous buffer system with 8% acrylamide running gel with pH 8.8 and 4% acrylamide stacking gel with pH 6.8. Running voltage was maintained at 80 volts for the first 15 minutes and increased to 200 volts for the remainder of the running period. Following electrophoresis, the gels were removed plates and placed in an enzyme reaction mixture to determine esterase (Harris & Hopkinson 1976). Bands on the gel were evaluated based on phenotype designations of Esbenshade and Triantaphyllou, 1985.

RESULTS

Interaction of *Meloidogyne incognita* race 3 with *Meloidogyne javanica* race 3

The diagnosis of nematode populations in susceptible pepper and tomato roots was made based on the Polyacrylamide Gel Electrophoresis method and it was determined which species caused more damage to the root. As a result of the first trial, the egg mass and galling index in the susceptible Falcon tomato, which was inoculated with root-knot nematode mixture (combination of 1000 J2 of *M. incognita* race 3 and 1000 J2 of *M. javanica* race 3) was found to be 4.75. The total number of females was 65.75 and the total number of transparent females was counted as 11.25. In order to determine the viability of the inoculum, the number of egg masses and galls in the roots of the Falcon tomato inoculated by *Meloidogyne incognita* race 3 and *M. javanica* race 3 were found as 4.00 based on the scale of 0 to 5. A total of 27 females and a total of 9 transparent females were determined on the tomato plant where 1000 second stage juvenile (J2) of *Meloidogyne incognita* race 3 was inoculated. After inoculation of 1000 J2 of *Meloidogyne javanica* race 3, a total of 27 females and a total of 6 transparent females were obtained. Both nematode species multiplied on the susceptible tomato plant (Falcon). According to the type diagnosis of the root-knot nematodes mixture, 21.00±4.63a *Meloidogyne incognita* race 3 and 13.00±2.79a *M. javanica* race 3 were found in plant roots (Figure 1). As a result of the first trial, since there was no difference between the data of two nematodes according to the Duncan multiple comparison test, it was determined that there was no interaction between *Meloidogyne incognita* race 3 and *M. javanica* race 3 on the roots of the susceptible Falcon tomatoes ($P \leq 0.05$) (Table 1).

As a result of the first trial of the susceptible Sena pepper plant, based on the average of 4 replications, the egg mass and root galling index value was 5.00, the total number of females in the roots was 167.50, the total number of transparent females was 40.75. After inoculation of 1000 J2 of *Meloidogyne incognita* race 3 to pepper plant both egg mass and root galling index was found to be 5.00, the total number of females was 135.00 and the number of transparent females was 6. Overall, 65 days after the inoculation of 1000 J2 of *Meloidogyne javanica* race 3 to the pepper plant, no egg mass and root galling were observed in the plant roots (0.00). Therefore, the reproduction of *M. javanica* race 3 on pepper (Sena) was not occurred. According to the PAGE results, it was determined that 167.50 females and 40.75 transparent females belonged to a single species were found (*M. incognita* race 3) in the pepper plant inoculated by mixed population (Figure 1, (Table 2).

According to the results of the second trial to determine the interaction of two mixed species in the Falcon tomatoes, the egg mass and root galling index was found to be 5.00 in tomato plant where mixture of both root nematode species were inoculated. A total of 105.50 females and 69.25 transparent females were found in the tomatoes roots inoculated by mixed nematode populations. To determine the viability of the inoculation, galling and root mass index of 5.00 was

found in susceptible tomato inoculated by 1000 J2 of each species. A total of 362.00 females and 45.00 transparent females were counted on the tomato plant inoculated by *Meloidogyne incognita* race 3.

Table 1. First trial results of the interaction of *Meloidogyne incognita* race 3 and *Meloidogyne javanica* race 3 on tomato

Çizelge 1. Domates bitkisinde Meloidogyne incognita ırk 3 ve Meloidogyne javanica ırk 3 etkileşiminin birinci deneme sonuçları

RKN Species	***FALCON (Susceptible tomato variety)***					
	Starting population (Pi)	**Egg mass index (0-5)**	**Gall index (0-5)**	**Number of females**	**Number of transparent females**	**PAGE result**
M. incognita race 3 + *M. javanica* race 3	1000+1000	4.75	4.75	65.75	11.25	21.00±4.63 a *M. incognita* 13.00±2.79 a *M. javanica*
Control *M. incognita* race 3	1000	4.00	4.00	27.00	9.00	*M. incognita*
Control *M. javanica* race 3	1000	4.00	4.00	27.00	6.00	*M. javanica*

0-5 egg mass and galling index, 0: no egg mass and galling, 1: 1-2 egg mass and galls, 2: 3-10 egg mass and galls, 3: 11-30 egg mass and galls 4: 31-100 egg mass and galls, 5: >100 egg mass and gall formation (Hartman and Sasser 1985). The different letters in the same column differ from each other according to Duncan multiple comparison test (P≤0.05)

Table 2. First trial results of the interaction of *Meloidogyne incognita* race 3 and *Meloidogyne javanica* race 3 in pepper plant

Çizelge 2. Biber bitkisinde Meloidogyne incognita ırk 3 ve Meloidogyne javanica ırk 3 etkileşiminin birinci deneme sonuçları

RKN Species	**SENA (Susceptible pepper variety)**					
	Starting population (Pi)	**Egg mass index (0-5)**	**Gall index (0-5)**	**Number of females**	**Number of transparent females**	**PAGE result**
M. incognita race 3 + *M. javanica race 3*	1000+1000	5.00	5.00	167.50	40.75	*M. incognita*
Control M. incognita race 3	1000	5.00	5.00	135.00	6.00	*M. incognita*
Control M. javanica race 3	1000	0.00	0.00	0.00	0.00	-

0-5 egg mass and galling index, 0: no egg mass and galling, 1: 1-2 egg mass and galls, 2: 3-10 egg mass and galls, 3: 11-30 egg mass and galls 4: 31-100 egg mass and galls, 5: >100 egg mass and gall formation (Hartman and Sasser 1985). The different letters in the same column differ from each other according to Duncan multiple comparison test (P≤0.05)

A total of 173.00 females and 85.00 transparent females were formed by the second-stage juvenile of 1000 *Meloidogyne javanica* race 3 inoculated susceptible tomato. Overall, 65 days after inoculation of 1000 second-stage *Meloidogyne incognita* race 3 juveniles and 1000 second-stage *M. javanica* 3 juvenile mixture in susceptible tomato plant, 33.00±8.59a *M. incognita* race 3, 26.50 ± 8.18a *M. javanica* race 3 was determined based on the results of Polyacrylamide Gel Electrophoresis. Duncan multiple comparison test indicated that there was no interaction between these two species in tomatoes roots (P≤0.05) (Table 3).

In the interaction of *Meloidogyne incognita* race 3 and *M. javanica* race 3 species on pepper host, the scale value of 0-5 egg mass formed in the roots by the mixture of two species was determined as 4.50 and the galling index was 4.25. In order to determine the viability of the inoculum, the egg mass index was determined as 5. And galling index was 4 on the control susceptible pepper plants inoculated with 1000 *Meloidogyne incognita* race 3. In the control plant, the total number of *Meloidogyne incognita* race 3 females was 135.00 and the number of transparent females was 9.00. It was observed that *Meloidogyne javanica* race 3 did not form any egg mass and galls on pepper roots. In the second experiment, no egg mass and galls formed on roots of pepper. Thus, it was confirmed that this species could not reproduce in pepper plants. It was determined that a total of 185.00 females and 11.00 transparent females formed in the roots of the pepper plant where the two species mixtures were inoculated based on the PAGE diagnosis method and no mutual interaction of these two species was observed in pepper host (Table 4).

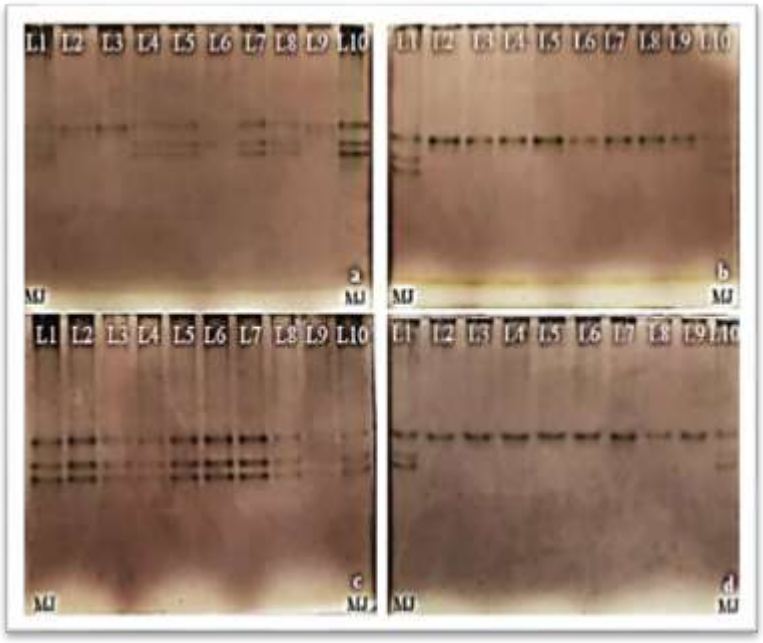

Figure 1. Esterase isoenzyme phenotypes formed on polyacrylamide gel; standard control *Meloidogyne javanica* (MJ) (L1 and L10) a) *M. incognita* race 3+*M. javanica* race 3 populations on tomato host; *M. incognita* race 3 (L2, L3, L9), *M. javanica* race 3 (L4, L5, L7, L8), b) *M. incognita* race 3+*M. javanica* race 3 populations on pepper; *M. incognita* race 3 (L2 and L9), c) *M. javanica* race 3 populations on control tomato (L2 and L9), d) *M. incognita* race 3 population on control pepper (L2 and L9).

Şekil 1. Poliakrilamid jel üzerinde oluşan esteraz izoenzim fenotipleri; standart kontrol Meloidogyne javanica (MJ) (L1 ve L10) a) Domates bitkisindeki M. incognita ırk 3+M. javanica ırk 3 popülasyonları; M. incognita ırk 3 (L2,L3,L9), M. javanica ırk 3 (L4,L5,L7,L8), b) Biber bitkisindeki M. incognita ırk 3+M. javanica ırk 3 popülasyonları; M. incognita ırk 3 (L2 ve L9), c) Kontrol domates bitkisindeki M. javanica ırk 3 popülasyonu (L2 ve L9), d) Kontrol biber bitkisindeki M. incognita ırk 3 popülasyonu (L2 ve L9).

Table 3. Second trial results of the interaction of *Meloidogyne incognita* race 3 and *Meloidogyne javanica* race 3 in tomato plant

Çizelge 3. Domates bitkisinde Meloidogyne incognita ırk 3 ve Meloidogyne javanica ırk 3 etkileşiminin ikinci deneme sonuçları

RKN Species	FALCON (Susceptible tomato variety)					
	Starting population (Pi)	Egg mass index (0-5)	Gall index (0-5)	Number of females	Number of transparent females	PAGE result
M. incognita race 3 + *M. javanica* race 3	1000+1000	5.00	5.00	105.50	69.25	33.00±8.59 a *M. incognita* 26.50±8.18 a *M. javanica*
Control *M. incognita* race 3	1000	5.00	5.00	362.00	45.00	*M. incognita*
Control *M. javanica* race 3	1000	5.00	5.00	173.00	85.00	*M. javanica*

0-5 egg mass and galling index, 0: no egg mass and galling, 1: 1-2 egg mass and galls, 2: 3-10 egg mass and galls, 3: 11-30 egg mass and galls 4: 31-100 egg mass and galls, 5: >100 egg mass and gall formation (Hartman and Sasser 1985). The different letters in the same column differ from each other according to Duncan multiple comparison test (P≤0.05)

Table 4. Second trial results of the interaction of *Meloidogyne incognita* race 3 and *Meloidogyne javanica* race 3 in pepper plant

Çizelge 4. Biber bitkisinde Meloidogyne incognita ırk 3 ve Meloidogyne javanica ırk 3 etkileşiminin ikinci deneme sonuçları

RKN Species	SENA (Susceptible pepper variety)					
	Starting population (Pi)	Egg mass index (0-5)	Gall index (0-5)	Number of females	Number of transparent females	PAGE result
M. incognita race 3 + *M. javanica* race 3	1000+1000	4.50	4.25	185.00	11.00	*M. incognita*
Control *M. incognita* race 3	1000	5.00	4.00	135.00	9.00	*M. incognita*
Control *M. javanica* race 3	1000	0.00	0.00	0.00	0.00	-

0-5 egg mass and galling index, 0: no egg mass and galling, 1: 1-2 egg mass and galls, 2: 3-10 egg mass and galls, 3: 11-30 egg mass and galls 4: 31-100 egg mass and galls, 5: >100 egg mass and gall formation (Hartman and Sasser 1985). The different letters in the same column differ from each other according to Duncan multiple comparison test (P≤0.05)

DISCUSSION and CONCLUSION

It has been reported in various studies that *Meloidogyne javanica* did not infect pepper varieties. All pepper lines and cultivars tested by Peixoto et al. (1995) against *Meloidogyne javanica* were found resistant. Özarslandan & Elekçioğlu (2003) determined that all 16 varieties of pepper plant were resistant to *Meloidogyne javanica* race 1. Pinheiro et al. (2020) examined the reaction of 37 pepper genotypes (*Capsicum annuum, C. chinense and C. frutescens*) against *Meloidogyne javanica* and found all pepper genotypes were resistant or immune to *M. javanica*. In this study, it was observed that *Meloidogyne javanica* did not compete life cycle or did not multiply in the pepper inoculated by mixed population and nevertheless only *M. incognita* race 3 increased in numbers.

Current study results indicated, there was no difference between the interaction of *Meloidogyne incognita* race 3 and *M. javanica* race 3 species on tomato plant. In previous studies, it was reported that the competition among *Meloidogyne* species, especially between *M. javanica* and *M. incognita* was fairly low (Eisenback 1985). Although the antagonistic interactions between *Meloidogyne javanica* and *M. incognita* are not intense, it has been reported that such interactions can occur in nature, which will affect the reproductive efficiency and population growth of nematodes (Khan & Haider 1991). It has been determined that the interaction between two nematodes may be hostile (antagonistic) for one or both species, may have no effect (neutral), or may be beneficial (mutualistic) for one or both species (Eisenback 1985; Khan & Haider 1991). In the interactions between *Meloidogyne javanica* and *M. incognita* species, it was stated that these species can live together closely, affect each other directly or indirectly (Norton 1978) and that one population may not also exclude the other (Gause 1934).

In a mixed population, *Meloidogyne javanica* was found to be able to survive more effectively than *M. incognita*, adapt or compete with *M. incognita*. Also, it has been reported that races 1 and 2 of *M. incognita* can compete life cycle more aggressively than races 3 and 4 (Khan & Haider 1991). Mixed populations of *Meloidogyne incognita* all four races and *M. javanica* did not have intense species interactions in tomato; however, it was determined that there was an intensive interaction between *M. incognita* races 2 and other remaining 3 races (Khan & Haider 1991). In this study, aggression of *Meloidogyne incognita* race 3 against *M. javanica* race 3 was not observed.

It can be thought that the fact that the two species in the experiment did not show a dominant feature to each other and that could be due to the fact of the environmental conditions (25±2 ºC temperature, 60±10% proportional humidity, 16/8 hours of light and darkness) which were remained steady for 65 days. In some studies, it was stated that *Meloidogyne incognita* was dominant in tomato plants at high temperatures and *M. javanica* as so at low temperatures in mixed populations. In addition, it has been determined that *Meloidogyne javanica* suppresses *M. incognita* and *M. hapla* as the temperature decreases, and *M. incognita* suppresses *M. javanica* and *M. hapla* as the temperature increase (Minz & Strich-Harari 1959). It has been reported that the entrance of *Meloidogyne hapla* and *H. schachtii* into the roots of tomato were maximized at 30°C and 26°C, respectively (Griffin 1985). From the plants inoculated with mix of *Meloidogyne incognita* and *M. hapla*, 90% of extracted females was *M. incognita* and 10% was *M. hapla* at

high temperatures, and only 57% that was *M. incognita* at low temperatures (Chapman 1965).

Haider (1989) reported that there is dominant interspecies interaction between *Meloidogyne* species and the races. It has been stated that two nematode species can co-exist when competition among individuals in a species is greater than competition between species (Brewer 1978). In this study, the neutrality of the interaction of *Meloidogyne incognita* race 3 and *M. javanica* race 3 may be thought to be due to the high competition between individuals within the species' own populations.

In nature, plant parasitic nematodes are rarely found as mono-specific populations. Instead, nematodes constantly interact with the plant, the environment, and other organisms. Mixed infections of the species on plants are common. In this study, trials for the invasion and development of *Meloidogyne incognita* race 3 and *M. javanica* race 3 species community on susceptible tomato and pepper plants were equally applied and carried out at appropriate climatic conditions. A certain degree of temperature in the climate chamber did not affect the dominance of the two species against each other and did not cause an interaction between these two root-knot nematode species. Therefore, it can be considered that environmental conditions are important in the competition of a nematode with other species. In addition, since only *Meloidogyne incognita* race 3 was found in the pepper host, the coexistence of *M. javanica* race 3 with a second species in the soil did not cause *M. javanica* to enter the roots of a non-host plant by competing with the other species. It can be concluded that the damage to the plant can be reduced by using pepper varieties in the crop rotation to manage the *Meloidogyne incognita* and *M. javanica* species found together in the field.

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Author's Contributions

The contribution of the authors is equal.

Statement of Conflict of Interest

The authors declare no conflict of interest.

REFERENCES

Abad P, Favery B, Rosso MN, Castagnone-Sereno P 2003. Root-knot nematode parasitism and host response: molecular basis of a sophisticated interaction. *Molecular Plant Pathology*, *1*(4):217-224. https://doi.org/10.1046/j.1364-3703.2003.00170.x.

Barker KR, Olthof THA 1976. Relationships between nematode population densities and crop responses. *Annual Review Phytopathology*, *14*, 327- 353. https://doi.org/10.1146/annurev.py.14.090176.001551.

Barker KR 1978. Relative sensitivity of flue-cured tobacco cultivars to four species of *Meloidogyne*. *Journal of Nematology*, *10*, 281-282.

Barros AF, Campos VP, Souza LN, Costa SS, Terra WC, Lessa JHL 2018. Morphological, enzymatic and molecular characterization of root-knot nematodes parasitizing vegetable crops. *Horticultural Brasileira*, *36*(4), 473-479. https://doi.org/10.1590/S0102-053620180408.

Brewer R 1978. Principles of Ecology. W. B. Saunders Company., Philadelphia, Penn. 299.

Curtis RHC 2008. Plant-nematode interactions: environmental signals detected by the nematode's chemosensory organs control changes in the surface cuticle and behavior Parasite, *15*(3), 310–316. https://doi.org/10.1051/parasite/2008153310.

Chapman RA 1965. Infection of single root systems by larvae of two coincident species of root-knot nematodes. *Nematologica, 12*, 89.

Eisenback JD 1985. Interactions among concomitant populations of nematodes. In Sasser, J.N., and Carter, C.C. Eds. An advanced treatise on *Meloidogyne*, *Volume 1*. Raleigh: North Carolina State University Graphics. 193-213.

Eisenback JD, Griffin GD 1987. Interactions with other nematodes. In Veech, J.A., and Dickson, D. Eds. Vistas on Nematology. *Society of Nematologists*. 313-320.

Esbenshade PR, Triantaphyllou AC 1985. Use of enzyme phenotypes for identification of *Meloidogyne* species (Nematoda: Tylenchida). *Journal of Nematology*, *17*(1): 6-20.

Ferris H 1974. Correlation of tobacco yield, value, and root-knot index with early to midseason and postharvest *Meloidogyne* population densities. *Journal of Nematology*, *6*(2):75-81. PMID: 19319371; PMCID: PMC2620044.

Gause GF 1934. The struggle for existence. Baltimore: Williams and Wilkins Company, 163. DOI: https://doi.org/10.5962/bhl.title.4489.

Griffin GD 1985. Interrelationship of *Heterodera schachtii* and *Meloidogyne hapla* on tomato. *Journal of Nematology*, *17*(4), 385-388.

Haider SR 1989. Studies on identity of *Meloidogyne* species and their interactions in vegetable crop pathosystem. PhD. Thesis. Aligarh Muslim University, Aligarh, India, 350.

Harris H, Hopkinson DA 1976. Handbook of enzyme electrophoresis in human genetics. Amsterdam: North Holland Pubi Company, *Volume 1*.

Hartman KM, Sasser JN 1985. Identification of *Meloidogyne* species on the basis of differential host test and perineal pattern morphology, '69-79'. An

Advanced Treatise on *Meloidogyne*, Volume II, Methodology, Eds.: Barker, K.R., Carter, C.C., and Sasser, J.N. North Carolina State University. Graphics, 223.

Hooper DJ 1986. Extraction of free-living stages from soil. In J.F. Southey, Ed. Laboratory Methods for Work with Plant and Soil Nematodes. *Ministry of Agriculture Fisheries and Food*, Reference Book, 402.

Johnson AW, Nusbaum CJ 1970. Interactions between *Meloidogyne incognita*, *M. hapla*, and *Pratylenchus bmchyurus* in tobacco. *Journal of Nematology*, *2*(4), 334-340.

Khan MW, Haider SR 1991. Interaction of *Meloidogyne javanica* with different races of *Meloidogyne incognita*. *Journal of Nematology*, *23*(3), 298.

Kinloch RA, Allen MW 1972. Interaction of *Meloidogyne hapla* and *M. javanica* infecting tomato. *Journal of Nematology*, *4*(1), 7-16.

Lucas GB 1975. Diseases of tobacco. *Biological Consulting Associates*, Raleigh, North Carolina. 621.

Minz G, Strich-Harari D 1959. Inoculation experiments with *Meloidogyne* spp. on tomato roots. *Ktavim Records of Agricultural Research Station, 9*, 275-279.

Mitkowski NA, Abawi GS 2003. Root-knot nematodes. *The Plant Health Instructor*. Online publication, 10. https://doi.org/10.1094/PHI-I-2003-0917-01.

Moens M, Perry R, Starr, J 2009. *Meloidogyne* species: a diverse group of novel and important plant-parasites. In: Root-Knot nematodes. Perry, R., Moens, M., and Starr, J. (Eds): CABI Nosworthy Way, Wallingford Oxfordshire OX10 8DE, UK. 1-17. https://doi.org/10.1079/9781845934927.0001.

Mukhtar T, Kayani MZ, Hussain MA 2013. Response of selected cucumber cultivars to *Meloidogyne incognita*. *Crop Protection*, *44*, 13-17. DOI: 10.1016/j.cropro.2012.10.015.

Norton DC 1978. Ecology of plant-parasitic nematodes. John Wiley and Sons, New York. 268.

Oostenbrink M 1966. Major characteristics of the relation between nematodes and plants. *Major characteristics of the relation between nematodes and plants.* Landbouw. Wageningen, 46.

Özarslandan A, Elekçioğlu İH 2003. Bazı hıyar, domates ve biber çeşitlerinin Kök-ur nematodları (*Meloidogyne javanica* Chitwood, 1949 ırk-1 ve *M. incognita* Chitwood, 1949 ırk-2) (Nemata: Heteroderidae)'na karşı dayanıklılıklarının araştırılması. *Türkiye Entomoloji Dergisi*, ISSN 1010-6960, *27*(4), 279-291.

Peixoto JR, Maluf WR, Campos VP 1995. Evaluation of red pepper genotypes for resistance to *Meloidogyne incognita* race-2 and *M. javanica*. *Horticultura Brasileira*, *13*, 154.

Pinheiro JB, Silva GOD, Macêdo AG, Biscaia D, Ragassi CF, Ribeiro CS, Reifschneider FJB 2020. New resistance sources to root-knot nematode in *Capsicum* pepper. *Horticultura Brasileira*, *38*(1), 33-40. https://doi.org/10.1590/S0102-053620200105.

Siddiqui Y, Ali A, Naidu Y 2014. Histopathological changes induced by *Meloidogyne incognita* in some ornamental plants. *Crop Protection*, *65*, 216-220. https://doi.org/10.1016/j.cropro.2014.08.001.

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Evaluation of Some Local and Registered Safflower (*Carthamus tinctorius* L.) Varieties Based on SRAP Markers

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ABSTRACT

Safflower (*Carthamus tinctorius* L.), a member of the Asteraceae family, is an important plant grown in the world as a source of vegetable oil. In addition, it is a versatile crop that is also used as biodiesel, animal feed, spice, dye, and medicinal plant. In this study, SRAP markers were used to determine the genetic diversity and relationships between four local and three registered safflower cultivars for use in cross-breeding programs. The twelve primer combinations yielded a total of 101 bands, including 33 polymorphic bands. The level of polymorphism of SRAP markers which were represented by the average number of total bands (NTB) (8.4), the average number of polymorphic bands (NPB) (2.8), polymorphic band ratios (PBR%) (34.5%), resolving power (RP) (1.48), effective multiplex ratio (EMR) (1.17), and marker index (MI) (0.43) was low. Conversely, polymorphism information content (PIC) (0.35), Nei's gene diversity (h) (0.36) and Shannon's information index (I) (0.55) showed a significant genetic variation in the safflower genotypes studied. The polymorphism information content of the SRAP primer combinations used in the study ranged from 0.24 to 0.46, with an average of 0.35. Genetic similarity was calculated according to Dice similarity and varied from 0.12 to 0.92, with a mean genetic similarity (GS) of 0.58. The cophenetic correlation between the Dice similarity matrix and corresponding dendrogram obtained by SRAP (r = 0.95) revealed very good compliance. The genetically close genotypes were Remzibey05 - TR64702 and TR49119 - TR42630 (GS=0.91). Also, Dinçer5-118 and Yenice5-38 were the most genetically distant varieties (GS=0.12). Dinçer5-118 was very different from other genotypes (GS=0.29).

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Bazı Yerel ve Tescilli Aspir Çeşitlerinin (*Carthamus tinctorius* L.) SRAP Markörleri ile Değerlendirilmesi

ÖZET

Asteraceae familyasının bir üyesi olan aspir (*Carthamus tinctorius* L.), bitkisel yağ kaynağı olarak dünyada yetiştirilen önemli bir bitkidir. Ayrıca biyodizel, hayvan yemi, baharat, boya ve tıbbi bitki olarak da kullanılan çok yönlü bir bitkidir. Bu çalışmada, melezleme programlarında kullanılmak üzere dört yerel ve üç tescilli aspir çeşidi arasındaki genetik çeşitliliği ve ilişkileri belirlemek için SRAP markörleri kullanılmıştır. On iki primer kombinasyonu, 33 polimorfik bant olmak üzere toplam 101 bant vermiştir. Primer başına düşen ortalama bant sayısı (NTB) (8.4), ortalama polimorfik bant sayısı (NPB) (2.8), polimorfik bant oranı (%PBR) (%34.5), çözümleme gücü (RP) (1.48), efektif multipleks oranı (EMR) (1.17) ve marker indeksi (MI) (0.43) ile incelenen SRAP markörlerinin polimorfizm seviyesi düşük bulunmuştur. Aksine, polimorfizm bilgi içeriği (PIC) (0.35), Nei'nin gen çeşitliliği (h) (0.36) ve Shannon'ın bilgi indeksi (I) (0.55), çalışılan aspir genotiplerinde önemli bir genetik varyasyon göstermiştir. Çalışmada kullanılan SRAP primer kombinasyonlarının polimorfizm bilgi içeriği (PIC) 0.24 ile 0.46 arasında değişmiş olup, ortalama 0.35 olarak tespit edilmiştir. Dice'ın benzerlik katsayısına göre hesaplanan genetik benzerlik, 0.12 ile 0.92

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Aspir
Carthamus tinctorius
SRAP markörleri
Genetik çeşitlilik
DNA parmak izi

arasında değişmiş ve ortalama genetik benzerlik (GS) 0.58 olarak belirlenmiştir. Dice benzerlik matrisi ile SRAP tarafından elde edilen dendrogram arasındaki kofenetik korelasyon (r = 0.95) çok iyi bir uyumu ortaya çıkarmıştır. Genetik olarak en yakın çeşitler Remzibey05-TR64702 ve TR49119- TR42630 (GS=0.91) olmuştur. Dinçer5-118 ve Yenice5-38 tescilli çeşitler genetik olarak en uzak çeşitler olarak bulunmuştur. (GS=0.12). Ayrıca Dinçer5-118 diğer çeşitlerden genetik olarak çok farklı olarak belirlenmiştir. (GS=0.29).

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INTRODUCTION

Safflower (*Carthamus tinctorius* L.) is a member of the Asteraceae family with yellow, red, orange, white, and cream-coloured flowers (Knowles, 1989). It has a taproot system that can go about 2-3m deep and secondary roots that can grow up to 60-90 cm. Therefore, it is more suitable to grow in arid conditions than other oilseed plants. Safflower has been used as oil, spice, tea, medicinal, and dye plant. Safflower seeds contain 25-45% oil, 90% of which is made up of unsaturated fatty acids (Weiss, 2000). Its oil is highly rich in tocopherol (vitamin E) (Weiss, 1971). *Carthamin* and *Carthamidin* pigments in their contents are used for food and textile applications (Yue et al., 2013; Golkar, 2018). Moreover, because of its chemical composition, it has pharmacological functions that include antioxidant, anti-inflammatory, analgesic, antidiabetic, hepatoprotective, and antihyperlipidemic activities (Asgarpanah and Kazemivash, 2013; Delshad et al., 2018). In recent years, it has also been heavily favoured as a raw material in biodiesel production (Yesilyurt et al., 2020; Nogales-Delgado et al., 2019).

Safflower is one of humanity's oldest plants. It has been cultivated in the Mesopotamian plains and the Eastern Anatolian Region and according to archaeological findings, its domestication is likely to date back to about 2500 B.C.E. (Prance and Nesbitt 2005). Commercial production began only in the 1940s. Currently, safflower is grown in different geographical regions of the world, primarily in Kazakhstan, USA, Russian Federation, Mexico, China, India, Argentina, and Turkey. These countries account for about 90% of the world's safflower production. Turkey is one of the World's ten largest safflower producers, it ranked 8th in 2020 with 21.325 tons of safflower seed production in approximately 15,114 hectares of land (FAOSTAT, 2022). The average safflower seed yield in Turkey is 14.10 hg/ha, which is over a global average of 9.05 hg/ha. Safflower cultivation in Turkey fluctuates from year to year. In the 1960s, 900-1000 ha plantation was made, while in 2000 it fell by as much as 30 ha, and in 2009 it was planted at 21.500 ha, increasing by about 16.000 ha compared to the previous year. During the 2014-2015 cultivation seasons, safflower plantation reached a record high with approximately 45.000 ha of plantation and 70,000 tons of production (FAOSTAT, 2022; TUIK, 2022). This plant was brought to Turkey via the Balkans by Bulgarian migrants in the 1940s and was first cultivated around Balıkesir, Bursa, and Kütahya (Baydar, 2021). Today, safflower agriculture is mostly done in 37 provinces including Ankara, Muş, Aksaray, Konya, Gümüşhane, Nevşehir, Kayseri, Afyon and Uşak (TÜİK, 2022).

The first studies of safflower plant breeding in Turkey were initiated in the early years of the Republic. In 1931, 'Yenice 5-38' safflower variety, which is composite of 5 spiny safflower varieties, was developed by the selection method. After a long time, through the selection method, "Dinçer 5-118" and "Remzibey 05" were registered in 1977 (Köse, 2017). In 2008-2009, safflower farming gained a lot of momentum and there were large increases in both cultivation areas and production, whereas breeding studies have been limited in developing different new safflower varieties with high grain yield and oil ratio and different oil compositions. New varieties (Balcı, Linas, Olas, Zirkon, Olein, Safir) developed with selection and hybridization breeding methods have been offered to the manufacturers of safflower since 2011. Turkey has increased safflower production by more than 50.000 tons in the years 2013-2015. The government's agricultural assistance for oilseed plants has been the biggest factor in the development of safflower agriculture. However, in 2016-2019, production decreased by more than 48,000 tons. This unforeseen decline is due to the marketing problem and the fact that the producers were not able to make a profit from safflower (Ilkdoğan, 2012). Another reason is that both seed yield and oil ratio remain low compared to other oil plants such as sunflower, sesame seeds, and rapeseeds with which safflower yield competes (Baydar & Erbaş, 2020). One of the main factors necessary for increasing productivity in safflower agriculture is the use of efficient and high-quality improved seeding. For safflower production to be

stable and sustainable in Turkey, alternative cultivation and breeding methods that will increase grain and oil yield and better cope with biotic and abiotic stress factors should be implemented considering the demands of producers, industrialists, and consumers.

The variation required for breeding studies is provided from proprietary varieties, local varieties, and wild relatives. Therefore, it is important to reveal genetic states of the cultivated populations for proper design of breeding programs and successful sustainability of populations to improve both yields and oil quality. Molecular markers are an important tool for assessing the levels and structure of genetic diversity and have been used to study genetic diversity in many breeding programs. Different molecular marker system could be used to assess germplasm diversity including SRAP (Sequence Related Amplified Polymorphism) markers, which have many advantages such as simplicity, reliability, flexibility, multiple-locus detection, genome-wide scopes, and cost-effectiveness (Li & Quiros, 2001; Li et al., 2013). It is a marker system that was developed to eliminate disadvantages related to AFLP and RAPD methods and was first used in *Brassica* species. SRAP markers are dominant, simple, and effective for amplification of open reading frames (ORFs), based on the amplification of forwards and reverse primers of 17-18 nucleotides. SRAP markers have widely been used to evaluate the genetic diversity and structure population in species, such as sesame (*Sesamum indicum* L.) (Zhang et al., 2010), soybean (*Glycin max* (L.) Merr.), peanut (*Arachis hypogaea* L.) (Baloch et al., 2010), safflower (*Carthamus tinctorius*) (Peng et al., 2008; Talebi et al., 2012), oilseed rape (*Brassica napus* L.) (Ahmad et al., 2014), flax (*Linum usitatissimum* L.) (Li et al., 2009). In this research, it was aimed to determine the relationships and genetic diversity of local and registered safflower genotypes and varieties to be used in crossbreeding programs with SRAP molecular markers.

MATERIALS and METHODS

Plant Materials

The material of the study consisted of the four local safflower genotype (TR 49119, TR 42630, TR 42670, and TR 64702) and three registered varieties (Yenice 5-38, Remzibey 05 and Dinçer 5-118) which were obtained from the Aegean Agricultural Research Institute, Izmir, Turkey. General information about the plant material (Table 1) is provided in a previous report (Giachino & Inan 2019). Molecular analyses were carried out at the laboratory of Ege University Application and Research Centre of Seed Technology (TOTEM).

DNA extraction

Genomic DNA was isolated from fresh leaves using the GenElute™ Plant Genomic DNA Miniprep Kit (Sigma-Aldrich). For this purpose, samples taken from the fresh leaves of young seedlings of 10-15 cm length were powdered with a mortar and pestle in liquid nitrogen. DNA quality and quantity were measured through 260:280 nm absorbance ratios with a spectrophotometer, and electrophoresis was conducted on 0.8% agarose gel in 1x TAE buffer at 100 V for 1 hours and stained with 0.5 µg/ml ethidium bromide (EtBr) and photographed under UV light. DNA samples were diluted to 10 ng/mL and stored at −20°C.

Sequence-related amplified polymorphism (SRAP) Analysis

The SRAP analyses were performed as described by Li & Quiros (2001) with some modifications. A total of 17 primer combinations (4 forward and 7 reverse) were screened and 12 suitable primer combinations were selected for amplification (Table 2) provided by ECS (Canada). The PCR reaction was performed in a total volume of 25 µl reaction mixtures consisting of 1X PCR buffer, 0.2 mM dNTP, 0.5 µM of each forward and reverse primer, 2 mM $MgCl_2$, 50 ng template DNA, 1 Unit Taq DNA Polymerase, and ddH_2O. DNA amplification reactions were performed in a Techne thermal cycler (Germany) using the following program: 94 °C initial denaturation for 5 min, then 5 cycles of 94 °C denaturation for 1 min, 35 °C annealing for 1 min, 72 °C elongation for 2 min, then 30 cycles of 94 °C denaturation for 1 min, 50 °C for 1 min, 72 °C for 2 min, followed by a 72 °C final extension for 5 min. The amplification products were separated by electrophoresis in 2% agarose gels in 1x TAE buffer (40 mM Tris-Acetate, 1 mM EDTA, pH: 8.0) at 100 V for 2-3 hours and stained with 0.5 µg/ml ethidium bromide (EtBr) and photographed under UV. Also, a 100 bp plus DNA ladder (Fermentas) was used as a standard marker for estimating the size of the PCR products.

Data Analysis

The SRAP bands of 12 primer combinations were graded according to their presence (1) or absence (0) in electrophoresis, and data was converted to the binary matrix using Microsoft Excel. For each SRAP primer combination, the total number of scored bands, the number of polymorphic bands, the number of monomorphic bands, and the percentage of polymorphism were determined. In addition, parameters such as polymorphism information content (PIC), resolving power (RP), effective multiplex ratio (EMR), and marker index (MI) were calculated. The PIC was calculated according to Anderson et al. (1993) by using the following formula for all primers:

$$PIC = 1 - \Sigma\, pi^2 \qquad (1)$$

where pi^2 is the frequency of the i th allele. The resolving power (RP) was calculated according to Prevost and Wilkinson (1999) for each primer

combination as follows:

$$RP = \Sigma\ I_b \quad (2)$$

where I_b is the band informativeness calculated with the following formula

$$I_b = 1 - [2 \times (0.5-p)] \quad (3)$$

and p is the proportion of seven genotypes containing the band. The effective multiplex ratio is the number of polymorphic bands detected per electrophoresis. The marker index was calculated as noted by Powell et al. (1996) and Milbourne et al. (1997) by multiplying PIC with the EMR (MI = EMR X PIC). Genetic diversity indicators such as Nei's gene diversity (h) and Shannon's information index (I) were calculated for each SRAP marker with POPGENE version 1.31 (Yeh et al., 1997). The dendrogram was generated by the Unweighted Pair Group Method Averages (UPGMA) with the NTSYSpc-2.02 software (Rohlf, 2000) using the Dice genetic similarity matrix (Dice, 1945). A principal coordinate analysis (PCoA) was also carried out using the same software. To determine the goodness-of-fit of the clustering compared with the basic data matrix, the cophenetic correlation coefficient was computed using the normalised Mantel's Z test (Mantel, 1967) via the COPH and MXCOMP procedures of NTSYS-pc, version 2.01e (Rohlf, 2000).

Table 1. General features of safflower genotypes used in the study*
Çizelge 1. Araştırmada kullanılan aspir genotiplerinin genel özellikleri

Local Genotypes	*Collection Year*	*Province*	*District*	*Altitude*	*Latitude*	*Longitude*
1-TR49119	1988	Isparta	Gelendost	860 m	380715N	0310055E
2-TR42630	1980	Edirne	Havsa	40 m	412054N	0265523E
3-TR42670	1980	Tekirdağ	Saray	240 m	412626N	0275519E
4-TR64702	1996	Mersin	Anamur	850 m	360442N	0325003E
Registered Varieties	*Registration Year*	*Breeding Method*	*Colour of Flower*	*Plant Length*	*Structure*	*Breeding Institution*
5-Yenice 5-38	1931	Selection	Red	100-120 cm	non-spiny	GKTAEM
6-Remzibey 05	2005	Selection	Yellow-orange	60-80 cm	spiny	GKTAEM
7-Dinçer 5-118	1977	Selection	Orange-red	90-110 cm	non-spiny	GKTAEM

(GKTAEM): Transitional Zone Agricultural Research Institute
*Taken from previous work by Giachino & Inan (2019)

Table 2. Sequence information of the SRAP primers used in this study
Çizelge 2. Çalışmada kullanılan SRAP primerinin dizi bilgisi

Forward primers	*5'→3' sequence*	*Reverse primers*	*5'→3' sequence*
Me1	TGAGTCCAAACCGGATA	Em1	GACTGCGTACGAATTAAT
Me3	TGAGTCCAAACCGGAAT	Em2	GACTGCGTACGAATTTGC
Me4	TGAGTCCAAACCGGACC	Em3	GACTGCGTACGAATTGAC
Me5	TGAGTCCAAACCGGAAG	Em4	GACTGCGTACGAATTTGA
		Em5	GACTGCGTACGAATTAAC
		Em7	GACTGCGTACGAATTATG
		Em8	GACTGCGTACGAATTAGC

RESULTS

Safflower genotypes were evaluated for determining genetic diversity using SRAP primer combinations. A total of 19 primer combinations were screened, of which 12 primer combinations yielded evaluable bands. The 12 primer combinations generated a total of 101 bands, including 33 polymorphic bands. The polymorphic band ratio is 32.6%. The number of total bands (NTB), number of polymorphic bands (NPB), polymorphic bands ratios (PBR %), PIC, RP, EMR, and MI values are presented in Table 3. The NTB amplified by each primer combination ranged from 2 (Me4xEm3) to 11 (Me3xEm2, Me4xEm1, Me5xEm5, and Me5xEm8) with an average of 8.4, and their molecular weights were between 80 and 1.600 bp. The NPB with each primer combination ranged from 1 (Me1xEm2, Me3xEm3, and Me4xEm3) to 6 (Me3xEm1) with an average of 2.8. The PBR of the primer combinations ranged from 13% (Me3xEm3) to 67% (Me3xEm1). The average percentage of the polymorphic bands was calculated as 34.5%. Only in 4 SRAP primer combinations (Me3xEm1, Me3xEm4, Me4xEm3, Me5xEm4), PBRs were found to be 50%, as well as, greater than 50%. The PBR of the other eight primer combinations was observed to be below 50%.

The PIC values for the 12 primer combinations varied from 0.24 to 0.46 with an average of 0.35. The same results were obtained for RAPD primers reported by Giachino and Inan (2019), PIC ranged from 0.24 to 0.46 with an average of 0.38. The lowest value was observed in three primer combinations, including Me1xEm2, Me3xEm4, and Me4xEm3, and the highest PIC value

was seen in Me3xEm1. Half of the SRAP primer combinations exceeded the average PIC values (0.35) (Table 3).

Table 3. Diversity parameters evaluated using SRAP markers to investigate the genetic diversity of safflower genotypes

Çizelge 3. Aspir genotiplerinin genetik çeşitliliğini araştırmak için SRAP belirteçleri kullanılarak değerlendirilen çeşitlilik parametreleri

Primer combination	*NTB*	*NPB*	*PBR%*	*PIC*	*RP*	*EMR*	*MI*	*h*	*I*
Me1xEm2	7	1	14	0.24	0.29	0.14	0.03	0.25	0.41
Me3xEm1	9	6	67	0.46	4.57	4.00	1.85	0.46	0.65
Me3xEm2	11	2	18	0.41	1.14	0.36	0.15	0.41	0.60
Me3xEm3	8	1	13	0.41	0.57	0.13	0.05	0.41	0.60
Me3xEm4	6	4	67	0.24	1.14	2.67	0.65	0.24	0.41
Me3xEm5	7	2	29	0.33	0.86	0.57	0.19	0.33	0.50
Me4xEm1	11	3	27	0.44	2.00	0.82	0.36	0.44	0.63
Me4xEm3	2	1	50	0.24	0.29	0.50	0.12	0.24	0.41
Me4xEm7	8	2	25	0.33	0.86	0.50	0.16	0.33	0.50
Me5xEm4	10	5	50	0.41	3.14	2.50	1.02	0.41	0.59
Me5xEm5	11	4	36	0.29	1.43	1.45	0.42	0.29	0.46
Me5xEm8	11	2	18	0.45	1.43	0.36	0.16	0.45	0.64
AV	**8.4**	**2.8**	**34.5**	**0.35**	**1.48**	**1.17**	**0.43**	**0.36**	**0.55**
Min.	**2**	**1**	**13**	**0.24**	**0.29**	**0.13**	**0.03**	**0.24**	**0.41**
Max.	**11**	**6**	**67**	**0.46**	**4.57**	**4.00**	**1.85**	**0.46**	**0.65**
Total	**101**	**33**	**32.6**	**-**	**-**	**-**	**-**	**-**	**-**

NTB: Number of total bands, NPB: Number of polymorphic bands, PBR%: Polymorphic band ratios, PIC: Polymorphism information content, RP: Resolving power, EMR: Effective multiplex ratio, MI: Marker index, h: Nei's gene diversity, I: Shannon's information index

The RP ranged from 0.29 (for primer combinations of Me1xEm2 and Me4xEm3) to 4.57 (for primer combinations of Me3xEm1) with an overall average of 1.48. Only three primer combinations (Me3xEm1, Me4xEm1, and Me5xEm4) exceeded the mean RP value. The highest EMR was 4.0, observed in Me3xEm1, but the lowest EMR was 0.13, obtained from Me3xEm3, and the average value was 1.17 per primer combination. The MI values ranged from 0.03 to 1.85, with an average of 0.43. Maximum MI was observed in the Me3xEm1 primer combination, and the minimum MI was obtained with SRAP primer combinations of Me1xEm2 (0.03) and Me3xEm3 (0.05).

The Nei's gene diversity values ranged from 0.24 to 0.46, with a mean of 0.36 (Table 3). The higher gene diversity was found in Me3xEm1 primer combination while the lower in Me3xEm4 and Me4xEm3 as 0.24. The Shannon's information index ranged from 0.41 (for primer combinations of Me1xEm2, Me3xEm4 and Me4xEm3) to 0.65 (for primer combination of Me3xEm1) with an overall average of 0.55.

When evaluated on the basis of SRAP primer pairs; the Me3xEm1 primer pair was the most informative by giving highest mean values of number of polymorphic bands (6), polymorphic band ratios (67), polymorphism information content (0.46), resolving power (4.57), effective multiplex ratio (4.00), marker index (1.85), Nei's gene diversity (0.46), and Shannon's information index (0.65), while the Me1xEm2 primer pair was least informative by reproducing low values of number of polymorphic bands (1), polymorphic band ratios (14), polymorphism information content (0.24), resolving power (0.29), effective multiplex ratio (0.14), marker index (0.03), Nei's gene diversity (0.25), and Shannon's information index (0.41).

The Dice similarity matrix was generated using NT-SYS software to analyse the SRAP data of safflower genotypes. Genetic similarity was calculated by making pairwise comparisons between all local and registered safflower genotypes by this matrix. Genetic similarity (GS) showed a wide distribution and varied from 0.12 to 0.92, with a mean similarity of 0.58 (Table 4). Genetically, the closest genotypes were Remzibey 05 and TR64702 with a value of 0.92, followed by TR49119 and TR42630 local genotypes with a value of 0.91, indicating a very close relationship. Dinçer 5-118 and Yenice 5-38 were the most genetically distant varieties with a value of 0.12. The cophenetic correlation coefficient, which is a measure of the correlation between the dendrogram, and similarity matrix calculated using the Z test (Mantel, 1967), was found to be 0.95, indicating that the clustering result shows very good compliance with the genetic similarity matrix.

Figure 1 shows the dendrogram based on the SRAP data. In the UPGMA dendrogram, safflower genotypes clustered into two main groups based on the Dice coefficient. Cluster I was further divided into three

subclusters: Subcluster 1 includes 3 local genotypes: TR 49119, TR 42630, and TR 42670. Of these, the TR49119 and TR42630 local genotypes were observed to be extremely similar with a coefficient of 0.91. Subcluster 2 includes TR 64702 landrace and Remzibey 05 cultivar which are the closest genotypes, with Dice values of 0.92. (Table 4). Subcluster 3 comprised a single variety, Yenice5-38. Cluster II also consisted of a single variety, Dinçer5-118. The distribution determined by UPGMA analysis also revealed that Dinçer5-118 registered variety was genetically very different from Yenice5-38 (0.12 GS) and from all other genotypes with 0.29 GS.

Table 4. The genetic similarity matrix based on the Dice coefficient calculated from SRAP data of safflower genotypes

Çizelge 4. Aspir çeşitlerinin SRAP verilerinden hesaplanan Dice katsayısına dayalı genetik benzerlik matrisi

	TR49119	*TR42630*	*TR42670*	*TR64702*	*Yenice5-38*	*Remzibey05*	*Dinçer5-118*
TR49119	1.00						
TR42630	**0.91**	1.00					
TR42670	0.79	0.78	1.00				
TR64702	0.67	0.63	0.79	1.00			
Yenice5-38	0.56	0.56	0.65	0.58	1.00		
Remzibey05	0.64	0.60	0.76	**0.92**	0.67	1.00	
Dinçer5-118	0.32	0.24	0.28	0.39	**0.12**	0.40	1.00

The bold values indicate the maximum and minimum genetic similarity values among the landraces

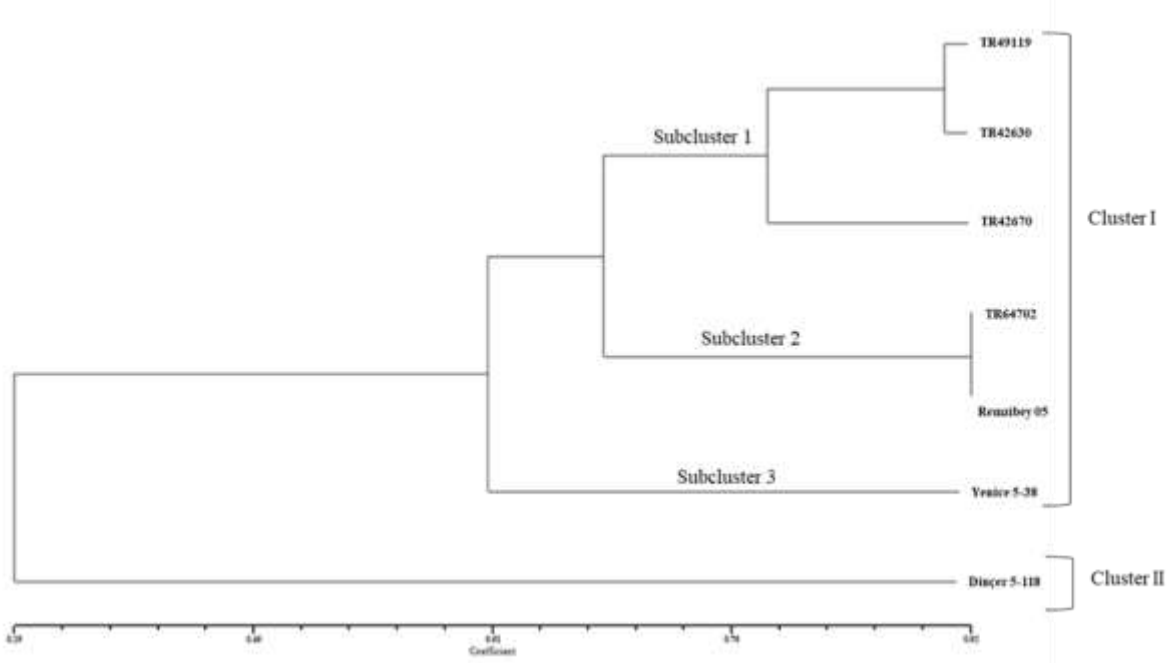

Figure 1. The UPGMA dendrogram of the safflower genotypes generated from SRAP data.
Şekil 1. Aspir çeşitlerinin SRAP verilerinden oluşturulan UPGMA dendrogramı

The results of PCoA for the SRAP data are presented in Fig. 2. The first two principal coordinates explained 66.5% and 13.5% of the total molecular variation, respectively. This corresponds to 80% of the total variation. The results of the PCoA analysis were in good agreement with the cluster analysis. The PCoA plot of SRAP clearly showed the main clusters of safflower genotypes.

DISCUSSION and CONCLUSIONS

In this research, the relationships and genetic diversity between local and registered safflower genotypes were determined by SRAP markers that are easy to apply and reliable. Twelve primer combinations yielded a total of 101 bands, including 33 polymorphic bands. The polymorphic band ratio is 34.5%. The level of polymorphism of SRAP markers, represented by the average number of total bands (8.4), average number of polymorphic bands (2.8), percentage of polymorphism (34.5 %), resolving power (1.48), effective multiplex ratio (1.17), and marker index (0.43), was low. Especially, the number of polymorphic bands detected with each primer combination is considerably lower than Peng et al.

(2008) (30), Talebi et al. (2012) (18.7), Mokhtari et al. (2013) (20.3) and slightly less than Golkar & Mokhtari (2018) (7.3). The possible explanation for the relatively low number of polymorphic bands may be associated with the investigated local and registered genotypes and different combinations of loci in the present research. The level of polymorphism is influenced by the number of markers, population size, and the type of plant material used in the study (Kiran et al., 2017).

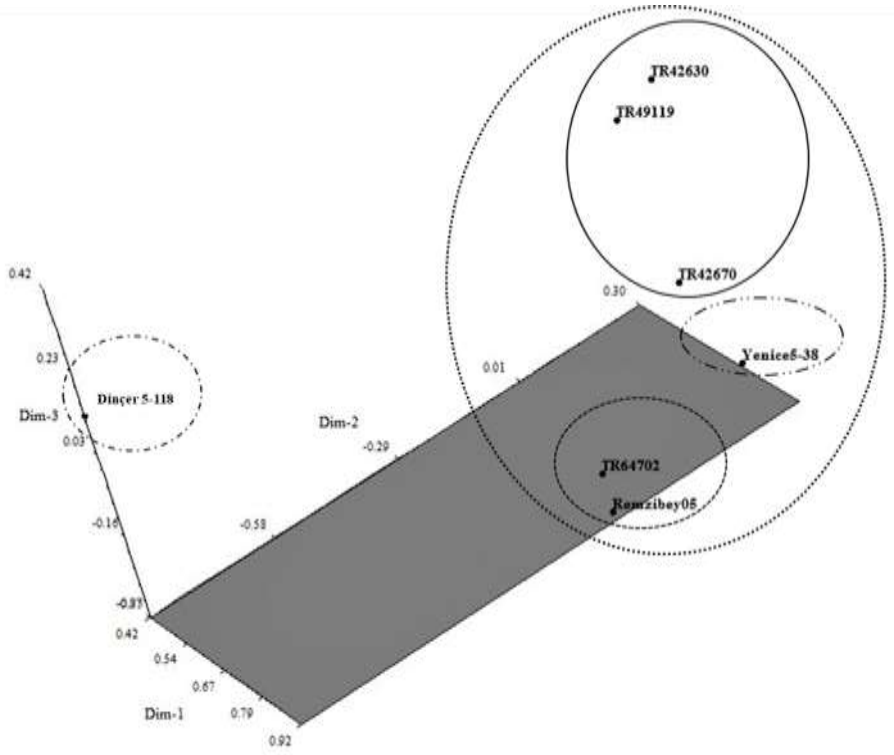

Figure 2. Principal coordinate analysis of safflower varieties based on the genetic similarity matrix generated from SRAP data.

Şekil 2. SRAP verilerinden oluşturulan genetik benzerlik matrisine dayalı olarak aspir çeşitlerinin temel koordinat analizi

A low level of polymorphism in safflower is also evident in previous studies. In our previous research with RAPD markers conducted on the same material of the current study, the number of polymorphic bands was similarly low (ranged from 2 to 9 with an average of 6) (Giachino & Inan, 2019). In addition, the number of polymorphic bands produced by the Me3xEm3, Me1xEm2, Me3xEm2, and Me5xEm8 primer combinations is considerably lower than that produced by the others, which may partly contribute to the low polymorphic fragments. Lee et al. (2014) observed an average of 2.8 alleles per SSR locus in a collection of 100 safflower accessions from different centres of similarity. Kiran et al. (2017) reported SSR alleles per locus ranged from 2 to 15 with an average of 3.6 in a collection of 148 safflower accessions representing 15 countries. Small polymorphic band numbers resulted in a relatively low average polymorphism percentage (34.5%). Similar results were also revealed by Lei et al. (2013) (35%). Several safflower genetic diversity studies with SRAP markers reported a greater result than the polymorphism ratio found in this study, including Peng et al. (2008) (57%), Talebi et al. (2012) (62.2%), Mokhtari et al. (2013) (82%), and Golkar & Mokhtari (2018) (76.3%). Also, in a previous RAPD study of Giachino & Inan (2019), the polymorphism ratio of the same varieties was observed as 63.9%. Indeed, Sehgal et al. (2009) reported a low average of polymorphic genes in the Turkish population (0.15 ratio) in a collection of 85 safflower accessions from different regional gene pools (originating from 24 countries). Furthermore, Tonguç et al. (2011) employed AFLP markers in 38 varieties and lines including three registered varieties used in the present study and reported that the average polymorphic percentage was 27.5%. Also, in the same research, AFLP analysis performed with 61 safflower ecotypes, 41 of which originating from Turkey and 13 from different countries, exhibited that the polymorphism ratio of the ecotypes was lesser than the polymorphism ratio of the cultivars and lines of studies and this paper (between 5.4-22.7% and average 14%).

The measure of PIC is an important component and one of the key information and statistical indicators in

the implementation of the planning of breeding programs (Chesnokov & Artemyeva, 2015). PIC reflects a discriminating ability of the marker and depends on the number of known alleles and their distribution frequency, thus representing genetic diversity (Giachino, 2020). A classification of the informativeness of dominant markers was proposed by Serrote et al. (2020) based on PIC values: low (0 to 0.10), medium (0.10 to 0.25), high (0.30 to 0.40), and very high (0.40 to 0.50). Accordingly, it is evident that the SRAP markers used in this study have high informative power with an average of 0.35. In addition, half of 12 primer pairs (Me3xEm1-0.46, Me5xEm8-0.45, Me4xEm1-0.44, Me3xEm2-0.41, Me3xEm3-0.41, Me5xEm4-0.41) have a very high distinguishing capacity, which may be more useful for genetic characterization in safflower as well as in other plants. The mean PIC was consistent with that of Golkar & Mokhtari (2018) (0.35). The findings of Talebi et al. (2012), Mokhtari et al. (2013), and Tonguç et al. (2011) revealed results of 0.33, 0.28, 0.29, respectively.

The RP ranged from 0.29 to 4.57 with an overall average of 1.48. Only three primer combinations (Me3xEm1, Me4xEm1, and Me5xEm4) exceeded the mean RP value. Different RP values were reported for RAPD (2.07), ISSR (2.44) (Safavi et al., 2010), ISSR (8.72) (Majidi & Zadhoush 2014), and RAPD (3.37) markers (Giachino & Inan 2019) which were used on safflower genotypes. The MI values ranged from 0.03 to 1.85, with an average of 0.43. Maximum MI was observed in the Me3xEm1 primer combination, and the minimum MI was obtained with SRAP primer combinations of Me1xEm2 (0.03) and Me3xEm3 (0.05). In safflower, different mean MI values have been reported for various RAPD, ISSR, AFLP markers (1.41, 0.70, 18.2, respectively) (Seghal and Raina 2005).

Genetic diversity of safflower genotypes generated by SRAP primers was measured by calculating indicators of genetic diversity such as Nei's gene diversity (h) and Shannon's information index (I). The average Nei's gene diversity values ranged from 0.24 to 0.46, with an average value of 0.36, which compatible with previous SRAP analysis results were reported by Mokhtari et al. (2013) for Sixty-two safflower accessions (0.26-0.44, av. h=0.36). Furthermore, obtained average Nei's gene diversity value was greater than the values found by Talebi et al. (2012) for SRAP markers (h=0.30), Ali et al. (2019) for İPBS- retrotransposon markers (h=0.21) and Yıldız et al. (2022) for POGP markers (h=0.27). Whereas Ali et al. (2020) studied 131 safflower accessions using ISSR markers and found Nei's gene diversity as 0.38, which is slightly higher than the value (0.36) obtained in this study. Shannon's information index values ranged between 0.41 and 0.65, with an average of 0.55. Similar results were reported by Ali et al. (2020) for ISSR markers (0.44–0.65, av. I=0.557). The mean Shannon's information index was found higher than previously reported studies of Hassani et al. (2020) and Talebi et al. (2012) revealed 0.43 and 0.17 respectively for SRAP markers in safflower. Golkar and Mokhtari (2018) for SRAP and SCoT markers, Ali et al. (2019) for IPBS markers, reported a lower Shannon information index mean (0.35, 0.33, respectively). Nei's gene diversity and Shannon's information index values are measures of genetic diversity, which explain the evolutionary pressure on alleles and the mutation rate (Bonneuil et al. 2012). Nei's genetic diversity and Shannon's information index values for all primers were 0.36 and 0.55 respectively, this indicates a prominent genetic diversity at the level of local and registered genotypes.

PCoA multivariate approach was used to complement the information obtained from the cluster analysis (Naik et al., 2017). The results of the PCoA analysis were in good agreement with the cluster analysis. The first two principal coordinates explained correspond 80% of the total variation. Accordingly, this result indicates the appropriate distribution of SRAP markers across the entire genome and confirms the results of the cluster analysis. Genetic similarity values showed a wide distribution ranging from 0.12 to 0.92, with a mean similarity of 0.58. Different genetic similarities have been reported previously among safflower. Peng et al. (2008) reported a mean similarity of 0.57 among 23 safflower populations. Golkar & Mokhtari (2018) reported that the similarity coefficient ranged from 0.53 to 1 with an average of 0.76 among one hundred safflower genotypes. The similarity matrix of genotypes used in the SRAP analysis by Talebi et al. (2012) ranged from 0.33 to 0.91, with an average of 0.51. In the UPGMA analysis which shows very good compliance with the genetic similarity matrix (r=0.95), local and registered genotypes were almost distinctly clustered. Safflower genotypes clustered into two main groups. Cluster I was further divided into three subclusters. Subcluster 1 includes 3 local genotypes: TR 49119, TR 42630, and TR 42670. Of these, the TR49119 and TR42630 local genotypes were observed to be extremely similar with a coefficient of 0.91. TR49119 is a local genotype collected from the 'Gelendost' district of 'Isparta' province. It is located in the transition zone between the Mediterranean climate and the continental climate that is dominant in Central Anatolia. It is situated on the coast of 'Lake Eğirdir' at an altitude of 860 m. TR42630 was collected from the 'Havsa' district of Edirne province at the altitude of 40 m (Table 1) in the Trakya Region of Turkey. It demonstrates a hybrid climate between the Mediterranean and Black Sea climates, which can be considered a mild oceanic climate (Akgün et al., 2013). There is an altitude difference of about 800 m and 800 km between these two varieties. Interestingly, although they have such

different geographical conditions, they show high similarities. The common point here is that both varieties are in the transitional zone. The other local genotype TR 42670 in subcluster 1 was collected from 'Saray' district of 'Tekirdağ' province, located in the Thrace region, at an altitude of 240 m. It shows high similarity with other local genotypes, with 0.78-0.79 GS. Consequently, varieties from different locations are grouped in the same cluster. A possible explanation for this may be the exchange of seeds materials among producers at different periods in different provinces of Turkey (Giachino, 2020). Moreover, these high similarity coefficients may be due to the narrowing of the genetic base by long-term selection. This is in accordance with some of the previous studies which concluded that local genotypes from different geographic regions may be genetically similar (Naik et al., 2017; Kiran et al., 2017). Subcluster 2 includes TR 64702 landrace and Remzibey05 cultivar which are the closest genotypes, with Dice values of 0.92. That was confirmed by RAPD markers (GS=0.85) similarly (Giachino and Inan 2019). TR 64702 landrace was collected from 'Anamur' district of 'Mersin' province at 850 m altitudes. Remzibey05 is a spiny variety with yellow flowers, relatively short height, and 25-35% oil content (with 2.5-3 times more oleic acid content than the other two varieties (Cosge et al., 2007)). It was improved by the selection method as a composite of landraces (Köse, 2017). Remzibey05 cultivar is probably from a common genetic origin with the TR 64702 local genotype. In other words, the very high similarity between the Remzibey05 cultivar and the TR 64702 landrace with a value of 0.92 can be explained by the fact that these two genotypes are descended from the same ancestor. Each of subcluster 3 and Cluster II comprised a single variety, Yenice5-38, and Dinçer5-118, respectively. The distribution determined by UPGMA analysis also revealed that Dinçer5-118 registered cultivar is very different genetically between themselves and from other genotypes, with 0.29 GS. Furthermore, an extreme variation was observed between these two cultivars, with a similarity of 0.12 (Table 4). This is predictable for two varieties with different characteristics, which were registered approximately 45 years apart. These two ancient cultivars were developed via selection as a composite of 5 non-spiny safflower cultivars. Dinçer5-118 stands out in terms of grain yield, especially in regions where the distribution of precipitation is regular throughout the growing season (Köse, 2017).

In this research, the relationships and genetic diversity between local genotypes and registered safflower varieties were determined by SRAP molecular markers that are easy to apply and reliable. Different marker parameters, viz number of total bands, number of polymorphic bands, polymorphic band ratios, resolving power, effective multiplex ratio and marker index of the SRAP markers were found to be low. In contrast, genetic diversity indicators such as polymorphism information content (0.35), Nei's gene diversity (0.36) and Shannon's information index (0.55) showed a significant genetic variation of the studied safflower genotypes. Genetic similarity values showed a wide distribution, and local genotypes originating from different geographical regions were genetically close. In addition, a very high genetic distance was determined between the old, registered cultivars Dinçer 5-118 and Yenice 5-38. Moreover, Dinçer 5-118 was classified as quite distant from all other genotypes. Dinçer 5-118 and Yenice 5-38 can be used as genitors in breeding studies. In future studies, safflower breeding programs can be planned by using more plant materials with various characteristics and advanced molecular breeding techniques.

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Conflict of interest

The author declares that they have no conflict of interest.

REFERENCES

Ahmad, R. Quiros, C.F., Rahman, H., & Swati, Z.A. (2014). Genetic diversity analyses of *Brassica napus* accessions using SRAP molecular markers. *Plant Genetic Resources: Characterization and Utilisation, 12*,14-21. https://doi.org/10.1017/S147926211300021X

Akgün, F., Akkiraz, M.S., Üçbaş, S.D., Bozcu, M., Kapan Yeşilyurt, S., & Bozcu, A. (2013). Oligocene vegetation and climate characteristics in north-west Turkey: Data from the South-western part of the Thrace Basin. *Turkish Journal of Earth Science, 22*, 277-303. https://journals.tubitak.gov.tr/earth/vol22/iss2/8

Ali, F., Yılmaz, A., Nadeem, M. A., Habyarimana, E., Subaşı, I., Nawaz, M. A., Chaudhary, H.J., Shahid, M.Q., Ercişli, S., Zia, M.A., Chung, G. & Baloch, F. S. (2019). Mobile genomic element diversity in world collection of safflowers (*Carthamus tinctorius* L.) panel using İPBS-retrotransposon markers. *PloS one*, 14(2), e0211985.

Ali, F., Nadeem, M. A., Habyarimana, E., Yılmaz, A., Nawaz, M. A., Khalil, I. H., Ercişli, S., Chung, G., Chaudhary, H.J.& Baloch, F. S. (2020). Molecular characterization of genetic diversity and similarity centers of safflower accessions with ISSR markers. *Brazilian Journal of Botany*, 43, 109-121.

Anderson, J.A., Churchill, G.A., Autrique, J.E., Tanksley, S.D., & Sorrells, M.E. (1993). Optimizing parental selection for genetic linkage maps. *Genome, 36*,181-186. https://doi.org/10.1139/g93-024

Asgarpanah, J., & Kazemivash, N. (2013). Photochemistry, pharmacology, and medicinal properties of *Carthamus tinctorius* L. *Chinese Journal of Integrative Medicine, 19*,153-159. https://doi.org/10.1007/s11655-013-1354-5

Amini, F., Saeidi, G., Arzani, A. (2008). Study of genetic diversity in safflower genotypes using agro-morphological traits and RAPD markers. *Euphytica, 163*,21-30. DOI: https://doi.org/10.1007/s10681-007-9556-6

Baloch, F.S., Kurt, C., Arıoğlu, H., & Ozkan, H. (2010). Assaying of diversity among soybean (*Glycin max* (L.) Merr.) and peanut (*Arachis hypogaea* L.) genotypes at DNA level. *Turkish Journal of Agriculture and Forestry,* 34, 285-301. https://doi.org/10.3906/tar-0907-281

Barati, M., & Arzani, A. (2012). Genetic diversity revealed by EST-SSR markers in cultivated and wild safflower. *Biochemical Systematics and Ecology, 44*,117-123. https://doi.org/10.1016/j.bse.2012.04.013

Baydar, H. (2021). Tarla Bitkileri Tarımı ve Endüstrisi (Ders Notu) Isparta Uygulamalı Bilimler Üniversitesi. Tbtf-tab- Isparta. p.293 (In Turkish).

Baydar, H., & Erbaş, S. (2020). Yerli ve Milli Aspir Çeşitlerimiz: Olein, Zirkon ve Safir. *Ziraat Fakültesi Dergisi Türkiye 13. Ulusal, I. Uluslararası Tarla Bitkileri Kongresi Özel Sayısı*: 233-237(In Turkish).

Bonneuil, C., Goffaux, R., Bonnin, I., Montalent, P., Hamon, C., Balfourier, F., & Goldringer, I. (2012). A new integrative indicator to assess crop genetic diversity. *Ecological Indicators*, 23, 280-289.

Chapman, M.A., Hvala, J., Strever, J., & Burke, J.M. (2010). Population genetic analysis of safflower (*Carthamus tinctorius*; Asteraceae) reveals a near eastern origin and five centres of diversity. *American Journal of Botany, 97*, 831-840. https://doi.org/10.3732/ajb.0900137

Chesnokov, Y.V., & Artemyeva, A.M. (2015). Evaluation of the Measure of Polymorphism Information of Genetic Diversity *Agricultural Biology, 50*, 571-578. https://doi.org/10.15389/agrobiology.2015.5.571

Cosge, B., Gurbuz, B., & Kiralan, M. (2007). Oil content and fatty acid composition of some safflower (*Carthamus tinctorius* L.) varieties sown in spring and winter. *International Journal of Natural and Engineering Sciences, 1*,11-15.

Delshad, E., Yousefi, M., Sasannezhad, P., Rakhshandeh, H., & Ayati, Z. (2018). Medical uses of *Carthamus tinctorius* L. (Safflower): a comprehensive review from Traditional Medicine to Modern Medicine. *Electron Physician, 10*, 6672-6681. http://dx.doi.org/10.19082/6672

Dice, L.R. (1945). Measures of the amount of ecologic association between species. *Ecology, 26*,297–302.

Ebrahimi, F., Majidi, M.M., Arzani, A., & Mohammadi-Nejad, G. (2017). Association analysis of molecular markers with traits under drought stress in safflower. *Crop and Pasture Science, 68*,167-175. https://doi.org/10.1071/CP16252

FAOSTAT, (2022). *Food and Agriculture Organization of the United Nations Statistical Databases*. http://www.fao.org/faostat/en/ [Erişim Tarihi: 15. 08.2022]

Giachino, R.R.A. (2020). Investigation of the genetic variation of anise (*Pimpinella anisum* L.) using RAPD and ISSR markers. *Genetic Resources and Crop Evolution, 67*, 763-780. https://doi.org/10.1007/s10722-019-00861-y

Giachino, R.R.A., & Inan, D. (2019). Assessment of genetic diversity in Safflower (*Carthamus tinctorius* L.) using RAPD markers. *Yuzuncu Yıl University Journal of Agricultural Sciences, 29*(2), 300-308. https://doi.org/10.29133/yyutbd.560936

Golkar, P., Arzani, A., & Rezaei, A.M. (2011). Genetic variation in safflower (*Carthamus tinctorious* L.) for seed quality-related traits and inter simple sequence repeat (ISSR) markers. *International Journal of Molecular Sciences, 12*, 2664-2677. https://doi.org/10.3390/ijms12042664

Golkar, P. (2018). Inheritance of carthamin and carthamidin in safflower (*Carthamus tinctorius* L.). *Journal of genetics, 97*, 331-336. https://doi.org/10.1007/s12041-018-0909-9

Golkar, P., & Mokhtari, N. (2018). Molecular diversity assessment of a world collection of safflower genotypes by SRAP and SCoT molecular markers. *Physiology and Molecular Biology of Plants, 24*,1261-1271. https://doi.org/10.1007/s12298-018-0545-0

Hamdan, Y.A.S., García-Moreno, M.J., Redondo-Nevado, J., Velasco, L., & Pérez-Vich, B. (2011). Development and characterization of genomic microsatellite markers in safflower (*Carthamus tinctorius* L.). *Plant Breeding, 130*,237-241. https://doi.org/10.1111/j.1439-0523.2010.01826.x

Hassani, S.M.R., Talebi, R., Pourdad, S.S., Naji, A.M., & Fayaz, F. (2020). Morphological description, genetic diversity and population structure of safflower (*Carthamus tinctorius* L.) mini core collection using SRAP and SSR markers. *Biotechnology & Biotechnological Equipment, 34*,1043-1055. https://doi.org/10.1080/13102818.2020.1818620

Ilkdoğan, U. (2012). *Türkiye'de Aspir Üretimi İçin Gerekli Koşullar ve Oluşturulacak Politikalar* (Tez no: 978-605-4672-04-2). [Doktora Tezi, Ankara Üniversitesi Fen Bilimleri Enstitüsü Tarım Ekonomisi Ana Bilim Dalı]. (In Turkish).

Johnson, R.C., Kisha, T.J., & Evans, M.A. (2007). Characterizing safflower germplasm with AFLP molecular markers. *Crop Science, 47*,1728-1736. https://doi.org/10.2135/cropsci2006.12.0757

Kiran, B.U., Mukta, N., Kadirvel, P., Alivelu, K., Kishore, P., Senthilvel, S., & Varaprasad, K.S. (2017). Genetic diversity in safflower (*Carthamus tinctorius* L.) germplasm as revealed by SSR markers. *Plant Genetic Resources: Characterization and Utilization, 15*,1-11. https://doi.org/10.1017/S1479262115000295

Knowles, P.F. (1989). Safflower. In: Downey RK, Robbelen G, Ashri A (eds) *Oil crops of the world*. New York 363-374.

Kobuk, M., Ekinci, K., & Erbaş, S. (2019). Aspir (*Carthamus tinctorius* L.) genotiplerinin fiziksel ve kimyasal özelliklerinin belirlenmesi. *Kahramanmaraş Sütçü İmam Üniversitesi Tarım ve Doğa Dergisi*, 22(1), 89-96. DOI:10.18016/ ksutarimdoga.vi.455408

Köse, A. (2017). Eskişehir Koşulları Altında Bazı Aspir (*Carthamus tinctorius* L.) Çeşitlerinin Tarımsal Performanslarının Belirlenmesi. *Selcuk Journal of Agriculture and Food Science*, 31, 1-7 (In Turkish).

Kumar, S., Ambreen, H., Murali, T.V., Bali, S., Agarwal, M., Kumar, A., Goel, S., & Jagannath, A. (2014). Assessment of genetic diversity and population structure in a global reference collection of 531 accessions of *Carthamus tinctorius* (safflower) using AFLP markers. *Plant Molecular Biology Reporter*, 33,1299-1313. https://doi.org/ 10.1007/s11105-014-0828-8

Lee, G.A., Sung, J.S., Lee, S.Y., Chung, J.W., Yi, J.Y., Kim, Y.Y., & Lee, M.C. (2014). Genetic assessment of safflower (*Carthamus tinctorius* L.) collection with microsatellite markers acquired via pyrosequencing method. *Molecular Ecology Resources, 14*, 69-78. https://doi.org/10.1111/1755-0998.12146.

Lei, J., Li, Y., Liu, B., Tang, T., Liu, H., Yan, X., Qin, R., & Li, G. (2013). Establishment and Optimization of SRAP-PCR and Preliminarily Analysis of Genomes in *Carthamus tinctorius* L. *Botanical Research, 2*, 62-66. http://dx.doi.org/ 10.12677/br.2013.22011

Li, G., & Quiros, C.F. (2001). Sequence-related amplified polymorphism (SRAP), a new marker system based on a simple PCR reaction: its application to mapping and gene tagging in Brassica. *Theoretical and Applied Genetics, 103*, 455-461. https://doi.org/10.1007/s001220100570

Li, D., Yadong, Z., Chao, L., Tu, S., & Li, M. (2009). Optimization of SRAP-PCR reaction system in flax. *Journal of Northeast Agricultural University, 16*, 17-22.

Li, G., McVetty, P.B.E., Quiros, C.F. (2013). SRAP molecular marker technology in plant science. In: Andersen SB (ed.) Plant Breeding from Laboratories to Fields. Rijeka, Croatia: InTech, pp.23-43.

Mahasi, M.J., Wachira, F.N., Pathak, R.S., & Riungu, T.C. (2009). Genetic polymorphism in exotic safflower (*Carthamus tinctorious* L.) using RAPD markers. *Journal of Plant Breeding and Crop Science, 1*, 008-012.

Majidi, M.M., & Zadhoush, S. (2014). Molecular and morphological variation in a world-wide collection of safflowers. *Crop Science, 54*, 2109-2119. https://doi.org/10.2135/cropsci2013.12.0850

Mantel, N. (1967). The detection of disease clustering and a generalized regression approach. *Cancer Research, 27*, 175-178.

Milbourne, D., Meyer, R., Bradshaw, J.E., Baird, E., Bonar, N., Provan, J., Powell, W., & Waugh, R. (1997). Comparison of PCR-based marker systems for the analysis of genetic relationships in cultivated Potato. *Molecular Breeding, 3*, 127-136. https://doi.org/10.1023/A:1009633005390

Mokhtari, N., Rahimmalek, M., Talebi, M., & Khorrami, M. (2013). Assessment of genetic diversity among and within Carthamus species using sequence-related amplified polymorphism (SRAP) markers. *Plant Systematics and Evolution, 299*, 1285-1294. https://doi.org/10.1007/s00606-013-0796-8

Naik, A., Prajapat, P., Krishnamurthy, R., & Pathak, J.M. (2017). Assessment of genetic diversity in *Costus pictus* accessions based on RAPD and ISSR markers. *3Biotech, 7*, 70. https://doi.org/10.1007/ s13205-017-0667-z

Nogales-Delgado, S., Encinar, J.M., & González, J.F. (2019). Safflower Biodiesel: Improvement of its Oxidative Stability by using BHA and TBHQ. *Energies, 12*, 1940:1-13. https://doi.org/10.3390/ en12101940

Peng, S., Feng, N., Guo, M., Chen, Y., & Guo, Q. (2008). Genetic variation of Carthamus tinctorius L. and related species revealed by SRAP analysis. *Biochemical Systematics and Ecology, 36*, 531–538. https://doi.org/10.1016/j.bse.2008.03.010

Powell, W., Morgante, M., Andre, C., Hanafey, M., Vogel, J., Tingey, S., & Rafalski, A. (1996). The unity of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. *Molecular Breeding, 2*, 25-238. https://doi.org/ 10.1007/BF00564200

Prance, G., & Nesbitt, M. (2005). The cultural history of plants. New York-London: Routledge. p.451.

Prevost, A., & Wilkinson, M.J. (1999). A new system of comparing PCR primers applied to ISSR fingerprinting. *Theoretical and Applied Genetics, 98*, 107-112. https://doi.org/10.1007/s001220051046

Rohlf, F.J. (2000). *NTSYS-pc. Numerical Taxonomy and Multivariate Analysis System, Version 2.2*. Exeter Publishing, Setauket, NY, USA.

Safavi, S.A., Pourdad, S.S., Taeb, M., & Khosroshahli, M. (2010). Assessment of genetic variation among safflower (*Carthamus tinctorius* L.) accessions using agro-morphological traits and molecular markers. *Journal of Food, Agriculture & Environment, 8* (3-4), 616-625. www.world-food.net

Sehgal, D., & Raina, S.N. (2005). Genotyping safflower (*Carthamus tinctorius*) cultivars by DNA fingerprints. *Euphytica, 146*, 67-76. https://doi.org/10.1007/s10681-005-8496-2

Sehgal, D., Rajpal, V.R., Raina, S.N., Sasanuma, T., & Sasakuma, T. (2009). Assaying polymorphism at DNA level for genetic diversity diagnostics of the safflower (*Carthamus tinctorius* L.) World germplasm resources. *Genetica, 135*, 457-470. https://doi.org/10.1007/s10709-008-9292-4

Serrote, C.M.L., Reiniger, L.R.S., Silva, K.B., dos Santos Rabaiolli, S.M., & Stefanel, C.M. (2020). Determining the polymorphism information content of a molecular marker. *Gene, 726*, 144175. https://doi.org/10.1016/j.gene.2019.144175

Talebi, M., Mokhtari, N., Rahimmalek, M., & Sahhafi, S.R. (2012). Molecular characterization of *Carthamus tinctorius* and *C. oxyacanthus* germplasm using sequence related amplified polymorphism (SRAP) markers. *Plant Omics Journal* ,*5*, 136-142.

Talebi, R., Nosrati, S., Etminan, A., & Naji, A.M. (2018). Genetic diversity and population structure analysis of landrace and improved safflower (*Carthamus tinctorious* L.) germplasm using arbitrary functional gene-based molecular markers. *Biotechnology & Biotechnological Equipment, 32*, 1183-1194. https://doi.org/10.1080/13102818.2018.1499443

Tonguç, M., Erbas, S., & Baydar, H. (2011). Aspirde Geliştirilen Rekombinant Saf Hat Populasyonunun Genetik Harita Populasyonu Olarak Kullanma İmkânlarının Araştırılması. *Süleyman Demirel Üniversitesi Ziraat Fakültesi Dergisi, 6* ,1-7 (In Turkish)

TÜİK (2022). *Turkish Statistical Institute*. Türkiye İstatistik Kurumu http://www.tuik.gov.tr_[Erişim Tarihi: 15.03.2022]

Weiss, E.A. (1971). Safflower. In: Weiss EA (ed.) *Castor, Sesame and Safflower*. New York: Barnes and Noble, pp. 529-744. https://doi.org/10.1017/S0014479700005366

Weiss, E.A. (2000). Safflower. In: Weiss EA (ed.) *Oilseed Crops*. Victoria, Australia: Blackwell Science Ltd. p.109.

Yaman, H., Tarıkahya-Hacıoglu, B., Arslan, Y., & Subasi, I. (2014). Molecular characterization of the wild relatives of safflower (*Carthamus tinctorius* L.) in Turkey as revealed by ISSRs. *Genetic Resources and Crop Evolution, 61*, 595-602. https://doi.org/10.1007/s10722-013-0061-6

Yang, Y.X., Wu, W., Zheng, Y.L., Chen, L., Liu, R.J., & Huang, C.Y. (2007). Genetic diversity and relationships among safflower (*Carthamus tinctorius* L.) analysed by inter-simple sequence repeats (ISSRs). *Genetic Resources and Crop Evolution, 54*, 1043-1051. https://doi.org/10.1007/s10722-006-9192-3

Yeh, F.C., Yang, R.C., Boyle, T.B.J., Ye, Z.H., & Mao, J.X. (1997). POPGENE, the user-friendly shareware for population genetic analysis, version 1.31. *Molecular Biology and Biotechnology Centre, University of Alberta*, Canada.

Yesilyurt, M.K., Cesur, C., Aslan, V., & Yilbasi, Z. (2020). The production of biodiesel from safflower (*Carthamus tinctorius* L.) oil as a potential feedstock and its usage in compression ignition engine: a comprehensive review. *Renewable and Sustainable Energy Reviews, 119,* 1-30. https://doi.org/10.1016/j.rser.2019.109574

Yıldız, M., Altaf, M.T., Baloch, F.S., Koçak, M., Sadık, G., Kuzğun, C., Nadeem, M.A., Ali, F., Bedir, M. & Tunçtürk, M. (2022). Assessment of genetic diversity among 131 safflower (*Carthamus tinctorius* L.) accessions using peroxidase gene polymorphism (POGP) markers. *Molecular Biology Reports*, 49(7), 6531-6539.

Yue, S.J., Tang, Y.P., Li, S.J., & Duan, J.A. (2013). Chemical and biological properties of quinochalcone C-glycosides from the florets of *Carthamus tinctorius*. *Molecules, 18,* 15220-15254. https://doi.org/10.3390/molecules181215220

Zhang, Y.X., Zhang, X.R., Hua, W., Wang, L.H., & Che, Z. (2010). Analysis of genetic diversity among indigenous landraces from sesame (*Sesamum indicum* L.) core collection in China as revealed by SRAP and SSR markers. *Genes Genome, 32*(3), 207-215. https://doi.org/10.1007/s13258-009-0888-6

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Arpa Unu İlavesinin Açık Ekmeğin Bazı Kimyasal ve Fonksiyonel Özelliklerine Etkisi

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ÖZET
Yapılan bu çalışmada arpa unu ilavesinin Şanlıurfa yöresine özgü "Açık (düz) Ekmek"in bazı kalite özelliklerine etkisi araştırılmıştır. Açık ekmek, düz ekmekler grubuna giren geleneksel bir ekmek çeşididir. Bu amaçla, buğday ununa % 0, 20, 40, 60, 80, 100 oranlarında arpa unu ilave edilerek açık ekmek üretilmiştir. Üretilen ekmeklerde bazı kimyasal (nem, protein, kül) ve fonksiyonel (besinsel lif, toplam fenolik madde, fitik asit) özellikler incelenmiştir. Arpa unu ilavesinin, ekmeklerin tüm kimyasal ve fonksiyonel özellikleri üzerine etkisi önemli ($p \leq 0.05$) bulunmuştur. Arpa unu ilavesine bağlı olarak ekmeklerin nem, kül ve protein içerikleri sırasıyla %27.41-36.68, %1.13-2.39 ve %9.41-11.97 aralıklarında tespit edilmiştir. Arpa unu ilave oranının artışına bağlı olarak ekmeklerin besinsel lif, toplam fenolik madde ve fitik asit değerlerinde önemli ($p \leq 0.05$) artışlar olmuştur. Besinsel lif değerleri %5.50-15.50 aralığında değişirken, fitik asit değerleri 0.24-3.95 mg g^{-1} aralığında, toplam fenolik madde değerleri ise 0.64-1.33 mgGAE g^{-1} aralığında değişmiştir. Elde edilen bulgular ışığında arpa ununun ekmek yapımında ve diğer başka gıdaların besinsel açıdan zenginleştirilmesinde doğal bir katkı olarak kullanılabileceği sonucuna varılmıştır.

Gıda Bilimi

Araştırma Makalesi

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Anahtar Kelimeler
Düz ekmek
Arpa
Fitik asit
Protein
Besinsel lif

The Effect of Barley Flour Addition on the Some Chemical and Functional Properties of "Açık Ekmek" (Flat Bread)

ABSTRACT
In this study, the effect of the addition of barley flour on some quality characteristics of "Açık Ekmek", which is native to the Sanliurfa region, was investigated. Açık ekmek is a traditional type of bread in the flat bread group. For this purpose, açık ekmek was produced by adding 0, 20, 40, 60, 80, 100 % barley flour to wheat flour. Some chemical (moisture, protein, ash) and functional (dietary fiber, total phenolic content, phytic acid) properties of the produced breads were examined. The effect of the addition of barley flour on all chemical and functional properties of breads was significant ($p \leq 0.05$). Depending on the addition level of barley flour, the moisture, ash and protein contents of the breads were determined as 27.41-36.68%, 1.13%-2.39% and 9.41-11.97%, respectively. There were significant ($p \leq 0.05$) increases in the dietary fiber, total phenolic content and phytic acid values of the breads due to the increase in the addition rate of barley flour. While dietary fiber values ranged from 5.50% to 15.50%, phytic acid values ranged from 0.24-3.95 mg g^{-1}, and total phenolic content values ranged from 0.64-1.33 mgGAE g^{-1}. In the light of the findings obtained, it was concluded that barley flour can be used as a natural additive in bread making and for nutritional enrichment of other foods.

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GİRİŞ

Arpa, buğdayla beraber kültüre alınan dünyanın ilk tahıllarındandır. Arpa başlangıçta insan gıdası olarak tüketilirken zaman içerisinde buğday ve pirince artan talepten dolayı arpanın insan gıdası olarak tüketimi azalmış ve arpa çoğunlukla hayvansal yem olarak kullanılmıştır (Baik & Ulrich, 2008). Türkiye'de de 2021 yılı verilerine göre ekmeklik buğday üretimi 17.7 milyon ton, arpa ise 5.8 milyon ton olarak gerçekleşmiştir. Türkiye'de üretilen arpanın %95'e yakını yem amaçlı, kalan kısmı maltlık olarak bira sanayinde ve gıda endüstrisinde kullanılmaktadır (TÜİK, 2021).

Ekmek; buğday, çavdar, arpa ve mısır gibi tahıl tane unlarının su ile muamele edilen hamurun pişirilmesiyle pazara arz edilen gıdadır. Açık yassı ekmek çeşidi olan lavaş ekmeği Türkiye'de özellikle Doğu ve Güney Doğu Anadolu bölgesinde geleneğe uygun yaygın üretimi olan ekmek çeşidindendir. Günümüzde, Türkiye'nin tüm bölgelerinde üretim ve tüketimi hayli artmış olan lavaş ekmeği, yapısal olarak pideden daha farklı uzun, oval, yassı ve esnek bir özelliğe sahiptir. Dünyada; Lavosh, Lahvosh, Lawaash ve Paraki olarak da isimlendirilmektedir (Akturfan, 2018). Zayıf pişme kalitesinden dolayı gıdalarda çok kullanılmayan arpa, protein, besinsel lif, nişasta olmayan polisakkaritler (ß-glukan, selüloz ve arabinoksilan), fitokimyasallar açısından diğer tahıllara nazaran daha zengin olduğundan arpanın son yıllarda gıdalarda kullanımı artmaya başlamıştır. Ekmek, kek, kurabiye gibi ürünlerin üretiminde sıklıkla kullanıldığı görülmektedir (Türker ve ark., 2021).

Yöre halkının günlük tüketiminde önemli bir yere sahip olan açık ekmek, içerdiği mineral madde ve proteinden dolayı önemli bir besin kaynağı konumundadır. Lokanta ve restoran gibi yerlerde açık ekmek (lavaş ekmeği) tüketimi diğer ekmek çeşitlerine nazaran daha fazladır (Köten & Ünsal, 2006). Şanlıurfa'da, açık ekmek oldukça yüksek düzeyde tüketilmektedir. Bu da açık ekmeğin farklı besleyici bileşenlerle zenginleştirilerek tüketiciye daha sağlıklı ve besleyici bir şekilde sunulması gerekliliğini düşündürmektedir. Yapılan bir çalışmada, buğday ununa kavuzsuz arpa unu ilavesinin bisküvilerde Se, Cu, Fe, Zn ve ß-glukan içeriklerini önemli ölçüde yükselttiği belirlenmiştir (Škrbić & Cvejanov, 2011). Arpa sahip olduğu protein, diyet lif, zengin ß-glukan ve nişasta ile gıda uygulamalarında oldukça ilgi çekmektedir (Çakır, 2020). Ayrıca arpanın bayatlamayı geciktirici, ekmeğin raf ömrünü uzatıcı etkileri olduğuna dair araştırmalar da mevcuttur (Elçi, 2022).

Dhingra ve Jood (2002), arpa ununun %15 oranında buğday unu yerine ikamesiyle ekmeklerin diyet lifi ve ß-glukan içeriğinin önemli ölçüde arttığını tespit etmişlerdir. Gupta ve ark. (2011), %15-20 oranında arpa unu ilavesiyle üretilen buğday ekmeğinin genel aroma, görünüm ve doku özelliklerinin duyusal açıdan kabul edilebilir olduğunu bildirmişlerdir. Al-Attabi ve ark. (2017) %10 oranında arpa unu katkılı ekmeğin şekil ve gözenek yapısının buğday unu ekmeğine benzer olduğunu, %15 ve %25 oranlarında arpa unu ilavesiyle ekmeklerde daha düzensiz ve daha büyük gözeneklerin oluştuğunu bildirmişlerdir. Kavuzsuz arpa ununun buğday ununa %40 oranında katılmasıyla ilgili yapılan araştırmada, arpa unu ilavesiyle ekmek hacminin azaldığı, toplam ve çözünür 1.4 ve 1.3 ß-D-glukan ile toplam arabinoksilan oranının arttığı belirlenmiştir (Trogh ve ark., 2004). Arpa ununun %15-30 oranında ilavesiyle ekmek özelliklerinin çok değişmediği de bildirmektedir (Ereifej ve ark., 2006).

Bu çalışmada, Şanlıurfa ve çevresinde hızla artan nüfusa bağlı olarak tüketimi de oldukça yüksek düzeyde olan açık ekmeğin besinsel özellik açısından zengin olan arpa unu ile zenginleştirilmesi ve elde edilen ekmeklerin bazı kalite özelliklerinin araştırılması amaçlanmıştır.

MATERYAL ve METOD

Materyal

Çalışmada Mardin ilinin Kızıltepe ilçesinde faaliyet gösteren buğday pazarından temin edilenTARM 92 arpa çeşidi kullanılmıştır., Arpanın bazı fiziksel özelliklerine ilişkin veriler Çizelge 1'de gösterilmiştir.

Arpanın öğütülmesi AACC metod 26-50'a göre yapılmıştır (AACC, 2010). Öğütme, BASTAK firmasına ait MAXI-C model, DC-4057 seri nolu 4 valsli değirmende gerçekleştirilmiştir. Öğütülen arpa; sırasıyla 8 no ipek (180 mikron), 15GG (1400 mikron), 32GG (600 mikron) ve 70GG (236 mikrondan) eleklerden elenerek % 68 randımanlı arpa unu elde edilmiştir. Ekmek üretiminde kullanılan buğday unu (İmsa, Adıyaman, Türkiye), pres yaş maya (Pakmaya, İzmit, Türkiye) ve tuz (Billur, İzmir. Türkiye) Şanlıurfa'daki yerel bir marketten temin edilmiş ve su olarak da şebeke suyu kullanılmıştır.

Çizelge 1. Arpanın bazı fiziksel özellikleri
Table 1. Some physical properties of barley

Arpa *Barley*	1000 Tane Ağırlığı (g) *1000 Kernel Weight (g)*	Hektolitre Ağırlığı (kg hl-1) *Hectoliter Weight (kg hl^{-1})*	Nem (%) *Moisture (%)*
TARM 92	38.95±0.35	66.50±0.71	13.90±0.18

Metod

Deneme deseni ve ekmek üretimi

Çalışmaya ait deneme deseni Çizelge 2'de verilmiştir. Buna göre; ekmeklik buğday unu ve arpa unu "100:0 (A)", "80:20 (B)", "60:40 (C)", "40:60 (D)", "20:80 (E) ve "00:100 (F)" (w/w) oranlarında karıştırılmış ve üretim tekniğine uygun olarak 6 adet açık ekmek üretilmiştir. Ekmek üretimi Köten ve Ünsal (2007)'ın bildirdiği metoda göre Şekil 1'de gösterildiği gibi yapılmıştır. Ekmek üretimleri, açık (düz) ekmek üretimi yapan yerel bir fırında gerçekleştirilmiştir.

Çizelge 2. Ekmek hamuru formülasyonuna ilave edilen bileşenler ve kullanım miktarları
Table 2. Ingredients added to the bread dough formulation and their usage amounts

Bileşenler *Ingredients*	A	B	C	D	E	F
Buğday unu (g) *Wheat flour (g)*	350	280	210	140	70	-
Arpa unu (g) *Barley flour (g)*	-	70	140	210	280	350
Su (ml) *Water (ml)*	225	245	251	252	264	273
Tuz (g) *Salt (g)*	3.5	3.5	3.5	3.5	3.5	3.5
Maya (g) *Yeast (g)*	5.25	5.25	5.25	5.25	5.25	5.25

A: Kontrol (%100 buğday unu); B: %20 arpa unu+ %80 buğday unu; C: %40 arpa unu+ %60 buğday unu; D: %60 arpa unu+ %40 buğday unu; E: %80 arpa unu+ %20 buğday unu; F: %100 arpa unu
A: Control (100% wheat flour); B: 20% barley flour + 80% wheat flour; C: 40% barley flour + 60% wheat flour; D: 60% barley flour + 40% wheat flour; E: 80% barley flour + 20% wheat flour; F: 100% barley flour

Un+Su+Maya+Tuz
(Maya tırnaklı ekmektekinin yarısı kadar konur)
↓
Yoğurma (~ 10 dakika)
↓
Kitle fermantasyonu (~ 30 dakika)
↓
Kesme- tartma (180 g. yumak şeklinde)
↓
2. fermantasyonu (5-10 dakika)
↓
Şekillendirme
(Hamur yumağını düz oval şekle getirme, un+su karışımından oluşan bulamaç sürme ~ 80 g.)
↓
Pişirme (350-400 ºC, 1.5-2 dakika)

Şekil 1. Açık ekmek üretimi
Figure 1. Açık ekmek production

Unlarda ve ekmeklerde yapılan kimyasal analizler

Ekmekler piştikten hemen sonra 10 dakika bez örtü arasında oda sıcaklığına soğutulmuştur. Daha sonra dilimlenip oda koşullarında kurutulduktan sonra (Şekil 2) öğütülmüş ve 840 mikronluk elekten geçirilerek analize hazırlanmıştır.

Buğday unu, arpa unu ve ekmek örneklerinin nem (standart no 110) ve kül (standart no 114) içeriklerinin belirlenmesinde ICC metotları kullanılmıştır (ICC, 2002). Protein içerikleri Leco marka FP-528 model cihaz ile Dumas yöntemi kullanılarak ölçülmüştür (Wiles ve ark., 1998).

Şekil 2. Kıyılmış ekmek örnekleri
Figure 2. Flat bread strips

Unlarda ve ekmeklerde yapılan fonksiyonel analizler

Besinsel lif analizi Köten (2021)'in bildirdiği yönteme göre yapılmıştır. Analizin yapılmasında toplam besinsel lif test kiti (Megazyme International Ireland Ltd., Bray Business Park, Bray, Co. Wicklow, Ireland) kullanılmıştır. Buna göre örnekler 10 ml MES-Tris tamponunda (pH 8.2) süspanse edildikten sonra, nişasta ve proteinlerin uzaklaştırılması amacıyla sırasıyla termal α-amilaz, proteaz ve amiloglukozidaz enzimleri ile muamele edilmişlerdir. Termal α-amilaz ile 100ºC'de nişasta, proteaz ile 60ºC'de proteinler hidrolize edilmiş ve amiloglukozidaz ile 60ºC'de nişastanın glukoz birimlerine parçalanması sağlanmıştır. Nişasta olmayan polisakkaritlerin (besinsel liflerin) çöktürülmesi, çözünür protein ve glukoz birimlerinin ortamdan uzaklaştırılması amacıyla örneklere % 95'lik etil alkol eklenmiş ve 60 dakika çökelmeye bırakılmıştır. Daha sonra önceden darası alınmış Por 3 sinter filtreli cam krozelerden filtrasyon işlemi gerçekleştirilmiştir. Erlenlerin içindeki kalıntı sırasıyla % 78'lik etil alkol, % 95'lik etil alkol ve aseton ile yıkanarak tekrar filtre edilmiştir. Besinsel lif miktarının belirlenmesi amacıyla cam krozeler 105ºC'de bir gece kurutulmuş ve tartılmıştır. Daha sonra cam krozelerdeki içerik 525ºC'de yakılarak bulunan kül miktarı önceden belirlenmiş toplam besinsel lif miktarından çıkarılarak kül doğrulaması yapılmıştır. Daha sonra toplam besinsel lif miktarı % olarak kuru madde üzerinden hesaplanmıştır.

Toplam fenolik madde (TFM) değerleri Çam ve İçyer (2015)'in bildirdiği yönteme göre Folin-Ciocalteu reaktifi kullanılarak belirlenmiştir. Analiz için öncelikle ekstraksiyon işlemi yapılmıştır. 1 gr örnek 10 ml % 80 metanol/su karışımında 2 saat 200 rpm'de 37°C'de çalkalamaya bırakılmıştır. Süre sonunda örnekler 4100 rpm'de 10 dakika santrifüj edilip filtre kağıdından geçirilen süzüntüden analiz yapılmıştır. TFM içeriği için Folin-Ciocalteau metodu kullanılmıştır. 100 µL örnek üzerine 900 µL su ilave edilip sonra 1 ml % 10 seyreltilmiş Folin-Ciocalteau ayracı (Merck, Almanya)ve 2 ml % 10'luk sodyum karbonat (Merck, Almanya) solüsyonu eklenip karıştırılmıştır. 1 saat oda sıcaklığında inkübasyona bırakılıp 765 nm'de spektrofotometrede (Biochrom Libra S60, UK) absorbans değerleri ölçülmüştür. TFM içeriği; okunan absorbans değerlerinin önceden gallik asit ile oluşturulan aşağıdaki absorbans/konsantrasyon standart grafiğinden elde edilen denklemde yerine konularak hesaplanmıştır. 1 gr örnek için mg gallik asit eşdeğer (GAE) miktarı olarak belirlenmiştir.

Fitik asit miktarı Köten (2021)'in bildirdiği metoda göre yapılmıştır. 0.1 gr un inceliğinde öğütülmüş örnek 15 ml'lik falkon tüpüne alınmış ve üzerine 10 ml 0.4 M HCI (Merck 100317) içinde çözündürülmüş %10'luk Na2SO4 (Merck 106649) eklenmiştir. Örnek ve çözelti tam olarak karışabilmesi için kısa bir süre vortekslendikten sonra 3 saat boyunca 175 RPM'de, oda sıcaklığında yatay çalkalayıcıda ekstraksiyona bırakılmıştır. Çalkalama sonunda karışım 4600 devir/dk da 20 dakika santrifüj edilmiştir. Santrifüj sonunda süpernatant (süzüntü) kısmından 1 ml alınarak (15 ml'lik falkon tüpüne) üzerine 2 ml ferik solüsyon ilave edilmiş ve sıcaklığı 95°C'ye ayarlı su banyosunda 30 dakika bekletilmiştir. Sıcak su banyosundan alınan örnekler soğuk su banyosuna konarak oda sıcaklığına kadar soğutulmuştur. Oda sıcaklığına ulaşan örnekler 10 dakika tekrar 4600 devir/dk da santrifüj edilmiştir. Santrifüj edilen örneklerden cam test tüplerine 1 ml alınmış ve üzerine 3 ml 2,2 bypiridine solüsyonu eklenmiştir (bypiridine eklendikten sonra örnekler pembemsi bir renk

almıştır). Oluşan pembe rengin 519 nm'de spektrofotometrede (Biochrom Libra S60, UK) absorbans değerleri ölçülmüştür. Fitik asitin sodyum tuzundan (Sigma P-8810) 0.2 g 100 ml destile suda çözündürülerek oluşturulan solüsyondan seyreltme ile 0, 50, 100, 200 mg L^{-1} (0, 50, 100 ve 200 ppm) lik standartlar hazırlanmıştır. Hazırlanan bu satandartların 519 nm'de okunan absorbanslarına göre excel programında bir eğri çizilmiştir (eğri x ekseninde absorbans değerleri, y ekseninde standartların ppm olarak konsantrasyonu olacak şekilde). Eğrinin denklemi bulunarak (y=ax+b şeklinde) denklemde x yerine örnekler için okunan absorbans değerleri konularak örneklerin fitik asit miktarı mg g^{-1} olarak bulunmuştur.

İstatistiksel analizler

Sonuçların değerlendirilmesinde JMP 2011 paket programı kullanılmış olup, ortalamalar arasındaki farklılık $p \leq 0.05$ önem seviyesinde LSD yöntemi ile test edilmiştir.

BULGULAR ve TARTIŞMA

Un örneklerinin bileşim özellikleri

Araştırmada kullanılan unların bazı kimyasal ve fonksiyonel özelliklerine ait veriler Çizelge 3'te verilmiştir.

Çizelge 3. Un örneklerinin bileşim özellikleri
Table 3. Composition of flour samples

	Nem (%) *Moisture (%)*	Kül* (%) *Ash (%)*	Protein* (%) *Protein (%)*	Besinsel lif (%) *Dietary Fiber (%)*	Fenolik Madde* (mgGAE/g) *Phenolic Matter (mgGAE g^{-1})*	Fitik asit* (mg/g) *Phytic acid (mg g^{-1})*
Buğday unu *Wheat flour*	13.20±0.10	0.79±0.03	11.10±0.18	6.14±0.34	0.23±0.01	2.13±0.06
Arpa unu *Barley flour*	13.80±0.20	2.20±0.08	13.79±0.30	16.91±0.44	1.00±0.10	7.16±0.25

*Kuru maddede hesaplanmıştır
** Calculated in dry matter*

Çizelge 3 incelendiğinde, buğday unu ile arpa ununun nem değerlerinin birbirine yakın bulunduğu görülmektedir. Kül miktarlarına bakıldığında arpa ununun kül değeri (%2.29) buğday ununun kül miktarından (%0.79) daha yüksek bulunmuştur. Protein içerikleri sırasıyla ekmeklik buğday ununda %11.10 olarak tespit edilirken, arpa ununda %13.79 olarak tespit edilmiştir. Sağlık açısından önemli bir bileşik olan besinsel lif miktarı ekmeklik buğday ununda %6.14 olarak saptanırken, arpa ununda oldukça yüksek bir değer olarak %16.91 şeklinde saptanmıştır. Benzer şekilde buğday unundaki fitik asit ve fenolik madde miktarı sırasıyla 2.13 mg g^{-1} ve 0.23 mgGAE g^{-1} olarak ölçülürken, arpa ununda bu değerler sırasıyla 7.16 mg g^{-1} ve 1 mgGAE g^{-1} olarak daha yüksek ölçülmüştür. Araştırmaya kaynak oluşturan arpa ununun kimyasal ve fonksiyonel özellikler açısından buğday ununa göre daha zengin bir içeriğe sahip olması birçok araştırmacının bulgularıyla da ortaya konmuştur. (Alu'Dat ve ark., 2012; Sharma & Gujral 2013; Blandino ve ark., 2015; Ünsal ve ark., 2016).

Ekmek örneklerinin kimyasal ve fonksiyonel özellikleri

Çalışma kapsamında üretilen açık ekmeklerin kimyasal ve fonksiyonel özelliklerine ait ortalama değerler ve oluşan gruplar Çizelge 4'te gösterilmiştir. Analiz sonuçlarına göre, arpa unu ilavesiyle ekmeklerin tüm kimyasal ve fonksiyonel değerlerinin önemli ($p \leq 0.05$) derecede arttığı tespit edilmiştir.

Farklı oranda arpa unu ilaveli açık ekmeklerin nem içeriklerinin %27.41 ile %36.68 arasında değiştiği görülmüştür (Çizelge 4). Sadece buğday unuyla yapılan kontrol ekmeğinin nem içeriği %27.41 olarak bulunurken, arpa unu ilavesiyle birlikte diğer tüm ekmeklerin nem içeriklerinin arttığı ve en yüksek nem değerine %36.68 ile %100 arpa unu ilaveli ekmekte ulaşıldığı tespit edilmiştir. Köten ve Ünsal (2006)'ın yaptıkları bir çalışmada, Şanlıurfa yöresine özgü açık ekmeklerde ortalama nem içeriğinin %26.53 olduğu belirlenmiş ve bu değerin kontrol ekmeklerde elde edilen nem değeriyle uyum içerisinde olduğu görülmüştür. Dizlek ve Gül (2007)'ün kepekli ekmeklerle ilgili yaptıkları bir çalışmada, artan kepek oranlarına karşılık unların su absorbsiyon değerlerinin %57.4'ten %65.7'ye yükseldiği ve buna karşılık ekmeklerdeki nem içeriklerinin 6. saat sonunda yapılan ölçümlerde % 34.7'den %38.4' e yükseldiği belirlenmiştir. Bu sonuç, yaptığımız çalışmada kül ve besinsel lif içeriği yüksek olan arpa unu ilavesiyle üretilen ekmeklerde görülen nem değerlerindeki artışla paralellik göstermekle birlikte benzer sınırlar içerisinde yer aldığı görülmektedir.. Çağlıyan (2008), İzmir piyasasında satılan bazı ekmek çeşitleri üzerine yaptığı bir çalışmada kepeğin, özellikle büyük boyutlu kepeğin su tutma

kapasitesinin yüksek olduğunu bildirmiştir. Mevcut çalışmada kontrol grubu açık ekmeklerin (A) nem içeriği %27.41±0.20 iken, sadece arpa unu kullanılarak üretilen örneklerde (F) bu değerin %36.68±0.30 olarak tespit edilmesi (Çizelge 4), arpa ununun kepek içeriğinin nispeten fazla olmasıyla açıklanabilir.

Çizelge 4. Açık ekmeklerin kimyasal ve fonksiyonel özellikleri*
*Table 4. Chemical and functional properties of açık (flat) breads**

Örnekler** *Samples*	Nem (%) *Moisture (%)*	Kül (%)*** *Ash (%)*	Protein (%)*** *Protein (%)*	Besinsel lif (%)*** *Dietary fiber (%)*	Fenolik madde (mgGAE/g)*** *Phenolic Matter (mgGAE g^{-1})*	Fitik asit (mg/g)*** *Phytic acid (mg g^{-1})*
A (Kontrol)	27.41±0.20^{c}	1.13±0.01^{e}	9.41±0.10^{e}	5.50±0.41^{d}	0.63±0.01^{d}	0.24±0.01^{d}
B	32.44±0.27^{b}	1.44±0.02^{d}	9.80±0.15^{de}	7.62±0.40^{c}	0.64±0.01^{d}	2.05±0.08^{c}
C	34.84±0.30^{ab}	1.66±0.03^{c}	10.36±0.25^{cd}	10.22±0.47^{b}	0.80±0.02^{c}	2.72±0.16^{bc}
D	33.32±0.23^{ab}	1.95±0.04^{b}	10.88±0.28^{bc}	11.22±0.57^{b}	0.81±0.03^{c}	2.51±0.04^{bc}
E	35.65±0.33^{ab}	2.19±0.03^{a}	11.48±0.14^{ab}	13.87±0.23^{a}	1.07±0.04^{b}	3.41±0.11^{ab}
F	36.68±0.30^{a}	2.39±0.06^{a}	11.97±0.30^{a}	14.96±0.70^{a}	1.33±0.06^{a}	3.95±0.21^{a}
D.K.(%)&	4.58	4.01	2.50	5.20	11.97	15.81
LSD (%5)	3.44	0.21	0.68	1.41	0.03	1.01

*Aynı sütun içerisinde aynı harfe sahip ortalamalar arasındaki fark önemsizdir (p> 0.05)
**The difference between means with the same letter in the same column is insignificant (p> 0.05)*
** A: Kontrol (%100 buğday unu); B: %20 arpa unu+ %80 buğday unu; C: %40 arpa unu+ %60 buğday unu; D: %60 arpa unu+ %40 buğday unu; E: %80 arpa unu+ %20 buğday unu; F: %100 arpa unu
*** A: Control (100% wheat flour); B: 20% barley flour + 80% wheat flour; C: 40% barley flour + 60% wheat flour; D: 60% barley flour + 40% wheat flour; E: 80% barley flour + 20% wheat flour; F: 100% barley flour*
*** Kuru maddede hesaplanmıştır
**** Calculated in dry matter*
& *Değişim Katsayısı.* &Coefficient of variation

Çizelge 4'e bakıldığında, farklı oranlarda ilave edilen arpa ununa bağlı olarak ekmeklerin kül içeriklerinin arttığı, en yüksek kül oranının %2.39 ile F örneğinde, en düşük kül oranı ise %1.13 ile A örneğinde tespit edilmiştir. Türker ve ark. (2021) arpa genotiplerindeki kül oranının %1.43-1.89 arasında olduğunu bildirmişlerdir. Bildirilen bu sonuçların bulgularımızdan daha düşük olduğu görülmüştür. Bu farklılığın kullanılan materyalin çeşit özelliğinden kaynaklanmış olabileceği düşünülmektedir. Yapılan çalışmalarda arpa unu ilave oranının artması sonucunda son üründe kül içeriğinin de önemli ölçüde arttığı Ünsal ve ark. (2016) ve Çakır (2020) tarafından rapor edilmiştir.

Ekmeklerin protein miktarları, farklı oranlarda arpa unu ilavesiyle doğru orantılı olacak şekilde artış göstermiş ve en yüksek protein oranı %11.97 ile F örneğinde, en düşük protein oranı ise %9.41 ile A örneğinde ölçülmüştür (Çizelge 4). Protein değerleri arasındaki farklılık istatistiksel açıdan önemli ($p\leq0.05$) bulunmuştur. Ekmeklik buğday ununa ilave edilen arpa unundaki artışla beraber üretilen ekmeklerin protein oranlarının arttığını bildiren araştırmacılarla (Dhingra & Jood, 2001; Skrbic & Cvejanov, 2011; Alu' dat ve ark., 2012) bulgularımız uyumlu bulunmuştur.

Ekmeklerin besinsel lif değerleri artan arpa miktarıyla birlikte artmıştır (Çizelge 4). Ekmeklerde tespit edilen besinsel lif değerleri %5.50 ile %14.96 arasında değişmiş ve bu değişim istatistiksel olarak önemli ($p\leq0.05$) bulunmuştur. En yüksek besinsel lif değeri %14.96 ile F örneğinde ölçülürken, en düşük besinsel lif değeri %5.50 ile A örneğinde ölçülmüştür. Türker ve ark. (2021), ekmeklik buğday ununa farklı miktarlarda arpa unu ilavesiyle üretilen ekmeklerin besinsel lif değerlerinin %3.00 ile %20.00 arasında değiştiğini bildirmişlerdir. Bulgularımız Pejcz ve ark. (2017) tarafından yapılan çalışmada bulunan sonuçlarla benzerlik göstermiştir. Besinsel lifin, gastrointestinal sistemin sağlıklı çalışmasına olumlu etki yaptığını (Knuckles ve ark., 1997; Dhingra & Jood., 2001; Alu' dat ve ark., 2012) bildirmektedirler. Ekmeklerde tespit edilen fenolik madde miktarı, ilave edilen arpa unun oran artışına bağlı olarak önemli derecede artmıştır ($p\leq0.05$). En yüksek fenolik madde içeriği 1.33 mgGAE g^{-1} ile F örneğinde gözlemlenirken, en düşük fenolik madde içeriği 0.63 mgGAE g^{-1} ile A örneğinde gözlemlenmiştir. Fenolik madde içeriğinin miktarı artıkça gıdanın besleyici özelliğinin geliştiği ve sağlığa olumlu etkisinin arttığı birçok araştırıcı (Zengin, 2015; De Vuyst ve ark., 2017; Papadimitriou ve ark., 2019) tarafından bildirilmiştir. Ayrıca, Lee ve ark. (2004) ve HoltekjØlen ve ark. (2008), fenolik madde içeren ürünleri tüketmenin kardiyovisküler hastalığa ile kansere yakalanma riskinin azaltıcı etkisinin olduğunu rapor etmişlerdir.

Fitik asit, mineralleri, proteinleri ve nişastayı dolaylı veya doğrudan bağlayabilmesi nedeniyle bir antinutrient olarak kabul edilmektedir. Bu bağlanma, bu besinlerin biyoyararlanımını veya sindirilebilirliğini olumsuz etkilemektedir. Bununla birlikte, fitik asidin antioksidan ve antikarsinojenik

etkiler dâhil olmak üzere bazı sağlıklı etkileri birçok araştırmacı tarafından bildirilmiştir. Ancak, yararlı etkiler ortaya çıkarmak için insanlar için dozaj bilgisi sınırlıdır (Aktaş & Levent, 2018).. Çizelge 4 incelendiğinde, ilave edilen arpa ununa bağlı olarak fitik asit değerlerinin yükseldiği, en yüksek fitik asit değerinin 3.95 mg g^{-1} ile F örneğinde, en düşük fitik asit değerinin 0.24 mg g^{-1} ile A örneğinde belirlendiği görülmektedir. Fitik asit miktarının randımanla ilişkili olduğu, artan lif ve kül içeriğine bağlı olarak miktarının arttığı, bazı esansiyel minerallerle kompleks oluşturarak bunların biyoyarayışlılığını azalttığı birçok araştırmacıların sonuçlarında görülmektedir (Özkaya, 2002; Şat & Keleş, 2004; Gupta ve ark., 2015; Ünsal ve ark., 2020). Ayrıca çimlenme, fermentasyon ve pişme gibi işlemler fitat hidrolizine neden olarak gerekli olan minerallerin kullanılabilirliğini arttırmaktadır (Bilgiçli, 2002; Steve, 2012; Ertaş & Türker, 2014; Gupta ve ark., 2015).

SONUÇ ve ÖNERİLER

Ekmek, hem diğer gıdalara çok iyi bir katık olması hem de dünyanın birçok ülkesinde diyetten sağlanan enerjinin büyük bir kısmını oluşturması açısından temel bir gıda maddesi konumundadır. Özellikle Şanlıurfa ve yöresinde yoğun olarak tüketilen düz ekmekler içerisinde yer alan açık ekmeğin besinsel açıdan zenginleştirilmesi, beslenme konusundaki eksikliklerin giderilmesi anlamında da büyük önem arz etmektedir.Çalışma kapsamında ekmeklik buğday ununa farklı oranlarda arpa unu ilave edilerek üretilen açık ekmeklerde kül, protein, besinsel lif, fitik asit ve fenolik madde içeriklerinin arttığı saptanmıştır. Bu sonuçlar çalışmanın amacı olan ekmeklerin besinsel açıdan zenginleştirilmesi sonucuna ulaşıldığını göstermektedir. Elde edilen bulgular ışığında arpa ununun açık ekmek yapımında ve diğer başka gıdaların besinsel açıdan zenginleştirilmesinde doğal bir katkı olarak kullanılabileceği sonucuna varılmıştır.

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Araştırmacıların Katkı Oranı Beyan Özeti

Yazarlar makaleye eşit oranda katkı sağlamış olduklarını beyan eder.

Çıkar Çatışması Beyanı

Tüm yazarlar makalelerinde, sonuçları veya yorumları etkileyebilecek herhangi bir maddi veya diğer asli çıkar çatışması olmadığını beyan ederler.

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KAYNAKLAR

AACC, (2010). Approved Methods of the American Association of Cereal Chemists. Approved Methods of Analysis 11th Edition, Cereals & Grains Association: St. Paul, MN, U.S.A.

Aktaş, K. & Levent, H. (2018). The effects of Chia (Salvia hispanica L.) and Quinoa Flours on The Qualıty of Rice Flour and Starch Based-Cakes. *Gıda*, 43(4), 644–654. doi:10.15237/gida.GD18032

Akturfan, M. (2018). *Lavaş Ekmeğine Farklı Düzeylerde Kinoa Tohumu Unu Katılmasının Hamur ve Ekmek Özellikleri İle Kimyasal Bileşim Üzerine Etkisi (Tez no 542696)*. [Yüksek Lisans Tezi, Selçuk Üniversitesi Sağlık Bilimleri Enstitüsü Besin Hijyeni ve Teknolojisi Ana Bilim Dalı]. Yükseköğretim Kurulu Ulusal Tez Merkezi.

Al-Attabi, Z.H., Merghani, T.M., Ali, A. & Rahman, M.S. (2017). Effect of Barley Flour Addition on the Physico-Chemical Properties of Dough and Structure of Bread. *Journal of Cereal Science, 75*, 61-68. https://doi.org/10.1016/j.jcs.2017.03.021

Alu' Dat, H.M., Rababah, T., Ereifej, K., Alli, I., Alrababah, A.M., Almajwal, A., Masadeh, N. & Alhamad, N.M. (2012). Effect of Barley Flour and Barley Protein Isolate on Chemical, Functional, Nutritional and Biological Properties of Pita Bread. *Food Hydrocolloids, 26*(1), 135-143. https://doi.org/10.1016/j.foodhyd.2011.04.018

Baik, B.K. & Ullrich, S.E. (2008). Barley for Food: Characteristics, Improvement, and Renewed Interest. *Journal of Cereal Science, 48*(2), 233-242. https://doi.org/10.1016/j.jcs.2008.02.002

Bilgiçli, N. (2002). Fitik Asitin Beslenme Açısından Önemi ve Fitik Asit Miktarı Düşürülmüş Gıda Üretim Metotları. *Selçuk Üniversitesi Ziraat Fakültesi Dergisi, 16*(30), 79-83.

Çağlıyan, B.İ., 2008. İzmir Piyasasında Satılan Bazı Ekmek Çeşitlerinin Nitelikleri ve Yapım teknikleri, [Doktora Tezi, Ege Üniversitesi Fen Bilimleri Enstitüsü Gıda Müh. Ana Bilim Dalı, 133 s.].

Çakır, E., Arıcı, M., Durak, M. (2020). Biodiversity and Techno-Functional Properties of Lactic Acid Bacteria in Fermented Hull-less Barley Sourdough. *Journal of Bioscience and Bioengineering, 130*(5), 450-456.https://doi.org/10.1016/j.jbiosc.2020.05.002

Çam, M. & İçyer, N.C. (2015). Phenolics of Pomegranate Peels: Extraction Optimization by Central Composite Design and Alpha Glucoside Inhibition Potentials. *Journal of Food Science and Technology, 52*(3): 1489-1497. https://doi.org/10.1007/s13197-013-1148-y

De Vuyst, L., Van Kerrebroeck, S. & Leroy, F. (2017). *Microbial Ecology and Process Technology of Sourdough Fermentation*. In S. Sariaslani & G.M. Gadd (Eds.), *Advances in Applied Microbiology.* Academic Press Inc., USA. https://doi.org/10.1016/bs.aambs.2017.02.003

Dhingra, S. & Jood, S. (2001). Organoleptic and Nutritional Evaluations of Wheat Breads Supplemented with Soybean and Barley Flour. *Food chemistry, 77*(4), 479-488. https://doi.org/10.1016/S0308-8146(01)00387-9

Dizlek, H. & Gül, H. (2007). Farklı Düzeylerde Kullanılan L-Askorbik Asidin Buğday Kepekli Ekmeklerin Bazı Nitelikleri Üzerindeki Etkileri. *Süleyman Demirel Üniversitesi Ziraat Fakültesi Dergisi, 2*(2), 1-10.

Elçi, M.A. (2022). *Arpa Unu İlavesinin Şanlıurfa Açık Ekmeğinin Kalitesine Etkisinin Araştırılması (Tez no 750386)*. [Yüksek Lisans Tezi, Harran Üniversitesi Fen Bilimleri Enstitüsü Gıda Mühendisliği Ana Bilim Dalı]. Yükseköğretim Kurulu Ulusal Tez Merkezi.

Ereifej, K., Al-Mahasneh, M. & Rababah, T. (2006). Effect of Barley Flour on Quality of Balady Bread. International *Journal of Food Properties. 9*(1), 39-49. https://doi.org/10.1080/10942910500471669

Ertaş, N. & Türker, S. (2014). Bulgur Processes Increase Nutrition Value: Possible Role in In-vitro Protein Digestability, Phytic Acid, Trypsin Inhibitor Activity and Mineral Bioavailability. *Journal of Food Science and Technology, 51*(7), 1401-1405. https://doi.org/10.1007/s13197-012-0638-7

Gupta, M., Bawa, A.S. & Abu-Ghannam, N. (2011). Effect of Barley Flour and Freeze-Haw Cycles on Textural, Nutritional and Functional Properties of Cookies. *Food and Bioproducts Processing, 89*(4), 520-527. https://doi.org/10.1016/j.fbp.2010.07.005

Gupta, R.K., Gangoliya, S.S. & Singh, N.K. (2015). Reduction of Phytic Acid and Enhancement of Bioavailable Micronutrients in Food Grains. *Journal of Food Science and Technology, 52*(2), 676-684. https://doi.org/10.1007/s13197-013-0978-y

Holtekjølen, A.K., Bævre, A., Rødbotten, M., Berg, H. & Knutsen, S.H. (2008). Antioxidant Properties and Sensory Profiles of Breads Containing Barley Flour. *Food chemistry, 110*(2), 414-421. https://doi.org/10.1016/j.foodchem.2008.02.054

ICC, 2002. Standard Methods of The International Association for Cereal Science and Technology (ICC). Printed by ICC, Vienna, Edition 2002.

Knuckles, B.E., Hudson, Ca., Chiu, M.M. & Sayre, R.N., 1997. Effect of β-Glucan Barley Fractions in High-Fiber Bread and Pasta. *Cereal Foods World, 42*(2), 94-99.

Köten, M. & Ünsal, A.S. (2006). Şanlıurfa Yöresine Özgü "Tırnaklı ve Açık (Lavaş)" Ekmeklerin Bazı Kimyasal Bileşimlerinin Belirlenmesi Üzerine Bir Araştırma. *Harran Üniversitesi Ziraat Fakültesi Dergisi, 10*(3/4), 57-62.

Köten, M. & Ünsal, A.S. (2007). Şanlıurfa Yöresine Özgü "Tırnaklı ve Açık" Ekmeklerin Geleneksel Üretim Yöntemleri. *Gıda, 32*(2), 81-85.

Köten, M., 2021. Influence of Roasted and Unroasted Terebinth (*Pistacia terebinthus*) on The Functional, Chemical and Textural Properties of Wire-Cut Cookies. *Food Science and Technology, 41*(1), 245-253. https://doi.org/10.1590/fst.17020

Lee, J., Koo, N. & Min, D.B. (2004). Reactive Oxygen Species, Aging and Antioxidative Nutraceuticals. *Comprehensive Reviews in Food Science and Food Safety, 3*(1), 21-33. https://doi.org/10.1111/j.1541-4337.2004.tb00058.x

Özkaya, B. (2002). Fitik Asit ve Tahıl Ürünlerindeki Önemi. Hububat Ürünleri ve Teknolojisi Kongre ve Sergisi, Gaziantep, Türkiye, 3-4 Ekim 2002, ss. 57-65.

Papadimitriou, K., Zoumpopoulou, G., Georgalaki, M., Alexandraki, V., Kazou, M. Anastasiou, R. & Tsakalidou, E. (2019). *Sourdough Bread*. In C.M. Galanakis (Ed.), *Innovations in Traditional Foods*, Elsevier, Woodhead Publishing, Sawston. https://doi.org/10.1016/b978-0-12-814887-7.00006-x.

Pejcz, E., Czaja, A., Wojciechowicz-Budzisz, A., Gil, Z. & Spychaj, R. (2017). The Potential of Naked Barley Sourdough to Improve the Quality and Dietary Fibre Content of Barley Enriched Wheat Bread. *Journal of Cereal Science, 77*, 97-101. https://doi.org/10.1016/j.jcs.2017.08.007

Skrbic, B. & Cvejanov, J. (2011). The Enrichment of Wheat Cookies with High-Oleic Sunflower Seed and Hull-less Barley Flour: Impact on Nutritional Composition, Content oh Heavy Elements and Physical Properties. *Food chemistry, 124*(4), 1416-1422. https://doi.org/10.1016/j.foodchem.2010.07.101

Steve, I.O. (2012). Influence of Germination and Fermentation on Chemical Composition, Protein Quality and Physical Properties of Wheat Flour (*Triticum aestivum*). *Journal of Cereals and Oil Seeds, 3*(3), 35-47

Şat, İ. & Keleş, F. (2004). Fitik Asit ve Beslenmeye Etkisi. *Gıda, 29*(6), 405-409.

Trogh, I., Courtin, C., Andersson, A., Aman, P., Sorensen, J. & Delcour, J. (2004). The Combined Use of Hullless Barley Flour and Xylanase as a Strategy for Wheat/Hull-Less Barley Flour Breads with Increased Arabinoxylan and (1→ 3, 1→ 4)-ß-D-Glucan Levels. *Journal of Cereal Science, 40*(3), 257-267. https://doi.org/10.1016/j.jcs.2004.08.008

TUİK, (2021). Türkiye İstastistik Kurumu 2021 Yılı Bitkisel Üretim İstatistikleri. (Alınma Tarihi: 05.06.2021).

Türker, M., Akkaya, M.R., Türker, B. & Kola, O. (2021). Bazı Arpa Genotipleri ile ß-glukan İçeriği Zenginleştirilmiş Ekmek Hamurunun Reolojik

Özellikleri. *Gaziosmanpaşa Bilimsel Araştırma Dergisi (GBAD), 10*(2), 105-117.

Ünsal, A.S., Atlı, A. & Köten, M. (2016). The Effect of Pearled and Unpearled Barley Flour Addition on Some Noodle Quality Parameters, IMCOFE 16. International Multidisciplinary Congress of Eurasia, Odessa, Ukraina, 11-13 July 2016, pp. 544-551.

Ünsal, A.S., Atlı, A. & Köten, M. (2020). Çimlendirilmiş Buğday Unundan Yapılan Tırnaklı (Düz Ekmek) ve Tava Ekmek Kalitesi Üzerine Bir Araştırma. *KSÜ Tarım ve Doğa Dergisi, 23*(5), 1209-1215. https://doi.org/10.18016/ksutarimdoga.vi.687817

Wiles, P.G., Gray, I.K. & Kissling, R.C. (1998). Routine Analysis of Proteins by Kjeldahl and Dumas Methods: Review and Interlaboratory Study Using Dairy Products. *Journal of AOAC International, 81*(3), 620-632. https://doi.org/10.1093/jaoac/81.3.620

Zengin, G. (2015). *Bazı İlkel Buğdaylarda Kalite Parametrelerinin Belirlenmesi Üzerine Bir Araştırma (Tez no 418868)*. [Yüksek Lisans Tezi, Selçuk Üniversitesi Fen Bilimleri Enstitüsü Gıda Mühendisliği Ana Bilim Dalı]. Yükseköğretim Kurulu Ulusal Tez Merkezi.

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https://doi.org/10.18016/ksutarimdoga.vi.1197761

Yağı Alınmış Nar Çekirdeklerinden Fenolik Antioksidanların Özütlenmesinde Ultrases Sisteminin Kullanımı

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ÖZET

Bu çalışmada yağı alınmış nar çekirdeklerin fenolik antioksidanların uygun şartlarda özütlenmesine odaklanılmıştır. Bu bağlamda özütleme adımında ultrases sistemi kullanılmış ve şartlar yanıt yüzey yöntemi ile optimize edilmiştir. Optimizasyon işleminde, özütleme süresinin (5-60 dk) ve ultrases cihazının genliğinin (%20-100) toplam fenolik madde miktarı üzerine etkisi araştırılmıştır. Maksimum toplam fenolik madde miktarı (TFM), özütleme süresinin 52 dk ve genliğin %88 olduğu noktada elde edilmiştir. Optimum koşullarda elde edilen özütlere ait özellikler klasik yöntemle (metanolik özütler) elde edilen özütlerle karşılaştırmalı olarak verilmiştir. Ultrases sistemi kullanılarak elde edilen özütlerin TFM (2.94 mg GAE g^{-1}), toplam flavanoid madde miktarı (TFMM) (0.36 mg KE g^{-1}) ve toplam hidrolize tanen madde miktarı (THTM) (22.07 mg TAE g^{-1}) metanolik özütlerden (2.60 mg GAE g^{-1}, 0.27 mg KE g^{-1}, 16.73 mg TAE g^{-1}) daha yüksek olduğu tespit edilmiştir. LC-ESI-MS/MS sonuçları yağsız nar çekirdeklerinin gallik asit ve ellajik asit açısından zengin olduğunu göstermiştir. Fenolik asitlerin baskınlığı FTIR spektroskopisi ile doğrulanmıştır. Üstün antioksidatif davranış optimum koşullarda hazırlanan özütlerde (DPPH: 105.26 µmol TEAC g^{-1}, ABTS: 57.65 µmol TEAC g^{-1}, FRAP: 13.03 µmol TEAC g^{-1}, CUPRAC: 8.91 µmol TEAC g^{-1}) tespit edilmiştir. Sonuçlar, meyve çekirdeklerden biyoaktif maddelerin özütlenmesinde ultrases sisteminin efektif bir uygulama olduğunu ortaya koymuştur.

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Anahtar Kelimeler
Yağı Alınmış Nar Çekirdeği
Ultrases Destekli Özütleme
LC-ESI-MS/MS
FTIR Spektroskopisi
Antioksidan Kapasite

Use of Ultrasound System in Extraction of Phenolic Antioxidants from Oil-Free Pomegranate Seeds

ABSTRACT

This study focused on the extraction of phenolic antioxidants from oil-free pomegranate seeds under suitable conditions. In this context, the ultrasound system was applied in the extraction step and the conditions were optimized by the response surface method. The effects of the extraction time (5-60 min) and the amplitude (20-100%) on total phenolic content were investigated. The optimum conditions were 52 min and 88% amplitude for providing maximum total phenolic content. The attributes of the extracts produced under optimum conditions were compared with the extracts obtained by the classical method (methanolic extracts). Total contents of phenolic (2.94 mg GAE g^{-1}), flavonoid (0.36 mg CE g^{-1}), and hydrolysable tannin (22.07 mg TAE g^{-1}) in the extracts prepared using ultrasound assisted system were superior than those of methanolic extracts (2.60 mg GAE g^{-1}, 0.27 mg CE g^{-1}, 16.73 mg TAE g^{-1}). LC-ESI-MS/MS results indicated that defatted pomegranate seeds were rich in gallic acid and ellagic acid. The predominance of phenolic acids was endorsed by FTIR spectroscopy. The extracts produced in the optimum conditions exhibited higher antioxidative behavior (DPPH: 105.26 µmol TEAC g^{-1}, ABTS: 57.65 µmol TEAC g^{-1}, FRAP: 13.03 µmol TEAC g^{-1}, CUPRAC: 8.91 µmol TEAC g^{-1}). The results indicated that ultrasound system created awareness in terms of the effective extraction of bioactive substances from fruit seeds.

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GİRİŞ

Artan nüfus artışı ve popülasyonların yaşam tarzındaki farklılıklara paralel olarak atık materyallerin değerlendirilmesi ve ekonomiye kazandırılması için son yıllarda küresel boyutta göz ardı edilemez bir çaba vardır. Atık yönetiminde, gıda işleme atıklarından katma değerli ürünlerin elde edilmesi bilimsel çalışmalar ve endüstri için öncelikli konular arasındadır. Daha önce yürütülmüş çalışmalar ve endüstriyel üretimler de bu yaklaşımı desteklemektedir. Gelişmiş (680 milyar dolar) ve gelişmekte (310 milyar dolar) olan ülkelerde, gıda atıklarından kaynaklı önemli maddi kayıpların olduğu önceki çalışmalarda ortaya konmuştur (Brito ve ark., 2022). Küresel olarak ortaya çıkan gıda orijinli atıkların hacminin 1,3 ile 1,4 milyar ton arasında değiştiği yapılan araştırmalar ile kanıtlanmış olup bu oranın önümüzdeki birkaç yıl içerisinde 2,6 milyar tona ulaşacağı tahmin edilmektedir (Sinha & Tripathi, 2021). Gıda kaynaklı bu atıkların yaklaşık %25-30'luk kısmını meyve-sebzelerin işleme ve hasat sonrası sürecinde açığa çıkan yan ürünler oluşturmaktadır (Sagar ve ark., 2018). Bu atıkların fonksiyonel özellik sergileme potansiyeli olan oluşumları yüksek miktarlarda yapılarında barındırdığı önceki çalışmalarda vurgulanmıştır (Méndez ve ark., 2022). Dolayısıyla meyve-sebze işleme atıklarından ekonomik değeri olan ürünlerin üretilmesi ve bu ürünlerin pazara kazandırılması ulusal/küresel bağlamda önem arz etmektedir.

Nar (*Punica granatum* L.) farklı bölgelerde kendisine yetişme alanı bulan en eski yenilebilir meyvelerden biridir (Çam ve ark., 2014). Nar meyvesi sofralık olarak tüketiminden ziyade endüstriyel olarak işlenmektedir. Meyve suyu, nar ekşisi, reçel, pekmez ve jöle narın girdi oluşturduğu gıdalara örnek olarak verilebilir (Abid ve ark., 2018; Bou Dargham ve ark., 2022). Narın, bu gıdalara işlenmesi esnasında yüksek miktarlarda yan ürünler (kabuk ve çekirdek) açığa çıkmaktadır (Qu ve ark., 2009). Farklı çalışmalarda nar kabuğunun ve nar çekirdeğinin fenolik antioksidanlar gibi biyolojik aktivite sergileyen özel yapıları içerdiği not edilmiştir (Paul & Radhakrishnan, 2020; Kaderides ve ark, 2021). Dolayısıyla bu özel yapıların ilgili atıklardan uygun yöntemlerle izole edilip katma değerli ürünlere dönüştürülmesi bilimsel literatür ve endüstri için gereklilik arz etmektedir. Bu yaklaşıma paralel olarak nar kabukları farklı çalışmalarda konu olarak işlenmiştir (Kaderides ve ark., 2021). Nar işleme atığı olan nar çekirdekleri ile ilgili de farklı çalışmalar mevcut olup bu çalışmalarda daha çok çekirdeklerden yağ eldesi ve elde edilen yağın karakterizasyonuna odaklanılmıştır (Paul & Radhakrishnan, 2020); ancak nar çekirdeklerinden yağ eldesinden sonra geriye kalan posa miktarı da göz ardı edilemeyecek kadar fazladır. Posanın farklı amaçlar için kullanımına yönelik çalışmalar kısıtlıdır. Bir nevi atığın atığı olan yağı alınmış nar çekirdeklerinden besinsel değeri olan materyallerin eldesi literatüre farklı bir bakış açısı kazandıracaktır. Çekirdeklerin kompleks bir yapıya sahip olduğu ve son yıllarda çevre dostu özütleme 'yeşil ekstraksiyon' yöntemlerinin ön plana çıktığı göz önünde bulundurulduğunda çekirdeklerden bu besinsel değeri olan fonksiyonel yapıların uygun tekniklerle elde edilmesi önem arz etmektedir. Bu teknikler arasında ultrases destekli özütleme sistemlerinin başarısı farklı çalışmalarda not edilmiştir (Da Porto ve ark., 2013). Corbin ve ark. (2015) keten tohumundan fenoliklerin özütlenmesi ile ilgili yürütmüş oldukları çalışmada 4 farklı yöntem (ultrases destekli, mikrogalga destekli, enzimatik destekli ve geleneksel alkali özütleme) kullanmış ve bulgular çekirdeklerden biyolojik aktiviteye sahip yapıların eldesinde ultrases sisteminin kullanımının daha makul olduğunu ortaya koymuştur. Başka bir çalışmada baobab çekirdeklerinden ultrases destekli sistemler kullanılarak elde edilen özütlerin geleneksel yöntemlerle elde edilen özütlere kıyasla fenolik maddelerce daha zengin olduğu rapor edilmiştir (Ismail ve ark., 2019). Tüm bu yaklaşımların ve sonuçların ışığı altında mevcut çalışmada:

(i) yağı alınmış nar çekirdeklerinden fenolik antioksidanları elde etmek amacıyla ultrases destekli özütleme yönteminin kullanılması ve özütleme koşullarının yanıt yüzey yöntemi ile optimize edilmesi,

(ii) özütlerin toplam fenolik madde miktarının (TFM), flavonoid madde miktarının (TFMM) ve hidrolize tanen miktarının (THTM) ortaya çıkarılması,

(iii) optimum koşullarda elde edilen yağı alınmış nar çekirdeği özütlerinin fenolik madde içeriğinin LC–ESI–MS/MS ile karakterize edilmesi,

(iv) özütlerdeki spesifik yapıların FTIR spektroskopisi ile tespit edilmesi,

(v) özütlerin antioksidatif davranışlarının belirlenmesi amaçlanmıştır.

MATERYAL ve METOD

Materyal

Çalışmada kullanılan nar çekirdekleri Isparta'da faaliyet gösteren Mavideniz Gıda A.Ş'den temin edilmiştir. Nar çekirdeklerini yağsız forma getirebilmek için laboratuvar ölçekli soğuk pres

makinesi (12 kg tohum saat^{-1}, tek kafa, 750 W güç, 12 mm çaplı çıkış ucu, 12 rpm hız ve 100 °C maksimum çıkış sıcaklığı) kullanılmıştır. Yağsız çekirdekler öğütülmüş (300-2000 µm parçacık boyutu) ve daha sonra özütleme aşamasına kadar +4 °C'de muhafaza edilmiştir. Analizde kullanılan kimyasallar analitik saflıkta olup aksi belirtilmedikçe Sigma-Aldrich (St. Louis, MO, ABD) ve Merck (Darmstadt, Almanya) firmalarından temin edilmiştir.

Örneklerin Hazırlanması

Ultrases destekli özütleme koşullarının optimizasyonu

Ultrases destekli özütleme işleminin optimizasyonu için 2 faktörlü ve 5 seviyeli toplam 12 farklı deneme noktası merkezi tümleşik tasarım ile yanıt yüzey yöntemi kullanılarak oluşturulmuştur (Çizelge 1). Bu aşamada 60 Hz frekansa ve 665 W güce sahip laboratuvar tipi ultrases cihazı (Wiseclean WUC-D10H) kullanılmış olup özütleme süresi (5, 13, 33, 52 ve 60 dk) ve cihaz genliğinin (%20, 32, 60, 88 ve 100) TFM üzerindeki etkileri araştırılmıştır.

İlk olarak, yağsız nar çekirdekleri saf su ile karıştırılmıştır (1:10, w/v). Daha sonra hazırlanan solüsyonlar ultrases cihazının ilgili bölümüne yerleştirilmiş ve özütleme işlemi, oluşturulan deneme noktaları dikkate alınarak oda sıcaklığında farklı sürelerde (5-60 dk) ve genliklerde (%20-100) yürütülmüştür. Son olarak, her bir deneme noktası için örnekler 4000 rpm'de 10 dk süre ile santrifüj edildikten sonra süpernatantlar toplanmış ve analizlere tabi tutulmuştur.

Geleneksel yöntem

Geleneksel özütleme yöntemi Alasalvar ve Yıldırım (2021)'e göre yapılmıştır. Bunun için yağsız nar çekirdekleri metanol içeren cam şişelere ilave edilmiş (1:10, w/v) ve özütleme işlemi laboratuvar tipi bir çalkalayıcıda 250 rpm'de oda sıcaklığında 24 s boyunca yürütülmüştür. Süre sonunda örnekler 4000 rpm'de 10 dk süre ile santrifüj edilmiş ve süpernatantlar toplanmıştır. Toplanan süpernatantlarda analizler gerçekleştirilmiştir.

Toplam Fenolik Madde Miktarı

TFM analizi için Folin–Ciocâlteu reaktifi kullanılmıştır (Singleton & Rossi, 1965). Seyreltilmiş özütler (0.4 mL), saf su (1.8 mL) ve Folin–Ciocâlteu reaktifi (0.2 mL) ile cam tüpler içerisinde karıştırılmıştır. Daha sonra tüpler içerisine %7.5'lik (w/v) sodyum karbonat solüsyonu (1.6 mL) ilave edilmiştir. Karanlık bir ortamda 60 dk'lık inkübasyondan sonra örneklerin absorbansı UV–Vis spektrofotometre (Model UV-1280, Shimadzu, Japonya) kullanılarak 765 nm'de okunmuştur. Sonuçlar g örnek başına mg gallik asit eşdeğeri (mg GAE g^{-1}) olarak ifade edilmiştir.

Toplam Flavanoid Madde Miktarı

TFMM analizi Zhishen ve ark. (1999)'a göre yürütülmüştür. On mL'lik tüpler içerisinde1 mL seyreltilmiş özüt, 4 mL saf su ve 0.3 mL sodyum nitrit (%5, w/v) karıştırılmış ve karışım 5 dk süre ile inkübasyona bırakılmıştır. Süre sonunda tüpler içerisine 0.3 mL alüminyum klorür (%10, w/v) ilave edilmiştir. Alüminyum klorür ilave edildikten 1 dk sonra, hazırlanan solüsyonların içerisine sırasıyla 2 mL sodyum hidroksit (1 M) ve 2.4 mL saf su eklenmiştir. Tüpler içerisindeki karışımların absorbans okumaları 510 nm'de UV–Vis spektrofotometre ile gerçekleştirilmiştir. Sonuçlar mg kateşin eşdeğeri g örnek^{-1} (mg KE g^{-1}) olarak verilmiştir.

Toplam Hidrolize Tanen Madde Miktarı

THTM analizinin yürütülmesinde daha önce literatürde not edilmiş bir çalışma referans alınmıştır (Willis, 1998). Seyreltilmiş örneklerden 1 mL alınmış ve 5 mL potasyum iyodat (%2.5, w/v) çözeltisi ile karıştırılmıştır. Kırmızı renkli karışımın absorbansı 550 nm'de UV–Vis spektrofotometre ile ölçülmüştür. Nihai sonuçlar g örnek başına mg tannik asit eşdeğeri (mg TAE g^{-1}) olarak sunulmuştur.

LC-ESI-MS/MS Analizi

Yağsız nar çekirdeği özütleri, ikili gradyan pompaya, bir otomatik enjeksiyon bloğuna (SIL-20AC), bir gaz gidericiye (DGU-20A3R) ve bir kolon termostatına (CTO-10ASVP) sahip Nexera Shimadzu UHPLC LC–ESI-MS/MS (Shimadzu, Japonya) cihazına enjekte edilmeden önce 0.45 µm gözeneklere sahip membran filtrelerden geçirilmiştir. Yağsız çekirdeklerdeki fenolik fraksiyonları ayırmada, sıcaklığı 40 °C'ye getirilmiş İnertsil ODS 4 (2 µm, 2.1 x 50 mm) kolonundan yararlanılmıştır. Mobil faz olarak formik asit-ultra saf su (%0.1, v/v) (A) ve formik asit-metanol (%0.1, v/v) (B) kullanılmıştır. Örnek enjeksiyon hacmi ve akışı sırasıyla 2 µL ve 0.4 mL dk^{-1} olacak şekilde ayarlanmıştır. Gradient koşullar: 0-4 dk (%95 B), 4.01-7 dk (%95 B) ve 7.01-12 dk (%5 B).

MS tespitinde, elektrosprey iyonizasyon (ESI) kaynağına sahip Shimadzu LC-MS/MS 8030 model üçlü tandem dört kutuplu kütle spektrometresi kullanılmıştır. Fraksiyonlara ayırma işlemi hem negatif hem de pozitif elektrosprey iyonizasyon modunda yürütülmüştür. Nebulize (3 mL/dk) ve kurutucu gaz (15 mL/dk) olarak azot kullanılmıştır. DL sıcaklığı, ısı bloğu sıcaklığı ve ara yüz voltajı sırasıyla 260 °C, 400 °C ve 4.5 kV'ye ayarlanmıştır. Verilerin değerlendirilmesinde LabSolutions programından (Shimadzu, Japonya) yararlanılmıştır.

FTIR Spektroskopisi

Özütlerdeki spesifik gruplar FTIR spektroskopisi

(Shimadzu, Japonya) kullanılarak araştırılmıştır. Özütler cihazın ilgili kısmına yerleştirildikten sonra 1 cm^{-1} çözünürlükte spektrum taraması (4000-600 cm^{-1}) yapılmıştır (Naji ve ark., 2022).

Antioksidan Kapasite

DPPH yöntemi: Seyreltme işlemi uygulanmış yağsız nar çekirdeği özütlerinden 0.1 mL alınmış ve 3.9 mL DPPH radikali (25 mg L^{-1}) içeren tüpler içerisine ilave edilmiştir. Tüpler karanlık ortamda 30 dk inkübasyona bırakılmış ve süre sonunda karışımların absorbansı 515 nm'de UV–Vis spektrofotometre kullanılarak okunmuştur (Çam ve ark., 2009).

ABTS yöntemi: Radikal solüsyonunu hazırlamak için 0.96 mg ABTS 25 mL'lik balon joje içerisinde 15 mL saf su ile tamamen çözündürülmüştür. Balon jojeye 5 mL potasyum persülfat (2.45 mM) ilave edilmiş ve nihai hacim saf su ile 25 mL'ye tamamlanmıştır. Daha sonra solüsyon 16 s karanlık ortamda inkübasyona bırakılmıştır. Radikal solüsyonun 734 nm'deki absorbansı sodyum fosfat tamponu (0.2 M, pH 7.4) ile 0.700 ± 0.02 olacak şekilde ayarlandıktan sonra ilgili solüsyon (2 mL) ve seyreltilmiş özütler (20, 40, 60 ve 80 µL) karıştırılmıştır. Karışımlar 6 dk karanlık ortamda bekletildikten sonra absorbans değerleri UV–Vis spektrofotometre ile 734 nm'de ölçülmüştür (Çam ve ark., 2009).

FRAP yöntemi: Özütleri (150 µL) içeren tüplere daha önceden hazırlanmış 2850 µL FRAP reaktifi (30 mM sodyum asetat, 10 mM 2,3,5-Trifeniltetrazolyum klorür, 20 mM demir (III) klorür) ilave edilmiştir. Hazırlanan karışımlar 30 dk karanlık ortamda inkübasyona bırakıldıktan sonra absorbans değerlerini okumak için dalga boyu 593 nm'ye ayarlanmış UV–Vis spektrofotometrenin ilgili kısmına kuvartz küvetler içerisinde yerleştirilmiştir (Benzie & Strain, 1996).

CUPRAC yöntemi: 1 mL bakır (II) klorür (0.01 M), 1 mL etanolik neocuproine (7.5×10^{-3} M), 1 mL amonyum asetat solüsyonu (1 M, pH 7), 0.4 mL örnek ve 0.7 mL saf su tüpler içerisinde homojen olacak şekilde karıştırılmıştır. Karışım karanlık ortamda 30 dk'lık inkübasyona bırakıldıktan sonra UV–Vis spektrofotometrenin dalga boyu 450 nm ayarlanmış ve absorbans okumaları yapılmıştır (Apak ve ark., 2008).

Antioksidan kapasite sonuçları µmol trolox eşdeğeri antioksidan kapasite g^{-1} (µmol TEAC g^{-1}) örnek olarak ifade edilmiştir.

İstatistik Analizler

Üretimler ve analizler en az 2 tekerrürlü olacak şekilde gerçekleştirilmiştir. OriginPro 8 (Origin Lab Inc.) programı kullanılarak grafikler oluşturulmuştur. Verilerin istatistiksel olarak değerlendirilmesi için SPSS 22 paket programı (SPSS Inc., Şikago, IL, ABD) kullanılmıştır. Ortalamalar arasındaki farklılıklar t testi ile tespit edilmiş olup $P<0.05$ sınır değeri olarak kabul edilmiştir. Özütleme işleminin optimizasyonunda deneme tasarımları Design Expert 7.0 (Stat-Ease Inc., Minneapolis, MN) programı ile oluşturulmuştur. Optimizasyon işleminde bağımlı değişken, ikinci dereceden bir polinom modeli elde etmek ve regresyon katsayılarını belirlemek için çoklu doğrusal regrasyonlara yerleştirilmiştir (Denklem 1).

$$Y = \beta_0 + \sum_{i=0}^{n} \beta_i X_i + \sum_{i=0}^{n} \beta_{ii} X_i^2 + \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} \beta_{ij} X_i X_j \quad (1)$$

Y: Bağımlı değişken, β_0: Kesişim regresyon katsayısı, β_i: Doğrusal regresyon katsayısı, β_{ii}: İkinci dereceden regresyon katsayısı, β_{ij}: Etkileşim regresyon katsayısı, $X_i X_j$: Ana değişkenler arasındaki etkileşim, n: Değişkenlerin sayısı.

BULGULAR ve TARTIŞMA

Ultrases Destekli Özütleme Koşullarının Optimizasyonu

Literatürde fenolik antioksidanların farklı materyallerden elde edilmesi ile ilgili çeşitli çalışmalar mevcuttur (Ben Yakoub ve ark., 2018; Pandey ve ark., 2018). Yürütülen çalışmaların hemen hemen hepsinde özütleme koşulları değiştirilerek maksimum verimde prosesin yürütülmesi amaçlanmıştır. Fenolik antioksidanların eldesi ile ilgili çalışmalarda proses üzerine özütleme sıcaklığının/süresinin (Tülek ve ark., 2021) ve çözücü tipinin/oranının (Zhang & Lee, 2021) etkili olduğu rapor edilmiştir. Ayrıca, bu biyolojik yapıların izole edilmesinde başarısı ispatlanmış metanol gibi toksik solventler yerine son yıllarda ultrases destekli, mikrodalga destekli ve süperkritik akışkan özütleme yöntemlerine bir yönelim vardır. Farklı çalışmalarda da bu tekniklerin başarısı ortaya konmuştur; ancak bu inovatif tekniklerde de makul bir özütleme süreci için cihaz şartlarının optimize edilmesi gerektiği yürütülen çalışmalarda vurgulanmıştır (Dairi ve ark., 2021; Mrkonjić ve ark., 2021; Pinto ve ark., 2021). Tüm bunlara ilaveten bilimsel literatürdeki yaklaşımlara bakıldığında özellikle çekirdek gibi kompleks yapılara sahip materyallerden fenolik antioksidanların izolasyon sürecinde toksik solventler yerine bu yeni tekniklerin kullanımının daha makul olduğu görülmektedir. Bu yaklaşımlardan yola çıkarak mevcut çalışmada yağı alınmış nar çekirdeklerinden fenolik antioksidanların eldesinde ultrases destekli özütleme yöntemi kullanılmıştır. Özütleme koşulları sistematik yaklaşımla optimize edilmiştir. Literatür verileri ve yapılan ön çalışmalar göz önünde bulundurularak özütleme süresinin (5-60 dk) ve ultrases cihazının genlik değerinin (%20-100) özütlerdeki TFM üzerine etkisi araştırılmıştır. Özütleme sıcaklığı, solvent tipi ve solvent oranı gibi parametreler izolasyon sürecinde sabit tutulmuş olup oluşturulan tasarıma ilave edilmemiştir. Yanıt yüzey yöntemi kullanılarak oluşturulan 12 deneme noktası ve bu noktalarda

bağımlı değişken (TFM) sonuçları Çizelge 1'de sunulmuştur. TFM'nin 0.11 mg GAE g^{-1} ile 3.45 mg GAE g^{-1} arasında değiştiği tespit edilmiştir. Genel olarak özütleme süresi ve ultrases cihazının genliğindeki düşüşle birlikte özütlerdeki TFM miktarının da düşme eğiliminde olduğu tespit edilmiştir. Bu durum, çekirdek materyalinin hücre duvarının kısa süre ve düşük genlikte yeterince parçalanmamasına paralel olarak hücre duvarında yer alan fenolik antioksidanların solvente difüzyon oranının sınırlı olması ile açıklanabilir.

Çizelge 1. Yağı alınmış nar çekirdeklerinden fenolik antioksidanların ultrases destekli özütleme işlemi ile ilgili deneysel tasarım

Table 1. The experimental design regarding the ultrasound-assisted extraction of phenolic antioxidants from defatted pomegranate seeds

Bağımsız değişkenler (*Independent variables*)		Bağımlı değişken *(Dependent variable)*
Özütleme süresi (dk) *(Extraction time) (min)*	Genlik (%) *(Amplitude) (%)*	Toplam fenolik madde miktarı (mg GAE g^{-1}) *(Total phenolic content) (mg GAE g^{-1})*
33	60	1.39
33	60	1.46
33	20	2.05
52	88	2.87
33	100	3.45
33	60	1.34
33	60	1.31
13	32	0.50
13	88	1.78
60	60	2.38
5	60	0.11
52	32	2.49

GAE: Gallik asit eşdeğeri *(Gallic acid equivalent)*.

Merkezi tümleşik tasarım kullanılarak yürütülen optimizasyon çalışması ile ilgili model istatistik sonuçları Çizelge 2'de sunulmuştur. Her bir deneme noktası için belirlenen TFM sonuçlarına göre optimizasyon işlemi için uygun olan model belirlenmiştir. Model belirleme aşamasında istatistiksel çıktılar (R^2: belirleme katsayısı, R^2_{Adj}: düzeltilmiş belirleme katsayısı, P değeri ve uyum eksikliği) dikkate alınmıştır. Bu parametreler arasında R2 ve R2Adj değerlerinin maksimum, P değerinin önem derecesinin yüksek ve uygum eksikliğinin 0.05'ten daha büyük olması uygun modelin seçiminde yol gösterici olmuştur. Çizelge incelendiğinde lineer ve 2FI (etkileşimli) modellerde arzu edilenin aksine R^2 ve R^2_{Adj} değerlerinin, P değerinin önem derecesinin düşük olduğu ve ayrıca uyum eksikliğinin de belirtilen sınırlar içerisinde olmadığı görülmektedir. Kübik modellerin yorumlanmasına gelince program üzerinde yapılan değerlendirmeler sonucunda ilgili modelin uygulanması ile birlikte veri kayıplarının olabileceği ve bu veri kayıpları neticesinde oluşturulan grafiklerin uygun olmayacağı tespit edilmiştir. Belirtilen yorumlar ve model istatistik sonuçları göz önünde bulundurulduğunda çalışmada bağımlı değişken olarak seçilen TFM'yi açıklamak için en uygun modelin kuadratik model olduğu belirlenmiştir.

Çizelge 2. Yağı alınmış nar çekirdeklerinden fenolik antioksidanların ultrases destekli özütleme işlemi ile ilgili model istatistik sonuçları

Table 2. Model summary statistics regarding the ultrasound-assisted extraction of phenolic antioxidants from defatted pomegranate seeds

Bağımlı değişken *(Response)*	Modeller *(Source)*	Standart sapma *(Std. Dev.)*	R^2 *(R^2)*	R^2_{Adj} *(R^2_{Adj})*	P-değeri *(P-value)*	Uyum eksikliği *(Lack of fit)*
Toplam fenolik madde miktarı *(Total phenolic content)*	Lineer (*Linear*)	0.62	0.6582	0.5822	0.0080**	0.0010
	2FI *(2FI)*	0.63	0.6776	0.5567	0.0229*	0.0009
	Kuadratik *(Quadratic)*	0.085	0.9956	0.9920	<0.0001***	0.2481
	Kübik *(Cubic)*	0.080	0.9974	0.9929	<0.0001***	0.1829

2FI: Etkileşimli *(Interactive)*. İstatistiksel anlamlılık dereceleri *(Statistical significance degrees)*: *P<0.05; **P<0.01; ***P<0.001.

Mevcut çalışma kapsamında bağımsız değişken olarak seçilen özütleme süresi ve genliğin TFM üzerine olan muhtemel etkilerini istatistiksel boyutta ortaya koymak için varyans analizi (ANOVA) sonuçları

incelenmiş ve ilgili sonuçlar Çizelge 3'te sunulmuştur. Her iki bağımsız değişkeninde TFM üzerine istatistiksel bağlamda önemli bir etkiye sahip olduğu belirlenmiştir (P<0.001). Benzer şekilde işlem parametrelerinin etkileşimleri (süre x genlik) de TFM üzerine P<0.01 düzeyinde etki ettiği tespit edilmiştir.

Çizelge 3. Yağı alınmış nar çekirdeklerinden fenolik antioksidanların ultrases destekli özütleme işlemi ile ilgili model katsayıları ve varyans analizi (ANOVA) sonuçları

Table 3. Model coefficients regarding the ultrasound-assisted extraction of phenolic antioxidants from defatted pomegranate seeds

Model katsayıları/değer *(Coefficient/value)*	Toplam fenolik madde miktarı (mg GAE g^{-1}) *(Total phenolic content) (mg GAE g^{-1})*
β_0	1.37***
Lineer	
β_1(Süre) *(Time)*	0.79***
β_2(Genlik) *(Amplitude)*	0.45***
Etkileşimli	
β_{12}(Süre × Genlik) *(Time × Amplitude)*	-0.22**
İkinci derece	
β_{11}(Süre × Süre) *(Time × Time)*	-0.087*
β_{22}(Genlik × Genlik) *(Amplitude × Amplitude)*	0.67***

GAE: Gallik asit eşdeğeri *(Gallic acid equivalent)*. İstatistiksel anlamlılık dereceleri *(Statistical significance degrees)*: *P<0.05; **P<0.01; ***P<0.001.

Model denklem *(Model equation)*: Total fenolik madde miktarı *(Total phenolic content)* = ß0 + ß1(Süre) *(Time)* + ß2(Genlik) *(Amplitude)* + ß12(Süre × Genlik) *(Time × Amplitude)* + ß11(Süre × Süre) *(Time × Time)* + ß22(Genlik × Genlik) (*Amplitude × Amplitude*).

Antioksidan Kapasite Toplam Fenolik Madde, Toplam Flavonoid Madde ve Toplam Hidrolize Tannen Madde Miktarı

Optimum koşullarda ve klasik yöntemle elde edilen yağı alınmış nar çekirdeklerinin TFM, TFMM ve THTM ile ilgili sonuçlar Çizelge 4'te verilmiştir. Optimum koşullarda elde edilen özütlerin (2.94 mg GAE g^{-1}) fenolik maddelerce metanolik özütlere (2.60 mg GAE g^{-1}) kıyasla daha zengin olduğu bulunmuştur (P<0.05). Benzer eğilim TFMM ve THTM sonuçlarında da tespit edilmiştir. Optimum koşullarda elde edilen özütler için TFMM ve THTM sırasıyla 0.36 mg KE g^{-1} ve 22.07 mg TAE g^{-1} iken; bu değerler metanolik özütlerde 0.27 mg KE g^{-1} ve 16.73 mg TAE g^{-1} olarak belirlenmiştir.

Fitokimyasalların özütlenmesinde solvent olarak su ile karşılaştırıldığında metanol kullanımının daha makul olduğu önceki çalışmalarda rapor edilmiştir (Elfalleh ve ark., 2019). Mevcut çalışmada literatür verilerinin aksine biyoaktif maddelerin ultrases sisteminde solvent olarak tercih edilen sulu faza difüzyon hızının daha fazla olduğu tespit edilmiştir. Bu durum ultrases destekli özütlemenin ne kadar etkili bir sistem olduğunu ortaya koymaktadır. Bir başka ifade ile toksik solventler prosese dahil edilmeden inovatif yöntemler kullanılarak sadece su ile biyoaktif yapıların efektif bir şekilde özütlenmesinin mümkün olabileceği görülmüştür. Bilimsel literatürde ultrases sisteminin kullanıldığı çalışmalarda benzer yorumlar yapılmıştır (Gisbert ve ark., 2021).

Çizelge 4. Yağı alınmış nar çekirdeklerinin toplam fenolik madde, toplam flavonoid madde ve toplam hidrolize tannen madde miktarı

Table 4. Total phenolic, total flavonoid and total hydrolysable tannin contents of defatted pomegranate seeds

Örnekler *(Samples)*	Toplam fenolik madde miktarı (mg GAE g^{-1}) *(Total phenolic content) (mg GAE g^{-1})*	Toplam flavanoid madde miktarı (mg KE g^{-1}) *(Total flavonoid content) (mg CE g^{-1})*	Toplam hidrolize tanen madde miktarı (mg TAE g^{-1}) *(Total hydrolysable tannin content) (mg TAE g^{-1})*
Optimum *(Optimum)*	2.94±0.03^{a}	0.36±0.01^{a}	22.07±0.86^{a}
Metanolik *(Methanolic)*	2.60±0.07^{b}	0.27±0.03^{a}	16.73±0.13^{b}

Aynı sütundaki farklı küçük harfler (a-b) istatistiksel farklılıkları gösterir (P<0.05) *(Different lowercase letters (a-b) in the same column indicate statistical differences)*. GAE: Gallik asit eşdeğeri *(Gallic acid equivalent)*, KE: Kateşin eşdeğeri *(Catechin equivalent)*, TAE: Tannik asit eşdeğeri *(Tannic acid equivalent)*.

Fenolik Fraksiyonları

Optimum koşullarda Materyallerin içermiş oldukları toplam biyoaktif madde içeriği kadar bu biyoaktif maddelerin fraksiyonlarının da bilinmesi üzerinde durulması gereken noktalardandır. Çünkü biyoaktif maddelerin fraksiyonları ilgili materyalin biyolojik

aktivitesi ile doğrudan ilişkilidir. Mevcut çalışmanın bu bölümünde LC-ESI-MS/MS ile optimum koşullarda ve metanol kullanılarak geleneksel yöntemle elde edilen özütlerin fenolik fraksiyonları (flavanoid ve fenolik asitler) araştırılmış ve sonuçlar Çizelge 5'te sunulmuştur. Her iki özütte de toplam 9 farklı fenolik fraksiyon belirlenmiştir. Yağsız nar çekirdeklerinde tespit edilen fenoliklerin 4 tanesi flavonoid (kuersetin, mirisetin, resveratrol ve luteolin) iken; 5 adedi fenolik asitler (gallik asit, asetohidroksamik asit, ellajik asit, salisilik asit ve p-hidroksibenzoik asit) grubunda yer almaktadır. Fenolik fraksiyonlar arasında flavanoidlere kıyasla fenolik asitlerin dominant olduğu tespit edilmiştir. Fenolik asitlerden, ultrases destekli sistem ve metanol kullanılarak elde edilen özütlerde gallik asitin (optimum: 35.49 µg g^{-1}; metanolik: 25.28 µg g^{-1}) ve ellajik asitin (optimum: 77.51 µg g^{-1}; metanolik: 72.30 µg g^{-1}) baskın olduğu belirlenmiştir. Nar çekirdeklerinin gallik asit ve ellajik asit açısından zengin olduğu önceki çalışmalarda da not edilmiştir (Mesías ve ark., 2013). Bu yapıların nar çekirdeğinde baskın olması ilgili materyal için önemli avantajları da beraberinden getirmektedir. Çünkü gallik asitin ve ellajik asitin sağlık üzerine olan faydalı etkileri *in vitro* ve *in vivo* çalışmalarda ortaya konmuştur (Andrade ve ark., 2021; Chen ve ark., 2022). Antioksidan, antiinflamatuar, antihiperlipidemik, antiviral, antimetastatik, antikanser, antidiyabetik aktivite bu bağlamda örnek olarak verilebilir (Aishwarya ve ark., 2021). Gallik asit ve ellajik asite ilaveten, fonksiyonel özellikleri farklı çalışmalara konu olmuş resveratrol da yağı alınmış nar çekirdeklerinden tespit edilmiştir.

Çizelge 5. Yağı alınmış nar çekirdeklerinin fenolik fraksiyonları
Table 5. Phenolic fractions of defatted pomegranate seeds

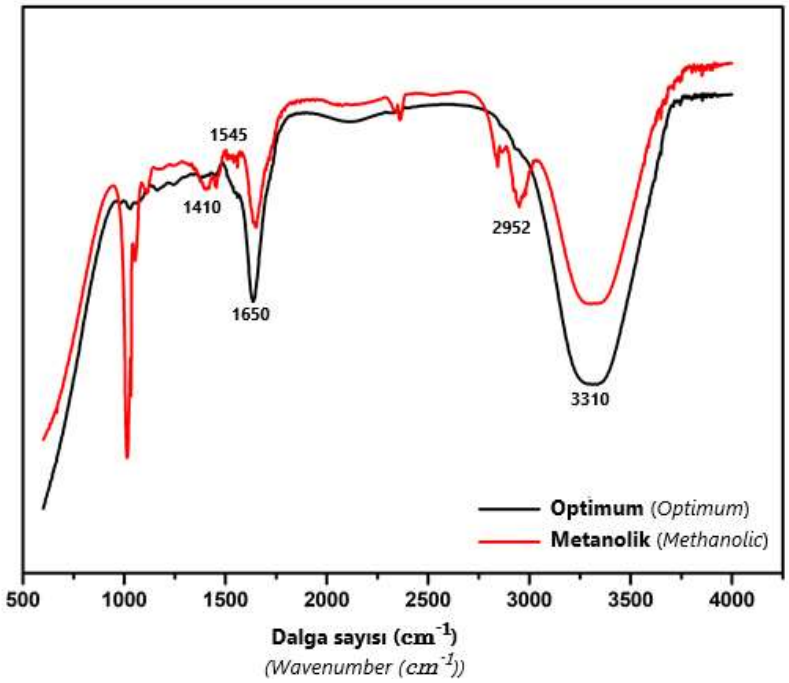
Fenolik bileşik (*Phenolic compound*)	MF (*MF*)	Öncü iyon (*m/z*) (*Precursor ion* (*m/z*))	Ürün iyon (*m/z*) (*Product ion* (*m/z*))	Optimum (µg g^{-1}) (*Optimum* (µg g^{-1}))	Metanolik (µg g^{-1}) (*Methanolic* (*µg g^{-1}*))
Gallik asit (*Gallic acid*)	$C_7H_6O_5$	169.1	124.9	35.79±0.95	25.28±1.01
Kuersetin (*Quercetin*)	$C_{15}H_{10}O_7$	301.1	178.9 150.9	0.13±0.02	0.42±0.04
Asetohidroksamik asit (*Acetohydroxamic acid*)	$C_2H_5NO_2$	76.1	58.0 44.0	5.52±0.46	5.52±0.74
Mirisetin (*Myricetin*)	$C_{15}H_{10}O_8$	317.0	150.9 179.1 136.9	0.13±0.03	0.22±0.02
Resveratrol (*Resveratrol*)	$C_{14}H_{12}O_3$	229.0	135.0 107.2	0.98±0.16	0.97±0.11
Ellajik asit (*Ellagic acid*)	$C_{14}H_6O_8$	301.1	228.9 145.1	77.51±1.25	72.30±1.42
Salisilik asit (*Salicylic acid*)	$C_7H_6O_3$	137.2	93.0 65.0	1.03±0.17	0.62±0.09
Luteolin (*Luteolin*)	$C_{15}H_{10}O_6$	285.0	150.9 133.0	0.19±0.04	10.22±0.32
p-Hidroksibenzoik asit (*p-Hydroxybenzoic acid*)	$C_7H_6O_3$	137.2	93.1 65.0	0.71±0.12	0.38±0.06

MF: Moleküler formül (*Molecular formula*).

FTIR Spektroskopisi

Özütlerdeki spesifik gruplar FTIR spektroskopisi aracılığıyla araştırılmış ve örneklere ait spektrumlar Şekil 1' de verilmiştir. Yaklaşık 3310 cm^{-1}'de gözlemlenen geniş ve yayvan pik özütlerdeki hidroksil (-OH) gruplarının germe titreşimine atfedilebilir. Farklı bitkisel özütler için de benzer pikler ilgili dalga sayısında rapor edilmiş ve doğrudan hidroksil grupları ile ilişkilendirilmiştir (Santiago-Adame ve ark., 2015). Bu dalga sayısında absorpsiyon bantlarının güçlü olması özütteki fenolik bileşiklerin varlığına atfedilmiştir (Wu ve ark., 2022). Her iki özüt içinde yaklaşık 2952 cm^{-1}'de görülen absorpsiyon bantları C-H gerilmesi ile ilgili olup CH_2 veya CH_3 gruplarının özütteki varlığına işarettir. Fenolik asitlerin varlığı ile ilişkilendirilen karboksil grupları 1650 cm^{-1}'de görülmüştür (Kashyap ve ark., 2022). Spektrum incelendiğinde özütlerde bu bantların güçlü olduğu görülmektedir ki bu durum LC-ESI-MS/MS sonuçları ile de desteklenmektedir. Çünkü fenolik bileşikler arasında yağı alınmış nar çekirdeklerinde fenolik asitlerden gallik asitin ve ellajik asitin baskın olduğu mevcut çalışma kapsamında tespit edilmiştir. Ayrıca, fenolik bileşiklerin varlığı ile ilişkilendirilen 3310 ve 1650 cm^{-1}'deki pikler optimum koşullarda elde edilen özütlerde daha güçlü bulunmuştur. LC-ESI-MS/MS sonuçlarına ek olarak TFM analizi de FTIR spektrumlarını desteklemektedir. Özetlerdeki C=O

karbonil ve -COOH grupları sırasıyla 1545 ve 1410 cm^{-1} dalga sayısı civarlarında belirlenmiştir (Hashemi ve ark., 2022; Kashyap ve ark., 2022). Fenolik yapıları temsil eden gruplar FTIR spektrumunda da net bir şekilde tespit edilmiş olup TFM ve LC-ESI-MS/MS sonuçları ile spektrumlar arasında bir korelasyonun olduğu belirlenmiştir.



Şekil 1. Yağ nar çekirdeği özütlerinin FTIR spektrumları
Figure 1. FTIR spectrums of defatted pomegranate seed extracts

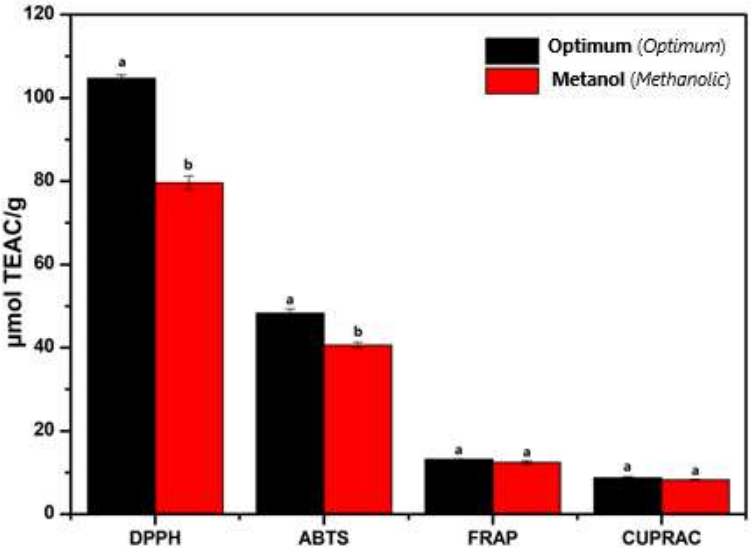
Antioksidan Kapasite

Bitkisel kaynaklı materyaller sahip oldukları biyoaktif yapılar sayesinde çeşitli fonksiyonel özellikler sergileme potansiyeline sahiptir. Mevcut çalışma kapsamında nar çekirdeklerinin içermiş olduğu başta gallik asit ve ellajik asit olmak üzere fenolik bileşikler bu kapsamda en makul örnekler olarak verilebilir. Çalışmanın fenolik fraksiyonları bölümde bu biyoaktif yapıların farklı biyolojik özellikleri vurgulanmıştır.

Ultrases destekli ve klasik yöntemle özütlenen yağı alınmış nar çekirdeklerinin antioksidatif davranışları bu çalışma kapsamında *in vitro* koşullarda belirlenmiştir. Analizler *in vitro* koşullarda gerçekleştirildiğinden ilgili materyalin antioksidan etkilerini tam olarak ortaya koymak adına tek bir metot yerine 4 farklı yöntem (Radikal süpürücü kapasite: DPPH ve ABTS; Demir ve bakır indirgeme gücü: FRAP ve CUPRAC) kullanılmıştır. Tüm analiz yöntemleri için sonuçlar trolox eşdeğeri antioksidan kapasite (TEAC) olarak ifade edilmiş olup analiz sonuçları Şekil 2'de sunulmuştur. Optimum koşullarda elde edilen özütlerin radikal süpürücü gücü metanolik özütlerden daha yüksek olduğu tespit edilmiştir ($P<0.05$). DPPH ve ABTS sonuçları ultrases destekli özütleme yöntemi ile üretilen özütlerde sırasıyla 105.26 µmol TEAC g^{-1} ve 57.65 µmol TEAC g^{-1} olarak belirlenmiştir. Bu değerler metanolik özütler için 80.77 µmol TEAC g^{-1} ve 41.10 µmol TEAC g^{-1} bulunmuştur. FRAP (optimum: 13.03 µmol TEAC g^{-1}; metanolik: 12.69 µmol TEAC g^{-1}) ve CUPRAC (optimum: 8.91 µmol TEAC g^{-1}; metanolik: 8.15 µmol TEAC g^{-1}) sonuçlarında ise örnekler arasında istatistiksel fark olmadığı tespit edilmiştir. Optimum koşullarda elde edilen özütlerde tespit edilen üstün antioksidan kapasite özütteki daha yoğun fenolik madde oranı ile açıklanabilir. Benzer yorumlara daha önce yürütülmüş çalışmalarda da yer verilmiştir (Ma ve ark., 2021; Karaçelik ve ark., 2022).

Mevcut çalışma ile benzer yaklaşımın sergilendiği bir çalışmada Çin'de kendisine yetişme alanı bulan 4 farklı nar çeşidine ait çekirdeklerde yağlar alınmış ve yağı alınmış materyallerde antioksidatif davranışlar araştırılmıştır. Yazarlar, yağı alınmış nar çekirdekleri için ABTS sonuçlarının 7.40 µmol TEAC g^{-1} ile 17.80 µmol TEAC g^{-1} arasında değerler aldığını bulmuştur (Jing ve ark., 2012). Başka bir çalışmada da nar çekirdekleri için antioksidan kapasite mevcut çalışmada elde edilen sonuçlardan daha düşük olduğu rapor edilmiştir (Peng, 2019). Nar çekirdeklerinden özütlenen serbest, esterifiye ve bağlı fenolik yapıların antioksidatif özelliklerinin araştırıldığı bir çalışmada ABTS sonuçları sırasıyla 18.30, 11.30 ve 11.0 µmol TEAC g^{-1} olarak bulunurken DPPH sonuçlarının 360, 430 ve 200 µmol TEAC g^{-1} olduğu not edilmiştir (Ambigaipalan ve ark., 2017). Literatür çalışmalarının birbiri arasında ve mevcut çalışma ile olan farklılıkları

kullanılan hammaddenin çeşidine (fiziksel ve kimyasal yapısı vb.) ve özütleme işleminde farklı yöntemlerin/solventlerin kullanılmasına bağlanabilir.



Şekil 2. Yağı alınmış nar çekirdeklerinin antioksidan kapasitesi
Figure 2. Antioxidant capacity of defatted pomegranate seeds
Her bir antioksidan kapasite analizi için farklı küçük harfler (a-b) istatistiksel farklılıkları gösterir (P<0.05) (*Different lowercase letters (a-b) for each antioxidant capacity analysis indicate statistical differences*). TEAC: Trolox eşdeğeri antioksidan aktivite (*Trolox equivalent antioxidant capacity*).

SONUÇ ve ÖNERİLER

Son yıllarda atık materyallerin değerlendirilip katma değerli ürünlere dönüştürülmesini konu alan farklı çalışmalar mevcuttur; ancak yapılan çalışmalarda atık materyaller değerlendirildikten sonra geriye kalan kısımları (atığın atığı) herhangi bir prosese dahil edilmeden bertaraf edilmektedir. Bu çalışma kapsamında atığın atığı olan materyallerin de hâlâ içerisinde önemli yapıları barındırdığı tespit edilmiştir. Ayrıca bilimsel literatürde ve endüstride, toksik özelliğe sahip solventlerin proseslerde kullanımını sınırlandırmak için göz ardı edilemez çabaların olduğu bilinmektedir. Bu bağlamda çalışma kapsamında inovatif tekniklerle bu problemlerin minimize edilebileceği sonucuna varılmıştır. Belirtilen bu noktalardan ötürü elde edilen sonuçlar ve çalışmaya olan yaklaşım ileride yürütülecek farklı çalışmalara yön verecek niteliktedir. Bu tarz materyallerle ilgili çalışmaları arttırmak ve bir pazar oluşturmak için elde edilen özütler farklı ürün gruplarında girdi olarak kullanılabilir.

Araştırmacıların Katkı Oranı Beyan Özeti

Makale tek yazarlı olup çalışmaya ait verilerin toplanmasından yazım aşamasına kadar tüm süreçler yazar tarafından organize edilmiştir.

Çıkar Çatışması Beyanı

Yazar bu çalışmada herhangi bir çıkar çatışması olmadığını beyan eder.

KAYNAKLAR

Abid, M., Yaich, H., Hidouri, H., Attia, H., & Ayadi, M. A. (2018). Effect of substituted gelling agents from pomegranate peel on colour, textural and sensory properties of pomegranate jam. *Food Chemistry*, *239*, 1047–1054. https://doi.org/10.1016/j.foodchem.2017.07.006

Aishwarya, V., Solaipriya, S., & Sivaramakrishnan, V. (2021). Role of ellagic acid for the prevention and treatment of liver diseases. *Phytotherapy Research*, *35*(6), 2925–2944. https://doi.org/10.1002/ptr.7001

Alasalvar, H., & Yildirim, Z. (2021). Ultrasound-assisted extraction of antioxidant phenolic compounds from Lavandula angustifolia flowers using natural deep eutectic solvents: An experimental design approach. *Sustainable Chemistry and Pharmacy*, *22*, 100492. https://doi.org/10.1016/j.scp.2021.100492

Ambigaipalan, P., de Camargo, A. C., & Shahidi, F. (2017). Identification of phenolic antioxidants and bioactives of pomegranate seeds following juice extraction using HPLC-DAD-ESI-MSn. *Food Chemistry*, *221*, 1883–1894. https://doi.org/10.1016/j.foodchem.2016.10.058

Andrade, S., Loureiro, J. A., & Pereira, M. do C. (2021). Influence of in vitro neuronal membranes on the anti-amyloidogenic activity of gallic acid: Implication for the therapy of Alzheimer's disease. *Archives of Biochemistry and Biophysics*, *711*, 109022. https://doi.org/10.1016/j.abb.2021.109022

Apak, R., Güçlü, K., Özyürek, M., & Çelik, S. E. (2008). Mechanism of antioxidant capacity assays and the CUPRAC (cupric ion reducing antioxidant capacity) assay. *Microchimica Acta*, *160*(4), 413–419. https://doi.org/10.1007/s00604-007-0777-0

Ben Yakoub, A. R., Abdehedi, O., Jridi, M., Elfalleh, W., Nasri, M., & Ferchichi, A. (2018). Flavonoids, phenols, antioxidant, and antimicrobial activities in various extracts from *Tossa jute* leave (*Corchorus olitorus* L.). *Industrial Crops and Products*, *118*, 206–213. https://doi.org/10.1016/j.indcrop.2018.03.047

Benzie, I. F. F., & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. *Analytical Biochemistry*, *239*(1), 70–76. https://doi.org/10.1006/abio.1996.0292

Bou Dargham, M., Matar Boumosleh, J., Farhat, A., Abdelkhalek, S., Bou-Maroun, E., & el Hosry, L. (2022). Antioxidant and anti-diabetic activities in commercial and homemade pomegranate molasses in Lebanon. *Food Bioscience*, *46*, 101540. https://doi.org/10.1016/j.fbio.2021.101540

Brito, T. B. N., Ferreira, M. S. L., & Fai, A. E. C. (2022). Utilization of agricultural by-products: Bioactive properties and technological applications. *Food Reviews International*, *38*(6), 1305–1329. https://doi.org/10.1080/87559129.2020.1804930

Çam, M., Hışıl, Y., & Durmaz, G. (2009). Classification of eight pomegranate juices based on antioxidant capacity measured by four methods. *Food Chemistry*, *112*(3), 721–726. https://doi.org/10.1016/j.foodchem.2008.06.009

Çam, M., İçyer, N. C., & Erdoğan, F. (2014). Pomegranate peel phenolics: Microencapsulation, storage stability and potential ingredient for functional food development. *LWT-Food Science and Technology*, *55*(1), 117–123. https://doi.org/10.1016/j.lwt.2013.09.011

Chen, D., Bai, R., Yong, H., Zong, S., Jin, C., & Liu, J. (2022). Improving the digestive stability and prebiotic effect of carboxymethyl chitosan by grafting with gallic acid: *In vitro* gastrointestinal digestion and colonic fermentation evaluation. *International Journal of Biological Macromolecules*, *214*, 685–696. https://doi.org/10.1016/j.ijbiomac.2022.06.170

Corbin, C., Fidel, T., Leclerc, E. A., Barakzoy, E., Sagot, N., Falguiéres, A., ... & Hano, C. (2015). Development and validation of an efficient ultrasound assisted extraction of phenolic compounds from flax (*Linum usitatissimum* L.) seeds. *Ultrasonics Sonochemistry*, *26*, 176-185. https://doi.org/10.1016/j.ultsonch.2015.02.008

Da Porto, C., Porretto, E., & Decorti, D. (2013). Comparison of ultrasound-assisted extraction with conventional extraction methods of oil and polyphenols from grape (*Vitis vinifera* L.) seeds. *Ultrasonics Sonochemistry*, *20*(4), 1076-1080. https://doi.org/10.1016/j.ultsonch.2012.12.002

Dairi, S., Dahmoune, F., Belbahi, A., Remini, H., Kadri, N., Aoun, O., Bouaoudia, N., & Madani, K. (2021). Optimization of microwave extraction method of phenolic compounds from red onion using response surface methodology and inhibition of lipoprotein low-density oxidation. *Journal of Applied Research on Medicinal and Aromatic Plants*, *22*, 100301. https://doi.org/10.1016/j.jarmap.2021.100301

Elfalleh, W., Kirkan, B., & Sarikurkcu, C. (2019). Antioxidant potential and phenolic composition of extracts from *Stachys tmolea*: An endemic plant from Turkey. *Industrial Crops and Products*, *127*, 212–216. https://doi.org/10.1016/j.indcrop.2018.10.078

Gisbert, M., Barcala, M., Rosell, C. M., Sineiro, J., & Moreira, R. (2021). Aqueous extracts characteristics obtained by ultrasound-assisted extraction from *Ascophyllum nodosum* seaweeds: Effect of operation conditions. *Journal of Applied Phycology*, *33*(5), 3297–3308. https://doi.org/10.1007/s10811-021-02546-5

Hashemi, Z., Shirzadi-Ahodashti, M., Mortazavi-Derazkola, S., & Ebrahimzadeh, M. A. (2022). Sustainable biosynthesis of metallic silver nanoparticles using barberry phenolic extract: Optimization and evaluation of photocatalytic, *in vitro* cytotoxicity, and antibacterial activities against multidrug-resistant bacteria. *Inorganic Chemistry Communications*, *139*, 109320. https://doi.org/10.1016/j.inoche.2022.109320

Ismail, B. B., Guo, M., Pu, Y., Wang, W., Ye, X., & Liu, D. (2019). Valorisation of baobab (*Adansonia digitata*) seeds by ultrasound assisted extraction of polyphenolics. Optimisation and comparison with conventional methods. *Ultrasonics Sonochemistry*, *52*, 257-267. https://doi.org/10.1016/j.ultsonch.2018.11.023

Jing, P., Ye, T., Shi, H., Sheng, Y., Slavin, M., Gao, B., Liu, L., & Yu, L. (Lucy). (2012). Antioxidant properties and phytochemical composition of China-grown pomegranate seeds. *Food Chemistry*, *132*(3), 1457–1464. https://doi.org/10.1016/j.foodchem.2011.12.002

Kaderides, K., Kyriakoudi, A., Mourtzinos, I., & Goula, A. M. (2021). Potential of pomegranate peel extract as a natural additive in foods. *Trends in Food Science & Technology*, *115*, 380–390. https://doi.org/10.1016/j.tifs.2021.06.050

Karaçelik, A. A., Şeker, M. E., & Karaköse, M. (2022). Determination of antioxidant activity of different extracts from bark of *Pinus* spp. grown in Giresun (Turkey) province–phenolic analysis by RP-HPLC-DAD. *Kahramanmaraş Sütçü İmam University Journal of Agriculture and Nature*, *25*(1), 10-18. https://doi.org/10.18016/ksutarimdoga.vi.875313

Kashyap, P., Riar, C. S., & Jindal, N. (2022). Effect of extraction methods and simulated *in vitro* gastrointestinal digestion on phenolic compound profile, bio-accessibility, and antioxidant activity of *Meghalayan* cherry (*Prunus nepalensis*) pomace extracts. *LWT-Food Science and Technology*, *153*, 112570. https://doi.org/10.1016/j.lwt.2021.112570

Ma, Y.-L., Sun, P., Feng, J., Yuan, J., Wang, Y., Shang, Y.-F., Niu, X.-L., Yang, S.-H., & Wei, Z.-J. (2021). Solvent effect on phenolics and antioxidant activity of Huangshan Gongju (*Dendranthema morifolium (Ramat) Tzvel. cv. Gongju*) extract. *Food and Chemical Toxicology*, *147*, 111875. https://doi.org/10.1016/j.fct.2020.111875

Méndez, D. A., Fabra, M. J., Odriozola-Serrano, I., Martín-Belloso, O., Salvia-Trujillo, L., López-Rubio, A., & Martínez-Abad, A. (2022). Influence of the extraction conditions on the carbohydrate and phenolic composition of functional pectin from persimmon waste streams. *Food Hydrocolloids*, *123*, 107066. https://doi.org/10.1016/j.foodhyd.2021.107066

Mesías, M., Navarro, M., Gökmen, V., & Morales, F. J. (2013). Antiglycative effect of fruit and vegetable seed extracts: Inhibition of AGE formation and carbonyl-trapping abilities. *Journal of the Science of Food and Agriculture*, *93*(8), 2037–2044. https://doi.org/10.1002/jsfa.6012

Mrkonjić, Ž., Rakić, D., Olgun, E. O., Canli, O., Kaplan, M., Teslić, N., Zeković, Z., & Pavlić, B. (2021). Optimization of antioxidants recovery from wild thyme (*Thymus serpyllum* L.) by ultrasound-assisted extraction: Multi-response approach. *Journal of Applied Research on Medicinal and Aromatic Plants*, *24*, 100333. https://doi.org/10.1016/j.jarmap.2021.100333

Naji, A. M., Başyiğit, B., Alaşalvar, H., Salum, P., Berktaş, S., Erbay, Z., & Çam, M. (2022). Instant soluble roselle (*Hibiscus sabdariffa* L.) powder rich in bioactive compounds: Effect of the production process on volatile compounds. *Journal of Food Measurement and Characterization*. https://doi.org/10.1007/s11694-022-01593-x

Pandey, A., Belwal, T., Sekar, K. C., Bhatt, I. D., & Rawal, R. S. (2018). Optimization of ultrasesic-assisted extraction (UAE) of phenolics and antioxidant compounds from rhizomes of *Rheum moorcroftianum* using response surface methodology (RSM). *Industrial Crops and Products*, *119*, 218–225. https://doi.org/10.1016/j.indcrop.2018.04.019

Paul, A., & Radhakrishnan, M. (2020). Pomegranate seed oil in food industry: Extraction, characterization, and applications. *Trends in Food Science & Technology*, 105, 273–283. https://doi.org/10.1016/j.tifs.2020.09.014

Peng, Y. (2019). Comparative analysis of the biological components of pomegranate seed from different cultivars. *International Journal of Food Properties*, *22*(1), 784–794. https://doi.org/10.1080/10942912.2019.1609028

Pinto, D., Vieira, E. F., Peixoto, A. F., Freire, C., Freitas, V., Costa, P., Delerue-Matos, C., & Rodrigues, F. (2021). Optimizing the extraction of phenolic antioxidants from chestnut shells by subcritical water extraction using response surface methodology. *Food Chemistry*, *334*, 127521. https://doi.org/10.1016/j.foodchem.2020.127521

Qu, W., Pan, Z., Zhang, R., Ma, H., Zhu, B., Wang, Z., & Atungulu, G. G. (2009). Integrated extraction and anaerobic digestion process for recovery of nutraceuticals and biogas from pomegranate marc. *Transactions of the ASABE*, *52*(6), 1997-2006. https://doi.org/10.13031/2013.29196

Sagar, N. A., Pareek, S., Sharma, S., Yahia, E. M., & Lobo, M. G. (2018). Fruit and vegetable waste: Bioactive compounds, their extraction, and possible utilization. *Comprehensive Reviews in Food Science and Food Safety*, *17*(3), 512–531. https://doi.org/10.1111/1541-4337.12330

Santiago-Adame, R., Medina-Torres, L., Gallegos-Infante, J. A., Calderas, F., González-Laredo, R. F., Rocha-Guzmán, N. E., Ochoa-Martínez, L. A., & Bernad-Bernad, M. J. (2015). Spray drying-microencapsulation of cinnamon infusions (*Cinnamomum zeylanicum*) with maltodextrin. *LWT-Food Science and Technology*, *64*(2), 571–577. https://doi.org/10.1016/j.lwt.2015.06.020

Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture*, *16*, 144–158.

Sinha, S., & Tripathi, P. (2021). Trends and challenges in valorisation of food waste in developing economies: A case study of India. *Case Studies in Chemical and Environmental Engineering*, *4*, 100162. https://doi.org/10.1016/j.cscee.2021.100162

Tülek, Z., Alaşalvar, H., Başyiğit, B., Berktas, S., Salum, P., Erbay, Z., Telci, I., & Çam, M. (2021). Extraction optimization and microencapsulation of phenolic antioxidant compounds from lemon balm (*Melissa officinalis* L.): Instant soluble tea production. *Journal of Food Processing and Preservation*, *45*(1). https://doi.org/10.1111/jfpp.14995

Willis, R. B. (1998). Improved method for measuring hydrolyzable tannins using potassium iodate. *The Analyst*, *123*(3), 435–439. https://doi.org/10.1039/a706862j

Wu, J., Wang, X., He, Y., Li, J., Ma, K., Zhang, Y., Li, H., Yin, C., & Zhang, Y. (2022). Stability evaluation of gardenia yellow pigment in presence of different phenolic compounds. *Food Chemistry*, *373*, 131441. https://doi.org/10.1016/j.foodchem.2021.131441

Zhang, J., & Lee, T. G. (2021). Optimization of phenolics and flavonoids extraction from the fruit of

Empetrum nigrum var. japonicum from Jeju Island in South Korea. *Journal of Industrial and Engineering Chemistry*, *98*, 350–357. https://doi.org/10.1016/j.jiec.2021.03.031

Zhishen, J., Mengcheng, T., & Jianming, W. (1999). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chemistry*, *64*(4), 555–559. https://doi.org/10.1016/S0308-8146(98)00102-2

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Effect of Hazelnut Pulp Addition on Physical and Chemical Properties of Tarhana

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ABSTRACT
In this study, hazelnut pulp was added to tarhana to increase its nutritional value. For this purpose, hazelnut oil was partially extracted by cold pressing method and hazelnut pulp was added to the tarhana formulation at varying concentrations (5, 10, 15, 20, 25 and 30%). According to the research data, as the hazelnut pulp ratio increased, pH and acidity values, redness (a*) and yellowness (b*) color values of tarhana increased, while the brightness (L*) value decreased. It was determined that the addition of hazelnut pulp increased the foaming capacity and foam stability of tarhana and decreased the viscosity values. Depending on the increase in hazelnut pulp, the protein, fat and ash ratios, total phenolic substance and antioxidant activity of tarhana also increased.

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Fındık Posasının Tarhananın Fiziksel ve Kimyasal Özelliklerine Etkisi

ÖZET
Bu çalışmada, besin değerini artırmak amacıyla tarhanaya fındık posası ilave edilmiştir. Bu amaçla fındık yağı soğuk pres yöntemi ile kısmen alınmış ve fındık posası belirli oranlarda (%5, 10, 15, 20, 25 ve %30) tarhana formülasyonuna katılmıştır. Araştırma verilerine göre fındık posası oranı arttıkça tarhananın pH ve asitlik değerleri ile kırmızılık (a*) ve sarılık (b*) renk değerleri yükselmiş, parlaklık (L*) değeri ise düşmüştür. Fındık posası ilavesinin tarhananın köpürme kapasitesini ve köpük stabilitesini arttırdığı, viskozite değerlerini düşürdüğü tespit edilmiştir. Fındık posası artışına bağlı olarak tarhananın protein, yağ ve kül oranları ile toplam fenolik madde ve antioksidan aktivitesinde de artış meydana gelmiştir.

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INTRODUCTION
Tarhana is a traditional fermented product made by combining wheat flour, yogurt, yeast, and various vegetables such as paprika, tomatoes, mint, onion, and salt, followed by fermentation, drying, and grinding. Tarhana is known and consumed under different names in many countries of the world; "Kishk" in Syria, Lebanon, and Egypt, "kushuk" in Iraq, "tahonya, talkuna" in Hungary and Netherlands, "tarhana" in Greece and "atole" in Scotland. The word tarhana was first included in the words of Kıpcak Turks in the form of tarhana in Turkish words (Çakıroğlu, 2008) but also claimed to be originated from Hittite civilization (Güveloğlu, 2019). Until recently, tarhana was primarily produced in rural areas of Türkiye. With the migration of people from rural to urban areas, and the increased participation of women in the labor force, the demand for ready-to-eat foods has increased, and traditional tarhana has taken its place among other instant soups.

The composition of tarhana changes with different formulations and varies depending on its raw material, the materials used in its production, and the variability of the production methods. Tarhana, which is usually dried and powdered, is preserved wet in some regions without drying, and it is dried as chips and consumed as a snack in others. In a study conducted by some researchers, the effects of different drying techniques such as tunnel type drying, freeze drying, microwave drying, spray drying, and hot air drying were investigated (Şengün, 2006). Koca et al. (2002) evaluated tarhana samples consisting of cow milk yogurt, soymilk yogurt, and cow and soymilk yogurt mixture in terms of pH, viscosity, color, and

sensory properties. They found that the samples with added soymilk had a higher viscosity than the samples with added cow milk yogurt. Bilgiçli (2004) examined the effect of yeast *(Saccharomyces cerevisiae)*, malt flour, and phytase enzyme additive on some nutritional parameters of tarhana. The mineral content, phytic acid amount, and protein bioavailability of tarhana are found to be very high, and the natural fermentation process is sufficient in this regard. In the study conducted by Gokmen (2009), the effects of adding quince to tarhana were investigated and it was found that the use of raw quince in making tarhana is more accepted in terms of acceptability scores. Additionally, tarhana is a product that is fairly rich in terms of mineral and protein content. Tarakçı et al. (2013) investigated the effect of blackberry substitution on some functional and physicochemical properties of tarhana and concluded that there was a decrease in acidity, dry matter, water holding capacity, foaming capacity and foam stability in tarhana with the addition of blackberry. They determined that the viscosity decreased with the temperature increase for all samples. Hazelnut pulp is rich in protein, ash, and fat contents (Yağcı & Göğüş, 2008) hence it is expected that inclusion of hazelnut pulp in tarhana will enhance the nutritional and functional properties of tarhana. The goal of this study was to produce a new type of tarhana by incorporating cold-pressed hazelnut pulp and adding tarhana with nutritional, functional, aroma, and structural properties.

MATERIALS and METHODS

For the production of tarhana samples, wheat flour, yogurt, tomato paste, fresh yeast, mint, red pepper, tomato, salt and hazelnut samples were purchased from the market in Ordu city.

Production of Tarhana samples

Natural hazelnuts were first broken down and divided into smaller pieces in a food processor. In the cold pressed oil extraction machine, the hazelnut oil rate is reduced. In the study, 0%, 5%, 10%, 15%, 20%, 25%, and 30% Hazelnut pulps were added to tarhana samples. The formulation specified in Table 1 refers to the control group (0%). Tarhana varieties were produced in 3 replicates.

Tarhana samples used in the production of tarhana are shown in Table 1. While the hazelnut ratio increased in the product formulations, the ratio of the other inputs was kept constant. Before chopping the onions in the food processor, tomato paste, dried mint, red pepper, and salt were added, and a mixture was obtained. After the mixture was pre-baked, water was added and then cooked for a while. When the temperature of the obtained mixture decreased to 20°C, flour, yogurt, yeast, and hazelnuts were added. To ensure a homogeneous dough structure, the mixture was kneaded for 10 minutes. The prepared tarhana doughs were allowed to ferment for 30 hours at 30°C. Fermented tarhana doughs were cut into Hazelnut-sized pieces and placed on the drying tray. The fermented tarhana doughs were dried in a convection oven (Nucleon, NST-120, Ankara) at 52°C until the moisture content was 12%, ground, and pulverized.

Table 1. Standard Tarhana formulation
Çizelge 1. Standart Tarhana formülasyonu

Material	(%)	Amount (g)
Wheat Flour	50	500
Yogurt	25	250
Onion	12	120
Tomato paste	6	60
Salt	4	40
Fresh yeast	1	10
Red pepper	1	10
Dry mint	1	10

Tarhana analyses

Determination of dry matter; the drying cups were kept at 105oC for 1 hour, tared and 5 g of sample were weighed into the drying cups and then left to dry in the oven (Nucleon, NST-120, Ankara) at 105ºC until they reached a constant weight. Results are expressed in %.
Ash analyses; James's (1995) method was modified and porcelain crucibles 3 g of sample was weighed at 550±5°C temperature until the formation of white color.

Color analyses; Color analysis of tarhana samples was performed with color measurement device (Minolta, CR-400, and Osaka, Japan).

Total fat analysis; Soxhelet extraction method was used to determine the fat content of the samples (James, 1995). The tarhana samples were boiled by distillation in the extraction apparatus (Velp Scientifica, SER 148, and Usmate, Italy).

The pH analysis; a 5 g tarhana dry sample was weighed into a beaker and the pH was measured with a digital pH meter to determine the pH (Mettler Toledo, Sevencompact S210) at 25°C (Ibanoğlu et al., 1995).

Titratable acidity analysis; titratable acidity was determined by adding 1% phenolphthalein to tarhana samples and titration with 0.1 N NaOH, and the results were determined in terms of lactic acid per 100 g sample (Ibanoğlu et al., 1999).

Protein analysis; The Kjeldahl method was used to determine the protein content (James, 1995).

Viscosity Analysis; 10 g of dry tarhana sample was weighed into a glass beaker and 150 ml of distilled water was added. The solution was cooked by stirring for 10 minutes, thereby gelatinizing the starch. The

samples were poured into the sample cup of the viscometer (AND, SV-10, Tokyo, Japan) while hot (Tarakçı et al. 2013).

Water absorption capacity and oil absorption capacity; tarhana (5.0 g) was thoroughly mixed with distilled water (25 mL) or sunflower oil in 50-mL centrifuge tubes. Dispersions were stirred at 15 min intervals over a 60 min period and then centrifuged at 4000 × *G* for 20 min. Water and oil absorption capacity values were expressed as grams of water or oil absorbed per gram of tarhana (Hayta et al. 2002).

Foaming capacity and foam stability; tarhana (10 g) was dispersed in distilled water and stirred for 20 min. The mixture was centrifuged at 4000 × *G* for 20 min. The obtained supernatant was filtered (Whatman no. 1), where it was stirred for 2 minutes on high speed. The solution was slowly poured into a cylinder, and the volume of the foam was recorded after 10 s. Foaming capacity was expressed as the volume (mL) of gas incorporated per mL of solution. Foam stability was recorded as the time passed until half of the original foam volume had disappeared (Hayta et al. 2002).

Total phenolic analysis: the method used by Xu and Chang (2007) in the total phenolic content analysis was modified. After homogenizing 3 g of tarhana with 10 ml of water, it was placed in a 25°C water bath for 30 minutes. Samples were centrifuged at 4000 x *G* for 10 min and filtered (Whatman no.1). After adding 300µl of the filtrate into the tubes, 4300µl of water and 100 µl of Folin Ciocalteu reagent were added and waited for 2 minutes. Then 300 µl 7.5% (w/v) Na_2CO_3 solution was added to the samples. The samples were vortexed and kept in the dark for 2 hours. The absorbance of the solutions was read at 760 nm by a spectrophotometer (UV-VIS Shimadzu UV mini-1240).

Antioxidant analysis; for the antioxidant activity analysis of tarhana samples, the method used by Demirkol and Tarakçı (2018) was modified. Three grams of tarhana samples were homogenized with 10 mL of methanol and then placed in a water bath of 25°C for 30 minutes. Samples that were centrifuged at 4000 rpm for 20 min were filtered through Whatman No.1 filter paper. DPPH (1, 1-Diphenyl-2-picrylhydrazyl radical) reagent was added to 1000 µl of the obtained filtrate. At the end of the period, absorbance values of the samples were measured with spectrophotometer at 515 nm wavelength.

Statistical Analysis

One-way ANOVA method was used with Minitab 18 package program for statistical analysis of the data of the analysis results of tarhana samples with hazelnut addition. Tukey multiple comparison test was used to compare the samples, which were found to be significant as a result of variance analysis.

RESULTS and DISCUSSION

The pH values changes in Tarhana Samples

Tarhana fermentation is performed by lactic acid bacteria (LAB) (*Streptococcus thermophilus*, *Lactobacillus bulgaricus*) coming from yogurt and bread yeast (*Saccharomyces cerevisiae*), which ensure the tarhana its characteristic aroma, acidic taste, and yeast flavor (Daglıoglu, 2000; Bilgiçli and Ibanoglu, 2007). In addition to *Streptococcus thermophilus* and *Lactobacillus bulgaricus* during the fermentation of tarhana, it also reported the presence of *L Lactobacillus casei, L Lactobacillus plantarum,* and *Lactobacillus brevis* in the product (Özbilgin, 1983). As a result, the metabolites produced reduce the pH of tarhana and extend its shelf life (Koca & Tarakçı, 1997). There are significant differences between the samples when the effect of hazelnut ratio on pH is investigated. The average pH value increased as the hazelnut rate increased and the lowest pH value was determined in control tarhana with 4.77±0.11, while the highest pH value was found with 5.13±0.16 and 30% hazelnut pulp (Table 3). The effect of hazelnut ratio on the pH value of tarhana were found statistically significant ($p<0.05$). In their study on the acceptability of tarhana, Ibanoglu et al. (1995) found that the pH value of tarhana varied between 4.3-4.8. Temiz et al. (1991) reported that the pH of the tarhana made by changing the type and amount of yoghurt was between 4.0-4.3. Bilgiçli et al. (2006) stated that the final pH of the samples increased as the amount of wheat seeds and bran added to the samples increased in the measurements made in tarhana containing wheat germ and bran.

Titratable Acidity Changes in Tarhana Samples

Yeast and lactic acid bacteria used in the production of tarhana soup are responsible for acid formation during fermentation. In our research, the acidity values of tarhana samples were observed in the control samples at the lowest hours at 0.46% and at the 0th hour, and the highest acidity values were found in the samples that used 30% hazelnut pulp (Table 2). Lactic acid bacteria and yeasts (*S. cerevisiae*) explain it by converting sugars into various organic acids (Erdoğrul & Erbilir 2006; Çelik et al., 2005). The titratable acidity value increased as the hazelnut rate increased, and the lowest acidity value was determined in control When the effect of hazelnut rate on acidity is investigated, there are significant differences between the rates ($p<0.05$). This can be explained by the fact that the free fatty acids formed by hydrolysis of the oil contained in hazelnut pulp, increase the acidity of hazelnut pulp tarhana. Bilgiçli (2009) reported that the addition of buckwheat increased tarhana acidity in his study. Ertaş et al. (2009) stated that the acidity value decreased in tarhana produced by using whey instead of yogurt. Bilgiçli et al. (2005) stated that the

addition of wheat germ and bran has higher titratable acidity values compared to the control tarhana sample.

Table 2. The pH, titratable acidity and color values of tarhana samples*
Çizelge 2. Tarhana örneklerinde pH, titre edilebilir asitlik ve renk değerleri

Samples	pH	Titratable acidity	*L**	*a**	*b**
FK	4.77±0.11^{g}	0.58±0.09^{g}	56.35±0.03^{a}	-1.23±0.11^{d}	30.73±0.46^{e}
F5	4.83±0.12^{f}	0.60±0.08^{f}	55.93±0.02^{b}	0.90±0.06^{c}	31.98±0.03^{d}
F10	4.90±0.13^{e}	0.63±0.09^{e}	54.71±0.02^{c}	1.45±0.04^{b}	32.20±0.10^{d}
F15	4.98±0.12^{d}	0.66±0.11^{d}	50.71±0.08^{d}	1.50±0.02^{b}	32.86±0.04^{c}
F20	5.02±0.13^{c}	0.69±0.11^{c}	49.73±0.05^{e}	1.55±0.02^{b}	33.68±0.04^{b}
F25	5.06±0.14^{b}	0.71±0.12^{b}	49.36±0.08^{f}	1.99±0.01^{a}	33.90±0.02^{b}
F30	5.13±0.16^{a}	0.74±0.12^{a}	47.73±0.09^{g}	2.01±0.01^{a}	34.74±0.02^{a}

Different superscript letters in the same column indicate a significant difference between the samples ($p<0.05$).
FK: 0%; F5: 5%; F10: 10%; F15: 15%; and F20: 20% hazelnut pulp containing tarhana samples

Color Changes in Tarhana Samples

In this system, L (brightness), a* (redness) and b* (yellowness) values are measured. The results of L* value in the samples of tarhana soup are given in Table 2. The effect of hazelnut ratio on L* value was statistically significant ($p<0.05$). When the L* values were examined, it was observed that as the ratio of hazelnut pulp increased, its brightness decreased. Hayta et al. (2002) demonstrated that there were significant differences in the color measurements of tarhana samples between the drying methods used in their study of the effect of drying methods on the functional properties of tarhana, suggesting that industrial microwave drying could be an alternative to classical drying methods. Bilgiçli (2009) investigated Tarhana with buckwheat flour and discovered that as the rate of buckwheat flour increased, the L* value decreased compared to control samples. L* values were determined between 71.72 and 78.51 in the study conducted by Erkan et al. (2006). According to the studies, it was observed that the L* value of tarhana supplemented with hazelnut pulp was lower.

Variance analysis results of a* values for tarhana soups are given in Table 2. The effect of hazelnut pulp ratio on a* value was statistically significant ($p<0.05$). With the control sample, it was determined that there were significant differences in a* values of hazelnut pulp tarhana ($p<0.05$). In control samples, the mean a* value, which is -1.23±0.11, increases with increasing hazelnut ratio, and the highest a* value is determined in tarhana as +2.01±0.01 for 30% hazelnut pulp. In the study conducted by Erkan et al. (2006), the use of barley in tarhana was investigated and stated that a values, ranged from +3.14 to +6.46. In their study on the use of different flours in tarhana, Köse et al. (2002) discovered a value of a ranging from +14.41 to +18.72. In our study, a value was found lower than other studies.

The effect of hazelnut pulp ratio on b* value of tarhana soups was found statistically significant ($p<0.05$). In control samples, the average b value of 30.73±0.46 increased as the hazelnut ratio increased and the highest b* value was determined in tarhana with 30% hazelnut pulp as34.74±0.02. (Table 3). Ertaş et al (2009) stated that in their study on the use of whey concentrate in tarhana, the b value ranged between 20.59 and 24.28. In the study conducted by Üçok et al (2019), quinoa flour was used in the production of tarhana and stated that the b values increased as the rate of quinoa flour increased (28.06-34.57). The b* values obtained in our study are higher than those of Ertaş et al. (2009) and are similar to those found by Üçok et al. (2019).

Viscosity changes in tarhana samples

Viscosity is a measure of the resistance of a fluid to flow under surface tension and is one of the main parameters for semi-liquid foods (Bourne, 2002). Norquist et al. (2012) examined the properties of gluten after its interaction with enzymatic hydrolysis or heat and markedly decreased viscosity with increasing frequency of hydrolysis. It inhibits the interactions between starch molecules by limiting the swelling of starch with its high-water absorption feature. Thus, it affects the viscosity of starch (Türksoy, 2018). The viscosity measurement results for tarhana pulp samples are shown in Table 3. The lowest viscosity value was measured containing 10.24 cp and 30% hazelnut pulp, while the highest viscosity was determined in control samples at 149.56 cp. According to the results obtained, it is seen that the viscosity value decreases as the hazelnut ratio increases. Viscosity is an indicator of the resistance of fluidity to followability. Viscosity changed inversely with the addition of hazelnut pulp. The lowest viscosity value was determined to be 24.67 cp in for samples containing 30% hazelnut pulp, while the highest was determined to be 105.08 cp in for control samples. With the addition of hazelnut pulp, increased fat content, reduced starch and gluten content, and loss of fermentation are thought to cause a significant reduction in baked tarhana viscosity. In their study on

the use of quinoa flour in the production of tarhana, Üçok et al. (2019) discovered that the viscosity decreased as the quinoa was added, and the viscosity values of tarhana samples ranged between 42.04 cp and 138.26 cp. Çağlar et al. (2012) reported that the viscosity of carob tarhana samples increased with the addition of carob, with results ranging from 98.4 to 117.65. Anıl et al. (2016) measured the viscosities of corn flour and baked corn flour tarhana and determined that the average viscosity value in baked corn flour was 4.13cp, while it was 121.35cp in non-baked corn flour samples. Current findings are compatible with the aforesaid study results.

Generally, molecules move more comfortably with increasing temperature, and viscosity decreases due to the decrease between intermolecular friction and thus hydrodynamic forces (Davis, 1995) and Çelik et al. (2005). In the study conducted by Ibanoğlu and Ibanoğlu (1998), the rheological properties of some traditional soups were determined, and it stated that the viscosity decreased with temperature. Heating can break down molecular entanglement and bonds stabilize the molecular structure and cause a decrease in viscosity. As the temperature increases, instability of protein-protein and protein-water interactions occurs, leading to a decrease in viscosity (Hayta et al., 2002).

Table 3. Viscosity, water-holding capacity, foaming capacity and foam stability values in tarhana*
Tablo 3. Tarhanada viskozite, su tutma kapasitesi, köpürme kapasitesi ve köpük stabilite değerleri

Hazelnut ratio (%)	Viscosity (cp)	Water holding capacity (ml g^{-1})	Foaming capacity (ml ml^{-1})	Foam stability (min.)
FK	105.08±27.58^{a}	0.74±0.02^{a}	0.01±0.01^{f}	0.01±0.02^{g}
F5	89.01±19.14^{b}	0.72±0.11^{a}	0.35±0.01^{e}	0.35±0.03^{f}
F10	78.19±18.04^{c}	0.85±0.07^{a}	0.67±0.01^{d}	0.85±0.02^{e}
F15	55.15±13.74^{d}	0.82±0.08^{a}	0.78±0.01^{c}	1.20±0.15^{d}
F20	43.47±11.49^{e}	0.73±0.10^{a}	0.92±0.03^{b}	1.56±0.05^{c}
F25	37.39±10.13^{f}	0.76±0.04^{a}	0.99±0.02^{a}	1.81±0.07^{b}
F30	24.67±7.47^{g}	0.70±0.06^{a}	1.03±0.02^{a}	2.26±0.13^{a}

Different superscript letters in the same column indicate a significant difference between the samples ($p<0.05$).
FK: 0%; F5: 5%; F10: 10%; F15: 15%; and F20: 20% hazelnut pulp containing tarhana samples.

Water holding capacity

Water holding capacity is considered as an important functional feature in viscous foods such as bakery products, and sauces (Hayta et al., 2002). Many parameters such as the size and shape of starch granules, the distribution of protein aggregates and pH, temperature, salt content have a significant effect on the water holding capacity (Muir et al., 2000). Proteins have a higher water holding capacity at pH values above and below the isoelectric point. The salt concentration below 1% increases the water retention capacity of the proteins. The water retention capacities of the proteins generally decrease with increasing temperature (Fennema, 1985). The analysis results regarding the water holding capacity are shown in Table 4. The lowest water holding capacity was 0.70 ml g^{-1} and 30% hazelnut pulp, the highest 0.85 ml g^{-1} and 10% hazelnut pulp samples. Hayta et al. (2002) reported that in their study on various drying techniques, the water holding capacity, drying, tunnel dryer and home-type microwave drying techniques were superior in industrial microwaves.

While water-holding capacity was determined as 0.74±0.02 (ml g^{-1}) in samples without hazelnut pulp, it was found to be 0.85±0.07 (ml g^{-1}) in samples with10% hazelnut pulp, indicating 14% increase (Table 3). It has been reported that the water holding capacity may increase by approximately 10%. As a reason for this increase, it has been observed that some hydrophobic groups in the protein are reduced by denaturation, and water is needed for protein hydrolysis (Fennema, 1985). The lowest water holding capacity was determined in samples using 30% hazelnut pulp (0.70 ± 0.06 ml g^{-1}). With the addition of hazelnut pulp, the proportional decrease in the amount of starch in the formulation of tarhana can have an effect on water absorption.

In the study conducted by Çelik et al. (2005), the effect of yeast on the functional properties and quality of tarhana was investigated. The water retention capacity, which is one of the important functional features of yeast added samples, has decreased. Aktaş (2018) has observed a regular increase in water absorption values as the dandruff substitution rate in tarhana samples increased and the water absorption values of the samples without dandruff added was 0.58 ml g^{-1}, while the water absorption average of the samples with bran added was 15%. The values indicated that the average increased by 44.8% to 0.84 ml g^{-1}. This parameter is 0.62-0.65 46 ml g^{-1}, according to the study performed by Tarakçı et al. (2013) in tarhana. The results obtained in our study show similarities with other studies.

Foaming Stability and Foam Capacity

Foam is defined as a two-phase system consisting of air cells separated by a thin layer of liquid and bubbled air in a liquid (Makri et al., 2005). Fermentation can cause significant or undesirable changes in the foaming properties of grain-based foods (İbanoğlu & İbanoğlu, 1999). The foaming capacity results of tarhana samples are given in Table 3. The foaming capacity results were determined to range from 0.00 to 1.06 (ml ml^{-1}). As hazelnut pulp ratio increased, foaming capacity increased. While the foaming capacity of the control samples averaged 0.01±0.01 (ml ml^{-1}), tarhana with 30% hazelnut pulp was determined as 1.03±0.02 (ml ml^{-1}) and the difference between them was found statistically significant ($p<0.05$). Proteins play an active role in foaming, and the fact that hazelnut is a protein-rich food has led to increased foaming capacity in tarhana.

Çağlar et al. (2012) reported in their study that the foaming capacity of tarhana increased with the addition of carob, with the highest foaming capacity of 0.91 ± 0.01 ml ml^{-1} in tarhana samples containing 8% carob. Bilgiçli (2008) discovered that complete replacement of wheat flour with buckwheat flour increased the foaming capacity from 0.750 ± 0.06 (ml ml^{-1}) to 1.91 ± 0.11 (ml ml^{-1}). The foaming capacity values recorded in this study were found compatible with other studies.

The presence and clustering of proteins as a thin layer on the foam surface is effective in improving foam stability. The stability increases as the small molecules formed by the hydrolysis of the proteins spread much better at the liquid-air interface. With fermentation, the protein molecule is broken down and results in an increase in the foam stability of compounds with smaller molecules (Ibanoğlu & Ibanoğlu, 1999). According to the results of foam stability given in Table 4, foam stability varies between 0.00 and 2.47 minutes. The effect of hazelnut pulp ratio on foam stability value was found statistically significant ($p<0.05$). While the foam stability in the control samples was 0.01±0.02 on average, this value was determined as 2.26±0.13 on the tarhana samples with 30% hazelnut pulp (Table 3.) and the difference between them was found to be statistically significant ($p<0.05$). High foam stability is due to the surfactant properties of continuous phase soluble proteins (Kaur and Singh, 2007). Hayta et al. (2002) reported that the foam stability varied between 1.37-6.17 min depending on the type of drying method, while Gokmen (2009) found this value as 0.35 min in tarhana. The results of foam stability obtained in our study are similar to other studies.

Protein Changes in Tarhana Samples

It is a low-quality protein source since it contains small amounts of amino acids such as flour, lysine, methionine, and threonine, which are the main components of tarhana. As the other main component, yogurt, these amino acids are present, so flour and yogurt in tarhana complement each other in terms of essential amino acids and are a source of higher quality protein. Many researchers have attempted to enrich tarhana with various protein sources due to the fact that vegetable proteins derived from wheat flour are more intense than animal proteins and have a lower bioavailability. Since the lactic acid bacteria in yoghurt in the composition of tarhana pre-digest the nutrients such as protein, carbohydrate, and fat in the environment, the digestibility and nutritional properties of tarhana increase (Bilgiçli & Türker, 2004). Table 4 shows the protein content of tarhana samples. The lowest protein content was found to be 12. 21% in the control tarhana sample, while the highest protein content was found in the sample containing 30% hazelnut pulp. The hazelnuts ratio effect on protein amount in tarhana samples was found statistically significant ($p<0.05$). As the hazelnut pulp rate increased, the protein amount of tarhana increased. While the average protein amount of the control samples was 12.211±0.2%, it was determined as 15.117±0.08% in the samples containing 30% hazelnut pulp (Table 5). In the study conducted by Bilgiçli and Türker (2004), it was discovered that the total protein amount increased as the yeast additive ratio increased, with the total protein amount in yeast-free samples having the lowest value with 15.019%, and the yeast additive yielding the highest total protein amount with 17.050%. Köse and Çağındı (2002) determined that protein content ranged between 8.8 and 22.5% in their study on the use of different flours in tarhana. Erkan et al. (2006) stated that in their study by adding barley to the tarhana formulation, the protein ratio varies between 10.1 and 15.9. In our study, the protein ratio in tarhana increased by 23.8% when 30% hazelnut pulp was added. As seen in table, hazelnut pulp contains a higher rate of protein than wheat flour. This difference is also reflected in the tarhana produced. In other words, as the hazelnut pulp ratio increased in tarhana samples, the protein content also increased.

Ash Changes in Tarhana Samples

Food ash is the inorganic part that remains after the combustion of organic matter. When organic matter is burned, water and CO_2 are formed, leaving the mineral-containing inorganic part. Minerals are obtained from plants, water and animal foods. Most minerals are found in foods due to organic matter (protein, fat, carbohydrate). The ash contents of the tarhana samples are given in Table 4. Control tarhana had the lowest ash content of 1.191 %, while tarhana with 30% hazelnut pulp had the highest protein rate of 1.778%. As the hazelnut pulp rate increased, the ash amount of tarhana increased. The average amount of ash in the control samples was 1.22 ± 0.02%, while the

amount of ash in the 30% hazelnut pulp added tarhana was 1.73 ± 0.04% and the difference was found to be statistically significant ($p<0.05$). The ash amount of the tarhana samples varies depending on the substances used in its formulation and the amount of these substances. In their study on cranberry tarhana, Koca et al. (2006) discovered that the ash rate ranged from 1.75 to 3.96%. In a study by Tamer et al. (2007), they stated that the ash rate of tarhana is between 1.36% and 9.4%. Bilgiçli et al. (2006) found that the ash content of tarhana increased with the addition of wheat seeds, reaching 3.26% with the addition of 50% wheat seeds; the ash content was the lowest in the control tarhana (1.36%). Dağcı et al. (2008) used soy yoghurt, which was obtained by processing soybeans, in the formulation of tarhana. They determined the ash content of the tarhana obtained to be 0.56%. The results obtained in this study Koca et al. (2006) and Tamer et al. (2007), however, Bilgiçli et al. (2006) and Dağcı et al. (2008) was found to be higher than the findings. This is due to the fact that the ash content of the ingredients in the formulation varies. As seen in the table, hazelnut pulp contains a higher rate of ash than wheat flour. This rate was reflected in the produced tarhana and as the rate of hazelnut pulp increased, the amount of ash increased.

Fat Changes in Tarhana Samples

Hazelnut contains 60-70% oil. Fatty acids with various functions and benefits are found in the composition of the oils for our organism. Hazelnut oil constitutes approximately 83% of the composition of fatty acids, oleic acid, and 12% of linoleic acid (Akdere, 2003). Table 4 shows the results of the amount of oil found in the tarhana samples. While the amount of oil in control tarhana was determined to be 2.39 %, the highest oil rate was determined to be 11.4 % for 30 percent% hazelnut pulp added tarhana. As hazelnut pulp ratio increased, the oil concentration of tarhana increased. While the average amount of oil in the control samples was 2.42±0.03%, it was 11.30±0.10% for the hazelnut pulp added tarhana, and the difference was found to be significant ($p<0.05$). Ertaş et al (2009) reported that oil concentrations ranged between 0.87% and 6.33% in their study on the use of whey concentrate in tarhana. Ibanoğlu et al (1995) found the fat content of tarhana between 3.7% and 5.6%. Koca and Tarakçı (1997) investigated the use of corn flour and whey in tarhana and found that the fat content was between 2.49% and 5.51% in their study. In our study, a higher rate of oil was detected than other study findings. The reason for this is the fat content of hazelnut pulp in the Table 4. This rate was reflected in the tarhana produced and the amount of oil increased as hazelnut pulp rate increased.

Total Phenolic Changes in Tarhana Samples

Phenolic compounds are substances which contain one or more hydroxyl groups, including functional derivatives attached to an aromatic ring. Phenolics are the most active natural antioxidants, and they achieve their antioxidant effects by binding free radicals, chelating metals, and inhibiting lipoxygenase enzyme. Phenolic compounds are the most important group of water-soluble antioxidants (Güleşçi & Aygül, 2016). Besides being rich in fat components, hazelnut is known to be a rich source of phenolic components with antioxidant potential. Table 4 shows the results of the total amount of phenolic substances found in tarhana samples. While the amount of phenolic substance was determined in control samples with the lowest 1.598 mgGAE g^{-1}, the highest amount of phenolic substance was determined in 2.178 (mgGAE g^{-1}) tarhana sample with 30% hazelnut pulp. As the hazelnut pulp ratio increased, the total phenolic content of tarhana increased. While the average amount of phenolic substance belonging to the control samples was 1.64±0.01(mg GAE g^{-1} sample), it was determined as 2.15±0.00 (mg GAE g^{-1} sample) in the samples using 30% hazelnut pulp and the difference was statistically significant ($p<0.05$). Demir (2018) stated that the total phenolic content of tarhana samples varied between 714.31±14.13 and 1521.08±15.65 (mg GAE/100g). Değirmencioğlu et al. (2016) investigated the effect of different drying methods (sun drying, oven drying, microwave drying) on total phenolic substance content and antioxidant activity in tarhana enriched with oat flour in various proportions, and determined that total phenolic content of the samples dried in kiln dryers at 55 °C was high. Kılcı and Göçmen (2014) added 10, 20, 30 and 40% oat crushing instead of wheat flour in the form of tarhana. They stated that the content of phenolic substances increased as it increased. In our study, the amount of phenolic substance increased as the hazelnut ratio increased. The reason for this is that hazelnut contains high levels of phenolic substances as seen in Table 4. The findings differed from those of previous studies. This is due to the fact that the tarhana ingredients contain varying amounts of phenolic substances.

Antioxidant Activity Changes in Tarhana Samples

Single electron parts that are not paired in atomic or molecular structures are called “free oxygen radicals”. These molecules, which can easily exchange electrons with other molecules, are also called “reactive oxygen particles”. These radicals interact easily with other molecules in the cell, causing oxidative stress. Oxidative stress causes damage to essential cell components and causes various ailments related to age. Nutrition with foods with high antioxidant content is important to protect against the effect of free radicals. Antioxidants in foods are “substances that have low concentrations compared to oxidizable substrates and prevent or delay oxidation of substrates” (Becker et al., 2004; Çağatay & Kayalı,

2006; Sönmez et al., 2010).

As the rate of hazelnut pulp increased, the antioxidant activity of tarhana increased (Table 4). While the antioxidant activity of the control samples was 0.15±0.01 (mgTroloxE g^{-1}), it was determined as 0.42±0.00 (mg TroloxE g^{-1}) in the samples containing 30% hazelnut pulp and the difference was statistically significant ($p<0.05$). Bilgiçli et al. (2006) determined the total antioxidant amount between 10.93-22.44 (mMol TroloxE g^{-1}) in his study with the addition of wheat germ and bran to tarhana. In the study conducted by Özmen (2011), different legume flours were used in the production of tarhana and the total amount of antioxidants were determined to be between 42.50 and 55.18 (mMol trolox g^{-1}). The reason for this is that hazelnut contains high levels of antioxidants as seen in Table 4. The results obtained were found different from other studies. This is due to the fact that the ingredients in tarhana contain antioxidant substances in varying proportions.

CONCLUSIONS

The addition of hazelnut pulp was found to have a significant effect on viscosity, foaming capacity and foam stability, but not on water holding capacity. The addition of hazelnut pulp causes a decrease in viscosity of tarhana, while foam stability and capacity increased. Hazelnut pulp contains a higher amount of phenolic substances than wheat flour. It was observed that with the increase of fermentation and hazelnut pulp, the total amount of phenolic substance increased together with the total antioxidant values. With this study, it was possible to present a food product with high antioxidant, phenolic, mineral, and protein content to social individuals who are more conscious about nutrition and health.

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Author contributions

MNO and ZT have designed the study and collected the data. MNO and ZT wrote the article, and critically reviewed by ZT. The authors contributed equally to the article.

Conflict of interest statement

Authors have declared no conflict of interest.

REFERENCES

Aktaş, K. (2018). *Farklı Kepek ve Süt Serum Proteinleri Kullanılarak Zenginleştirilen Tarhananın Bazı Özelliklerinin Belirlenmesi*. Selçuk Üniversitesi, Fen Bilimleri Enstitüsü, Doktora Tezi, 175 sy.

Anıl, M., Durmuş, Y., & Tarakçı, Z. (2016). Farklı Gamlar Içeren Mısır Unlu ve Fırınlanmış Mısır Unlu Tarhanaların Viskozitelerinin Kıyaslanması. *Ordu Üniversitesi Bilim ve Teknoloji Dergisi 6(2),* 128-135.

Becker, E.M., Nissen, L.S., & Skibsted, L.H. (2004). Antioxidant Evaluation Protocols: Food Quality or Health Effects. *European Food Research and Techonology 219,* 561–571.

Bilgiçli, N. (2004). *Tarhananın Fitik Asit Içeriği ve Bazıbesin Ögeleri Üzerine Maya, Malt ve Fitaz Katkılarının Etkileri*. Selçuk Üniversitesi Fen Bilimleri Enstitüsü, Doktora Tezi, 108 sy.

Bilgiçli, N. & Türker, S. (2004). Tarhanada Sindirilebilir Protein ve Kül Miktarı Üzerine Maya, Malt Unu ve Fitaz Katkılarının Etkileri. *Selçuk Üniversitesi Ziraat Fakültesi Dergisi 18(33),* 90–97.

Bilgiçli, N., Elgun, A., Herken, E.N., Türker, S., Ertaş, N., & İbanoğlu, Ş. (2006). Effect of Wheat Germ/Bran Addition on the Chemical, Nutritional and Sensory Quality of Tarhana, a Fermented Wheat Flour-Yoghurt Product. *Journal of Food Engineering 77,* 680–686.

Bilgiçli, N. (2009). Effect of Buckwheat Flour on Chemical and Functional Properties of Tarhana. *LWT-Food Science and Technology 42,* 514–518.

Bilgiçli, N., Aktaş, K., & Levent, H. (2014). Utilization of Citrus Albedo in Tarhana Production. *Journal of Food and Nutrition Research 53(2),* 162-170.

Bourne, M.C. (2002). *Food Texture and Viscosity: Concept and Measurement. 2nd Edition. Academic Press,* San Diego.

Çağlar, A., Erol, N., & Elgün, M.S. (2012). Effect of Carob Flour Substitution on Chemical and Functional Properties of Tarhana. *Journal of Food Processing and Preservation 37,* 670-675.

Çakıroğlu, F.P. (2008). *Geleneksel Tarhananın Modern Yolculuğu. Ankara Türkiye. Atatürk Kültür Dil ve Tarih Yüksek Kurumu Yayını, Maddi Kültür,* I: 339-351.

Çelik, I., Işık, F., Şimşek, Ö., & Gürsoy, O. (2005). The Effects of The Addition of Baker's Yeast on The Functional Properties and Quality of Tarhana, A Traditional Fermented Food. *Czech Journal of Food Sciences 23(50),* 190-195.

Dağcı, E.K., Dığrak, M., & Dayısoylu, K.S. (2008). Soya Yoğurdunun Geleneksel Kahramanmaraş Tarhanası Yapımında Kullanılması. 10. Gıda Kongresi: 669-670.

Dağlıoğlu, O. (2000). Tarhana As a Traditional Turkish Fermented Cereal Food, Its Recipe, Production and Composition. *Nahrung 44 (2),* 85-88.

Değirmencioğlu, N., Gürbüz, O., Herkeni, E.N., & Yurdunuseven Yıldız, A. (2016). The Impact of Drying Techniques on Phenolic Compound, Total Phenolic Content and Antioxidant Capacity of Oat Flour Tarhana. *Food Chemistry 194,* 587-594.

Demir, K.M. (2008). Geleneksel Tarhana Üretiminde Tam Buğday Ununun Kullanımı. *Akademik Gıda 16(2),* 148-155.

Demirkol, M. & Tarakci, Z. (2018). Effect of Grape (Vitis Labrusca L.) Pomace Dried by Different Methods on Physicochemical, Microbiological and Bioactive Properties of Yoghurt. *LWT-Food Science and Technology 97,* 770-777.

Davis, E.A. (1995). Functionality of sugars: physicochemical interactions in foods. *Journal of American Chemical Nutrition 62,* 170-177.

Erbaş, M. (2003). *Yaş Tarhananın Üretim ve Farklı Saklama Koşullarında Bileşimindeki Değişmeler.* Akdeniz Üniversitesi Fen Bilimleri Enstitüsü, Doktora Tezi, 189 sy.

Erbaş, M., Certel, M., Uslu, M.K. (2004). Yaş ve Kuru Tarhananın Şeker İçeriğine Fermantasyon ve Depolamanın Etkisi. *Gıda 29(4),* 299-305.

Erdoğrul, Ö. & Erbilir, F. (2006). Isolation and Characterization of Lactobacillus bulgaricus and Lactobacilus casei from Various Foods. *Turk Journal of Biology 30,* 39-44.

Erkan, H., Çelik, S., Bilgi, B., & Köksel, H. (2006). A New Approach for the Utilization of Barley in Food Products: Barley Tarhana. *Food Chemistry 97,* 12–18.

Ertaş, N., Sert, D., Demir, M.K., & Elgün, A. (2009). Effect of Whey Concentrate Addition on the Chemical, Nutritional and Sensory Properties of Tarhana (A Turkish Fermented Cereal-Based Food). *Food Science and Technology Research 15(1),* 51 – 58.

Fennema, O. (1985). Food Chemistry. New York: Marcel Dekker. 2. Edition.

Gökmen, S. (2009). *Çiğ-Pişmiş ve Kurutulmuş Ayva Katkısının Tarhana Üzerine Olan Etkisi. Yüksek* Lisans Tezi, Afyon Kocatepe Üniversitesi, Fen Bilimleri Enstitüsü, Gıda Mühendisliği Ana Bilim Dalı, Afyonkarahisar.

Güleşçi, N., & Aygül, İ. (2016). Beslenmede Yer Alan Antioksidan ve Fenolik Madde Içerikli Çerezler. *Gümüşhane Üniversitesi Sağlık Bilimleri Dergisi 5(1),* 109-129.

Hayta, M., Alpaslan, M., & Baysar, A. (2002). Effect of Drying Methods on Functional Properties of Tarhana: A Wheat Flour-Yogurt Mixture. *Journal of Food Science 67*, 740-744.

İbanoğlu, S., Ainsworth, P., Wilson, G., & Hayes, G.D. (1995). The Effect of Fermentation Conditions on the Nutrients and Acceptability of Tarhana. *Food Chemistry 53(2),* 143–147.

İbanoğlu, Ş. & İbanoğlu, E. (1998). Rheological Characterization of Some Traditional Turkish Soups. *Journal of Food Engineering 35,* 251-256.

James, C.S. (1995). Analytical Chemistry of Food. Publisher Blackie Academic and Professional, London, 176s.

Kaur, M. & Singh, N. (2007). Characterization of Protein Isolates from Different Indian Chickpea (Cicer Arietinum L.) Cultivars. *Food Chemistry 102(1),* 366-374.

Kılcı, A. & Göçmen, D. (2014). Phenolic acid composition, antioxidant acitvity and phenolic content of tarhana supplemented with oat flour. *Food Chemistry 151,* 547-553.

Koca, A.F. & Tarakçı, Z. (1997). Tarhana Üretiminde Mısır Unu ve Peyniraltı Suyu Kullanımı. *Gıda 22(4),* 287-292.

Koca, A.F., Yazıcı, F., & Anıl, M. (2002). Utilization of Soy Yoghurt in Tarhana Production. *European Food Research and Technology 215,* 293–297.

Koca, A.F., Koca, I., Anıl, M. & Karadeniz, B. (2006). Kızılcık Tarhanasının Fiziksel, Kimyasal ve Duyusal Özellikleri. 9. Gıda Kongresi: 377-380.

Makri, E., Papalamprou, E. & Doxastakis, G. (2005). Study of Functional Properties of Seed Storage Proteins from Indigenous European Legume Crops (Lupine, Pea, Broad Bean) in Admixture with Polysaccharides. *Food Hydrocolloids 19(3),* 583-594.

Muir, D.D., Tamime, A.Y., & Khaskheli, M. (2000). Effect of Processing Conditions and Raw Materials on The Properties of Khisk. 2. Sensory Profile and Micostructure. *Lebensmittel-Wissenschaft Technologie 33,* 452-461.

Nordqvist, P., Lawther, M., Malmström, E., & Khabbaz, F. (2012). Adhesive Properties of Wheat Gluten After Enzymatic Hydrolysis or Heat Treatment – A Comparative Study. *Industrial Crops and Products 38,* 139–145.

Özbilgin, S. (1983). *The Chemical and Biological Evaluation of Tarhana Supplemented with Chickpea and Lentil*. Ph.D. Thesis, Cornell University, 125 p., New York, U.S.A.

Özmen, F.H. (2011). *Çölyak Hastaları Için Baklagil Unları ile Zenginleştirilmiş Pirinç Tarhanası.* Yüksek Lisans Tezi, Hacettepe Üniversitesi, Fen Bilimleri Enstitüsü. Ankara.

Sönmez, C., Erbaş, G., Okur, Ö.D. & Seydim, Z.G. (2010). UHT Sütlerin Bazı Kalite Kriterlerinin ve Antioksidan Aktivitelerinin Belirlenmesi. *Akademik Gıda 8(1),* 13-16.

Tamer, C.E., Kumral, A., Aşan, M. & Şahin, I. (2007). Chemıcal Composıtıons of Tradıtıonal Tarhana Having Different Formulations. *Journal of Food Processing and Preservation 31,* 116–126.

Tarakçı, Z., Anıl, M., Koca, I. & İslam, A. (2013). Effects of Adding Cherry Laurel (Laurocerasus Officinalis) on Some Physicochemical and Functional Properties and Sensorial Quality of Tarhana. *Quality Assurance and Safety of Crops and Foods 5(4),* 347–355.

Temiz, A. & Pirkul, T. (1991). Farklı Bileşimlerde Üretilen Tarhanaların Fiziksel ve Kimyasal Bileşimleri. *Gıda 16(1),* 7-13.

Türksoy, S. (2018). Tam Tane Baklagil Unlarinin Kimyasal, Fonksiyonel ve Reolojik Özelliklerinin Belirlenmesi. *The Journal of Food 43(1),* 78-89.

Üçok, G., Cankurtaran, T., & Demir, M.K. (2019). Geleneksel Tarhana Üretiminde Kinoa Ununun

Kullanımı. *Harran Tarım ve Gıda Bilimleri Dergisi 23(1),* 22-30.

Yağcı, S. (2018). *The Use of Durum Clear Flour in Combination with Hazelnut Cake and Different Pomaces in the Production of Extruded Food.* Gaziantep Üniversitesi, Fen Bilimleri Enstitüsü, Doktora tezi, 145 sy.

Yağcı, S. & Göğüş, F. (2008). Response surface methodology for evaluation of physical and functional properties of extruded snack foods developed from food-by-products. *Journal of Food Engineering 86(1),* 122-132.

Yücel Şengün, İ. (2006). *Ege Bölgesinin Bazı Yörelerinde Yapılan Geleneksel Tarhana ve Bileşenlerinin Bakteri Florasanının Tanımlanması,* Ege Üniversitesi, Fen Bilimleri Enstitüsü, Doktora Tezi, 212sy.

Xu, B.J. & Chang, S.K. (2007). A Comparative Study on Phenolic Profiles and Antioxidant Activities of Legumes as Affected by Extraction Solvents. *Journal of Food Science 72(2),* 159-166.

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Effect of Hazelnut Skin Addition on Quality Characteristics of Functional Crackers

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ABSTRACT

Hazelnut skin, an innovative by-product, has been classified as one of the richest sources of edible phenolic compounds in recent studies. In this study, hazelnut skin powder was used in cracker formulation at 5, 10, 15 and 20% ratios replaced with wheat flour, and some technological, chemical properties and bioactive components of cracker samples were determined. The hazelnut skin powder used as raw material has 5.1, 4.8, 3.9, 11.6, 1244 and 20 times higher ash, phytic acid, total phenolic content, DPPH, FRAP, CUPPRAC values than wheat flour, respectively. Increasing hazelnut skin powder in cracker production increased the darkness and redness of the cracker. High hazelnut skin powder usage ratios improved the spread ratio and reduced the hardness of the crackers. Increasing use of hazelnut skin powder in cracker increased the amount of ash, fat, phytic acid and resistant starch from 1.59%, 13.63%, 246.58 mg/100g and 0.97% up to 2.13%, 16.53%, 581.54 mg/100g and 2.15%, respectively. Antioxidant (DPPH, FRAP and CUPRAC) and phenolic substances (free, bound and total) increased significantly ($p<0.05$) at all hazelnut skin powder usage ratios. The high utilization ratios (15-20%) of hazelnut skin powder negatively affected overall acceptability of the crackers.

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Fındık Kabuğu İlavesinin Fonksiyonel Krakerlerin Kalite Özelliklerine Etkisi

ÖZET

Yenilikçi bir yan ürün olan fındık zarı, son yıllarda yapılan çalışmalarda en zengin yenilebilir fenolik bileşik kaynaklarından biri olarak sınıflandırılmıştır. Bu çalışmada, kraker formülasyonunda buğday unu yerine %5, 10, 15 ve 20 oranlarında fındık zarı tozu kullanılmış ve kraker numunelerinin bazı teknolojik, kimyasal özellikleri ve biyoaktif bileşenleri belirlenmiştir. Hammadde olarak kullanılan fındık zarı tozu, buğday ununa kıyasla sırasıyla 5.1, 4.8, 3.9, 11.6, 1244 ve 20 kat daha fazla kül, fitik asit, toplam fenolik içerik, DPPH, FRAP, CUPPRAC değerlerine sahiptir. Kraker üretiminde artan oranda fındık zarı tozu kullanımı, krakerin koyuluğunu ve kırmızılığını artırmıştır. Yüksek fındık zarı tozu kullanım oranları, yayılma oranını artırmış ve krakerlerin sertliğini azaltmıştır. Fındık zarı tozunun krakerde artan oranda kullanımı, kül, yağ, fitik asit ve dirençli nişasta miktarını sırasıyla %1.59, %13.63, 246.58 mg/100g ve %0.97'den %2.13, %16.53, 581.54 mg/100g ve %2.15'e yükselmiştir. Tüm fındık zarı tozu kullanım oranlarında antioksidan (DPPH, FRAP ve CUPRAC) ve fenolik maddeler (serbest, bağlı ve toplam) önemli ölçüde ($p<0,05$) artmıştır. Fındık zarı tozunun yüksek kullanım oranları (%15-20) krakerlerin genel kabul edilebilirliğini olumsuz yönde etkilemiştir.

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INTRODUCTION

Corylus avellana, known as hazelnut belonging to the Betulacae family, with an annual average production of 1 million tons, is a very popular tree nut due to its pleasant flavors and health-promoting effects (Pelvan et al., 2018). A small amount (10%) of hazelnut is

consumed as a snack, and the rest is used in many different ways, including in the production of chocolate, dessert, pastry products and cooking oil (Platteau et al., 2011; Bozoğlu et al., 2019). After being harvested, the hazelnut goes through the stages of cracking, peeling and roasting, and during these processes, by-products are produced, including the hazelnut skin (Odabaş and Koca, 2016). After the hard skin is removed, the hazelnut obtained is surrounded by a brown perisperm (hazelnut skin) layer. This by-product, which is separated as waste during the roasting process, is responsible for 2.5% of the hazelnut kernel weight. Hazelnut skin is a by-product rich in of fiber (65%), polyphenolics, and proanthocyanins. These components make hazelnut skins an important by-product. For this reason, researchers are conducted to evaluate hazelnut skin in the production of food products in order to provide functional properties with antioxidant, phenolic and dietary fiber components of the end product (Anil, 2007; Durmus et al., 2021). In addition, Özdemir et al. (2014) demonstrated its potential to be used as a brown colored functional component in their studies. Dinkçi et al. (2021) used hazelnut shell as a functional additive in yoghurt in their research.

Cereal-based foods, prepared in different forms such as baked goods, pasta, snacks and others, are consumed as staple foods worldwide to meet energy and nutritional needs (Xu et al., 2020). Crackers are thin and brittle products prepared by using soft wheat flour together with fat, salt, and leavening agents, respectively. It is a snack food that is frequently preferred by consumers due to its unique taste, easy preservation, and cheapness (Polat et al., 2020). Also, as baked goods, crackers are seen as a healthy snack over deep-fried or sugar-filled alternatives. The increasing demand of consumers for healthy and functionally rich foods has increased the variety in these products, and in response to this increasing demand, various natural raw materials such as food industry by-products have started to take their place in the cracker formulation (Batista et al., 2019).

The aim of this study is to reveal the nutritional and functional properties of hazelnut skin through a snack product. More specifically, to reveal the effect of increased use of crust powder in cracker dough on the chemical composition and quality parameters (texture, diameter, thickness, spread rate and color) of crackers.

MATERIAL and METOD

Materials

Soft wheat flour, shortening, salt, powdered sugar, baking powder and baker's yeast were purchased from a market (Konya, Türkiye) and the protease enzyme was purchased from Vatan Enzim (Istanbul, Türkiye). The hazelnut skin was achieved from a local producer (Gürsoy) in Ordu, Türkiye. The hazelnut skin, which emerged as a product burned during the roasting of hazelnuts at 150 °C, was ground to 500 µm using a coffee grinder and stored at -18 °C until use.

Methods

Cracker production

The crackers were made with minor modifications to the procedure reported by Davidson (2016). The ingredients of crackers are displayed in Table 1. For control sample preparation; wheat flour (100 g), shortening (20 g), table salt (1.6 g), powdered sugar (1.5 g), baking powder (1.5 g), baker's yeast (0.2 g) and protease (0.01 g) were mixed in the kneader (Hobart N50, Offenburg, Germany) until a homogeneous dough was obtained. The dough was fermented in a chamber (Fimak FMD16, Konya, Turkey) for 20 minutes at 30 °C and 75-80 % relative humidity. Then, the fermented dough was formed into a 1 mm thick layer between two glass plates and shaped with a 50 mm diameter biscuit mold. It was baked in an oven (Fimak Rokon Classic FRN10G, Konya, Turkey) for 11 minutes at 180°C. Other crackers were formulated by replacing wheat flour with 5, 10, 15, and 20% levels of hazelnut skin powder. The crackers are displayed in Figure 1.

Figure 1. Samples of cracker containing 0-20% hazelnut skin powder
Şekil 1. %0-20 oranında fındık zarı tozu içeren krakerler örnekleri

Table 1. Formulation of crackers rich in hazelnut skin powder
Çizelge 1. Fındık zarı tozu açısından zengin kraker formülasyonu

	Control	Cracker with Hazelnut skin powder
Wheat flour	100	95, 90, 85, 80
Hazelnut skin powder	-	5, 10, 15, 20
Shortening	20	20
Table salt	1.6	1.6
Powdered sugar	1.5	1.5
Baking powder	1.5	1.5
Baker's yeast	0.2	0.2
Protease	0.01	0.01

Color properties

Color measurement of raw and cracker samples was performed using the Minolta CR 400 (Chroma Meter, Osaka, Japan). The measurement was made on the ground raw materials and at five different points on the surface of the crackers. L* (lightness, darkness), a* (red, green) and b* (yellow, blue) values were measured in raw materials and cracker samples. Hue (color essence) value was calculated with arctan (b*/a*) formula and SI (saturation index) value was calculated with $(a^{*2}+b^{*2})^{1/2}$ formula.

Physical properties

The diameter, thickness, spread ratio and textural properties of the end products were determined. The diameter and thickness were measured using five sample pieces by a caliper (Mitutoyo, Tokyo, Japan) according to the AACC method 10-54 (AACC, 2010), and values were reported in millimeters. The cracker spread ratio was determined by dividing the diameter by thickness.

The hardness and fracturability value of the crackers were analyzed by three-point bending (HDP/3 PB) tests on a TA-XT plus texture analyzer (Stable Micro Systems, Surrey, UK) equipped with a 5 kg loading cell. The measurement values of the texture analyzer were as follows: pre-test speed, 1.0 mm/s; test speed 1.0 mm/s; post-test speed, 10.0 mm/s. In the hardness and fracturability value measurements, 5 measurements were made for each sample and it was studied in 2 replications.

Proximate composition

Hazelnut skin powder, wheat flour and cracker samples were tested for their moisture (method 44–19), ash (method 08-01), protein (method 46-10) and fat content (method 30-10) (AACC, 1999). Resistant starch value of the samples was determined using Megazyme kit method (K-RSTAR 09/14, Megazyme International Ireland, Wicklow, Ireland) following manufacturer's instructions. The phytic acid value in the raw materials and cracker samples was extracted with 0.2 N hydrochloric acid solution and then treated with a certain amount of Fe+3 solution and precipitated. The amount of iron remaining in the serum part was determined spectrophotometrically, and the amount of phytic acid was calculated from the results obtained. Results are given in mg/100g (Haug and Lantzsch, 1983).

Analysis of antioxidant activity

The extraction method described by Yılmaz and Koca (2017) was used to determine the antioxidant activities of the samples. Extraction was performed by mixing 1 g of sample with 80% methanol, but without 1% acidification, as in free phenolic extraction.

Three methods were used in the antioxidant activity analysis of the samples.[1]For the analysis of the samples with the DPPH (2-2-Diphenyl-2-picrylhydrazil) antioxidant activity method, the method described by Beta et al. (2005) was used and the results were calculated as mg Trolox Equivalent/kg. [2]For the analysis of the samples with the FRAP (ferric reducing antioxidant power) antioxidant activity method, the method described by Gao et al. (2000) was used and the results were calculated as µmol Trolox Equivalent/g. [3]For the analysis of the samples with the CUPRAC (cupric ion reducing antioxidant capacity) antioxidant activity method, the method described by Apak et al. (2008) was used and the results were calculated as µmol Trolox Equivalent/g.

Analysis of free, bound and total phenolic content

Free and bound phenolic content was extracted defined to the method specified by Vitali et al. (2009). For the free phenolic extraction; raw materials and cracker samples (1 g) were mixed 10 ml of 1% acidified (HCl) methanol: water solution (80:20, v/v). Extraction was carried out by shaking the mixture at room temperature (24±1 ˚C) for 2 h. After extraction, the mixture was centrifuged at 3000 rpm to obtain the supernatant for analysis, and the separated supernatant was stored at -20°C for analysis. For bound phenolic extraction; 20 ml of methanol/H_2SO_4 (10:1) was added to the residue remaining after free phenolic extraction and the mixture was incubated in a shaking water bath for 20 hours at 85°C, then the

cooled supernatant was separated by centrifugation was stored at -20°C until analysis.

The free and bound phenolic content of each extract was analyzed according to the Folin-Ciocalteu colorimetric method as performed by Naczk and Shahidi (2004). Total phenolic content was obtained by summing the free and bound phenolic content. Phenolic content was expressed as gallic acid equivalents (mg of GAE/ kg).

Sensory evaluation

Sensory evaluation was performed 24 hours after cooking by 12 people selected from the Engineering faculty of Necmettin Erbakan University, who were informed in advance. Sensory evaluation selected representative features (color, taste, odor, appearance, brittleness and overall acceptability) of crackers were assessed. These features were evaluated using 7 hedonic scales as described by Meilgaard et al. (1999). Scores ranged from 1 "unacceptable" to 7 "excellent". Informed consent was obtained from the panelist prior to their participation in the panel, and their individual judgments were kept confidential.

Statistical analysis

SPSS statistical program version 22.0 (SAS Institute Inc., Cary, NC, USA) was used for statistical data analysis. Tukey test was used to determine significant differences. p values <0.05 were regarded as significant.

RESULTS and DISCUSSION

The color value of raw and cracker samples

The color value of soft wheat flour, hazelnut skin powders and crackers are shown in Table 2. The color properties (L*, a* and b*) of hazelnut skin powder and refined wheat flour were determined as 44.21, 8.27 and 11.90 and 93.40, -5.24 and 15.44, respectively. The hazelnut skin powder demonstrated a lower L* and b*, higher a* value in comparison with refined wheat flour. Similarly, Durmuş et al. (2020) stated that the hazelnut skin color is darker than wheat flour. This may be related to the polyphenolic compounds that contribute to the high phenolic value of hazelnut skin. The a* and b* color values of raw materials were used in Hue and SI calculations, and Hue and SI for wheat flour and hazelnut skin were found to be 108.74 and 16.31 and 63.93 and 18.82, respectively.

Table 2. Color values of hazelnut skin powder-enriched crackers[1]
Çizelge 2. Fındık zarı tozu ile zenginleştirilmiş krakerlerin renk değerleri[1]

	*L**	*a**	*b**	Hue	SI
Raw materials					
Wheat flour	93.40±0.06	-5.24±0.04	15.44±0.08	108.74±0.02	16.31±0.09
HSP[2]	44.21±0.39	8.27±0.01	11.90±0.14	63.93±0.23	18.82±0.12
HSP ratio (%)					
0	77.10±0.58a	-2.25±0.13d	28.28±0.49a	94.59±0,24a	28.38±0,50a
5	53.85±1.01b	5.14±0.53c	16.90±0.16b	73.07±0,62b	17.67±0,19b
10	45.23±0.36c	5.87±0.13bc	13.61±0.30c	66.67±0,26c	14.82±0,27c
15	40.55±1.14d	6.55±0.18ab	11.93±0.24d	61.24±0,20d	13.61±0,12d
20	37.75±0.16e	6.78±0.27a	10.86±0.09e	58.02±0,45e	12.81±0,33d

[1]Means with the same letter within a column are not significantly different (p > 0.05). Hue: Hue angle, SI: Saturation index. HSP: Hazelnut skin powder.

When the color properties of the crackers were examined, it was found that the supplementary of hazelnut skin in the cracker formulation increased the darkness and redness values on the other hand b* value decreased. It has been reported that this increase may be relevant to the color characteristics and high phenolic value of the hazelnut skin powder (Ha et al., 2011). Similarly, Velioğlu et al. (2017) determined that the L* and a* values of bread, cake and cookie samples were significantly affected when wheat flour was replaced by hazelnut skin. Researchers reported that the use of 6% hazelnut skin decreased the L* value and increased the a* value in all samples.

Physical properties of cracker samples

Table 3 presents the physical properties of hazelnut skin powder enriched-crackers. The average diameter, thickness and spreading ratio values of the crackers were determined as 47.56 mm, 6.02 mm and 9.48, respectively (Table 3). While the addition of 10% or more hazelnut skin powder in cracker formulations decreased the diameter and thickness values, the spread rate increased with the use of 20% hazelnut skin powder and reached the highest value. Decrease in cracker diameter value was attributed to the high water holding capacity of the hazelnut skin powder. This situation increased the viscosity of the dough and made it difficult to spread the cookies (Park et al., 2015). Gluten is responsible for the increase in the thickness of the biscuit (Handa et al., 2012), and the decreasing gluten content may have caused a decrease in the thickness of the crackers. The spreading rate is a marker of cookie quality and a high spreading rate is desired for cookies and similar products (Barak et al., 2013). The texture properties of samples were

displayed in Table 3. The textural character of samples are affected by the gluten strength, damaged starch ratio and water absorption capacity of the flour (Liu et al, 2021). The presence of hazelnut skin powder reduced the hardness value of the crackers. Fracturability value of the cracker containing 20% hazelnut skin powder was lower than the control sample. The lowest hardness values were found numerically in the crackers with 20% hazelnut skin powder addition, and the hardness values of the crackers with 15% and 20% hazelnut skin powder addition ratios were statistically in the same group. The decreased firmness value may be related to the insufficient formation of the gluten network due to the competition of dietary fiber, sugar and flour proteins for water (Kulthe et al., 2014).

Table 3. Physical properties of hazelnut skin powder - enriched crackers[1]
Çizelge 3. Fındık zarı tozu ile zenginleştirilmiş krakerlerin fiziksel özellikleri[1]

HSP ratio (%)	Diameter (mm)	Thickness (mm)	Spread ratio (W/T)	Hardness (g)	Fracturability (mm)
0	48.78±0.26a	8.52±0.96a	5.76±0.67c	4556.25±41.4a	36.99±0.95a
5	48.34±0.06a	6.80±0.42ab	7.14±0.41bc	3863.45±85.6b	36.30±1.41ab
10	46.68±0.25b	5.84±0.79b	8.03±1.08bc	3532.66±32.8c	35.41±0.15ab
15	46.44±0.34b	5.56±0.65b	8.40±0.93b	3003.31±57.8d	34.75±1.01ab
20	46.34±0.23b	3.52±0.40c	13.20±1.36a	2945.76±6.8d	34.00±0.44b

1Means with the same letter within a column are not significantly different ($p > 0.05$). HSP: Hazelnut skin powder.

Chemical composition of crackers

Chemical and bioactive component of crackers are displayed in Table 4 and 5. The moisture amount of wheat flour and hazelnut skin powder were determined as 10.25% and 7.52%, respectively. Ash, fat, phytic acid and resistant starch contents of hazelnut skin were determined considerably higher than that of refined wheat flour. A small numerical difference was determined between the protein contents of wheat flour and hazelnut skin powder. The ash, protein and fat contents hazelnut skins are in line with Özdemir et al. (2014) and Tunçil (2020) reports. Phytic acid and resistant starch contents of hazelnut skin powder were 4.8 and 1.8 times higher compared to wheat flour, respectively. Phytic acid is one of the important bioactive components of hazelnuts. Compared to cereal and legumes, phytic acid amount in nuts ranges from 0.1 to 9%, while in cereal and legumes this value varies between 0.06% - 2.2% and 0.2 - 2.9%, respectively. The phytate content in peanuts is affected not only by different hazelnut botanical varieties, but also according to factors such as environmental conditions, soil type, farming techniques and ripening stage (Schlemmer et al., 2009). Chemical compositions of crackers were compared according to ratio factor (Table 4). The moisture value of cracker samples changed from 4.34 to 4.94%. Crackers formulated with 20% hazelnut skin powder had the highest ash content than the other cracker samples, which may be due to the higher ash existence of hazelnut skin powder (2.68%) compared to wheat flour (0.53%). The addition of increasing hazelnut skin powder into the cracker formulation slightly reduced the protein amount of the control cracker from 9.41% to 9.10%, and the reduction was statistically insignificant ($p > 0.05$). These results according to the lower protein content of hazelnut skin powder (7.52%) than refined wheat flour (7.83%). Cracker samples prepared with hazelnut skin powder showed higher fat content compared to control cracker samples. As the hazelnut skin level increased in the formulation of crackers fat content increased from 16.63 to 19.53%. The phytic acid value of cracker samples changed between 246.58 and 581.54 mg/100 g. The phytic acid content of cracker samples increased with the use of hazelnut skin powder. Phytic acid chelates minerals, especially Ca, Mg, Fe and Zn, and limits the absorption of starch, amino acids, and proteins (Oatway et al., 2001). For this reason, foods with low phytic acid content are seen as more important in terms of nutrition. However, recent studies have reported that phytic acid contributes significantly to antioxidant activity. Barbhai and Hymavathi (2022) stated that phytic acid is a natural antioxidant source that promotes health and prevents diseases due to oxidative stress. The fact that hazelnut skin is a better source of resistant starch than wheat flour is also reflected in the cracker samples prepared with hazelnut skin powder addition. Replacing 20% of wheat flour with hazelnut skin powder increased the RS content of control crackers from 0.97% to 2.15%. The higher RS value of cracker samples than the raw materials used in the formulation may be related to the rich polyphenolics and proanthocyanins content of hazelnut skin. Deng et al. (2021) stated that the formation of amylose-proanthocyanidin and starch-polyphenol complexes increased the resistant starch content by decreasing the digestibility of starch. Khan et al. (2013) reported that the polyphenolic content of sorghum flour was responsible for the increase in the resistant starch amount of pasta samples.

Antioxidant activity and phenolic content of raw materials and cracker samples

Among the raw materials, antioxidant activity (DPPH, FRAP and CUPRAC) free, bound and total phenolic

content of hazelnut skin powder were found to be higher compared to wheat flour. In the literature, it has been stated that hazelnut by-products are rich materials of natural antioxidants and polyphenolic (Locatelli et al. 2010). Also, Gu et al. (2003) reported that among the nuts, hazelnuts are rich in phenols and especially proanthocyanidins. Alasalvar et al. (2009) found that Turkish Tombul hazelnut skin showed high antioxidant/antiradical activity.

Table 4. Chemical properties of hazelnut skin powder-enriched cracker[1]
Çizelge 4. Fındık zarı tozu ile zenginleştirilmiş krakerlerin kimyasal özellikleri[1]

	Moisture (%)	Ash (%)	Protein (%)	Fat (%)	Phytic acid (mg/100 g)	RS (%)
Raw materials						
Wheat flour	10.25±0.07	0.53±0.01	7.83±0.13	0.78±0.03	247.24±4.92	1.04±0.03
HSP	7.52±7.52	2.68±0.03	7.52±0.18	11.37±0.04	1175.26±14.77	1.85±0.07
HSP ratio (%)						
0	4.94±0.04a	1.59±0.03d	9.41±0.31a	16.63±0.11e	246.58±8.92e	0.97±0.02d
5	4.79±0.02b	1.66±0.03d	9.27±0.25a	17.07±0.05d	388.27±2.46d	1.05±0.04cd
10	4.61±0.03c	1.82±0.00c	9.19±0.62a	18.36±0.13c	447.47±12.31c	1.24±0.06bc
15	4.49±0.03c	1.98±0.02b	9.14±0.06a	18.92±0.31b	494.48±4.93b	1.43±0.05b
20	4.34±0.05d	2.13±0.07a	9.10±0.25a	19.37±0.12a	581.54±2.46a	2.15±0.19a

[1]Means with the same letter within a column are not significantly different (p > 0.05). RS: Resistant starch, HSP: Hazelnut skin powder.

Table 5. Antioxidant activity and free, bound and total phenolic content of the hazelnut skin powder-enriched cracker samples[1]
Çizelge 5. Fındık zarı tozu ile zenginleştirilmiş krakerlerin antioksidan aktivite, serbest, bağlı ve toplam fenolik içeriği[1]

	DPPH (mg TE/kg)	FRAP (umol TE/g)	CUPRAC (umol TE/g)	FPC (mg GAE/ kg)	BPC (mg GAE/kg)	TPC (mg GAE/kg)
Raw materials						
Wheat flour	175.96±12.87	0.47±0.09	2.32±0.02	1481.44±3.64	2856.76±67.94	4338.20±75.83
HSP	2037.15±75.10	584.69±12.54	46.99±0.19	2250.70±50.78	5526.30±48.66	16779.80±84.63
HSP ratio(%)						
0	293.24±16.68d	1.36±0.08e	5.06±0.26e	1132.63±48.02e	2070.49±21.35c	3203.12±18.83c
5	426.56±42.25c	3.20±0.17d	9.30±0.51d	1294.54±73.02d	2348.43±44.87bc	3642.98±27.82c
10	516.79±74.89c	6.55±0.20c	16.94±0.24c	1617.75±84.26c	2618.70±38.34bc	4236.45±34.69b
15	1253.81±37.13b	14.89±0.32b	46.18±1.33b	1876.97±43.97b	2878.22±98.30ab	4755.19±23.20b
20	1707.68±15.31a	26.25±0.16a	59.49±1.85a	2201.55±25.14a	3393.19±10.89a	5594.75±34.69a

[1]Means with the same letter within a column are not significantly different (p > 0.05).DPPH; 2.2- diphenyl-1-picrylhydrazyl. FRAP; Ferric reducing antioxidant potential. CUPRAC; Cupric ion reducing antioxidant capacity. FPC; Free phenolic content. BPC; Bound phenolic content. TPC; Total phenolic content. HSP: Hazelnut skin powder.

When the antioxidant activity of crackers containing hazelnut skin powder was compared with the control, the use of increasing hazelnut skin powder increased the DPPH, FRAP and CUPRAC values from 293.24 up to 1707.68 mg TE/kg, 1.36 upto 26.25 umol TE/g and 5.06 upto59.49 umol TE/g, respectively. This may be due to the high antioxidant activity of hazelnut skin powder. Condensed tannins in hazelnut skin contribute greatly to the antioxidant content of hazelnut skin (Lainas et al., 2016). Similarly, Pelvan et al. (2018) stated that the antioxidant component in hazelnut skin was high as a result of the analysis they performed on hazelnut and hazelnut skin using DPPH, ORAC and ABTS methods. Parallel to the antioxidant capacity values, the phenolic content of the cracker samples increased by 1.9, 1.6 and 1.9 with the use of 20% hazelnut skin. Pelvan et al. (2018) stated that the roasted hazelnut skin has about 710 times more total phenolic acid content than the roasted hazelnut, in which most of the phenolic content is in the skin. Hazelnut skin was rich in total phenolics than wheat flour, which may have been reflected in the cracker samples.

Sensorial analysis

Sensorial properties of crackers are demonstrated in Figure 2. The color characteristics of the cracker formulated with 5% hazelnut skin powder was higher than the other samples. Usage of hazelnut skin powder at a high rate (15-20%) caused a decrease in appearance scores and the cracker sample with 20% hazelnut skin addition was evaluated with the lowest

score of appearance. The use of 5% hazelnut skin was similar to the control and provided a higher taste score than all other cracker samples. The use of 10–20% hazelnut skin powder in formulations caused a decrease in the odor score compared to control. The brittleness values of the cracker samples were evaluated with numerically close scores, and the samples with 20% hazelnut skin powder addition were found to be less brittle statistically. The use of 5% hazelnut skin powder provided the highest overall acceptability score among all cracker samples.

Figure 2. Sensory attributes of crackers containing hazelnut skin powder.
Şekil 2. Fındık zarı tozu içeren krakerlerin duyusal özellikleri.

CONCLUSION

Hazelnut skin occurs as a by-product during the roasting phase of roasted hazelnut production. Since the hazelnut skin is rich in polyphenolic content, consist of phenolic acids, flavonoids and flavonols, which have a health-promoting effects, it can be preferred for enrichment of foods. Evaluating the use of such a valuable ingredient in food formulations is highly valuable. The addition of hazelnut skin powder into crackers improved the resistant starch, antioxidant activity and phenolic content. The significant reduction in L* and b* color value of cracker samples might be due to the color properties of hazelnut skin powders. With the addition of hazelnut, the hardness values of the crackers decreased and the spreading rate increased. The data revealed that the addition of up to 10% hazelnut skin could be considered a potential ingredient for producing functionally crackers.

The results obtained from this study may be a precursor to the use of a low-value industrial by-product in functional food formulations that contribute to the formation of resistant starch as a rich source of phenolic compounds and as a natural coloring agent. Data on chemical content obtained from analyzes of hazelnut shells showed that this by-product is very rich in health-promoting antioxidants and phenolics. More studies are needed to investigate the effects of hazelnut shell powder in different food formulations.

Declaration of Competing Interest

The author declares that she has no known competing financial interests or personal relationships that may appear to affect the work reported in this article.

REFERENCES

AACC, (1999). American Association of Cereal Chemists (AACC), Method 14–50.01, in Approved Methods of the AACC. AACC, St Paul, MN.

AACC, (2010). Approved Methods of Analysis, 11th Ed. Methods 10-52, 10-54, and 56-11. Available online only. AACC International: St. Paul, MN.

Alasalvar, C., Amaral, J. S., Satır, G., & Shahidi, F. (2009). Lipid characteristics and essential minerals of native Turkish hazelnut varieties (Corylus avellana L.). *Food Chemistry, 113*(4), 919-925. https://doi.org/10.1016/j.foodchem.2008.08.019

Anil, M. (2007). Using of hazelnut testa as a source of dietary fiber in breadmaking. *Journal of Food Engineering, 80*(1), 61-67. https://doi.org/10.1016/j.jfoodeng.2006.05.003

Apak, R., Güclü, K., Özyürek, M., & Celik, S. E. (2008). Mechanism of antioxidant capacity assays and the CUPRAC (cupric ion reducing antioxidant capacity) assay, *Microchimica Acta, 160* (4), 413-419. DOI 10.1007/s00604-007-0777-0

Barak, S., Mudgil, D., & Khatkar, B. S. (2013). Relationship of gliadin and glutenin proteins with dough rheology, flour pasting and bread making performance of wheat varieties. *LWT-Food Science*

and Technology, 51(1), 211-217. https://doi.org/10.1016/j.lwt.2012.09.011

Barbhai, M. D., & Hymavathi, T. V. (2022). Nutrient, phytonutrient and antioxidant potential of selected underutilized nutri-cereal brans. *Journal of Food Measurement and Characterization, 16*(3), 1952-1966. https://doi.org/10.1007/s11694-022-01301-9

Batista, A. P., Niccolai, A., Bursic, I., Sousa, I., Raymundo, A., Rodolfi, L., ... & Tredici, M. R. (2019). Microalgae as functional ingredients in savory food products: application to wheat crackers. *Foods, 8*(12), 611. https://doi.org/10.3390/foods 8120611

Beta, T., Nam, S., Dexter, J. E., & Sapirstein, H. D. (2005). Phenolic content and antioxidant activity of pearled wheat and roller-milled fractions, *Cereal Chemistry, 82* (4), 390-393. https://doi.org/10.1094/ CC-82-0390.

Bozoğlu, M., Başer, U., Topuz, B. K., & Eroğlu, N. A. (2019). An overview of hazelnut markets and policy in Turkey. *Kahramanmaraş Sütçü İmam Üniversitesi Tarım ve Doğa Dergisi, 22*(5), 733-743. DOI:10.18016/ ksutarimdoga.v22i45606.532645

Davidson, I. (2016). Biscuit baking technology: processing and engineering manual. Academic press. 35-48.

Deng, N., Deng, Z., Tang, C., Liu, C., Luo, S., Chen, T., & Hu, X. (2021). Formation, structure and properties of the starch-polyphenol inclusion complex: A review. *Trends in Food Science & Technology, 112,* 667-675. https://doi.org/10.1016/ j.tifs.2021.04.032

Dinkçi, N., Aktaş, M., Akdeniz, V., & Sirbu, A. (2021). The Influence of Hazelnut Skin Addition on Quality Properties and Antioxidant Activity of Functional Yogurt. *Foods, 10*(11), 2855. https://doi.org/10.3390/ foods10112855

Durmus, Y., Anil, M., & Simsek, S. (2021). Effects of hazelnut skin, cross-linked starch, and oxidized starch on wheat flour and dough quality. Journal of Food Processing and Preservation, 45(2), e14919. https://doi.org/10.1111/jfpp.14919

Gu, L., Kelm, M. A., Hammerstone, J. F., Beecher, G., Holden, J., Haytowitz, D., & Prior, R. L. (2003). Screening of foods containing proanthocyanidins and their structural characterization using LC-MS/MS and thiolytic degradation. *Journal of Agricultural and Food Chemistry, 51*(25), 7513-7521. https://doi.org/10.1021/jf034815d

Ha, B. C., Park, Y. K., & Cho, S. (2011). Suppliers' affective trust and trust in competency in buyers: Its effect on collaboration and logistics efficiency. *International Journal of Operations & Production Management, 31*(1), 56-77.

Handa, C., Goomer, S., & Siddhu, A. (2010). Performance and fatty acid profiling of interesterified trans free bakery shortening in short dough biscuits. International *Journal of Food Science & Technology, 45*(5), 1002-1008. https://doi.org/10.1111/j.1365-2621.2010.02222.x

Haug, W., & Lantzsch, H. J. (1983). Sensitive method for the rapid determination of phytate in cereals and cereal products. *Journal of the Science of Food and Agriculture, 34*, 1423-1426. https://doi.org/ 10.1002/jsfa.2740341217.

Khan, I., Yousif, A., Johnson, S. K., & Gamlath, S. (2013). Effect of sorghum flour addition on resistant starch content, phenolic profile and antioxidant capacity of durum wheat pasta. *Food Research International, 54*(1), 578-586. https://doi.org/ 10.1016/j.foodres.2013.07.059

Kulthe, A. A., Pawar, V. D., Kotecha, P. M., Chavan, U. D., & Bansode, V. V. (2014). Development of high protein and low calorie cookies. *Journal of Food Science and Technology, 51*(1), 153-157. https://doi.org/10.1007/s13197-011-0465-2

Lainas, K., Alasalvar, C., & Bolling, B. W. (2016). Effects of roasting on proanthocyanidin contents of Turkish Tombul hazelnut and its skin. *Journal of Functional Foods, 23*, 647-653. https://doi.org/ 10.1016/j.jff.2016.03.029

Liu, Y., Li, M., Liu, Y., Guan, E., & Bian, K. (2021). Effects of superheated steam treatment of wheat on physicochemical properties of wheat flour and cracker quality. *Journal of Cereal Science, 97*, 103165. https://doi.org/10.1016/j.jcs.2021.103165

Locatelli, M., Travaglia, F., Coïsson, J. D., Martelli, A., Stévigny, C., & Arlorio, M. (2010). Total antioxidant activity of hazelnut skin (Nocciola Piemonte PGI): Impact of different roasting conditions. *Food Chemistry, 119*(4), 1647-1655. https://doi.org/ 10.1016/j.foodchem.2009.08.048

Meilgaard, M. C., Carr, B. T., & Civille, G. V. (1999). Sensory evaluation techniques, 4th ed.; CRC Press LLC: New York, 2007.

Naczk, M., & Shahidi, F. (2004). Extraction and analysis of phenolics in food. *Journal of Chromatography A, 1054*(1-2), 95-111. https:// doi.org/10.1016/j.chroma.2004.08.059

Oatway, L., Vasanthan, T., & Helm, J. H. (2001). Phytic acid. *Food Reviews International, 17*(4), 419-431. https://doi.org/10.1081/FRI-100108531

Odabaş, H. İ., & Koca, I. (2016). Application of response surface methodology for optimizing the recovery of phenolic compounds from hazelnut skin using different extraction methods. Industrial Crops and Products, 91, 114-124. https://doi.org/ 10.1016/j.indcrop.2016.05.033

Özdemir, K. S., Yılmaz, C., Durmaz, G., & Gökmen, V. (2014). Hazelnut skin powder: A new brown colored functional ingredient. *Food Research International, 65*, 291-297. https://doi.org/10.1016/j.foodres. 2014. 01.060

Park, J., Choi, I., & Kim, Y. (2015). Cookies formulated from fresh okara using starch, soy flour and hydroxypropyl methylcellulose have high quality

and nutritional value. LWT-*Food Science and Technology, 63*(1), 660-666. https://doi.org/10.1016/j.lwt.2015.03.110

Pelvan, E., Olgun, E. Ö., Karadağ, A., & Alasalvar, C. (2018). Phenolic profiles and antioxidant activity of Turkish Tombul hazelnut samples (natural, roasted, and roasted hazelnut skin). *Food Chemistry, 244*, 102-108. https://doi.org/10.1016/j.foodchem.2017.10.011

Platteau, C., De Loose, M., De Meulenaer, B., & Taverniers, I. (2011). Detection of allergenic ingredients using real-time PCR: a case study on hazelnut (*Corylus avellena*) and soy (*Glycine max*). *Journal of Agricultural and Food Chemistry, 59*(20), 10803-10814. https://doi.org/10.1021/jf202110f

Polat, H., Capar, T. D., Inanir, C., Ekici, L., & Yalcin, H. (2020). Formulation of functional crackers enriched with germinated lentil extract: A Response Surface Methodology Box-Behnken Design. LWT, 123, 109065. https://doi.org/10.1016/j.lwt.2020.109065

Schlemmer, U., Frølich, W., Prieto, R. M., & Grases, F. (2009). Phytate in foods and significance for humans: food sources, intake, processing, bioavailability, protective role and analysis. *Molecular Nutrition & Food Research, 53*(S2), S330-S375. https://doi.org/10.1002/mnfr.200900099

Tunçil, Y. E. (2020). Dietary fibre profiles of Turkish Tombul hazelnut (*Corylus avellana* L.) and hazelnut skin. *Food Chemistry, 316*, 126338. https://doi.org/10.1016/j.foodchem.2020.126338

Velioğlu, S. D., Güner, K. G., Velioğlu, H. M., & Çelikyurt, G. (2017). Fındık Zarının Fırıncılık Ürünlerinde Kullanımı. *Tekirdağ Ziraat Fakültesi Dergisi, 14*(3), 127-139.

Vitali, D., Dragojević, I. V., & Šebečić, B. (2009). Effects of incorporation of integral raw materials and dietary fibre on the selected nutritional and functional properties of biscuits. *Food Chemistry, 114*(4), 1462-1469. https://doi.org/10.1016/j.foodchem.2008.11.032

Xu, J., Zhang, Y., Wang, W., & Li, Y. (2020). Advanced properties of gluten-free cookies, cakes, and crackers: A review. *Trends in Food Science & Technology, 103*, 200-213. https://doi.org/10.1016/j.tifs.2020.07.017

Yilmaz, V. A. (2019). Effects of different cooking and drying methods on phenolic acids, carotenoids, and antioxidant activity of emmer (*Triticum turgidum* ssp. dicoccum) bulgur. *Cereal Chemistry, 96*(6), 1093-1102. https://doi.org/10.1002/cche.10219

Yilmaz, V. A., & Koca, A. F. (2017). Effect of different production techniques on bioactive compounds and antioxidant capacity of einkorn (Triticum monococcum L.) and durum (*Triticum turgidum* subsp. durum) bulgur. *Journal of the Science of Food and Agriculture, 97*(1), 269-277. https://doi.org/10.1002/jsfa.7724.

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Isolation and Characterization of Collagen from the Invasive Sea Urchin (*Diadema setosum* L., 1778) in North-Eastern Mediterranean Sea, Türkiye

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ABSTRACT

In the present study, collagen was obtained from tissues of the invasive sea urchin (*Diadema setosum*) rapidly spread in the Mediterranean Sea. As a result, the yield of collagen isolated from sea urchin was determined to be 23.78±1.33% (dry weight). As a result of SDS-Page analysis, it was determined that it contains (α1)2α2, (Molecular Weight (MA) 124, 114 kDa) and ß chains (MA 245 kDa) similar to calfskin collagen. In the amino acid analysis of collagen, it was determined that the imino acid (proline+hydroxyproline) content was 196.1 residues/1000 residues. The functional bands of amide A (3301 cm^{-1}), B (2924 cm^{-1}), I (1643 cm^{-1}), II (1550 cm^{-1}), and III (1242 cm^{-1}) functional bands were visualized in the FTIR spectrum. These results were like other collagen sources. Invasive sea urchin was used as a source of collagen for the first time in the present study. An alternative source of collagen to mammalian collagen, which is used commercially in many industries such as biomedicine, food and cosmetics, was isolated for the first time from *D. setosum*. It was proposed that marine collagen can be used as an alternative source of collagen and a functional component in areas including food, cosmetics, and pharmaceutical industries.

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Türkiye Kıyılarında Dağılım Gösteren İstilacı Denizkestanesinden (*Diadema setosum* L., 1778) Kolajen Ekstraksiyonu ve Karakterizasyonu

ÖZET

Bu çalışmada, Akdeniz'de hızla yayılan istilacı denizkestanesi (*Diadema setosum*) dokularından kolajen elde edilmiştir. Sonuç olarak, denizkestanesinden elde edilen kolajenin verimi %23.78±1.33 (kuru ağırlık) olarak belirlenmiştir. SDS-Page analizi sonucunda, dana derisi kolajenine benzeyen $(\alpha_1)_2\alpha_2$, (Molekül Ağırlığı (MA) 124, 114 kDa) ve ß zincirleri (MA 245 kDa) içerdiği tespit edilmiştir. Kolajenin amino asit analizinde imino asit (prolin+hidroksiprolin) içeriğinin 196.1 kalıntı/1000 kalıntı olduğu belirlenmiştir. FTIR spektrumuna göre Amid A (3301 cm^{-1}), Amide B (2924 cm^{-1}), Amide I (1643 cm-1), Amide II (1550 cm^{-1}) ve Amide III (1242 cm^{-1}) fonksiyonel bantları görüntülenmiştir. İstilacı denizkestanesinden kolajen ilk defa bu çalışma ile elde edilmiştir. Biyotıp, gıda ve kozmetik gibi birçok endüstride ticari olarak kullanılan memeli kolajenine alternatif olarak istilacı denizkestanesinden (*D. setosum*) kolajen ilk kez elde edilmiştir. Elde edilen bu kolajeninin gıda, kozmetik ve ilaç endüstrileri gibi alanlarda alternatif bir kolajen kaynağı ve fonksiyonel bir bileşen olarak kullanılabileceği önerilmiştir.

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INTRODUCTION

Collagen is found in many tissues including skin, tendons, and connective tissues of vertebrates, and comprise about 30% of the total protein in the body (Muthumari et al. 2016). Approximately 29 different types of collagens have been described with unique amino acid orders and molecular constructions in vertebrate tissues (Ali et al. 2018). It usually has a triple helix (Gly-X-Y) structure wrapped around each other, consisting of Glycine (Gly), proline (X), and hydroxyproline (Y). Collagens are widely used in many fields such as food, cosmetics, tissue engineering, and the pharmaceutical industry due to their properties such as biocompatibility and biodegradability (Muthumari et al., 2016). Collagen is obtained from the skin and bones of terrestrial animals such as cows, pigs, and chickens. However, since communicable diseases such as bird flu, bovine spongiform encephalopathy (BSE), and foot and mouth disease (FMD) have increased in recent years, collagens obtained from terrestrial animals cause disease concerns (Gharagheshlagh et al., 2020). Therefore, marine collagens have become the focus of attention to meet the need for collagen. In addition to their use as a food source, marine species are important resources that can be used in fields such as agriculture, chemical industry, and cosmetics. In recent years, there is a tendency toward marine resources for the production of collagen, which has been rapidly increasing for use, especially in health and cosmetics.

Marine collagen sources are generally vertebrate and invertebrate species such as fish, octopus, cuttlefish, shrimp, sea cucumber, and sea urchin. Skins, tissues or marine animal waste parts of economically important species constitute an important part of marine collagen resources (Ahmad & Benjakul, 2010; Muthumari et al., 2016; Ali et al., 2018; Nurilmala et al., 2019; Nurubhasha et al., 2019; Gharagheshlagh et al., 2020; Li et al., 2020; Sulaiman & Sarbon, 2020). Research on collagen production from species with low or no economic value has increased because of the increase in collagen use and demand (Barzideh et al., 2014; Iswariya et al., 2018). The fact that marine animals that do not have economic value are not used as a resource, there is no hunting pressure on these species, and their rapid spread creates negative effects on the ecosystem and native marine species.

The Mediterranean region is a good example of this case, especially due to the rapidly increasing foreign species inflows in the last decade. The entry of foreign species into the Mediterranean Sea can be through the Suez Canal, the Strait of Gibraltar, or by ship transport. However, the most intense foreign species entry is through the Suez Canal. To date, 80 fish and 123 invertebrates have been introduced from these alien marine species, which are called the lessepsian species (Çınar et al., 2020). Pufferfish are harmful invaders that have spread throughout the Mediterranean Sea within the last 20 years (Kayhan et al., 2021). In a similar way, the lionfish (*Piterois miles*) has been spreading rapidly (Hüseyinoğlu et al., 2021). One of the species that enters the Mediterranean Sea and has a serious invasion potential is the sea urchin *Diadema setosum*.

The invasive sea urchin (*D. setosum*) is a species of Indo-Pacific origin and has a wide distribution in the Red Sea (Gulf of Suez, Gulf of Aqaba, Northern and Southern Red Sea), east coast of Africa, Japan, and Australia (Lessios et al., 2001). It was first reported on the Turkish coasts in 2006 on the Kaş Peninsula of Antalya, and then on the Iskenderun Bay, the Aegean Sea, and the Marmara Sea coasts (Turan et al., 2011; Yapıcı 2018; Artüz & Artüz, 2019). *D. setosum* reaches large sizes due to its unique feeding habits and reproductive behaviors a great threat to endemic species with the increase in population density. Due to the morphological structure of this species (long spines), it damages the fishing gear used in fishing, hindering hunting activities. In addition, its long and black spines cause adverse effects such as painful injury, swelling, and redness when penetrating human skin.

The present study aimed to extract collagen from the invasive sea urchin (*D. setosum*) for the first time and to determine the physical, chemical, and structural properties of this collagen. Thus, the collagen extracted from the sea urchin species can be used in cosmetics, tissue engineering, pharmaceutical industry, etc. This invertebrate, which is not consumed in Türkiye, will be brought to the country's economy as a biomaterial with high economic value.

MATERIAL and METHODS

Materials

In the study, *D. setosum* samples were collected with nets from the coast of Iskenderun Bay in August 2021. (Figure 1). The sea urchin samples collected were brought to the laboratory in sealed plastic boxes. Morphometric measurements of the samples were made with the help of a digital caliper with a precision of 0.01 mm after their weighing using a digital scale with a precision of 0.01 g. The mean total weight, gonad weight, test weight, and test diameter of the individuals were calculated as 84.64±2.77 g, 3.04±0.11 g, 32.72±1.21 g, and 51.8±1.18 mm, respectively (Mean±SD). Then all soft tissues were taken and stored at -80°C until the time of analysis.

Collagen Preparation

The method of Sivakumar & Chandrakasan (1998) was modified for the isolation of collagen from the soft tissues of invasive sea urchin (*D. setosum*). The whole procedure was carried out at 4°C. Soft tissues were cut into small pieces using scissors and kept in 0.2 N NaOH solution for two days (NaOH solution was changed daily). The precipitate was washed three times with distilled water and then lyophilized. The dried precipitate was melted in 1 M acetic acid two days. Then 5% (w/v) pepsin (Sigma p7000), according to the lyophilized weight was added and hydrolyzed for 48 h. The mixture was then centrifuged at 8000xg for 1h at 4 ° C, and the precipitate was collected. The precipitate was salted by adding NaCl to a last concentration of 0.7 M and was followed by precipitation by adding NaCl to a last concentration of 2.3 M in 0.05 M Tris-HCl (pH 7.5). The precipitate was divided by centrifugation at 9000×g for 1 h at 4 °C. The precipitate was then melted in 0.5 M acetic acids, dialyzed in contrast to 0.1 M acetic acid, distilled water, and lyophilized.

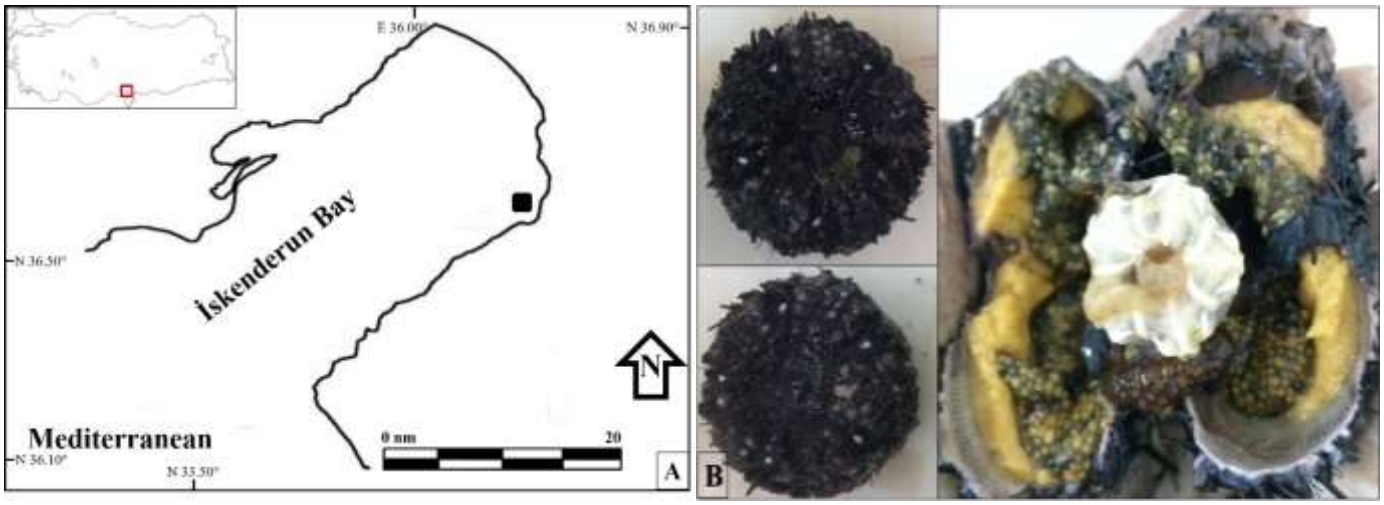

Figure 1 A) Study area, B) Invasive Sea Urchin *D. setosum* species.
Şekil 1 A) Çalışma Alanı, B) İstilacı denizkestanesi *D. setosum* türü

Yield of collagen

The initial weight of the soft tissues of sea urchins was used to calculate the collagen yield and was calculated with the following Eq. 1.

Yield of collagen= (Weight of lyophilized collagen)/(Initial wet weight of tissues)x100 (1)

Proximate analysis

The ash and protein values of the samples taken from the soft tissues of the sea urchin were determined according to the Association of Official Analytical Chemists (AOAC) (2000) method, the lipid analyses according to Bligh & Dyer (1959) and the total crude protein analysis was carried out using the Kjeldahl method (Bligh & Dyer, 1959; AOAC 2000). All analyses were performed in triplicate.

SDS-Page analysis

The collagen was dissolved in 5 mg mL^{-1} 0.1 M acetic acid by agitating at room temperature for four hours. Then it was dialyzed against PBS (phosphate buffered saline) and loaded into the well in 100, 200, and 400 ng wells, respectively, by denaturation using a buffer at 95 °C for 5 minutes. The sample in the first well was 100 ng of BSA (Bovine Serum Albumin). Separation gel was 8% and no stacking gel was used. The sample was marked with Coomassie Brilliant Blue R250 and then destained.

Amino Acid analysis

The amino acid analysis of collagen obtained from sea urchin soft tissues was performed according to the D.05.G106 (UFLC-UV) method (PITC 1999; Dimova 2003). The amino acid content was stated as residues/1000 residues.

FTIR analysis

The FTIR spectra (SHIMADZU F-TIR-IRAffinity-1S) of collagen obtained from soft tissues of sea urchins were performed using the ATR method under dry conditions. All spectra readings were performed between 400 and 4000 cm^{-1} and at a data acquisition rate of 4 cm^{-1}.

SEM analysis

A SEM device was used to examine the surface area and internal structure of sea urchin collagen. Before collagen imaging, the collagen was coated with gold-palladium (Au-Pd) using a POLARON SC7620 sputter coating device. The sample was imagined under SEM (Scanning Electron Microscope) (JEOL JSM-638OLA) using 15 kV.

Statistical analyses

Data were analyzed using Microsoft Office Excel 2016. All samples were analyzed in triplicate. All quantitative results are given as mean±standard deviation.

RESULTS and DISCUSSION

In the last ten years, very few studies have been found on the study of invasive and poisonous *D. setosum* on the Mediterranean coast, such as growth, reproduction, and accumulation of metal. Previous studies have generally been concerned with species registration, availability, distribution in the region, biological properties and extraction of chitin and chitosan (Rahman et al., 2012; Fitriyani et al., 2022; Uğurlu & Duysak, 2022). Furthermore, there are studies on the components of nutrients (protein, lipid, fatty acid), antibacterial effects, cytotoxic activity, and accumulation of metals in tissues consumed such as gonads of this species (Flammang et al., 1997; Marimuthu et al., 2015; Tulandi et al., 2021). Examining studies on the species in Türkiye in recent years, it was seen that they were carried out on the Mediterranean Sea, Aegean Sea and Marmara Sea coasts and showed only the first records (Yokes & Galil, 2006; Turan et al., 2011; Yapıcı 2018; Artüz & Artüz, 219; Bilecenoğlu et al., 2019). There are studies of collagen extraction on different species of sea urchins in the literature. Collagen obtained in these studies had two structures of fibrillar collagen (α_1 and α_2) structures (Trotter & Kobb, 1994; Omura et al., 1996; Cluzel et al., 2000; Nagai & Suzuki, 2000). It has the potential to be an alternative marine collagen source for use in several areas such as food, medicine, and cosmetics (Nagai & Suzuki, 2000). It is a promising biomaterial for tissue and regenerative medicine and is environmentally friendly and economically sustainable (Shimizu et al., 1990). However, no biomaterial production studies related to *D. setosum were found.*

Yield in sea urchin collagen

For the collagen obtained from the soft tissues of sea urchins, 17.5 g collagen was obtained from 75 g of soft tissue (dry weight) (Figure 2). The mean collagen yield was calculated as 23.78±1.33%. It has been reported that the mean collagen yields extracted from the skin and bones of fish vary between 2% and 29% (Ahmad & Benjakul, 2010; Muthumari et al., 2016; Ali et al., 2018; Nurubhasha et al., 2019; Gharagheshlagh et al., 2020). The wide range of differences between collagen yields was associated with the species of fish or tissues used, their ecological environments, and different extraction methods. Nagai & Suzuki (2000) have reported a yield of approximately 35% (dry weight) of the collagen obtained from the shells of purple sea urchin (*Anthocidaris crassispina*). Ferrariro et al. (2020) have reported the mean yield of the collagen obtained from the soft tissue of *Paracentrotus lividus* as 4.93±2.22%. The differences in collagen yields obtained from *D. setosum* were associated with the difference in species or the difference in the tissues from which the collagen is obtained.

Figure 2 The collagen obtained from the *D. setosum* (original images).

Şekil 2 D. setosum'dan elde edilen kolajen (orijinal görüntü)

Proximate analysis

It was determined that the total lipid and ash contents of the collagen extracted from *D. setosum* were lower than the total lipid and ash contents of the raw tissue. This was associated with the process of removing collagen from inorganic substances and fat. In the present study, the protein, lipid and ash content of sea urchin tissues were calculated as 20.99±0.33%, 15.78±0.34%, 18.35±0.56%, respectively, while the protein, lipid and ash content of collagen was calculated as 49.5±0.26 and 2.7±0.25% and 1.72 ±0.12%, respectively.

Nurilmala et al. (2019) have reported the protein, lipid, and ash contents of the collagen obtained from *Thunnus albacares* skin as 36.09%, 1.08%, and 2.25%, respectively. Sulaiman & Sarbon (2020) calculated the protein, oil, and ash values of collagen extracted from *Decapterus macrosoma* fish waste as 22.86%, 0.38%, and 60.9%, respectively. Li et al. (2020) have reported the protein, lipid and ash levels of collagen extracted from the body wall of *H. cinerascens* as 10.3±0.4%, 0.3±0.0%, and 0.9±0.1%, respectively. The difference in protein, lipid, and ash content of collagen obtained from *D. setosum* was associated with the fact that it lived in different habitats or with the difference in the tissues from which collagen was obtained.

SDS-Polyacrylamide Gel Electrophoresis (SDS-Page)

SDS-Page analysis of collagen obtained from sea urchin is presented in Figure 3. The collagen extracted from the sea urchin comprised α_1, α_2, and high molecular weight chains ß and γ chains (Figure 3; Lane

1). The molecular weight of this collagen, the α_1, α_2, and ß chains, was determined to be approximately 124 kDa, 114 kDa and 245 kDa, respectively. In addition, it was determined that the collagen has an $(\alpha_1)_2\alpha_2$ molecular structure (Figure 3).

It has been reported that collagen obtained from the test portion of *Asthenosoma ijimai* has an $(\alpha_1)_2\alpha_2$ heterotrimer structure, like that determined in the present study (Omura et al., 1996). Shimizu et al. (1990) have reported that there are four α chains as α_1 α_2 α_3 α_4 in the *A. ijimai*, while Trotter & Koob (1994) have reported that *Eucidaris tribuloides* cidaroid sea urchin has heterotrimer collagen with an $(\alpha_1)_2\alpha_2$ molecular structure. The sea urchin collagen in the present study was found to have an $(\alpha_1)_2\alpha_2$ molecular structure, similar to the collagen of different species in similar studies (Liu et al., 2012; Zhang et al., 2014; Asaduzzaman et al., 2020). As a result, the α_1 and α_2 chains in the sea urchin collagen confirm that it is like type I collagen.

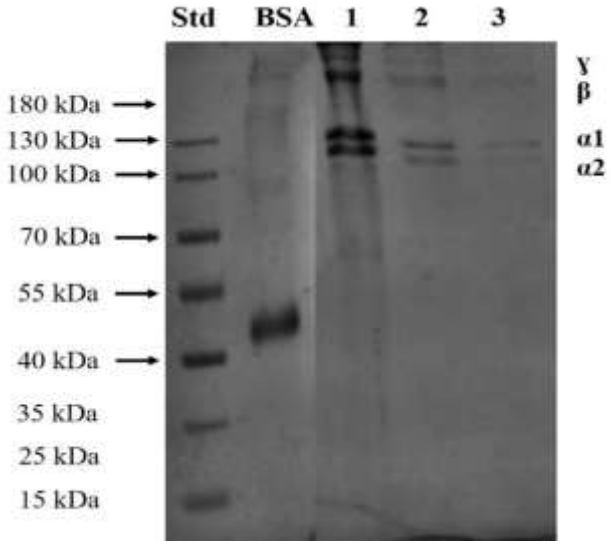

Figure 3 Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-Page) of collagen from D. *setosum*. Std: Protein markers; BSA: 100 ng BSA (Bovine Serum Albumin); lane 1: 400 ng collagen; lane 2: 200 ng collagen; lane 3: 100 ng collagen.

Şekil 3 D. setosum kolajenin Sodyum Dodesil Sülfat Poliakrilamid Jel Elektroforezi (SDS-Page). Std: Protein markır; BSA: 100 ng BSA (Sığır Serum Albümin); Şerit 1: 400 ng kolajen; Şerit 2: 200 ng kolajen; Şerit 3: 100 ng kolajen.

Amino Acid Composition of Collagens from the Soft Tissues of *D. setosum*

The amino acid content of the collagen extracted from *D. setosum* is shown in Table 1 as residue/1000 residues. In the present study, proline and hydroxyproline, which are unique in their amino acid content, were determined in collagen extracted from *D. setosum* soft tissues and glycine was determined as the amino acid with the highest amount (Table 1). In general, the amino acid glycine comprises about one-third of the total amount of amino acids. In the present study, 326.94 residues/1000 residues of glycine were found in the collagen extracted from *D. setosum* (Table 1).

Table 1 Amino acid composition of collagen from *D. setosum* (residues/1000 resiudes).

Çizelge 1 D. setosum kolajenin amino asit içerikleri (kalıntı/1000kalıntı)

Amino Acids	*D. setosum*
Alanine (ALA)	101.6
Arginine (ARG)	35.9
Aspartic acid (ASP)	69.3
Cysteine (CYS)	0
Glutamic acid (GLU)	32.4
Glycine (GLY)	326.9
Histidine (HIS)	9.8
Isoleucine (ILE)	11.4
Leucine (LEU)	25.8
Lysine (LYS)	11.2
Methionine (MET)	15.8
Phenylalanine (PHE)	16.2
Proline (PRO)	117.5
Serine (SER)	58.2
Threonine (THR)	56.6
Tyrosine (TYR)	9.9
Valine (VAL)	22.1
Hydroxyproline (HYP)	78.6
Imino acid (HYP + PRO)	196.1

In the literature, the amount of glycine has been reported to be the leading amino acid in collagen samples and comprises approximately 30-35% of the amino acid content in Saurida spp, *Mugil cephalus* and *Cypselurus melanurus* (Kumar et al., 2012; Veeruraj et al., 2013; Kozlowska et al., 2015). It has been reported that the ASC and PSC collagens obtained from the scales of Saurida spp. contained 335 and 338 residues/1000 residues glycine, respectively.

In the collagen extracted from *D. setosum*, alanine, aspartic acid, threonine, and serine amino acids were found to be 101.6, 69.3, 56.6, and 58.2 residues/1000 residues, respectively. No cystine was detected in the *D. setosum* collagen (Figure 4). Similarly to the present study, Senaratne et al. (2006) did not detect cystine in collagens extracted from different marine organisms, and cystine was not found in amino acid analyses of collagen material in other studies (Iswariya et al., 2018) The amino acid content of proline and alanine of collagen obtained from *D. setosum* is expected values for sea urchin, which is a tropical and subtropical species and explains the higher denaturation temperatures compared to cold-climate species.

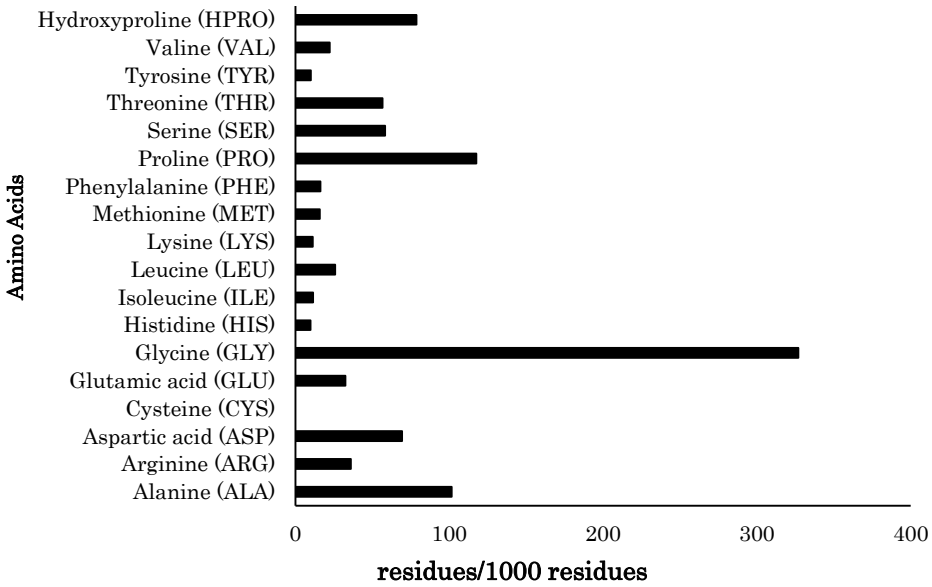

Figure 4 Amino acid composition of sea urchin collagen.
Şekil 4 Denizkestanesi kolajenin amino asit kompoziyonları

In the present study, proline and hydroxyproline contents of the collagen extracted from sea urchin were determined to be 117.5 and 78.6 residues/1000 residues, and the total imino acid content was determined to be 196.1 residues/1000 residues. Sea urchin collagen has higher than those of the Chrysaora sp. (149 residues/1000 residues) (Barzideh et al., 2014), *A. ijimai* (84 residues/1000 residues), *Strongylocentrotus nudus* (86 residues) residues/1000 residues) and *Strongylocentrotus intermedius* (85 residues/1000 residues) sea urchin species (Omura et al., 1996). Imino acid provides structural integrity in collagen. It has been known that the reason for the different amounts of imino acid content is due to the habitat differences in which the animals live and different habitat temperatures.

FTIR spectrum

The FTIR spectrum analysis of *D. setosum* collagen is presented in Figure 5. The positions of characteristic Amide A, Amid B, Amid I, Amid II, and Amid III bands are shown in Table 2. The IR spectrum results of sea urchin collagen showed the typical bands of Amide A, Amide B, Amide I, Amide II and Amide III for Type I collagen. The amide A band is generally associated with the extending vibrations of the N-H group. A free N-H extending vibration occurs in the range of between 3400 and 3440 cm^{-1}. The Amide A band of collagen extracted from *D. setosum* was determined to be 3301 cm^{-1} (Table 2). A shift of the wavenumber to a lower frequency, i.e., 3300 cm^{-1} of the N-H extending vibration, typically indicates that the N-H group has more hydrogen bonds (Gharagheshlagh et al., 2020).

Table 2 The major peak assignments of the FTIR spectra for collagen from *D. setosum.*
Çizelge 2 D. setosum kolajenin FTIR spektrumu için major pikleri.

Region	Peak wavenumber (cm^{-1})	Assignment
Amide A	3301	N-H stretch and H bond
Amide B	2924	CH2 asymmetric stretch
Amide I	1643	C=O stretch/hydrogen bond coupled with COO
Amide II	1550	NH bond coupled with CN stretch
Amide III	1242	NH bond coupled with CN stretch

The Amid B band, which corresponds to the asymmetric extending of the alkanyl C-H group and represents the NH_3^+ group, is observed at a wavelength of between 2850 and 2950 cm^{-1}. In the present study, the Amid B band was observed at 2924 cm^{-1} in the sea urchin collagen. The amide I band has characteristic frequencies of between 1600 and 1700 cm^{-1}, is associated with extending vibrations of the carbonyl group (C=O), and is the most important factor in determining the secondary structure of a protein. The Amide I band was observed at 1643 cm^{-1} in *D. setosum* collagen. The amide II band is associated with the N-H bond due to C-N extending vibrations in the range of between 1550 and 1600 cm^{-1} and its shift to

lower wavelengths indicates a hydrogen bond formation. The Amide II band of collagen obtained from the soft tissues of sea urchins was determined to be 1550 cm^{-1} (Figure 5).

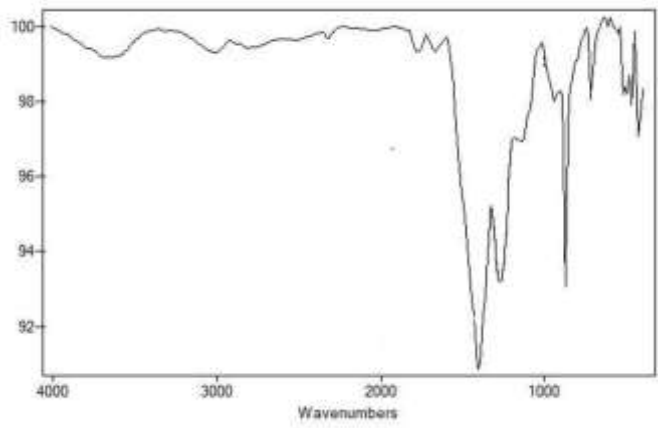

Figure 5 The FTIR spectrum of collagen from *D. setosum*.
Şekil 5 D. setosum kolajenin FTIR spektrumu

Finally, the Amide III band has a characteristic frequency between 1236 cm^{-1} and 1452 cm^{-1}. It shows the combination levels between C-H extending vibrations and N-H deformation of triple helix collagen and is considered a collagen fingerprint. The Amide III band was observed at 1242 cm^{-1} in *D. setosum* collagen. The present study determined that the FTIR results of collagens obtained from different marine organisms were similar to those reported in the literature (Ahmad & Benjakul, 2010; Ali et al., 2018; Nurubhasha et al., 2019; Gharagheshlagh et al., 2020). The FTIR spectrum confirmed that *D. setosum* collagen has a natural triple helix structure.

Scanning Electron Microscope (SEM)

The morphological structure of collagen from *D. setosum* is shown in Figure 6. To the naked eye, collagen is soft, white, and porous. However, examined by SEM, it was determined that it consisted of interconnected, multilayered, scaly, dense, and irregular layers (Figure 6). Due to the excellent properties of collagen, its three-dimensional structure is of great importance. In the present study, the morphological structure of collagen obtained from sea urchin was investigated for the first time and it was concluded that it can be utilized in many fields such as cosmetics, tissue engineering, and biomedicine.

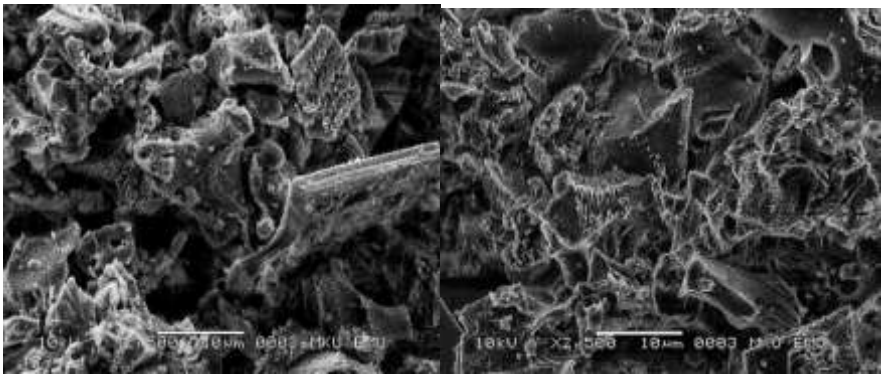

Figure 6. The SEM images of collagen from *D. setosum.*
Şekil 6. D. setosum kolajenin SEM görüntüleri

CONCLUSION

Collagen material was obtained for the first time from *D. setosum* sea urchin. The collagen obtained from *D. setosum* was similar to Type I collagen, which has a wide application area. Invasive sea urchin collagen showed similarities to those of terrestrial vertebrates and marine species, and it was found that it can be used as an alternative source of marine collagen. It can be proposed that alternative marine collagen extracted from *D. setosum* can be used as a biomaterial in fields such as biomedicine, tissue engineering, and cosmeceuticals.

As a result, using the sea urchin, an invasive species, as a collagen material will provide a high added value to Türkiye's economy by meeting the need for collagen biomaterial, which has been a popular research topic recently. On the other hand, in the case of its use in different industrial areas, *D. setosum* will be needed on an industrial scale, and its catch volume will eventually increase. Thus, the sea urchin population will be indirectly controlled. It will be ecologically beneficial and its dangerous situation in terms of tourism will be eliminated.

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Researchers' Contribution Rate Declaration Summary

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

Conflicts of Interest Statement

Authors have declared no conflict of interest.

REFERENCES

Ahmad, M., & Benjakul, S. (2010). Extraction and characterisation of pepsin-solubilised collagen from the skin of unicorn leatherjacket (*Aluterus monocerous*). *Food Chemistry 120*(3), 817-824. doi: 10.1016/j.foodchem.2009.11.019

Ali, A.M.M., Benjakul, S., Prodpran, T., & Kishimura H (2018). Extraction and characterisation of collagen from the skin of golden carp (*Probarbus jullieni*), a processing by-product. *Waste Biomass Valorization 9*(5), 783-791. doi: 10.1007/s12649-017-9841-0

AOAC (2000). *Official Methods of Analysis of the AOAC International* (17th). Association of Official Analytical Chemists, Washington DC, USA. p.

Artüz, M.L., & Artüz, O.B. (2019). First and northernmost record of *Diadema setosum* (Leske, 1778) (Echinodermata: Echinoidea: Diadematidae) in the Sea of Marmara. *Thalassas: An International Journal of Marine Sciences 35*(2), 375-379. doi: 10.1007/s41208-019-00137-3

Asaduzzaman, A. K. M., Getachew, A. T., Cho, Y. J., Park, J. S., Haq, M., & Chun, B. S. (2020). Characterization of pepsin-solubilised collagen recovered from mackerel (Scomber japonicus) bone and skin using subcritical water hydrolysis. *International journal of biological macromolecules, 148*, 1290-1297.

Barzideh, Z., Latiff, A.A., Gan, C.Y., & Benjakul, S (2014). Karim AA. Isolation and characterisation of collagen from the ribbon jellyfish (C hrysaora sp.). *International Journal of Food Science & Technology 49*(6), 1490-1499. doi: 10.1111/ijfs.12464

Bilecenoğlu, M., Yokes, M.B., & Draman M (2019). The invasive sea urchin *Diadema setosum* provides shelter for coastal fish–first observations from the Mediterranean Sea. *Zoology in the Middle East, 65*(2), 183-185. doi: 10.1080/09397140.2019.1571746

Bligh, E.G., & Dyer, W.J. (1959). A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology, 37*(8), 911-917.

Çınar, M.E., Bilecenoğlu, M., Yokeş, M.B., Öztürk, B., Taşkin, E., Bakir, K., Dogan, A., & Açık, Ş (2021). Current status (as of end of 2020) of marine alien species in Turkey. *Plos One 16*(5), e0251086. doi: 10.1371/journal.pone.0251086

Cluzel, C., Lethias, C., Garrone, R., & Exposito JY (2000). Sea urchin fibrillar collagen 2α chain participates in heterotrimeric molecules of (1α)22α stoichiometry. *Matrix Biology, 19*(6), 545-547. doi: 10.1016/S0945-053X(00)00109-8

Dimova, N (2003). RP-HPLC analysis of amino acids with UV-detection. Comptes rendus *de l'Académie bulgare des Sciences, 56*(12), 12-75. 2003CRABS..56l..75D

Ferrario, C., Rusconi, F., Pulaj, A., Macchi, R., Landini, P., Paroni, M., Colombo, G., Martinello, T., Melotti, L., Gomiero, C., Candia Carnevali, M.D., Bonasoro, F., Patruno, M., & Sugni, M. (2020). From food waste to innovative biomaterial: Sea urchin-derived collagen for applications in skin regenerative medicine. *Marine drugs, 18*(8), 414.

Fitriyani, F., Santoso, D., & Karnan K (2022). Abundance and Distribution Patterns of Sea Urchins (Echinoidea) at Lakey Beach, Hu'u District, Dompu Regency. *Jurnal Biologi Tropis, 22*(1), 277-288. doi: 10.29303/jbt.v22i1.3119

Flammang, P., Warnau, M., Temara, A., Lane, D.J., & Jangoux M (1997). Heavy metals in *Diadema setosum* (Echinodermata, Echinoidea) from Singapore coral reefs. *Journal of Sea Research, 38*(1-2), 35-45. doi: 10.1016/S1385-1101(97)00033-6

Gharagheshlagh, S.N., Fatemi, M.J., Jamili, S., Sharifi, A.M., & Nourani MR (2020). Isolation and characterization of acid-soluble collagen from the skin of *Rutilus Frisii* Kutum (Kamensky) of the Caspian Sea. *Iranian Journal of Fisheries Sciences, 19*(2), 768-779. doi: 10.22092/ijfs.2019.118957

Hüseyinoğlu, M.F., Demir, V., Arda, Y., Draman, M., & Yokes MB (2021). Spatio-temporal distribution of lionfish, *Pterois miles* (Bennett, 1828) in Kas-Kekova special environmental protected area, Turkey. *Estuarine, Coastal and Shelf Science, 254*, 107331. doi: 10.1016/j.ecss.2021.107331

Iswariya, S., Velswamy, P., & Uma TS (2018). Isolation and characterization of biocompatible collagen from the skin of puffer fish (*Lagocephalus inermis*). *Journal of Polymers and the Environment, 26*(5), 2086-2095. doi: 10.1007/s10924-017-1107-1

Kayhan, F.E., & Yön Ertuğ, N.D. (2021). Invasive Toxic Pufferfish (*Lagocephalus sceleratus* Gmelin, 1789) in Turkish Coasts. *Doğanın Sesi 8*, 35-46 (in Turkish with an abstract in English).

Kozlowska, J., Sionkowska, A., Skopinska-Wisniewska, J., & Piechowicz, K. (2015). Northern pike (Esox lucius) collagen: Extraction, characterization and potential application. *International journal of biological macromolecules, 81*, 220-227.

Kumar, N.S., Nazeer, R.A., & Jaiganesh R (2012). Wound healing properties of collagen from the bone of two marine fishes. *International Journal of*

Peptide Research and Therapeutics, 18(3), 185-192. doi: 10.1007/s10989-012-9291-2

Lessios, H.A., Kessing, B.D., & Pearse JS (2001). Population structure and speciation in tropical seas: global phylogeography of the sea urchin Diadema. *Evolution, 55*(5), 955-975. doi: 10.1111/j.0014-3820.2001.tb00613.x

Li, P.H., Lu, W.C., Chan, Y.J., Ko, W.C., Jung, C.C., Le, T.D., & Ji YX (2020). Extraction and characterization of collagen from sea cucumber (*Holothuria cinerascens*) and its potential application in moisturizing cosmetics. *Aquaculture 515*, 734590. doi: 10.1016/j.aquaculture. 2019. 734590

Liu, D., Liang, L., Regenstein, J. M., & Zhou, P. (2012). Extraction and characterisation of pepsin-solubilised collagen from fins, scales, skins, bones and swim bladders of bighead carp (Hypophthalmichthys nobilis). *Food Chemistry, 133*(4), 1441-1448.

Marimuthu, K., Gunaselvam, P., Rahman, A., Xavier, R., Arockiaraj, J., Subramanian, S., Yusoff, F.M., & Arshad A (2015). Antibacterial activity of ovary extract from sea urchin *Diadema setosum*. *European Review for Medical and Pharmacological Sciences, 19*(10), 1895-1899.

Muthumari, K., Anand. M., & Maruthupandy M (2016). Collagen extract from marine finfish scales as a potential mosquito larvicide. *Protein Journals, 35*(6), 391-400. doi: 10.1007/s10930-016-9685-7

Nagai, T., & Suzuki N (2000). Partial characterization of collagen from purple sea urchin (*Anthocidaris crassispina)* test. *International Journal of Food Science & Technology, 35*(5), 497-501. doi: 10.1046/j.1365-2621.2000.00406.x

Nurubhasha, R., Kumar, N.S., Thirumalasetti, S.K., Simhachalam, G., & Dirisala VR (2019). Extraction and characterization of collagen from the skin of *Pterygoplichthys pardalis* and its potential application in food industries. *Food Science and Biotechnology 28*(6), 1811-1817. doi: 10.1007/ s10068-019-00601-z

Nurilmala, M., Pertiwi, R.M., Nurhayati, T., Fauzi, S., Batubara, I., & Ochiai Y (2019). Characterization of collagen and its hydrolysate from yellowfin tuna *Thunnus albacares* skin and their potencies as antioxidant and antiglycation agents. *Fisheries Science, 85*(3), 591-599. doi: 10.1007/s12562-019-01303-5

Omura, Y., Urano, N., & Kimura S (1996). Occurrence of fibrillar collagen with structure of (α1) 2α2 in the test of sea urchin *Asthenosoma ijimai. Comparative Biochemistry and Physiology B, 115*(1), 63-68. doi: 10.1016/0305-0491(96)00085-5

Rahman, M.A., Amin, S.M.N., Yusoff, F.M., Arshad, A., Kuppan, P., & Nor Shamsudin M (2012). Length weight relationships and fecundity estimates of long-spined sea urchin, *Diadema setosum,* from the Pulau Pangkor, Peninsular Malaysia. *Aquatic Ecosystem Health & Management, 15*(3), 311-315. doi: 10.1080/14634988.2012.712027

Senaratne, L.S., Park, P.J., & Kim SK (2006). Isolation and characterization of collagen from brown backed toadfish (*Lagocephalus gloveri*) skin. *Bioresource Technology, 97*(2), 191-197. doi: 10.1016/j.biortech. 2005.02.024

Sivakumar, P., & Chandrakasan, G (1998). Occurrence of a novel collagen with three distinct chains in the cranial cartilage of the squid Sepia officinalis: comparison with shark cartilage collagen. *Biochimica et Biophysica Acta (BBA)-General Subjects, 1381*(2), 161-169.

Shimadzu Corporation, Instructions, Phenylisothiocyanate (PITC), 26922, Pierce Chemical Company, 6/1999, U.S.A

Shimizu, K., Amemiya, S., & Yoshizato K (1990). Biochemical and immunological characterization of collagen molecules from echinothurioid sea urchin *Asthenosoma ijimai*. *Biochimica et Biophysica Acta (BBA) - Protein Structure and Molecular Enzymology, 1038*(1), 39-46. doi: 10.1016/0167-4838(90)90007-3

Sulaiman, A.W., & Sarbon NM (2020). Characterization of acid soluble collagen (ASC) and pepsin soluble collagen (PSC) extracted from shortfin scad (*Decapterus macrosoma*) waste. *Food Research, 4*(6), 2272-2280. doi: 10.26656/ fr.2017.4(6).267.

Tulandi, S.M., Tanzil, L., & Ulfa DM (2021). Analysis of Bioactive Compounds from Methanol Extract of Diadema setosum Sea Urchin Gonads using Gas Chromatography-Mass Spectrometry. *Research Journal of Pharmacy and Technology, 14*(3), 1629-1634. doi: 10.5958/0974-360X.2021.00289.4

Turan, C., Erguden, D., & Uygur N (2011). On the occurrence of *Diadema setosum* (Leske, 1778) in Antakya Bay, Eastern Mediterranean Sea. *Journal of Black Sea/Mediterranean Environment, 17*(1), 78-82.

Trotter, J.A., & Koob TJ (1994). Biochemical characterization of fibrillar collagen from the mutable spine ligament of the sea-urchin *Eucidaris tribuloides*. *Comparative Biochemistry and Physiology B, 107*(1), 125-134. doi: 10.1016/0305-0491(94)90234-8

Uğurlu, E., & Duysak, Ö. (2022). A study on the extraction of chitin and chitosan from the invasive sea urchin Diadema setosum from Iskenderun Bay in the Northeastern Mediterranean. Environmental Science and Pollution Research, 1-9. https://doi.org/10.1007/s11356-022-23728-9

Veeruraj, A., Arumugam, M., & Balasubramanian, T. (2013). Isolation and characterization of thermostable collagen from the marine eel-fish (Evenchelys macrura). Process biochemistry, 48(10), 1592-1602.

Yapıcı, S (2018). Unusual observation of the alien sea urchin Diadema setosum (Leske, 1778) in the Aegean Sea: recent and recorded occurrences. Thalassas: An International Journal of Marine Sciences, 34(2), 267-269. doi: 10.1007/ s41208-017-0060-z

Yokes, B., & Galil BS (2006). The first record of the needle-spined urchin *Diadema setosum* (Leske, 1778) (Echinodermata: Echinoidea: Diadematidae) from the Mediterranean Sea. *Aquatic Invasions, 1*(3), 188-190. doi: 10.3391/ai.2006.1.3.15.

Zhang, J., Duan, R., Huang, L., Song, Y., & Regenstein, J. M. (2014). Characterisation of acid-soluble and pepsin-solubilised collagen from jellyfish (Cyanea nozakii Kishinouye). *Food Chemistry, 150*, 22-26.

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Investigating Relationships Between Catch Per Unit Effort (*CPUE*) and Some Angler Characteristics in the Turkish Inland Recreational Fisheries: A Case Study from Uluabat Lake

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ABSTRACT

In this study it is aimed that determining effect of some social and demographic properties on Catch Per Unit Effort (*CPUE*) in Uluabat Lake anglers. A total of 375 interview applied with angler from April 2015 to May 2016 monthly period. Mean *CPUE* values of each angler was estimated as 1.36 fish/hr (0.08-5.67). Generalized additive models (*GAMs)* was used for evaluating the data set. Variables used in model were age of angler (*X1*), experience of angler (*X2*), annual total fishing day (*X3*), total value of fishing equipment (*X4*), monthly total income of anglers (*X5*), household number of anglers (*X6*) and *CPUE* of anglers (*Y*). Effect of all variables on the *CPUE* were founded not significant ($p>0.05$), except "Annual total fishing day" ($p>0.05$). The variables such as monthly total income of anglers, experience of angler, total value of angling equipment and annual total fishing day positively affected *CPUE*.

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Bazı Amatör Balıkçı Özelliklerinin, Türkiye İç Su Amatör Balıkçılığında Birim Çabaya Düşen Av Miktarı (*CPUE*) İle İlişkisinin Araştırılması: Uluabat Gölü Örneği

ÖZET

Bu çalışmada Uluabat Gölü amatör balıkçılarının bazı sosyal ve demografik özelliklerinin Birim Çabaya Düşen Av Miktarı (*CPUE*) üzerine etkilerinin belirlenmesi amaçlanmıştır. Nisan 2015 ile Mayıs 2016 döneminde aylık olarak yürütülen saha çalışmalarında amatör balıkçılar ile toplam 375 anket yapılmıştır. Ortalama CPUE her bir balıkçı için 1.36 balık/saat (0.08 – 5.67) olarak tahmin edilmiştir. Verilerin değerlendirilmesinde *Genelleştirilmiş Eklemeli Model* kullanılmıştır. Modelde kullanılan değişkenler; balıkçı yaşı (*X1*), balıkçı tecrübesi (*X2*), yıllık toplam avcılık günü (*X3*), balıkçılık ekipmanlarının toplam değeri (*X4*), amatör balıkçının aylık gelir durumu *(X5*), hanedeki kişi sayısı (*X6*) ve *CPUE* (*Y*)' dir. Yıllık toplam avcılık günü (*X3*) hariç diğer tüm değişkenlerin *CPUE* üzerindeki etkisi istatistiksel olarak önemli bulunmuştur ($p<0.05$). Amatör balıkçıların aylık gelir düzeyleri, balıkçılık ekipmanlarının toplam değeri ve yıllık toplam avcılık günü *CPUE* değeri üzerinde olumlu etki göstermiştir.

Su Ürünleri

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INTRODUCTION

In the global perspective, "*recreational fishing is defined as fishing of aquatic animals (mainly fish) that do not constitute the individual's primary resource to meet basic nutritional needs and are not generally sold or otherwise traded on export, domestic or black markets*" (FAO, 2012). This term (recreational fishing) identified as "A type of fisheries activity aimed for recreation, sport or vacation, there is no goal of financial and commercial gain and caught fish not sold" in scope of Turkish national fisheries legislation (Anonymous, 2020). That is very popular activity both global and national scale. Estimated number of recreational fishers in global scale vary widely from 220 million (World Bank, 2012) to 700 million (Cooke & Cowx 2004). Total number of registered Turkish

recreational fishers was reported as 245137 (Ateşşahin & Cilbiz, 2018). However, there is no legal registering obligation for recreational fishers in Turkey, so only 45.3 % angler registered (Ateşşahin & Cilbiz, 2018). Total global recreational catches is reported as 900 000 tonnes in 2014 by Freire et al. (2020). Same year total captured based commercial marine production was occurred as 81 549 353 tonnes and inland waters captured production was 11 895 881 tonnes (FAO, 2016). Angling is the most common recreational fishing technique in all around the world (Soykan & Cerim, 2018). To participants in recreational angling is referred as anglers (Arlinghaus et al., 2007).

Recreational fisheries have crucial effect on both the ecosystem and the economy in Türkiye (Soykan & Cerim, 2018). The total annual economic value of recreational fishing in Europe, which has a high market share, is estimated to be over €25 billion (Dillon, 2004; Pawson et al., 2008). Since the amount of fish produced by commercial fishing always have more importance for management authorities, managers have mostly pushed aside amateur fishing (Lloret et al., 2008). However, the decrease in fish stocks, in contrast to the increase in world population, has compelled managers to regulate amateur fishing. Management of amateur fisheries can be enhanced through cooperation between scientists, managers, and recreational fisheries (Dedual et al., 2013). In terms of management, it varies according to the development level of the countries.

One of the most important central management goal for both recreational and commercial fisheries is preventing of the overfishing (Allen et al., 2013). There are some legal regulations in Turkish inland recreational fishing for both limitation of the catch effort and protecting of the species, such as banned species, minimum landing size, limitation of caught (both number and weight), close season, banned area and location, limitation of fishing gear (most of them commercially used), banned fishing technique (explosive - chemical using etc…) number of fishing line (maximum four) and number of hook (maximum three for each fishing line), limitation of boat length (maximum 7.5 m) (Anonymous, 2020).

One of the most fundamental elements of fisheries management is undoubtedly Catch Per Unit Effort (*CPUE*). Any studies on the *CPUEs* of inland fishermen in Türkiye have been limited. However, numerous studies have been conducted on this subject in different parts of the world such as River Gallo - Spain (Almodóvar & Nicola, 1998); Kleiner Döllnsee, Germany (Kuparinen et al., 2010); Merced River - USA Wilberding and Hafs (2013); Lake Opinicon, Canada (Moraga et al., 2015); Karakaya Dam Lake, Türkiye (Ateşşahin et al., 2015; Ateşşahin & Cilbiz, 2019). The effect of the fishers' characteristics on *CPUE* has not been examined in most of these studies. In one of the rare studies conducted on this subject, Kuparinen et al. (2010) investigated some abiotic and fishing-related correlates on catch rates of pike (*Esox lucius*) in angling by using the generalized additive model (*GAM*). Scientific investigation of the reasons that push recreational fishers towards fishing more will be very useful for management of fisheries.

Uluabat Lake is one of the most rich lakes of Türkiye with plankton, bottom organism, aquatic plants, fishes & bird populations, where was announcement by Ministry of Environment as *RAMSAR* protected area at 1998 (Bulut et al., 2010). Shoreline of lake shows differences in a year connected with differences of the water depth. Uluabat Lake is located in Bursa province, which is fourth most crowded city of Türkiye with 3 million population. Lake is very close the Bursa city centrum (almost 40 km) so which have seriously potential in terms of recreational fisheries.

Besides amateur fishing, commercial fishing is also carried out by fishermen in Ulubalat Lake. 398 fishermen, with a mean age of 52, were using fiber-boats that were 6-7 meters in length and powered by 13 HP engines to fishing in the lake (Anonymous 2013). Gillnets, trammel nets, fyke nets and longlines are commonly used in fishing by fishermen. Commercial fisheries based annual fish production was almost 159.2 tonnes in 2022 (11.6 t *Cyprinus carpio*; 135.6 t *Carassius gibelio* and 12 t *Esox lucius*) in the lake.

The purpose of this study is to investigate the catch compositions, some socio-economic characteristics, and the effects of these variables on the *CPUE* of recreational fishers that are engaged in recreational fisheries at Lake Ulubat.

MATERIAL and METHODS

Study Area

The Uluabat Lake is located in north-western part of the Republic of Türkiye (Figure 1) It is ninth-largest lake of Türkiye with 160 km^2 surface area, average depth of the lake is 2.5 m (Yurtseven & Randhir, 2020).

Data collecting process

A face-to-face survey method was used for obtain of targeted data. Simple random sampling method was used for determining of the simple size. As a mass population (16207), official records of Bursa Directorate of Provincial Agriculture and Forestry was used. The following sampling formula [*1*] was used to compute the number of anglers to be surveyed (Elbek et al. 2006).

$$n=\frac{N.t^2p.q}{d^2.(N-1)+t^2p.q} \qquad I$$

Where;
N: mass population, ***t***: standard normal distribution value, ***d***: error value for

population, p: likelihood,
q: unlikelihood

The angler number was computed as 375 in the confidence interval with 95% and margin of error 5%. Questionnaire studies were conducted monthly (except the close seasons) between April 2015 and May 2016, and were administered to 375 amateur fishermen. Survived fishermen number and age information are given in Table 1. Used questionnaire forms were included in some question about social, economic and demographic status of angler besides applied fishing pressure on fish populations of lake.

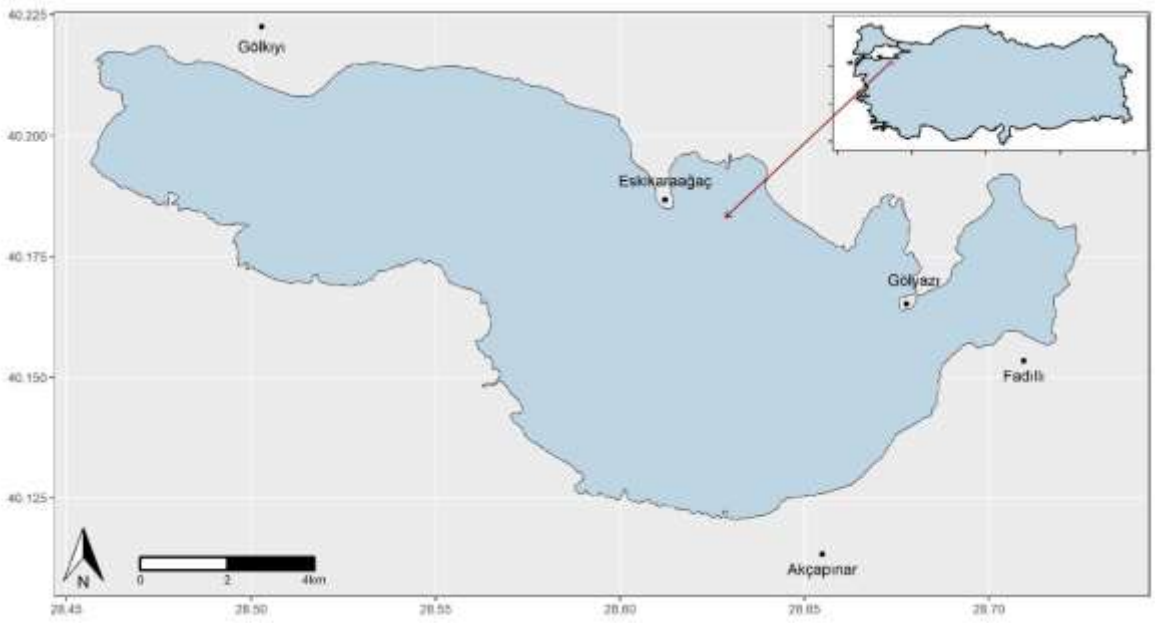

Figure 1. Uluabat Lake
Şekil 1. Uluabat Gölü

Table 1. Survived amateur fishermen numbers and its age distributions
Çizelge 1. Çalışmaya dahil olan amatör balıkçıların sayı ve yaş dağılımları

Month	N	Age		
		Min	Max	Mean
January	4	26	36	32
April	54	14	62	33.407
May	69	14	67	35.725
August	54	18	71	37.167
September	50	14	64	34.2
October	55	13	72	40.964
November	60	18	67	44.167
December	29	24	63	39.069

Estimating of *CPUE*

Catch Per Unit Effort (*CPUE*) was used evaluating of the fishing effect. Angler statements were based for fish production. Mean *CPUE* value was calculated for each angler with formula [*II*] given below (Aydın, 2011; Godøy et al., 2003):

$$CPUE = \frac{\sum n}{\sum Hook\ number\ x \sum (fishing\ trials\ x\ angling\ time)} \quad II$$

Modelling approaches

The effect of variables on the *CPUE*, was examined by means of Generalized additive models, (*GAMs*) techniques (Hastie & Tibshirani, 1990). Restricted maximum likelihood (*REML*) was used as the smoothing parameter estimation method. The statistical modeling was performed in R software using the "mgcv" package v1.8-38 (Wood, 2003; Wood, 2004; Wood, 2011; Wood, Pya & Saefken, 2016, Wood, 2017). Six social, economic and fisheries-based covariates were considered for inclusion in the model, namely *X1* (Age of angler), *X2* (Monthly total income of anglers (*TL*), *X3* (Number of household members), *X4* (Experience of angler (year), *X5* (Total value of angling equipment (*TL*), *X6* (Annual total fishing day). The finally full model for analysing the *CPUE* (*Y*) data of anglers is represented as follows:

$$(Y1 \sim \beta_0 + s(X1, k=5) + s(X2, k=5) + s(X3, k=5) + s(X4, k=5) + s(X5, k=5) + s(X6, k=7)) + \varepsilon_j)$$

where β_0 is the intercept, ε_j is a random error term. *k*-

index values were optimised by using "gam.check ()" function, finally *GAMs* model distributional assumptions were met as possible as. Average exchange rate of dollar was 2.92 TL in survey period.

RESULTS

Survived fishermen number is shown significant differences by the month (Table 1.) We did not receive any questionnaires in February and March, and very few in January and December. This is because these months fall within the closed season for *E. lucius* fishing, the main target species for amateur fishermen. Due to this lack of homogeneity in the data across seasons, we were unable to use the season component in the *GAMs* analysis.

Angler profile

The anglers, who include in study, age range are change between 13 and 72, mean value (±SE) computed as 37±0.7 (Table 2). Fishing experience (year) of anglers are founded from 1 to 62, also mean experience (±SE) is estimated as 14±0.7 years. Most of angler were male (99.2%) and 74.7% of married. Considering educational status of the anglers, 44.3% of secondary school graduate and 26.1% of primary school graduate. Number of household members was founded between 1-14 while mean value (±SE) was computed as 3±0.07.

Table 2. Descriptive statistic some factors used in *GAMs* model
Çizelge 2. *GAMs* modelde kullanılan bazı faktörlerin tanımlayıcı istatistik bilgileri

Variables	*Code*	*Mean*	*Min.*	*Max.*	*Median*
Age of angler	*X1*	37	13	72	35
Monthly total income of anglers (*TL*)*	*X2*	1801.46	0.00	8000.00	1500.00
Number of household members	*X3*	3	1	14	4
Experience of angler (year)	*X4*	14	1	62	10
Total value of angling equipment (*TL*)*	*X5*	202.35	4.00	2000.00	100.00
Annual total fishing day	*X6*	13	3	111	9
CPUE of anglers (fish/hr)	*Y*	1.36	0.08	5.67	1

*Average exchange rate of dollar was 2.92 TL in survey period

CPUE

Mean *CPUE* values of each angler was estimated as 1.36 fish/hr (0.08-5.67). Estimated *CPUE* values were found to range between 0.08 – 5.66 fish/hr (mean 1.36 fish/hr), and the reason for this wide range may be the differences in preferred fishing point.

Cath compositions

Common carp (*Cyprinus carpio*), northern pike (*Esox lucius*) ve gibel carp (*Carassius gibelio*) were expressed by angler as main target due to more delicious and which have higher economic value relatively. These three species consist of 53.8% total catch (8.83%, 19.73% and 25.23% for *C. carpio*, *E. lucius* and *C. gibelio*, respectively). Remainder of the total catch (46.2%) arise from shemaya (*Alburnus chalcoides*), roach (*Rutilus rutilus*) ve rudd (*Scardinius erythrophthalmus*) that have fewer commercial value and less consumption in local community. To the question asked about the consumption of the catch, answered as completely consumed by 74.7% of anglers. On the one hand, remainder part (25.3%) was expressed that catch and released to the lake (*I*), gave to other angler end of the fishing trial (*II*) and sold (*III*). As Lake Uluabat is shallow, it is very difficult for amateur fishermen to fish from the shore due to lack of sufficient depth. In order to overcome this problem, amateur fishermen prefer to fish on the sides of streams entering or leaving the lake, where the depth of the lake is more suitable for fishing. Fishermen who own or rent a boat fish at the middle parts of the lake where there is more depth. This leads to serious differences between species composition and fishing yields. While fish with low economic value (*A. chalcoides, R. rutilus, S. erythrophthalmus*) are generally caught where the streams connect to the lake, more valuable species (*E. lucius, C. carpio*) are caught in the off shore.

Interaction between *CPUE* and angler characteristic

The scatter plot made to observe of interaction between variables used in *GAMs* models is given Figure 2. In generally it is seen that all correlations were observed as weak, besides all of them were founded as insignificant (p>0.05) except "*X4-X1", "X6-X1", "X5-X2", "X6-X4", "X6*-Y" compare. *CPUE* value is mainly shown a change between 0-2 n/hour, it is in increasing trend connected with increasing of the Annual total fishing day *(X6)* (Figure 2). The angler experience (*X4*) is shown increasing with rising of the Age of angler (*X1*). It is observed that monthly total income of anglers (*X2*) is positive effected on total value of angling equipment (*X5*) (Figure 2).

Used *GAMs* model parameters to compare *CPUE* and other response (such as, Age of angler, Monthly total income of anglers (*TL*), Number of household members, Experience of angler (year), Total value of angling equipment (*TL*) Annual total fishing day) are

given Table 3. Estimated total *d.f.*, *REML* score, *AIC* factor, *p* and β_0 value of used model were found as 12.49, 520.69, 1018.99, < 0.001 and 1.37 respectively. Only two response (*X1*- Age of angler and *X6*-Annual total fishing day) shown statistical difference by angler *CPUE*.

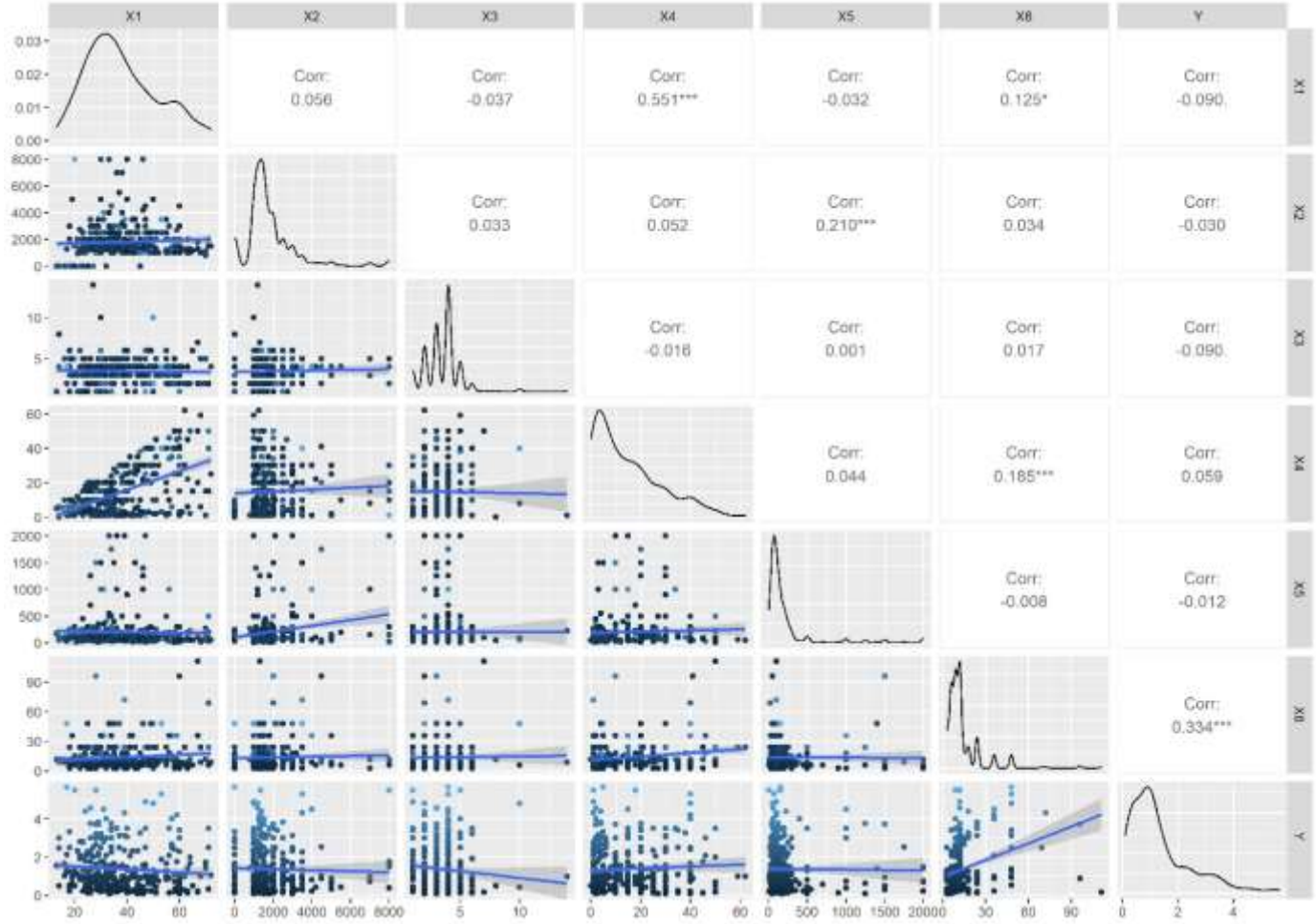

Figure 2. Scatter plot matrix for predictors used *GAMs* analysis
Şekil 2. *GAMs* analizinde kullanılan değişkenlerin saçılım grafiği matrisi

Table 3. Summary of the individual contribution of selected variables to the *CPUE* based *GAMs*
Çizelge 3. *CPUE*'ye dayalı *GAMs* için seçilen değişkenlerin bireysel katkılarına ilişkin özet bilgiler

Response	*df*	*F*	*p*
(*X1*)-Age of angler	1.285	3.936	0.049
(*X2*)-Monthly total income of anglers	1.967	2.504	0.099
(*X3*)-Number of household members	1.708	3.192	0.102
(*X4*)-Experience of angler	1.001	2.324	0.128
(*X5*)-Total value of angling equipment	1.001	0.715	0.398
(*X6*)-Annual total fishing day	5.624	21.526	<0.001

GAMs estimated effect of angler characteristics on *CPUE* for Uluabat Lake recreational fisheries are given Figure 3. Increasing of angler age have been caused negative effect on the *CPUE* (Figure 3-s(X1)). When monthly total income of anglers was fell into between 0-3500.00 TL (0-1198.63 $USD), it has shown that negative effect on *CPUE*. However, monthly total income has shown positive effect on *CPUE* when in the range of 3500.00 to 8000.00 TL (0-2739.73 $USD) (Figure 3-s(X2)). In generally number of household members effect on *CPUE* is founded as negative, it is modelled that there is no any effect of higher than 10 members of household on *CPUE* (Figure 3-s(X2)). A liner increasing is observed in *CPUE* on Figure 3-s(X4) by increasing of the experience of angler. Similarly, total value increasing of angling equipment reflected as positive on *CPUE* (Figure 3-s(X5)). Annual total fishing day is positive effected on *CPUE* as certain point ((~70 day), but after that point the effect turns negative (Figure 3-s(X6)).

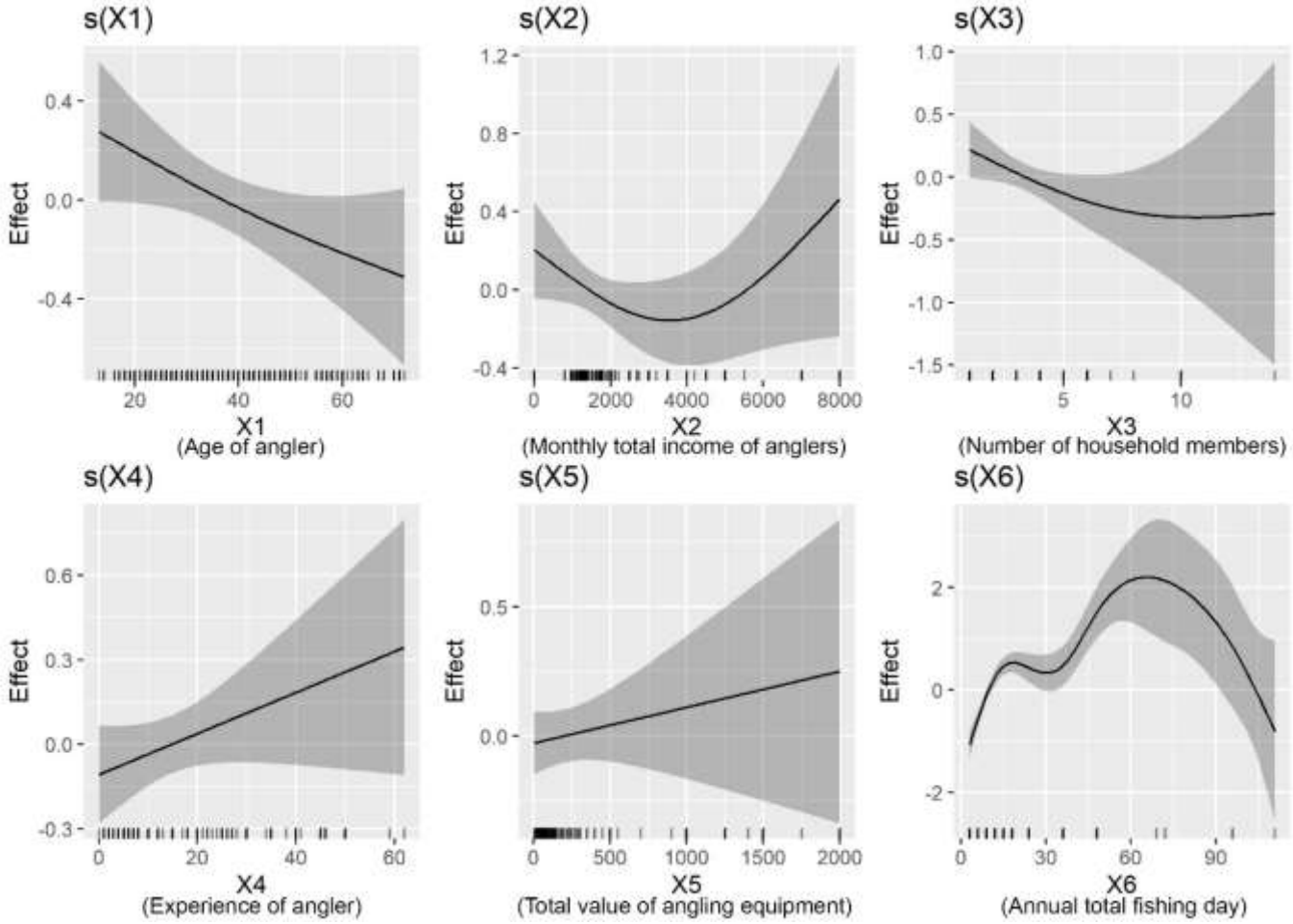

Figure 3. The relationships between *CPUE* and other factors from fitted *GAMs*
Şekil 3. *GAMs* tarafından uyarlanmış *CPUE* ve diğer faktörler arasındaki ilişkiler

DISCUSSION

Catch per unit effort (*CPUE*) is one of the basic components for effectively manage the fish stocks (Makwinja et al., 2021), so estimating of this value very important both commercial and recessional fisheries. In this study, mean *CPUE* values of each angler was estimated as 1.36 fish/hr (0.08-5.67). In other studies carried on inland waters, the *CPUE* value was found to be 1.59-2.06 fish/hr (for *Salmo trutta* in River Gallo - Spain) by Almodóvar and Nicola (1998); 1.27 fish/hr (for *Oncorhynchus spp.* in Merced River, California - USA) by Wilberding and Hafs (2013); 0.174 – 0.307 fish/hr (for *Oncorhynchus mykiss* in Karakaya Dam Lake, Türkiye) by Ateşşahin et al. (2015); 0.120- 0.136 fish/hr (for *Luciobarbus mysteceus* and *Luciobarbus esocinus* in Keban Dam Lake, Türkiye) by Ateşşahin (2021).When the average *CPUE* value of current research is compared with the values found in other studies, it is similar to the values reported by Almodóvar and Nicola (1998) and Wilberding and Hafs (2013), but it is higher than other studies. This may be due to the differences in the target species, fishing season, and the fishing area (Aydın & İlkyaz, 2021). In addition, it can be argued that factors such as the quantity of the target species in the fishing area and the fishing method have quite an effect on the fishing yield. The *Cyprinidae* family is generally found in the deeper parts of the lake away from the shore, except for during reproductive season, and the *CPUE* value of the anglers who fish at the shore during this period is very low. However, as the water warms up in the spring, these fish start to go closer to the shore where more vegetation is found due to their reproductive instinct, so the probability of catching them increases during this period, increasing the *CPUE* values of the anglers.

Age range of angles were determined as 13 – 72. In a study conducted by Ateşşahin and Cilbiz (2019) across Türkiye, the age range of inland anglers was reported to be 14 – 69. In this context, it can be stated that the age range of the inland angers at Lake Ulubat is quite compatible with those across Türkiye. In this study, which was conducted in the inland waters of Türkiye, it was reported that 74.7% of the survey participants used the fish they caught for nutritional purposes. Global freshwater systems that the consumption of

caught fish varies by species and country, despite the limited economic impact of recreational fishing worldwide, it remains an important source of nutrition for people in freshwater areas throughout the world (Embke et al. 2022).

In the study, it was found that a higher age has a negative effect on *CPUE* (Figure 3). This can be explained by the fact that young anglers tend to be more ambitious and eager. However, McCormick and Porter (2014) found in their study that younger anglers had lower fishing success compared to older angers in rainbow trout fishing, which is contrary to our findings. The difference between the two fishing areas may be due to the differences in used fishing methods. In addition, most of the older and retired anglers merely want to have fun in their free time, while young anglers, most of whom have jobs, use their free days to be able to go fishing. Because of this, it is possible that they want to make the most of the limited time they can spare for fishing.

The average monthly income was found to be 1801.46 TL (~616.94 $USD), and a monthly income in the range of 0-3500.00TL (0-1198.63 $USD) was found to have a negative effect on the *CPUE*, while a monthly income in the range of 3500.00 - 8000.00 TL (1198.63-2739.73 $USD) was found to have a positive effect. This may be due to the increase in the budget allocated for angling and purchasing and using more effective fishing equipment in parallel with the monthly income. For example, in the context of amateur fishing at Lake Ulubat, boats can be used for fishing in the deeper parts of the lake and not only the fish that come close to the shore, but also the fish in the deeper parts of the lake can be caught. Of course, only the amateur fishermen who have a higher income have the opportunity to invest in boats and can benefit from this. By Monk & Arlinghaus (2018), combination of fishing location and lure type may be an important predictor of angling success.

The number of household members was found to have a negative effect on *CPUE* in the range of 0 – 5, and it was found have no effect at higher numbers (Figure 3). This factor was included in the model to examine whether the number of people in the household who need to be provided has any effect on *CPUE*. The fact that the effect was found to be partially negative at the beginning and non-existent after a certain number may be an indication that amateur fishermen go fishing at Lake Ulubat for fun rather than catching a certain amount of fish. It was frequently observed that, especially crowded angler groups, fishing or not, turned the activity into a picnic (where they consume beverages and eat the food they brought).

The fishing experience of amateur fishermen has a clear positive effect on *CPUE* (Figure 3). It can be argued that the experience gained over time about matters regarding fishing gear, bait, fishing area, fishing time, etc., all of which are needed for maximum efficiency, are effective in this respect. According to the findings of Bellanger and Levrel (2017), amateur fishermen with more experience and more enthusiasm are likely to achieve higher yield rates, which is in line with our findings. Heermann et al. (2013) reported that "fishing experience had a large influence on angling success, with anglers having a long history of fishing (≥ 40 years) being the most successful."

In the study, a higher budget allocated for fishing gear was found to have a positive effect on *CPUE* (Figure 3). A higher budget may have a positive impact on catch yield since it allows purchasing modern and efficient gear or replacing worn-out gear. Pita et al. (2018) Galicia (Spain) reports that the budget allocated for fishing gear corresponds to approximately 31.6% of total angling expenses. As can be seen, the budget allocated for fishing gear is one of the most important expense items in angling activities.

While the total annual number of days spent fishing was found to have a positive effect on *CPUE* in the range of 0-65 days, it was found to have a negative effect after that (Figure 3). It can be argued that this situation is due to the ecological characteristics of the target species and some environmental factors (reproduction, migration, decrease in water level, commercial fishing conflict, etc.), and the fact that fishing is productive in some periods and unproductive in others. The fishermen, most of whom are local anglers, may prefer not to fish in unproductive periods, as they know which period is productive and which period is unproductive. The *CPUE* of less frequent angling activities carried out only in productive periods will naturally be higher than the *CPUE* of more frequent angling activities carried out in both productive and unproductive periods. Another important factor in this situation is the fishing activities of commercial fishermen. As the increase in fishing pressure during certain periods (for example, market demands, fish prices, weather conditions, etc.) will reduce the fish abundance in the lake, it is likely to have a negative impact on *CPUE* of the anglers. By Heermann et al. (2013), angling catchability of Eurasian perch (*Perca fluviatilis*) might depend on lake's nutrient status, size and morphometry, in addition it should also be influenced by other ecological factors, such as food availability or season.

In the study, it has been observed that Lake Uluabat receives a large influx of amateur fishermen depending on the season and especially on the weekends, due to being very close to one of the metropolitan cities of Türkiye. Angler *CPUE* is a reliable measure of fish population abundance (Erisman et al., 2011), and in this context, considering that the angler *CPUE* values estimated for Lake Ulubat are similar to the values found in other studies, it is thought that the exploitation rates of target species are similar.

Overfishing have occurred in commercial marine fisheries in terms of high-profile cases of recruitment, but it can also occur in freshwater recreational fisheries (Allen et al., 2013; Post et al., 2002). A recreational fisheries based on recruitment overfishing reported by (Sullivan 2003) from Alberta lakes (Canada) for walleyes (*Sander vitreus*)(Sullivan, 2003). In this direction, the *CPUE* data should be monitored regularly, especially in freshwater areas where both commercial and recreational fishery activities are carried out simultaneously. Additional measures may need to be taken to reduce fishing effort in order to protect stocks of target species.

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Statement of Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

Author's Contributions

The contribution of the authors is equal.

REFERENCES

Allen, M. S., Ahrens, R. N. M., Hansen, M. J., & Arlinghaus, R. (2013). Dynamic angling effort influences the value of minimum-length limits to prevent recruitment overfishing. *Fisheries Management and Ecology*, *20*(2-3), 247-257. https://doi.org/10.1111/j.1365-2400.2012.00871.x

Almodóvar, A., & Nicola, G. G. (1998). Assessment of a brown trout Salmo trutta population in the River Gallo (central Spain): Angling effects and management implications (Salmonidae). *Italian Journal of Zoology*, *65*(SUPPL.), 539-543. https://doi.org/10.1080/11250009809386881

Anonymous (2020) The circular No. 2020/21, regulating the amateur fisheries in 2020-2024, 17 pp., The Ministry of Agriculture and Forestry, Ankara, Türkiye, Official paper no: 31221 (In Turkish). https://www.mevzuat.gov.tr/ mevzuat?MevzuatNo=34822&MevzuatTur=9&MevzuatTertip=5

Anonymous (2013). A socio-ecomomic analysis of center and west anatolian inland fishermen. (Unpublished project final report). Republic of Türkiye, Ministry of Agriculture and Forestry, General Directory of Agricultural Research and Policies. 90p. Isparta.

Arlinghaus, R., Cooke, S. J., Lyman, J., Policansky, D., Schwab, A., Suski, C., Sutton, S. G., & Thorstad, E. B. (2007). Understanding the Complexity of Catch-and-Release in Recreational Fishing: An Integrative Synthesis of Global Knowledge from Historical, Ethical, Social, and Biological Perspectives. *Reviews in Fisheries Science*, *15*(1-2), 75-167. https://doi.org/10.1080/10641260601149432

Ateşşahin, T. (2021). The effect of artificial lure type and environment conditions on the short-time post-release mortality of two recreational fished Luciobarbus species. *Journal of Applied Ichthyology*. https://doi.org/10.1111/jai.14278

Ateşşahin, T., & Cilbiz, M. (2018). Amateur Fishing License in Turkish Inland Amateur Fishery'. *Journal of Limnology and Freshwater Fisheries Research*, *4*(2), 103-111. https://doi.org/10.17216/LimnoFish.374113

Ateşşahin, T., & Cilbiz, M. (2019). Some Socio-Demographic Characteristics of Inland Amateur Fishermen: Case of Turkey [in Turkish]. *Turkish Journal of Agriculture - Food Science and Technology*, *7*(1), 134-141.://doi.org/10.24925/turjaf.v7i1.134-141.2326

Ateşşahin, T., & Cilbiz, M. (2019). The effect of hook size, spinner colour and fishing season on catching efficiency in angling for rainbow trout, Oncorhynchus mykiss (Walbaum, 1792).*Pakistan Journal of Zoology*, *51*(5), 1937-1942.

Ateşşahin, T., Duman, E., & Cilbiz, M. (2015). Selectivity and Catch Efficiency of Three Spinner Hook Sizes in Angling for Rainbow Trout (*Oncorhynchus mykiss* Walbaum, 1792) in Karakaya Dam Lake (Eastern Turkey). *Turkish Journal of Fisheries and Aquatic Sciences*, *15*(4), 851-859. https://doi.org/10.4194/1303-2712-v15_4_08

Aydın, C., & İlkyaz, A. T. (2021). Catching Performance and Catching Efficiency of Siliconized Baits in Handline Fishery. *Journal of Agricultural Sciences (Tarim Bilimleri Dergisi)*, *27*(2), 219-230. https://doi.org/10.15832/ankutbd.606513

Aydın, İ. (2011). Is natural bait type a stochastic process for size and condition of fishes in the recreational fishery of Izmir Bay? *Mediterranean Marine Science*, *12*(2), 390-400. https://doi.org/10.12681/mms.39

Bellanger, M., & Levrel, H. (2017). A cost-effectiveness analysis of alternative survey methods used for the monitoring of marine recreational fishing in France. *Ocean & Coastal Management*, *138*, 19-28. https://doi.org/10.1016/j.ocecoaman.2017.01.007

Bulut, C., Ramazan, A., Uysal, K., Esengül, K., & Çınar, Ş. (2010). Uluabat Gölü yüzey suyu kalitesinin değerlendirilmesi. *Aquatic Sciences and Engineering*, *25*(1), 9-18.

Cooke S.J. & Cowx I.G. (2004) The role of recreational fisheries in global fish crises. BioScience 54, 857–859, https://doi.org/10.1641/0006-3568(2004)054[0857:TRORFI]2.0.CO;2

Dedual, M., Sague Pla, O., Arlinghaus, R., Clarke, A., Ferter, K., Geertz Hansen, P., Gerdeaux, D.,

Hames, F., Kennelly, S. J., Kleiven, A. R., Meraner, A., & Ueberschär, B. (2013). Communication between scientists, fishery managers and recreational fishers: lessons learned from a comparative analysis of international case studies. *Fisheries Management and Ecology*, *20*(2-3), 234-246. https://doi.org/10.1111/fme.12001

Dillon B. A bio-economic review of recreational angling for Bass (*Dicentrachus labrax*). Scarborough Centre for Coastal Studies, University of Hull, 2004.

Elbek A.G., Oktay E.& Saygı H. (2006) Basic statistic in aquaculture [in Turkish], İzmir, Ege Üniversitesi Yayımları, No:19. 308.

E Embke, H. S., Nyboer, E. A., Robertson, A. M., Arlinghaus, R., Akintola, S. L., Atessahin, T., Badr, L. M., Baigun, C., Basher, Z., Beard, T. D., Boros, G., Bower, S. D., Cooke, S. J., Cowx, I. G., Franco, A., Gaspar-Dillanes, M. T., Granada, V. P., Hart, R. J., Heinsohn, C. R., . . . Lynch, A. J. (2022). Global dataset of species-specific inland recreational fisheries harvest for consumption. Scientific Data, 9(1), 488. https://doi.org/10.1038/s41597-022-01604-y

Erisman, B. E., AllenLarry, G., ClaisseJeremy, T., Pondella, D. J., Miller, E. F., Murray, J. H., & Walters, C. (2011). The illusion of plenty: hyperstability masks collapses in two recreational fisheries that target fish spawning aggregations. *Canadian Journal of Fisheries and Aquatic Sciences*, *68*(10), 1705-1716. https://doi.org/10.1139/f2011-090

FAO. (2012). *Recreational Fisheries, FAO Technical Guidelines for Responsible Fisheries No. 13. Rome*.

FAO. (2016). *The State of World Fisheries and Aquaculture 2016. Contributing to food security and nutrition for all. Rome, 200*

Freire, K. M. F., Belhabib, D., Espedido, J. C., Hood, L., Kleisner, K. M., Lam, V. W. L., Machado, M. L., Mendonça, J. T., Meeuwig, J. J., Moro, P. S., Motta, F. S., Palomares, M.-L. D., Smith, N., Teh, L., Zeller, D., Zylich, K., & Pauly, D. (2020). Estimating Global Catches of Marine Recreational Fisheries. *Frontiers in Marine Science*, *7*(12). https://doi.org/10.3389/fmars.2020.00012

Godøy, H., Furevik, D., & Løkkeborg, S. (2003). Reduced bycatch of red king crab (Paralithodes camtschaticus) in the gillnet fishery for cod (Gadus morhua) in northern Norway. *Fisheries Research*, *62*(3), 377-384. https://doi.org/10.1016/S0165-7836(02)00281-3

Hastie, T. J., & Tibshirani, R. J. (1990). Generalized Additive Models, Monographs on Statistics and Applied Probability 43. Chapman & Hall (Vol. 9).

Heermann, L., Emmrich, M., Heynen, M., Dorow, M., König, U., Borcherding, J., & Arlinghaus, R. (2013). Explaining recreational angling catch rates of Eurasian perch, Perca fluviatilis: the role of natural and fishing-related environmental factors. *Fisheries Management and Ecology*, *20*(2-3), 187-200. https://doi.org/10.1111/fme.12000

Kuparinen, A., Klefoth, T., & Arlinghaus, R. (2010). Abiotic and fishing-related correlates of angling catch rates in pike (Esox lucius). *Fisheries Research*, *105*(2), 111-117. https://doi.org/10.1016/j.fishres.2010.03.011

Lloret J., Zaragoza N., Caballero D. & Riera V. (2008) Biological and socioeconomic implications of recreational boat fishing for the management of fishery resources in the marine reserve of Cap de Creus (NW Mediterranean). Fisheries Research 91, 252–259, https://doi.org/10.1016/j.fishres.2007.12.002.

Makwinja, R., Mengistou, S., Kaunda, E., Alemiew, T., Phiri, T. B., Kosamu, I. B. M., & Kaonga, C. C. (2021). Modeling of Lake Malombe Annual Fish Landings and Catch per Unit Effort (*CPUE*). *Forecasting*, *3*(1), 39-55. https://doi.org/10.3390/forecast3010004

McCormick, J. L., & Porter, T. K. (2014). Effect of Fishing Success on Angler Satisfaction on a Central Oregon Rainbow Trout Fishery: Implications for Establishing Management Objectives. *North American Journal of Fisheries Management*, *34*(5), 938-944. https://doi.org/10.1080/02755947.2014.932869

Monk, C. T., & Arlinghaus, R. (2018). Eurasian perch, Perca fluviatilis, spatial behaviour determines vulnerability independent of angler skill in a whole-lake reality mining experiment. *Canadian Journal of Fisheries and Aquatic Sciences*, *75*(3), 417-428. https://doi.org/10.1139/cjfas-2017-0029

Moraga, A. D., Wilson, A. D. M., & Cooke, S. J. (2015). Does lure colour influence catch per unit effort, fish capture size and hooking injury in angled largemouth bass? *Fisheries Research*, *172*, 1-6. https://doi.org/10.1016/j.fishres.2015.06.010

Pawson, M. G., Glenn, H., & Padda, G. (2008). The definition of marine recreational fishing in Europe. *Marine Policy*, 32(3), 339-350. https://doi.org/10.1016/j.marpol.2007.07.001

Pita, P., Hyder, K., Gomes, P., Pita, C., Rangel, M., Veiga, P., Vingada, J., & Villasante, S. (2018). Economic, social and ecological attributes of marine recreational fisheries in Galicia, Spain. *Fisheries Research*, *208*, 58-69. https://doi.org/10.1016/j.fishres.2018.07.014

Post, J. R., Sullivan, M., Cox, S., Lester, N. P., Walters, C. J., Parkinson, E. A., Paul, A. J., Jackson, L., & Shuter, B. J. (2002). Canada's Recreational Fisheries: The Invisible Collapse? *Fisheries*, *27*(1), 6-17. https://doi.org/10.1577/1548-8446(2002)027<0006:CRF>2.0.CO;2

Soykan, O., & Cerim, H. (2018). General Aspects of Recreational Angling and Some Estimations on Catch Amounts in Turkey. *Düzce University Journal of Science & Technology*, *6*, 1441-1452,

https://doi.org/10.29130/dubited.393756.

Sullivan, M. G. (2003). Active Management of Walleye Fisheries in Alberta: Dilemmas of Managing Recovering Fisheries. *North American Journal of Fisheries Management*, *23*(4), 1343-1358. https://doi.org/10.1577/M01-232AM

Wilberding, M. C., & Hafs, A. W. (2013). Angler catch-per-unit-effort in restored and reference sections of the Merced River, California: A preliminary analysis. *California Fish and Game*, *99*(3), 149-154. http://www.scopus.com/inward/record.url?eid=2-s2.0-84891946448&partnerID=40&md5=64d2626bbe9a8b1b13d7dfa12ef9a3ed

Wood S.N., Pya, N. & Saefken, B. (2016) Smoothing parameter and model selection for general smooth models (with discussion). *Journal of the American Statistical Association, 111*:1548-1575, https:// doi.org/10.1080/01621459.2016.1180986.

Wood, S.N. (2003) Thin-plate regression splines. Journal of the Royal Statistical Society (B) 65(1):95-114, http://www.jstor.org/stable/3088828.

Wood, S.N. (2004) Stable and efficient multiple smoothing parameter estimation for generalized additive models. Journal of the American Statistical Association. 99:673-686.

Wood, S.N. (2011) Fast stable restricted maximum likelihood and marginal likelihood estimation of semiparametric generalized linear models. Journal of the Royal Statistical Society (B) *73*(1):3-36, http://www.jstor.org/stable/41057423.

Wood, S.N. (2017) Generalized Additive Models: An Introduction with R (2nd edition). Chapman and Hall/CRC, https://doi.org/10.1201/9781315370279.

World Bank (2012) Hidden Harvest: The Global Contribution of Capture Fisheries. Report No. 66469-GLB. Washington, DC: International Bank for Reconstruction and Development, 71 pp.

Yurtseven, I., & Randhir, T. O. (2020). Multivariate assessment of spatial and temporal variations in irrigation water quality in Lake Uluabat watershed of Turkey. *Environmental Monitoring and Assessment*, *192*(12), 793. https://doi.org/10.1007/s10661-020-08723-2

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Aeromonas veronii biovar veronii Infection in Cultured European Seabass (*Dicentrarchus labrax*) in Türkiye

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ABSTRACT
Fish in aquaculture systems are susceptible to infectious agents as they are kept in a densely populated and often physically restricted environment. Genus *Aeromonas* include well-known pathogens, and the member *Aeromonas veronii* has been reported to cause diseases in both humans and animals, either as primary infection or as mixed infection with other pathogens. This study describes a low mortality epizootic caused by *Aeromonas veronii* biovar veronii in European seabass (*Dicentrarchus labrax*) farmed in the Aegean Sea. The gills, kidneys and livers of moribund fish were pale. Erythema, haemorrhage and superficial ulcerative lesions were detected on the skin. In addition, petechial haemorrhage was observed on the tongue, maxilla, and operculum. The spleen was enlarged and multiple granulomas were detected in both the kidney and the spleen. Some fish had skin depigmentation, ecchymosis in the liver, and a bloody exudate in the abdominal cavity. The intestinal walls were lined with a clear yellowish fluid. Twenty-nine motile, Gram-negative bacterial isolates were obtained from the internal organs of diseased fish. According to morphology, biochemical properties and 16S rRNA gene sequencing results, all isolates were identified as *Aeromonas veronii* bv. veronii. All isolates were resistant to amoxicillin and ampicillin, and sensitive to oxytetracycline, enrofloxacin, ciprofloxacin, florfenicol, and flumequine.

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Türkiye'de Yetiştiriciliği Yapılan Levrek Balıklarında (*Dicentrarchus labrax*) *Aeromonas veronii* biovar veronii Enfeksiyonu

ÖZET
Yetiştiricilik sistemlerindeki balıklar, stok yoğunluğu yüksek ve genellikle fiziksel olarak kısıtlı bir ortamda tutuldukları için enfeksiyöz ajanlara karşı hassastırlar. *Aeromonas* cinsinin içinde pek çok patojen tür içerdiği bilinmektedir ve bu cins üyelerinden *Aeromonas veronii* bakterisinin hem insanlarda hem de hayvanlarda birincil enfeksiyon şeklinde veya diğer patojenlerle karışık enfeksiyon olarak hastalıklara neden olduğu bildirilmiştir. Bu çalışma, Ege Denizi'nde yetiştirilen levrek balıklarında (*Dicentrarchus labrax*) *Aeromonas veronii* biovar veronii bakterisinin neden olduğu düşük ölüm oranına sahip bir epizootiği tanımlamaktadır. Hasta balıklarda solungaç, böbrek ve karaciğerin solgun olduğu tespit edilmiştir. Deride eritem, hemoraji ve yüzeyel ülseratif lezyonlar görülmüştür. Ayrıca dil, maksilla ve operkulumda peteşiyal hemoraji gözlenmiştir. Dalağın büyüdüğü ve hem böbrek hem de dalakta çoklu granülomalar tespit edilmiştir. Dalak ve böbrekte granülomlar gözlenmiştir. Bazı balıklarda deri depigmentasyonu, karaciğerde ekimoz ve karın boşluğunda kanlı eksüda görülmüştür. Bağırsak duvarları berrak ve sarımsı bir sıvıyla kaplı olarak bulunmuştur. Hasta balıkların iç organlarından 29 adet Gram negatif hareketli bakteri izolatı elde edilmiştir. Morfoloji, biyokimyasal özellikler ve 16S rRNA gen dizileme sonuçlarına göre tüm izolatlar *Aeromonas veronii* bv. veronii olarak tanımlanmıştır. Elde edilen

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Anahtar Kelimeler
Aeromonas veronii biovar veronii
Dicentrarchus labrax
Levrek balığı
Histopatoloji

izolatların amoksisilin ve ampisiline dirençli ve oksitetrasiklin, enrofloksasin, siprofloksasin, florfenikol ve flumekine duyarlı olduğu tespit edilmiştir.

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INTRODUCTION

Aquaculture is arguably the fastest growing industry in the global food animal production and this growth is expected to continue in the near future. According to the latest data published by FAO (the Food and Agriculture Organization), the total aquaculture production worldwide is 85.3 million tons and %66 of this production volume (approximately 56.3 million tons) is obtained from finfish farming. The trade volume from marine fish farming alone has a calculated value of approximately 14 billion US dollars (FAO, 2021).

The intensive culture condition that is required for this rapid growth and expansion cause, on the other hand, some negative effects on fish health. Among these, fish disease and mortality are notable factors that limit the production volume in aquaculture systems. In addition, although the mortality rate may be very low in some diseases, the external lesions caused by these diseases make the fish unmarketable. Many fish disease-causing bacteria have been identified to date. Most of them have been identified in and isolated from farmed species rather than from wild fish. Fish in aquaculture systems, unlike their wild counterparts, are kept in a densely populated and often physically restricted environment, making them much more susceptible to pathogens (Toranzo et al., 2005; Fryer & Rohovec, 2021).

Members of the *Aeromonas* genus are Gram-negative, facultative anaerobic, non-spore forming bacteria that are ubiquitous in both terrestrial and aquatic environments. But the genus also includes pathogens that have are well known to human and veterinary medicine (Brenner et al., 2005; Janda & Abbott, 2010). *Aeromonas veronii* was first isolated as a potential causative agent from a patient by Hickman-Brenner et al. (1987) and has been subsequently reported as a pathogen from many organisms. This bacterium has been isolated from many fish species to date, either as the primary agent or in mixed infections, including cyprinids (Rahman et al., 2002; Yu et al., 2010; Sun et al., 2016; Zhu et al., 2016), cichlids (Dong et al., 2017), members of Siluriformes (Rahman et al., 2002; Nawaz et al, 2010; Cai et al., 2012; Hoai et al., 2019), gilthead seabream (*Sparus aurata*) (Gashgari & Selim, 2015) and European seabass (*Dicentrarchus labrax*) (Smyrli et al, 2017). In Türkiye, *A. veronii* infections were reported in marine cages cultured European seabass in the Black Sea (Uzun & Oğut, 2015) and in the Aegean Sea (Tanrıkul & Dinçtürk, 2021).

The aim of the present study was to determine the likely cause of an epizootic that occurred in June in European seabass (*Dicentrarchus labrax*) cultured in the Aegean Sea.

MATERIALS and METHODS

The recorded mortality during this epizootic that affected European seabass (*Dicentrarchus labrax*) was less than %1. Ten moribund fish (approx. 250 g) were sampled from offshore floating cages and examined by standard procedures (Whitman, 2004). Sample material was taken from liver, spleen and kidney and streaked onto Tryptic Soy Agar (TSA) medium containing 1.5% NaCl and incubated at 22°C for 48 h. Pure bacterial cultures were obtained from the colonies on primary plates by repeated streaking. Tissue samples for histopathology were taken from gills, liver, kidney, spleen and processed after fixation in %10 buffered formalin solution and then embedded into paraffin blocks. Histological sections of 5µm were stained with hematoxylin and eosin, Ziehl-Neelsen staining, and examined by light microscopy (Culling, 1963).

The morphological and biochemical characteristics of the isolated bacteria were determined by routine laboratory methods including API 20E kits (bioMérieux). For bacterial identification, the isolates were inoculated into Marine Broth 2216 (Difco). After incubation overnight at 22°C, genomic DNA was extracted from the isolates by using the GeneJET Genomic DNA Purification Kit (Thermo) according to the manufacturer's instructions. A universal bacteria primer set S-D-Bact-0008-a-S-20 (5' AGAGTTTGATCCTGGCTCAG 3') and S-*-Univ-0536-a-A-18 (5' GWATTACCGCGGCKGCTG 3') were used to amplify a partial fragment of the 16S rRNA gene (Suau et al., 1999).

The PCR mixture included template DNA (approx. 50 ng) 0.4 µM of each primer, PCR master mix (2X) (Thermo Scientific) and DNase/RNase-free distilled water (Thermo Scientific). Amplification was done using a thermal cycler (Biometra, TAdvanced) programmed as follows: 95°C for 3 min (initial denaturation) followed by 30 cycles of amplification

(95°C for 30 s for denaturation, 56°C for 1 min for annealing, 72°C for 1 min for extension) and 72°C for 4 min for a final extension step. PCR products obtained from the amplification were visualized by gel electrophoresis [%1.5 agarose (w/v) in 1X TAE buffer, containing EtBr (0.5 µg ml^{-1})] and running for 45 min at 100 V. All PCR products were purified and sequenced in both directions by a local sequencing company. Editing and analysis of the sequences were performed in Bioedit v7.0.0 (Hall, 1999) using the BLASTN (v2.2.20) (Larkin et al., 2007) and ClustalX (v2.1) (Zhang et al., 2000) algorithms. All sequences obtained in this study have been deposited in the GenBank database under accession numbers OP522255-OP522261.

All bacterial isolates were also tested for antimicrobial susceptibility by the Kirby-Bauer disk diffusion method (Bauer et al., 1966). The isolates were plated onto Mueller-Hinton agar (Oxoid) with eight antimicrobial disks (Oxoid) (amoxicillin, ampicillin, enrofloxacin, ciprofloxacin, oxytetracycline, sulfamethoxazole/trimethoprim, florfenicol, flumequine) and then incubated at 20ºC for 48-96 h and the results were interpreted according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2010).

RESULTS

Gross Pathology

In the examined fish, the gills, kidneys and livers were pale. Erythema, haemorrhage and superficial ulcerative lesions in the skin, petechial haemorrhage on the tongue, upper jaw and operculum as well as in the visceral fat were observed (Figure 1a-d). The spleen was enlarged and had multiple abscesses (Figure 1e). Some fish had ecchymosis and fatty degeneration in the liver (Figure 1f), bloody exudates in the abdominal cavity, and a transparent intestinal wall. Other observations were yellowish liquid in the intestinal tract, multiple granulomas in the spleen (Figure 1g), and the kidney (Figure 1h).

Bacteriological Findings

A total of twenty-nine Gram-negative motile bacterial isolates were obtained from the visceral organs of ten diseased fish. According to their morphological and biochemical characteristics (Table 1) and 16S rRNA gene sequencing results (%100 similarity), all isolates were identified as *Aeromonas veronii* bv. veronii.

a

b

c

d

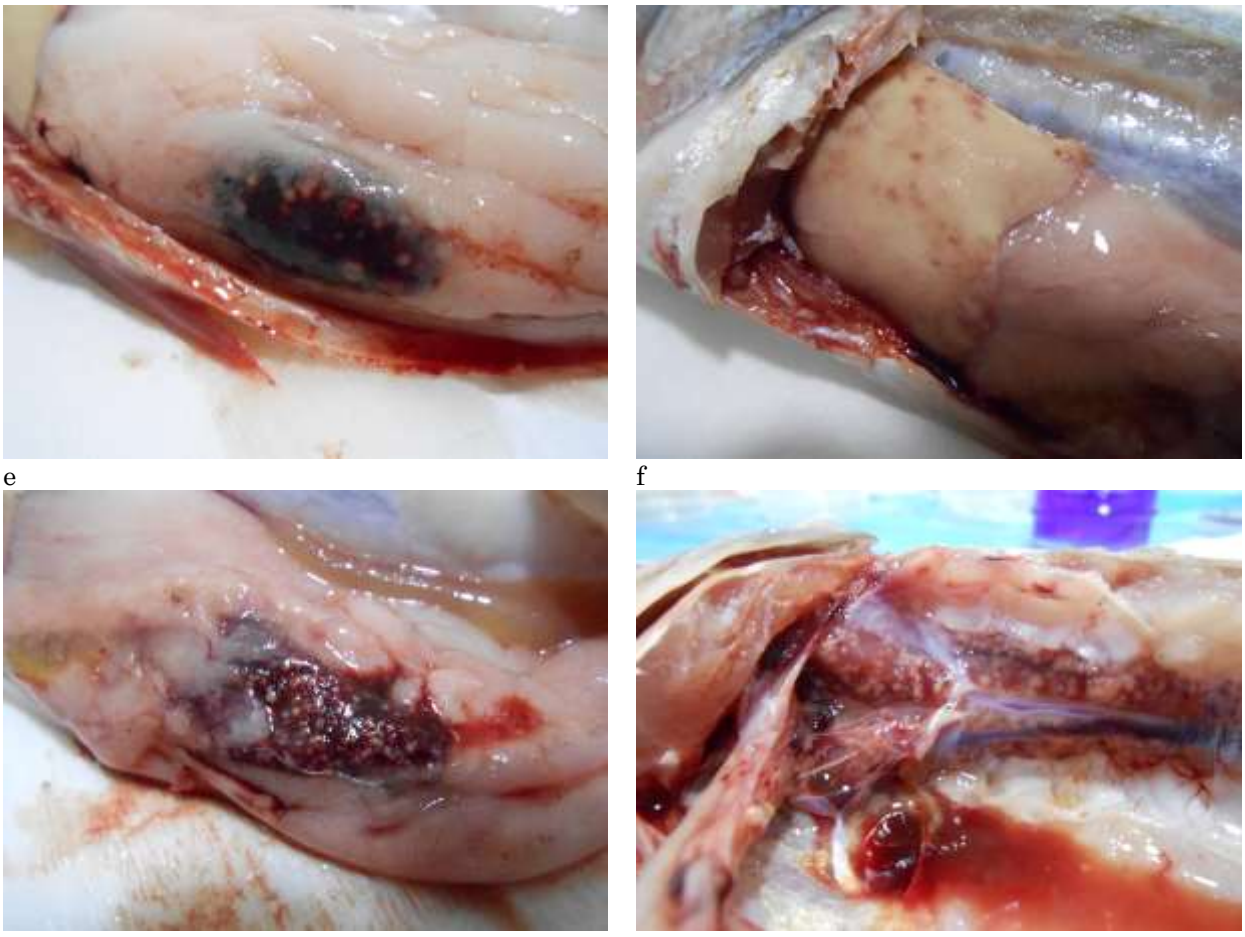


Figure 1. Moribund fish exhibited (a) corneal opacity, haemorrhage and superficial ulcerative lesions in the skin, (b) petechial haemorrhage on the tongue, (c) haemorrhage, haemorrhagic ulcers on the ventral side of the body and between the pelvic fins, mild prolapse, (d) haemorrhage on the upper jaw and operculum, (e) multiple abscesses in the enlarged spleen, (f) pale liver with ecchymosis, (g) transparent intestinal wall and granulomas in the spleen and (h) granulomas in the kidney.

Şekil 1. Hasta balıklarda balıklarda (a) deride korneal opaklık, hemoraji ve yüzeysel ülseratif lezyonlar, (b) dilde peteşiyal kanama, (c) kanama, vücudun ventral tarafında ve pelvik yüzgeçler arasında hemorajik ülserler, hafif prolapsus, (d) üst çene ve kapakçıkta kanama, (e) genişlemiş dalakta çoklu nodüller, (f) ekimoz ile soluk karaciğer, (g) şeffaf bağırsak duvarı ve dalakta granülomlar ve (h) böbrekteki granülomlar.

According to antimicrobial susceptibility testing results, all isolates were determined to be sensitive to oxytetracycline, enrofloxacin, ciprofloxacin, florfenicol and flumequine and to be resistant to amoxicillin and ampicillin as well as intermediate resistant against sulfamethoxazole/trimethoprim.

Histopathological Findings

Vacuolar degeneration, haemorrhage and necrosis in parenchyma cells of the liver, hemosiderin deposits and depletion of white blood cells in the spleen and hyperaemia and depletion of haemopoietic tissue in the anterior kidney were observed in histological sections. Other findings were melting lamellae, necrosis in the secondary gill lamellae and epithelial cell hyperplasia in the gills. Granulomas were observed in spleen tissue of some of the examined fish (Figure 2 a-f). These granulomas were stained with Ziehl-Neelsen acid-fast staining to determine if they contained any acid-resistant bacilli, but no bacilli were found (Figure 2g). In addition, a blood parasite, *Trypanosoma* sp., was detected in the histopathological spleen section in one fish.

DISCUSSION

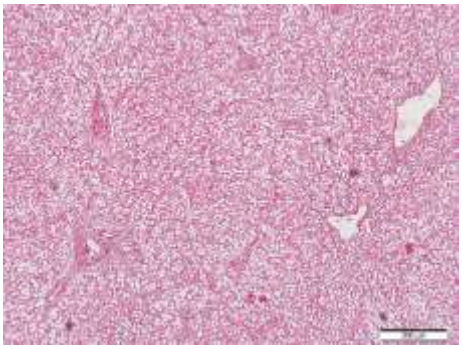
Motile Aeromonads (MAS) are commonly found in terrestrial and aquatic environments as natural members of both the environment and the microbiota of animals, including fish. In the aquaculture industry, this group of bacteria is regarded as opportunistic and often causes diseases under stressful conditions. Besides that, based on disease reports, *Aeromonas veronii* appears to have a wider host range than other fish pathogenic motile *Aeromonas* species (Smyrli & Katharios, 2020).

Table 1. Morphological and biochemical characteristics of the examined *Aeromonas veronii* isolates (n=29)
Çizelge 1. İncelenen Aeromonas veronii izolatlarının morfolojik ve biyokimyasal özellikleri (n=29)

Morphology	Rod	Growth on	
Motility	+	4°C	-
Gram staining	-	37°C	+
Cytochrome oxidase	+	44°C	-
Catalase	+	%0 NaCl	+
O/F	F	%1.5 NaCl	+
Indole	+	%3 NaCl	+
MR test	-	%5 NaCl	-
VP reaction	+	TCBS	+
B-Galactosidase	-	McConkey agar	+
0/129 (10µg)	R	Acid Production from	
0/129 (150µg)	R	Glucose	+
Arginine dihydrolase	+	Fructose	+
Lysine decarboxylase	+	Lactose	+
Ornithine decarboxylase	+	Sucrose	+
Nitrate reduction	+	Mannose	+
Esculin hydrolysis	+	Maltose	+
Citrate utilization	-	Inositol	-
Urease	+	Sorbitol	-
Production of H_2S	-	Arabinose	-
API 20E profile	716712757	Xylose	-

F, Fermentative; +, Positive; -, Negative; R, Resistant.

It is known that *A. veronii* isolates vary phenotypically and the species has been divided into two different biovars: *A. veronii* bv. veronii and *A. veronii* bv. sobria. Studies have been reported that *A. veronii* bv. sobria is negative for esculin hydrolysis and ornithine decarboxylase tests, whereas *A. veronii* bv. veronii is positive. The latter biovar is arginine dihydrolase negative but, produces acid from salicin and utilizes tartrate (Abbott et al., 2003). While degradation of urea, Voges-Proskauer reaction, esculin hydrolysis and



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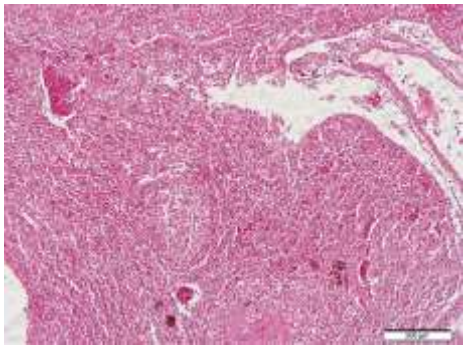

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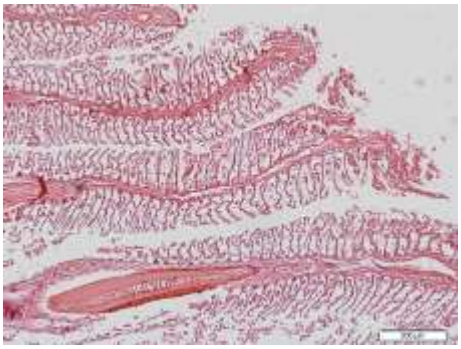

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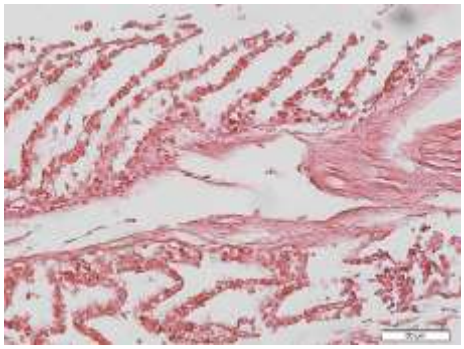

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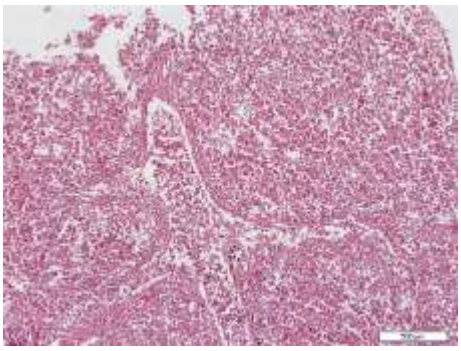

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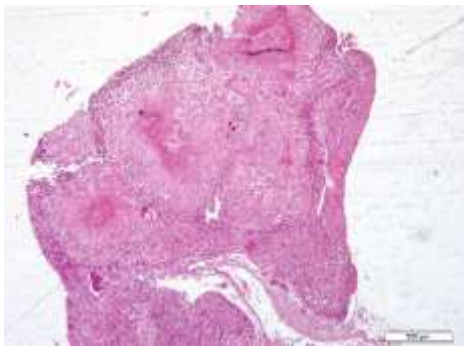

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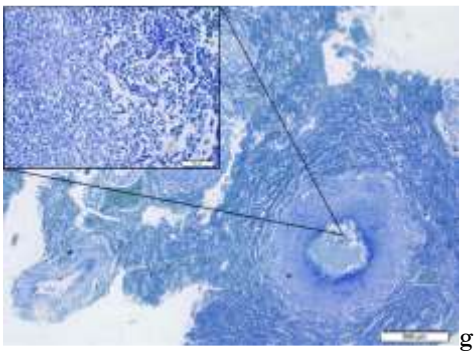

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Figure 2. Findings observed in photomicrographs of tissue sections: (a) vacuolation and necrosis in the parenchyma cells and haemorrhage in the liver, (b) hemosiderin deposits and depletion of white blood cells in the spleen, (c) melting lamella and epithelial cells hyperplasia in the gills (arrows), (d) fusion of the gill lamella (arrows), (e) hyperaemia and depletion of hematopoietic tissue in the anterior kidney, (f) granulomas (stars) in the spleen, (g) Ziehl-Neelsen negative staining result of granuloma in the spleen.

Şekil 2. Doku kesitlerinin fotomikrograflarında gözlenen bulgular (a) karaciğerde hemoraji ve parankim hücrelerinde vakuolasyon ve nekroz, (b) dalakta hemosiderin birikintileri ve beyaz kan hücrelerinde boşalma, (c) solungaç lamelalarında erime ve epitel hücre hiperplazisi (okla gösterildi), (d) lamellar füzyonu (okla gösterildi), (e) ön böbrekte hiperemi ve hematopoetik dokuda boşalma, (f) dalakta granülomlar (yıldızla gösterildi) ve (g) dalakta granülomanın Ziehl-Neelsen negatif boyama sonucunu.

ornithine decarboxylase were positive in agreement with previous reports, our arginine dihydrolase tests for the isolates were positive. Taken together with the 16S rRNA gene sequencing results, these isolates were identified as *A. veronii* bv. veronii (Janda & Abbott, 2010; Shameena et. al, 2020).

The studied disease outbreak occurred during summer (June), with elevated water temperature, similar to other reports in European seabass so far (Smyrli et al., 2019; Tanrıkul & Dinçtürk, 2021). Ulcers, abdominal distention, hemorrhage and in some cases exophthalmia and fin rot/tail rot are reported as a general clinical finding in disease (both in marine and freshwater species) caused by *A. veronii* (Smyrli & Katharios, 2020; Tanrıkul & Dinçtürk, 2021). However, clinical findings vary between biovar types and is dependent on isolate virulence, co-infections and fish host species (Smyrli et al., 2019). In fact, some studies suggest that *Aeromonas veronii* bv. sobria is more virulent than *Aeromonas veronii* bv. veronii (Shameena et al., 2020).

Studies on the histopathology of diseases caused by *Aeromonas veronii* in fish tissues are very limited. Chen et al. (2019) reported in crucian carp (*Carassius auratus gibelio*) that multiple organs and tissues of the diseased fish displayed intense hemorrhaging, infiltrating inflammatory cells and necrosis. Severe intravascular congestion, swelling of liver cells, cell necrosis and karyolysis, as well as hyperplasia of the gill lamellae have been reported. Another study, found that the liver of largemouth bass (*Micropterus salmoides*) was hemorrhagic and had necrotic lesions Pei et al. (2021). Numerous inflammatory cells infiltrated the kidney, as well as necrosis in the glomeruli. The aforementioned study also found that the spleens of diseased fish had large amounts of hemosiderin granules, and secondary gill lamellae were an important sign of necrosis. Similar to these findings; we observed hemorrhages in the liver, hemosiderin deposits in the spleen and necrosis in the gill lamellae of diseased fish. In contrast to previous studies, depletion and hyperemia in the hematopoietic tissue in the anterior kidney were detected in examined fish. Our study also revealed intravascular hyperemia in many organ tissue sections from diseased fish. Some other histopathological findings

were similar to previously reported *A. veronii* epizootics in European seabass described by Tanrıkul and Dinçtürk (2021). These researchers reported lymphocytic cell infiltration in spleen tissue and granuloma, hyperemia, hemorrhages and necrotic tissue in the liver. They also found that the gill epithelium had lamellar epithelial hypertrophy and hyperplasia with degenerative changes as those observed in the present study.

In previous disease reports, the susceptibility of *A. veronii* isolates to antibiotics varies according to the fish host and the environment. Researchers have generally reported that *A. veronii* isolates are resistant to many antibiotics including ampicillin, amoxicillin, carbenicillin, chloramphenicol, clindamycin, enrofloxacin, kanamycin, lincosamide, nalidixic acid, pipemidic acid, teicoplanin and vancomycin (Vila et al., 2002; Cai et al., 2012; Liu et al., 2016). Uzun and Öğüt (2015) reported that biovar *A. veronii* bv. sobria isolates were resistant to ampicillin, sulfadiazine, tilmicosin, trimethoprim, penicillin-G, streptomycin and vancomycin antibiotics. Herein, all *A. veroni* bv. veronii isolates from European seabass were found to be sensitive to oxytetracycline, enrofloxacin, ciprofloxacin, florfenicol and flumequin, but, resistant to ampicillin and amoxicillin.

CONCLUSION

In this work, we identified *Aeromonas veronii* biovar veronii as the causative agent behind an epizootic that affected European seabass (*Dicentrarchus labrax*) cultured in Türkiye. Chronic disease characteristics observed in histopathology are similar to previously reported findings. Accumulated mortality below %1 support that this is a low virulence biovar. Out of the many antibiotics tested, the examined isolates of *Aeromonas veronii* biovar veronii were only resistant to ampicillin and amoxicillin, and sensitive to oxytetracycline, enrofloxacin, ciprofloxacin, florfenicol and flumequine.

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Author's Contributions

The authors declare that they contributed equally to this article.

Conflict of Interest

Authors declare that there is no conflict of interest.

REFERENCES

Abbott, S. L., Cheung, W. K., & Janda, J. M. (2003). The genus *Aeromonas*: biochemical characteristics, atypical reactions, and phenotypic identification schemes. *Journal of Clinical Microbiology*, *41*(6), 2348-2357.

Bauer, A. W., Kirby, W. M., Sherris, J. C. & Turck, M. (1966). Antibiotic susceptibility testing by a standardized single diffusion method. *American Journal of Clinical Pathology*, 45, 493-496.

Brenner DJ, Boone DR, Garrity GM, Goodfellow M, Krieg NR, Rainey FA., Schleifer, K., Staley, J. T., & Vos, P. (2005). *The Proteobacteria: Part C: The Alpha-, Beta-, Delta-, and Epsilonproteobacteria. Bergey's Manual Trust*. Springer, New York.

Cai, S. H., Wu, Z. H., Jian, J. C., Lu, Y. S., & Tang, J. F. (2012). Characterization of pathogenic *Aeromonas veronii* bv. veronii associated with ulcerative syndrome from Chinese longsnout catfish (*Leiocassis longirostris* Günther). *Brazilian Journal of Microbiology*, *43*(1), 382-388.

Chen, F., Sun, J., Han, Z., Yang, X., Xian, J., Lv, A., Hu, X., & Shi, H. (2019). Isolation, identification and characteristics of *Aeromonas veronii* from diseased crucian carp (*Carassius auratus gibelio*). *Frontiers in Microbiology, 10*, 2742.

Culling, C. F. A. (1963). *Handbook of Histopathological Techniques (including museum technique)* 533 pp. London, Butterworths.

Dong, H. T., Techatanakitarnan C, Jindakittikul P, et al (2017). *Aeromonas jandaei* and *Aeromonas veronii* caused disease and mortality in Nile tilapia, *Oreochromis niloticus* (L.). *Journal of Fish Diseases, 40*(10), 1395-1403.

FAO (2021). *FAO Yearbook. Fishery and Aquaculture Statistics 2019* Rome. ISBN: 978-92-5-135410-0 https://doi.org/10.4060/cb7874t

Fryer, J. L. & Rohovec, J. S. (2021). *Bacterial diseases of fish*. In *Pathobiology of Marine and estuarine Organisms* (pp. 53-83), CRC Press, Florida.

Gashgari, R. M. & Selim, S. A. (2015). Detection and characterization of antimicrobial resistance and putative virulence genes in *Aeromonas veronii* biovar Sobria Isolated from Gilthead sea bream *(Sparus aurata L.). Foodborne Pathogens and Disease, 12*(9), 806-811.

Hall, T. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. 95-98. In: Nucleic Acids Symposium Series.

Hickman-Brenner, F. W., Macdonald, K. L., Steigerwalt, A. G., Fanning, G. R., Brenner, D. J., & Farmer 3rd, J. J. (1987). *Aeromonas veronii*, a new ornithine decarboxylase-positive species that may cause diarrhea. *Journal of Clinical Microbiology, 25*(5), 900–906

Hoai, T. D., Trang, T. T., Van Tuyen, N., Giang, N. T. H., & Van Van, K. (2019). *Aeromonas veronii* caused disease and mortality in channel catfish in Vietnam. *Aquaculture, 513*, 734425.

Janda, J. M. & Abbott, S. L. (2010). The genus *Aeromonas*: taxonomy, pathogenicity, and infection. *Clinical Microbiology Reviews, 23*(1), 35-73.

Larkin, M. A., Blackshields, G., Brown, N. P., Chenna, R., McGettigan, P. A., McWilliam, H., Valentin, F., Wallace, I. M., Wilm, A., & Lopez, R. (2007). Clustal W and Clustal X version 2.0. Bioinformatics, 23, 2947-2948.

Liu, D., Geng, Y., Wang, K., Chen, D., Huang, X. L., Ou, Y., He, C. L., Zhong, Z. J., & Lai, W. (2016). *Aeromonas veronii* infection in cultured channel catfish*, Ictalurus punctatus,* in Southwest China. *The Israeli Journal of Aquaculture-Bamidgeh, 68*, 1-8.

Nawaz, M., Khan, S. A., Khan, A. A., Sung, K., Tran, Q., Kerdahi, K., & Steele, R. (2010). Detection and characterization of virulence genes and integrons in *Aeromonas veronii* isolated from catfish. *Food Microbiology*, *27*(3), 327-331.

Pei, C., Song, H., Zhu, L., Qiao, D., Yan, Y., Li, L., Zhao, X., Zhang, J., Jiang, X., & Kong, X. (2021). Identification of *Aeromonas veronii* isolated from largemouth bass *Micropterus salmoides* and histopathological analysis. *Aquaculture, 540*, 736707.

Rahman, M., Colque-Navarro, P., Kühn, I., Huys, G., Swings, J., & Möllby, R. (2002). Identification and characterization of pathogenic *Aeromonas veronii* biovar sobria associated with epizootic ulcerative syndrome in fish in Bangladesh. *Applied and Environmental Microbiology, 68*(2), 650-655.

Shameena, S. S., Kumar, K., Kumar, S., Kumar, S., & Rathore, G. (2020). Virulence characteristics of *Aeromonas veronii* biovars isolated from infected freshwater goldfish *(Carassius auratus)*. *Aquaculture, 518*, 734819.

Smyrli, M., Prapas, A., Rigos, G., Kokkari, C., Pavlidis, M., & Katharios, P. (2017). *Aeromonas veronii* infection associated with high morbidity and mortality in farmed European seabass *Dicentrarchus labrax* in the Aegean Sea, Greece. *Fish Pathology, 52*(2), 68-81.

Smyrli, M., Triga, A., Dourala, N., Varvarigos, P., Pavlidis, M., Quoc, V. H., & Katharios, P. (2019). Comparative study on a novel pathogen of European seabass. Diversity of *Aeromonas veronii* in the Aegean Sea. *Microorganisms, 7*(11), 504.

Smyrli, M., Katharios, P. (2020). 12. *Aeromonas* spp. 107-116. In: Zrncic S (Ed), *Diagnostic Manual for the main pathogens in European seabass and Gilthead seabream aquaculture*. CIHEAM, Zaragoza.

Suau, A., Bonnet, R., Sutren, M., Godon, J. J., Gibson, G. R., Collins, M. D., & Doré, J. (1999). Direct analysis of genes encoding 16S rRNA from complex communities reveals many novel molecular species within the human gut. *Applied and Environmental Microbiology, 65*(11), 4799-4807.

Sun, J., Zhang, X., Gao, X., Jiang, Q., Wen, Y., & Lin, L. (2016). Characterization of virulence properties of *Aeromonas veronii* isolated from diseased Gibel Carp *(Carassius gibelio). International Journal of Molecular Sciences, 17*(4), 496.

Tanrıkul, T. & Dinçtürk, E. (2021). A New Outbreak in Sea Bass Farming in Turkey: *Aeromonas veronii*. *Journal of the Hellenic Veterinary Medical Society, 72*(3), 3051-3058.

Toranzo, A. E., Magariños, B., & Romalde, J. L. (2005). A review of the main bacterial fish diseases in mariculture systems. *Aquaculture, 246*(1-4), 37-61.

Uzun, E. & Ogut, H. (2015). The isolation frequency of bacterial pathogens from sea bass *(Dicentrarchus labrax)* in the Southeastern Black Sea. *Aquaculture, 437*, 30-37.

Vila, J., Marco, F., Soler, L., Chacon, M., & Figueras, M. J. (2002). In vitro antimicrobial susceptibility of clinical isolates of *Aeromonas caviae, Aeromonas hydrophila* and *Aeromonas veronii* biotype sobria. *The Journal of Antimicrobial Chemotherapy, 49*(4), 701-702.

Wayne, P. A. (2010). Clinical and Laboratory Standards Institute: *Performance standards for antimicrobial susceptibility testing: 20th informational supplement*. CLSI document M100-S20.

Yu, J. H., Han, J. J., Kim, H. J., Kang, S. G., & Park, S. W. (2010). First report of *Aeromonas veronii* infection in farmed Israeli carp *Cyprinus carpio* in Korea. *Journal of Fish Pathology, 23*(2), 165-176.

Zhang, Z., Schwartz, S., Wagner, L., & Miller, W. (2000). A greedy algorithm for aligning DNA sequences. *Journal of Computational Biology, 7*(1-2), 203-214.

Zhu, M., Wang, X. R., Li, J., Li, G. Y., Liu, Z. P., & Mo, Z. L. (2016). Identification and virulence properties of *Aeromonas veronii* bv. *sobria* isolates causing an ulcerative syndrome of loach *Misgurnus anguillicaudatus*. *Journal of Fish Diseases, 39*(6), 777-781..

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Antalyadaki Yarasa Türlerinin Akustik Olarak Araştırılması

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ÖZET

Bu çalışma Antalya Akdeniz Üniversitesi Yerleşkesi'nin 8 farklı noktasına ses kayıt cihazı yerleştirilerek toplam 30 gün veri toplanmıştır. Ses kayıtlarının analizi ve görsel tespitler sonucunda yerleşkede beslenen 3 farklı familyaya ait toplam 12 farklı yarasa türü tespit edilmiştir. Bunlar sırasıyla, Vespertilionidae familyasından *Myotis blythii, Nyctalus noctula, Nyctalus leisleri, Eptesicus serotinus, Pipistrellus pipistrellus, Pipistrellus pyqmaeus, Pipistrellus kuhlii, Pipistrellus nathusii, Hypsugo savii, Barbastella barbastellus*, Miniopteridae familyasından *Miniopterus schreibersii* ve Pteropodidae familyasından *Rousettus aegyptiacus*'tur. Anova analizi sonuçlarına göre; istasyonlar ve yarasa türlerine ait toplam çağrı sayıları arasında anlamlı bir farklılık olmadığı tespit edilmiştir. Korelasyon analizine göre ise, göstergelerin farklı düzeylerde birbirleriyle ilişkili olduğu sonucuna ulaşılmıştır.

Zooloji

Araştırma Makalesi

Makale Tarihçesi
Geliş Tarihi : 17.08.2022
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Anahtar Kelimeler
Yarasa
Tür Çeşitliliği
Antalya

Acoustic Investigation of Bat Species in Antalya

ABSTRACT

In this study, a total of 30 days of data were collected by placing a voice recorder at 8 different points of the Antalya Akdeniz University Campus. As a result of the analysis of the sound recordings and visual determinations, a total of 12 different bat species belonging to 3 different families were identified. These are, respectively, from the family Vespertilionidae *Myotis blythii, Nyctalus noctula, Nyctalus leisleri, Eptesicus serotinus, Pipistrellus pipistrellus, Pipistrellus pyqmaeus, Pipistrellus kuhlii, Pipistrellus nathusii, Hypsugo savii, Barbastella barbastellus*; from the family Miniopteridae *Miniopterus schreibersii* and from the family Pteropodidae *Rousettus aegyptiacus*. According to the results of Anova analysis; it was determined that there was no significant difference between the total number of calls belonging to the stations and bat species. According to the correlation analysis, it was concluded that the indicators were related to each other at different levels.

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GİRİŞ

Yarasalar, tarım alanlarında potansiyel zararlı böcekleri tüketerek, tohumları dağıtarak, tozlaşmayı sağlayarak ve çevresel değişimin biyobelirteçleri olarak birçok ekosistemde kritik rol oynarlar. Yarasalar tipik gece aktif hayvanlarda olduğu gibi gün batımı sırasında ve hemen sonrasında ortaya çıkarlar ve çok dikkat çekmezler. Hem kentsel hem de ormanlık alanlarda yiyecek aramak için tüneklerinden çıktıklarında böceklerle beslendiklerinin ilk saatlerinde çıplak gözle bile gözlemlenebilmektedirler. Ancak yarasaların, gün boyu saklandıkları tüneme alanlarının mevcudiyetini etkileyebilecek artan antropojenik arazi kullanımı ve kentleşme nedeniyle tehdit altında olabilir (Schimpp et al., 2018). Sürekli çoğalan bu tehditler göz önüne alındığında biyologların yarasaları incelemek için daha fazla alternatif yollar araştırması gerekmektdir (Blackburn & Unger, 2019).

Akustik araştırmalar günümüzde, yarasaları araştırmak için maliyeti düşük canlıya en az zararı olan yöntemdir. Bu araştırmalar, yön bulma ve böcek ararken yayılan ultrasonik çağrıları kullanan yarasaların biyolojisine dayanmaktadır (Fenton & Simmons, 2014). Yarasalarda dış görünüşe bakarak tür teşhisi yapmak zor olduğu için bu teknolojinin

kullanımı oldukça önemlidir.[1] Bu nedenle, ilkel yöntemlerinden ziyade ileri teknolojiler kullanılarak türlerin tespiti daha kolay ve zararsızdır. Uzun süre kayıt yapma ve aynı anda birçok sesi kaydetme yeteneği, geleneksel yakalama yöntemlerine kıyasla veri toplama verimliliğini büyük ölçüde arttırmaktadır (Britzke et al., 2013). Yarasa yoğunluklarındaki ciddi azalmalar, yarasaların yakalanmasını daha da zorlaştırmakta ve özellikle nesli tehlike altında olan türlerde rahatsızlık oluşturmaktadır. Bu açıdan da akustik izleme günümüzde oldukça önem kazanmıştır.

Yapılan literatür taramalarında, bugüne kadar Antalya ilinde yarasalar ile ilgili ultrasonik ses kaydı alınarak yapılmış bir tür tespiti çalışmasına rastlanılmamıştır. Bu nedenle daha önce herhangi bir araştırmaya konu edilmemiş Antalya Akdeniz Üniversitesi kampüsü çalışma alanı olarak seçilmiştir. Antalya şehir merkezi tüm Türkiye'de olduğu gibi hızla betonlaşmaya doğru giden, nüfusu en hızlı artan büyük kentlerden olmasına rağmen, yer yer korunan doğal yapısıyla kentin ortasında yer alan Akdeniz Üniversitesi yerleşkesi yarasa faunası açısından bilinmeyen bir bölgedir. Dolayısıyla Akdeniz Üniversitesi yerleşkesindeki yarasa türlerinin ve aktivite yoğunluklarının müdahalesiz yöntemle tespit edilmesi oldukça önemlidir. Çalışmada yarasa türlerinin kış uykusu süreleri göz önünde bulundurularak aktif oldukları zaman aralığı olan Nisan ve Kasım ayları arasında alan çalışması gerçekleştirilmiştir.

Çalışma sonucunda, IUCN kriterlerine göre sınıflandırılan, bölgedeki türler ilk kez tespit edilmiş olup literatüre katkısı açısından da ilk olma özelliğindedir. Ayrıca çalışma sırasında tespit edilen, farkında olmadan yarasa ölümlerine yol açan yerleşkedeki yoğun tarım ilacı kullanımının da azaltılması, yarasa varlığının öneminin daha iyi anlaşılması ve bu konuda üniversite yönetiminde ve öğrencilerde bir farkındalık yaratılması konusunda da etkili olacağı düşünülmektedir.

MATERYAL ve METOD

Çalışma Alanı

Akdeniz Üniversitesi yerleşkesi ülkemizin güneyi; 36º53 kuzey enlemi, 30º40 boylamı ile Antalya İli' nin batısında yer almaktadır. Yerleşke alanının büyüklüğü 360 hektar (3600 dönüm), arazi yüzölçümü 615.105 m²'dir. Deniz seviyesinden 50-60 metre yüksekliğindedir. Makilik bitki örtüsü hakim olmasına rağmen yer yer kültür bitkilerle alan çeşitlendirilmiştir. Yerleşke alanı herdem yeşil bodur maki formasyonu ile kaplıdır (Ünal & Gökçeoğlu, 2003). Ayrıca, yerleşke içerisinde yarasaların böcek avlayabileceği şehir özelliğini temsil eden bol ışıklı (karanlıkta böcekleri çekmesi açısından) binalar ve doğal barınak özelliği sunan, kovuk, oyuk ve kayalık alanlar ile küçük mağaralar tespit edilmiştir. Bu alanda, ortaya çıkan ve yiyecek arayan yarasalar daha önceden yapılan arazi çalışmalarıyla görsel olarak gözlemlenmiş ve bu alan çalışılacak ideal bir yer olarak seçilmiştir (Şekil 1).

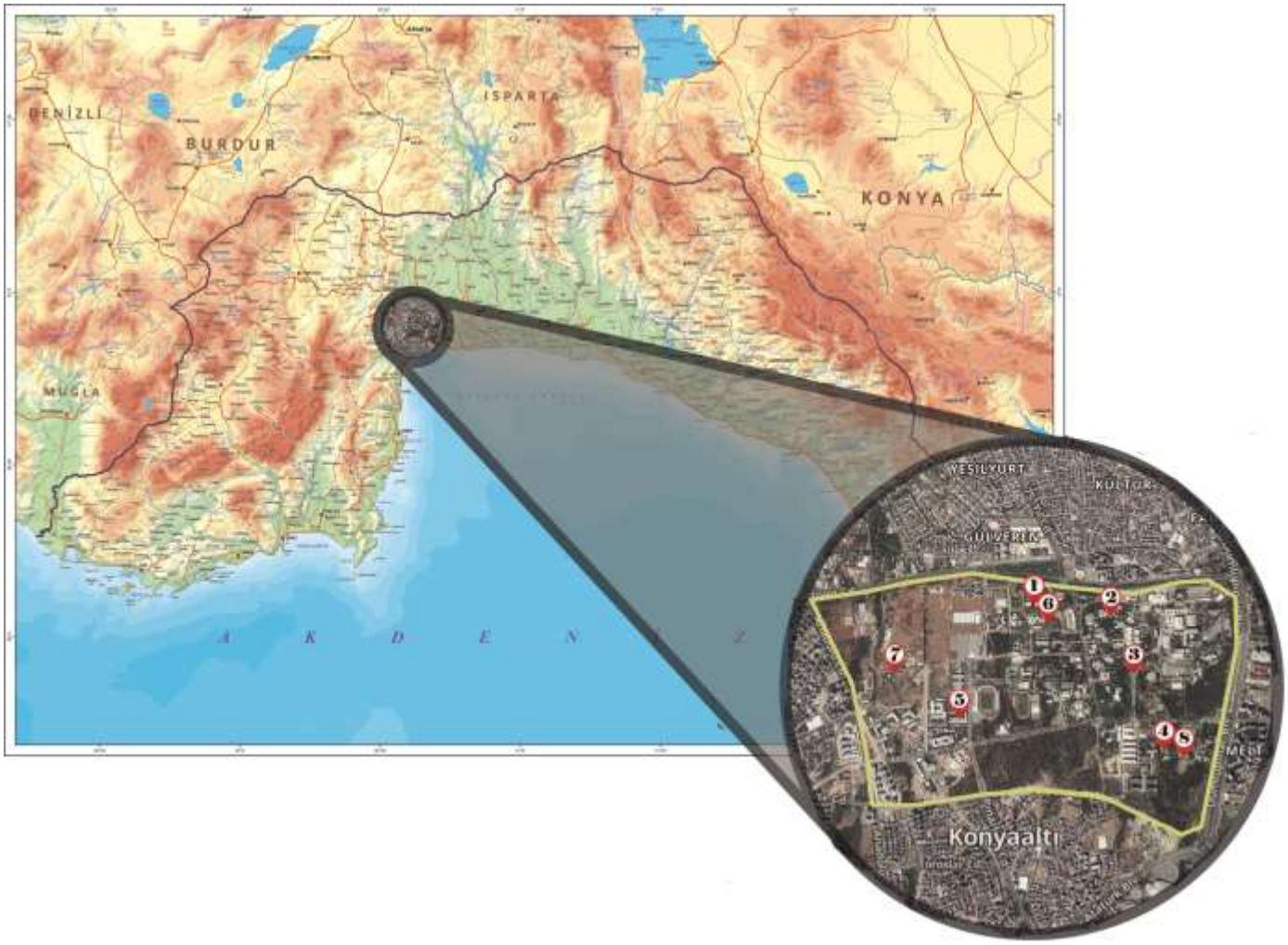
Arazi Çalışmaları

Arazi çalışmaları yarasaların aktif olduğu zaman aralığı olan Nisan ve Kasım 2018 tarihleri arasında 8 farklı noktaya, farklı zamanlarda Batcorder ses kayıt cihazı takılarak gerçekleştirilmiştir. Cihaz her ayın belirli tarihlerinde hava şartları göz önüne alınarak, akşam alacakaranlığından sabah gün doğumuna kadar kayıt alma işlemi yapacak şekilde programlanmıştır. Batcorder cihazı kullanırken kayıt alacağı alanın önünün açık olmasına, yarasa türlerinin görülme ihtimali yüksek (çalılık, fundalık ağaçlar, mağara, terkedilmiş binalar, su kütlesinin olduğu alanlar veya çevresi, sokak lambaları gibi) ve gece değişen hava şartlarına karşı kayıt almasının aksamaması için korunaklı yerlere takılmasına özen gösterilmiştir.

Kullanılan Ekipmanlar

Bu çalışmada sesleri kaydedebilmek için 16-150 kHz aralığında ultrasonik sesleri kaydedebilen Batcorder cihazı kullanılmıştır. Bu cihaz: Radyoya benzer bir görünüme sahip olup, sabit bir noktaya akşamdan sabaha kadar bağlandığında, ultrasonik sesleri algılayıp, gerçek zamanlı (Real-time) kaydedebilen bir özelliktedir. Batcorder, kaydettiği seslerin filtrelenmesi, çağrı sayıların belirlenmesi, veri tabanındaki ses frekansları ile eşlenmesi, seslerin analizi ve seslerin tür düzeyinde teşhislerini yapabilmek için lisanslı bcAdmin, bcAnalyze ve batIdent programları, programları çalıştırabilmek için bu programlara uygun OSX 10.10.1 işletim sistemine sahip bir lap top kullanılmıştır.

[1] Batcorder cihazı ile ultrasonik sinyalleri gerçek zamanlı ve dijital olarak kaydedip, 500 kHz örnekleme hızında, 16 bit genlikte, 53 dB veya daha az sistem gürültüsüyle kayıt yapmaktadır. Tür teşhisi için seslerin analizi lisanslı bcAdmin, batIdent ve bcAnalyze2 programları kullanılarak yapılmaktadır. Çeşitli şekilde yazılımın sistemine kaydedilmiş ekolokasyon çağrıları kullanarak yarasa türlerinin tanımlanması yardımcı olur. Otomatik arama bulucu, batIdent ile otomatik kimlik (tür ismi) için kalite gereksinimlerini en doğru ve en net sonuç vermek için diğer sosya çağrı gibi çağrıları, tüm aramaları ayıklar. Aramalar ve batIdent sonuçları çeşitli araçlar kullanılarak görselleştirilir. Ana arayüz, hızlı kontrol için çağrılara genel bir bakış içerir. Manuel olarak görselleştirilen çağrılara bakarak tür doğruluğu hakkında yorum yapılabilir. Ayrıca yapılan analiz sonucunda %90 oranında sesler dikkate alınarak ve tür düzeyinde değerlendirilmiştir.



Şekil 1. Akdeniz Üniversitesi yerleşkesi ve ses kaydı alınan istasyonlar (İstasyon 1; Ziraat Fak., İstasyon 2; Fen Fak., İstasyon 3; Rektörlük, İstasyon 4; Sağ.Bil.MYO, İstasyon 5; Eğit. Fak., İstasyon 6; Ziraat Fak. 2, İstasyon 7; Kek İstas., İstasyon 8; Lojman)

Figure 1. Akdeniz University campus and sound recording stations (Station 1; Agriculture Fac., Station 2; Science Fac., Station 3; Rectorate, Station 4; Health SVS, Station 5; Education Fac., Station 6; Agriculture Fac.2, Station 7; Partridge Station, Station 8; Housıng)

Yöntem

Akdeniz Üniversitesi'ndeki çalışma alanında uçuş sırasında yarasaları dinlemek, kaydetmek ve tanımlamak için otomatik ultrason kayıt ünitesi (batcorder) kullanıldı. Müdahalesiz yöntem canlıya temas etmeden, yuvalanma alanlarına zarar vermeden, beslenme aktivitelerine engel olmadan türlerin teşhis edilmesini sağlamaktadır. Uzun süre boyunca sürekli izlemeye izin veren otomatik ultrason kayıt ünitesi (batcorder, ecoObs) yarasaların aktivitesini izlemeye imkân sağlamıştır. Batcorder cihazı ile ultrasonik sinyalleri gerçek zamanlı ve dijital olarak kaydedip (500 kHz, 16 bit) yarasa besin arama seslerini ve diğer (böcek sesi vs.) orijinli ultrasonik sinyalleri ayırt etmek için çevrimiçi bir analiz sistemi kullanılmıştır. Bu sistemin avantajı, farklı cihazlar (kalibre edilmiş hassasiyet) ve mikrofonun çok yönlü sonuçlarının karşılaştırılabilmesidir (Anonymous, 2022).

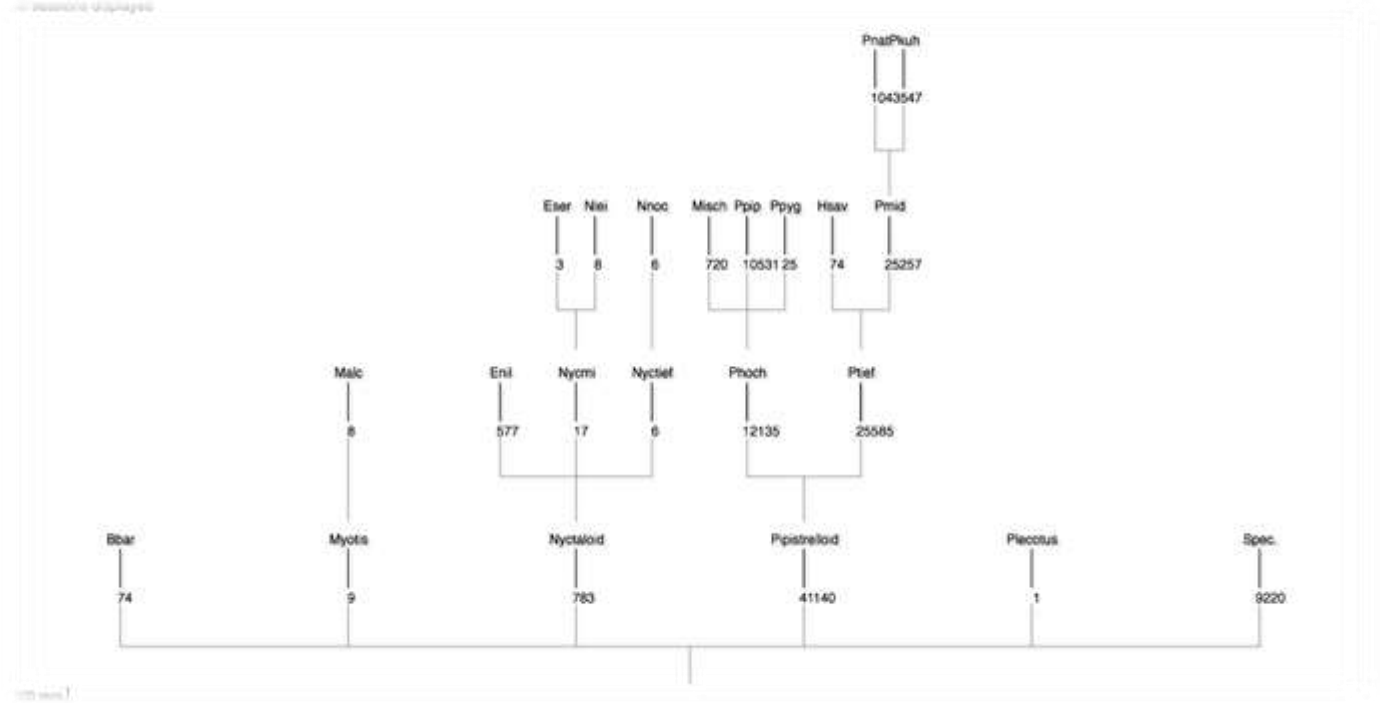
BULGULAR

Yarasaların kış uykusundan uyanma aktif zamanları olan Nisan ayından Kasım ayına kadar (2018 yılında) Akdeniz Üniversitesi merkez yerleşkesi ve çevresinde yapılan çalışmalar sonucunda Chiroptera takımının türleri Batcorder cihazı kullanılarak toplam 30 tam gece ultrasonik ses kayıtları alınarak türler tespit edilmiştir (Şekil 2).

Tür Çeşitliliği

Yarasa türlerine ait toplam 49571 adet ses kaydı alınmıştır. Bu seslerden 41140'ı Pipistrelloid, 783'ü Nyctaloid, 9'u Myotis, 1'i Plecotus, 74'ü Barbastellus'a ait olup 9220 ses tanımlanamamıştır. Ses kayıtlarının dijital analizi sonucunda 2 familyaya ait toplam 11, görsel olarak doğrudan gözlem ile 1 familya ait 1 yarasa türü tespit edilmiştir. Bunlar sırasıyla, Vespertilionidae familyasından *Myotis blythii,*

Nyctalus noctula, Nyctalus leisleri, Eptesicus serotinus, Pipistrellus pipistrellus, Pipistrellus pyqmaeus, Pipistrellus kuhlii, Pipistrellus nathusii, Hypsugo savii, Barbastella barbastellus, Miniopteridae familyasından *Miniopterus schreibersii*' ve Pteropodidae familyasından *Rousettus aegyptiacus*'tur. Ayrıca analiz programının tespit ettiği, soy ağacında yer alan *Eptesicus nilssonii* türünün Türkiye'de yayılışı bulunmamaktadır (Şekil 2). Karşılaştırmalı olarak yapılan sonogram analizlerinde Türkiye'de yayılışı bulunmayan türün aslında *Eptesicus serotinus* olduğu (Widerin & Reiter, 2017); Antalya'da yayılışı bulunmayan *Myotis alcathoe* türünün ise aslında *Myotis blythii* olduğu (Heim et al., 2020) tespit edilmiştir.[2] Bu başlık altında, yalnızca araştırmadan elde edilen bulgular sunularak, konuyla ilgili daha önceden gerçekleştirilmiş benzer ve dolaylı çalışmalarla atıf yapmak kaydıyla bulgular karşılaştırılır. Benzer ve farklı yanlar vurgulanır ve yayına sunulan çalışmada diğer çalışmalara göre neden farklı bir bulgu elde edildiği tartışılır. Sonrada bu tartışma üzerinden araştırmada elde edilen bulgular istikametinde alanın uzmanı olarak yorum yapılır. Bu bölümde, deneysel sonuçların net bir sunumu yapılmalıdır (Şekil 3).



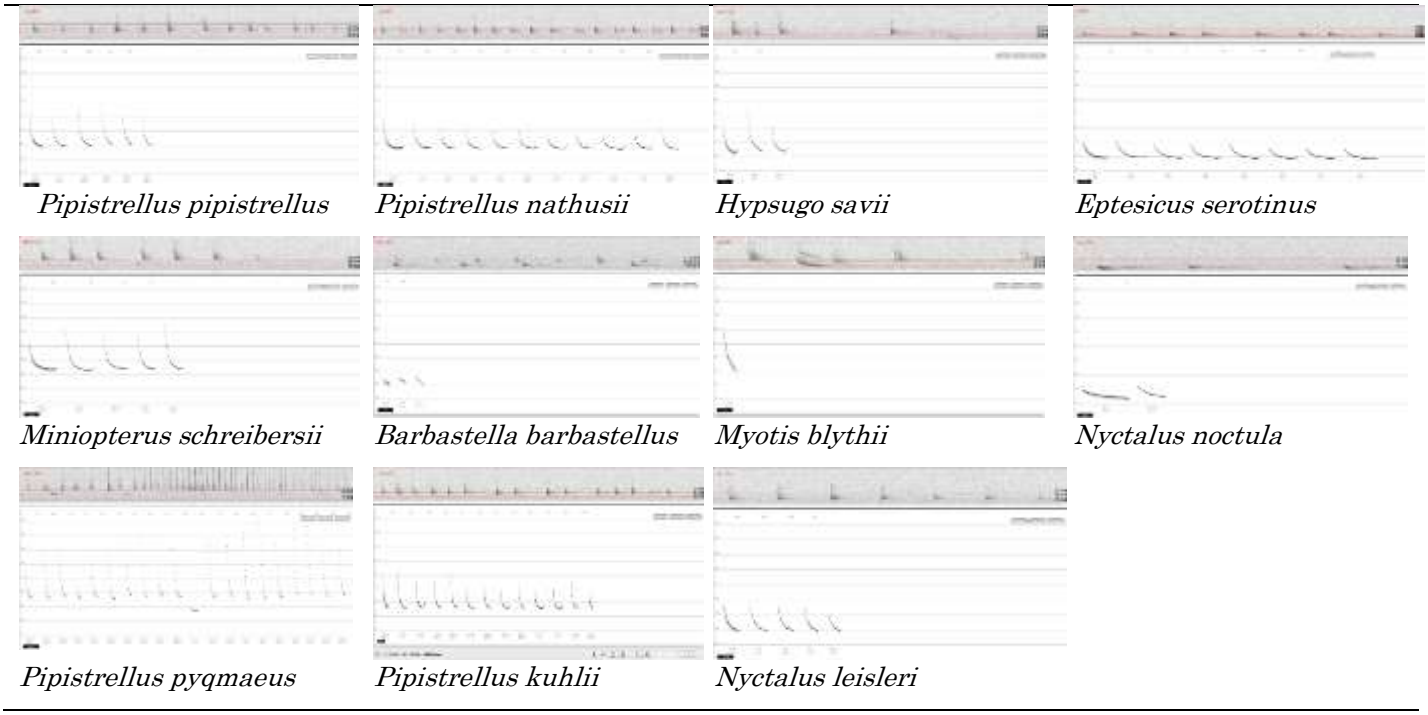
Şekil 2. Nisan-Kasım 2018 tarihlerinde alınan ses kayıtlarının analizi sonucunda saptanan türlere ait soyağacı
Figure 2. The pedigree of the species determined as a result of the analysis of the sound recordings taken between April-November 2018

Ultrasonik ses kayıtları ile 11 tür, doğrudan gözlem ile 1 tür gözlemlenmiştir. Alınan ses kayıtlarının incelenmesi sonucunda türlere ait ses sonogramları Şekil 3'de; çalışma yapılan aylar ve kaydedilen günlük toplam ses sayıları ile tespit edilen türler Çizelge 1'de verilmiştir.

Pipistrellus pipistrellus, %90 tahminlerle en sık ses kaydı alınan tür ve *Rousettus aegyptiacus*'in en sık doğrudan gözlemlenen yarasa tür olduğu görülmüştür. Her iki yarasa türü araştırma yapılan zaman aralığındaki tüm aylarda görülmüştür. Aylara göre en az ses kaydedilen yarasalar Mayıs ayında *Nyctalus noctula* ve Eylül ayında ise *Nyctalus leisleri* türleri olduğu belirlenmiştir.

Tür teşhisi için seslerin analizi lisanslı bcAdmin, batIdent ve bcAnalyze2 programları kullanılarak %90'nın üzerindeki tahminler esas alınmış ve tür düzeyinde değerlendirilmiştir. Buna bağlı olarak tür çeşitliliği yeniden değerlendirilmiş ve sesler ayıklanmıştır. Tür çeşitliliğinde değişiklik olmaz iken çağrı sayıları ve yoğunlukları farklılık göstermiştir. İstasyonlarda en fazla tür çeşitliliğine 12 tür ile II numaralı istasyon, en az tür çeşitliliğine tek tür ile VII numaralı istasyon sahiptir. Çağrı sayıları açısından; II numaralı istasyon 18738 çağrı sayısı ile en yüksek, VII numaralı istasyon 2 çağrı sayısı ile en düşük çağrı sayısına sahiptir (Çizelge 2).

[2] Cihaz analizinde "*M. myotis / blythii* " şeklinde verilen türü cihaz ayırt edemediği için çalışma sonucunda yer verilmemiştir. Bunun dışında "*Myotis alcathoe"* olarak verilen tür karşılaştırma yapılarak %90 güven aralığındaki ses sonogramları dahil edilerek tespit edilmiştir (Heim ve ark. 2020).



Şekil 3. Tespit Edilen Türlere Ait Ses Sonogramları[3]
Figure 3. Sound Sonograms of Detected Species

Şekil 4(a)'te türlerin çağrı sayıları ve istasyonlara göre dağılımı % olarak kaydedilmiş olup, en çok çağrı sayısı 17.09.2018 ve 18.09.2018'de, en az çağrı sayısı 11.04.2018 ve 13.04.2018 'de kayıt edilmiştir (Şekil 4(b)).

Tür Yoğunluğu

Türlerin yoğunluklarını tespit etmek amacıyla yapılan gözlemlerde elde edilen ses kayıt süreleri %90 güven aralığında tablolaştırılmıştır (Çizelge 3).

Tür yoğunluğu açısından, 19920,9 saniye ile II numaralı istasyon en yüksek yoğunluğun görüldüğü yerdir. En düşük yoğunluk 2,31 saniye ile VII numaralı istasyonda görülmüştür. Türlerin çağrı süreleri göz önüne alınarak istasyonlara göre dağılımları Şekil 5(a)'da gösterilmiştir. Kayıt alınan tarih ve istasyonlara göre aktivite yoğunlukları Şekil 5'da gösterilmiştir. Aktivite yoğunlukları en çok 17.09.2018 ve 18.09.2018'de kayıt edilmiştir. En az aktivite 11.04.2018 ve 13.04.2018' te gerçekleşmiştir (Şekil 5 (b)).

Yarasa aktivesi, Nisan-Kasım 2018 tarihlerinde mevsimsel açıdan değerlendirilecek olursa; bahar aylarında aktivitenin başladığı (üreme), yaz aylarında aktivite yoğunluğun en yoğun haliyle gerçekleştiği (beslenme), sonbahar aylarında aktivite yoğunluğunun azaldığı (beslenme-kış hazırlığı) ve kış aylarında aktivitenin durma noktasına geldiği (kışlama) gözlenmektedir (Şekil 6). Akdeniz üniversitesi yerleşkesinde yapılan çalışmalar sonucunda 4 önemli tür tespit edilmiştir. Bu türler Akdeniz ölçeğinde IUCN kırmızı listesinde tehdit altına girebilir (NT) kategorisinde yer alan *Barbastella barbastellus, Miniopterus Schreibersii, Myotis blythii* ve *Rousettus aegyptiacus* türleridir.

İstatistiksel Analizler

Çalışmada istatistiki analizlere geçmeden önce tanımlayıcı (descriptives) analizler ile verilerin normal dağılım gösterip göstermediğine Shapiro-Wilk testi ile bakılmıştır. Ortalamalara ait varyansların normal dağılmadığı görülmüştür ($W=0.523$, $p<.001$). Buna göre, istasyonlar ile toplam çağrı sayıları arasında farklar Anova (Kruskal-Wallis); Toplam çağri sayilari, ortalama çağri sayilari ve istasyonlar arasindaki farklar ise korelasyon testi ile analiz edilmiştir. Tüm istatiksel analizler %95 güven aralığında yapılmıştır.

İstasyonlar ile yarasa türlerine ait toplam çağrı sayıları arasında anlamlı fark olup olmadığına ilişkin yapılan Non-Parametrik Tek yönlü ANOVA testi yapilmiştir. Testin non-parametrik olmasının nedeni verilen normal dağılmamasından ($p<.001$) kaynaklanmaktadır. Buna Anova analizi sonuçları Çizelge 4'de verielmiştir (N=12).

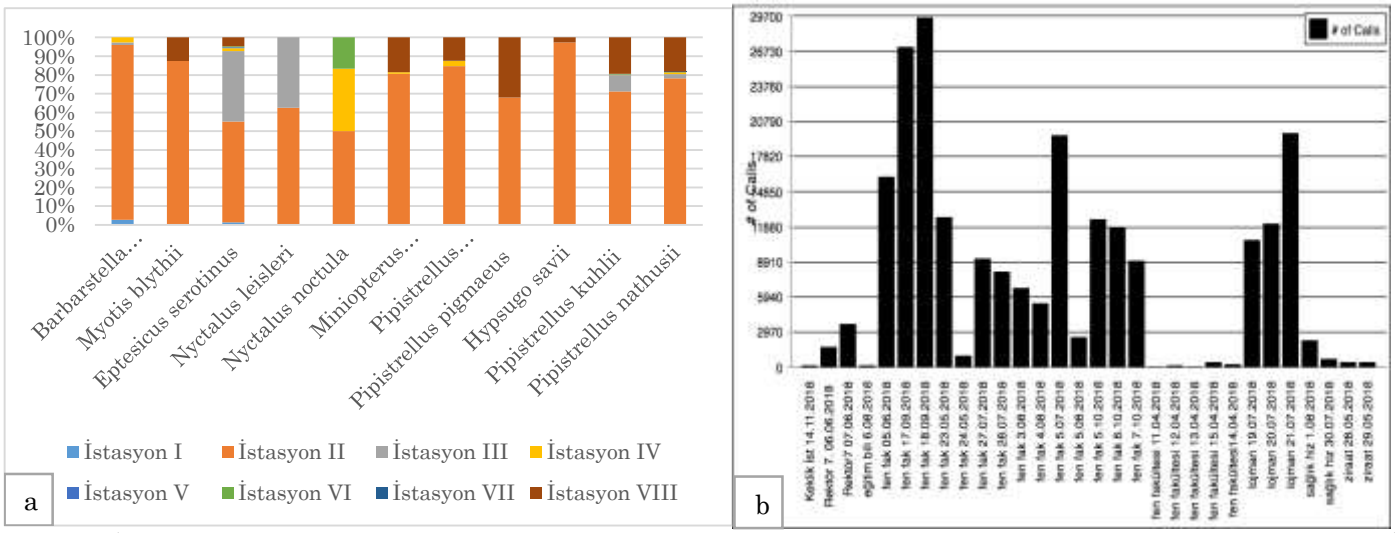
[3] Rousettus aegyptiacus tam bir ultrasonik ses dalgası çıkarmayıp sadece dil hareketleri ile ekolokasyon benzeri sesler çıkarmaktadır (Salles, 2022). Kullanılan cihaz sadece Avrupa da üretilip sadece Avrupa yarasalarını kapsamaktadır. Bunda ötürü Rousettus aegyptiacus türü ile ilgili herhangi bir ses kaydı verilmemiştir.

Çizelge 1. Yapılan çalışma günleri ve kayıt alınan/gözlemlenen türler
Table 1. Working days and registered/observed species

AYLAR / *MONTHS*	GÜN / *DAY*	KAYIT / *RECORD*	TÜRLER / *SPECIES*											
			Barbarstella barbastellus	*Pipistrellus*	*Pipistrellus*	*Pipistrellus kuhlii*	*Pipistrellus*	*Miniopterus*	*Hypsugo Savii*	*Nyctalus noctula*	*Nyctalus Leisleri*	*Eptesicus*	*Myotis blythii*	*Rousettus*
Nisan / April	11	15												X
	12	45		X										X
	13	27		X	X									X
	14	110	X	X	X									X
	15	190		X	X	X								X
Mayıs / May	23	2563	X	X	X	X	X	X	X	X	X			X
	24	396	X	X	X									X
	28	81	X	X	X			X				X		X
	29	76		X	X	X				X		X		X
Haziran/ June	5	2799	X	X	X	X	X	X	X			X		X
	6	414	X	X	X	X								X
	7	910		X	X	X					X	X		X
Temmuz / July	5	4053	X	X	X	X					X	X		X
	19	1466		X	X	X	X	X	X			X	X	X
	20	1762		X	X	X	X	X				X		X
	21	2405		X	X	X		X	X			X	X	X
	27	2172	X	X	X	X	X	X	X	X	X	X		X
	28	2101	X	X	X	X	X	X	X			X		X
	30	122	X	X	X	X		X		X		X		X
Ağustos / August	1	553	X	X	X	X		X				X		X
	3	2585		X	X	X	X	X	X	X	X	X		X
	4	1836	X	X	X			X	X		X	X		X
	5	1327	X	X	X	X		X	X			X		X
	6	21		X	X	X						X		X
Eylül / September	17	6286	X	X	X	X		X	X		X	X	X	X
	18	5509	X	X	X	X		X	X					X
Ekim / October	5	3487	X	X	X	X		X	X			X		X
	6	3477	X	X	X	X		X	X			X		X
	7	2736	X	X	X	X		X				X		X
Kasım / November	14	47	X											X

Çizelge 2. İstasyonlarda tespit edilen türler ve çağrı sayıları
Table 2. Species detected in stations and call numbers

Tür / *Species*	İstasyon / *Station*							
	I	II	III	IV	V	VI	VII	VIII
Barbarstella barbastellus	2	69	1	2	0	0	0	0
Myotis blythii	0	7	0	0	0	0	0	1
Eptesicus serotinus	8	312	218	9	3	3	0	27
Nyctalus leisleri	0	5	3	0	0	0	0	0
Nyctalus noctula	0	3	0	2	0	1	0	0
Miniopterus schreibersii	2	579	0	6	0	0	0	133
Pipistrellus pipistrellus	14	8909	4	286	1	8	0	1309
Pipistrellus pigmaeus	0	17	0	0	0	0	0	8
Hypsugo savii	0	72	0	0	0	0	0	2
Pipistrellus kuhlii	0	603	74	2	1	3	0	164
Pipistrellus nathusii	7	8162	220	111	6	23	2	1904
Toplam çağrı sayısı *Total number of calls*	**33**	**18738**	**520**	**418**	**11**	**38**	**2**	**3548**



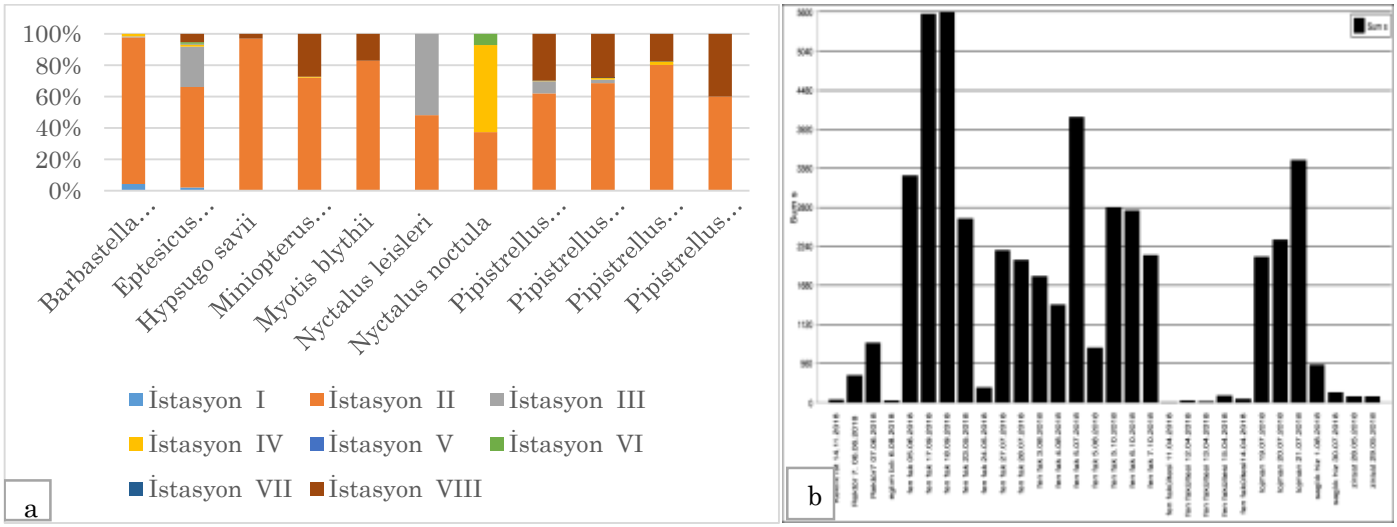
Şekil 4. a) Nisan-Kasım 2018 tarihleri arasında yarasaların bıraktığı çağrı sayısının istasyonlara göre dağılımı; b) yarasaların bıraktığı çağrı sayısı

Figure 4. a) The distribution of the number of calls left by bats by stations between April and November 2018; b) number of calls left by bats

Çizelge 3. İstasyonlara göre türlerin çağrı süreleri

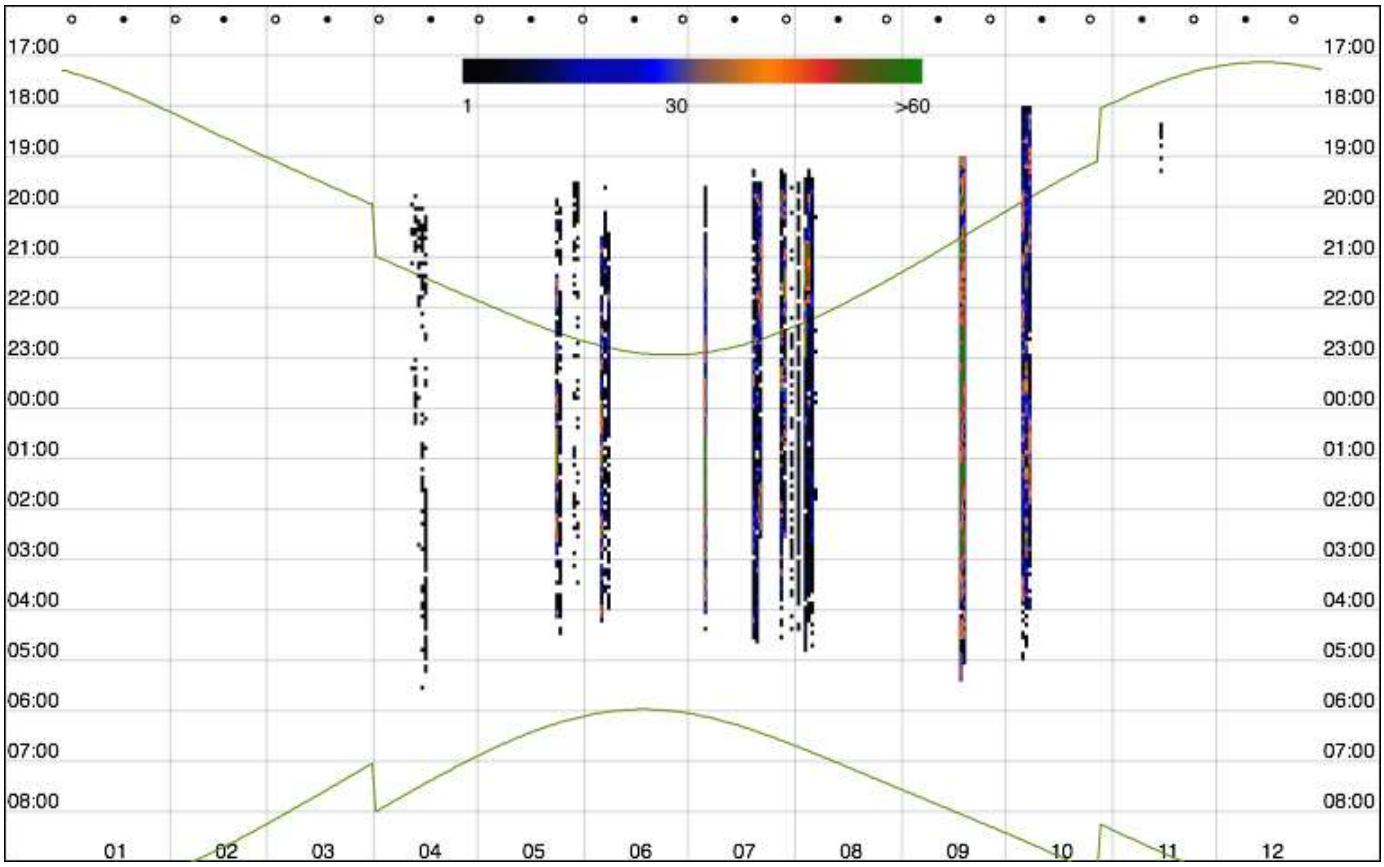
Table 3. Call durations of types according to stations

Tür / *Species*	**İstasyon / *Station***							
	I	**II**	**III**	**IV**	**V**	**VI**	**VII**	**VIII**
Barbastella barbastellus	2.89	63.9	0.46	1.03	0	0	0	0
Eptesicus serotinus	15.7	509.08	204.12	11.83	2.29	8.47	0	42.1
Hypsugo savii	0	160.41	0	0	0	0	0	4.99
Miniopterus schreibersii	1.92	497.58	0	4.56	0	0	0	189.07
Myotis blythii	0	8.63	0	0	0	0	0	1.79
Nyctalus leisleri	0	6.58	7.06	0	0	0	0	0
Nyctalus noctula	0	3.27	0	4.88	0	0.62	0	0
Pipistrellus kuhli	0	838.9	105.78	2.39	1.48	2.45	0	401.8
Pipistrellus nathusii	8.69	9163.89	278.28	135.8	10.8	30.6	2.31	3725.4
Pipistrellus pipistrellus	14.5	8649.94	5.88	230.7	1.21	8.54	0	1885.4
Pipistrellus pygmaeus	0	18.67	0	0	0	0	0	12.47
Toplam süre (saniye) ***Total time (seconds)***	**43.6**	**19920.9**	**601.58**	**391.2**	**15.8**	**50.7**	**2.31**	**6263**



Şekil 5. a) Çağrı süresi ve tür grafiği, b) Nisan-Kasım 2018 tarihlerinde yarasa aktivite yoğunluğu

Figure 5. a) Call duration and species graph, b) Bat activity intensity between April-November 2018



Şekil 6. Yarasa aktivitesinin aylara göre yoğunlaştığı saatler
Figure 6. Hours of intensification of bat activity by months

Çizelge 4. Akdeniz Üniversitesi kampüsündeki istasyonlar ve yarasa türlerinin toplam çağrı sayılarına ilişkin Anova sonuçları

Table 4. Anova results regarding the total number of calls of stations and bat species on the Akdeniz University campus

	χ^2	df	p	ε^2
istasyon1	10.03	9	**0.348**	0.912
istasyon2	10.92	9	**0.281**	0.993
istasyon3	9.58	9	**0.386**	0.871
istasyon4	10.33	9	**0.325**	0.939
istasyon5	11.00	9	**0.276**	1.000
istasyon6	11.00	9	**0.276**	1.000
istasyon7	11.00	9	**0.276**	1.000
istasyon8	10.26	9	**0.330**	0.933

Elde edilen bulgular ışığında gerçekleştirilen Oone-Way Anova (Kruskal-Wallis) testi neticesinde, istasyonlar ve yarasa türlerine ait toplam çağrı sayıları arasında anlamlı bir farklılık olmadığı tespit edilmiştir.

Toplam çağri sayıları, ortalama çağrı sayıları ve istasyonlar arasındaki farklar ise korelasyon testine ilişkin veriler aşağıda verilmiştir (çizelge 5).

Analizde 8 istasyon ve tespit edilen 12 yarasa türüne ait veriler kullanılmıştır (N=12). Değişkenlerden herhangi birinde kayıp değere rastlanmamıştır. Değişkenler arasındaki farkın normal dağılım gösterip göstermediği Shapiro-Wilk testi ile kontrol edilmiş ve verilerin normal dağılım göstermediği sonucuna ulaşılmıştır. Bu nedenle Korelasyon testinde değişkenleri normal dağılmadığı ve örneklem sayısının az olduğu analizlerde kullanılan Kendall's tau-b korelasyon katsayısı uygulanmıştır.

Çizelge 5. Toplam çağrı sayıları, ortalama çağrı sayıları ve istasyonlar arasındaki farklara ilişkin korelasyon testi sonuçları
Table 5. Correlation test results regarding total number of calls, average number of calls and differences between stations

		toplam çağrı sayısı	istasyon1	istasyon2	istasyon3	istasyon4	istasyon5	istasyon6	istasyon7	istasyon8	ortalama çağrı sayısı
toplam çağrı sayısı	Kendall's Tau B	—									
	p-value	—									
istasyon1	Kendall's Tau B	0.622 *	—								
	p-value	0.010	—								
istasyon2	Kendall's Tau B	0.985 ***	0.594 *	—							
	p-value	< .001	0.013	—							
istasyon3	Kendall's Tau B	0.508 *	0.486	0.465 *	—						
	p-value	0.032	0.057	0.047	—						
istasyon4	Kendall's Tau B	0.652 **	0.828 **	0.626 **	0.539 *	—					
	p-value	0.006	0.001	0.008	0.031	—					
istasyon5	Kendall's Tau B	0.596 *	0.520	0.587 *	0.852 **	0.655 *	—				
	p-value	0.016	0.052	0.017	0.001	0.012	—				
istasyon6	Kendall's Tau B	0.528 *	0.477	0.520 *	0.654 *	0.725 **	0.843 **	—			
	p-value	0.030	0.069	0.030	0.010	0.005	0.002	—			
istasyon7	Kendall's Tau B	0.339	0.318	0.334	0.464	0.373	0.545	0.500	—		
	p-value	0.191	0.257	0.192	0.089	0.173	0.057	0.075	—		
istasyon8	Kendall's Tau B	0.807 ***	0.448	0.826 ***	0.398	0.514 *	0.658 **	0.603 *	0.428	—	
	p-value	< .001	0.070	< .001	0.098	0.033	0.009	0.015	0.105	—	
ortalama çağrı sayısı	Kendall's Tau B	1.000 ***	0.622 *	0.985 ***	0.508 *	0.652 **	0.596 *	0.528 *	0.339	0.807 ***	—
	p-value	< .001	0.010	< .001	0.032	0.006	0.016	0.030	0.191	< .001	—

Note. * p < .05, ** p < .01, *** p < .001

Buna göre:

- İstaston 1 ile toplam çağrı sayıları arasında pozitif ve iyi düzeyde korelasyon(0.622),
- İstasyon 2 ile toplam çağrı sayıları arasında pozitif ve mükemmel düzeyde korelasyon (0.985); istasyon 1 ile ise pozitif ve orta düzeyde korelasyon (0.594),
- İstasyon 3 ile toplam çağrı sayıları (0.508) ve istasyon 2 (0.465) arasında pozitif ve orta düzeyde korelasyon,
- İstasyon 4 ile toplam çağrı sayıları (0.652), istasyon 1 (0.828) ve istasyon 2 (0.626) arasında pozitif ve iyi düzeyde; istasyon 3 (0.539) ile pozitif ve orta düzeyde korelasyon,
- İstasyon 5 ile toplam çağrı sayısı (0.596), istasyon 2 (0.587) ve istasyon 4 (0.655) arasında pozitif ve orta düzeyde; istasyon 3 ile pozitif ve iyi düzeyde korelasyon,
- İstasyon 6 ile toplam çağrı sayısı (0.528), istasyon 2 (0.520) ve istasyon 3 (0.654) arasında pozitif ve orta düzeyde; istasyon 4 (0.725) ve istasyon 5 (0.843) arasında pozitif ve iyi düzeyde korelasyon,
- İstasyon 8 ile toplam çağrı sayıları (0.807) ve istasyon 2 (0.826) arasında pozitif ve mükemmel düzeyde korelasyon; istasyon 5 (0.658) ile pozitif ve iyi düzeyde korelasyon, istasyon 4 (0.514) ve istasyon 6 (0.603) ile arasında pozitif ve orta düzeyde korelasyon,
- Ortalama çağrı sayıları ile toplam çağrı sayıları, istasyon2 ve istasyon 8 arasında pozitif ve mükemmel düzeyde korelasyon; istasyon 4 ile pozitif ve iyi düzeyde korelasyon; istasyon 1, istasyon 3, istasyon 5 ve istasyon 6 ile pozitif ve orta düzeyde korelasyon olduğu tespit edilmiştir.

12 yarasa türünden alınan verilerle toplam çağrı sayıları, istasyonlar ve ortalama çağrı sayıları arasındaki ilişki incelenmiştir. Yukarı da verildiği gibi göstergelerin farklı düzeylerde birbirleriyle ilişkili olduğu sonucuna ulaşılmıştır.

TARTIŞMA

Türkiyede yarasa çeşitliliği en fazla olan yerler Trakya ve Akdeniz Bölgesidir. Akdeniz Bölgesi özellikle ılıman iklimi, habitat çeşitliliği, içerdiği mağara ve kayalık alanlar ile yarasalar açısından öne çıkmaktadır. Akdeniz Bölgesi'nde Orta ve Doğu Akdeniz'de yarasa çeşitliliği konusunda çeşitli çalışmalar gerçekleştirilmiş olsa da Batı Akdeniz'de özellikle Antalya ve çevresindeki çalışmalar oldukça sınırlıdır (Yorulmaz, 2010; Yavuz, 2013; Yavuz & Tunç 2016). Yavuz ve Tunç (2016) Phaselis Antik Kenti ve yakın çevresinin memeli faunası üzerine gerçekleştirdikleri çalışmada, alanda 8 yarasa türünün varlığı belirlenmiştir. Bu türler *Rhinolophus ferrumequinum, Rhinolophus hipposideros, Rhinolophus euryale, Rhinolophus mehelyi, Myotis capaccinii, Miniopterus schreibersi, Pipistrellus pipistrellus, Eptesicus serotinus*'tir. IUCN kriterlerine göre tehdit durumları değerlendirildiğinde: 4'ü düşük risk (LC), 2'si tehdit altına girebilir (NT), 2'si hassas (VU) kategorilerinde yer almaktadır. Yavuz ve Tunç (2016) yaptığı çalışmada bu türlerden *Miniopterus schreibersi, Pipistrellus pipistrellus, Eptesicus serotinus* türleri yerleşkede müdahalesiz yöntem ile de tespit edilerek varlığı desteklenmiştir. Bu ortak türlerden *Miniopterus schreibersi* tehdit altına girebilir (NT) kategorisinde yer alırken diğer ikisi düşük risk (LC) kategorisinde yer almaktadır. Yerleşkede bu türlerin yanında *Barbarstella barbastellus, Pipistrellus nathusii, Pipistrellus kuhlii, Pipistrellus pigmaeus, Hypsugo Savii, Nyctalus noctula, Nyctalus leisleri, Myotis blythii, Rousettus aegyptiacus* türleri de tespit edilmiştir. Bu türlerden *Barbarstella barbastellus* ve *Myotis blythii* tehdit altına girebilir (NT) kategorisinde yer alırken diğer 7 tür düşük risk (LC) kategorisinde yer almaktadır. *Rhinolophus ferrumequinum, Rhinolophus hipposideros, Rhinolophus euryale, Rhinolophus mehelyi, Myotis capaccinii* türleri Yavuz ve Tunç (2016) çalışma yaptığı alanda bulunmakta olup yerleşkede bu türlerin varlığına rastlanılmamıştır. Bu türlerin buradaki varlığı çalışma yapıldığı habitat; mağara, oyuk ve ormanlık alanların yaygın olduğundan kaynaklandığı düşünülmektedir. Bu türlerden Rhinolophus euryale tehdit altına girebilir (NT), *Rhinolophus mehelyi* ve *Myotis capaccinii* hassas (VU) kategorisinde yer alırken diğer iki türde düşük risk (LC) kategorisinde yer almaktadır. Yavuz (2013) Antalya ilindeki mağaralar ve yarasa çeşitliliği üzerine gerçekleştirdiği çalışmada Antalya'da yarasalar için 10 uygun mağaranın bulunduğunu ve 14 yarasa türünün de bulanabileceğini belirtmiştir. Bununla birlikte Antalya il merkezinde yarasa çeşitliliği hakkında çalışma bulunmamaktadır. Bu çalışma Akdeniz Üniversitesi yerleşkesi ve yakın çevresi müdahalesiz yöntem ile yarasa çeşitliliğinin tespit edildiği ilk çalışmadır.

Yarasaların tür teşhisleri yapabilmek için müdahaleli ve müdahalesiz yöntemler kullanılır (Flaquer et al., 2009). Müdahaleli yöntemler de doğrudan yarasa atrap veya sis ağı gibi araçlar ile yakalanarak teşhis anahtarı yardımıyla kulak yapısı, burun yapısı, kafa yapısı, kuyruk yapısı, vücut büyüklüğü gibi morfometrik ölçümler alınarak tür teşhisi yapılır. Hatta bazı türlerin teşhisi için yarasanın öldürülerek kafatası çıkartılıp gerekli ölçümler alınmasıyla tür teşhisi yapılmaktadır (Albayrak & Coşkun 2000). Bu nedenle müdahaleli yöntemler canlıya zarar vermekte ve bazen ölüme sebep olmaktadır. Bu çalışmada kullanılan müdahalesiz yöntemde ise yarasanın yakalanmasına gerek olmayıp ses kayıtları alınarak teşhis edilir ve bu bakımdan da canlıya zarar vermemektedir (Skalak et al., 2012). Bununla birlikte,

müdahelesiz yöntemler yardımıyla bir alanın yarasa çeşitliliğinin ve aktivitesinin belirlenmesi ve izlenmesi (Georgiakakis et al., 2010; Deshpande & Kelkar, 2015; Dalhoumi et al., 2017; Widerin & Reiter, 2017), yakalaması mümkün olmayan ve özellikle alan için transit göçer türlerin belirlenmesi (Benda et al., 2012; Slough et al., 2014; Blejwas et al., 2014; Lausen, 2014), insan yapımı yapıların birey ve populasyon düzeyinde etkisinin belirlenmesi (Lagerveld et al., 2014) gibi farklı birey ve populasyon düzeyindeki çalışmalarda kullanılmaktadır. Bu bakımdan bölgede daha önce tespit edilemeyen *Barbarstella barbastellus, Pipistrellus nathusii, Pipistrellus kuhlii, Pipistrellus pigmaeus, Hypsugo Savii, Nyctalus noctula, Nyctalus leisleri, Myotis blythii* ve *Rousettus aegyptiacus* türlerinin varlığına dair veriler uygulanan yöntemle elde edilmiştir.

Gözütok (2022), müdahalesiz yöntem ile otomatik ve manuel akustik ses kayıt cihazları kullanarak, doğrudan ve dolaylı gözlemlerinin yanında güncel literatürü de tarayarak Bartın ilinde 7 tür tespit etmiştir. Bu türler *Pipistrellus pipistrellus, Nyctalus noctula, Myotis blythii, Myotis daubentonii, Rhinolophus hipposideros, Rhinolophus ferrumequinum* ve *Rhinolophus euryale*"dır. Bu türler küresel ölçekte IUCN kriterlerine göre değerlendirildiğinde, Rhinolophus euryale tehdit altına girebilir (NT) diğerleri ise düşük veri (LC) kategorilerinde yer almaktadır. Özetle bu çalışmadan farklı olarak *Myotis daubentonii, Rhinolophus hipposideros, Rhinolophus ferrumequinum* ve *Rhinolophus euryale* türleri tespit edilmiştir.

Şeker & Keleş (2022), Artvin ili memeli faunasu üzerine yaptığı çalışmada, doğrudan gözlem ile bir ölü Pipistrellus pipistrellus türü ve literatür taraması ile bölgede toplam 14 tür olduğunu belirlemiştir. Bu türler *Plecotus macrobullaris, Tadarida teniotis, Eptesicus serotinus, Rhinolophus hipposideros, Rhinolophus ferrumequinum, Pipistrellus pipistrellus, Pipistrellus savii, Myotis myotis, Myotis blythii, Myotis nattereri, Myotis mystacinus, Miniopterus schreibersi, Myotis bechsteini* ve *Barbarstella barbastellus*'tur. Küresel ölçekte IUCN kriterlerine göre, *Miniopterus schreibersi, Myotis bechsteini* ve *Barbarstella barbastellus* tehdit altına girebilir (NT) kategorisinde, diğer türler ise düşük risk (LC) kategorisinde yer almaktadır. Çalışmada yerleşkede tespit edilen yarasalardan farklı olarak da *Tadarida teniotis, Rhinolophus hipposideros, Rhinolophus ferrumequinum, Pipistrellus savii, Myotis myotis, Myotis nattereri, Myotis mystacinus, Myotis bechsteini, Plecotus macrobullaris* türleri bulunmaktadır. Artvin ilinde bulunan yarasa türlerinden farklı olarak *Pipistrellus nathusii, Pipistrellus kuhlii, Pipistrellus pigmaeus, Hypsugo savii, Nyctalus noctula, Nyctalus leisleri* ve *Rousettus aegyptiacus* türleri de yerleşkede bulunmaktadır.

İlemin (2022), Manisa ilinde yaptığı fauna çalışmasında 10 tür tespit etmiştir. Müdahalesiz yöntem ile akustik ses kayıt cihazı kullanmış ve doğrudan gözlem ile bölgede barınabilecekleri kale, mağara, eski bina, gibi ortamlara girerek tüneklerinde türleri ve dışkıları fotoğraflamış ve literatür taramıştır. Çalışmada tespit edilen türler: *Rhinolophus euryale, Rhinolophus ferrumequinum, Rhinolophus hipposideros, Pipistrellus pipistrellus, Eptesicus serotinus, Miniopterus schreibersii, Myotis blythii, Myotis emarginatus, Myotis myotis* ve *Myotis mystacinus* olarak listelenmiştir. Bu türlerden *Rhinolophus euryale* tehdit altına girebilir (NT) kategorisinde, diğer türler ise düşük risk (LC) kategorisinde yer almaktadır. Araştırmanın gerçekleştirildiği yerleşkedeki yarasalardan farklı olarak çalışma yapılan alanda: *Rhinolophus euryale, Rhinolophus ferrumequinum, Rhinolophus hipposideros, Myotis emarginatus, Myotis myotis, Myotis mystacinus* türleri bulunmaktadır. Manisa İlinde yerleşkeden farklı olarak *Rousettus aegyptiacus, Nyctalus noctula, Hypsugo savii, Pipistrellus pigmaeus, Pipistrellus kuhlii, Pipistrellus nathusii*, ve *Barbarstella barbastellus* türleri bulunmaktadır.

Baş & Arslan (2021), müdahalesiz yöntem ile akustik ses kayıt cihazı (batcorder) kullanılarak Konya ili, Selçuklu ilçesinde 12 tür tespit etmiştir. Ayıklama ve analiz çalışmaları sonrası tür sayısı 7 olarak saptanmıştır. Bu türler *Myotis myotis/blythi, Barbastella barbastellus, Pipistrellus pipistrellus, Hypsugo savii, Eptesicus serotinus* ve *Miniopterus schreibersii* dir. IUCN kriterlerine göre *Barbastella barbastellus* ve *Eptesicus serotinus* çalışılan bölgede ilk kez kayda geçmiştir. Baş & Arslan (2021) tarafından *Myotis myotis/blythi* türleri tespit edilmiş iken yerleşkede yapılan çalışmada bu türlere rastlanılmamıştır. IUCN kriterlerine göre küresel ölçekte *Myotis myotis/blythi* düşük veri (LC) kategorilerinde yer almaktadır. Bu türlerden IUCN kriterlerine göre küresel ölçekte *Barbarstella barbastellus* ve *Miniopterus schreibersi* tehdit altına girebilir (NT), *Pipistrellus pipistrellus*, *Eptesicus serotinus, Hypsugo savii* türleri düşük risk (LC) kategorisinde yer almakta olup, aynı yöntem kullanılarak yerleşkede yapılan çalışmada da bu türler tespit edilmiştir. Antalya ilinin yakın bölgesindeki yapılan bu çalışma birbirini destekler niteliktedir.

Selçuk & Kefelioğlu (2020), müdahalesiz yöntem ile otomatik akustik ses kayıt cihazları kullanarak Samsun, Amasya, Tokat ve Eskişehir illerinde memeli faunası üzerine yapmış oldukları çalışmada 11 tür tespit etmiştir. Bu çalışma ile Samsun ilinde *Rhinolophus ferrumequinum, Rhinolophus hipposideros, Myotis daubentonii, Myotis emarginatus, Eptesicus serotinus, Pipistrellus*

pipistrellus türlerini, Amasya ilinde *Pipistrellus pipistrellus* türünü, Tokat ilinde *Rhinolophus ferrumequinum, Rhinolophus hipposideros, Rhinolophus euryale, Myotis myotis, Eptesicus serotinus, Pipistrellus pipistrellus, Myotis blythii, Myotis aurascens* türlerini ve Eskişehir ilinde *Rhinolophus ferrumequinum, Rhinolophus hipposideros, Myotis myotis* türlerini saptamıştır. Bu türlerden *Rhinolophus euryale* IUCN kapsamında tehdit altına girebilir (NT) kategorisinde diğer türler düşük risk (LC) kategorisinde yer almaktadır. *Barbarstella barbastellus, Pipistrellus nathusii, Pipistrellus kuhlii, Pipistrellus pigmaeus, Miniopterus schreibersii, Hypsugo savii, Nyctalus noctula, Nyctalus leisleri* ve *Rousettus aegyptiacus* türleri yerleşkede bulunmakta fakat yılında yapılan ilgili çalışmada bu türlere rastlanılmamıştır. *Rhinolophus ferrumequinum, Rhinolophus hipposideros, Rhinolophus euryale, Myotis daubentonii, Myotis emarginatus, Myotis myotis*, ve *Myotis aurascens* türleri Selçuk & Kefelioğlu (2020)'nun yaptığı çalışmada bulunurken bu çalışmada tespit edilememiştir.

Urker & Yorulmaz (2020) tarafından, müdahalesiz yöntem ile otomatik ve manuel akustik ses kayıt cihazları kullanarak, doğrudan ve dolaylı gözlemlerle Muğla ili, Köyceğiz-Dalyan Sığla ormanlarında yapılmış olan çalışmada 13 kayıt alınmış olup 2'si cins düzeyindedir. Bu türlerden *Myotis daubentonii, Myotis emarginatus, Pipistrellus nathusii, Pipistrellus pygmaeus, Plecotus macrobullaris* çalışılan bölgede ilk kez kayda geçmiştir. Tespit edilen türler; *Rhinolophus ferrumequinum, Myotis daubentonii, Myotis emerginatus, Pipistrellus pipistrellus, Pipistrellus pygmaeus, Pipistrellus kuhlii, Pipistrellus nathusii, Hypsugo savii, Plecotus macrobullari, Miniopterus schreibersii, Myotis sp., Nyctalus sp.*' dir. Küresel ölçekte IUCN kriterlerine göre *Miniopterus schreibersii* tehdit altına girebilir (NT) kategorisinde diğer türlerde düşük risk (LC) kategorisinde yer almaktadır. Yerleşkedeki yarasalardan farklı olarak çalışma yapılan alanda *Rhinolophus ferrumequinum*, *Myotis daubentonii*, *Myotis emerginatus* ve *Plecotus macrobullari* türleri de bulunmaktadır.

Gözütok (2017), Bursa ilinde yaptığı fauna çalışmasıyla 14 tür tespit etmiştir. Doğrudan veya dolaylı gözlemler yaparak arazi çalışmaları ile *Pipistrellus pipistrellus* ve *Rhinolophus hipposideros* türlerini, literatür çalışmaları ile *Hypsugo savii*, *Pipistrellus kuhli*, *Nyctalus lasiopterus*, *Myotis blythii*, *Myotis mystacinus*, *Myotis myotis*, *Myotis daubentonii*, *Myotis capaccinii, Rhinolophus ferrumequinum, Rhinolophus euryale, Rhinolophus blasii, Miniopterus schreibersii* türlerinin varlığını tespit etmiştir. *Rhinolophus euryale*, *Miniopterus schreibersii* ve *Rhinolophus blasii* yakın gelecekte tehdit altına girebilir (NT) kategorisinde, *Myotis capaccinii* ve *Nyctalus lasiopterus* hassas (VU) kategorisinde ve diğer türler düşük risk (LC) kategorisinde yer almaktadır. *Pipistrellus pipistrellus, Pipistrellus kuhli*, *Hypsugo savii, Miniopterus schreibersii* ve *Myotis blythi* yerleşkedeki türler ile ortak iken *Rhinolophus ferrumequinum*, *Rhinolophus hipposideros, Rhinolophus euryale, Rhinolophus blasii, Myotis capaccinii, Myotis daubentoni, Myotis myotis, Myotis mystacinus, Nyctalus lasiopterus* türleri Bursa'da gözlemlenirken yerleşkede gözlenmemiştir.

İlkbaharda 9 tür, yaz aylarında 9, sonbaharda 9 tür kaydedilmiş kış aylarında kayıt alınmamıştır. Bütün mevsimlerde *Barbarstella barbastellus, Pipistrellus nathusii, Pipistrellus pipistrellus, Pipistrellus kuhlii, Miniopterus schreibersii, Eptesicus serotinus, Rousettus aegyptiacus* türleri gözlemlenmiştir. *Rousettus aegyptiacus* türü doğrudan gözlem ile kayıtlara geçerken diğer türler ses kaydı alınarak tespit edilmiştir. *Pipistrellus pigmaeus* türü ilkbahar ve yaz aylarında ses kaydı alınmışken sonbahar aylarında gözlemlenmemiştir*. Hypsugo savii* ve *Myotis blythii* türü yaz ve sonbaharda gözlemlenirken ilkbaharda gözlemlenmemiştir. *Nyctalus noctula* türü sadece ilkbahar, *Nyctalus leisleri* türü de sadece sonbaharda gözlemlenmiştir (Çizelge 6).

Önceki çalışmalara bakıldığında, kısa dalga boyunda UV ışığı yayan ve yüksek böcek yoğunluklarını çeken "Cıva Buharı" lambaları gibi beyaz sokak lambalarının sırayla P. Pipistrellus başta olmak üzere yarasaları çektiği tespit edilmiştir (Stone et al., 2012). Ancak yeni nesil LED ışıkların ise tam tersi bir etki gösterdikleri yani yarasa faaliyetlerini azalttıkları bilidirilmiştir (Bolliger et al., 2020). İstasyonlara göre tür çeşitliliği açısından değerlendirildiği zaman en fazla tür çeşitliliği 12 tür ile II numaralı istasyon sahiptir. Bölgenin besin açısından zengin olması; bina yakınında vektör çalışmalarının yapılması, ışık kaynağının yer alması ve ağaçlık ve çalılık alanların olması yarasaların beslenmesi açısından elverişli bir alan oluşturmaktadır (Stone et al., 2012). Bu açıdan tür sayısının fazla olduğu düşünülmektedir. En az tür çeşitliliği iki tür ile VII numaralı istasyonda gözlenmiştir. Etrafta böcek çekici ışık kaynağının olmaması daha çok LED ışıklarının olması nedeniyle yarasa tür sayısının az olduğu düşünülmekedir (Bolliger et al., 2020).

Görüldüğü gibi ışık kaynağına bağlı olarak böceklerin çekilmesi bu sebepten dolayı da yarasaların belli bölgelere çekilmesi söz konusudur. Ancak çeşitli kaynaklara göre böcekler ve buna bağlı olarak yarasalar belli bölgelerde yoğunlaşsalarda ışık türlerine dalga boylarına göre belli böcek takımları belli ışık kaynaklarına çekilmekte ve buraya gelen yarasalar yalnızca bu türlerle tek tip beslenmekte diğer böceklerin sayısı artmaktadır (normal doğal bir ortamda etrafta bulunan tüm böcek türleri ile

beslenecek) (Mostafaeipour et al., 2018; Giavi et al., 2021). Birçok araştırmacı ışık kirliliğinin biyoçeşitlik üzerindeki olumsuz etkisinden bahsetmekle birlikte bunun daha çok araştırmaya muhtaç bir konu olduğu görüşünde birleşmektedir (Sanders et al., 2021). Hatta yapılan bir araştırmada ışık kaynakları insan yerleşim alanlarının yoğun olduğu bölgelerde daha fazla bulunmakta ve buralarda da insektisit kullanımının yaygın olması yüzünden ilaçlı böceklerle beslenen yarasa türleri zarar görmektedir (Oliveira et al., 2020).

Çizelge 6. Yerleşkedeki yarasa türlerinin aylara ve gözlem şekline göre dağılımı
Table 6. Distribution of bat species in the campus according to months and type of observation

TÜRLER / S*PECIES*	İLKBAHAR *SPRING*		YAZ *SUMMER*			SONBAHAR *AUTUMN*			GÖZLEM ŞEKLİ *OBSERVATION METHOD*	
	Nisan	Mayıs	Haziran	Temmuz	Ağustos	Eylül	Ekim	Kasım	Ses Kaydı	Doğrudan Gözlem
Barbarstella barbastellus	X	X	X	X	X	X	X		X	
Pipistrellus nathusii	X	X	X	X	X	X	X	X	X	
Pipistrellus pipistrellus	X	X	X	X	X	X	X		X	
Pipistrellus kuhlii		X	X	X	X	X	X		X	
Pipistrellus pigmaeus		X	X						X	
Miniopterus schreibersii		X	X	X	X	X	X		X	
Hypsugo savii			X	X		X	X		X	
Nyctalus noctula		X							X	
Nyctalus leisleri						X			X	
Eptesicus serotinus		X	X	X	X	X	X		X	
Myotis blythii				X		X			X	
Rousettus aegyptiacus	X	X	X	X	X	X	X	X		X

Bu çalışmaya paralel bir sonuç Akdeniz Üniversitesi yerleşkesinde yapılan çalışmada da gözlenmiştir. Yerleşke içerisinde bulunan yolları ikiye bölen uzun refüjlerde meyve ağaçları bulunmakta ve bu ağaçlar yaz boyunca peryodik olarak ilaçlanmaktadır. Bu uzun refüjler boyunca da böcek çeken civa buharlı lambalarla aydınlatılma yapılmaktadır. Bu konuda bir zehirlenme araştırması yapılmamakla birlikte Oliveira et al. (2020) sonuçlara dayanarak tesadüfi olarak bu yol üzerinde birçok kez ölü yarasa bireyleri bulunmasını büyük ihtimalle zehirlenmeye bağlanabilmektedir.

SONUÇ ve ÖNERİLER

Artan tarımsal üretim, dünya genelinde hem insan hem de çevre sağlığını tehdit eden pestisit kullanımını artırmıştır. Son araştırmalar, arılardan memelilere kadar birçok hedef olmayan organizmanın, pestisit maruziyetinin, davranış, gelişme ve üreme bozuklukları dahil olmak üzere çok çeşitli toksik etkileri olduğunu göstermektedir. Memeliler arasında, yarasalar ekotoksikolojik çalışmalar arasında genellikle ihmal edilen bir taksondur (Oliveira et al., 2020).

Böcekçil yarasalar, agroekosistemlerdeki haşerelerin etkili avcılarıdır. Bu haşere kontrol hizmetinin dünya çapında tarım için milyarlarca dolar değerinde olduğu tahmin edilmektedir. Çok çeşitli beslenme alışkanlıkları göz önüne alındığında, yarasalar, yiyecek veya su kontaminasyonu yoluyla veya tünedikleri alanlarda doğrudan cilt teması yoluyla çevresel kirleticilere maruz kalırlar.

Doğal alanların insan faaliyetleri sonucu tahribi son yıllarda artarak hızla devam etmektedir. Özellikle artan nüfusa bağlı olarak enerji, barınma ve beslenme ihtiyaçlarının artışı bu sorunun temelini oluşturmaktadır. Pek çok bitki ve hayvan türü bu durumdan olumsuz etkilenmektedir. Diğer taraftan, bazı türler de şehir ekosistemleri uyum sağlayarak şehir ekosistemlerde yaşamlarını sürdürmektedir. Bu bakımdan, şehir ekosistemleri pek çok hayvan türü için barınma ve beslenme alanları sağlamaktadır. Bazı yarasa türlerinin tüneller, tarihi yapılar, köprü altları, ahırlar, terkedilmiş binalar, park ve bahçeler vb. insan yapımı yapılarda konakladıkları bilinmektedir (Lausen & Barclay, 2006). Özellikle geniş alan kaplayan park ve bahçeler yarasalar için şehir ekosistemlerinde önemli yer tutmaktadır. Dahası, şehir ışıklandırmaları da yarasaların beslendikleri bazı böcek türlerini çektiği için yarasalar bu alanlarda beslenmektedir (Rydell, 1992; Avila-Flores & Fenton, 2005). Bununla birlikte, şehir bitki çeşitliliği de yarasa beslenme faaliyetlerini etkilemektedir (Jung & Kalko, 2010). Sekiz aylık yapılan çalışma sonucunda en çok ses kaydı Eylül ve Ekim aylarında kaydedilmiştir. En

çok ses 41140 kayıt ile pipistreloid grubuna aittir. Tür düzeyinde en çok ses 10531 kayıt ile *Pipistrellus pipistrellus'tür*. En az çağrı ise 1 kayıt ile Plecotus grubundan alınmıştır. 9220 kayıt ise tanımlanamamıştır. *Rousettus aegyptiacus* türü 4 mevsim doğrudan gözlemlenmiştir. Akdeniz Üniversitesi yerleşkesi içerdiği farklı habitat tipleri, bitki çeşitliliği ve özellikle *Rosettus aegyptiacus* ve diğer tespit edilen türlerin tercih ettiği çeşitli kültür bitkileri ile yarasalar için önemli bir beslenme alanıdır.

Bu çalışma ile yerleşkedeki yarasa türleri müdahelesiz yöntemlerle belirlenmiş olup yerleşkenin gerek yarasa çeşitliliği gerekse de koruma biyolojisi kapsamında önemli bir alan olduğu tespit edilmiştir. Sonraki çalışmalarda yıl boyunca aküstik kayıt yaparak yarasaların yıl boyunca aktivelerinin izlenmesi, yarasa türleri hakkındaki daha geniş verilere ulaşılmasını sağlayacaktır. Ayrıca yarasa türlerinin besin tercihlerinin belirlenmesi de biyolojik mücadelede açsından faydalı olacaktır. Her yıl belirli dönemlerde zararlı böcek mücadelesi için çeşitli pestisitlerin yerleşke içerisinde kullanıldığı bilinmektedir. Bu bağlamda, bu pestisitlerin yarasalar üzerine olası olumsuz etkilerinin incelenmesi gerekmektedir. Diğer taraftan, yaşayan yarasaların üzerindeki zoonotik canlıların tespit edilip insanlara hastalık tehditi oluşturup oluşturmayacağı da ele alınması gerekliliğini ortaya koymaktadır.

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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.

Etik Kurul Beyanı

Çalışma klinik ve deneysel amaçla, insan ve hayvanlar üzerinde yapılmadığı için etik kurul izni gerekmemektedir.

KAYNAKLAR

Albayrak, İ. & Coşkun, Ş. (2000). Miniopterus Schreibersi'nin (Kuhl, 1819) Türkiye'deki (Chiroptera: Vespertilionidae) Coğrafi Varyasyonları ve Taksonomik Durumu. *Türk Zooloji Dergisi 24(2)*, 125-134. https://journals.tubitak.gov.tr/zoology/vol24/iss2/2/

Anonymous, (2022). *The Batcorder-System*. https://ecoobs.com/. (Alınma tarihi: 06.08.2022)

Avila-Flores, R. & Fenton, MB. (2005). Use of Spatial Features by Foraging Insectivorous Bats In a Large Urban Landscape. *Journal Of Mammalogy 86(6)*, 1193-1204. https://doi.org/10.1644/04-MAMM-A-085R1.1.

Baş, M., & ARSLAN, A. (2021). Acoustical Investigation of Bats in Selçuklu District of Konya Province. *Kahramanmaraş Sütçü İmam Üniversitesi Tarım ve Doğa Dergisi, 24(1)*, 186-195

Benda, P., Ivanova, T., Horacek, I., Hanak, V., Cerveny, J., Gaisler, J., & Vohralik, V. (2003). Bats (Mammalia: Chiroptera) Of The Eastern Mediterranean. Part 3. Review Of Bat Distribution In Bulgaria. *Acta Societatis Zoologicae Bohemicae, 67(4),* 245-357. https://www.academia.edu/download/30182115/acta_soc_zool_bohem_2003-04.

Benda, P., Faizolâhi, K., Andreas, M., Obuch, J., Reiter, A., Sevcik, M., & Ashrafi, S. (2012). Bats (Mammalia: Chiroptera) Of The Eastern Mediterranean and Middle East. Part 10. Bat Fauna of Iran. *Acta Societatis Zoologicae Bohemicae 76(1/4)*, 163-582. http://www.nm.cz/admin/files/PM/download/zivotopisy-publikace/benda2012aszb.pdf

Bolliger, J., Hennet, T., Wermelinger, B., Bösch, R., Pazur, R., Blum, S., Haller, J., & Obrist, MK. (2020). Effects Of Traffic-Regulated Street Lighting on Nocturnal Insect Abundance and Bat Activity. *Basic and Applied Ecology, 47*, 44-56. https://doi.org/10.1016/j.baae.2020.06.003.

Britzke, ER., Gillam, EH., & Murray, KL. (2013). Current State of Understanding of Ultrasonic Detectors for the Study of Bat Ecology. *Acta Theriologica, 58(2)*, 109-117. https://doi.org/10.1007/s13364-013-0131-3.

Blejwas, KM. (2014). Acoustic Monitoring Provides First Records of Hoary Bats (Lasiurus Cinereus) and Delineates The Distribution of Silver-Haired Bats (Lasionycteris Noctivagans) in Southeast Alaska. *Northwestern Naturalist, 95(3)*, 236-250. https://doi.org/10.1898/13-34.1.

Çağlar, M. (1969). Türkiye'nin Yarasaları II (Bats of Turkey II). *Türk Biyoloji Dergisi 19(2-4)*, 88-106. http://actabiologicaturcica.com/index.php/abt/article/view/686.

Danford, C. & Alston, E. (1877). On The Mammals of Asia Minor. *Proceedings of The Zoological Society*, London, İngiltere, 1877, ss. 11.

Dalhoumi, R., Morellet, N., Aissa, P., & Aulagnier, S. (2017). Seasonal Activity Pattern and Habitat Use by the Isabelline Serotine Bat (Eptesicus Isabellinus) In An Arid Environment of Tunisia. *Acta Chiropterologica, 19(1)*, 141-153. https://doi.org/10.3161/15081109ACC2017.19.1.011

Deshpande, K. & Kelkar, N. (2015). Acoustic Identification of Otomops Wroughtoni and Other Free-Tailed Bat Species (Chiroptera: Molossidae) From India. *Acta Chiropterologica, 17(2)*, 419-428. https://doi.org/10.3161/15081109ACC2015.17.2.018

Flaquer, C., Puig-Montserrat, X., Goiti, U., Vidal, F., Curco, A., & Russo, D. (2009). Habitat Selection in Nathusius Pipistrelle (Pipistrellus Nathusii): The Importance of Wetlands. *Acta Chiropterologica, 11(1)*, 149-155. https://doi.org/10.3161/150811009 X465767

Georgiakakis, P., Vasilakopoulos, P., Mylonas, M., & Russo, D. (2010). Bat Species Richness and Activity over an Elevation Gradient in Mediterranean Shrublands of Crete. *Hystrix, 21(1)*. https://doi.org/10.4404/hystrix-21.1-4485.

Giavi, S., Fontaine, C., & Knop, E. (2021). Impact of artificial light at night on diurnal plant-pollinator interactions. *Nature communications, 12(1)*, 1-5. 10.1038/s41467-021-22011-8.

Gözütok, S. (2017). Bursa İli Memeli (Classis: Mammalia) Faunası ve Türlerin Koruma Statüleri. *Uluslararası Tarım ve Yaban Hayatı Bilimleri Dergisi 3(2)*, 120-130. https://doi.org/10.24180/ ijaws.320870.

Gözütok, S. (2022). Bartın İlindeki Memeli (Classis: Mammalia) Türleri ve Korunma Durumları. *Uluslararası Tarım ve Yaban Hayatı Bilimleri Dergisi, 8(3)*, 528-541. DOI: 10.24180/ijaws. 1178230.

Harrison, D. L. (1964). The mammals of Arabia. *Benn publishing*, London.

Heim, O., Heim, D. M., Marggraf, L., Voigt, C. C., Zhang, X., Luo, Y., & Zheng, J. (2020). Variant maps for bat echolocation call identification algorithms. *Bioacoustics 29(5)*, 557-571. https://doi.org/10.1080/09524622.2019.1621776.

Helversen, O.V. (1989). New Records of Bats (Chiroptera) From Turkey. *Zoology ın the Middle East, 3(1)*, 5-18. https://doi.org/10.1080/ 09397140.1989.10637569.

Hutson, A. M., Marnell, F., & Torv, T. (2015). A Guide to the Implementation of The Agreement on the Conservation of Populations of European Bats (Eurobats). *Eurobats*, Bonn.

İlemin, Y. (2022). Manisa Memeli Faunası Üzerine Bir Araştırma. *Commagene Journal of Biology*, 6 (1), 6-10. DOI: 10.31594/commagene.1052286

Jung, K. & Kalko, E. K. (2010). Where Forest Meets Urbanization: Foraging Plasticity of Aerial Insectivorous Bats in an Anthropogenically Altered Environment. *Journal Of Mammalogy, 91(1)*, 144-153. https://doi.org/10.1644/08-MAMM-A-313R.1.

Lagerveld, S., Poerink, B. J., Haselager, R., & Verdaat, H. (2014). Bats In Dutch Offshore Wind Farms In Autumn 2012. *Lutra, 57(2)*, 61-69. https://tethys. pnnl.gov/sites/default/files/publications/Lagerveld-et-al-2014.pdf.

Lausen, C. L. & Barclay, R. M. (2006). Benefits of Living in a Building: Big Brown Bats (Eptesicus Fuscus) in Rocks Versus Buildings. *Journal Of Mammalogy, 87(2)*, 362-370. https://doi.org/ 10.1644/05-MAMM-A-127R1.1.

Lausen, C. L. (2014). Eastern Red Bat (Lasiurus Borealis) Occurrence in Northern Alberta. *Northwestern Naturalist, 95*, 219–227. https:// doi.org/10.1898/13-32.1.

Lehmann, E. V. (1966). Taxonomische Bemerkungen zur Saugerausbeute der Kumerloeve-schen Orientreisen 1953-1965. *Zoologische Beitrcige, 12*, 251-317. https://archive.org/details/bonner-zoologische-beitraege-30-0027-0031/page/n5/mode/2up.

Mostafaeipour, A., Goli, A., & Qolipour, M. (2018). Prediction of Air Travel Demand Using a Hybrid Artificial Neural Network (ANN) With Bat and Firefly Algorithms: a Case Study. *The Journal of Supercomputing, 74(10)*, 5461-5484. https://doi.org/ 10.1007/s11227-018-2452-0.

Osborn, D. J. (1963). New Distributional Records of Bats From Turkey. *Mammalia, 27(2)*, 210-217. https://doi.org/10.1515/mamm.1963.27.2.210.

Oliveira, K., Araujo, T., Rotti, A., Mothe, D., Rivals, F., & Avilla, L. S. (2020). Fantastic Beasts and What They Ate: Revealing Feeding Habits And Ecological Niche of Late Quaternary Macraucheniidae From South America. *Quaternary Science Reviews 231*, 106178. https://doi.org/10.1016/j.quascirev.2020. 106662.

Rydell, J. (1992). Exploitation of Insects Around Streetlamps By Bats in Sweden. *Functional Ecology*, 744-750. https://doi.org/10.2307/2389972.

Sanders, D., Frago, E., Kehoe, R., Patterson, C., & Gaston, K. (2021). A Meta-Analysis of Biological Impacts of Artificial Light at Night. *Nature Ecology & Evolution 5(1)*,74-81. https://doi.org/10.1038/ s41559-020-01322-x.

Salles, A. (2022). *Bats:* Vision or echolocation, why not both? *Current Biology, 32(7)*, R318-R320.

Selçuk, A. Y., & Kefelioğlu, H. (2020). Samsun, Amasya, Tokat ve Eskişehir İlleri Memeli Faunası ve Türlerin Koruma Statüleri. *Kahramanmaraş Sütçü İmam Üniversitesi Tarım ve Doğa Dergisi, 23(2)*, 379-387.

Skalak, S. L., Sherwin, R. E., & Brigham, R. M. (2012). Sampling Period, Size and Duration Influence Measures of Bat Species Richness From Acoustic Surveys. *Methods in Ecology and Evolution, 3(3)*, 490-502. https://doi.org/10.1111/j.2041-210X.2011. 00177.x.

Slough, B. G. (2014). Acoustic Surveys Reveal Hoary Bat (Lasiurus Cinereus) and Long-Legged Myotis (Myotis Volans) In Yukon. *Northwestern Naturalist, 95(3)*, 176-185. https://doi.org/10.1898/ 13-08.1.

Stone, E. L., Jones, G., & Harris, S. (2012). Conserving Energy at a Cost to Biodiversity? Impacts of LED

Lighting on Bats. *Global Change Biology, 18*, 2458-2465. https://doi.org/10.1111/j.1365-2486.2012.02705.x.

Şeker, P. S., & Keleş, G. A. (2022). Kamilet Vadisi (Arhavi, Artvin) memeli türleri ve koruma. *Anadolu Orman Araştırmaları Dergisi, 8(2)*, 33-39.

Urker, O. & Yorulmaz, T. (2020). Köyceğiz-Dalyan Özel Çevre Koruma Bölgesi'ndeki Anadolu sığla ormanlarında yarasa (Chiroptera) aktivitesinin belirlenmesi. *Ormancılık Araştırma Dergisi, 7 (1)*, 88-103. DOI: 10.17568/ogmoad.651223.

Ünal, O. & Gökçeoğlu, M. (2003). Akdeniz Üniversitesi Kampüs Florası. *Akdeniz Üniversitesi Ziraat Fakültesi Dergisi 16(2)*, 143-154. https://dergipark.org.tr/en/download/article-file/18264.

Widerin, K. & Reiter, G. (2017). Bat Activity at High Altitudes in the Central Alps, Europe. *Acta Chiropterologica, 19(2)*, 379-387. https://doi.org/10.3161/ 15081109ACC2017.19.2.014.

Yavuz, M. (2013). Antalya İlindeki Mağaralar ve Yarasalar (Sözlü bildiri). Türkiye Yarasaları Sempozyum I Bildiriler Kitabı, Balıkesir, Türkiye 25-26 Ekim 2013, ss 7.

Yavuz, M. & Tunç, M. R. (2016). Phaselis Antik Kenti ve Yakın Çevresinin Memeli Faunası: 2014-2015 Yılları Çalışmaları. *Journal of Interdisciplinary Mediterranean Studies, 2*. 10.18367/Pha.16012.

Yorulmaz, T. (2010). *K.K.Ü. Güneydoğu Türkiye Yarasaları (Tez no 258936)*. [Doktora Tezi, Kırıkkale Üniversitesi Fen Bilimleri Enstitüsü Biyoloji Ana Bilim Dalı]. Yüksek Öğretim Kurulu Ulusal Tez Merkezi.

Zimmermann, K. (1953). Das Gesamtbild Der Sauger-Fauna Kretas. *Zeitschrift für Säugetierkunde, 67*, 1-72. https://archive.org/stream/zeitschri171919531 954deut/zeitschri171919531954deut_djvu.txt.

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Impact of Different Methods of Wheat Cultivation on the Occurrence of Carabidae Family Representatives (*Carabidae*, *Coleoptera*)

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ABSTRACT

The aim of this study was to evaluate the impact of different methods of wheat cultivation on the occurrence of Carabidae (Coleoptera, Carabidae). The collection was carried out during a three-year period from 2016 to 2018, on research areas in Borovce, which belong to the Research Institute of Plant Production (VÚRV) in Piešťany. The collection of epigeic material was done in five variants: V1: mulching technique; V2: conventional technique; V3: minimization technique; V4: no-till technique; and ECO: ecological technique. During the three-year period, we obtained 11,362 specimens from eight epigeic groups. Out of all the epigeic groups, the dominant order was Coleoptera with the number of individuals 7593. We addressed the Coleoptera order in more detail. It was represented by six families, of which the *Carabidae* family was dominant. We recorded 6656 specimens from the mentioned family, of which we determined 20 species. The eudominant species were *Pterostichus melanarius* (Illiger, 1798) and *Pseudoophonus rufipes* (DeGeer, 1774) throughout the whole period on all types of variants. Based on the specimens obtained, we calculated the values of dominance, frequency, species similarity of communities, diversity of communities and statistically evaluated the correlation of the impact of meteorological factors and the occurrence of Carabidae.

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INTRODUCTION

The ecology and taxonomy of beetle species is well known. These invertebrates have high demands on the habitat, which they live in, and they are very sensitive to the changes that take place in it. This is also the reason, why they are used as bioindicators of the environment (Rainio and Niemelä, 2003). The diversity and abundance of insect predators in agricultural areas is influenced by the type of agriculture and the presence of natural habitats (Pfiffner and Luka, 2000). Ground beetles such as the Carabidae family feed in agricultural ecosystems on predators, such as ants, aphids, caterpillars or insect eggs (Ekschmitt et al., 1997). The occurrence of this family may reduce some populations of prey in agroecosystems (Edwards et al., 1979; Lang et al., 1999). Due to their sensitive response to pesticides, herbicides, toxic substances and fertilizers, they are used as a model group in ecological studies. Through this group, we monitor changes in the environment, as well as the human environment (Hůrka, 1996). One of the examples of changes in the environment is indicated by the species *Pseudoophonus rufipes* (DeGeer, 1774), which may indicate degrading effects of human activity (Peterkova, 2014). Vician et al. (2018) examined the Carabidae family in the areas with different soil management, and found that the ecological conditions were the most satisfactory. Soil with agri-environmental maintenance contained more macro and microelements, more humus and had a more neutral pH than the conventionally cultivated soil. The occurrence of Carabidae is also affected by the use of agricultural sprays and fertilizers. Multiple studies have shown that the Carabidae family is negatively affected by heavy metals, such as zinc or cadmium, which in *Poecilus cupreus* (Linnaeus, 1758) have affected individuals' size and growth (Ermakov, 2004). The high number of species of Carabidae family may not necessarily indicate the preservation of the ecosystem. In agricultural areas, the number of Carabidae species is usually higher than in undisturbed forest habitats. Therefore, we do not consider the number of species to be decisive, but rather their ecological demands. Preserved ecosystems are dominated by species with higher ecological demands (Bohac, 2007). The Carabidae family belongs

to polyphagous species. Due to the predation of invertebrates at different development stages, they play an important role in the formation of organic matter and the circulation of substances in the ecosystem (Vavara, 2010). Deep plowing is one of the agrotechnical techniques that reduce the diversity of Carabidae on arable land (Swaminathan, 2014). The aim of this study was to evaluate the occurrence of Carabidae (Coleoptera, Carabidae) in various methods of wheat cultivation.

MATERIAL and METODS

Epigeic material collection was carried out in the form of pitfall traps, which consisted of 700 ml glass container with a bait (soft cheese) in 2016 to 2018 during the given period (March to October) in the crops of a hybrid of *Triticum aestivum x Triticum spelta*. Pitfall traps were placed annually into the winter wheat, whose placing was changed every year as shown on the Figure 1. Pitfall traps placement for the ECO area was not changed in the course of 2016 to 2018. Every research area was marked for the respective option as V1, V2, V3, V4 and ECO. Every trap was marked with a number - for V1 (1-6), V2 (7-12), V3 (13-18), V4 (19-24) and for EKO (1-6). On every research field, six traps were placed - four traps at the ends and two in the middle of the research area. Consequently, the traps were emptied every 24 hours with an instant classification of the material according to a key (Hůrka, 1996; Pokorný, 2002). Same procedure was maintained during the whole research period. We managed to determine the abundance, dominance, species similarity, diversity index and frequency of Carabidae family. Furthermore, we managed to statistically determine the impact of meteorological factors on Carabidae communities. The data collected were recorded every month into a table (Microsoft Excel), which serves as a basis for this work. The statistically processed data were subjected to an analysis in the Statistica Programme, version 12.

Study area

The monitored area is located in the municipality of Borovce, district of Piešťany, falling under the Trnava self-governing region. In terms of landscape structure, Borovce is divided into the agriculture area, forest area and urbanised area. The research areas are located in the mild temperature climate, in the altitude of 167 m above sea level, with an average temperature of 9,2 °C and average annual rainfall of 593 mm (Remenar, 2017). Average wind speed on the monitored area is approximately 4,2 m/s, with the northwest and north flows direction (Mazur and Luknis, 1980).

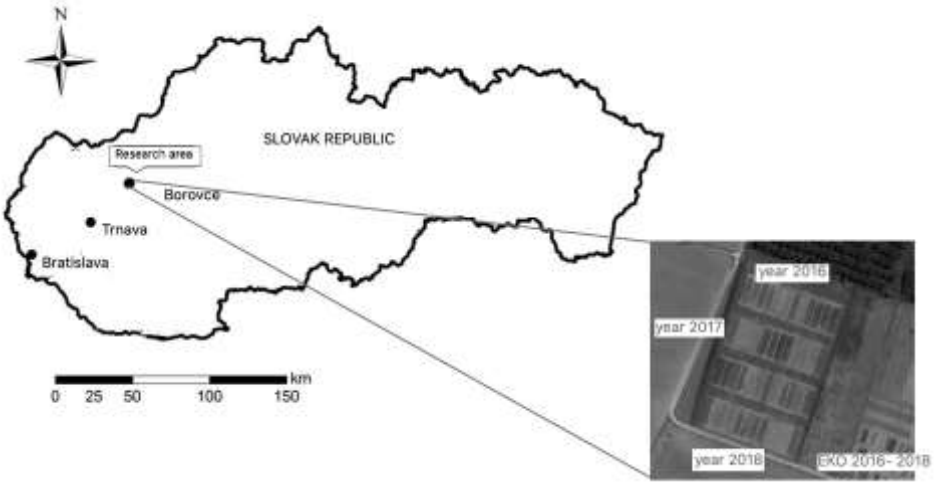

Figure 1 Map of study area – Borovce

RESULTS

Species composition and dominance

During the three-year monitored period, we managed to collect 11 365 specimens from eight epigeic groups and six families. On every monitored area, groups of Coleoptera were present at the eudominant level with the average of 67,26 %, Arachnidae group with the average of 10,66 % and Hymenoptera with the average of 13,29 %. Other epigeic groups were present at a lower level. No epigeic group was present at the dominant level. Group of Larvae was present at the subdominant level with the average of 4,58 %. Groups of Acarina with the average of 1,81 % and Dermaptera with 1,73 % were present at the recedent level. The group of Amphipoda with the average of 0,29 % and Chilopoda 0,44 % were present at the subdominant level. We recorded six families from the beetles collected. On average, families of Carabidae (88,15 %) and Staphylinidae (11,43 %) were present at the eudominant level on all three monitored periods. Other families were present at the subrecedent level - Cerambicidae (0,73 %), Chrysomelidae (0,86 %), Scarabaeidae (0,14 %) and Cicindelidae (0,11 %). In the years 2016-2018, a total of 6656 individuals of the

Carabidae family was collected in the research areas. Individuals were classified into 20 species. During the research period, we collected 1622 individuals from 19 species on the V1 area. We collected 1432 individuals from 19 species on the V2 research area. We collected 1095 individuals from 14 species on the V3 research area. On the V4 research area, we collected 1576 individuals from 17 species and on the ECO area, we collected 931 individuals belonging to 14 species. During the years 2016 to 2018, the number of species in individual areas fluctuated. The largest number of species was in the variant V1 in all three evaluated years. Eudominant species in all monitored areas during the three-year period were *Pterostichus melanarius* (Illiger, 1798) and *Pseudoophonus rufipes* (DeGeer, 1774). In the research area V1, the species *P rufipes* was significantly eudominant during all three years. The dominance of this species ranged from 39 to 54 %. Another eudominant species on the V1 research area was *P. melanarius*. The dominance of this species during the three-year monitored period ranged from 24 to 36 %. In the research area V2, the species *P. rufipes* and *Pterostichus melanarius* were significantly eudominant in all three monitored years. Their values ranged from 32 to 46 % for *P. rufipes* and 33 to 43 % for *Pterostichus melanarius*. Eudominant species in the V3 research area in the three-year monitored period were *P. rufipes* with the representation from 36 to 43 % and *Pterostichus melanarius* with the representation from 35 to 40 %. The V4 research area was represented by two eudominant species *P. rufipes* and *P. melanarius*. Their values in the three-year period ranged from 33 to 40 % for the *P. rufipes* species and 31 to 38 % for the *P. melanarius* species. In the ECO research area, *P. rufipes* was in the range of 33 to 44 % and *P. melanarius* in the range of 35 to 43 %. Both species occurred as eudominant. Other species were at a lower level on all research areas during the whole monitored period. In Table 1, we evaluate the comparison of the species dominance in the years 2016-2018 in all monitored areas. The table shows that *P. melanarius* and *P. rufipes* were characteristic species during the monitored period for each area in all three years.

Frequency

The highest frequency or recurrence of species of the Carabidae family in the V1 research area was achieved by the *Pterostichus melanarius* (Illiger, 1798) and *Pseudoophonus rufipes* (DeGeer, 1774) species. Based on the frequency, we can consider the *P. rufipes* to be constant, in 2018 on the V1 monitored area even euconstant. The *P. melanarius* species can be considered constant, in 2017 it occurred at the accessory level in the V1 monitored area. The highest frequency in the V2 research area was achieved by the *P. melanarius* and *P. rufipes* species. The data obtained show that the frequency of *P. rufipes* was at a euconstant level in 2018, and in 2016 and 2017 it was at a constant level. In the area V3, the frequency of occurrence of *P. melanarius* and *P. rufipes* was at a constant level during all three monitored years. In the V4 research area, *P. melanarius* and *P. rufipes* occurred at a constant level in the three-year monitored period. In the ECO research area, *P. melanarius* and *P. rufipes* were at a constant level in all three monitored years. The data show that the *P. melanarius* and *P. rufipes* species were constant to euconstant in all monitored areas throughout the observed period. To assess the differences between the individual research areas, we calculated the Sőrensen similarity index of species. The similarity of research areas in 2016 ranged from 86 to 100 %. The ECO research area had the highest similarity in the given year together with the V3 research area - the species similarity reached the value of 100 %. In 2017, the similarity of communities in the research areas was lower than in the previous year, it ranged from 70 to 96 %. In the given year, the ECO research area had the highest species similarity together with the V3 area, reaching the value of 96 %. In the last monitored year, the values of species similarity ranged from 80 to 100 %, similarly to 2016. Also in 2018, the ECO research area achieved the highest species similarity together with the V3 research area. The similarity value between the ECO and V3 area was 100 %. Table 2 shows the results of the Sőrensen similarity index on V1-ECO areas in the three-year monitored period.

Species similarity

The Carabidae community in the research area V1 reached a similarity of 77 to 86 %, in all monitored periods. Year-on-year similarity indices were higher in the research area V2 than in the research area V1. The research area V2 achieved year-on-year differences in species similarity from 81 to 88 %. By comparing the species similarity of Carabidae communities on the research area V3, we found a slight decrease in the value of the similarity index than in the previous variants. Year-on-year similarity indices reached values in the range of 66 to 84 %. The V4 research area achieved similarity indices in the range of 70 to 84 %. By comparing the similarity indices of individual variants, in 2016 and 2017, we found the similarity between the research areas V1 and the area V2, which ranged from 91 to 97 %. The research areas V1 and V2 were located next to each other. Similarity in 2016 and 2017 was also achieved by areas V2 and V4, whose similarity indices were around 91 %. The ECO research area and the V3 research area achieved a very high similarity index in all three monitored periods in the range from 96 to 100 %. Based on a statistical t-test comparison, we assessed the species similarity of research areas V2 and V3 with the ECO area. These areas are managed in a similar way, using deep plowing and shallow tillage. Comparison of the V2 and

Table 1 Comparison of species dominance in 2016-2018 on the monitored areas (ED- eudominant, D- dominant, SD- subdominant, R- recedent, SR- subrecedent)

Species	V1			V2			V3			V4			EKO		
	2016	2017	2018	2016	2017	2018	2016	2017	2018	2016	2017	2018	2016	2017	2018
Amara aenea	SD	SD	SD	SD	SD	SD	SD	D	SD	SD	D	SD	SD	D	SD
Amara aulica	D	SD	SD	D	D	SD	D	R	SD	D	D	SD	D	R	SD
Amara communis	D	D	SD	D	D	SD	D	D	D	D	D	D	D	D	D
Amara familiaris	SR	SR	SR	R	SR	SR	SR	SR	R	R	R	SR	SR	SR	R
Bradycellus caucasicus	SR	SR	SR	SR	SR	SR	SR	R	SR	SR	SR	SR	SR	R	SR
Brachinus crepitans	SR	SR	R	SR	SR	SR	R	SR	R	SR	SR	SR	R	R	R
Cicindela campestris	SR	SR	SR	SR	SR	SR	R	SR	SR	SD	SR	SR	R	SR	SR
Harpalus affinis	SR	SR	SR	SR	SR	R	SR	SR	SR	SR	SR	SD	SR	SR	SR
Harpalus hospes	SR	SR	SR	SR	R	SR	SR	SR	SR	SR	R	SR	SR	SR	SR
Lebia chlorocephala	SR	R	R	SR	SR	SR	SR	SR	R	SR	SR	SR	SR	SR	R
Nebra brevicollis	SR	SR	R	SR	SR	R	SR	SR	SR	SR	SR	R	R	R	SR
Ophonus rupicola	SR	SR	SR	SR	SR	SR	SR	SR	SR	SR	SR	SR	SR	SR	SR
Poecilus cupreus	R	SR	SR	SD	SR	SR	SR	SR	SR	SD	R	R	SR	SR	SR
Pseudoophonus rufipes	ED	ED	ED	ED	ED	ED	ED	ED	ED	ED	ED	ED	ED	ED	ED
Pterostichus incommodus	SR	SR	SR	SR	SR	SR	SR	SR	SR	SR	SR	SR	SR	SR	SR
Pterostichus melanarius	ED	ED	ED	ED	ED	ED	ED	ED	ED	ED	ED	ED	ED	ED	ED
Pterostichus niger	SR	SR	SR	SR	SR	SR	SR	SR	SR	SR	SR	SR	SR	SR	SR
Pterostichus nigrita	SR	SR	SR	SR	SR	SR	SR	SR	R	SR	SR	SR	SR	SR	R
Pyrrhocoris apterus	SR	R	R	SR	SR	SR	R	SR	SR	SR	R	R	SR	SR	SR
Zabrus tenebrioides	SR	R	R	SR	SR	R	SR	SR	SR	R	SR	SD	SR	SR	SR

Table 2 Results of the Sőrens similarity index on V1-ECO areas throughout the three-year monitored period

Locality	V1/2017	V1/2018	V2/2016	V2/2017	V2/2018	V3/2016	V3/2017	V3/2018	V4/2016	V4/2017	V4/2018	EKO/2016	EKO/2017	EKO/2018
V1/2016	86,49	86,49	91,89	97,3	84,21	81,25	75	84,85	88,89	83,33	80	81,25	72,73	84,85
V1/2017	-	77,78	77,78	94,44	75,68	77,42	70,97	68,75	74,29	85,71	70,59	77,42	68,75	68,75
V1/2018	-	-	77,78	88,89	86,49	70,97	70,97	87,5	74,29	74,29	82,35	70,97	68,75	87,5
V2/2016	-	-	-	88,89	81,08	83,87	77,42	81,25	91,43	85,71	76,47	83,87	68,75	81,25
V2/2017	-	-	-	-	86,49	77,42	77,42	81,25	85,71	91,43	82,35	77,42	75	81,25
V2/2018	-	-	-	-	-	75	75	84,85	77,78	77,78	85,71	75	72,73	84,85
V3/2016	-	-	-	-	-	-	84,62	74,07	86,67	80	82,76	100	81,48	74,07
V3/2017	-	-	-	-	-	-	-	66,67	80	80	82,76	76,92	96,3	66,67
V3/2018	-	-	-	-	-	-	-	-	77,42	70,97	80	74,07	64,29	100
V4/2016	-	-	-	-	-	-	-	-	-	70,59	84,85	86,67	77,42	77,42
V4/2017	-	-	-	-	-	-	-	-	-	-	84,85	80	77,42	70,97
V4/2018	-	-	-	-	-	-	-	-	-	-	-	82,76	80	80
EKO/2016	-	-	-	-	-	-	-	-	-	-	-	-	81,48	74,07
EKO/2017	-	-	-		-	-	-	-	-	-	-	-	-	64,29

ECO areas in 2016 is shown in Fig 1. The graph also shows that the difference in the occurrence of Carabidae in these areas is not statistically significant (p = 0.491730).

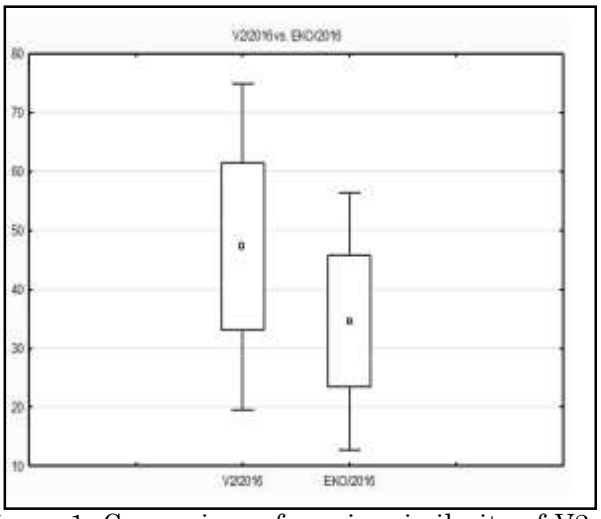

Figure 1. Comparison of species similarity of V2 and ECO research area in the monitored year 2016

By comparing the V2 and ECO areas in 2017, we reached the same result (p = 0.334654). Fig 2 shows a comparison of the species similarity of the research area V2 and ECO in 2017.

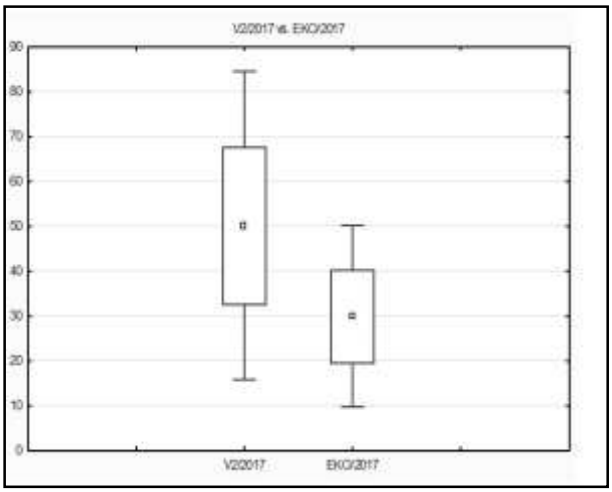

Figure 2 Comparison of species similarity of V2 and ECO research area in the monitored year 2017

Even in 2018, we did not notice a statistically significant difference between the V2 and ECO areas (p = 0.350437). Fig 3 shows a comparison of the species similarity of the research area V2 and ECO in 2018.
We also compared the V3 and ECO areas in individual years with the same test. Even by comparing these areas, we did not find statistically significant differences. In 2016, the value of evidence was (p = 0.726472), in 2017, we recorded the value of evidence (p = 0.701478) and in 2018, the value of the t-test was (p = 0.774668). These results fully correspond to the results of the Sőrensen similarity indices.

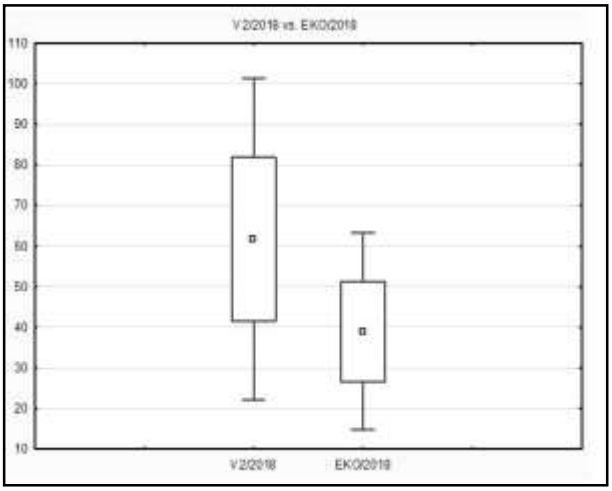

Figure 3 Comparison of species similarity of V2 and ECO research area in the monitored year 2017

Diversity index

In the V1 research area, the diversity index reached 1.63 bits in 2016, 1.42 bits in 2017 and 1.45 bits in 2018. In the V1 research area, the community reached the limit of ecological carrying capacity in 2017 and 2018, in 2016 the community was ecologically balanced. The average value of the diversity index for the research area V1 was 1.51 bits. Based on the average value, we can conclude that the community in the research area V1 was ecologically balanced. The number of individuals in the research area V1 was 505 in 2016. In 2017, the number increased to 611 and in 2018, it decreased again to 506 individuals. The change in the diversity index in 2016 could be due to an increase in the number of species that year. The diversity index on the V2 research area reached 1.58 bits in 2016, in 2017 the value was 1.51 bits and 1.51 bits in 2018. The average value of the diversity index on the V2 research area is 1.53 bits, which indicates ecological balance of the community. The V3 research area reached a diversity index of 1.50 bits in 2016, 1.42 bits in 2017 and 1.47 bits in 2018. The average value of the diversity index for the V3 research area is 1.47 bits. The community in the V3 research area was on the verge of ecological carrying capacity. In the V4 research area, the values of the diversity index were at the level of 1.69 bits. Based on the average value of the diversity index for the V4 area, we can consider the community to be ecologically balanced. The values of the diversity index of the ECO research area reached 1.49 bits in 2016, 1.43 bits in 2017 and 1.50 bits in 2018. In the ECO research area, the Community reached the limit of ecological carrying capacity during the three-year monitored period. The average value of the diversity index for the ECO research area is 1.47

bits. Based on the average value, we can conclude that the community in the ECO research area reached the limit of ecological carrying capacity. Fig 4 shows the values of the diversity index in the V1-ECO research areas in the observed period 2016 to 2018.

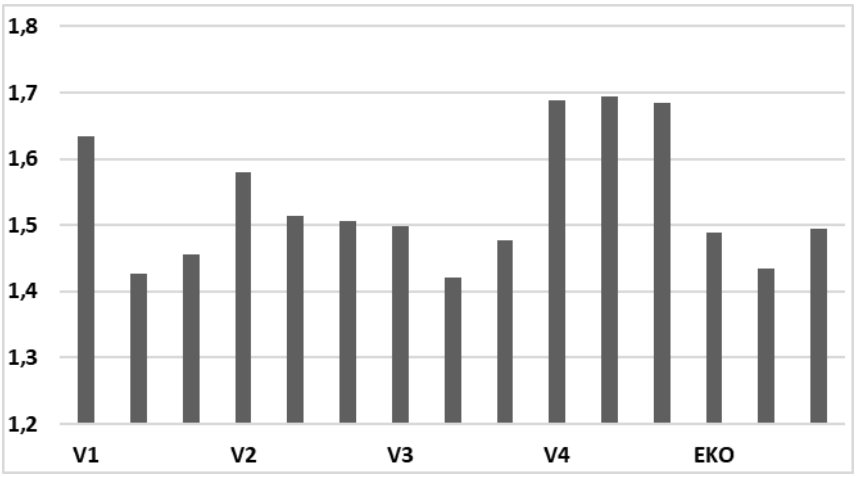

Figure 4 Values of the diversity index on research areas V1-ECO in the monitored period 2016 - 2018

Impact of Meteorological Factors

In 2016, the occurrence of Carabidae species correlated with temperature in the research area V2, V3, V4 and ECO. The correlation between the occurrence of Carabidae and temperatures was highly statistically demonstrated for the area V2 ($p = 0.000$), the area V3 ($p = 0.000$), the area V4 ($p = 0.001$) and the research area ECO ($p = 0.000$). Another factor we assessed was the total rainfall. In 2016, on all monitored areas, the total rainfall did not correlate with the occurrence of the Carabidae family. In 2017, the temperature correlated with the occurrence of the Carabidae family in the research area V2 ($p = 0.001$), the area V3 ($p = 0.000$), the area V4 ($p = 0.001$) and the ECO research area ($p = 0.000$). The influence of temperature on the abundance of the Carabidae family was highly demonstrated in the observed year in the areas V2, V3, V4 and ECO. The occurrence of Carabidae did not correlate with the total rainfall on any of the monitored areas. The correlation of the occurrence of Carabidae with the temperature in 2018 was positive in the area V1 ($p = 0.010$). The correlation of the abundance of Carabidae with temperature was statistically significant in the monitored area V1. The effect of rainfall on the occurrence of Carabidae did not mutually correlate.

DISCUSSION and CONCLUSION

In the three-year monitoring period, we recorded 11,362 specimens of epigeic groups of animals in the research areas of the Research Institute of Plant Production (VÚRV) in Borovce. Individual species were classified into eight epigeic groups. Porhajasova et al. (2007) report the Coleoptera order as dominant in almost all types of ecosystems, including agroecosystems. The data obtained by us confirmed the dominance of the Coleoptera order (7593 individuals), the presence of this order contributes to the increased diversity of agroecosystems, and at the same time, serves as a food source for other invertebrates and vertebrates. Out of the Coleoptera order, we determined six families, of which the family Carabidae (6656 individuals) had a dominant representation. During the three-year period, we collected 6656 individuals of this family. The number of individuals in the individual research areas ranged from 931 to 1622 species. We recorded the lowest number of individuals in the ECO research area and the highest in the V1 area. Melnychuk et al. (2003) also did not note statistically significant differences in the number of individuals, when comparing commercial and ecological agriculture systems. During all three years of the monitored period, we recorded two significantly eudominant species, *P. melanarius* and *P. rufipes*, in all research areas. The dominance values of these species ranged from 32 to 54 % during the monitored period. Twardovski et al. (2006) state that the impact of intensive agriculture does not negatively affect the presence of dominant species *P. melanarius* and *P. rufipes*. Other species were observed as recurrent or subrecedent, which fully corresponds to the data obtained by us. Kos et al. (2011) identified *P.melanarius* and *P. rufipes* as the most common species in maize fields in three locations in Croatia. The data obtained by us suggest a positive effect of intensive agriculture on the predominance of *P. melanarius* and *P. rufipes*. We also conclude that intensive agriculture has a negative effect on the abundance of other species of the *Carabidae* family. Lacko-Bartosova et al. (2005) came to similar conclusions. Rusch et al. (2013) assessed the dominance of *Carabidae* in agroecosystems in Sweden before and after the introduction of a pesticide risk reduction programme. No different species composition of the community was demonstrated, but differences in species dominance and community

functional composition were demonstrated. The data obtained by us did not confirm the differences in the dominance of species in ecological and conventional agriculture, which could be caused by the surrounding agricultural areas with conventional agricultural management and the termination of the ECO research area in 2018. Vician et al. (2010) identified eudominant species of *P. melanarius* and *P. rufipes* on different management of agricultural areas, without observing significant differences in dominance. By evaluating the frequency of occurrence of individual species, we conclude that the *P. melanarius* and *P. rufipes* species were constant during the three-year monitored period. The probability of occurrence of these species was 30 to 43 %, the occurrence of other species was accessory or accidental with low abundance, which could be related to their migratory ability from the surrounding country or accidental occurrence. Indices of species similarity of individual variants in 2016 and 2017 reached similarity between the research areas V1 and V2, which ranged from 91 to 97 %. The similarity in the occurrence of Carabidae could be due to the proximity of research areas and the migratory ability of individuals. In 2016 and 2017, similarity was also achieved on the areas V2 and V4, whose diversity indices were around 91 %. The highest similarity of 96-100 % was achieved during all three monitored periods on the research areas V3 and ECO. Based on the findings, we can assume that the V3 and ECO research areas provided very similar conditions for Carabidae, which may be related to the agrotechnological procedures used. These results were also confirmed by a comparison of the above areas using a t-test, whereas we showed no statistically significant difference between the areas in any year. Our finding corresponds with the finding of Melnychuk et al. (2003), who also did not find statistically significant differences in the number of individuals in their study when comparing commercial and organic farming systems. Gallardo et al. (2011) argue that disturbed ecosystems are characterized by a lower value of species similarity. The diversity index is used to assess the species diversity of Carabidae. Petřvalský (1993) considers as ecologically balanced communities those, whose value of the diversity index is in the range of 1.51-2.0 bits. Communities above 2.0 bits are considered environmentally stable. The values obtained by us in the three-year monitored period ranged from 1.42 to 1.69 bits. Based on this, we can conclude that none of the monitored research areas represented an ecologically stabilized community. The low value of the diversity index could be caused by agrotechnical interventions. Sustek et al. (2011) monitored the Žitavský lúh Reservation, calculating a species diversity index of 2.5092, which represents a highly stabilized ecosystem. Horakova et al. (2005) monitored the Carabidae family in the areas of the Moravian Karst Protected Landscape Area and found that the least anthropogenically affected areas were characterized by the highest index of species diversity, which represented a value of about 2.90 bits. On the V4 monitored research area, the value of species diversity reached the value of 1.69 bits, representing the highest value of all monitored areas. It follows that the no-till technique used in the V4 research area could have had a positive effect on the species diversity of Carabidae communities. Kosewska et al. (2014) dealt with the impact of various agricultural techniques on the occurrence of Carabidae in Poland. They concluded that plowing negatively affects the species variability of Carabidae, with zoophagous and medium-sized species predominating. The data we found also showed a negative impact of plowing on the diversity of Carabidae. The low diversity on the ECO variant could be caused by plowing, while we did not have a research variant with no-till management in ecological agriculture. Based on the evaluated data, we found that the temperature was positively correlated in individual areas with the abundance of the family of Carabidae. The data also show that the impact of total rainfall did not correlate in any of the monitored years and in any research area. The effect of temperature on the abundance of Carabidae was statistically significant in 2018 ($p = 0.010$) and highly statistically demonstrated in 2016 ($p = 0.000$-0.001) and 2017 ($p = 0.000$-0.001).

CONCLUSION and RECOMMENDATIONS

The aim of this work was to evaluate the occurrence of brassicas in different wheat cultivation methods, focusing on the determination of individual brassicas species. We collected epigeic material during a three-year period between 2016 and 2018, in research plots in Borovce, which belong to the Research Institute of Plant Production (RIPP) in Piešt'any. The collections were carried out within 5 variants of the research plots: V1: mulching technique; V2: conventional technique; V3: minimization technique; V4: no-till technique and ECO: ecological technique with ploughing using the earth trap method, using bait, with 24-hour emptying.

During the 3-year study period, we collected 11362 specimens, from eight epigeic groups. Of all epigeic groups, Coleoptera were dominant (7593 specimens). The order was represented by six families, of which the family Carabidae was dominant. We recorded 6656 specimens from the aforementioned family, from which we determined 20 species. In all study plots, we recorded two strongly eudominant species, *P. melanarius* and *P. rufipes*, over the three-year study period. The permanence of occurrence of these species was 30-43%, while the occurrence of the other species was accessory or accidental with low abundance, which could be related to their migratory ability from the surrounding landscape or accidental occurrence.

The highest species similarity of the bryozoan community, 96-100%, was recorded in all three study periods at study plots V3 and EKO. Based on the findings, we can conclude that research plots V3 and research plot EKO provided very similar conditions for sedges, which may be related to the agrotechnological practices used. The species diversity of the sedge communities in the research plots we monitored was highest in plot V4, with a value of 1.69 bits. Which means that the no-till technique used in research plot V4 positively influences the species diversity of sedge communities in the agroecosystem.

The data obtained by us can serve as a baseline material for the detection of species diversity in different wheat cultivation methods in organic farming with subsequent comparison with the data obtained by us. The seasonal dynamics increased in the month of May, which corresponds to the developmental cycle of the brassicas. Individual research plots reached their maximum values in the months of June-August. The decrease in values occurred in the months September-October, which is related to the survival of imagoes and the appearance of overwintering stages. From the evaluated data, we found a positive correlation of temperature in the individual plots with the abundance of the bryozoan family. The data also showed that the effect of rainfall was not correlated in any of the years studied. The effect of temperature on bryophyte abundance was statistically significant in 2018 and highly statistically demonstrated in 2016 and 2017.

Evaluating the results of our work, we conclude that the no-till technology is the most suitable wheat cultivation method in terms of promoting the diversity of brassica species. Of the plots studied, the highest similarity was achieved by abundance in plots V3 and EKO, in these plots deep tillage with subsoiling was used and also for this reason the abundance of sedges as well as their species diversity was lower. The organically cultivated area was used only with ploughing and for this reason we could not evaluate the abundance of sedges in the no-till variant, without the use of chemical preparations. We recorded the highest abundance and diversity in plot V1, where mulching is used to aerate the soil, this confirms the assumption that the no-till technology is more acceptable for sedge populations.

Researchers' Contribution Rate Declaration Summary

The authors declare that they contributed equally to the article.

Conflict of Interest Declaration

The authors declare that there is no conflict of interest between them.

REFERENCES

Bohac, J., Moudry, J., & Desetova, L. (2007). Biodiverzita a zemedelstvi. *Zivotne prostredi. 41* (1), 24-29.

Edwards, C.A., Sunderland, K.D., & George, K.S. (1979). Studies of polyphagous predators of cereal aphids. *Journal of Applied Ecology 16*(3), 811-823.

Ekschmitt, K., Weber, M., & Wolters, V. (1997). Spiders, carabids, and staphylinids: the ecological potential of predatory macroarthropods. In: Benckiser, G. (Ed.). Fauna in Soil Ecosystems. New York: Marcel Dekker, 1997. p.307-362.

Ermakov, A.I. (2004). Structural changes in thecarabid fauna of forest ecosystems under a toxicimpact. In. *Russian Journal of Ecology*, *35*, 403-408.

Gallardo, B., Gascón, S., Quintana, X. a Comín, F. A. (2011). How to choose a biodiversity indicator–Redundancy and complementarity of biodiversity metrics in a freshwater ecosystem. *Ecological indicators, 11*(5), 1177-1184.

Horakova, J., Hula, V., Pikula, J. (2005). Contribution to fauna of invertebrates of sink holes within the agricultural landscape of the Moravian Karst Protected Area. Part one: Carabidae (Coleoptera). Acta univ. agric. et silvic. *Mendel. Brun., LIII* (5), 53–62.

Hurka, K. (1996). Carabidae Ceské a Slovenske republiky. Zlin: Kabourek. 9-35 s. (a) ISBN 80-901466-2-7.

Kosewska, A., Skalski, T., Nietupski, M. (2014). Effect of conventional and non-inversion tillage systems on the abundance and some life history traits of carabid beetles (Coleoptera: Carabidae) in winter triticale fields. *European Journal of Entomology, 111*(5), 27-31.

Kos, T. , Bazok R., Barcic, J. (2011). Abudance and frequency of ground beetles in three maize fields in *Croatia. 12* (3), 894–902.

Lacko Bartosova, M. (2005). Udrzatelne a ekologicke polnohospodarstvo, SUA in Nitra, 575 p. ISSN 80-8069-556-3.

Lang, A.; Filser, J., Henschel, J.R. (1999). Predation by ground beetles and wolf spiders on herbivorous insects in a maize crop. Agriculture, *Ecosystems and Environment, 72*, 189-199.

Mazur, E., Lukniss, M. (1980). Geomorfologicke jednotky. In Mazúr, E., ed Atlas Slovenskej socialistickej republiky. Bratislava (SAV a SÚGK), 54-55 s.

Melnychuk, N.A., Olfert, O., Yongs, B., Gillott, C. (2003), Abundance and diversity of Carabidae (Coleoptera) in different farming systems. In: *Agriculture, Ecosystems and Environment. 95,* 69–72.

Petrvalsky, V. (1993). Zoocenozy ako indikator zatazenia ekosystemov. In: Kongres slovenskej vedy 1993 – sekcia Polnohospodarstvo, S. 46 – 47.

Pfiffner, L., Luka, H. (2000). Overwintering of arthropods in soils of arable fields and adjacent semi-natural habitats. *Agriculture, Ecosystems and Environment, 78*, 215-222.

Pokorny, V., Paseka, (2002). Atlas brouku. Praha– Litomysl, 144 s. ISBN 80-7185-484-0.

Rainio J., Nemelä J. (2003). Ground beetles (Coleoptera: Carabidae) as bioindicators. *Biodiversity and Conservation. 12*, 487– 506.

Remenar, M. (2017). Rekreaccna oblast Jazero Borovce, dokumentacia pre uzemne konanie (CM PROJECT). S. 60-65.

Rusch, A., Bommarco, R., Chiverton, P., Öberg, S., Wallin, H., Wiktelius, S., Ekbom, B. (2013). Response of ground beetle (Coleoptera, Carabidae) communities to changes in agricultural policies in Sweden over two decades. *Agriculture, Ecosystems & Environment, 176*, 63-69.

Swaminathan, R. (2014). Ground Beetles (Coleopetra: Carabidae): Their potential as bio-agents in agroecosystems. in: basic applied aspects of biopesticides. 225-233 s. DOI:10.1007/978-81-322-1877-7.

Sustek, Z., Porhajasova, J., (2011). Priestorova struktura spolocenstiev bezstavovacov s dorazom na celad Carabidae v prirodnej rezervacii Zitavsky luh. Nitra: SPU, 2011. 77 s., 2011.

Twardowski, J., Hurej, M., Jaworska, T. (2006). An effect of strip management on carabid beetles (Coleoptera, Carabidae) in sugar beet crop. In. *Journ. Of Plant Prot. Research. 1*, 116-125. ISSN 1427-4345.

Varvara, M. (2010). Thegenus Carabus (Coleoptera: Carabidae) in some poteto crops from Romania, 1978-1999. In: Muzeul Olteniei Craiova. Oltenie. Studii si comunicari. *Stiintele Naturii. 26*(2), 137-146 s. ISSN 1454-6914.

Vician, V., Stasiov, S., Kocík, K., Hazuchova L. (2010). Carabidae (Coleoptera) structure on variously managed agricultural land of podpoľanie area. In: *Acta Facultatis Ecologiae. 22*, 133-146.

Vician, V., Svitok, M., Michalkova, E., Lukacik, I., Stasiov, S. (2018). Influence of tree species and soil properties on ground (Coleopetra: Carabidae) communities. In: *Acta Oecologica. 91*, 120-126 s. https://doi.org/10.1016/ j.actao.2018. 07.005.

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Yeni Bir Tür Tanımı ile Türkiye'deki *Mesiotelus* Simon, 1897 Cinsine Katkılar (Araneae: Liocranidae)

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ÖZET
Bu çalışmada *Mesiotelus* Simon, 1897 cinsinin yeni türü, *Mesiotelus mersinensis* sp. n., Akdeniz Bölgesi'nden (Mersin) bir erkek üzerinden tanımlandı. Karakteristik yapıların çizimleri ve fotoğrafları sunuldu. Ayrıca, Türkiye'de oldukça zayıf bir dağılıma sahip olan bu cinsin *Mesiotelus scopensis* Drensky, 1935 türü, farklı bir lokaliteden kaydedildi. Türlerin Türkiye'deki mevcut dağılımı harita üzerinde gösterildi.

Zooloji

Araştırma Makalesi

Makale Tarihçesi
Geliş Tarihi : 16.12.2022
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Anahtar Kelimeler
Mesiotelus
Araneae
Yeni tür
Mersin
Türkiye

Contributions to the Genus *Mesiotelus* Simon, 1897 in Turkey, with the Description of a New Species (Araneae: Liocranidae)

ABSTRACT
In this study, a new species of the genus *Mesiotelus* Simon, 1897, *Mesiotelus mersinensis* sp. n., was described on the basis of a male from the Mediterranean Region (Mersin). Photos and drawings of characteristic structures were presented. Besides, the species *Mesiotelus scopensis* Drensky, 1935 of this genus, which has a very poor distribution in Turkey, was recorded from a different locality. The current distribution in Turkey of species was shown on the map.

Zoology

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GİRİŞ
Liocranidae, dünya genelinde 35 cins ve 335 tür içerir (World Spider Catalog, 2023). Aile üyeleri Avrupa'da geniş bir dağılıma sahipken, Türkiye'de oldukça zayıf bir dağılım sergiler. Liocranidae, Türkiye'de 10 cins ve 18 tür ile temsil edilir (Danışman ve ark., 2023). Türkiye'den *Mesiotelus* Simon, 1897 cinsine ait dört tür bilinmektedir: *Mesiotelus caucasicus* Zamani & Marusik, 2021, *Mesiotelus deltshevi* Naumova, 2020, *Mesiotelus scopensis* Drensky, 1935 ve *Mesiotelus tenuissimus* (L. Koch, 1866) (Elverici ve ark., 2013; Senckenberg, 2022; Coşkun ve ark., 2023; Danışman ve ark., 2023). Bu çalışmada, Türkiye'den *Mesiotelus* cinsine ait yeni bir tür, erkek örnek temelinde tanımlanmıştır. Ayrıca ilk olarak Ege Bölgesi'nden (Muğla) kaydedilen *Mesiotelus scopensis* Drensky, 1935 türü, Güneydoğu Anadolu Bölgesi'nden (Gaziantep) yeni lokalite kaydı olarak verilmiştir.

MATERYAL ve METOD
Mesiotelus mersinensis sp. n., Akdeniz Bölgesi'nden (Mersin) ve *Mesiotelus scopensis* Drensky, 1935

Güneydoğu Anadolu Bölgesi'nden (Gaziantep) el aspiratörü ile toplanmıştır. Daha sonra % 70' lik etil alkol içine konularak Bayburt Üniversitesi bünyesinde müze materyali olarak muhafaza altına alınmıştır (BAYU LIO-02/01-02). Tüm çizimler, bir çizim tüpüne sahip Olympus SZX-16 stereomikroskop aracılığıyla yapılmıştır. Fotoğraflar, Olympus Camedia C-5060 fotoğraf makinesi kullanılarak çekilmiştir. Tüm ölçümler milimetre cinsinden verilmiştir. Bacak segmentlerinin uzunluğu dorsalden ölçülmüştür (Ta IV eksik olduğundan dolayı ölçülememiştir) (Çizelge 1). Yeni türün tanımlanmasında, çiftleşme organı yapısı ve çiftleşme organı terminolojisi incelemelerinde Zamani ve Marusik (2021) yayını takip edilmiştir. Türlerin Türkiye'deki mevcut dağılımı harita üzerinde gösterilmiştir (Şekil 3).

Metin ve şekillerde kullanılan kısaltmalar: Ale, ön yan göz; Ame, ön medyan göz; Em, embolus; Fe, femur; Ma, medyan apofiz; Mt, metatarsus; Pa, patella; Ple, arka yan göz; Pme, arka medyan göz; Pt - Rt, prolateral ve retrolateral tegular apofizler; Rta, retrolateral tibyal apofiz; St, subtegulum; Ta, tarsus; Ti, tibya.

Çizelge 1. *Mesiotelus mersinensis* sp. n., bacak ölçümleri
Table 1. Mesiotelus mersinensis sp. n., Leg measurements

Holotip (♂) Bacaklar	Fe	Pa	Ti	Mt	Ta	Toplam (koksa and trokanter hariç)
I	2.20	1.00	2.35	2.00	1.20	8.75
II	2.10	0.90	2.00	1.80	1.05	7.85
III	1.90	0.40	1.60	1.95	1.00	6.85
IV	2.00	0.40	1.45	1.85	-	-

BULGULAR ve TARTIŞMA

***Mesiotelus* Simon, 1897**

***Mesiotelus mersinensis* sp. n.**

Materyal: Holotip ♂, Türkiye: Mersin ili, Mut ilçesi, Hacıahmetli köyü, 36°51'45"K, 33°59'21"D 19.IX.2016, 1100 m, A. Topçu.

Etimoloji: Türün adı, türün bulunduğu tip lokalitesinden türetilmiştir.

Tanımlama: Toplam uzunluk 3.5; Karapaks uzunluk 1.3, genişlik 1.2. Keliser uzunluk 1.0. Abdomen uzunluk 2.2, genişlik 1.1. Göz boyutları: Ale: 0.12, Ple: 0.09, Ame: 0.1, Pme: 0.08. Sternum uzunluk 1.1, genişlik 0.7.

Karapaks, sternum, keliser, maksilla ve labyum açık kahverengi. Keliser, medyal boyunca dizilmiş 3 küçük promarjinal dişe ve 2 büyük retromarjinal dişe sahip (biri keliser distaline yakın, diğeri medyale yakın). Göz bölgesi siyah. Abdomen bej, önde yoğun tüylü. Bacaklar sarımsı kahverengi (Şekil 1A-G). Bacak segmentlerinin uzunluğu Çizelge 1'de verilmiştir.

Simbyum boyu (0.8) eninden yaklaşık 3 kat daha uzun. Retrolateral tibyal apofiz (Rta) boyu (0.3), palpal tibyanın terminal genişliğinden yaklaşık 1,5 kat daha uzun. Bulbus boyu (0.5) eninden yaklaşık 2 kat daha uzun. Medyan apofiz (Ma), bulbusun orta hattına yerleşmiş ve terminal kısmı uca doğru kıvrık. Prolateral tegular apofiz (Pt) geniş ve uçta sivri. Embolus (Em) ve retrolateral tegular apofiz (Rt) sivri uçlu; Retrolateral tibyal apofiz (Rta) simbyumun neredeyse yarısına kadar uzanmakta. Kondüktör (Co) yarı saydam.

Karşılaştırma: Yeni türün erkeği, *M. caucasicus*'un erkeğine uzun bir bulbus ve küçük medyan apofiz ile benzer, ancak medyan apofizin yerleşimi, keliserdeki diş sayısı, medyan apofizin terminal kısmının kıvrılış şekli, prolateral tegular apofizin şekli ve retrolateral tibyal apofizin uzunluğu ile kolayca ayırt edilebilir (Şekil 1D-G).

Ayrıca, *Mesiotelus mersinensis* sp. n., *M. caucasicus* türünden vücut, keliser ve bacak uzunlukları ile de ayrılır.

Dağılım: Sadece tip materyalinin bulunduğu tip lokalitesinden bilinmektedir.

***Mesiotelus scopensis* Drensky, 1935**

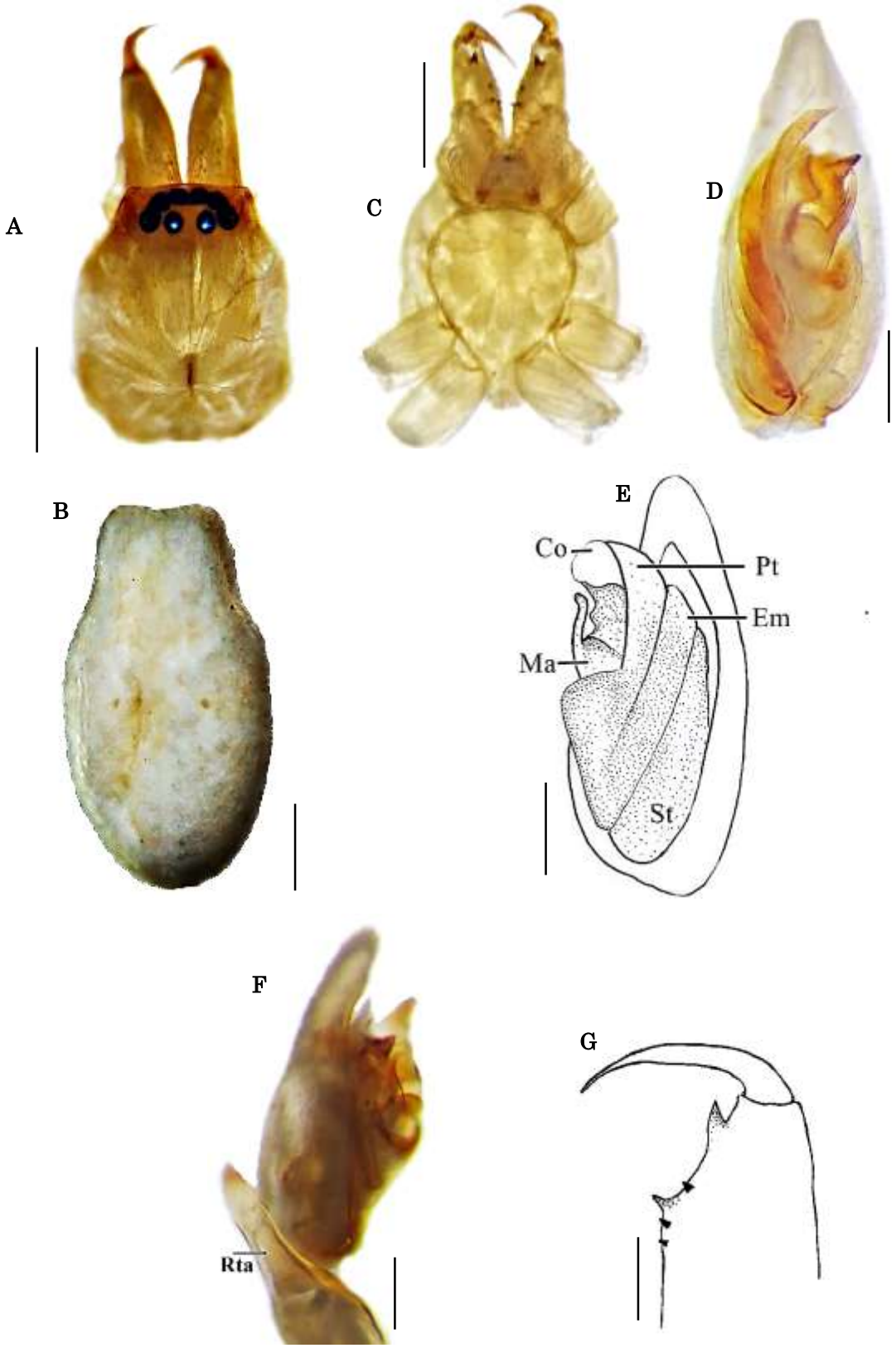
Materyal: ♀, Türkiye: Gaziantep ili, Islahiye ilçesi, 37°04'25"K, 36°50'07"D, 02.V.2007, 510 m, A. Topçu.

Tanımlama: Toplam uzunluk 6.0; Karapaks uzunluk 2.0, genişlik 1.6. Abdomen uzunluk 4.0, genişlik 2.0. Karapaks kahverengi, bacaklar sarı, abdomen koyu gri renkte (Şekil 2A, B).

Türkiye'deki ilk kaydı Muğla ili (Elverici ve ark., 2013).

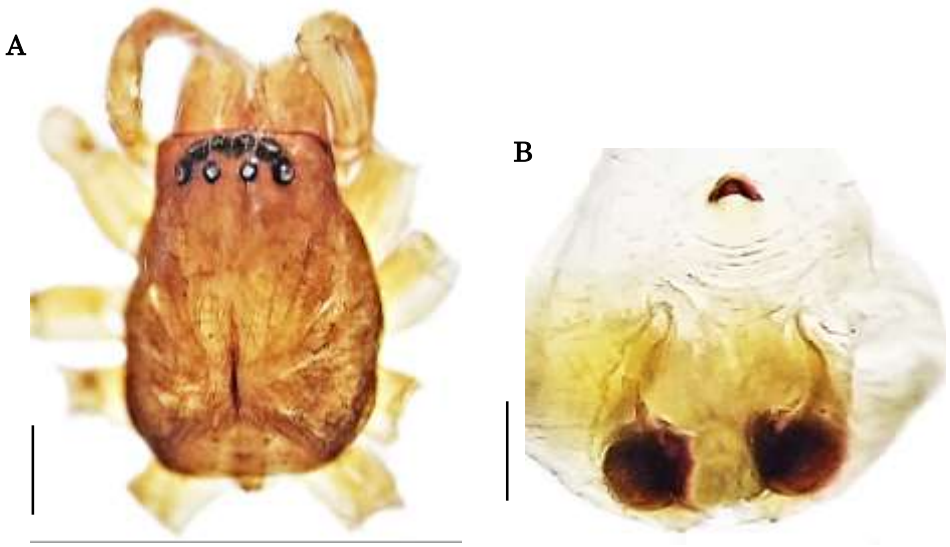
SONUÇ ve ÖNERİLER

Bu çalışma ile *Mesiotelus* Simon, 1897 cinsinin Türkiye'deki tür sayısı 5'e yükselmiştir. Dünyada ise, yeni türle birlikte bu cinse ait toplam tür sayısı 17'ye ulaşmıştır. Ayrıca, çalışmada, daha önce Ege Bölgesi'nden kaydedilen *Mesiotelus scopensis* Drensky, 1935 türünün farklı bir lokaliteden kaydı verilmiştir. Yeni katkılar, Türkiye'de oldukça nadir rastlanan bu cinsin dağılımı açısından önemlidir. Cinsin habitat özellikleri göz önüne alındığında yapılacak faunistik çalışmalarla birlikte yeni katkıların artacağı düşünülmektedir



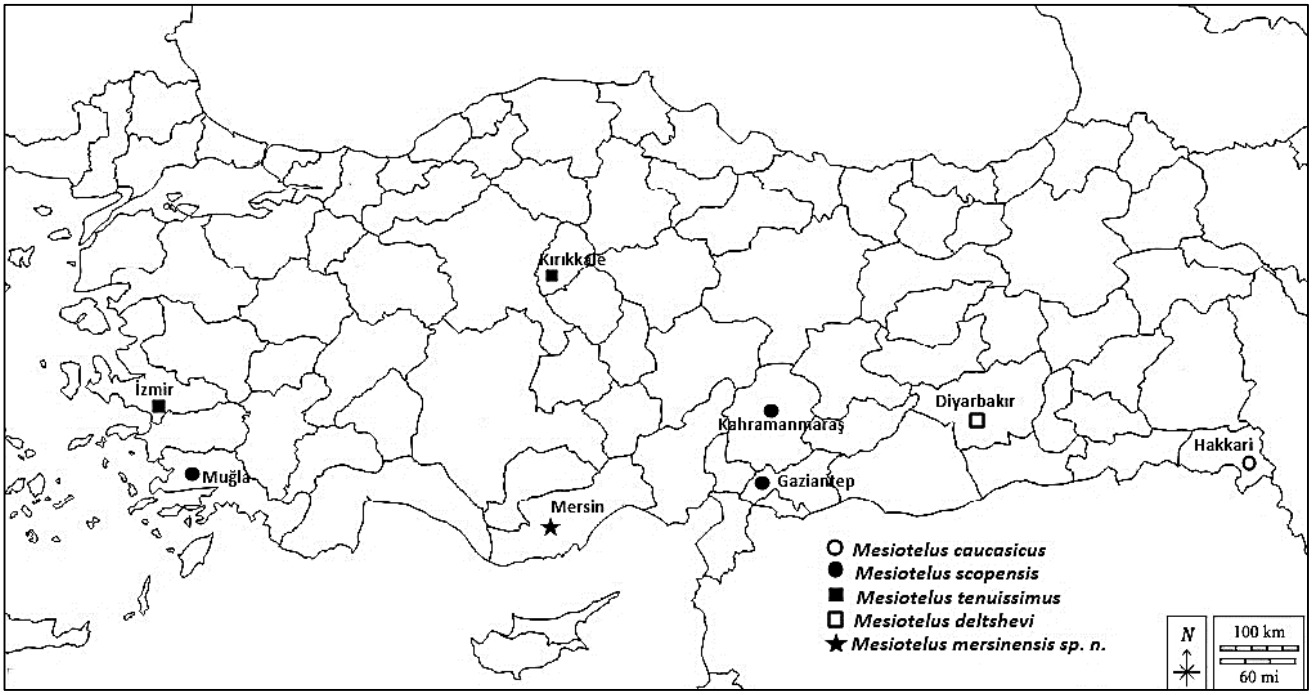
Şekil 1. *Mesiotelus mersinensis* sp. n., erkek A, B. Habitus, dorsal görünüş C. Prosoma, ventral görünüş; D. Sol palp, ventral görünüş; E, F. Sağ palp, prolateral ve retrolateral görünüş G. Keliser, dorsolateral görünüş. A-C. Ölçek çubukları: 0.5 mm, D-G. Ölçek çubukları: 0.2 mm.

Figure 1. Mesiotelus mersinensis sp. n., male A, B. Habitus, dorsal view C. Prosoma, ventral view; D. Left palp, ventral view; E, F. Right palp, prolateral view and retrolateral view G. Chelicera, dorsolateral view. A-C. Scale bars: 0.5 mm, D-G. Scale bars: 0.2 mm.



Şekil 2. *Mesiotelus scopensis* Drensky, 1935, dişi A. Prosoma, dorsal görünüş B. Epijin, ventral görünüş. Ölçek çubukları; A. 0.5 mm, B. 0.2 mm.

Figure 2. Mesiotelus scopensis Drensky, 1935, female A. Prosoma, dorsal view B. Epigyne, ventral view. Scale bars; A. 0.5 mm, B. 0.2 mm.



Şekil 3. Türkiye'deki *Mesiotelus* cinsinin dağılımı

Figure 3. Distribution of the genus Mesiotelus in Turkey

TEŞEKKÜR

Çalışmadaki türlerin incelenmesi ve fotoğraflanmasında Niğde Ömer Halisdemir Üniversitesi bünyesinde yer alan Olympus SZX-16 stereomikroskop kullanılmıştır. Sağladığı imkanlardan dolayı Prof. Dr. Aydın Topçu'ya teşekkür ederim.

Çıkar Çatışması Beyanı

Makale yazarı herhangi bir çıkar çatışması olmadığını beyan eder.

KAYNAKLAR

Coşar, İ., Danışman, T. & Erdek, M. (2023). The genus *Mesiotelus* Simon, 1897 (Araneae: Liocranidae) in Turkey. *Caucasian Entomological Bulletin 19*(1), 9-13.

Danışman, T., Kunt, K. B. & Özkütük, R. S., (2023). The Checklist of the Spiders of Turkey. Version 2023, Online at http://www.spidersofturkey.info, accessed on 22.03.2023.

Elverici, M., Özkütük, R. S. & Kunt, K. B. (2013). Two new liocranid species records from Turkey (Araneae: Liocranidae). *Munis Entomology and*

Zoology 8(1), 305-308.

Senckenberg, (2022). Collection Arachnology SMF. Occurrence dataset https://doi.org/10.15468/fiz2j5 accessed via GBIF.org on 11.12.2022.

World Spider Catalog, (2023). World Spider Catalog. Version 24. Natural History Museum Bern, online at http://wsc.nmbe.ch, accessed on accessed on 20.03.2023.

Zamani, A. & Marusik, Y. M. (2021). Two new species of Liocranidae (Arachnida: Aranei) from the Caucasus and northern Iran. *Arthropoda Selecta 30*(4), 557-564.

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The Effects of Fipronil on Glutathione and Histology of Freshwater Snails

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ABSTRACT

Fipronil ($C_{12}H_4Cl_2F_6N_4OS$, CAS No: 120068-37-3) is frequently used in agricultural fields and veterinary medicine as an insecticide and acaricide. It is known to contaminate aquatic ecosystems by mixing with surface waters and to accumulate in abiotic matrices. In this study, the effects of fipronil are investigated using freshwater snails *Viviparus contectus* (Millet, 1813). After exposure of snails to 1, 10 and 100 mg L^{-1} fipronil for 7 days, all body tissues were taken. As a result of the study of glutathione, one of the tissue antioxidant parameters, a significant increase was observed in the control group, which was administered 1 mg L^{-1} fipronil, compared to the other dose groups ($P<0.05$). Exposure to different concentrations of fipronil resulted with degenerations and necrosis of the digestive gland tubules of snails, histologically. The damages in the digestive gland tissue were increased with increasing of the concentration. Since snails are an important species for freshwater ecosystems, it can be emphasized that pesticides such as fipronil pose a potential risk to these organisms.

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Tatlı Su Salyangozlarında Fipronilin Glutatyon ve Histolojisine Etkileri

ÖZET

Fipronil ($C_{12}H_4Cl_2F_6N_4OS$, CAS No: 120068-37-3) insektisit ve akarisit olarak tarımsal alanlarda ve veteriner hekimlikte sıklıkla kullanılmaktadır. Sucul ekosistemler, yüzey sularına karışması yolu ile kontamine ettiği ve abiyotik matrikslerde birikim gösterdiği bilinmektedir. Bu çalışmada, fipronilin etkileri tatlı su salyangozları *Viviparus contectus* (Millet, 1813) kullanılarak incelenmektedir. Salyangozların 7 gün süreyle 1, 10 ve 100 mg L^{-1} fipronile maruz kalmasını takiben tüm vücut dokuları alınmıştır. Doku antioksidan parametrelerinden glutatyon incelemesi sonucunda 1 mg L^{-1} fipronil uygulanan grupta kontrol de diğer doz gruplarına göre önemli bir artış gözlenmiştir ($P<0.05$). Farklı fipronil konsantrasyonlarına maruz kalmak, salyangozların histolojik olarak sindirim bezi tübüllerinin dejenerasyonuna ve nekroza neden olmuştur. Konsantrasyonun artmasıyla sindirim bezi dokusundaki hasarlar artmıştır. Salyangozların tatlı su ekosistemleri için önemli bir tür olması nedeniyle fipronil gibi pestisitlerin bu canlılara karşı potansiyel bir risk oluşturduğu vurgulanabilir.

Zooloji

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Anahtar kelimeler
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Histopatoloji
Fipronil

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INTRODUCTION

The application of pesticides in agriculture, industry, domestic and veterinary medicine is very important in pest control and prevention of pest-borne diseases (Perkins et al. 2021; Tang et al. 2021a; Shen et al. 2022). After application of these areas, the pesticides are transported into aquatic environments by different

ways including run-off water (Perkins et al. 2021). Due to the bioaccumulation and biomagnification tendency of pesticides and their metabolites, they are important contaminant of the aquatic environment (Tongo et al. 2022). There are many studies in the aquatic environment where pollutants and their metabolites including pesticides, are encountered in water, sediment and tissues of aquatic organisms (Arisekar et al. 2019; Adeyeye et al. 2021; Tyohemba et al. 2021; Arslan & Ozeren 2022; Şimşek & Bilgili 2022). For example, fipronil and its metabolites ranged from 0.5 to 1.6 ng L^{-1} water samples, 4.05 ng g^{-1} of fish muscle and 19.91 ng g^{-1} of fish liver samples of River Elbe (Germany) (Michel et al. 2016). Likewise, fipronil, an insecticide, were found 0.69 ng g^{-1} in sediment samples of River Sutlej (India) (Kaur et al. 2019).

Fipronil, which is detected as a residue in different compartments in the aquatic ecosystem, has a wide range of use as an ectoparasite agent (Perkins et al. 2021). It shows its effect on insects by blocking the γ-aminobutyric acid (GABA)-gated chloride channel (Michel et al. 2016). It is reported that fipronil has toxic effects of non-target organisms such as fish and aquatic invertebrates (Wirth et al. 2004; Nillos et al. 2009; Qu et al. 2014; Qian et al. 2017).

Pesticides including fipronil are the main producers of reactive oxygen species (ROS) in the cells that damage on lipids, proteins and DNA (Stara et al. 2021; Dash and Rahman 2022; Sule et al. 2022). A multicomponent antioxidant complex largely regulates the balance between the production and elimination of ROS, as well as their potential detrimental effects (Gostyukhina et al. 2022). Glutathione, an antioxidant defense mechanism, is a main component to protect the cell from oxidative stress. As a reducing agent, it traps free radicals and acts as a substrate for some enzymes (Ali et al. 2020). Besides oxidative stress effects, research reported that pesticides also have adverse effects on histological alterations in the aquatic animal tissues (Ghaffar et al. 2018; Farhan et al. 2021; Tang et al. 2021b; Arslan & Ozeren 2022; Merola et al. 2022).

In studies with aquatic invertebrates, it has been observed that different subjects such as their ecology, biological properties, distribution and responses to aquatic pollutants are emphasized (Graf & Cummings 2021; Stara et al. 2021; Arslan et al. 2022). The freshwater snail *Viviparus contectus*, one of the aquatic invertebrates, is a cosmopolitan species that lives in freshwater ecosystems including rivers, lakes and swamps. In addition to feeding with detritus, they also feed by filtering the water. Thus, they show the feature of cleaning the abiotic parts of the aquatic ecosystem (Kocabaş et al. 2022; Kutluyer & Kocabaş 2022). Therefore, it may be used as an alternative non-target organism in toxicological studies in freshwater systems.

So far, to our knowledge, no studies have assessed the toxic effects of fipronil on freshwater snails. In this regard, the present study aimed to get knowledge about the biochemical and histological effects of fipronil on *V. contectus*.

MATERIALS and METHODS

Tested Organisms and Chemical

In the current study, the freshwater snails *Viviparus contectus* (Millet, 1813) was used as a model organism. Snails were collected from Hoyran, Eğirdir Lake located in Isparta, Turkey. A total of 100 snails (mean length 1.36±0.05 cm and mean weight 0.5±0.01 g) were acclimated to laboratory conditions as well as depuration period for two weeks.

24-h before the experiments, the stock solution of fipronil ($C_{12}H_4Cl_2F_6N_4OS$, CAS No: 120068-37-3, purity 98%) was prepared in dimethyl sulfoxide (DMSO) kept in +4ºC.

Experimental Design and Samples Collection

After the acclimation and depuration period, snails were randomly transferred to 15 L experimental aquariums (15 organisms/aquarium). There were three fipronil applied groups (1, 10, and 100 mg L^{-1}) and two control groups (control: water and snails; solvent control: water, snails and DMSO) in duplicate. The snails from each group were collected on the 7th day of exposure. For the biochemical analysis, 10 organisms in each group were dissected as whole body and immediately frozen in liquid nitrogen. Then, the samples were kept in -80ºC until the analysis. For the histological analysis, 5 organisms in each group were taken as whole body in tissue cassette and fixed into Davidson solution.

Biochemical Analysis

Frozen whole-body tissues were weighed as 100 mg on ice and homogenized in metaphosphoric acid (0.5 M, pH:8) using Miccra D-1 homogenizer (Germany). The homogenates were centrifuged in a refrigerated centrifuge (Hettich Zentrifugen Micro 220 R) at +4ºC 3500 rpm for 10 min. The levels of glutathione were evaluated according to the procedure of Ellman (1959). The main principle of this method is to determine the reaction of a thiol-selective DTNB reagent with free sulfhydryl groups to form a colored product at 420 nm. Total protein of tissues was measured at 595 nm using bovine serum albumin (BSA) as a standard and the Bradford method (Bradford, 1976).

Histological Analysis

After fixing the whole-bodies for 24-h, the tissues were put into ethyl alcohol series and embedded in paraffin blocks. The blocks were cut into 5 µm thickness and stained with hematoxylin and eosin (Luna 1968). The slides were observed under a light microscope

according to Benli et al. (2008).

Statistical Analysis

The glutathione values in the graphs were expressed as mean (µgM mg^{-1} protein) ± SEM. The GraphPad Prism program (version 5, USA) was used for the statistical analysis. One-way ANOVA was used to evaluate the differences between the control and exposed ($P < 0.05$).

RESULTS and DISCUSSION

Biochemical Assay

Glutathione activity increased in the 1 and 10 mg L^{-1} fipronil exposed groups compared to the control groups, while it decreased in the 100 mg L^{-1} fipronil exposed group. Glutathione activity was 2.8 times higher in the low-dose fipronil group compared to the control group ($P<0.001$). In addition, glutathione activity in the 1 mg L^{-1} fipronil exposed showed a significant increase of 1.9 ($P<0.01$) and 4.3 ($P<0.001$) times compared to the glutathione activities in the 10 mg L^{-1} and 100 mg L^{-1} fipronil exposed groups, respectively. Similarly, 10 mg L^{-1} fipronil exposed group compared to 100 mg L^{-1} fipronil exposed group significantly increased 2.2 times ($P<0.05$). The glutathione values of control and fipronil exposed groups is shown in Figure 1.

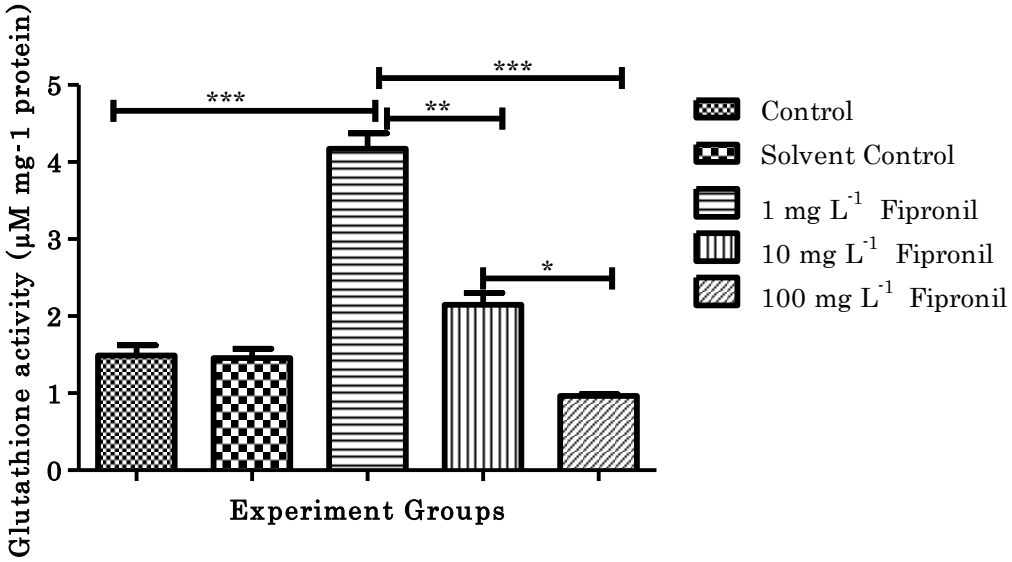

Figure 1. The glutathione activities of control and fipronil-exposed groups
(* indicates P<0.05, ** indicates P<0.01, *** indicates P<0.001)
Şekil 1. Kontrol ve fipronil uygulanan grupların glutatyon aktiviteleri
(P<0.05'i gösterir, ** P<0.01'i gösterir, P<0.001'i gösterir)*

By causing oxidative stress in organisms and speeding the generation of ROS, environmental pollutants including pesticides have impacts on antioxidant responses and harm cellular macromolecules (Stara et al. 2021). The results obtained in this study showed that the levels of glutathione, an antioxidant defense system, occur in freshwater snail tissues. The toxic effect of fipronil was more markedly increased in snails exposed to the lower concentration tested. This result obtained in the study differs from other ecotoxicological studies with freshwater snails in the literature. In a study conducted with another freshwater snail species, *Lymnaea luteola*, observed that the glutathione activities in digestive glands decreased in the azoxystrobin exposed groups for 24-h and 96-h (Ali et al. 2021). Similarly, another study reported that the glutathione levels decreased in the nanoparticle exposed groups of *L. luteola* (Al-Abdan et al. 2021). It has been reported that a decrease in glutathione level occurred in the freshwater snail *Pila virens* exposed to a nanoparticle substance for 24-h and 48-h (Srikanth et al. 2021). These results point to a critical role for antioxidants in the control of cell metabolism in the case of an imbalance brought on by xenobiotics injury.

Histopathological Analysis

The whole tissues of snails did not show any histological changes in the control and DMSO added control groups. Histopathological alterations were observed in the digestive gland tissues of *V. contectus* after exposure to different fipronil concentrations (Table 1). The control group digestive gland tissues were normal appearance with tubules (Fig 2a). The digestive gland tissues of snails were exhibited degenerations of the tubules and necrosis (Fig 2b, c,

and d) increased with increasing of the fipronil concentrations. There was no study regarding the histopathological effects of fipronil or xenobiotics to *V. contectus* in the open literature. The digestive gland, analogue of the liver in vertebrates, is beneficial tissue for determination of the health and toxicological exposures to aquatic invertebrates (Klobucar et al. 2001, Faggio et al. 2018). Exposure to toxic compounds has also previously been resulted with tubule degenerations in the digestive gland tissues of aquatic invertebrates and non-specific histological alterations to various xenobiotics (Cengiz et al. 2005, Karakaş & Otludil 2020, Tresnakova et al. 2020, Balamurugan et al, 2021). Similar to the results of the present study, exposure to 0.264 mgL^{-1} and 0.528 mgL^{-1} fipronil for 48-h and 7-d resulted with accumulated lipofuscin aggregates and caused mild degeneration of digestive tubules in freshwater mussels (Arslan & Gunal 2023). Exposure to fipronil also resulted with some tissue damages in other aquatic species and mamalian organisms. Qureshi et al. (2016) determined hemorrhagia, hyperplasia and nuclear hypertrophy of *Cyprinus carpio* after exposed to 400 µgL-1 for 4 days. Oral exposure to 6.46, 12.12 and 32.33 $mgkg^{-1}$ body weight/daily of fipronil for 90 days resulted with hypertrophy of hepatocytes in Wistar albino rats (Kartheek & David, 2019).

Table 1. Histopathological findings of digestive gland tissue of *V. contectus* after exposure to fipronil
Çizelge 1. Fipronile maruz kalan V. contectus'un sindirim bezi dokusunda histopatolojik bulgular

	Experimental Groups				
Histopathology	Control	Solvent control	1 mgL^{-1} fipronil	10 mgL^{-1} fipronil	100 mgL^{-1} fipronil
Tubul degeneration	-	-	+	++	++
Necrosis of digestive tubules	-	-	-	++	+++

* The histopathological alterations were scored as "(-) none (no histopathological alterations), which represents normal histological structure; (+) histopathology in > 20% of fields (mild); (++) histopathology in 20-60 % of fields (moderate) and (+++) histopathology in < 60% of fields (severe)"

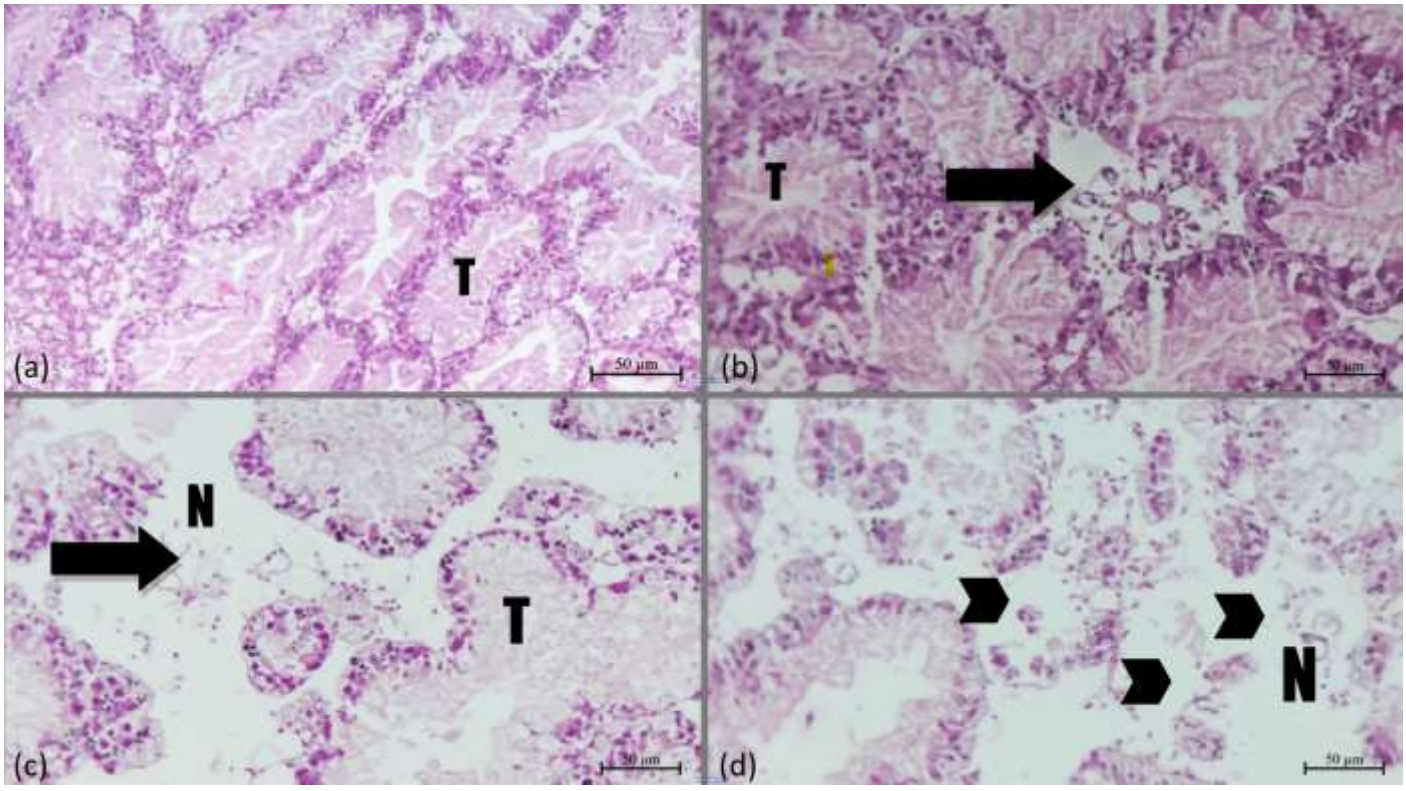

Figure 2. Digestive gland tissue of freshwater snail, *Viviparus contectus*, a) control, b) degenerations of the digestive tubules (black arrow) after exposure to 1 mg L^{-1} fipronil for 7 days, c) degeneration and necrosis of the digestive tubules (black arrow) after exposure to 10 mg L^{-1} fipronil for 7 days d) degeneration and necrosis of the digestive tubules after exposure to 10 mg L^{-1} fipronil for 7 days (black arrow heads) (H&E) T: tubule; N: necrosis

Şekil 2. Tatlı su salyangozu Viviparus contectus'un sindirim bezi dokusu, a) kontrol, b) 7 gün boyunca 1 mg L^{-1} fipronile maruz kaldıktan sonra sindirim tübüllerinin dejenerasyonu (siyah ok), c) 7 gün boyunca 10 mg L^{-1} fipronile maruz kaldıktan sonra sindirim tübüllerinin dejenerasyonu ve nekrozu (siyah ok) d) 7 gün boyunca 10 mg L^{-1} fipronile maruz kaldıktan sonra sindirim tübüllerinin dejenerasyonu ve nekrozu (siyah ok başları) (H&E) T: tübül; N: nekroz

CONCLUSION

The present study showed the first ecotoxicological effects of fipronil on the freshwater snail *V. contectus*. The glutathione activity results indicated that there was no dose-dependent concentration correlation in fipronil toxicity in snails. The histological results were revealed the effects of fipronil and *V. contectus* can be used as good candidates of determination of freshwater bodies as bioindicator organisms. The future experiments with other antioxidant stress parameters are required to measure the toxicological effects of fipronil on snails.

ACKNOWLEDGEMENT

The results of glutathione exposed to fipronil in this present study were presented as oral presentation at ESENIAS & DIAS Conference 2022.

Author's Contributions

The contribution of authors is equal.

Declaration of Interest

The authors declare that they do not have any competition and any conflicts of interest.

REFERENCES

Adeyeye, E. I., Ibigbami, O.A., Adesina, A. J., Azeez, M. A., Olaleye, A. A., Olatoye, R. A., & Gbolagade, Y. A. (2021). Assessment of pesticides residues in water, sediment and fish parts: case study of fish pond in Ado-Ekiti, Nigeria. *Asian Journal of Microbiology Biotechniology Environmental Science, 23*(1), 42-50.

Al-Abdan, M. A., Bin-Jumah, M. N., Ali, D., & Alarifi, S. (2021). Investigation of biological accumulation and eco-genotoxicity of bismuth oxide nanoparticle in fresh water snail *Lymnaea luteola*. *Journal of King Saud University-Science, 33*(2), 101355.

Ali, D., Ibrahim, K. E., Hussain, S. A., & Abdel-Daim, M. M. (2021). Role of ROS generation in acute genotoxicity of azoxystrobin fungicide on freshwater snail *Lymnaea luteola* L. *Environmental Science and Pollution Research, 28*(5), 5566-5574.

Ali, S. S., Ahsan, H., Zia, M. K., Siddiqui, T., & Khan, F. H. (2020). Understanding oxidants and antioxidants: Classical team with new players. *Journal of Food Biochemistry, 44*(3), e13145.

Arisekar, U., Shakila, R. J., Jeyasekaran, G., Shalini, R., Kumar, P., Malani, A. H., & Rani, V. (2019). Accumulation of organochlorine and pyrethroid pesticide residues in fish, water, and sediments in the Thamirabarani river system of southern peninsular India. *Environmental Nanotechnology, Monitoring & Management, 11,* 100194.

Arslan, P., Ozeren, S.C. (2022). Physiological and histopathological alterations in *Capoeta baliki* and *Squalius pursakensis* after caused by some environmental pollutants. *Environmental Monitoring and Assessment, 194,* 183.

Arslan, P., Gül, G. & Günal, A.Ç. (2022). The effects of invasive epibiont Dreissena polymorpha on the narrow-clawed crayfish (*Astacus leptodactylus*, Astacidae) in Eğirdir Lake, Turkey: a case study. *Environmental Monitoring and Assessment, 194,* 590.

Arslan, P., Günal, A.Ç. (2023). Does fipronil affect on aquatic organisms? Physiological, biochemical, and histopathological alterations of non-target freshwater mussel species. *Water, 15,* 334.

Balamurugan, S., Subramanian, P. (2021) Histopathology of the Foot, Gill, and Digestive Gland Tissues of Freshwater Mussel, *Lamellidens marginalis* Exposed to Oil Effluent. *Austin Journal of Environmental Toxicology, 7*(1), 1033.

Benli, A.C.K., Köksal, B., Özkul, A. (2008) Sublethal ammonia exposure of Nile tilapia (*Oreochromis niloticus* L.): Effects on gill, liver and kidney histology. *Chemosphere, 72*(9), 1355-1358.

Bradford, M.M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry, 72,* 248–254.

Cengiz, E.I., Yildirim, M.Z., Otludil, B., Ünlü, E. (2005) Histopathological effects of Thiodan® on the freshwater snail, *Galba truncatula* (Gastropoda, Pulmonata). *Journal of Applied Toxicology, 25*(6), 464–469.

Dash, M. K., & Rahman, M. S. (2022). Molecular and biochemical responses to tributyltin (TBT) exposure in the American oyster: Triggers of stress-induced oxidative DNA damage and prooxidant-antioxidant imbalance in tissues by TBT. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology,* 109523.

Ellman, G.l. (1959). Tissue sulfhydryl groups. *Archieves of Biochemistry and Biophysiology, 82*(1), 70-77.

Faggio, C., Tsarpali, V., Dailianis, S. (2018) Mussel digestive gland as a model tissue for assessing xenobiotics: an overview. *Science of the Total Environment, 636,* 220-229.

Farhan, M., Wajid, A., Hussain, T., Jabeen, F., Ishaque, U., Iftikhar, M., ... & Noureen, A. (2021). Investigation of oxidative stress enzymes and histological alterations in tilapia exposed to chlorpyrifos. *Environmental Science and Pollution Research, 28*(11), 13105-13111.

Ghaffar, A., Hussain, R., Abbas, G., Kalim, M., Khan, A., Ferrando, S., ... & Ahmed, Z. (2018). Fipronil (Phenylpyrazole) induces hemato-biochemical, histological and genetic damage at low doses in

common carp, *Cyprinus carpio* (Linnaeus, 1758). *Ecotoxicology, 27*(9), 1261-1271.

Graf, D. L., & Cummings, K. S. (2021). A 'big data'approach to global freshwater mussel diversity (Bivalvia: Unionoida), with an updated checklist of genera and species. *Journal of Molluscan Studies, 87*(1), eyaa034.

Gostyukhina, O. L., Andreyeva, A. Y., Chelebieva, E. S., Vodiasova, E. A., Lantushenko, A. O., & Kladchenko, E. S. (2022). Adaptive potential of the Mediterranean mussel *Mytilus galloprovincialis* to short-term environmental hypoxia. *Fish & Shellfish Immunology, 131,* 654-661.

Karakaş, S.B. & Otludil, B. (2020) Accumulation and histopathological effects of cadmium on the great pond snail *Lymnaea stagnalis* Linnaeus, 1758 (Gastropoda: Pulmonata). *Environmental Toxicology and Pharmacology, 78,* 103403.

Kaur, N., Singh, P., Bedi, J. S., & Gupta, A. (2019). Studies on persistent organic pollutants residue in water, sediment and fish tissues of River Sutlej, India. *Journal of Environmental Biology, 40*(2), 258-264.

Kartheek, R.M., David, M. (2018) Assessment of fipronil toxicity on wistar rats: A hepatotoxic perspective. *Toxicology Reports, 5,* 448-456.

Klobucar G.I., Lajtner J., Erben R. (2001) Increase in number and size of kidney concretions as a result of PCP exposure in the freshwater snail *Planorbarius corneus* (Gastropoda, Pulmonata). *Diseases of Aquatic Organisms, 44*(2), 149-54.

Kocabaş, F. K., Kocabaş, M., & Akça, A. (2022). Morphometric Parameters Comparison of *Viviparus contectus* (Millet, 1813) in Demirköprü Dam Lake, Turkey using multivariate statistical methods: Morphometric Parameters Comparison. *Sustainable Aquatic Research, 1*(2), 126-132.

Kutluyer, F., & Kocabaş, M. (2022). Demirköprü Baraj Gölü'nde İlk *Viviparus contectus* (Millet, 1813) Kaydı ve Bazı Biyometrik Parametrelerinin Değerlendirilmesi. *Kahramanmaraş Sütçü İmam Üniversitesi Tarım ve Doğa Dergisi, 25*(Ek Sayı 1), 263-269.

Luna, L.G., 1968. Manual of histologic staining methods of the Armed Forces Institute of Pathology. New York: Blackiston Division, McGraw-Hill.

Merola, C., Fabrello, J., Matozzo, V., Faggio, C., Iannetta, A., Tinelli, A., ... & Perugini, M. (2022). Dinitroaniline herbicide pendimethalin affects development and induces biochemical and histological alterations in zebrafish early-life stages. *Science of The Total Environment, 828,* 154414.

Michel, N., Freese, M., Brinkmann, M., Pohlmann, J. D., Hollert, H., Kammann, U., ... & Hanel, R. (2016). Fipronil and two of its transformation products in water and European eel from the river Elbe. *Science of The Total Environment, 568,* 171-179.

Nillos, M. G., Lin, K., Gan, J., Bondarenko, S., & Schlenk, D. (2009). Enantioselectivity in fipronil aquatic toxicity and degradation. *Environmental Toxicology and Chemistry: An International Journal, 28*(9), 1825-1833.

Perkins, R., Whitehead, M., Civil, W., & Goulson, D. (2021). Potential role of veterinary flea products in widespread pesticide contamination of English rivers. *Science of The Total Environment, 755,* 143560.

Qian, Y., Wang, C., Wang, J., Zhang, X., Zhou, Z., Zhao, M., & Lu, C. (2017). Fipronil-induced enantioselective developmental toxicity to zebrafish embryo-larvae involves changes in DNA methylation. *Scientific Reports, 7*(1), 1-11.

Qu, H., Wang, P., Ma, R. X., Qiu, X. X., Xu, P., Zhou, Z. Q., & Liu, D. H. (2014). Enantioselective toxicity, bioaccumulation and degradation of the chiral insecticide fipronil in earthworms (*Eisenia feotida*). *Science of The Total Environment, 485,* 415-420.

Qureshi,Z.I., Bibi, A., Shahid, S., Ghazanfar, M. (2016) Exposure to sub-acute doses of fipronil and buprofezin in combination or alone induces biochemical, hematological, histopathological and genotoxic damage in common carp (*Cyprinus carpio* L.). *Aquatic Toxicology, 179,* 103-114.

Shen, C., Pan, X., Wu, X., Xu, J., Dong, F., & Zheng, Y. (2022). Predicting and assessing the toxicity and ecological risk of seven widely used neonicotinoid insecticides and their aerobic transformation products to aquatic organisms. *Science of The Total Environment, 847,* 157670.

Srikanth, K., Raju, N. V., Pamanji, R., & Nutalapati, V. (2021). Pila virens as sentinel of silica nanoparticles toxicity induced oxidative stress. *Materials Letters, 300,* 130185.

Stara, A., Pagano, M., Albano, M., Savoca, S., Di Bella, G., Albergamo, A., ... & Faggio, C. (2021). Effects of long-term exposure of *Mytilus galloprovincialis* to thiacloprid: A multibiomarker approach. *Environmental Pollution, 289,* 117892.

Sule, R. O., Condon, L., & Gomes, A. V. (2022). A common feature of pesticides: oxidative stress—the role of oxidative stress in pesticide-induced toxicity. *Oxidative Medicine and Cellular Longevity,* 5563759.

Şimşek, İ., & Bilgili, A. (2022). Investigation of monthly residues of polycyclic aromatic hydrocarbons in water and sediment samples from Kirikkale Kizilirmak River Basin. *Environmental Monitoring and Assessment, 194*(683), 1–11.

Tang, F. H., Lenzen, M., McBratney, A., & Maggi, F. (2021a). Risk of pesticide pollution at the global scale. *Nature Geoscience, 14*(4), 206-210.

Tang, J., Wang, W., Jiang, Y., & Chu, W. (2021b). Diazinon exposure produces histological damage, oxidative stress, immune disorders and gut microbiota dysbiosis in crucian carp (*Carassius*

auratus gibelio). *Environmental Pollution, 269,* 116129.

Tongo, I., Onokpasa, A., Emerure, F., Balogun, P. T., Enuneku, A. A., Erhunmwunse, N., ... & Ezemonye, L. (2022). Levels, bioaccumulation and biomagnification of pesticide residues in a tropical freshwater food web. *International Journal of Environmental Science and Technology, 19*(3), 1467-1482.

Třešňáková, N., Günal, A.Ç., Başaran Kankılıç, G., Paçal, E., Uyar, R., Erkoç, F. (2020) Sub-lethal toxicities of zinc pyrithione, copper pyrithione alone and in combination to the indicator mussel species *Unio crassus* Philipsson, 1788 (Bivalvia, Unionidae). *Chemistry and Ecology, 36*(4), 292-308.

Tyohemba, R. L., Pillay, L., & Humphries, M. S. (2021). Bioaccumulation of current-use herbicides in fish from a global biodiversity hotspot: Lake St Lucia, South Africa. *Chemosphere, 284*, 131407.

Wirth, E. F., Pennington, P. L., Lawton, J. C., DeLorenzo, M. E., Bearden, D., Shaddrix, B., ... & Fulton, M. H. (2004). The effects of the contemporary-use insecticide (fipronil) in an estuarine mesocosm. *Environmental Pollution, 131*(3), 365-371.

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Cortinarius bovinus (Şişörümcekmantarı): Türkiye Mikotası İçin Yeni Bir Kayıt

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ÖZET

Cortinarius bovinus'un bazidiyokarpları Türkiye'den ilk kez toplanmış, incelenmiş ve burada ilgili fotoğraflar ve kısa bir tartışma ile birlikte sunulmuştur. Yeni kayıt konik, dışbükey, tepe çıkıntılı, turuncumsu, grimsi veya kırmızımsı kahverengi veya toprak boyası renginde ve higroskopik şapka; seyrek, geniş, soluk grimsi kahverengi, turuncumsu kahverengi veya passı kahverengi lameller; çomak biçiminde ve tabanda soğansı sap; geniş eliptik veya yuvarlağımsı, az veya çok dikenli, 8–10.5 × 5–7 µm ve açık limon rengi bazidiyosporlar ile yakın türlerden ayırt edilir.

Mikoloji

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Anahtar Kelimeler
Bazidiyomikota
Cortinariaceae
Taksonomi
Trabzon

Cortinarius bovinus (Şişörümcekmantarı): A New Record for the Turkish Mycota

ABSTRACT

Basidiocarps of *Cortinarius bovinus* were collected and examined for the first time from Turkey, and are presented herein with relevant photographs and a short discussion. The new record is distinguished from closely related species by conical, convex, umbonate, orangeish, greyish or reddish brown or ocher colored and hygroscopic pileus; sparse, broad, pale greyish-brown, orange-brown or rust-brown lamellae; rod shaped and bulbous based stipe; broadly elliptical or roundish, more or less spiny, 8–10.5 × 5–7 µm and light lemon colored basidiospores.

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GİRİŞ

Cortinarius (Pers.) Gray cinsi içerdiği takson sayısı ile en büyük şapkalı mantar cinslerinden birisidir. Cins üyelerinin ortak özelliklerinden birisi genç üyelerin şapkası ile sapları arasında kortinaya sahip olmalarıdır. Kortina tül perde, *Cortinarius* perdeli anlamına gelir. Kortina liflerinin çoğu kısa ömürlüdür, sap ve / veya şapka kenarında geçici kalıntılar şeklinde kendini belli eder ve cinsin teşhisine yardımcı olur. Tüm cins üyeleri pas rengi veya kahverengimsi kırmızı spor izi üretir. Bazı üyeleri yüksek oranda zehirlidir ve tüketildiği zaman böbrek harabiyetine neden olur. Bu nedenle bu cins mantarları beslenme amaçlı tüketmemek veya çok dikkatli olmak en doğrusudur. İstisnai olarak bazı türler, örneğin *Cortinarius praestans* (Cordier) Gillet ve *C. caperatus* (Pers.) Fr. yenilebilir. İç zar genç üyelerde lamelleri korur ve mantar büyüdükçe genellikle çok az iz bırakarak kaybolur. Doğada saptanan bir mantarın *Cortinarius* cinsine ait olduğunu saptamak kolay olmakla birlikte, hangi tür olduğunu belirlemek genellikle zordur. Bu cinsteki hemen hemen tüm mantarlar çeşitli ağaçlar ile mikorizal yaşar. Bazı *Cortinarius* türleri renklidir ve genellikle boya yapımında kullanılır. Günümüzde Cortinariaceae familyası dünyada yaklaşık altı bin civarında, Türkiye'de ise 145 kayıt ile temsil edilmektedir (Kirk ve ark., 2008; Akata ve ark., 2014; Keleş ve ark., 2014; Sadullahoğlu & Uzun, 2019; Sesli ve ark., 2020; Doğan ve ark., 2021; Uzun & Kaya, 2022). Elbette bu kayıtlar gerçek takson sayısını göstermez fakat *Cortinarius*'un mantarlar âleminin en zengin cinslerinden birisi olduğunu ortaya koyar. Bu çalışmanın amacı daha önce Türkiye'den rapor edilmemiş *Cortinarius bovinus* Fr. türüne ait

bazidiyokarpları morfolojik yöntemlerle inceleyip şekillerle tanıtarak Türkiye mikotasına katkı sağlamaktır.

MATERYAL ve METOD

Araştırmanın materyalini Trabzon, Maçka, Mataracı Mahallesi'nden toplanan bazidiyokarplar oluşturmaktadır. Toplama sahasında doğu ladini, gürgen, yabani fındık ve çeşitli çalılar yetişmektedir. İlk olarak bazidiyokarpların arazide resimleri çekildi, genel özellikleri ve olası mikorizal ilişkileri kaydedildi ve birkaç tanesi toplanarak kese kağıtlarına konulup laboratuvara getirildi. Spor izleri elde edildikten sonra geriye kalan numuneler radyatör üzerinde birkaç saatte kurutuldu ve kataloglanarak fungaryum dolabında ilgili bölüme yerleştirildi. Daha sonra kuru bazidiyokarbın lamel, şapka ve sap yüzeyinden binoküler mikroskop altında ince kesitler alındı. Kesitler %5'lik amonyak çözeltisi içerisinde, birkaç dakika bekletildikten sonra Axio Imager A2 görüntüleme sistemi sayesinde fotoğrafları çekildi, bazidiyum, kenar hücreleri ve şapka derisi hiflerinin boyutları ölçüldü. Bazidiyosporların elde edilebilmesi için bazidiyokarptan yaklaşık bir santimetreküplük bir parça kesildi, %5'lik amonyak çözeltisi içerisinde 3-5 dakika bekledildikten sonra lam üzerinde pens yardımı ile birkaç defa sıkılıp bırakıldı. Bazidiyospor karakterleri incelendikten sonra mikrofotografi sistemi sayesinde fotoğrafları çekilmiştir. Türün teşhisi ilgili kaynaklara göre yapılmıştır (Breitenbach & Kränzlin, 2000; Høiland & Holst-Jensen, 2000; Roux, 2006; Knudsen & Vesterholt, 2008). Yeni kayda Türkçe isim verilmesi sürecinde Ali Nihat Gökyiğit Vakfı Nezahat Gökyiğit Botanik Bahçesi'nin ilgili veritabanından faydalanılmıştır.

Örnekleme Yöntemi

Mevcut çalışmanın konusu olan *Cortinarius* cinsi üyeleri mikorizal olduğundan materyal toplama sahası olarak ormanlık alanlar ve çalılıklar seçilmiştir. Toplama alanları rastgele belirlenmiş ve mümkün olduğunca çeşitli gelişim aşamasındaki früktifikasyon organlarından ayrı ayrı örnekler alınmıştır.

Laboratuvar çalışmaları

Çalışmada makromantar teşhisinde esas olan konvansiyonel yöntemler kullanılmıştır. Bazidiyokarpların şapka üstünden yüzeysel kesitler ve lamellerinden de enine kesitler alınmış, %5'lik amonyak çözeltisi ile işlemden sonra bazidiyum, kenar hücreleri ve pileipellis yapıları incelenmiş, sayısal değerler teşhiste kullanılmak amacı ile not edilmiştir. Bazidiyosporların boyutlarını belirlemek amacı ile üç ayrı bazidiyokarptan elde edilen numunelerden 35'er ölçüm yapılmış, kenar hücreleri ve şapka derisi hiflerinin boyutlarını belirlemek amacı ile 20'şer ölçüm yapılmış ve çıkan rakamların aritmetik ortalaması alınmıştır. Kesitlerin alımı için Carl Zeiss Stemi 2000C Model trinoküler mikroskop, incelemeler için ise Zeiss Axio Imager A2 araştırma mikroskobu kullanılmıştır.

BULGULAR ve TARTIŞMA

Cortinariaceae Singer / Örümcekmantarıgiller

Cortinarius bovinus Fr., Epicr. Syst. Mycol.: 297 (1838) / Şişörümcekmantarı

Şapka konik, dışbükey veya nispeten düz, turuncumsu veya kırmızımsı kahverengi, 25–85 mm, genellikle küt tepe çıkıntılı, higroskopik; yüzeyi lifli, donuk veya parlak görünümde ve kenarı uzun süre içeriye kıvrıktır. Lameller geniş, seyrek, ayrık veya sapa genişliği ölçüsünde bağlı, soluk kahverengi, turuncumsu kahverengi veya passı kahverengi ve kenarları hafif dişli görünümdedir. Eti ince, bej kahvesi, grimsi kahverengi veya kirli beyaz, turp kokulu ve hafif tatlıdır. Sap çomak biçiminde, tabanda soğansı, dolu, 50–95 × 10–30 mm; yüzeyi grimsi kahverengi, aşağıda soluk zemin üzerinde kahverengi lifli, yukarıda beyazımsıdır. Yüzük kirli veya grimsi beyaz veya grimsi kahverengi misel kalıntısı biçimindedir. Bazidiyumlar çomak biçiminde, 2 veya 4 sporlu, 30–36 × 8–10 µm ve kancalıdır. Bazidiyosporlar geniş eliptik veya yuvarlağımsı, orta derecede veya yoğun dikenli, (6.9–)7–9.5(–10) × (4.8–)5–7(–7.2) µm ve saman rengindedir. Kenar hücreleri silindir veya çomak biçiminde ve 15–35 × 5–8 µm'dir. Şapka derisi düzgün ve paralel, kancalı ve ortalama 3–10 µm genişliğinde hiflerden oluşmuştur (Şekil 1).

İncelenen örnekler

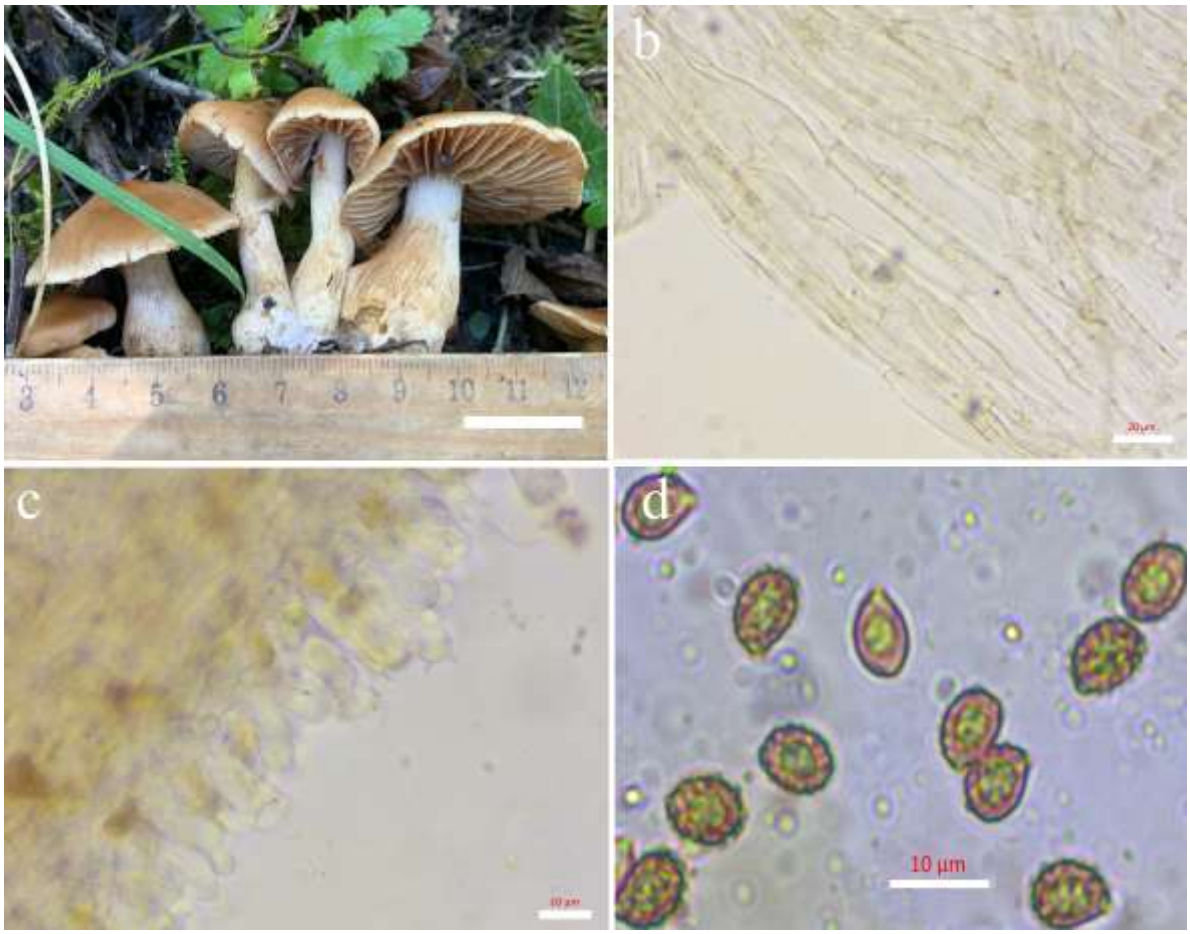
Türkiye, Trabzon, Maçka, Mataracı, 40°50'56.85" K ve 39°37'40.19" D, 819 m, 18.10.2022. Yaz sonlarından sonbahar sonlarına doğru iğne yapraklı ağaç ormanlarında, özellikle doğu ladini bazen de meşe altında, kireçli topraklarda, öbekler halinde yayılış gösterir. E. Sesli 4527.

Yeni kayıt turuncumsu veya kırmızımsı kahverengi, tepe çıkıntılı, 85 mm büyüklüğe ulaşabilen higroskopik şapkası; geniş ve seyrek lamelleri; çomak biçiminde, tabanda soğansı ve yüzeyi lifli sapı; geniş eliptik veya yuvarlağımsı, orta derecede veya yoğun dikenli, 7–9.5 × 5–7 µm ve saman rengi bazidiyosporlar; silindir veya çomak biçiminde ve 15–35 × 5–8 µm büyüklüğündeki kenar hücreleri ile yakın türlerden ayırt edilebilir. En çok benzerlik gösterdiği tür *Cortinarius bulbosus* (Sow.:Fr.) Fr. daha küçük (en fazla 60 mm) ve tepe çıkıntısı bulunmayan şapkası; daha küçük bazidiyosporları (6–8.5 × 4–5.5 µm) ile farklılık gösterir. Diğer benzer fakat farklı bir tür *C. brunneofulvus* Fr. tepe çıkıntısız, kenarı dalgalı, koyu kırmızımsı kahverengi veya kehribar kahverengi şapkası; oldukça kısa çomak biçimindeki kenar

hücreleri ile farklılık gösterir. Daha az benzer diğer bir takson *C. brunneus* (Pers.: Fr.) Fr. var. *brunneus* daha büyük (50–100 mm), koyu kırmızımsı, grimsi, siyahımsı kahverengi veya bej kahvesi şapka yapısı ile fark eder. *C. damascenus* Fr., *C. bovinus*'tan daha büyük ve kırmızımsı, turuncumsu kahverengi veya kehribar rengi şapka; uç kısmı incelmiş sap ve daha küçük (6.5–10 × 4–5.5 µm) bazidiyosporlara sahiptir. *C. duracinus* Fr. var. *duracinus* karışık ağaçlı ormanlarda yetişir; kırmızımsı kahverengi ve kenarı dalgalı şapka ile çomak veya iğ biçiminde ve tabanda köksü ve beyazımsı sap içerir. *C. duracinus* Fr. var. *raphanicus* Mos. daha küçük (25–50 mm), kırmızımsı veya bej kahvesi şapka; köke benzeyen sap ve nispeten küçük (7–10 × 4.5–6 µm) bazidiyosporlara sahiptir (Breitenbach & Kränzlin, 2000; Knudsen & Vesterholt, 2008)

SONUÇ ve ÖNERİLER

Bu çalışma sayesinde *Cortinarius bovinus* Türkiye için yeni kayıt olarak saptanmış ve Türkiye mikotasına katkıda bulunulmuştur. *Cortinarius* grubu mantarları diğer cinslerden ayırmak oldukça kolay, fakat cins içerisindeki taksonları birbirinden ayırt etmek hayli zordur. Fakat dikkatli bir gözlemle, birbirinden oldukça farklı renkteki bazidiyokarpları, mikorizal ilişkileri, kenar hücreleri, şekilleri ve üzerindeki süsler yönünden farklı olabilen bazidiyosporları, renk değiştirebilme özellikleri, şapka derisinin mikroskopik yapısı, kokusu ve benzeri birçok kullanılabilir karakterleri sayesinde teşhis edilebilecekleri söylenebilir. Fungaryum materyali haline getirilip uzun süre bozulmadan saklanabilmeleri kolaydır. Bu çalışma kapsamında gerçekleştirilen gözlemlere göre her mevsim yetişebilmelerine rağmen en yoğun olarak sonbaharda dikkat çekerler.



Şekil 1. *Cortinarius bovinus*: a- bazidiyokarplar, b- şapka derisi kesiti, c- bazidiyumlar ve kenar hücreleri, d- bazidiyosporlar (ölçek çubukları: a: 20 mm, b: 20 µm, c ve d: 10 µm)

Figure 1. Cortinarius bovinus: a-basidiocarps, b- section from the pileipellis, c-basidia and marginal cells, d-basidiospores (scale bars: a: 20 mm, b: 20 µm, c and d: 10 µm)

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Herhangi bir kurum veya kişi ile çıkar çatışması bulunmamaktadır

Etik Kurul Beyanı

Bu makalenin kapsamı çerçevesinde etik kurul kararına gerek yoktur

KAYNAKLAR

Akata, I., Uzun, Y. & Kaya, A. (2014). Macromycetes determined in Yomra (Trabzon) district. *Turk J Bot.*, *38*(5), 999-1012.

Breitenbach, J. & Kränzlin, F. (2000). *Fungi of Switzerland*, vol: 5, Agarics 3. Part. Verlag Mykologia CH-6000 Luzern 9, Switzerland, 338 pp.

Doğan, H.H., Öztürk, Ö. & Şanda, M.A. (2021). The mycota of Samanlı Mountains in Turkey. *TUJNS*, *22*(2), 215-243.

Høiland, K. & Holst-Jensen, A. (2000). *Cortinarius* phylogeny and possible taxonomic implications of ITS rDNA sequences. *Mycologia 92*(4), 694-710.

Keleş, A., Demirel, K., Uzun, Y. & Kaya, A. (2014). Macrofungi of Ayder (Rize/Turkey) High Plateau. *Biodicon*, *7*(3), 177-183.

Kirk, P.M., Cannon, P.F., Minter, D.W. & Stalpers, J.A. (2008). *Dictionary of the Fungi* (10th ed.). CAB International, Wallingford, UK.

Knudsen, H. & Vesterholt, J. (2008). *Funga Nordica. Agaricoid, Boletoid and Cyphelloid Genera*. Nordsvamp, Denmark.

Roux, P. (2006). *Mille et un Champignons*. Édition Roux, Sainte-Sigolène.

Sadullahoğlu, C. & Uzun, Y. (2019). Karz Dağı (Tatvan-Bitlis) ve çevresinde belirlenen makrofunguslar. *The Journal of Fungus, 11*(1), 1-11.

Sesli, E., Asan, A., Selçuk, F. (eds), Abacı Günyar, Ö., Akata, I., Akgül, H., Aktaş, S., Alkan, S., Allı, H., Aydoğdu, H., Berikten, D., Demirel, K., Demirel, R., Doğan, H.H., Erdoğdu, M., Ergül, C.C., Eroğlu, G., Giray, G., Halikî Uztan, A., Kabaktepe, Ş., Kadaifçiler, D., Kalyoncu, ...& Yoltaş, A. (2020). *Türkiye mantarları listesi*. Ali Nihat Gökyiğit Vakfı Yayını, İstanbul.

Uzun, Y. & Kaya, A. (2022). Macromycetes determined in Tonya (Trabzon) District. *KSU J. Agric Nat*, *25*(1), 66-77. DOI: 10.18016/ksutarimdoga.vi.857201.