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Evaluation of Anticholinergic, Antidiabetic and Antioxidant Activity of *Astragalus dumanii*, an Endemic Plant

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ABSTRACT

The research was conducted to separately evaluate and detect the possible *in vitro* antioxidant, antimicrobial activity of ethanol extracts prepared from aerial parts and roots of *Astragalus dumanii* and anti-cholinesterase and α-glucosidase inhibitory activity from only aerial parts of its The antioxidant capacity was tested by scavenging of DPPH and ABTS free radicals. Compared with the standard antioxidant compound gallic acid; Root and aerial part extract showed lower DPPH radical scavenging activity, however aerial part extract demonstrated higher ABTS radical scavenging activity. The phenolic contents were detected as 5.31 ± 0.03 and 13.23 ± 0.05 mg gallic acid equivalent g^{-1} extract, flavonoid contents were found as 8.26 ± 0.004 and 7.93 ± 0.005 mg Qercetin equivalent g^{-1} extract. In addition, the effects of the extracts obtained from aerial parts of the plant on acetylcholinesterase, butyrylcholinesterase and a-glycosidase enzymes were investigated in vitro and IC_{50} values were obtained as 1.47, 0.83 and 0.48 µg mL^{-1}, respectively. When these values were compared with standard substances, it was seen that Astragalus *dumanii* could be a good enzyme inhibitory agent. Antimicrobial activity of the plant extracts were determined using the microdilution method and the extracts was not observed to have any antimicrobial activities..

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Keywords
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Sivas da Yetişen Endemik Bir Bitki Olan *Astragalus Dumanii*'nin Antikolinerjik, Antidiyabetik ve Antioksidan Aktivitesinin Değerlendirilmesi

ÖZET

Araştırma, *Astragalus dumanii* bitkisinin topraküstü ve köklerinden hazırlanan etanol ekstrelerinin olası *in vitro* antioksidan, antimikrobiyal ve yalnızca topraküstü etanol ekstrelerinin anti-kolinesteraz ve α-glikozidaz inhibitör aktivitesini ayrı ayrı değerlendirmek ve tespit etmek için yapılmıştır. Antioksidan kapasitesi, DPPH ve ABTS serbest radikalleri temizleme metoduyla test edildi. Standart antioksidan bileşik gallik asit ile karşılaştırıldığında; Kök ve toprak üstü kısım ekstreleri düşük DPPH radikal süpürücü aktivite gösterirken, toprak üstü kısmı daha yüksek ABTS radikal süpürücü aktivite göstermiştir. Fenolik içerikleri 5,31 ± 0,03 ve 13,23 ± 0,05 mg gallik asite eşdeğer g^{-1} ekstre, flavonoit içerikleri ise 8,26 ± 0,004 ve 7,93 ± 0,005 mg kersetine eşdeğer g^{-1} özüt olarak bulunmuştur. Ayrıca bitkinin toprak üstü kısımlarından elde edilen ekstrelerin asetilkolinesteraz, butirilkolinesteraz ve a-glikosidaz enzimlerine etkisi in vitro araştırıldı ve sırasıyla IC_{50} değerleri: 1.47, 0.83 ve 0.48 µg mL^{-1} elde

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Antidiyabetik
Asetilkolinesteraz
Butirilkolinesteraz

edildi. Bu değerler stadart maddelerle karşılaştırıldığında da *Astragalus dumanii*'nin iyi bir enzim inhibe edici ajan olabileceği görülmüştür. Bitki ekstrelerinin antimikrobiyal aktiviteleri mikrodilüsyon yöntemi kullanılarak belirlendi ve ekstrelerde herhangi bir antimikrobiyal aktivite gözlemlenmedi.

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INTRODUCTION

Plants have been used from ancient times for maintaining good health and to cure various ailments. The genus *Astragalus* (Leguminosae) is represented by 2500 taxa and widely distributed in the world. It is also among the largest genus in Turkey, known as 'geven' in Turkish language and represented by 439 species in 61 sections (Chamberlain and Malthews, 1970; Ekici and Aytaç,2001). The genus is best represented in the steppe areas in the Irano-Turanian phytogeographic region of Turkey. The flowering plant of *Astragalus* genus, common name "milkvetches" or "locoweed" ("loco" is Spanish for crazy), was well known to western European botanists of the 17th century (Pistelli, 2002). The roots of *Astragalus* sp. have been shown to contain different types of secondary metabolites such as triterpene saponins, izoflavonoids, polysaccharides, amino acids, and trace elements (Juan et al., 2014; Jia et al., 2016; Zhang et al., 2016). Various biological activities have so far been reported on *Astragalus* species. The roots of Astragalus membranaceus, a well-known species, included in the official drug list of Traditional Chinese Pharmacopeia and prescribed mainly as tonic and for treatment of nephritis and diabetes (Pistelli, 2002). The compounds of this genus are used in the treatment of many diseases (Gökalp; 2020; 2021).

In vertebrates, the enzymes hydrolyse acetylcholine (ACh) exist in two diverse forms. Acetylcholinesterase enzyme (EC 3.1.1.7; AChE) terminates ACh activity at the post-synaptic membrane in the neuromuscular linkage (Aksu et al., 2016; Taslimi et al., 2017; Kocyigit et al., 2017). The other key enzyme hydrolyses ACh is in multitude in comparison to other esters but has no recorded physiological function. It is called nonspecific cholinesterase, cholinesterase (EC 3.1.1.8), butyrylcholinesterase, and pseudocholinesterase. In this study, it is called butyrylcholinesterase (BChE), while cholinesterases refer to both BChE and AChE. In vertebrate cells, both enzymes have key roles and they are inhibited by 10-5 with Meserine, a property which distinguishes them from nonspecific esterases. BChE and AChE enzymes can be specifically inhibited by N,N'-di-isopropylphosphorodiamidic anhydride and BW284C51 (Garibov et al., 2016). BChE is within the scope of attention of pharmacologists, because of the role of the enzyme in the succinylcholine hydrolysis (SCh), having a potential to have an a short-acting blocking on the ACh receptor. For specific patient groups, slow SCh hydrolyzation might result in prolonged apnea, possibly related to a genetic alteration in the BChE (Taslimi et al., 2017). It is not clear whether AChE enzyme within such locations as the membrane of red blood cell, early myotendinous bond, and migrating neurocrest cells plays a vital role (Çağlayan et al., 2019; Erdemir et al., 2019; Yamalı et al., 2020). It has been reported that in embryonic extension, a successive or organizing pattern of AChE and BChE gives rise to the suggestion of BChE functioning as an embryonic AChE (Caglayan et al., 2019; Taslimi et al., 2019). Protein sequencing, as well as the newly recorded cDNA clones and determined amino acid sequences for both enzymes allow a better evaluation of BChE and AChE (Sujayev et al.,2016; Topal et al., 2016; Öztaskın et al., 2017).

About 3-7% of the aggregate population of all humans can be categorized within the group of the people diagnosed with diabetes mellitus (DM), as the leading endocrine disease, leading to mortality and morbidity (Demir et al., 2019). Through the action of pancreatic α–amylase, it is possible to hydrolyze and absorb starch molecule as glucose in the small intestine by α–glucosidase enzymes (Taslimi et al., 2018; Demir et al., 2018). In addition to its utilization of multiple methods, α-glucosidase inhibitors (AGIs) can be grouped into the possible methods in treatment (Paslimi et al., 2017; Demir et al., 2020). The dietary carbohydrates as sucrose and maltose are taken by certain traits of α-glucosidases viz. sucrase, maltase, isomaltase, and glucoamylase existing within the intestines. As a result, inhibitory process by such enzymes has the potential to reduce the postprandial hyperglycemia and thus may be among the vital approaches in the treatment of DMs (Türker et al., 2017; Taslimi et al., 2018). The AGIs were extracted from the natural sources as microbes, nutrients, and plants (Choi et al., 2005; Inyushkina et al., 2007; Noh et al.,2011; Kanget al., 2012). In this study, the aim was to characterize the antioxidant and antimicrobial activities of ethanol extracts

prepared from aerial parts and roots of *Astragalus dumanii* and anti-cholinesterase and α-glucosidase inhibitory activity of only aerial part *extracts* of its.

MATERIALS and METHODS

Plant material and extraction

Aerial parts and roots of *Astragalus dumanii* (Fabaceae) were collected from Turkey: B6 square, Sivas-Kangal-Gürün road inter-section, 1560 m, 39°07'53.1" N; 37°14'32.9" E, and identified by Dr. Mehmet Tekin, Trakya University, Edirne. Dried specimens were deposited at the herbarium of Faculty of Science, Cumhuriyet University, under the collector number M. Tekin, 1494. The aerial parts and roots of *A. dumanii* were air dried at room temperature with shade and grounded with laboratory type grinder. 100g of powdered plant material (aerial parts and roots separately) macerated with aqueous ethanol (80: 20, v/v) for one day and filtered through filter paper. The filtrate was evaporated in vacuum (via Buchi rotary evaporator R-100). Ethanolic extract from aerial parts and roots afforded the yield of 28.15% and 16.18% (w/w), respectively. The resulting product was kept at -20ºC.

Antioxidant activity

Radical scavenging activity

The scavenging potential of ethanol extracts for 1,1-diphenyl-2-picryl-hydrazil (DPPH) radicals was determined by means of the altered approach developed by Sannigrahi et al (2010). 3 mL of test samples in various concentrations were blended with 1mL of 0.1mM DPPH solution in MeOH. The mixture was incubated at 25ºC. The absorbing status was determined at 517 nm utilizing a UV-VIS spectrophotometer after 30 min. The radical scavenging activity was determined through decreasing absorbance rate by the equation below:

% Scavenging activity = (Ac-As)/Ac × 100,

where As is the sample absorbing value, and Ac is the control absorbing value (without extract)

ABTS [2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)] radical cation scavenging potential

In measurement of radical scavenging potential of the ABTS cation, the specific method was utilized (Re et al., 1999). Interacting 7 mM ABTS solution with 2.45 mM potassium persulfate, subsequently keeping the mixture at 25ºC for 12–16 h in dark, ABTS radical cation was attained. As a preliminary step of the assay, ABTS working solution was attained by diluting the stock ABTS solution with methanol to give the absorbance of 0.700 ± 0.02 at 734 nm. 1mL of sample solution was blended with 1 mL of ABTS working solution. The absorbing capacity of resulting compost was ascertained at 734 nm following 7 min incubation period at room temperature. The results were expressed as IC_{50}.

Total phenolic content (TPC)

In line with the procedure described by Adedapo et al., 2008, TPC was detected with the Folin- Ciocalteu (F-C) approach. 1mL of test solutions with different concentrations and 4 mL of 7.5% Na_2CO_3 solution, and 5mL of 10% Folin- Ciocalteu reagent were mixed. After vortexing, the mixture was allowed to stand for 2h for color development. Then the absorbing capacity was evaluated at 760 nm with UV-VIS spectrophotometer. Expression of total phenolic content was determined as mg gallic acid equivalent per gram of extract.

Total flavonoid content (TFC)

TFC of the extracts was detected with aluminum chloride colorimetric method descried by Erygur et al. (Eruygur et al., 2017) with some modifications. On to the 0.5 mL of test sample (2 mg mL^{-1} in methanol) in test tubes, 4.3 mL of 80% ethanol, 0.1 mL of 1 M sodium acetate, and 0.1 mL of 10% aluminum chloride (w/v) were added. Following a 30min period left for incubating at 25ºC, the absorbing capacity was detected at 415 nm in comparison to the curve ofquercetin. The TFC was determined in mg of quercetin equivalents (QE) per gram of extract.

Antimicrobial activity

The minimum inhibitory concentration (MIC) of *A. dumanii* aerial parts and roots were determined using the broth microdilution method in 96-well microtiter plates (Eloff, 1998). The bacterial and yeast test strains used in this study were *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 29213), *Streptococcus pyogenes* (ATCC 19615), *Klebsiella pneumonia* (ATCC 13883), *Pseudomonas aeruginosa* (ATCC 27853), *Enterococcus faecalis* (ATCC 29212), *Candida albicans* (ATCC 10231) and *Candida tropicalis* (DSM 11953) (Eruygur et al., 2017). Mueller-Hinton broth (Accumix®AM1072) was used as a culture media for bacteria and Sabouraud Dextrose Broth (Himedia ME033) was used for *Candida spp.* (CLSI, 2002, CLSI, 2012).

The extracts were dissolved in the broth (512 mg mL^{-1}). 100 µL of the extract including broth was added to the first row of the microtiter plates. 50 µL of media was added the remaining wells and serial two-fold dilutions were prepared. The 11^{th} wells were used as the reproductive controls and 100 µL broth was added. The concentration of the extract in the wells was ranging from 256 to 0.5 mg mL^{-1}. The bacteria and fungi suspensions (50µL) were added to prepared samples. The final inoculum size was 5×10^5 CFU/mL in the bacteria wells and $0.5\text{-}2.5\times10^3$ CFU/mL in the

Candida sp. wells (CLSI, 2002, CLSI, 2012). The incubation of plates took place at 37°C for 24h for bacteria and at 30°C for yeasts. Then 2 mg mL^{-1} of 2, 3, 5-Triphenyltetrazolium chloride (TTC) sterile solution was blended and further incubated at 37°C for 1h (Eloff, 1998). The presence of a red pellet located at the well bottom with formation of formazan indicated microbial growth.

AChE/BChE activity determination

The inhibition potential of aerial parts *extracts* of *A. dumanii* on AChE/BChE were determined conforming to spectrophotometric method of Ellman et al.(Ellman et al., 1961). For substrates for the reactions mentioned above, acetylthiocholine and butyrylthiocholine iodides (AChI/BChI) were utilized. Namely, after dissolving through deionized water at different concentrations, 750 µL of solution, 50 µL BChE/AChE (5.32×10^{-3} U) solution and 100 µL of Tris/HCl buffer (1 M, pH 8.0) were mixed and incubated for 8 min at 20 ºC. Then, 50 µL of DTNB (0.5 mM) was added to the solution. For the activities of AChE/BChE, 5,5'-Dithio-bis(2-nitro-benzoic) acid (DTNB) acted as the measurement criteria. Addition of 50 µL of BChI/AChI initiated the reaction (Giacobini, 2003). The spectrophotometric monitoring of BChI/AChI substrates hydrolysis indicated that maximum absorption took place at 412 nm wavelength (Silman and Sussman, 2005).

α-glucosidase inhibition assay

In line with the procedure of Tao et al. (Tao et al., 2013) using p-nitrophenyl-D-glycopyranoside (pNPG) as the substrate, α-Glycosidase inhibiting potential for aerial parts *extracts* of *A. dumanii* was performed. 10 mg dissolved in 10 ml (EtOH:H_2O) was the procedure for sample preparation. As the preliminary step, in phosphate buffer (0.15 U mL^{-1}, pH 7.4) and 10-100 µL of the sample, 100 µL of phosphate buffer was blended with 20 µL of the enzyme solution. Multiple solutions in phosphate buffer were prepared in order to attain a complete inhibitory effect. Then, it was pre-incubated at 35 ºC for 12 min prior to the addition of the p-NPG to initiate reaction. 50 µL of p-NPG in phosphate buffer (5 mM, pH 7.4) was added after preincubation and re-incubated at 37 ºC. The absorbing capacities were spectrophotometrically measured at 405 nm. The IC_{50} amount was calculated from activity (%) versus plant concentration plots (Demir, 2019;, Demir, 2020).

Statistical analysis

The data were analysed by using MS Excel 2007 and presented as mean ± SD of three replicates. One-way analysis of variance (ANOVA) and Tukey tests were performed by using SPSS ((Version 20.0, SPSS Inc.,Chicago, IL, USA) to determine significant group differences and means were considered as statistically significant if $p < 0.05$.

RESULTS and DISCUSSION

Antioxidant activity

The present research focuses on finding naturally occurring natural antioxidants from plant origin. The results of different antioxidant assays on *A. dumanii* were showed in Table 1. Both of the radical scavenging activity was assessed by measuring the reduction in their absorbance at 734 nm for ABTS and 517 nm for DPPH. The DPPH radical are soluble in methanol while the ABTS is water soluble radical. As seen from the data given in Table 1, IC_{50} values for ABTS are lower than DPPH. It can be explained that the antioxidants present in the extract are relatively polar compounds (Soare et al., 2007). IC_{50} value for DPPH radical scavenging activity of ethanol extract from root (1008.88 µg mL^{-1}) was higher than aerial part (1398.08 µg mL^{-1}). On the other hand, ABTS radical scavenging activity of aerial part extract (1.18 µg mL^{-1}) higher than root extract (82.25 µg mL^{-1}).

Table 1. Radical scavenging activity (IC_{50} in µg ml^{-1}), total phenol and flavonoid content of ethanol extracts from aerial parts and roots of *A. dumanii* (values are given as mean ± SD of 3 measurements)

Tablo 1. A. dumanii'nin (sultan geveni) topraküstü ve köklerinden elde edilen etanol ekstrelerinin radikal süpürme aktivitesi (IC_{50} µg ml^{-1} cinsinden), toplam fenol ve flavonoit içeriği (değerler 3 ölçümün ortalama ± standard sapma (SD) olarak verilmiştir)

	Radical scavenging activities		Total phenolic content (mg GAE/g)	Total flavonoid content (mg QE/g)
	DPPH assay	ABTS assay		
*R*oot ethanol extract	1008.88 ± 0.04[a]	82.25 ± 0.01[a]	5.31 ± 0.03	8.26 ± 0.004
*A*erial part ethanol extract	1398.08 ± 0.03[b]	1.18 ± 0.002[b]	13.23 ± 0.05	7.93 ± 0.005
Gallic acid	5.59 ± 0.06[c]	56.01 ± 0.004[ab]		

Different letters in the same column were significantly different ($p < 0.05$).

Based on these results, it was suggested that the root of *A. dumanii* has a potential candidate for polar radical scavenger. Lim et al. reported that *A. sinicus* aceton extract showed strong DPPH radical scavenging activity than other extracts, showed 95.1% scavenging activity at 10 mg mL^{-1} concentration (Lim et al., 2011). Asgarpanah reported that the IC_{50} value of DPPH radical scavenging activity of methanol

extract from *A. squarrosus* is 1220 µg mL^{-1}(Asgarpanah et al., 2011). These results are in consistent with the results obtained in this study.

Phytochemical analysis revealed the presence of volatile oils, phenolic, flavonoids, sterols, and tannins in methanol extracts of *Astragalus* species. Plant phenolic constituents exhibit important biological activities. Phenolic compounds and flavonoids are known as potential antioxidants for their scavenging free radicals and chelating metal ions in the biological reaction chain. (Demir et al., 2017, Özaslan et al., 2018). Phenolics and flavonoids concentration in the extract are indicated as mg of gallic acid and mg of quercetin per g of extracts, respectively. The higher concentrations of polyphenolics were found in extract of aerial parts (13.23 ± 0.05 mg GAE g^{-1}) than roots (5.31 ± 0.03 mg GAE g^{-1}), whereas the content of flavonoids are higher in the roots (8.26 ± 0.004 mg QE g^{-1}) than aerial parts (7.93 ± 0.005 mg QE g^{-1}) and it is probably responsible for their free radical scavenging activity.

Antimicrobial activity

The antimicrobial activities against five bacteria and two yeast of the raw aqueous ethanol extract prepared from aerial parts and roots of *A. dumanii* were detected using the microdilution technique at the concentration range 32 to 256 mg mL^{-1} (Table 2).

Table 2. Antimicrobial activities of *A. dumanii* aerial parts and root extracts (MIC values, mg mL^{-1})
Tablo 2. A. dumanii toprak üstü ve kök ekstrelerinin antimikrobiyal aktiviteleri (MIC değerleri, mg mL^{-1})

	E. coli	*S. aureus*	*S. pyogenes*	*K. pneumoniae*	*P. aeruginosa*	*E. faecalis*	*C. albicans*	*C. tropicalis*
A. dumanii aerial part extract	256	32	128	256	128	32	32	256
A. dumanii root extract	256	128	256	256	256	64	64	256

According to Holetz et al. (2002) if the extracts displayed an MIC less than 100 µg mL^{-1}, the antimicrobial activity is good; from 100 to 500 µg mL^{-1} the antimicrobial activity is moderate; from 500 to 1000 µg mL^{-1} the antimicrobial activity is weak; over 1000 µg/ml the extract is considered inactive.

According to these criteria, the raw aqueous ethanol extracts of *A. dumanii* not showed antimicrobial activities on tested microorganisms.

Enzyme inhibition studies of the extract

Alzheimer (AD) is characterized as an advanced, neurodegenerative disease predominantly inflicting the people over 60 years of age and it is estimated to responsible for 50-60% of cases of dementia in humans within and above this age limit. The important symptoms associated with the later phases of AD involve cognitive dysfunction, primarily memory loss (Türkeş et al., 2019; Gündogğdu et al., 2019). In the brain of mammals, two main forms of ChEs exist, that is, AChE and BChE. Among the considerable biochemical changes in AD patients, diminution of ACh amounts in the cortex and hippocampus of the brain is the most noticeable (Işık et al., 2019). Thus, inhibition of AChE enzyme, hydrolyzing ACh at the cholinergic synapse is presently the most accepted approach to treatment of AD (Işık, 2019; Topal, 2019). The important side effects caused by licensed drugs utilized in treatment of AD have forced researchers to consider extracting safer BChE or AChE inhibitors from natural sources. Various plants and their constituents have long been benefited in traditional medicinal practices for improving cognitive function and alleviating other symptoms of AD, including depression (Öztaşkın et al., 2015;Istrefi et al., 2020).

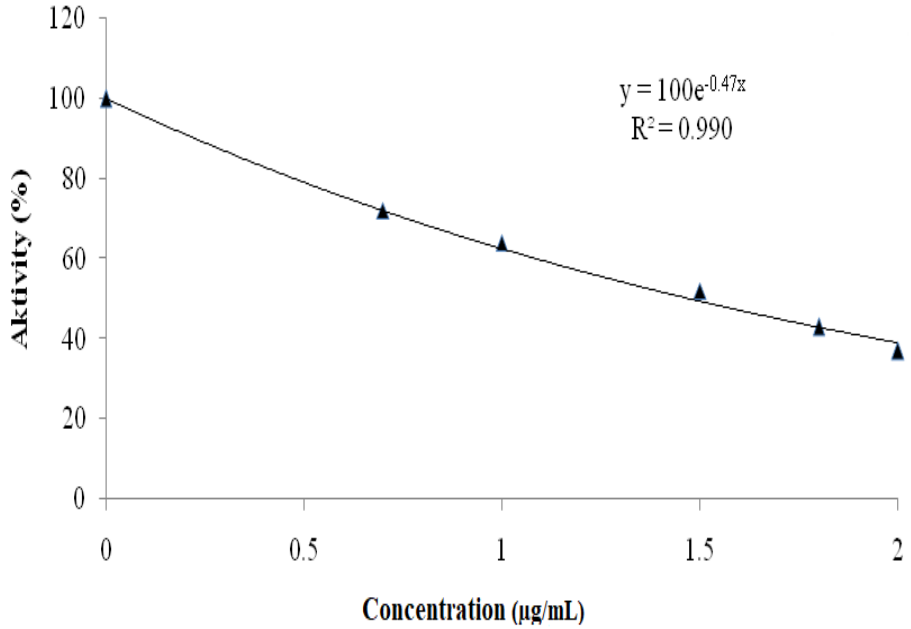
It is very important to discover new inhibitors that have less side effects than currently used cholinesterase inhibitor drugs, are cheaper and easily obtainable. Therefore, the discovery of new inhibitors is one aspect of this study. The effect of *A. dumanii aerial parts extracts* on enzyme activities was investigated spectrophotometrically under in vitro conditions and the results were compared with standard substances.

Aerial parts extracts of A.*dumanii* plant effectively resulted in the inhibition of AChE and BchE (Figure 1. and Table 3). TAC (9-amino-1,2,3,4-tetrahydroacridine) reversibly inhibits of BChE and AChE and it can be categorized as the first drug to be recommended for the placative treatment of AD. IC_{50} values for these enzymes were obtained 1.47 µg mL^{-1} (r^2 : 0.990) for AChE and 0.83 µg mL^{-1} (r^2 : 0.982) for BChE. Moreover, Tacrine (TAC) was used as positive standard BChE and AChE inhibitor with IC_{50} values 19.11 µg mL^{-1} (r^2 : 0.981) and 12.36 µg mL^{-1} (r^2 : 0.958) , respectively. These results showed us *Aerial parts extracts of* A.*dumanii* is a potential cholinesterase inhibitor.

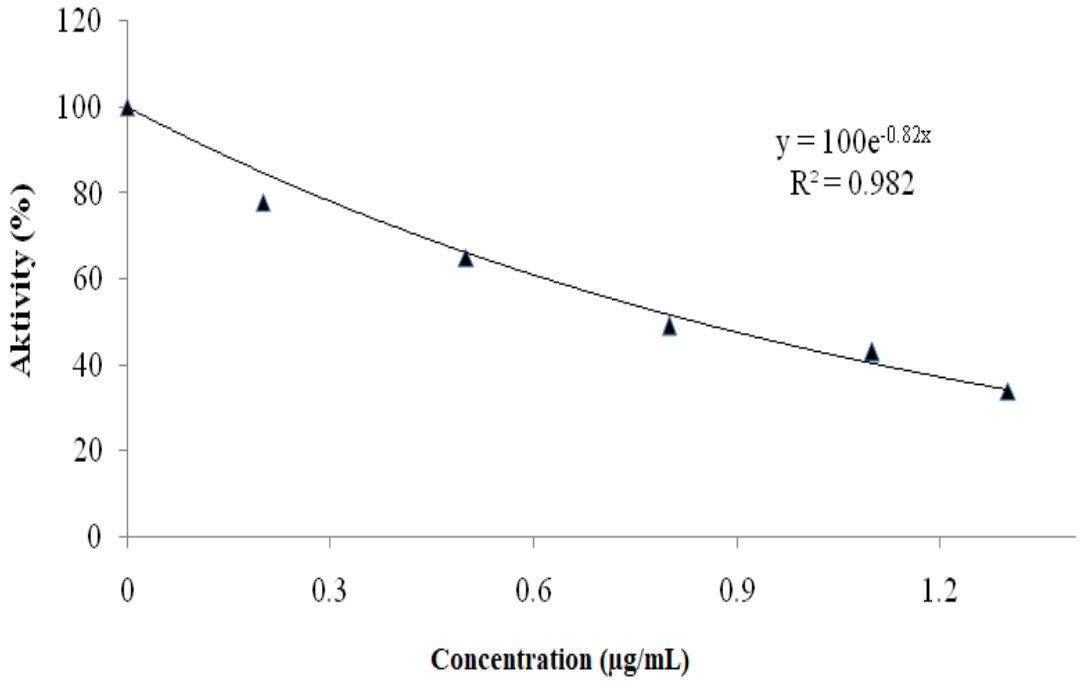
Due to their modulatory physiological effects in the cure and prevention of obesity and diabetes, functional food profiles and plant-based medicines propelling a new surge of interest. Accordingly, the highly beneficial and attractive purposes as in vitro

inhibition of enzymes α-amylase and α-glucosidase are presently under wide scrutiny (Burmaoglu et al., 2019). Reports considering the safety and efficiency of natural nutritious supplements and specific herbs which have long been used for treating diabetic disease in traditional medicine and both academic and public interest in such reports have been accumulating (Gondolova et al., 2018).

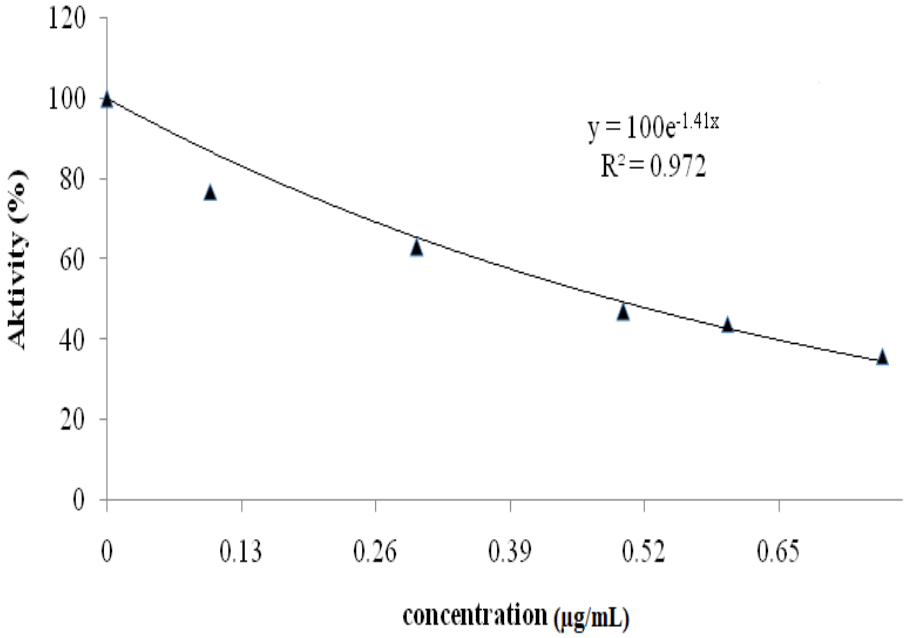
In the current study, it was also studied inhibition effect of extract of A.dumanii on α-glycosidase. IC_{50} value was found 0.48 µg mL^{-1} (r^2 : 0.972) for α-glycosidase. The results demonstrate that *Aerial parts extracts of* A.dumanii inhibited α-glucosidase more effectively than acarbose, which had IC_{50} values of 22.80 (r^2 : 0.985)) µg mL^{-1} for a-glucosidase.



A



B



C

Figure 1. The IC_{50} graphs of aerial parts extracts of *A. dumanii* against A) AChE, B) BChE, C) α-glucosidase enzymes

Şekil 1. A. dumanii'nin (sultan geveni) toprak üstü kısım ekstresinin A) AChE, B) BChE, C) α-glukosidaz enzimlerine karşı IC_{50} grafikleri

Table 3. The enzyme inhibition results of aerial parts extracts of *A. dumanii* against AChE, BChE, and α-glycosidase enzymes.

Tablo 3. A. dumanii'nin (sultan geveni) toprak üstü kısım ekstrelerinin AChE, BChE ve α-glukosidaz enzimlerine karşı enzim inhibisyon sonuçları

	α-glucosidase		AChE		BChE	
Compounds	IC_{50} (µg mL^{1})	r^2	IC_{50} (µg mL^{-1})	r^2	IC_{50} (µg mL^{-1})	r^2
A. dumanii	0.48	0.972	1.47	0.990	0.83	0.982
Tacrine#	-	-	12.36	0.958	19.11	0.981
Acarbose*	22.8	0.985	-	-	-	-

#Tacrine was used as positive control for AChE and BChE enzymes and determined
*Acarbose was used as positive control for α-glycosidase enzymes and determined

CONCLUSION

Evaluation of anticholinergic, antidiabetic and antioxidant activity of *A. dumanii*, an endemic plant, is very important. Inhibition of active enzyme activity has become a prominent target in the therapy or management of many chronic disorders, particularly Alzheimer's disease, cancer, and diabetes. The results show that Astragalus dumanii is a natural source of antioxidants and is also a good enzyme inhibitory agent against acetylcholinesterase, butyrylcholinesterase and α-glycosidase (IC_{50} values: 1.47, 0.83 and 0.48 µg mL^{-1}, respectively) when compared with standard substances.In addition, the obtained results can provide a pharmacological basis for further research on the bioactivity isolation of active compounds.

CONFLICT of INTEREST

The authors report no declarations of interest.

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Determination of Antimicrobial Activity of *Nasturtium officinale* and Its Content of Volatile Organic Compounds and Fatty Acids

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ABSTRACT
Due to the side effects of antibiotics used in the treatment of diseases caused by pathogenic bacteria, and antibiotic resistance that develops due to the misuse of antibiotics, scientists have turned to the search for alternative antimicrobial compounds. Plants and antimicrobial compounds in plants are widely researched because they are natural and have been a familiar resource in the field of complementary medicine for centuries. In this study, antimicrobial activities of the methanol and water extracts of *Nasturtium officinale* prepared at different concentrations were investigated on gram-positive bacteria, gram-negative bacteria, and fungi by the disc diffusion method. In addition, volatile organic compound and fatty acidcontent of the plant were determined. For this purpose, fatty acids were determined by converting them to methyl esters in GC-FID (gas chromatography flame ionization detector), volatile compounds were determined by SPME (Solid-phase microextraction) method in GC-MS (gas chromatography-mass spectrometry). In addition, the amounts of volatile components in different parts of the plant were shown comparatively within the scope of the research. According to the results obtained; it was revealed that *Nasturtium officinale* has an antimicrobial effect on *Bacillus megaterium*, *Escherichia coli*, *Candida albicans*, *Pseudomonas aeruginosa*, *Bacillus Spizizenii*, *Klebsiella pneumoniae*, *Staphylococcus aureus* bacteria. The plant showed a stronger antimicrobial effect, especially on *P. aeruginosa*, *C. Albicans, and E. coli*. It has also been determined that *Nasturtium officinale* has important essential fatty acids as well as many volatile components. In the analyzes made, it was determined that the main volatile component of *Nasturtium officinale* was alpha-Terpinolene.

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Nasturtium officinale'nin Antimikrobiyal Aktivitesinin ve İçeriğindeki Uçucu Organik Bileşikler ve Yağ Asitlerinin Belirlenmesi

ÖZET
Patojen bakterilerin neden olduğu hastalıkların tedavisinde kullanılan antibiyotiklerin gerek yan etkileri, gerekse yanlış kullanımına bağlı olarak gelişen antibiyotik direnci nedeniyle bilim insanları alternatif antimikrobiyal bileşik arayışına yönelmiştir. Doğal olması ve yüzyıllardır tamamlayıcı tıp alanında tanıdık bir kaynak olması sebebiyle bitkiler ve bitkilerdeki antimikrobiyal bileşenler yaygın bir şekilde araştırılmaktadır. Bu çalışmada, *Nasturtium officinale*'nin farklı konsantrasyonlarda hazırlanan metanol ve su ekstraktlarının gram-pozitif bakteriler, gram-negatif bakteriler ve mantar üzerindeki antimikrobiyal aktivitesi disk difüzyon yöntemiyle araştırıldı. Bunun yanısıra bitkinin içeriğindeki uçucu organik bileşikler ve yağ asitleri tespit edildi. Bu amaçla GC-FID'de (gaz kromatografisi alev iyonizasyon dedektörü) yağ asitleri metil esterlere dönüştürülerek belirlenirken, GC-MS'de (gaz

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Yağ asitleri

kromatografisi - kütle spektrometrisi) SPME (Katı fazlı mikroekstraksiyon) yöntemi ile uçucu bileşenleri belirlendi. Ayrıca araştırma kapsamında bitkinin farklı kısımlarındaki uçucu bileşenler karşılaştırmalı olarak ele alındı. Elde edilen sonuçlara göre; *Nasturtium officinale*'nin *Bacillus megaterium, Escherichia coli, Candida albicans, Pseudomonas aeruginosa, Bacillus Spizizenii, Klebsiella pneumoniae, Staphylococcus aureus* bakterileri üzerinde antimikrobiyal etkisi olduğu ortaya çıktı. Bitki özellikle *P. aeruginosa*, *C. Albicans ve E. Coli* üzerinde daha güçlü antimikrobiyal etki göstermiştir. Ayrıca *Nasturtium officinale*'nin önemli esansiyel yağ asitlerinin yanısıra çok sayıda uçucu bileşenlere de sahip olduğu tespit edildi. Yapılan analizlerde *Nasturtium officinale*'nin ana uçucu bileşeninin alfa-Terpinolen olduğu belirlendi.

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INTRODUCTION

N. officinale, a member of the *Brassicaceae* family, is a rare aquatic plant with thin and fibrous roots, and oval baby leaves (Daniel, 2009). *N. officinale* is considered a valuable traditional medicinal plant due to its many health-beneficial components such as vitamins B, C, and E, pro-vitamin A, folic acid, carotenoids, glucosinolates (Gonçalves et al., 2009; Pourhassan-Moghaddam et al, 2014). It is known that watercress (*N. officinale*) leaves are used in anti-inflammatory, diuretic, expectorant, hypoglycemic, antihypertensive, urinary tract infections, and cardiovascular diseases (Amiri, 2012; Shahani et al., 2016).

Plants that have been used in the treatment of various diseases for centuries are still recommended in the treatment of many diseases, especially as a supplement. Due to the high cost of drugs used in the treatment of diseases, as well as the risk of unwanted side effects, researchers around the world are trying to identify effective drugs with minimal side effects to overcome the difficulties associated with the unreliability of modern pharmaceuticals (Adokoh et al., 2019; Abd Rashid et al., 2021). Recently, ways to eliminate many bacteria that cause various infections and food poisoning without using antibiotics are being investigated (Şengün and Öztürk, 2018).

The use of herbal products in the industry, health, textile, food, cosmetics, and the increasing trend towards natural products have led to the examination of plants from all aspects (Varlı et al., 2020). For this purpose, plants and their bioactive components, fatty acids, volatile components, and their effects have begun to be investigated more (Varlı et al., 2020). Some volatile compounds obtained from aromatic plants are used in medicine and pharmacology as antimicrobial, anti-inflammatory, antioxidant, expectorant, analgesic, and in the treatment of many ailments and are also effective in defense against herbivores and pathogens (Pichersky et al., 2006; Maffei et al., 2011; Yip et al., 2019). It has been shown in many studies that these compounds, which are synthesized by plants to attract pollinators and fight pests, have many beneficial effects such as anticarcinogen, anti-inflammatory, antidiabetes and neuroprotective, as well as being used as fragrance and food additives (Maffei et al., 2011; Pichersky et al., 2006). ; Vieira et al., 2018; Yip et al., 2019).

On the other hand, fatty acids are also among the important compounds contained in plants. It is also known that essential fatty acids, which cannot be synthesized in the body of humans and other mammals and must be taken from outside, play a key role in the prevention of many diseases such as heart attack, cardiovascular diseases, depression, migraine, arthritis, diabetes, high cholesterol, blood pressure, allergies, and cancer (Santos et al., 2017; Wassell et al., 2010). The essential fatty acids α-linolenic acid and linoleic acid are required for the synthesis of various molecules that affect vital functions (Das, 2006). According to previous research reports, it has been shown that polyunsaturated fats such as omega-3 play an important role in the prevention of coronary heart disease and some cancers (Pretorius and Schőnfeldt, 2021). Linolenic acid, one of the polyunsaturated fatty acids, has anticancer, antiosteoporotic, antioxidant, anti-inflammatory, and coronary protective properties (Santos et al., 2017; Martins et al., 2018). In addition to the many benefits of essential oils, many negative situations arise in the lack of these essential oils. It is important to reveal the volatile compounds and fatty acids contained in

plants to find out their valuable aspects. Therefore, in this study, the volatile components, fatty acids, and antimicrobial effects of *N. officinale* were investigated.

MATERIAL and METHOD

Plant material

The *N. officinale* used in this study was collected from the natural environment in Kayseri, Turkey, and the plant samples were authenticated by Prof. Dr. Hasan AKAN. It is stored in herbarium number 6363 at Harran University.

Chemicals

Nutrient agar medium, broth medium (Condalab brand), amikacın (30µg), ampicillin-sulbactam (20 µg), rifampin (5 µg), and erythromycin (15 µg) were purchased from Bioanalyse. Methanol, DMSO (dimethylsulfoxide), and KOH were purchased from Sigma- Aldrich.

Tested Microorganisms

Tested Microorganisms; *Klebsiella aerogenes* ATCC 13048, *Pseudomonas aeruginosa* ATCC 9027, *Bacillus megaterium* ATCC 14581, *Escherichia coli* ATCC 11229, *Klebsiella pneumoniae* ATCC 13883, *Staphylococcus aureus* ATCC 25923, *Candida albicans* ATCC 10231, *Bacillus subtilis subsp. spizizenii* ATCC 6633, strains are purchased from Microbiologics.

Preparation and Analysis of Plant Extracts for Antimicrobial Activity Determination

To investigate the antimicrobial effects of *N. officinale*, two separate extractions were prepared with distilled water and methanol. Water extract was prepared by adding 200 mL of distilled water to 20 grams of plant samples, and methanol extract was prepared by adding 200 mL of methanol to 20 grams of plant samples. After filtration through filter paper, the water extract from the solutions was lyophilized while the methanol extract was evaporated. To determine the antimicrobial effect of the plant, solutions were prepared of lyophilized water extract and evaporated methanol extract with dimethyl sulfoxide (DMSO) at different concentrations (20, 40, 60 mg mL). The Disc Diffusion method was used to determine antimicrobial activity (Wayne, 1997). Microorganisms were incubated at 37°C in nutrient broth (NB) until 0.5 McFarland (1.5x108 Kob mL) turbidity occurred. Turbidity control was performed in a spectrophotometer at a wavelength of 625 nm with a between absorbance of 0.08-0.10. 100 µl of prepared test microorganisms were taken and inoculated in nutrient agar solid plates. Then, the solutions of the lyophilized water extracts and the evaporated methanol extracts prepared were absorbed into sterile discs in the inoculated plate. For *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus, Bacillus megaterium, Klebsiella pneumoniae, Bacillus spizenii,* and *Klebsiella aerogenes* bacteria 24 hours of incubation at 37°C were measured and for *Candida albicans* incubation at 30°C, inhibition was measured after 48 hours of incubation. The same procedure was repeated for the positive control erythromycin (15 µg), rifampin (5 µg), amikacin (30 µg), ampicillin-sulbactam (SAM) (20 µg), and the negative control (DMSO).

Determination of Volatile Components Using SPME-GCMS

The solid-phase microextraction (SPME) method is a simple method that allows direct volatile component analysis without extraction of the plant (Panighel and Flamini, 2014). 0.6 grams of *N. officinale* and leaf and stem parts of *N. officinale* were weighed each to determine the volatile organic compound content by the SPME (Solid Phase Micro Extraction) method in Gas Chromatography-Mass Spectrometry (GC-MS). Volatile organic compounds were determined by the SHIMADZU QP2020 brand GC-MS device. The column used in the analysis is DB-HEAVYMAX (60 m x 0.25 mm x 0.25 µm). The injection temperature is 250°C and the injection mode is split, the total flow rate is 1.05 mL min. The incubation temperature was set to 40°C. The SPME fiber used is 100 µm PDMS (polydimethylsiloxane).

Determination of Fatty Acid Methyl Ester Using GC-FID

To determination of fatty acid methyl ester in gas chromatography flame ionization detector (GC-FID), 20 grams of *N. officinale* plant was taken and 200 mL of hexane was added and mixed with a magnetic stirrer at room temperature for 12 hours. The solution was then filtered through a filter paper and the solvent was evaporated at 35°C in the evaporator until the final volume of the solution was 20 mL. It was taken from this extract 0.1 mL and mixed with 10 mL hexane and 0.5 mL of 2N KOH prepared in methanol was added and kept in the dark for 1-2 hours. Fatty acid methyl ester analysis was performed with SHIMADZU QP2020 brand GC-FID (Gas Chromatography-Flame Ionization Detector). Fatty acids were analyzed by converting them into methyl ester derivatives in the determination of fatty acids in GC-FID branded SHIMADZU QP2020. Rtx-2330 RESTEK (60 m x 0.25 mm x 0.2 µm) column was used for analysis. Injection temperature was set to 250°C, injection mode Split, pressure 100 kPa, Split Ratio 100. The injection volume was set to 1 µL (AOAC, 2012).

Statistical Analysis

All measurements were carried out in triplicate and the results were presented as mean values ± SD (standard deviations). Statistical analyzes between groups of antimicrobial analyzes were revealed by one-way ANOVA. $p<0.05$ was considered significant.

RESULTS and DISCUSSION

Antimicrobial Activity Results

Antimicrobial Efficacy of Methanol Extracts

Antimicrobial activity of the plant on gram-positive bacteria (*S. aureus, B. subtilis subsp. Spizizenii, B. megaterium*), gram-negative bacteria (*E. coli, K. pneumoniae, P. aeruginosa, K. Aerogenesis*), and fungi (*C. albicans)* have been examined. Negative control (DMSO) showed no antibacterial effect. The methanol extracts showed an antimicrobial effect on *K. pneumoniae, B. megaterium, E. coli, C. albicans, P. aeruginosa, B. Spizizenii, S. aureus* bacteria. Methanol extracts antimicrobial activity results (inhibition zone diameters) are given in Table 1.

Antimicrobial Efficacy of Water Extracts

Negative control (DMSO) did not show an antibacterial effect. The antimicrobial activity results (inhibition zone diameters) of the water extracts of *N. officinale* are given in Table 2.

Table 1. Antimicrobial activity results of the methanol extracts (mm)
Çizelge 1. Metanol ekstraktlarının antimikrobiyal aktivite sonuçları (mm)

Methanol extracts	Amikacin (AK-30)	Ampicillin -sulbactam (SAM- 20)	Rifampin (RD-5)	Erythromycin (E-15)	20 mg mL methanol extract	40 mg mL methanol extract	60 mg mL methanol extract
P. aeruginosa	18.02±0.01	15.23±0.01	10.9±0.03	12.61±0.03	9.39±0.07	10.63±0.03	11.69±0.05
B. megaterium	19.05±0.15	26.42±0.32	22.78±0.17	25.36±0.33	8.29±0.03	8.29±0.01	8.8±0.04
C. albicans	21.79±0.45	27.43±0.69	17.00±0.41	14.58±0.04	7.54±0.01	8.35±0.07	8.75±0.05
K. pneumoniae	19.01±0.07	17.30±0.21	12.52±0.03	16.41±0.09	-	7.67±0.03	7.86±0.09
E. coli	14.95±0.08	21.8 ±1.24	9.82±0.01	12.77±0.05	7.74±0.05	9.39±0.05	11.44±0.12
K. aerogenes	17.06±0.03	15.25±0.12	9.37±0.07	18.34±0.12	-	-	-
B. spizizenii	24.34±0.42	25.42±0.21	19.37±0.15	26.87±1.17	6.84±0.07	6.95±0.05	7.31±0.04
S. aureus	12.74±0.02	27.8±1.45	22.34±0.45	19.27±0.03	-	6.42±0.04	6.48±0.02

(-): No inhibition, ± SD (standard deviation)
(-): İnhibisyon yok, ± SD (standart sapma)

Table 2. Antimicrobial activity results of water extracts (mm)
Çizelge 2. Su ekstraktlarının antimikrobiyal aktivite sonuçları (mm)

Water extracts	Amikacin (AK-30)	Ampicillin -sulbactam (SAM- 20)	Rifampin (RD-5)	Erythromycin (E-15)	20 mg mL water extract	40 mg mL water extract	60 mg mL water extract
P. aeruginosa	20.75±0.12	16.7±0.07	10.9±0.08	12.61±0.07	-	9.16±0.04	10.41±0.07
B. megaterium	18.7±0.04	26.99±0.45	22.7±0.53	24.63±0.71	8.06±0.06	8.8±0.03	9.71±0.02
C. albicans	25.26±0.32	20.97±0.06	17.74±0.75	15.18±1.69	7.41±0.04	8.05±0.04	9.31±0.12
K. pneumoniae	20.12±0.09	16.12±0.05	11.84±0.01	15.42±0.26	-	-	-
E. coli	17.69±0.01	19.35±0.01	10.21±0.05	15.49±0.05	-	7.51±0.05	8.81±0.09
K. aerogenes	18.17±0.05	12.87±0.02	9.62±0.09	17.78±0.35	-	-	-
B. spizizenii	19.96±0.21	20.93±0.21	19.37±0.01	28.64±1.24	6.62±0.04	7.49±0.03	8.29±0.07
S. aureus	13.08±0.06	28.72±0.06	23.71±0.04	18.43±0.52	-	-	-

(-): No inhibition, ± SD (standard deviation)
(-): İnhibisyon yok, ± SD (standart sapma)

According to the results given in Table 2, the water extract of the plant has an antimicrobial effect on *B. megaterium, E. coli, C. albicans, P. aeruginosa, B. spizizenii* bacteria. However, the water extract started to inhibit *E. coli, P. aeruginosa* bacteria at a concentration of 40 mg mL. When the results are examined, it is seen that the methanol extracts of the plant have an antimicrobial effect on *K. pneumoniae, B. megaterium, E. coli, C. albicans, P. aeruginosa, B. spizizenii* bacteria. When the inhibition diameters and percent inhibition amounts were examined, water extracts of *N. officinale* showed more antimicrobial effect than methanol extracts on gram-positive bacteria except for *S. aureus*. Methanol extracts, on the other hand, showed more antimicrobial effect on gram-negative bacteria than water extracts, and also inhibited *S. aureus*, a gram-positive bacteria. While the water extracts of the plant did not inhibit *K. pneumoniae, S. aureus* bacteria, it began to inhibit the methanol extracts at a concentration of 40 mg mL. Comparison of antimicrobial activity results of methanol and water extracts of *N. Officinale* is given in Figure 1.

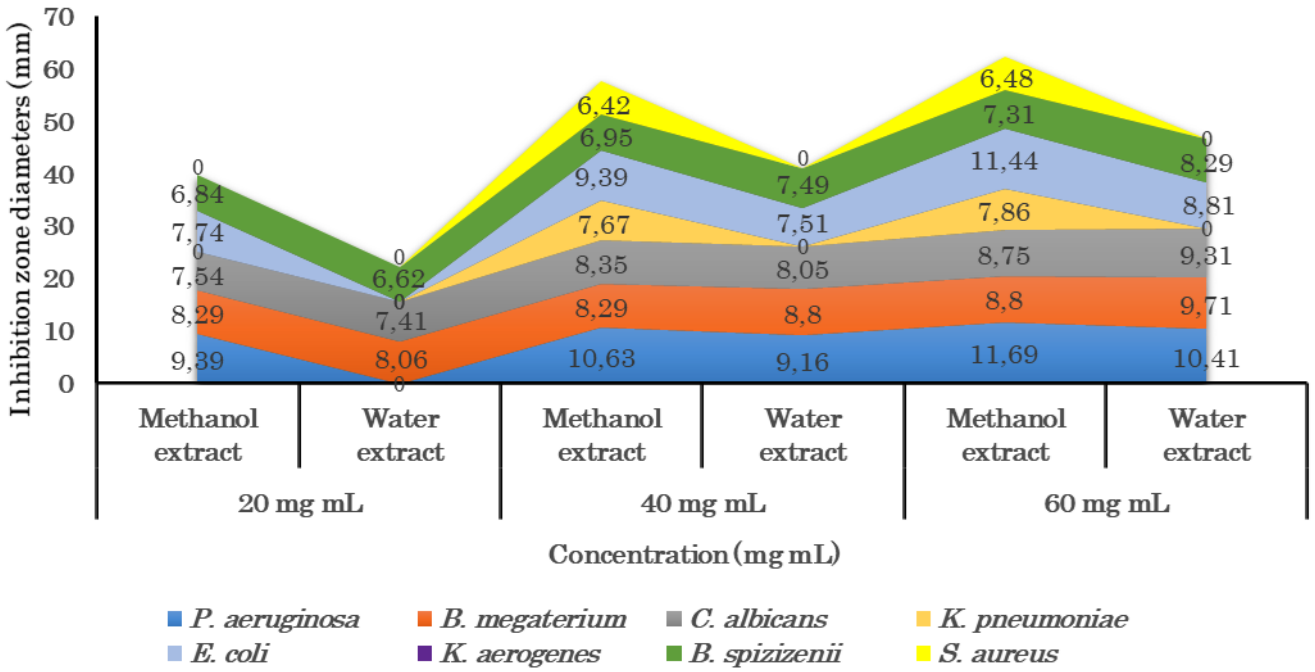

Figure1. Comparison of antimicrobial activities of methanol and water extracts of N. Officinale
Şekil 1. N. Officinale'nin metanol ve su ekstraktlarının antimikrobiyal aktivitelerinin karşılaştırılması

According to these results, methanol extracts have more antimicrobial effects because they inhibit more bacteria. Components that dissolve better in alcohol may have been effective on this result. From this point of view, it can be said that components with antimicrobial properties have a more apolar structure. In addition, methanol extracts at a concentration of 60 mg mL showed a stronger antimicrobial effect on *P. aeruginosa* and *E. coli* bacteria than Rifampin antibiotic. As a result, *N. officinale* showed an antimicrobial effect on *B. megaterium, E. coli, P. aeruginosa, B. Spizizenii, K. pneumoniae, S. aureus* bacteria, and *C. albicans*. The plant showed a better antimicrobial effect especially against *C. albicans, P. aeruginosa*, and *E. coli.* In a study on *Escherichia coli, Salmonella typhimurium*, *Staphylococcus aureus,* and *Listeria monocytogenes* bacteria, it was reported that aqueous and alcoholic extracts of *N. officinale* were more effective on gram positives and did not inhibit gram-negative bacteria (Derhami et al., 2017). In experiments, it was found that *N. officinale* was also effective on *K. pneumoniae, E. coli P. aeruginosa.* The difference between these results may be due to the time the plant was collected, the place where it was collected, as well as the possibility that the plant samples may lose some of their components during drying.

Volatile Organic Component Determination Results

The volatile organic compounds of *N. officinale* were determined and shown in Table 3.

According to these results, it was determined that *N. officinale* is rich in volatile components and contains many terpenes and terpenoid volatile components. It has been observed that most of these volatile components are composed of monoterpenes and *N. officinale* contains alpha-terpinolene (54.46%), which is a monoterpene, as the main component. It contains many monoterpenes such as D-limonene, gamma-terpinene, beta-phellandrene other than alpha-terpinolene, and many sesquiterpenes such as cadina-1(2), 4-diene, caryophyllene, ß-sesquiphellandrene. Monoterpenes are among the volatile organic components of many medicinal plants. Very few volatile components were detected in the previous volatile component analysis of *N. Officinale* (Amiri, 2012). The reason for this may be that the plant loses its volatile components depending on the collection time or storage conditions. Because the plant samples used in this study are freshly dried samples.

Many monoterpenes have antimicrobial, antioxidant, anti-inflammatory, and anti-carcinogenic effects. In addition, it has been shown in previous studies that monoterpenes such as limonene prevent breast, lung and other cancers and monoterpenes are effective in cancer treatment (Gould, 1997). It has been reported that ß-myrcene, a monoterpene, acts like estrogen activity, which is particularly important for women and also exhibits cardiotonic and diuretic properties (Chappell, 1995; Koziol et al., 2014; Kweka, 2009). ß-Myrcene also has antibacterial properties on *Staphylococcus aureus, Escherichia coli, Salmonella enterica* as well as some plant pathogenic bacteria

Table 3. Volatile organic component analysis results of the *N. officinale*
Çizelge 3. N. officinale'nin uçucu organik bileşen analiz sonuçları

Compounds *Bileşikler*	RI	%	Molecular formula	Classification *Sınıflandırma*
2-Isobutyl-4,4-dimethyl-1,3-dioxane	549	0.05	$C_9H_{19}O_2$	acetal
Furan, 2,3-dihydro-	730	0.57	C_4H_6O	enol ether
Ethanol	921	0.15	C_2H_5OH	alcohol
(1R)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene (1R-α-Pinene)	1062	0.57	$C_{10}H_{16}$	monoterpene
alpha-Thujene	1068	0.03	$C_{10}H_{16}$	monoterpene
Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methyl- (beta pinene)	1136	0.18	$C_{10}H_{16}$	monoterpene
l-Phellandrene	1152	0.62	$C_{10}H_{16}$	monoterpene
beta-Myrcene	1154	1.64	$C_{10}H_{16}$	monoterpene
D-Limonene	1189	6.43	$C_{10}H_{16}$	monoterpene
Cyclohexane, 1-methylene-4-(1-methylethenyl (p-Mentha-1(7),8-diene)	1193	0.05	$C_{10}H_{16}$	monoterpene
beta-Phellandrene	1198	6.69	$C_{10}H_{16}$	monoterpene
cis-Ocimene	1233	0.29	$C_{10}H_{16}$	monoterpene
gamma-Terpinene	1242	5.28	$C_{10}H_{16}$	monoterpene
Benzene, methyl(1-methylethyl)- (cymene)	1268	1.23	$C_{10}H_{14}$	monoterpene
alpha-Terpinolene	1283	54.46	$C_{10}H_{16}$	monoterpene
Bicyclo[2.2.1]hept-2-ene, 1,7,7-trimethyl- (Bornylene)	1287	0.07	$C_{10}H_{16}$	monoterpenoid
Octanal	1290	0.09	$C_8H_{16}O$	aldehyde
Tetradecane	1400	0.04	$C_{14}H_{30}$	alkan
3,8-dimethylene-1-cyclooctene	1432	0.21	$C_{10}H_{16}$	monoterpene
Benzene, 1-methyl-4-(1-methylethenyl)- (p-Cymenene)	1451	0.66	$C_{10}H_{12}$	monoterpene alkylbenzene
Epoxyterpinolene	1480	0.69	$C_{10}H_{16}O$	monoterpenic ether
1-Hexanol, 2-ethyl-	1491	0.06	$C_8H_{18}O$	alcohol
Copaene	1500	0.08	$C_{15}H_{24}$	sesquiterpene
Benzaldehyde, 2,5-bis[(trimethylsilyl)oxy]-	1521	0.06	$C_{13}H_{22}O_3Si_2$	aldehit
3,5-Octadien-2-one	1527	0.06	$C_8H_{12}O$	oxygenated hydrocarbon
Benzaldehyde	1538	0.04	C_6H_5CHO	aldehyde
Cyclohexane, 2-ethenyl-1,1-dimethyl-3-methylene	1560	0.10	$C_{10}{}^{13}CH_{16}D_2$	cyclic hydrocarbon
Ethanone, 1-(6,6-dimethylbicyclo[3.1.0]hex-2 en	1562	0.08	$C_{10}H_{14}O$	ketone
beta-Cyclocitral	1565	0.10	$C_{10}H_{16}O$	monoterpenoid
alpha-Bergamotene	1597	0.16	$C_{15}H_{24}$	sesquiterpene
Heneicosane	1600	0.02	$C_{21}H_{44}$	alkan
Caryophyllene	1614	3.21	$C_{15}H_{24}$	sesquiterpene
Cyclohexanol, 2,6-dimethyl-	1622	0.04	$C_8H_{16}O$	alcohol
Naphthalene, 1,2,3,4,4a,7-hexahydro-1,6-dimethyl(Cadina-1(2),4-diene, cis)	1625	6.86	$C_{15}H_{24}$	sesquiterpene
16-Methyl-heptadecane-1,2-diol,trimethylsilyl ether	1640	0.12	$C_{24}H_{54}O_2Si_2$	alcohol
Farnesol	1673	0.24	$C_{15}H_{26}O$	sesquiterpenoid
cis-thujan-10-oic acid methyl ester	1686	0.15	$C_{10}H_{16}O_2$	sesquiterpenoid
alpha-Humulene	1690	0.14	$C_{15}H_{24}$	sesquiterpene
1,8-menthadien-4-ol	1696	1.07	$C_{10}H_{16}O$	monoterpenoid
1H-Cyclopenta[1,3]cyclopropa[1,2]benzene, o (beta cubebene)	1714	0.28	$C_{15}H_{24}$	sesquiterpenoid
Germacrene-D	1730	0.11	$C_{15}H_{24}$	sesquiterpene
beta-Bisabolene	1740	0.16	$C_{15}H_{24}$	sesquiterpene
3,5-Nonadien-7-yn-2-ol, (E,E)-	1777	0.06	$C_9H_{12}O$	alcohol

Cyclohexene,3-(1,5-dimethyl-4-hexenyl)-6-methylene(ß-Sesquiphellandrene)	1785	1.95	$C_{15}H_{24}$	sesquiterpene
Ethanone, 1-(4-methylphenyl)-	1795	0.05	$C_9H_{10}O$	alkyl-phenylketone
Bicyclo[3.1.1]hept-2-en-6-ol,2,7,7-trimethyl-(Chrysanthenol, cis)	1814	0.16	$C_{10}H_{16}O$	monoterpenoid
Benzenemethanol, .alpha,alpha,4-trimethyl-(p-Cymen-8-ol)	1858	0.92	$C_{10}H_{14}O$	oxygenated monoterpene
5,9-Undecadien-2-one, 6,10-dimethyl-, (Z)-(cis-Geranylacetone)	1866	0.08	$C_{13}H_{22}O$	monoterpene ketone
Naphthalene, 1,2,3,4-tetrahydro-1,6-dimethyl-(cis calamenene)	1876	0.07	$C_{15}H_{22}$	sesquiterpene
Propanoic acid, 2-methyl-, 1-(1,1-dimethylethyl ester	1891	0.23	$C_8H_{16}O_2$	ester
Neophytadiene	1927	0.15	$C_{20}H_{38}$	sesquiterpenoid
5-Isopropenyl-2-methyl-7-oxabicyclo[4.1.0]heptan-2 ol	1948	0.15	$C_{10}H_{16}O_2$	alcohol
3-Buten-2-one, 4-(2,6,6-trimethyl-1-cyclohexe (ß-Ionone)	1958	0.06	$C_{13}H_{20}O$	sesquiterpenoid
1-Nonadecanamine, N,N-dimethyl-	1993	0.15	$C_{21}H_{45}N$	amine
Diphenyl ether	2039	0.09	$(C_6H_5)_2O$	ether
5,7-Octadien-3-ol, 2,4,4,7-tetramethyl-	2044	0.07	$C_{12}H_{22}O$	monoterpenoid
Benzenepropanenitrile	2063	0.33	C_9H_9N	nitrile
Squalene	2153	1.55	$C_{30}H_{50}$	triterpene
1H-Purin-6-amine, [(2-fluorophenyl)methyl]-	2159	0.05	$C_{12}H_{10}FN_5$	amine
2-Cyclohexen-1-one,2-methyl-5-(1-methylethyl)(Carvotanacetone)	2203	0.65	$C_{10}H_{16}O$	menthane monoterpenoid
Cinnamaldehyde, alpha - pentyl-	2255	0.09	$C_{14}H_{18}O$	phenylpropanoid

RI: Retention index, %: Percentage of volatile component in total volatile components (w/w)
RI: Alıkonma indeksi, %: Uçucu bileşenin, toplam uçucu bileşen içindeki yüzdesi (w/w)

(Abdel Rasoul et al., 2012; Wang et al., 2019; Połeć et al., 2020). Squalene is a triterpene that is a precursor to the biosynthesis of cholesterol and other steroids. It has been reported to be anticarcinogenic and reduce tumor growth (Reddy and Couvreur, 2009). ß-Sesquiphellandrene has been reported to have anti-proliferative effects in leukemia, multiple myeloma, and colorectal cancer cells (Denyer et al., 1994; Tyagi et al., 2015; Siripoltangman and Chickos, 2019). ß-Caryophyllene is a sesquiterpene with anti-inflammatory, anti-spasmodic, antimicrobial effects as well as beneficial effects such as curing asthma and inhibiting hypersensitive immune reaction. (Kim et al., 1998; Cho et al., 2007; Galdino et al., 2012; Dahham et al., 2015; Yoo and Jwa, 2019).

N. officinale contains large amounts of alpha-terpinolene. Terpinolene, a flavored ingredient, is also known to have an anti-fungal function (Hammer et al., 2004). On the other hand, it has been suggested that these monoterpenes suppress NF-κB (Nuclear Factor kappa B) activity and that terpinolene and α-phellandrene may contribute to the treatment of wounds by reducing inflammation and oxidative stress (Scherer et al., 2019). It has also been proven that terpinolene is non-genotoxic and exhibits a wide variety of properties such as antioxidant, antiproliferative, anticancer, antifungal, and larvicide (Dorman et al., 2000; Hammer et al., 2004; Conti et al., 2012; Harada et al., 2012). All these findings reinforce that terpinolene is a good and safe natural antioxidant as well as a potential anticancer agent (Aydın et al., 2013). In the light of all this information, many useful components were determined in *N. Officinale* by volatile component analysis.

In addition, the results of the volatile component analysis of the leaf and stem of *N. officinale* are given in Table 4 comparatively.

When the results regarding the volatile components of the leaves and stems of *N. officinale* given in Table 4 are examined, it is seen that the leaf part of the plant is richer in volatile components than the stem part. Alpha-terpinolene is the main volatile component of both the leaf and stem of the plant. On the other hand, compounds such as D-limonene, gamma-terpinene, p-cymenene, cymene, benzenepropanenitrile, squalene, beta-phellandrene were found more in the stem part.

Fatty Acid Methyl Ester Analysis Results

Analysis results of fatty acid methyl ester of *N. officinale* are given in Table 5.

The total unsaturated fatty acid ratio of the plant is 58.75%, and the total saturated fatty acid ratio is 41.25%. The plant contains alpha-linolenic acid,

arachidic acid, linolenic acid, elaidic acid and palmitic acid. It has been observed that *N. officinale* can be an important nutrient in daily nutrition because it is rich in alpha-linolenic acid (omega 3), an essential fatty acid with important functions for the human body (Santos et al., 2017; Martins et al., 2018). It also contains linolenic acid, elaidic acid, and palmitic acid. The plant stands as a precious food with this content.

Table 4. Volatile component analysis results of leaves and stems of *N. officinale*
Çizelge 4. N. officinale'nin yaprak ve gövdesinin uçucu bileşen analiz sonuçları

	Compounds *Bileşikler*	Leaf Analysis % *Yaprak analizi %*	Stem Analysis % *Gövde analizi %*
1	Furan, 2,3-dihydro-	0.5	0.43
2	Borane-methyl sulfide complex	0.05	-
3	Acetone	0.07	0.10
4	N-Methylene-tert-butylamine	0.05	-
5	Butanal, 2-methyl-	0.02	-
6	Pentanal	0.1	0.03
7	Octane, 3,5-dimethyl-	0.03	-
8	1R-α-pinene	0.69	0.66
9	Alpha-Thujene	0.03	0.02
10	Beta pinene	2.94	2.88
11	Hexanal	0.06	0.03
12	Alpha terpinene	0.11	0.10
13	D-Limonene	7.2	7.49
14	p-Mentha-1(7),8-diene	0.05	0.05
15	Beta-Phellandrene	9.3	10.8
16	2-Hexenal, (E)-	0.09	-
17	cis-Ocimene	0.37	-
18	Gamma-Terpinene	7.85	10.19
19	Cymene	1.6	1.81
20	Alpha-terpinolene	51.78	49.67
21	Octanal	0.04	0.07
22	1,5-Cyclooctadiene, 3-t-butyl-	0.57	-
23	2-Methylisoborneol	-	0.49
24	1,3,8-p-Menthatriene	-	0.21
25	3,8-dimethylene-1-cyclooctene	0.17	0.20
26	p-Cymenene	0.78	1.06
27	Epoxyterpinolene	0.57	0.77
28	Copaene	0.1	-
29	3,5-Octadien-2-one	0.09	-
30	Benzaldehyde	0.11	-
31	Cyclohexane, 2-ethenyl-1,1-dimethyl-3-methy	0.09	0.10
32	2-Isopropylidene-3-methylhexa-3,5-dienal	-	0.06
33	Ethanone, 1-(6,6-dimethylbicyclo[3.1.0]hex-2	0.05	-
34	Bicyclo[3.1.1]hept-2-ene, 2,6-dimethyl-6-(4-m	0.14	0.11
35	Hexadecane	0.02	-
36	Caryophyllene	3.3	2.57
37	Cyclohexanol, 2,6-dimethyl-	0.14	0.04
38	Cadina-1(2),4-diene, cis	4.48	3.28
39	beta-Cyclocitral	0.09	-
40	Cyclohexane, 1-ethenyl-1-methyl-2-(1-methyl	0.26	-
41	cis-beta-Farnesene	0.16	0.11
42	cis-thujan-10-oıc acıd methyl est	0.11	0.24
43	alpha-Humulene	0.15	0.11
44	1,8-menthadien-4-ol	0.76	1.62
45	Beta cubebene	0.18	0.16
46	Benzoic acid, 4-methyl-, 2-hydroxy-2-phenyl -	-	0.12
47	Cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-m -	-	0.69
48	Ethanone, 1-(3-methylphenyl)--	-	0.06
49	Benzenemethanol, .alpha.,.alpha.,4-trimethyl--	-	1.26

50	1s,cis-calamenene	0.05	0.05
51	Neophytadiene	0.42	0.05
52	5-Isopropenyl-2-methyl-7-oxabicyclo[4.1.0]he	0.27	0.28
53	Benzenepropanenitrile -	-	0.10
54	Cyclooctanone	0.06	0.07
55	Squalene	-	1.56
56	3,5-Octadien-2-ol	-	0.05
57	Germacrene-d	0.13	-
58	beta-Bisabolene	0.15	-
59	alpha-Farnesene	0.05	-
60	Benzenemethanol, .alpha.,4-dimethyl-	0.08	-
61	beta-Sesquiphellandrene	2.09	-
62	Ethanone, 1-(4-methylphenyl)-	0.06	-
63	Selina-3,7(11)-diene	0.04	-
64	Chrysanthenol, cis	0.16	0.25
65	Benzenemethanol, alpha, alpha, 4-trimethyl-	0.96	-
66	3-Buten-2-one, 4-(2,6,6-trimethyl-1-cyclohexe	0.09	-
67	Diphenyl ether	0.08	-
68	5,7-Octadien-3-ol, 2,4,4,7-tetramethyl-, (E)-	0.06	-

%: Percentage of volatile component in total volatile components (w/w)
%: Uçucu bileşenin, toplam uçucu bileşen içindeki yüzdesi (w/w)

Table 5. Analysis results of fatty acid methyl ester of *N. officinale*
Çizelge 5. N. officinale'nin yağ asidi metil ester analiz sonuçları

Peak	R.T	Fatty acid	Concentration	Units	Area
1	18.482	Palmitic Acid C16:0	14.422	%	435
2	22.814	Elaidic Acid C18:1n9t	11.890	%	358
3	24.818	Linolenic Acid C18:2n6t	16.368	%	493
4	25.685	Arachidic Acid C20:0	26.827	%	808
5	26.336	Alpha-Linolenic Acid C18: 3n-3	30.493	%	919
				Total	3013

R.T.: Retention time, Total: Total peak area, %: Percentage of the component in total peak area (w/w)
R.T.: Alıkonma zamanı, Total : Toplam pik alanı, %: Bileşenin, toplam pik alanındaki yüzdesi (w/w)

CONCLUSION

In the light of all these results, it has been determined that the *N. officinale* is an important plant that contains essential fatty acids and many volatile organic compounds and also has antimicrobial effects. Methanol extract of *N. officinale* showed more antimicrobial effect than water extract. In the volatile component analysis results, in which we determined the volatile components of different parts of the plant, it was determined that *N. officinale* contains important components such as alpha-terpinolene, ß-Myrcene, ß-Caryophyllene, Squalene, ß-Sesquiphellandrene. In addition, according to the results of the fatty acid analysis, it was determined that it contains important fatty acids such as omega-3 in its structure. As a result, it has been shown that the plant is a species that can be used in new applications with both its antimicrobial activity and the important components it contains.

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Author's Contributions

Author 1: Writing-original draft preparation, data collection, data curation, visualization, analysis, data interpretation. Author 2: Conceptualization, methodology, validation, writing-review, and editing, supervision, provision of analysis tools. All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

Statement of Conflict of Interest

The authors state no conflict of interest.

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Partial Purification and Characterization of Polyphenol Oxidase Enzyme from Common-Morning Glory (*Ipomoea purpurea*)

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ABSTRACT

This study aimed to purify and biochemically characterize polyphenol oxidase (PPO) enzyme from the plant *Ipomoea purpurea* (*I. purpurea*) for the first time. For this purpose, the crude extract sample obtained from the extraction of *in vitro* cultured plant leaves under optimum conditions (25 mgmL^{-1} Polyvinylpolypyrrolidone, pH 7.0) was subjected to three-phase partitioning, and the PPO enzyme was 10.5-fold purified with a 57% activity recovery. The optimum pH and temperature values were determined as 7.0 and 30°C, respectively. Laccase, peroxidase, and catechol oxidase activities were observed after activity staining of partially purified enzyme. From stability tests, it was noted that more than 75% and 65% of its original activity were maintained at temperatures 20℃-40℃ and pH 7.0-9.0, respectively.

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Gündüz Sefası (*Ipomoea purpurea*) Bitkisinden Polifenol Oksidaz Enziminin Kısmi Saflaştırılması ve Karakterizasyonu

ÖZET

Bu çalışmada, polifenol oksidaz (PPO) enziminin *Ipomoea purpurea* (*I. purpurea*) bitkisinden ilk kez saflaştırılması ve enzimin biyokimyasal özelliklerinin belirlenmesi amaçlandı. Buna göre, in vitro olarak üretilen bitkiye ait yapraklardan optimum koşullarda (25 mg ml-1 Polivinilpolipirrolidon, pH 7.0) gerçekleştirilen ekstraksiyon sonrası elde edilen ham ekstrakt örneğinden PPO enzimi üçlü faz ayırma tekniği ile yaklaşık %57 aktivite geri kazanımla 10,5 kat saflaştırıldı. Enzimin optimum pH ve sıcaklık değerleri sırasıyla 7.0 ve 30°C olarak belirlendi. Aktivite boyama sonrası kısmen saf enzimde lakkaz, peroksidaz ve katekol oksidaz aktiviteleri tespit edildi. Ayrıca, 20°C ile 40°C arasında enzim aktivitesinin ≥%75, pH 7.0 ile 9.0 arasında ≥%65 oranında korunduğu tespit edildi.

Biyokimya

Araştırma Makalesi

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Anahtar Kelimeler
Polifenol oksidaz
Üçlü-faz ayırma
Enzim saflaştırma
Karakterizasyon
Ipomoea purpurea

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INTRODUCTION

Polyphenol oxidases (PPOs) (E.C. 1.14.18.1, E.C. 1.10.3.1, or E.C. 1.10.3.2) are a group of copper proteins found in nearly all prokaryotic and eukaryotic cells. PPOs are capable of oxidizing a considerable amount of phenolic and non-phenolic aromatic compounds. In most cases, its physiological functions are either pigmentation or protection from the harmful effects of the environment. Despite the uncertainty in the classification of PPOs, they can be divided into three groups according to the literature: laccase (*p*-diphenol: oxygen-oxidoreductase, E.C. 1.10.3.2), catechol oxidase (*o*-diphenol: oxygen-oxidoreductase, E.C. 1.10.3.1) and tyrosinase (monophenol-monooxygenase, E.C. 1.14.18.1) (Kocabas et al., 2011; Panadare and Rathod, 2018).

PPO activity is vital to determine food products' quality and shelf life; therefore, a fast, reliable, and

precise method is desired to estimate PPO activity in agricultural and horticultural crops. Biosensors are perfect for this purpose. Industrially, PPOs have an extensive range of application areas, including medicine, pharmaceutical and chemical industries, analytical devices (biosensor preparation), and the food industry (Panadare and Rathod, 2018).

In countries where it is essential to produce economically significant amounts of fruits and vegetables, green plants are used as a source of PPO. There are many studies on plant PPOs especially from fruits and leaves, in the scientific literature (Kocabas et al., 2011).

Ipomoea purpurea (L.) Roth (Common-morning glory, also known as Tall Morning-glory) is an annual ornamental plant. *I. purpurea* naturally spread throughout Central America but has become naturalized in most parts of the world due to its ornamental value. It is also used medicinally. The stems, seeds, roots, and flowers of *I. purpurea* have been utilized as laxative and hallucinogenic agents. Also, several parts of the species have been used to treat rheumatism, fungal and urinary infections, acne, diarrhea and constipation, infertility, liver diseases, and syphilis (Srivastava, 2017; Muhammad et al., 2019). Although it has wide use, studies on *I. purpurea* are pretty limited. Kıran and Acemi (2019) investigated the effects of chitosan on photosynthetic pigment, protein, and dry matter contents of *I. purpurea* to determine chitosan's indirect impact on plant leaves. Atala et al. (2014) performed three separate greenhouse experiments to test the twining induced in *I. purpurea* by applying different levels of artificial damage (mechanical) and natural damage with snails. Park et al. (2007) showed that phytomelanins are mainly found in the outer epidermis and palisade layers in wild type *I. purpurea*.

This study aimed to purify and biochemically characterize the PPO enzyme from leaves obtained from *in vitro* cultured *I. purpurea* for the first time. *I. purpurea* was selected as a PPO source due to its broad ornamental and medicinal uses and the limited number of studies in the literature. We have used the three-phase partitioning (TPP) technique to extract PPO enzyme from plant extract since TPP is a cost-efficient and straightforward method that purifies protein of interest in a single step (Panadare and Rathod, 2018).

MATERIALS and METHODS

Materials

The seeds of Common-Morning Glory (*Ipomoea purpurea*) were purchased from Anadolu Tohum Production and Marketing Incorporated company. The chemicals used during the experiment with the highest purity grade were purchased from BioRad (California, USA), Biolife (Viale Monza, Milan, Italia), Duchefa Biochemie (RV Haarlem, The Netherlands) and Sigma-Aldrich (St. Louis, MO, USA).

Preparation of Crude Extracts

The seeds of *I. purpurea* were germinated in LS (Linsmaier and Skoog, 1965) medium after surface disinfection treatments (Kıran Acemi and Acemi, 2019). The leaves from *in vitro*-raised individuals were collected after 30 d of the incubation period. They were used in experiments within a few hours after collection or stored at -80 °C until used. The leaves were thoroughly washed with dH_2O before use in the experiment. In the crude extract preparation, 30 g of leaf tissue was homogenized by thumping in 200 ml 0.1 M sodium phosphate buffer (pH 7.0) solution in a mortar at +4°C. Polyvinylpolypyrrolidone (PVPP) was added to a final concentration of 25 mg ml^{-1} (optimized in the present study) to remove the phenolic substances, and the mixture was filtered through cheesecloth and centrifuged at 10000×g for 30 min at +4°C (Kocabas et al., 2011). The resulting supernatant was collected and used as the crude enzyme extract for further purification.

PPO Assay and Protein Amount

PPO activity was measured by observing quinone production at 420 nm at room temperature on a spectrophotometer. In the calculation of the enzyme activity, the sample cuvette contained 500 µl substrate (pyrocatechol) at 100 mM concentration, 1 ml buffer solution of sodium phosphate (100 mM, pH 7.0), and appropriately-diluted 500 µl enzyme solution (Alici and Arabaci, 2016). The control (blank) cuvette contained the same chemicals but without the enzyme. One unit of the enzyme was the amount of enzyme producing 1 µmole of quinone per minute.

Protein concentration was performed according to the Bicinchoninic Acid (BCA) method. Bovine serum albumin (BSA) was used to draw the standard graph (Smith et al., 1985).

PPO Purification

The PPO enzyme was purified using a TPP system consisting of ammonium sulfate, t-butanol, and crude enzyme extract. The method is based on the appearance of three phases (bottom aqueous phase, protein-rich middle phase, and top t-butanol phase) after adding t-butanol to the crude enzyme extract saturated with ammonium sulfate (Dennison and Lovrien, 1997).

For TPP systems, different amounts of $(NH_4)_2SO_4$ (from 20% to 70% w/v adding in intervals of ten) were added to 2 ml samples of the crude enzyme (CE)

extract to bring different ammonium sulfate saturation, and the mixtures were vortexed gently until the ammonium sulfate was completely dissolved. Then, t-butanol was added at ratios of 1.0:0.5, 1.0:1.0, 1.0:1.5 and 1.0:2.0 (v/v) to CE extract. The mixture was vortexed gently on a magnetic stirrer for 1 min and kept at room temperature for 60 min for phase separation. After standing complete phase separation was attained by centrifugation at 4500×g for 10 min (Duman and Kaya, 2014; Alici and Arabaci, 2016). The t-butanol-containing upper phase, where the protein was not anticipated to be shown, was expelled by cautious pipetting. One ml of 0.1 M sodium phosphate buffer solution (pH 7.0) was used to dissolve the PPO enzyme-containing precipitate (middle phase). Afterward, activity and protein determination experiments were carried out at 420 nm using a spectrophotometer (Cary 60, Agilent) at room temperature on the middle and bottom phase samples. The parameters such as ammonium sulfate concentration, crude extract: t-butanol ratio, and system pH have crucial roles in the TPP system were also optimized to test their effects on the selective separation of biomolecules. CE extract activity for *in vitro*-cultured plant (6560 U) was taken as 100%. A blank system was set up, including $(NH_4)_2SO_4$, dH_2O, and t-butanol (excluding the CE extract).

Characterization of the PPO Enzyme

Activity staining

The activity staining procedure was carried out to identify the PPO activity through polyacrylamide gel electrophoresis (Rescigno et al., 1997). The SDS-free polyacrylamide gel contained 10% (w/v) separation gel and 4% (w/v) stacking gel. The electrophoresis was carried out at 150 V for 45 min at 4°C (Kaptan, 2004). After that, 20 ml of 0.1 M potassium phosphate buffer solution (pH 6.0) was used to wash the separation gel for 5 min. For the determination of laccase activity, the gel was treated with 40 mM 4-amino-N-N diethylaniline (ADA) prepared in 10 mM HCl solution. Then, 10 mM H_2O_2 for peroxidase activity determination was applied, and finally, it was exposed to 40 mM 4-tert-butyl catechol (tBC) prepared in 10 mM acetic acid solution for the determination of catechol oxidase activity. Before each treatment, the gel was washed with dH_2O. The existence of pink-red bands after ADA and H_2O_2 and dark blue bands after tBC were analyzed (Rescigno et al., 1997).

Effect of pH on enzyme activity and stability

The effect of different pH on *I. purpurea* PPO activity was determined by using 100 mM pyrocatechol as substrate at several pH values ranging from 4.0 to 9.0. The buffer solutions used in the experiment were 0.1 M citrate buffer for reactions between pH 4.0-5.0, 0.1 M phosphate buffer for pH 6.0-7.0, 0.1 M Tris buffer solution for pH 8.0, and 0.1 M glycine-sodium hydroxide buffer for pH 9.0 (Kavrayan and Aydemir, 2001; Gülçin et al., 2005; Kocabas et al., 2011). Percent relative activities were calculated by dividing the enzyme activity measured at each pH value by the maximum enzyme activity multiplied by 100.

To determine the stability of *I. purpurea* PPO against the same pH range, the PPO sample was incubated in the buffers mentioned above for 30 and 60 min in a water bath. The enzyme activity was measured at the end of the incubation period using the standard experimental procedure. Percent residual activities were calculated by dividing the post-incubation enzyme activity by the pre-incubation activity multiplied by 100.

Effect of temperature on enzyme activity and stability

In order to detect the effect of temperature on *I. purpurea* PPO activity, enzyme activity was measured by setting a spectrophotometer temperature between 20°C and 70°C. The enzyme stability temperature was measured after keeping the PPO samples in a water bath at different temperatures between 20°C and 70°C (20, 30, 40, 50, 60 and 70°C) for both 30 and 60 min (Kavrayan and Aydemir, 2001; Gülçin et al., 2005; Kocabas et al., 2011). After the incubation, enzymes were first kept on ice, and then polyphenol oxidase activity measurements were made in a spectrophotometer device. Percent relative and residual activities were estimated as described above.

Statistical Analysis

All experiments were performed in triplicate. The data are represented as mean ± standard deviation (SD). The statistical differences between the means were compared using Duncan's multiple range test (DMRT) at $P \leq 0.05$ through the software "The Statistical Package for the Social Sciences" (SPSS, version 22, IBM Inc., Chicago, IL, USA).

RESULTS and DISCUSSION

Optimization of PPO Extraction Conditions

For PPO isolation from *I. purpurea*, the first step was optimizing extraction conditions, including PVPP concentration and pH of the extraction medium. PVPP is a compound capable of preventing hydrogen bonding between phenolics and PPO enzyme (Smith and Montgomery, 1985). For this reason, PVPP was used during extraction, and its concentration was optimized to improve PPO yield. PVPP was added to the extraction medium in a 5-75 mg PVPP ml^{-1} concentration range. As seen in Table 1, 25 mg PVPP ml^{-1} appeared to be a suitable concentration to remove phenolics for all samples tested. This was consistent

with previous reports where PVPP was used at a broad range of 10-60 mg ml^{-1} concentrations (Rocha and Morais, 2001; Kocabas et al., 2011; Pelalak et al., 2021). 25 mg PVPP ml^{-1} was chosen for further extraction optimization analysis.

Table 1 PVPP optimization results
Çizelge 1. PVPP optimizasyon bulguları

PVPP concentration (mg ml^{-1})	PPO activity (U ml^{-1})
5	1937±95 ^{d}
12.5	2310±108^{c}
25	3252±163^{a}
37.5	3007±136^{b}
50	1876±95^{d}
62.5	3028±151^{b}
75	1510±78^{e}

^{a,b,c} Values within a row with different superscripts differ significantly at P≤0.05

In order to optimize the pH of the environment, extraction media was prepared under conditions ranging from pH 4.0 to 9.0. Afterward, extracts were obtained, and enzyme activity was determined under standard conditions in the spectrophotometer. According to the results given in Table 2, the highest activity was observed in the extraction medium with pH 7.0. Thus, further screening experiments were performed at pH 7.0. In general, it is seen that the preferred pH during extraction of PPO from different plants falls in the range of 6.0-7.0. For example, pH 6.0 was used for artichoke (*Cynara scolymus* L.) (Aydemir, 2004), while pH 6.5 and 7.0 were chosen for cotton (*Gossypium hirsutum* L.) (Kouakou et al., 2009) and grape (*Vitis vinifera*) (Öztan, 2007), respectively.

Table 2 Extraction pH optimization results
Çizelge 2. Ekstraksiyon pH'sının optimizasyon bulguları

pH	PPO activity (U ml^{-1})
4.0	0^{g}
5.0	532±30^{f}
6.0	2201±102^{b}
6.5	3000±112^{c}
7.0	3328±164^{a}
8.0	1943±73^{d}
9.0	1250±61^{e}

^{a,b,c} Values within a row with different superscripts differ significantly at P≤0.05

Purification of PPO by TPP

The second step to obtain the PPO enzyme was the application of three-phase partitioning to the crude extract. Compared with other conventional purification methods, TPP is a simple purification technique with a short processing time and is economical (ammonium sulfate and t-butanol are readily available). This technique does not denature proteins during purification and generally works at room temperature (Rachana and Jose, 2014). Although TPP was chosen for many scientists to purify various important enzymes (Nadar and Rathod., 2017), studies on its use for PPO purification are restricted (Panadare and Rathod, 2018).

For TPP systems, it is vital to optimize the system components like ammonium sulfate and t-butanol concentrations and the pH to extract a sufficient amount of saturated protein of interest from the complex mixture (CE extract) with the complex mixture minimal interaction with contaminating materials. In general, optimization trials are started with solutions prepared at 20% (w/v) saturation. The desired protein concentration in the middle or bottom phases is determined (Dennison and Lovrien, 1997). Therefore, the experiments were started with 20% (w/v) $(NH_4)_2SO_4$, since it was recommended in various reports (Niphadkar and Rathod, 2015, Panadare and Rathod, 2018). In the experiments, $(NH_4)_2SO_4$ was tested up to 70% (w/v) at pH 7.0 and room temperature on crude enzyme extract to t-butanol (1.0:1.0; v/v). Figure 1 reveals the effect of $(NH_4)_2SO_4$ concentration on PPO partition from the crude extract.

Ammonium sulfate saturations below 20% (w/v) usually lead to poor protein recovery, but higher concentrations cause protein to move from bottom to top (Bayraktar and Önal, 2013). Likewise, in this study, it was observed that the PPO enzyme accumulated in the bottom phase when the crude extract was saturated with 20%-40% (w/v) $(NH_4)_2SO_4$ (Figure 1). On the other hand, 50%, 60%, and 70% saturations resulted in PPO movement from the bottom to the middle, but the purification fold decreased. The highest fold purification of 8.2-fold along with 57% activity recovery of PPO in the bottom phase was obtained with 30% (w/v) $(NH_4)_2SO_4$.

Crude extract:t-butanol ratio is also an essential parameter in TPP systems and should be optimized. t-Butanol shows higher deactivation and lower interfacial precipitation through hydrophobic interactions with proteins than other organic solvents such as n-butanol, n-propanol, or isopropanol and does not denature proteins. Therefore, it is the most preferred organic solvent in TPP systems. Its lower concentrations might be insufficient to exert a synergistic effect with the salt used. At the same time, its higher amounts are expected to inhibit intermolecular interactions due to the increased viscosity of the TPP mixture (Dennison and Lovrien, 1997).

TPP systems were set up containing the crude extract saturated with 30% $(NH_4)_2SO_4$. t-Butanol was added into the solutions of crude extracts saturated with

$(NH_4)_2SO_4$ at ratios varying between 1.0:0.5 and 1.0:2.0 to determine the appropriate optimal volume added to the solution. As observed from Figure 2, the best PPO purification fold (8.2) and highest activity recovery (57%) were achieved from the bottom phase of the TPP system prepared by the addition of an equal volume of t-butanol to the crude extract saturated with 30% (w/v) $(NH_4)_2SO_4$. Also, in Figure 2, with ratios of crude extract to t-butanol of 1.0:0.5 and 1.0:2.0, the purification fold of the PPO enzyme at the bottom phase was decreased. On the other hand, with a 1.0:1.5 ratio, activity recovery was slightly increased.

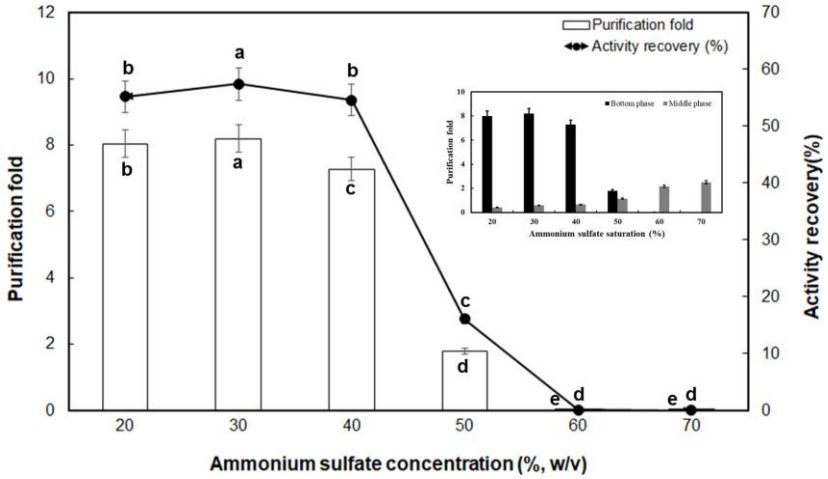

Figure 1. The effect of different $(NH_4)_2SO_4$ concentrations on PPO enzyme distribution in TPP systems. The specific activity of *I. purpurea* crude extract is 4686 U mg^{-1} and the total protein amount is 1.4 mg. Equal volumes of t-butanol were added to the mixtures prepared with different $(NH_4)_2SO_4$ saturation at a ratio of 1.0:1.0 crude extract: t-butanol. The bottom phase formed in all systems were collected and analyzed. Insets, Effect of ammonium sulfate saturation on PPO partition into the bottom and middle phases.

Şekil 1. Farklı $(NH_4)_2SO_4$ konsantrasyonlarının TPP sisteminde PPO enzim dağılımına etkisi. I. purpurea ham ekstraktının spesifik aktivitesi 4686 U mg^{-1}, toplam protein miktarı ise 1.4 mg olarak hesaplandı. Farklı *$(NH_4)_2SO_4$ doygunluğu ile hazırlanan karışımlara ham ekstrakt: t-butanol oranı 1.0:1.0 olacak şekilde eşit hacimlerde t-butanol ilave edildi. Tüm sistemlerde oluşan alt fazlar toplandı ve analiz edildi. Ekli küçük resim, Amonyum sülfat doygunluğunun PPO'nun alt ve orta fazlara dağılımına etkisi.*

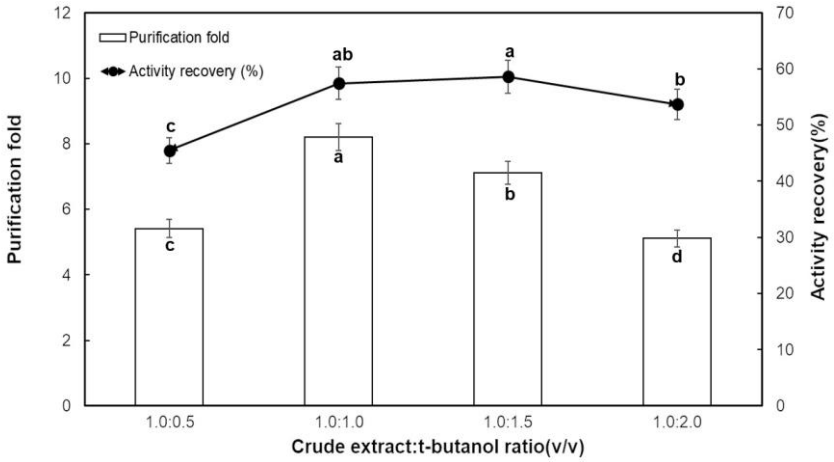

Figure 2. The effect of different crude extract: t-butanol ratios on PPO enzyme distribution in TPP systems. The specific activity of *I. purpurea* crude extract is 4686 U mg^{-1}. The total protein amount is 1.4 mg. t-Butanol was added to the mixtures prepared at 30% (w/v) $(NH_4)_2SO_4$ saturation to obtain different crude extract: t-butanol ratio. The bottom phase formed in all systems were collected and analyzed.

Şekil 2. Farklı ham ekstrakt:t-butanol otanlarının TPP sisteminde PPO enzim dağılımına etkisi. I. purpurea ham ekstraktının spesifik aktivitesi 4686 U mg^{-1}, toplam protein miktarı ise 1.4 mg olarak hesaplandı. %30 (w/v) $(NH_4)_2SO_4$ doygunluğunda hazırlanan karışımlara t-Butanol ilave edilerek farklı ham ekstrakt:t-butanol oranları elde edildi. Tüm sistemlerde oluşan alt faz toplandı ve analiz edildi.

The last important parameter affecting the protein enrichment and purification efficiency of TPP systems is the system pH. This effect is generally associated with changes in amino acid residues on the surface of proteins due to pH changes. In general, the cleavage of the target protein into the middle or aqueous phase in the TPP system is mainly based on the isoelectric point (Yan et al., 2018). The $(NH_4)_2SO_4$ concentration and crude extract: the t-butanol ratio was kept constant in the systems to investigate the effect of the system pH on the separation of PPO enzyme in phases. Then, the system pH was adjusted to the desired pH using 0.1 M sodium hydroxide (NaOH) and 0.1 M hydrochloric acid (HCl). Figure 3 indicates that the highest purification (10.5-fold) and the highest recovery (57%) were observed at pH 7.5. As shown in Figure 3, at pH 7.0 and 7.5 values, activity recovery remained unchanged, but the purification fold was increased when system pH was increased to 7.5. The moderate activity was also recovered when the system pH adjusted to pH 5.0, 6.0, or 8.0. Below pH 5.0, however, poor protein recovery and purification fold were observed. This can be explained by more hydrogen ions competing with the protein of interest for interaction with water molecules (Chew et al.,2019).

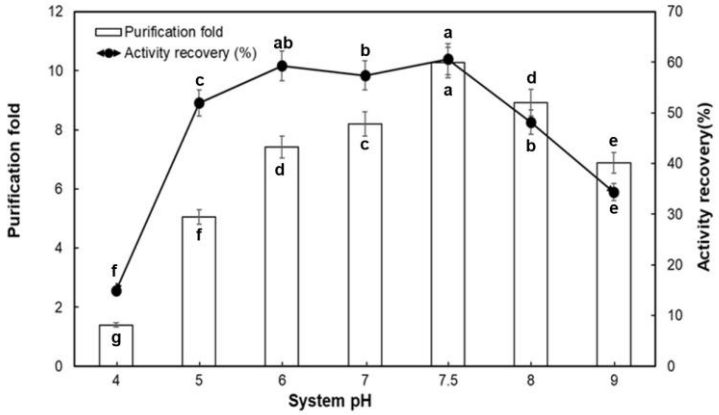

Figure 3. The effect of the system pH on PPO enzyme distribution in TPP systems. The specific activity of *I. purpurea* crude extract is 4686 U/mg, and the total protein amount is 1.4 mg. Prepared systems containing 1.0:1.0 crude extract t-butanol saturated with 30% (w/v) $(NH_4)_2SO_4$ were adjusted to different pH. The bottom phase formed in all systems were collected and analyzed.

Şekil 3. Sistem ph'sının TPP sisteminde PPO enzim dağılımına etkisi. I. purpurea ham ekstraktının spesifik aktivitesi 4686 U mg^{-1}, toplam protein miktarı ise 1.4 mg olarak hesaplandı. %30 (w/v) $(NH_4)_2SO_4$ doygunluğunda ve *1.0:1.0 oranında ham ekstrakt:t-butanol içerecek şekilde hazırlanan sistemler farklı pH'a ayarlandı. Tüm sistemlerde oluşan alt faz toplandı ve analiz edildi.*

In summary, the best purification of the PPO enzyme was achieved from a TPP system consisting of 2 ml of the crude enzyme (extracted from *I. purpurea* leaves) saturated with 30% (w/v) $(NH_4)_2SO_4$ and 2 ml of t-butanol at pH 7.5. A few PPO enzymes have been purified through TPP with different purification folds and yields. Niphadkar and Rathod (2015) and Alici and Arabaci (2016) have reported 70% and 69% activity recovery values of PPO corresponding to 6.3- and 3.6-fold purifications, respectively. Yuzugullu Karakus et al. (2020) and (2021) have reported 230% and 120% activity recoveries of PPO corresponding to 14- and 20-fold purifications, respectively. In this study, PPO was purified to a higher fold than potato and borage PPO enzymes. However, the activity recovery value of the enzyme was the lowest among PPOs from other plants.

Activity Staining of PPO

Activity staining was performed to detect the enzyme activity on a native gel. After SDS-free polyacrylamide gel electrophoresis (Kaptan, 2004), the gel was stained according to the method reported by Rescigno et al. (1997). The protocol was applied as described in the Materials and Methods section.

As seen in Figure 4, a light pink band was observed after staining with ADA (laccase substrate), supporting the presence of laccase activity. Further treatment of the gel with H_2O_2 (peroxidase substrate) leads to an amaranth pink band appearance corresponding to where laccase activity was observed on ADA-stained gel. This indicated the presence of peroxidase activity. Lastly, the gel was exposed to tBC, resulting in dark blue color without any extra band. The dark blue color indicates that tBC was oxidized to corresponding quinones, and catechol oxidase activity was detected at the same position as laccase and peroxidase bands. Considering all results obtained, it can be concluded that *I. purpurea* PPO exhibited laccase, peroxidase, and catechol oxidase activities.

Effect of Reaction pH on PPO Activity and Stability

The reaction pH is an important parameter that affects enzyme activity by altering the enzyme's net charge, thereby affecting the enzyme's solubility, binding ability with different substrates/inhibitors, and folding. Enzymes mostly become inhibited at extreme pH values because they lose their ability to fold (Panadare and Rathod, 2018). The effect of pH on PPO activity was investigated at a broad pH range of 4.0 to 9.0 and presented in Figure 5A. Accordingly, optimum pH was found at 7.0 using catechol as substrate. Similarly, using catechol, PPO enzymes from chest nut kernel, buriti, rosemary, mango, banana, and peppermint were optimum at pH 7.0 (Table 4). The PPO enzyme maintained more than 60% of its initial activity over the pH range 5.0-9.0 (Figure 5A).

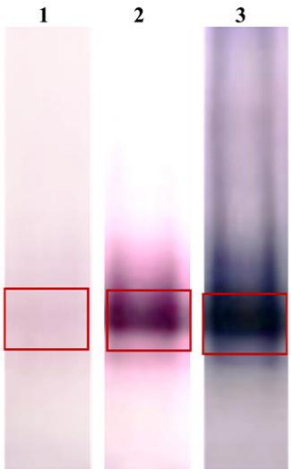

Figure 4. Gel view showing the activity staining results of *I. purpurea* PPO with ADA (1), H_2O_2 (2), and tBC (3).
Şekil 4. I. purpurea PPO'sunun ADA (1), H_2O_2 (2), ve tBC (3) ile aktivite boyama sonuçlarını gösteren jel

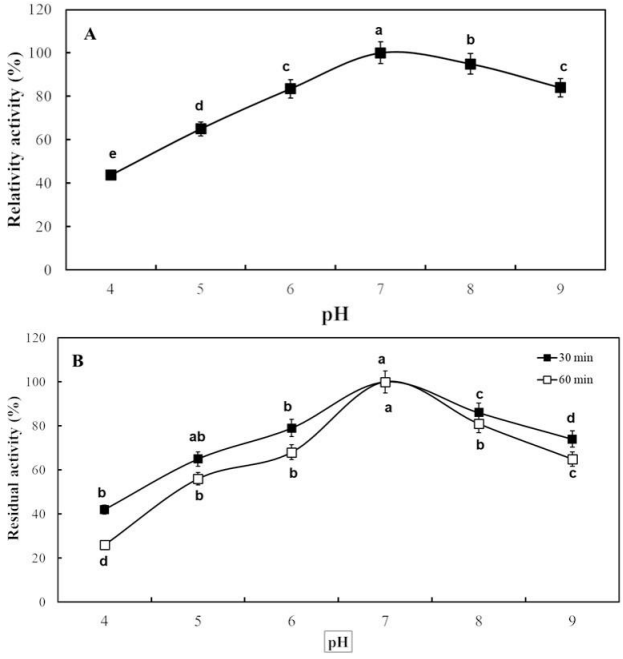

Figure 5. Effects of reaction pH on *I. purpurea* PPO activity (A) and stability (B). (A) Percent relative activities measured at various pH values (4.0-9.0) at room temperature in citrate, phosphate, Tris-HCl, and glycine-NaOH buffers. (B) Percent residual activities measured after pre-incubation of the PPO at pH values between 4.0 and 9.0. The values are presented as means ± SD.

Şekil 5. Reaksiyon pH'sının I. purpurea PPO aktivitesi (A) ve stabilitesi (B) üzerine etkisi. (A) Sitrat, fosfat, Tris-HCl ve glisin-NaOH tamponları kullanarak farklı pH değerlerinde oda sıcaklığında ölçülen yüzde relatif aktivite değerleri. (B) 4.0 ile 9.0 arasındaki pH değerlerinde hazırlanan tamponlarda PPO'nun ön inkübasyonundan sonra ölçülen bağıl aktivite yüzde değerleri. Değerler, ortalama ± SD olarak sunuldu.

The pH stability of the PPO was also investigated by preincubating the enzyme for 30 and 60 min at various pH values between 4.0 and 9.0 and measuring the residual activity at 30°C and pH 7.0 under standard experimental conditions. As is shown in Figure 5B, the enzyme was the most stable at pH 7.0. An increase or decrease in pH above or below the stability range leads to decreased enzyme activity. From Figure 5B, it is also seen that more than 65% of the residual activity was recovered at pH values of 6.0-9.0. However, only 26% and 56% of its residual activity was maintained at pH 4.0 and 5.0, respectively. This means the enzyme is more stable in neutral and alkaline pH rather than acidic pH.

Table 4 Comparison of different plant PPOs in terms of pH and temperature optima
Çizelge 4. pH ve sıcaklık optimumları açısından farklı bitki PPO'larının karşılaştırılması

Source	Temperature	pH	Reference
Snake fruit	30℃	6.5	Zaini et al., 2013
Chestnut kernel	40℃	7.0	Gong et al., 2015
Honeydew peach	40℃	6.5-7.0	Liu et al., 2015
Buriti palm	35℃	7.0	de Oliveira Carvalho and Orlanda, 2017
Blueberry	35℃	6.1-6.3	Siddiq and Dolan, 2017
Plums	25℃	6.0	Ioniță et al., 2017
Apricot	45℃	4.5	Derardja et al., 2017
Rosemary	30℃	7.0	Yuzugullu Karakus et al., 2020
Fennel	30℃	6.0	Yuzugullu Karakus et al., 2021
Mango	30℃	7.0	Wang et al., 2007
Banana	30℃	7.0	Ünal et al.,2007
Peppermint	30℃	7.0	Kavrayan and Aydemir, 2001
Morning glory	30℃	7.0	Current study

According to literature studies, crude PPO extracted from peppermint (Kavrayan and Aydemir, 2001), pawpaw (Bello et al., 2011), grape (Kaya and Bağci, 2021), artichoke (Aydemir et al., 2003), and rosemary (Yuzugullu Karakus et al., 2020) showed that the PPO enzyme is more stable in the pH ranges of 6.0-7.0, 6.0-8.0, 7.0, 6.0-8.0, 6.0-9.0, respectively. These observations are similar to the findings of I. purpurea plant, which shows that the enzyme is stable between pH 6.0 and 9.0. However, it was observed that PPO obtained from bush mango (*Irvingia gabonnensis*) had more activity in the pH stability range of 3.5-5.5 (Bello et al., 2011). This is presumably due to the existence of isoenzymes. Therefore, enzyme pH stability may vary depending on the material.

Effect of Reaction Temperature on PPO Activity and Stability

The reaction temperature is another critical factor that plays an essential role in oxygen solubility in reaction medium and affects reaction kinetics (Panadare and Rathod, 2018). The effect of temperature on PPO activity was tested at temperatures between 20 and 70°C. Figure 6A exhibited the optimum temperature for *I. purpurea* PPO enzyme was 30°C when catechol was used as substrate. Similarly, PPO enzymes from snake fruit, rosemary, fennel, mango, banana, and peppermint were optimum at 30°C using catechol (Table 4). On the other hand, the optimum temperature for some plant PPOs can change from 25°C to 65°C depending on the enzyme source (Table 4). In this study, 93% and 87% of initial PPO activities were recovered at 50°C and 60°C, respectively, while the enzyme maintained 59% of its activity at 70°C (Figure 6A).

The thermal stability of the TPP-partitioned PPO was tested by incubating the enzyme without substrate at different temperatures for 30 and 60 min. The results are given in Figure 6B, from which it can be seen that the PPO enzyme is stable between 20 and 40℃ and maintained 40% of its residual activity after incubation at 50°C for 60 min. On the other hand, the enzyme was inhibited entirely after incubation for 60 min at 60°C. Likewise, cotton PPO was reported to be inhibited after incubation for only 10 min at 60°C (Kouakou et al., 2009).

CONCLUSIONS

In this study, the leaves of in vitro grown *I. purpurea* plant, which is used for ornamental and medicinal purposes, were screened for their PPO activities. Crude extract samples were obtained under optimized conditions where the extraction medium was prepared at pH 7.0 in the presence of 25 mg ml^{-1} PVPP. Then, using the TPP system, the PPO enzyme was purified 10.5-fold, with 57% recovery in a single step. Optimum conditions for TPP included adding an equal volume of t-butanol to the crude extract saturated with 30% (w/v) $(NH_4)_2SO_4$ at pH 7.5. Activity staining results indicated that the enzyme exhibited laccase, peroxidase, and catechol oxidase activities, supporting that the PPO enzyme from I.

purpurea is highly functional. Biochemical characterization studies revealed that the effect of temperature and pH on enzyme activity resembles other PPO enzymes obtained from different plant sources. On the other hand, its stability in an alkaline pH environment gives the enzyme advantage for industrial use over other PPO enzymes.

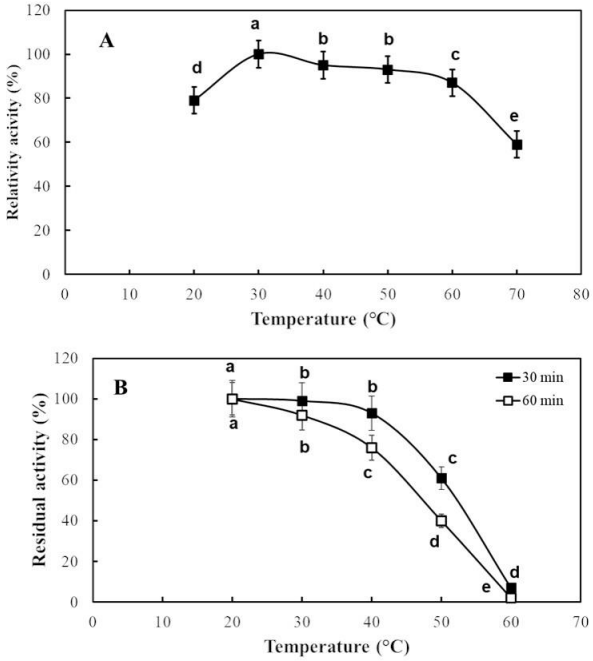

Figure 6. The changes in *I. purpurea* PPO activity (A) and stability (B) due to the reaction temperature. (A) A range of reaction temperatures between 20 and 70 ℃ at pH 7.0 were applied to test percentage relative activity changes. (B) Multiple temperatures ranging from 20 to 70˚C were applied on PPO to test percent residual activity changes. The values are presented as means ± SD.

Şekil 6. Reaksiyon sıcaklığına bağlı olarak I. purpurea PPO aktivitesi (A) ve stabilitesinde (B) gözlenen değişiklikler. Yüzde relatif aktivite değerindeki değişmleri test etmek için pH 7.0'da 20 ila 70 ℃ arasında bir dizi reaksiyon sıcaklığı uygulandı. (B) Yüzde bağıl aktivite değerindeki değişikliklerini test etmek için PPO'ya 20 ila 70°C arasında değişen çoklu sıcaklıklar uygulandı. Değerler, ortalama ± SD olarak sunuldu.

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Author's Contributions

BM prepared the crude extract from in vitro cultured *I. purpurea* leaves, purified the PPO enzyme, and performed biochemical characterization experiments. EK carried out statistical analysis. AA & YYK created the project. YYK guided the work and wrote the paper.

Statement of Conflict of Interest

Authors have declared no conflict of interest.

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Effects of *Pistacia terebinthus* L. Subsp. *palaestina* and *Rhus coriaria* L. Plants on Some Biochemical Parameters of Brain Tissue of Sprague-Dawley Rats in Experimental Breast Cancer Model

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ABSTRACT

In this study, the therapeutic effects of *Pistacia terebinthus* L. subsp. *Palaestina* (terebinth) and *Rhus coriaria* L (sumac) plants on DMBA-induced breast cancer in 66 female Sprague-Dawley rats were investigated through biochemical analysis. The rats were divided into 6 groups as Control, DMBA (7.12-Dimethylbenzanthracene), PT (terebinth), RC (sumac), PT+DMBA and RC+DMBA. DMBA was administered to 8-week-old rats via gavage, a single dose of 80 mg/kg according to body weight. Aqueous extracts of terebinth and sumac were given orally to rats in antioxidant groups 3 days a week.

In biochemical studies, changes in activities of the antioxidant enzymes CAT (catalase), GST (glutathione transferase), and SOD (superoxide dismutase) in brain tissues as well as total protein, MDA (malondialdehyde), GSH (glutathione), fatty acid, and vitamin levels were determined. Total protein levels generally reduced in the DMBA group compared to the control group ($p<0.05$)while the levels of the MDA in the DMBA brain tissue groups elevated compared to the control group and reduced in antioxidant groups ($p<0.01$; $p<0.001$). Cholesterol levels and lipophilic vitamins were determined by HPLC. Their grades were different in the DMBA and antioxidant groups. Fatty acids were analyzed by GC. As a result of analysis, fatty acids such as palmitic, palmitoleic, stearic, oleic, linoleic, arachidonic, and docosahexaenoic were high in the tissues examined ($p<0.01$; $p<0.001$). The fatty acid levels were also found to vary in the DMBA and antioxidant tissue groups. These data suggest that *Pistacia terebinthus* and *Rhus coriaria* plants can be used against breast cancer and to reduce its negative outcomes, and studies should be continued for their safe medical application.

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Sprague-Dawley Sıçanlarda Deneysel Meme Kanseri Modelinde *Pistacia terebinthus* L. Subsp *palaestina* ve *Rhus coriaria* L. Bitkilerinin Beyin Dokusunun Bazı Biyokimyasal Parametreleri Üzerindeki Etkileri

ÖZET

Bu çalışmada, dişi 66 Sprague-Dawley ratında DMBA ile indüklenen meme kanserine karşı *Pistacia terebinthus* L. subsp *Palaestina* (terebinth) ve *Rhus coriaria* L. (sumak)'nin iyileştirici etkileri biyokimyasal olarak incelendi. Sıçanlar, Kontrol, DMBA (7.12-Dimetilbenzantrasene), PT (menengiç), RC (sumak), PT+DMBA ve RC+DMBA olmak üzere 6 gruba ayrıldı. DMBA, vücut ağırlıklarına göre, tek doz 80 mg/kg olacak şekilde, gavaj yoluyla, 8 haftalık dişi Sprague-Dawley sıçanlara uygulandı. Antioksidan gruplarındaki ratlara oral yolla menengiç ve sumak'ın sulu ekstraktları verildi. Biyokimyasal çalışmalarda beyin dokusunda antioksidan enzimler olan CAT (katalaz), GST (glutatyon transferaz) ve SOD (süperoksit dismutaz) aktivitelerindeki değişiklikler, total protein, MDA,

Biochemistry

Araştırma Makalesi

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Anahtar Kelimeler
Beyin dokusu
Biyokimyasal parametreler
Meme kanseri
Pistacia terebinthus
Rhus coriaria

(malondialdehit) GSH (glutatyon), yağ asidi ve vitamin düzeyleri belirlendi. Toplam protein seviyelerinin genellikle DMBA grubunda kontrole göre azaldığı gözlendi ($p<0.05$). DMBA beyin dokusu gruplarındaki MDA seviyeleri kontrol grubuna göre arttığı; antioksidan gruplarında azaldığı saptandı ($p<0.01$; $p<0.001$). Kolesterol seviyeleri ve lipofilik vitaminler HPLC ile belirlendi. DMBA ve antioksidan gruplarında bunların derecelerinin farklı olduğu görüldü. Yağ asitleri GC ile analiz edildi. Analiz sonucunda dokularda palmitik, palmitoleik, stearik, oleik, linoleik, araşidonik, dokosaheksaenoik gibi yağ asitlerinin yüksek olduğu bulundu ($p<0.01$; $p<0.001$). Bu yağ asidi seviyeleri DMBA ve antioksidan doku gruplarında değişiklikler gösterdi. Bu veriler, *Pistacia terebinthus* ve *Rhus coriaria* bitkilerinin meme kanseri ve olumsuzluklarına karşı bir uygulama olarak kullanılabileceği, güvenli tıbbi uygulaması için çalışmaların sürdürülmesi önerilir.

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INTRODUCTION

International Agency for Research on Cancer reports that breast cancer is the most common cancer diagnosed among women and this cancer type is one of leading causes of death in them (Globocan, 2018; Sheokand et al., 2019).

The mortality rate of female breast cancer is 11.6% all over the world (Globocan, 2018). In the majority of countries (154 countries), breast cancer is the most common cancer in women in terms of new cases. The mortality profile among women was more heterogeneous, and breast cancer is the leading cause of cancer deaths in 103 countries (Globocan, 2018).

Some chemotherapy medications (paclitaxel, cyclophosphamide, carboplatin, and cisplatin) have also been commonly used in breast cancer treatments (Guerrero et al., 2010). Breast cancer cells tend to resist chemotherapeutic agents with different signal responses (Mundhe et al., 2015).

Epidemiological studies on diet and cancer have led to investigate anticarcinogenic agent-like nutraceuticals (Sheokand et al., 2019). Most cancer treatments include surgery, radiotherapy, chemotherapy, and immunotherapy. New chemotherapeutic agents and molecular-targeted drugs contribute to cancer treatment, but their toxicity and drug resistance result in the failure of chemotherapy. Therefore, researchers strive to discover few toxic and efficient bio-components for cancer therapy. In this regard, some herbs have played a leading role as an alternative in the discovery of new cancer preventative molecules (İçen et al., 2015).

Among medicinal plants, *Pistacia terebinthus* L. and *Rhus coriaria* L. species belong to the Anacardiaceae family, which is used in alternative medicine. Extracts obtained from *Rhus coriaria* are used as pharmaceuticals (Verzele et al., 1985). It is reported that *R. coriaria* has anti-cancer activity and is a promising alternative treatment candidate (El Hasasna et al., 2016).

The effects of *Pistacia terebinthus* subsp. palaestina (terebinth), that it has the high antioxidant capacity and antioxidant properties, and effects of *Rhus coriaria* (sumac), that it has a chemotherapeutic treatment potential with its strong anti-breast cancer activity on biochemical parameters in brain tissue in DMBA-induced breast cancer model in rats were aimed in this work.

MATERIALS and METHODS

Animals and experimental protocols used in the study were approved by the Local Animal Experiments Ethics Committees of Firat University (Elazig, Turkey). Animal maintenance and experimental protocols were carried out in accordance with the NIH Guide for the Care and Use of Laboratory Animals (NIH publication no. 12.10.2016/181). Sixty-six healthy adult female Sprague-Dawley rats, aged 8 weeks were obtained from Firat University Experimental Research Center (Elazig, Turkey). The animals were kept in the polycarbonate cages with a 12-h/12-h day/night cycle, at temperature of 22±3°C, with humidity of 45% to 65%. Throughout the experiment, the animals were regularly fed with a commercial diet (Elazig Food Corporation, Elazig, Turkey) *ad libitum*.

One of the groups was the control group. The other

groups were DMBA group (n=15), *Pistacia terebinthus* group (PT) (n=7), *Rhus coriaria* group (RC) (n=7), DMBA+ *Pistacia terebinthus* group (DMBA+PT) (n=15), DMBA+ *Rhus coriaria* group (DMBA+RC) (n=15). In the DMBA group 80 mg kg^{-1} 7.12-dimethylbenz(α)anthracene was administered as a single dose according to Mundhe et al., (2015).

Pistacia terebinthus and *Rhus coriaria* extracts of 20 mg kg^{-1} were added to 500 mL drinking water of the rats once per week (Chakraborty et al., 2009; Saglam et al., 2014). These treatments continued for 16 weeks and then each subject was decapitated with ether. Their brain tissue samples were dissected and stored at -85°C until biochemical analyses.

Preparation of homogenates

Tissue samples were homogenized in Tris-HCl buffer (pH 7.4) and centrifuged at 9050xg at 4°C for 15 min. Supernatants were gathered, processed, and stored at -70°C until they were used to identify MDA, glutathione (GSH), antioxidant enzymes (CAT, SOD and GST), and total protein. The pellets were used for ADEK vitamins, cholesterol, and fatty acid analysis.

Determination of MDA-TBA level

Lipid peroxidation was calculated as specified by Ohkawa et al., (1972).

Determination of GSH level in tissue samples

Reduced glutathione (GSH) was measured according to Ellman's (1959).

Lipid extraction

Hexane-isopropanol (3:2 v v^{-1}) was used for lipid extractions of tissue samples according to Hara and Radin (1978). Fatty acids in the lipid extracts were converted into methyl esters including 2% sulphuric acid (v v^{-1}) in methanol (Christie, 1999). Analysis of fatty acid methyl ester was performed in a Shimadzu GC-17A instrument gas chromatograph equipped with a flame ionization detector (FID) and a 25 m, 0.25 mm i.d.permabond fused-silica capillary column (Macherey-Nagel, Germany). The oven temperature was set between 145-215°C, 4°C/min. Temperatures Injector and FID were 240 and 280°C, respectively.

The results were calculated as mg/g tissue.

Saponification and extraction

Alpha-tocopherol and cholesterol were obtained from the lipid extracts according to Sanchez-Machado et al., (2004). They were identified by HPLC device.

Total protein assay

Total protein of the brain tissue was measured according to Lowry et al., (1951). For this purpose, BSA (Bovin serum albumin) was used as standard. The absorbance was measured at 750nm as spectrophotometric.

Antioxidant Enzymes Analysis

Superoxide dismutase (SOD) (EC 1.15.1.1) activity was performed in terms of its capacity to inhibit the oxygen-dependent oxidation of adrenalin (epinephrine) to adrenochrome by xanthine oxidase plus xanthine (Panchenko et al., 1975).

Glutathione S-transferase (GST) (EC 2.5.1.18) activity was determined spectrophotometrically at 340 nm (Bell et al., 1985). As a result of decomposition of hydrogen peroxide (H_2O_2) with catalase, the decrease in absorbance at 240 nm was utilized to calculate catalase activity (Aebi, 1984).

Statistical analysis

One-way analysis of variance (ANOVA) and Post Hoc Tukey-HSD test were used to determine differences between the groups. Results are presented as mean ±S.E.M. Values were considered statistically significant at the level of $p<0.05$. The SPSS/PC program (Version 15.0; SPSS, Chicago, IL) was used for the statistical analysis.

RESULTS and DISCUSSION

Protein Values

Table 1 shows protein values of the brain tissue. When compared with the control group, a significant decrease ($p<0.05$) was observed in the DMBA group, but the changes in other groups were not statistically significant ($p>0.05$).

Table 1. Protein content of brain tissue (mg g^{-1})
Çizelge 1. Beyin dokusunun protein içeriği (mg g^{-1})

GROUPS	PROTEIN (mg g^{-1})
C	30.97±0.76
DMBA	$27.88±0.50^{b}$
PT	$31.11±1.13^{a}$
PT+DMBA	$29.95±0.55^{a}$
RC	$30.01±0.59^{a}$
RC+DMBA	$29.29±0.37^{a}$

d: $p<0.001$; c: $p<0.01$; b: $p<0.05$; a: $p>0.05$

The findings of the present study indicated that total protein levels of brain tissue significantly decreased in the DMBA group compared to the control group ($p<0.05$). Also, the differences in the other groups were not statistically significant ($p>0.05$). In another study, DMBA administration decreased total protein, albumin, and globulin levels while organoselenium compounds significantly increased total protein and albumin levels (Özdemir et al., 2007). In a study, the effects of cat's claw (*Uncaria tomentosa*), rosemary (*Salvia rosmarinus*), and hops (*Humulus lupulus*)

plants against hepatic toxicity induced by 7.12-DMBA were determined (El Kholy et al., 2013). As a result of the DMBA applications, serum and liver total proteins, total albumin, and globulin amounts significantly decreased. There was a significant decrease in serum total proteins, total albumin, globulin and liver total protein in the DMBA group. On the other hand, in addition to serum albumin and globulin, a remarkable improvement was observed in serum and hepatic total protein with the supplementation of these plants to rats treated with DMBA (El Kholy et al., 2013). In this study, total protein decreased with the application of DMBA. When DMBA and plant extracts were applied together, it was understood that the total protein content increased.

MDA Values

Table 2 shows MDA values of the brain tissue. While a significant decrease was found in MDA values of RC, PT+DMBA and RC+DMBA groups ($p<0.01$; $p<0.001$), there was a significant increase in the MDA value of DMBA group ($p<0.05$). However, the difference in the PT group was insignificant ($p>0.05$).

Table 2. MDA contents of the brain tissue (nmol gr^{-1})
Çizelge 2. Beyin dokusunun MDA içeriği (nmol gr^{-1})

GROUPS	MDA (nmol gr^{-1})
C	170.70±12.74
DMBA	181.13±6.66^{b}
PT	174.02±17.42^{a}
PT+DMBA	140.01±8.08^{c}
RC	**110.79±9.37^{d}**
RC+DMBA	132.31±7.98^{c}

d: $p<0.001$; c: $p<0.01$; b: $p<0.05$; a: $p>0.05$

When the MDA level of the brain tissue was examined in the present study, an important increase was observed in the DMBA group when compared to the control group ($p<0.05$). There were significant decreases in some antioxidant groups ($p<0.01$; $p<0.001$). The amount of malondialdehyde detected in the circulation is higher in advanced stages of breast cancer than the value reported in its early stages (Zarrini et al., 2016). It was also stated that the amount of the MDA determined in breast cancer patients was higher than the value of the control group patients (Coughlin, 2018). In the present study,, the increase in the amount of the MDA in the DMBA groups was associated with oxidative stress. Oxidative stress plays a role in the development and metastasis of breast cancer (Jezierska-Drutel et al., 2013). It was also determined that the MDA level decreased in the antioxidant groups. *P. terebinthus* are effective in the fight against oxidative stress.

GSH Values

Table 3 shows GSH values of the brain tissue. When examining GSH values based on the control group, it was found that there was a significant decrease in the DMBA, PT, PT+DMBA and RC+DMBA groups ($p<0.001$; $p<0.01$), but no statistically significant difference in the RC group ($p>0.05$).

Table 3. GSH values of the brain tissue (µg gr^{-1})
Çizelge 3. Beyin dokusunun GSH değerleri (µg gr^{-1})

GROUPS	GSH (µg gr^{-1})
C	121.91±6.69
DMBA	96.15±1.77^{c}
PT	106.64±7.24^{b}
PT+DMBA	99.82±3.36^{c}
RC	126.46±13.68^{a}
RC+DMBA	**92.13±4.45^{d}**

d: $p<0.001$; c: $p<0.01$; b: $p<0.05$; a: $p>0.05$

Glutathione level in brain tissue significantly decreased in the DMBA group ($p<0.01$). Likewise, a significant decrease ($p<0.001$) was detected in the RC+DMBA group. While GSH deficiency or a decrease in GSH/GSSG ratio causes an increased susceptibility to oxidative stress in advanced stage cancer. High GSH levels in most cancer cells support the antioxidant capacity and thus the defense mechanism against oxidative stress (Traverso et al., 2013). The fact that the amount of glutathione in the brain tissue in the DMBA groups was less than the amount of the control group is evidence of oxidative stress in the cancer groups.

Antioxidant Enzymes Activities

Catalase, glutathione S transferase and superoxide dismutase enzyme levels of the brain tissue were examined (Table 4). When the catalase enzyme was examined in the groups, the analysis revealed that the amount of enzyme decreased in all groups and this decrease was more significant in the RC+DMBA group ($p<0.001$). When the GST enzyme of the groups was compared with the value of the control group, it was observed that while the DMBA group showed a significant decrease ($p<0.001$), no statistical differences were found in the other treatment groups ($p>0.05$).

SOD enzyme activities were calculated as % inhibition and unit. There was a significant decrease in PT+DMBA and RC+DMBA groups ($p<0.01$) and a significant increase in the PT group in terms of the SOD level ($p<0.05$); whereas, there was no statistical difference in the RC group ($p>0.05$).

Antioxidant enzymes have important tasks against the negative effects of free radicals in cells. GST, one of these enzymes, is effective in the detoxification of carcinogenic and reactive oxygen species (Shokrzadeh

et al., 2019). It has been reported that the GSTP1 protein level is very low in the human breast cancer cell line MCF-7 (Dong et al., 2019). Similarly, in the study, it was determined that the GST activity in the brain tissue was lower in the DMBA groups than the control group and this finding is compatible with the literature.

Table 4. CAT, GST and SOD activities of brain tissue
Çizelge 4. Beyin dokusunun KAT, GST ve SOD aktiviteleri

GROUPS	Catalase (μg^{-1} ml^{-1}dk)	Glutathione Transferase (μg^{-1} ml^{-1}dk)	Superoxide dismutase (% İnhibition)	Superoxide dismutase (Unit)
C	202.79±17.20	14.72±1.21	32.65±2.41	16.72±1.26
DMBA	156.77±4.85^{c}	**8.45±0.43^{d}**	28.60±0.87^{b}	14.91±0.56^{b}
PT	152.32±20.52^{c}	13.82±1.77^{a}	36.58±3.07^{b}	19.72±1.95^{b}
PT+DMBA	165.90±5.93^{b}	14.07±0.93^{a}	25.76±1.15^{c}	13.23±0.55^{c}
RC	166.18±10.90^{b}	11.91±1.23^{b}	30.46±1.38^{a}	15.68±0.72^{a}
RC+DMBA	**152.08±6.53^{d}**	13.36±0.77^{a}	25.74±0.84^{c}	13.34±0.35^{c}

d: p<0.001; c: p<0.01; b: p<0.05; a: p>0.05

Another enzyme, CAT, acts as a protector against damage that occurs under oxidative stress conditions. Catalase frequently decreases in tumor tissues compared to normal tissues. Available data indicate that catalase activity severely reduces in human breast carcinoma cell line MCF-7 cells compared to normal healthy ones (Glorieux et al., 2018). In the present study, it was concluded that the level of catalase in the brain tissues was lower in the DMBA groups than the control group. The results of the present study are consistent with the literature.

In a study investigating the role of superoxide dismutase (SOD), an important member of enzymatic antioxidants in breast cancer, it was found that metformin significantly reduced the ROS levels and upregulated the SOD isoforms (Sharma and Kumar, 2018). The results of the present study indicated that the SOD level in the brain tissue was lower in the DMBA groups when compared to the control group. In the antioxidant groups, higher levels of the SOD enzyme activity were determined.

Cholesterol levels and ADEK vitamins

Table 5 shows cholesterol levels and vitamins A, D, E, K in brain tissue. When the groups were examined in terms of these variables, it was detected that while a significant increase was found in the levels of D3, α-tocopherol, and ß – Sterol (p<0.01; p<0.001), a significant decrease was found in the levels of δ-tocopherol, ergosterol, and stigmasterol (p<0.05); however, no statistical difference was seen in the level of D2 (p>0.05).

The PT group showed a significant increase in terms of α-tocopherol, D2, cholesterol, ergosterol, ß-sterol levels (p<0.01; p<0.001) and a significant decrease in stigmasterol level (p<0.05).

The PT+DMBA group showed a significant increase in terms of α-tocopherol, K2, ß-Sterol levels and a significant decrease in amounts of K1, cholesterol, δ-tocopherol, and stigmasterol (p<0.001; p<0.01).

A significant decrease was found in the RC group in terms of K1, cholesterol, and δ-tocopherol levels (p<0.001); on the other hand, the same group had a significant increase in stigmasterol, ß-Sterol, and retinol levels; besides, no statistical difference was found in the changes of K2, D2, D3, α-tocopherol, and ergosterol levels (p>0.05).

While there was a significant increase in D3, α-tocopherol levels of the RC+DMBA group, a significant decrease was detected in its cholesterol and δ-tocopherol amounts (p<0.001; p<0.01).

Some diseases are related to vitamin levels. A study reported that there was a relationship between breast cancer and vitamin D concentrations in both animal models and cell lines (De La Puente-Yagüe et al., 2018). In addition, high amounts of 25-hydroxyvitamin D cause a significant decrease in the postmenopausal incidence of breast cancer (Abbas et al., 2009). And lipophilic vitamins are important for many physiological events especially in immunity.

Vitamin D induces apoptosis, stimulates cell differentiation, and provides inhibition of angiogenesis, invasion and metastasis with its anti-inflammatory and antiproliferative properties (De La Puente-Yague et al., 2018). There is a correlation between vitamin D deficiency and different types of diseases including oncological diseases such as breast, colorectal, and prostate cancer (Garland et al., 2006).

Fatty Acids Values

The PT group showed a significant decrease in 15:1, 16:1, 18:0, 18:1, 24:0, 24:1 levels (p<0.01; p<0.001) and had a significant increase in 14:0, 22:0 levels (p<0.05) (Table 6).

There was a significant decrease in the PT+DMBA group in terms of 15:1, 16:1, 17:1, 18:0, 18:1, 18:2 levels (p<0.001; p<0.01; p<0.05); whereas, a significant increase was found in 18:1, 22:0, 24:1

levels of this group. Moreover, no statistical significance was found in the changes of 14:0, 16:0, 17:0 levels ($p>0.05$).

A significant increase was found in the RC group in terms of 14:0, 18:2, 22:0 levels; on the other hand, there was a significant decrease in this group's 16:1, 18:1 and 24:1 fatty acid amounts ($p<0.001$; $p<0.05$).

While there was a significant decrease in 15:1, 16:1, 18:0, 18:1, 18:2, 24:1 levels in the RC+DMBA group ($p<0.01$); a significant increase was observed in 16:0, 18:1 and 22:0 levels.

Table 5. Cholesterol and A, D, E, K vitamins values of the brain tissue (μg g^{-1})
Çizelge 5. Beyin dokusunun kolesterol ve A, D, E, K vitemin değerleri (μg g^{-1})

Vitamins/Sterols	CONTROL	DMBA	PT	PT+DMBA	RC	RC+DMBA
K-2	3.72±0.25	3.95±0.36^{a}	3.95±0.39^{a}	5.11±0.46^{b}	4.19±0.38^{a}	4.74±0.29^{b}
δ-Tocopherol	5.71±0.99	4.03±0.62^{b}	4.63±0.44^{a}	3.79±0.14^{c}	3.24±0.43^{c}	3.45±0.27^{c}
D-2	1.02±0.12	1.40±0.19^{a}	1.98±0.49^{c}	1.29±0.12^{a}	0.96±0.21^{a}	0.94±0.14^{a}
D-3	0.36±0.05	0.94±0.11^{c}	0.68±0.32^{a}	0.57±0.07^{a}	0.47±0.08^{a}	**1.27±0.16^{d}**
α-Tocopherol	10.87±1.23	22.95±3.69^{c}	**33.08±3.98^{d}**	**27.47±4.30^{d}**	10.14±1.14^{a}	20.16±1.73^{c}
Ergosterol	20.25±5.03	15.93±1.78^{b}	25.59±1.76^{b}	21.85±1.67^{a}	17.16±1.73^{a}	21.81±1.51^{a}
K-1	2.69±0.23	**1.38±0.25^{d}**	2.72±0.18^{a}	2.18±0.17^{b}	**1.27±0.31^{d}**	2.49±0.19^{a}
Cholesterol	1038.55±2.77	**847.31±0.60^{d}**	**1121.57±3.61^{d}**	**940.22±0.57^{d}**	**984.90±2.46^{d}**	**940.30±0.73^{d}**
Stigmasterol	6.71±3.21	1.95±0.51^{b}	1.81±0.33^{b}	1.02±0.32^{c}	14.95±8.21^{c}	5.33±4.46^{a}
β - Sterol	0.15±0.11	**8.95±3.62^{d}**	2.37±0.73^{b}	1.24±0.29^{b}	5.94±1.27^{c}	1.18±0.25^{b}
Retinol	0.03±0.002	0.03±0.001^{a}	0.02±0.005^{a}	0.03±0.007^{a}	0.06±0.015^{c}	0.03±0.001^{a}

d: $p<0.001$; c: $p<0.01$; b: $p<0.05$; a: $p>0.05$

Table 6. Fatty acids values of brain tissue (%)
Çizelge 6. Beyin dokusunun yağ asidi değerleri (%)

Fatty Acids	CONTROL	DMBA	PT	PT+DMBA	RC	RC+DMBA
14:0	0.11±0.006	0.12±0.005^{a}	0.14±0.01^{b}	0.11±0.008^{a}	0.13±0.01^{b}	0.10±0.005^{a}
15:1	2.27±0.05	1.79±0.13^{c}	1.94±0.15^{b}	**1.47±0.10^{d}**	2.02±0.17^{a}	**1.47±0.07^{d}**
16:0	17.62±0.26	17.15±0.11^{b}	17.97±0.15^{a}	17.64±0.17^{a}	17.94±0.16^{a}	18.04±0.13^{b}
16:1. n-7	1.03±0.05	**0.81±0.02^{d}**	0.93±0.05^{b}	**0.76±0.04^{d}**	0.88±0.03^{b}	**0.73±0.03^{d}**
17:0	0.32±0.02	0.29±0.009^{b}	0.32±0.01^{a}	0.34±0.02^{a}	0.33±0.002^{a}	0.32±0.01^{a}
18:0	16.41±0.29	9.13±2.29^{c}	10.85±3.27^{c}	11.94±2.41^{c}	16.91±0.37^{a}	14.63±1.63^{b}
18:1. n-7	15.25±4.82	12.97±3.20^{b}	9.12±3.76^{c}	19.59±1.60^{c}	**3.17±0.12^{d}**	21.42±0.13^{c}
18:1. n-9	20.31±0.34	17.82±0.63^{b}	16.41±0.92^{b}	16.97±0.44^{b}	20.69±0.14^{a}	17.30±1.21^{b}
18:2. n-6	0.90±0.04	0.75±0.02^{c}	0.90±0.06^{a}	0.87±0.04^{b}	**1.18±0.29^{d}**	0.79±0.02^{c}
22:0	0.30±0.03	0.33±0.02^{a}	0.42±0.07^{b}	0.48±0.01^{c}	**0.57±0.05^{d}**	0.40±0.02^{b}
24:0	1.36±0.19	**0.45±0.07^{d}**	**0.43±0.11^{d}**	**0.44±0.02^{d}**	**0.38±0.04^{d}**	0.79±0.22^{c}
24:1	0.68±0.07	0.68±0.04^{a}	0.50±0.04^{d}	0.78±0.19^{c}	**0.48±0.02^{d}**	0.60±0.03^{c}

d: $p<0.001$; c: $p<0.01$; b: $p<0.05$; a: $p>0.05$; myristic acid (14:0); pentadecanoic acid (15:0); pentadecenoic acid (15:1); palmitic acid (16:0); palmitoleic acid (16:1 n-7); heptadecanoic acid (17:0); heptadecenoic acid (17:1); stearic acid (18:0); oleic acid (18:1 n-9); linoleic acid (18:2 n-6); linolenic acid (18:3 n-3); behenic_acid (22:0); nervonic acid (24:1); saturated fatty acids (SFA); monounsaturated fatty acids (MUFA); polyunsaturated fatty acids (PUFA); unsaturated fatty acids (USFA)

Polyunsaturated fatty acids in cholesterol esters, phospholipids, and triglycerides are affected by oxidation caused by free radicals and participate in chain events that cause damage to biomolecules. Brain tissue has a large amount of cholesterol. The present results of the study indicated that the level of cholesterol in the brain tissue of cancerous rats decreased in the groups containing plant extracts than in the control group and plant extracts reversed this situation.

Lipids are substances with a wide structural diversity covering many cytological and pathological metabolisms such as breast cancer.

Most lipids act as secondary messengers, as in tumors. In this way, lipids can be used for pathophysiology, therapy, and diagnosis of diseases. Changes in lipid metabolism can be detected in cancer cells in the early stages of malignancy (Kawashima et al., 2013).

In a study on the development of human breast cancer cells and lipid profile, it was reported that omega-6 polyunsaturated fatty acids (n-6 PUFA) promoted development of breast tumor and metastasis while long-chain n-3 polyunsaturated fatty acids exhibited suppressive effects. In addition, the ratio of n-6 to n-3 fatty acids was seen as an important factor in the control of tumor growth. Dietary intake of n-3 PUFA may alter breast cancer risk factors. The addition of fish oil n-3 PUFAs such as eicosapentaenoic acid (20:5 n-3, EPA) and

docosahexaenoic acid (22:6 n-3, DHA) to culture media or animal diets suppress tumor cell proliferation and increase apoptosis by multiple mechanisms (Ge et al., 2002).

Linoleic and linolenic acids are known as essential fatty acids. The essential fatty acid consists of Δ6 and Δ5 desaturase enzymes. Δ6 desaturation is added to this acid. As a result of the activities of these enzymes, γ-linoleic, eicosatrienoic, arachidonic, docosapentaenoic, and docosahexaenoic acids formed. Researchers reported that linoleic acid and linolenic acid should be taken with diet and arachidonic acid would be synthesized from 18:2 and 18:3 (Rule et al., 1994). Polyunsaturated fatty acids are important structural components by imparting fluidity and selective permeability to membranes (Horrobin 1993).

CONCLUSION

Oxidative stress occurs if antioxidants cannot scavenge free oxygen radicals (Zarrini et al., 2016), resulting in cancer and other chronic diseases. Breast cancer develops as a result of abnormal changes that occur in breast cells (Kumar et al., 2013). Treatment options for breast cancer include surgery, radiotherapy, chemotherapy, and immunotherapy. New chemotherapeutic agents and molecular-targeted drugs contribute to cancer treatment. However, the toxicity of these drugs and the drug resistance prevent chemotherapy from achieving the desired result. Less toxic and more effective bio-compounds have gained importance for treatment. Medicinal plants have come to the fore for the development of new anticancer agents (İçen et al., 2015).

Pistacia terebinthus L. and *Rhus coriaria* L. are species belonging to the Anacardiaceae family (cashew family) and they are used in alternative medicine. Extracts derived from *Rhus coriaria* are used as pharmaceuticals (raw materials of medicines) (Verzele et al., 1985). *R. coriaria* has an anticancer activity and is a promising alternative treatment candidate (El Hasasna et al., 2016). *Pistacia terebinthus* L. fruit extracts are used for anticarcinogenic, antioxidant, antimicrobial, and antimutagenic purposes in alternative medicine (Germano et al., 2002; Tesoriere et al., 2007; Kulisic et al., 2012). This study revealed the effects of *P. terebinthus* and *R. coriaria* on some biochemical parameters of brain tissue of rats with experimentally-induced breast cancer.

The results of the present study showed that the herb suspensions exerted anti-cancer effects and consequently may alleviative brain damage caused by DMBA-induced breast cancer. However, it was observed that they were not sufficiently effective especially on enzymatic activities at molecular level. In the light of the findings of the present study, it is concluded that these plants can be used for follow-up and treatment of cancer patients. *Pistacia terebinthus* and *Rhus coriaria* plants can be used in the fight against oxidative stress in breast cancer. Both plants can give positive results in similar cancer cases.

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The authors declare no conflict of interest. This study complies with research and publishing ethics and rules.

Researchers Contribution Rate Declaration Summary

The authors declared that they contributed equally to the article.

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Characterization of Spatiotemporal Variations in Mert Stream Water Quality by Phytoplankton Community and Biological Indices

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ABSTRACT

In order to determine the water quality of the Mert Stream, algal indicators and some biological indices (TDI, IDG, PTI, Palmer, DAIpo) based on phytoplankton species were used. Phytoplankton samples were performed monthly from six different sites at a depth of 0.5 meter using a one-litre water sampler between July 2011 and June 2012. After phytoplankton samples were placed in 250-mL dark bottles and fixed with Lugol's solution until processed in the laboratory, they were identified and counted in the tubular plankton counting chambers using an inverted microscope according to Utermöhl method. Bacillariophyta members became the dominant division in the phytoplankton with an abundance rate of 83.3% and 71 taxa. *Lindavia glomerata*, *Navicula cryptocephala*, *Cyclotella planctonica*, and *Navicula veneta* were determined as the most dominant species among phytoplanktonic taxa of Mert Stream. Trophic diatom index (TDI) and Generic diatom index (IDG) based on planktonic diatoms were used to determine the trophic status of the Mert Stream. Pollution tolerance index (PTI), Diatom assemblage index (DAIPo) and Palmer index were also included to assess the organic load-based sabrobity level of the Mert Stream. According to the results of mean TDI and IDG (55 and 13, respectively), the trophic status of the Mert Stream is a mesotrophic structure with moderate nutrients and good water transparency. According to the average PTI result (2.7), Mert Stream is in the ß-mesosaprobic class, which corresponds to moderate pollution. Considering DAIpo indice, the saprobity of Mert stream is α-oligosaprobic level and the stream is not exposed to a serious organic-based pollution. However, according to the Palmer index, all stations of the Mert Stream except the 6th station are under the threat of high organic pollution. In present study, it was seen that the best biological index that reflects the station-based trophic structure of the stream is IDG, and the best biological index that reflects the station-based organic pollution of the stream is PTI.

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Mert Irmağı Su Kalitesi'ndeki Mekansal ve Zamansal Değişimlerin Fitoplankton Topluluğu ve Biyolojik İndeksler Yardımıyla Belirlenmesi

ÖZET

Mert Irmağı'nın su kalitesini belirlemek için, algal indikatörler ve fitoplankton temelli bazı biyolojik indeksler (TDI, IDG, PTI, Palmer, DAIpo) kullanılmıştır. Fitoplankton örneklemeleri, Temmuz 2011 ile Haziran 2012 arasında bir litrelik su örnekleyici kullanarak altı farklı noktada 0.5 metre derinlikten aylık olarak gerçekleştirildi. Fitoplankton örnekleri 250 mL'lik koyu renkli kaplara konup laboratuvara taşınıncaya kadar Lugol çözeltisi ile fikse edildikten sonra Utermöhl metoduna göre inverted mikroskoba yerletirilen tüpsü plankton sayım çemberlerinde teşhisleri ve sayımları yapıldı. Bacillariophyta üyeleri % 83.3'lük bolluk oranı ve 71 takson ile fitoplanktonun baskın divizyosu olmuştur. *Lindavia glomerata*,

Çevre Bilimi

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Anahtar Kelimeler
Mert Irmağı
Fitoplankton
Biyolojik indeks
Su Kalitesi

Navicula cryptocephala, *Cyclotella planctonica* ve *Navicula veneta* Mert Irmağı'ndaki fitoplanktonik taksonlar arasında en baskın türler olarak belirlenmiştir. Mert Irmağı'nın trofik durumunu belirlemek için planktonik diyatome temelli Trofik diatom indeksi (TDI) ve Genel diatom indeksi (IDG) kullanıldı. Mert Irmağı'nın organik yük temelli sabrobite seviyesini değerlendirmek için ise Kirlilik tolerans indeksi (PTI), Diatom topluluk indeksi (DAIPo) ve Palmer indeksi dahil edildi. Ortalama TDI ve IDG sonuçlarına göre (sırasıyla 55 ve 13), Mert Irmağı trofik durumu, orta düzey besin ve iyi su şeffaflığına sahip mezotrofik bir yapıdır. Ortalama PTI sonucuna göre (2.7), Mert Irmağı orta düzey kirliliğe karşılık gelen ß-mezosaprobik sınıftadır. DAIpo indeksi dikkate alındığında, Mert Irmağı'nın saprofikliği α-oligosaprobik düzeyde olup ırmakta organik kaynaklı ciddi bir kirlilik söz konusu değildir. Fakat, Palmer indeksine göre Mert Irmağı'nın 6. istasyon haricinde tüm istasyonları yüksek organik kirlilik tehdidi altındadır. Bu çalışmada, ırmağın istasyon bazlı trofik yapısını en iyi yansıtan biyolojik indeksin IDG, istasyon bazlı organik kirliliği en iyi yansıtan biyolojik indeksin ise PTI olduğu görülmüştür.

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INTRODUCTION

The algae co-occur even though each species has a specific niche based on its physiological requirements and the constraints of the environment. These are many detailed descriptions of phytoplankton succession being correlated with changes in environmental parameters particularly temperature, light, nutrients availability and mortality factors such as grazing and parasitism. Because the variation of phytoplankton succession is strongly linked to meteorological and water stratification mixing processes, patterns in temperate ecosystems differ considerably from those of tropical waters (Wetzel, 2001). The dynamics of phytoplankton are a function of some environmental processes that affect species diversity. The abundance of algae of different kinds is rather closely associated with restricted seasonal periodicity, differing of course in widely separated geographical locations (Smith, 1951).

All of the physical, chemical and biological properties of the water constitute a water quality (Anonymous, 1999). The phytoplankton in a freshwater source is an important biological indicator of the water quality. Phytoplankton and algae monitoring is crucial because monitoring based only on physical and chemical analysis can be insufficient at times. Over the last few decades, there has been much interest in the processes influencing the development of phytoplankton communities, primarily in relation to water quality (Martín et al., 2010; Solak 2011; Tokatlı and Dayıoğlu, 2011; Delgado et al., 2012; Atıcı and Udoh, 2016; Temizel et al., 2017; Maraşlıoğlu et al., 2017; Maraşlıoğlu and Soylu, 2018; Tokatlı et al., 2020; Maraşlıoğlu et al., 2020).

Bioindicators have a number of advantages over chemical assessments when it comes to environmental monitoring has some general advantages. The main advantages can be summarised as the following; firstly, lowering the cost of sampling and analysis on a regular basis (Wu et al., 2010), secondly, the equipment is reasonably priced (Kienzl et al., 2003), thirdly, a fairly simple analysis (Zbikowski et al., 2007), fourthly, the likelihood to discover both short-term changes in water quality and long-term changes in the environment, and lastly, sensitivity to a variety of environmental influences (Stein et al., 2007). As a result, biological criteria are increasingly being used in environmental assessment and pollution monitoring around the world (Wu et al., 2010).

The use of algae for biological monitoring of stream water quality has a long history in Europe (e.g. Butcher, 1947; Fjerdingstad, 1964). Trophic Diatom Index (TDI), Generic Diatom Index (IDG), Pollution Tolerance Index (PTI), Palmer Index, and Diatom Assemblage Index are some of the regionally scaled biocenotic indices to organic water pollution (DAIpo). The trophic Diatom Index (TDI) was developed for English streams and rivers (Kelly et al., 1995; revised by Kelly et al., 2001) and has since been used in Australia, Europe, North, and South America, and

Asia (Prygiel and Coste, 1993; Lobo et al., 1995; Kelly and Whitton, 1995; Jüttner et al., 1996; Gómez and Licursi, 2001; Newall and Walsh, 2005), and Turkey (Gürbüz and Kıvrak, 2002; Solak, 2011; Tokatlı, 2013; Ongun-Sevindik and Küçük, 2016; Temizel et al., 2017; Tokatlı et al., 2020; Maraşlıoğlu et al., 2020). Rumeau and Coste (1988) proposed the generic diatom index (IDG), which is likewise based on the generic composition of assemblages. The pollution tolerance index for diatom assemblages (PTI) shows how nutrient concentration affects diatom assemblages and water trophic status (Anonymous, 2002). The PTI is similar to the trophic diatom index (Kelly, 1998) and other diatom indices which use relative abundance and eutrophication-tolerance values assigned to taxa (Lange-Bertalot, 1979; Watanabe et al., 1986). With the equation promoted by Watanabe et al. (1981), the diatom assemblage index to organic water pollution (DAIpo) is determined considering the relative abundances of taxa belonging to saprophilous and saproxenous species. The Palmer index is based upon the existence of algal genera that have allowance to organic pollution in aquatic structures (Palmer, 1969). Algal genera that are sensitive to organic pollution are given a fewer number, while algal genera that are tolerant to organic pollution are given a greater number.

Mert Stream is the most significant lotic ecosystem for the Central Black Sea Region of Turkey and it is well documented that this system is being exposed to partial anthropogenic pressure by means of agricultural and industrial applications conducted around the watersheds (Bektaş, 2016; Maraşlıoğlu et al., 2018; Maraşlıoğlu et al., 2020). The goal of this qualitative study is to use phytoplankton assemblages and biological indices (TDI, IDG, PTI, DAIpo, Palmer) for estimating trophic structure, water quality, and amount of organic pollution in Mert Stream. It is expected that the research will contribute to current knowledge of Turkey's ecology and freshwater algal flora.

MATERIAL and METHODS

Study Site

The Mert Stream flows through the province of Samsun in Turkey's Central Black Sea Region (between 41°09'02"-41°17'04" N and 35°48'04"-36°21'50" E). The stream is bordered on the west by the Kızılırmak River Basin, on the south by the Yeşilırmak River Basin, and on the east by the Abdal Creek Basin. Mert stream is vital to the region since it serves as an irrigation source for several of the settlements along the path. During the summer, the stream's depth drops to less than 50 cm, but it rises to 4-5 meters in the winter (Bakan and Şenel, 2000).

Sampling Strategy

From July 2011 to June 2012, water samples were performed monthly from 0.5-m depth at six sites using a one-litre water sampler. Figure 1 depicts the six sampling stations chosen from the Mert Stream to represent the whole stream as well as the stream's location. The sampling locations were chosen based on the basin's potential for point and non-point pollution loads, primarily from agricultural and light industrial activities. Phytoplankton samples were placed in 250-mL dark bottles and fixed with Lugol's solution (IKI) until processed in the laboratory. After water samples taken from the field for counting delivered to the laboratory, they were placed in 100 mL measuring cylinders for settling and 1-2 more drops of Lugol's solution were dropped on them.

Phytoplankton Analyses

Water samples put into tubular plankton chambers depending on phytoplankton density, after standing overnight by dropping lugol's solution, counting phytoplankton were made by using inverted microscope (Utermöhl, 1958). The average of three countings from each station was utilized in the evaluations. Every colony and threadlike creature was treated as a distinct unit during the counting process. Except for Bacillariophyta, the remaining portion of the water sample was filtered using Whatman GF/A glass fibre filter paper with a pore size of 55 m, and the residue on the filter paper was utilized to identify the algae. Among the planktonic algae that were counted in the counting chambers, some diatom species that could not be underdiagnosed due to the organic matter on frustules were identified on permanent slides which had been prepared according to the method of Della Bella and Mancini (2009).

For the idetification of algal species Hustedt (1985), Round et al. (1990), Hartley et al. (1996), Krammer and Lange-Bertalot (1991a; 1991b; 1999a; 1999b), and John et al. (2002) were used. AlgaeBase web (Anonymous, 2021) and Turkishalgae web (Anonymous, 2022) were used for classification and verification of currently-accepted taxonomic names of algae. The water quality indicators of taxa in phytoplankton were classified in three categories as sensitive (S), tolerant (T) and facultative (H/T) based on the phytoplankton composition metrics in the lakes reported by Phillips et al. (2010) at the wiser project.

Biological Indices

Trophic Diatom Index (Kelly and Whitton, 1995; Kelly et al., 2001) and Generic Diatom Index (based on genera) (Coste and Ayphassorho, 1991) were used to characterize the planktonic diatoms at each site. The organic pollution level in Mert Stream was

determined by Diatom Pollution Tolerance Index (Muscio, 2002), Diatom Assemblage Index to organic water pollution (Watanabe, 1981), and Palmer index (Palmer, 1969). The organic pollution indices (PTI, DAIpo, Palmer) are based on the presence of algal species or genus, which have the organic pollution tolerance in water bodies. Ecological and pollution classification belonging to the TDI, IDG, PTI, Palmer and DAIpo values were given in Table 1.

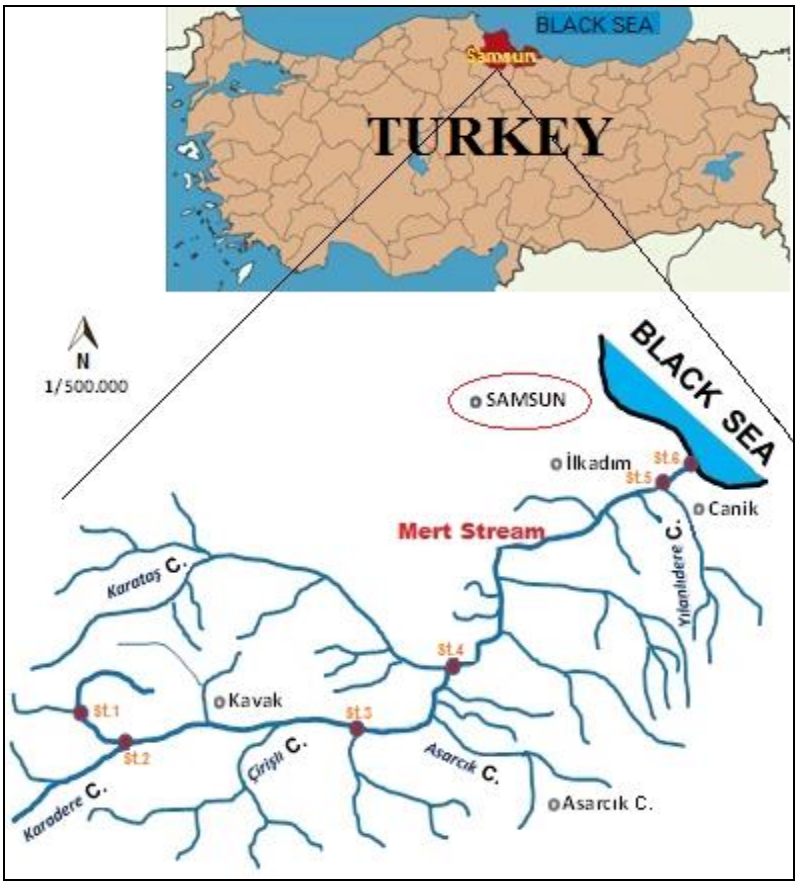

Figure 1. Location and sampling stations of the Mert Stream
Şekil 1. Mert Irmağı'nın konumu ve örnek alma istasyonları

Table 1. Ecological and pollution classification of some biological indices (TDI, IDG, PTI, Palmer and DAIpo)
Çizelge 1. Bazı biyolojik indekslerin (TDI, IDG, PTI, Palmer ve DAIpo) ekolojik ve kirlilik sınıflandırması

Ecological classification				Pollution classification			
TDI	IDG	Ecological class	Trophic status	PTI	Palmer	DAIpo	Saprobic level
< 35	> 17	High	Oligotrophic	> 4	< 5	100-70	ß-oligosaprobic
35 –50	15–17	Good	Oligo-Mesotrophic	4	5-10	70-50	α-oligosaprobic
50 – 60	12 –15	Moderate	Mesotrophic	3	10-15	50-30	ß-mesosaprobic
60 – 75	9–12	Low/ Poor	Eutrophic	2	15-19	30-15	α-mesosaprobic
> 75	< 9	Bad	Hypertrophic	1	≥ 20	15-0	polysaprobic

RESULTS

Algal community structure

A total of 122 taxa belonging to 8 divisions was found in the phytoplankton of Mert Stream throughout the study period. In the phytoplankton, divisio Bacillariophyta dominated in the community being presented by a total of 71 taxa, followed by the divisions Chlorophyta (16 taxa), Euglenozoa (11 taxa), Charophyta (10 taxa), Cyanobacteria (9 taxa), Miozoa (3 taxa), Ochrophyta (1 taxon), and Rhodophyta (1 taxon).

Algal abundance

In the phytoplankton, Bacillariophyta was the most dominant phylum in the stream with a total organism rate of 83%. Cyanobacteria with 7% and Ochrophyta

with 5.3% were secondary important phyla, even though they did not reach a very important percentage in terms of the total organism. *Dinobryon divergens*, non-diatom taxa, from Ochrophyta became one of the dominant organisms in phytoplankton by peaking at the second station in September 2011. Dominant organisms in phytoplankton are all diatoms except one species from Ochrophyta. Dominant and subdominant organisms of phytoplankton are *Lindavia glomerata*, *Navicula cryptocephala*, *Cyclotella planctonica*, *Navicula veneta*, *Dinobryon divergens*, *Ulnaria ulna*, and *Brachysira exilis*. The abundance rates of dominant and subdominant taxa in phytoplankton were shown in Figure 2.

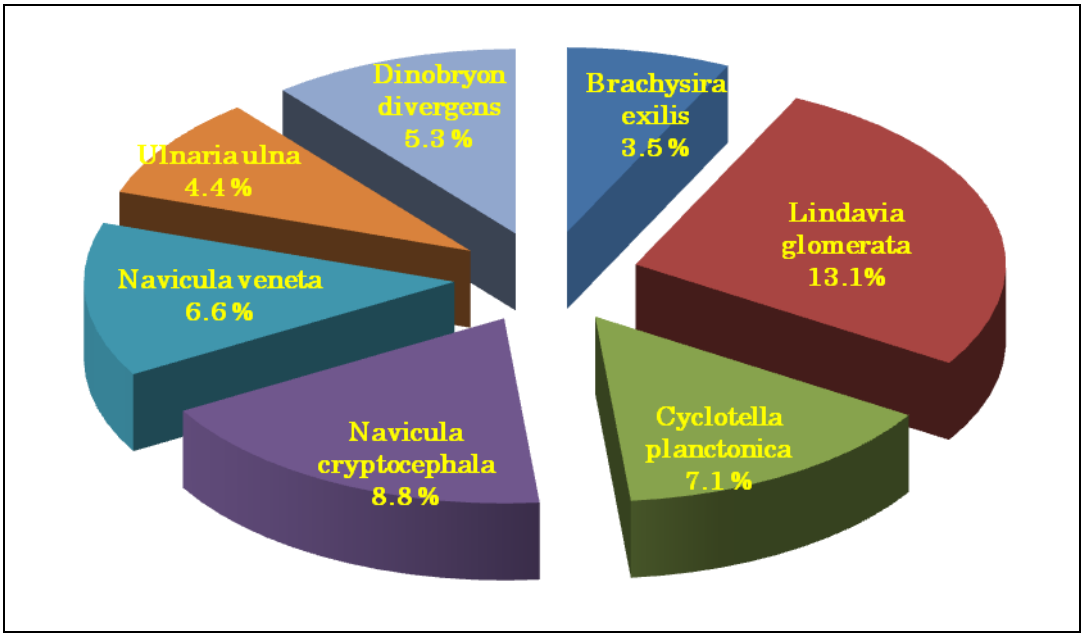

Figure 2. The abundance rates of dominant and subdominant taxa in phytoplankton
Şekil 2. Fitoplanktondaki dominant ve subdominant taksonların bolluk oranları

Spatiotemporal variations in phytoplankton commnity

The highest species diversity in phytoplankton was seen at the 2nd station with 82 taxa. On the other hand, the least species diversity in phytoplankton was seen at the 6th station with 60 taxa. The phytoplankton at 1st and 2nd stations was characterized by similar dynamics with diversity maximum in February with 30 identified species affiliated primarily to Bacillariophyceae and Cyanobacteria. For the entire period, the lowest species diversity was detected in August at both stations. At the 3rd and 5th stations, around 65 taxa were recorded at both stations, distributed as follows: 27 taxa in the June samples (3rd and 5th stations) with the pronounced dominance of Bacillariophyceae. In the samples from 4th station, 71 different taxa were identified for the studied period with twenty-nine species registered in February and thirty-four in September, October, and April. At the 6th station, the phytoplankton community was presented by 31 taxa in April.

The montly variatons in phytoplankton abundance at the sampling stations of Mert Stream were reported in Figure 3. In terms of total organisms, the highest phytoplankton density was found in September and November 2011 in the Mert stream, while the lowest number of organisms was detected in May 2012. While all stations contributed equally to the phytoplankton peak registred in November, the peak in September was caused by the 2nd and 3rd stations. While station-based highest phytoplankton abundance was counted at the 2nd and 3rd stations, the least number of organisms was found at the 1st station. When the seasonal variation of the phytoplankton in the Mert stream was analyzed, it was seen that the season with the highest cell density in the stream was autumn (234.330 cells ml^{-1}). The number of organisms detected in the autumn season constituted 60% of the number of organisms throughout the year.

Water quality and biological metrics

When the water quality status of the taxa is analyzed on the basis of the Wiser report (Phillips et al., 2010), it was seen that the tolerant taxa in phytoplankton had the highest rate with a rate of 40%, even if they were not at a overdominance level. It was observed that facultative taxa were important at the second level with a rate of 34%, while sensitve species corresponded to a rate of 26%.

TDI results ranged from 50-62, with an average of 55. The TDI value of the 1st station separated from the TDI values of the other stations with exceeding 60. The average IDG values were around 13 and only stations 2 and 3 had low scores (around 11). The PTI results of the stations are close to each other, only the PTI value of the 3rd station (2.5) was below that of the other stations. DAIpo index results ranged from 53.0 to 61.8, with an average DAIpo value of 57. Palmer index scores were recorded the lowest value at 6th station (12) and the highest value at the 4th station (32). Index results of sampling stations based on trophy and sabrophy were shown in Table 2.

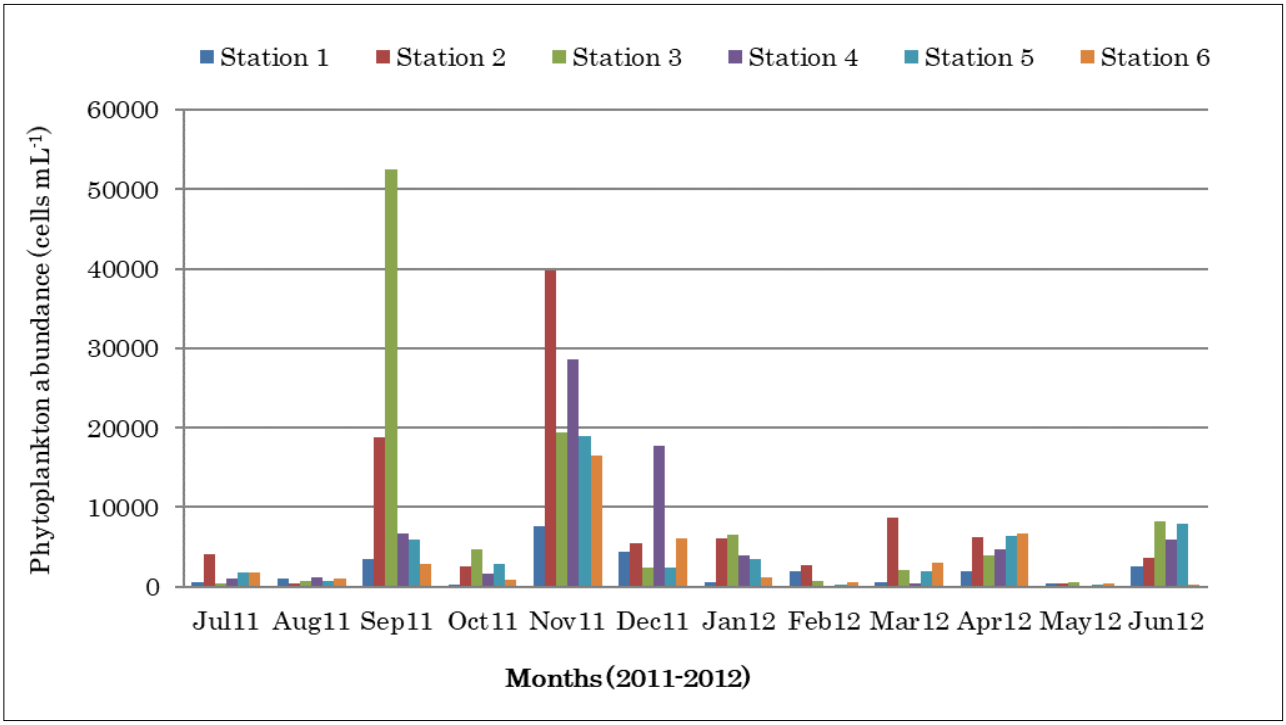

Figure 3. Monthly variations in phytoplankton abundance at the sampling stations of the Mert Stream
Şekil 3. Mert Irmağı örnekleme istasyonlarındaki fitoplankton bolluğunun aylık değişimleri

Table 2. Index results of sampling stations based on trophy and sabrophy (blue: high, green: good, yellow: moderate, orange: poor, red: bad)
Çizelge 2. Örnekleme istasyonlarının trofi ve saprofi temelli indeks sonuçları (mavi: yüksek, yeşil: iyi, sarı: orta, turuncu: zayıf, kırmzı: kötü)

	Trophic status		Saprobic level		
	TDI	IDG	PTI	Palmer	DAIpo
St.1	eutrophy	mesotrophy	ß-mesosaprobic	polysaprobic	α-oligosaprobic
St.2	mesotrophy	meso-eutrophy	ß-mesosaprobic	α-mesosaprobic	α-oligosaprobic
St.3	mesotrophy	meso-eutrophy	α-mesosaprobic	polysaprobic	α-oligosaprobic
St.4	mesotrophy	mesotrophy	ß-mesosaprobic	polysaprobic	α-oligosaprobic
St.5	mesotrophy	mesotrophy	ß-mesosaprobic	polysaprobic	α-oligosaprobic
St.6	mesotrophy	mesotrophy	ß-mesosaprobic	ß-mesosaprobic	α-oligosaprobic

DISCUSSION

In the study, we have identified a hundred and twenty-two taxa in which Bacillariophyta was dominant with a rate of 83%. Most of the taxa collected from Mert Stream have a cosmopolitan distribution. Bacillariophyta were also dominant in other Turkish rivers (Altuner, 1988; Altuner and Gürbüz, 1989; Soylu and Gönülol, 2003). Generally, in lotic systems, there is a dominance of diatoms originating from benthic systems. The author has proven that the diatoms dominate in the community and the phytoplankton fluctuations are highly seasonal dependent. The water and temperature regimes have a significant impact on the development of phytoplankton. The high summer temperatures reduce the intensity of diatoms and the temperature decrease leads to increase of their number respectively.

When we examined the seasonal variation of phytoplankton, it was seen that 60% of the total

organism in all stations of the stream was detected in the autumn months. Considering that 83% of the total organisms detected in phytoplankton are members of Bacillariophyta, it can be said that the increase in organisms in the autumn months is normal. This prevalence of diatoms during the autumn months are generated a higher flow of nutrients and suspended particles into this area than elsewhere, creating changes in both diatom abundance and the community structure between regions (Ben Brahim et al., 2015). In the autumn season, the increase in the number of organisms in September and November was remarkable. It was also detected in Yeşilırmak river that Bacillariophyta members increased in September in autumn, then decreased in October and then increased in numbers again in November (Soylu and Gönülol, 2003). While all stations contributed equally to the increase of the total organism in November, approximately 60% of the increase of organism in September accounted for the 3rd station. This has made the 3rd station where the most species and organisms in phytoplankton are detected. The main reason for this is the poultry facilities operating near the 3rd station. In the study area, after the 3rd station the most remarkable station was 2nd station in terms of total organism. The number of organisms at the 2nd station in November constituted 40% of the total number of organisms in this station and 30% of the total organism in November. It is thought that the agriculture activities with chemical fertilizers in the environment are effective in the increase of the total organism in the 2nd station. According to Lavoie et al. (2008), the integration duration varies depending on the stream's trophic state and nutrient concentration variations. The diatom communities of oligotrophic streams are more sensitive to nutrient changes and are directly affected by nutrient increases, whereas the diatom communities of eutrophic rivers are less susceptible to nutrient fluctuations and substantial changes take longer to be integrated into index values. Although the increase in nutrient originating from agricultural and poultry activities near the 2nd and 3rd stations of the Mert Stream with a meso-eutrophic structure caused an increase in the numbers of organisms of the phytoplankton (diatoms) in these stations, it did not cause a serious variation in the species diversity of the diatoms.

Agreeing with what has already been pointed out by Claps (1996), a reduction in the algal population can be seen after the spring rains. The planktonic algae of the Mert Stream were also affected in the same way by rains in spring. Similiar conditions were also observed in Meram (Yıldız, 1985) Karasu (Altuner and Gürbüz, 1989), and Yeşilırmak (Soylu and Gönülol, 2003) streams. However, spring rains can sometimes affect algal communities of rivers in different ways. In the study area, while the spring rains depleted the number of organisms in the phytoplankton, it caused an increase in the algal abundance of the benthic environment. On the other hand, in the Pampean River of Argentina, the spring rains caused the opposite situation (Solari and Claps, 1996). The phytoplankton was enriched whereas on the sediments an impoverishment took place.

Lindavia glomerata, *Cyclotella planctonica*, *Navicula cryptocephala*, and *N. veneta* were the most common species in Mert Stream. *Lindavia glomerata* (*Cyclotella glomerata*), one of the dominant species of phytoplankton, constituted 13.1% of the total organism and became the species with the highest number of organisms in the stream. Species of genus *Cyclotella* are primarily planktonic and frequent in the freshwaters. *Cyclotella* species generally bloomed from spring to autumn or early winter (Cho, 1996). As a matter of fact, *Cyclotella* species in the phytoplankton of the Mert stream reached the highest number of organisms in September and November. *Cyclotella* species more rich and diverse than before the construction of estuarine dam. Cho (1996) stated that the richness and diversity of *Cyclotella* species decreased due to the water became eutrophic after the construction of estuarine dam at Naktong River. A few *Cyclotella* species were recorded in 5th and 6th stations of the Mert Stream (the part of the stream within the city), where the water flow rate is very low. *N. cyrptocephala*, one of the symmetric biraphid diatoms, has been the secondary important organism in the stream with a rate of approximately 9%. It was reported that *Navicula* species are included in facultative or unregistered species and can be found widely and abundantly in both organic matter rich and organic matter poor environments (Van Dam et. al., 1994). It was observed that 20.2% of the dominant taxa in the study consisted of pelagic origin and 28.8% of them consisted of diatoms of benthic origin. This shows that benthic origin organisms are more common in the phytoplankton. This result is a normal for streams that do not have much pelagic zone, such as the Mert Stream.

Even if the abundance of Ochrophyta members didn't reach a very serious level, the fact that the one species from Ochrophyta reached an abundance of 5.3% made this division a section of secondary importance in the phytopankton. The presence of golden algae such as *Dinobryon*, which favor water with a lower inorganic phosphorus level, suggests oligotrophic conditions throughout a majority of the lake (Barinova et al., 2006). In the stream, *D. divergens* species, which is the only representative of the *Dinobryon* genus, peaked at the 2nd station. This station has a mesotrophic character with low level of pollution in terms of index results.

The fact that only 7% of Cyanobacteria members represented in the Mert Stream shows that there is no serious pollution in the stream, except for part-time pressures. Among the members of the Cyanobacteria division, the only taxon found in all stations was the toxic *Microcystis aeruginosa* species with an abundance of 2.5%. One of the most important biological stressors in the aquatic environment is cyanobacterial blooms. *M. aeruginosa* blooms can be seen in natural and artificial water bodies of eutrophic character all over the world.

In the study area, 11 species from Euglenozoa division were identified. The fact that the members of the Euglenozoa division, which are found in high numbers in areas with organic pollution, are represented by only 1.2% in the study area, also shows that there is no serious pollution due to sewage waste in the area. There was no organism that became prominent in terms of the total number of organisms in the Euglenozoa group. *Lepocinclis acus* from the Euglenozoa group was the only taxon present in all stations.

Other divisions (Chlorophyta, Charophyta, Rhodophyta) detected in phytoplankton of Mert Stream could not reach a very serious abundance rate. Although the Chlorophyta division was the one with the most species after the diatoms (16 taxa), its abundance rate remained at only 2%. Charophyta division was represented by 10 taxa and Rhodophyta division by a taxon.

Indicator organisms are species that are sensitive to changes in water quality and react in predictable ways to changes in their surroundings. Algae are an example of this type of organism. The degree of stress a stream is under can be evaluated by the organisms that reside in that stream, because different algae species have different levels of pollution tolerance. By eliminating sensitive creatures and increasing the number of tolerant ones, environmental degradation reduces the number of diverse sorts of organisms in a group. This reduces the stream's biodiversity (the amount of various types of species). The indicator organisms are classified into three groups as tolerant, facultative and sensitive based on their pollution tolerance (Muscio, 2002). When the water quality indicator ratios of the phytoplanktonic taxa in Mert Stream are examined, it is seen that the pollution-tolerant algae dominate the area with a ratio of 40%, even if not at a dominant level. The heartiest organisms, they are tolerant of pollution. In large numbers, they point to poor water quality conditions, but can also be present in good and fair water quality. The rate of taxa that can prefer both tolerant and sensitive environments, which we call facultative, is close to the rate of tolerant species in the area and is at the level of 34%. These organisms can survive in a greater variety of water quality settings than sensitive organisms. As a result, they can be found in waters other than poor- and high-quality waters. The rate of pollution-sensitive taxa in Mert Stream remained at the level of 26%. Because sensitive water creatures cannot thrive in contaminated environments, their existence usually signifies good water quality. According to all these ratios, it can be said that there is pollution pressure especially in certain stations, even if it is not serious in the area. Because tolerant species are more dominant than sensitive species in the area, the result is that the trophic structure of the Mert Stream is a meso-eutrophic structure.

In present study, two different index types were used to determine both trophic status and saprobic level in the stream. Station-based trophic status of the stream was determined using TDI and IDG indices, and station-based saprobic level of the stream was determined using PTI, Palmer and DAIpo indices. The least tolerant group has the highest index value, while the most tolerant group has the lowest. The number of indicator species present in each group determines a stream's index score.

Based on all this information, according to the mean result of TDI and IDG (55 and 13, respectively), the trophic status of the Mert Stream is a mesotrophic structure with moderate nutrients, dominated by plants or algae and good water transparency. According to the TDI index result, 1st station is an eutrophic class. This means that frequent algal blooms due to high nutrients and moderate to poor water transparency should be seen at 1st station. However, such typical characters of eutrophic class were not observed in the 1st station of the Mert Stream. According to the IDG index result, 2nd and 3rd stations are a meso-eutrophic class. Both transparency and quality of the water are poor due to the poultry farms near the 3rd station and the intensive farming activities around the 2nd station. Indeed, the phytoplankton community structure in 2nd and 3rd stations of the stream also reflected this situation. In the TDI studies based on planktonic diatoms conducted by Shaimaa et al. (2017) and Amal (2012) to determine the trophic structure of the Tigris and Shatt AL-Arab rivers, trophic structure results determined as mesotrophic were similar to the TDI results in the Mert Stream.

The results acquired in the saprobic system can be used to classify trophic states (Dokulil, 2003). As a result, phytoplankton species identification is important for determining the trophic condition of aquatic environments. Saprobic indicators in phytoplankton are commonly employed in European and Asian countries to assess water quality (Walley et al., 2001; Barinova et al., 2004). The quantity of saprobic activity determines the classification of a water body in the saprobic system. The index aims to

organize water bodies on a numerical scale based on their saprobity (Heckman et al., 1990). The saprobic technique is only useful for assessing organic pollution that is being decomposed by bacteria, and it is not ideal for assessing toxin or other pollution. This index is relevant to both natural and man-made minor water bodies. It also applies to all organic matter contaminants found in freshwater and marine environments. In summary, this technique can be employed in a variety of aquatic environments, including water quality assessments for drinking water, industrial, and surface water contamination (Dokulil, 2003). According to the average PTI results (2.7), Mert Stream is at ß-mesosaprobic level with moderately polluted. Considering PTI index results, only the 3rd station differed from the others sampling points with α-mesosaprobic level, which corresponds to poor water quality. This was an expected result in the 3rd station, where organic pollution originating from chicken farms is high. Station 3 is also an area where water turbidity and BOD value are high due to bacterial activity density. The DAIpo saprobity index ranged from 53.0 to 61.8. Accordingly, Mert Stream is at α-oligosaprobic level, which corresponds to good water quality. Considering DAIpo index result, which is calculated by proportioning the cleanliness indicator saproxenous species to the pollution indicator saprophilous species, there is no organic pollution load at any station of the stream. Considering all of saprobity index values between 1.5-2.5, the Kenozero waters is classified as oligo-ß-mesosaprobic state or class II of water quality, which matches moderate content of organic substances (Abakumov, 1992). According to the Palmer index, which is based on the presence of algal genera with organic pollution tolerance in water bodies, all sites of the Mert Stream except the 6th station are under the threat of high organic pollution. Considering the Palmer index scores, the highest organic load was found at the 3rd and 4th stations. The organic pollution load at the 6th station is at the ß-mesosaprobic level, which corresponds to moderate pollution. The quality of the water in Nhu Yriver was characterised by highly organically polluted conditions because the Palmer index values were over 20 at all sites (Te et al., 2018). The ten most tolerant species stated by Palmer (1969) were not dominant or subdominant in Mert Stream indicates that the nutrient richness of the stream is not very high. It has been observed that genus such as *Anomoeoneis*, *Brachysira*, *Brebissonia*, *Craticula*, *Gomphonella*, *Sellaphora*, *Ulnaria*, *Chroococcus*, *Leptolyngbya*, *Lyngbya*, *Limnothrix*, *Microcystis*, *Pseudanabaena*, *Spirogyra*, *Cladophora*, *Desmodesmus*, *Pediastrum*, *Ulothrix*, *Volvox*, *Messastrum*, *Ceratium*, and *Trachelomonas*, which are not among pollution-tolerant genera of the Palmer index (1969), are mostly found in polluted waters (Maraşlıoğlu et al., 2005; Phillips et al., 2010; Te et al., 2018). Similar genera were recorded in the present investigation. *Cyclotella* species, which were found to be the most active participants at all stations in Mert Stream, may be good indicators of less contaminated water bodies as similar observations were recorded by Willen (1991), Hornstrom et al. (1993), Saros and Anderson (2014).

CONCLUSION

The results of this study revealed the benefits of using biotic factors (phytoplankton community and index) together in water quality assessment studies and showed that minor changes in environmental conditions may cause major effects in the phytoplankton communities. In present study, it was seen that the best biological index that reflects the station-based trophic structure of the stream is IDG, and the best biological index that reflects the station-based organic pollution load of the stream is PTI. Another result of this study is that the use of indicator organisms in phytoplankton gave good results in determining the trophic structure and water quality level of the stream. While more research is needed for the assessment of quality status of the investigated water ecosystem, the results of the present research do have the characteristics of a preliminary research with the aim of providing resources for any future bioindication investigation in the region.

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Researchers' Contribution Rate Statement Summary

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

Conflict of Interest Statement

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

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Evaluation of Antibacterial Effect of Honey on ESBL and Biofilm-Producing Enterobacterales

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ABSTRACT

Mastitis is a mammary gland inflammatory disease that causes milk yield reduction and economic losses. Mastitis is bacteriological and antibiotics are usually used for treatment. Alternative natural treatment methods such as bee products, phytotherapy, and essential oils were evaluated to reduce the use of antibiotics in the treatment of mastitis. In this study, the in vitro antibacterial effect of flower and oak honey samples dissolved in distilled water and boric acid (2%) on ESBL and biofilm-producing Enterobacterales pathogens was investigated. The aim was to establish the usability of honey/boric acid solution against mastitis as a natural antiseptic solution for bovine udder surfaces. Honey samples were studied by dissolving in distilled water and boric acid (2%) solvents. There was no significant statistical difference between honey solutions using distilled water and boric acid ($p>0.05$). Antibacterial effects were increased according to the increasing honey proportion in flower honey solutions. However, the antibacterial activity of oak honey dissolved in boric acid solution was higher than dissolved in distilled water. As a result of the statistical correlation analysis between flower and oak honey samples, antibacterial effects of flower honey samples were determined to be higher than oak honey samples ($p<0.05$) ($R=0.825$). An alternative formulation for mastitis treatment with honey and boric acid was developed for the first time in the literature.

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Balın GSBL ve Biyofilm Üreten Enterobacterales Üzerindeki Antibakteriyel Etkisinin Değerlendirilmesi

ÖZET

Mastitis, süt veriminin düşmesine ve ekonomik kayıplara neden olan bir meme bezi hastalığıdır. Mastitis bakteriyolojiktir; bu nedenle tedavilerinde genellikle antibiyotikler kullanılır. Mastitis tedavisinde antibiyotik kullanımının azaltılması için arı ürünleri, fitoterapi, uçucu yağlar gibi alternatif doğal tedavi yöntemleri değerlendirilmektedir. Bu çalışmada, distile su ve borik asit (%2) solventlerinde çözündürülen çiçek ve meşe balı örneklerinin ESBL ve biyofilm üreticisi Enterobacterales patojenleri üzerindeki in vitro antibakteriyel etkisi araştırıldı. Amaç bal/borik asit solüsyonunun büyükbaş göğüs yüzeylerinde doğal bir antiseptik solüsyon olarak mastitise karşı kullanılabilirliğini ortaya koymaktır. Borik asit ve distile su çözücülü bal çözeltileri arasında istatistiksel olarak anlamlı bir fark yoktur. Çiçek balı çözeltilerinde artan bal oranına göre antibakteriyel etkiler artmıştır. Ancak borik asit çözeltisinde çözülen meşe balının antibakteriyel etkinliği distile suda çözünenden daha yüksekti. Çiçek ve meşe balı örnekleri arasındaki istatistiksel ilişki analizi sonucunda çiçek balı örneklerinin antibakteriyel etkileri meşe balı örneklerine göre daha yüksek bulunmuştur ($p<0.05$) ($R=0,825$). Bu çalışma ile literatürde ilk defa bal ve borik asit ile mastitis tedavisine alternatif bir formülasyon geliştirilmiştir.

Mikrobiyoloji

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INTRODUCTION

Mastitis is an essential inflammation of the mammary gland that affects milking and dairy production and causes economic losses (Tepeli and Zorba, 2017; Nadia and Kheira, 2018; Krömker et al., 2019). Mastitis occurs in two types as clinical and subclinical. Clinical mastitis causes a noticeable difference in raw milk or udder quarters that can be identified by farmers. However, the subclinical version of the disease cannot be easily identified by visual symptoms (Leitner et al., 2018; Tepeli, 2020). As a result of pathologic and bacteriological changes in the mammary gland, milking channels can block continuously, and milking yield and milk quality decrease dramatically (Abdalhamed et al., 2018). Coliforms, *Streptococcus* sp., coagulase-positive *Staphylococcus* (especially *S. aureus*), and coagulase-negative *Staphylococcus* (CNS) are the main mastitis pathogens (Abdalhamed et al., 2018). Furthermore, gram-negative bacteria (mostly *E. coli*, *Klebsiell*a spp, and *Enterobacter* spp.) are an important cause of bovine mastitis around the world (Dahmen et al., 2013; Schukken et al., 2012). Subclinical mastitis infections can be prevented by early diagnosis of the disease, post-milking teat disinfection, and adequate pre-milking hygiene (Das et al., 2017). Antibiotics have been used in the treatment of mastitis for many years (Nadia and Kheira, 2018). The extensive and inappropriate use of antibiotics in dairy farms induces resistance among pathogens in mastitis. Antibiotics such as ß-lactam antibiotics (cephalosporins, penicillin), oxytetracycline, and aminoglycosides are used for the treatment of mastitis or the other bacterial infections among dairy cattle on the farm (Santioago et al., 2015; Ibrahim et al., 2016). Pathogenic bacteria, which belong to the order Enterobacterales, produce the ß-lactamase enzyme inactivating ß-lactam antibiotics (Das et al., 2017). The most clinically essential enzymes in the order Enterobacterales are known as extended-spectrum ß-lactamases (ESBLs). CTX-M, TEM and SHV are the predominant ESBL families encountered. Moreover, *bla*$_{\text{CTX-M-1}}$ is the most common genotype in animals according to available studies (EFSA, 2011; Ögedey et al., 2016; Das et al., 2017; Yıldırım and Pehlivanoğlu, 2018).

Alternative natural treatment methods such as bee products, phytotherapy, and essential oils were evaluated to reduce the use of antibiotics in the treatment of mastitis (Bal, 2011; Nadia and Kheira, 2018). Honey and bee products were used in folk medicine since ancient times due to biological activities such as antimicrobial, antioxidant, anti-inflammatory, immune modulator, and antitumor effects (Kwakman et al., 2008; Cinar, 2020; Kolaylı et al., 2020). Moreover, honey is an important antibacterial agent in traditional medicine containing polyphenols (flavonoid, benzoic, and cinnamic acid), sugar, acid, and hydrogen peroxide and having osmotic pressure effect (Weston, 2000; Baykam, 2007; Mercan et al., 2007; Erturk et al., 2014; Al-Masaudi et al., 2021). Honey and bee products, which have an important place in Turkey, are used in food, agriculture, medicine, cosmetics, paint, and many other fields. In 2020, honey production in Turkey reached 104,077 tons, while it was 1716 tons in Çanakkale. China (24.0%) ranked first in honey production in the world in 2019 with 444 thousand tons of honey production. Turkey (6.2%) ranked second with 114 thousand tons of honey production. Since most of the honey produced in this country is consumed in the domestic market, only 6,011 tons of honey produced in 2020 were exported (Anonymous, 2021a).

Boron is a natural element which is mainly mined in Turkey (67%). Other important producers are USA and China. This element is not carcinogenic and mutagenic. Boric acid is an important boron base weak acid. Its formula is H_3BO_3. Boric acid is used as antimicrobial, antiseptic, pesticide, and food additive (E284) (İpek, 2017; Demircan and Velioğlu, 2020; Anonymous, 2021b). Moreover, boric acid was used to treat fungal infections in humans for one hundred years (Liu et al., 2021). Boric acid has broad-spectrum antibacterial and therapeutic effects, and is considered a non-antibiotic alternative treatment option for superficial bacterial, fungal, and protozoal infections of the eye, ear, and vagina because of lower expense, being widely available, easy to use, and causing little irritation (Schmidt, 2017; Liu et al., 2021; Parin et al., 2021)

In this study, the in vitro antibacterial effect of flower and oak honey samples dissolved in distilled water and boric acid (2%) on ESBL (*bla*$_{\text{CTX-M}}$ gene positive) and biofilm-producing Enterobacterales pathogens was investigated. In this research aim was to establish the usability of honey/boric acid solution against the bovine inflammatory disease mastitis as a natural antiseptic solution for bovine udder surfaces.

MATERIAL and METODS

Honey Samples

Flower and oak honey samples were provided by Çanakkale Beekeepers' Association from the Kaz Mountain region (Mount İda), Çanakkale province in the west of Turkey. Physicochemical analyses were completed by Çanakkale Beekeepers Association at Ege University Drug Development and Pharmacokinetic Research and Application Center (Table 1). The main oak cultivars grown in Kazdağı National Park and its surroundings are Turkey oak (*Quercus cerris L.*), kermes oak (*Quercus coccifera* L.), Hungarian oak (*Quercus frainetto* Ten.), Aleppo oak (*Quercus infectoria* Oliv. subsp. *Infectoria*), Georgian oak (*Quercus petraea* subsp. *iberica* (Steven ex M. Bieb.) Krassiln.), downy oak (*Quercus pubescens* Willd.) and common oak (*Quercus robur* L) as determined by Deniz and Selvi (2021). Polat and Selvi (2011) examined plants visited by bees in their study of the region that includes Kaz Mountains. It was determined that the plants most visited by honeybees are taxa belonging to the families *Asteraceae*, *Boraginacea*e, *Fabaceae*, *Lamiaceae*, and *Cistaceae*. In addition, honeybees mostly visited species including *Echium plantagineum*, *Helianthus nnuus*, *Origanum* spp., *Paliurus spina-christi*, *Vitex agnus-castus*, *Cistus* spp., *Trifolium* spp., and *Cercis siliquastrum*.

Honey samples were dissolved in distilled water and powdered boric acid (2%) (CID: 7628, 99.5%, Merck, Germany) at final concentrations of 30%, 60%, and 90% (w v^{-1}).

Table 1. Some physicochemical properties of flower and oak honey
Çizelge 1. Çiçek ve meşe balının bazı fizikokimyasal özellikleri

Analysis *(Analiz)*	Flower honey *(Çiçek balı)*	Oak honey *(Meşe balı)*
Delta C13-Proteins (*Delta C13 protein)*	-26.30 ± 0.26‰	-25.70 ± 0.26‰
Delta C13-Raw product (*Delta C13 Hambal)*	-26.50 ± 0.27‰	-25.20 ± 0.25‰
Difference protein-honey (*Protein bal farkı)*	0.20‰	-0.50‰
C4% sugar (*% C4 şeker)*	0.00%	3.13%
Activity of diastase (*Diastaz aktivitesi)*	15.1 ± 2.6 ds dn^{-1}	19.2 ± 3.3 ds dn^{-1}
Electrical conductivity (*Elektrik iletkenliği)*	0.606 ± 0.018 mS cm^{-1}	1.083 ± 0.032 mS cm^{-1}
Hydroxymethylfurfural (*Hidroksimetilfurfural)*	6.7 ± 0.7 mg/kg	Not detected
Moisture (*Nem)*	18.2 ± 0.5%	17.1 ± 0.5%
pH (*pH)*	3.90 ± 0.03	4.47 ± 0.03
Proline (*Proline)*	974.0 ± 155.8 mg kg^{-1}	705.0 ± 112.8 mg kg^{-1}
Free acidity (*Serbest asitlik)*	32 ± 5 meq kg^{-1}	27 ± 4 meq kg^{-1}
Fructose (*Fruktoz)*	43.1 ± 9.9 g $100g^{-1}$	35.4 ± 8.1 g $100g^{-1}$
Glucose (*Glukoz)*	37.8 ± 7.9 g $100g^{-1}$	29.7 ± 6.2 g $100g^{-1}$
Sucrose (*Sakkaroz)*	Not detected	Not detected
Maltose (*Maltoz)*	Not detected	Not detected
Fructose/Glucose Ration (*Fruktoz/Glikoz Oranı)*	1.13	1.19

ESBL-producing (*bla*$_{CTX-M}$ gene positive) Enterobacterales Isolates

Isolates were identified as Enterobacterales causing mastitis in raw milk with subclinical mastitis by Tepeli and Zorba (2017) in a previous study. In this previous study, the ESBL activity of isolates was determined by the agar disk diffusion method according to European Committee on Antimicrobial Susceptibility Testing Standards (EUCAST, 2013). Moreover, detection of *bla*$_{CTX-M}$ gene was carried out by polymerase chain reaction (PCR). The *bla*$_{CTX-M}$ F-5-TCTTCCAGAATAAGGAATCCC-3, R- 5-CCGTTTCCGCTATTACAAAC-3, 909 bp primers were used for genotypic identification by Tepeli et al. (2018). For this study, seven *Escherichia coli strains*, two *Morganella morganii* strains, one *Serratia liquefaciens*, and *Citrobacter braakii bla*$_{CTX-M}$ positive strains were chosen. *Klebsiella pneumoniae* ATCC 700603 was used as ESBL positive control, and *E. coli* ATCC 25922 was used as ESBL negative control. Reference cultures were provided by Çanakkale Onsekiz Mart University Food Engineering Department microbiology culture collection.

Biofilm Assay

The crystal violet 96-well microtiter plate method was used to evaluate the biofilm formation status of isolates. For this, 20 µL overnight, 10^8 CFU mL^{-1} cell density (0.5 MacFarland) isolates were dispensed into each well, containing 100 µL of TSB (tryptone soya broth, Merck, Germany). The 96-well microtiter plate was incubated at 30 °C for 24 h. The incubated microtiter plate was washed twice with distilled, sterile water, dried, and fixed in an airflow cabinet. Each well was dyed with 120 µL of 1% (v v^{-1}) crystal violet solution (Sigma-Aldrich, USA). The crystal violet solution was discarded, and wells were washed twice with distilled, sterile water, and dried at room temperature for 30 min. Then, 120 µL of ethanol (96%) was added to each well, and OD values were

read by spectrophotometer (Multiscan FC; Thermo Fisher Scientific, NY, ABD) at OD_{600}. According to the scale of Sepanovic et al. (2000), OD values were evaluated as $OD_{control} < OD \leq 2xOD_{control}$ weak, $2xOD_{control} < OD \leq 4xOD_{control}$ moderate and $4xOD_{control} < OD$ strong biofilm producers. Control was evaluated as the negative control (NC).

Agar-Wells Diffusion method

Flower and oak honey samples with three different concentrations (30, 60, 90% w v^{-1}) were prepared with distilled water and boric acid (2%). ESBL-producing (*bla*$_{CTX-M}$ gene positive) Enterobacterales and reference culture isolates stored in TSB containing 16% glycerol at –20 °C were incubated overnight at 37 °C in TSB. Overnight TSB cultures were suspended in 5 mL of sterile saline (0.85% NaCl) until 0.5 MacFarland (1–2 × 10^8 CFU mL^{-1}) standard suspension was reached (DEN-1, MacFarland densitometers, Britain). The 0.5 MacFarland standard suspensions were added to Muller Hinton agar (MHA, HiMedia, India) with sterile swabs. After inoculum absorption, 6 mm wells were prepared on MHA, and 100 µL of honey samples (at each concentration) were added to each well. Plates were incubated at 35 °C for18-24 h in a flat position. Inhibition zone diameters were measured with a digital caliper (PM, China). Positive (*K. pneumoniae* ATCC 700603) control and negative control *(E. coli* ATCC 25922), cefotaxime antibiotic (2 mg L^{-1}) and boric acid (2%) were used in the present study (Valgas et al., 2007; Boorn et al., 2010).

Statistical Analysis

Statistical analysis was done with the SPSS 23.0 (SPSS Inc., Chicago, IL, USA) program. The differences between means of concentrations were determined by two-way ANOVA analysis; comparisons were made with the Tukey test ($p<0.05$). Differences between concentrations of oak and flower honey samples were examined with the Paired Sample t-test.

RESULT and DISCUSSION

According to the results of the biofilm assay, in this study all *E. coli* strains (except *E.coli* II and *E. coli* III), *S. liquefaciens,* and *C. braakii* were weak biofilm producers, while *M. morganii* II and *E. coli* III produced moderate biofilms (Fig. 1). Additionally, *E. coli* II was classified as a strong biofilm producer.

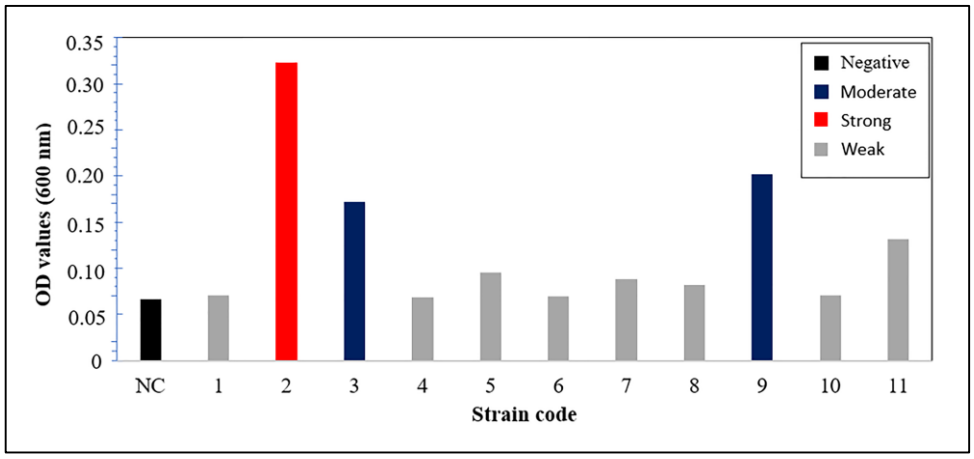

Figure 1. Biofilm status of ESBL producing Enterobacterales isolates (1-7: *E. coli*, 8-9: *M. morganii*, 10: *S. liquefaciens,* 11: *C. braakii, respectively*).
Şekil 1. GSBL üreten Enterobacterales *izolatlarının biofilm durumları*

Solutions of flower and oak honey prepared with distilled water and boric acid (2%) solvents were antibacterial against ESBL (*bla*$_{CTX-M}$ gene positive) at different levels for biofilm-producing Enterobacterales isolates (Fig. 2). The highest antibacterial activity was obtained with 90% (w v^{-1}) flower honey-distilled water solution against *C. braakii*, *M. morganii* II and all *E. coli* strains (Table 2). The highest antibacterial effect for *M. morganii* I strain was determined for the 60% flower honey-distilled water solution. However, 90% (w v^{-1}) flower honey-boric acid (2%) solution showed antibacterial activity against all strains (except *S. liquefaciens*) (Table 3).

No statistically significant difference was found between the antibacterial effect of flower honey - distilled water solutions and flower honey-boric acid (2%) solutions ($p>0.05$). Statistically significant differences were identified between both solvent concentrations ($p<0.05$). An increase in antibacterial activities for both solvents was seen when the honey amount increased.

The highest antibacterial activity was determined from 90% (w v^{-1}) oak honey-distilled water solution, except for *E. coli* II and *S. liquefaciens* (Table 4). In

addition, there was a statistically significant difference between concentrations of oak honey-distilled water samples ($p<0.05$). For oak honey-boric acid (2%), the highest antibacterial activity was determined for 30% (w v^{-1}) concentration against *M. morganii* I and *E. coli* VII. Also, the antibacterial activity of the 90% (w v^{-1}) concentration was evaluated as the highest activity against all other microorganisms (except *S. liquefaciens*) (Table 5). Differences between concentrations of oak honey–boric acid (2%) samples were determined to be statistically significant ($p<0.05$). Antibacterial activity increased with the increase in the amount of honey in oak honey–boric acid (2%) solutions. Differences between antibacterial activities of both solvents were reported to be statistically significant (distilled-water and boric-acid 2%) ($p<0.05$). Therefore, oak honey solutions prepared with boric acid (2%) had higher antibacterial activities. The antibacterial effects of oak and flower honeys were compared. The difference between the two honeys was analyzed statistically and a statistically significant difference was found ($p<0.05$) ($R=0.825$). Flower honey exhibited more antibacterial effect than oak honey.

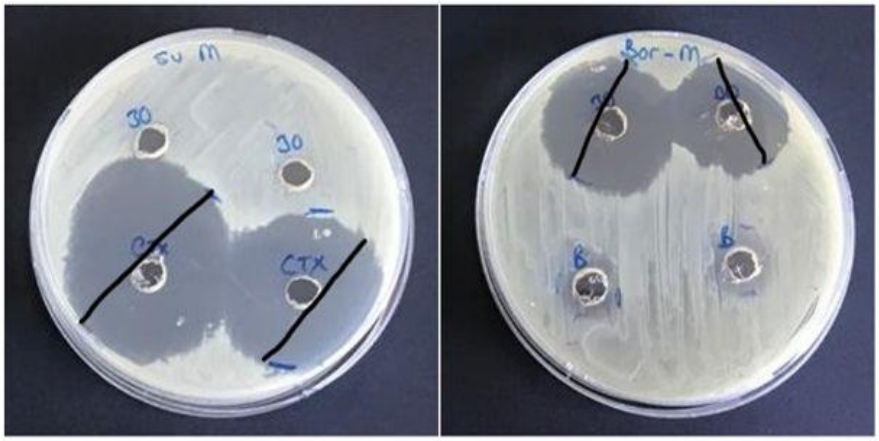

Figure 2. Inhibition zone diameter for agar-well diffusion method.
Şekil 2. Agar-kuyucuk difüzyon yöntemi inhibisyon zon çapı

Table 2. Antibacterial activity of flower honey-distilled water solutions
Çizelge 2. Çiçek balı-saf su solüsyonunun antibakteriyel etkisi

Isolate code *(İzolat kodu)*	Strain name *(Suş ismi)*	Cefotaxime 2 mg L^{-1} *(Sefotaksim)*	30% (w v^{-1})	60% (w v^{-1})	90% (w v^{-1})
1	*E. coli* I	20.13±0.53Ab	20.60±1.00Ac	15.66±0.34Acd	31.49±0.1Ac
2	*E. coli* II	18.29±0.33Ab	29.20±1.35Ac	22.99±0.17Acd	30.18±0.47Ac
3	*E. coli* III	39.59±1.58Ab	16.97±0.62Ac	27.62±0.57Acd	35.80±0.00Ac
4	*E. coli* IV	19.40±0.14Ab	15.62±2.23Ac	22.02±0.37Acd	31.33±0.32Ac
5	*E. coli* V	17.10±0.28Acb	≤ 6.00Acc	21.42±0.47Acd	21.62±0.16Acc
6	*E.coli* VI	43.27±0.37Ab	19.02±0.53Ac	20.07±0.97Acd	23.05±1.11Ac
7	*E. coli* VII	44.20±0.55Ab	22.93±0.49Ac	22.78±1.00Acd	13.51±0.47Ac
8	*M. morganii* I	42.57±0.25Ab	20.28±0.23Ac	22.56±0.12Acd	18.77±0.83Ac
9	*M. morganii* II	39.42±0.55Ab	25.72±1.14Ac	25.73±0.02Acd	35.52±1.46Ac
10	*S.liquefaciens*	45.44±0.83Adb	≤ 6.00Adc	≤ 6.00Adcd	≤ 6.00Adc
11	*C. braakii*	21.05±0.38Ab	24.85±1.38Ac	25.82±0.58Acd	35.75±0.71Ac
E.coli ATCC 25922 (ESBL negative control)		43.89±0.26Aeb	≤ 6.00Aec	≤ 6.00Aecd	21.07±1.01Aec
K. pneumoniae ATCC 700603 (ESBL positive control)		30.20±0.23Abb	≤ 6.00Abc	≤ 6.00Abcd	≤ 6.00Abc

* Capital letters (A) indicate that the difference between microorganisms is statistically significant ($p<0.05$). Lower case letters (b) show that the difference between concentrations is statistically significant ($p< 0.05$).

Antibiotics are used to treat mastitis. However, the inappropriate use of antibiotics on dairy farms can lead to resistance in mastitis pathogens. Therefore, researchers have begun to researching alternative treatment methods. Some researchers investigated alternative therapies such as ozone, essential oils, clay treatment, homeopathy, and phototherapy against microbial agents of mastitis to limit the use of antibiotics (Bal, 2011; Oral et al., 2014; Tepeli, 2020). In the present study, the in vitro antibacterial effects

of flower and oak honey samples dissolved in distilled water and boric acid (2%) were evaluated on *bla*CTX-M positive and biofilm-producing Enterobacterales isolated from bovine mastitis.

Table 3. Antibacterial activity of flower honey -boric acid solutions (2%)
Çizelge 3. Çiçek balı- borik asit (2%) solüsyonunun antibakteriyel etkisi

Isolate code *(İzolat kodu)*	Strain name *(Suş ismi)*	Boric acid 2% m V^{-1} *(Borik asit)*	Cefotaxime 2 mg L^{-1} *(Seftaksim)*	30% (w v^{-1})	60% (w v^{-1})	90% (w v^{-1})
1	*E. coli* I	13.94±0.86Aa	20.13±0.53Ab	≤ 6.00Aa	21.43±0.96Acb	28.16±0.67Ab
2	*E.coli* II	14.49±0.37AB	18.29±0.33ABb	28.91±1.36ABa	21.99±0.55ABcb	30.26±0.00ABb
3	*E. coli* III	15.72±0.13BA	39.59±1.58BAb	18.93±0.68BAa	31.79±0.43BAcb	34.95±0.35BAb
4	*E. coli* IV	≤ 6.00ABa	19.40±0.14ABb	19.87±0.19ABa	18.51±1.27ABcb	22.74±1.68ABb
5	*E. coli* V	≤ 6.00Aa	17.10±0.28Ab	≤ 6.00Aa	22.81±2.55Acb	28.50±2.33Ab
6	*E. coli* VI	≤ 6.00Aa	43.27±0.37Ab	≤ 6.00Aa	24.42±2.38Acb	28.72±1.55Ab
7	*E. coli* VII	≤ 6.00Aa	44.20±0.55Ab	≤ 6.00Aa	29.87±0.79Acb	36.14±2.06Ab
8	*M.morganii* I	≤ 6.00Aa	42.57±0.25Ab	15.43±0.48Aa	28.09±1.61Acb	29.50±0.02Ab
9	*M. morganii* II	13.05±0.64Aa	39.42±0.55Ab	≤ 6.00Aa	28.02±3.94Acb	35.75±0.25Ab
10	*S. liquefaciens*	15.44±0.54Aa	45.44±0.83Ab	≤ 6.00Aa	≤ 6.00Acb	≤ 6.00Ab
11	*C. braakii*	≤ 6.00Aa	21.05±0.38Ab	≤ 6.00Aa	26.71±1.97Acb	35.65±0.43Ab
E. coli ATCC 25922 (ESBL negative control)		≤ 6.00Aa	43.89±0.26Ab	≤ 6.00Aa	17.08±0.19Acb	23.64±1.04Ab
K. pneumoniae ATCC 700603 (ESBL positive control)		≤ 6.00ACa	30.20±0.23ACb	≤ 6.00ACa	≤ 6.00ACcb	≤ 6.00ACb

* Capital letters (A) indicate that the difference between microorganisms is statistically significant ($p< 0.05$).
Lower case letters (b) show that the difference between concentrations is statistically significant ($p< 0.05$).

Table 4. Antibacterial activity of oak honey - distilled water solutions
Çizelge 4. Meşe balı- saf su solüsyonunun antibakteriyel etkisi

Isolate code *(İzolat kodu)*	Strain name *(Suş ismi)*	Cefotaxime 2 mg L^{-1} *(Sefotaksim)*	30% (w v^{-1})	60% (w v^{-1})	90% (w v^{-1})
1	*E. coli* I	20.13±0.53Ab	≤ 6.00Aa	13.47±0.93Aa	26.86±0.60Ac
2	*E. coli* II	18.29±0.33Ab	21.60±0.06Aa	≤ 6.00Aa	≤ 6.00Ac
3	*E. coli* III	39.59±1.58Ab	≤ 6.00Aa	17.57±1.45Aa	26.07±0.96Ac
4	*E. coli* IV	19.40±0.14Ab	≤ 6.00Aa	≤ 6.00Aa	21.04±0.56Ac
5	*E. coli* V	17.10±0.28Ab	≤ 6.00Aa	≤ 6.00Aa	24.54±0.61Ac
6	*E. coli* VI	43.27±0.37Ab	≤ 6.00Aa	≤ 6.00Aa	18.99±0.36Ac
7	*E. coli* VII	44.20±0.55Ab	17.17±0.19Aa	13.43±0.95Aa	14.24±0.56Ac
8	*M. morganii* I	42.57±0.25Ab	18.59±1.20Aa	14.15±0.72Aa	15.31±0.46Ac
9	*M. morganii* II	39.42±0.55ACb	≤ 6.00ACa	19.02±0.44ACa	26.69±3.71ACc
10	*S. liquefaciens*	45.44±0.83Ab	≤ 6.00Aa	≤ 6.00Aa	≤ 6.00Ac
11	*C. braakii*	21.05±0.38Ab	≤ 6.00Aa	20.23±0.08Aa	26.61±0.73Ac
E. coli ATCC 25922 (ESBL negative control)		43.89±0.26Ab	≤ 6.00Aa	≤ 6.00Aa	10.98±0.48Ac
K. pneumoniae ATCC 700603 (ESBL positive control)		30.20±0.23ABb	≤ 6.00ABa	≤ 6.00ABa	≤ 6.00ABc

* Capital letters (A) indicate that the difference between microorganisms is statistically significant ($p< 0.05$).
Lower case letters (b) show that the difference between concentrations is statistically significant ($p< 0.05$).

Bacterial biofilms are structured communities of cells. These structures often occur along with the production of extracellular polymers by microorganisms as a response to different environmental conditions in order to survive. These biofilms formed on surfaces are a long-term source of contamination of foodstuffs on dairy process lines and surfaces due to the available nutrients and humidity (Čabarkapa et al., 2015; İpek and Zorba, 2018). Laranjo et al. (2018) investigated the in vitro activity of propolis ethanol extracts (PEE) against biofilms produced by staphylococci isolated from the milk of small ruminants with mastitis. They and some researchers stated that biofilms associated with mammary infection should be controlled due to the fact that bacteria growing in a biofilm can become 10 to 1000 times more resistant to antimicrobials. The biofilm structure is defined as a 3-dimensional exopolysaccharide matrix in which microorganisms can interact with each other. Horizontal gene

transfers were reported in this structure between resistant bacteria in food, milk, etc., which are excellent nutrients for microorganisms. Accordingly, biofilm structures can easily form on food-related surfaces, and resistance can transfer from one microorganism to another (Magesh et al., 2013; Cengiz et al., 2014; İpek, 2017). Determination of biofilm-formation capacity is an important piece of information to understand resistance transferability status. In the current study, *E. coli* II was a strong biofilm producer, and *E. coli* II and *M. morganii* I were moderate biofilm producers. Antibacterial effects of distilled water and boric acid solutions of flower-honey samples were determined against microorganisms forming biofilms. Only 30% of flower honey-boric acid solution had an antimicrobial effect on *M. morganii* II which can form biofilms. Antibacterial effects of oak honey solutions were reported to be lower than flower honey solutions against biofilm-producing microorganisms. Milanov et al. (2015) indicated that the biofilm-producing strains might play an important role in the spread of microorganisms in the environment and milking systems. Cengiz et al. (2014) suggested that antibiotic resistance profiles in herds should be monitored. Moreover, they stated that the main cause of mastitis treatment failure is the development of antibiotic resistance. They detected that 67.8% biofilm-producing *E.coli* strains originated from cow mastitis.

Table 5. Antibacterial activity of oak honey-boric acid (2%) solutions
Çizelge 5. Meşe balı-borik asit (2%) solüsyonunun antibakteriyel etkisi

Isolate code *(İzolat kodu)*	Strain name *(Suş ismi)*	Boric acid 2% m V^{-1} *(Borik asit)*	Cefotaxime 2 mg L^{-1} *(Sefotaksim)*	30% (w v^{-1})	60% (w v^{-1})	90% (w v^{-1})
1	*E. coli* I	13.94±0.86Aa	20.13±0.53Ab	≤ 6.00Aac	16.84±0.38Acd	26.80±1.55Ad
2	*E. coli* II	14.49±0.37Aa	18.29±0.33Ab	≤ 6.00Aac	18.95±1.24Acd	28.40±1.96Ad
3	*E. coli* III	15.72±0.13Aa	39.59±1.58Ab	28.40±0.52Aac	17.18±0.62Acd	26.97±0.18Ad
4	*E. coli* IV	≤ 6.00ABa	19.40±0.14ABb	≤ 6.00ABac	≤ 6.00ABcd	23.36±0.64ABd
5	*E. coli* V	≤ 6.00ACa	17.10±0.28ACb	≤ 6.00ACac	21.12±0.44ACcd	22.80±0.22ACd
6	*E. coli* VI	≤ 6.00Aa	43.27±0.37Ab	17.75±0.17Aac	21.18±0.12Acd	24.66±1.03Ad
7	*E. coli* VII	≤ 6.00Aa	44.20±0.55Ab	19.96±0.09Aac	16.23±0.01Acd	15.97±0.29Ad
8	*M. morganii* I	≤ 6.00Aa	42.57±0.25Ab	20.66±0.36Aac	16.39±0.50Acd	15.97±1.04Ad
9	*M. morganii* II	13.05±0.64Aa	39.42±0.55Ab	12.16±0.23Aac	20.72±0.66Acd	30.23±1.03Ad
10	*S. liquefaciens*	15.44±0.54ADa	45.44±0.83ADb	≤ 6.00ADac	≤ 6.00ADcd	≤ 6.00ADd
11	*C. braakii*	≤ 6.00Aa	21.05±0.38Ab	14.36±0.96Aac	19.29±0.36Acd	26.64±0.20Ad
E. coli ATCC 25922 (ESBL negative control)		≤ 6.00Aa	43.89±0.26Ab	14.84±0.09Aac	19.79±0.37Acd	23.58±0.52Ad
K. pneumoniae ATCC 700603 (ESBL positive control)		≤ 6.00AEa	30.20±0.23AEb	30.20±0.23AEac	≤ 6.00AEcd	≤ 6.00AEd

* Capital letters (A) indicate that the difference between microorganisms is statistically significant ($p< 0.05$).
Lower case letters (b) show that the difference between concentrations is statistically significant ($p< 0.05$).

The antimicrobial activity of honey was firstly observed in 1892. There are many studies about the antibacterial activity of honey (Baltrušaitytė et al., 2007; Süerdem et al., 2018; Çakır et al., 2020; Yalazi and Zorba, 2020; Al-Masaudi et al., 2021). Basically, the antimicrobial activity of honey is associated with acidity, pH, osmotic pressure caused by sugar content, hydrogen peroxide produced enzymatically by glucose oxidase, and phenolic compounds (Ulusoy et al., 2010; Güneş et al., 2016; Nolan et al., 2019; Çil et al., 2020). The antibacterial activities of flower honey samples were higher than oak honey samples in this research. The concentration of phenolic acids and flavonoids, which differ according to plant flora constituting the source of honey, are important due to their antimicrobial effects (Nisbet and Aker, 2020). In studies conducted about the polyphenol contents of different types of honey in Turkey, catechin, luteolin, and syringic acid were detected in flower honey. However, catechin, vanillic acid, syringic acid, daidzein, and luteolin were not identified in oak honey from Thrace (Güneş et al., 2016; Kolayli et al., 2016; Nolan et al., 2019). Differences in polyphenol contents of honey can explain antibacterial activity levels.

Özkirim et al. (2021) reported the antibacterial activity of oak honey against biofilm-producing, antibiotic-resistant clinical *E. coli* ATCC 35218 strains. Similarly, in this study, oak honey samples prepared with distilled water and boric acid solvent had antibacterial activity against the weak, medium, and strong biofilm producing *E. coli* strains. Süerdem et al. (2018) reported that oak honey was the more effective honey type because activity was measured against some bacterial strains (*E. faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 29213, *S. aureus* ATCC 29213, *S. aureus* ATCC 6538P, *Listeria monocytogenes*, and *Salmonell*a Typhimurium ATCC 51812). Wasihun and Kasa (2016) reported the antibacterial activity of red and white honey samples

against multi-drug resistant clinical *E. coli* and *K. pneumonia* strains. Kwakman et al. (2008) determined bactericidal activity of 10-40% (v v^{-1}) Revamil honey prepared with phosphate buffer against ESBL-producing *E. coli* and *K. oxytoca.* Bacayo et al. (2018) studied the antibacterial activity of 25% (w v^{-1}) concentrate prepared with Mueller Hinton broth and Tualang honey against *K. pneumonia,* and they identified antibacterial activity. Mercan (2007) reported that honey samples from the Sivas region had antimicrobial effects on *M. morganii* strains. Similar to the results of these studies, antibacterial activities of honey samples were determined against *E. coli*, *K. pneumonia,* and *M. morganii* in this research.

In the literature, no study evaluated the antibacterial activity of boric acid against the strains tested in this study. Boric acid contains boron, a natural element, which has been found in many foods like pistachio nuts, plums, tea and coffee. Boron amounts were determined in the order 13.8-18 µg g^{-1}, 21.5-27 µg g^{-1}, 21.5-27 µg g^{-1}, and 14.33 µg g^{-1} (İpek, 2017). Boric acid at 4 g kg^{-1} can be used as preservative for fish caviars. It was emphasized that there was no cause for concern in terms of genotoxicity (EFSA, 2013; Demircan and Velioğlu 2020; Liu et al., 2021). In practice, 2% boric acid solutions are usually used for human inflammatory treatments and 2% boric acid solutions are not cytotoxic as an inflammatory surface antiseptic agent (Anonymous, 2006; Anonymous, 2019; Liu et al., 2021). In this, 20 mg mL^{-1} boric acid was prepared as 2% solution against the animal inflammatory disease mastitis as a natural antiseptic solution for udder surface of the animal. Schmidt (2017) stated that intact skin does not absorb boric acid and boric acid in elemental form is safe for oral ingestion up to 18 mg day^{-1} for an adult. The Panel on Food Additives and Nutrient Sources organized by the European Food Safety Authority (EFSA) concluded that boric acid does not raise concerns in terms of genotoxicity (EFSA, 2013). Furthermore, İlhan et al. (2019) reported the antibacterial activity of boric acid against antibiotic-resistant *L. monocyto*genes and *S. aureus*. This study by İlhan et al. (2019) provided significant information about the antibacterial activity of boric acid against different types of resistant bacteria. Liu et al. (2021) measured the therapeutic effect of 3% boric acid on skin microflora. They concluded that boric acid inhibited candida, reduced microbial diversity, and improved the microecological flora of mouse skin. Parin et al. (2021) reported the antimicrobial activity of polyamide 6/honey fibers loaded with boric acid against *Escherichia coli* and *Staphylococcus aureus*. Almost all common bacteria or fungi were inhibited with 10–20 mg mL^{-1} boric acid (Hui et al., 2016). The specific mechanisms of boric acid versus cells are not clear. But studies indicate that boric acid increases the permeability of the pathogen cell wall, destroys cell membranes, and inhibits cell membrane formation (Liu et al., 2021). Nevertheless, the effect of boric acid on microflora was rarely reported.

CONCLUSIONS

Today, the use of natural products for the treatment of antibiotic resistant bacteria and the diseases caused by these pathogens has attracted the attention of researchers. This study showed that flower and oak honey samples dissolved in distilled water and boric acid (2%) solvents had significant antibacterial activity potential against ESBL and biofilm producing Enterobacterales. In conclusion, it is thought that honey/boric acid solutions will be an alternative method instead of antibiotics for the prevention or treatment of mastitis caused by environmental pathogens. This is a preliminary study before cytotoxicity studies. Further studies are needed about the applicability of honey/boric acid solutions.

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Author(S) Contribution

S. Özdikmenli Tepeli participated conception and design of the work, data collection, data analysis and interpretation, performing the analysis, drafting the article, critical revision, final approval of the version to be published. B. Kaya was involved in data collection, data analysis and interpretation, performing the analysis, drafting the article. D. İpek was involved in data analyses, critical revision and final approval of the version to be published.

Conflict of Interest

The authors declare no conflict of interest.

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Cytogenetic Characteristics of *Gerbillus dasyurus* and *Meriones tristrami* (Rodentia: Gerbillinae) from Kilis, Turkey: Conventional and C Banded Karyotypes

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ABSTRACT
In this study, diploid chromosome number and constitutive heterochromatin distributions of chromosomes were determined in *Gerbillus dasyurus* and *Meriones tristrami* species. The diploid chromosome numbers (2n) and autosomal chromosome arms (NFa) of *G. dasyurus* and *M. tristrami* were determined as 2n=60, NFa= 68, and 2n=72, NFa= 73, respectively. It was found that there were differences in autosomal chromosomal arms (NFa) of two different gerbil species, the karyotypes of which were obtained in this study, compared to previously conducted studies in Türkiye. Heteromorphic chromosome pair (submetacentric/acrocentric) was found in the autosomal set of *M. tristrami.* There was an enlarged heterochromatin block on the short arm of submetacentric chromosome in heteromorphic chromosome pair.

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Gerbillus dasyurus ve *Meriones tristrami* (Rodentia: Gerbillinae) Türlerinin Sitogenetik Özellikleri: Standart ve C Bantlı Karyotipler

ÖZET
Bu çalışmada, *Gerbillus dasyurus ve Meriones tristrami* türlerinin diploid kromozom sayısı ve kromozomların konstitütif heterokromatin dağılımları belirlenmiştir. *G. dasyurus* ve *M. tristrami* diploid kromozom sayısı ve otozomal kromozom kol sayıları sırasıyla 2n=60, NFa= 68 ve 2n=72, NFa= 73 şeklindedir. Karyotipleri elde edilen iki farklı gerbil türünün otozomal kromozom kol sayılarında (NFa) Türkiye'den daha önce gerçekleştirilen çalışmalara kıyasla farklılık olduğu belirlenmiştir. *M. tristrami*'nin otozomal kromozom setinde heteromorfik kromozom çiftinin (submetasentrik/akrosentrik) olduğu belirlenmiştir. Heteromorfik kromozom çiftinde submetasentrik kromozomun kısa kolunda genişlemiş heterokromatin blok bulunmaktadır.

Biyoloji

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INTRODUCTION
The *Meriones* and *Gerbillus*, which are classified within Gerbillinae subfamily, are two rodent genera and their representatives are distributed in steppe and semi-arid habitats (Kryštufek and Vohralik, 2009). The genus *Gerbillus* is represented with one species (*Gerbillus dasyurus*) in Türkiye, while the genus *Meriones* is represented with six species (*Meriones persicus*, *M. vinogradovi*, *M. dahli*, *M. crassus*, *M. libycus* and *M. tristrami*) (Kryštufek and Vohralík, 2009).

Meriones and *Gerbillus* species may show a large number of chromosome variations. These species draw attention with their variation fundamental number arms (NFa) or constitutive heterochromatin distributions (Lay et al., 1975; Lay, 1983; Qumsiyeh

et al., 1986; Volobouev et al., 1995; Aniskin et al., 2006; Abiadh et al., 2010; Kaya and Çoşkun, 2012; Mahmoudi et al., 2020). There are limited numbers of chromosome studies conducted on *G. dasyurus* in Türkiye (Yiğit et al., 1997). The first record of *G. dasyurus* from Türkiye, the northernmost distribution border of the species, was reported by Yiğit et al. (1997). In this study, the standard karyotype characteristics and morphological characteristics of this species were reported. In the studies carried out within the distribution borders of *G. dasyurus* species, different researchers have reported variations in the number of chromosomal arm (NFa) of the samples (Qumsiyeh et al., 1986). A similar situation is also true for *M. tristrami* species, which is largely distributed in Anatolia, Transcaucasia and Middle East (Korobitsyna, 1975; Korobitsyna and Korablev, 1980). In the studies conducted to find out the karyological characteristics of *M. tristrami*, this species has been found to have different number of chromosomal arms in Anatolia population (Kefelioğlu, 1997; Yiğit and Çolak, 1998; Kaya and Çoşkun, 2012; Mahmoudi et al., 2020).

The aim of this study is to perform a chromosome banding analysis of the karyotype of *G. dasyurus* and *M. tristrami* from Türkiye with the use of C-banding and to compare the results with previous studies regarding the species.

MATERIAL and METHODS

One specimen (female) of *G. dasyurus* species and one specimen (male) of *M. tristrami* species were collected using live animal catch traps from the province of Kilis (N 36° 43' 00" E 37° 16' 00", Southeast Anatolia, Turkish-Syrian border) between November 1 and 10, 2020. Chromosome preparations were obtained from the femoral bone marrow cells of colchicine treated animals (Ford and Hamerton, 1956). The diploid chromosome number (2n), fundamental number of autosomal arms (NFa) and sex chromosomes of the specimens used in the study were identified. The constitutive heterochromatin distribution was determined using techniques from Summer (1972). From each specimen, 10 to 20 slides were prepared and at least 10 well-spread metaphase plates were analysed. Tissue samples the skins prepared as standard museums materials and karyotype preparations of two species were stored in Ondokuz Mayıs University Cytogenetic laboratory for further studies (Museum sample no: *G. dasyurus* : 41-AYS; *M. tristrami* : 42-AYS). This study was carried out with the permission of Ondokuz Mayıs University local ethics committee for animal experiments (permission number: E-68489742-604.01.03.-12821).

RESULTS

The diploid chromosome number of *G. dasyurus* was 2n=60, NFa= 68 and NF=72. While five pairs of chromosomes (chromosomes no: 1-5) were biarmed chromosomes in autosomal chromosomes, 24 pairs of chromosomes are acrocentric of different sizes (chromosome no:6-29). X chromosome was a large biarmed chromosome (metacentric or submetacentric) (Figure 1).

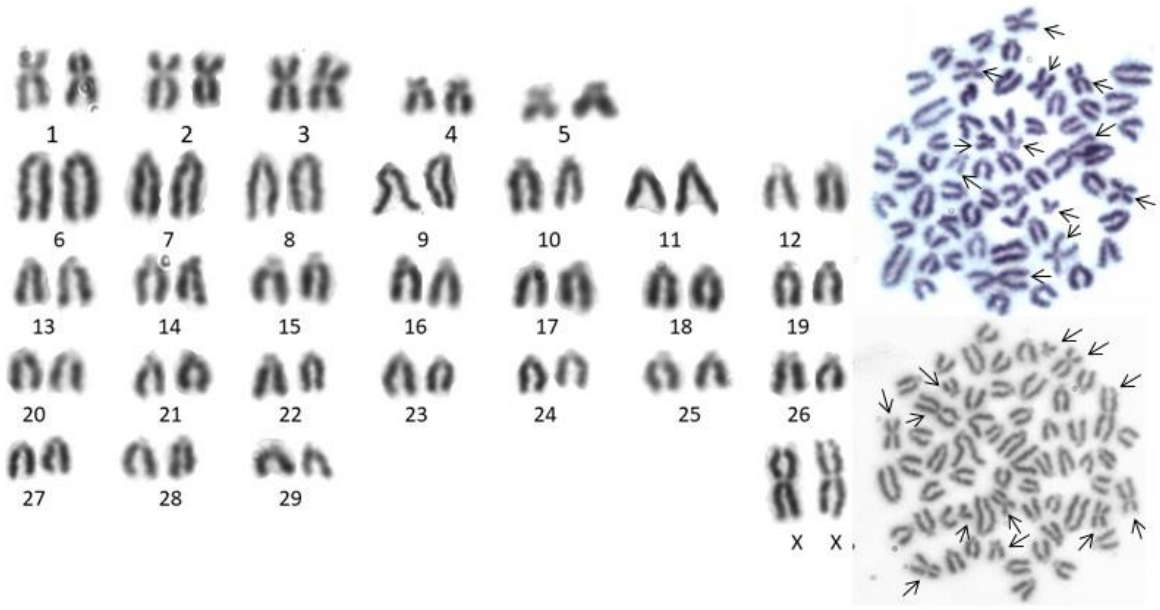

Figure 1. Conventional karyotype of *G. dasyurus* (female) from Kilis (Southeast Anatolia), arrow: biarmed chromosomes in metaphase plates

Şekil 1. G. dasyurus' un (dişi) standart karyotipi, ok: çift kollu kromozomlar

The diploid chromosome number of *M. tristrami* was 2n=72, NFa= 73 and NF=77. Autosomal chromosomes had a pair of biarmed chromosomes (chromosomes no: 1), a pair of heteromorphic (chromosome no:2, submetacentric/acrocentric) and 33 pairs of different sizes of acrocentric (chromosome no:3-35) chromosomes. Of the sex chromosomes, the X and Y chromosomes were biarmed chromosome (metacentric or submetacentric) (Figure 2).

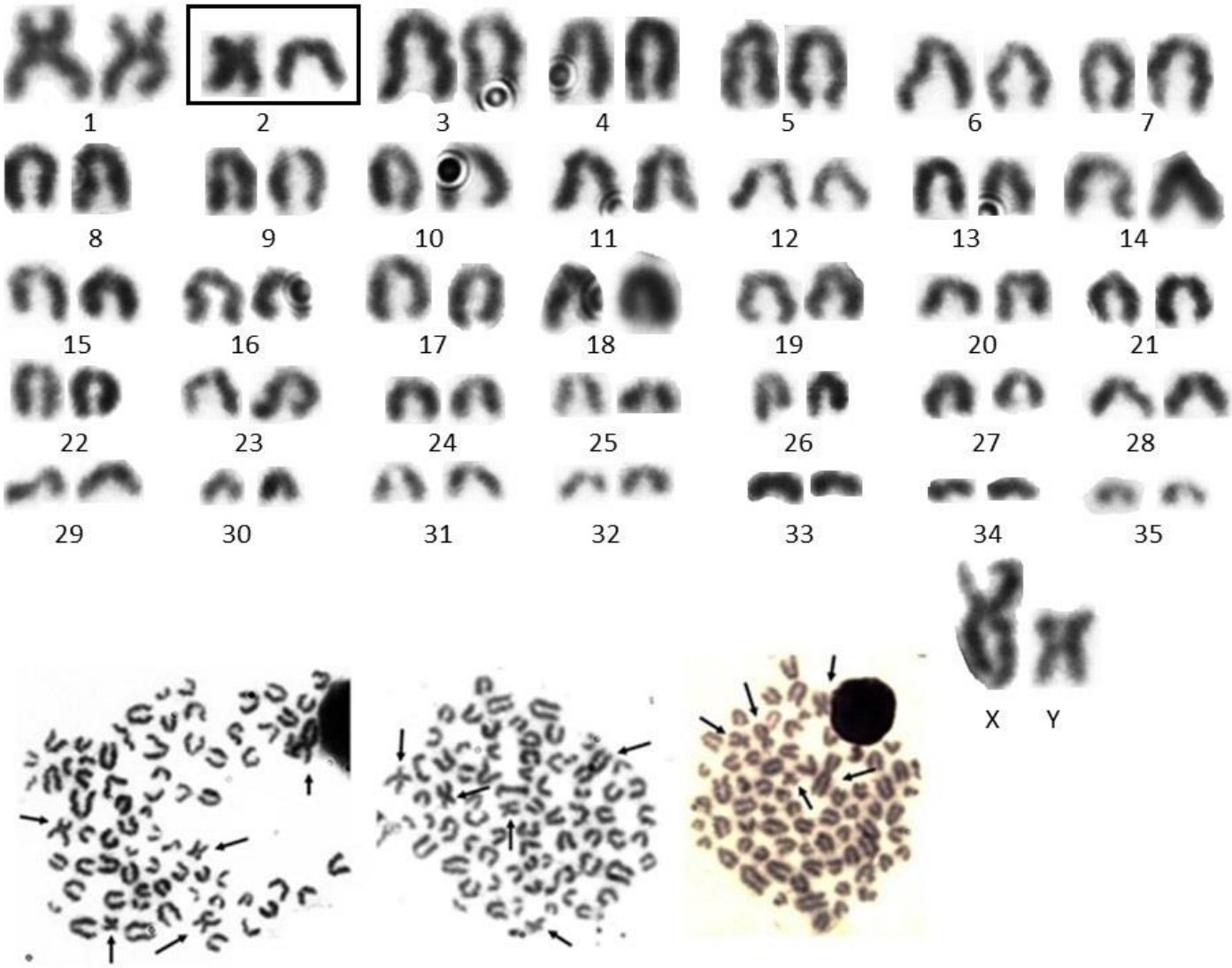

Figure 2. Conventional karyotype of *M. tristrami* (male) from Kilis (Southeast Anatolia) and heteromorphic chromosome no:2, arrow: biarmed chromosomes in metaphase plates

Şekil 2. M. tristrami' nin (erkek) standart karyotipi ve heteromorfik 2. kromozom çifti, ok: çift kollu kromozomlar

In the C-banded karyotype of *G. dasyurus*, the constitutive heterochromatins were in the centromere region, and they were notably distinct. The X chromosome was distinctively C-band positive (Figure 3).

In the C-banded karyotype of *M. tristrami*, positive constitutive heterochromatins were in the centromere region. Contrary to this, some acrocentric autosomes had not heterochromatin block. The X chromosome was C-band positive. In addition, there was an enlarged heterochromatic region on the short arm of submetacentric chromosome in heteromorphic chromosome pair (chromosome no:2) (Figure 4).

DISCUSSION

In studies conducted to find out the karyotype characteristics of *G. dasyurus* distributed in the Middle East, Arabic peninsula, Egypt and Türkiye's southeast region (Palestine, Wahrman and Zahavi, 1955; Israel, Wahrman et al., 1988; Egypt, Wassif et al., 1969; Lay et al., 1975; Jordan, Qumsiyeh et al., 1986, Abu Baker et al., 2009; Türkiye, Yiğit et al., 1997; current study), it was found that this species had stable diploid chromosome number (2n=60),

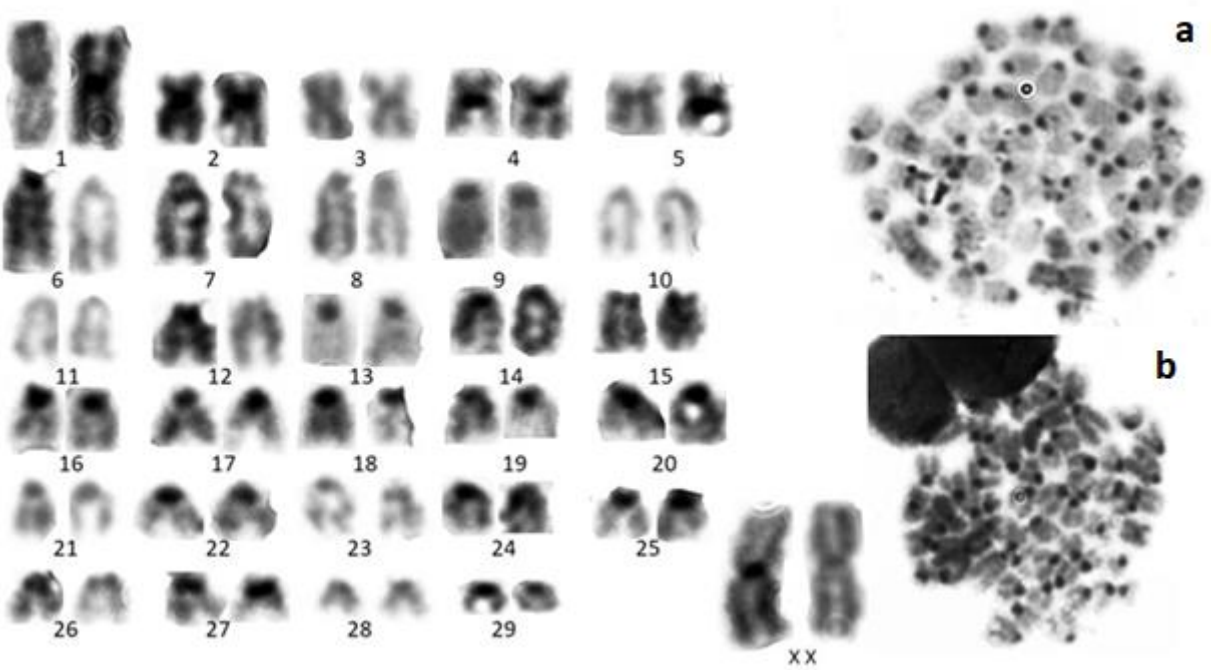

Figure 3. C-banded karyotype of *G. dasyurus* (female) and metaphase plates (a,b)
Şekil 3. G. dasyurus' un (dişi) C bant karyotipi ve metafaz plakları (a,b)

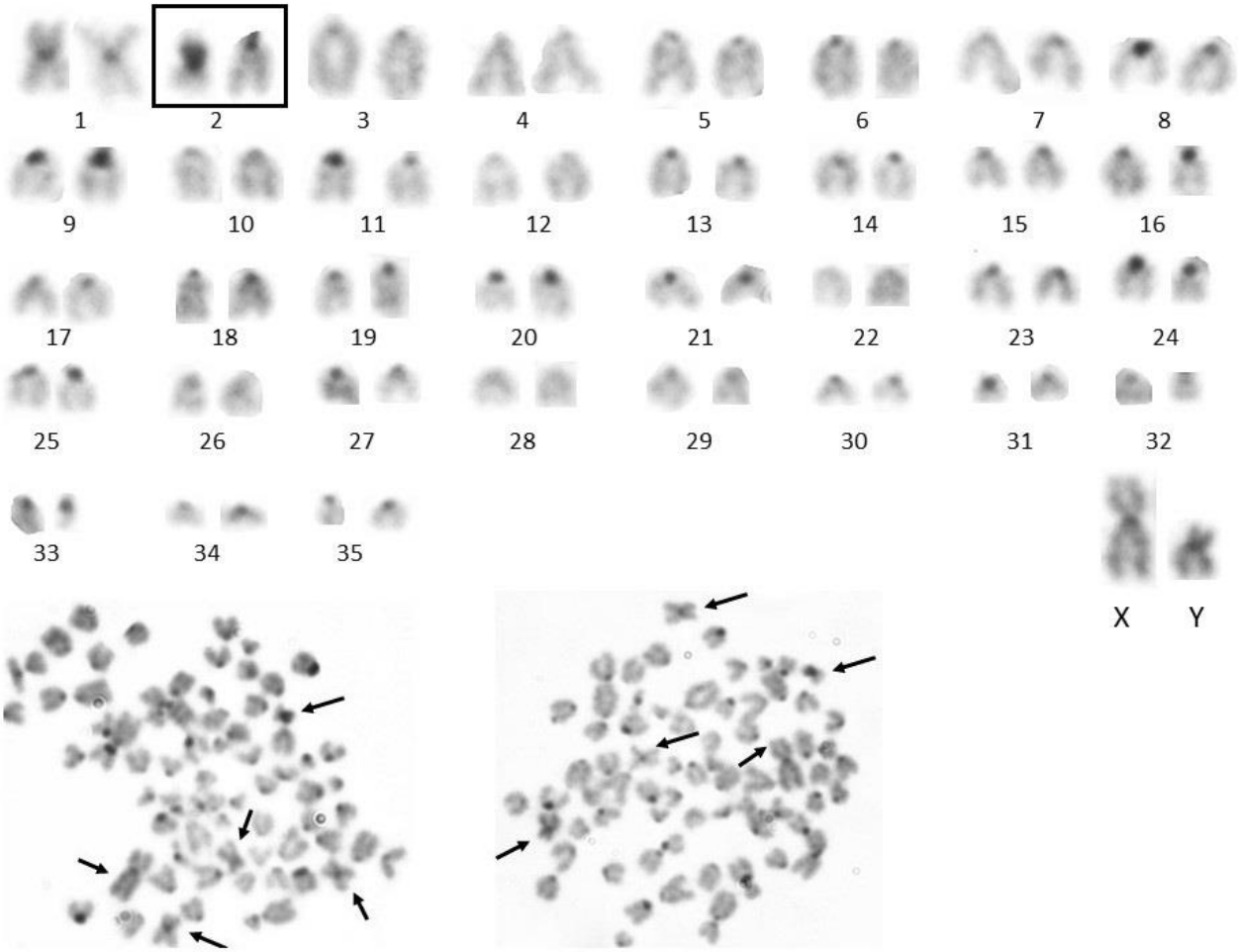

Figure 4. C-banded karyotype of *M. tristrami* (male) and heteromorphic chromosome no:2, arrow: biarmed chromosomes in metaphase plates
Şekil 4. M. tristrami' nin (erkek) C bant karyotipi ve heteromorfik 2. kromozom çifti, Ok. Çift kollu kromozomlar

while variation (NFa= 66, 68, 70) was found in the number of autosomal chromosome arm resulting from the variation in the number of biarmed chromosome (Qumsiyeh et al., 1986). Yiğit et al. (1997) reported that the karyotype of *G. dasyurus* was NFa=66. However, the number of autosomal chromosome arms found was NFa=68 in this study (Table 1). While constitutive heterochromatin distribution may constitute nearly half of the total length of chromosomes in karyotypes of *Gerbillus* species (*G. nigeriae* and *G. aureus*, Pequignot-Viegas et al., 1984; Volobouev et al., 1995), it may also show low rates of heterochromatin density (Aniskin et al., 2006). According to the results obtained this study (Figure 3a), heterochromatin regions in *G. dasyurus* were in centromere regions.

Table 1. Karyological characteristics of *G. dasyurus* and *M. tristrami* species from Türkiye, Southeast Anatolia (Batman, Diyarbakır, Gaziantep and Kilis), Central Anatolia (Ankara, Konya, Kırıkkale, Karaman), West Anatolia (İzmir), East Anatolia (Ağrı), North Anatolia (Kastamonu). Metacentric (M), Submetacentric (SM), Acrocentric (A) chromosome

Çizelge 1. G. dasyurus ve *M. tristrami türlerinin Türkiye' den belirlenen karyolojik özellikleri,* Güneydoğu Anadolu (Batman, Diyarbakır, Gaziantep ve Kilis), Merkez Anadolu (Ankara, Konya, Kırıkkale, Karaman), Batı Anadolu (İzmir), Doğu Anadolu (Ağrı), Kuzey Anadolu (Kastamonu). Metasentrik (M), Submetasentrik (SM), Akrosentrik (A) kromozom

Species *Türler*	**Method** *Yöntem*	**Locality** *Lokasyon*	**2N** *2N*	**NFa** *NFa*	**X** *X*	**Y** *Y*	**References** *Kaynakça*
G.dasyurus	Conventional	Kilis	60	66	SM	A	Yiğit et al., 1997
G.dasyurus	**Conventional, C-banding**	**Kilis**	**60**	**68**	**biarmed**	**-**	**In this study**
M.tristrami	Conventional	İzmir	72	72	M	M	Yiğit et al., 1998
M.tristrami	**Conventional, C-banding**	**Kilis**	**72**	**73**	**biarmed**	**biarmed**	**In this study**
M.tristrami	Conventional	Kilis	72	74	SM	SM	Yiğit and Çolak, 1998
M.tristrami	Conventional	Gaziantep	72	74	SM	-	Kaya, 2010
M.tristrami	Conventional	Various locality	72	76	SM	SM	Kefelioğlu, 1997
M.tristrami	Conventional	Karaman, Kastamonu	72	78	SM	SM	Yiğit et al., 1998
M.tristrami	Conventional	Various locality	72	80	SM	SM	Kefelioğlu, 1997
M.tristrami	Conventional	Kırıkkale	72	80	M	M	Demirbaş and Pamukoğlu, 2008
M.tristrami	Conventional	Unknown	72	80	SM	SM	Arslan and Zima, 2014
M.tristrami	Conventional, Ag-NOR banding	Ankara, Kırıkkale	72	80	M	-	Aşan et al., 2010
M.tristrami	Conventional, C-banding, Ag-NOR banding	Konya	72	80	SM	M	Mahmoudi et al., 2020
M.tristrami	Conventional	Diyarbakır	72	82	M	SM	Kaya and Coşkun, 2012
M.tristrami	Conventional	Ağrı	72	82	A	A	Yiğit et al., 2006
M.tristrami	Conventional, Ag-NOR banding	Batman	72	86	M	M	Ulutürk, 2022

In the studies conducted to find out the karyotype characteristics of *M. tristrami* species distributed in Transcaucasia, a part of Middle East and Anatolia (Armenia and Azerbaijan, Korobitsyna and Korablev, 1980; Palestine, Hermann, 1973; Jordan, Qumsiyeh et al., 1986, Sözen et al., 2008; Iran, Mahmoudi et al., 2020; Anatolia, Table 1), this species was found to have a stable diploid chromosome number (2n=72) and X chromosome was biarmed (except Yiğit et al., 2006). However, the number of biarmed chromosomes

of *M. tristrami* was reported in extensive variation (Matthey, 1957; Korobitsyna, 1975; Korobitsyna and Korablev, 1980; Zima and Král, 1984; Mahmoudi et al., 2020; current study, Table 1). The intraspecific variation of fundamental number of autosomal arms (NFa= 70-86) in *M. tristrami* resulted from the presence/absence of heterochromatic arms (Zima and Král, 1984; Korobitsyna and Korablev 1980; Qumsiyeh et al., 1986). In addition to this, previously conducted studies have reported that heteromorphic chromosome pairs in the autosomal chromosome set of *M. tristrami* species (speciemens from Jordan, Sözen et al., 2008; Azerbaijan and Armenia, Korobitsyna and Korablev, 1980; Türkiye, current study).

Constitutive heterochromatin distribution in *M. tristrami* bi-armed autosomes was C-positive as in previous studies (Korobitsyna and Korablev, 1980; Mahmoudi et al., 2020). In the karyotype of *M. tristrami*, C-heterochromatin distribution of small biarmed chromosomes can be in pericentromeric region, while it may also be in the form of complete C-heterochromatic arm (Mahmoudi et al., 2020). In the results of a previous study performed on the specimens from Transcaucasia (Korobitsyna and Korablev, 1980) and the present study (Figure 4), there was an enlarged heterochromatin block on the short arm of chromosome in heteromorphic chromosome pair.

As a conclusion, it was found that there were differences in the autosomal chromosome arm rates (NFa) of two different gerbil species (*G. dasyurus* and *M. tristrami*), the karyotypes of which were obtained in the present study, when compared with previously conducted studies in Türkiye. Furthermore, autosomal chromosome arm polymorphisms need to be better characterized cytogenetically in similar localities, particularly for *Meriones tristrami.*

Author Contributions

The contribution of the authors is equal

Conflict of Interest

The authors declare that they do not have any competition and any conflicts of interest.

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Rapid Biosynthesis of Silver Nanoparticles by *Celtis tournefortii* LAM. Leaf Extract; Investigation of Antimicrobial and Anticancer Activities

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ABSTRACT
The usage of metallic nanoparticles are very common. Environmentally friendly approaches in obtaining nanoparticles attract a lot of attention because of their advantages. In this study, an easy and economical biosynthesis of silver nanoparticles (AgNPs) was made with the extract of *Celtis tournefortii* LAM. leaf. For the characterization of synthesized nanoparticles, Spectrophotometer (UV-vis), Transmission Electron Microscope (TEM), Field Emission Scan Electron Microscopy (FE-SEM), Atomic Power Microscopy (AFM), Electron Disperse X-ray (EDX) Fourier Transformation Infrared Spectroscopy (FT-IR), X-ray Diffraction (XRD), Thermogravimetric and Differential Thermal Analysis (TGA-DTA), Zeta Sizer and Zeta Potential Analysis data were used. As a result of the data analysis, it was determined that the AgNPs had a spherical appearance, an average size distribution of 4.8 nm, had a maximum absorbance at a wavelength of 482.13 nm, a crystal nanosize of 10.95 nm, and a surface charge of -21.6 mV. Inhibition activities of AgNPs on the growth of pathogenic strains were determined by the microdilution method. The results showed that the nanoparticles were effective even at low concentrations. The Minimum Inhibitory Concentration (MIC) value of the tested materials on the growth of the strains was found between 0.03-1.00 µg mL^{-1}. Anticancer activity of AgNPs was investigated on CaCo-2, U118, Skov3 cancer cell lines and healthy cell line HDF by the MTT method.

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Celtis tournefortii LAM. Yaprak Özütüyle Gümüş Nanopartiküllerin Hızlı Biyosentezi; Antimikrobiyal ve Antikanser Aktivitelerinin İncelenmesi

ÖZET
Metalik nanopartiküllerin kullanım alanları çok yaygındır. Nanopartiküllerin elde edilmesinde çevre dostu yaklaşımlar getirdiği avantajlar ile oldukça fazla ilgi görmektedir. Bu çalışmada, Celtis tournefortii LAM yaprağı özütü ile gümüş nanopartiküllerin (AgNP'ler) kolay ve ekonomik bir biyosentezi yapıldı. Sentez ile elde edilen nanopartiküllerin karakterizasyonu için Spektrofotometre (UV-vis), Geçirmeli Elektron Mikroskopu (TEM), Alan Emisyon Taramalı Elektron Mikroskobu (FE-SEM), Atomik Güç Mikroskopisi (AFM), Enerji Dağılımlı X-Ray Floresans Spektrometre Cihazı (EDX), Fourier dönüşüm kızılötesi spektroskopisi (FT-IR), X-ışını difraksiyon (XRD), termogravimetrik ve diferansiyel termal analizi (TGA-DTA), Zetasizer ve zeta potansiyeli analiz verileri kullanıldı. Analiz verileri sonucunda AgNP'lerin küresel bir görünüme sahip olduğu, ortalama boyut dağılımının 4.8 nm olduğu, 482.13 nm dalga boyunda maksimum absorbansa, 10.95 nm kristal nano boyutuna ve -21.6 mV yüzey yüküne sahip olduğu belirlendi. AgNP'lerin patojen suşların üremesi üzerindeki inhibisyon aktiviteleri mikrodilusyon yöntemi ile belirlendi. Elde edilen sonuçlar nanopartiküllerin düşük konsantrasyonlarda bile etkili olduklarını gösterdi. Test edilen

Biyoloji

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Anahtar Kelimeler
AgNP'ler
Antimikrobiyal
Antikanser
Nanotıp
FE-SEM

materyallerin suşların büyümesi üzerindeki Minimum İnhibitör Konsantrasyon (MIC) değeri 0.03-1.00 µg mL^{-1} arasında bulundu. AgNP'lerin antikanser aktivitesi, MTT yöntemi ile CaCo2, U118, Skov3 kanser hücre hatları ve sağlıklı hücre hattı HDF üzerinde araştırıldı.

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INTRODUCTION

Particles between 1-100 nm are known as nanoparticles (NPs) (Kumar et al., 2017). These particles have a large surface area, superior physical and chemical properties. Among the metallic nanoparticles, Silver (Ag) (Baran 2019a), Gold (Au) (Baran 2020 et al., ; Keskin et al., 2021), Zinc (Zn) (Doğaroğlu et al., 2019), Titanium (Ti) (Baran et al., 2019b) are the commonly used ones. Among these metallic nanoparticles, AgNPs are used in many nanomedical applications, such as anticancer (Abu-Dief et al., 2020), anti-inflammatory (Kumar et al., 2019), antimicrobial (Mohammadi et al., 2019), antioxidant agent (Khalil et al., 2019). In addition, AgNPs can be used in bioremediation applications (Francis et al., 2017), in catalysis studies (Thomas et al., 2018), in cosmetics (Arroyo et al., 2020), and in many different areas including the food industry (Velmurugan et al., 2014). The AgNPs can be obtained by various methods. Among these methods physical, chemical and environmentally friendly biological methods are most used. Biological methods offer several advantages over physical and chemical methods, such as being environmentally friendly, low in cost, and not using toxic chemicals in the synthesis stages (Patil et al., 2018; Rolim et al., 2019). Synthesis studies made by plant sources among biological methods, besides the ease of processing and do not require special conditions stand out with synthesizing more products. (Al-ogaidi et al., 2017).

Antibiotic resistance is an important problem and numberless used antibiotics for treatment are unfortunately ineffective on microorganisms. The development resistance of microorganisms to antibiotics also increases the needing for new effective antimicrobial agents. The antimicrobial effects of AgNPs obtained by environmentally friendly synthesis methods have been demonstrated in many studies (Singh et al., 2018; Baran, 2018; Oliveira et al., 2019; Baran 2019a; Mohammadi et al., 2019; Aktepe et al., 2021a).

Cancer is one of the common diseases of the age and the treatment process is very troublesome. New treatment methods and the discovery of new anticancer agents for this disease are continued. There are some studies on the usability of AgNPs as anticancer agents. (Remya et al., 2015; Sarkar et al., 2018; Satpathy et al., 2018; Pandiyan et al., 2019; Baran et al., 2021; Gomes et al., 2021).

Celtis tournefortii, known as "Eastern fenugreek, Dardağan, Dıgdıge, Taok, Ingires or Dağdağan", is a deciduous tree species that grows in high temperate, tropical regions, and grows in high temperate, tropical regions. It grows in countries such as Turkey, Greece, Croatia, Iran, Ukraine, Iraq, and Azerbaijan. The edible fruits of the tree are often consumed in these countries. *Celtis tournefortii* fruit is used in traditional folk medicine for shortness of breath, chest pain, strengthening and polishing tooth, and healing wound (Ural, 2001; Keser et al., 2017; Kawarty et al., 2020). Studies on the *Celtis* genus reported that have many phytochemicals such as coumarins, tannins, flavonoids, terpenoids, alkaloids, coumaroyl tyramines, steroids, and phenolics are included in their chemical profile (Keser et al., 2017; Yıldırım et al., 2017; Gecibesler 2019). Phenolic compounds show antioxidant activity through the OH groups in their structures. In addition to their antioxidant properties; they have antiallergenic, antimutagenic, anticarcinogenic, antimicrobial, anti-inflammatory and antithrombotic effects. In addition, it is stated that regular nutrition with foods containing high levels of flavonoids reduces the incidence of diseases such as prostate and breast cancer in the society (Demir and Akpınar, 2020).

This study was aimed to synthesize and characterization of the AgNPs with the extract of *Celtis tournefortii* leaves and to determine their antimicrobial and anticancer effects.

MATERIAL and METHOD

Preparation of Plant Extract and Silver Nitrate Solution

Plant leaves (Figure 1B) were collected from Mardin Kızıltepe Region (37°17' 07.3" N and 40° 29' 03.0" E) (Figure 1A) at the end of the summer period (end of August). Plant samples were identified by Dr Cumali KESKİN. Plant samples were washed several times using tap water and then distilled water and it was dried in room condition. 100 grams of dried plant

samples were placed in a flask containing 500 ml of distilled water and allowed to boil. The obtained extract was cooled at room conditions and filtered for the using synthesis of nanoparticles.

Biosynthesis of AgNPs

A solution with a concentration of five millimolar (mM) was prepared from silver nitrate ($AgNO_3$; Sigma Aldrich) salt for the biosynthesis of AgNPs.

100 ml plant extract and 100 ml AgNO3 solution were mixed in a flask. The time depending colour changes were observed at the synthesizing period. To evaluate the formation and presence of AgNPs depending on the color change, samples were taken and wavelength scans were performed to determine the maximum absorbances with the UV-vis spectrophotometer.

Characterization of AgNPs

To detect the formation and presence of AgNPs, samples were taken with the color change due to the reaction, and the maximum absorbance values were examined by scanning in the wavelength range of 200-800 nm using the UV-vis Spectrophotometer (Perkin Elmer One). With FT-IR (Perkin Elmer One) spectroscopy, the spectrum changes of the frequencies of the extract and the reaction liquid obtained after the synthesis in the range of 4000-400 cm-1 were evaluated to determine the functional groups responsible for biosynthesis. The XRD (Rigaku Miniflex 600) device was used to determine the crystal patterns and sizes of synthesized AgNPs at 2θ in the range of 20-80. The below Debye-Scherrer equation was used to compute the crystal sizes (Baran 2018; Baran et al., 2020).

$$D = K\lambda / (\beta \cos\theta) \quad \text{(Eq. 1)}$$

(D = particle size, K = constant value, λ = X-ray wavelength value, β = half of the FWHM value of the maximum peak, θ = Bragg angle of the high peak).

SEM (EVO 40 LEQ), FE-SEM, TEM (Jeol Jem 1010) microscopes were used to determine the morphological appearance of AgNPs as a result of biosynthesis. In addition, the shape and topographic distributions of AgNPs were also shown with micrographs acquired by the AFM (Park System XE-100) device. Elemental compositions of the synthesized particles were evaluated using EDX (RadB-DMAX II computer-controlled) device data. The resistance of AgNPs to heat treatment was determined by thermogravimetric and differential thermal analysis (TG-DTA) are performed on DTG-60H Simultaneous DTA-TG Apparatus Shimadzu. The samples were heated to 25-900 °C at the rate of 10 °C min-1, individually in the atmosphere of dry air and N_2(g) flow rate of 20 cm^3/min. Zeta potential and zeta sizer analysis (Malvern, UK) data were also used to determine the surface charges and size distributions of AgNPs.

Antimicrobial Activities of AgNPs

The antimicrobial activities of synthesized AgNPs were determined against the growth of the pathogenic strains by the microdilution method. Gram positive bacteria *Bacillus subtilis* ATCC 11774 (*B. subtilis*) and *Staphylococcus aureus* ATCC 29213 (*S. aureus*), gram negative bacteria *Pseudomonas aeruginosa* ATCC27833 (*P. aeruginosa*) and *Escherichia coli* (*E. coli*) ATCC25922 were used for MIC assay. In addition, the antifungal effect of AgNPs was determined on *Candida albicans* (*C. albicans*) yeast with the same method. The *C. albicans*, *S. aureus*, and *E. coli* microorganisms were procured from İnönü University Medical Faculty Hospital Microbiology Laboratory (in Malatya, Turkey), and B. subtilis and P. aeruginosa microorganisms were procured from Mardin Artuklu University Microbiology Research Laboratory (Mardin, Turkey).

Gram-positive and gram-negative bacteria (Nutrient agar) and yeast (Sabora dextrose agar) were incubated in the growing medium at 37 °C overnight. McFarland solution was prepared with a turbidity value of 0.5 by using bacteria and yeast strains grown on the medium plates (Emmanuel et al., 2015). Then, Mueller-Hinton Broth (bacteria) and Roswell Park Memorial Institute 1640 broth (yeast) medium were transferred to 96 well microplates in appropriate amounts for microorganisms. Some wells were designated for sterilization and control steps. Solutions containing AgNPs at different concentrations were prepared and transferred into microplates. Afterwards, a series of micro dilutions were applied to the wells to ensure the distribution of AgNPs in the medium. After this process, microorganism solutions prepared according to McFarland 0.5 were added into the microplate wells. At the end of the administration, the prepared microplates were kept in incubation at 37 °C for one night. After the incubation period, the microplates were examined for the growth of microorganisms. The concentration of the well where growth occurred was determined as the MIC value.

Anticancer Effects of AgNPs

Anticancer effects of AgNPs obtained by biosynthesis were carried out in Dicle University Scientific Research Center, Cell Culture Laboratory (Diyarbakir, Turkey), using the MTT method. Cell lines used in the experimental application were obtained from the American Type Culture Collection (ATCC). The cytotoxic effects on Glioblastoma (U118), Human Colorectal Adenocarcinoma (Caco-2), Human Ovarian sarcoma (Skov-3) cancer cell lines used in the application, as well as on healthy cell line Human Dermal Fibroblast (HDF) cell lines were investigated.

CaCo2, U118, and HDF cell lines were grown in 75 t-flasks in Dulbecco's Modified Eagle (DMEM) medium containing 2 mM L-Glutamine, 10% FBS, 100 U/ml Penstrep. The Skov-3 cell line was also grown in RPMI media with 10% FBS, 100 U/ml Penstrep in 75 t-flasks. The flasks of the cultured cell lines were kept in an oven at 37 °C with 5% CO_2, 95% air, and humidity conditions for cell growth. Then, it was examined whether the cell lines were at 80% confluence with a hemocytometer and resuspended at different concentrations. Suspended cell lines were transferred to 96-well microplates and incubated overnight. At the end of the period, AgNPs with concentrations ranging from 25 µg ml^{-1} to 200 µg ml^{-1} were added to the wells cultured with cell lines and allowed to interact for 48 hours. After the interaction period, MTT solution was added to the microplate wells and waited for 3 hours. Then DMSO (Dimethyl sulfoxide) was added and waited for 15 minutes. After these procedures, the absorbance data of the cells were evaluated at a wavelength of 540 nm by the MultiScan Go, Thermo device. The Absorbance values of the cell lines and the concentrations of AgNPs that suppressed the % viability on the cell lines were calculated using Equation 2 given below (Remya et al., 2015)

$$\%viability = U/C*100 \quad (2)$$

In the equation, U; The absorbance values of the cells after interaction with AgNPs, C; represents the absorbance values of control cells.

RESULT and DISCUSSION

UV-vis spectrophotometric data

A rapid color changes off yellow to brown were observed after the 5 minutes from mixing solution. This color change and the 482.13 nm maximum absorbance bands (Figure 1) that show surface plasma resonance vibrations (SPR) due to the formation of AgNPs are characteristic data of AgNPs (Luna et al., 2015; Eren and Baran 2019).

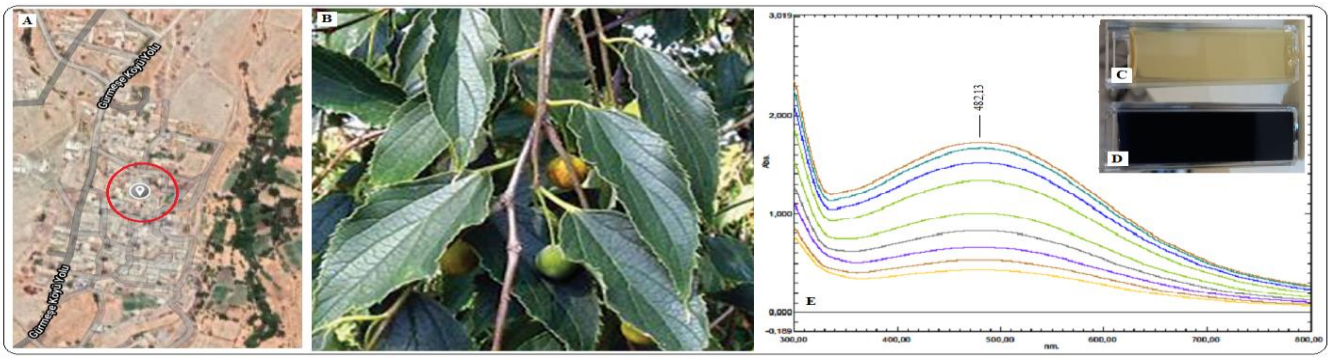

Figure 1. A. *C. tournefortii* geographical location of the growing area, **B**. leaves of the plant, **C.** plant extract image, **D**. color change as a result of the synthesis of AgNPs, and **E**. UV-vis Spectrophotometric data showing the presence of AgNPs

Şekil 1. **A**. *C. tournefortii*'nin yetiştiği alana ait coğrafi konum, **B**. bitkinin yaprakları, **C.** bitki özütü görüntüsü, **D**. AgNP'lerin sentezi sonucunda meydana gelen renk değişimi ve **E.** AgNP'lerin varlığını gösteren UV-vis spektrofotometrik verileri

XRD analysis

XRD analysis data performed to evaluate the crystal pattern and dimensions of AgNPs, the spectra were taken at 111°, 200°, 220°, and 311° at 2θ. The values of these spectra determined as 38.06, 44.35, 64.35, and 77.26 respectively (Figure 2). XRD data show that the crystal pattern of AgNPs are cubic and they have 10.95 nm crystal size. (Krishnaraj et al., 2010; Patra et al., 2016; Rani et al., 2020).

FTIR analysis

FTIR spectra of the plant extract and the reaction liquid after synthesis were used in the evaluation of the functional groups of phytochemicals that may be responsible for the reduction of the Ag^+ form to the Ag^o form. Frequency shift was detected at three points (Figure 3). The shifts in the FTIR spectrum at 3337. 81-3330. 20 cm^{-1}, 2122. 95-2122. 80 cm^{-1}, and 1635. 48-1635. 33 cm^{-1} are due to the bioreduction of hydroxyl groups (O-H stretch), aromatic groups (C=C stretch), flavonoid and phenolic groups (C=O stretching) has shown to be effective (Kumar et al., 2015; Khan et al., 2018; Hemmati et al., 2019; Jebril et al., 2020). In addition, the frequency shift occurring at 163548 cm^{-1} showed the effectiveness of proteins as a capping agent in AgNPs in ensuring the stability of AgNPs in synthesis (Hemmati et al., 2019).

FESEM and TEM micrographs of AgNPs

The images of the morphological appearances of AgNPs in FE-SEM and TEM micrographs were determined. As seen in Figure 4, TEM and FESEM micrographs showed that AgNPs exhibited a spherical appearance (Kumar et al., 2016, Othman et al., 2017, Thomas et al., 2018, Wongpreecha et al., 2018). AgNPs were evaluated to have an average size

of 5.28-10.80 nm in TEM micrographs (Gopinath et al., 2016; Atalar et al., 2021).

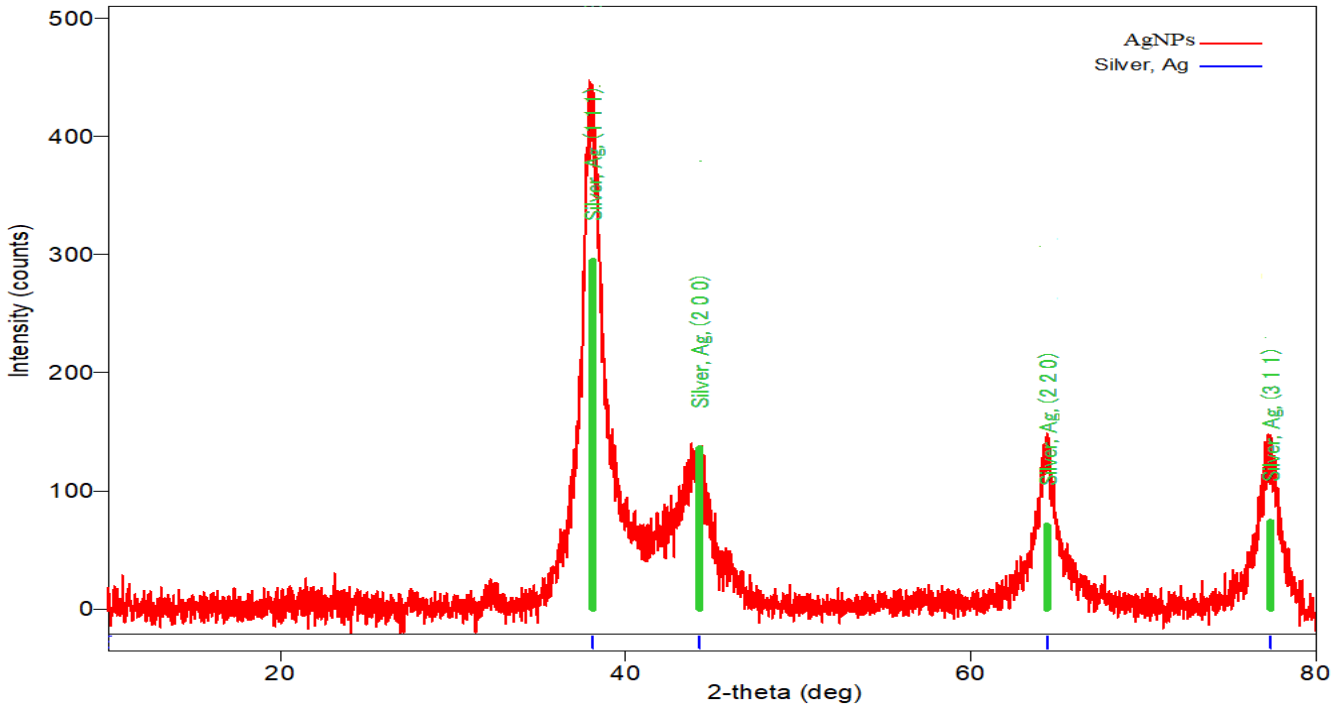

Figure 2. X-ray diffraction of the crystal patterns of biosynthesized AgNPs
Şekil 2. *Biyosentezi yapılan AgNPlerin kristal desenlerine ait X-ray diffractionu*

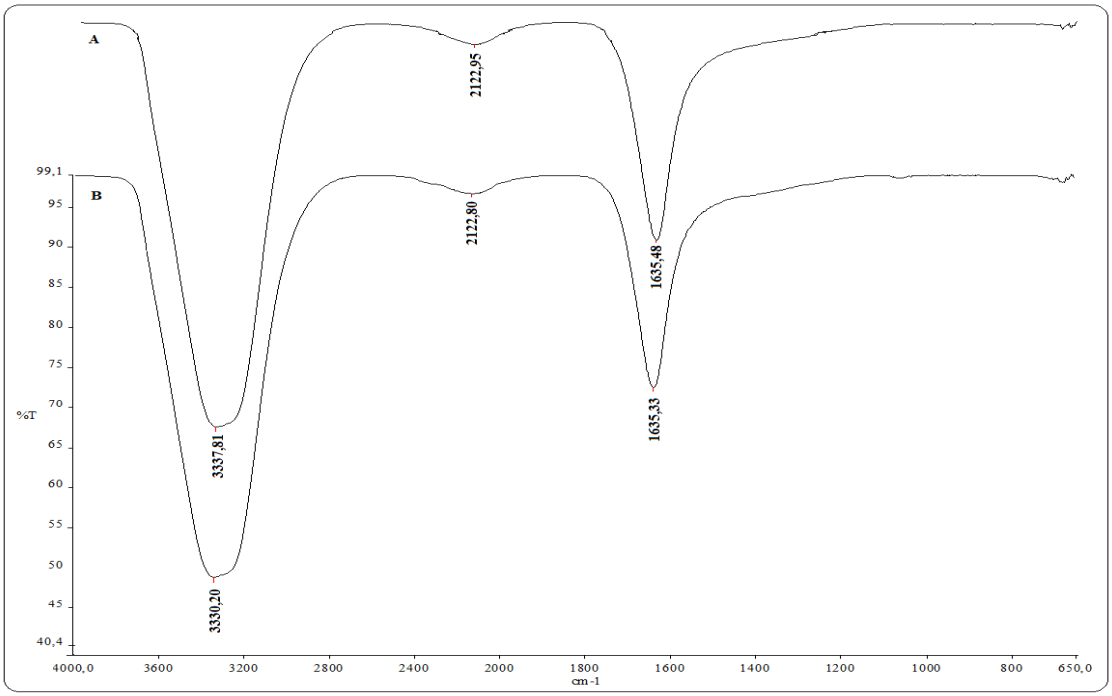

Figure 3. FTIR spectra of *C. tournefortii* plant extract **(A)** and reaction liquid obtained as a result of Synthesis **(B)**
Şekil 3. *FTIR spektrumları; **A.** C. tournefortii bitki özütü ve **B.** Sentez sonucunda elde edilen reaksiyon sıvılarının ait*

EDX analysis

EDX data was used for the evaluation of the elemental compositions of nanoparticles synthesized with *C. tournefortii* leaf extract. It was determined that almost all of the particles were formed by AgNPs with the presence of strong peaks belonging to the silver field (Pallela et al., 2018). The low peaks of elements such as carbon and oxygen are due to the phytochemicals in the plant extract, which act as a capping agent that provides stability on the surface of AgNPs (Vastrad 2016; Kumar et al., 2019; Das et al., 2021). The frequency shifts in the FTIR spectra in Figure 3 also supports this situation.

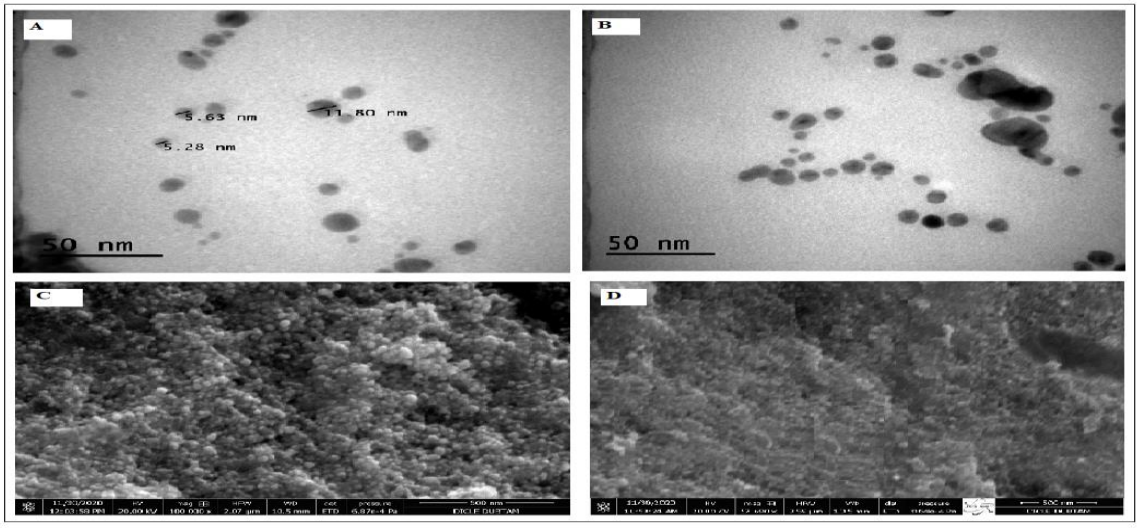

Figure 4. Morphological appearances of AgNPs after biosynthesis with *C. tournefortii* leaf extract; **A** and **B** TEM, **C** and **D** FESEM micrographs

Şekil 4. *C. tournefortii yaprak özütü ile biyosentez sonrası AgNP'lerin morfolojik görünümleri; **A** ve **B** TEM, **C** ve **D** FESEM mikrografları*

TGA-DTA analysis

TGA-DTA data obtained at 25-1000 °C was used to determine the resistance of AgNPs to temperature changes. In the graphics, it was seen that 2.47%, 9.38% and 6.38% mass losses occurred at 18.4 °C, 423.72 °C and 878.41 °C, respectively (Figure 5). The first mass loss is due to the loss of water adsorbed at 18.4 °C. The second mass loss at 423.72 °C and the third at 878.41 °C is due to the phytochemicals found on the surface of AgNPs (Baran 2019c; Rolim et al., 2019; Keskin et al., 2021). The weak C and O peaks in the EDX profile in Figure 6 also confirm this situation.

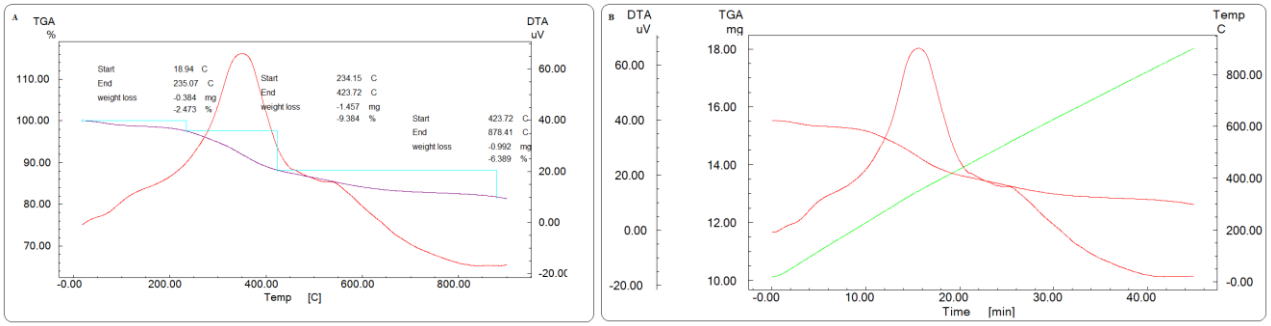

Figure 5. AgNPs Thermal resistance temperature points with **A** and **B** TGA-DTA data of AgNPs after biosynthesis

Şekil 5. *Biyosentez sonrası AgNP'lerin **A** ve **B** TGA-DTA datalarıyla termal direnç sıcaklık noktaları*

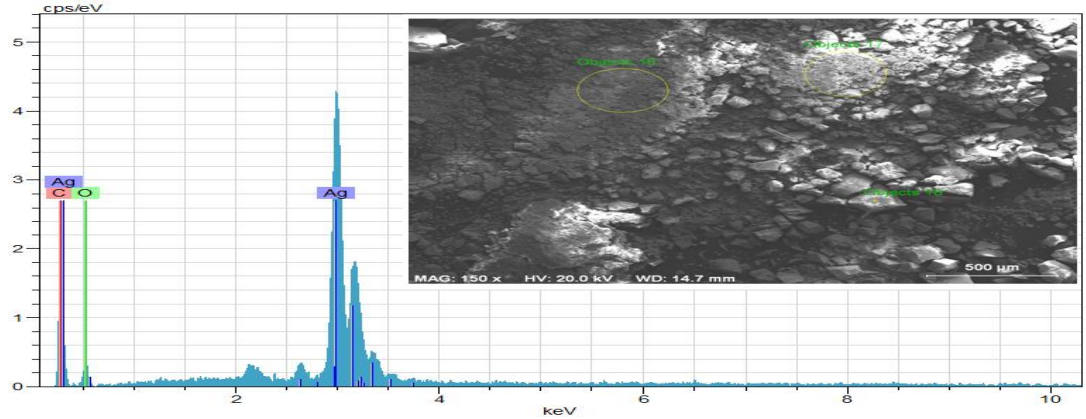

Figure 6. EDX profile of elemental compositions of post-biosynthesis particles with *C. tournefortii* plant extract

Şekil 6. *C. tournefortii* bitki özütü ile biyosentez sonrası partiküllerin element kompozisyonlarının EDX profili

Zeta size and zeta potential distributions of AgNPs

The zeta size and zeta potential analyses were performed to determine the surface charges and size distributions of the biosynthesized AgNPs, it was determined that AgNPs has an average size distribution of 4.8 nm and a surface charge of -21.6 mV. (Figures 7A and 7B). There is no standard size in nanoparticle synthesis, different sizes of nanoparticles can be synthesized. Remya et al., 2015, Alkhulaifi et al., 2020, and Singh et al., 2018 reported the average nanoparticle sizes as 27-32 nm, 59.74 nm and 5-10 nm, respectively. On the other hand, synthesized AgNPs exhibit only negative charge distribution is important data showing that AgNPs are stable. Green way synthesized AgNPs show better negative charge distribution compared to conventional (Chemical and physical way) synthesis studies. In this polymer matrix, the presence of oxidized hydroxyl groups of free electrons is the factor that makes the system more stable in colloidal form. The negative charge distribution is due to the phytochemicals present on the surface of AgNPs (Pugazhendhi et al., 2018; Oliveira et al., 2019; Jebril et al., 2020). The results of FTIR spectra, EDX profile, and TGA-DTA graphs given in Figure 3, Figure 5, Figure 6, support this data. The different surface charges of AgNPs cause problems such as fluctuation and aggregation due to electrostatic attraction between nanoparticles (Al-ogaidi et al., 2017; Satpathy et al., 2018) and this situation negatively affects their stable structure (Al-ogaidi et al., 2017; Satpathy et al., 2018; Patil et al., 2018). AgNPs obtained by biosynthesis exhibited a stable structure with the surface charge distribution of -21.6 mV. In other studies reported that the obtained AgNPs show -19 mV (Al-ogaidi et al., 2017; Oliveira et al., 2019) and −22 ± 5 mV (Ferreyra Maillard et al., 2018) zeta potential distribution.

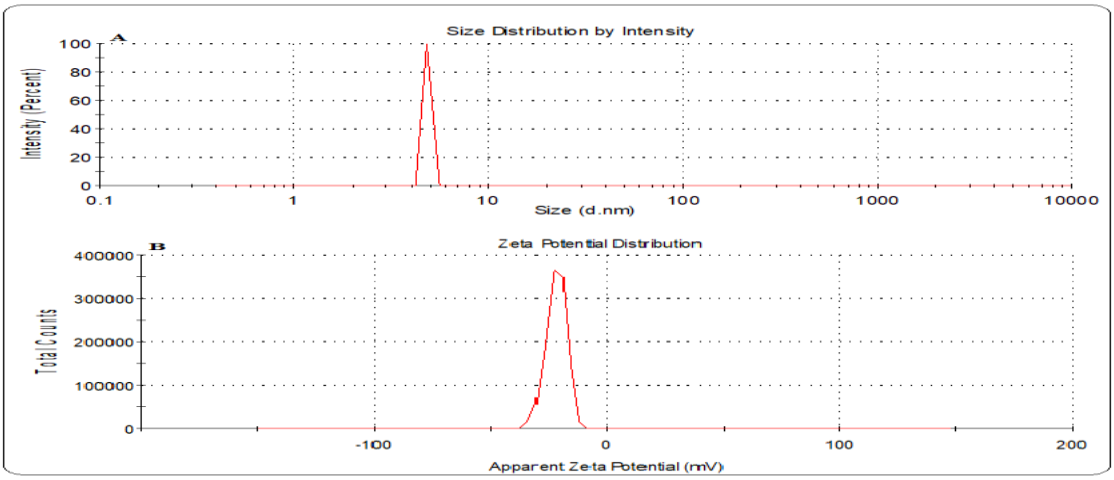

Figure 7. AgNPs after biosynthesis; A. Density-dependent size distribution, B. Zeta potential charge distribution graphs

Şekil 7. *Biyosentez sonrası AgNPlerin; A. Yoğunluğa bağlı boyut dağılımı, B. Zeta potansiyeli yük dağılımı grafikleri*

AFM micrographs of AgNPs

Topographic distributions and structures of the biosynthesized AgNPs were evaluated by AFM. As seen in Figure 8, it was seen that AgNPs were spherical, showed monodisperse properties and had dimensions below 15 nm (Swamy et al., 2015; Kumar et al., 2017; Rauf et al., 2021).

Antimicrobial effects of AgNPs

The effects of AgNPs synthesized with *C. tournefortii* leaf extract on pathogen strains were determined by defining MIC by the microdilution method. To compare this effect, the same application steps were performed in standard antibiotics and $AgNO_3$ solution. The data show that concentrations of 0.03-1.00 µg mL-1 have a suppressive effect on the growth of microorganisms (Table 1 and Figure 9). 0.06-0.13 µg mL^{-1} and 0.50-1.00 µg mL^{-1} AgNPs concentrations showed effective suppressor activity on gram-positive *B. subtilis*, *S. aureus* and gram-negative bacteria *P. aeruginosa*, *E. coli* respectively. AgNPs showed effectiveness on *C. albicans* growth with 0.03 µg mL^{-1} at a much lower concentration than antibiotics and silver nitrate solution. AgNPs are ionized in a liquid medium and tend to show high reactivity. After being ionized in the aqueous environment, they interact with the microorganisms in the same environment depending on the electrostatic attraction force (Narayan and Dipak 2015; Ahmed et al., 2016; Aina et al., 2018). After the interaction, they cause an increase in reactive oxygen species (ROS) within the microorganisms. Biomolecules with a high affinity for ROS (such as cell membrane, DNA, RNA, some enzymes) are adversely affected by this situation. The functions and structure of adversely affected

biomolecules are impaired. Due to these reasons, the death of the microorganism occurs (Emmanuel et al., 2015; Shao et al., 2018). In Table 2, some biosynthesis studies using the green synthesis method are given.

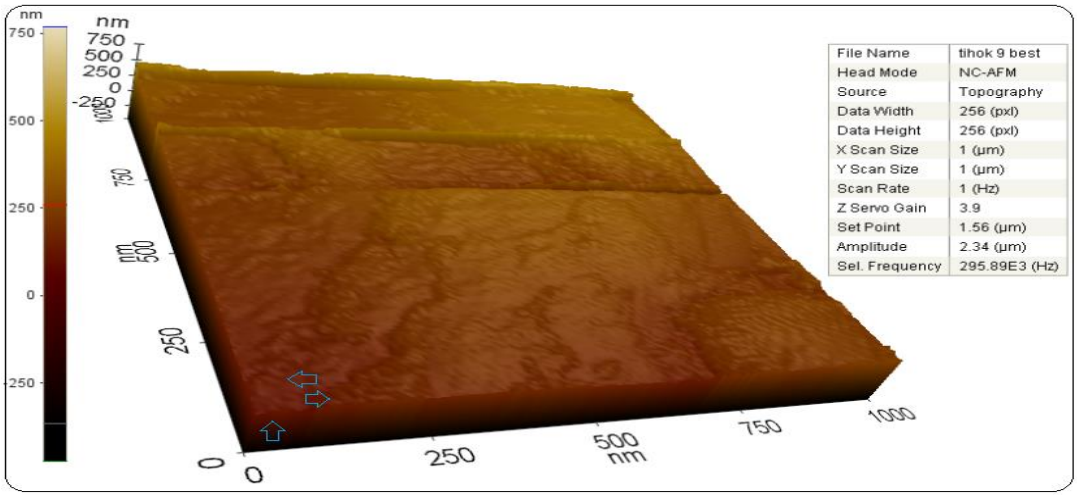

Figure 8. AFM micrograph of topographic structures and shapes of AgNPs
Şekil 8. AgNP'lerin topografik yapı ve şekilerinin AFM mikrografisi

Table 1. Antimicrobial effect concentrations of AgNPs, antibiotics and silver nitrate solution on the growth of microorganisms
Tablo 1. *AgNP'lerin, antibiyotiklerin ve gümüş nitrat çözeltisinin mikroorganizmaların üremeleri üzerinde antimikrobiyal etki gösterdikleri konsantrasyonları*

TESTED ORGANISM	AgNPs µg mL^{-1}	Silver Nitrate µg mL^{-1}	Antibiotic µg mL^{-1}
S. aureus	0.13	2.00	2.00
B. subtilis	0.06	1.00	0.50
E. coli	1.00	4.00	4.00
P. aeruginosa	0.50	2.00	4.00
C. albicans	0.03	0.50	1.00

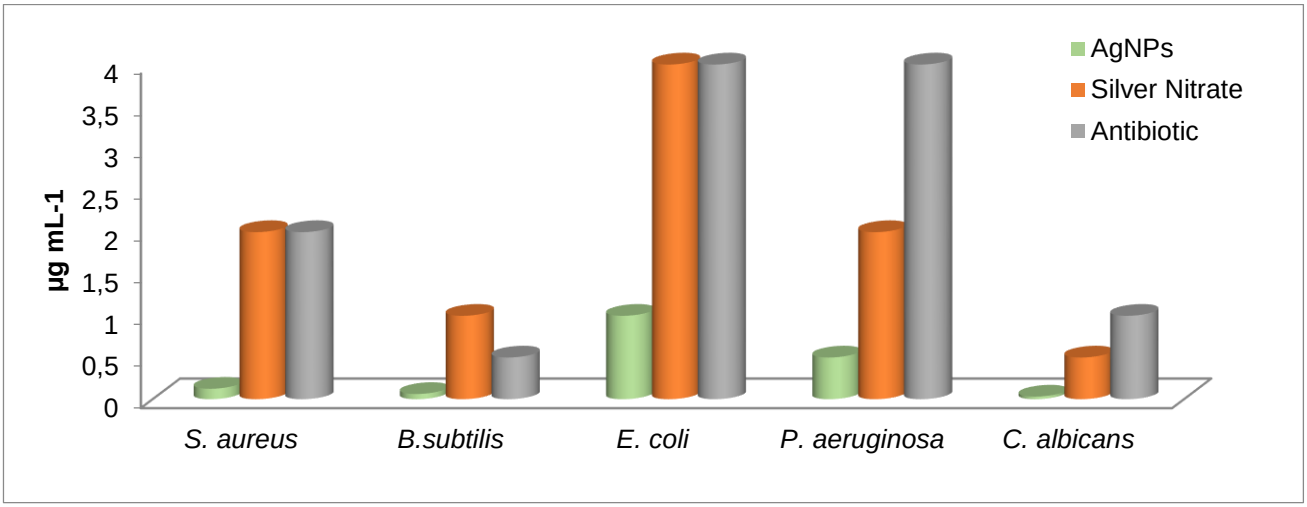

Figure 9. Comparison of Antimicrobial Effects of AgNPs with $AgNO_3$ and antibiotics
Şekil 9. AgNP'lerin Antimikrobiyal etiklerinin $AgNO_3$ ve antibiyotiklerle karşılaştırılması

Anticancer Effects of AgNPs

The most important advantage of plant-based synthesis is that there is no need to use chemicals. Phytochemicals (phenols, amines, flavonoids, terpenes), especially phenolic compounds, in their structure facilitate the synthesis. In addition, these phytochemicals can be shown as the source of many other biological activities of nanoparticles. The functional groups of these phytochemicals surrounding nanoparticles are preferred in anticancer studies due to their bonding properties (Demir at al., 2020; Aktepe et al., 2021b). The viability-suppressing

effects of biosynthesized AgNPs were investigated using the MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyl tetrazolium Bromide) method on U118, CaCo-2, and Skov-3 cancer cell lines and healthy cell line HDF. As seen in Table 3 and Figure 10, AgNPs have a very low toxic effect on healthy cell line HDF at a concentration of 25 µg mL^{-1} with a viability rate of 79.45%. In cancer cell lines, it was observed that it was most effective on the CaCo-2 cell line with an inhibition rate of 58.99%. On the U118, Skov-3 cell lines, it was determined that the viability rate of 24.83% and 30.86%, respectively, was suppressed at 200 µg mL^{-1} concentration.

Table 2. Antimicrobial effects of AgNPs obtained by biosynthesis in green synthesis studies
Tablo 2. *Green sentez çalışmalarında elde edilen AgNP'lerin antimikrobiyal etkileri*

Biological Source	Average Size (nm)	Gram-Negative µg mL^{-1}	Gram-Positive µg mL^{-1}	References
Camellia sinensis	23	7-30	30-250	(Rolim et al., 2019)
Chitosan	>20	39.1	312.5	(Wongpreecha et al., 2018)
Fritillaria	10	2-8	1-4	(Hemmati et al., 2019)
Citrullus lanatus	21.27	0.13-0.25	0.50-1.00	(Aktepe and Baran, 2021a)
Acalypha indica	20-30	10	10	(Krishnaraj et al., 2010)
Hawthorn leaf	16.50	0.11	0.25	(Baran, 2019b)
Madhuca longifolia	30-50	80-90	40-60	(Patil et al., 2018)
Abelmoschus esculentus	19.05	0.12-0.50	0. 03-0.12	(Hatipoğlu, 2021)
Prunus amygdalus L. (almond)	14.67	1.00-2.00	0.13	(Aktepe and Baran 2021b)
Pistacia terebinthus	15	0.8	0.32	(Baran 2018)
C. tournefortii leaf extract	10	0.06-0.13	0.50-1.00	This study

Table 3. % viability rates as a result of interactions of AgNPs with cell lines
Tablo 3. *Hücre hatlarının AgNP'lerle etkileşimleri sonucunda % canlılık oranları*

Cell Line	25 µg mL-1	50 µg mL-1	100 µg mL-1	200 µg mL-1
HDF	79.45	67.55	67.55	41.00
U118	93.93	84.57	78.86	75.17
CaCo-2	41.01	34.22	32.42	26.69
Skov-3	100	95.76	90.88	69.14

The AgNPs have high oxidative effects and tend to settle on some biomolecules (such as cell membrane, nucleus). They are localized to these biomolecules and cause toxic effects by causing damage to biomolecules with the increase of ROS, and also by stimulating apoptosis and directing the cell to death (Gliga et al., 2014; Remya et al., 2015; Morais et al., 2020). As a result of these effects, the toxic effects of Ag^{+} ions released in living environments should be examined and evaluated (Wongpreecha et al., 2018). Some properties of nanomaterials play a decisive role in their toxic effect. These include properties such as concentration, shape, charge, exposure time, chemistry of the surface composition, degree of deposition, and size (Rolim et al., 2019).

In environmentally friendly synthesis studies, it has been stated that AgNPs have toxic effects on CaCo-2 from cancer cell lines at concentrations of 3.75 µg mL^{-1} (Mohmed et al., 2017) and 5 µg mL-1 (Zein et al., 2020) in suppressing viability. In studies with HDF and Skov-3 cells, it was stated that 25 µg mL-1 concentration suppressed the viability of the cells (Aktepe et al., 2021a; Baran et al., 2021). Various properties of nanoparticles such as interaction times, sizes, surface charges, concentrations, shapes, deposition degrees play a role in determining their toxic activities (Remya et al., 2015; Swamy et al., 2015).

CONCLUSION

This is the first study on biomedical applications of silver nanoparticles obtained using *Celtis tournefortii* leaf extract. The biosynthesis of AgNPs was carried out in an environmentally friendly, easy and cost-effective way. The characteristic properties of the synthesized AgNPs were determined by UV-vis, FT-IR, FE-SEM, TEM, AFM, EDX, XRD, TGA-DTA, Zeta sizer, and Zeta potential analysis data. It was determined that AgNPs had an average size of 10 nm, a spherical morphological appearance, a maximum absorbance of 483.13 nm, and a surface charge of -21.16 mV. The results of analyzing data such as TGA-DTA, EDX, AFM showed that these AgNPs have only a negative charge with -21.6 mV and that AgNPs

have a stable structure. The Microdilution method was determined that synthesized AgNPs showed the antimicrobial effect on pathogen strains even at very low concentrations. The MTT assay data demonstrated the suppressive effect of AgNPs (25 µg mL^{-1}), especially on the CaCo-2 cancer cell line, with a survival rate of 58.995%. In addition, in the examinations of AgNPs to evaluate their toxic effect on healthy cells lines (HDF), it was seen that it has a quality that can eliminate the toxic effect concerns with its 79.45% viability rate when used as an anticancer agent. It is thought that the application steps of biosynthesized AgNPs will contribute to the search for antimicrobial and anticancer agents, especially in biomedical applications.

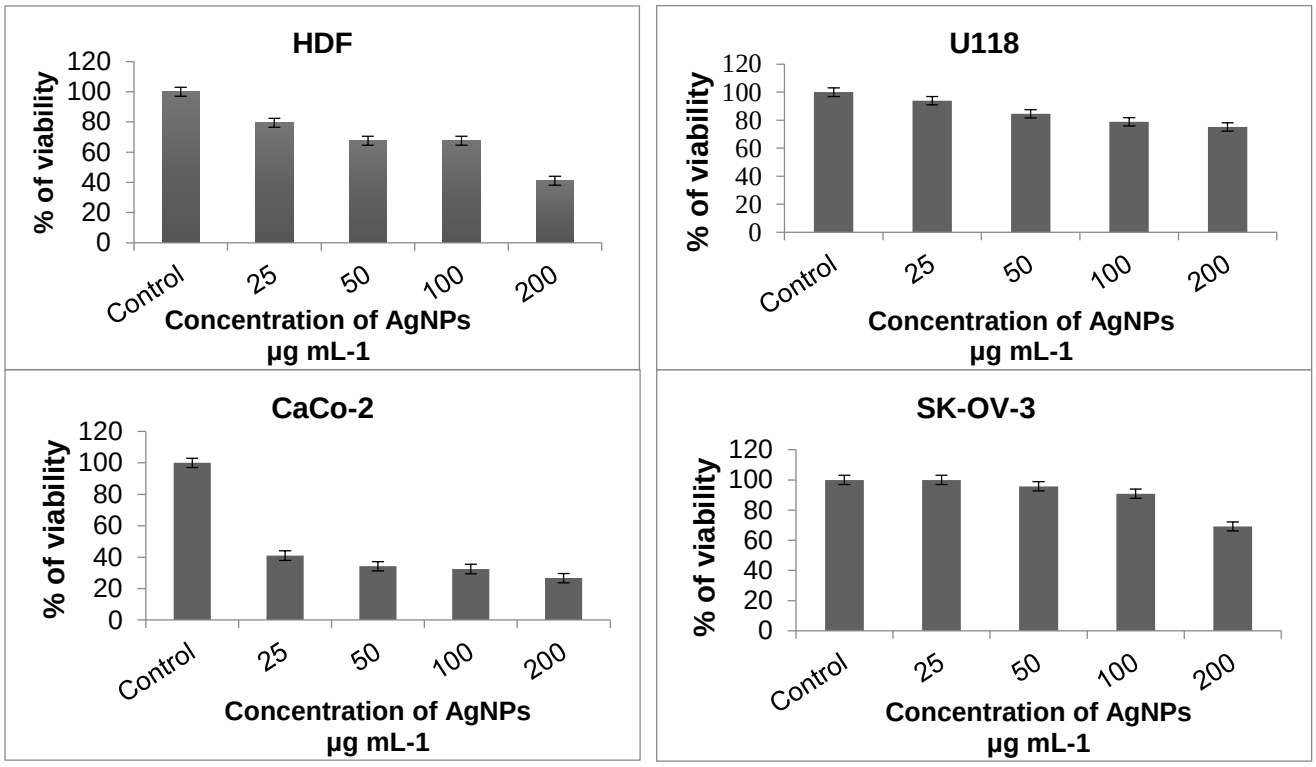

Figure 10. % viability rates resulting from the interaction of AgNPs on cell lines for 48 hours with the MTT method

Şekil 10. *AgNP'lerin MTT metoduyla hücre hatları üzerinde 48 saat etkileşimleri sonucu canlılık oranları*

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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.

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Contribution to Knowledge on The Anatomy of The Genus *Noccaea* Moench (Brassicaceae) From Türkiye

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ABSTRACT
The anatomical properties of *Noccaea camlikensis* and *N. cariensis* were determined in this study. The roots have a secondary root type. The stem cross-sections have a single-layered epidermis, parenchymatous cortex, one layered distinct endodermis, vascular bundles with sclerenchymatic caps, and parenchymatic pith cells. Equifacial and bifacial mesophyll types are observed in the leaves, with multiple-layered palisade tissue, and the spongy parenchyma is well-developed. Vascular bundles are small in the leaves except in the leaf midrib. Anisocytic stomata type are observed in the surface sections. The assessment of anatomical characteristics of the studied *Noccaea* species, such as the number of cortex in the stem and the shape of midvein of leaf, are of taxonomical value.

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Türkiye'den *Noccaea* Moench (Brassicaceae) Cinsi Anatomisi Üzerine Katkılar

ÖZET
Bu çalışmada, *Noccaea camlikensis* ve *N. cariensis*'in anatomik özellikleri belirlenmiştir. Kökler, sekonder kök tipine sahiptir. Gövde enine kesitlerinde, tek tabakalı epidermis, parankimatik korteks, tek tabakalı belirgin endodermis, sklerenkimatik başlıklı iletim demetleri ve parankimatik öz hücreleri vardırYapraklarda çok tabakalı palizat parankiması ve 3-4 sıralı sünger parankiması bulunan ekvifasiyal mezofil tipi gözlemlenmiştir. İletim demetleri orta damar hariç küçüktür. Yüzeysel kesitlerde anizositik tip stoma gözlemlenmiştir. İncelenen *Noccaea* türlerinin gövde korteksinin sayısı ve yaprak orta damarının şekli gibi anatomik özelliklerinin değerlendirilmesi taksonomik değere sahiptir.

Biyoloji

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INTRODUCTION
The family Brassicaceae, or mustard family, is a monophyletic group of about 338 genera and 3709 species with global distribution (Hall et al. 2002; Bailey et al. 2006). An evaluation of its morphology and generic circumscriptions, and a new tribal alignment was proposed by Al-Shehbaz (Al-Shehbaz, 2012).

In the past, the generic and subgeneric concepts of the genus *Thlaspi* L. (Brassicaceae) have changed several times and have been the subject of partly polemically debated classifications. Meyer (1973, 1979; summary in 1991), who introduced a classification largely based on the seed-coat anatomical characters, which were considered to be conservative and thus apt for obtaining a more natural system, while the siliquae characters proved to have evolved convergent evolution, to split *Thlaspi* into 12 genera. These proposals were rejected by the Med-Checklist (Greuter et al., 1986) and the most recent standard Floras, such as Flora Europaea (Clapham and Akeroyd, 1993), Flora Iberica (Pujadas Salvá, 1993), Flora of Turkey Supplement 1 (Davis et al., 1988) Flora Hellenica (Artelari, 2002), and, by inference, also Flora of Turkey Supplement 2 (Yildirimli, 2000). In contrast, they were convincingly supported by the molecular-based studies of Mummenhoff and Zunk (1991), Koch et al. (1993), Mummenhoff and Koch (1994), Mummenhoff et al. (1997a, b) and Koch and Mummenhoff (2001).

When Aytac et al. (2000) described *Noccaea camlikensis* Aytaç, Nordt and Parolly as a new species for the genus *Noccaea*, they largely adopted Meyer's classification and generic concepts. Indeed, recent floristic studies (e.g., Al-Shehbaz, 2010, 2012; Al-Shehbaz and Watson, 2011) have recognized *Noccaea* as distinct from *Thlaspi*. Later, Al-Shehbaz (2014) announced a synopsis of the genus *Noccaea*, and *Thlaspi cariensis* Carlström was accepted as *Noccaea cariensis* (Carlström) Parolly, Nordt and Aytaç. In his *Noccaea* synopsis, Al-Shehbaz (2014) used a broad concept for delimitation of that genus and transferred all Meyer's segregates to *Noccaea*, with the exception of *Thlaspi* s.str. and *Noccidium*. The aim of the present study is not to discuss those controversial issues and in this study the generic concept of *Noccaea* adopted by Al-Shehbaz (2014) is accepted. Threated categories of *N. camlikensis* and *N. cariensis* are evaluated as critically endangered (CR) (IUCN, 2001).

For years, anatomical characters have been of crucial importance in detecting the taxonomic and phylogenetic relationships of particular plant groups and have been successfully used in the Brassicaceae (Atçeken et al., 2016; Karaismailoğlu, 2019; Şirin and Karaismailoğlu, 2020; Çıtak and Dural, 2020).Metcalfe and Chalk (1957) indicated that the important discriminative anatomical traits of Brassicaceae include stomata and epidermal cell type and structures of the vascular bundles, which may supply insight into many taxonomical characters displayed to be significant in the species classification (Stace, 1984). Some notes on *Thlaspi* genus were declared include the pattern of epidermal cell and mesophyll layers, the number and size of vascular bundles, and the thickness of the cortex and endodermis (Karaismailoğlu and Erol, 2020). However, there has been no taxonomic research conducted on the anatomy of the genus *Noccaea* in Turkey. Thus, the main aims of this study were to 1) identify and examine the anatomical characteristics of Turkish *Noccaea camlikensis and N. cariensis* and 2) elucidate the systematic value of the these traits.

MATERIAL and METHODS

Species collection

The specimens of *N. camlikensis* and *N. cariensis* were collected from the below-mentioned localities. The plant specimens of the studied species were stored at the herbarium of the Department of Biology, University of Selçuk (KNYA).

N. camlikensis: C4 Konya: Derebucak, Çamlık Village, Kızıldağ, stony places, 1400–1500 m., 21.05.2015, *H.Dural*-3569-*B.Çıtak*
N. cariensis: C2 Muğla: Marmaris, stony places, 1000 m., 03.06.2015, *H.Dural*-3590-*B. Çıtak*.

Anatomical analysis

The paraffin method was applied to the vegetative organs of both studied *Noccaea* species for taking the cross-sections (Johansen, 1940). The handmade cross-sections of the stems and superficial sections of the stomata were stained with phloroglusinol-HCl. On average, 20 preparations were made for each type of section for the 20 pieces of plant material, and 30 cell groups were measured. The measurements of the cells were made using Kameram 21 software (Argenit, Istanbul, Turkey). For vessel grouping in the xylem, the Metcalfe and Chalk (1957) classification was used.

RESULTS and DISCUSSION

Noccaea camlikensis

Root anatomy

The secondary root structure was observed in the root cross-sections of *N. camlikensis* with the peridermis, cortex, phloem and xylem (Fig. 1-A). The peridermis was a protective tissue composed of disintegrating or squashed cells. The width of the peridermis cells was 57.24±11.03 µm (Table 1). The cortex was 4–5 layered, and followed the periderm towards the center. The phloem was well-developed, and the cambium was not distinguished clearly (Fig. 1-B). Vessels in the xylem were irregular, according to Metcalfe and Chalk (1957) classification of vessel grouping. The center of the roots in the cross sections was covered with xylem (Fig 1A-C).

Stem anatomy

The cross-sections of the stem had an epidermis layer in the outermost surface. The cortex was 6–7 layered and characterized by parenchymatic cells (Fig. 2-A,B). Their dimension was 26.99 ± 5.77 µm (Table 1). The endodermis was rowed and fusiform-shaped (Fig. 2-A,B). The phloem and xylem were well-developed. Above the phloem, sclerenchymatic cells were present (Fig. 2-C). The diameter of the tracheas was 17.29 ± 2.86 µm (Table 1). The pith region of the stem consisted of large parenchymatic cells (Fig. 2-A).

Leaf anatomy

The cross sections of the leaf showed that the upper and lower epidermis were made up of rectangular cells with adaxial and abaxial cuticles (Fig. 3A-B). Cells of the lower epidermis (52.61 ± 31.9 µm wide × 37.96 ± 16.9 µm long) were wider than those of the upper epidermis (39.13 ± 12.6 µm wide × 41.11 ± 14.9 µm long) (Table 1)(Fig. 3B-C). The leaf was amphistomatic and bifacial. Vascular bundles were composed of phloem and xylem (collateral type). The stomata type was anisocytic (Fig. 3A-B).

Table 1. The anatomical measurements of *N. camlikensis* and *N. cariensis* (values in µm).
Çizelge 1. N. camlikensis ve N. cariensis'in anatomik ölçümleri (değerler mikrometre)

Species/Anatomic characters		*Noccaea camlikensis* Length Min-Max	Mean±SD	Width Min-Max	Mean±SD	*Noccaea cariensis* Length Min-Max	Mean±SD	Width Min-Max	Mean±SD
Root	Peridermis	-	-	43.41-72.46	57.24±11.03	-	-	32.38-55.62	43.34±7.73
	Cortex	21.71-39.24	30.64±6.88	51.68-118.64	85.97±25.15	10.44-21.71	15.6±3.54	28.98-59.05	39.58±10.53
	Vessel	-	-	87.33-110.04	101.2±6.99	-	-	23.48-68	42.9±12.86
Stem	Cuticle			2.81-4.65	3.77±0.66			5.08-8.81	7.64±1.54
	Epidermis	11.92-18.67	15.52±2.22	16.96-27.42	21.98±3.40	15.47-23.53	19.83±2.94	20.26-31.18	24.17±3.49
	Cortex	35.02-58.76	45.93±7.38	44.76-76.29	55.13±9.72	14.15-23.21	18.64±3.27	22.34-35.83	28.35±4.25
	Vessel			13.87-25.62	21.22±5.41			16.45-29.35	22.57±3.86
	Pith cell			70.28-118.91	92.42±13.93			31.96-61.4	41.88±8.78
Leaf	Cuticle on upper epidermis			3.99-6.96	5.17±1.20			3.90-5.24	4.61±0.52
	Cuticle on lower epidermis			2.56-5.19	3.54±1.00			3.90-6.3	5.08±0.93
	Upper epidermis	23.86-66.81	41.11±14.9	26.85-61.44	39.13±12.6	18.19-52.85	29.75±10.88	16.15-44.87	29.67±9.35
	Lower epidermis	17.76-58.09	37.96±16.9	29.04-105.65	52.61±31.9	27.16-41.27	31.98±5.63	23.93-48.31	37.68±9.17
	Palisade parenchyma	39.54-73.05	55.94±9.6	14.08-30.93	21.57±5.48	26.52-57.83	38.76±8.79	11.77-21.63	16.63±2.62
	Spongy parenchyma			22.36-52.27	33.51±9.4			19.89-37.74	30.8±6.57

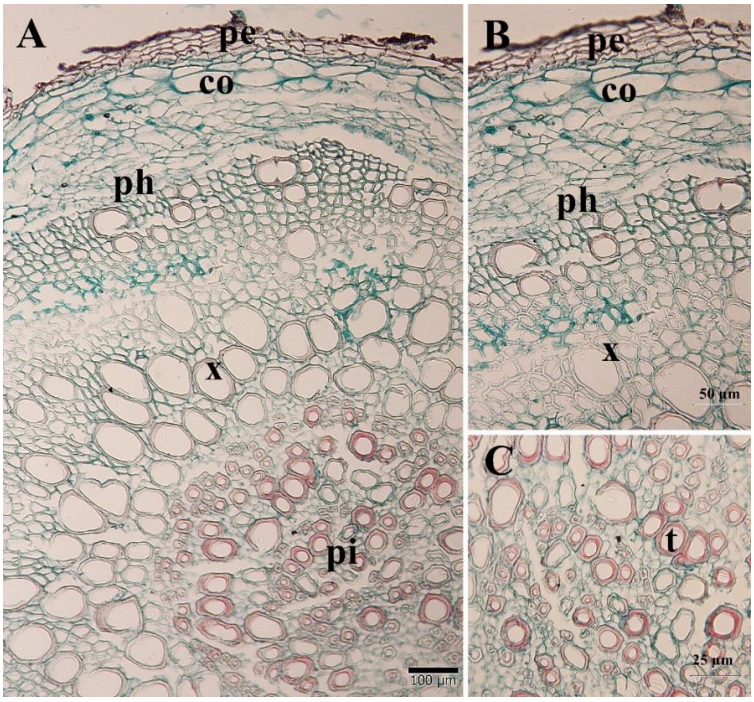

Figure 1. The root cross sections of *Noccaea camlikensis*. A. General view of root pe: peridermis, co: cortex, ph: phloem, x: xylem, pi: pith region, B. Close view of peridermis, cortex and, phloem, C. Tracheal elements t: trachea

Şekil 1. Noccaea camlikensis'in kök enine kesitleri. A. Kök genel görünüşü pe: peridermis, co: korteks, ph: floem, x: ksilem, pi: öz bölgesi, B. Peridermis, korteks ve floemin yakın görünüşü, C. Trakeal elemanlar t: trake

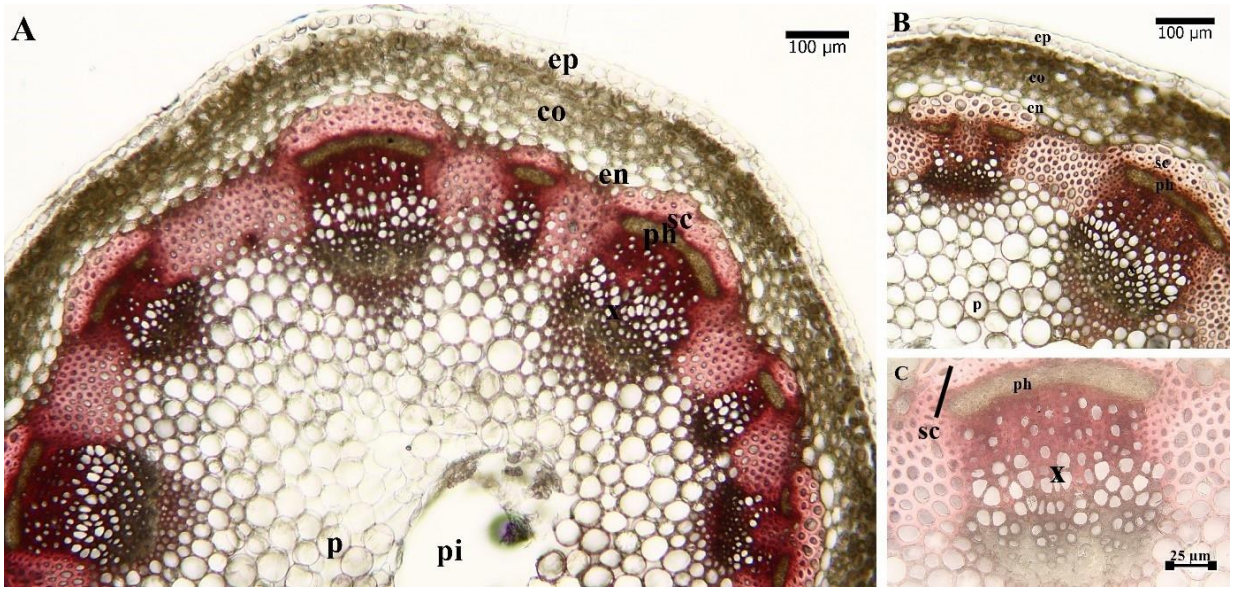

Figure 2. The stem cross sections of *Noccaea camlikensis*. A. General view of stem ep: epidermis, co: cortex, en: endodermis, sc: sclerenchyma, ph: phloem, x: xylem, p: parenchyma, pi: pith region, B. Close view of epidermis, cortex and vascular bundles, C. Close view of vascular bundle.

Şekil 2. Noccaea camlikensis'in gövde enine kesitleri. A. Gövde genel görünüşü ep:epidermis, co:korteks, en:endodermis, sc:sklerenkima, ph:floem, x:ksilem, p:parenkima, pi:öz bölgesi, B. Epidermis, korteks ve iletim demetleri yakın görünüşü, C. İletim demeti yakın görünüşü

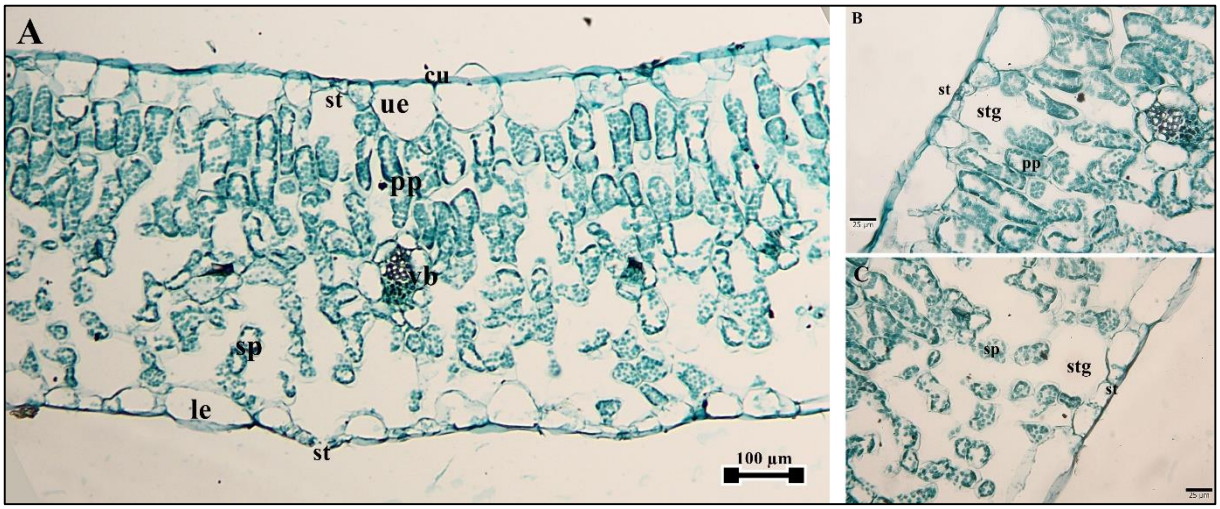

Figure 3. The leaf cross sections of *N. camlikensis*. A. General view of leaf cu: cuticle, ue: upper epidermis, le: lower epidermis, pp: palisade parenchyma, sp: spongy parenchyma, vb: vascular bundle, st: stomata, B. Close view of upper epidermis stg: stomatal gap C. Close view of lower epidermis

Şekil 3. N. camlikensis'in yaprak enine kesitleri. A. Yaprağın genel görünüşü cu:kütikul, ue: üst epidermis, le:alt epidermis, pp: palizat parankiması, sp: sünger parankiması, vb: iletim demeti, st: stoma, B. Üst epidermisin yakın görünüşü stg: stomata boşluğu C. Alt epidermisin yakın görünüşü

Noccaea cariensis

Root anatomy

The secondary root structure was observed in the root cross-sections of *N. cariensis* with the peridermis, cortex, phloem, and xylem (Fig 5-A). The peridermis was a protective tissue composed of disintegrating or squashed cells. The width of the peridermis cells was 43.34 ± 7.73 µm (Table 1). The cortex was 5–6 layered, and followed periderm towards the center. The phloem was well developed, and the cambium was not distinguished clearly (Fig. 5-B). Vessels in xylem were irregular, according to the Metcalfe and Chalk (1957) classification of vessel grouping. The center of the roots in the cross sections was covered with xylem (Fig. 5-C).

Stem anatomy

The cross-sections of the stem had an epidermis layer in the outermost surface (Fig. 6-A). The cortex was 8–9 layered and characterized by parenchymatic cells.

Their dimension was 45.93±7.38×55.13±9.72 µm. The endodermis was rowed and fusiform-shaped. The phloem and xylem were well developed. Above the phloem, sclerenchymatic cells were present (Fig. 6-B). The diameter of the tracheas was 21.22 ± 5.41 µm (Table 1). The pith region of the stem consisted of large parenchymatic cells (Fig. 6-C).

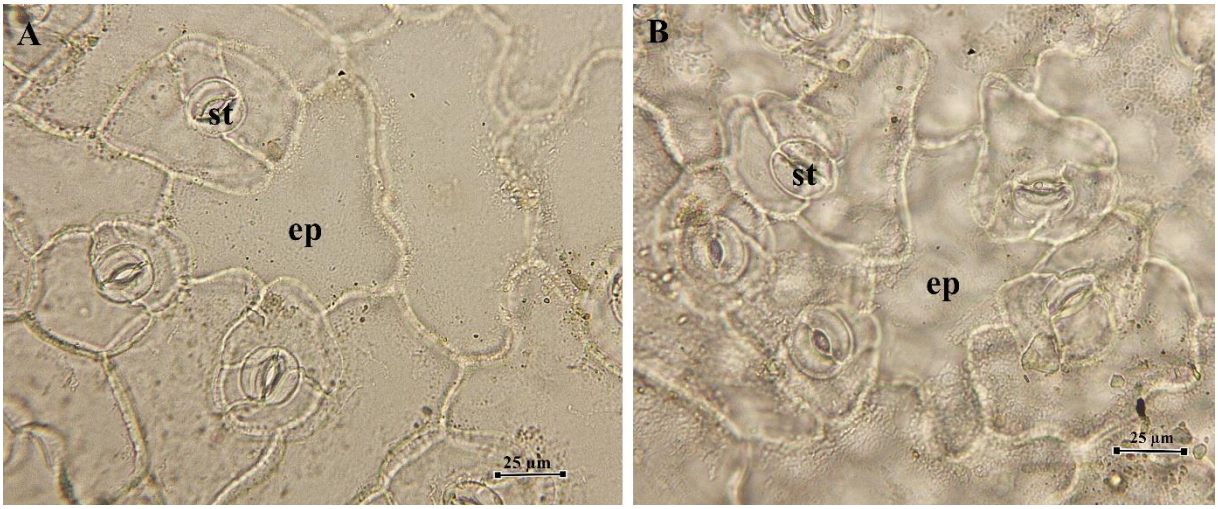

Figure 4. The cross sections of leaves of *N. camlikensis*. A. Upper surface B. Lower surface st: stomata ep: epidermis

Şekil 4. N. camlikensis'in yapraklarının yüzeysel kesitleri. A. Üst yüzey B. Alt yüzey st: stoma, ep: epidermis

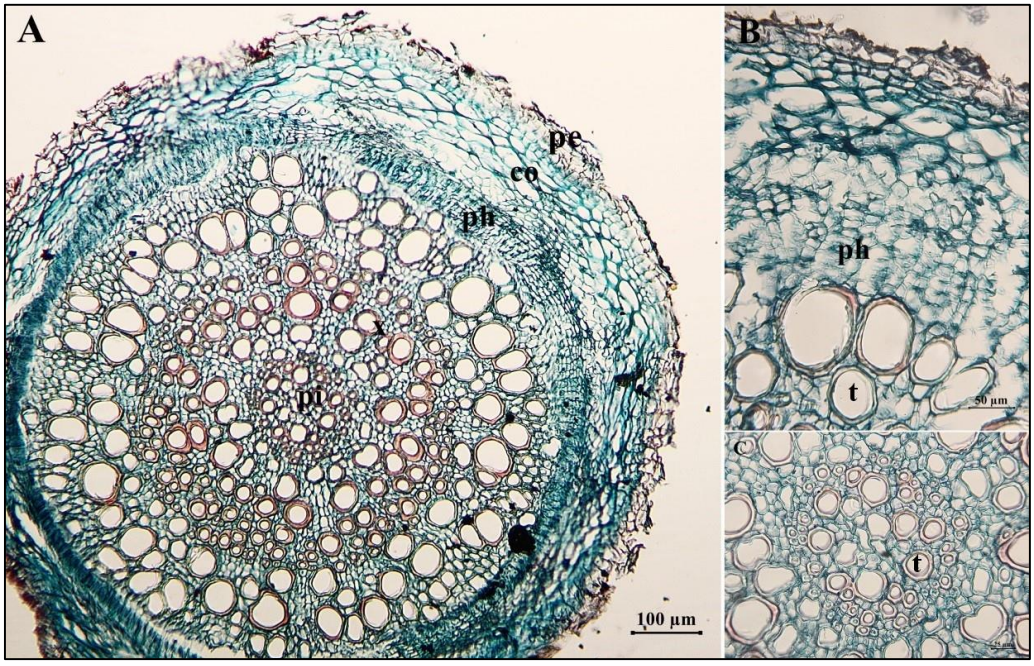

Figure 5. The root cross sections of *Noccaea cariensis*. A. General view of root pe: peridermis, co: cortex, ph: phloem, x: xylem, pi: pith region, B. Close view of peridermis, cortex and, phloem, C. Tracheal elements t: trachea.

Şekil 5. Noccaea cariensis'in kök enine kesitleri. A. Kök genel görünüşü pe: peridermis, co: korteks, ph: floem, x: ksilem, pi: öz bölgesi, B. Peridermis, korteks ve floemin yakın görünüşü, C. Trakeal elemanlar t: trake

Leaf anatomy

The cross sections of the leaves showed that the upper epidermis was made up of rectangular cells with adaxial and abaxial cuticles, and the lower epidermis was oval-rectangular shaped (Fig. 7-A). Cells of the lower epidermis (27.16–41.27 µm long × 23.93 ± 48.31 µm wide) were wider than those of the upper epidermis (18.19–52.85 µm long × 16.15–44.87 µm wide) (Table 1). The mesophyll was equifacial. The palisade parenchyma was on both sides of the leaves.

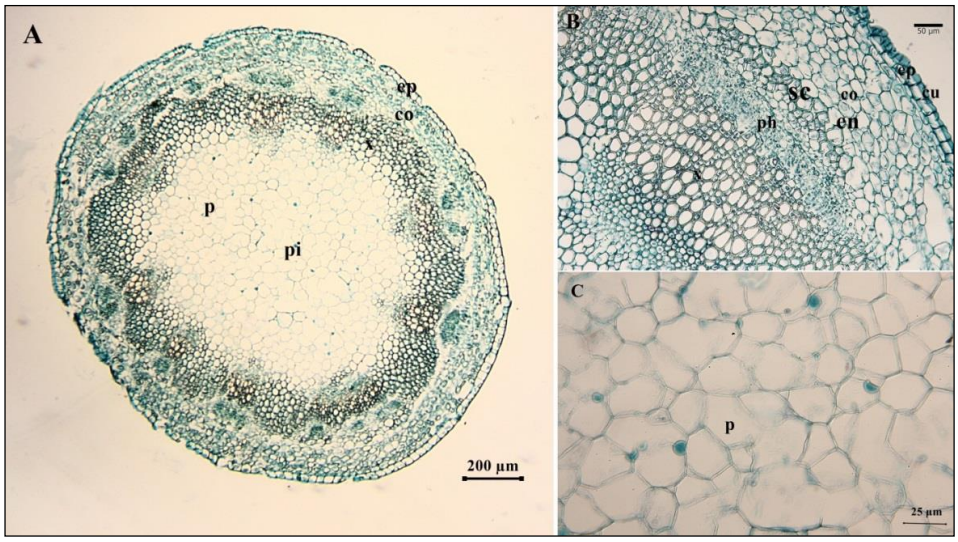

Figure 6. The stem cross sections of *Noccaea cariensis*. A. General view of stem cu: cuticle, ep: epidermis, co: cortex, sc: sclerenchyma, en: endodermis, ph: phloem, x: xylem, p: parenchyma, pi: pith region, B. Close view of epidermis, cortex and vascular bundles, C. Close view of vascular bundle.

Şekil 6. Noccaea cariensis'in gövde enine kesitleri. A. Gövde genel görünüşü ep:epidermis, co:korteks, en:endodermis, sc:sklerenkima, ph:floem, x:ksilem, p:parenkima, pi:öz bölgesi, B. Epidermis, korteks ve iletim demetleri yakın görünüşü, C. İletim demeti yakın görünüşü.

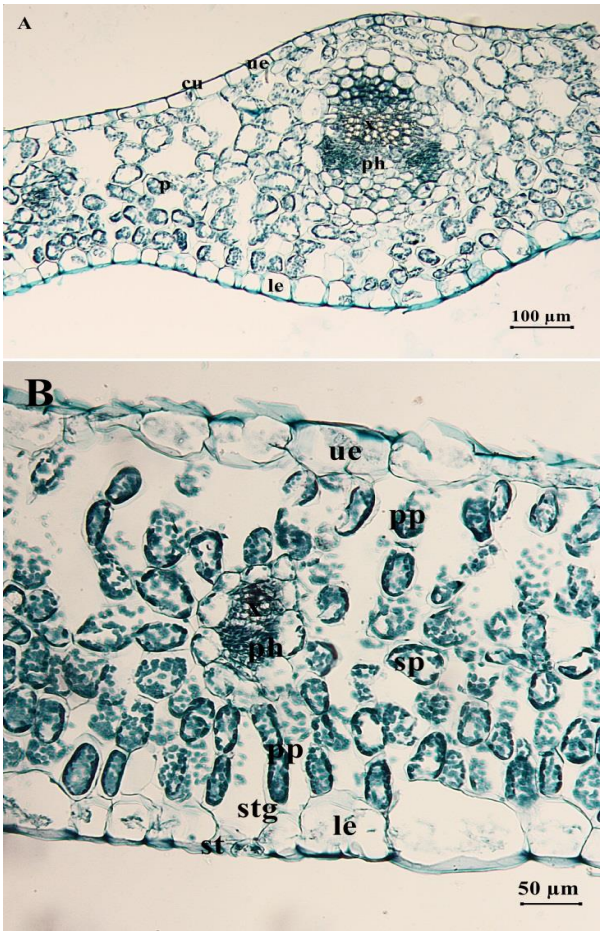

Figure 7. The leaf cross sections of *N. cariensis*. A. General view of midrib cu: cuticle, ue: upper epidermis, le: lower epidermis, p: parenchyma, x: xylem, ph: phloem, B. Close view of lamina st: stomata, pp: palisade parenchyma, sp: spongy parenchyma, stg: stomata gap.

Şekil 7. N. cariensis'in yaprak enine kesitleri. A. Orta damarın genel görünüşü cu:kütikul, ue: üst epidermis, le:alt epidermis, p: parankima, x:ksilem, ph: floem, B. Laminanın yakın görünüşü pp:palizat parankiması, sp: sünger parankiması, st:stoma, stg: stoma boşluğu.

N. camlikensis and *N. cariensis* were selected to determine their anatomical characteristics for the first time and it was aimed to confirm their systematic position.

The root anatomy of the studied species showed that there was a secondary root structure with a peridermis, cortex, phloem, and xylem. The cortex parenchymatic cells were more or less oval-shaped. The phloem and xylem were well-developed and the centre of the roots were covered with xylem elements.

In the root cross-sections, the studied taxa had a similar secondary structure with regards to their peridermis, cortex parenchyma, xylem, phloem, and sclerenchymatic pith region, as reported in the root anatomy of the family Brassicaceae (Tekin et al., 2013; Atçeken et al., 2016; Çıtak and Dural, 2020). Most species have a single cambium, wherein the growth rings are inconspicuous, with narrow vessels ranging from 16–71 µm in the wood anatomy of Brassicaceae (Carlquist, 1971), as in studied species.

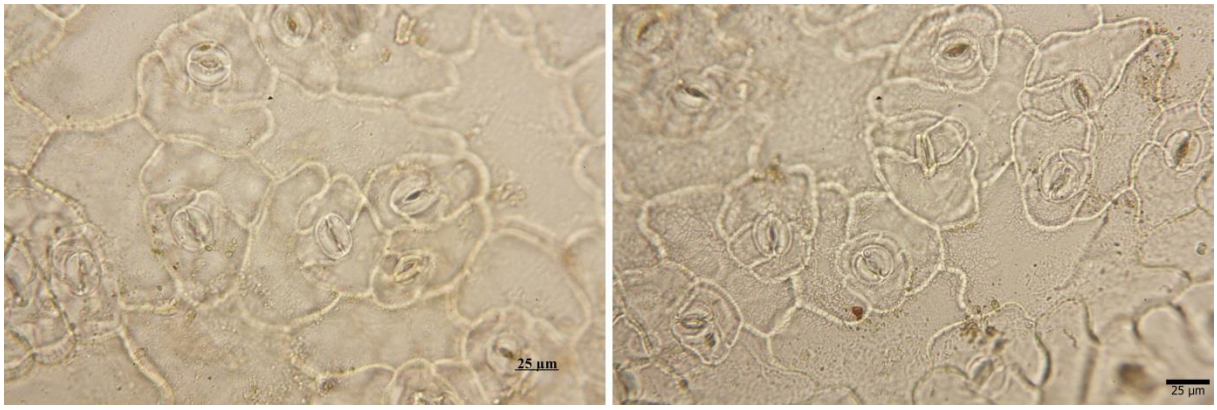

Figure 8. The cross sections of leaves of *N. cariensis*. A. Upper surface B. Lower surface st: stomata ep: epidermis
Şekil 8. *N. cariensis'in yapraklarının yüzeysel kesitleri. A. Üst yüzey B. Alt yüzey st: stoma, ep:epidermis*

The studied species shared similar stem anatomical characteristics, which were characterized by a single-layered epidermis, containing chlorophyll pigments in the cortex parenchyma, well-developed phloem, and xylem and pith cells in the center, as in the other members of Brassicaceae (Tekin et al., 2013; Atçeken et al., 2016; Çıtak and Dural, 2020). The contour of the stem cross-sections was rounded with collenchymatic ridges, ovoid, or polygonal in the family Brassicaceae and also rounded in the studied *Noccaea* species. The rounded-shape cross-sections of the stem in *N. camlikensis* and *N. cariensis* were observed to have the general characteristics of the primary stem.

The family Brassicaceae includes unifacial, bifacial, and equifacial mesophyll in its leaf anatomy (Tekin et al., 2013; Atceken et al., 2016; Çıtak and Dural, 2020). The cross-sections of shapes of leaves of *N. camlikensis* were linear-shaped, while they were v-shaped in *N. cariensis*, and the median vascular bundle was larger than the others, with a bifacial mesophyll in the examined species.

CONCLUSION

With this study, the anatomical characteristics of *Noccaea camlikensis* and *N. cariensis* were determined and these characteristics were found to be not specific for the species taxonomic position. Nevertheless, the anatomical traits can be more valuable if other species of *Noccaea* are also investigated.

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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.

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Megacollybia rodmanii: Türkiye Mikotası için Yeni Bir Kayıt

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ÖZET

Megacollybia rodmanii R.H. Petersen, K.W. Hughes & Lickey'nin meyvemsileri Türkiye'den bu çalışmada ilk kez toplandı, morfolojik olarak incelendi ve burada arazi ve mikroskopik resimler ve kısa bir tartışma ile birlikte verildi. Yeni kayıt kahverengi, zeytini kahverengi veya soluk grimsi kahverengi ve 50–155 mm şapka, sapa az veya çok bağlı lameller, beyazımsı ve 50–115 × 15–25 mm sap, eliptik ve 6–10 × 5–8 µm bazidiyosporlar ile yakın akraba türlerden ayrılır.

Biyoloji

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Anahtar Kelimeler
Biyoçeşitlilik
Sistematik
Şapkalı mantar
Trabzon

Megacollybia rodmanii: A New Record for the Turkish Mycota

ABSTRACT

The fruiting bodies of *Megacollybia rodmanii* R.H. Petersen, K.W. Hughes & Lickey were collected from Turkey for the first time in this study. They were examined morphologically and presented herein with field and microscopic pictures and a brief discussion. The new record is distinguished from the closely related species with brown, olive brown or pale greyish brown and 50–155 mm pileus, more or less attached lamellae, with whitish and 50–115 × 15–25 mm stipe, elliptical and 6–10 × 5–8 µm basidiospores.

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GİRİŞ

Megacollybia Kotl. & Pouzar günümüzde dünyada yaklaşık 12 ve Türkiye'de ise bu çalışmadan önce sadece bir kayıt ile temsil edilen küçük bir cinstir (Kirk ve ark., 2008; Sesli ve ark., 2020). Bu cins karakız mantarlarına (*Tricholoma*) benzer görünümde, grimsi kahverengi, 50–150 mm, lifli ve pullu şapka; az veya çok sapa birleşik, seyrek ve beyaz lameller; beyazımsı veya grimsi kahverengi sap; yuvarlağımsı, 7–11 × 6–9 µm bazidiyosporlar ve çürükçül yaşayan bireyleri ile karakterize edilir (Knudsen ve Vesterholt, 2008). Günümüzde dünyada yayılış gösterdiği tespit edilmiş taksonlar *M. clitocyboidea* R.H. Petersen, *M. fallax* (A.H. Sm.) R.H. Petersen & J.L. Mata, *M. fusca* J.L. Mata, Aime & T.W. Henkel, *M. marginata* R.H. Petersen, O.V. Morozova & J.L. Mata, *M. platyphylla* (Pers.) Kotl. & Pouzar / Kökbacak, *M. rimosa* V. Coimbra & Wartchow, *M. rodmanii* R.H. Petersen / Yaş kökbacak, K.W. Hughes & Lickey, *M. subfurfuracea* R.H. Petersen, *M. texensis* R.H. Petersen & D.P. Lewis ve *M. virosa* Manim. & K.B. Vrinda'dır. Bu çalışmadan önce *M. platyphylla* (Pers.) Kotl. & Pouzar / Kökbacak Karadeniz Bölgesinde tespit edilmiştir (Kirk ve ark., 2008; Akata ve ark., 2010; Sesli ve ark., 2020). Bu araştırmanın materyali 20.07.2018 tarihinde Bolu ili Gerede ilçesindeki ormanlık alandan Amatör mikolog Eralp Aytaç tarafından toplanarak herbaryumumuza gönderilmiştir. Toplama sahasındaki ormanlar ortalama 1000 metreye kadar kayın, meşe, göknar, karaçam, akçaağaç, ıhlamur, dişbudak, karaağaç, karaçam ve kızılağaçlardan, 1000 - 2000 metre yükseltiler arasında sarıçam, gürgen, meşe, şimşir, göknar ve doğu kayınından, 2000 metreden sonraki yükseltilerde ise daha bodur ağaççıklar ve çalılardan

oluşmaktadır. Yapılan araştırmalara göre Bolu'da otsu ve odunsu türlerden 1200 civarında bitki bulunmaktadır. Bu bitkilerden 88 tanesi endemiktir. Soğanlı bitkilerden özellikle mavi çiçekli Abant Çiğdemi ile sarıçiçekli Ankara Çiğdemi yörede görülen endemik bitkilerdendir. Bu araştırmanın amacı Türkiye'den ilk kez toplanarak teşhisi yapılan *Megacollybia rodmanii* R.H. Petersen, K.W. Hughes & Lickey / Yaş kökbacak türünün makroskopik ve mikroskopik özelliklerini tanıtarak Türkiye mikotasına katkı sağlamaktır.

MATERYAL ve METOD

Megacollybia rodmanii R.H. Petersen, K.W. Hughes & Lickey'nin meyvemsileri 20.07.2018 tarihinde, Bolu ili Gerede ilçesi ormanlarından toplanmıştır. Materyalin olası mikorizal ilişkileri not edilip fotoğrafı çekildikten sonra birkaç tanesi sökülerek kese kağıtlarına konulmuş, laboratuvara getirilerek spor izleri alınmış, kurutucuya yerleştirilerek birkaç saatte herbaryum materyali haline getirilmiş ve nihayet kataloglanarak fungaryum dolaplarına yerleştirilmiştir. Bazidiyum, kanca, sistit ve diğer hifal yapıların gözlenebilmesi için keskin jilet ile lamel ve şapka yüzeyinden ince kesitler alınmış, %5'lik amonyak içerisinde 3-5 dakika bekletilerek incelenebilecek duruma getirilmiştir. Mikroskopik ölçümler için iki farklı meyvemsiden 30 ölçüm yapılmış ve bunların ortalaması alınmıştır. Yapısal hücreler mikrofotografi yöntemi ile görüntülenmiş ve mikroskoba bağlı bilgisayara kaydedilmiştir. Bazidiyosporlar için ortalama otuz ölçüm yapılmış, onların ortalaması alınmış ve ayrıca ilgili kamera sistemi sayesinde fotoğrafları çekilmiştir. Teşhisler elde edilen morfolojik verilerin ilgili literatürlerle karşılaştırılması sonucu yapılmıştır (Hughes ve ark. 2007; Knudsen ve Vesterholt, 2008; Kuo, 2010). Kurutulmuş meyvemsiler Trabzon Üniversitesi Fatih Eğitim Fakültesindeki kişisel fungaryumda saklanmaktadır.

BULGULAR ve TARTIŞMA

Porotheleaceae Murrill

Megacollybia rodmanii R.H. Petersen, K.W. Hughes & Lickey / Yaş kökbacak (Şekil 1)

Şapka konveks veya tabak biçiminde, 50–150 mm, yüzeyi kuru, kahverengi, zeytini kahverengi veya soluk grimsi kahverengidir. Lameller sapa genişliği ölçüsünde veya daralarak bağlı, beyazımsı genellikle sık ve bazen de seyrektir. Dokusu beyazımsı, hafif tatlı ve kokusu pek belirgin değildir. Sap silindirik, 50–110 × 15–25 mm, beyazımsı, tabana doğru daha geniş ve tabanda miselyumludur. Bazidiyumlar ince çomakçık biçiminde, kancalı, 4 sporlu ve 40–55 × 8–10 µm'dir. Bazidiyosporlar eliptik, düz yüzeyli ve 5–10 × 5–8 µm'dir. Ceylosistityumlar çomakçık biçiminde ve 40–80 × 10–22 µm'dir. Şapka derisi paralel, 4–8 µm genişliğinde, kancalı, ve bazıları çomakçık biçiminde yüzeye doğru uzayan hiflerden oluşmuştur. Türkiye'de günümüze değin gerçekleştirilen çalışmalarda Çatalca-Kocaeli Bölümünde saptanmıştır. İlkbahardan sonbahar sonlarına değin ağaçların gömülü kısımları üzerinde çürükçül yaşar.

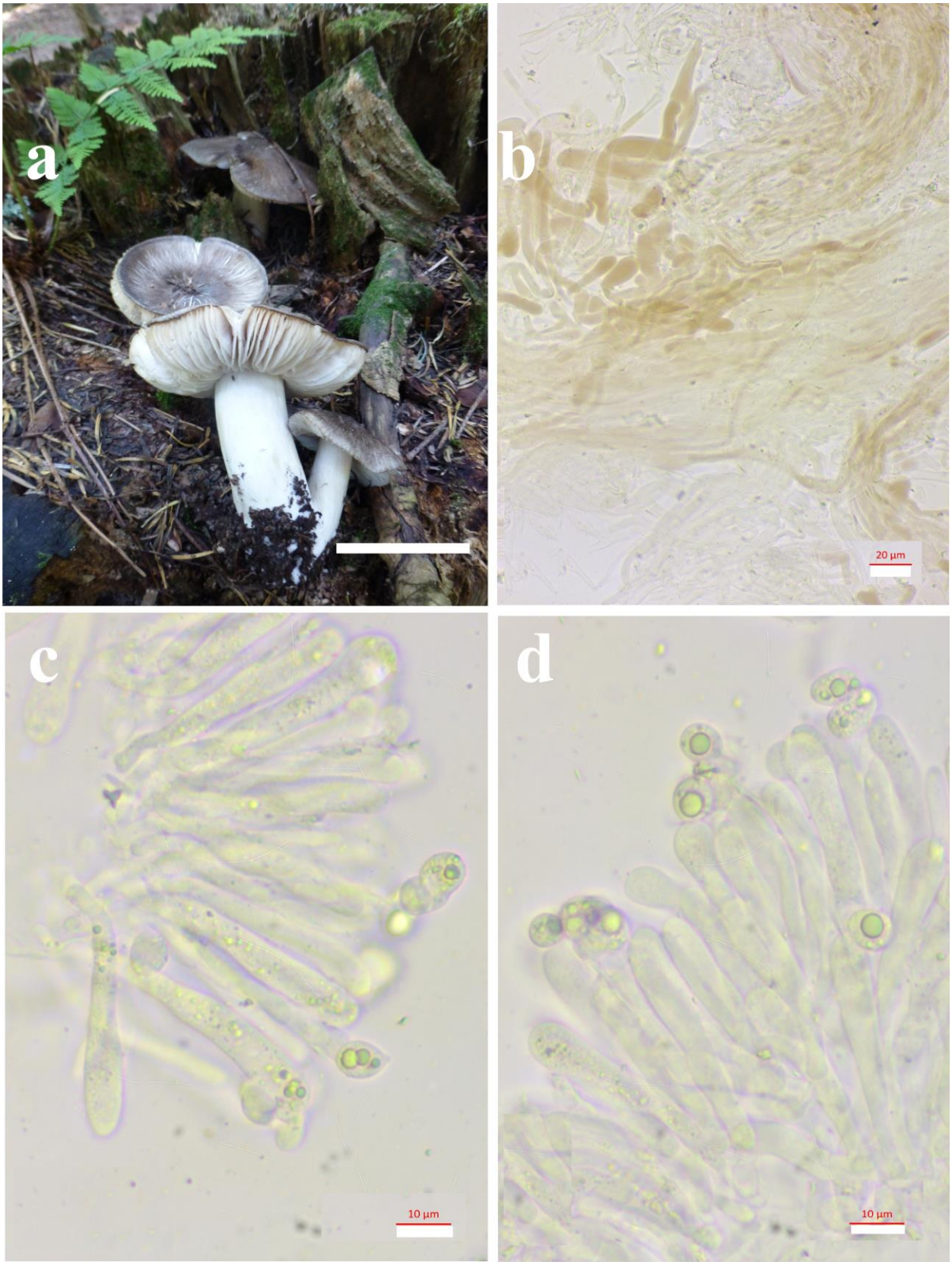
İncelenen örnekler: Türkiye, Bolu, Gerede, 40°49'05.94" K ve 32°10'59.60" D, 1664 m, 20.07.2018, gruplar halinde, Aytaç 3926.

Megacollybia Kotl. & Pouzar küçük bir cins olduğundan teşhisi kolaydır. Mevcut koleksiyonumuzun yakın akraba olduğu tür *M. platyphylla* (Pers.) Kotl. & Pouzar / Kökbacak'dır. Bu tür yarımküre, çan şeklinde veya konveks, 50–120 mm, grimsi veya zeytini kahverengi şapka; beyazımsı veya krem rengi, sapa tüm yüzeyi ile bağlı veya sap üzerine dökük lameller; silindirik, tabana doğru daha geniş, 50–150 × 15–25 mm, beyazımsı veya krem rengi sap ve daha küçük ceylosistityumlar (30–65 × 12–20 µm) ile koleksiyonumuzdan farklılık gösterir (Breitenbach ve Kränzlin, 1991; Hughes ve ark. 2007; Knudsen ve Vesterholt 2008). Bu çalışma sayesinde Türkiye mikotasında yer alan *Megacollybia* tür sayısı ikiye çıkmıştır.

SONUÇ ve ÖNERİLER

Bu araştırma sonucunda *Megacollybia rodmanii* R.H. Petersen, K.W. Hughes & Lickey / Yaş kökbacak türü Türkiye'den teşhis edilerek Türkiye mikotasına yeni kayıt olarak eklenmiştir. Genç araştırıcılara önerim geniş alanlarda seyrek arazi çalışması gerçekleştirmek yerine, daha küçük bölgelerin sık sık taranarak yeni meyvemsi oluşup oluşmadığını sürekli gözlemeleridir. İklim ve fiziki koşulları zor olan arazilerde daha ilginç türler bulunabileceğinden bu yöreleri öncelikli olarak incelemelerini öneririz. Araziden toplanan meyvemsilerin birkaç saat içerisinde kurutulması moleküler çalışmalar açısından yararlı olacaktır. Büyük boyutlu meyvemsileri kuruturken küflenme olmaması için dilimleme yöntemi uygulanmalıdır. Sonbahar aylarında veya kışa doğru mono veya bisporik bazidiyumlar normalden büyük bazidiyosporlar oluşturabileceğinden teşhislerde bu durumu dikkate almak gerekir. Küçük boyutlu meyvemsiler bazen birbirine yakın büyümesine rağmen farklı türlere ait olabileceğinden gereğinden fazla materyal toplamanın da olumsuz sonuçlara yol açabileceği unutulmamalıdır. Ölçümlerde daha doğru sonuçlara ulaşabilmek için farklı meyvemsilerden örnek alınarak onların ortalaması alınmalıdır. Bazidiyospor ölçümlerinde normalde spora ait çıkıntılar hesaba dahil edilmez. Eğer apikulus, diken, siğil ve benzeri çıkıntıları ölçüme dahil edilecekse bunu muhakkak belirtmek gerekir. Herbaryum örnekleri böceklenmemesi için muhakkak -40 °C'lik ortamda en

az bir hafta süre ile bekletilmeli ve bu işlem her yıl tekrar edilmelidir.



Şekil 1: Megacollybia rodmanii. a- meyvemsiler, b- şapka derisi kesiti, c ve d- bazidiyumlar ve bazidiyosporlar (ölçekler: a: 70 mm, b: 20 µm, c ve d: 10 µm)

Figure 1: Megacollybia rodmanii. a- fruiting bodies b- section from pileipellis, c and d - basidia and basidiospores (scale bars: a: 70 mm, b: 20 µm, c and d: 10 µm)

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Çıkar Çatışması Beyanı

Herhangi bir çıkar çatışması yoktur

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Sodyum Pirition'un (Sodyum Omadin) Sıçan Karaciğer ve Böbrek Dokularındaki Akut Etkileri

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ÖZET
Bu araştırmada intraperitonal yolla uygulanan sodyum pirition (35 ve 70 mg kg^{-1}, ip) ve serum fizyolojik (salin) (%0.09 NaCl 0.5 ml kg^{-1}, ip)uygulanmasının sıçanlar üzerine 24 ve 96 saatlik sürelerde böbrek ve karaciğer dokuları üzerine histolojik etkileri araştırılmıştır. Araştırma sonucunda, kontrol grubu ile karşılaştırıldığında intraperitonal yolla uygulanan sodyum piritionun doz ve süre artışına bağlı histopatolojik değişimlere neden olan sitotoksik etkisinin olabileceği kanısına varılmıştır.

Zooloji

Araştırma Makalesi

Makale Tarihçesi
Geliş Tarihi : 14.07.2021
Kabul Tarihi : 23.12.2021

Anahtar Kelimeler
Sıçan
Sodyum pirition
Karaciğer
Böbrek
Histopatoloji

Acute Effects of Sodium Pyrithione (Sodium Omadine) on Rat Liver and Kidney Tissues

ABSTRACT
In this study, the histological effects of intraperitoneally applied sodium pyrithione (35 and 70 mg kg^{-1}, ip) and saline (0.09% NaCl 0.5 ml kg^{-1}, ip) on the kidney and liver tissues of rats in 24 and 96 hours were investigated. As a result of the study, it was concluded that intraperitoneally applied sodium pyrithione may have a cytotoxic effect that causes histopathological changes due to the increase in dose and duration ,when compared to the control group,.

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GİRİŞ
Çevresel kirleticiler, çevre sağlığını ve toplumların sağlık düzeyini önemli ölçüde etkilemektedir. Ekolojik çevrenin korunması, düzenlenmesi ve geliştirilmesi tüm sağlık hizmetlerinin iyileştirilmesinde hayati önem taşımaktadır. Bazı organik maddeler, petrol ve türevleri, endüstriyel atıklar, yapay tarımsal gübreler, pestisitler, inorganik tuzlar, ağır metaller, deterjanlar, radyoaktivite ve yapay organik kimyasallar ekolojik dengeyi bozan kirletici unsurlardır. Bu maddeler doğal dengeyi olumsuz yönde tehdit eden unsurlardır. Bütün bu maddeler canlı sistemlerinde birikebilirler ve besin zinciri yoluyla her basamakta daha yüksek derişimlere ulaşabilirler. İnsanların, oral, inhalasyon ve dermal yolla birçok çevresel kirletici maddeye maruz kaldıkları bilinmektedir.

Sodyum pirition yaygın olarak kozmetik, madencilik ve yakıt sektörlerinde kullanılan antimikrobiyal ve antifungusit ajandır (Dinning ve ark., 1998). Bunun mantar ve bakterilerde substratın taşınma işlemlerini inhibe ettiği bilinmektedir (Chandler ve Segel, 1978). Kısıtlı literatür verilerine göre canlılar üzerinde olumsuz etkileri gösterilmektedir. Fizyolojik, hematolojik ve histolojik araştırmalar oldukça kısıtlıdır. Sodyum pirition kolayca uygulanan, gastrointestinal kanaldan ve sağlam deriden emilen, toksisitesi bilinen bir maddedir (Mitoma ve ark., 1983). Sitotoksik olduğundan, sadece düşük konsantrasyonlar test edilebilmektedir. Tek veya birden fazla doz sodyum pirition verilen sıçanlarda, farelerde ve tavşanlarda zehirlenmenin tipik belirtisi olarak arka uzuvlarda geri dönüşümlü olan felç

oluştuğu bildirilmektedir (Gibson ve ark., 1982). Geri dönüşü olmayan göz hasarı, tapetum luciduma sahip türlerde görülmüştür (Gibson ve ark., 1982; Borg-Neczak ve Tjälve, 1991). Sodyum piritionun farelere oral uygulamasından sonra kanserojen etkileri tespit edilmiştir. Yüksek doz seviyelerdeki sodyum piriton uygulamasının genotoksik olduğu gösterilmiştir.

Sıçanlar, tavşanlar ve maymunlarda oral uygulamadan sonra, sodyum piritionun hızlı bir şekilde gastrointestinal sistemden absorbe edildiği ve sıçanlarda oral uygulamadan sonra emilimin %88-100 olduğu tespit edilmiştir (Olin Corporation, 1989). Farelerde radyoaktif olarak etiketlenmiş sodyum piriton oral ya da intraperitonal uygulama sonrasında uygulanan radyoaktivitenin % 0.15-0.6 karaciğer %0.4-0.8 diğer organlarda tespit edilmiştir (Ziller, 1977; Borg-Neczak ve Tjälve, 1991). Radyoaktif sodyum piritionun tek bir doz dermal uygulaması sonucunda, uygulama yerine yakın kas ve karaciğerde tespit edildiği bildirilmektedir. Bu araştırmada sodyum piritionun akut sürede (24 ve 96 saat) sıçanlar üzerine histolojik etkilerinin belirlenmesi amaçlanmıştır.

MATERYAL ve METOD

Deneylerde Uygulanacak Kimyasal Maddeler ve İlaçlar

Deneyde sodyum piriton (35 ve 70 mg kg^{-1}, ip), serum fizyolojik (salin) (% 0.09 NaCl 0.5 mg kg^{-1}, ip) ve dietileter inhalasyonu uygulanılmıştır.

Deney Hayvanları

Sıçanlar kafeslerde standart sıçan yemi ve su verilerek ad libitum olarak beslenilmiştir. 21-25 °C oda sıcaklığında, 12:12 saat aydınlık-karanlık periyodu uygulanılmıştır. Vücut ağırlığı 238-287 gram arasında değişen erişkin erkek Wistar albino sıçanlar kullanılmıştır (Çizelge 1).

Table 1 Weight of Animals Used in Experiments
Çizelge 1. Deneylerde Kullanılan Hayvanların Ağırlığı

Gruplar	Ortalama±Standart Hata (Gram)
Kontrol grubu (24.saat)	265.2±12.2
Kontrol grubu (96.saat)	256.2±16.4
35 (mg kg^{-1}, ip) Sodyum Piriton (24.saat)	261.9±16.4
35 (mg kg^{-1}, ip) Sodyum Piriton (96.saat)	258.7±13.8
70 (mg kg^{-1}, ip) Sodyum Piriton (24.saat)	256.7±14.3
70 (mg kg^{-1}, ip) Sodyum Piriton (96.saat)	265.8±15.0

Deney grupları ve bu gruplardaki hayvan sayıları aşağıdaki şekilde oluşturulmuştur.

1. grup (n: 10): kontrol grubu serum fizyolojik (salin) (% 0.09 NaCl 0.5 mg kg^{-1}, ip) (24. saat kontrolü).
2. grup (n: 10): kontrol grubu serum fizyolojik (salin) (% 0.09 NaCl 0.5 mg kg^{-1}, ip) (96. saat kontrolü).
3. grup (n: 10): 35 mg kg^{-1}, ip sodyum piriton enjeksiyonu. (24. Saatlik deney grubu)
4. grup (n: 10): 70 mg kg^{-1}, ip sodyum piriton enjeksiyonu. (24. Saatlik deney grubu)
5. grup (n: 10): 35 mg kg^{-1}, ip sodyum piriton enjeksiyonu. (96. Saatlik deney grubu)
6. grup (n: 10): 70 mg kg^{-1}, ip sodyum piriton enjeksiyonu. (96. Saatlik deney grubu).

Deney Protokolü

Bütün deneylerin gerçekleştirileceği laboratuvarın sıcaklığı 21-25 °C civarında tutulmuştur. Deneylere saat 09.00'da başlanmış, sodyum piriton (35 ve 70 mg kg^{-1}, ip) ve serum fizyolojik (salin, %0.09 NaCl 0.5 mg kg^{-1}, ip) uygulamaları yapılmış ve takip eden 24 saat ve 96 saat sonra örnekler alınmıştır. Deney protokolü Kırıkkale Üniversitesi Hayvan Deneyleri Yerel Etik Kurulu tarafından onaylanmış ve deneyler Kırıkkale Üniversitesi Hayvan Deneyleri Merkezinde gerçekleştirilmiştir.

Histolojik İncelemeler

Uygulama sonrasında diseksiyonla alınan karaciğer ve böbrek doku parçaları doku takip kasetleri içerisine konularak etiketlenmiş ve fiksatif (% 10'luk formaldehit) çözeltisine konulmuştur. En az 24 saat fiksasyon için fiksatifte bekletilen doku takip kasetleri fiksatiften çıkarılarak, 24 saat hafif akan çeşme suyunda yıkanmıştır. Daha sonra Leica TP1020 model doku takip cihazına konulan kasetler doku suyunun giderilmesi, saydamlaştırma ve parafin emdirme işlemleri yapılmıştır. Cihazdan çıkarıldıktan sonra Leica EG1160 model doku bloklama (gömme) cihazı kullanılarak uygun parafin bloklar hazırlanmıştır.

Hazırlanan bloklar Leica RM 2125RTS model döner mikrotomda 5-7 µm kalınlığında kesitler alınarak lama yapıştırılmıştır. Lamlar Harris Hematoksilen Eozin boyama yöntemine uygun işlemlerle boyanmıştır. Hazırlanan preparatlar entellan ile kapatıldıktan sonra Leica DM3000 model ışık mikroskobunda incelenmiştir. Histolojik incelemeler araştırmacıların kişisel desteği ile yapılmıştır.

BULGULAR ve TARTIŞMA

Kontrol grubu karaciğer dokuları incelendiğinde, hepatosit hücrelerinin düzlenlenmesinin ve sinozoitlerin normal yapıda olduğu gözlenmiştir. Sodyum piritiona maruziyetinde doz ve süre artımına bağlı olarak şiddeti artan hepatositlerde vakuolleşme, pasif hiperemi, mononükleer hücre infiltrasyonu ve hepatoselüler dejenerasyon tespit edilmiştir (Çizelge 2 ve Şekil 1-6).

Table 2 Distribution of Lesions in Liver
Çizelge 2. Karaciğer Dokularında Görülen Lezyonların Dağılımı

Karaciğer dokusunda gözlenen histopatolojik lezyonlar	Kontrol		35 (mg kg^{-1}, ip) Sodyum Piriton		70 (mg kg^{-1}, ip) Sodyum Piriton	
	24 Saat	96 Saat	24 Saat	96 Saat	24 Saat	96 Saat
1- Hepatositlerde vakuolleşme	-	-	+	++	+	++
2- Pasif hiperemi	-	-	-	-	-	+
3- Mononükleer hücre infiltrasyonu	-	-	-	-	-	+
4- Hepatoselüler dejenerasyon	-	-	+	++	+	++

+: Histopatolojik değişiklik olduğu görülmüştür.

Kontrol grubu böbrek dokuları incelendiğinde, glomerulus yapılarının aynı olduğu, epitelin kesintiye uğramadığı ve proksimal ve distal tübüllerin normal yapıda olduğu gözlenmiştir. Sodyum piritiona maruz kalınan gruplarda doz ve süreye bağlı olarak şiddeti artan glomerulusta atrofi (parietal ve viseral yapraklar arasında mesafe artışı), proksimal tübüllerde dejenerasyon, distal tübüllerde dejenerasyon, mononükleer hücre infiltrasyonu, ve konjesyon (kanama) tespit edilmiştir (Çizelge 3 ve Şekil 7-12).

Table 3 Distribution of Lesions in Kidney
Çizelge 3. Böbrek Dokularında Görülen Lezyonların Dağılımı

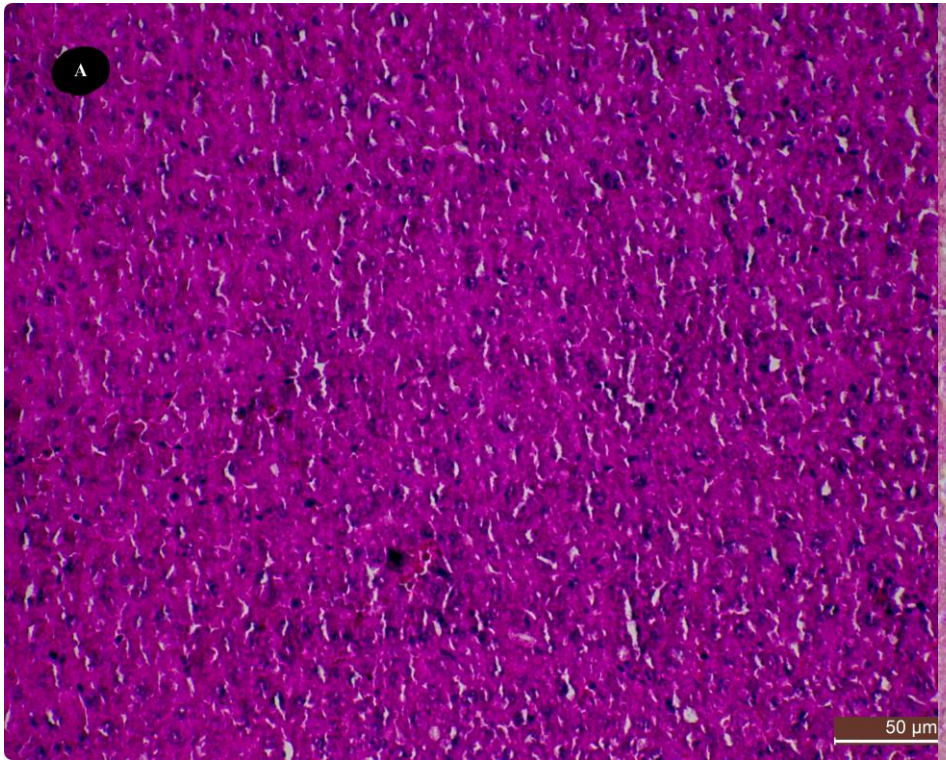
Böbrek dokusunda gözlenen histopatolojik lezyonlar	Kontrol		35 (mg kg^{-1}, ip) Sodyum Piriton		70 (mg kg^{-1}, ip) Sodyum Piriton	
	24 Saat	96 Saat	24 Saat	96 Saat	24 Saat	96 Saat
1-Glomerulusta Atrofi (Parietal ve viseral yapraklar arasında mesafe artışı)	-	-	+	++	+	++
2-Proksimal tübüllerde dejenerasyon	-	-	+	++	+	++
3-Distal tübüllerde dejenerasyon	-	-	+	++	+	++
4-Mononükleer hücre infiltrasyonu	-	-	+	++	+	++
5-Konjesyon (Kanama)	-	-	+	++	+	++

+: Histopatolojik değişiklik olduğu görülmüştür.

Genellikle sodyum, çinko veya bakır tuzu şeklinde uygulanan piriton, geniş spektrumlu mantar ve antimikrobiyal bir ajan olup, kozmetik, madencilik, boya ve yakıt sektörlerinde kullanılmaktır (Ross ve Lawhorn, 1990; Knox ve ark., 2008). Sodyum piriton kolayca gastrointestinal kanaldan ve sağlam deriden emilebilir ve organizmalar üzerinde doza ve süreye bağlı toksikolojik etkileri olduğu bilinmektedir (Mitoma ve ark., 1983). Yapılan araştırmalarda, farklı organizmalarda nörotoksik, sitotoksik, genotoksik, immünotoksik ve histopatolojik etkileri üzerinde durulmuştur.

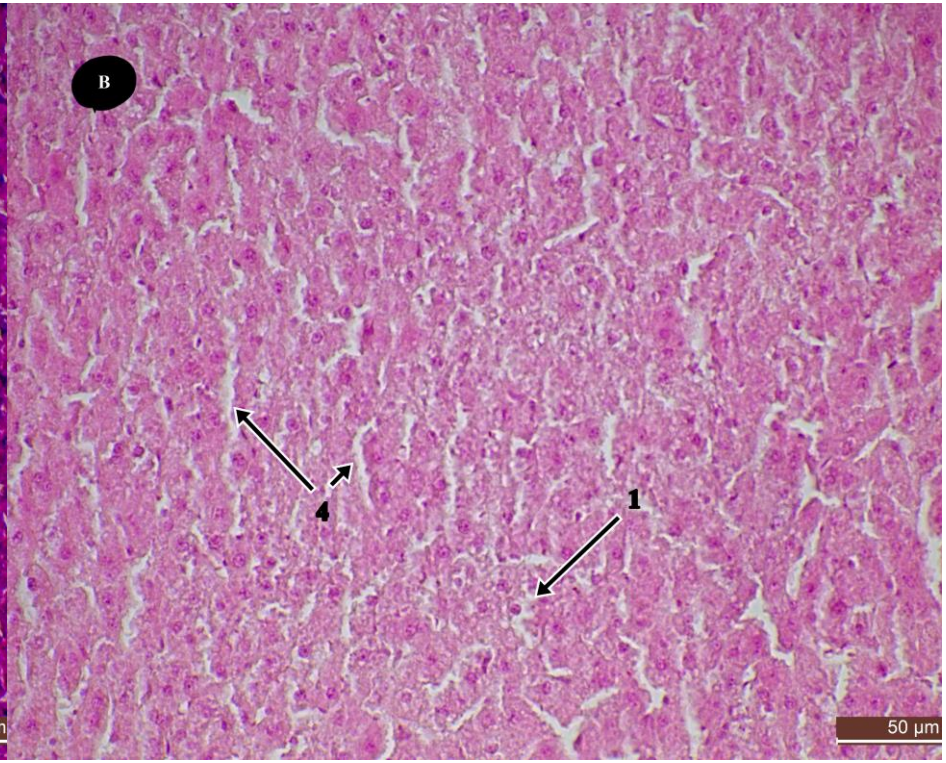
Pirition kemirgenlerde geri dönüşümlü nörotoksisiteye neden olduğu, bu etkinin nöromüsküler kavşakta sinaptik bulaşmanın başarısız olması (Ross ve Lawhorn, 1990) ve periferik motor sinirlerdeki biyokimyasal ve histopatolojik değişiklikler ile karakterize olduğu bildirilmektedir (Snyder ve ark., 1979). Kemirgen modellerinde, piritionların nörotoksik etkileri üzerinde birincil odak olmasına rağmen, diğer türlerin de hassas olduğu bildirilmiştir (Lansdown, 1991). Piritionların kemiricilere *in vivo* uygulamasının güçsüzlüğe neden olduğu bildirilmiştir (Gibson ve ark., 1982). Sodyum piritionun tek veya birden fazla doz verildiği sıçanlar, fareler ve tavşanlarda zehirlenme tipik belirtisi, arka uzuvların geri dönüşümlü olan felçe neden olduğu belirtilmiştir (Gibson ve ark., 1982). Sıçanların çinko piritiona (ZnPt) tekrarlı maruz bırakılmasının, aksoplazmik nakil oranını azalttığı ve motor nöronların distal sinir terminallerinde tubulovesiküler profillerin birikmesine yol açtığı gösterilmiştir (Sahenk ve Mendell, 1980). Sodyum piritionun maruziyeti ile indüklenme benzer şekilde [Ca^{2+}]'nin yaklaşık 3 kat artışı, mikrotübül bütünlüğünün ve aksonal akımı etkilediği gösterilmiştir (Chan ve ark., 1980; Mattson ve ark., 1991). Sodyum piritionun Aplysia'nın torba hücre nöronlarında [Ca^{2+}] yükseldiğini, bu yükselmesinin dış ortamdan kalsiyum iyonlarının akışı sonucu olduğu gösterilmiştir (Knox ve ark., 2004). Kalsiyumdaki geçici artışlar, iyon kanallarının modülasyonu, nörotransmitter salınımı ve gen transkripsiyonunu içeren fizyolojik süreçleri kontrol eder. Anormal derecede uzun bir yükselmenin, nöronal hücre ölümüne yol açan hücresel süreçlerle ilişkili olduğu bildirilmektedir (McConkey ve Orrenius, 1997; Nicotera ve Orrenius, 1998).

Kısıtlı araştırmalarda sıçanlara intraperitonal enjeksiyon ile 30 gün uygulanan 50 mg/kg sodyum piriton dozlarının herhangi bir patolojik değişiklik oluşturmadığı bildirilmektedir (Moe ve ark., 1960).



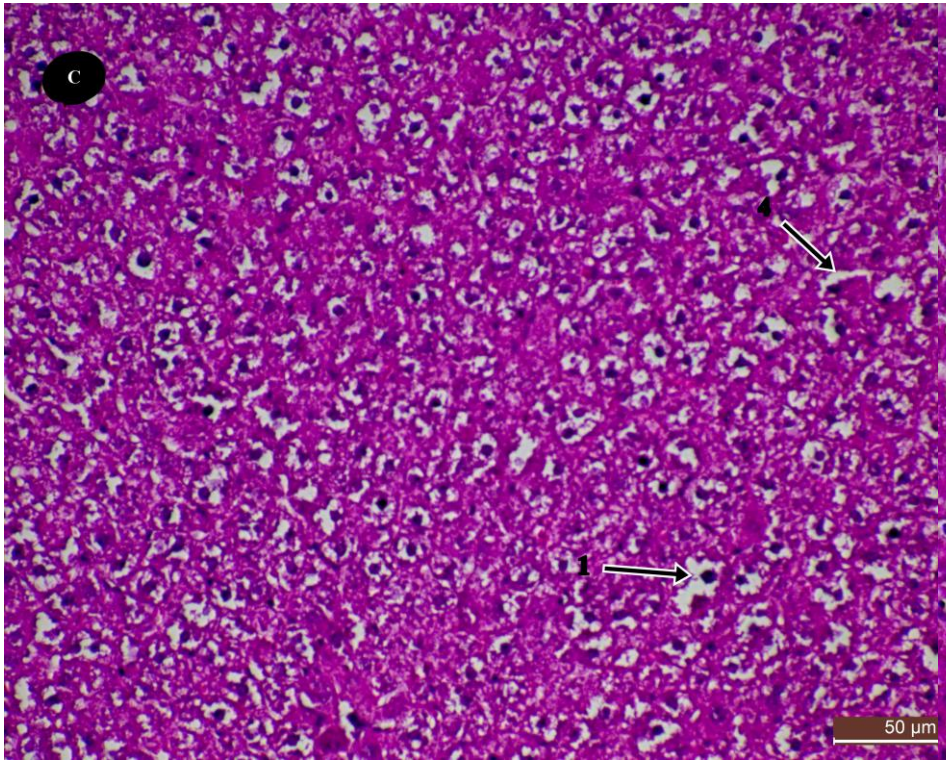
Şekil 1. Kontrol Grubunun (24 saat) Karaciğer Dokusunda Meydana Gelen Histopatolojik Değişiklikler. HE X200

Figure 1. Histopathological Changes in the Liver Tissue of the Control Group (24 hours). HE X200



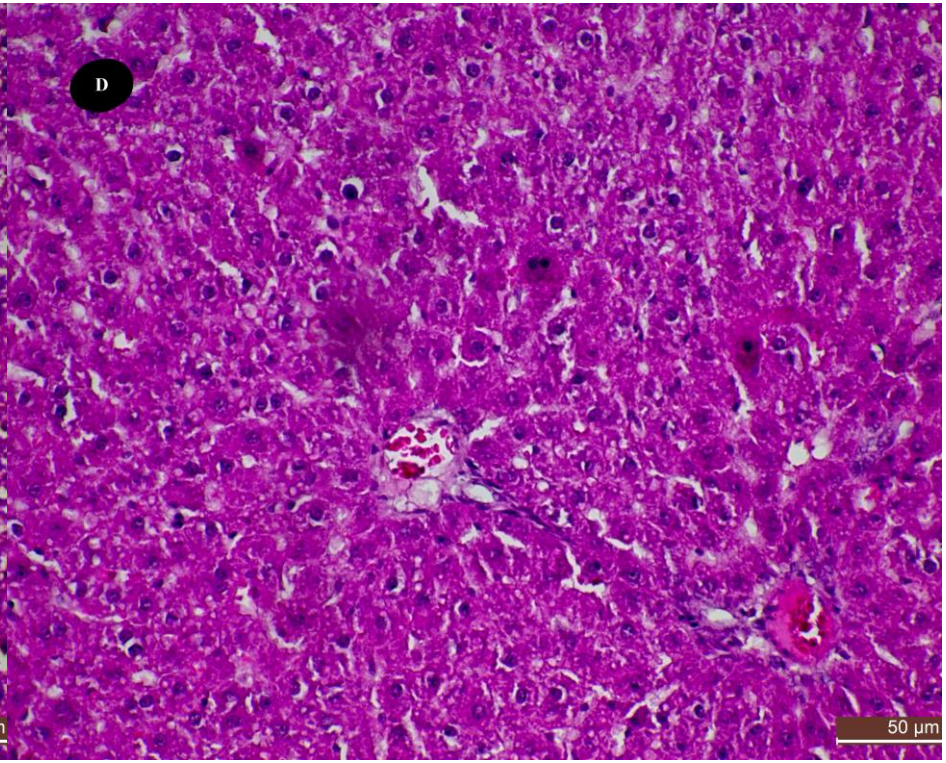
Şekil 2. Doz 1 Grubunun (24 saat) Karaciğer Dokusunda Meydana Gelen Histopatolojik Değişiklikler.
(1- Hepatositlerde vakuolleşme, 4- Hepatoselüler dejenerasyon) HE X200

Figure 2. Histopathological Changes in Liver Tissue of the Dose 1 Group (24 hours).
(1- Vacuolization in hepatocytes, 4- Hepatocellular degeneration) HE X200



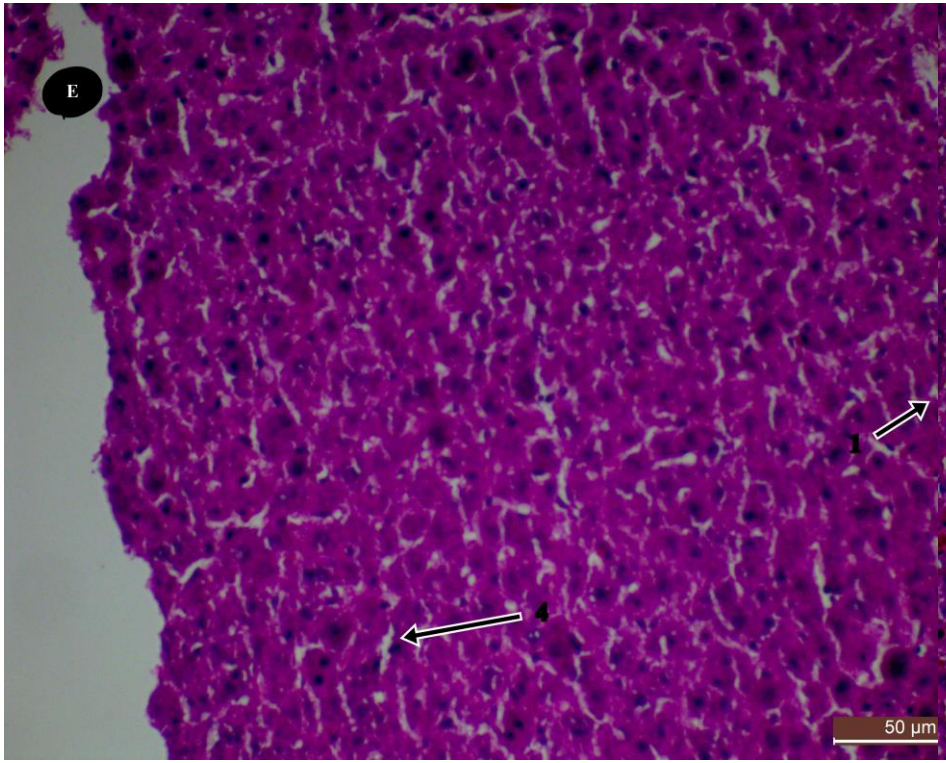
Şekil 3. Doz 2 Grubunun (24 saat) Karaciğer Dokusunda Meydana Gelen Histopatolojik Değişiklikler.
(1- Hepatositlerde vakuolleşme, 4- Hepatoselüler dejenerasyon) HE X200

Figure 3. Histopathological Changes in Liver Tissue of the Dose 2 Group (24 hours).
1- Vacuolization in hepatocytes, 4- Hepatocellular degeneration) HE X200



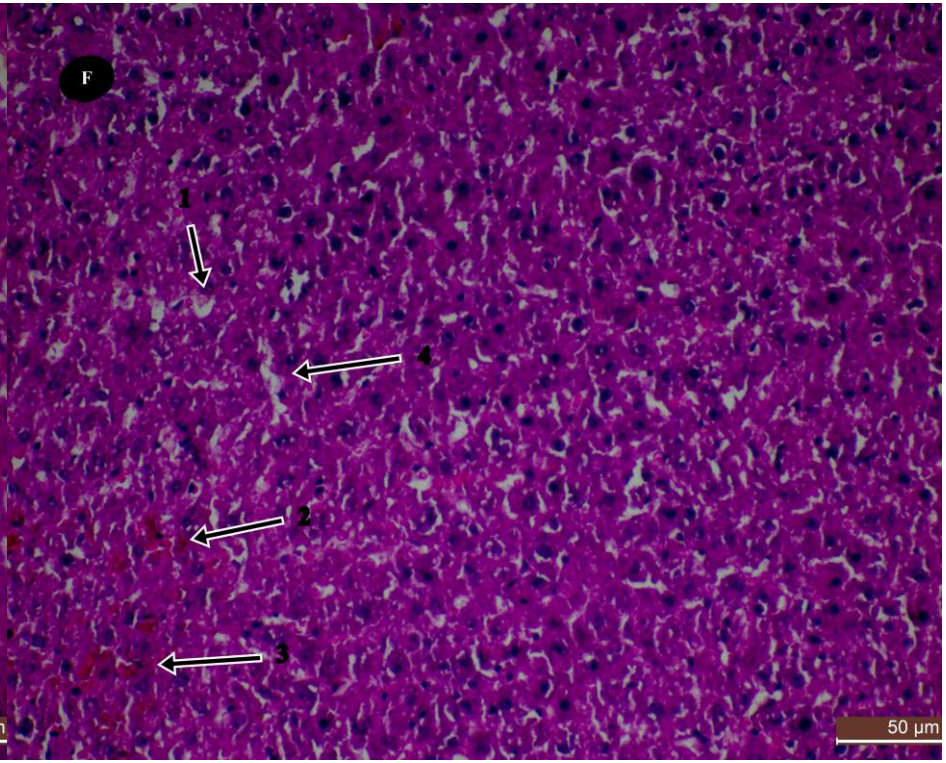
Şekil 4. Kontrol Grubunun (96 saat) Karaciğer Dokusunda Meydana Gelen Histopatolojik Değişiklikler. HE X200

Figure 4. Histopathological Changes in Liver Tissue of the Control Group (96 hours). HE X200



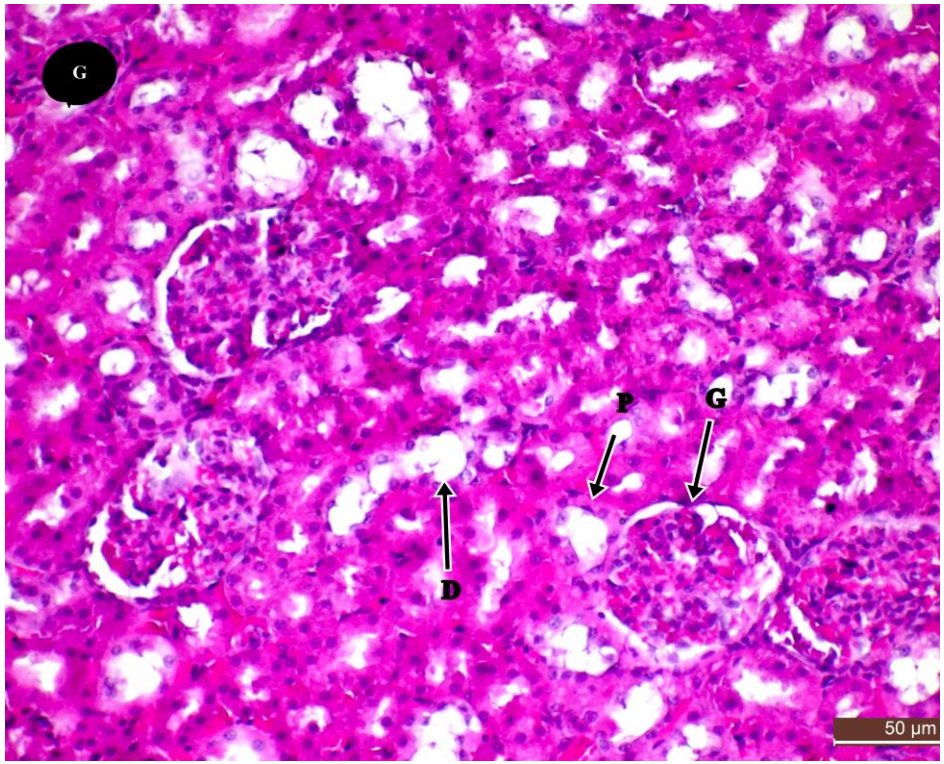
Şekil 5. Doz 1 Grubunun (96 saat) Karaciğer Dokusunda Meydana Gelen Histopatolojik Değişiklikler.
(1- Hepatositlerde vakuolleşme, 4- Hepatoselüler dejenerasyon) HE X200

Figure 5. Histopathological Changes in Liver Tissue of the Dose 1 Group (96 hours).
(1- Vacuolization in hepatocytes, 4- Hepatocellular degeneration) HE X200



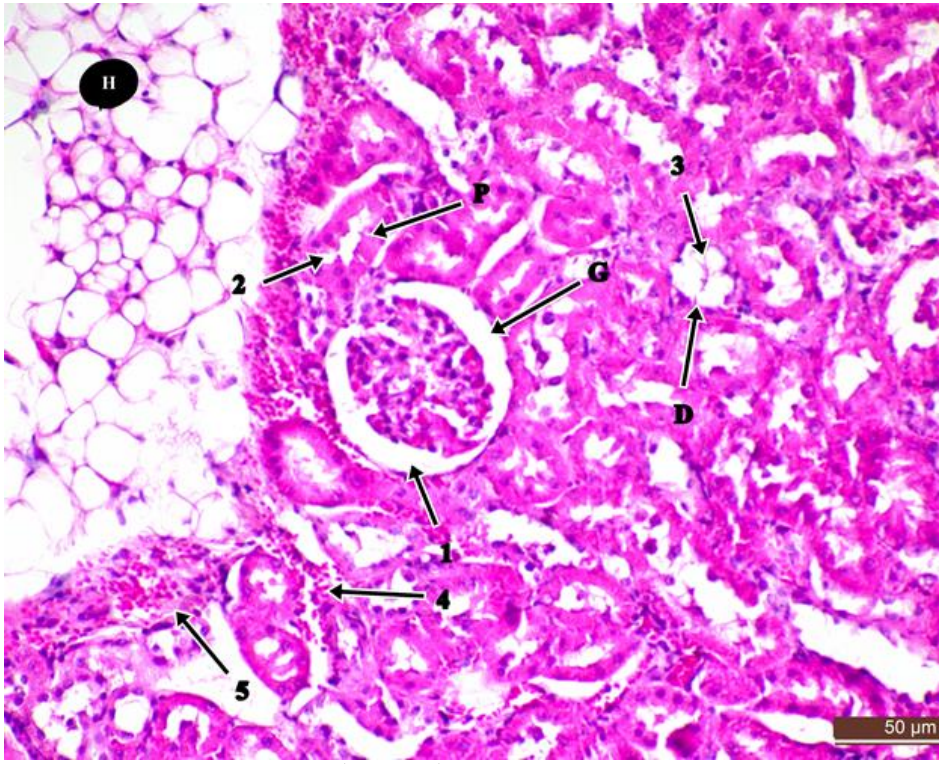
Şekil 6. Doz 2 Grubunun (96 saat) Karaciğer Dokusunda Meydana Gelen Histopatolojik Değişiklikler.
(1- Hepatositlerde vakuolleşme, 2- Pasif Hiperemi, 3- Mononükleer hücre infiltrasyonu, 4- Hepatoselüler dejenerasyon) HE X200

Figure 6. Histopathological Changes in Liver Tissue of the Dose 2 Group (96 hours).
*(1- Vacuolization in hepatocytes, 2- Passive Hyperemia, 3- Mononuclear cell infiltratio*n, *4- Hepatocellular degeneration) HE X200*



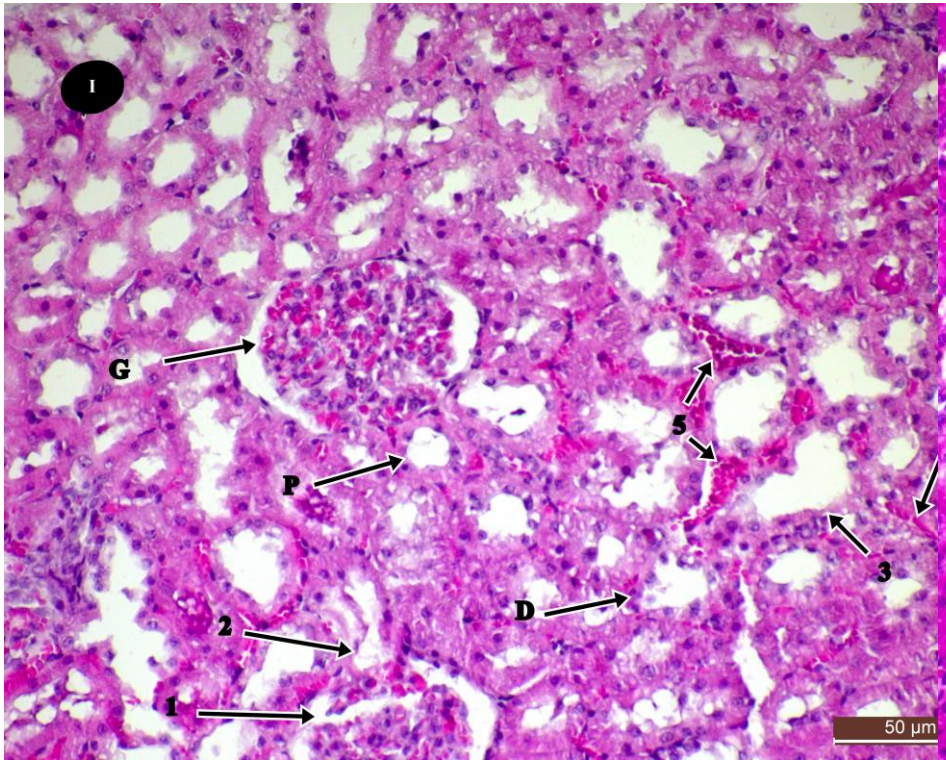
Şekil 7. Kontrol Grubunun (24 saat) Böbrek Dokusunda Meydana Gelen Histopatolojik Değişiklikler.
(G: Glomerulus, P: Proksimal Tübül, D: Distal Tübül) HE X200.

Figure 7. Histopathological Changes in Kidney Tissue of the Control Group (24 hours).
(G: Glomerulus, P: Proximal Tubule, D: Distal Tubule) HE X200.



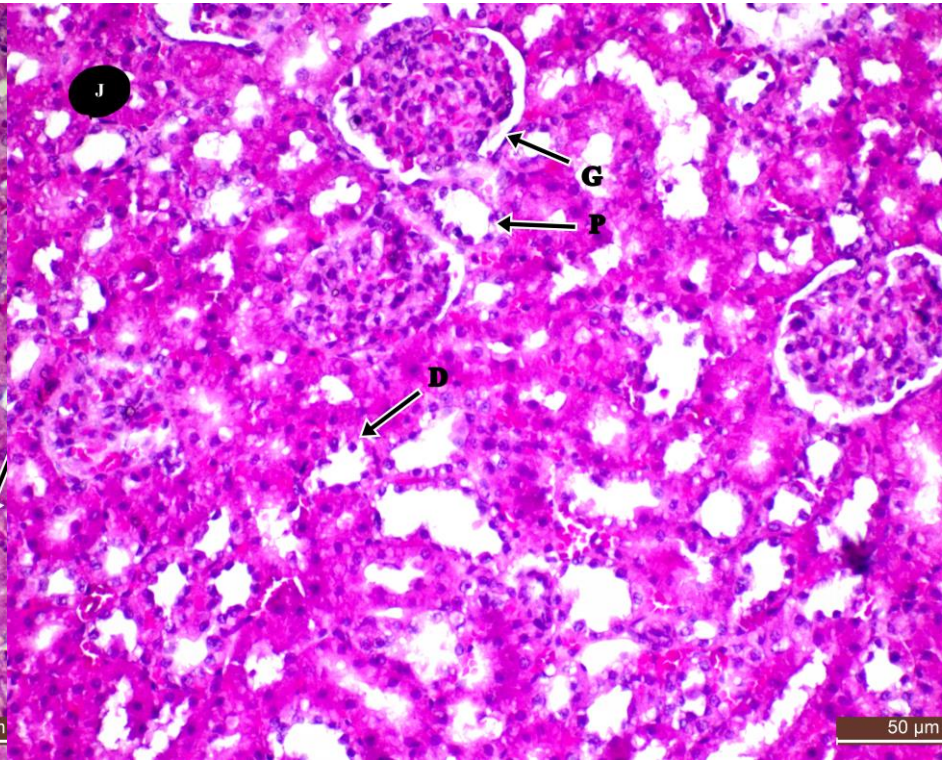
Şekil 8. Doz 1 Grubunun (24 saat) Böbrek Dokusunda Meydana Gelen Histopatolojik Değişiklikler.
(G: Glomerulus, P: Proksimal Tübül, D: Distal Tübül) (1- Glomerulusta Atrofi (Parietal ve viseral yapraklar arasında mesafe artışı), 2- Proksimal tübüllerde dejenerasyon, 3- Distal tübüllerde dejenerasyon 4- Mononükleer hücre infiltrasyonu, 5- Konjesyon (Kanama)) HE X200.

Figure 8. Histopathological Changes in Kidney Tissue of the Dose 1 Group (24 hours).
(G: Glomerulus, P: Proximal Tubule, D: Distal Tubule) (1- Glomerulus Atrophy (increased distance between parietal and visceral layers), 2- Degeneration in proximal tubules, 3- Degeneration in the distal tubules 4- Mononuclear cell infiltration, 5-Congestion (Bleeding)) HE X200.



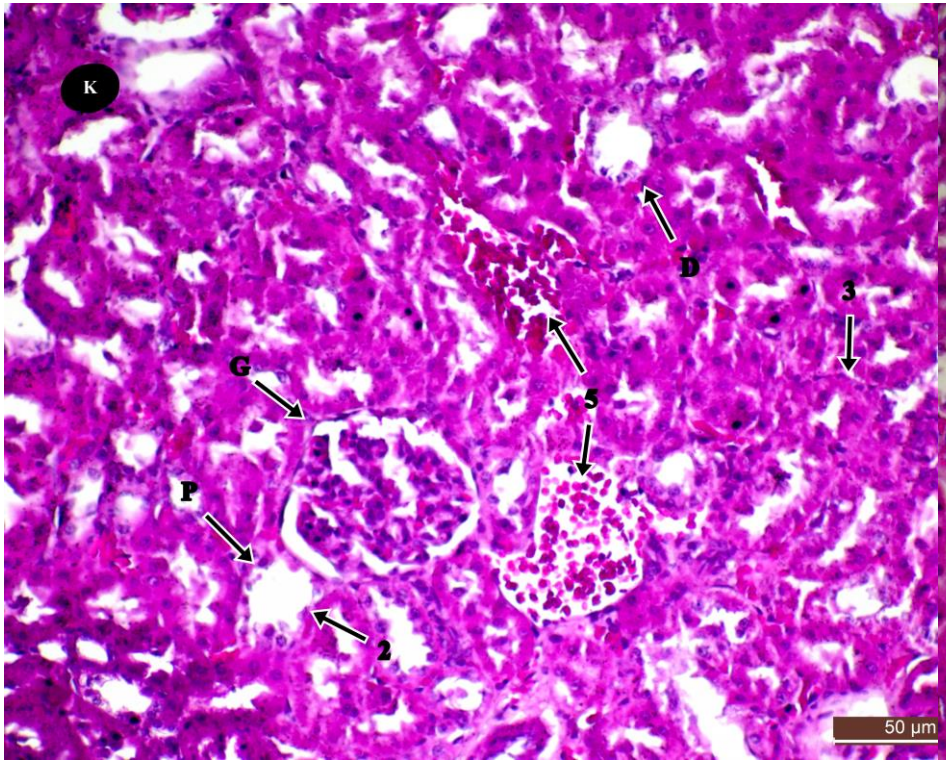
Şekil 9. Doz 2 Grubunun (24 saat) Böbrek Dokusunda Meydana Gelen Histopatolojik Değişiklikler.
(G: Glomerulus, P: Proksimal Tübül, D: Distal Tübül) (1- Glomerulusta Atrofi (Parietal ve viseral yapraklar arasında mesafe artışı), 2- Proksimal tübüllerde dejenerasyon, 3- Distal tübüllerde dejenerasyon 4- Mononükleer hücre infiltrasyonu, 5- Konjesyon (Kanama)) HE X200.

Figure 9. Histopathological Changes in Kidney Tissue of the Dose 2 Group (24 hours).
(G: Glomerulus, P: Proximal Tubule, D: Distal Tubule) (1- Glomerulus Atrophy (increased distance between parietal and visceral layers), 2- Degeneration in proximal tubules, 3- Degeneration in the distal tubules 4- Mononuclear cell infiltration, 5-Congestion (Bleeding)) HE X200.



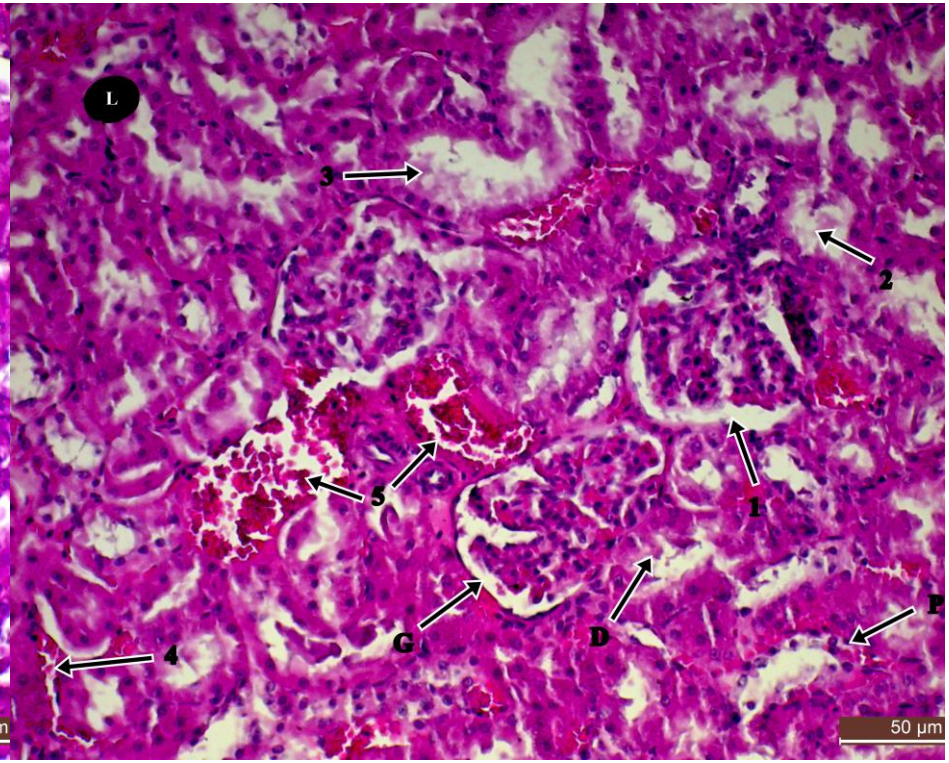
Şekil 10. Kontrol Grubunun (96 saat) Böbrek Dokusunda Meydana Gelen Histopatolojik Değişiklikler.
(G: Glomerulus, P: Proksimal Tübül, D: Distal Tübül) HE X200.

Figure 10. Histopathological Changes in Kidney Tissue of the Dose 2 Group (24 hours).
(G: Glomerulus, P: Proximal Tubule, D: Distal Tubule) HE X200.



Şekil 11. Doz 1 Grubunun (96 saat) Böbrek Dokusunda Meydana Gelen Histopatolojik Değişiklikler.
(G: Glomerulus, P: Proksimal Tübül, D: Distal Tübül) (2- Proksimal tübüllerde dejenerasyon, 3- Distal tübüllerde dejenerasyon, 5- Konjesyon (Kanama)) HE X200.

Figure 11. Histopathological Changes in Kidney Tissue of the Dose 1 Group (96 hours).
(G: Glomerulus, P: Proximal Tubule, D: Distal Tubule) (2- Degeneration in proximal tubules, 3- Degeneration in the distal tubules, 5-Congestion (Bleeding)) HE X200.



Şekil 12. Doz 2 Grubunun (96 saat) Böbrek Dokusunda Meydana Gelen Histopatolojik Değişiklikler.
(G: Glomerulus, P: Proksimal Tübül, D: Distal Tübül) (1- Glomerulusta Atrofi (Parietal ve viseral yapraklar arasında mesafe artışı), 2- Proksimal tübüllerde dejenerasyon, 3- Distal tübüllerde dejenerasyon 4- Mononükleer hücre infiltrasyonu, 5- Konjesyon (Kanama)) HE X200.

Figure 12. Histopathological Changes in Kidney Tissue of the Dose 2 Group (96 hours).
(G: Glomerulus, P: Proximal Tubule, D: Distal Tubule) (1- Glomerulus Atrophy (increased distance between parietal and visceral layers), 2- Degeneration in proximal tubules, 3- Degeneration in the distal tubules 4- Mononuclear cell infiltration, 5-Congestion (Bleeding)) HE X200.

Yapılan bu çalışmada karaciğer histopatolojik incelemeleri sonucunda doz ve süre artımına bağlı olarak şiddeti artan hepatositlerde vakuolleşme, pasif hiperemi, mononükleer hücre infiltrasyonu ve hepatoselüler dejenerasyon tespit edilmiştir (Çizelge 2 ve Şekil 1). Böbreklerde, sodyum piritiona maruz kalınan gruplarda doz ve süreye bağlı olarak şiddeti artan glomerulusta atrofi (parietal ve viseral yapraklar arasında mesafe artışı), proksimal tübüllerde dejenerasyon, distal tübüllerde dejenerasyon, mononükleer hücre infiltrasyonu ve konjesyon (kanama) tespit edilmiştir (Çizelge 3 ve Şekil 2). Çalışma sonucu elde edilen bulgular sitotoksik etkileri olan diğer toksikolojik ajanların oluşturduğu bulgularla benzerlikler göstermektedir (Möller ve ark., 2002; Durmaz ve Giray, 2013). Doz ve süre artışına bağlı bu bulguların, sodyum piritionun sitoksik etkisinden kaynaklanmış olabileceğini göstermiştir.

SONUÇ

Bu araştırma sonucunda, sıçanlara intraperitonal yolla uygulanan sodyum pirition (35 ve 70 mg kg^{-1}, ip) 24 ve 96 saatlik sürelerde sitotoksik etkisinin doz ve süre artışına bağlı histopatolojik değişimlere neden olduğu görülmüştür. Bu araştırma sonucu sodyum pirition üzerine doz ve süreye bağlı araştırmaların etkilerini belirlemeye yönelik ileri araştırmalara ihtiyaç duyulduğunu göstermektedir.

Araştırmacıların Katkı Oranı Beyan Özeti

Yazarlar makaleye eşit oranda katkı sağlamış olduklarını beyan eder.

Çıkar Çatışması Beyanı

Makale yazarları aralarında herhangi bir çıkar çatışması olmadığını beyan ederler.

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Alanya Ekolojik Koşullarında Yetişen *Cistus creticus* L. Bitkisinin Taze ve Kuru Yaprak Örneklerinde Bazı Biyoaktif Bileşenlerin, Pigment İçeriği ve Antioksidan Kapasitenin Belirlenmesi

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ÖZET
Cistus creticus L. Türkiye'nin kuzey, güney ve batı kıyıları boyunca yayılım gösteren önemli tıbbi bitkilerden birisidir. Antik çağlardan beri antimikrobiyal, antiinflamatuar, sitotoksik ve antiülserojenik özellikleri nedeniyle halk tıbbında kullanılmaktadır. Bitkinin taze ve kuru yaprakları genellikle çay olarak tüketilmektedir. Bu çalışma, Alanya ekolojik koşullarında doğal olarak yetişen *Cistus creticus* L. bitkisinin taze ve kurutulmuş yapraklarının bazı biyokimyasal özelliklerini araştırmayı amaçlamaktadır. Bu amaçla bitkinin vejetatif gelişiminin en yüksek olduğu ilkbahar döneminde hasat edilen yaprak örneklerinde; toplam askorbik asit, toplam fenolik, toplam flavonoid, toplam klorofil ve toplam karotenoid içerikleri ile antioksidan aktivite araştırılmıştır. Toplam askorbik asit miktarı dışındaki tüm parametreler kuru yaprak örneklerinde taze yaprak örneklerinden daha yüksek bulunmuştur. Bitkinin yüksek derecede biyoaktif bileşen içermesi ve % 50 civarında radikal süpürücü aktiviteye sahip olması bakımından tüketiminin önemli olduğu düşünülmektedir.

Bitki Fizyolojisi

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Anahtar Kelimeler
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Fenolik
Karotenoid
Klorofil

Determination of Some Bioactive Components, Pigment Content and Antioxidant Capacity in Fresh and Dry Leaf Samples of *Cistus creticus* L. Grown in Alanya Ecological Conditions]

ABSTRACT
Cistus creticus L. is one of the important medicinal plants spreading along the northern, southern and western coasts of Turkey. It has been used in folk medicine since ancient times for its antimicrobial, anti-inflammatory, cytotoxic and antiulcerogenic properties. The fresh and dry leaves of the plant are generally consumed as tea. This study aims to investigate some biochemical properties of fresh and dried leaves of *Cistus creticus* L., which grows naturally in Alanya ecological conditions. For this purpose, in leaf samples harvested in the spring period when the vegetative development of the plant is highest; total ascorbic acid, total phenolic, total flavonoid, total chlorophyll and total carotenoid contents and antioxidant activity were investigated. Except for the total ascorbic acid content, all parameters were higher in dry leaf samples than in fresh leaf samples. It is thought that the consumption of the plant is important because it contains a high degree of bioactive component and has a radical scavenging activity of around 50%.

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GİRİŞ

İnsanlar yüzyıllar boyunca bitkilerin çiçek, yaprak, dal ve sap gibi farklı kısımlarından çeşitli şekillerde hastalıklarına çözüm bulmaya çalışmışlardır. Hastalıkların bitkiler ile tedavi edilme yöntemlerinin başarılı sonuçlar vermesi ile tıbbi ve aromatik bitkilerin önemi artmıştır. Dünya Sağlık Örgütüne göre çeşitli hastalıkların tedavisi için kullanılan ilaçların %25'i tıbbi bitkilerden üretilmektedir ve dünya genelinde tıbbi ve aromatik bitkilere olan talep artmaktadır (Acıbuca ve Budak, 2018).

Türkiye sahip olduğu ekolojik ve coğrafik koşullar sayesinde önemli tıbbi ve aromatik bitkileri barındırmaktadır. İçerisinde önemli tıbbi bitkiler barındıran familyalardan bir tanesi de Cistaceae (Ladengiller) familyasıdır ve 8 cins ile yaklaşık olarak 175 türün çalı ve otsu formlarını içerir (Riehle ve ark. 2013). Familyanın tıbbi özellikli en önemli türleri *Cistus* cinsi içerisinde yer almaktadır. *Cistus* cinsinin üyeleri Türkiye'de doğal olarak yetişen tıbbi bitkilerdendir. Türkiye'de 5 *Cistus* türü yetişmektedir. Bu türler *Cistus creticus* L., *C. parviflorus* L., *C. salviifolius* L., *C. laurifolius* L. ve *C. monspeliensis* L. olup ağırlıklı olarak Türkiye'nin kuzey, güney ve batı kesimlerinde yayılım göstermektedir (Davis, 1965). Bu türler halk arasında 'Kaya gülü', 'Pamucak', 'Pamukluk', 'Karağan', 'Karahan', 'Davşanotu' ve 'Tavşancıl' gibi farklı isimler ile anılmaktadır (Sekeroglu ve Gezici, 2021). *Cistus* sp. genellikle bozulmuş alanlarda yayılış göstermektedir ve türlerin tohum çimlenmesinin yangın sıcaklığı ile teşvik edildiği düşünülmektedir (Riehle ve ark. 2013). Bu bitkiler sahip olduğu antiviral, antimikrobiyal, antidiyabetik, antispazmotik, antienflamatuar ve yüksek antioksidan özellikleri nedeni ile yüzyıllardır çeşitli hastalıkların tedavisinde ve yaraların iyileştirilmesinde kullanılmakla birlikte hoş kokuları nedeni ile parfüm eldesinde ya da görünümleri nedeni ile süs bitkisi olarak da tercih edilmektedir (El Euch ve ark. 2015, Sekeroglu ve Gezici, 2021). Bağışıklık sistemini güçlendirici ve iltihab azaltıcı olarak kullanılmaktadır. Aynı zamanda cilt ve mide hastalıklarının tedavisinde ve mantar enfeksiyonlarına karşı etkisinin bulunduğu bildirilmiştir (Güvenc ve ark. 2005).

Yurtdışında yapılan çalışmalarda bitki ekstraktının koronavirüs gibi zarflı virüslere karşı güçlü antiviral rol oynadığı bildirilmiştir. Özellikle virüsle temas eden ekstraktın yüksek oranda antiviral etkinliği nedeniyle *Cistus*'lu pastiller geliştirilmiştir. Bu pastillerdeki bitki özütünün ağız ve yutak yüzeyi üzerinde koruyucu bir bariyer oluşturarak virüse karşı etki gösterdiği bildirilmiştir. Türkiye'de de 2021 yılında yerli *Cistus*'lu pastil üretimi gerçekleştirilmiştir (Anonim, 2021).

Cistus creticus L. (Pembe laden) Türkiye'de doğal olarak yetişen ve halk arasında tıbbi amaçla kullanılan bir türdür. Ülkemizin batı, güney ve kuzey kıyı kesimlerinde yaygın olarak bulunan ve genellikle çay olarak tüketilen *Cistus creticus* bitkisinin antibakteriyal, antioksidan ve DNA interaksiyon aktivitelerine sahip olduğu bilinmektedir (Davis, 1965, Kilic ve ark., 2019).

Bu çalışmanın amacı ülkemizde Alanya ekolojik koşullarında doğal olarak yetişen *Cistus creticus* bitkisinde bazı biyoaktif bileşenlerin, pigment içeriğinin ve antioksidan özelliklerin belirlenmesidir. Bu amaçla bölgeden toplanan bitkilerin hem taze hem de kurutulmuş yaprak örneklerinde; toplam fenolik, toplam flavonoid ve toplam askorbik asit miktarı, 3 farklı yöntemle antioksidan kapasite tayini (DPPH radikali süpürücü aktivite, FRAP demir iyonu indirgeyici antioksidan güç ve ABTS radikali süpürücü aktivite) ile pigment içerikleri incelenmiştir.

MATERYAL ve METOD

Çalışmada kullanılan bitki örnekleri Alanya bölgesinden ilkbahar döneminde toplanmıştır. Taze olarak çalışılacak örnekler polietilen torbalar içerisinde çalışılıncaya kadar -80 °C'de bekletilmiş, kuru olarak çalışılacak örnekler ise oda sıcaklığında kurutulmuş ve sonrasında polietilen torbalar içerisinde çalışılıncaya kadar -80 °C'de bekletilmiştir.

Ekstrakt hazırlanması

Cistus creticus bitkisine ait 5 gr taze ve 5 gr kurutulmuş yaprak örneği 50 ml metanol ile homojenize edildikten sonra homojenat 1 gece 4 °C'de, çalkalamalı inkübatörde bekletilmiş ve ardından 10.000 rpm'de 10 dk santrifüj edilerek süpernatan elde edilmiştir. Toplam askorbik asit analizi için ise çözücü olarak methanol yerine okzalik asit kullanılmıştır (Murathan ve Kaya, 2020).

Toplam fenolik madde analizi

Toplam fenolik madde miktarının belirlenmesi için Spanos ve Wrolstad (1992)'ın Folin-Ciocalteu yönteminde küçük değişiklikler yapılmış, absornabs ölçümü 765 nm'de gerçekleştirilmiş ve gallik asit standart grafiğinden yararlanılarak fenolik madde miktarı mg GAE (gallik asit) 100 g^{-1} olarak hesaplanmıştır.

Toplam flavonoid madde analizi

Toplam flavanoid madde miktarının belirlenmesi için Quettier ve ark. (2000)'nın metodunda küçük değişiklikler yapılmış, absorbans ölçümü 415 nm'de gerçekleştirilmiş, epikateşin standart grafiğinden yararlanılarak flavanoid madde miktarı mg epikateşin (EC) 100 g^{-1} olarak hesaplanmıştır.

Toplam askorbik asit analizi

Toplam askorbik asit miktarı AOAC (1990)'a göre belirlenmiş, absorbans ölçümü 520 nm'de gerçekleştirilmiş ve askorbik asit miktarı askorbik asit standart grafiğinden yararlanılarak mg 100 g^{-1} olarak hesaplanmıştır.

Antioksidan kapasite tayini

Örneklerin antioksidan kapasiteleri DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate), ABTS (2,2-Azino-bis-3-ethylbenzothiazoline-6-sulfonic Acid) ve FRAP (demir iyonu indirgeyici antioksidan güç) metotları ile tespit edilmiştir.

DPPH yöntemi

Örneklerin DPPH radikal süpürücü aktiviteleri Bakhshi ve Arakawa (2006)'ya göre belirlenmiştir. Karışımların absorbans değerleri 515 nm'de tespit edilmiş ve %DPPH=(Akontrol-Aörnek)/Akontrol x100 formülü kullanılarak hesaplanmıştır.

ABTS yöntemi

Örneklerin ABTS radikal süpürücü aktiviteleri Re ve ark. (1999)'na göre belirlenmiştir. Absorbans değerleri 734 nm'de tespit edilmiş ve %ABTS=(Akontrol-Aörnek)/Akontrol x 100 formülü kullanılarak hesaplanmıştır.

FRAP yöntemi

Örneklerin FRAP değerleri Benzie ve Strain (1996)'e göre belirlenmiştir. Absorbans ölçümü 593 nm'de yapılmıştır. Sonuçlar FeSO4 standardından (100-1000 µl) yararlanılarak µmol Fe (II)g^{-1} olarak belirlenmiştir.

Pigment içeriğinin belirlenmesi

Örneklerin pigment değerleri De-Kok ve Graham (1980)'a göre belirlenmiştir. Absorbanslar 470, 645 ve 662 nm'de ölçülmüş, örneklerin Klorofil a, klorofil b, toplam klorofil ve karatenoid miktarları Lichtenthaler and Welburn (1983)'a göre hesaplanmıştır.

İstatistiksel analizler

Çalışmada analizler üç tekrarlı olarak yapılmış ve ortalama değerler hesaplanmıştır. Bu amaçla SPSS 20.0 paket programı kullanılmıştır. Gruplar arasındaki farklılıklar bağımsız t testi ile $p<0.05$ önem düzeyinde belirlenmiştir.

BULGULAR ve TARTIŞMA

Örneklerin toplam fenolik madde, toplam flavonoid madde, toplam askorbik asit ve pigment içerikleri Çizelge 1.'de verilmiştir. Çizelgede görüldüğü gibi toplam fenolik madde içeriği taze ve kuru yaprakta istatistiksel olarak farklılık göstermiştir ($p<0.05$). Toplam fenolik madde içeriği taze yaprakta 568.9 mg GAE 100 g^{-1}, kuru yaprakta ise 776.6 mg GAE 100 g^{-1} olarak bulunmuştur. Toplam flavonoid madde içeriği fenolik madde içeriğine benzer bir değişim göstermiş ve taze yaprakta 24.8 mg EC 100 g^{-1}, kuru yaprakta ise 123.1 mg EC 100 g^{-1} olarak belirlenmiştir. Kurutulmuş örneklerde toplam fenolik ve flavonoid madde miktarlarındaki artışın kurutmayla birlikte bitki hücre duvarlarının parçalanması ve bu bileşiklerin ekstraksiyonunun kolaylaşması olduğu bildirilmiştir (Kamiloglu ve Capanoglu, 2015). Kilic ve ark. (2019) *C. creticus* kuru yaprak ekstraktlarında toplam fenolik madde ve toplam flavanoid madde miktarlarını sırasıyla 130.3 mg GAE g^{-1} ve 83.9 mg QE g^{-1} olarak bildirmişlerdir. Yine Lahcen ve ark. (2020) *C. creticus* yapraklarında toplam flavanoid madde içeriğinin 53 mg QE g^{-1} olduğunu belirlemişlerdir. Piluzza ve ark. (2011) bu değeri 19.80 mg EQ g^{-1} olarak tespit etmişlerdir. Amensour ve ark. (2010) *C. ladaniferus* bitkisinin kuru yapraklarının etanol ve metanol ekstraktlarında toplam fenolik madde içeriğinin 11.90 mg GAE g^{-1} ve 18 mg GAE g^{-1} olduğunu, Kose ve ark. (2017) ise aynı bitkide toplam fenolik madde içeriğinin 520 mg GAE g^{-1} olduğunu bildirmişlerdir. Dimcheva ve Karsheva (2017) *C. incanus* kuru yapraklarının etanolik ekstraktlarında toplam fenolik madde içeriğini 41.73 ile 98.69 mg GAE g^{-1} olarak, Gawel-Beben ve ark. (2020) 297.71 mg GAE g^{-1} olarak belirlemişlerdir. Aynı araştırmada toplam flavanoid madde miktarı 44.77 mg QE g^{-1} olarak tespit edilmiştir. *C. ladanifer* kuru yapraklarının metanol ekstraktında ise toplam fenolik madde miktarını 142.81 mg GAE g^{-1}, toplam flavanoid madde miktarını 27.91 mg QE g^{-1} olarak bildirilmiştir. Literatür sonuçlarıyla karşılaştırıldığında çalışmada kullanılan örneklerin toplam fenolik ve flavonoid madde içeriklerinin daha düşük olduğu belirlenmiştir. Bu durum farklı çalışmalarda kullanılan bitki örneklerinin genetiksek farklılıklarından kaynaklanabileceği gibi bitkilerin yetiştiği ekolojik ve coğrafik koşulların farklılığından veya metotsal farklılıklardan da kaynaklanabilmektedir.

Örneklerin toplam askorbik asit içeriği taze ve kuru yaprakta istatistiksel olarak farklı tespit edilmiştir ($p<0.05$). Diğer parametrelerin aksine taze yapraktaki toplam askorbik asit içeriği kuru yaprağa oranla daha yüksek bulunmuştur. Taze yaprakta bu değer 162.4 mg 100g^{-1} olarak belirlenirken, kuru yaprakta 96.7 mg 100 g^{-1} olarak belirlenmiştir (Çizelge 1). Bitkilere uygulanan kurutma yöntemlerinin birçoğunda askorbik asit kaybı görülmektedir. Bu kaybın nedeni kurutma sırasında hem askorbik asitin okside olması hem de polifenollerin oksidasyonunu korumak için kullanılması olarak gösterilebilir (Toor ve Sevage,

2006; Joshi ve ark., 2011). Guimarães ve ark. (2009) *C. ladanifer* kuru yapraklarında toplam askorbik asit içeriğini 647.64 µg g^{-1}olarak belirlemiştir. Viapiana ve ark. (2017) farklı *Cistus* genotiplerinin kuru yapraklarının metanolik ekstraktlarında toplam askorbik asit değerlerinin 0.09 ile 0.89 µg g^{-1}arasında olduğunu bildirmişlerdir. Çalışmada kullanılan örneklerin toplam askorbik asit sonuçlarının literatürde bildirilen sonuçlardan daha yüksek olduğu belirlenmiştir. Bu durumun genetiksel, iklimsel ve metotsal farklılıklara ek olarak bitki kurutma metodunun farklılığından da kaynaklanabileceği düşünülmektedir. Nitekim Nindo ve ark. (2003) daha uzun süreli ve daha yüksek sıcaklık kullanılarak yapılan kurutma işlemlerinin askorbik asit oksidasyonunu kolaylaştırması nedeniyle daha yüksek degredasyona neden olduğunu bildirmişlerdir.

Çizelge 1. Yaprak *ö*rneklerinin toplam fenolik madde, toplam flavonoid madde, toplam askorbik asit ve pigment içerikleri

Table 1. *Total phenolic compounds, total flavonoid compounds, total ascorbic acid and pigment contents of leaf samples*

		Taze yaprak	Kuru yaprak
Toplam fenolik madde	mg $100g^{-1}$	568.9±22.3[b]	776.6±23.5[a]
Toplam flavonoid madde	mg $100g^{-1}$	24.8±2.2[b]	123.1±23.3[a]
Toplam askorbik asit	mg $100g^{-1}$	162.4±12.5[a]	96.7±7.8[b]
Kla	µg g^{-1}	3.86±0.06[b]	9.94±0.011[a]
Klb	µg g^{-1}	0.84±0.06[b]	4.19±0.01[a]
Toplam karoten	µg g^{-1}	0.46±0.04[b]	2.1±0.007[a]
Toplam klorofil	µg g^{-1}	5.16± 0.06[b]	14.14±9.03[a]

Aynı satırda gösterilen farklı harfler (a-b) t testine göre istatistiksel olarak farklılıkları göstermektedir ($p<0.05$)

Örneklerin pigment içerikleri kuru örnekte taze örneğe kıyasla daha yüksek olarak belirlenmiştir. Kla değeri taze ve kuru örnekte sırasıyla 3.86 ve 9.94 µg g^{-1}, Klb değeri ise sırasıyla 0.84 ve 4.19 µg g^{-1} olarak tespit edilmiştir. Toplam klorofil içeriği taze yaprakta 5. 158 µg g^{-1}, kuru yaprakta 14.14 µg g^{-1}olarak belirlenmiştir. Benzer şekilde toplam kartenoid içeriği taze yaprakta 0.46 µg g^{-1}, kuru yaprakta 2.1 µg g^{-1}olarak saptanmıştır (Çizelge 1). Kurutmayla birlikte bitki ağırlığı azalmakta ve hücrelerin içeriğindeki su uzaklaştırılmaktadır. Ekstrakt hazırlanırken kullanılan örnek miktarı sabit tutulduğundan bitkinin toplam kuru maddesi içeriğinde bulunan klorofil miktarı da taze örneklere oranla daha yüksek bulunacaktır. Yilmaz ve ark. (2021) oda sıcaklığında kurutulmuş *Thymus vulgaris* L. bitki örneklerinde klorofil içeriğinin taze örneklerden daha yüksek olduğunu belirlemişlerdir. Soysal ve Oztekin (1998) düşük sıcaklıklarda uzun süre kurutulan bitki örneklerinde toplam klorofil kaybının çok az olduğunu bildirmişlerdir. Yine Kumar ve ark. (2014) dondurarak veya oda sıcaklığında kurutma metodlarının klorofil kaybına en az neden olan metotlar olduğunu belirlemişlerdir. Bitkilerin kapalı ortamda düşük sıcaklıklarda kurutulması sırasında azalan oksijenin karotenoidlerin stabilitesini artırdığı bildirilmiştir (Liu ve ark., 2016).

Bitkilerde bulunan fenolik bileşikler ve klorofiller antioksidan potansiyele sahip en önemli bileşenlerdendir (Lanfer-Marquez ve ark. 2005). Çalışmada antioksidan aktivite analizi 3 farklı metotla belirlenmiştir. DPPH radikali süpürücü aktivite taze yaprakta % 49.5, kuru yaprakta %56.9, ABTS radikali süpürücü aktivite taze yaprakta % 42.8, kuru yaprakta % 58.1 ve FRAP değeri ise taze yaprakta 181.7 µmol g^{-1}, kuru yaprakta 428.4 µmol g^{-1} olarak saptanmıştır (Çizelge 2). Farklı yöntemlerden elde edilen bu sonuçlara göre antioksidan kapasite kuru yapraklarda taze yapraklara kıyasla daha yüksek bulunmuştur. Bu durumun kuru örneklerde antioksidan kapasiteye sahip olan fenolik bileşenlerin ve pigment miktarlarının hacme oranla fazla olmasından kaynaklandığı düşünülmektedir. Viapiana ve ark. (2017) farklı *Cistus* genotiplerinin kuru yapraklarının metanolik ekstraktlarında FRAP değerinin 3.16 ile 169.30 µmol g^{-1}olarak bildirmişlerdir. Akkol ve ark. (2012) *C. laurifolius* yapraklarında DPPH radikali süpürücü aktivite oranını % 22.23 olarak, Barrajón-Catalán ve ark. (2010) ise *C. ladanifer* ve *C. populifolius* kuru yaprak ekstraktlarında FRAP değerini 117.72 ve 179.10 mmol 100 g^{-1} olarak tespit etmişlerdir.

Çizelge 2. Yaprak örneklerinin antioksidan aktiviteleri

Table 2. *Antioxidant activities of leaf samples*

	FRAP (µmol g^{-1})	ABTS (%)	DPPH (%)
Taze yaprak	181.7±12.5[b]	42.8±1.26[b]	49.5±5.7[b]
Kuru yaprak	428.4±21.9[a]	58.1±1.3[a]	56.9±5.2[a]

Aynı satırda gösterilen farklı harfler (a-b) t testine göre istatistiksel olarak farklılıkları göstermektedir ($p<0.05$).

SONUÇ ve ÖNERİLER

Sonuç olarak *C. creticus* bitkisinin yapraklarının yüksek derecede biyoaktif bileşen içermesi ve % 50 civarında radikal süpürücü aktiviteye sahip olması nedeniyle halk tarafından kullanılması önerilmektedir. Aynı zamanda daha önce yapılan çalışmalar bitkinin antiviral özelliğinin olduğunu da göstermektedir. Son dönemde gündemde olan Covid-19 virüsüne karşı korunmada bitkinin etkili olabileceği düşünülmektedir. Bu yönde çalışmaların devam ettiği de bilinmektedir. Bitkinin içerdiği biyoaktif bileşenlerin ve antiradikal etkinin bağışıklık sistemini koruyucu etki yaparak dolaylı yönden de virüse karşı koruyucu etkisinin olduğu düşünülmektedir. Ancak bilindiği gibi bitkilerin içerdiği bazı bileşenler belirli bir dozun üzerinde alındığında mutajenik etki göstererek organizmanın DNA'sına zarar verip, sitotoksik etkiye neden olmakta ve nesilden nesile aktarılmaktadır. *Cistus* bitkisinin yaprakları tıbbi amaçlı olarak çeşitli şekillerde kullanılmaktadır. Ancak hangi dozda kullanılmasının uygun olduğu, hangi dozun üzerinde kullanan organizmada hasar oluşturduğu bilinmemektedir. Bu nedenle daha sonra yapılacak çalışmalarla bitkinin mutajenik aktivitesinin olup olmadığının belirlenmesi de önemlidir.

Araştırmacıların Katkı Oranı Beyan Özeti

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Practicality of Seed Morpho-anatomical Characters for the Identification of Species *Alyssum* (Brassicaceae) in Turkey: a Systematic Approach

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ABSTRACT

In this work, morphological and anatomical features of seeds of 15 *Alyssum* taxa (*A. caricum*, *A. davisianum*, *A. filiforme*, *A. haussknechtii*, *A. murale* subsp. *murale* var. *murale*, *A. simplex*, *A. sibiricum*, *A. desertorum*, *A. hirsutum* subsp. *hirsutum*, *A. minutum*, *A. strictum*, *A. strigosum* subsp. *strigosum*, *A. szowitsianum*, *A. dasycarpum* and *A. linifolium* var. *linifolium*) from Turkey were examined with the cluster analysis, and the systematic importance of these characteristics was assessed. The findings exhibited that there were variations among the taxa with respect to seed form, color, and wing existence. The seed dimensions were between 0.43 - 2.12 mm in length, and between 0.34 - 2.05 mm in width. *A. davisianum* and *A. filiforme* were remarkably distinct from others in terms of seed dimension, the first having the smallest and the second having the largest seeds. The surface ornamentation was categorized as nine types; reticulate-foveate, scalariform, tuberculate, alveolate, reticulate-alveolate, aculate, ruminate, verrucate and rugose. The most common type was alveolate, however; tuberculate and rugose types were taxon specific. Also, formation and thicknesses in testa and endosperm layers were studied anatomically. Seed morphological and anatomical features with a few exclusions revealed variety and they were of the taxonomic significance in discrimination of the studied taxa.

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Türkiye'de *Alyssum* (Brassicaceae) Türlerinin Tanımlanmasında Tohum Morfo-anatomik Karakterlerinin Uygulanabilirliği: Sistematik Bir Yaklaşım

ÖZET

Bu çalışmada, Türkiye'den 15 *Alyssum* taksonunun (*A. caricum*, *A. davisianum*, *A. filiforme*, *A. haussknechtii*, *A. murale* subsp. *murale* var. *murale*, *A. simplex*, *A. sibiricum*, *A. desertorum*, *A. hirsutum* subsp. *hirsutum*, *A. minutum*, *A. strictum*, *A. strigosum* subsp. *strigosum*, *A. szowitsianum*, *A. dasycarpum* ve *A. linifolium* var. *linifolium*) tohumlarının morfolojik ve anatomik özellikleri küme analizi ile incelenmiş ve bu özelliklerin sistematik önemi değerlendirilmiştir. Bulgular, taksonlar arasında tohum şekli, rengi ve kanat varlığı açısından farklılıklar olduğunu ortaya çıkarmıştır. Tohum boyutları 0,43 - 2,12 mm uzunluğunda ve 0,34 - 2,05 mm genişliğinde olmuştur. *A. davisianum* ve *A. filiforme*, tohum boyutu açısından diğerlerinden oldukça farklıydı, birincisi en küçük, ikincisi en büyük tohumlara sahipti. Yüzey ornamentasyonları dokuz tip olarak sınıflandırılmıştır; ağsı-foveat, skalariform, tüberkülat, alveolat, ağsı-alveolat, akulat, ruminat, verrukat ve rugoz. En yaygın tip alveolattı, buna karşın; tüberkülat ve rugoz tipleri taksona özgüydü. Ayrıca testa ve endosperm tabakalarındaki oluşum ve kalınlıklar anatomik olarak incelenmiştir. Tohum morfolojik ve anatomik özellikleri, birkaç istisna dışında çeşitliliği ortaya koydu ve incelenen taksonların ayrımında taksonomik öneme sahipti.

Botanik

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INTRODUCTION

Brassicaceae (Cruciferae) is one of the biggest Spermatophyta families, involving 340 genera and 3740 taxa dispersed through the areas mostly on mild areas of the Northern Hemisphere (Khalik and Maesen, 2002; Hohmann et al., 2015; Karaismailoğlu, 2017). The genus *Alyssum* L. has over 195 taxa in worldwide with major spread in Turkey and Eastern Europe (Warwick et al., 2006; Bülbül et al., 2019). The genus is represented by more than 100 taxa in Turkey (Güner et al., 2012).

The genus *Alyssum* and many genera in the family are taxonomically problematic, due to very variable in habit, fruit and floral morphology (Dudley, 1965; Karaismailoğlu, 2018). This situation triggers some difficulties in classification of the genus; and so extra characteristics are required in the classification of the genus in addition to traditional characters.

Micromorphological characters are systematically important in separation of the plant taxa (Brochmann, 1992), in defining of the evolutionary links and in explaining of the systematic difficulties (Khalik and Maesen, 2002; Karaismailoğlu, 2019a). Also, forms of the epidermal cells are excellent identification features at species level (Barthlott, 1981; Khalik and Maesen, 2002; Karaismailoğlu, 2019b). In addition, seed anatomical characteristics are utilized in separating of closely related taxa in the genera (Karaismailoğlu, 2016 and 2019a). However, there are only few systematic studies on seeds features of *Alyssum* (Bülbül et al., 2019; Şirin, 2019). Thus, the purpose of this investigation is to assess seed morpho-anatomical features of the examined *Alyssum* taxa, and their utilize in infrageneric delimitation.

MATERIAL and METHOD

The seeds belonging to 15 *Alyssum* taxa were used for the morpho-anatomical examinations. The samples were gathered from native inhabitants and collected in Siirt University Fauna and Flora center (SUFAF) (Table 1). Evaluations were made on 50 mature seeds for each taxon.

Morphological characters like shape, dimension and color of seeds were analyzed utilizing stereomicroscope and Kameram Imaging Software (KIS). To observe micromorphological structures, samples were arranged for Scanning Electron Microscopy (SEM) by sticking with silver-paste on the stub, and covered with gold (Karaismailoğlu, 2015).

The cross-sections were obtained with an automatic microtome from the mid-section of seeds. The samples were put in FAA for 24 hours. Afterward, they were dehydrated via ethanol and xylene sets, and dyed with Hematoxylin-Eosin Y, and were covered with entellan to see anatomical elements (Karaismailoğlu, 2015). The anatomical features involving testa and endosperm were examined, and their pictures were taken with utilizing Olympus CX21FS1 light microscope and KIS.

The terminology of morpho-anatomical features of seeds was suitable with Stearn (Stearn,1985).

Clustering of taxa was done with applying the grouping assessment approach (UPGMA) as per 59 characteristics in Tables 2-3 [Characteristics utilized in statistical examination; seed color: brown or dark brown (1), clear brown (2), dark brown-black (3), shape: ellipticus (4), ovatus (5), circularis (6), circularis-transverse (7), ovatus-late (8), ovatus-transverse late (9), surface: coarse protrusions (10), smooth (11), slightly striped (12), reticulate (13), sizes: length (14), width (15), raphe presence (16), surface ornamentation: alveolate (17), scalariform (18), reticulate-alveolate (19), aculate (20), ruminate (21), verrucate (22), tuberculate (23), rugose (24), reticulate-foveate (25), anticlinal cell wall: raised (26), sunken (27), unclear (28), periclinal cell wall: concave (29), convex (30), unclear (31), epidermal cell structures: rectangular (32), pentagonal (33), alveolate (34), protrusion (35), polygonal (36), crushed polygonal (37), oval (38), unclear (39), the anatomical structures of outer epidermis of outer testa: flat (40), elongated rectangular (41), cubic (42), polygonal (43), oval (44), inner epidermis presence (45), inner epidermis structure: flat (46), rectangular (47), elongated rectangular (48), crushed (49), outer testa thickness (50), inner testa presence (51), inner testa structure: flat (52), rectangular (53), crushed (54), inner testa thickness (55), parenchyma structure: flat (56), rectangular (57), parenchyma thickness (58), presence of mucilage cell (59)]. Furthermore, the dissimilarity matrix of the analyzed taxa was created with MVSP (Kovach, 2007) (Table 4).

RESULTS

The seed features containing color, dimension, shape and surface characters are macromorphologically assessed (Figure 1 and Table 2). Seed colors of examined taxa are observed as brown, clear brown, dark brown, black-dark brown. Brown is the most

Table 1. The analyzed taxa and their locations.
Çizelge 1. Analiz edilen taksonlar ve lokasyonları.

No	Sections *Seksiyonlar*	Taxa *Taksonlar*	Location *Lokasyon*	Collection number *Koleksiyon numarası*
1	*Odontarrhena*	**Alyssum caricum* T.R.Dudley & Hub.-Mor.	Muğla, Marmaris, 13.8.2016	Karaismailoğlu 331
2	*Odontarrhena*	**A. davisianum* T.R.Dudley	Kütahya, Gediz, 16.6.2016	Karaismailoğlu 279
3	*Odontarrhena*	**A. filiforme* Nyár.	Gümüşhane, Kürtün, 13.7.2014	Karaismailoğlu 85b
4	*Odontarrhena*	**A. haussknechtii* Boiss.	Bolu, Abant, 21.5.2016	Karaismailoğlu 247
5	*Odontarrhena*	*A. murale* Waldst. & Kit. subsp. *murale* var. m*urale*	Gümüşhane, Kürtün, 13.7.2014	Karaismailoğlu 74b
6	*Odontarrhena*	*A. simplex* Rudolph	İstanbul, Büyükçekmece-Çatalca, 8.7.2016	Karaismailoğlu 312
7	*Odontarrhena*	*A. sibiricum* Willd.	Kütahya, Gediz, 24.6.2016	Karaismailoğlu 290
8	*Alyssum*	*A. desertorum* Stapf.	Ankara, Keçiören, 08.2016	Karaismailoğlu 337
9	*Alyssum*	*A. hirsutum* M.Bieb. subsp. *hirsutum*	Mersin, Mut, 27.7.2012	Karaismailoğlu 8
10	*Alyssum*	*A. minutum* Schlecht. ex DC.	Bursa, Uludağ, 1.7.2016	Karaismailoğlu 295
11	*Alyssum*	*A. strictum* Willd.	Niğde, Çamardı, 12.6.2016	Karaismailoğlu 271
12	*Alyssum*	*A. strigosum* Banks & Sol. subsp. *strigosum*	Bursa, Uludağ, 1.7.2016	Karaismailoğlu 296
13	*Alyssum*	*A. szowitsianum* Fisch. & C.A.Mey.	Gümüşhane, Zigana, 27.3.2015	Karaismailoğlu 115b
14	*Psilonema*	*A. dasycarpum* Stephan ex Willd.	Ankara, Haymana, 10.8.2016	Karaismailoğlu 330
15	*Meniocus*	*A. linifolium* Stephan ex Willd. var. *linifolium*	Konya, Cihanbeyli-Yavsan, 11.7.2016	Karaismailoğlu 316

* = endemic taxon
**= endemik takson*

Table 2. The seed macro and micro morphological features of the examined taxa (L=length, W=Width, +=present, -=absent, ±=standard deviation).
Çizelge 2. İncelenen taksonların tohum makro ve mikro morfolojik özellikleri (L=boy, W=en, +=mevcut, -=yok, ±= standart sapma).

Taxa *Taksonlar*	**Color** *Renk*	**Shape** *Şekil*	**Seed surface** *Tohum yüzeyi*	**Seed dimensions** *Tohum boyutları*		**Seed ornamentation** *Tohum ornamentasyonu*	**Anticlinal cell wall** *Antiklinal hücre duvarı*	**Periclinal cell wall** *Periklinal hücre duvarı*	**Epidermal cell form** *Epidermal hücre formu*
				L (mm)	**W (mm)**				
A. caricum	Dark Brown	Ellipticus	Coarse protrusions	1.32±0.18	1.05±0.12	Scalariform	Raised	Concave	Rectangular or Pentagonal
A. davisianum	Dark Brown	Ovatus	Smooth	0.43±0.08	0.34±0.06	Scalariform	Sunken	Convex	Rectangular
A. filiforme	Clear Brown	Circularis	Smooth	2.12±0.18	2.05±0.12	Alveolate	Sunken	Concave	Alveolate
A. haussknechtii	Brown	Ovatus	Slightly striped	0.75±0.24	0.47±0.16	Reticulate-Alveolate	Raised	Concave	Polygonal or Alveolate
A. murale* subsp. *murale* var. m*urale	Brown	Circularis-Transverse	Smooth	2.07±0.15	1.91±0.08	Aculate	Sunken	Convex	Protrusion
A. simplex	Black-Dark Brown	Ovatus-transverse late	Smooth	1.22±0.12	1.07±0.08	Ruminate	Unclear	Unclear	Unclear
A. sibiricum	Brown	Ovatus-late	Reticulate	1.02±0.04	0.95±0.04	Verrucate	Sunken	Convex	Oval
A. desertorum	Clear Brown	Circularis	Smooth	1.15±0.14	1.09±0.15	Tuberculate	Sunken	Convex	Unclear
A. hirsutum* subsp. *hirsutum	Dark Brown	Ovatus	Smooth	1.41±0.15	1.21±0.12	Aculate	Sunken	Convex	Alveolate
A. minutum	Brown	Ovatus-transverse late	Reticulate	1.38±0.18	1.29±0.15	Tuberculate	Sunken	Convex	Unclear
A. strictum	Brown	Circularis	Reticulate	1.08±0.06	1.01±0.04	Alveolate	Sunken	Concave	Oval
A. strigosum* subsp. *strigosum	Black-Dark Brown	Ovatus	Smooth	1.93±0.12	1.41±0.15	Verrucate	Sunken	Concave	Unclear
A. szowitsianum	Clear Brown	Ellipticus	Smooth	1.09±0.18	0.95±0.10	Rugose	Sunken	Convex	Crushed polygonal
A. dasycarpum	Brown	Ellipticus	Slightly striped	1.02±0.21	0.79±0.18	Reticulate-Foveate	Sunken	Convex	Polygonal
A. linifolium* var. *linifolium	Dark Brown	Ellipticus	Reticulate	1.28±0.21	1.05±0.10	Ruminate	Unclear	Unclear	Unclear

Table 3. The seed anatomical features of the examined taxa (+=present, -=absent, ±standart deviation).
Çizelge 3. İncelenen taksonların tohum anatomik özellikleri (+=mevcut, -=yok, ±= standart sapma).

Taxa *Taksonlar*	**Outer testa** *Dış testa*			**Inner testa** *İç testa*		**Parenchyma layer** *Parenkima tabakası*	
	Outer epidermis structures *Dış epidermis yapısı*	**Inner epidermis structure** *İç epidermis yapısı*	**Thickness** *Kalınlık* (µm)	**Structure** *Yapı*	**Thickness** *Kalınlık* (µm)	**Structure** *Yapı*	**Thickness** *Kalınlık* (µm)
A. caricum	1-2 layers, flat cells	-	28.39±2.06	-	-	1 layer, flat cells	20.18±1.24
A. davisianum	1 layer, elongated rectangular cells	1 layer, flat cells	59.86±4.03	-	-	1 layer, flat cells	33.85±2.39
A. filiforme	2-3 layers, polygonal cells	1 layer, flat cells	122.77±5.86	-	-	1 layer, flat cells	25.42±1.88
A. haussknechtii	1 layer, flat cells	2-3 layer, flat cells	67.42±3.24	-	-	1 layer, flat cells	27.17±0.99
A. murale subsp. ***murale*** var. m***urale***	1 layer, cubic or polygonal cells	1 layer, flat cells	56.27±2.51	-	-	1 layer, flat cells	19.76±2.54
A. simplex	1 layer, oval cells	1 layer, flat cells	69.27±3.46	1 layer, flat cells	22.38±1.28	1 layer, flat cells	27.52±2.41
A. sibiricum	1-2 layers, polygonal cells	1 layer, flat cells	72.19±2.45	1 layer, flat cells	39.72±2.87	1 layer, flat cells	35.28±3.17
A. desertorum	1 layer, cubic cells	1 layer, flat cells	50.24±2.11	-	-	1 layer, flat cells	21.16±3.07
A. hirsutum subsp. ***hirsutum***	2-3 layers, polygonal cells	1-2 layers, elongated rectangular cells	133.15±4.64	-	-	1 layer, flat cells	32.14±1.14
A. minutum	1 layer, elongated rectangular cells	1 layer, elongated rectangular cells	86.25±3.35	-	-	1 layer, flat cells	24.83±1.56
A. strictum	1 layer, elongated rectangular cells	1 layer, elongated rectangular cells	130.21±4.98	1 layer, rectangular cells	24.26±2.15	1 layer, flat cells	24.76±2.05
A. strigosum subsp. ***strigosum***	2-3 layers, polygonal cells	1-2 layers, crushed cells	138.49±3.77	-	-	1 layer, rectangular cells	29.44±3.29
A. szowitsianum	1-2 layers, polygonal cells	1-2 layers, elongated rectangular cells	115.89±2.54	1 layer, crushed cells	19.21±2.54	1 layer, flat cells	50.66±2.97
A. dasycarpum	1 layer, elongated rectangular cells	1 layer, flat cells	67.81±5.42	-	-	1 layer, flat cells	24.47±1.10
A. linifolium var. ***linifolium***	2-3 layers, polygonal cells	-	60.28±3.88	-	-	1 layer, flat cells	22.19±0.77

Table 4. The dissimilarity matrix of the examined taxa.
Çizelge 4. *Çalışılan taksonların benzemezlik matrisi.*

Taxa *Taksonlar*	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**
A. caricum **(1)**	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A. davisianum **(2)**	**1.06**	0	-	-	-	-	-	-	-	-	-	-	-	-	-
A. filiforme **(3)**	4.23	3.76	0	-	-	-	-	-	-	-	-	-	-	-	-
A. haussknechtii **(4)**	4.85	8.43	6.25	0	-	-	-	-	-	-	-	-	-	-	-
A. murale **subsp.** ***murale*** **var.** ***murale*** **(5)**	3.54	3.61	1.89	7.24	0	-	-	-	-	-	-	-	-	-	-
A. simplex **(6)**	4.19	8.08	11.75	10.75	6.67	0	-	-	-	-	-	-	-	-	-
A. sibiricum **(7)**	3.68	5.65	4.27	6.08	5.39	8.18	0	-	-	-	-	-	-	-	-
A. desertorum **(8)**	8.95	2.19	5.16	6.74	5.63	8.43	4.15	0	-	-	-	-	-	-	-
A. hirsutum **subsp.** ***hirsutum*** **(9)**	2.01	4.59	1.15	6.18	1.88	8.11	3.58	4.32	0	-	-	-	-	-	-
A. minutum **(10)**	2.48	4.96	4.32	5.34	5.18	8.54	3.14	5.58	3.19	0	-	-	-	-	-
A. strictum **(11)**	5.86	4.57	4.09	6.33	5.93	8.46	1.88	5.51	2.97	1.21	0	-	-	-	-
A. strigosum **subsp.** ***strigosum*** **(12)**	4.21	4.83	4.11	6.03	6.16	7.76	1.86	6.03	3.27	1.09	1.29	0	-	-	-
A. szowitsianum **(13)**	7.38	7.27	5.21	7.46	5.35	9.46	5.19	2.01	4.76	4.62	4.19	6.84	0	-	-
A. dasycarpum **(14)**	12.67	11.32	12.65	11.42	11.64	**12.95**	11.73	11.51	12.09	11.95	11.18	12.33	11.54	0	-
A. linifolium **var.** ***linifolium*** **(15)**	10.52	9.89	9.55	9.74	8.96	8.87	9.02	8.55	8.61	8.69	8.44	8.51	8.49	7.55	0

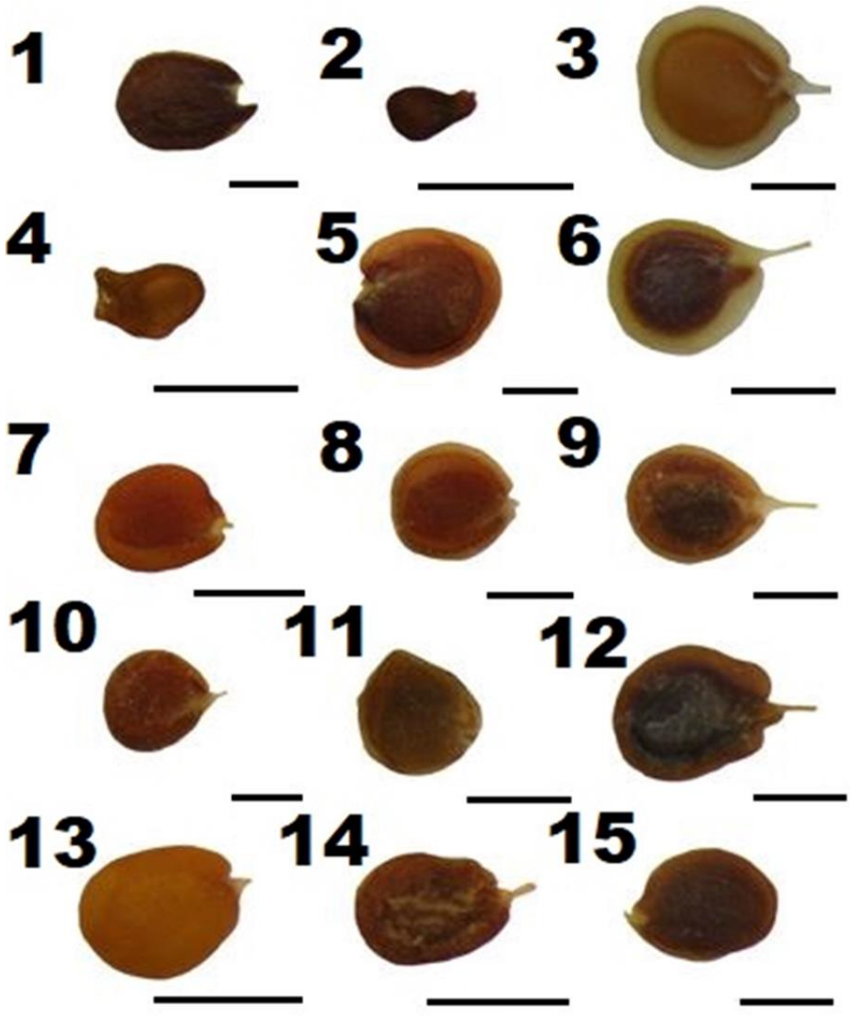

Figure 1. The seeds of the analyzed taxa; 1: *Alyssum caricum*, 2: *A. davisianum*, 3: *A. filiforme*, 4: *A. haussknechtii*, 5: *A. murale* subsp. *murale* var. *murale*, 6: *A. simplex*, 7: *A. sibiricum*, 8: *A. desertorum*, 9: *A. hirsutum* subsp. *hirsutum*, 10: *A. minutum*, 11: *A. strictum*, 12: *A. strigosum* subsp. *strigosum*, 13: *A. szowitsianum*, 14: *A. dasycarpum*, 15: *A. linifolium* var. *linifolium* (Scale bars=1 mm).

Şekil 1. Analiz edilen taksonların tohumları; 1: Alyssum caricum, 2: A. davisianum, 3: A. filiforme, 4: A. haussknechtii, 5: A. murale subsp. murale var. murale, 6: A. simplex, 7: A. sibiricum, 8: A. desertorum, 9: A. hirsutum subsp. hirsutum, 10: A. minutum, 11: A. strictum, 12: A. strigosum subsp. strigosum, 13: A. szowitsianum, 14: A. dasycarpum, 15: A. linifolium var. linifolium (Ölçekler= 1mm).

ordinary color, noticed in 6 of the studied taxa. 6 different seed shapes are found: ellipticus, ovatus, circularis, circularis-transverse, ovatus-late and ovatus-transverse late. Ovatus and ellipticus are the more common than other taxa. Ovatus-late and circularis-transverse types are characteristic for *A. sibiricum* and *A. murale* subsp. *murale* var. *murale*, respectively (Figure 1 and Table 2). The seed dimension varies between 0.43 - 2.12 mm in length, between 0.34 - 2.05 mm in width. *Alyssum davisianum* and *A. filiforme* are markedly distinct from other taxa in terms of seed dimension. Seed surface structure has demonstrated differences: smooth in 8 taxa, reticulate in 4 taxa, slightly striped in 2 taxa and coarse protrusion in 1 taxon (Figure 1 and Table 2). In addition, the presence of raphe has an important role in separation of some taxa with the same population appearance.

The seed ornamentation types, periclinal-anticlinal cell wall structures, and epidermal cell forms of the examined taxa are micromorphologically analysed. Seed ornamentation has recorded in 9 different types: alveolate, scalariform, reticulate-alveolate, aculate, ruminate, verrucate, tuberculate, rugose and reticulate-foveate (Figure 2 and Table 2). The most common types are scalariform, alveolate, aculate, ruminate, verrucate and tuberculate (each is seen in 2 taxa). Some types such as reticulate-alveolate (in *A. haussknechtii*), rugose (in *A. szowitsianum*) and reticulate-foveate (in *A. dasycarpum*) are specific to only 1 taxon. The anticlinal cell walls are in raised, sunken and unclear structures. The anticlinal cell wall of the ruminate ornamentation type does not contain special structure. Periclinal cell wall is convex (8 taxa), concave (5 taxa), or unclear (2 taxa) in form (Table 2). Also, the cell forms on surface are very distinct: rectangular, pentagonal, alveolate, protrusion, polygonal, crushed polygonal, oval, and unclear. The most common cell form is unclear, while protrusion is the rarest type (Table 2).

The outcomes of the seed anatomical examination are displayed in Figure 3 and Table 3. Generally, the seeds of the analysed samples are comprised of 4 layers, involving the outer epidermis, the inner epidermis (outer testa), the inner testa, and the endosperm. The outer epidermis consists of the elongated rectangular, flat, cubic, polygonal, and oval cell shapes, with the number of layers varying between 1 and 3 (Table 3). The most frequent type is polygonal, while the rarest one is the oval form (Figure 3). The inner epidermis consists of 1-3 layers of flat, crushed, and elongated rectangular cells. There is no the inner epidermis layer in *A. caricum* and *A. linifolium* var. *linifolium* taxa. The thickness of the epidermis layers (outer testa) varies between 28.39 µm (in *A. caricum*) - 138.49 µm (in *A. strigosum* subsp. *strigosum*). The inner testa, which is mostly a squeezed tissue below the outer testa layers, has 1-layered of flat, rectangular, or crushed cells in the studied 4 taxa (*A. strictum*, *A. simplex*, *A. szowitsianum* and *A. sibiricum*). Thickness of this layer ranges from 19.21 µm (in *A. szowitsianum*) to 39.72 µm (in *A. sibiricum*) (Table 3). The endosperm of the analysed samples is 1-layered, and contains mainly flat and rarely rectangular cells. Endosperm thickness ranges from 19.76 µm to 50.66 µm. The broadest endosperm is observed in *A. szowitsianum*; however, *A. murale* subsp. *murale* var. *murale* is of the narrowest. Mucilage cells are noticed in the outer testa layers of seeds of the examined taxa, outside of *A. caricum* (Figure 3 and Table 3).

The statistical evaluation of the seed morpho-anatomical features allows the produce of a dendrogram revealing the similarities and differences between the taxa studied. A dendrogram is created as a consequence of the cluster testing based on the difference and similarity of 59 characteristics in 15 Alyssum taxa. The co-phenetic relationship coefficient has computed to obtain the correlation between the dissimilarity matrix and dendrogram (Table 4 and Figure 4). The co-phenetic relationship between dissimilarity matrix and dendrogram has been determined as 0.59. The cluster analysis show that there are 2 main clusters as A and B: Cluster A1 contains *A. linifolium* var. *linifolium*, *A. szowitsianum* and *A. desertorum*. Cluster A2 consists of 2 subsets as A21 (*A. hirsutum* subsp. *hirsutum*, *A. filiforme*, *A. murale* subsp. *murale* var. *murale*, *A. davisianum*, *A. caricum*) and A22 (*A. sibiricum*, *A. strictum*, *A. minutum* and *A. strigosum* subsp. *strigosum*). Cluster B contains *A. haussknechtii* and *A. simplex*. *Alyssum dasycarpum* has created a clade branch outside of clusters in dendrogram (Figure 4). *A. caricum* and *A. davisianum* are the most closely related taxa (dissimilarity coefficient: 1.06), as *A. dasycarpum* and *A. simplex* are the most distantly related taxa (dissimilarity coefficient: 12.95) (Table 4).

DISCUSSION

The seed morphological data such as color, dimension and surface are valuable in explaining evolutionary relationships, solving taxonomic problems and separating closely related taxa in the family Brassicaceae (Vaughan and Whitehouse, 1976; Corner, 1976; Karaismailoğlu, 2016, 2019a, 2019b). The seed macromorphological features have demonstrated differences among the *Alyssum* species. The color is helpful in separating of some closely correlated taxa in terms of population appearance, flower and fruit characters, like *A. strictum* (brown) - *A. szowitsianum* (clear brown), *A. simplex* (black-dark brown) - *A. sibiricum* (brown) and *A. desertorum* -

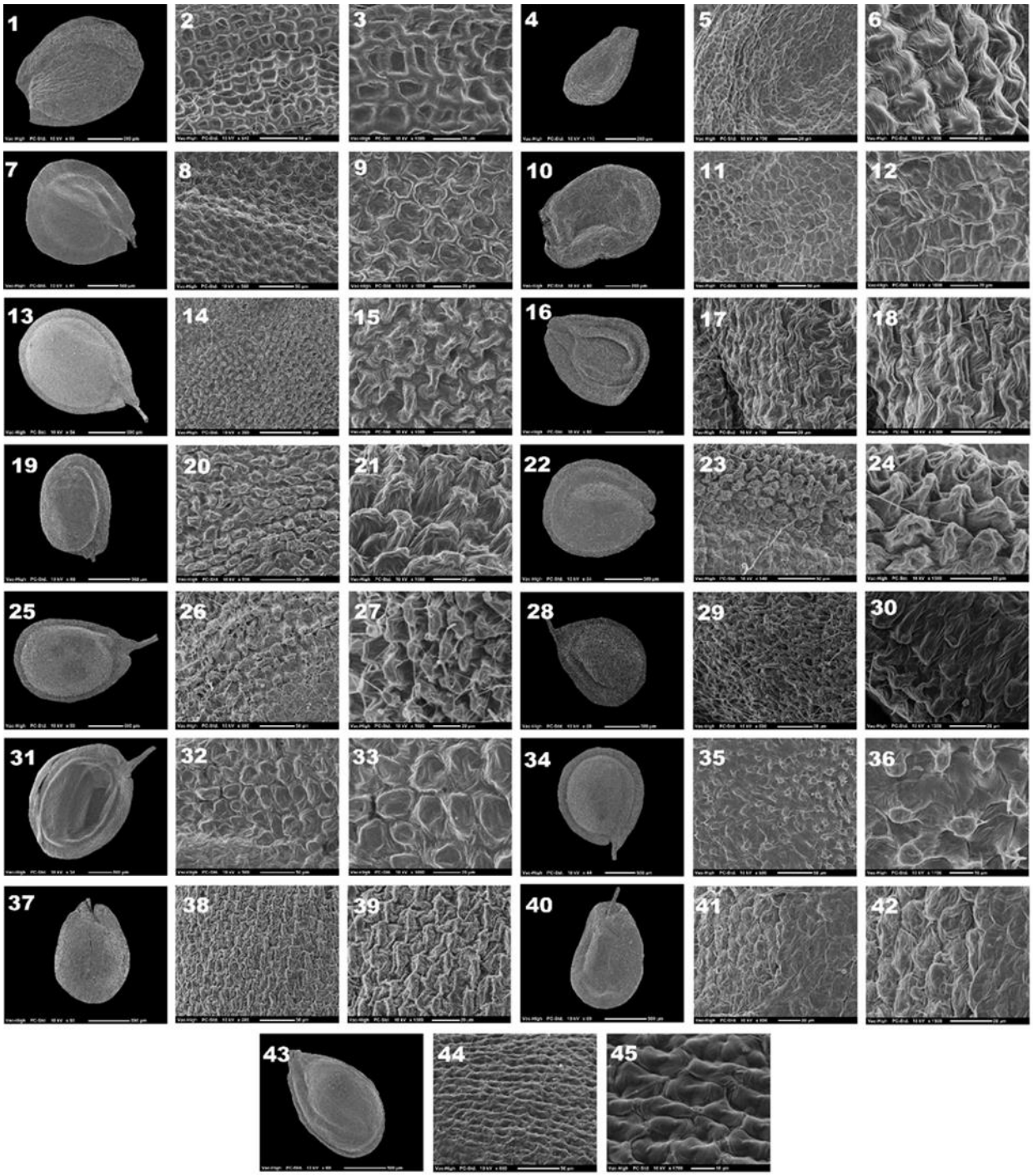

Figure 2. SEM pictures of the examined taxa: 1-3: *Alyssum caricum*, 4-6: *A. davisianum*, 7-9: *A. filiforme*, 10-12: *A. haussknechtii*, 13-15: *A. murale* subsp. *murale* var. *murale*, 16-18: *A. simplex*, 19-21: *A. sibiricum*, 22-24: *A. desertorum*, 25-27: *A. hirsutum* subsp. *hirsutum*, 28-30: *A. minutum*, 31-33: *A. strictum*, 34-36: *A. strigosum* subsp. *strigosum*, 37-39: *A. szowitsianum*, 40-42: *A. dasycarpum*, 43-45: *A. linifolium* var. *linifolium*.

Şekil 2. İncelenen taksonların SEM resimleri: 1-3: Alyssum caricum, 4-6: A. davisianum, 7-9: A. filiforme, 10-12: A. haussknechtii, 13-15: A. murale subsp. murale var. murale, 16-18: A. simplex, 19-21: A. sibiricum, 22-24: A. desertorum, 25-27: A. hirsutum subsp. hirsutum, 28-30: A. minutum, 31-33: A. strictum, 34-36: A. strigosum subsp. strigosum, 37-39: A. szowitsianum, 40-42: A. dasycarpum, 43-45: A. linifolium var. linifolium.

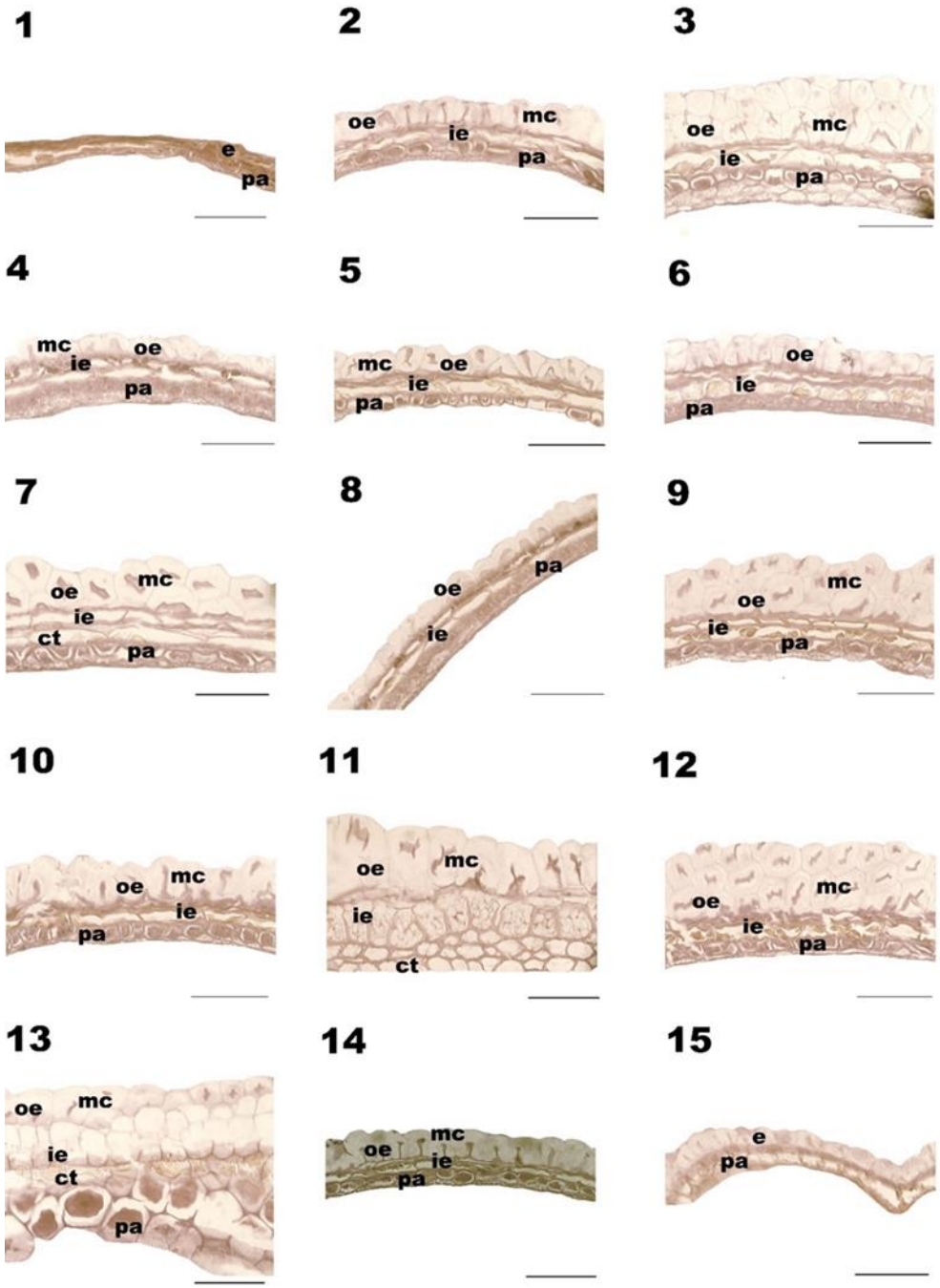

Figure 3. The anatomical structures of the seeds of the analyzed taxa; 1: *Alyssum caricum*, 2: *A. davisianum*, 3: *A. filiforme*, 4: *A. haussknechtii*, 5: *A. murale* subsp. *murale* var. *murale*, 6: *A. simplex*, 7: *A. sibiricum*, 8: *A. desertorum*, 9: *A. hirsutum* subsp. *hirsutum*, 10: *A. minutum*, 11: *A. strictum*, 12: *A. strigosum* subsp. *strigosum*, 13: *A. szowitsianum*, 14: *A. dasycarpum*, 15: *A. linifolium* var. *linifolium* (e: epidermis, oe: outer epidermis, ie: inner epidermis, pa: parenchyma, ct: compressed tissue=inner testa, mc: mucilages cells, scale bars=100 µm).

Şekil 3. Analiz edilen taksonların tohumlarının anatomik yapıları; 1: Alyssum caricum, 2: A. davisianum, 3: A. filiforme, 4: A. haussknechtii, 5: A. murale subsp. murale var. murale, 6: A. simplex, 7: A. sibiricum, 8: A. desertorum, 9: A. hirsutum subsp. hirsutum, 10: A. minutum, 11: A. strictum, 12: A. strigosum subsp. strigosum, 13: A. szowitsianum, 14: A. dasycarpum, 15: A. linifolium var. linifolium (e: epidermis, oe: dış epidermis, ie: iç epidermis, pa: parenkima, ct: baskılanmış doku=iç testa, mc: musilaj hücreleri, ölçekler=100 µm).

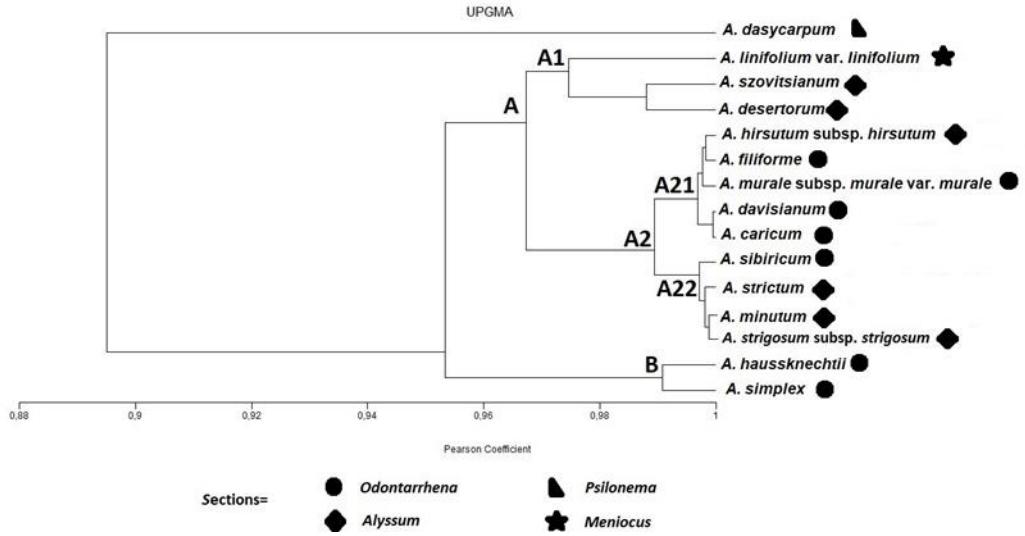

Figure 4. The dendrogram created with UPGMA of studied taxa.
Şekil 4. Çalışılan taksonların UPGMA ile oluşturulan dendrogramı.

(clear brown) *A. hirsutum* subsp. *hirsutum* (dark brown). Seed surfaces mirror ecological knowledge and adaptation of the species. So, they are diverse among species and are of the systematic importance (Brochmann, 1992; Karaismailoğlu, 2015, 2016, 2019a; Karaismailoğlu and Erol, 2018). In this work, seed surfaces of the studied species are in different structures: slightly striped, reticulate, smooth or coarse protrusions. This diversity has been found to be one of the reliable characters that distinguish taxa from each other. The obtained macro-morphological outcomes are coherent with the earlier findings, which are concerned on seed exomorphic features realized on some genus within the family (Kaya et al., 2011; Bona, 2013; Karaismailoğlu and Erol, 2018; Bülbül et al., 2019; Karaismailoğlu 2019a, 2019b; Şirin, 2019; Şirin and Karaismailoğlu, 2020).

The seed micromorphological features are of the high taxonomic significance (Karaismailoğlu and Erol, 2018; Şirin and Karaismailoğlu, 2020). The significance and effectiveness of scanning electron microscopy in resolving systematic problems within the family Brassicaceae has been shown in many studies performed on seed microstructures (Kaya et al., 2011; Kasem et al., 2011; Bona, 2013; Bülbül et al., 2019; Karaismailoğlu 2019b). The tested taxa have been analyzed for the first time, except for *A. caricum* (reticulate), *A. sibiricum* (reticulate-foveate) and *A. strictum* (ruminate) (Bülbül et al., 2019). In this work, 9 different ornamentation types have found as alveolate, scalariform, reticulate-alveolate, aculate, ruminate, verrucate, tuberculate, rugose and reticulate-foveate. The most common types are scalariform, alveolate, aculate, ruminate, verrucate and tuberculate (each is seen in 2 taxa), as reported in Tantawy et al. (2004) and Karaismailoğlu and Erol (2018). The surface ornamentation type is effective in separating of some closely related taxa; *A. strictum* (alveolate) - *A. szowitsianum* (rugose), *A. simplex* (ruminate) - *A. sibiricum* (verrucate) and *A. desertorum* (tuberculate) - *A. hirsutum* subsp. *hirsutum* (aculate). Earlier seed morphology reports have indicated that the structures of the anticlinal-periclinal cell walls are great problem-solving parameters within genus (Barthlott, 1981; Tantawy et al., 2004; Bona, 2013). Also, the forms of anticlinal-periclinal cell walls are useful in discrimination of taxa. As, anticlinal cell wall is in raised, sunken or unclear structures, the form of periclinal cell wall is concave, convex or unclear. *Alyssum* species are mostly distinct in terms of the epidermal cells, which may be rectangular, pentagonal, alveolate, protrusion, polygonal, crushed polygonal, oval and unclear structures. Generally, scanning electron microscope findings have revealed that the exhaustive analysis of seed features of *Alyssum* species is very helpful in identification and separation of taxa.

Studies of seed testa anatomy in the Brassicaceae family have enabled systematic problems to be overcome (Vaughan et al., 1976; Ghaempanah et al., 2013; Karaismailoğlu and Erol, 2018; Karaismailoğlu, 2019a; Karaismailoğlu and Erol, 2020; Şirin and Karaismailoğlu, 2020). The testa anatomical features of some seed belonging to family have been reported by Vaughan et al. (1976), Karaismailoğlu and Erol (2018) and Karaismailoğlu (2019a). But still there are not enough studies explaining evolutionary relationships and including a phylogenetic perspective. The seed testa anatomical structures of the studied taxa have been revealed for the first time in this study. The seed testa mostly contains of 4 layers as the outer epidermis, the inner epidermis (outer testa), the inner testa, and the endosperm

(Ghaempanah et al., 2013; Karaismailoğlu and Erol, 2018). Vaughan and Whitehouse (1971) have analyzed the testa anatomical forms of seeds of 200 taxa belonging to 90 genera in the family and debated their usage as systematic characteristics, and discovered 15 form of epidermis cells. Besides, Karaismailoğlu and Erol (2018) have reported 4 different type in the seeds of *Thlaspi*. In this study, the outer and inner epidermis structures quite differ among taxa. They contain the elongated rectangular, flat, cubic, polygonal, crushed and oval cell shapes, with 1-3 layered. As flat and polygonal cells are commonly observed, oval cells are uncommon in the studied taxa. The inner testa has 1-layered of flat, rectangular or crushed cells in the studied 4 taxa (*A. sibiricum*, *A. simplex*, *A. szowitsianum* and *A. strictum*). The endosperm parenchyma of seed has showed differences among taxa in terms of structure and thickness. The endosperm of taxa is 1-layered and contains mostly flat and seldom rectangular cells. The widest endosperm is observed in *A. szowitsianum*; however, *A. murale* subsp. *murale* var. *murale* has the narrowest. Mucilage cells are noticed in the outer testa layers of seeds of the examined taxa, outside of *A. caricum*. Furthermore, mucilage presence on seed surfaces is acceptable as an ecological response to water shortage (Young and Martens, 1991). Since, the examined taxa are not observed in wetlands.

CONCLUSION

A dendrogram is designed to assess the seed morpho-anatomical features of the studied *Alyssum* taxa with UPGMA test. The dendrogram, representing 2 main groups, is somewhat congruent with the results of Dudley (1965). The seed morpho-anatomical variations have been seen in inter-species level, especially in closely related taxa like *A. strictum* - *A. szowitsianum*, *A. simplex* - *A. sibiricum* and *A. desertorum* - *A. hirsutum* subsp. *hirsutum*. As a result, assessing the seed morpho-anatomical characters of the studied taxa of *Alyssum* presents substantial contributions to the taxonomy of the genus.

Conflicts of Interest

No conflict of interest was declared by the author.

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Anatomical, Micromorphological, Karyological and Biochemical study of *Scutellaria orientalis* subsp. *virens* and *Scutellaria salviifolia*

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ABSTRACT

In this study, the anatomical and micromorphological structure, karyological characteristics and biochemical content of *Scutellaria orientalis* subsp. *virens* and endemic *Scutellaria salviifolia*, whose distributions areas overlap, were compared. Some anatomical and micromorphological differences were observed on the taxa; scleranchymatic pericycle layer on the stem, stomata density, distribution of trichomes, as well as the main vascular bundle and general shape of the petiole. The chromosome numbers of both taxa were determined as 2n = 22. However, there was a difference between chromosome length range and total chromosome length. The chromosome numbers and chromosome morphologies of these species have been defined for the first time in this paper. Differences in biochemical content were observed between species. Chlorophyll a (Chl a), total chlorophyll (Total Chl), total carbohydrate and malondialdehyde (MDA) contents were determined higher in leaf and stem samples of *S. orientalis* subsp. *virens* than *S. salviifolia*. There was no significant difference between the two taxa in terms of chlorophyll b (Chl b) content. Carotenoid (Car) content was detected higher in leaves samples of *S. orientalis* subsp. *virens*, but no significant difference was found between stems samples. Also, the effect of taxa on biochemical contents in relation to the habitat they live in is given in this study.

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Scutellaria orientalis subsp. *virens* ve *Scutellaria salviifolia* üzerinde Anatomik, Mikromorfolojik, Karyolojik ve Biyokimyasal bir çalışma

ABSTRACT

Bu çalışmada, yayılış alanları örtüşen *Scutellaria orientalis* subsp. *virens* ve endemik *Scutellaria salviifolia*'nın anatomik ve mikromorfolojik yapısı, karyolojik özellikleri ve biyokimyasal içeriği karşılaştırılmıştır. Taksonlarda bazı anatomik ve mikromorfolojik farklılıklar gözlenmiştir. Bunlar; gövdede skleranchymatik periskl tabakası, stoma yoğunluğu, trikomların dağılımı, petiyolün genel şekli ve ayrıca ana iletim demetinde birtakım farklılıklar şeklindedir. Her iki taksonun kromozom sayıları 2n=22 olarak belirlenmesine rağmen kromozom uzunluk aralığı ile toplam kromozom uzunluğu arasında fark görülmüştür. Bu türlerin kromozom sayıları ve kromozom morfolojileri ilk kez bu çalışmada tanımlanmıştır. Türler arasında biyokimyasal içerik farklılıkları da gözlenmiştir. *S. orientalis* subsp. *virens*'in yaprak ve gövde örneklerinde klorofil a (Chl a), toplam klorofil (Toplam Chl), toplam karbonhidrat ve malondialdehit (MDA) içeriğinin *S. salviifolia*'ya göre daha yüksek olduğu belirlenmiştir. Klorofil b (Chl b) içeriği açısından iki takson arasında önemli bir fark tespit edilmemiştir. *S. orientalis* subsp. *virens*'in yaprak örneklerinde karotenoid (Car) içeriği daha yüksek saptanmış, ancak gövde örnekleri arasında önemli bir fark bulunmamıştır. Çalışmada taksonların yaşadıkları habitata göre biyokimyasal içerikleri üzerindeki etkisi de verilmiştir.

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INTRODUCTION

Lamiaceae is the third largest family in terms of number of taxa in Turkey. Lamiaceae family is represented by 48 genera and 782 taxa (603 species, 178 subspecies and varieties) in Türkiye. 44% of these taxa are endemic. In addition, the family has 23 hybrid species and 19 of them are endemic (%83). Studies of Turkey's Lamiaceae result has proven to be one of the centers of diversity in the Old World. In addition, in Turkey, there are about 10% of all species of Lamiaceae (Celep and Dirmenci, 2017). Being from the Lamiaceae family, the *Scutellaria* L. genus is one of the semi-cosmopolitan genera of the Lamiaceae family with its 471 species identified (Yilmaz, et al., 2020). Due to the suspicious species status of some taxa defined from the Soviet Union and China, in reality this number is close to 360 (Paton, 1990). The *Scutellaria* species usually grow in stony and rocky slopes in Turkey. The genus *Scutellaria* has 39 taxa (17 species, 23 subspecies, 2 varieties and 1 hybrid) were represented in Turkey (Güner et al., 2012, Yilmaz et al., 2020). 17 taxa are endemic (44%) in Turkey (Davis, 1988; Duman, 2000; Güner et al., 2012; Celep and Dirmenci, 2017).

Chlorophylls are the main pigments that drive photosynthesis by absorbing light and converting it into chemical energy (Agathokleous et al., 2020). Carotenoids absorb certain wavelengths of light energy in the photosynthetic system and then transfer it to the chlorophyll molecule and thus contribute to the photosynthesis process. In addition, according to some researchers, carotenoids prevent the breakdown of chlorophyll (photo oxidation) in an environment with excessive light and oxygen and protect the plant against physiological tissue injuries caused by excessive light (Leiva-Ampuero et al., 2020; Zhang et al., 2020).

Carbohydrates are direct photosynthetic activity products and are structural building blocks as well as energy sources and metabolites. They act as a source of energy and provide carbon necessary to produce new tissue (Trouvelot et al., 2014; Homayoonzadeh et al., 2020). Lipid peroxidation is the reaction that occurs in unsaturated fatty acids of cell membrane phospholipids. Products such as malondialdehyde resulting from lipid peroxidation event determine the severity of peroxidation (Abdelrahim et al., 2020; Álvarez-Robles et al., 2020).

The genus *Scutellaria* is taxonomically complex because it has many species and especially some taxa closely in morphologically. Therefore, anatomical findings, karyo-morphological analysis of taxa are important for the systematics. Also, the effect of taxa on biochemical contents in relation to the habitat they live in is given in this study. This study aims to reveal the anatomical structure and karyomorphological features of *S. orientalis* L. subsp. *virens* (Boiss. & Kotschy) J.R. Edm. and *S. salviifolia* Benth. taxa whose overlapping areas of distribution, and to improve the knowledge on their biochemical content. Thus, the results obtained from this study will provide data for future studies.

MATERIAL and METHOD

Scutellaria taxa, the study material, were collected from their natural habitats in the field. The localities of taxa are given in Table 1 and Figure 1. The collected samples have been turned into herbarium material and are kept in the herbarium of Munzur University.

Table 1. The localities and collector number of taxa
Çizelge 1. Taksonların lokaliteleri ve numaraları

Taxon	Locality
S. orientalis subsp. *virens*	B7 Tunceli: Between Tunceli center and Ovacık, roadsides, 1000 m, May 2020, MA 2000
S. salviifolia	B7 Tunceli: Between Tunceli center and Ovacık, Aktuluk neighborhood, the roadsides May 2020, 950 m, MA 2001

Anatomical studies was made on samples kept in 70% ethyl alcohol. And performed on at least two individuals to represent the population for each taxon. Anatomical sections were carried out manually. In order to better distinguish the tissues and cells were stained with safranin-fastgreen. After, it was made into a permanent preparation with entellan (Tardif and Conciatori, 2015). Anatomical examinations were made under an Olympus BX53 microscope. Nutlet was examined in SEM (JCM-5000) and microphotographs were taken.

Nutlets of the studied taxa were collected from their

natural habitats for karyological analysis. In order to obtain somatic chromosomes, the germination environment was provided by sowing in an oven at 22 ° C. After the seed germinated, the cut root tips were kept in colchicine solution for 2 hours at room temperature (Elçi, 1982; Gedik et al., 2014). Root tips kept in colchicine solution for 2 hours were taken from this solution and placed in Farmer's solution (3: 1). Root tips were fixed in acetic alcohol at +4 ° C in a refrigerator for 24 hours. At the end of 24 hours, the root tips were hydrolyzed in 1N HCl for 8-10 minutes in an oven set at 60 ° C. After the hydrolysis process was completed, the root tips were dyed with feulgen dye for 1 hour at 22 °C in a dark environment. The meristems taken for chromosome examinations were broken up in a drop of aceto orcein dye with a sharp razor blade and the coverslip was closed (Levan et al., 1964). Levan's naming system was used in determining the location of the centromere (Levan et al., 1964). Intra-chromosomal asymmetric index (A1) and inter-chromosomal asymmetric index (A2) were calculated according to Zarco (1986).

Figure 1. General view of taxa. A,B- *Scutellaria orientalis* subsp. *virens* C,D- *S. salviifolia*
Şekil 1. Taksonların genel görünümü. A,B- Scutellaria orientalis subsp. virens C,D- S. salviifolia

Biochemical analysis

Plant parts were stored at -80° C until analysis. Pigment analysis, total carbohydrate and lipid peroxidation analysis were determined.

Photosynthetic Pigment Analysis

Method suggested by De-Kok and Graham (1980) was used for pigment analysis. 0.5 gr of each leaf and stem samples ground in the blender were taken for extraction in 3 replicates for each sample and placed in a glass mortar and homogenized in 25 ml of acetone. They were homogenized in a shaking oven for 30 minutes. These samples were then stored for 24 hours at 4 ° C. Then samples were filtered and 1/5 water was added. Samples were centrifuged at 3000 rpm for 10 minutes. The absorbance values of samples were read at 470 nm, 645nm and 662 nm according to Lichtenthaler and Welburn (1983) and Chl-a, Chl-b, Car and Total Chl contents were determined.

Total Carbohydrate Content

The total carbohydrate content was assayed according to Rosenberg (1980). Anthrone method was used for colorimetric method of determining the concentration of the total carbohydrate. Absorbance was measured at 620 nm (Shimadzu UV-1201V). Glucose values have been calculated corresponding to the standard values entered on the computer in the Slide program.

Lipid Peroxidation Analysis

The method was done according to Heath and Packer (1968). 0.5 gram of leaf tissue was homogenized in 5 mL of 0.1% TCA. The homogenate was centrifuged at 10,000 g for 10 minutes. 2 mL of 0.5% TBA (prepared in 20% TCA) was added to 2 mL of this solution and kept in a water bath at 95 ° C for 30 min. than samples were centrifuged at 10,000 gram for 15 min again. MDA content was calculated at 532 nm and 600 nm.

Statistical Analysis

Statistical evaluation of the obtained data was made using the SPSS 21.0 program. In this program, variance analysis was performed and Duncan test (Duncan, 1955) was applied within the scope of significance test.

RESULTS AND DISCUSSION

Stem anatomy and micromorphology

The stem anatomical structure of taxa shows the general characteristics of the family. The stem has 4 corners and vascular bundles at the corners are developed (Figure 2-3). In addition, developed collenchyma layer was seen in the corners. *S.*

orientalis subsp. *virens*, scleranchymatic pericycle surround partly the stem, whereas in *S. salviifolia* there is no scleranchymatic pericycle layer. Endodermis cannot be distinguished from parenchyma tissue completely. Between the corners, parenchyma tissue in *S. salviifolia* occupies relatively more area. Although the collenchymatic hypodermis layer is seen between the corners in both taxa, it was seen more in *S. orientalis* subsp. *virens*. In addition, although vascular cambium was observed in *S. salviifolia*, it is not obvious in *S. orientalis* subsp. *virens*. Non-glandular trichomes and glandular trichomes have seen in both taxa; Non-glandular trichomes have been observed as a multicellular with micropapillae and developed cell wall. Non-glandular trichomes cell wall thickness more in *S. orientalis* subsp. *virens*. The glandular trichomes are; both taxa were observed as Labiatae type (peltate) and capitate type glandular trichome. Capitate glandular trichomes are of three subtypes. Rounded head with a short stalk cell and a broad round head shape (A type) and with a neck or without neck structure and with 2 stalk cells and multicellular round head shape (B1 type) and long 2-3 cell stalk and with neck or without neck structure and rounded head shape (B2 type). Although these two types of glandular trichomes are observed in taxa, relatively long capitate (B1 type) is more dense in *S. salviifolia*. Short large headed capitate (A type) was more intense in *S. orientalis* subsp. *virens*. Besides, in the stem structure, B2 type only observed in *S. orientalis* subsp. *virens*. Only in *S. salviifolia,* a cup-shaped head structure was commonly seen after releasing secretion B1 type capitate trichomes. However, this head cell shape was not seen in *S. orientalis* subsp. *virens*.

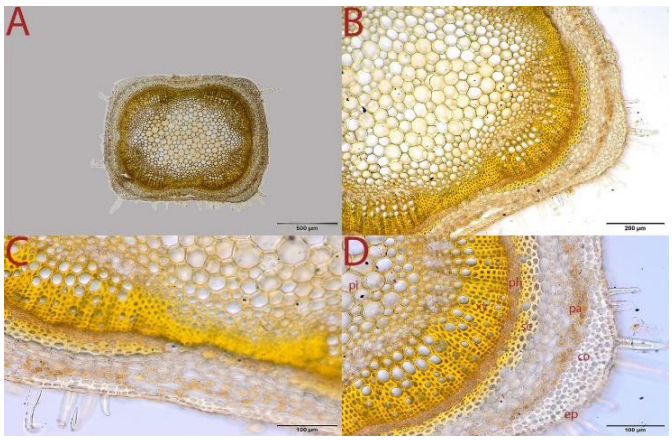

Figure 2. The anatomical structure of the stem *S. orientalis* subsp. *virens*. ep: epidermis, co: collenchyma, pa: parenchyma, sc: sclerenchyma, ph: phloem, xy: xylem, pi: pith

Şekil 2. S. orientalis subsp. Virens' in kök anatomisi. co: kollenkima, pa: parankima, sc: skleranchima, ph: floem, xy: ksilem, pi: öz

Leaf anatomy and micromorphology

Leaf anatomical structure of taxa differs more than stem anatomical structure. Generally, in both taxa midrib has developed and swollen in abaxial direction

Figure 3. The anatomical structure of the stem *S. salviifolia* e: epidermis, co: collenchyma, pa: parenchyma, ph: phloem, xy: xylem

Şekil 3. S. salviifolia' nın kök anatomisi. co: kollenkima, pa: parankima, sc: skleranchima, ph: floem, xy: ksilem, pi: öz

(Figure 4-5). Midrib area; it is arranged as, below the upper epidermis, collenchyma, below it parenchyma, xylem, phloem, parenchyma, again collenchyma and lower epidermis. Collenchyma layer is less developed in *S. salviifolia*. In both taxa, the mesophyll consists of only the palisade parenchyma, while in *S. orientalis* subsp. *virens* it consists of 4-5 rows and the parenchyma cells in the abaxial direction can be a little more oval. In *S. salviifolia*, there are 4 rows of palisade parenchyma. While the epidermis is mostly rectangular shape in *S. orientalis* subsp. *virens*, it is more oval shape in *S. salviifolia*. In addition, stomata are located on both the upper and lower surfaces in both taxa, while it is dense on the upper surface in *S. orientalis* subsp. *virens*, while it is dense on the lower surface in *S. salviifolia* While in *S. orientalis* subsp. *virens* the leaf bottom surface is densely covered with non-glandular trichome, it is not so in *S. salviifolia*. Both non-glandular trichome and glandular trichome were observed in both taxa, and the non-glandular trichome is denser in *S. orientalis* subsp. *virens* and the glandular trichome is denser in *S. salviifolia*. In addition, B2 type has been observed only in *S. salviifolia* and P type is quite intense.In addition, while the non-glandular trichomes are curved in *S. orientalis* subsp. *virens*, they are relatively not curved in *S. salviifolia*.

Petiole anatomy and micromorphology

The petiole is flattened in the adaxial direction in *S. orientalis* subsp. *virens*. In *S. salviifolia*, it is hollowed in the adaxial direction and swollen and ribbed from

the edges. There are 3 vascular bundles in both taxa. One is in the middle and the other two are on the edges. The middle vein is developed. Both taxa are in arc shape and consist of almost two lobes in *S. salviifolia*. Chlorenchymatic cells were seen in the corners (Figure 6-7).

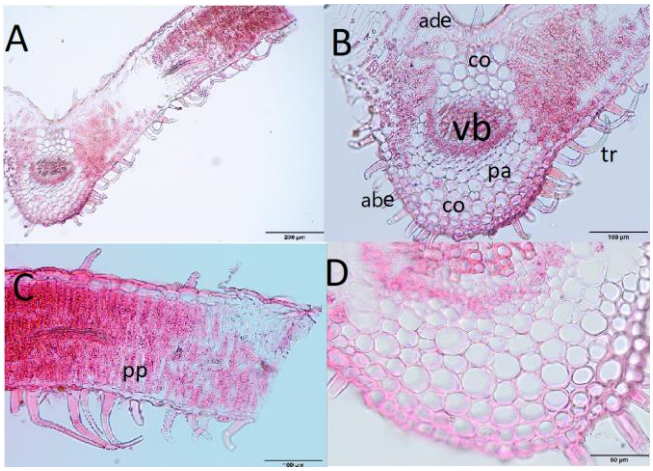

Figure 4. *S. orientalis* subsp. *virens*, anatomical structure of the leaf ade: adaxial epidermis, abe: abaxial epidermis, co: collenchyma, vb: vascular bundle, pa: parenchyma, pp: palisade parenchyma, tr: trichome

Şekil 4. S. orientalis subsp. Virens yaprağının anatomik yapısı. ade: adaksiyel epidermis, abe: abaksiyel epidermis, co: kollenkima, vb: vasküler demet, pa: parankima, pp: palisat parankiması, tr: trikom

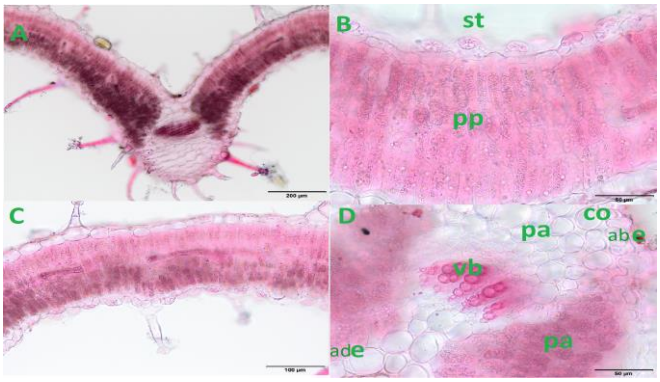

Figure 5. S.*salviifolia* , anatomical structure of the leaf ade: adaxial epidermis, abe: abaxial epidermis, co: collenchyma, vb: vascular bundle, pa: parenchyma, pp: palisade parenchyma, tr: trichome

Şekil 5. S.salviifolia yaprağının anatomik yapısı. ade: adaksiyel epidermis, abe: abaksiyel epidermis, co: kollenkima, vb: vasküler demet, pa: parankima, pp: palisat parankiması, tr: trikom

Vascular bundles in the corners are surrounded by a parenchymatic sheath. Both non-glandular and glandular trichomes were observed in both taxa, while A, P, B1 and B2 types were observed in *S. orientalis* subsp. *virens*; A, B1 and B2 types were observed in *S. salviifolia* and P type was not observed (Figure 8).

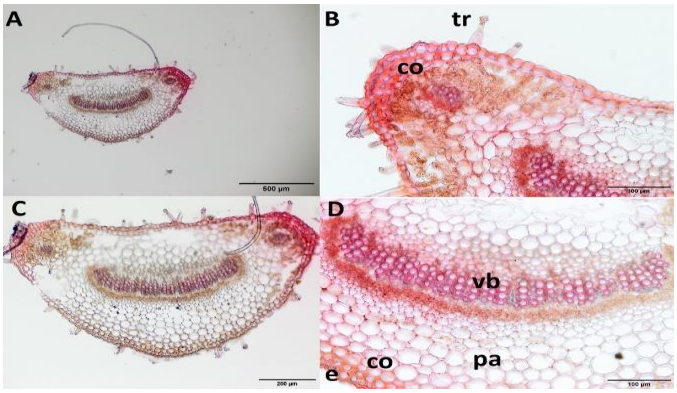

Figure 6. S. *orientalis* subsp. *virens,* anatomical structure of petiole, A and C- General view, B-, D- Middle vein, e: epidermis, co: collenchyma, vb: vascular bundle, pa: parenchyma, pp: palisade parenchyma, tr: trichome

Şekil 6. S. orientalis subsp. Virens. Yaprark sapının anatomik yapısı. Ave C genel görünüş, B-, D köşe görünümü

e: epidermis, co: kollenkima, vb: vasküler damar, pa: parankima, pp: palizet parankiması, tr: trikıom

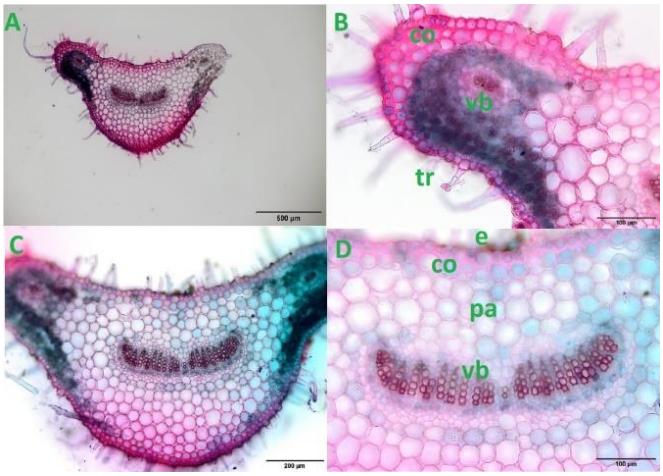

Figure 7. *S. salviifolia,* anatomical structure of petiole, A and C- General view, B- Corner view, D- Middle vein, e: epidermis, co: collenchyma, vb: vascular bundle, pa: parenchyma, pp: palisade parenchyma, tr: trichome

Şekil 7. S. salviifolia. Yaprark sapının anatomik yapısı. Ave C genel görünüş, B-, D köşe görünümü

e: epidermis, co: kollenkima, vb: vasküler damar, pa: parankima, pp: palizet parankiması, tr: trikom

Nutlet Structure

The nutlet surface structure of taxa could not be observed since it is covered with dense trichomes. However, the non-glandular trichomes differ from each other. In *S. orientalis* subsp. *virens*, surface completely covered with ashy hairlets. In *S. orientalis* subsp *virens*, there are curved non-glandular trichomes; It is flat in *S. salviifolia*. Nutlet shape is obovate in both taxa.

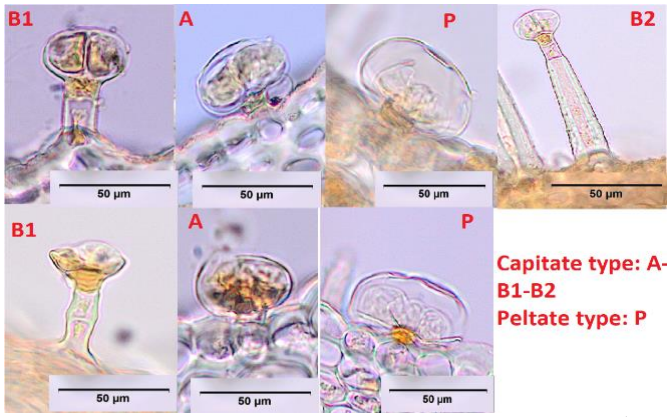

Figure 8. Glandular trichome structure of taxa (top; *S. orientalis* subsp. *virens,* under; *S. salviifolia*

Şekil 8. Taksonların glandüler trikom yapısı (üstte; S. orientalis subsp. *virens,* altta; *S. salviifolia)*

Karyomorphological finding are given in Figure 9-10 and Table 2-3.

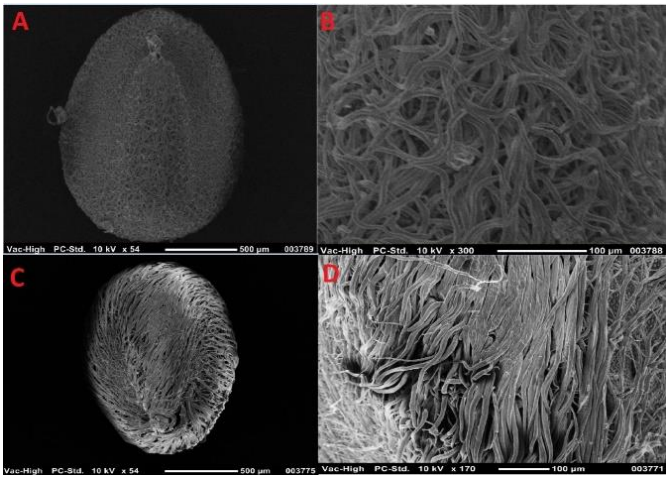

Figure 9. A and C- General view of *S. orientalis* subsp. *virens* and *S. salviifolia*, B and D- Nutlet surface of *S. orientalis* subsp. *virens* and *S. salviifolia*

Şekil 9. A ve C- S. orientalis subsp. *virens ve S. salviifolia' nın genel görünüşü. B ve D S. orientalis* subsp. *virens ve S. salviifolia' nın nutlet yüzeyi*

Karyomorphological Findings

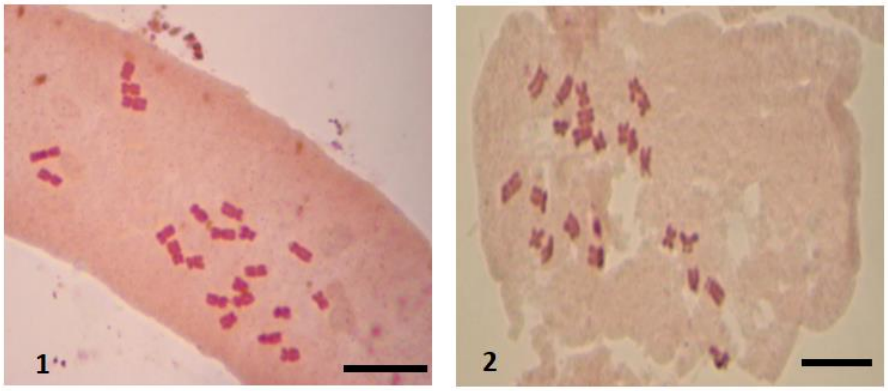

Figure 11. Metaphase chromosomes belonging to the taxa studied; 1. S. orientalis subsp. virens 2. S. salviifolia (scale bar 10 µm)

Şekil 11. Incelenen taksonlara ait metafaz kromozomları; 1. S. orientalis subsp. virens 2. S. salviifolia (scale bar 10 µm)

Table 2. Somatic chromosome number, polyploid level, karyotype formula, chromosome length range, total chromosome length (TKL) and asymmetric index (A1, A2) of the examined taxa.

Çizelge 2. İncelenen taksonların somatik kromozom sayısı, poliploid düzeyi, karyotip formülü, kromozom uzunluk aralığı, toplam kromozom uzunluğu ve asimetrik indeks (A1, A2)

Taxon	2n	Polyploid level	Karyotype formula	Chromosome length range (µm)	TKL (µm)	A1	A2
S. orientalis subsp. *virens*	22	2x	1M+10m	2.18-3.72	30.50	3.6	2.77
S. salviifolia	22	2x	2M+ 2sm+8m	2.38-3.65	34.24	15.8	3.11

Pigmentation Results

As analysis of pigment was evaluated in *S. salviifolia*, Chl a content in leaves and stems samples were determined respectively 6.45 µg g^{-1} and 2.32 µg g^{-1}.

Chl b content was found 2.60 µg g^{-1} in the leaves and 0.45 µg g^{-1} in the stems. Total Chl content in leaves and stems samples were measured as 9.05 µg g^{-1} and 2.76 µg g^{-1} respectively. Car content was found as 0.71 µg g^{-1} in leaves and 0.44 µg g^{-1} in stems samples. When the Chl a content in leaves and stem samples in *S. orientalis* subsp. *virens* were determined respectively 7.37 µg g^{-1} and 2.54 µg g^{-1}. The concentration of Chl b was contained in leaves at 2.58 µg g^{-1} and stems at 0.48 µg g^{-1}. Total Chl content was calculated in leaves and stems samples as 9.95 µg g^{-} and 3.01 µg g^{-1} respectively. Car concentrations were detected on the leaves as 0.82 µg g^{-1} and on the stems as 0.48 µg g^{-1}. When we compared the Chl a content in *S. salviifolia* and *S. orientalis* subsp. *virens*, the Chl a and Total Chl contents were determined higher in the leaves and stems samples of *S. orientalis* subsp. *virens*. There was no significant difference between the two plants in terms of Chl b content. When the Car content was evaluated, it was determined higher in leaves samples of *S. orientalis* subsp. *virens*, but no significant difference was found between stems patterns ($p < 0.05$) (Figure 12).

Table 3. Karyomorphological parameters of the examined taxa: (NB: Relative length, L / S: arm ratio, CI: centromere index, SD: Centromere status,M:median point, m: median, sm: submedian)

Çizelge 3. Incelenen taksonların karyomorfolojik parametreleri: (NB: nispi boy, L / S: kol oranı, CI: sentromer indeksi, , SD: sentromer durumu, M:noktalı medyan, m: medyan, sm: submedyan)

S. orintalis* subsp. *virens							
Haploid	**Total Lenght**	**Long Arm L**	**Small Arm S**	**Arm Ratio (AR)**	**Centromere İndex İ=100*(S/C)**	**Relative Length N.P.**	**Centromere Status S.D**
1	3.72	1.90	1.82	1.04	48.92	12.20	m
2	3.38	1.78	1.60	1.11	47.34	11.08	m
3	3.12	1.60	1.52	1.05	48.72	10.23	m
4	3.05	1.58	1.47	1.07	48.20	10.00	m
5	2.91	1.50	1.41	1.06	48.45	9.54	m
6	2.85	1.47	1.38	1.07	48.42	9.34	m
7	2.75	1.40	1.35	1.04	49.09	9.02	m
8	2.47	1.25	1.22	1.02	49.39	8.10	m
9	2.18	1.05	1.13	0.93	51.83	7.15	m
10	2.04	1.02	1.02	1.00	50.00	6.69	M
11	2.03	1.03	1.00	1.03	49.26	6.66	m
S. salviifolia							
Haploid	**Total Lenght**	**Long Arm L**	**Small Arm S**	**Arm Ratio (AR)**	**Centromere İndex İ=100*(S/C)**	**Relative Length N.P.**	**Centromere Status S.D**
1	3.65	2.40	1.25	1.92	34.25	10.66	sm
2	3.50	2.28	1.22	1.87	34.86	10.22	sm
3	3.95	2.15	1.80	1.19	45.57	11.54	m
4	3.25	1.92	1.33	1.44	40.92	9.49	m
5	3.72	1.86	1.86	1.00	50.00	10.86	M
6	3.18	1.63	1.55	1.05	48.74	9.29	m
7	2.91	1.45	1.46	0.99	50.17	8.50	m
8	2.74	1.37	1.37	1.00	50.00	8.00	M
9	2.53	1.32	1.21	1.09	47.83	7.39	m
10	2.43	1.28	1.15	1.11	47.33	7.10	m
11	2.38	1.25	1.13	1.11	47.48	6.95	m

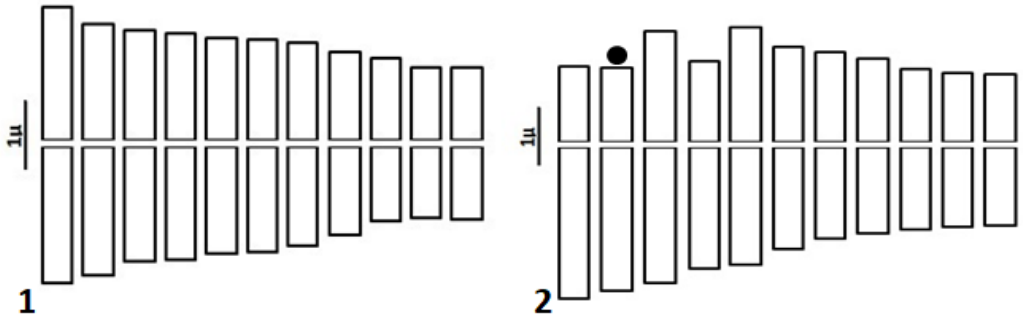

Figure 12. Haploid idiograms belonging to the taxa studied; 1. *S. orientalis* subsp. *virens* 2. *S. salviifolia*
Şekil 12. Incelenen taksonlara ait haploid idiyogramlar; 1. S. orientalis subsp. virens 2. S. salviifolia

Total Carbohydrate Results

The total carbohydrate content of *S. salviifolia* plant was determined as 1.27 µg g^{-1} and 0.55 µg g^{-1}, respectively, in leaves and stems samples. The total carbohydrate content of *S. orientalis* subsp. *virens* leaf and stem samples was found to be 1.46 µg g^{-1} and 0.69 µg g^{-1} respectively. When we compared the total carbohydrate content in *S. salviifolia* and *S. orientalis* subsp. *virens*. Total carbohydrate content in *S. orientalis* subsp *virens* was found higher in leaves and stems samples. Statistically, these changes were determined significant ($p < 0.05$) (Figure 13).

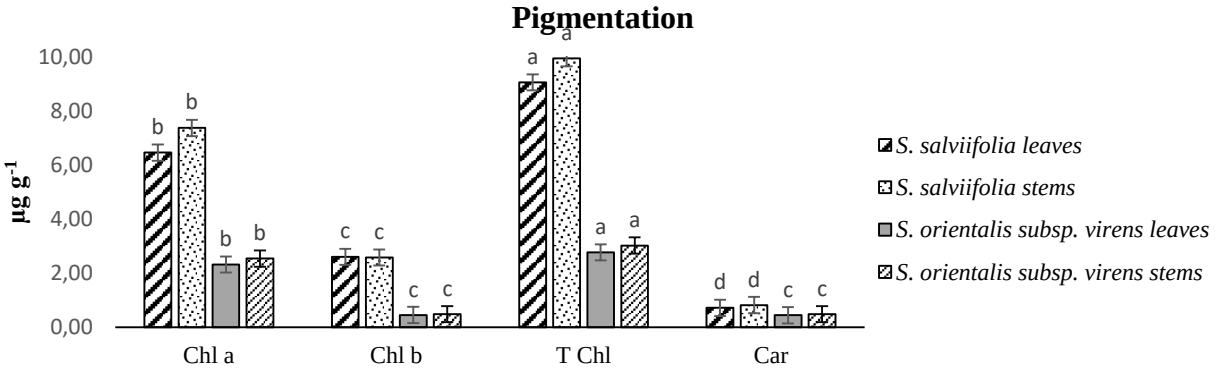

Figure 13. Changes in pigment contents in *S. salviifolia* and *S. orientalis* subsp. *virens*. The values shown with different letters were found to be statistically significant ($p < 0.05$), the values shown with the same letters were found to be insignificant (Duncan, 1955)

Şekil 13. *S. salviifolia* ve *S. orientalis* subsp. *virens*' in pigment içeriğindeki değişiklikler. Farklı harflerle gösterilen değerler istatiksel olarak anlamlı ($p < 0.05$),aynı harflerle gösterilen değerler anlamsız bulundu (Duncan, 1955)

MDA Results

The MDA content of *S. salviifolia* plant was found as 2.55 µmol MDA/ g fresh weight (FW) and 1.07 µmol MDA/ g FW, respectively, in leaves and stem samples. The MDA content of *S. orientalis* subsp *virens* was determined as µmol MDA/ g FW in leaves and µmol MDA/ g fresh weight in stems samples. When we evaluated the MDA content in *S. salviifolia* and *S. orientalis* subsp. *virens*, MDAcontent in *S. orientalis* subsp. *virens* was found higher in leaves and stems samples. Statistically, these changes were found to be significant ($p < 0.05$) (Figure 14).

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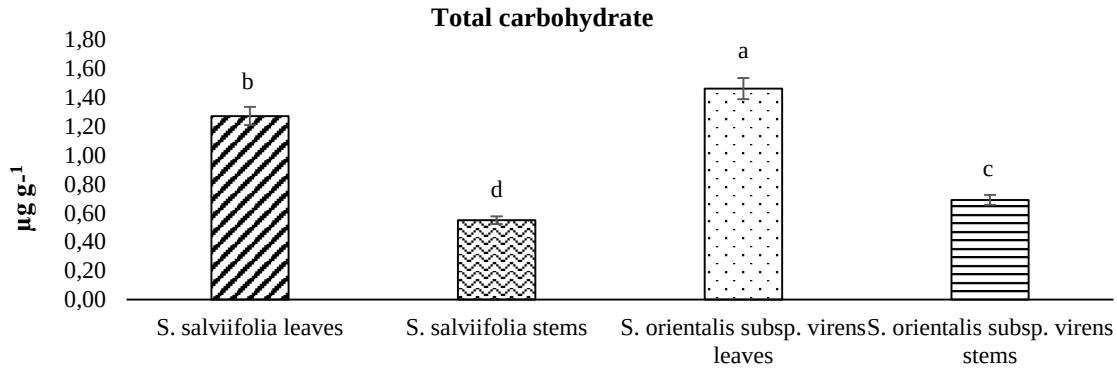

Figure 14. Changes in Total carbohydrate contents in *S. salviifolia* and *S. orientalis* subsp. *virens*. The values shown with different letters were found to be statistically significant ($p < 0.05$), the values shown with the same letters were found to be insignificant (Duncan, 1955)

Şekil 14. S. salviifolia ve S. orientalis subsp. virens' deki toplam karbonhidrat içeriğindeki değişiklikler. Farklı harflerle gösterilen değerler istatiksel olarak anlamlı (p <0.05),aynı harflerle gösterilen değerler anlamsız bulundu (Duncan, 1955)

In this study; the anatomical and karyological characteristics and biochemical content of the samples taken from *S. orientalis* subsp. *virens* and *S. salviifolia* belonging to the genus of *Scutellaria*, Lamiaceae family, which are frequently used in traditional medicine, pharmacology, cosmetics and food industry and are of great economic importance, were investigated.

Anatomical and micromorphological studies conducted on Lamiaceae family recently can provide useful characters in revealing their similarities and differences in distinguishing taxa (Açar and Satıl, 2019; Ecevit-Genç et al., 2018; Polat et al., 2017; Kaya et al., 2013; Selvi et al., 2013; Satıl and Kaya, 2007; Satıl et al., 2011).

The vascular structure of the petiole carries a taxonomic character. In the cross sections, the middle vein curves in the form of a half-moon or it creates an annular structure by further curling; the arrangement of small bundles of vascular vessel or circular structure of small bundles; The number and sequence of small vascular bundles at the ends of the petiole (wing) are systematically important characters in identifying genera and species (Metcalfe and Chalk, 1950).

According to the study by Akçın et al. (2011), the *S. salviifolia* petiole gave similar results to the research we conducted, but the vascular bundle in the middle part in this study differs in that it consists of almost two parts and the absence of peltate-type hair. Çalı (2017) stated in his study that there were sclerenchymatic cells in the stem of *S. salviifolia*, but sclerenchyma was not found in this study. The fact that the mesophyll is made entirely of palisade is similar to the stoma being on both surfaces. In addition, the fact that the petiole main vascular bundle consists of one piece reveals its difference from in this study. In addition, type II C glandular hair seen only in calyx in his study was not observed in this leaf stem and petiole. Özdemir and Altan (2005) stated in their study that the vascular bundles in *Scutellaria orientalis* subsp. *santolinoides* and *S. orientalis* subsp. *bicolor* petioles were surrounded by scleranchymatic cells, but in this study, these scleranchymatic cells were not observed in the other subspecies of this species*, S. orientalis* subsp *virens*. In the stem, it has been stated that the vascular bundles are surrounded by scleranchymatic cells, and in the case it is seen that it is in partly form. It is also stated that the leaf mesophyll is bifacial, in the case it is unifacial. In *S. orientalis* subsp. *virens*, the leaf consists only of the palisade parenchyma. However, in some observations, it was also observed that the palisades on the lower surface were slightly more oval.

Karyological studies have been carried out on some species belonging to the genus *Scutellaria*. The chromosome number of *S. tomentosa* Bertol., *S. theobromina* Rech.f., *S. araxensis* Grossh., *S. platystegia* Juz., *S. nepetifolia* Benth*., S., S. persica* Bornm. and *S. pinnatifida* has been reported as 2n = 2x = 22 (Ranjbar and Mahmoudi, 2013) In another study, the chromosome number of endemic *S. orientalis* subsp*. bicolor* species was determined as 2n = 22 (Gedik et al., 2016). However, there is no information about the chromosome number and structure of *S. salviifolia* species, which is an endemic taxon.

The chromosome number of *S. salviifolia* species, which is an endemic species was determined as 2n = 22. It is seen that the total chromosome lengths of

this species vary between 2.38-3.65 µm and arm ratios between 1.11-1.92 µm. The karyotype formula is 7m + 2sm + 2M. The total chromosome length is 34.24 um. It has been determined that this species has a satellite on its second chromosome. The karyology of the *S. salviifolia* species was first determined in this study (Figure 11-12).

The chromosome number of *S. orientalis* subsp. *virens* was determined as 2n = 22. It is seen that the total chromosome lengths vary between 2.08-3.72 µm and arm ratios between 1.03-1.04 µm. The karyotype formula is 10m + 1M. Total chromosome length is 30.50 µm. The *S. orintalis* subsp. *virens* taxon was first discussed in terms of chromosome number and chromosome morphology in this study (Table 2).

Chlorophylls absorb light energy of certain wavelengths and either convert this energy into another wavelength used in photosynthesis or transfer it directly to the compounds required for photosynthesis. Also they act like a catholyzer in the stages of photosynthesis. The spectral distribution of light sources in crop production plays an important role in finding photomorphogenic reactions. It has been noted that the plant grows in direct proportion as the pigment system absorbs the sunlight (Amoozgar et al., 2017; Izzo et al., 2019). Carotenoids are not only one of the plant pigments but also important antioxidants that play a role in oxidative stress tolerance. There are studies showing that the carotenoid oxidation products as stress signals for plants (Berru et al., 2021; Xia et al., 2021). In this study, the contents of Chl a and Total Chl were higher in the leaves and stem samples of *S. orientalis* subsp. *virens*, There was no significant difference in the content of Chl b between the two plants. Car content was dound higher in the leaf samples of *S. orientalis* subsp. *virens*, but no significant difference was found between the patterns of stems. Statistically, it was observed that these changing were substantial ($p < 0.05$) (Figure 13).

The most important product of lipid peroxidation is MDA. It leads to binding of ion permeability and enzyme it causes negative consequences such as change of activity. Due to this feature of MDA, it can be used to protect DNA with nitrogen bases. React and hence mutagenic, cell genotoxic and carcinogenic for cultures (Hafez et al., 2020; Khoubnasabjafari and Jouyban, 2020; Nilsson and Liu, 2020). MDA content in *S. orientalis* subsp. *virens* was found higher in leaves and stems samples than *S. salviifolia*. Statistically, it was observed that these changing were substantial ($p < 0.05$) (Figure 14).

Carbohydrates are organic compounds containing carbon, oxygen and hydrogen atoms in their structures. It is also stated that carbohydrates act as signal molecules similar to hormones (Shah et al., 2019; Smeekens et al., 2000). Total carbohydrate content in *S. orientalis* subsp. *virens* was found higher in leaves and stems samples than *S. salviifolia*. Statistically, these changes have been determined to be significant ($p < 0.05$) (Figure15). Pigment, total carbohydrate and MDA contents are used as important markers in plants under stress conditions. The biochemical characteristics of the plant are important in the response and adaptation to stress in ecological conditions. In this study, it was determined that the biochemical composition of *S. orientalis* subsp. *virens* was found higher than that of *S. salviifolia*.

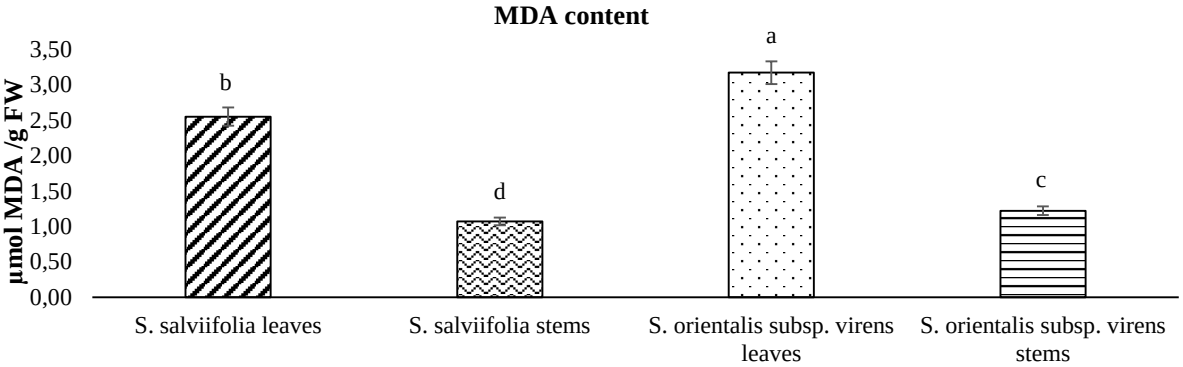

Figure 15. Changes in MDA contents in *S. salviifolia* and *S. orientalis* subsp. *virens*. The values shown with different letters were found to be statistically significant ($p < 0.05$), the values shown with the same letters were found to be insignificant (Duncan, 1955)

Şekil 15. *S. salviifolia* ve *S. orientalis* subsp. *virens*' de MDA içeriğindeki değişiklikler. Farklı harflerle gösterilen değerler istatiksel olarak anlamlı ($p < 0.05$),aynı harflerle gösterilen değerler anlamsız bulundu (Duncan, 1955)

CONCLUSION

In this study, *S. orientalis* subsp. *virens* and endemic *S. salviifolia* species were investigated in terms of anatomical, karyological and biochemical content.

The anatomical structure of the species has been determined. Biochemical content has been described. The chromosome number and chromosome morphology of the species were first revealed. It was found that the chromosome number of both study samples was the same, but there was a difference in terms of chromosome structures and chromosome morphologies. The results increase the knowledge of these species. This information will contribute to the determination of the systematic location of the species and other biological researches.

Author's Contributions

The contribution of the authors is equal.

Statement of Conflict of Interest

Authors have declared no conflict of interest.

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Iğdır ve Çevre İllerde Hububat Ekiliş Alanlarında Süne, *Eurygaster* spp. (Hemiptera: Scutelleridae)'nin Ergin Parazitoitleri ve Parazitleme Oranlarının Belirlenmesi

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ÖZET

Bu çalışma, 2015-2016 yıllarında Ağrı, Erzurum, Iğdır, Kars ve Van illeri hububat ekiliş alanlarında süne, *Eurygaster* spp. (Hemiptera: Scutelleridae) erginlerinden elde edilen parazitoit türleri, bunların popülasyonlar içerisindeki dağılımları ve parazitleme oranlarının belirlenmesi amacıyla yürütülmüştür. Çalışma sonucunda, süne ergin parazitoitleri olarak; *Elomya lateralis* (Meigen, 1824), *Ectophasia oblonga* (Robineau-Desvoidy, 1830) ve *Phasia subcoleoptrata* (Linnaeus, 1767) türleri belirlenmiştir. Parazitoit popülasyonu içerisindeki türlerin dağılımları ise %57'si *E. lateralis*, %23'ü *P. subcoleoptrata* ve %20'si ise *E. oblonga* olarak kaydedilmiştir. Bu türlerden *E. lateralis*'in Ağrı ve Van illerinde, *E. oblonga*'nın Iğdır ve Kars illerinde *P. subcoleoptrata*'nın ise Erzurum'da yaygın tür olduğu tespit edilmiştir. Ağrı, Iğdır ve Van illerinde 2015-2016 yıllarında hububat alanlarında toplanan kışlamış ergin sünelerde toplam parazitlenme oranı sırasıyla %8.73, %1.2 ve %3.6, Erzurum ve Kars illeri hububat ekiliş alanlarında ise 2016 yılında toplanan ergin sünelerde sırasıyla %2.5 ve %0.68 parazitlenme meydana gelmiştir. En yüksek parazitlenme %8.73 oranıyla Ağrı'da tespit edilmiştir.

Entomoloji

Araştırma Makalesi

Makale Tarihçesi
Geliş Tarihi : 23.09.2021
Kabul Tarihi : 22.01.2022

Anahtar Kelimeler
Süne
Tachinidae (Diptera)
Hububat
Iğdır
Çevre iller

Determination of Adult Parasitoids of Sunn pest *Eurygaster* spp. (Hemiptera: Scutelleridae) and their Parasitization Rates in Cereal Cultivation Areas in Iğdır and Neighboring Provinces

ABSTRACT

This study was conducted to determine parasitoid species identified from adults of Sunn pest *Eurygaster* spp. (Hemiptera: Scutelleridae), their distribution in populations and their parasitization rates on cereal cultivation areas in Ağrı, Erzurum, Iğdır, Kars and Van provinces in the years of 2015 and 2016. As a result of the study, *Elomya lateralis* (Meigen), *Ectophasia oblonga* (Robineau-Desvoidy) and *Phasia subcoleoptrata* (Linnaeus, 1767) species were identified as the adult Sunn pest *Eurygaster* species. The distribution of the species within the parasitoid population was recorded as 57 %*E. lateralis*, 23% *P. subcoleoptrata* and 20% *E. oblonga*. Among these species, *E. lateralis* was found to be common in Ağrı and Van, *E. oblonga* in Iğdır and Kars provinces, and *P. subcoleoptrata* in Erzurum. The total parasitization rate was 8.73%, 1.2% and 3.6%, respectively. in the overwintered adult Sunn pests collected in the cereal fields of Ağrı, Iğdır and Van provinces in 2015-2016, and 2.5% and 0.68%, respectively, in the adult Sunn pests collected in the cereal cultivation areas of Erzurum and Kars provinces in 2016. The highest parasitism rate was observed in Ağrı as 8.73%.

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GİRİŞ

Tahıl, insan ve hayvan gıdası olarak önemli bir yere sahiptir. Çoğu ülkede olduğu gibi Türkiye'de üretilen tahıllar içerisinde buğday ilk sırayı almaktadır. Türkiye'de, 2019 yılı verilerine göre, buğday ekiliş alanı 68.5 milyon da ve üretim miktarı 19 milyon tondur; Doğu Anadolu Bölgesi 6. milyon da ekiliş alanıyla %8.75 ve 1. milyon ton üretim ile %5.26'lık bir paya sahiptir (TUİK, 2021).

Türkiye'de geniş alanlarda üretimi yapılan hububatta, ürün ve kalite kayıplarına neden olan en önemli zararlı süne, *Eurygaster* spp. (Hemiptera: Scutelleridae)'dir (Gözüaçık ve Yiğit, 2014). Sünenin birçok doğal düşmanı bulunmaktadır. Süne popülasyonunu sınırlayan en önemli doğal düşmanı yumurta parazitoiti *Trissolcus* (Ashmead, 1893) (Hymenoptera: Scelionidae) türleridir. Tachinidae (Diptera) familyasına bağlı süne nimf ve ergin parazitoitleri de sünenin popülasyonlarını sınırlamada belirli bir paya sahiptir (Lodos, 1961).

Tachinidae familyasına ait sinek türlerinin, Lepidoptera, Coleoptera, Hemiptera ve Orthoptera takımına bağlı böceklerin parazitoitleri olduğu ve Avrupa'da yaklaşık 750-800 kadar türü parazitlediği bildirilmektedir (Greiner ve Liljesthröm, 1992). Aynı familyaya bağlı Phasiinae alt familyası türlerinin sadece Heteroptera türlerini parazitlediği, ergin süneleri kısırlaştırdığı ve popülasyonlarını baskı altına aldığı bildirilmektedir (Dubina, 1974; Belyaeva, 1975; Tchorsing ve Herting, 1994; Kıvan, 1996; İslamoğlu ve Kornoşor 2004).

Süne ergin parazitoitleri olarak Güneydoğu Anadolu Bölgesi'nde yapılan çalışmalarda *Heliozeta helluo* (Fabricius, 1805), *Phasia subcoleoptrata* (Linnaeus, 1767), *Clytiomya helluo* (Fabr, 1805), *Helomyia lateralis* (Meig, 1824), *Phasia* crassipennis (Fabricius, 1794), *E. crassipenis* (Fabricius, 1794), *Elomya lateralis* (Meigen, 1824) ve *Ectophasia oblonga* (Robineau-Desvoidy, 1830)(Lodos, 1961; Brown, 1962; Yüksel, 1968; Öncüer, 1991; Gözüaçık ve ark., 2010; Gün, 2010; Duman, 2015), Akdeniz Bölgesi'nde *P. crassipennis*, *E. oblonga, P. subcoleoptrata*, *H. helluo* ve *E. lateralis* (Zwölfer, 1942; Şimşek ve ark., 1994; İslamoğlu, 2003; Çolak, 2004; Keçeci ve ark. 2007; Gün, 2010), Marmara Bölgesi'nde *E. oblonga, H. helluo, P. subcoleoptrata* ve *E. lateralis* (Öncüer ve Kıvan, 1995; Kıvan, 1996; Çetin ve Hantaş, 2011), İç Anadolu Bölgesi'nde *E.lateralis, Gymnosoma desertorum* (Rohdendorf, 1947) *P. subcoleoptrata, E. oblonga* ve *H. helluo* (Brown, 1962; Memişoğlu ve Özer, 1994; Atay and Kara, 2014) ve Karadeniz Bölgesi'nde (Tokat) *G. desertorum* (Atay and Kara, 2014) türlerinin bulunduğu bildirilmiştir.

Bu çalışma, 2015-2016 yıllarında Ağrı, Erzurum, Iğdır, Kars ve Van illerinde hububat ekiliş alanlarında yürütülmüş ve süne ergin parazitoit türleri, parazitoit türlerinin popülasyon içerisinde dağılımı ve parazitlenme oranlarını belirlemek amacıyla ele alınmıştır.

MATERYAL ve METOD

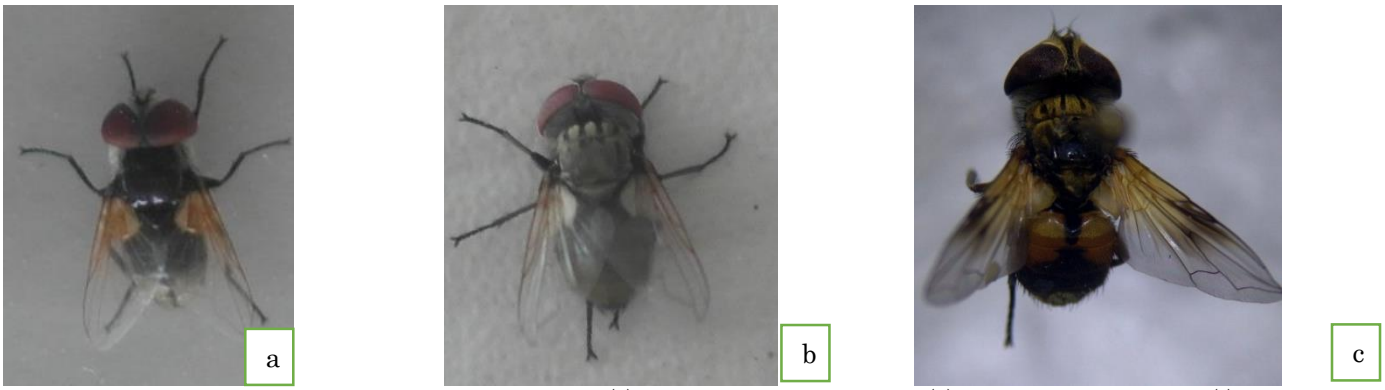
Çalışma 2015-2016 yıllarında nisan-ağustos aylarında Iğdır (Aralık, Merkez, Karakoyunlu ve Tuzluca ilçelerine bağlı 57 köy), Ağrı (Doğubayazıt, Diyadin, Eleşkirt, Hamur, Merkez, Taşlıçay, Tutak ve Patnos ilçelerine bağlı 29 Köy), Erzurum (Horasan, Köprüköy, Pasinler ve Yakutiye ilçelerine bağlı 9 köy), Kars (Arpaçay, Digor, Kağızman, Merkez, Sarıkamış, Selim ve Susuz ilçelerine bağlı 26 köy) ve Van (Başkale, Çaldıran, Edremit, Erciş, İpekyolu, Muradiye, Özalp, Saray ve Tuşba ilçelerine bağlı 27 köy) 'de toplam 32 ilçede ve 148 tarlada yapılmıştır. Örnekleme her tarlanın 10 farklı yerinde 10'ar (toplam 100 atrap) atrap süpürme yoluyla yapılmıştır. Her lokasyonda toplanan kışlamış ergin süneler, laboratuvarda ayrı ayrı plastik kaplarda 25°C ± 1 sıcaklık ve %65 ± 5 nem ortamında kültüre alınmıştır (Şekil 1a). Ergin sünelere besin olarak taze buğday bitkisi verilmiş ve bitkiler 2-3 günde bir değiştirilmiştir. Sünelerden elde edilen parazitoitler öldükten sonra iğnelenip etiketlenmiştir (Şekil 1b). Ayrıca, elde edilen ergin parazitoit türlerin süne popülasyonu içerisindeki dağılımı ve parazitlenme oranları (%) hesaplanmıştır. Süne ergin parazitoitlerin teşhisi, Gaziosmanpaşa Üniversitesi Ziraat Fakültesi, Bitki Koruma Bölümü Öğretim Üyesi Doç. Dr. Turgut ATAY tarafından yapılmıştır.

BULGULAR ve TARTIŞMA

Iğdır İli ve Çevresinde Hububat Alanlarında Bulunan Parazitoit Türleri ve Popülasyon İçerisindeki Yüzde (%) Dağılımlar

Çalışmanın yürütüldüğü 2015-2016 Ağrı, Erzurum, Iğdır, Kars ve Van illerinde süne ergin parazitoiti olarak, Tachinidae (Diptera) familyasına ait *Elomya lateralis* (Meigen, 1824) (Şekil 2a), *Phasia subcoleoptrata* (Linnaeus, 1767) (Şekil 2b) ve *Ectophasia oblonga* (Robineau-Desvoidy, 1830) (Şekil 2c) türleri belirlenmiştir. Ağrı ve Van illerinde *E. lateralis* ve *P. subcoleoptrata*, Erzurum ilinde *P. subcoleoptrata*, Iğdır ilinde *E. lateralis, P. subcoleoptrata* ve *E. oblonga,* Kars ilinde *E. oblonga* türlerinin bulunduğu belirlenmiştir (Çizelge 1).

Şekil 1. Ergin sünelerin laboratuvarda kültüre alınması (a) ve parazitoitler öldükten sonra iğneleme ve etiketlenmesi (b)
Figure 1. Laboratory culture of adult Sunn pests (a) *and pinning and labeling after parasitoids have died* (b)



Şekil 2. Ergin parazitoitler türleri; *Elomya lateralis* ♀ (a), *Phasia subcoleoptrata* ♀ (b), *Ectophasia oblonga* ♀(c)
Figure 2. Types of adult parasitoids; Elomya lateralis ♀ (a), *Phasia subcoleoptrata* ♀ (b), *Ectophasia oblonga* ♀(c)

Çizelge 1. Iğdır ili ve çevresinde hububat alanlarında bulunan parazitoit türleri ve sayısı
Table 1. Parasitoid species and number found in cereal fields in and around Iğdır province

Yıl	İl	İlçe	Yer	Tahıl	Ergin Parazitoiti türü	Sayısı
2015	Iğdır	Tuzluca	Ağabey	Buğday	*Ectophasia oblonga*	2
			Üçkaya	Buğday	*Elomya lateralis*	1
			Eğrekdere	Buğday	*E. lateralis*	2
			Eğrekdere	Buğday	*E. oblonga*	1
			Karabulak	Arpa	*Phasia subcoleoptrata*	1
	Ağrı	Merkez	Yolugüzel	Arpa	*E. lateralis*	5
			Yoncalı	Buğday	*E. lateralis*	1
		Tutak	Dereköy	Arpa	*E. lateralis*	1
			Merkez	Y.Ot	*P. subcoleoptrata*	1
2016	Iğdır	Tuzluca	Üçkaya	Buğday	*E. oblonga*	2
			Üçkaya	Buğday	*E. lateralis*	1
			Küçükova	Buğday	*E. oblonga*	1
			Küçükova	Buğday	*P. subcoleoptrata*	1
			Eğrekdere	Arpa	*E. oblonga*	1
			Eğrekdere	Arpa	*P. subcoleoptrata*	1
			Merkez	Buğday	*E. lateralis*	1
			Merkez	Buğday	*P. subcoleoptrata*	1
			Buruksu	Buğday	*E. oblonga*	1
	Ağrı	Eleşkirt	Ergözü	Arpa	*E. lateralis*	2
			Uzunyazı	Arpa	*E. lateralis*	1
			Güvence	Arpa	*E. lateralis*	1
		Merkez	Yolugüzel	Buğday	*E. lateralis*	2
		Tutak	Merkez	Arpa	*E. lateralis*	2
			Merkez	Arpa	*P. subcoleoptrata*	1
	Van	Merkez	Çakırbey	Buğday	*P. subcoleoptrata*	1
			Irgat	Buğday	*P. subcoleoptrata*	2
			Güvençli	Buğday	*E. lateralis*	3
		Muradiye	Ünseli	Buğday	*E. lateralis*	2
	Kars	Kağızman	Denizgölü	Buğday	*E. oblonga*	1
	Erzurum	Yakutiye	Çiftlikköy	Arpa	*P. subcoleoptrata*	1
					Toplam	44

Ağrı, Erzurum, Iğdır, Kars ve Van illerinde 2015-2016 yıllarında hububat ekiliş alanlarında toplanan kışlamış ergin sünelerden elde edilen toplam 46 adet parazitoit pupasından %95.65'lik bir ergin çıkışıyla 44 adet ergin parazitoiti elde edilmiştir. Elde edilen parazitoitlerden *E. lateralis* Ağrı (15 adet), Iğdır (5 adet) ve Van (5 adet), *P. subcoleoptrata* Iğdır (4 adet), Ağrı (2 adet), Van (3 adet) ve Erzurum (1 adet) ve *E. oblonga* Iğdır (8 adet) ve Kars (1 adet), parazitoitlerin 25'i *E. lateralis*, 10'u *P. subcoleoptrata* ve 9'u *E. oblonga* olduğu belirlenmiştir (Çizelge 1).

Parazitoit popülasyonu içerisindeki türlerin dağılımı, %57 oranıyla *E. lateralis*, %23 oranıyla *P. subcoleoptrata* ve %20 oranıyla *E. oblonga*'nın olduğu saptanmıştır. Türkiye'de yapılan çalışmalarda Güneydoğu Anadolu Bölgesi'nde hâkim ve yaygın türün *H. helluo* olduğu bunları *P. subcoleoptrata*, *E. oblonga* ve *E. lateralis*'in izlediği (Gözüaçık ve ark., 2010), Akdeniz Bölgesi'nde, *H. helluo ve P. subcoleoptrata* türlerini bölgede yaygın tür oldukları (Şimşek ve ark.,1994), Marmara Bölgesi'nde 1994 yılında *H. helluo*, 1995 yılında ise *P. subcoleoptrata*'nın yaygın tür olduğunu *E. oblonga* ve *E. lateralis* izlediği belirlenmiştir (Kıvan, 1996), İç Anadolu Bölgesi'nde hâkim türün %43.75 ile *P. subcoleoptrata,* %25 ile *H. helluo,* %18.75 ile *E. oblonga* ve %12.5 ile *E. lateralis* izlediği belirlemişlerdir (Memişoğlu ve Özer, 1994). Yapılan çalışmalarda görüldüğü gibi diğer bölgelerde hâkim tür olan *H. helluo* bu çalışmanın yapıldığı illerde rastlanmamıştır. Diğer bölgelerde çok az olarak bulunan *E. lateralis*'in ise Ağrı ve Van illerinde hâkim tür olduğu, *E. oblonga*'nın Iğdır ve Kars illerinde hâkim tür olduğu ve *P. subcoleoptrata* ise Erzurum'da hâkim tür olduğu tespit edilmiştir. Süne türü olarak *Eurygaster integriceps*'in baskın olduğu Ağrı ve Van illerinde *E. lateralis* ve *E. maura*'nın baskın tür olduğu Iğdır ve Kars illerinde ise *E. oblonga* hâkim tür olarak tespit edilmiştir. Tespit edilen türler iller için yeni kayıt niteliğindedir.

Iğdır İli ve Çevresinde Hububat Alanlarındaki Kışlamış Ergin Sünelerin Parazitlenme Oranları

Ağrı, Erzurum, Iğdır, Kars ve Van illerindeki hububat alanlarında 2015-2016 yılında Ağrı ilinde toplam 206 adet (144♀-62♂) birey, Erzurum ilinde toplam 40 adet (34♀-6♂ birey), Iğdır ilinde toplam 1.432 adet (1.077♀-355♂ birey), Kars ilinde 145 adet (75♀-70♂ birey), Van ilinde toplam 250 adet (160♀-90♂ birey) kışlamış ergin süne toplanmıştır. Toplam parazitlenme oranları 2015-2016 yıllarında Ağrı ilinde %8.73, Iğdır'da %1.2, Van'da %3.6 olarak belirlenmiştir. Parazitoitler 2015 yılında bulunmayıp 2016 yıllında bulunan Erzurum ve Kars illerinde %2.5 ve %0.68 oranlarında belirlenmiştir. En yüksek parazitlenme 2015-2016 yıllarında Ağrı'da elde edilmiştir (Çizelge 2).

Çizelge 2. Ağrı, Erzurum, Iğdır, Kars ve Van illeri hububat alanlarında 2015-2016 yıllarında toplanan kışlamış süne sayısı, parazitlenme oranı, elde edilen parazitoit türü ve sayısı

Table 2. Number of overwintered Sunn pests collected in the grain fields of Ağrı, Erzurum, Iğdır, Kars and Van Provinces in 2015-2016, parasitizatian rate, parasitoid species and number obtained

İl	Toplam Ergin Süne Sayısı (adet)	Parazitli Süne Sayısı (adet)	Parazitoit Türü	Parazitleme Oranı (%)	Genel Parazitleme Oran (%)
Ağrı	206	18	*E. lateralis* *P. subcoleoptrata*	7.27 0.97	8.73
Erzurum	40	1	*P. subcoleoptrata*	2.50	2.50
Iğdır	1.432	17	*E. oblonga* *E. lateralis* *P. subcoleoptrata*	0.60 0.30 0.30	1.20
Kars	145	1	*E. oblonga*	0.70	0.70
Van	250	9	*E. lateralis* *P. subcoleoptrata*	2.00 1.20	3.60

Çizelge 2'de görüldüğü üzere 2015-2016 yıllarında Ağrı ilinde 206 kışlamış ergin süneden 18 tachinid birey elde edilmiş %8.73'lik bir oran ile diğer illere göre en yüksek parazitlenme olduğu görülmüştür. Van ilinde 250 adet kışlamış ergin süneden 9 tachinid birey elde edilmiş ve parazitlenme oranının %3.6 olduğu belirlenmiştir. Erzurum ilinde 40 adet kışlamış ergin süneden 1 tachinid birey elde edilmiş ve %2.5 parazitlenme olduğu saptanmıştır. Iğdır ilinde 1.432 adet kışlamış ergin süneden 17 tachinid birey ve %1.2 parazitlenme oranı tespit edilmiştir. Kars ilinde 145 adet kışlamış ergin sünede 1 tachinid birey elde edilmiş ve parazitlenme oranının %0.7 olduğu görülmüştür. Farklı yıllarda ve yerlerde yapılan çalışmalarda benzer parazitlenme oranları elde edilmiştir. Gözüaçık ve ark. (2010)'ın 2005-2006 yıllarında Güneydoğu Anadolu Bölgesi'nde yaptıkları çalışmalarda, hububat tarlalarında parazitlenme

oranlarının sırasıyla Diyarbakır'da %6.4-5.7, Adıyaman'da %9.4-15.0, Siirt'te %12.6-7.8, Batman'da %5.0-5.4, Mardin'de %9.3-11.2, Şırnak ilinde %2.1 ve Şanlıurfa'da %12.2-7.3 olarak saptanmışlardır. Duman (2015) Diyarbakır ve Şanlıurfa illerinde yapmış olduğu çalışmada hububat alanlarında 2013 yılında %15.9-20.2; 2014 yılında %14.6-20.00 olarak belirlemiştir, Şimşek ve ark. (1994), yapmış oldukları çalışmada Adana'da %11.25, Gaziantep% 1.08, Hatay'da %1.55 Antalya'da %3.28 ve Kahramanmaraş'ta %10.3 parazitlenme oranı elde etmişlerdir, İslamoğlu ve Kornoşor (2003), ortalama parazitlenme oranlarının Gaziantep'te, 2001 yılında %9.25; 2002 yılında %11.0, Kilis'de 2001 yılında %16.5; 2002 yılında %19.0 olarak belirlemişlerdir. Kıvan (1996), Tekirdağ'da hububat alanlarında parazitlenme oranlarının 1994-1995 yıllarında sırasıyla %7.70 ve 4.58 olduğunu, Memişoğlu ve Özer (1994)'de Ankara'da *E. maura*'da parazitlenmenin 1981, 1982, 1983 ve 1984 yıllarında sırasıyla %3.35, % 2.88, %0.83 ve %1.67 oranlarında olduğunu bildirmişlerdir. Maafi, (1991)'nin İran'da yaptığı çalışmalarda, süne erginlerinin parazitlenme oranlarının 1989 yılında Saeid Abad'da %52.89, Fashand'da %66.92; 1990 yılında ise Saeid Abad'da %64.72; Fashand'da %62.59 olduğu tespit edilmiştir. Yunanistan'ın Boeotia (Voitia) ve Atina bölgelerinde yapılan çalışmada ise, %18.0-53.0 arasında parazitlenmenin olduğunu bildirilmiştir (Stavraki, 1977).

Parazitoitlerin süne üzerindeki baskısını arttıracak önlemlerin alınması gerekmektedir. Kışlakta ve hububat alanlarının kenarlarında yabancı otlar, çalılık alanlar korunmalı ve hedef dışı alanlar kesinlikle ilaçlanmamalıdır. Parazitoitler için besin kaynağı olarak tespit edilen bitkilerin korunması gerekmektedir. Süne yumurta parazitoitleri *Trissolcus* spp'lerin yanı sıra Tachinidae'lerin de korunması süne ile doğal biyolojik mücadelede önemli katkılar sağlanabilecektir.

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Araştırmacıların Katkı Oranı Beyan Özeti

Yazarlar makaleye eşit oranda katkı sağlamış olduklarını beyan eder.

Çıkar Çatışması Beyanı

Makale yazarları aralarında herhangi bir çıkar çatışması olmadığını beyan ederler.

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Uygulama Sonrası Zararlı Bulaşmasından Depolanmış Buğdayın Korunması Bakımından *Beauveria bassiana* Etkinliğinin Kalıcılığı

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ÖZET

Bu çalışma, üç yerel *Beauveria bassiana* izolatının spor etkinlik sürelerinin belirlenmesi amacıyla, yaygın olan depolanmış ürün zararlılarından *Sitophilus oryzae* L. (Coleoptera: Curculionidae) *Rhyzopertha dominica* F. (Col.: Bostrichidae) ve *Oryzaephilus surinamensis* L. (Col.: Silvanidae) erginlerine karşı uygulanarak yürütülmüştür. Biyolojik testler için buğday danelerine 1000 ppm (w/w) konsantrasyonunda *B. bassiana* sporu karıştırılmış ve 1, 15 ve 28 gün sonra 20'şer ergin salımı yapılmıştır. *Sitophilus oryzae* ile yürütülen testlerde 3 izolat için genel olarak; 7. ve 14. gün ölüm oranı başlangıçta %33.3-41.6 ve %68.3-76.6 olup 28 gün sonraki salımlarda %6.6-18.3 ve %13.3-21.6'ya düşmüştür. *Rhyzopertha dominica* başlangıç ölüm oranları 7. ve 14. gün için %46.6-50.0 ve %93.3-95.0'dır ve 28 gün sonraki salımlar sonucunda %10.0-18.3 ve %16.6-28.3 olmuştur. *Oryzaephilus surinamensis* başlangıç ölümleri ise 7. ve 14. gün için %41.6-46.6 ve %70.0-85.0'den 28 gün sonraki salımda %8.3-15.0 ve %13.3-20.0'a düşmüştür. Buğdayda sporların bekleme süresi uzadıkça, tüm fungus izolatları ve böcek türleri için her inkübasyon süreci sonrasında ölüm oranları önemli derecede düşmüştür. İnkübasyon süreçlerinin tümünde tüm izolatlar için benzer ölüm oranları belirlenmiş ve etkinlik kayıplarının benzer olduğu gözlenmiştir. Tüm sonuçlar *B. bassiana* izolatlarında zamana bağlı aktivite kaybından sorumlu faktörlerin belirlenmesi ve bu veriler doğrultusunda önlem alınması gerektiğini göstermektedir.

Entomoloji

Araştırma Makalesi

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Anahtar Kelimeler
Biyolojik mücadele
Mikrobiyal mücadele
Entomopatojen fungus
Beauveria bassiana
Depolanmış ürün zararlıları

Persistence of *Beauveria bassiana* Efficacy on Wheat Grains to Protect Stored-Grains from Post-Treatment Pest Infestations

ABSTRACT

This study was carried out to determine the post-treatment efficacy of three local *Beauveria bassiana* isolates by applying them against adults of three common stored-product pests, *Sitophilus oryzae* L. (Coleoptera: Curculionidae) *Rhyzopertha dominica* F. (Col.: Bostrichidae) and *Oryzaephilus surinamensis* L. (Col.: Silvanidae).

For the biological tests, wheat grains were mixed with fungal spores at 1000 ppm (w/w) concentration then 20 adults were released 1, 15 and 28 days after the treatments. For the tests carried out with *S. oryzae*; the mortality rate was initially 33.3-41.6% and 68.3-76.6%; and declined to 6.6-18.3% and 13.3-21.6% on the 7th and 14th day, respectively, when released 28 days later. The initial mortality of *R. dominica* was 46.6-50.0 % and 93.3-95%; and became 10-18.3% and 16.6-28.3% when released 28 days later. *Oryzaephilus surinamensis* mortality on the 7th and 14th day was initially 41.6-46.6% and 70.0-85.0%, and decreased to 8.3-15% and 13.3-20.0%, respectively, when released 28 days later. Mortality rates at the end of each incubation period, for all testing isolates and insect species, were significantly reduced when waiting time of spores on wheat was prolonged. For all incubation periods, similar mortality rates were obtained from all

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isolates and similar efficiacy loss was observed. All the results together indicate that the responsible factors for time-dependent loss of activity in *B. bassiana* isolates should be determined, and in line with these data, precautions need to be taken.

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INTRODUCTION

Cereals are among the most important food sources. Grain production in Turkey was 37.187.508 tons in 2020 (Anonymous, 2021). The climatic conditions and inproper storage facilities in Türkiye often create favorable environments for the development of insect pests. Stored-cereals are commonly exposed to stored-product insect pests. These pests are responsible for qualitative and quantitative loss due to feeding activities reducing weight, nutrient content and germination as seeds while contaminating cereals due to their existance and waste accumulation, consequently decreasing market value of the commodity (Sewify et al., 2014). Among pests of stored-cereals, the rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae), saw toothed grain beetle, *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae) and the lesser grain borer, *Rhyzopertha dominica* (Coleoptera: Bostrychidae) are considered notorious species worldwide (Kaur et al., 2014). Damage increases rapidly when several generations develop during the storage period. There are some insecticides registered for use against these pests. However, as a result of excessive pesticides usage, significant problems arise including insecticide resistance, hazards to human, livestock and environment. Scientists have been seeking alternative pest management strategies for stored-product pests to avoid such adverse effects of insecticides. Entomopathogenic fungi can control stored-grain insect pests effectively according to Batta (2016). Around the World, fungal isolates have been tested against various stored-product pest species both under controlled conditions and in field experiments. High mortality rates from applications were reported by several authors who tested *B. bassiana* on various insects (Heviefo et al. 2020). Batta (2003) mentioned that different fungal isolates of the same species can vary in infectivity to a given host species. Wakil et al. (2020) suggested that a mixture of biological control agents and natural or chemical substances exhibiting insecticidal properties has a potential to provide high protection of stored-cereals for short or long storage periods. Batta and Kavallieratos (2018) as a result of their work emphasized that obtaining new and effective isolates of entomopathogenic fungi for biological control of stored-product pest insects and improving their formulations are important requirements for success. Another area requiring more studies is their post-application status. This study was carried out to determine the post-treatment efficacy of three local *B. bassiana* isolates against three coleopteran pests (*R. dominica, S. oryzae* and *O. surinamensis*) of stored grains.

MATERIAL and METHODS

Insect cultures

Insect cultures were maintained in the laboratory, Department of Plant protection at University of Kahramanmaraş Sütçü İmam. *Oryzaephilus surinamensis* cultures were maintained on wheat with oat flakes, the *S. oryzae* and *R. dominica* cultures were maintained on wheat grains at 25±2°C and 65±5% relative humidity in darkness. Adults were left in clean food for three days for oviposition. After this period all adults removed and cultures incubated for the emergence of new generation adults. Seven-ten days old adults were used for testing.

Fungal cultures

Beauveria bassiana isolates were previously obtaied from stored-grain pests collected from grain storages in Adana and Kahramanmaraş province in Turkey. Isolate identifications; carried out using morphological and molecular techniques. Three *B. bassiana* isolates (1-1, 22-1, 42-1) used in this study were quite successful in terms of mortality and reduction in new generation emergence of three coleopteran pests (*R. dominica*, *S. oryzae* and *O. surinamensis*). Potato Dextrose Agar (PDA) was used for growing fungal isolates in Petri dishes (Ø = 90 mm). After sealing the Petri dishes with Parafilm they were incubated at 26°C ± 1°C in a dark incubator for 10 days. The cultures that completed sporulation were left open one night to reduce moisture. Then, the conidia of the fungi were gathered by vacuming. They were kept at +4°C on silica gel in Eppendorf tubes until used. Germination test was performed for fungal spores before experiments. Fungal spores were diluted in 0.01% Tween 80 solution and spread on PDA medium. After 24 hours at 25°C the spores were examined under a microscope (×40) and those with a

germ tube equal or longer than the spore were considered germinated. The spores used in the experiments had germination rate of 96-98%.

Experimental design

Wheat grains were homogenously mixed with *B. bassiana* spores at 1000 ppm (w/w) concentration and 40 g was placed in each centrifuge tube (50ml capacity). One, 15 and 28 days after the treatment of wheat grains, twenty adults were release into each tube. As controls, pests were released simultaneously into clean grains without fungal spores. All were incubated under the same conditions at 25±2°C, 65±5% relative humidity in darkness. The experiment had 3 repetitions and mortality rates were assessed on the 7th and 14th days after insect releases.

Statistical analysis

Adult mortality rates were corrected by using Abbott's formula (Abbott, 1925) before Arcsine transformation. The data were subjected to one-way ANOVA, and Duncan multiple comparison test at 5% significance level to determine differences between treatments. The statistical analysis were performed by using SPSS15.0.

RESULTS and DISCUSSION:

This study was to investigate post-treatment efficacy of three *Beauveria bassiana* isolates against adults of *Sitophilus oryzae*, *Rhyzopertha dominica* and *Oryzaephilus surinamensis* under laboratory conditions. The results for *S. oryzae, R. dominica* and *O. surinamensis* were presented in Table 1, 2 and 3, respectively. They confirmed the pathogenic characteristic of the *B. bassiana* isolates (1-1, 22-1, 42-1) to the tested stored-grain pests. The mortality effects of the testing isolates were statistically the same in majority of the experimental units (Tables 1,2,3). The only exceptions were 7th day mortalities of *S. oryzae* for insect release 15 days after fungus application (AFA) ($F_{2,6}$=6.45, P< 0.05) and 14th day mortalities of *R. dominica* for insect release 28 days AFA ($F_{2,6}$=12.30, P<0.01). In both cases, isolate 42-1 killed significantly lower insects than the other two *B. bassiana* isolates. The mortality of *S. oryzae* adults was 33.3-41.6% and 68.3-76.6% on the 7th and 14th day, respectively, when the insects were released one day AFA. The mortalities declined gradually with extended time gap between fungus application and insect release. The mortality was 6.6-18.3% and 13.3-21.6% on the 7th and 14th day, respectively, when the insects were released 28 days AFA. Mortality rates segragated for each time gap between fungus application and insect release, particularly more clear for the results obtained on 14th day post-release. The trend was the same for the other two tested insect species. The mortality of *R. dominica* adults was 46.6-50.0% and 93.3-95.0% on the 7th and 14th day, respectively, when the insects were released one day AFA. These mortality rates became 10.0-18.3% and 16.6-28.3% when the time gap was increased to 28 days. *Oryzaephilus surinamensis* adult mortality on 7th and 14th day was initially 41.6-46.6% and 70.0-85.0%, and decreased to 8.3-15.0% and 13.3-20.0% for 1 and 28 days AFA, respectively.

All the obtained data showed clearly that mortality rates at the end of both incubation periods were significantly reduced with prolonged time between treatment of wheat with fungal spores and release of the insects. This was the case for all three fungal isolates and for all pest species tested (Tables 1- 3). Each two-weeks of time gap caused mostly a reduction of 35-60%; in one case, an almost 70% decline in *R. dominica* mortality between 15th and 28th days for isolate 42-1.

One of the most important factors in pathogenicity is the virulence of the pathogen, and each isolate has a specific innate capacity (Soetopo, 2004). Er et al. (2016), in the search for biological agents, reported that wild fungi comprise genetic and adaptive diversity. They show utility of selecting single spores from wild fungal isolates for studies towards developing more virulent microbial control agents. As a result of the trials carried out with *S. oryzae* to determine the rate of decrease in the number of individuals in following generation, Korkmaz (2017) determined that only isolate 1-1 caused a decrease of 100% amongst several isolates. Spore viability is another important factor that influences the efficacy of entomopathogenic fungi, and thus maintaining viability of spores is as important as the virulence of the fungus against targeted pest (Glare et al., 2012, Moore et al.,2000; Batta, 2004). Aregger (1992) demonstrated that viability loss of *B. bassiana* spores varies among isolates. However, mortality effect of fungal isolates is not solely dependent on their virulence and their spore viability, but also on the conditions in which they are applied.

Ambient temperature and humidity, aeration, light, air as well as the condition of the host itself have significant effects on the pathogenicity of entomopathogenic fungi (Padmini and Padmaja 2010). Kim et al (2019) studied the effect of temperature on storing *B. bassiana* for a long-term and found that *B. bassiana* can tolerate a wide range of temperatures between 4°C and 30°C. However, in long-term storage of entomopathogenic fungi, fungal isolate, ambient conditions during its production and storage influence the viability (Faria et al., 2009; Blanford et al., 2012). Therefore, it seems that stability of insecticides based on entomopathogenic fungi is a major obstacle for commercialization. Several studies have been conducted for stabilization of isolates under a range of temperature and

Table 1. Mortality rates on days 7 and 14 following release of *Sitophilus oryzae* adults on wheat treated with 1000 ppm *Beauveria bassiana* spores.

Çizelge 1. Buğdayın 1000 ppm fungus sporu ile muamelesinden 1, 15, 28 gün sonra Sitophilus oryzae erginlerinin düzeneklere bırakılmasını izleyen 7. ve 14. günlerdeki ölüm oranları

Isolate no. (İzolat no)	7th day mortality rates (%) ± SEM *(7. gün ölüm oranı(%) ± S.Hata)*			F ve P values *(F ve P değeri)*
	Released 1 day after treatment *(1 gün sonra salım)*	Released 15 days after treatment *(15 gün sonra salım)*	Released 28 days after treatment *(28 gün sonra salım)*	
1-1	41.6 ± 0 Aa	23.3 ± 1.6Ab	18.3 ± 1.6Ab	$F_{2,6}$=29.17 P<0.001
22-1	33.3 ± 3.3Aa	21.6 ± 6ABb	18.3 ± 1.6Ab	$F_{2,6}$=51.43 P<0.001
42-1	36.6 ± 4.4Aa	16.6 ± 7.2Bab	6.6 ± 3.3Ab	$F_{2,6}$=6.88 P<0.05
Control(Kontrol)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	
F and P values *(F ve P değeri)*	$F_{2,6}$= 1.27 P= 0.3462	$F_{2,6}$=6.45 P< 0.05	$F_{2,6}$=4.14 P=0.0742	
Isolate no. (İzolat no)	14th day mortality rates (%) ± SEM *(14. gün ölüm oranı(%) ± S.Hata)*			F ve P values *(F ve P değeri)*
	Released 1 day after treatment *(1 gün sonra salım)*	Released 15 days after treatment *(15 gün sonra salım)*	Released 28 days after treatment *(28 gün sonra salım)*	
1-1	76.6 ± 3.3Aa	41.6 ± 1.6Aab	18.2 ± 3.1Ab	$F_{2,6}$=84.39 P<0.0001
22-1	70.0 ± 2.8Aa	38.3 ± 4.4Ab	21.6 ± 1.6Ac	$F_{2,6}$=69.43 P<0.001
42-1	68.3 ± 3.3Aa	30 ± 2.8Ab	13.3 ± 1.6Ac	$F_{2,6}$=91.52 P<0.0001
Control *(Kontrol)*	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	
F and P values *(F ve P değeri)*	$F_{2,6}$= 1.97 P=0.2203	$F_{2,6}$= 3.55 P= 0.0962	$F_{2,6}$= 3.42 P=0.1019	

*Different capital letters in each column and different lowercase letters in each line constitute statistically different groups according to Duncan multiple comparison tests (P ≤ 0.05).

Table 2. Mortality rates on days 7 and 14 following release of *Rhyzopertha dominica* adults on wheat treated with 1000 ppm *Beauveria bassiana* spores

Çizelge 2. Buğdayın 1000 ppm fungus sporu ile muamelesinden 1, 15, 28 gün sonra Rhyzopertha dominica erginlerinin düzeneklere bırakılmasını izleyen 7. ve 14. günlerdeki ölüm oranları

Isolate no. (İzolat no)	7th day mortality rates (%) ± SEM *(7. gün ölüm oranı(%) ± S.Hata)*			F and P values (F ve P değeri)
	Released 1 day after treatment *(1 gün sonra salım)*	Released 15 days after treatment *(15 gün sonra salım)*	Released 28 days after treatment *(28 gün sonra salım)*	
1-1	50.0 ± 5.0Aa	33.3 ± 3.3Aab	18.3 ± 6.0Ab	$F_{2,6}$=7.06 P<0.05
22-1	50.0 ± 5.7Aa	28.3 ± 4.4Ab	18.3 ± 4.4Ab	$F_{2,6}$=10.46 P<0.05
42-1	46.6 ± 1.6Aa	35.0 ± 5.0Aa	10.0 ± 2.8Ab	$F_{2,6}$=28.03 P<0.001
Control(Kontrol)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	
F and P values (F ve P değeri)	$F_{2,6}$= 0.11 P=0.2203	$F_{2,6}$= 0.56 P= 0.5990	$F_{2,6}$= 1.20 P= 0.3637	
Isolate no. (İzolat no)	14th day mortality rates (%) ± SEM *(14. gün ölüm oranı(%) ± S.Hata)*			F and P values (F ve P değeri)
	Released 1 day after treatment *(1 gün sonra salım)*	Released 15 days after treatment *(15 gün sonra salım)*	Released 28 days after treatment *(28 gün sonra salım)*	
1-1	93.3 ± 6.6Aa	60.0 ± 2.8Ab	28.3 ± 1.6Ac	$F_{2,6}$=29.03 P<0.001
22-1	95.0 ± 5.0Aa	46.6 ± 4.4Ab	23.3 ± 1.6Ac	$F_{2,6}$=46.97 P<0.001
42-1	95.0 ± 2.8Aa	53.3 ± 6.0Ab	16.6 ± 1.6Bc	$F_{2,6}$=62.91 P<0.0001
Control(Kontrol)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	
F and P values (F ve P değeri)	$F_{2,6}$= 0.04 P=0.9638	$F_{2,6}$=2.08 P= 0.2062	$F_{2,6}$=12.30 P<0.01	

*Different capital letters in each column and different lowercase letters in each line constitute statistically different groups according to Duncan multiple comparison tests (P ≤ 0.05).

Table 3. Mortality rates on days 7 and 14 following release of *Oryzaephilus surinamensis* adults on wheat treated with 1000 ppm *Beauveria bassiana* spores

Çizelge 3. Buğdayın 1000 ppm fungus sporu ile muamelesinden 1, 15, 28 gün sonra Oryzaephilus surinamensis erginlerinin düzeneklere bırakılmasını izleyen 7. ve 14. günlerdeki ölüm oranları

Isolate no. (İzolat no)	7thday mortality rates (%) ± SEM *(7. gün ölüm oranı(%) ± S.Hata)*			F and P values (F ve P değeri)
	Released 1 day after treatment *(1 gün sonra salım)*	Released 15 days after treatment *(15 gün sonra salım)*	Released 28 days after treatment *(28 gün sonra salım)*	
1-1	46.6 ± 3.3 Aa	26.6 ± 1.6 Ab	15 ± 5.7 Ab	$F_{2,6}$=11.37 P < 0.01
22-1	46.6 ± 4.4 Aa	23.3 ± 1.6 Aa	8.3 ± 4.4 Ab	$F_{2,6}$=13.41 P < 0.01
42-1	41.6 ± 3.3 Aa	11.6 ± 6 Ab	8.3 ± 1.6 Ab	$F_{2,6}$=7.82 P < 0.05
Control(Kontrol)	0.0 ± 0.0	0.0±0.0	0.0 ± 0.0	
F and P values (F ve P değeri)	$F_{2.6}$= 0.60 P=0.9638	$F_{2,6}$=2.61 P= 0.1527	$F_{2,6}$= 0.67 P=0.5456	
Isolate no. (İzolat no)	14thday mortality rates (%) ± SEM *(14. gün ölüm oranı(%) ± S.Hata)*			F and P values (F ve P değeri)
	Released 1 day after treatment *(1 gün sonra salım)*	Released 15 days after treatment *(15 gün sonra salım)*	Released 28 days after treatment *(28 gün sonra salım)*	
1-1	83.3 ± 3.3Aa	39.6 ± 1.7Ab	20.0 ± 2.8Ac	$F_{2,6}$=96.06 P < 0.0001
22-1	85 ± 6.6Aa	36.2 ± 6.2Ab	20.0 ± 0Ab	$F_{2,6}$=18.66 P < 0.01
42-1	70.0 ± 6.6Aa	29.3 ± 4.5Ab	13.3 ± 1.6Ac	$F_{2,6}$=31.14 P < 0.001
Control(Kontrol)	0.0 ± 0.0	3.3 ± 1.6	0.0 ± 0.0	
F and P values (F ve P değeri)	$F_{2.6}$= 1.39 P=0.3190	$F_{2,6}$= 1.38 P= 0.3411	$F_{2,6}$=4.15 P=0.0738	

*Different capital letters in each column and different lowercase letters in each line constitute statistically different groups according to Duncan multiple comparison tests ($P \leq 0.05$).

ultraviolet light, and for obtaining thermotolerant fungal cultures (Kim et al., 2011; Santos et al., 2011; Shin et al., 2017).

In the present study, the tested three *B. bassiana* isolates expressed virulence against the pest species, but reduction in viability of their spores for long periods was their weakness. The factors responsible for the reduction of *B. bassiana* efficacy during the time after application should be determined and taken into account while spore production or application. Should they be developed as biocontrol agents as they are, their application needs to be repeated as required in time. According to Jackson (1997) and Rangel et al. (2015), it is possible to find more virulent and tolerant fungal isolates by screening. They also emphasize the importance of optimization of nutritions and physical manipulations while growing fungi for conidial vigor. St. Leger and Wang (2010) proposed using genetic engineering to increase the resistance of fungi to environmental factors.

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Conflict of Interest

The author declares that there is no conflict of interest in the study.

Author's Contributions

The contribution of the authors is equal.

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Pathogenicity of Different *Rhizobium radiobacter* (*Agrobacterium tumefaciens*) Isolates and Their Identification with Conventional Methods

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ABSTRACT

Rhizobium radiobacter is a significant causal agent that ranks among the top ten bacteria of molecular plant pathology in the world, has the largest range of hosts among plant pathogenic bacteria, and limits production and leads to economic losses in agriculture. The distinctive feature of the causal agent from other bacteria is the Ti plasmid, the extrachromosomal structure present in all virulent types. In this study, virulence of five *R. radiobacter* isolates (1A, 1B, 2A, 2B and RK 473) isolated from different rootstocks was tested in carrot slices, squash fruits, kalanchoe leaves, tomato and sunflower seedlings, and GF677, M9 and MM106 rootstocks, and hypersensitive response tests were conducted in tobacco plant. The isolates were diagnosed with biochemical and physiological tests by classical methods. All isolates formed tumors in carrot slices and squash fruits. 1A, 1B, 2A and 2B isolates formed tumors in the stem of GF677 peach rootstock, while it did not form any tumors on the stems of M9 and MM106 rootstocks. Tumor formation was observed in 1B isolate in the root application of GF677 peach rootstock, while no tumor formation was observed in other isolates. RK 473 isolate became pathogenic in M9 and MM106 apple rootstocks, while it was observed that the other isolates did not form any tumors. It was seen that none of the isolates became pathogenic in tomato and sunflower root and stem, and kalanchoe leaf applications. According to the virulence test results, 1B isolate was found out to be the most virulent isolate. Biochemical and physiological tests revealed the differences between isolates.

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Farklı *Rhizobium radiobacter* (*Agrobacterium tumefaciens*) İzolatlarının Patojeniteleri ve Konvensiyonel Yöntemlerle Tanımlanması

ÖZET

Rhizobium radiobacter, dünyada moleküler bitki patolojisinde ilk on bakteri içerisinde yer alan, bitki patojeni bakteriler içerisinde en geniş konukçu dizisine sahip olan, fidan yetiştiriciliğinde üretimi sınırlayan ve ekonomik kayıplara neden olan önemli bir hastalık etmenidir. Etmeni diğer bakterilerden ayırıcı özelliği tüm virülent türlerinde bulunan ekstrakromozomal yapı olan Ti plazmitidir. Bu çalışmada farklı fidanlardan izole edilmiş beş *R. radiobacter* izolatının (1A, 1B, 2A, 2B ve RK 473) virülanslıkları havuç dilimi, kabak meyvesi, kalonşe yaprağı, domates ve ayçiçeği fideleri, GF677, M9 ve MM106 anaçlarında test edilmiş ve tütün bitkisinde aşırı duyarlılık testi yapılmıştır. İzolatların biyokimyasal ve fizyolojik testler ile klasik yöntemlerle tanısı yapılmıştır. Tüm izolatlar havuç dilimi ve kabak meyvesinde ur oluşturmuştur. 1A, 1B, 2A ve 2B izolatları GF677 şeftali anacının gövdesinde ur oluştururken, M9 ve MM106 elma anaçlarının gövdesinde ur oluşturmamıştır. GF677 şeftali anacının kök uygulamasında 1B izolatında ur oluşumu gözlemlenirken diğer izolatlarda ur oluşumu gözlemlenememiştir. RK 473 izolatı M9 ve MM106 elma anaçlarında patojen olurken, diğer izolatların ur oluşturmadığı tespit edilmiştir. Tüm izolatların domates kök ve

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gövde, ayçiçeği gövde ve kalonşe yaprak uygulamalarında patojen olmadıkları görülmüştür. Virülanslık test sonucunda 1B izolatı en virülant izolat olarak belirlenmiştir. İzolatlar arasında biyokimyasal ve fizyolojik test sonuçlarında farklılıklar bulunmuştur.

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INTRODUCTION

Human nutrition of physiological and biological aspects with the discovery, it was revealed that nutrients such as carbohydrates, fats, proteins, vitamins, minerals and water in the structure of fruits are important for health (Yamankaradeniz, 1981).

In world, 65.220.334.00 hectares (ha) area 865.590.060 tons of fresh fruit was produced with China, India, Brazil and the USA placed on the top. Turkey ranks 5th with a fruit production capacity of 23.6 million tons, accounting for 2.68% of the world's total fruit production (FAO, 2019).

Türkiye has an important place in fruit growing as it has vast and fertile agricultural land and different environmental conditions, and is the homeland of various fruits produced in the world (Agaoglu et al., 1997).

In the world and Türkiye have an important position fruit growing sector is faced with several biotic/abiotic factors that may lead to loss of productivity and quality in terms of plant production throughout the process from seed stage to post-harvest stage. It is known that ~36% of the world's plant production are lost due to plant diseases, pest and weeds. 60-75% of these losses was grouped to arise from fungal and bacterial diseases, 10-15% from viral diseases, and 10% from other pathogens and environmental factors (Agrios, 1997). *Rhizobium radiobacter* (*Agrobacterium tumefaciens*) (Smith and Townsend) Conn., which leads to crown gall, is the most important causal agent resulting in great production and economic losses (Lippincott et al., 1981). Known to have spread to ~77 countries, the causal agent ranks among the top ten bacteria of molecular plant pathology in the world (EPPO, 2019). It has been reported that ~140 species of dicotyledons in more than 100 plant families [fruit trees (almond, apple, peach, cherry, apricot, pear, plum, walnut etc.), berries (raspberries, blueberries etc.), grapes and various ornamental plants (chrysanthemums, roses, poplars etc.)] were susceptible to crown gall and incurred great economic loss (Moore and Canfield, 1996; Marti et al., 1999; Collins, 2001; Gupta et al., 2010 Lacroix and Citovsky, 2016). The disease is tumor formation in consequence of uncontrolled growth, division and overmultiplication of parenchyma cells in the section of the host plant where the bacteria are present. In addition to tumor formation, it may lead to symptoms such as dwarfing and giving small and chlorotic leaves. These symptoms ultimately cause the plants to dry and die (De Cleene and De Ley, 1976; Saygılı et al., 2008).

R. radiobacter is a rod-shaped, gram-negative, motile, soil bacterium of *Rhizobiaceae* family, which has an approximate length of 1,5-3 µm and a diameter of 0.4-0.8 µm as well as 2-4 peritrichous flagella, is often found individually or in short chains, grows at an optimum temperature of 25-28ºC and loses its virulence at 37ºC, and does not form spores (Romanenko and Perepnikhatka, 1984; Collins, 2001).

The distinctive feature of *R. radiobacter* from other bacteria is that it is a plant pathogenic bacteria with circular Ti plasmid (Tumor Inducing Plasmid), which is present in all virulent types, known as extrachromosomal structure, and can transfer oncogenic DNA segment to susceptible plant cells and induce tumor formation (Roy, 2015).

Ti plasmid consists of one or more T-DNA (Transfer DNA) region(s) integrating into the plant genome, a vir region, a replication center, a conjugative transfer region, and regions containing genes required for opine catabolism. T-DNA contains the region to be integrated into the plant genome. In order that the causal agent can infect a plant, it should enter through a scratch or lenticel on the plant (Penyalver et al., 2000). The causal agent penetrating through plant tissues modifies the genetic structure of the plant, activates auxin-cytokinin group hormones and opine synthesis, and cause uncontrolled cell multiplication and formation of tumors in the roots (Zambryski, 1998; Kerr, 1991).

R. radiobacter is generally characterized and defined with morphological, biochemical, pathogenicity, antibiotic susceptibility and molecular methods. In this study, isolates were aimed to characterized by pathogenicity test, hypersensitivity test, biochemical and physiological tests.

MATERIALS and METHODS

Potential Pathogen and Bioagent Bacterial Isolates

Five different *R. radiobacter* isolates, which were

isolated from apple (RK 473), apricot (2A, 2B) and peach (1A, 1B) rootstocks were used.

Potential Pathogenic Bacteria Isolates Virulence Tests and Hypersensitive Responses

Carrot slices (*Daucus carota* L.) and squash fruits (*Cucumis pepo*), tomato (*Solanum lycopersicum* L.) and sunflower (*Helianthus annus* L.) seedlings, kalanchoe (*Kalonchoe daigremontiana* Raym.-Hamet and H. Perrier) leaves, and GF677 (*Prunus persica* x *P. amygdalus*) peach and M9 and MM106 (*Malus domestica* L.) apple rootstocks were used to test the pathogenicity of five potential pathogenic bacteria isolates, and to identify the most virulent isolate among them.

Virulence Test on Carrot Slices

The application was made according to Ryder et al. (1985). Then, 100 µL of each bacterial inoculum prepared according to Eastwell et al. (2006) was inoculated on the surface of carrot slices. 9 carrot slices were used in total, by repeating the application 3 times for each bacterium and using 3 carrot slices for each repetition. Sterile water was used for control. Petri dishes were evaluated according to expansion on the surface of carrot slices at the end of 30 days (1. no tumor formation, 2. tumor formation slightly expanding on the surface, 3. tumor formation starting around conducting tissue, 4. apparent tumor formation around conducting tissue and slightly expanding on the surface, 5. apparent tumor formation around conducting tissue and expanding on the surface, 6. dense tumor formation covering the whole surface), and the most virulent isolate was identified by analyzing the results with JMP 5.0.1 statistical analysis program (Limanska et al., 2015).

Virulence Test in Squash Fruits

The application was made according to Tolba and Soliman (2013). 12 wounds were inflicted on squash fruits with the help of a sterile toothpick, and 10 µL of bacterial inoculum prepared according to Eastwell et al. (2006) was applied on each wound. The squash fruits were placed in sterile transparent boxes covered with blotting paper, and kept waiting at ambient temperature in 16 hours light/8 hours dark. Sterile water was used for control. The size and number of tumors formed within 10-15 days after the inoculation were evaluated. Evaluations were made according to the earliest tumor formation and tumor diameters.

Virulence Test on Kalanchoe Leaves

Leaves of ~10-20 cm from young kalanchoe plants were used, and pathogenic bacteria isolates grown in NA for 48 hours were inoculated along opposite veins of the leaves with the help of a toothpick. Sterile water was used for control. 3 leaves were used for each pathogenic bacteria isolates, and 2 wound were inflicted on each leaf. Evaluations were made according to the presence/absence of tumors (Minnemayer et al., 1991).

Virulence Test on Tomato and Sunflower Seedlings and Rootstocks

Under greenhouse conditions, ~5 week old tomato and sunflower seedlings and ~30 cm peach (GF677) and apple (M9 and MM106) rootstocks were injured (Jaeger, 1974), and according to Eastwell et al. (2006) 100 µL of pathogenic bacteria inoculum was inoculated. Sterile water was used for control, and 3 wounds were opened on each of the 5 plants for each application. In seedlings presence/absence of tumor 8 weeks after inoculation; A scale was prepared according to the size of the tumors formed in the wounds of 6 months after the application of the rootstocks. According to the scale; 1. no change in the wound, 2. the wound is width of 0.5-1 cm, 3. the wound is width of 1-2 cm, 4. the wound is width of 2-3 cm, and 5. the wound is width of 3-5 cm evaluations were made (Jaeger, 1974). In addition, the roots of the seedlings and rootstocks were applied by immersing them in the pathogen solution. In root applications, evaluations were made according to the presence/absence of tumors.

Hypersensitive Response Test

A suspension with a density of 10^8 kob ml^{-1} was prepared in sterile dH_2O with pathogenic bacteria isolates grown for ~48 hours in NA medium. Each of the suspensions prepared were inoculated on the area between two side veins of tobacco plant (*N. tabacum* var. *Samsun*) with the help of a syringe. After 24-48 hours, formation of a transparent appearance in the area of bacteria inoculation was considered positive, while sterile dH_2O was used for negative control (Lelliot and Stead, 1987).

Identification of Potentially Pathogenic Bacterial Isolates by Conventional Methods

In order to support the diagnosis of potential pathogen isolates (1A, 1B, 2A, 2B, RK 473) with classical methods, biochemical and physiological tests were performed. These tests; growth at 23 and 35°C, 3-ketolactose production from lactose, growth on medium containing 2% NaCl, potassium hydroxide (KOH), acid purification in PDA+$CaCO_3$ medium, ferric ammonium citrate usage, alkaline formation from malonic, mucic and tartaric acid, acid production from melezitose, erythritol and sucrose, citrate usage, litmus milk reaction (Moore et al., 2001), and oxidase test (Kovacs, 1956) were made.

RESULTS and DISCUSSION

Potential Pathogen Bacteria Isolates Virulence Tests and Hypersensitivity Reaction Test Results

Pathogenic isolates virulence test results are given in Table 1. All isolates were pathogenic in carrot slices and squash fruits. 1A, 1B, 2A and 2B isolates formed tumors in the stem of GF677 rootstock, while they did not form any tumors in the stems of M9 and MM106 rootstocks. 1B isolate caused tumors but other isolates no tumor formation in the root application of GF677. RK 473 was only pathogenic isolate in M9 and MM106 stem applications, while other isolates no tumor formation. All of isolates were not pathogenic in tomato and sunflower root and stem, kalanchoe leaf.

According to virulence test results, 1B isolate was found out to be the most virulent isolate (Figure 1).

Figure 1. Tumors on carrot slices, squash fruit and GF677 rootstock caused by 1B isolate
Şekil 1. *1B patojen izolatının havuç dilimleri, kabak meyvesi ve GF677 anacında oluşturduğu urlar*

Many species in *Rhizobium* genus have a large range of hosts. However, it is recently known that some strains display very high host specifity based on Ti plasmid. (Knauf et al., 1982). Moreover, Owens and Cress (1985) showed that in consequence of tests conducted on different genotypes of soybeans, different *Rhizobium isolates* might display variations in tumor formation. They indicated that this might vary according to environmental factors and hormonal conditions, and these factors might play a key role in tumor formation. Anderson and Moore (1979) used 11 different plants to test the virulence of different isolates of *Agrobacterium* species, and observed that each isolate was pathogenic in different hosts. They indicated that these differences might arise from host susceptibility, virulence degree or interaction between the two. Deng and Nester (1998) also indicated that *R. radiobacter* isolate, which has a large range of hosts, could not cause tumor formation in all plants.

Theoretically, tumor formation may not occur due to reasons such as weak bacterial growth, bacteria's inability to survive at the scratch, lack of vir gene inducers or receptors required for bacterial connection in plants, or defective vir genes in T-DNA transfer. Moreover, it is known that tumor formation may not be induced due to failed T-DNA integration into the plant, absence of a full T-DNA gene set, or weak or excessive expression of T-DNA genes (Deng and Nester, 1998). In consequence of pathogenicity tests conducted in carrot slices, squash fruits, kalanchoe leaves, tomato and sunflower seedlings, and rootstocks in order to identify the most virulent among five different *R. radiobacter* isolates, which is the first objective of the study, it was observed that the isolates did not cause tumor formation in all test plants; in other words, there were differences in the pathogenicity of isolates (Table 1).The plants we used in pathogenicity tests are commonly used in *R. radiobacter* pathogenicity tests (Burr et al., 1995). In pathogenicity tests conducted for isolates, tumor formation was observed in some test plants and not observed in others, which showed that there were differences among hosts in terms of their pathogenicity. It was found out in the literature research that there were no rules indicating that an isolate might become pathogenic in all plants, and that there might be differences, as in the situations explained above. Among the plants used in pathogenicity tests, squash fruits (15 days) gave the clearest and quickest results, followed by carrot slices (30 days). Therefore, it was concluded that the use of squash fruits and carrot slices in pathogenicity tests were convenient (Figure 1). In the pathogenicity tests on 138 isolates of *Agrobacterium* species obtained from tissues with tumor, Yuzbasıoglu (2014) observed that some isolates did not become pathogenic in any plants except the one they were isolated from, some isolates became pathogenic in all indicator plants, and the

-Table 1. Virulence test results of potential pathogen isolates

Çizelge 2. Potansiyel patojen izolatlarının virülanslık test sonuçları

	Potantial Pathogen Bacteria Isolates Virulence Test Results (Potansiyel Patojen İzolatlarının Virülanslık Test Sonuçları)													
Test Plants *(Test Bitkileri)*	***RK473***		***1A***		***1B***		***2A***		***2B***		***Control***		**CV**	**LSD**
Carrot slices (*Havuç dilimleri*)	3.67±0.43	C	4.67±0.29	AB	4.89±0.24	A	4.00±0.48	C	4.22±0.29	BC	0.00±0.0	D	0.18	0.60
Squash fruits (Kabak meyveleri)	0.33±0.06	D	2.25±0.06	C	6.75±0.18	A	3.75±0.21	B	1.00±0.18	D	0.00±0.0	D	0.59	1.13
GF677 stem application (*GF677 gövde uygulama*)	0.00±0.0	D	1.86±0.72	B	2.73±1.29	A	1.33±0.47	AB	1.00±0.44	C	0.00±0.0	D	0.70	1.72
M9 stem application (*M9 gövde uygulama*)	2.47±1.02	A	1.00±0.0	B	1.00±0.0	B	1.00±0.0	B	1.00±0.0	B	1.00±0.0	B	0.34	0.31
MM106 stem application (*M9 gövde uygulama*)	2.39±0.96	A	1.00±0.0	B	1.00±0.0	B	1.00±0.0	B	1.00±0.0	B	1.00±0.0	B	0.41	0.36
GF677 root application (*GF677 Kök uygulama*)	-		-		+		-		-		-			
M9 root application (*M9 kök uygulama*)	-		-		-		-		-		-			
MM106 root application (*MM106 kök uygulama*)	-		-		-		-		-		-			
Sunflower seedling root and stem application (*Ayçiçeği fidesi kök ve gövde uygulama*)	-		-		-		-		-		-			
Tomato seedling root and stem application (*Domates fidesi kök ve gövde uygulama*)	-		-		-		-		-		-			
Kalonche leaves application (*Kalonşe yaprak uygulama*)	-		-		-		-		-		-			
Hypersensitive response test (*Aşırı duyarlılık reaksiyon test*)	+		+		+		+		+		+			

*****There is no statistically significant difference between the values expressed with similar letters on the same line ($p<0.01$), **+:** positive, **-:** negative

remaining isolates became pathogenic in one or two indicator plants. These results also support the conclusion that the pathogenic isolates used in the the study might not become pathogenic in all plants. Moreover, the necessity of using multiple plants instead of a single indicator plant in pathogenicity studies of *R. radiobacter* isolates was revealed.

Isolates (1A, 1B, 2A, 2B, RK 473) hypersensitive response test results are given in Figure 2. All isolates were showed slight yellowing and typical chlorosis after 48 hours. Kado and Crosa (1994) indicated that pathogenic R. radiobacter isolates mostly do not cause hypersensitive response, and only cause slight chlorosis in filtration areas.

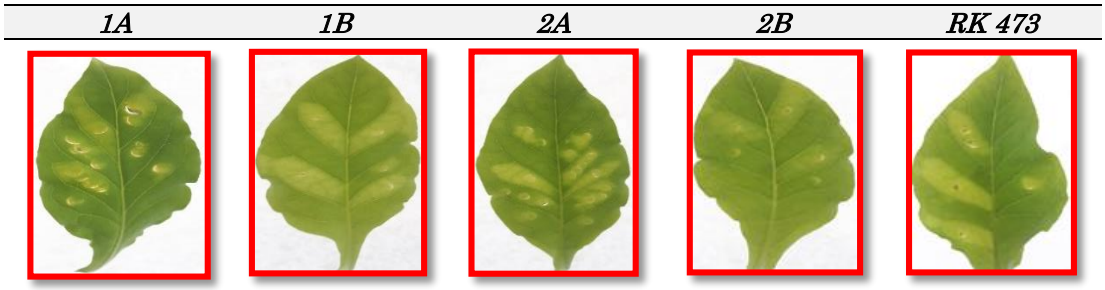

Figure 2. Hypersensitive reaction test result of potential pathogenic bacterial isolates in tobacco leaves
Şekil 2. *Potansiyel patojen bakteri izolatlarının tütün bitkisinde oluşturduğu aşırı duyarlılık reaksiyon test sonucu*

Potential Pathogen Bacterial Isolates Identification by Conventional Methods Results

Biochemical and physiological features have been the most useful features in the classification of tumorigenic *Rhizobium* species (Moore et al., 2001). Biochemical and physiological test results are given in Table 2.

According to biochemical and physiological test results, there are differences between *R. radiobacter* isolates.

It was found out that the isolates gave similar results growth in different temperature values (23 and 35°C), growth in medium containing 2% NaCl, KOH, acid purification in PDA+$CaCO_3$ medium, acid production from erythritol, litmus milk reaction, and oxidase tests (Table 2).

It was found out that they showed different reactions in the biochemical tests for 3-ketolactose production from lactose, ferric ammonium citrate usage, alkaline formation from malonic, mucic acid and tartaric acid, citrate usage, and acid production from melezitose and sucrose (Table 2).

While the test results for 3-ketolactose production from lactose were found out to be positive in relevant literature, test results for four of the isolates (1A, 1B, 2A ve 2B) used were found out to be negative (Table 2). The researchers also stated in their studies that 3-ketolactose production test might be negative (Lippincott and Lippincott, 1969; Kerr, 1969).

According to ferric ammonium citrate test usage, formation of a brick-colored layer on the tube was considered positive, and only RK 473 test result positive. (Table 2). Moore et al. (2001) said that isolates have might be differences at the level of 80%. In malonic and mucic acid tests, only RK 473 gave negative result and it was similar in literatüre research (Moore et al., 2001). In tartaric acid test, 1B and 2A isolates showed positive results while 1A, 2B and RK 473 gave negative results (Table 2).

RK 473 produced acid from melezitose and it was similar with Moore et al., (2001). All isolates except 2B produced acid from sucrose, this result was similar Moore et al. (2001) (Table 2).

According to citrate usage test results, only RK 473 isolate showed negative reaction (Table 2). Moore et al. (2001) said that *R. radiobacter* isolates may give different results.

Pulawska (2010) indicated that these differences were common among *Rhizobium* isolates forming tumors or fibrous roots. Similarly, Canik (2013) revealed that *R. vitis* isolates from vines showed different results in most of the biochemical and physiological tests.

Puławska et al. (2016) pointed out in the relevant study that the tests might give different results for strains belonging to the same species, and in some cases, same results for strains belonging to different taxons. As Pulawska explained, most important reason is that the bacterium generally uses different carbon sources in its metabolism (opine metabolism). Therefore, it was found out that a single definition system could not be sufficient for bacteria causing crown gall *R. radiobacter* (Puławska et al., 2016).

CONCLUSION and RECOMMENDATIONS

The study demonstrated that there may be differences the isolates which cause crown gall. It is important to make molecular identification in order to support their diagnosis with classical methods.

Table 1. Potential pathogen bacterial isolates biochemical and physiological test results
Çizelge 2. *Potansiyel patojen bakteri izolatlarının biyokimyasal ve fizyolojik test sonuçları*

Tests	***1A***	***1B***	***2A***	***2B***	***RK 473***
Growth on 35°C (*35°C gelişme*)	+	+	+	+	+
Growth on 23°C (*23°C gelişme*)	+	+	+	+	+
3-ketolactose production (*Laktozdan 3-ketolaktoz üretimi*)	-	-	-	-	+
Growth on medium 2% NaCl *(%2 NaCl besiyerinde gelişme*)	+	+	+	+	+
KOH test (*KOH testi*)	+	+	+	+	+
PDA+$CaCO_3$ acid purification (*PDA+$CaCO_3$ asit temizleme*)	-	-	-	-	-
Ferric ammonium citrate (*Demir amonyum sitrat kullanımı*)	-	-	-	-	+
Alkaline formation from malonic (*Malonik asitten alkali oluşturma*)	+	+	+	+	-
Alkaline formation from mucic (*Mucic asitten alkali oluşturma*)	+	+	+	+	-
Alkaline formation from tartaric (*Tartarik asitten alkali oluşturma*)	-	+	+	-	-
Acid production from melezitose (*Melezitozdan asit üretimi*)	-	-	-	-	+
Acid production from erythritol (*Eritritolden asit üretimi*)	-	-	-	-	-
Acid production from sucrose (*Sükrozdan asit üretimi*)	+	+	+	-	+
Citrate usage (*Sitrat kullanımı*)	+	+	+	+	-
Litmus milk reaction (*Litmus milk'te reaksiyon*)	Alk	Alk	Alk	Alk	Alk
Oxidase test (*Oksidaz testi*)	+	+	+	+	+

+: 80% and above positive, **-:** 80% and above negative, **V:** 21-79% between pozitive, **Alk:** alkaline

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Author's Contributions

The contribution of the authors is equal.

Statement of Conflict of Interest and Author's Contributions

Authors have declared no conflict of interest.

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Determination of Entomopathogenic Nematode Persistency with Surface Spraying and Soil Injection Applications in a Peach Orchard

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ABSTRACT

Entomopathogenic nematodes (EPN) are effective biological control agents against underground and cryptic pests. Persistency and survival of EPN in soil after soil application is important for long term success of management programs. In this study, it was aimed to determine the soil persistency of 4 native EPN species after surface spraying and soil injection applications in a peach orchard. In the study, *S. feltiae* (96), *S. carpocapsae* 1133, *H. bacteriophora* 1144 and *S. affine* 47 species were applied in 30 l water with 140.000 IJ/tree dose per tree by surface spraying and soil injection methods. EPN were applied to soil with a watering can in surface spraying method and with a pulverizator into 5-15 cm depth in soil injection method. After the monthly application, soil samples were collected and EPN presence was tested with *G. mellonella* larvae and White traps in the laboratory. The study was conducted for 2 times in 2018 and 2019. At the end of the study, EPN persistency in soil was found to be 90 days in 2018 and 150 days in 2019.

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Spreyleme ve Toprak Enjeksiyonu Yöntemleri ile Toprağa Uygulanan Entomopatojen Nematodların Şeftali Bahçesinde Kalıcılığının Belirlenmesi

ÖZET

Entomopatojen nematodlar (EPN'ler), toprak ve korunaklı habitatlarda yaşayan zararlılara karşı etkili biyolojik mücadele etmenleridir. EPN'lerin toprağa uygulanmasından sonra topraktaki kalıcılıkları ve yaşamlarını sürdürebilmeleri mücadele çalışmalarında uzun süreli başarı için önemlidir. Bu çalışmada 4 yerel EPN türünün spreyleme sulama ve toprak enjeksiyonu yöntemleri ile şeftali bahçesinde uygulanmasından sonra topraktaki kalıcılık sürelerinin belirlenmesi amaçlanmıştır. Çalışmada, *Steinernema feltiae* 96, *S. carpocapsae* 1133, *Heterorhabditis bacteriophora* 1144 ve *S. affine* 47 türleri ağaç başına 30 L su içinde 140.000 IJ/ağaç dozunda spreyleme ve toprak enjeksiyonu yöntemleri ile uygulanmıştır. Spreyleme yönteminde, EPN'ler sulama bidonu ile toprak yüzeyine uygulanırken, toprak enjeksiyonu yönteminde bir pülverizatör ile toprağın 5-15 cm derinliğine uygulanmıştır. Uygulamadan sonra aylık olarak toprak örnekleri alınmış ve laboratuvarda EPN varlığı *G. mellonella* tuzağı yöntemiyle test edilmiştir. Çalışma 2018 ve 2019 yıllarında 2 tekrarlı olarak gerçekleştirilmiştir. Araştırma sonucunda 2018 yılında EPN'lerin toprakta kalıcılıklarının 90 gün, 2019 yılında ise 150 gün sürdüğü belirlenmiştir.

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INTRODUCTION

Entomopathogenic nematodes (EPN) are important biological control agents that can be used to control many agricultural pests. They are especially important as an alternative to chemical control of pests in isolated places like soil or cryptic habitats. Also they can be applied just like insecticides and there are many studies to develop successful EPN preparations (Caamano et al., 2008).

Even though there are approximately 40 nematode families that are associated with insects, only Heterorhabditidae and Steinernematidae families are suitable to use in biological control applications (Gaugler and Kaya, 1990).

When we look at the number of EPN species identified, according to Bhat et al. (2020) from the first identified EPN *Steinernema kraussei* (Steiner, 1923) to *S. riojaense* (Puza et al., 2020), there are 100 steinernematid species. In *Heterorhabditis* genus, after the identification of *Heterorhabditis bacteriophora* (Poinar, 1976) to *H. noenieputensis* (Malan et al., 2014) a total of 16 species were identified.

First heterorhabditid species in Turkey was *H. bacteriophora* from *Aelia rostrata* (Hemiptera: Pentatomidae) by Kepenekçi et al (1999). Other EPN species identified in Turkey are *S. carpocapsae*, *S. feltiae*, *S. affine*, *S. websteri*, *S. anatoliense*, *S. weiseri*, *S. bicornutum*, *S. kraussei, H. marelatus* and *H. megidis* (Kepenekçi and Susurluk, 2000; Kepenekçi, 2002; Hazır et al., 2003; Ünlü et al., 2007; Yılmaz et al., 2009; Ertürk et al., 2014; Gökçe et al., 2015; Canhilal et al., 2016; 2017).

Entomopathogenic nematodes are dependent on their symbiotic bacteria to survive and reproduce. Bacteria not only help the EPN to kill their host, but they also help by killing competing organisms, maintaining a suitable environment for EPN survival, making nutrients from host body digestible for EPN and by directly being food for EPN. In return, bacteria live inside the nematode's body and are protected from environmental elements, also they use the suppression of the host's defenses by EPN to their advantage for reproduction (Akhurst and Boemare, 1990; Forst and Clarke, 2002; Hazır et al., 2003; Stock and Goodrich-Blair, 2008).

EPN enter the host from natural orifices or from wounds on the skin. Also, Heterorhabdits can penetrate the host's skin where it is thinner like the skin between segments by using their dorsal tooth (Bedding and Molyneux, 1982). Bacteria released from EPN guts into the insect haemocoel kill the host insect with septicemia in 24-48 hours. After 2-3 generations of reproduction in the host body, IJ that are more resistant to environmental conditions are generated with the decline of nutrients in the host body. These IJs emerge from the host cadaver and search for new hosts to infect (Kaya and Gaugler, 1993; Koppenhöfer and Gaugler, 2009).

In this study, we aimed to determine the soil persistency of four native EPN species of Turkey, *Steinernema carpocapsae*; *S. feltiae*; *S. affine* (Rhabditida: Steinernematidae), and *H. bacteriophora* (Rhabditida: Heterorhabditidae) in a peach orchard after surface spraying and soil injection application methods.

MATERIALS and METHODS

The study was conducted in an 8-year-old peach orchard (*Prunus persica* L.) (Rosaceae) (40°23'39 " N, 26°44'48 " E, 8 m) in Lapseki district in Çanakkale province. The orchard consist of Black Hale, Royal Glory and Abdos cultivars. Before choosing the orchard for the study soil samples were taken to eliminate the chance of naturally occurring EPN species in the area (Bedding and Akhurst 1975; Griffin et al. 2000).

Production of Entomopathogenic nematodes

Entomopathogenic nematodes were produced in vivo on mature *G. mellonella* larvae in the laboratory. The species used in the experiment were *Steinernema feltiae* 96 (Bursa), *S. carpocapsae* 1133 (Sakarya), *H. bacteriophora* 1144 (Sakarya) and *S. affine* 47 (İstanbul), which were prepared in the dose of 140.000 IJ/tree in a 50 cc falcon tubes in distilled water before the experiment. Then they were transferred to the experiment orchard in an ice box. The experiments were designed with 3 replications in randomized block design, with the blocks consisting of tree lines. The study was conducted for two years in 2018 and 2019.

Soil Injection Application

In soil injection application, EPN were applied with a 15 L capacity manual pulverizator directly into 5-15 cm depth of soil (Figure 1a, b) by penetrating the soil surface with the tip of the pulverizator. Before the application in the orchard, the ability of EPN to pass through the nozzle of the pulverizator was tested in the laboratory and the EPN were confirmed to be alive under the microscope. Using this pulverizator, 140.000 IJ/tree dose was applied with 30 l of water into the soil around each tree.

Surface Application with Watering Can

In surface application, EPN were applied to the soil with a watering can. Before the application the ability of EPN to pass through the holes of the watering can was tested in the laboratory and the EPN were confirmed to be alive under the microscope. The watering can had a capacity of 10 l and 140.000 IJ/tree dose was applied with 30 l of water per each tree (Figure 1c, d).

Determination of EPN Persistency in Soil in Natural Conditions

Soil sampling was conducted monthly around the trees to determine the persistency of EPN in soil. Soil samples were collected from 5-30 depth where EPN were applied around the trees (Stock et al., 1999). Soil samples were placed into polyethylene bags and transferred to the laboratory in an ice box (Kaya and Stock, 1997). After thoroughly mixing the samples, they were placed into 500 ml volume plastic boxes and 6-8 *G. mellonella* larvae were also put into the soil in petri dishes with wire mesh lids (Bedding and Akhurst, 1975). After four days, larvae were checked to collect the dead ones and the cadavers were placed on White traps. These cadavers were checked daily to observe EPN emergence. Soil sampling in the experimental orchard was continued until EPN persistence in soil has ended.

Şekil 1. Toprak Enjeksiyonu (a, b), Yüzey Sulama Uygulaması (c, d)
Figure 1. Soil injection (a, b), Surface Spraying Application (c, d)

RESULTS and DISCUSSION

In 2018, first EPN application was done on 18th of September. On this date, soil and air temperature were recorded as 21.2°C and 22°C, respectively. EPN persistency in soil after the application was determined as 3 months. Last EPN isolated from the soil samples were on 17th of December. The soil and air temperature on this date were recorded as 10.3°C and 9.7°C, respectively. With this data in mind, it is thought that the number of EPN declined after the 1st of January with soil temperatures falling below 7.9°C. From the soil sample of 17th of January, no living EPN has emerged.

In 2019, the second year of the study, first EPN application was conducted on 24th of July. On this date, soil and air temperature were recorded as

23.9°C and 23.8°C, respectively. Applications have started 60 days before the previous year. Soil persistency of EPN was found to be 150 days. Last EPN isolation from the soil samples was on 27th of December and the soil and air temperature on this date was recorded as 6.5°C and 9.9°C, respectively.

According to several studies, *Steinernema feltiae* is species that is adapted to colder climates and can infect its host between 8-28°C temperatures, while it can reproduce between 8-25°C temperatures (Hazır et al., 2001; Grewal et al., 1996; Umana, 2014). According to Griffin (1993) and Grewal et al. (1994), many EPN species cannot survive temperatures under 8°C. Similar to these studies, in this results confirm that EPN presence and persistency in soil is closely related to climatic conditions. Insects from different orders (Diptera, Orthoptera, Coleoptera) were observed in soil during soil samplings, which may be used as hosts by EPN. This host presence is also known to be closely linked to EPN persistency and survival in soil.

When we look at some studies about EPN persistency in natural conditions, Martinez de Altube et al., (2008) reported that *S. carpocapsae* can live up to 170 days in soil. In another study, Guo et al. (2013) have determined a 70-day persistency from *S. carpocapsae* and *H. bacteriophora*. Under suitable conditions, IJs of *H. bacteriophora* were active for 22 months in soil (Susurluk and Ehlers, 2008). However, Morton and Garcia Del Pino (2008) have reported a much shorter period of persistency, such as 2 weeks on soil surface and 6 weeks in 14-20 cm depth for *H. bacteriophora*. In addition, some studies have reported higher infectivity and persistency in soil from EPN produced in vivo than EPN produced in vitro (Perez et al., 2003; Shapiro-Ilan et al., 2003). Thus, we think that the reason for high EPN persistency in this study may be because of they are produced in vivo.

Ishibashi and Kondo (1986) have examined the persistency of *S. feltiae* and *S. glaseri* in sandy soil and bark compost. At the end of the study, they have determined that the longest persistency was in sterilized soil and compost with 8 weeks. According to their results, the longer persistency in sterilized material was caused by the competition between EPN and other microorganisms in unsterilized materials. Same researchers have also found the persistency of *S. feltiae* in soil as 14 weeks (Ishibashi and Kondo, 1987). In the same study they also reported that EPN infectivity is higher in sandy soil than clay soil, but persistency in clay soil is higher than sandy soil because of the higher water holding capacity of the former. Thus, we think that the high persistency of EPN in the experimental orchard may also be caused by the high-water holding capacity of the soil's loamy clay texture.

CONCLUSIONS

It is predicted that EPN will increase their share on the market for biological control agents because of their high adaptation, effectiveness against multiple hosts, fast host finding ability, mass production in artificial mediums, high reproduction capacity and high potential for the control of agricultural pests, in addition to being harmless to non-target organisms. Thus, it is important to increase the number of studies on EPN to devise better application programs.

In this study, we determined the soil persistency of four native EPN species as 90 days in 2018 and 150 days in 2019. EPN reisolated from the soil samples were tested for their infectivity and found to be still infective.

This study is one of the few studies in Turkey to determine the persistency and reestablishment of native EPN species into the soil. We think that it is important to focus on adaptation to different ecological conditions and increasing the effectiveness of EPN against pests.

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Declaration of researcher contribution

We declare that all researchers have equal amount of contribution

Declaration of Conflict of interest

We declare no conflict of interest

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Kiraz ve Nektarin Bahçelerinde Kiraz Sirkesineği, *Drosophila suzukii*' nin Matsumura 1931 (Diptera: Drosophilidae) Ergin Popülasyon Değişimi ve Zarar Oranının Belirlenmesi

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ÖZET

Drosophila suzukii Matsumura 1931 (Diptera: Drosophilidae) meyvelerde ekonomik anlamda zararlara yol açan istilacı bir türdür. Bu çalışmada *D.suzukii*'nin Adana ve Mersin illerinde nektarin ve kiraz bahçelerinde ergin popülasyon değişimi ve zarar oranı belirlenmiş, ayrıca tercih ettiği meyve olgunluk dönemleri tespit edilmiştir. Adana ve Mersin illerinde 2017-2018 yıllarında, dörder adet nektarin ve kiraz bahçesinde yürütülen çalışmada elma sirkesi ile hazırlanan tuzaklar kullanılmıştır. Çalışma sonucunda *D. suzukii* popülasyonunun ilkbaharda meyvelere ben düşme (sarı olum) olarak nitelendirilen meyvelerin renk değiştirerek şekerlenmeye başladığı dönem ile hasat dönemi arasında ve sonbahar aylarında arttığı tespit edilmiştir. Zararlı erginlerinin yumurta bırakmak için meyvelerin pembe-kırmızı ve koyu kırmızı olgunluk dönemini tercih ettiği belirlenmiş, bununla birlikte zararın da bu dönemde meydana gelidiği tespit edilmiştir. Erkenci nektarin çeşitlerinde herhangi bir zarar tespit edilmezken, kirazlarda ilk yıl % 2 ve ikinci yıl % 62 oranında zarar tespit edilmiştir.

Bitki Koruma

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Anahtar Kelimeler
Drosophila suzukii
Popülasyon takibi
Zarar oranı
Nektarin
Kiraz

Determination of Adult Population Fluctuation and Damege Rate of *Drosophila suzukii* Matsumura 1931 (Diptera: Drosophilidae) in Cherry and Nectarine Orchards

ABSTRACT

Drosophila suzukii Matsumura 1931 (Diptera: Drosophilidae) is an invasive species causing significant economic damage in fruits. In this study, the change of adult population and damage rate as well as the preference of fruit maturity periods in the nectarine and cherry orchards were determined. Prepared apple cider vinegar traps were used in four nectarine and four cherry orchards in Adana and Mersin provinces between 2017-2018. As a result of the study, it was found that the *D. suzukii* population increased between the period when the fruits, which were described as becoming juicy (yellow maturation) in the spring, began to sugar by changing color, and the harvest period and in autumn months. It was also observed that the pest adults preferred the pink-red and dark red maturity period of the fruits to lay eggs and with this, the damage also occurred during this period. While no damage was detected in early nectarine varieties, 2% damage was detected in the first year and 62% in the second year in cherries.

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GİRİŞ

Dünyada istilacı bir tür olarak tanınan Kiraz sirkesineği, *Drosophila suzukii* Matsumara 1931 (Diptera: Drosophilidae) toleransı sıfır bir karantina etmenidir (Eppo 2021a). Asya orjinli bir zararlı olan *D. suzukii*, ilk olarak 1916 yılında, Japonya'da tespit edilmiştir (Kanzawa,1939). Daha sonra Çin, Kore, Amerika Birleşik Devletleri ve İspanya'da varlığı

ortaya çıkarılmıştır (Kanzawa, 1936; Guo, 2007; Grassi ve ark., 2009, Walsh ve ark. 2011,; Calabria ve ark., 2012; Asplen ve ark., 2015). Polifag bir tür olan bu zararlı çilek, ahududu, böğürtlen, kiraz, erik, şeftali, nektarin, kaysı, elma, armut, yaban mersini ve üzüm olmak üzere geniş bir konukçu potansiyeline sahiptir (Kanzawa, 1939; Rossi Stacconi ve ark., 2015; Eppo, 2021b). *Drosophila suzukii*'nin ekonomik anlamda en önemli zararı kiraz ile üzümsü meyvelerde görülmektedir (Kanzawa, 1939; Kawase ve ark., 2007; Lee ve ark., 2011; Goodhue ve ark., 2011; Walsh ve ark., 2011; Bellamy ve ark., 2013; Cini ve ark., 2012; Asplen ve ark., 2015; Wiman ve ark., 2016). Özellikle Amerika Birleşik Devletleri'nin batı eyaletlerinde yaklaşık olarak her yıl 500 milyon dolar, doğusunda ise 207 milyon doların üzerinde ekonomik kayıplara neden olmaktadır (Wiman ve ark., 2016). Fransa ve İtalya'da kiraz, çilek, ahududu, böğürtlen, yabanmersini elma ve şeftali gibi ürünlerde özellikle %100'e varan oranda zarar yaptığı belirtilmiştir (Cini ve ark., 2012; Weydert ve Mandrin, 2013). Türkiye'de ilk olarak 2014 yılında Erzurum'da çilekte saptanmış (Lengyel ve ark., 2015; Orhan ve ark., 2016), daha sonra Karaman, Adana, Bolu, Çanakkale gibi bir çok yerde tespit edilmiştir (Öğür ve ark., 2018, Efil 2018, Arıdıcı-Kara ve Ulusoy, 2019, Kaçar ve Koca 2020). *Drosophila suzukii* yüksek üreme potansiyeline sahip olduğundan hızlı bir şekilde popülasyonu artmakta ve ortam koşullarına bağlı olarak yılda yaklaşık 13 döl verebilmektedir (Kanzawa, 1939; Wiman ve ark., 2016).

Drosophila suzukii dişileri testere benzeri ovipozitör yardımı ile yumurtalarını, olgunlaşmış sağlıklı meyvelerin içine bırakırlar. Yumurtadan çıkan larvalar meyve kabuğunun altında meyvenin etli ve sulu kısmında beslenerek meyveye esas zararı vermektedir. Gelişmesini tamamlayan larva çoğunlukla meyvede pupa dönemine geçer. Zarar gören meyvelerde yumuşama ve çökme görülerek, etrafa ağır bir sirke kokusu yayılır. Diğer saprofit türlerinde meyvelerde beslenmesi ile birlikte meyveler hasat edilemez duruma gelir (Rossi Stacconi ve ark. 2015).

Kısa sürede hızlı bir şekilde çoğalabilen ve karantina etmeni olan *D. suzukii*'nin popülasyonunu etkileyen kriterlerin başında yüksek nem ve sıcaklık gelmektedir (Wiman ve ark., 2016). Türkiye'de meyve yetiştiriciliği yönünden önemli bir paya sahip olan Doğu Akdeniz Bölgesi, iklim özellikleri ve ürün zenginliği açısından *D. suzukii*'nin yıl boyunca zarar yapmasına ve popülasyon yoğunluğunda önemli artışa olanak sunmaktadır. Bu çalışma ile meyve ihracatı için büyük bir tehdit oluşturan *D. suzukii*'nin zarar oranı, tercih ettiği meyve olgunluk dönemleri ile ergin popülasyon değişimi belirlenerek, zararlının mücadelesinde kullanılabilecek bazı önemli özellikler belirlenmeye çalışılmıştır.

MATERYAL ve METOD

Drosophila suzukii Matsumara'nin Ergin Popülasyon Değişiminin Belirlenmesi

Çalışma, 2017- 2018 yıllarında Adana (Seyhan Yalmanlı ve Dervişler Mahahallelerinde 13 ve 14 da, Saimbeyli ilçesi Kalesekisi Mahallesinde 11 ve 25 da alana sahip bahçeler) ve Mersin'de (Toroslar ilçesi Çopurlu ve Karaisalı Mahallelerinde12 ve 10 da, Atlılar Mahallesinde 25 ve 5 da alana sahip bahçeler), iki'şer adet nektarin (Laura çeşidi) ve kiraz bahçesi (Ziraat 0900 çeşidi) olmak üzere toplam sekiz adet bahçede yürütülmüştür (Çizelge 1). Çalışmada elma sirkesinden hazırlanan tuzaklar kullanılmıştır.

Bu amaçla, 500 ml'lik pet şişelerin ağız kısmına yakın 3 mm çapında 8-10 adet delik açılmış ve içerisine 250 ml elma sirkesi konularak oluşturulan tuzaklar kullanılmıştır. Tuzaklardan, her bahçeye 4'er adet olacak şekilde ağaçların kuzey yönüne, yerden yaklaşık 1,5 m yüksekliğe ve ağacın dışa bakan dallarına asılmıştır (Wang ve ark., 2016). Tuzaklar kiraz (12 nisan 2017) ve nektarinde (20 Şubat 2017) çiçeklenme döneminde asılmış ilkbahar, yaz ve sonbahar aylarında haftada bir, kışın ise iki haftada bir yenisi ile değiştirilerek yıl boyunca bahçelerde bırakılmıştır. Deneme bahçelerinden toplanan tuzaklar laboratuvara getirilmiş ve sirke süzüldükten sonra yakalanan *D.suzukii* erginleri stereobinoküler mikroskop altında erkek / dişi ayrımı yapılarak sayılmıştır (Wang ve ark., 2016). Çalışmanın yürütüldüğü bahçelere ait meteorolojik veriler hobo cihazı ile günlük olarak kaydedilmiştir. Bahçelerin özellikleri ve nektarin bahçelerinde thrips, meyvekurtları ile kiraz bahçelerinde Kiraz sineği ve *D.suzukii*' ye karşıyapılan insektisit uygulamaları Çizelge 1'de verilmiştir. Deneme bahçelerinde üreticiler tarafından yapılan uygulamalara müdehale edilmemiştir.

Drosophila suzukii Matsumara'nin Zarar Oranı ve Tercih Ettiği Meyve Olgunluk Dönemlerinin Belirlenmesi

Drosophila suzukii ergin popülasyonunun izlendiği Adana ve Mersin illeri nektarin ve kiraz bahçelerinde, hasat öncesinde kurtlu meyve oranını tespit etmek amacıyla, deneme parselini temsil edecek şekilde rastgele seçilen 25 ağacın 4 yönünden nektarinde birer ve kirazlarda ikişer adet olmak üzere nektarinlerde 4 (toplam 100 adet meyve) ve kirazda ise 8 adet meyve (toplam 200 adet meyve) gözle ve lup yardımı ile kontrol edilmiştir. Ayrıca kirazlardan sağlam görünen meyvelerden toplanarak laboratuvarda bir gün süreyle %10'luk tuz eriyiği içerisinde bekletilmiş ve larva çıkışına göre zarar görüp görmedikleri tespit edilmeye çalışılmıştır (Timmeren ve ark. 2017).

Çizelge 1. Denemenin yürütüldüğü bahçelerin özellikleri
Table 1. Features of fruit orchards where the trial was conducted

Bahçe	Meyve	Alan (da)	İnsektisit uygulaması
Adana Seyhan Yalmanlı (1. bahçe)	Nektarin Laura	13	1. yıl 01.03.17 Spinetoram 10.04.17Cypermethrin + Spinetoram 01.05.17 Cypermethrin 2. yıl 18.02.18 Spinetoram 07.03.18 Spinetoram 28.04.18 Cypermethrin +Spinetoram 01.05.18 Cypermethrin
Adana Seyhan Dervişler (2. bahçe)	Nektarin Laura	14	1. yıl 01.02.17 Spinetoram%25 WG 10.03.17 Spinosad+ Spinetoram 01.04.17 Spinosad 15.04.17 Spinosad 2.yıl 18.02.18 Spinetoram %25 WG 07.03.18 Spinetoram %25 WG 28.03.18 Spinosad + Spinetoram 01.04.18 Spinosad
Adana Saimbeyli Kalesekisi (1. bahçe)	Kiraz Ziraat 0900	11	*1.yıl 05.05.17 Deltamethrin 30.05.17 Cypermethrin, 05.06.17 Cypermethrin, 10.06.17 Malathion 16.06.17 Malathion 2.yıl 2.05.18 Deltamethrin 18.05.18 Deltamethrin 30.05.18 Thiacloprid 15.05.18 Thiacloprid
Adana Saimbeyli Kalesekisi (2. bahçe)	Kiraz Ziraat 0900	25	
Mersin Toroslar Çopurlu (1. bahçe)	Nektarin Laura	12	*1. yıl 24.02.17 Deltamethrin 25.03.17 Thiacloprid 20.04.17 Thiacloprid 28.04.17 Thiacloprid 20.05.17 Deltamethrin +Malathion 2.yıl 24.02.18 Deltamethrin 22.03.18 Thiacloprid 20.04.18 Thiacloprid 1.05.18 Deltamethrin +Malathion
Mersin Toroslar Karaisalı (2. bahçe)	Nektarin Laura	10	
Mersin Toroslar Atlılar (1. bahçe)	Kiraz Ziraat 0900	25	*1. yıl 24.02.17 Deltamethrin 22.03.17 Thiacloprid 20.04.17 Thiacloprid 28.04.17 Thiacloprid 05.05.17 Thiacloprid 20.05.17 Deltamethrin +Malathion 20.07.17 Thiacloprid 2.yıl 02.05.18 Deltamethrin 18.05.18 Deltamethrin 30.05.18 Thiacloprid 15.06.18 Thiacloprid
Mersin Toroslar Atlılar (2. bahçe)	Kiraz Ziraat 0900	5	

* Aynı üreticilere ait bahçelerde aynı uygulama yapılmıştır.

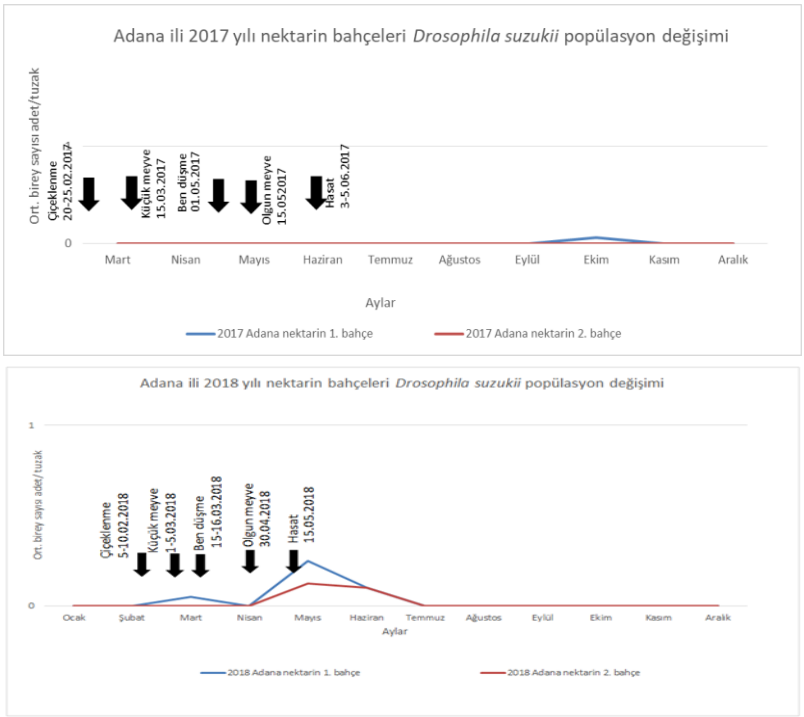
Kontroller kiraz meyvelerinin farklı fenolojik dönemleri olan yeşil - sarı - pembe kırmızı ve koyu kırmızı olgunluk dönemlerinde yapılarak yumurta koymak için hangi meyve dönemini tercih ettiği saptanmaya çalışılmıştır. Bahçede yapılan meyve kontrollerinde vuruklu olduğundan şüphelenilen meyveler kese kâğıdına konularak laboratuvara getirilmiş burada meyvelerde vuruk sayısı, larva sayısı belirlenerek, kültüre alınmıştır. Bu şekilde meyve zarar oranı tespit edilmeye çalışılmıştır.

BULGULAR ve TARTIŞMA

Drosophila suzukii Matsumara'nin Nektarin Bahçelerinde Ergin Popülasyon Değişimi

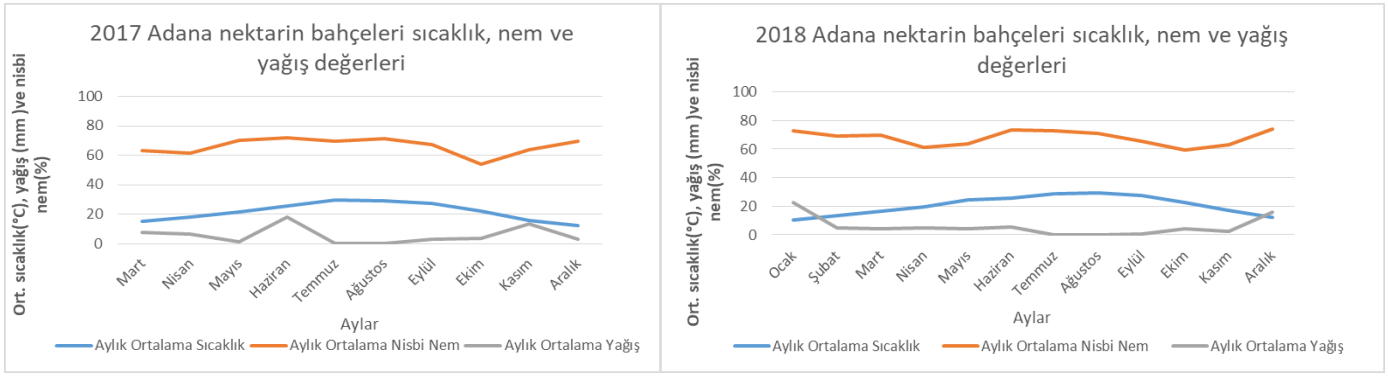
Adana ve Mersin'deki nektarin deneme bahçelerinde ilk yıl tuzakların asıldığı tarihten hasat sonuna kadar gerek tuzaklarda gerekse kültüre alınan meyvelerde zararlının herhangi bir biyolojik dönemine rastlanmamıştır. Ürün hasat edildikten sonra Adana birinci bahçede (Yalmanlı) 1 dişi (6 Ekim 2017) tuzaklarda yakalanmıştır (Şekil 1). Adana nektarin bahçesine ait ortalama sıcaklık, nisbi nem ve yağış değerleri Şekil 2'de verilmiştir. Mersin ikinci bahçede (Karaisalı) 1 dişi ve 1 erkek birey (6 Eylül 2017) tuzaklarda tespit edilmiştir (Şekil 3). Mersin nektarin bahçesine ait ortalama sıcaklık, nisbi nem ve yağış değerleri Şekil 4'de verilmiştir. Tuzaklarda yakalanan bu erginlerin çevrede bulunan olgunlaşma dönemindeki hurma, turunçgil, nar ile yabani böğürtlen gibi konukçulardan veya bahçe içerisinde bırakılan meyve atıklarından kaynaklanmış olacağı kanısına varılmıştır. Aynı bahçelerde çalışmanın ikinci yılında (2018) ise; Adana'da ilk ergin birinci bahçede 1 dişi (9 Mart 2018) meyveler ceviz büyüklüğünde, ikinci bahçede (Dervişler) ise 1 dişi (11 Mayıs 2018) meyvelerin ben düşme döneminde yakalanmıştır (Şekil 1).

Mersin'deki nektarin bahçelerinde çalışmanın ikinci yılında (2018) *D. suzukii* erginleri ilk olarak, 1 dişi (11 Nisan 2018) meyvelere ben düşme döneminde yakalanmış olup, en fazla ergin (1 dişi, 1 erkek) (2 Mayıs 2018) meyvelerin olgunlaşma döneminde belirlenmiştir (Şekil 3). Meyve hasadı nedeniyle 1 Mayıs 2018'de kimyasal mücadele uygulamaları sonlandırılmış ve 15 Mayıs 2018 tarihinde hasat yapılmış olup hasat zamanı ve sonrasında tuzaklarda yakalanan ergin sayısı hasat öncesinde elde edilen sayılara benzer bulunmuştur. Nitekim Çopurlu'daki bahçede hasattan sonra 4 Temmuz 2018 tarihinde (2 adet dişi) tuzaklarda tespit edilmiştir (Şekil 3).



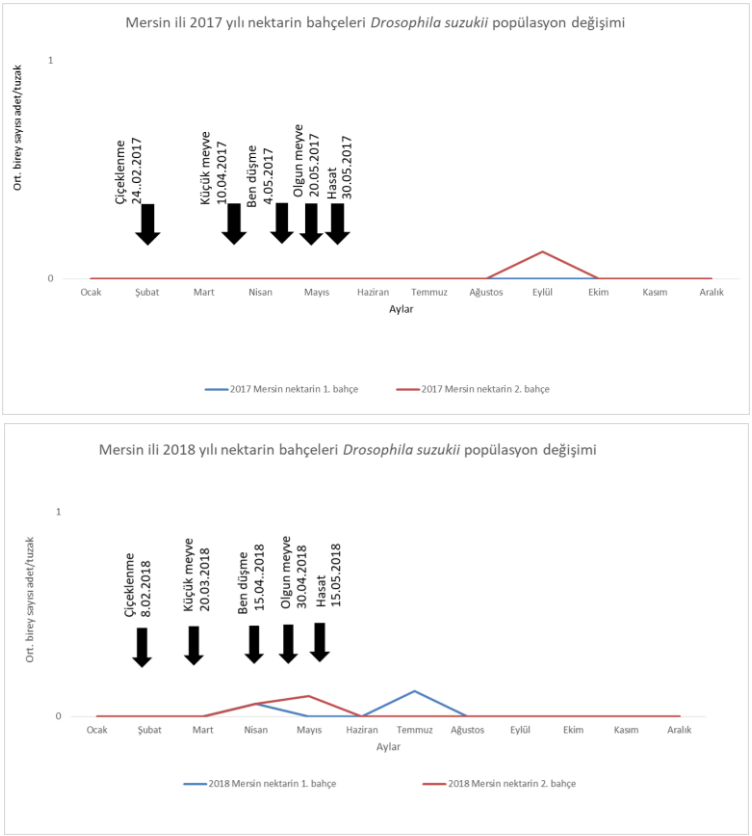
Şekil 1. Adana ilindeki nektarin bahçelerinde,2017-2018 yıllarında *Drosophila suzukii* Matsumara'nin ergin popülasyon değişimi

Figure 1. Fluctuation of adult population of Drosophila suzukii Matsumara in the nectarine orchards in Adana in 2017-2018



Şekil 2. Adana ilindeki nektarin bahçelerinde, 2017-2018 yıllarında ortalama sıcaklık, nisbi nem ve yağış değerleri

Figure 2. Average temperature, relative humidity and precipitation values in nectarine orchards in Adana in 2017-2018



Şekil 3. Mersin ilindeki nektarin bahçelerinde, 2017-2018 yıllarında *Drosophila suzukii* Matsumara'nin ergin popülasyon değişimi

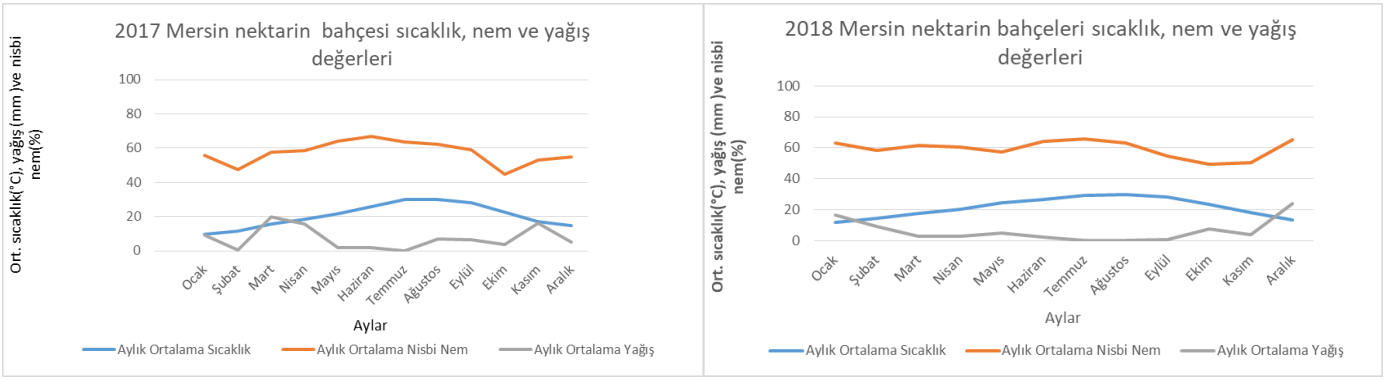
Figure 3. Fluctuation of adult population of Drosophila suzukii Matsumara in the nectarine orchards in Mersin in 2017-2018

Sonuç olarak nektarin bahçelerinde yapılan çalışmalarda, *D. suzukii* popülasyonunun çok düşük olduğu ve erkenci nektarin meyvelerinde ekonomik anlamda zararın ortaya çıkmadığı belirlenmiştir. Bu sebeple zararlının tercih ettiği meyve olgunluk dönemleri belirlenememiştir (Şekil 1). Bu durumun deneme kurulan alanlardaki gözlem ve üreticilerden edinilen bilgilere dayanarak özellikle erkenci nektarinlerde zarar yapan *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae) ve *Anarsia lineatella* Zeller (Lepidoptera; Gelechiidae) gibi zararlılara karşı yapılan kimyasal uygulamaları (Çizelge 1) meyvelerin yola dayanması ve raf ömrünün uzatılması gibi nedenlerden dolayı meyvelerin yarı olgun dönemde hasat edilmesinden kaynaklandığı kanaatine varılmıştır.

Kiraz Bahçelerinde *Drosophila suzukii* Matsumara'nin Ergin Popülasyon Değişimi

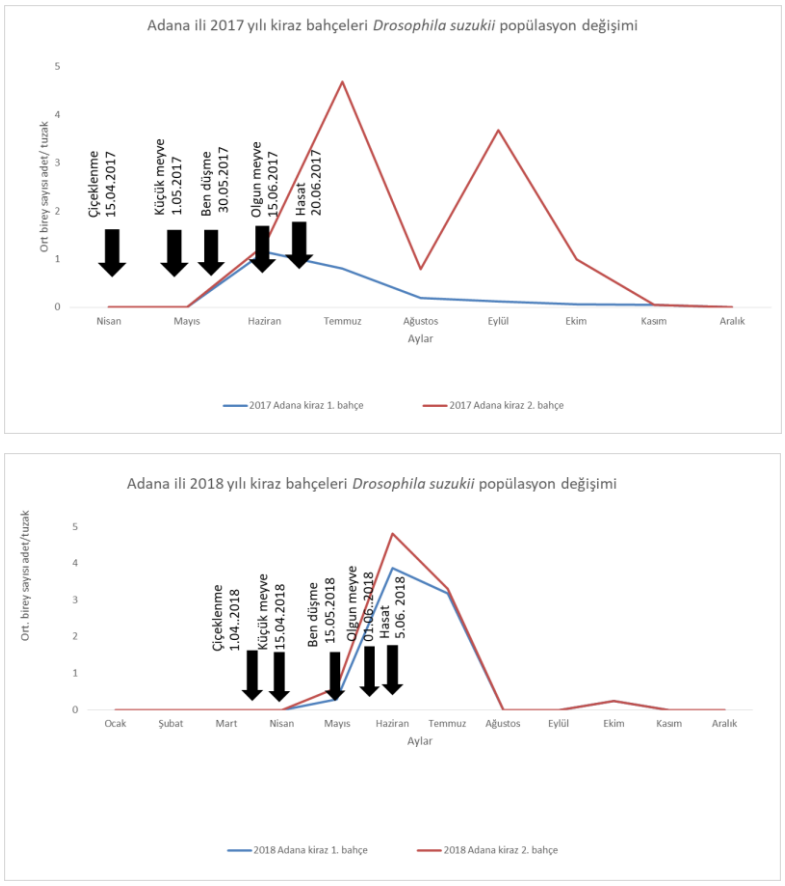
Adana ili kiraz bahçelerinde *D. suzukii* ergin

popülasyon takibi 2017-2018 yıllarında Saimbeyli (Kalesekisi)'de iki bahçede yürütülmüştür. Denemenin kurulduğu ilk yıl, tuzaklarda *D. suzukii* erginleri her iki bahçede de kirazlara ben düşme (sarı meyve) döneminde (15 dişi, 2 erkek) (8 Haziran 2017) yakalanmıştır (Şekil 5). Bahçelerde, meyvelerin ben düşme döneminden itibaren özellikle Kiraz sineği (*Rhagoletis cerasi* L., Diptera: Tephritidae)'ne ve *D. suzukii*'ye karşı Zirai Mücadele Teknik Talimatı (Anonim, 2008) dışı yapılan ve nerdeyse hasattan bir hafta öncesine kadar uygulanan (4-5 kez) kimyasal mücadeleden dolayı zararlı erginleri tuzaklarda yakalanmamıştır. Nitekim hasat sonrasında bahçeler üreticiler tarafından terk edildiği için bu dönemde kimyasalların baskısı ortadan kalktıktan sonra *D. suzukii* erginleri tuzaklarda yakalanmaya başlamıştır (Şekil 5).



Şekil 4. Mersin ilindeki nektarin bahçelerinde, 2017-2018 yıllarında ortalama sıcaklık, nisbi nem ve yağış değerleri

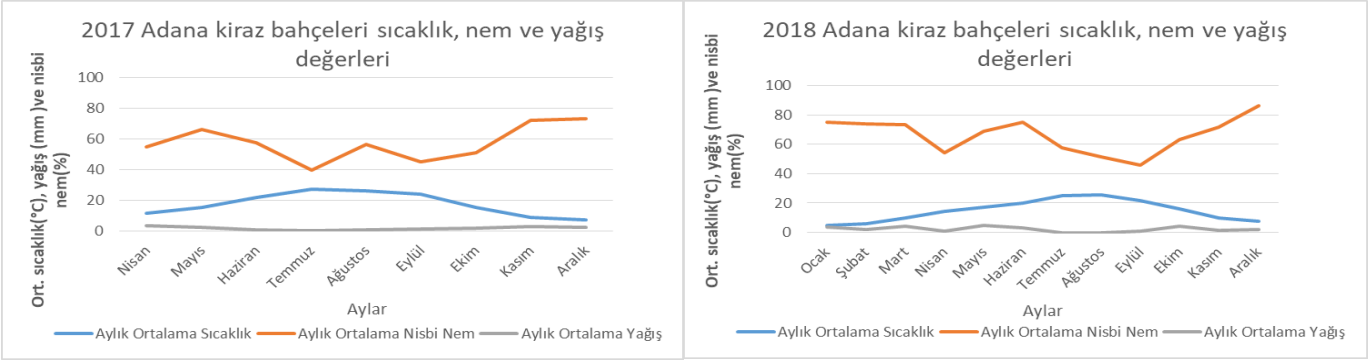
Figure 4. Average temperature, relative humidity and precipitation values, in nectarine orchards in Mersin in 2017-2018



Şekil 5. Adana ilindeki kiraz bahçelerinde, 2017-2018 yıllarında *Drosophila suzukii* Matsumara'nin ergin popülasyon değişimi

Figure 5. Fluctuation of Adult population of Drosophila suzukii Matsumara in the cherry orchards in Adana in 2017-2018

Denemenin ikinci yılında da (2018) *D. suzukii* erginlerinin iklime bağlı olarak kirazların ben düşme döneminde (1 dişi) (10 Mayıs 2018) ilk tespiti yapılmıştır. Kirazlar pembe kırmızı olum ve özellikle koyu kırmızı olum döneminden itibaren hasada kadar birinci bahçede 92 dişi, 37 erkek 28 Haziran 2018; ikinci bahçede 119 dişi, 41 erkek 7 Haziran kimyasal uygulamalarına rağmen nispeten fazla sayıda *D. suzukii* ergini tespit edilmiştir (Şekil 5). Hasat sonrasında zararlının ergin popülasyonu ikinci tepe noktasına birinci bahçede (39 dişi, 17 erkek) 28 Haziran ve12 Temmuz tarihlerinde, ikinci bahçede ise (39 dişi, 14 erkek) 28 Haziran ve 19 Temmuz tarihlerinde olmak üzere iki kez ulaşmıştır (Şekil 5). Nitekim ben düşme dönemi öncesi itibari ile Saimbeyli'deki üreticilerin yoğun olarak (Çizelge 1) kimyasallarla mücadele yaptığı belirlenmiştir. Çalışma süresince ben düşme- hasat arasına denk gelen bir ay gibi kısa sürede 5-6 kez kimyasal uygulaması yapılmıştır. Saimbeyli çevresinde ve denemelerin yürütüldüğü kiraz bahçelerinde 2017 yılına göre 2018 yılında gerek zararlı popülasyonunda görülen artışın ve gerekse üreticilerin yaptığı kimyasal uygulama sayısındaki artışın bölgede hemen hemen her günün yağışlı geçmesinden kaynaklanmış olabileceği kanısına varılmıştır (Şekil 5 ve 6). Nitekim bu zararlılara karşı uygulanan ruhsatlı insektisitlerin kontak etkili olması nedeniyle yağmur ile insektisit kalıntılarının yıkanarak kısa sürede etkisini kaybetmesinden kaynaklandığı düşünülmektedir.



Şekil 6. Adana ilindeki kiraz bahçelerinde, 2017-2018 yıllarında ortalama sıcaklık, nisbi nem ve yağış değerleri
Figure 6. Average temperature, relative humidity and precipitation values, in cherry orchards in Adana in 2017-2018

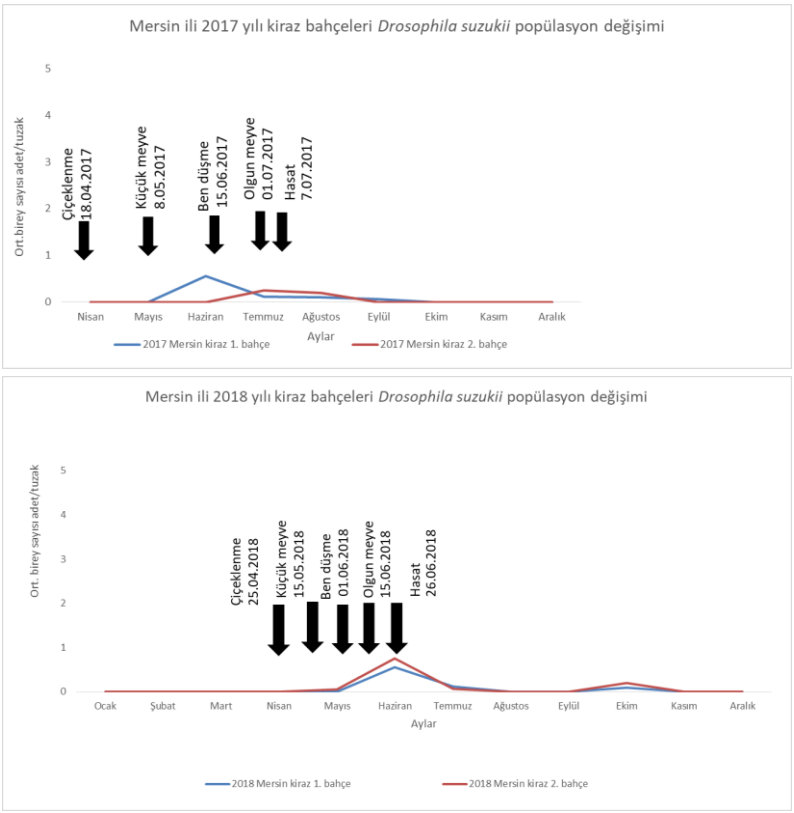
Sonuç olarak bahçelere tuzak asılma tarihinin fenolojik olarak ben düşme döneminin başlangıcı olması, Kiraz sirkesineği (*D. suzukii*) için kritik bir dönem olup bu dönem aşıldığında özellikle Kiraz sirkesineği ile mücadele kaçırılmış olunmaktadır. Ben düşme dönemi öncesinde yapılan uygulamaların kirazlarda zararlı olan bu tür ile mücadelede herhangi bir yararının olmadığı tespit edilmiştir.

Mersin ili kiraz bahçelerinde *D. suzukii* ergin popülasyon takibi 2017-2018 yıllarında Toroslar (Atlılar)'da iki bahçede yürütülmüştür. Mersin de çalışmanın birinci yılı (2017) için her iki bahçede de yakalanan ergin sayısında belirgin bir artış gözlenmemiştir (Şekil 7). Birinci bahçede ilk erginler ben düşme (sarı meyve) döneminde (1 dişi) (14 Haziran 2017), ikinci bahçede ise hasat sonrasında (3 dişi, 1 erkek) (12 Temmuz 2017) tespit edilmiştir (Şekil 7). Her yıl düzenli olarak yapılan kimyasal mücadele uygulamaları ile özellikle meyve olgunlaşma ve hasat döneminde bahçede dolu zararına karşı net (tül) uygulamasından dolayı tuzaklarda ergin yakalanmamıştır. Özellikle meyvelerin olgunlaşmaya başladığı dönemde Kiraz sirkesineği ve Kiraz sineği'ne karşı (Çizelge 1) yoğun olarak kimyasal uygulama yapıldığı gözlenmiştir.

Denemenin ikinci yılında her iki bahçe için de belirgin ergin artışı görülmezken, ikinci bahçede meyve olum (yeşil meyve) döneminde tuzaklarda (1 dişi, 2 Mayıs 2018) ilk erginler tespit edilmiştir. Her iki bahçede de meyve olgunlaşma (kırmızı meyve) döneminde (8 dişi, 13 Haziran 2018) ergin sayısında artış gözlenmiştir (Şekil 7). İkinci bahçede özellikle olgunlaşma dönemi içerisinde tuzaklarda (5 dişi, 20 Haziran 2018) nispeten ikinci en yüksek ergin sayısı gözlenirken, takip eden haftalarda tuzakta yakalanan ergin sayısının giderek düştüğü gözlenmiştir (Şekil 7). Popülasyonun hasat başlangıcında yavaş yavaş artarak hasat sonrasında tepe noktası oluşturması bahçelerde yapılan kimyasal mücadelenin sonlandırılmasından, ayrıca ağaç üzerinde ve yerde kalan meyvelerden ve çevre bahçelerdeki çilek, geçci şeftali ve böğürtlen gibi konukçulardan kaynaklanmıştır. (Şekil 7). Çalışmanın ikinci yılında (2018) birinci yıla (2017) göre yağış, nem ve sıcaklık değerleri daha yüksek olmuştur (Şekil 8).

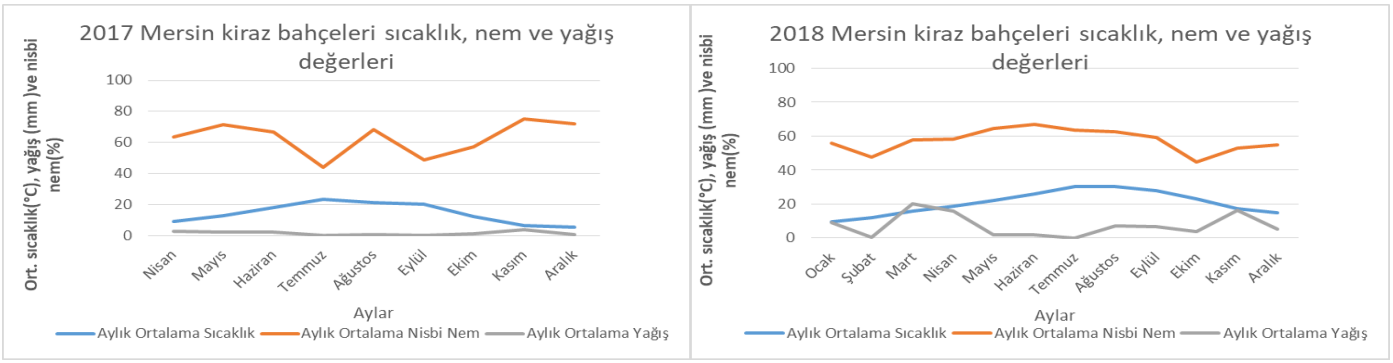
Sonuç olarak bahçelere tuzak asılma tarihinin fenolojik olarak ben düşme döneminin başlangıcı

olması, Kiraz sirkesineği (*D. suzukii*) için kritik bir dönem olup bu dönem aşıldığında özellikle Kiraz sirkesineği ile mücadele kaçırılmış olunmaktadır. Ayrıca bahçede kimyasal mücadeleye ek olarak yapılan net (tül) uygulamasının *D. suzukii* popülasyonunun düşük olmasını etkilediği tespit edilmiştir.



Şekil 7. Mersin ilindeki kiraz bahçelerinde, 2017-2018 yıllarında *Drosophila suzukii* Matsumara'nın ergin popülasyon değişimi

Figure 7. Fluctation of Adult population of Drosophila suzukii Matsumara in the cherry orchards in Mersin in 2017-2018



Şekil 8. Mersin ilindeki kiraz bahçelerinde, 2017-2018 yıllarında ortalama sıcaklık, nisbi nem ve yağış değerleri

Figure 8. Average temperature, relative humidity and precipitation values, in cherry orchard in Mersin in 2017-2018

Genel olarak Adana ve Mersin illerinde yürütülen *D. suzukii* popülasyon takibi çalışmasında, meyve olgunlaşma ve hasat dönemi ile ilkbahar ve sonbahar aylarında *D. suzukii* popülasyonunda artış gözlenmiştir (Şekil 1, 3, 5, 7). Wang ve ark. (2016) tarafından 2013-2014 yıllarında Kaliforniya (ABD)'da kiraz bahçelerinde, elma sirkesi yardımıyla *D. suzukii*'nin popülasyon gelişmesini belirlemek amacı ile yürüttükleri çalışmada, ilkbahar ve sonbahar mevsiminde tuzaklarda en yüksek sayıda *D. suzukii* ergini yakalandığını bununla beraber tuzaklardaki ergin sayısının hasadın olduğu haziran ayında en yüksek seviyeye ulaştığını bildirmişlerdir.

Benzer şekilde Kasap ve Özdamar (2019), Çanakkale ilindeki üzüm bağlarında 2014 ve 2017 yıllarında *D. suzukii*'nin popülasyon gelişiminin belirlenmesi

amacı ile yaptıkları çalışmada, *D. suzukii* popülasyonun hasat zamanı olan sonbaharda artış gösterdiğini bildirmişlerdir. Benzer özellik gösteren diğer bir çalışma Živković ve ark. (2020) tarafından, Hırvatistan'da üç farklı lokasyonda ve erik, kayısı, kiraz, elma, şeftali, böğürtlen, ahududu bahçelerinde *D. suzukii*'nin popülasyon dinamiğini belirlemek amacı ile 2017 yılında yürütülmüştür. Yapılan çalışma sonucunda popülasyonun özellikle sonbahar ayında arttığını ve iklimin popülasyon üzerine çok etkili olduğunu bildirmişlerdir. Sözü edilen çalışmalarda elde edilen sonuçlar ile nektarin ve kiraz bahçelerinde yapılan bu çalışma bulguları *D. suzukii* popülasyonun yoğun olduğu dönemler açısından benzerlik göstermektedir. Denemenin kurulduğu bahçelerde üretici tarafından yoğun kimyasal uygulama yapılmasına rağmen söz konusu dönemlerde zararlının popülasyonunda artış gözlenmiştir. Diğer yandan Mersin kiraz alanlarında kimyasal uygulamasına ek olarak net (tül) uygulamasının yapılması ile Adana kiraz alanlarına göre daha düşük *D. suzukii* popülasyonu tespit edilmiştir. Net uygulaması ile ilgili olarak yapılan çalışmalarda da özellikle Kuzey Karolina (ABD)'da 2013-2014 yıllarında ahududu alanlarında yapılan çalışmada kimyasal mücadele ile kombine olarak yapılan net uygulamalarının *D. suzukii* popülasyon yoğunluğunu düşürdüğü ve üç haftaya kadar meyve zararında gecikme olduğu bildirilmiştir (Leach ve ark. 2016). Ayrıca yapılan birçok çalışmada da ahududu böğürtlen gibi meyve alanlarında yapılan net uygulamasının *D. suzukii* popülasyonunu azaltması konusunda ümit var olduğu bildirilmiştir (Link 2014, Cormier ve ark. 2015, Rogers ve ark. 2016). Sözü edilen çalışma sonuçları bu çalışmadan elde edilen sonuçlar birbirini destekler niteliktedir.

Diğer taraftan Drummond ve ark. (2019) yabanmersini alanlarında, 2012-2018 yılları arasında yaptıkları çalışmada zararlının ilk ergin çıkışının kış koşullarına bağlı olarak zararlının fizyolojik gelişmesine bağlı olduğunu, ilk ergin çıkışını takiben zararlının kısa sürede epidemi yapacak popülasyona ulaştığını, yıl içerisindeki uçuş aktivitesinin pestisit uygulama sıklığına göre değiştiğini belirtmişlerdir. Ayrıca yapılan çalışmada bahçe kenarı veya yakınında yabani meyve yoğunluğunun bulunmasının da *D. suzukii* popülasyonunun artmasına neden olduğunu bildirmişlerdir. Nitekim sekiz bahçede yapılan bu çalışmada da zararlının popülasyon yoğunluğunun, özellikle meyve hasadına kadar üreticiler tarafından yapılan yoğun kimyasal uygulamalar ile düşük seviyelerde kaldığı tespit edilmiştir. Diğer yandan bahçe yakınında bulunan *D. suzukii* konukçularından çilek, elma, böğürtlen, erik gibi meyvelerin de popülasyon artışını etkilediği tespit edilmiştir. Gerek yurtdışı gerekse yurtiçinde yapılan çalışma bulguları incelendiğinde, Adana ve Mersin illerinde *D. suzukii*'nin popülasyon takibini belirlemek amacı ile yapılan bu çalışmanın bulguları benzerlik göstermektedir. Adana ve Mersin illeri sıcaklığın yüksek derecelerde seyrettiği ve oransal nemin %60'ın üzerinde olduğu bir bölgedir (Şekil 2, 4, 6, 8). *Drosophila suzukii* açısından bu iklim koşullarının ve konukçu çeşitliliğinin, zararlının popülasyon yoğunluğu üzerinde pozitif bir etkiye sahip olduğu söylenebilir. Ayrıca bölgede kış ayları süresince, 10°C'nin altında geçen gün sayısının çok az olmasının da *D. suzukii* popülasyonunun artmasında etkili olacağı beklenebilir. Nitekim Tochen ve ark. (2016) Amerika'da yaptıkları bir çalışmada yüksek nem koşullarında *D. suzukii* popülasyonunun arttığını bildirmişlerdir. Diğer taraftan Shearer ve ark. (2016) *D. suzukii* popülasyonlarında mevsimsel fenotipi tanımlamak amacı ile yaptıkları bir çalışmada, yazdan kışa geçiş döneminde kış fenotipi olarak adlandırılan popülasyon oranında artış eğilimi gözlendiğini, bu fenotipin daha koyu pigmentasyon ve daha uzun kanat yapısı ile karakterize edildiğini belirtmişlerdir. Bu sebeple Doğu Akdeniz Bölgesi'nde özellikle yüksek rakımlı bölgelerde düşük sıcaklıklara rağmen bölgede kışı geçiren ve soğuk toleransı yüksek olan kış fenotipinin popülasyon artış sebeplerinden biri olduğu düşünülmektedir.

Drosophila suzukii'nin Zarar Oranının ve İlk Yumurta Bırakma Zamanının Belirlenmesi

Çalışma sonunda, nektarin bahçesinin erkenci çeşit olması, meyvelerin olgunlaşma başlangıcında hasat edilmesi ve özellikle tomurcuklanma başlangıcından itibaren trips ve şeftali güvesine karşı kimyasal mücadelenin yapılması nedenleri ile bu bahçelerde zarar tespit edilmemiştir.

Kirazlarda ise, yeşil ve sarı olum dönemlerindeki meyvelerde zararlının herhangi bir biyolojik dönemine rastlanmamıştır. Diğer taraftan zararlının kırmızı ve koyu kırmızı olum dönemindeki meyvelere yumurta bıraktığı bu meyvelerde larvaların gelişimini tamamlayarak pupa oldukları ve ergin çıkışı olduğu belirlenmiştir. Sonuç olarak *D. suzukii*'nin yumurta bırakmak için olgun meyveleri tercih ettiği tespit edilmiştir.

Kirazda hasat öncesi yapılan sayımlar sonucunda, ilk yıl 100 meyvede 2 meyve (%2), ikinci yıl ise 100 meyvede 62 meyvede (%62) zarar tespit edilmiştir. Zarar tespit edilen her bir meyvede 3-5 adet larva olduğu belirlenmiştir. Sonuç olarak çalışmanın yapıldığı birinci yıl ile ikinci yıl arasında zarar oranı açısından büyük bir fark ortaya çıkmıştır. Bu farkın, çalışmanın yapıldığı ikinci yıl meyve olgunlaşma zamanında, hava şartlarının aşırı yağışlı, sıcak ve nem yoğunluğunun yüksek olmasından kaynaklandığı düşünülmektedir. Hava şartları özellikle *D. suzukii* ergin popülasyonunda ve fungal hastalık zararında da yoğun artışlara yol açmıştır

(Şekil 2, 4, 6, 8). Özellikle yapılan kimyasal mücadelenin yağışlar nedeniyle etkili olmadığı görülmüştür. Günlük ortalama oransal nemin 2017 Haziran ayının 9 gününün, 2018 Haziran ayında ise 26 gününün % 60 ve üzeri nem oranında olduğu saptanmıştır (Şekil 2, 4, 6, 8). Bolda (2010), 2008 yılında Amerika Birleşik Devlatleri'nde yaptığı çalışmasında, *D. suzukii*'nin yaban mersininde %50, kirazda %33 ve çilekte %20 oranında zarara neden olduğunu bildirmiştir. Goodhue ve ark. (2011) ise, Kaliforniya (ABD)'da yaptıkları çalışmada, zararlının çileklerde %20, ahududunda %37 oranında zarar yaptığını kaydetmişlerdir. Diğer taraftan Fransa çilek alanlarında 2010 yılında yapılan çalışmada % 80 üzerinde *D. suzukii* zararı tespit edilmiştir (Farnsworth ve ark., 2017; Mazzi ve ark., 2017). Yapılan çalışmalarda elde edilen sonuçlar ile Doğu Akdeniz Bölgesi'nde yapılan bu çalışmada elde edilen bulgular arasında, zarar oranı açısından fark olduğu görülmektedir. Doğa koşullarında yürütülen çalışmaları etkileyen birçok faktör bulunmaktadır ki bunlardan iklim etkeni en büyük paya sahiptir. Bu farkın öncelikle iklim koşullarından ve üreticilerin yaptıkları kimyasal mücadele başta olmak üzere diğer yapılan bakım şartlarından ileri geldiği söylenebilir.

SONUÇ ve ÖNERİLER

Doğu Akdeniz Bölgesi'nde meyvelerde zararlı *D. suzukii*'nin popülasyon takibi ile zarar oranını belirlemek amacı ile yapılan çalışma sonunda *D. suzukii* popülasyonunun ilkbahar ve sonbahar ayları ile meyve olgunlaşma- hasat dönemi arasında iklim faktörlerine bağlı olarak arttığı, aynı zamanda insektisit, net(tül) uygulamaları ile bakım koşulları gibi faktörlerin çevrede bulunan *D. suzukii* için konukçuluk yapacak ürünlerin popülasyon artışını ve zarar oranını etkilediği belirlenmiştir.

Drosophila suzukii ile ilgili mücadele kriterlerinden olan zararlının popülasyon takibinin yapıldığı bu çalışmanın sonuçları ışığında aşağıda belirtilen konular önem kazanmaktadır.

1. Zararlı ile mücadele takvimini, *D. suzukii* popülasyonunun arttığı ilkbahar, sonbahar, olgunlaşma ve hasat dönemine uygun olarak düzenlemek zararlı ile etkin mücadele yapmak açısından faydalı olacaktır.

2. *Drosophila suzukii* olgunlaşma ve hasat zamanında meyveye zarar verdiği için kalıntı ve çevre sağlığı nedeni ile bu dönemde zararlı mücadelesinde kültürel önlemler, biyoteknik ve biyolojik mücadele uygulamalarına yönelmeli, ileri zamanda bu konularda yapılacak çalışmalara ağırlık verilmelidir.

TEŞEKKÜR

Bu çalışma Ç.Ü. Araştırma ve Projeler Birimi ile Tarım ve Orman Bakanlığı Tarımsal Araştırmalar ve Politikalar Genel Müdürlüğü (TAGEM) tarafından desteklenen doktora tezi kapsamında yürütülmüş olup, tezin bir bölümünü kapsamaktadır. Desteklerinden ötürü Ç.Ü. Araştırma ve Projeler Birimi ve Tarım ve Orman Bakanlığı TAGEM'ne teşekkürlerimi sunmaktan onur duyarım.

Araştırmacıların Katkı Oranı Beyan Özeti

Yazarlar bu çalışmada eşit oranda katkı sağlamıştır.

Çıkar Çatışması Beyanı

Yazarlar arasında hiçbir şekilde çıkar çatışması bulunmamaktadır.

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First Report of Root-Knot Nematode, *Meloidogyne arenaria* on Lemon Balm (*Melissa officinalis* L.) in Turkey

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ABSTRACT
Lemon palm (*Melissa officinalis* L.), a perennial plant from Lamiaceae family, is cultivated in all Mediterranean countries and coastal regions of Türkiye. It can be attacked by several pathogens like nematodes which reduce its yield and quality. In this study, morphometric measurements, morphological and molecular identifications were done using juveniles and females obtained from galled roots of lemon balm collected from Balıkesir province of Türkiye. As a result, *M. arenaria* was the only identified species in analyzed samples. This is the first report of *M. arenaria* detected on lemon balm in Türkiye.

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ÖZET
Lamiaceae familyasından çok yıllık bir bitki olan melisa (*Melissa officinalis* L.), tüm Akdeniz ülkelerinde ve Türkiye'nin kıyı bölgelerinde yetiştirilmektedir. Melisa bitkileri nematodlar gibi verim ve kalitede düşüşe yol açan çeşitli patojenler tarafından saldırıya uğramaktadır. Bu çalışma kapsamında Balıkesir ilinden toplanan melisa bitkilerinin urlu köklerinden elde edilen larvalar ve dişi bireyler kullanılarak morfometrik ölçümler, morfolojik ve moleküler tanılamalar yapılmıştır. Sonuç olarak, *M. arenaria*, analiz edilen örneklerde tespit edilen tek tür olmuştur. Bu çalışma Türkiye'de melisa üzerinde *M. arenaria*'nın ilk tespitidir.

Bitki Koruma

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Anahtar Kelimeler
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INTRODUCTION

Medicinal and aromatic plants have been used in pharmaceuticals, perfumery, cosmetics, and food industries and there is a current significant increasing demand in tropical and subtropical regions of the world (Pandey, 2017). *Melissa officinalis* belongs to Lamiaceae family and is distributed in all Mediterranean countries. This plant has many common names such as garden balm, lemon mint, sweet balm in Türkiye (Mill, 1982; Baytop, 1994). This plant is widely cultivated due to its content of aromatic, culinary and medicinal compound (Verma et al. 2015). In addition, it has been known that lemon balm essential oils can be used as antioxidant and antitumoral agents for the treatment of Alzheimer's disease and have a positive effect on human nervous system (Akhondzadeh et al. 2003).

Lemon balm hosts several pests and pathogens which reduce its yield and quality (Bokor et al. 2008). Plant parasitic nematodes, particularly root-knot nematodes, cause serious economic losses in vegetables, horticultural crops, and medicinal and aromatic plants (Koshy et al. 2005; Karssen et al. 2013; Ataş et al. 2021).

Meloidogyne arenaria (Neal, 1889) Chitwood, 1949 was reported on lemon balm in Greece (Karanastasi

et al. 2008). Also, *Meloidogyne incognita* (Kofoid & White 1919) Chitwood 1949 and *M. arenaria* on *M. officinalis* was reported in Cuba (Kindelan et al. 1990). In several other studies, *Melissa officinalis* was found to be the host of *M. paranaensis* n. sp. (Carneiro et al. 1996), and *M. incognita* race 3 (Walker, 1995; Mendonça et al. 2017). However, no reports were found on root-knot nematodes in lemon balm in Türkiye.

MATERIAL and METHODS

A survey was caried out in lemon balm growing areas in Balıkesir province of Türkiye in 2020. Root samples were taken from plants with wilting and drying symptoms and examined under a binocular microscope. Egg masses were separately taken from roots of lemon balm by a needle. Then, they were incubated and transferred to sieve included water for second stage juveniles (J2s) hatching. Based on Seinhorst's (1959) method, J2s hatched from the egg were fixed in TAF (triethanolamine formalin) for permanent slides. Overall, measurements of 25 J2s were taken place under the Leica DM1000 stereomicroscope as described by Karssen (2002). For morphological diagnosis, females were taken from the roots by a needle and scalpel under binoculars. Females were prepared in glycerin by cutting in 45% lactic acid to obtain perineal patterns (Hooper, 1986). The population was morphologically identified by the comparing to morphometric measurements of Jepson (1987) and Karssen (2002). DNA was extracted from J2s for molecular identification using isolation kit (High Pure PCR Template Preparation Kit, Roche) and were performed with species-specific primers; Inc-K14F/Inc-K14R (Randig et al., 2002), MincF1/MincR1 (Devran et al. 2018), Fjav/Rjav (Zijlstra et al. 2000); Far/Rar (Zijlstra et al. 2000), JMV1/JMV2/JMVhapla (Wishart et al. 2002). PCR reactions were carried out according to our previous studies (Özalp et al. 2020; Ataş et al. 2021).

RESULTS and DISCUSSION

The results of morphometric measurements and morphological identification showed that the overall morphology of this population appeared to be similar to *M. arenaria* (Table 1, Figure. 1).

Table 1. *Morphometric measurements of Meloidogyne arenaria J2s on Melissa officinalis*
Çizelge 1. *Melissa officinalis'den elde edilen Meloidogyne arenaria'ya ait J2s'lerin morfometrik ölçümleri*

Diagnostic characters	*Current study*	*Whitehead, 1968*	*Cliff and Hirschmann, 1985*
Body length	405.31 ± 9.68 (389.67-430.12)	450-490	391.6-605.2
Greatest body width	14.58 ± 1.37 (12.29-18.20)		12.8-17.8
Body width at stylet base	8.69 ± 0.60 (7.12-9.99)		
Body width at anus	9.34 ± 0.82 (7.59-11.34)		9.7-12.8
Stylet length	12.54 ± 1.29 (10.27-13.95)	10	10.1-11.9
DGO	3.34 ± 0.32 (2.68-3.84)	3	2.7-4.7
Tail length	47.61 ± 4.31 (40.87-56.66)		43.6-69.4
Excretory pore to head end	80.08 ± 5.96 (66.25-101.01)		75.0-105.2
Body width at excretory pore	12.15 ± 0.79 (10.79-13.61)		
A	27.28 ± 2.59 (23.15-33.71)	26-32	22.4-40.5
B	3.79 ± 0.23 (3.27-4.22)		
C	8.35 ± 0.72 (7.22-9.93)	6-7.5	7.5-10.9
c'	5.13 ± 0.59 (4.18-6.63)		

Note: All measurements are in µm and in the form: mean ± s.d. (range)

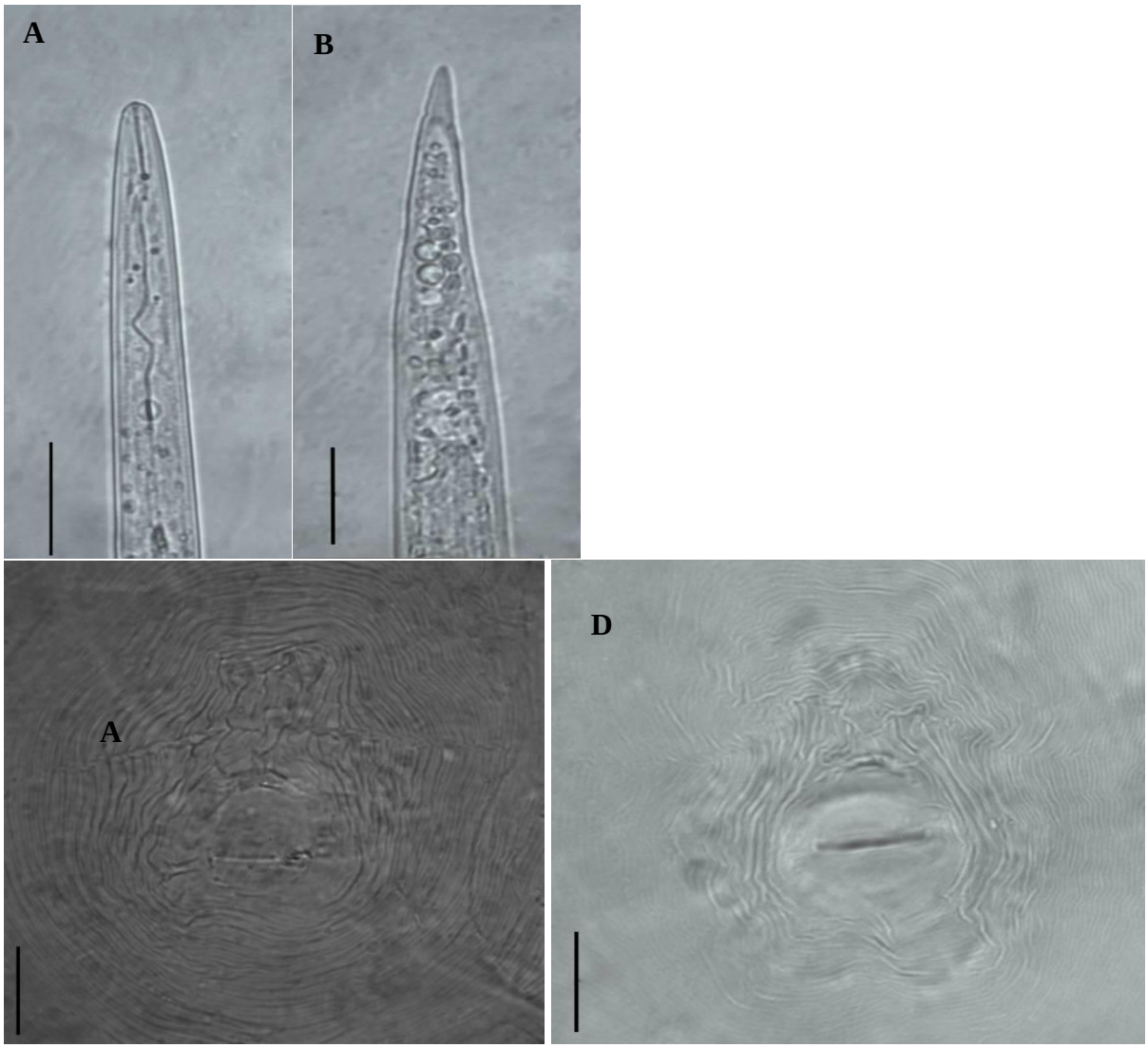

Figure 1. *Meloidogyne arenaria* obtained from *Melissa officinalis* A: Anterior end region, B: Tail region, C-D: Perineal pattern (Scale bar: 20 µm)

Şekil 1. *Melissa officinalis* 'den elde edilen *Meloidogyne arenaria* A: Anterior end region, B: Tail region, C-D: Perineal pattern (Skala bar: 20 µm)

Figure 2. PCR products obtained with *Meloidogyne arenaria*-specific Far/Rar primers. M: DNA Ladder (Hibrigen 100 bp); BM1-BM4: Samples; K18: *M. arenaria* (positive control); W: Water

Şekil 2. *Meloidogyne arenaria*-spesifik Far/Rar primerleri kullanılarak elde edilen PCR ürünleri. M: DNA Ladder (Hibrigen 100 bp); BM1-BM4: Örnekler; K18: *M. arenaria* (pozitif kontrol); W: Su

In molecular identification, PCR with *Meloidogyne arenaria* specific Far/Rar primer sets produced an expected approximately 420 bp, but the other species-specific primers failed to amplify any products (Figure. 2).

This is the first report of the detection of *M. arenaria* on lemon balm grown as a medicinal and aromatic plant in Türkiye. The damage and prevalence of plant parasitic nematodes has been currently increasing in medicinal and aromatic plants. With these findings, the damage of root-knot nematodes could be eliminated by crop rotation, using non-host plants or resistant cultivars.

Autor's Contributions

Authors declares the contribution of the authors is equal.

Statement of Conflict of Interest

Authors have declared no conflict of interest.

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The Effect of Farm Manure on Yield and Some Soil Properties in a Pear Garden in Yozgat

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ABSTRACT
The aim of this study is to determine the effect of farm manure applied at two doses on some chemical and physical properties of the soil. In addition, it was conducted to determine the relationships between the contribution of improved soil health and quality to the development of pear trees. For this purpose, farmyard manure of Etruşka variety grafted on a 6-year-old OHF 333 rootstock planted in Gedikhasanlı Research and Application Center was applied to the crown projection of the trees in three different doses (control, half dose: 5 kg tree^{-1} and full dose: 10 kg tree^{-1}). At the end of the study, while there was no statistical difference between applications in 2018 and 2019 measurements in tree height measurements, 5 kg tree^{-1} and 10 kg tree^{-1} farm manure applications affected 2020 measurements significantly. When the number of shoots was examined, there was no statistical difference in the year of application (2018) and one year after (2019), while in 2020, 5 kg tree^{-1} and 10 kg tree^{-1} farm manure applications were higher than control. With the application of 10 kg farm manure per tree, the organic matter content of the soil was increased from "low" level (1.02%) to "medium" level (3.03%) at the end of the first year. A similar situation was valid for 5 kg tree^{-1} dose application, although the increase in the amount of organic matter was lower (2.45%). At the end of the second year, while the level of organic matter in soils where high dose application was applied was preserved as "medium" (2.14%), the low dose application lost its effectiveness and the level of organic matter fell back to the "low" class (1.45%). The most effective application reducing bulk density and penetration resistance was 10 kg tree^{-1} dose.

Horticulture

Araştırma Makalesi

Makale Tarihçesi
Geliş Tarihi : 29.06.2021
Kabul Tarihi : 08.03.2022

Anahtar Kelimeler
Pear
Farm manure
Organic matter
Plant growth and yield
Soil physical properties

Yozgat'ta Bir Armut Bahçesinde Çiftlik Gübresinin Verim ve Bazı Toprak Özelliklerine Etkisi

ÖZET
Bu çalışmanın amacı iki farklı dozlarda uygulanan çiftlik gübresinin toprağın bazı kimyasal ve fiziksel özellikleri üzerine etkisini belirlemektir. Ayrıca iyileşen toprak sağlığı ve kalitesinin armut ağaçlarının gelişimine katkısı arasındaki ilişkileri tespit etmektir. Bu amaçla Gedikhasanlı Araştırma ve Uygulama Merkezi'nde dikili olan 6 yaşındaki OHF 333 anacına aşılı Etruşka çeşidi armut bahçesinde ihtimar edilmiş çiftlik gübresi, ağaçların taç izdüşümüne iki farklı dozda (yarım doz: 5 kg ağaç$^{-1}$ ve tam doz: 10 kg ağaç$^{-1}$) uygulanmıştır. Çalışma sonunda armut ağaç boyu 2018 ve 2019 yılları ölçümlerinde uygulamalar arasında istatistiksel fark yokken 2020 yılı ölçümlerinde yarım doz ve tam doz çiftlik gübresi uygulamaları kontrole göre önemli bulunmuştur. Sürgün sayısı incelendiğinde uygulamanın yapıldığı yıl (2018) ve bir yıl sonrasında (2019) istatistiksel fark yokken, 2020 yılında yarım doz ve tam doz çiftlik gübresi uygulamaları kontrole göre daha yüksek bulunmuştur. Ağaç başına 10 kg çiftlik gübresi uygulaması ile toprağın organik madde içeriğini birinci yılın sonunda "düşük" seviyeden (%1.02) "orta" seviyeye (%3.03) çıkarılmıştır. Benzeri bir

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Toprak fiziksel özellikleri

durum organik madde miktarındaki artış daha düşük olmakla birlikte 5 kg ağaç$^{-1}$ doz uygulaması için de geçerlidir (%2.45). İkinci yılın sonunda ise yüksek doz uygulaması yapılan topraklarda organik madde seviyesi "orta" olarak korunurken (%2.14) düşük doz uygulaması etkinliğini yitirerek organik madde seviyesi yeniden "düşük" sınıfına gerilemiştir (%1.45). Hacim ağırlığı ve penetrasyon direncini azaltmada en etkili uygulama 10 kg ağaç$^{-1}$ dozu olmuştur.

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INTRODUCTION

With its increasing commercial value, pear is grown widely in countries such as China, America, Italy, Argentina, and Spain, along with Turkey. Particularly concentrated in temperate climates, this species is systematically included in the *Pyrus* genus, which includes about 20 pear species within the *Pomoideae* subfamily of the *Rosaceae* family. The species in the *Pyrus* genus, which is economically grown; classified as eastern and western pears (Gökmen, 1973). The western pear group includes *Pyrus communis* species grown in Europe, North and South America, and Africa, while the eastern pear group includes *P. ussuriensis*, *P. bretschneideri* and *P. sinkiangensis* grown in China, and *P. pyrifolia* species growing in China and Japan (Bell, 1990).

According to the data of FAO (2020), world pear production in 2019 is 23.919.075 tons. China is first place with 17 million tonnes in production when followed by the USA (661.340 tonnes), Argentina (595.427 tonnes), Turkey (530.723 tonnes), Italy (429.290 tons), South Africa (407.212 tonnes) and the Netherlands (373.000 tons). Turkey ranks fourth in the world in terms of pear production quantity and pear production area (530.723 tonnes and 26.299 ha).

The dense planting systems that European countries have been implementing for years in today's fruit growing have been increasingly preferred by the growers of Turkey in recent years. Saplings are the most basic material of modern orchards established as a garden with sustainable production techniques. A healthy and standard well-branched sapling is the most important step in profitability in a garden facility (Wertheim et al., 2001). It has been reported that the number of side branches of the seedlings, the angle of the side branches, and the height of the seedlings is very effective in the early yield and high yield of the garden (Hrotko et al., 1996). In the modern orchard facility, obtaining high quality and high amount of fruit by the saplings as soon as possible depends on the nutrition of the plant. Because there are very close and important relationships between pear nutrition and product quantity and quality as in other soft seed species (Başar, 2001).

In Yozgat and similar conditions, in fruit trees and especially in saplings grafted on clonal rootstocks, the formation of the side branch and consequently the crown structure takes longer than other temperate regions, which delays the time of the saplings to lay on the fruit. Despite regular cultural practices in the pear garden, which was established in 2012, tree development and yield remained low. It is thought that this situation is caused by the soil health below a certain level and low soil quality in the cultivated garden. Soil quality or health is a product of genetics such as parent material, climate and topography, and human-induced interactions such as tillage and crop rotation. Soil quality can be evaluated by determining the changes in soil properties affected by management (Aziz et al., 2009). Since organic matter affects the physical, chemical and biological properties of the soil, it has been the top priority in almost every study on soil quality. In addition to organic matter, soil properties such as soil pH, salinity, bulk weight, and resistance to penetration have also been accepted as indicators of the dynamic quality of the soil (Wienhold et al., 2009; Ding et al., 2011). The aims of this study are to determine the farm manure to be applied in different three doses to the soil of the research area which has some problems in terms of horticultural crop cultivation; (i) to determine the effect on some chemical properties, which are indicators of soil health, (ii) to determine the effect on some physical properties, which are indicators of soil quality, and (iii) to determine the relationship between the contribution of improved soil health and quality to the vegetative growth and yield of pear trees.

MATERIALS and METHODS

Plant and soil materials

This study was carried out in the pear garden established with Etryoshka pear variety grafted to the rootstock of OHF333 were planted at 4 m x 5 m in-row and row spacing in 2012, in Gedikhasanlı Research and Application Center, located in Sorgun district, Yozgat between 2018 and 2020. The general

characteristics of the soil in the land where the pear orchard is located are as follows: it is a sandy clayey loam (clay, slt and sand contents are 299,89 g kg^{-1} and 612 g kg^{-1}, respectively), it has sufficient exchangeable K content (215 µg g^{-1}), its total N content is 0.05% and available P content is 5.76 µg g^{-1}. It has no salt and slihtly alkaline. Its organic matter content level is very low (0.99%) and total $CaCO_3$ content is 5.76% (Yakupoğlu, 2018). By applying different doses of farm manure, its effectiveness on the vegetative growth and yield of the tree was investigated by improving the health and quality of the soil. Gedikhasanlı village is connected to Sorgun district and has an altitude of 1050 m and is located at 39°58′69″ N - 35°15′95″ E coordinates (Figure 1).

The average monthly temperature and precipitation values determined throughout the study were obtained from the Yozgat Meteorology Provincial Directorate (Figure 2 and Figure 3).

Methods

In this study, three different doses of farm manure (control, full dose: 10 kg $tree^{-1}$ and half dose: 5 kg $tree^{-1}$) were applied to the crown projection of the trees by being mixed into the soil after being left in circular canals to be opened in 15 cm width and 15 cm depth (Kacar and Katkat, 1998). Organic N concentration of the manure is 6.60%, and its NH_4^+-N and NO_3^--N contens are 170 µg g^{-1} and 2100 µg g^{-1}, respectively. The P and K contents of manure, which can be considered as rich in phosphorus and potassium, are 2.86% and 3.55%, respectively. 10 kg of farm manure was given in full dose application per tree. The reason for choosing half dose is that routine chemical fertilizer applications will be performed.

Before the vegetation started, pruning of pear saplings was carried out in March 2019 and 2020. Chemical fertilization, which is carried out every year, was made with Ammonium sulphate (21% N) at a rate of 200 g per tree in 2019 and 2020, and the fertilizer was mixed with the soil. Chemical fertilizers were not preferred for phosphorus fertilization because phosphorus supplementation from farm manure was taken into account. Also, no chemical potassium fertilization was done because the soil has a sufficient exchangeable K content.

Figure 1. The Gedikhasanlı Research and Application Center
Şekil 1. Gedikhasanlı Araştırma ve Uygulama Merkezinin konumu

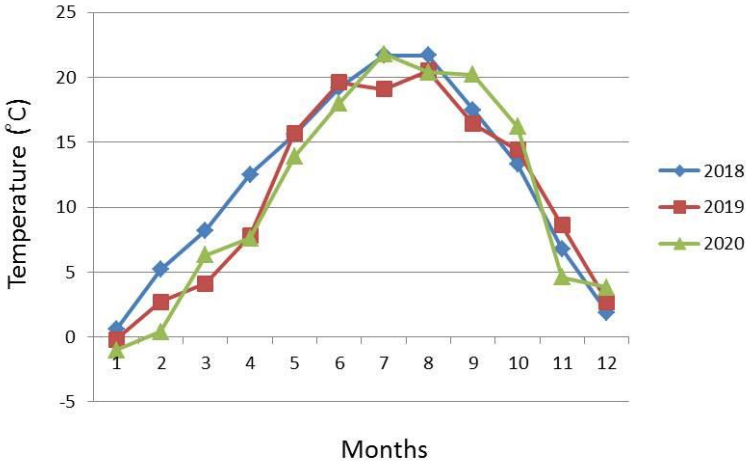

Figure 2. Monthly average air temperature values of the trial area for the years 2018-2020.
Şekil 2. 2018-2020 yılları için deneme alanına ait aylık ortalama hava sıcaklık değerleri.

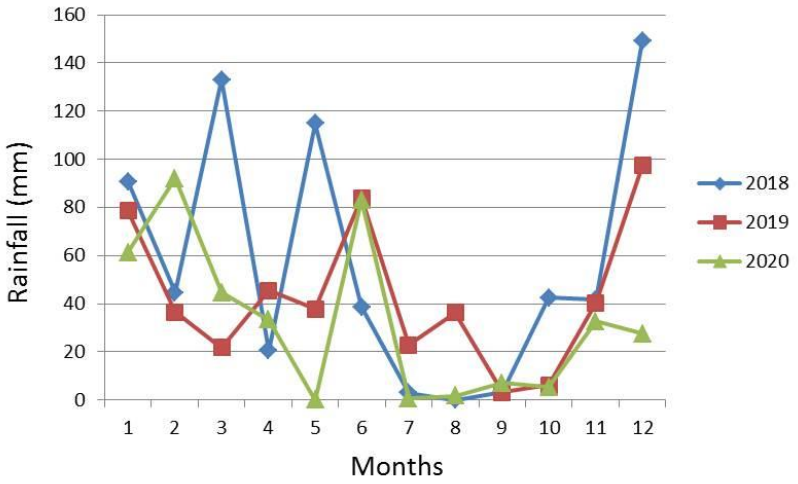

Figure 3. Monthly average precipitation values of the trial site for the years 2018-2020
Şekil 3. 2018-2020 yılları için deneme alanına ait aylık ortalama yağış değerleri.

The following measurements were made on plants for 2 years to determine the effect of farm manure applications on the vegetative growth of trees: Average tree height (cm), average trunk diameter (mm), average tree crown width (cm), average number of annual shoots, average annual shoot length (cm), average annual shoot diameter (mm), leaf chlorophyll content readings (measured using the Konica Minolta SPAD-502 Plus Brand Chlorophyll Meter), leaf anthocyanin amount (measured by (Opti Science ACM–200 Plus Anthocyanin Meter), leaf area determination (determined by ADC Bio Scientific Area Meter AM300 device, cm^2). In fruit measurements, 15 fruits in 3 replications were used in fruit measurements. Fruit weight (g), total soluble solids (brix,%), total acidity (g / 100 ml), fruit firmness (kg/cm^2), and yield (g) were measured.

The following variables were measured to determine farm manure applications on selected soil properties: Soil organic matter (OM): This variable was determined by using the Walkley-Black method (Kacar, 1994). Soil reaction (pH): It was determined by pH-meter with glass electrode in saturation sludge (Kacar, 1994). Total salt: In the saturation paste, the electrical conductivity was determined with a glass electrode Hanna EC-meter and the salt content was calculated (Bayraklı, 1987). Bulk density (BD): In the undisturbed soil samples taken with standard steel cylinders of 100 cm^3, and calculations were based on oven dry weight (Demiralay, 1993). Infiltration ratio rate (IR): Measured according to the double-cylinder infiltrometer method (Soil quality Ins. Staff., 1999). Penetration resistance (PNTR): Penetration measurements in the field were carried out according to Herrick and Jones (2002) by using hand penetration with 30˚ peak angle at 0-15 cm soil depth with 5 replications. PNTR results have been standardized using Equation 2 as reported in Aksakal and Öztaş (2010).

$$PNTR=M/A \quad \text{[Equation 1]}$$

PNTR: Penetration resistance, M: Manometer reading, A: Base area of the conical tip used

$$Y_c = Y_0 e^{\left(\frac{X-0.1}{0.132}\right)} \quad \text{[Equation 2]}$$

Yc: Corrected penetration resistance (kPa), Y0: Measured penetration resistance (kPa)

X: Soil moisture at the time of measurement (kg kg-1), 0.1: Moisture value selected for standardization (kg kg-1)

Statistical analysis

The experiment was set up in a randomized block design, with 3 doses (control, 5 kg $tree^{-1}$ and 10 kg $tree^{-1}$), with 3 replications and using 3 trees in each replication. Effects of subjects on measured variables Comparison of subject averages with ANOVA was performed with Duncan ($\alpha = 0.05$) and the comparison of two breeding years over measured variables was performed with t test. These statistical evaluations were made using IBM SPSS 20.0 package program and the means were separated according to Duncan's Multiple Range Test.

RESULTS and DISCUSSION

Changes in Plant Characteristics

Fertilization and fertilizer application play a vital role in the growth and yield of fruit trees. A well-nourished fruit tree not only provides good yields but also improves the quality and stays in a healthy and fertile state longer. Nitrogen and farm manure are important for the normal growth of plants. Nitrogen is the basic element of amino acid structure and plays an important role in protein synthesis, increasing chlorophyll content and speed of photosynthesis. Vegetative growth mainly depends on the nitrogen

supply and helps the root system to develop better. Nitrogen, which enhances the assimilation process through glycolysis and fatty acid synthesis, greatly increases the effectiveness of inorganic fertilizers when applied with farm manure, because it helps to keep organic matter in the root zone of urea and to make phosphate and potash suitable for the plant (Khan and Sharma, 2018).

Tree height, crown width, trunk diameter, shoot number, shoot length and shoot diameter were measured in November 2018, 2019, and 2020 in trees in the resting period in order to determine the effect of farm manure applications on the vegetative growth of trees (Table 1).

Table 1. The effect of manure application on some vegetative growth characteristics of pear trees.
Çizelge 1. Armut ağaçlarında çiftlik gübresi uygulamasının bazı vejetatif büyüme özelliklerine etkisi

		Mean ± Std. Deviation		
Features	Applications	2018	2019	2020
Tree Height (cm)	Control	229.44±43.45 NS	235.17 ± 43.62 NS	231.50±39.03 b*
	5 kg tree^{-1}	250.89±33.78	255.86 ± 38.11	292.75±25.21 a
	10 kg tree^{-1}	240.22±32.47	258.89 ± 48.65	263.75±20.69 ab
	Mean	237.5±38.73	246.00 ± 44.00	262.67±37.26
Crown Width (cm)	Control	109.33±37.64 NS	109.00 ± 31,63 NS	116.75±23.20 NS
	5 kg tree^{-1}	117.00±43.39	112.63 ± 40.05	142.50±45.86
	10 kg tree^{-1}	99.00±20.30	108.89 ± 16.21	125.50±13.53
	Mean	108.67±35.42	109.8 ± 29.92	128.25±29.92
Trunk Diameter (mm)	Control	52.93±9.01 NS	60.89 ± 5.90 NS	61.82±6.27 NS
	5 kg tree^{-1}	52.90±10.03	62.99 ± 7.08	67.84±3.61
	10 kg tree^{-1}	56.80±5.37	64.48 ± 4.88	66.44±3.83
	Mean	53.89±8.48	62.29 ± 5.98	65.36±5.05
Number of Shoots (Piece)	Control	17.78±10.36 NS	18.06 ± 9.69 NS	15.00±2.16 b*
	5 kg tree^{-1}	18.67±6.96	19.13 ± 6.56	20.50±7.94 ab
	10 kg tree^{-1}	16.11±7.11	20.00 ± 14.28	23.25±7.09 a
	Mean	17.58±8.70	18.09 ± 10.61	19.25±6.97
Shoot length (cm)	Control	28.50±13.59 NS	19.64 ± 11.69 b*	23.55±11.70 b*
	5 kg tree^{-1}	26.18±8.84	28.63 ± 10.40 a	29.25±13.32 ab
	10 kg tree^{-1}	27.62±13.76	27.78 ± 13.14 a	32.55±19.19 a
	Mean	27.69±12.58	22.73 ± 12.40	22.84±13.95
Shoot diameter (mm)	Control	7.35±1.47ab*	8.55 ± 0.83 b*	8.67±0.41 c*
	5 kg tree^{-1}	7.00±1.10 b	9.08 ± 0.58 a	9.97±0.41 a
	10 kg tree^{-1}	7.68±2.30 a	8.88 ± 0.80 ab	9.03± 0.83 b
	Mean	7.35±1.65	8.71 ± 0.81	8.89±1.21

NS. The difference between the applications is not significant.
* There is no statistically difference between the averages indicated with the same letter (P <0.05)

While there was no statistical difference between applications in 2018 and 2019 measurements in tree height measurements, half-dose, and full-dose farm manure applications were found to be significant compared to the control in 2020 measurements.

Crown width and trunk diameter were not found statistically different in the measurements made in three years. When the number of shoots was examined, there was no statistical difference in the year of application (2018) and one year after (2019), while in 2020, half-dose and full-dose farm manure applications were found to be higher than control. While there was no difference between trees in the measurements of shoot length in the year of application (2018), a statistically significant difference was found in half-dose and full-dose applications one year later (2019) and the following year (2020) compared to the control.

Shoot diameter was determined in the trees with the lowest half dose in the year of application, while the shoot diameter was found to be statistically different in trees selected as full dose and control. In the following first year (2019), the shoot diameter of the trees that were applied half dose and full dose increased, while the second year (2020) was the highest in the trees that were applied half dose. Cheng et al. (2001) found that leaf and shoot development in pear trees in spring was closely related to reserve nitrogen from seedlings. Finding and using nitrogen reserves in trees is as important as taking nitrogen from the soil (Titus and Kang, 1982; Tromp, 1983). With the development of spring, nitrogen is extracted from tree reserves in pears and only a small part of the nitrogen applied before flowering grows into the newly formed tissues.

Nitrogen application from the soil towards the end of summer is recommended to create a reserve for the following development period (Hart et al., 1997).

Most of the nitrogen required for flowering and fruit formation comes from the reserves stored in the tree from the previous development period. For this reason, it should be noted that for a good nutrition, a good nutrition is needed from the previous year. In some regions, nitrogen applications are made after harvest to meet the needs of the next season. However, the application must be done after the product is collected, otherwise the nitrogen application made close to the harvest in the summer period will negatively affect the fruit quality and storage life (Bright, 2005).

Kumar et al. (2013) reported that the application of NPK (600: 400: 400 g) + 20 kg farm fertilizer / plant in the Gola pear variety significantly increased the percentage of plant growth, plant spread and fruit set compared to control. Khan et al. (2016) observed that the application of 800 grams of nitrogen and 90 kg of farmyard manure per plant in pear varieties significantly improved shoot length, number of leaves per branch, fruit set and fruit retention.

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In other measurements made on leaves in 2019 and 2020 (leaf chlorophyll content (Spad value), anthocyanin and leaf area), a statistically significant difference was found between applications (Table 2). Leaf chlorophyll content was higher in half-dose and full-dose applications compared to the control. Leaf anthocyanin content was found to be higher in full dose application compared to the other two applications. While there was a statistically significant difference between applications in terms of leaf area in 2019, there is no difference in 2020. In the measurements made in 2019, half dose and full dose applications were found to be higher than control.

Table 2. Leaf chlorophyll content (Spad value), anthocyanin measurement and leaf area values
Çizelge 2. Yaprak klorofil içeriği (Spad değeri), antosiyanin ölçümü ve yaprak alanı değerleri

Applications	Leaf chlorophyll content (Spad value)*		Leaf anthocyanin content (LAC) *		Leaf area (cm^2)*	
	2019	2020	2019	2020	2019	2020
Control	42.6±6.5 b	41.2 ± 5.1 b	7.4 ± 1.8 b	6.8±1.2 b	30.7 ± 7.5 b	28.3 ± 6.7 NS
5 kg tree^{-1}	44.5 ±4.5 a	47.2 ± 3.6 a	7.8 ±1.5 b	6.7 ± 0.8 b	32.2 ± 8.1 ab	28.7 ± 5.6
10 kg tree^{-1}	44.0 ± 4.9 ab	46.4 ± 1.9 ab	8.8 ± 5.9 a	7.2 ± 0.9 a	33.9 ± 7.7 a	28.5 ± 4.4
Mean	43.4 ± 5.7	44.9 ± 4.3	7.9 ± 3.4	6.9 ± 0.9	31.9 ± 7.8	28.2 ± 5.6

* There is no statistically difference between the averages indicated with the same letter ($P < 0.05$) NS. The difference between the applications is not significant

Measurements and analyzes were made on the yield and fruit characteristics of the trees where half and full dose applications of farm manure were applied (Table 3). While there was no statistically significant difference between the applications in 2019 in terms of efficiency values, half and full dose applications were found to be significant in 2020 compared to the control. Fruit weight and fruit firmness properties were not statistically significant in both years.

According to Özbek (1981), nitrogen deficiency is seen more in soft stone fruit types due to their high nitrogen needs. In nitrogen deficiency, fruits remain small, ripen early and at the same time early fruit drop occurs and as a result, the fruit amount is significantly reduced. According to the same researcher, despite the continuous development of strong shoots in young apple and pear trees with nitrogen excess, flower bud formation decreases very much and the amount of product decreases. In addition, low nitrogen level affects fruit yield negatively (Kacar and Katkat, 1998).

In terms of fruit weight, Kumar and Chandel (2004) measured the maximum fruit weight of Red Bartlett pear variety with the application of NPK (700: 300: 600 g / tree). Kumar et al. (2013) reported that the application of NPK (600: 400: 400 g) + 20 kg farm fertilizer / plant significantly increased fruit weight, number of fruits per tree and fruit yield in pear compared to control. Khan et al. (2016) observed that 600 g of nitrogen treatment combined with 90 kg of farm manure significantly improved fruit set (6.95%),

fruit yield (25.14%), fruit weight (113.87 g) and fruit count. Arba et al. (2017), in their study investigating the effects of different nitrogen and phosphorus levels on pears, found that nitrogenous mineral fertilization increased fruit yield, especially fruit size (weight and size).

Table 3. Measurement values of fruit yield and characteristics
Çizelge 3. Meyve verimi ve meyve özelliklerinin ölçüm değerleri

Features	Applications	Mean ± Std. Deviation	
		2019	2020
Fruit Yield (g/tree)	Control	4659.7 ± 1443.9 NS	5696.1 ± 855.9 b*
	5 kg tree^{-1}	6250.0 ± 1117.9	8996.7 ± 2983.4 a
	10 kg tree^{-1}	6318.0 ± 1675.5	8221.7 ± 2831.9 a
	Mean	5742.6 ± 1481.9	7607.3 ± 2720.4
Fruit Weight (g)	Control	139.9± 22.4 NS	139.7±18.7 NS
	5 kg tree^{-1}	125.8 ± 19.7	130.6±29.4
	10 kg tree^{-1}	141.7 ± 9.2	139.8±25.9
	Mean	135.8 ±17.3	136.7±25.2
Total Soluble Solids (brix, %)	Control	14.7 ± 0.12 b*	11.7±1.24 b*
	5 kg tree^{-1}	15.2 ± 0.06 a	15.7±1.23 ab
	10 kg tree^{-1}	13.9 ± 0.12 c	17.6±1.20 a
	Mean	14.6 ± 0.57	15.0±3.2
Titratable acidity (g/100 ml)	Control	0.22 ± 0.05 NS	0.17±0.02 a*
	5 kg tree^{-1}	0.20 ± 0.00	0.08±0.01 b
	10 kg tree^{-1}	0.19 ± 0.02	0.10±0.00 b
	Mean	0.20 ± 0.03	0.12±0.04
Fruit Firmness (kg/cm^2)	Control	7.1 ± 0.2 NS	5.3±0.8 NS
	5 kg tree^{-1}	6.6 ± 1.5	3.9±0.7
	10 kg tree^{-1}	7.8 ± 0.6	3.5±0.7
	Mean	7.2 ± 0.9	4.2±1.4

NS. The difference between the applications is not significant
* There is no statistically difference between the averages indicated with the same letter (P <0.05)

According to the pomological analysis results made on fruits in 2019 and 2020, while there was a significant difference between applications in Total Soluble Solids (TSS) measurements, titratable acidity (TA) values were found to be statistically significant only in 2019. The TSS value was determined to be the highest in half-dose administration in 2019, half and full-dose administration in 2020. Control application was found to be statistically significant in both years. While TA was not statistically different in 2019, it was higher in the control application in the 2020 analysis. There was no statistical difference between the applications in both years in terms of flesh firmness. Akçay et al. (2009) In a pear cultivar trial conducted with a total of 13 varieties including Deveci cultivar between 1995 and 2002 under Yalova conditions, the TSS. They determined the value as 13.50%. Kappel et al. (1995) determined for the ideal pear, TSS reports that their values are between 13.6 and 17.2%. Kingston (1992) reported that lower titratable acidity values in pears were associated with increased nitrogen applications. Nava et al. (2008) stated in the study they carried out in Brazil by applying nitrogen from 0 to 200 kg per hectare in apples and that there was no change in titratable acidity values with increasing nitrogen doses.

In the Bagugosha pear variety, Yadav and Bist (2003) observed that increasing nitrogen levels did not have a significant effect on the soluble solids content (TSS) of fruits. However, the acidity of the fruits was significantly reduced in fruits obtained from trees given 60 g / tree / year and 90 g / tree / year of nitrogen. Kumar and Chandel (2004) observed that different nitrogen levels did not affect solubility and acidity in pear. It gives the acidity taste of the fruits in reasonable limits and the lack of optimum concentration causes an unpleasant taste. However, excess can make fruits tasteless even if other ingredients are optimal. In the Bagugosha pear variety, Yadav and Bist (2003) noticed that nitrogen had no significant effect on total sugar. Similar results were noted by Kumar and Chandel (2004) for the Red Bartlett pear variety, TSS and sugars, the highest at 800 g nitrogen and 90 kg farm fertilizer / plant application, and the minimum at control. Khan et al. (2017) applied different levels of nitrogen and farm manure on pears and it was observed that 600 g of nitrogen and 90 kg of farm manure increased the sugar content of fruits.

Changes in Soil Properties

Some descriptive statistics related to soil properties determined in Etryoshka pear garden are given in Table 4. According to the aforementioned table, the

pH values of the soils varied between 7.35 and 8.45 in the first year, while this variable varied between 7.33 and 7.99 in the second year. While the average organic matter value was 1.90% in the first year, this value decreased to 1.43% in the second year. Bulk density values of soils in the first year varied between 1.06-1.44 g cm^{-3}, and in the second year between 1.20-1.44 g cm^{-3}.The average penetration value was measured as 2.01 MPa for the first year, and the average infiltration rate value was measured as a very low value such as 3.68 mm h^{-1} for the first year. The average penetration value increased to 2.06 MPa in the second year, and the average infiltration rate decreased further and decreased to 3.36 mm h^{-1} compared to the first year.

Table 4. Descriptive statistics for measured soil variables
Çizelge 4. Ölçülen toprak değişkenleri için tanımlayıcı istatistikler

	Variables	N	Lowest	Highest	Mean	Std. Deviation
2019	pH	36	7.35	8.45	8.02	0.253
	Salt (%)	36	0.06	0.37	0.13	0.063
	OM (%)	36	0.72	3.30	1.90	0.917
	BD (g cm^{-3})	36	1.06	1.44	1.24	0.110
	PNTR (MPa)	36	1.06	2.75	2.01	0.519
	IR (mm h^{-1})	36	2.50	4.90	3.68	0.661
2020	pH	36	7.33	7.99	7.66	0.150
	Salt (%)	36	0.10	0.32	0.18	0.045
	OM (%)	36	0.92	2.38	1.43	0.470
	BD (g cm^{-3})	36	1.20	1.44	1.30	0.055
	PNTR (MPa)	36	1.56	2.63	2.06	0.349
	IR (mm h^{-1})	36	2.20	4.70	3.36	0.464

OM: Soil organic matter, BD: Dry bulk weight, PNTR: Penetration resistance, IR: Infiltration rate

ANOVA results showing the effects of farm manure application on measured soil variables are given in Table 5. As it can be understood from the examination of the table, first year farm manure application affected the salt variable at the level of P <0.01, while the effects of the applications on the other measured variables in the first year were found to be significant at the level of P <0.001. Second year applications did not affect salt and IR variables, its effect on pH was found to be P <0.05, and its effects on other variables were found to be significant at P <0.001.

The results of Duncan test comparing the average of soil variables over farm manure application doses are presented in Table 6. When the first year is examined in the Table 6, the average pH value in the soil of pear trees selected as control was 7.84c, this value increased to 8.08b with 5 kg $tree^{-1}$ dose of farm manure and 8.32a with 10 kg $tree^{-1}$ dose application. These values are statistically different from each other.

While the 5 kg dose of $tree^{-1}$ of the farm manure applications did not change the salt content of the soil statistically, the 10 kg dose of $tree^{-1}$ reduced the salt concentration to 0.08b% and the difference is statistically significant. The highest organic matter content was achieved with high dose administration (3.03a), followed by low dose administration (2.54b). The organic matter value measured for the control is at the lowest level (1.02c). As can be seen, all the averages determined for the organic matter variable are statistically different from each other.

The volume weight and penetration resistance variables changed to reflect this change in organic matter, and as the organic matter content increased, these values decreased, and the differences between the averages were found to be statistically significant. In terms of IR, there was no difference between control and 10 kg $tree^{-1}$ dose administration, while the highest value for this variable was measured for 5 kg $tree^{-1}$ dose administration and it is statistically different from the others (4.70a mm h^{-1}).

Table 5. ANOVA results showing the effects of farm manure application on measured soil variables
Çizelge 5. Çiftlik gübresi uygulamasının ölçülen toprak değişkenleri üzerindeki etkilerini gösteren ANOVA sonuçları

	Variables and significance levels					
	pH	Salt	OM	BD	PNTR	IR
2019	***	**	***	***	***	***
2020	*	NS	***	***	***	NS

OM: Soil organic matter, BD: Dry bulk density, PNTR: Penetration resistance, IR: Infiltration ratio. ^{NS}The difference between the applications is not significant

Table 6. Comparison of the means of soil variables over the farm manure application doses with Duncan test (α = 0.05)

Çizelge 6. Duncan testi ile çiftlik gübresi uygulama dozları üzerinden toprak değişkenlerinin ortalamalarının karşılaştırılması (α = 0.05)

Years	Application	Variable means					
		pH	Salt (%)	OM (%)	BD (g cm^{-3})	PNTR (MPa)	IR (mm h^{-1})
2019	Control	7.84c	0.16a	1.02c	1.34a	2.49a	3.28b
	5 kg tree^{-1}	8.08b	0.15a	2.54b	1.22b	1.78b	4.70a
	10 kg tree^{-1}	8.32a	0.08b	3.03a	1.09c	1.32c	3.47b
2020	Control	7.60b	0.18	1.06c	1.34a	2.34a	3.23
	5 kg tree^{-1}	7.68ab	0.17	1.45b	1.28b	1.86b	3.63
	10 kg tree^{-1}	7.76a	0.16	2.14a	1.24c	1.69c	3.55

OM: Soil organic matter, BD: Dry bulk density, PNTR: Penetration resistance, IR: Infiltration ratio

When Table 6 is analyzed over the second year values, it is seen that the applications made lose their power to change the salt concentration and infiltration rate. Both application doses showed similar effects on soil reaction. Although there were various changes in the averages of the other measured variables compared to the first year, the direction of the effects of the applications on the variables and the statistical significance of the differences between them were similar to the first year. The fact that the effectiveness of organic fertilizer application on the IR variable disappeared in the second year can be attributed to the fact that the applied manure lost its role on aggregation together with decomposition in the soil. In this case, since the pore continuity in the soil will change, the IR values in the manure applied plots were also measured similar to those of the control plot.

According to the results of this study, where different doses of farm manure were applied in order to improve some physical and chemical soil properties, which are also used as soil quality indicators in the Etryoshka variety pear garden, improvements were observed in the measured soil properties, especially at the end of the first year. The application of 10 kg of farm manure per tree contributed more to the positive effects obtained than the application of 5 kg. Both doses increased the pH value of the soils. The ability of farm manure to affect pH depends on the amount of various substances in it (Uçgun et al., 2019). Alagöz et al. (2006), statistically significant increases in soil pH were achieved with organic wastes of different origin. The comparison of the means of soil variables belonging to the first year and the second year with the t test is given in Table 7.

Table 7. Comparison of the means of soil variables of the first year and the second year with the t test

Çizelge 7. İlk yıl ve ikinci yıl toprak değişkenlerinin ortalamalarının t testi ile karşılaştırılması

pH	Salt	OM	BD	PNTR	IR
***	**	**	**	NS	*

OM: Soil organic matter, BD: Dry bulk density, PNTR: Penetration resistance, IR: Infiltration ratio
^{NS}. The difference between the applications is not significant

Accordingly, first year and second year pH averages are different from each other and this difference is statistically significant at P <0.001 level. The first year and the second year are also different from each other in terms of total salt concentration, total organic matter content and bulk density, and all of these differences were statistically significant at the P <0.01 level. While there was no statistically significant difference in penetration resistance between the two years of the trial, the infiltration ratio differences between the two years were significant at the P >0.05 level. The differences between years can be attributed to the possibility that the applied organic fertilizer may have decomposed in the soil.

At the Etryoshka pear garden, salt content of the soils was reduced with a dose of 10 kg tree^{-1} at the end of the first year, but the farm manure applied in the second year lost its effectiveness on this variable. This can be attributed to the fact that the organic material may have decomposed substantially by the end of the first year and that chemical fertilizer supplementation on each tree may have increased salinity in the environment. Applied wastes of organic origin may affect the salt concentration of soils in different directions and levels depending on environmental factors, the properties of the organic regulator and anthropogenic applications. In this study, the organic matter content of the soil was increased from "low" level (1.02%) to "medium" level (3.03%) at the end of the first year with the application of 10 kg of farm manure per tree. A similar situation is valid for 5 kg tree^{-1} dose application, although the increase in the amount of organic matter is lower (2.45%). At the end of the

second year, while the level of organic matter in soils where high dose application was applied was preserved as "medium" (2.14%), the low dose application lost its effectiveness and the level of organic matter fell back to the "low" class (1.45%). These results show that high dose application can be successful in maintaining the organic matter level of the soil subject to the study in the second breeding year. The effects of organic-based regulators in increasing soil organic matter content have been known for a long time. In another study conducted on the subject (Yakupoğlu and Özdemir, 2007), it was reported that various organic wastes applied to eroded soils increased the organic matter contents of soils after a certain incubation period depending on the application dose and this increase had a positive effect on the useful microelement contents of soils. In this study carried out in Etryoshka pear garden, it was observed that one-year incubation was suitable for the application of 5 and 10 kg tree^{-1} dose of farm manure, but when the time increased to two years, the low dose lost its effectiveness.

The effects of farm manure applications on pH, total salt and organic matter, which are chemical soil quality indicators, were examined above and changes in chemical soil fertility were discussed. However, the physical productivity of soils is as important as their chemical efficiency in terms of their productivity and sustainable use (Yakupoğlu and Özdemir, 2012). At the end of the first harvest season, a 16% decrease in bulk density compared to the control was achieved with the application of 10 kg tree^{-1} dose of farm manure in the Etryoshka pear garden. This decrease remained at approximately 9% in 5 kg tree^{-1} dose application. After the second harvest season, an increase in the weight of the soil of the trees treated with farm manure was observed, but it was found that the effectiveness of the applications continued depending on the application dose. The decrease in the volume weight reduction effect of farm manure at the end of the second year can be explained by the decomposition of organic material as a result of microorganism activities. By increasing the organic matter level of the soil, soil physical properties can be improved (Barzegar et al., 2002; Anikwe et al., 2003). Depending on the type and characteristics of the organic stabilizer applied to the soil, different levels of reduction in the volume weight of the soil can be achieved or sometimes the volume weight may not change depending on the application dose insufficiency or soil properties. For example, Alagöz et al. (2006), while the application of litter compost and processed chicken manure did not affect the volume weight of the soil, the researchers found that the effect of leonardite on the volume weight was significant at the level of $P < 0.05$.

The penetration value of the soils was reduced from 2.49 MPa to 1.32 MPa with a dose of 10 kg per tree at the end of the first year and it was reduced to 1.78 MPa with 5 kg dose application. At the end of the second year, although the dose effects were the same as the first year, there were increases in the average penetration resistance values. Penetration limit value for ideal cultivation in agricultural land is 2 MPa (Gupta et al., 1990). When the penetration resistance exceeds this value, various problems arise, and when this value exceeds 3, root growth is limited in different rates depending on other factors (Busscher and Sojka, 1987; Yakupoğlu et al., 2013). With farm manure applications, the volume weight could be reduced below the limit value of 2 MPa in the Etryoshka pear garden. This situation can be explained by the decrease in the volume weight of farm manure application. At the end of the first year, the dose of 10 kg tree^{-1}, which was evaluated as a full dose application, was not successful in increasing the infiltration rate of the soil, while the dose of 5 kg tree^{-1} farm manure, which was a half dose application, slightly increased the infiltration rate of the soil and this increase was found to be statistically significant. This complex result actually indicates that the applications do not affect the infiltration rate, but that the change is caused by other factors, and the results of the infiltration measurements made at the end of the second harvest season show that this variable is not affected by the farm manure application.

It is thought that soil compaction is the leading cause of the other factors. In a study carried out in a berry fruits orchard with an Etryoshka pear garden (Balcı and Yakupoğlu, 2018), a serious soil compaction is pointed out, although it is divided in area. Agricultural activities are an important factor in top soil compaction. Especially one of the biggest effects of agricultural traffic and processing tools on soil is soil compaction. The data of this study carried out in the Etryoshka pear garden also show a serious middle depth compression in the field.

CONCLUSIONS

As a result, when the literature is examined, it shows that farm manure and nitrogenous fertilization rate are the most important agricultural inputs that largely define pear yield and quality. When applied together with farm manure, the effectiveness of inorganic fertilizers increases greatly. Farmyard manure is a valuable soil improver that heals and restores a number of natural properties, including soil fertility. Nitrogen application is extremely important at a certain level and excessive nitrogen application reduces the tolerance of pear to pests and diseases. It was concluded that the most successful manure application in reducing the bulk density and penetration resistance, which is used as the

evaluation criteria of soil compaction, was 10kg tree^{-1} for both years. The applied manure lost its effect on increasing the infiltration rate in the second year.

In the light of the results of this study carried out in Etryoshka pear garden and in the light of the discussions above, in the pear garden in the semi-arid climate zone in the Gedikhasanlı region, the application of at least 10 kg of farm manure per tree every year to improve the physical and chemical properties of the soil physical and chemical will improve its properties. Even increasing the application dose to 12-14 kg per tree may increase the infiltration rate.

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Authorship Contribution Statements

The contribution of the authors is equal.

Conflict of Interest

The authors have declared no conflict of interest.

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Molecular Characterization and Assessment of Population Structure of Hulled Wheats

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ABSTRACT
Analysis of genetic diversity among genotypes and differentiation among populations are crucial for determination of conservation strategies and one of the best plant breeding approaches. In this study, ISSR markers were used to determine genetic variation and population structure in 23 hulled wheats. Together with control durum wheat and bread wheat registered cultivars, 32 wheat genotypes were analyzed with 14 ISSR markers located throughout the wheat genome. Number of alleles per locus ranged from 3 to 13 and the polymorphism information content (PIC) value ranged from 0.27 for the UBC-852 to 0.37 for the UBC-824 with an average of 0.33. High levels of polymorphism ratio (100%) were observed for ISSR primers. Mean number of polymorphic alleles (N), expected heterozygosity (He), PIC, number of effective allele (Ne), Shanon's information index (I) and genetic variation (F_{ST}) were determined as 10.21, 0.42, 0.33, 1.78, 0.61 and 0.63, respectively. UPGMA analysis based on dice genetic similarity ranged between 0.981 and 0.112 showing the high genetic diversity among hulled wheat genotypes. Results showed that the ISSR markers provided reliable and reproducible fingerprinting profiles for assessment of population structure and genetic diversity of hulled wheat genotypes. These molecular variations obtained from present study can be used in parent choosing for breeding studies.

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Kavuzlu Buğdayların Moleküler Karakterizasyonu ve Popülasyon Yapısının Değerlendirilmesi

ÖZET
Genotipler arasındaki genetik çeşitliliğin ve popülasyonlar arasındaki farklılaşmanın analizi, koruma stratejilerinin belirlenmesi ve en iyi bitki ıslah yaklaşımlarından biri için çok önemlidir. 23 kavuzlu buğdayda genetik varyasyonu ve popülasyon yapısını belirlemek için ISSR markörleri kullanılmıştır. Kontrol makarnalık buğday ve ekmeklik buğday tescilli çeşitleriyle birlikte, 14 ISSR markörü ile 32 buğday genotipi analiz edildi. Lokus başına alel sayısı 3 ile 13 arasında ve polimorfizm bilgi içeriği (PIC) değeri UBC-852 için 0.27 ile UBC-824 için 0.37 arasında ve ortalama 0.33 olarak belirlenmiştir. ISSR primerleri için yüksek düzeyde polimorfizm oranı (%100) gözlenmiştir. Ortalama polimorfik alel sayısı (N), beklenen heterozigotluk (He), PIC, etkili allel sayısı (Ne), Shanon bilgi indeksi (I) ve genetik varyasyon (F_{ST}) sırasıyla 10.21, 0.42, 0.33, 1.78, 0.61 ve 0,63 olarak belirlenmiştir. Dice genetik benzerliğine dayanan UPGMA analizi, kavuzlu buğday genotipleri arasındaki yüksek genetik çeşitliliği göstermiş olup 0.981 ile 0.112 arasında değişmiştir. Sonuçlar, ISSR markörlerinin kavuzlu buğday genotiplerinin popülasyon yapısı ve genetik çeşitliliğinin değerlendirilmesi için güvenilir ve tekrarlanabilir parmak izi profilleri sağladığını göstermiştir. Mevcut çalışmadan elde edilen bu moleküler varyasyonlar, ıslah çalışmaları için ebeveyn seçiminde kullanılabilir.

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INTRODUCTION

Hulled wheats are one of the first domesticated crop species. The grains belong to emmer (*Tiriticum dicoccon* L.) and einkorn (*T. monoccocum* L.) wheats discovered in Cayonu excavation dated back to 6500-7000 B.C. in Turkey (Harlan, 1998). Hulled wheats contain all ploidy levels such as diploid, tetraploid and hexaploid. They are known as transitional positions between wild and cultivated forms of wheat during the evolutionary process. Diploid hulled wheat, *T. monoccocum* var. *monoccocum*, is considered as a new genetic resource to improve *T. durum* and *T. aestivum* that contain a genome in their genetic structures (Heun et al., 1997). Currently, admixture of emmer and einkorn populations is generally cultivated in rural regions of Kastamonu, Sinop and Cankırı provinces of Turkey. However, the cultivated hulled wheat acreage has been decreasing (Karagoz, 1996). A small number of Turkish einkorn and emmer wheat together with durum and bread wheat landraces was characterized (Karagoz and Zencirci, 2005).

Genetic diversity is a cornerstone for any plant breeding program (Khush, 2002). Genetic diversity studies conducted with molecular markers are advantageous because they are not affected from environmental factors and genetic variation can be estimated using a small amount of DNA (Prasad et al., 2000). Different molecular markers such as RAPD, SSR, AFLP and ISSR have been used for genetic characterization of diverse cereal species containing wheat accessions. These molecular markers had been used in wheat for detecting genetic variation, genotype identification, and genetic mapping (Najaphy et al., 2011; Abou-Deif et al., 2013). Inter simple sequence repeats (ISSRs) have become broadly used for different goals in plant genetic researches (Karaca and Izbirak, 2008). The ISSR marker is one of the universal DNA markers amplified regions among microsatellite sequences by the polymerase chain reaction (PCR) (Gupta et al., 1994). The technique is commonly used in studies of cultivar identification, genetic diversity, genetic mapping, evolution and molecular ecology (Yang et al., 1996). Najaphy et al. (2012) showed that ISSR markers together with agronomic and morphological characters of wheat can be used to determine molecular variation in wheat genotypes. The first aim of this study was to evaluate genetic diversity in hulled wheat land races collected from Kastamonu province of Turkey by ISSR markers. The second aim was to evaluate the informativeness of ISSR markers for detecting molecular variation in hulled wheat.

MATERIAL and METHOD

Plant Material

Twenty three (23) hulled wheat populations used in this study were collected in different parts of Kastamonu province. Also, four registered durum (*Tiriticum durum* L.) wheat cultivars named as Kızıltan-91, Ç-1252, Sarıçanak-98, Y.popülasyon and five registered bread wheat (*Tiriticum aestivum* L.) cultivars named as Doğankent-1, Kıraç-66, İkizce-96, Bayraktar-2000, Bezostaja-1 were used in this study. Seeds of hulled wheat which were pre-tested in field conditions was planted in viols with durum and bread wheat cultivars. Plants germinated from seed were used as genotypes for molecular identification.

DNA Extraction

Wheat seeds were grown in plastic container in room temperature. Fresh leaves belonging to each wheat genotype were harvested for DNA isolation. DNA extraction was carried out using the CTAB method modified by Gulsen et al. (2005). Genomic DNA was suspended with 50 µl 1x TE (Tris Edta) buffer for stock solution and stored at -80 ^{o}C till use. DNA concentration of each genotype was measured through both agarose gel (1%) and NanoDrop (BioSpec-nano Shimadzu Biotech). Final DNA concentration was adjusted to 5 ng μL^{-1} to be used in ISSR-PCR amplification and then DNA samples were stored at -20 °C until use.

Molecular Analyzes

Fifty-four ISSR markers were firstly screened for consistency and their ability to produce polymorphism in wheat using DNA of randomly selected eight wheat genotypes. From 54 ISSR primers, 14 were selected for PCR amplification due to their polymorphic, clear, strong and reproductive bands (Table 1). PCR volume for each ISSR primer to characterize the thirty two wheat genotypes was adjusted to 15 µl total reaction volume including; 1.8 µl 10X PCR buffer, 1.5 µl $MgCl_2$ (25 mM), 6.5 µl dH_2O, 2 µl of 2 mM dNTP, 1.3 µl of 0.6 mM primer, 0.4 µl Taq polymerase (5 U μl^{-1}) and 1.5 µl of 20 ng μl^{-1} DNA. Amplified PCR products were separated on 2% agarose gel and agarose gel was stained with ethidium bromide. Then, it was photographed under UV light and ISSR band polymorphism visually evaluated. A 100 bp DNA

ladder was used in order to estimate molecular weight of ISSR bands.

ISSR band profiles were scored for each ISSR primer to create a binary format matrix. Only reliable and reproducible polymorphic bands were recorded. The ISSR binary data matrix obtained was used to calculate the dice similarity coefficient. Different genetic diversity parameters of individual ISSR markers were estimated. While effective alleles number (Ne) and Shannon's Information Index (I) was calculated using PopGene software 1.32 version, polymorphism information content (PIC) and gene diversity (He) were estimated with PowerMarker software ver. 3.25. The F_{ST} values of ISSR markers were computed by Arlequin software. Cluster analysis was conducted by complete linkage method using NTSYS-pc software version 2.02 (Rohlf, 2000). A dendrogram was constructed based on binary data of dice similarity matrix by unweighted pair group method with arithmetic average (UPGMA) cluster analysis. Principal component analysis (PCA) was also performed via this software. To assess wheat population structure and assign individuals to wheat populations, ISSR data were analyzed using a Bayesian approach in Structure v.2.3.4 (Pritchard et al., 2000). The number of supposed clusters (K) was set from two to nine. The data obtained from Structure software was then uploaded to Structure Harvester which is an online tool. The best K value (K subpopulations) was determined according to the protocol of Evanno et al. (2005). In addition, genetic diversity parameters for each population were assessed in terms of He, F_{ST}, Nm and Shanon's information index. He and F_{ST} values for populations were obtained from Structure software v.2.3.4. In addition, Nm and Shanon's information index were calculated by PopGene software 1.32 version.

RESULT and DISCUSSION

Different marker systems based on DNA had been used in wheat genetic studies such as RAPD, RFLP, AFLP, SSR, and ISSR. ISSR markers are widely polymorphic and could be used for wheat cultivars identification as many authors reported (El Maati et al., 2004; Motawei et al., 2007; Karaca and Izbirak, 2008; Sofalian et al., 2008; Carvalho et al., 2009). Furthermore, Motawei et al. (2007) also made genotypic identification in emmer and durum varieties by using ISSR primers. Fahmy et al. (2016) showed that ISSR markers could be used as fairly informative markers for gene tagging and genome mapping. We carried out molecular characterization using ISSR DNA marking technique for wheat genotypes includeing 9 emmer (*T. dicoccum*) and 14 einkorn (*T. monococcum*) and 9 registered cultivars. Fifty-four ISSR primers were tested and 14 of them were selected for the genetic diversity study (Table 1) and they were used to characterize and evaluate the genetic variation of the wheat genotypes. The selected primers generated totally 148 bands ranging from 3 to 18, with average 10.21 (95.42%) bands per primer; these values show high genetic variability (Table 2).

Table 1. Eigenvalues of first three main components of PCA
Çizelge 1. PCA'nın ilk üç ana bileşeninin değerleri

Main components	Eigenvalue	Percent	Cumulative values
1.	17.29	54.05	54.05
2.	8.32	26.02	80.07
3.	1.05	3.28	83.36

Allele length of ISSR markers was ranged from 180 bp to 980 bp. The maximum number of alleles was observed at UBC-852 and their size ranged from 200 to 880 bp. An average of 10.8 polymorphic alleles per locus was detected for the thirty-two wheat genotypes. Primers UBC843, UBC822, UBC840, UBC823, UBC851, UBC852, UBC818, UBC815 and UBC826 were the most informative and they have 100% polymorphism ratio. With 75% polymorphism ratio, primer UBC824 showed the lowest number of bands (Table 1). These polymorphism ratios were quite high than the polymorphism rates reported by Gulbitti et al. (2007) for *T. dicoccoides*, *T. monococcum* ssp. *boeoticum*, and *T. urartu,* 32.34%, 42.63% and 27.71%, respectively. However, the polymorphism levels were lower than those obtained by Sofalian et al. (2008) using ISSR markers on 27 wheat genotypes including 18 spring landraces and 9 cultivars. Du et al. (2002) reported 87% of polymorphism in 47 hybrid wheats with 11 ISSR markers. Najaphy et al. (2011) observed that 10 ISSR primers generated 80.2% polymorphism among 30 wheat accessions. Morgante et al. (2002) showed that the polymorphism of ISSR markers depends on the microsatellite frequency and distribution throughout the genome of the species. PIC of ISSR markers recorded mean value of 0.33, with a variation ranging from 0.27 to 0.37. The lowest and the highest PIC values were obtained for primer UBC852 and UBC824, respectively. In the present study, the mean PIC value was higher than ISSR markers used by Najaphy et al. (2011). This difference is probably due to use of different genotypes and ISSR markers. The highest value of Nei's genetic diversity (He) for ISSR

primers was observed for the UBC824 (0.49) and the lowest value was observed for the UBC852 (0.33). Ne (Number of effective alleles) values ranged from 1.59 (UBC852) to 1.96 (UBC824). UBC822, UBC851, UBC818 and UBC815 presented the highest Ne values. UBC852 has the lowest Shannon's index (I) ranging from 0.51 to 0.68, with mean 0.61 (Table 1).

Table 2. Genetic diversity parameters of ISSR markers used for assessment of genetic variation of wheat genotypes

Çizelge 2. Buğday genotiplerinin genetik varyasyonunun değerlendirilmesinde kullanılan ISSR markörlerinin genetik çeşitlilik parametreleri

No	Primers	SP	N	AL	PB	He	PIC	I	Ne	F_{ST}
1	UBC–843	$(CT)_8RA$	11	810–380	100	0.42	0.33	0.61	1.75	0.59
2	UBC–853	$(CT)_8RT$	15	950–280	93.7	0.43	0.34	0.63	1.83	0.63
3	UBC–846	$(CA)_8RT$	11	950–300	91.6	0.39	0.30	0.57	1.72	0.61
4	UBC–824	$(TC)_8G$	3	920–700	75	0.49	0.37	0.68	1.96	0.70
5	UBC–815	$(CT)_8G$	12	920–180	100	0.45	0.35	0.65	1.85	0.41
6	UBC–852	$(CT)_8RA$	18	880–200	100	0.33	0.27	0.51	1.59	0.43
7	UBC–813	$(CT)_8T$	5	900–450	83.3	0.40	0.31	0.59	1.71	0.47
8	UBC–845	$(CT)_8RG$	12	980–180	92.3	0.36	0.28	0.54	1.65	0.47
9	UBC-840	$(GA)_8YT$	12	920–220	100	0.41	0.32	0.60	1.76	0.63
10	UBC–823	$(TC)_8C$	10	920–490	100	0.43	0.34	0.63	1.81	0.42
11	UBC–851	$(GT)_8YG$	8	740–250	100	0.46	0.35	0.66	1.87	0.99
12	UBC–826	$(AC)_8C$	12	850–350	100	0.41	0.33	0.61	1.72	0.68
13	UBC–818	$(CA)_8G$	8	880–230	100	0.45	0.35	0.65	1.86	0.85
14	UBC–822	$(TC)_8A$	6	920–310	100	0.46	0.36	0.66	1.88	0.98
Total			143							
Mean			10.21		95.42	0.42	0.33	0.61	1.78	0.63

SP, Sequence of primers; N, Number of polymorphic allels; AL, Allel length (bp); PB, Percentage of polymorphic bands (%); He, Nei's (1973) gene diversity index; PIC, Polymorphism information content; I, Shannon's information index; Ne, Effective alleles number; FST, Fixation index

In order to investigate genetic relationships among emmer and einkorn wheat genotypes cluster analysis were performed as based on dice similarity coefficients. Dice similarity matrix based on ISSR markers was used to classify the wheat genotypes. The mean value of dice similarity coefficient was 0.553 and genetic similarity ranged from 11% to 98% for all wheat genotypes based on ISSR analysis. The similarity values clearly revealed significant differences among the hulled wheat genotypes (Kastamonu=KST). In terms of genetic similarity, KST18, KST10 and KST7 genotypes were the closest genotypes with 0.981 similarity coefficient. On the contrary, some genotypes showed low genetic similarity, such as KST12-KST13 (0.1157) and KST10-KST13 (0.1148). KST6-KST13 with 0.112 dice coefficient had the lowest genetic similarity (Figure 1). Furthermore, KST6-KST10 with 0.9541 similarity coefficient and KST6-KST16 with 0.9009 similarity coefficient were determined to be the closest to each other (Figure 2). The dendrogram resulting from UPGMA cluster analysis showed that two main clusters (A and B clusters) were classified into thirty-two wheat genotypes (Figure 1). Cluster A included generally *T. dicoccum* genotypes, but cluster B consists of mainly *T. monococcum* genotypes. The first main group (A) was divided into two sub groups and the first sub-group (A1) consisted of emmer wheats (*T. dicoccum* L.) population excluding only one emmer wheat genotype (Figure 2). On the other hand, the second sub-group of cluster A (A2) comprises tetraploid and hexaploid registered cultivars. The durum wheat genotypes were clustered much closer to the crustacean tetraploid subgroups (A1) than the hexaploid varieties (Figure 1). Based on ISSR dendrogram, the first group (PopA) includes 11 genotypes (KST10, KST8, KST7, KST6, KST16, KST1, KST3, KST12, KST14, KST4 and KST11) along with 9 registered cultivars. The second main group B was also formed mainly diploid hulled wheats (*T. monoccocum* var. *monoccocum*). The group B includes KST15, KST2, KST20, KST23, KST21, KST5, KST8, KST13, KST9, KST22, KST19 and KST17. Among the registered cultivars, İkizce-96 and Bayraktar-2000 grouped close to each other in the UPGMA dendrogram. The UPGMA dendrogram obtained in present study distinguished the wheat genotypes according to their ploidy level (tetraploid and diploid). Gurcan et al (2017) demonstrated that SSR markers effectively grouped hulled wheats (emmer and einkorn) according to their ploidy level. Our results show that the ISSR marker system is effective to mainly distinguish wheat genotypes, consistently with their ploidy levels. Einkorn (*T. monococcum*) and emmer (*T. diccoccum*) wheat generally were classified separately. Also Motawei et al. reported that ISSR and RAPD primers divided

wheat genotypes into two main groups based on their pedigrees. Carvalho et al. (2009) stated that ISSR primers group wheat genotypes based on their ploidy levels. Their results are consistent with this results.

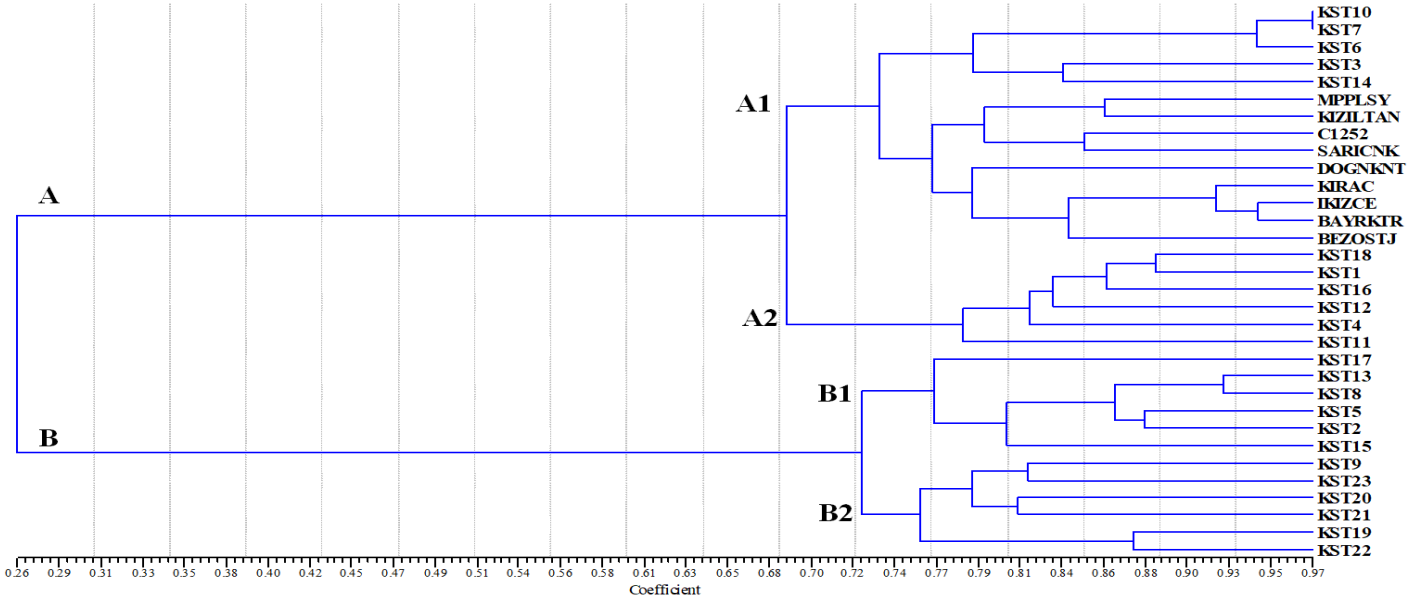

Figure 1. UPGMA dendrogram generated by using dice similarity index
Şekil 1. Dice benzerlik indeksi kullanılarak oluşturulan UPGMA dendrogramı

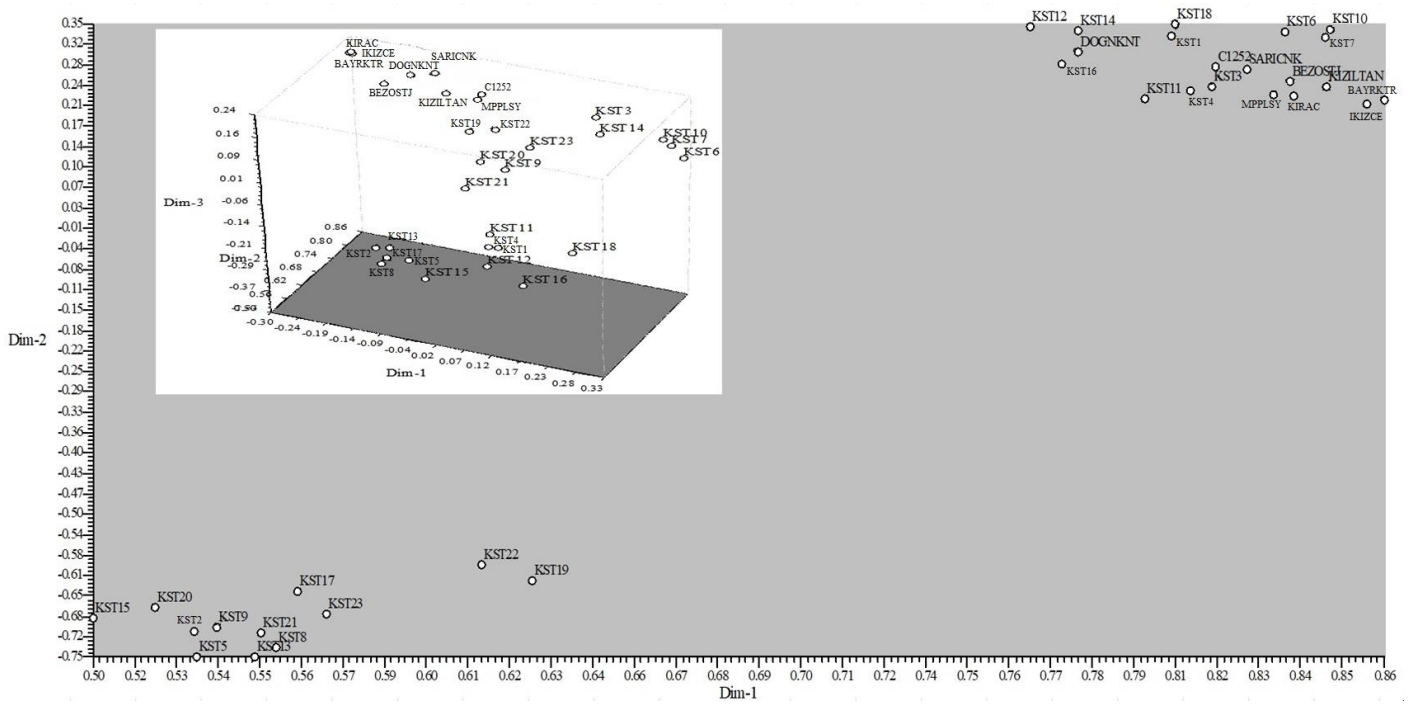

Figure 2. Principal component analysis based on ISSR data of 32 wheat genotypes
Şekil 2. 32 buğday genotipinin ISSR verilerine dayalı temel bileşen analizi

In this study, the principal component analysis (PCA) has also been performed to reveal the genetic diversity. Two and three-dimensional graphics were created by using NTSYS-pc software version 2.02. The results of PCA are demonstrated in Figure 2 and showed that all wheat genotypes are classified genetically into two groups similarly to UPGMA dendrogram. Overall, *T. monococcum* (einkorn) and *T. dicoccum* (emmer) were fairly separated by the PCA. First group in PCA include einkorn wheat genotypes except for KST17 (emmer). Second group consists of emmer, bread and durum wheat genotypes. The cumulative sum of the eigenvalues of first three divisions for two and three-dimensional graphs in

basic components analysis explains 83.36 of total variation. First component of PCA with Eigenvalues was explained 54.05 of molecular variation. Principal Component Analysis (PCA) based on molecular data was performed to reveal the genetic difference between genotypes. According to the PCA result, the additive sum of the first three main components was determined as 83.36% (Table 1).

Analysis of genetic structure of 32 genotypes using bayesian approach in Structure software explained to population structure in wheat. Analysis of ISSR data produced the highest log likelihood scores when number of populations was set at two, which was consistent with clustering based on genetic distance. All the wheat genotypes were classified into two populations as PopA and PopB. Wheat genotypes are represented by vertical columns colored red and green (Figure 3).

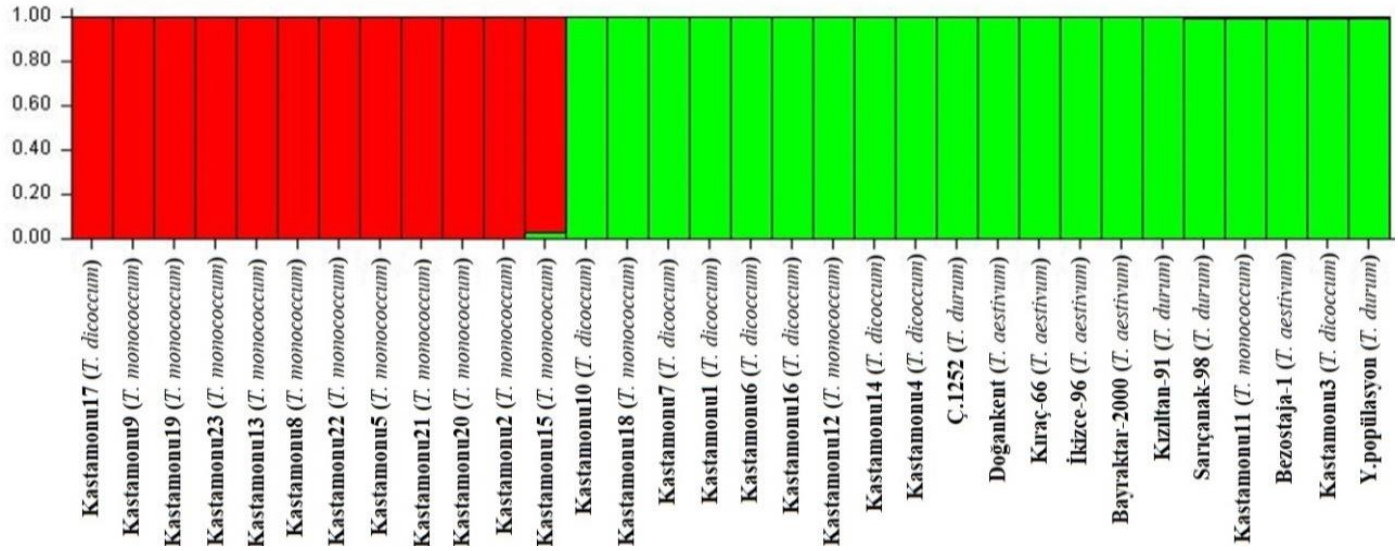

Figure 3. Population structure of 32 wheat genotypes for K=2
Şekil 3. K=2 için 32 buğday genotipinin popülasyon yapısı

T. monoccuccum and *T. diccocum* genotypes clustered in PopA and PopB, respectively. PopB includes both durum and bread wheat genotypes. According to membership coefficient, 18 genotypes were assigned into the largest subpopB (PopB) included *T. diccocum* (einkorn) genotypes mostly from Kastamonu. According to the structure program, genotypes with population membership coefficient below 80% were assumed to be hybrid, and genotypes above 80% were assumed to be pure (Gurcan et al., 2017). All wheat genotypes in this study were considered as pure genotypes since population membership coefficients based on sutrucrure analysis were above 0.80. Each wheat genotype was shown by a coloured bar according to estimated membership to each of PopA and PopB. The pairwise Fst values are statistically significant for the comparisons between subpopulations. The expected heterozygosity, FST, Nm and I were estimated to analyse the genetic structure of wheat populations by PopGene32 software. PopA (0.67) and PopB (0.63) have FST values approximately resemble to each other. The average of expected heterozygosity (He) and Shannon's Index (I) for two populations were found to be 0.1834 and 0.265 respectively. The highest and the lowest Nei's gene diversity (He) were related to PopB (0.188) and PopA (0.178), respectively. Shannon's diversity index was higher for *T. diccocum* (PopB). Average of Nm referring gene flow was found to be 0.135. The low genetic divergence was observed within subpopulations (Table 3).

Table 3. Genetic variation parameters among wheat genotypes
Çizelge 3. Buğday genotipleri arasındaki genetik varyasyon parametreleri

Subpopulation	He	F_{ST}	Nm	I
PopA	0.1786	0.6787	0.12	0.26
PopB	0.1882	0.6308	0.15	0.27
Average	0.1834	0.6547	0.135	0.265

He, Nei's (1973) gene diversity index; F_{ST}, Fixation index; Nm, Estimate of gene flow from F_{ST}; I, Shannon's information index

CONCLUSION

The present study definitely indicated that the 32 wheat genotypes could be separated with ISSR primers having a high level of polymorphism ratio. As result, the present study demonstrates that ISSR markers are useful for molecular characterization and analysis of population of hulled wheats. In addition, the ISSR markers identified can provide useful information for breeding programs to select the individuals. However, the utility of ISSR markers in separating wheats according to ploidy level should be confirmed by future cytological studies.

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Author Contribution Rates

The authors declare that they contribute equally to the article.

Conflict of Interests

Authors declare that there is no conflict of interests.

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Meteorolojik Faktörler Yardımıyla Bazı Buğday Çeşitlerinde Verim Tahmini: Şanlıurfa Ceylanpınar Örneği

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ÖZET

Buğday dünya genelinde açlığın izlemesinde temel gösterge kabul edilen en önemli ürünlerden biridir ve artan nüfusun ihtiyacını karşılayacak miktarda buğday üretiminin sağlanması bir zorunluluktur. Bu çalışmanın amacı; Şanlıurfa Ceylanpınar yöresinde meteorolojik faktörlerin kuru koşullarda yetiştirilen buğday çeşitlerinin verimleriyle arasındaki ilişkilerin büyüme dönemlerine göre ortaya konulması ve çeşit bazlı verim tahmin modellerinin geliştirilmesidir. Araştırmada buğday büyüme dönemleri; Çimlenme/Çıkış, Kardeşlenme, Sapa Kalkma, Başaklanma, Tane Oluşumu ve Olgunlaşma olarak ele alınmış, toplam vejetasyon süresi ortalaması Dinlenme periyoduyla birlikte 235 gün olarak belirlenmiştir. Yörede araştırma periyodu içinde en çok ekilen buğday çeşitlerinden bazıları olan Pandas, Fırat-93, Pehlivan ve Çeşit-1252 araştırmanın konusuna dâhil edilmiştir. Çalışmada Pandas ve Fırat-93 çeşitlerinde vejetasyon süresi yağışlarının (sırasıyla r=0.74 ve r=0.73), Pehlivan çeşidinde ortalama rüzgâr hızının (r=0.74) ve Çeşit-1252 çeşidinde Çimlenme/Çıkış dönemi yağışlarının (r=0.88) verimler üzerinde en yüksek etkili değişkenler olduğu belirlenmiş, çeşit bazlı tahmin modelleri geliştirilmiş ve ilgili yıllar için verim tahminleri yapılmıştır.

Tarımsal Yapılar ve Sulama

Araştırma Makalesi

Makale Tarihçesi
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Anahtar Kelimeler
Şanlıurfa Ceylanpınar
Buğday Çeşitleri
Büyüme Dönemleri
Meteorolojik Faktörler
Verim Tahmin Modeli

Yield Estimation in Some of the Wheat Varieties with the Help of Meteorological Factors: The Case of Şanlıurfa Ceylanpınar

ABSTRACT

Wheat is one of the most important products that is accepted as a primary indicator in the monitoring of hunger worldwide and it is a necessity to ensure that the amount of wheat production meets the needs of the increasing population. This study aims to reveal the relationship between meteorological factors and yields of wheat varieties grown in dry conditions in Şanlıurfa Ceylanpınar region by growth periods and develop variety-based yield estimation models. In the research, wheat growth periods have been determined as Establishment, Tillering, Head Development, Flowering, Yield Formation and Ripening, and the average vegetation period together with Winter Dormancy was determined as 235 days. Pandas, Fırat-93, Pehlivan and Çeşit-1252, which are some of the most cultivated wheat varieties in the region during the research period, were included in the subject of the study. In the study, it was determined that vegetation period rainfall (r=0.74 and r=0.73, respectively) in Pandas and Fırat-93 varieties, average wind speed (r=0.74) in Pehlivan variety and Establishment period rainfall (r=0.88) in Çeşit-1252 varieties were the most effective variables on yields. Besides, in the scope of the studyvariety-based yield estimation models were developed, and yield estimates were made for the relevant years.

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GİRİŞ

İnsanların temel besin kaynağını oluşturan buğday, tarihte tarımı ve ıslahı yapılan ilk bitkilerdendir. Dünyanın hemen her bölgesinde yetişmesine olanak sağlayan adaptasyon yeteneğine sahip olması çok sayıda ıslah çalışmasının yapılmasına neden olmuştur. Buğday bu sayede dünya geneline yayılmış ve 'stratejik' olarak değerlendirilen bir ürün haline gelmiştir. Dünyada buğday üretiminde başta yer alan ülkeler Hindistan, AB, Rusya, Çin ve ABD şeklindedir. 2020-21 dönemi itibariyle dünya buğday ekim alanının yaklaşık %55'ini, dünya buğday üretiminin ise yaklaşık %65.4'ünü bu ülkeler oluşturmaktadır (Anonim, 2020a). Türkiye'de ise 2019 yılı verilerine göre toplam buğday ekilişi 68.5 milyon dekar olup, bunun yaklaşık %84'ü (57.5 milyon dekar) ekmeklik, geri kalanı (yaklaşık 11 milyon dekar) ise makarnalık buğdaydan oluşmaktadır (Anonim, 2020b). Birleşmiş Milletler Gıda ve Tarım Örgütü (FAO) tarafından paylaşılan istatistiklere göre; Türkiye'de 1999 yılında toplam buğday ekim alanı yaklaşık 92.7 milyon dekar ve ortalama buğday verimi 194.1 kg da^{-1} iken 2019 yılına gelindiğinde, buğday ekim alanı yaklaşık 68.3 milyon dekara gerilemiş olmasına rağmen, ortalama buğday verimi %43.3'lük bir artışla 278.1 kg da^{-1} olarak gerçekleşmiştir (FAO, 2021). Öte yandan, sanayi devriminin gerçekleşmesinin ardından günümüze kadar geçen sürede doğal afetlerin sayı ve şiddetlerinde meydana gelen artışlar insanoğlunu iklim değişikliği gerçeğiyle yüzleşmeye zorlamaktadır. İklim değişikliği nedeniyle son yıllarda sıklıkla görülmeye başlanan en etkili doğal afet ise, Türkiye'de önde gelen gündem maddelerinden biri olan kuraklıktır. Bunun nedeni, yağış, sıcaklık, güneşlenme gibi meteorolojik faktörlere bağımlı olarak tarım yapılan yörelerde kuraklığın verimde temel belirleyici konumunda olmasıdır. Tarımsal üretimde verim azalmalarının yanı sıra, bitki hastalıklarında artışa, ürün kalitelerinde düşüşe neden olan kuraklık uzun süreli durumlarda kıtlığa yol açan büyük bir felakete dönüşebilmektedir. Sık ve uzun süreli kuraklık dönemlerinde, toprak ve bitki örtüsü büyük zararlar görmekte, tarım arazileri çölleşme ve erozyon tehdidi altında özelliklerini yitirmeye başlamaktadır. Kuraklıkla mücadele ise oldukça maliyetlidir ve Türkiye'de özellikle Orta ve Güney Doğu Anadolu'da son yıllarda sıklıkla görülmektedir. Çalışmada Ceylanpınar Ovası özelinde ele alınan Güneydoğu Anadolu Bölgesi, Türkiye'nin yüzölçümü olarak en küçük bölgesidir. Bölge Gaziantep, Diyarbakır, Şanlıurfa, Batman, Adıyaman, Siirt, Mardin, Kilis ve Şırnak illerinden oluşmaktadır. Orman Genel Müdürlüğü (OGM) verilerine (OGM, 2020) göre yaklaşık 1.34 milyon ha (%5.8) orman varlığı ile Türkiye'nin coğrafi bölgeleri içinde son sırada yer alan Güneydoğu Anadolu Bölgesi, Ceylanpınar, Harran ve Birecik gibi büyük verimli ovaları sayesinde geniş bir tarımsal ürün çeşitliliğine sahiptir. Arpa, buğday, pamuk, mercimek, susam, Antep fıstığı, üzüm, zeytin, karpuz, domates gibi birçok ürünün yetiştirildiği bölgede TUİK verilerine göre, 2020 yılında buğday hasat alanı (durum buğdayı hariç) yaklaşık 7.1 milyon da, üretim miktarı 2.6 milyon ton ve dekar başına alınan verim 394 kg olarak gerçekleşmiştir (TUİK, 2021).

Yörede buğday genellikle sonbaharda ekilmekte ve devam eden serin-soğuk dönemde dinlenme periyoduna girerek soğuklanma gereksinimini (vernalizasyon) karşılamaktadır. Buğday için soğuklanma, soğuk havanın bitkiye nüfuz ettiği süreç olarak tanımlanabilir. Geçit (2016), buğday gibi serin iklim tahıllarında soğuklanmanın 2-5 °C sıcaklık aralığında, 15-20 gün ile 90-120 gün arasında gerçekleştiğini bildirmiştir. Buğdayda dinlenme süresi ekildiği yörenin iklimine göre daha kısa ya da uzun olabilmektedir. Bu dönemde bitkinin generatif devreye geçebilmesi (çiçeklenmesi) için gerekli olan enzimler salgılanmaktadır. Bu nedenle dinlenme dönemindeki iklim faktörleri tahmin modellerine değişken olarak eklenmiştir.

Buğday vejetasyon süresinin ilk dönemlerinde yüksek sıcaklıklar yerine daha düşük sıcaklıklar, daha yüksek nemlilik, daha düşük güneşlenme tercih etmektedir. Sapa Kalkma (1c) dönemiyle birlikte sıcaklık ve ışık (güneşlenme) isteği artmakta, havadaki nem isteği ise azalmaya başlamaktadır. Başaklanma (2) döneminden Olgunlaşmaya (4) kadarki dönemde ise genel olarak 20-30 °C arasında hava sıcaklığı, %65'den daha düşük nispi nem ve 20 bin lüksten daha yüksek ışık isteği uygun yetişme koşullarını sağlamaktadır (Geçit, 2016).

Ülkeler sosyal, ekonomik veya teknolojik olarak hangi düzeyde gelişmiş olurlarsa olsunlar sürdürülebilir bir yapıya sahip olmaları için tarımsal üretimlerinin kendilerine yetiyor olması gerekmektedir. Bu ise sadece tarımsal üretimin sürdürülebilirliği ile mümkün olmaktadır. Tarımda hedeflenen üretim miktarlarının veya ürün verimlerinin elde edilmesi bu noktada büyük önem

arz etmektedir. Ülkeler üretim planlamalarını öncelikle kendi ihtiyaçlarını karşılayacak, sonrasında ise ihracat ile küresel piyasalarda rekabete girmeye olanak sağlayacak şekilde yapmaktadırlar. Üretim planlaması ne kadar doğru yapılırsa ülkesel ekonomiye katkı da o derecede yüksek olmaktadır. Bu nedenlerden dolayı verim tahmin çalışmaları, özellikle de iklim değişikliğinin etkisi altındayken daha fazla önem kazanmaktadır.

Türkiye'de ve Dünyada yapılmış çok sayıda verim tahmin çalışması bulunmaktadır. Rudorff ve Batista (1991) Landsat uydu verileri ve agrometeorolojik verileri kullanarak Brezilya'da buğday için verim tahmini çalışması yapmışlardır. Çalışmada bitki örtüsü indeksi ile agrometeorolojik verilerin tek bir modelde ortak kullanılmasının sonuçları önemli ölçüde iyileştirdiği kanaatine varmışlardır. Kodal ve ark. (1987) buğday veriminin Orta Anadolu koşullarında tahmini üzerine yaptıkları çalışmada; Polatlı, Altınova, Gözlü ve Konuklar Tarım İşletmelerini çalışma sahası olarak belirlemişlerdir. Çalışmada bağımsız değişkenler olarak meteorolojik faktörlerin yanında bir zaman faktörü, bağımlı değişken olarak ise işletmelerin araştırma periyodundaki buğday verimleri kullanılmıştır. Geliştirilen tahmin modelleri ile 1986 ve 1987 yılları için buğday verim tahminleri yapılmıştır. Aküzüm ve Kodal (1988) Orta Anadolu koşullarında arpanın verim tahmini üzerine bir çalışma yapmışlardır. Çalışmada meteorolojik faktörler ve zaman faktörü bağımsız değişkenler, arpa verimi ise bağımlı değişken olarak ele alınmıştır. Aşamalı Çoklu Regresyon Yöntemi (Multiple Stepwise Regression Method) kullanılarak tahmin modelleri geliştirilmiş ve 1988 yılı için arpa verim tahmini yapılmıştır. Sönmez ve Sarı (2004) verim tahmin çalışmalarında yeni yaklaşımları konu aldıkları çalışmalarında çeşitli uzaktan algılama tekniklerinin ve agrometeorolojik verilerin kullanılma olanakları tartışmışlardır. Çalışmada agrometeorolojik elemanlar ile uzaktan algılama tekniklerinin birlikte kullanıldığı ve farklı ekolojik bölgelere hitap edebilecek metotların geliştirilmesinin ve uygulanmasının bir zorunluluk olduğu sonucuna varmışlardır. Esfandiary ve ark. (2009) Erdebil İlçesi için agrometeorolojik yaklaşımlarla buğday verim tahmini çalışması gerçekleştirmişlerdir. Çalışmada meteorolojik parametreler olarak maksimum sıcaklık, minimum sıcaklık, günlük yağış, buharlaşma ve güneşlenme süresinden yararlanarak agrometeorolojik indeksleri (büyüme derece gün vb.) hesaplamışlardır. Geliştirdikleri model yardımıyla 2005 ve 2006 yılları için verim tahmini yapmışlardır. Yan ve ark. (2018) agroklimatolojik kaynaklar ile kışlık buğday verimi arasındaki ilişkiyi araştırdıkları çalışmalarında; iklim faktörlerinin buğdayın büyüme dönemlerine göre etkilerini analiz etmeyi amaçlamışlardır. Araştırma sonuçlarında kışlık buğday verimi üzerinde en büyük etkinin bölgedeki yağışlı geçen kış dönemi olduğunu, tane oluşumu ve olgunlaşma dönemlerinde ise sıcaklık ve güneşlenme süresinin verim üzerinde önemli etkisi olduğunu bulmuşlardır.

Bu çalışmanın amacı ise; meteorolojik faktörlerden maksimum sıcaklık, minimum sıcaklık, minimum 5 cm toprak sıcaklığı, güneşlenme şiddeti, güneşlenme süresi, rüzgâr hızı, nispi nem ve yağışın kuru koşullarda yetiştirilen buğday çeşitlerinin verimleriyle arasındaki ilişkinin büyüme dönemlerine göre ortaya konulması ve çeşit bazlı verim tahmin modellerinin geliştirilmesidir.

MATERYAL ve METOD

Materyal

Araştırma alanı olan Ceylanpınar Tarım İşletmesi, Güneydoğu Anadolu Bölgesinde, Şanlıurfa İli Ceylanpınar İlçesi sınırları içerisinde yer almaktadır. Karasal iklim koşullarının hâkim olduğu yöre yaz aylarında fazla yağış almamaktadır (Anonim, 2020c). Ceylanpınar İlçesi, Erinç iklim sınıflandırmasına göre kurak, De Martonne ve Thorntwaite iklim sınıflandırmalarına göre yarı kurak, Trewartha iklim sınıflandırmasına göre ise kışları serin, yazları çok sıcak iklim sınıfında yer almaktadır (MGM, 2016). Bu nedenle Ceylanpınar Ovası, meteorolojik faktörlerin kuru tarımı yapılan bitkiler üzerinde verim açısından etkilerinin görüldüğü bir alandır. Yörede 1990-2020 yılları arası ortalama iklim değerleri Çizelge 1'de görülmekte olup, ortalama yağış miktarı 274.0 mm olarak gerçekleşmiştir.

Çizelge 1. Şanlıurfa Ceylanpınar 1990-2020 yılları ortalama iklim değerleri
Table 1. Şanlıurfa Ceylanpınar average climate values for the years 1990-2020

	1	2	3	4	5	6	7	8	9	10	11	12	Yıllık
Yağış-*Precipitation (mm)*	47.2	43.9	35.5	35.8	17.2	3.1	0.3	0.3	3.6	21.0	29.2	36.9	274.0
Sıcaklık-*Temperature (°c)*	5.6	7.4	11.5	16.5	22.7	29.0	32.2	31.2	26.4	20.2	12.3	7.2	18.5
Min. Sıcaklık-*Min.Temperature (°c)*	-12.3	-10.0	-7.2	-2.1	3.0	10.6	14.7	10.4	6.4	0.0	-5.2	-10.0	-12.3
Max. Sıcaklık-*Max.Temperature (°c)*	19.8	25.3	32.2	37.5	42.1	45.4	48.2	47.0	48.8	38.8	37.0	27.4	48.8
Min 5 cm Top. Sıc. -*Soil Temp. (°c)*	-8.4	-2.5	-0.1	3.0	11.0	15.2	24.5	15.9	10.7	0.0	0.0	-2.6	-8.4
Güneş. Süresi-*Sunshine duration (h)*	3.8	4.9	6.2	7.5	9.0	11.4	11.1	10.6	9.0	6.8	5.0	4.0	7.4

Kaynak: Meteoroloji Genel Müdürlüğü kayıtları (MGM 2020)

Ceylanpınar Tarım İşletmesi 1984 yılından itibaren Tarım İşletmeleri Genel Müdürlüğü (TİGEM) bünyesinde faaliyetlerini sürdürmektedir. İşletmenin arazi varlığı 1 635 928 dekar (da) olup, kuru tarım alanlarında ise sertifikalı tohumluk amaçlı nadas sistemi ile çalışmanın da konusunu oluşturan buğday üretimi yapılmaktadır (Anonim, 2020c). İşletmede ekimi gerçekleştirilen ve çalışmaya konu edilen buğday çeşitleri Pandas (ekmeklik), Fırat-93 (makarnalık), Pehlivan (ekmeklik) ve Çeşit-1252 (makarnalık) şeklindedir. İşletme toprakları tekstürü genel olarak killi (C sınıfı) olup, toplam tuz oranı 0-150 cm arası tüm katmanlarda %0.045 ve daha azdır. Katmanlardaki pH değerleri 7.65 ile 7.99, kireç miktarı %53.4 ile %74.9 ve organik madde miktarı ise %0.13 ile %1.74 arasında değişmektedir (TİGEM, 2019).

Çalışmada Meteoroloji Genel Müdürlüğünden (MGM) temin edilen ve 2000-2019 yıllarını kapsayan iklim verileri ile Tarım İşletmeleri Genel Müdürlüğünden (TİGEM) temin edilen, Ceylanpınar Tarım İşletmesine ait buğday (kuru) çeşitlerinin verim ve müşahede kayıtları ile toprak tekstür bilgilerinden (TİGEM, 2019) yararlanılmıştır.

Metod

Yörede ortalama buğday büyüme dönemlerinin belirlenmesinde Şanlıurfa Ceylanpınar Tarım İşletmesi müşahede kayıtlarından yararlanılmıştır. Kayıtlara göre; buğday ekimleri araştırma periyodunda genellikle 15-25 Ekim, hasatlar ise 8-15 Haziran tarihleri arasında gerçekleştirilmektedir. Buğdayın dinlenme dönemi için ilk ve son ortalama don tarihlerinin belirlenmesinde ise 'Meteoroloji Genel Müdürlüğü Zirai Don Takvimi' (MGM, 2019) kitabından yararlanılmıştır. Büyüme dönemleri skalası olarak ve Kardeşlenme (1a) ile Sapa Kalkma (1c) dönemleri belirlenmesinde Kodal (2011)'ın Doorenbos ve Kassam (1979)'dan yararlanarak düzenlediği duyarlılık aşamaları uzunlukları kullanılmıştır. Türkiye'nin güneyinde sıcak ve kurak-yarı kurak iklim kuşağında yer alan Şanlıurfa Ceylanpınar yöresinde buğdayın vejetasyon süresi daha kısa olmaktadır. Örneğin, İç Anadolu Bölgesi'nde buğdayın vejetasyon süresi 270 gün iken, bu süre Güneydoğu Anadolu Bölgesinde 211 güne kadar gerilemektedir (Anonim, 2017). Bu nedenle Kardeşlenme (1b) ve Sapa Kalkma (1c) sürelerinin belirlenmesinde alt sınır değerler temel alınmıştır. Ortalama ekim-hasat tarihlerinin 18 Ekim-9 Haziran şeklinde belirlendiği yörede, buğdayın büyüme dönemleri Dinlenme (1b) periyodu ile birlikte Çimlenme/Çıkış (0), Kardeşlenme (1a), Sapa Kalma (1c), Başaklanma (2), Tane Oluşumu (3) ve Olgunlaşma (4) olarak yedi aşamada incelenmiştir. Kardeşlenme (1a), Dinlenme (1b) ve Sapa Kalkma (1c) dönemleri Vejetatif Gelişme (1) aşamasının ara dönemleri olarak ele alınmıştır.

Araştırma periyodu ortalamaları Çıkış (0) için 32, Kardeşlenme (1a) için 17, Dinlenme (1b) için 59, Sapa Kalkma (1c) için 40, Başaklanma (2) için 30, Tane Oluşumu (3) için 33 ve Olgunlaşma (4) için 24 gün olarak belirlenmiştir. Buna göre ortalama vejetasyon süresi 235 gün olarak gerçekleşmiştir.

Araştırmada çeşit bazlı verim tahmin modellerinin geliştirilmesinde; bağımlı değişkenler olarak; buğday çeşitlerinin verimleri kullanılmıştır. Tarım İşletmesi Genel Müdürlüğünden temin edilen ve Ekim 2000-Haziran 2019 dönemini kapsayan buğday (kuru) verim kayıtlarından sıkça ekilen çeşitler belirlenmiştir. Tahmin modellerinde bağımsız değişkenler olarak ise; büyüme dönemlerine göre iklim faktörleri ve bir 'zaman faktörü' kullanılmıştır. Yörede çeşit farkı gözetmeksizin buğday için belirlenen yedi aşama ve toplam vejetasyon süresine göre iklim faktörlerinin ortalamaları alınarak bağımsız değişkenler elde edilmiştir. Çizelge 2'de maksimum sıcaklık 'Tx', minimum sıcaklık 'Tn', minimum 5cm toprak sıcaklığı 'Ts', ortalama nem 'Rh', yağış 'Rf', rüzgâr hızı 'W', güneşlenme süresi 'Sd' ve güneşlenme şiddeti 'Sr' sembolleriyle, büyüme dönemleri ise sırasıyla 0, 1a, 1c, 2, 3, 4, Dinlenme periyodu 1b ve vejetasyon süresi bir başka ifadeyle; büyüme sezonu 'S' sembolüyle gösterilmiştir. İklim faktörü ve hangi dönemde olduğu bu iki sembol grubunun birlikte kullanılmasıyla ifade edilmiştir. Örneğin; 'Tx2' ifadesi başaklanma dönemindeki maksimum sıcaklık anlamına gelmektedir. Zaman faktörü araştırmanın başlangıcı olan 2000 yılı temel alınarak, 'X_1=T-2000' eşitliği ile belirlenmiştir. Burada, 'T' hasat edilen yılı temsil etmektedir. Zaman Faktörü, araştırmada verim üzerinde etkili olan ancak göz önüne alınmayan tarım teknikleri, toprak hazırlığı, ekim, gübreleme, ilaçlama, tarımsal mekanizasyon gibi girdilerin yıllar içindeki olumlu etkilerine karşılık olarak modele eklenmiştir.

Meteorolojik veriler 'Şanlıurfa Ceylanpınar TİGEM (İstasyon no:17968)' meteoroloji gözlem istasyonundan günlük olarak alınmıştır. Verim tahmininde buğday verimine etkili olduğu düşünülen meteorolojik faktörler; maksimum sıcaklık (°C), minimum sıcaklık (°C), minimum 5 cm toprak sıcaklığı (°C), nispi nem (%), yağış miktarı (mm), rüzgâr hızı (m s^{-1}), güneşlenme süresi (Saat) ve güneşlenme şiddeti (MJ m^{-2} $gün^{-1}$) şeklinde belirlenmiştir.

Buğday verimleriyle bağımsız değişkenler arasındaki ilişki düzeyleri 'Korelasyon Yöntemi' ile buğday çeşitlerinin verim tahminlerinde kullanılacak eşitlikler ise 'Aşamalı Çoklu Regresyon Yöntemi' ile belirlenmiştir (Aküzüm ve Kodal, 1988). Aşamalı çoklu regresyon, her aşamada en önemli değişkeni modele ekleme ya da en az önemli değişkeni

modelden çıkarma prensibine göre gerçekleştirilmektedir. İlk aşamada bağımlı değişken üzerindeki en yüksek etkili değişken seçilerek bir denklem kurulur, sonraki aşamada kalan değişkenler arasından en etkili olan tekrar seçilir ve ilk seçilenle beraber bir kez daha denklem kurulur. Bu süreç esnasında önceki aşamalarda modele girmiş olan ancak ilerleyen aşamalarda ilişkiyi açıklamadaki etkisi azalan bir değişken varsa modelden çıkması söz konusu olur. Değişkenlerin aşamalar halinde, denkleme eklenip çıkarılmaları regresyon modeli fit hale gelene kadar devam eder. Modelin fit olması, Determinasyon Katsayısının (R^2) 1'e en yakın değeri bulması şeklinde ifade edilir.

Çizelge 2. Araştırmada kullanılan değişkenler
Table 2 Variables used in the research

Bağımlı Değişkenler *(Dependent Variables)*					
Y_1	Pandas Verimi, Ypan *(Pandas Yield)*	kg da^{-1}	Y_3	Pehlivan Verimi, Ypeh *(Pehlivan Yield)*	kg da^{-1}
Y_2	Fırat-93 Verimi, Yfır *(Fırat-93 Yield)*	kg da^{-1}	Y_4	Çeşit-1252 Verimi, Yçes *(Ç.-1252 Yield)*	kg da^{-1}
Bağımsız Değişkenler *(Independent Variable*					
X_1	Zaman Faktörü *(Time Factor)*	(T-2000)	X_{34}	Yağış miktarı [0], Rf0	mm
X_2	Maksimum Sıcaklık [0], Tx0	°C		*(Rainfall)*	
	(Maximum Temperature)		X_{35}	Yağış miktarı [1a], Rf1a	mm
X_3	Maksimum Sıcaklık [1a], Tx1a	°C	X_{36}	Yağış miktarı [1b], Rf1b	mm
X_4	Maksimum Sıcaklık [1b], Tx1b	°C	X_{37}	Yağış miktarı [1c], Rf1c	mm
X_5	Maksimum Sıcaklık [1c], Tx1c	°C	X_{38}	Yağış miktarı [2], Rf2	mm
X_6	Maksimum Sıcaklık [2], Tx2	°C	X_{39}	Yağış miktarı [3], Rf3	mm
X_7	Maksimum Sıcaklık [3], Tx3	°C	X_{40}	Yağış miktarı [4], Rf4	mm
X_8	Maksimum Sıcaklık [4], Tx4	°C	X_{41}	Yağış miktarı [S], RfS	mm
X_9	Maksimum Sıcaklık [S], TxS	°C	X_{42}	Rüzgâr Hızı [0], W0	m s^{-1}
X_{10}	Minimum Sıcaklık [0], Tn0	°C		*(Wind Speed)*	
X_{11}	*(Minimum Temperature)*		X_{43}	Rüzgâr Hızı [1a], W1a	m s^{-1}
	Minimum Sıcaklık [1a], Tn1a	°C	X_{44}	Rüzgâr Hızı [1b], W1b	m s^{-1}
X_{12}	Minimum Sıcaklık [1b], Tn1b	°C	X_{45}	Rüzgâr Hızı [1c], W1c	m s^{-1}
X_{13}	Minimum Sıcaklık [1c], Tn1c	°C	X_{46}	Rüzgâr Hızı [2], W2	m s^{-1}
X_{14}	Minimum Sıcaklık [2], Tn2	°C	X_{47}	Rüzgâr Hızı [3], W3	m s^{-1}
X_{15}	Minimum Sıcaklık [3], Tn3	°C	X_{48}	Rüzgâr Hızı [4], W4	m s^{-1}
X_{16}	Minimum Sıcaklık [4], Tn4	°C	X_{49}	Rüzgâr Hızı [S], WS	m s^{-1}
X_{17}	Minimum Sıcaklık [S], TnS	°C	X_{50}	Güneşlenme Süresi [0], Sd0	h
X_{18}	Minimum 5 cm Toprak Sıcaklığı [0], Ts0	°C		*(Sunshine Duration)*	
	(Minimum 5 cm Soil Temperature)		X_{51}	Güneşlenme Süresi [1a], Sd1a	h
X_{19}	Minimum 5 cm Toprak Sıcaklığı [1a], Ts1a	°C	X_{52}	Güneşlenme Süresi [1b], Sd1b	h
X_{20}	Minimum 5 cm Toprak Sıcaklığı [1b], Ts1b	°C	X_{53}	Güneşlenme Süresi [1c], Sb1c	h
X_{21}	Minimum 5 cm Toprak Sıcaklığı [1c], Ts1c	°C	X_{54}	Güneşlenme Süresi [2], Sd2	h
X_{22}	Minimum 5 cm Toprak Sıcaklığı [2], Ts2	°C	X_{55}	Güneşlenme Süresi [3], Sd3	h
X_{23}	Minimum 5 cm Toprak Sıcaklığı [3], Ts3	°C	X_{56}	Güneşlenme Süresi [4], Sd4	h
X_{24}	Minimum 5 cm Toprak Sıcaklığı [4], Ts4	°C	X_{57}	Güneşlenme Süresi [S], SdS	h
X_{25}	Minimum 5 cm Toprak Sıcaklığı [S], TsS	°C	X_{58}	Güneşlenme Şiddeti [0], Sr0	MJ m^{-2} d^{-1}
X_{26}	Ortalama Nem [0], Rh0	%		*(Solar Radiation)*	
	(Average Humidity)		X_{59}	Güneşlenme Şiddeti [1a], Sr1a	MJ m^{-2} d^{-1}
X_{27}	Ortalama Nem [1a], Rh1a	%	X_{60}	Güneşlenme Şiddeti [1b], Sr1b	MJ m^{-2} d^{-1}
X_{28}	Ortalama Nem [1b], Rh1b	%	X_{61}	Güneşlenme Şiddeti [1c], Sr1c	MJ m^{-2} d^{-1}
X_{29}	Ortalama Nem [1c], Rh1c	%	X_{62}	Güneşlenme Şiddeti [2], Sr2	MJ m^{-2} d^{-1}
X_{30}	Ortalama Nem [2], Rh2	%	X_{63}	Güneşlenme Şiddeti [3], Sr3	MJ m^{-2} d^{-1}
X_{31}	Ortalama Nem [3], Rh3	%	X_{64}	Güneşlenme Şiddeti [4], Sr4	MJ m^{-2} d^{-1}
X_{32}	Ortalama Nem [4], Rh4	%	X_{65}	Güneşlenme Şiddeti [S], SrS	MJ m^{-2} d^{-1}
X_{33}	Ortalama Nem [S], RhS	%			

Çalışmada değişkenleri eşitliğe ekleme ve çıkarmada %95 güven aralığı kullanılmış, bir başka ifadeyle α-değeri (anlam seviyesi) 0.05 olarak belirlenmiştir. Kocabaş ve ark. (2013), α-değerinin belirlenmesi konusunda herhangi bir kural olmamakla birlikte, bu değerin biyoloji, fen ve uygulamalı bilimlerde 0.01 ve/veya 0.05 olarak belirlenebileceğini bildirmişlerdir. Bu değer küçüldükçe güvenilirlik derecesi artmaktadır.

Bu çalışmada korelayon ve regresyon analizleri MİNİTAB paket programı kullanılarak yapılmıştır. Verim tahminlerinin yapılmasında, çalışmada kullanılan çeşitlere dair veriler 'Eğitim Seti' ve 'Deneme Seti' olarak ayrılmıştır. Eğitim seti, eldeki verinin modelin kurulmasında kullanılan kısmını, deneme seti ise modelin tahminlerinin test edildiği kısmını ifade etmektedir. Deneme setleri verileri eğitim setlerine dâhil edilmemiştir. Modeller eğitim setleri ile kurulmuş ve kurulan modeller ilk önce eğitim setlerinin tahmini verimleri hesaplanarak test edilmiş, yani gerçek verimlerle arasındaki hatalar değerlendirilmiş ve sonrasında deneme setlerindeki yıllar için verimlerin tahmin edilmesinde kullanılmıştır. Tahmin edilen verimlerle gerçek verimler karşılaştırılmış ve modeller değerlendirilmiştir.

BULGULAR ve TARTIŞMA

Meteorolojik parametrelerin verimlerle olan ilişkilerinin boyutunu istatistiksel olarak anlamlı olup olmadığının anlaşılması için parantez içinde korelasyon değerlerinin (r) yanına %95 güven aralığına göre hesaplanmış olan P-değerleri eklenmiştir. P-değerinin 0.05'ten küçük olması istatistiksel olarak anlamlı olduğunu göstermektedir.

Sıcaklık-Verim İlişkisi

Ceylanpınar yöresinde gerçekleşen günlük maksimum, minimum ve minimum 5m toprak sıcaklıklarının yörede buğdayın büyüme dönemlerinde yıllara göre kuruda yetiştirilen buğday çeşitlerinin verimleri ile arasındaki ilişkiler incelenmiş olup, maksimum sıcaklıklar ile buğday çeşitlerinin verimleri arasında Olgunlaşma (4) dönemi hariç zıt yönlü ilişkiler görülmüştür. Vejetasyon süresi (sezon) ele alındığında en yüksek ilişki düzeyinin zıt yönlü (r=-0.71, P=0.022) olarak Çeşit-1252'de olduğu belirlenmiştir. Bunu yine zıt yönlü olarak Pehlivan (r=-0.52, P=0.070) çeşidi takip etmiştir. Büyüme dönemlerine göre en yüksek ilişki Fırat-93 çeşidinde Olgunlaşma (4) döneminde aynı yönlü (r=0.55, P=0.051) olarak belirlenmiştir. Araştırmada dikkate alınan buğday çeşitlerinin tümünde vejetasyon süresinde zıt yönlü ilişkiler belirlenmiştir. Buğdayın generatif devresinin sonlarına doğru yüksek sıcaklık isteği artmaktadır. Bu nedenle Olgunlaşma (4) döneminde tüm çeşitlerde maksimum sıcaklıklarla aynı yönlü ilişkiler belirlenmiştir. Bu dönemde en düşük ilişki düzeyi (r=0.23, P=0.380) Pandas çeşidi buğdayda belirlenmiştir.

Minimum sıcaklıklar ile buğday çeşitlerinin verimleri arasındaki ilişkiler vejetasyon süresine göre değerlendirildiğinde; Fırat-93 çeşidi hariç tüm çeşitlerde zıt yönlü ve zayıf kabul edilebilecek düzeylerde ilişkiler dikkat çekmektedir. En yüksek ilişki zıt yönlü olarak (r=-0.39, P=0.260) Çeşit-1252'de belirlenmiştir. Büyüme dönemlerine göre en yüksek ilişki Pehlivan çeşidinde Kardeşlenme (1a) döneminde zıt yönlü (r=-0.44, P=0.128) olarak belirlenmiştir.

Toprak sıcaklığı bitkinin gelişimine etki eden önemli faktörlerdendir. Toprakta su dengesinin sağlanmasına, toprağın havalanmasına, mikroorganizma faaliyetlerinin artmasına ve daha hızlı çimlenmeye yardımcı olmaktadır. Ekimlerin toprak sıcaklığının 8-10 °C dönemde gerçekleştirilmesi ise hızlı kök gelişmesine katkı sağlamaktadır (Süzer, 2012). Minimum 5 cm toprak sıcaklıkları ile buğday çeşitlerinin verimleri arasındaki ilişki vejetasyon süresine göre ele alındığında; en yüksek ilişki düzeyinin zıt yönlü (r=-0.28, P=0.439) olarak Çeşit-1252'de olduğu belirlenmiştir. Bunu zıt yönlü ilişki düzeyiyle (r=-0.27, P=0.296) Pandas çeşidi takip etmiştir. Büyüme dönemlerine göre en yüksek ilişkiler sırasıyla Pehlivan çeşidinde Kardeşlenme (1a) döneminde zıt yönlü (r=-0.46, P=0.115), Fırat-93 çeşidinde Çimlenme/Çıkış (0) döneminde zıt yönlü (r=-0.42, P=0.156) ve Çeşit-1252 çeşidinde Olgunlaşma (4) döneminde zıt yönlü (r=-0.40, P=0.258) olarak belirlenmiştir.

Nemlilik-Verim İlişkisi

Vejetasyon süresinde nemlilik ile buğday çeşitlerinin verimleri arasındaki en yüksek ilişkinin zıt yönlü olarak (r=-0.29, P=0.332) Pehlivan çeşidinde olduğu belirlenmiştir. Büyüme dönemlerine göre en yüksek ilişkiler sırasıyla Pehlivan çeşidinde Tane Oluşumu (3) döneminde zıt yönlü (r=-0.58, P=0.038) ve Çeşit-1252 çeşidinde Olgunlaşma (4) döneminde aynı yönlü (r=0.49, P=0.154) olarak belirlenmiştir. Olgunlaşma döneminde Çeşit-1252 hariç diğerlerinde zıt yönlü ilişkiler dikkat çekmektedir.

Rüzgâr-Verim İlişkisi

Rüzgâr hızındaki dönemsel değişmelerin bitki verimi üzerindeki etkilerini incelemek için yapılan analiz neticesinde, vejetasyon süresi genelinde en yüksek ilişki düzeyinin aynı yönlü (r=0.74, P=0.004) olarak Pehlivan çeşidinde olduğu belirlenmiştir. Bunu aynı yönlü ilişki düzeyiyle (r=0.66, P=0.037) Çeşit-1252

çeşidi takip etmiştir. En düşük ilişki düzeyi ise aynı yönlü olarak (r=0.09, P=0.767) Fırat-93 çeşidinde belirlenmiştir. Büyüme dönemleri ve dinlenme periyotlarına göre göze çarpan en yüksek ilişkiler Çeşit-1252 çeşidinde Dinlenme (1b) döneminde aynı yönlü (r=0.75, P=0.013), Çimlenme/Çıkış (0) döneminde aynı yönlü (r=0.73, P=0.015) ve Pehlivan çeşidinde Dinlenme (1b) döneminde aynı yönlü (r=0.70, P=0.008) olarak belirlenmiştir. Pandas ve Fırat-93 çeşitlerinde ise Olgunlaşma (4) dönemlerinde önemli düzeyde ilişkiler (r=0.56, P=0.021 ve r=0.52, P=0.52) dikkat çekmektedir.

Güneşlenme-Verim İlişkisi

Araştırmada güneşin bitkisel üretimde verimliliğe etkileri güneşlenme şiddeti (solar radyasyon) ve güneşlenme süresi açısından ele alınmıştır. Yörede güneşlenme şiddeti ile buğday çeşitlerinin verimleri arasında hem büyüme dönemleri hem de vejetasyon süresi yönünden genellikle zıt yönlü ilişkiler dikkat çekmektedir. Vejetasyon süresi boyunca en yüksek ilişki düzeyinin zıt yönlü (r=-0.60, P=0.050) olarak Fırat-93'te olduğu belirlenmiştir. Bunu zıt yönlü ilişki düzeyiyle Pandas (r=-0.34, P=0.255) çeşidi takip etmiştir. Büyüme dönemlerine göre göze çarpan en yüksek düzeyli ilişkiler Pandas çeşidinde Çimlenme/Çıkış (0) döneminde zıt yönlü (r=-0.54, P=0.056), Çeşit-1252 çeşidinde Olgunlaşma (4) döneminde zıt yönlü (r=-0.45, P=0.221) ve Fırat-93 çeşidinde Çimlenme/Çıkış (0) döneminde zıt yönlü (r=-0.40, P=0.226) olarak belirlenmiştir.

Güneşlenme süresi ile buğday çeşitlerinin verimleri arasında toplam vejetasyon süresine göre yüksek düzeyde değerlendirilebilecek ilişkiler belirlenmemiş olup, en yüksek ilişki düzeyinin aynı yönlü (r=0.28, P=0.353) olarak Pehlivan çeşidinde olduğu görülmektedir. Bunu aynı yönlü ilişki düzeyiyle Çeşit-1252 (r=0.13, P=0.727) çeşidi takip etmiştir. Büyüme dönemleri açısından en yüksek düzeyli ilişkiler Çeşit-1252 çeşidinde Olgunlaşma (4) döneminde zıt yönlü (r=-0.48, P=0.156) ve Fırat-93 çeşidinde Çimlenme/Çıkış (0) döneminde aynı yönlü (r=0.26, P=0.391) olarak belirlenmiştir.

Yağış-Verim İlişkisi

Buğday tarımında yüksek verimin alınmasında toprakta depolanan su miktarı önemli rol oynamaktadır. Topraktaki su miktarının en büyük belirleyicisi gerçekleşen yağışlardır. Buğday normal şartlarda yıllık yağışı 250-1750 mm olan yerlerde yetiştirilmektedir (Gommes et al, 2010).

Araştırmada yağış ile buğday çeşitlerinin verimleri arasında hem büyüme dönemleri hem de vejetasyon süresi yönünden aynı yönlü önemli ilişkiler göze çarpmaktadır. Vejetasyon süresi ele alındığında en yüksek ilişki düzeyinin aynı yönlü (r=0.80, P=0.006) olarak Çeşit-1252 çeşidinde olduğu belirlenmiştir. Bunu aynı yönlü ilişki düzeyleriyle sırasıyla Pandas (r=0.74, P=0.001), Fırat-93 (r=0.73, P=0.004) ve Pehlivan (r=0.49, P=0.093) çeşitleri takip etmiştir. Büyüme dönemlerine göre göze çarpan en yüksek ilişkiler Çeşit-1252 çeşidinde Çimlenme/Çıkış (0) ve Başaklanma (2) dönemlerinde aynı yönlü (r=0.88, P=0.001 ve r=0.70, P=0.858), Fırat-93 çeşidinde Sapa Kalkma (1c) döneminde aynı yönlü (r=0.62, P=0.025), Pehlivan çeşidinde Sapa Kalkma (1c) döneminde aynı yönlü (r=0.52, P=0.068), ve Pandas çeşidinde Başaklanma (2) döneminde aynı yönlü (r=0.50, P=0.040) olarak belirlenmiştir.

Zaman Faktörü-Verim İlişkisi

Buğday üretiminde verime etkili olan ancak gözönüne alınmayan faktörleri temsil eden zaman faktörüyle tüm çeşitlerin verimleri arasında zıt yönlü ilişkiler belirlenmiştir. Zaman faktörü ile korelasyonlar (r) Pehlivan'da -0.73 (P=0.005), Fırat-93'te -0.68 (P=0.011), Pandas'ta -0.65 (P=0.004) ve Çeşit 1252'de -0.46 (P=0.180) düzeylerinde gerçekleşmiştir. Zaman faktörünün zıt yönlü çıkmasının nedeni, teknolojideki gerilemeler değil, iklim faktörlerinin etkisiyle verimde gerçekleşen azalış trendidir.

Buğday Çeşitlerinde Verim Tahmini

Buğday çeşitlerinin verim tahmin eşitlikleri aşamalı çoklu regresyon yöntemi ile belirlenmiştir. Elde edilen modeller eğitim setindeki yılların verimleriyle test edilmiş ve Çizelge 3'deki sonuçlar bulunmuştur. Çizelgede yıllara göre çalışma konusunu oluşturan çeşitlerin verim tahminlerinde kullanılan yıllar (year), gerçek verimler (Ya), modellerden tahmin edilen verimler (Ye) ve ikisi arasındaki farkı (Ya-Ye) ifade eden Hata (R) sütunları yer almaktadır. Çizelgede ayrıca R (Test) olarak adlandırılan sütun ise modellerin kurulduğu yıllardaki gerçek verimlerle modelin bu yıllar için belirlediği verimler arasındaki farkı göstermektedir. Bu işlem modelin test edilmesi olarak ifade edilmektedir. Burada elde edilen sonuçlara göre modelin kullanılıp kullanılmamasına karar verilerek ilgili yıllar için tahminler yapılmıştır. Buna göre; en yüksek değerde hata Pandas çeşidi tahmin modelinde belirlenmiştir. Çalışmanın devamında tahmin modelleri buğday çeşitleri için uygulanmış ve aşağıda görülen sonuçlar elde edilmiştir (Çizelge 3).

Tahmin modellerinin geliştirilmesinde çeşitlerin verimleri üzerine etkili değişkenler ayrı ayrı belirlenmiştir. Aşağıdaki çizelgelerde korelasyon değeri, r>0.50 olan değişkenlere yer verilmiştir. Aşamalı çoklu regresyon yöntemiyle geliştirilen eşitliklere yüksek ilişkili olan değişkenlerin yerine daha düşük ilişkili ancak daha açıklayıcı olanların da girebileceği unutulmamalıdır.

1

Pandas çeşidinde verim tahmini

Pandas soğuğa ve kuraklığa orta dayanıklı ekmeklik buğday çeşididir. Ortalama bitki boyu 90-100 cm olup, yatmaya dayanıklıdır. Ortalama dane verimi 553 kg da^{-1} civarındadır (Anonim, 2021a). Araştırmada Pandas çeşidi buğday verimine en yüksek düzeyde etki eden bağımsız değişkenler Çizelge 4'deki gibi belirlenmiştir. P-değeri<0.005 olanlar * ile işaretlenmiştir.

Çizelge 3. Buğday çeşitlerinde yıllara göre verim tahminleri ve hatalar (kg da-1)
Table 3. Yield estimations and residuals in wheat varieties by years (kg da-1)

Yıl	Pandas				Fırat-93				Pehlivan				Çeşit-1252			
Year	R(Test)	Ya	Ye	R	R(Test)	Ya	Ye	R	R(Test)	Ya	Ye	R	R(Test)	Ya	Ye	R
2003													0.1			
2004																
2005									0.4							
2006					-1.8				0.5							
2007					-1.1				0.5				0.1			
2008																
2009	39.9				21.6											
2010	0.1				6.2				0.4							
2011	-0.1				-12				0.6				0.1			
2012	0.2				-1.5				0.3							
2013	0.0				11.8				0.5				0.1			
2014		59.0	64.8	-5.7	-10.9								0.0			
2015		220.8	218.6	2.2		195.7	193.3	2.4		141.5	146.4	-4.9	0.1			
2016		100.3	109.9	-9.6		100.2	124.9	-24.7					0.2			
2017		85.0	65.5	19.5		34.3	81.1	-46.7		85.0	91.5	-6.5		49.3	44.2	5.1
2018		30.2	123.3	-93.1						31.2	103.9	-72.7		47.8	29.8	17.9
2019										179.9	172.1	-7.7		210.1	176.0	34.1

Çizelge 4. Pandas verimi üzerine etki eden değişkenler
Table 4. Variables affecting Pandas yield

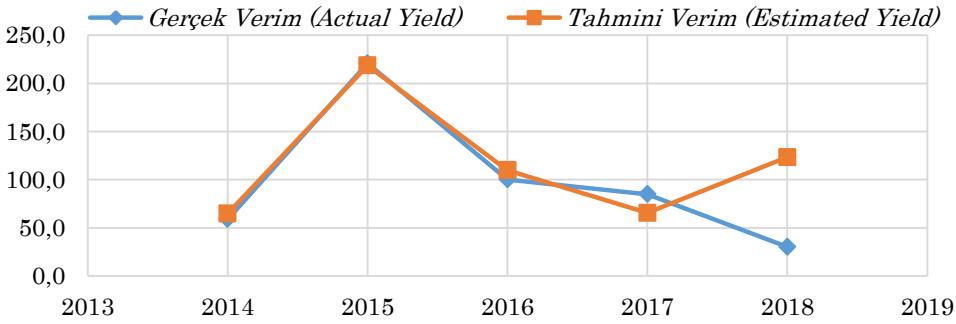
Değişken *Variable*	Sembol *Symbol*	Korelasyon *Correlation*	P-Değeri *P-Value*
Çimlenme/Çıkış Dönemi Ortalama Güneşlenme Şiddeti (Sr0), MJ m^{-2} gün^{-1} *Establishment Period Average Solar Radiation (Sr0), MJ m^{-2} day^{-1}*	X_{58}	-0.54	0.056
Olgunlaşma Dönemi Ortalama Rüzgâr Hızı (W4), m s^{-1} *Ripening Period Average Wind Speed (W4), m s^{-1}*	X_{48}	0.56	*0.021
Başaklanma Dönemi Ortalama Yağış Miktarı (Rf2), mm gün^{-1} *Flowering (Anthesis) Period Average Rainfall (Rf2), mm day^{-1}*	X_{38}	0.50	*0.040
Vejetasyon Dönemi Ortalama Yağış Miktarı (RfS), mm gün^{-1} *Vegetation Period Average Rainfall (RfS), mm day^{-1}*	X_{41}	0.74	*0.001
Zaman Faktörü *Time Factor*	X_1	-0.65	*0.004

**P-Değeri<0.05, P-Value<0.05*

Pandas çeşidinde iklim faktörlerinden güneşlenme şiddeti, rüzgâr hızı ve yağışın verimde etkili temel faktörler olduğu görülmektedir. Verimle en yüksek ilişkinin aynı yönlü (r=0.74) olarak vejetasyon dönemi yağışlarıyla, zıt yönlü en yüksek ilişkinin (r=-0.65) ise zaman faktörüyle olduğu belirlenmiştir. Buna göre, Pandas çeşidi için verim tahmin modeli 'Y_1=-316.7+460.59(X_{41})-3.952(X_1)' şeklindedir. Yukarıda belirlenen eşitliğe göre 2009-2013 döneminde gerçekleşen verimlerle modelin testleri gerçekleştirilmiştir ve 2014, 2015, 2016, 2017 ve 2018 yılları için verim tahminleri yapılmıştır. Elde edilen tahmin sonuçları ve hatalar Çizelge 3'deki gibidir. Model yardımıyla tahmin edilen verim değerlerinde 2018 yılı hariç başarılı sonuçlar elde edilmiştir (Şekil 1).

2018 yılındaki hatanın büyük olmasının nedeninin dönemsel olarak meydana gelebilen extrem hava olayları olduğu kanaatine varılmıştır. Nitekim yağış verilerine göre yörede 2018 Ekim ayında ilk yağışların geciktiği, Mayıs ayında ise kısa sürede yüksek miktarda yağışların gerçekleştiği belirlenmiştir. Meteoroloji Genel Müdürlüğü kayıtlarından (MGM, 2018) edinilen bilgiye göre ise, büyük kısmını Tarım İşletmesi arazilerinin oluşturduğu Ceylanpınar İlçesinde kuvvetli mevzii sağanak yağışlar sebebiyle ani taşkın ve sel felaketleri yaşanmıştır. Gerçekleşen kuvvetli yağışların, ekiliş sürecinde yeterli yağış alamayan olgunlaşma öncesi dönemdeki buğdayları devirerek

fiziksel zararlarla birlikte verimde kayıplara yol açmış olabileceği sonucuna varılmıştır. Yukarıda bahsi geçen durum nedeniyle modelin yağış miktarını genel ortalama içinde değerlendirebileceği bu nedenle extrem olayları yakalamada eksik kalabileceği öngörülmektedir. Modele göre yaşanan verim kaybı yaklaşık %75.5 civarındadır.



Şekil 1. Pandas çeşidinde gerçek ve tahmin edilen verimlerin grafiksel gösterimi
Figure 1. Graphical representation of actual and estimated yields of Pandas variety

Fırat-93 çeşidinde verim tahmini

Fırat-93 yazlık, orta erkenci ve kuraklığa dayanıklı makarnalık buğday çeşididir. Orta boylu ve sağlam saplıdır. Ortalama verimi 450 kg/da civarındadır (Anonim, 2021b). Araştırmada Fırat-93 çeşidinde iklim faktörlerinden maksimum sıcaklık, rüzgâr hızı, güneşlenme şiddeti, yağış ve zaman faktörünün verimde etkili temel faktörler olduğu görülmektedir. Verimle en yüksek ilişkinin aynı yönlü (r=0.73) olarak vejetasyon dönemi yağışlarıyla, zıt yönlü en yüksek ilişkinin (r=-0.68) ise zaman (yıl) faktörüyle olduğu belirlenmiştir (Çizelge 5).

Çizelge 5. Fırat-93 verimi üzerine etki eden değişkenler
Table 5. Variables affecting Fırat-93 yield

Değişken *Variable*	Sembol *Symbol*	Korelasyon *Correlation*	P-Değeri *P-Value*
Olgunlaşma Dönemi Ortalama Maksimum Sıcaklık (Tx4), °C *Ripening Period Average Maximum Temperature (Tx4), °C*	X_8	0.55	0.380
Olgunlaşma Dönemi Ortalama Rüzgâr Hızı (W4), m s^{-1} *Ripening Period Average Wind Speed (W4), m s^{-1}*	X_{48}	0.52	0.210
Vejetasyon Dönemi Ortalama Güneşlenme Şiddeti (SrS), MJ m^{-2} $gün^{-1}$ *Vegetation Period Average Solar Radiation (SrS), MJ m^{-2} day^{-1}*	X_{65}	-0.60	0.255
Dinlenme Dönemi Ortalama Yağış Miktarı (Rf1b), mm $gün^{-1}$ *Winter Dormancy Period Avarage Rainfall (Rf1b), mm day-1*	X_{36}	0.52	0.422
Sapa Kalkma Dönemi Ortalama Yağış Miktarı (Rf1c), mm $gün^{-1}$ *Head Development Period Avarage Rainfall (Rf1c), mm day^{-1}*	X_{37}	0.62	0.109
Vejetasyon Dönemi Ortalama Yağış Miktarı (RfS), mm $gün^{-1}$ *Vegetation Period Average Rainfall (RfS), mm day^{-1}*	X_{41}	0.73	*0.001
Zaman Faktörü *Time Factor*	X_1	-0.68	*0.004

** P-Değeri<0.05, P-Value<0.05*

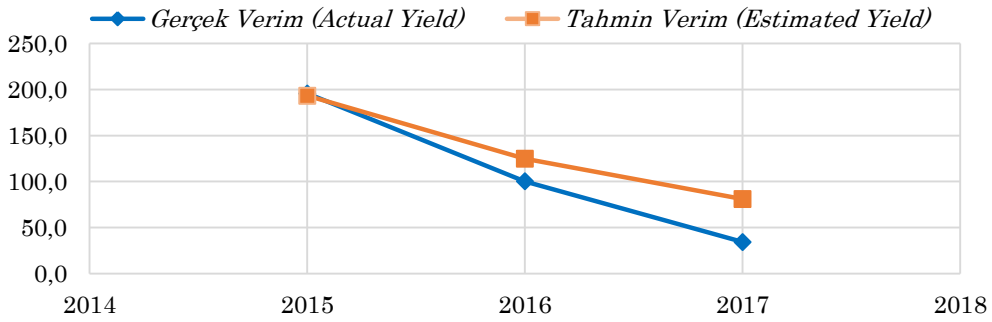
Fırat-93 verimindeki değişiklikleri en iyi tanımlayan değişkenler; vejetasyon süresi ortalama yağış miktarı, dinlenme dönemi ortalama yağış miktarı ve çıkış/çimlenme dönemi maksimum sıcaklıkları olarak belirlenmiştir. Buna göre, Fırat-93 çeşidi için verim tahmin modeli 'Y_2=-397.64+194(X_{41})+119(X_{36})+8(X_2)' şeklindedir. Eşitliğe göre 2006-2014 döneminde gerçekleşen verimlerle modelin testleri gerçekleştirilmiş, hatalar belirlenmiş ve model uygulanarak 2015, 2016 ve 2017 yılları için verim tahminleri yapılmıştır (Çizelge 3). Model yardımıyla tahmin edilen verim değerleri içinde en büyük hata 2017 tahmininde gerçekleşmiştir (Şekil 2).

Yapılan incelemede 2017 yılında hem Dinlenme döneminde (59.8 mm) hem de vejetasyon süresi boyunca (161.4 mm) gerçekleşen yağış miktarının diğer yıllara oranla önemli düzeyde düşük, çıkış/çimlenme dönemi maksimum sıcaklıklarının (28.7 °C) ise yüksek olduğu belirlenmiştir. Bilindiği gibi yağışların normalinden düşük ve sıcaklıkların normalinden yüksek seyretmesi en temel kuraklık göstergesidir. Ceylanpınar'ın uzun yıllar yağış ortalamasının 274 mm olduğu düşünüldüğünde, modelin mevsim normallerinde başarılı sonuçlar

verdiği ancak kurak geçen dönemlerde hata oranının yükseldiği görülmektedir. Fırat-93 çeşidi için geliştirilen söz konusu tahmin eşitliğinin kullanımında bu husus dikkate alınmalıdır.

Pehlivan çeşidinde verim tahmini

Pehlivan soğuğa karşı çok iyi, kuraklığa karşı ise iyi dayanıklı ekmeklik buğday çeşididir. Yatmaya karşı dayanıklı olup, ortalama verim potansiyeli 450-700 kg/da arasındadır (Anonim, 2021c). Pehlivan çeşidinde iklim faktörlerinden maksimum sıcaklık, nispi nem, rüzgâr hızı, yağış ve zaman faktörünün verimde etkili temel faktörler olduğu görülmektedir. Verimle en yüksek ilişkilerin aynı yönlü (r=0.74) olarak vejetasyon dönemi rüzgâr hızıyla ve zıt yönlü (r=-0.73) olarak zaman faktörüyle olduğu belirlenmiştir (Çizelge 6)..



Şekil 2. Fırat-93 çeşidinde gerçek ve tahmin edilen verimlerin grafiksel gösterimi
Figure 2. Graphical representation of actual and estimated yields of Fırat-93 variety

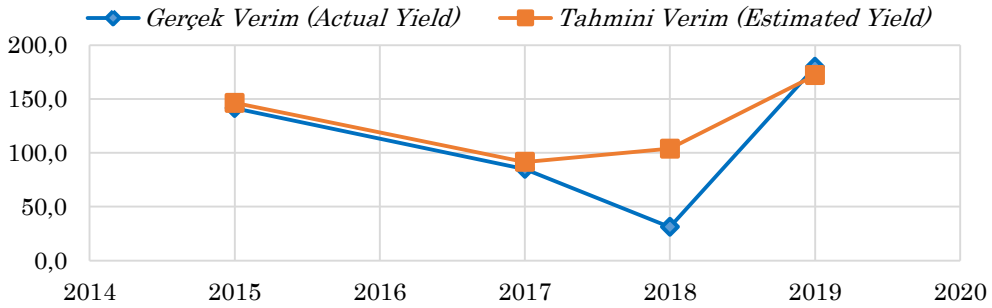
Çizelge 6. Pehlivan verimi üzerine etki eden değişkenler
Table 6. Variables affecting Pehlivan yield

Değişken *Variable*	Sembol *Symbol*	Korelasyon *Correlation*	P-Değeri *P-Value*
Kardeşlenme Dönemi Ortalama Maksimum Sıcaklık (Tx1a), °C *Tillering Period Avagare Maximum Temperature (Tx1a), °C*	X_3	-0.51	0.073
Vejetasyon Dönemi Ortalama Maksimum Sıcaklık (TxS), °C *Vegetation Period Avarage Maxium Temperature (TxS), °C*	X_9	-0.52	0.070
Tane Oluşumu Dönemi Ortalama Nispi Nem (Rh3), % *Yield Formation Period Avarage Relative Humididy, %*	X_{31}	-0.58	*0.038
Çimlenme/Çıkış Dönemi Ortalama Rüzgâr Hızı (W0), m s^{-1} *Establishment Period Avarage Wind Speed (W0), m s^{-1}*	X_{42}	0.51	0.072
Dinlenme Dönemi Ortalama Rüzgâr Hızı (W1b), m s^{-1} *Winter Dormancy Period Avarage Wind Speed (W1b), m s^{-1}*	X_{44}	0.70	*0.008
Sapa Kalkma Dönemi Ortalama Rüzgâr Hızı (W1c), m s^{-1} *Head Development Period Avarage Wind Speed (W1c), m s^{-1}*	X_{45}	0.55	0.051
Başaklanma Dönemi Ortalama Rüzgâr Hızı (W2), m s^{-1} *Flowering (Anthesis) Period Avarage Wind Speed (W2), m s^{-1}*	X_{46}	0.54	*0.050
Tane Oluşumu Dönemi Ortalama Rüzgâr Hızı (W3), m s^{-1} *Yield Formation Period Avagare Wind Speed (W3), m s^{-1}*	X_{47}	0.63	*0.020
Olgunlaşma Dönemi Ortalama Rüzgâr Hızı (W4), m s^{-1} *Ripening Period Avarage Wind Speed (W4), m s^{-1}*	X_{48}	0.67	*0.012
Vejetasyon Dönemi Ortalama Rüzgâr Hızı (WS), m s^{-1} *Vegetation Period Avarage Wind Speed (WS), m s^{-1}*	X_{49}	0.74	*0.004
Sapa Kalkma Dönemi Ortalama Yağış Miktarı (Rf1c), mm $gün^{-1}$ *Head Development Period Avarage Rainfall (Rf1c), mm day^{-1}*	X_{37}	0.52	0.068
Zaman Faktörü *Time Factor*	X_1	-0.73	*0.005

**P-Değeri<0.05, P-Value<0.05*

Pehlivan çeşidine dair tahmin modelinde verimdeki değişiklikleri en iyi tanımlayan değişkenler; Dinlenme dönemi rüzgâr hızı, çıkış/çimlenme dönemi yağışı, sapa kalkma dönemi güneşlenme şiddeti,

vejetasyon süresi minimum sıcaklığı olarak belirlenmiştir. Buna göre, Pehlivan çeşidi için verim tahmin modeli 'Y_3=269.9+149.4(X_{44})+19.95(X_{34})-25.8(X_{61})-9.41(X_{17})+17.1(X_{45})' şeklindedir. Yukarıda belirlenen eşitliğe göre 2005-2013 yılları arasında gerçekleşen verimlerle modelin testleri gerçekleştirilmiş ve hatalar belirlenmiştir. Model 2015, 2017, 2018 ve 2019 yılları için uygulanarak verim tahminleri yapılmıştır (Çizelge 3). Model yardımıyla tahmin edilen yıllar içinde en büyük hata 2018'de gerçekleşmiştir (Şekil 3).



Şekil 3. Pehlivan çeşidinde gerçek ve tahmin edilen verimlerin grafiksel gösterimi
Figure 3. Graphical representation of actual and estimated yields of Pehlivan variety

Bunun nedeninin Pandas çeşidinde açıklananla aynı olduğu düşünülmektedir. Bu nedenle Pehlivan çeşidi için geliştirilen bu modelin yağış miktarını ortalama içinde değerlendirebileceği ancak extrem olayları yakalamada eksik kalabileceği öngörülmektedir. Bu yıl Pehlivan çeşidinde gerçekleşen verim kaybının modele göre yaklaşık %70 seviyelerinde olduğu görülmektedir.

Çeşit-1252 çeşidinde verim tahmini

Çeşit-1252 soğuğa ve kışa dayanıklılığı iyi, yatmaya dayanıklı, orta boylu ve sağlam saplı makarnalık buğday çeşididir. Ortalama verimi kuru koşullarda 250-350 kg/da, sulu şartlarda 350-500 kg/da arasındadır. Su stresinin olmadığı alanlarda verim potansiyeli yüksektir (Anonim, 2021d). Çeşit-1252 çeşidinde iklim faktörlerinden maksimum sıcaklık, rüzgâr hızı ve yağışın verimde etkili temel faktörler olduğu görülmektedir (Çizelge 7).

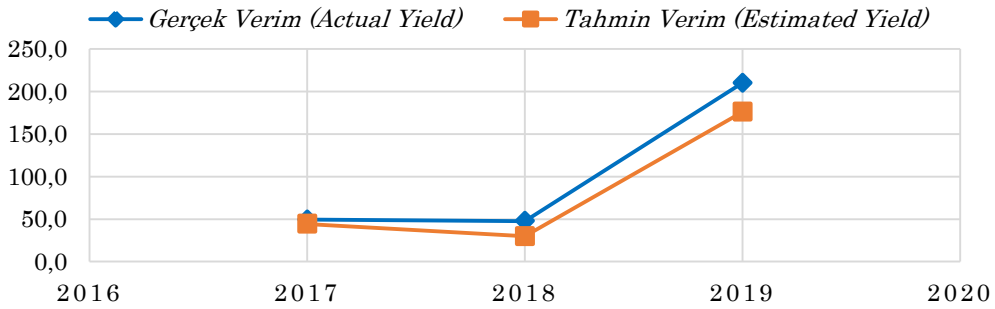
Çizelge 7. Çeşit-1252 verimi üzerine etki eden değişkenler
Table 7. Variables affecting Çeşit-1252 yield

Değişken *Variable*	Sembol *Symbol*	Korelasyon *Correlation*	P-Değeri *P-Value*
Vejetasyon Dönemi Ortalama Maksimum Sıcaklık (TxS), °C *Vegetation Period Avarage Maximum Temperatıre (TxS), °C*	X_9	-0.71	*0.022
Çimlenme/Çıkış Dönemi Ortalama Rüzgâr Hızı (W0), m s^{-1} *Establishment Period Avarage Wind Speed (W0), m s^{-1}*	X_{42}	0.73	*0.015
Dinlenme Dönemi Ortalama Rüzgâr Hızı (W1b), m s^{-1} *Winter Dormancy Period Avarage Wind Speed (W1b), m s^{-1}*	X_{44}	0.70	*0.013
Vejetasyon Dönemi Ortalama Rüzgâr Hızı (WS), m s^{-1} *Vegetation Period Avarage Wind Speed (WS), m s^{-1}*	X_{49}	0.66	*0.037
Çimlenme/Çıkış Dönemi Ortalama Yağış Miktarı (Rf0), mm $gün^{-1}$ *Establishment Period Avarage Rainfall (Rf0), mm day^{-1}*	X_{34}	0.88	*0.001
Sapa Kalkma Dönemi Ortalama Yağış Miktarı (Rf1c), mm $gün^{-1}$ *Head Development Period Avagare Rainfall (Rf1c), mm day^{-1}*	X_{37}	0.58	0.077
Başaklanma Dönemi Ortalama Yağış Miktarı (Rf2), mm $gün^{-1}$ *Flowering (Anthesis) Period Avarage Rainfall (Rf2), mm day^{-1}*	X_{38}	0.70	*0.024
Vejetasyon Dönemi Ortalama Yağış Miktarı (RfS), mm $gün^{-1}$ *Vegetation Period Avarage Rainfall (RfS), mm day^{-1}*	X_{41}	0.80	*0.006

** P-Değeri<0.05, P-Value<0.05*

Verimle en yüksek ilişkinin aynı yönlü (r=0.88) olarak çıkış/çimlenme dönemi yağış miktarıyla, zıt yönlü en yüksek ilişkinin (r=-0.71) ise vejetasyon dönemi maksimum sıcaklıklarıyla olduğu belirlenmiştir. Buna göre verim tahmin eşitliği 'Y_4=245.17+61.61(X_{34})+65.46(X_{37})-12.52(X_{16})-0.54(X_{30})-

5.74(X_{40})' şeklindedir. Modelde Çeşit-1252 verimindeki değişiklikleri en iyi tanımlayan değişkenler; vejetasyon süresi yağışları, sapa kalkma dönemi yağışları, olgunlaşma dönemi minimum sıcaklığı, başaklanma dönemi nemliliği, olgunlaşma dönemi yağışları olarak belirlenmiştir. Elde edilen eşitliğe göre gerçekleşen verimlerle modelin testleri gerçekleştirilmiş ve yıllara göre hataları belirlenmiştir. Hataların çok düşük olması neticesinde modelin uygulanmasına karar verilmiş 2017, 2018 ve 2019 yılları için verim tahminleri yapılmıştır (Çizelge 3). Model yardımıyla tahmin edilen verim değerleri içinde en büyük hata 2019 yılında gerçekleşmiştir (Şekil 4).



Şekil 4. Çeşit-1252 çeşidinde gerçek ve tahmin edilen verimlerin grafiksel gösterimi
Figure 4. Graphical representation of actual and estimated yields of Çeşit-1252 variety

Hatanın temel nedeni tahmin denklemine giren başaklanma dönemi nemliliğinin ortalamanın oldukça üstünde gerçekleşmesidir. Aynı zamanda 2019 yılı olgunlaşma döneminde hiç yağış gerçekleşmemesine rağmen, araştırma periyodu içindeki en yağışlı yıl olma özelliğini taşımaktadır. Çizelge 8'de tahmin yapılan diğer yıllarla karşılaştırma görülmektedir.

Elde edilen sonuçlara göre su stresine (kuraklık) çok dayanıklı olmayan Çeşit-1252 çeşidi buğdayın, kurak geçen 2017 ve 2018 yıllarındaki verimi düşük, nemli geçen 2019 yılında ise verimi yüksek gerçekleşmiştir. Bu bilgiler ışığında geliştirilen tahmin modeli kullanılabilir olarak değerlendirilmiştir.

Çizelge 8. Verim tahmin yıllarında değişkenlerinin karşılaştırılması
Table 8. Comparison of variables over the yield estimation years

Sezon	Rh2	RfS	Rf4
2016-17	66.3	161.4	16.8
2017-18	53.8	190.6	1.4
2018-19	88.0	570.0	0.0

Rh2: Başaklanma Dönemi nispi nemi, RfS:Mevsimlik yağış miktarı, Rf4:Olgunlaşma Dönemi yağış miktarı.

SONUÇ ve ÖNERİLER

İklim değişikliğinin etkilerinin sıkça görüldüğü bir yer olan Şanlıurfa Ceylanpınar'da yaygın olarak yetiştirilen buğday çeşitleri için meteorolojik faktörlerin yardımıyla tahmin modellerinin geliştirilmesi amacıyla gerçekleştirilen bu çalışmada, buğday çeşitlerinde iklim istekleri genetik ve dönemsel olarak farklılıklar göstereceği için çalışmanın büyüme dönemlerine göre yapılmasının daha iyi sonuçlar vereceği düşünülmüştür. Gerçekleştirilen analizler sonucunda elde edilen çeşit temelli tahmin eşitlikleri Çizelge 9'daki gibidir

Kurulan verim modellerine dahil olan değişkenler, %95 güvenilirlik derecesine göre verim üzerine olan etkileri açıklamada anlamlıdırlar. Buna göre; Pandas verim denkleminde X_{41} ve X_1 değişkenlerinin P-değerleri sırasıyla 0.001 ve 0.013, Fırat-93 verim denkleminde X_{41}, X_{36} ve X_2 değişkenlerinin P-değerleri sırasıyla 0.000, 0.000 ve 0.020, Pehlivan verim denkeleminde X_{44}, X_{34}, X_{61}, X_{17} ve X_{45} değişkenlerinin P-değerleri sırasıyla, 0.001, 0.006, 0.007, 0.029 ve 0.049, son olarak Çeşit-1252 verim denkleminde X_{34}, X_{37}, X_{16}, X_{30} ve X_{40} değişkenlerinin P-değerleri sırasıyla 0.000, 0.001, 0.003, 0.006 ve 0.024 olarak gerçekleşmiştir. Bu bilgiler ışığında tahmin modelleri kullanılabilir olarak değerlendirilmiştir. Ancak tahmin modellerinin güçlü yanları olduğu gibi bazı eksik ya da zayıf yönleri olabilmektedir. Bu bağlamda yukarıda Pandas ve Pehlivan çeşitleri için belirlenen tahmin modellerinin yapılan incelemede özellikle dolu, sel veya taşkına neden olabilecek yağışlar neticesinde meydana gelen kayıpları öngörmekte yeterli olamayabileceği düşünülmektedir. Fırat-93 çeşidi için geliştirilen modelde ise mevsim normalleri için tutarlı sonuçlar verirken, kurak dönemlerde modelde hata oranının yüksek olacağı öngörülmektedir. Çeşit-1252 çeşidi buğday için geliştirilen modelde ise nemliliğin ve dönem içindeki yağışların miktarlarının yöre normallerinin üzerinde seyretmesi neticesinde hata oranının arttığı diğer dönemler için kabul edilebilir tahminler yapabileceği sonucuna varılmıştır. .

Çizelge 9. Buğday çeşitleri için verim tahmin eşitlikleri
Table 9. Yield estimation equations for wheat varieties

Çeşit *Variety*	Tahmin Eşitlikleri *Estimation Equals*	S *S*	R^2 (Düzeltilmiş) *R^2 (Adjusted)*
Pandas	Y_1=-316.7+460.59(X_{41})-3.952(X_1)	0.172	100.00
Fırat-93	Y_2=-397.64+194(X_{41})+119(X36)+8(X_2)	16.100	97.99
Pehlivan	Y_3=269.9+149.4(X_{44})+19.95(X_{34})-25.8(X_{61})-9.41(X_{17})+17.1(X_{45})	0.241	100.00
Çeşit-1252	Y_4=245.17+61.61(X_{34})+65.46(X_{37})-12.52(X_{16})-0.54(X_{30})-5.74(X_{40})	0.122	100.00

Araştırma sonuçları, büyüme dönemlerine göre verim tahmin modellerinin geliştirilebileceğini ve bu modeller yardımıyla başarılı tahminlerin yapılabileceğini göstermektedir. Ancak bu noktada büyüme dönemlerinin net şekilde belirlenebilmesinde fenolojik gözlemler büyük önem taşımaktadır. Çalışmada, meteorolojik faktörler yardımıyla geliştirilen verim tahmin modelleriyle, yöre için ortalama sayılabilecek iklim koşullarında tutarlı tahminler yapılabilmektedir. Ancak dönemsel olarak yaşanabilecek sel, taşkın, dolu benzeri ekstrem olayların etkilerini yansıtmada eksiklikler göze çarpmaktadır. Bu nedenle ekstrem olayların değişkenler olarak hesaba katıldığı modellerin geliştirilmesi durumunda, daha başarılı sonuçların sağlanabileceği kanaatine varılmıştır.

Araştırmacıların Katkı Oranı Beyan Özeti

Yazarlar makaleye eşit oranda katkı sağlamış olduklarını beyan eder.

Çıkar Çatışması Beyanı

Makale yazarları aralarında herhangi bir çıkar çatışması olmadığını beyan ederler.

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Effect of Brewing Conditions on Sensorial and Antioxidant Properties of Sage Tea

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ABSTRACT
In this study, the effects of infusion time and temperature on the sensorial properties and antioxidant capacity of sage tea were evaluated by using the response surface methodology. The obtained quadratic models explained more than 90% variability in the responses. The infusion temperature showed significant negative effect on the sensorial properties whereas it had significant positive effect on the total phenolic content and antioxidant capacity ($p<0.05$). Moreover, the infusion time showed significant negative effects ($p<0.05$) on the responses. The best combination of brewing conditions was determined as 75-80 ºC and 2-4 min.

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Demleme Koşullarının Adaçayının Duyusal ve Antioksidan Özellikleri Üzerine Etkisi

ÖZET
Bu çalışmada, infüzyon süresi ve sıcaklığının adaçayının duyusal özellikleri ve antioksidan kapasitesi üzerine etkileri yanıt yüzey yöntemi kullanılarak değerlendirilmiştir. Elde edilen ikinci dereceden modeller %90'dan fazla değişkenliği açıklamıştır. İnfüzyon sıcaklığı adaçayının duyusal özellikleri üzerinde anlamlı negatif etki gösterirken toplam fenolik madde içeriği ve antioksidan kapasite üzerinde anlamlı pozitif etki göstermiştir ($p<0.05$). Ayrıca, infüzyon süre parametreler üzerinde anlamlı negatif etki göstermiştir ($p<0.05$). En iyi demleme koşulunun 75-80 ºC ve 2-4 dk olduğu belirlenmiştir.

Gıda Bilimi

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INTRODUCTION

Herbal tea infusions prepared from the leaves, flowers, fruits, stems or root of plant species have been used for a long time. Sage, one of the most consumed herbal teas, is extremely popular in the folk medicine (Kamiloğlu et al., 2016). It is used in traditional remedies for the treatment of mild dyspepsia, excessive sweating, and inflammation of throat (Ghorbani and Esmaeilizadeh, 2017). Hypoglycemic (Khattab et al., 2012), antiinflammatory (Baricevic et al., 2001), antioxidant, and antibacterial properties (Bozin and Mimica-Dukić, 2007; Hayouni et al., 2008) of the sage have also been reported.

Salvia species are native to Middle East and Mediterranean areas. *Salvia fruticosa* (synonym: *S. triloba*), *S. cryptantha*, *S. multicaulis*, *S. sclarea* and *S. tomentasa* are the species grown in the natural flora of Turkey. *S. fruticosa* and *S. tomentasa* were mostly collected from the nature and exported as tea and spice (Aydın et al., 2019).

Sage contains terpenoids and phenolic compounds (Vosoughi et al., 2018). These compounds may contribute to the pharmacological properties of sage. Phenolic compounds are reported to exhibit antioxidant properties. The beneficial effects of herbal tea infusions could be attributed to their antioxidant capacity. The antioxidant capacity of herbal tea may depend on the extracted compounds from the herb to herbal tea infusion. The extracted antioxidant compounds may also impact on the sensorial properties due to the organoleptic properties of some

antioxidant compounds. Brewing conditions may have impact on the antioxidant capacity and sensorial properties of herbal tea infusions. Some studies revealed that infusion time and temperature are the most important parameters affecting antioxidant capacity of tea beverages (Hajiaghaalipour et al., 2016; Sharpe et al., 2016; Kelebek et al., 2019).

Brewing conditions may affect consumer preference because of their impacts on the sensorial properties and antioxidant capacity of herbal tea infusions. In the literature, available studies on the effects of brewing conditions on the sensorial properties and antioxidant capacity of sage tea infusion are limited. Therefore, the objective of this study was to evaluate the effects of infusion time and temperature on the sensorial properties, total phenolic content, and antioxidant capacity of sage tea using response surface methodology (RSM).

MATERIAL and METHOD

Experimental Design

Central composite design was applied for the RSM study. Infusion time (2-6-10 min) and temperature (75℃-85℃-95℃) were selected as independent variables.Ten experimental settings were generated (Table 1). Duplicate analyses were performed at each design point. The regression analysis, stastical significance and response surface were analysed ($p< 0.05$). Modde Pro software (Umetrics, Sweden) was used for the experimental design and data analysis.

Preparation of Herbal Tea Infusion

Dried sage (*Salvia triloba* L.) was purchased from a local market. According to the information obtained from its producer company (Beşikçi Spices Limited Company), it was harvested from Aegean region in August (2018), dried with traditional techniques at 35 ℃, and stored in the insulated container. The sage was ground with a laboratory mill (IKA M20, Germany) and passed through a 250-micron sieve. The infusion rate was selected as 1% with respect to the literature survey and the sensorial evaluation of the tea prepared at different infusion rates (1-1.5-2 %). 1 g of sage was weighed in a glass beaker (200 mL) and 100 mL of water at 75℃- 85℃-95℃ was added. After filling the water, the beaker was immediately placed in the water bath. It was brewed at the experimental conditions in the water bath and then cooled and and filtered through filter paper (Whatman Grade 1) The herbal infusion was stored at 4 ºC and was analysed in 24 hours.

Sensorial Properties

Consumer preference test was performed. Authorization for research with human subjects was obtained from Ethical Commission of Gümüşhane University (Date: 30/10/2018- Number :2018/8). A total of 50 healthy volunteers were included for the evaluation of the colour, flavour, taste, and overall acceptability of the analysed tea samples. They were not allowed to eat anything within 1 h before the session. The tea samples (20 mL) were served in the cups coded with three random digit numbers. Water was given to the panellists to rinse their mouth between samples. A nine-point scale was used for evaluation (1: dislike extremely, 9: like extremely). Sensory analysis was performed in three-stage.

The students of Department of Nutrition and Dietetics evaluated the samples (female : 30 and male: 20). The age of the students ranged from 18 to 22 years old.

Total Phenolic Content

Total phenolic content was determined using the Folin Ciocalteu method. A 50-µL of sample was mixed with Folin-Ciocalteu reagent (500 µL), sodium carbonate (1 M, 400 µL), and water (4 mL). The absorbance was measured at 760 nm after 1 hour. The calibration curve was prepared with gallic acid ranging from 0 to 1 mg mL^{-1}. The TPC were expressed as mg gallic acid per L of tea.

Antioxidant Activity Analysis

Ferric reducing antioxidant power (FRAP) and 2,2-diphenyl-1-picrylhydrazyl (DDPH) radical scavenging activity assays were performed to determine antioxidant capacity

FRAP Assay

The FRAP was determined according to Benzie and Strain (1996). FRAP reagent was prepared by mixing several solutions (10:1:1) acetate buffer solution (pH = 3.6), TPTZ solution in 40 mM HCI (10 mM), and $FeCI_3$ (20 mM) solution. A total of 50-µL of the diluted sample (1:9) was mixed with 1450 µL of FRAP reagent and the absorbance was measured at 595 nm after 20 min. The results were expressed as micromoles of Trolox.

DPPH Radical Scavenging Activity Assay

The DPPH radical scavenging activity was determined according to Brand-Williams et al. (1995). DPPH radical scavenging assay, 50-µL of the diluted sample was mixed with 1450 µL of DPPH radical solution (100 µM). The absorbance was measured at 515 nm after 60 min at room temperature.The results were expressed as micromoles of Trolox.

Statistical Analysis

The regression analysis and analysis of variance (ANOVA) were analysed ($p< 0.05$). Experimental design and data analyses were performed using the

Modde Pro (Umetrics, Sweden) software.

RESULTS and DISCUSSION

Sensorial Properties

The sensorial scores of the sage tea infusions are presented in Table 1. The scores of the colour, flavour, taste, and overall acceptability ranged from 4.3 to 5.8, from 4.4 to 5.6, from 3.1 to 4.3, and from 3.4 to 4.6.

Table 2 showed that the regression was significant ($p<0.05$) for the colour and flavour scores of the sage tea. Moreover, the quadratic models of these variables had no lack of fit value ($p>0.05$). The obtained quadratic models explained more than 90% variability in the responses ($R^2>0.90$) so they can be used to explain the effect of the studied variables on these responses.

As can be from Table 3, the most significant independent variable was temperature for the sage tea. The linear term of temperature (T) had negative impact on the colour and flavour scores of the sage tea while the quadratic term of temperature (T×T) presented significant positive impact ($p<0.10$).

Table 1. Central composite design and responses
Çizelge 1. Merkezi dizayn ve yanıtlar

Experiment no *Deney no*	Uncoded (coded) levels *Seviyeler* Temperature (°C) *Sıcaklık*	Time (min) *Süre*	Colour *Renk*	Flavour *Koku*	Taste *Tat*	Overall acceptability *Genel beğeni*	TPC (mg L^{-1}) *TFM*	DPPH (µmol) *DPPH*	FRAP (µmol) *FRAP*
1	75(-1)	2(-1)	5.24	5.35	4.10	4.57	486.40	5463.33	6399.39
2	75(-1)	6(0)	5.76	5.57	3.92	4.39	504.81	5165.00	6110.00
3	75(-1)	10(1)	5.65	5.45	3.69	4.18	457.26	5255.00	6095.86
4	85(0)	2(-1)	4.41	4.43	3.51	3.69	483.06	6403.75	7358.89
5	85(0)	6(0)	4.86	4.80	4.33	4.57	471.26	5841.67	6686.26
6	85(0)	6(0)	4.69	4.41	3.82	4.14	469.04	5633.33	6557.98
7	85(0)	10(1)	4.41	4.63	3.41	3.96	447.13	5910.00	6668.08
8	95(1)	2(-1)	4.34	4.62	3.38	3.48	581.40	5583.33	6795.86
9	95(1)	6(0)	4.40	4.70	3.50	3.84	610.05	5100.00	6367.07
10	95(1)	10(1)	4.52	4.64	3.12	3.38	531.98	5493.33	6717.58

TFM: toplam fenolik madde, DPPH: 2,2-diphenyl-1-picrylhydrazyl radikal yakalama aktivitesi, FRAP: Demir indirgeyici antioksidan güç.

Table 2. Analysis of variance (ANOVA) of sage tea
Çizelge 2. Adaçayının varyans analizi (ANOVA)

Factor *Faktör*	DF	Sum of Square *Kareler toplamı*	Mean square *Kareler ortalaması*	F-value	*p*-value
Colour *Renk*					
Regression *Regresyon*	5	2.485	0.497	17.555	**0.008**
Residual *Artık*	4	0.113	0.028		
Lack of fit *Uyum Eksikliği*	3	0.098	0.032	2.091	0.461
Pureerror *Hata*	1	0.016	0.016		
R^2: 0.96					
Flavour *Koku*					
Regression *Regresyon*	5	1.580	0.316	14.454	**0.011**
Residual *Artık*	4	0.087	0.022		
Lack of fit *Uyum Eksikliği*	3	0.011	0.004	0.046	0.981
Pureerror *Hata*	1	0.077	0.077		
R^2: 0.95					
TPC *TFM*					
Regression *Regresyon*	5	25501.800	5100.350	20.834	**0.006**
Residual *Artık*	4	979.236	244.809	133.087	0.064
Lack of fit *Uyum Eksikliği*	3	976.789	325.596		
Pureerror *Hata*	1	2.447	2.447		
R^2: 0.96					
DPPH					
Regression *Regresyon*	5	1300590.000	260118.000	11.917	**0.016**
Residual *Artık*	4	87313.800	21828.500		
Lack of fit *Uyum Eksikliği*	3	65611.100	21870.400	1.008	0.607
Pureerror *Hata*	1	21702.700	21702.700		
R^2: 0.94					
FRAP					
Regression *Regresyon*	5	1102690.000	220538.000	7.224	**0.039**
Residual *Artık*	4	122110.000	30527.400		
Lack of fit *Uyum Eksikliği*	3	113882.000	37960.600	4.614	0.327
Pureerror *Hata*	1	8227.850	8227.850		
R^2: 0.90					

Bold values are significant at 95% confidence of level. TFM: toplam fenolik madde, DPPH: 2,2-diphenyl-1-picrylhydrazyl radikal yakalama aktivitesi, FRAP: Demir indirgeyici antioksidan güç.

Table 3. Regression coefficients of sage tea
Çizelge 3. Adaçayının regrasyon katsayıları

Factor *Faktör*	**Colour** *Renk* Regression coefficient *Regrasyon katsayısı*	*p*-value *p-değeri*	**Flavour** *Koku* Regression coefficient *Regrasyon katsayısı*	*p*-value *p-değeri*	**TPC** *TFM* Regression coefficient *Regrasyon katsayısı*	*p*-value *p-değeri*	**DPPH** *DPPH* Regression coefficient *Regrasyon Katsayısı*	*p*-value *p-değeri*	**FRAP** *FRAP* Regression coefficient *Regrasyon katsayısı*	*p*-value *p-değeri*
Mean *Ortalama*	4.718	**0.000[a]**	4.624	**0.000[a]**	481.729	**0.000[a]**	5765.220	**0.000[a]**	6654.650	**0.000[a]**
Temperature (L) *Sıcaklık*	-0.462	**0.001[a]**	-0.332	**0.003[a]**	37.780	**0.002[a]**	40.855	0.453	174.630	**0.040[a]**
Temperature (Q) *Sıcaklık*	0.283	**0.018[a]**	0.330	**0.007[a]**	42.971	**0.003[a]**	-441.092	**0.002[a]**	-301.407	**0.017[a]**
Time (L) *Süre*	0.081	0.223	0.043	0.430	-15.731	**0.039[a]**	-110.320	**0.089[b]**	-146.881	**0.065[b]**
Time (Q) *Süre*	-0.161	**0.094[b]**	-0.067	0.360	-17.960	**0.058[b]**	240.702	**0.020[a]**	213.686	**0.049[a]**
Interaction *Interaksiyon*	-0.039	0.528	-0.013	0.803	-3.414	0.548	20.185	0.703	37.777	0.552

L Linear, *Q* Quadratic, [a]Bold values are significant at 95% confidence of level, [b]Bold values are significant at 90% confidence of level. TFM: toplam fenolik madde, DPPH: 2,2-diphenyl-1-picrylhydrazyl radikal yakalama aktivitesi, FRAP: Demir indirgeyici antioksidan güç.

The colour and flavour scores of the sage tea decreased with increasing temperature until the midpoint was reached and then it changed slightly. It changed slightly with increasing time (Fig. 1 and 2).

The flavour scores of the studied herbal tea infusions were found to decrease with increasing brewing temperature, which may be attributed to their volatile compounds. *Salvia officinialis* includes α-thujone, ß-thujone, 1-8-cineole, α-pinene, camphor, caryophyllene, germacrene D, viridiflorol, elemene, α-humulene, linalool, borneol, and ledene as volatile compounds (Sharifi-Rad et al., 2018). The panellists could perceive more volatile compounds with increasing temperature, leading to a less flavour score.

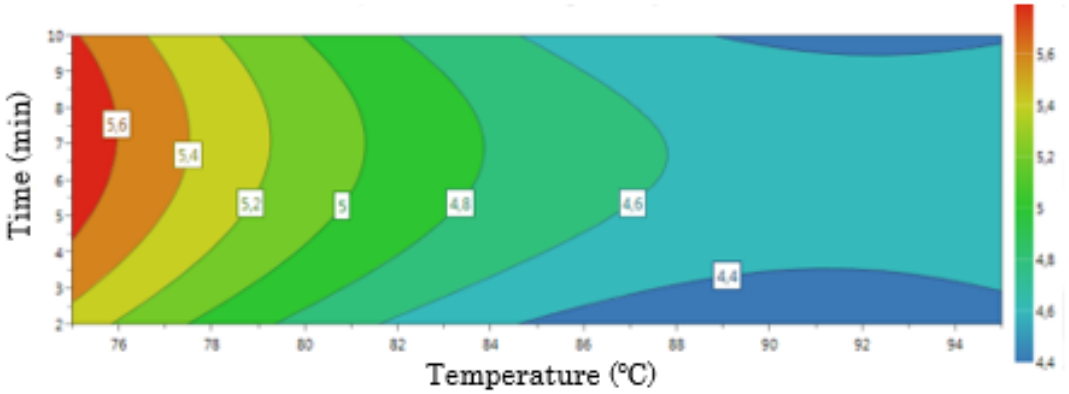

Figure1. Contour plot of colour
Şekil 1. Kontur grafiği: renk

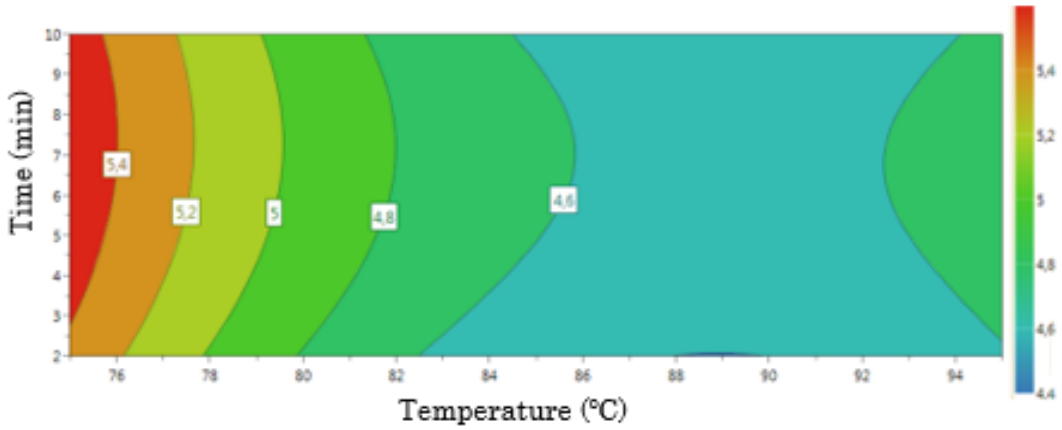

Figure 2. Contour plot of flavour
Şekil 2. Kontur grafiği: koku

Total Phenolic Content

Total phenolic content (TPC) of the herbal infusion samples is presented in Table 1. The TPC ranged from 447 to 610 mg L^{-1} for the sage tea.

Table 2 showed that the regression was significant ($p<0.05$) and the quadratic model had no lack of fit value ($p>0.05$). The quadratic model explained 96% variability (R^2 : 0.96) so it can be used to explain the effects of the studied variables on the TPC.

The regression coefficients of the generated models are shown in Table 3. The most significant independent variable was temperature for the sage tea. The linear term of temperature (T) and the quadratic of temperature (T×T) had significant positive impacts on the TPC of the sage tea ($p<0.05$) whereas the linear term of time (t) and the quadratic term of time (t×t) showed significant negative impacts on the TPC ($p<0.10$).

The TPC of the sage tea increased with increasing temperature after the midpoint was reached. It decreased with increasing time after the midpoint was reached (Fig. 3).

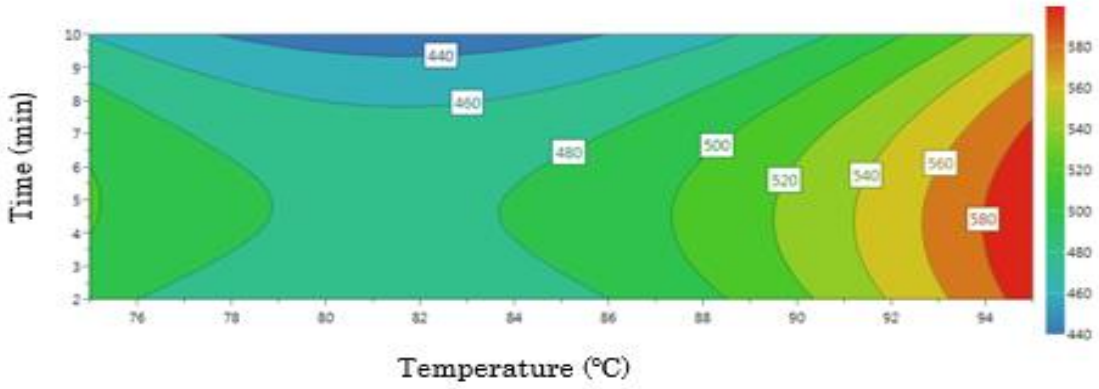

Figure 3. Contour plot of TPC
Şekil 3. Kontur grafiği: TFM

The TPC of the sage tea presented an increasing trend between 83 ºC and 95ºC. According to Sun et al. (2017), the TPC of the wolfberry infusion showed an increased trend with increasing temperature, ranging from 60 ºC to 100 ºC. Komes et al. (2010) found that the green tea samples reached their maximum TPC at 100 ºC when they were brewed at 60 ºC, 80 ºC, and 100 ºC. Dent et al. (2012) reported that the TPC of sage's water extract increased at higher temperatures from 60ºC to 90ºC. A higher temperature may improve the solubility of phenolic compounds and enhance the permeability of cell walls, increasing the diffusion coefficients of phenolic compounds, which may explain the results of this study (Harbourne et al., 2013).

The TPC of the sage tea decreased with increasing time between 6 and 10 min, which could be attributed to the decomposition of phenolic compounds with a longer infusion time (Sentkowska et al., 2016).

DPPH Radical Scavenging Activity

The DPPH values of the tea samples are presented in Table 1. The DPPH value ranged from 5100 to 6404 µmol TE for the sage tea.

Table 2 showed that the regression was significant ($p<0.05$) and the generated model had no lack of fit value ($p>0.05$). The model explained 94% variability in the DPPH value (R^2: 0.94), so it can be used to explain the effects of the studied variables on the DPPH radical scavenging activity.

The regression coefficients of the generated models are shown in Table 3. The most significant independent variable was time. It can be seen from Table 3, the quadratic term of time (t×t) had significant positive impact on the DPPH value of the sage tea whereas the linear term of time (t) and the quadratic of temperature (T×T) showed significant negative impacts on the DPPH value ($p<0.10$).

The DPPH value of the sage tea increased with increasing temperature until the midpoint was reached and then it decreased. It decreased with increasing time until the midpoint was reached (Fig. 4).

Similar findings were found in the literature. Sotiropoulou et al (2020) found that the antioxidant capacity of sage extract increased from 25 to 80 °C and decreased between from 80 to 100 °C. The antioxidant activity of the herbal tea extracts such as sage, chamomile, linden, lingia and gyokuro were determined to decrease at the highest temperature (Horžic et al., 2009; Stagos et al., 2012; Cvetanovic et al., 2019). A reduction in the DPPH value of the sage tea was observed after it increased up to the certain extent, which may be related to the degradation of antioxidant compounds or alterations in their

molecular structure with a higher infusion temperature. A reduction in the antioxidant capacity was previously attributed to a loss of antioxidant compounds due to intense thermal treatment (Sotiropoulou et al., 2020). Furthermore, the polymerization of phenolic compounds may reduce antioxidant activity (Fu et al., 2018).

The DPPH value of the sage tea decreased with increasing infusion time. The decomposition of antioxidant compounds could lead to a reduction in the DPPH radical scavenging activity.

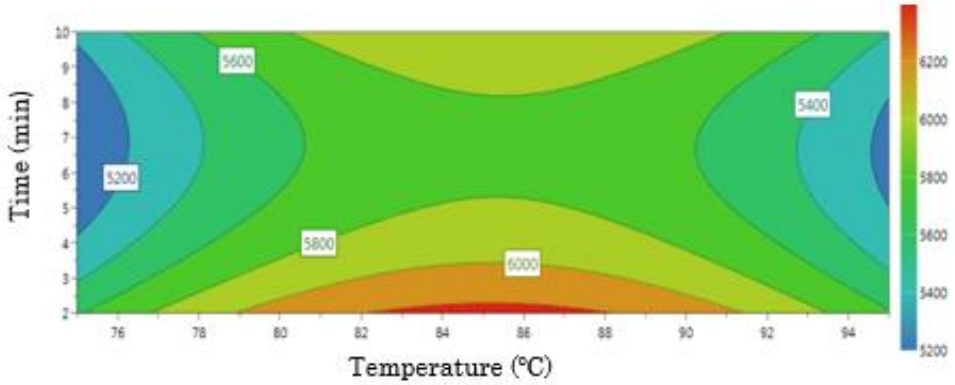

Figure 4. Contour plot of DPPH
Şekil 4. Kontur grafiği: DPPH

FRAP

The FRAP values of the tea samples are presented in Table 1. The FRAP value ranged 6110 to 7359 µmol TE for the sage tea.

Table 2 showed that the regression was significant ($p<0.05$) and the generated model had no lack of fit value ($p>0.05$). The model explained 90% variability in the FRAP value (R^2 : 0.90) so it can be used to explain the effects of the studied variables on the FRAP.

The regression coefficients of the generated models are shown in Table 4. The most significant independent variable was temperature for the sage tea. The linear term of temperature (T) and the quadratic term of time (t×t) had significant positive impacts on the FRAP value of the sage tea ($p<0.05$) whereas the quadratic term of temperature (T×T) and the linear term of time (t) presented significant negative impacts on the FRAP value ($p<0.10$).

The FRAP value of the sage tea increased with increasing temperature until the midpoint was reached and then it changed slightly. The FRAP value of the sage tea decreased with increasing time until the midpoint was reached and then it changed slightly (Fig. 5).

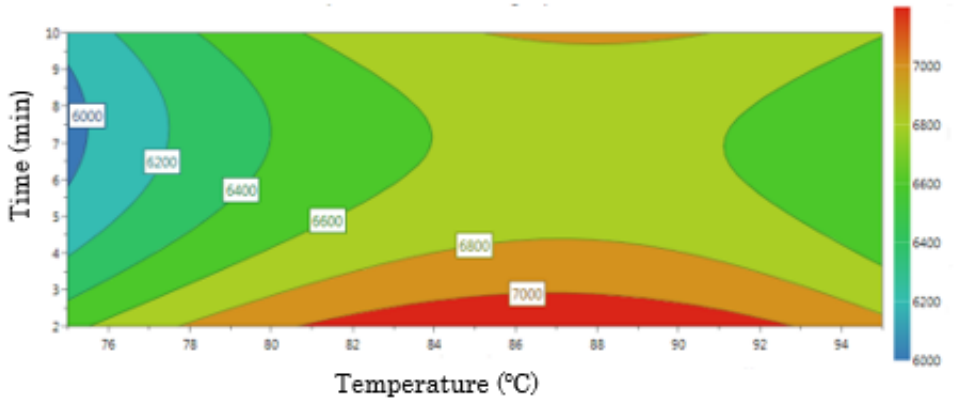

Figure 5. Contour plot of FRAP
Şekil 5. Kontur grafiği: FRAP

The FRAP value of the sage tea increased to reach the midpoint of the response surface, and then there was no further increase. Similar results were reported for black tea. Black tea showed a linear increase between 20 °C and 70 °C, and with no further increase between 70 °C and 90 °C (Langley-Evans, 2000). With increasing infusion temperature, an increase in the TPC could be resulted in an increase in the antioxidant activity.

The FRAP value of the sage tea decreased with

increasing infusion time, which may be related to the decomposition of antioxidant compounds (Sentkowska et al., 2016).

CONCLUSION

The results of this study revealed that the infusion temperature had negative effect on the sensorial properties and infusion time had negative effect on the antioxidant capacity at the studied brewing conditions (75-95 °C and 2-10 minutes). It can be concluded that the best brewing conditions were the lowest water temperature (75-80ºC) and the shortest brewing time (2-4 min) for the studied brewing conditions. Lower infusion temperature and shorter infusion time can be recommended to obtain the most admired sage tea infusion with higher antioxidant capacity.

Author Contribution

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

Conflict of Interest

The authors declare that there is no conflict interest

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Determination of the Effect of Garden Cress (*Lepidium sativum* L.) on the Ripening Parameters of White Cheese

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ABSTRACT
The aim of this study is to reveal the effect of *Lepidium sativum* L. on the ripening characteristics of White cheese. A control White cheese without garden cress and White cheeses with four different concentrations of garden cress (0.6-1.2-1.8-2.4%) were produced and their dry matter, fat, and protein contents; pH and titratable acidity levels; water-soluble nitrogen (WSN) and trichloroacetic acid-soluble nitrogen (TCA-SN) were determined and electrophoretograms showing α- and ß-casein degradation rates were obtained for the storage of 90 days. The addition of garden cress did not affect WSN and TCA-SN values significantly (P>0.05). Both α- and ß- casein degradations increased with storage; the latter being relatively less broken down. The degradation levels for α-casein were similar for all cheese types while ß-casein was more degraded when garden cress was included in the cheeses. There was no statistically significant difference between cheese samples in terms of sensory properties (P>0.05). The results suggest that garden cress may carry a ß-casein specific protease and could be conveyed with White cheese successfully. This study will contribute to the limited knowledge on the utilization of garden cress in food products. Also, the application of garden cress is thought to expand the product variety in the market.

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Tere Otunun (*Lepidium sativum* L.) Beyaz Peynirin Olgunlaşma Parametrelerine Etkisinin Belirlenmesi

ÖZET
Bu çalışmanın amacı, tere otunun (*Lepidium sativum* L.) Beyaz peynirin olgunlaşması üzerine etkisini belirlemektir. Kontrol Beyaz peynir ve dört farklı tere otu konsantrasyonu (%0.6-1.2-1.8-2.4) içeren Beyaz peynirler üretilmiş ve bunların kurumadde, yağ ve protein içerikleri; pH ve titre edilebilir asitlik seviyeleri; suda çözünür azot (WSN) ve triklorasetik asit (TCA)'te-çözünür azot (TCA-SN) oranları belirlenmiş ve 90 günlük depolama süresince α- ve ß-kazein parçalanma oranlarını gösteren elektroforetogramlar elde edilmiştir. Tere otu katkısının WSN ve TCA-SN oranları üzerinde istatistiksel olarak anlamlı bir etkisi tespit edilememiştir (P>0.05). Hem α- hem de ß- Kazein parçalanma düzeyi, depolama periyodu boyunca artarken ß-Kazeinin α-Kazeine kıyasla daha az parçalandığı gözlenmiştir. α-Kazein için parçalanma seviyeleri tüm peynir türleri için benzer iken, tere otu ilave edilen peynirlerde ß-Kazeinin daha fazla parçalandığı belirlenmiştir. Duyusal özellikler açısından peynir örnekleri arasında istatistiksel olarak önemli bir farka rastlanmamıştır (P>0.05). Sonuçlar, tere otunun ß-Kazeine özgü bir proteaz taşıyabileceğini ve Beyaz peynirin tere otu tüketiminde taşıyıcı olarak başarılı bir şekilde kullanılabileceğini göstermektedir. Bu çalışma, tere otunun gıda ürünlerinde kullanımı hakkındaki sınırlı bilgiye katkıda bulunacaktır. Ayrıca, tere otunun

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INTRODUCTION

Spices and herbs are usually not consumed alone but mostly used as an additive to make the foods more tasty, appealing, and presentable. With over 200 years of history, Turkish Otlu cheese is one of the best examples of herby foods containing approximately 25 different kinds of herbs including *Allium vinarale, Chaerophyllum macropodum,* and *Antriscus nemorosa*. These herbs not only give the characteristic flavor to Otlu cheese but also extend its shelf life, owing to their antimicrobial properties (Hayaloglu and Fox, 2008; Tarakci and Temiz, 2009). Also, the addition of spices to cheese reduces the need for salt thereby limits the structural problems generated due to salt (Ayar and Akyüz, 2003). Different cheese products have been fortified with a variety of spices and herbs including chili pepper, thyme, mint, cumin, nutmeg, clove, cinnamon, black pepper, cumin, oregano, and fennel to bring antimicrobial properties (Masatcıoğlu and Avşar, 2005; Shan et al., 2011; Akarca et al., 2016).

Lepidium sativum L., a member of the *Brassicaceae (Cruciferae*) family, has a short vegetation time. Except for very hot seasons, it can be grown pretty much all year long everywhere in Turkey and also in India, China, and Africa (Yanmaz et al., 2010). Annual garden cress production of Turkey was 31 tons in 2005 and with an increasing trend, it reached 2762 tons in 2019 (TSI, 2020). It is usually combined with other foods, especially when cooked, to suppress its bitter taste. The leaves are consumed either raw in salads or as cooked with vegetable curries and used as a garnish, especially in Indian and Iranian cuisines due to its spicy, tangy, and peppery flavors (Mali et al., 2007; Sharma and Agarwal, 2011). The leaves are not only important sources of macro elements such as sodium, potassium, calcium, magnesium, and phosphorus, but also rich in some trace elements such as iron, zinc, and manganese. They are also rich in vitamins A, B, C, and K (Yanmaz et al., 2010; Hassan et al., 2011). Garden cress was one of the 26 vegetables tested from the Iranian diet with an extraordinarily high antioxidant activity, even higher than that of quercetin (Souri et al., 2004). The volatile compounds acquired from crushed leaves were found to have antibacterial activity against *Bacillus subtilis* and *Micrococcus pyogenes* var. *aureus* and they also showed lower activity against *Escherichia coli* (Sharma and Agarwal, 2011). Therefore, the leaves are used as therapeutics for a variety of health issues including asthma and cough. The leaves are mildly stimulant, diuretic, and useful in scorbutic diseases and liver complaints (Mali et al., 2007). It also helps in the alleviation of anemia due to its high iron content (Jain and Grover, 2018). Finally, *L. sativum* leaves are rich in essential amino acids; isoleucine, leucine, phenylalanine, tyrosine, and valine but poor in sulfur-containing amino acids; methionine and cysteine. Due to relatively rich essential amino acid content, e.g., methionine, cheese could be a potential complementary conveyor of garden cress to fulfill the need of consuming garden cress with other foods as indicated by Hassan et al. (2011).

Recently, there has been a growing interest in the utilization of natural additives that can accelerate the ripening while contributing to the sensory properties of cheese. We believe that this is the first work on the use of garden cress aiming to improve the ripening characteristics of cheese. To present an alternative functional cheese product to the consumers, this research aims to investigate the effects of *L. sativum* L. on some chemical, biochemical properties, and ripening characteristics of White cheese.

MATERIALS and METHODS

Preparation of garden cress

Fresh *L. sativum* L. was purchased from a local bazaar in Ordu, Turkey. Only its leaves were picked for further processing. After washing under tap water, the leaves were sliced into short strips using a knife and added to curd in this form (Figure 1). The garden cress concentrations applied to cheeses have been determined in preliminary trials.

Cheese production

Cheese production was carried out twice based on the method given by Tarakçı and Deveci (2019). White cheeses were produced from pasteurized (85 °C, 20 s) cow milk in a local dairy plant (Sül-Meh-Ser Körpe LLC, Samsun, Turkey). The pasteurized milk was cooled down to 32-35 °C, then the starter culture (%0.1 milk basis; a mixture of *Lactococcus lactis* subsp. *lactis* and *Lactococcus lactis* subsp. *cremoris*), calcium chloride (0.02% milk basis), and calf rennet (1:16000 MCU/mL) was added followed by an incubation period of approximately one hour at the

already indicated temperature range. Coagulum was cut into approximately 1 cm^3 cubes and let it set. Once it reached the required pH range between 5.1 and 5.4, the whey was removed, and the curd pieces were collected in a cheesecloth. The curds were pressed slightly in order to remove some of the remaining whey. Then, the whole curd was divided into 5 equal portions. Simultaneously, *L. sativum* L. strips were added into the curds at four different concentrations (0% (C), 0.6% (T1), 1.2% (T2), 1.8% (T3), and 2.4% (T4), curd weight basis) and gently mixed until a homogenous pile was obtained. An additional manual pressing step (0.2 kgf/cm^2) was applied approximately 2h until a firm structure was obtained. The curds were shaped in a circular form (15 cm diameter and 4 cm height) and dry-salted (3%, w/w) to make it suitable for vacuum-packaging and also, enhance the rind formation. Finally, they were vacuum-packed and stored at 4 °C for 3 months (Figure 1). All the analyses were performed on the 1st, 30th, 60th, and 90th days of storage.

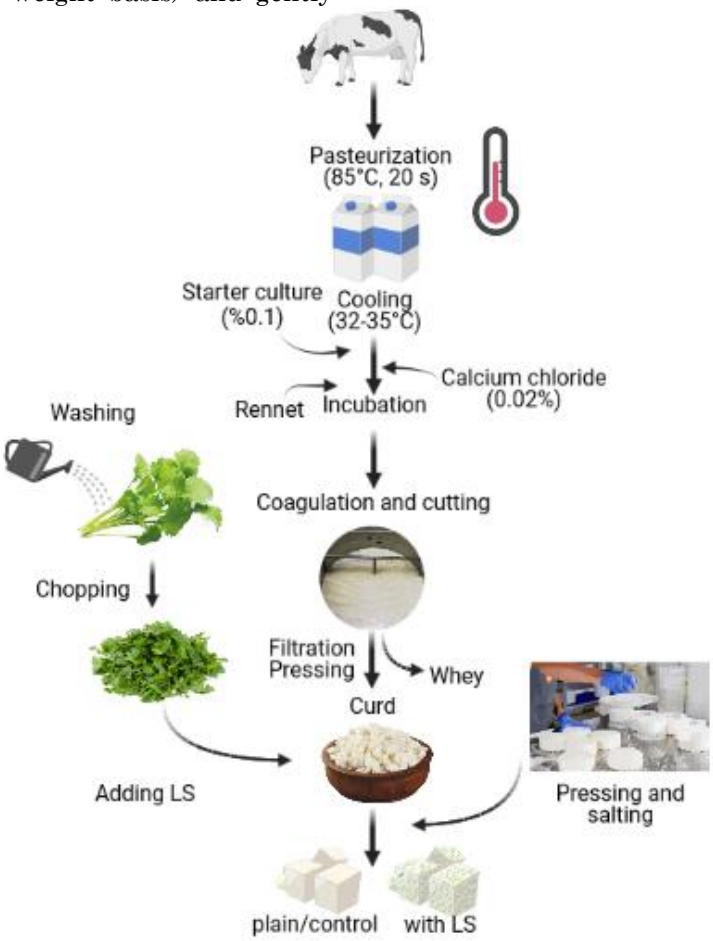

Figure 1. A flow diagram for the production of LS-added White cheeses
Şekil 1. LS-katkılı Beyaz peynirlere ilişkin üretim akış diyagramı

Chemical analysis

The dry matter ratio of cheeses was determined using a gravimetric method according to IDF (2004). The Gerber-van Gulik method was followed to determine the fat content (Nielsen, 2003). To determine titratable acidity and pH, cheese slurries (10%, w/v) were prepared, and the filtrates were used. The filtrates were titrated with 0.1 N NaOH, in the presence of phenolphthalein indicator to determine the total titratable acidity as lactic acid % (AOAC, 1990) and a pH meter (model Starter 3100; OHAUS, NJ, USA) probe was immersed into the filtrates for pH measurements. The salt content was determined according to the Mohr method. Briefly, the previously prepared cheese filtrates were titrated with 0.1 N $AgNO_3$ using potassium chromate as the indicator (Nielsen, 2003).

An automatic distillation unit (UDK-149; VELP Scientifica, Usmate, Italy) was used to determine the total nitrogen contents of the cheese samples based on the Kjeldahl method (IDF, 1993). The nitrogen concentrations were multiplied by a factor of 6.38 in order to determine total protein concentration (%). The filtrate used for pH and titratable acidity was used for determination of water-soluble nitrogen (WSN%) while this filtrate was treated with 12% trichloroacetic acid (TCA) for 2 hours at room temperature to determine TCA-soluble nitrogen

(TCA-SN%). They were determined using the same setup and the values were expressed as the percentage of total nitrogen (Bütikofer et al., 1993).

Proteolysis and urea–polyacrylamide gel electrophoresis (Urea-PAGE)

Urea-PAGE was performed in a vertical type electrophoresis system (SE600X Chroma Deluxe; Hoefer Inc., MA, USA) according to the method of Ardö and Polychroniadou (1999) modified by Celik et al (2018). The cheese samples (100 mg) were dissolved in 2 mL sample buffer (1.5 g tris methylamine, 49 g urea, 0.15 g bromophenol blue, 2 mL 2-mercaptoethanol, and 0.4 mL HCl per 100 mL). Samples were run in an electrode buffer (0.3% tris methylamine, 1.46% glycine, pH=8.4) using a 1.5 mm thick stacking (4% acrylamide, pH 7.6) and separating (12.5% acrylamide, pH 8.9) polyacrylamide gels, solidified with ammonium persulphate and TEMED solutions, until the tracking dye front reached to the bottom of the gel slab at 300 V (ELITE 300, Wealtec Corp., NV, USA). An aqueous solution of Coomassie Briliant Blue G250 (0.2%, w/v) was applied for staining and images of the gels were captured with a scanner (Epson V750 Pro, Seiko Epson Corp., Japan) and digitized for quantification of the intensity of the bands using the Total Lab 1D (v12.2; Phoretix, Newcastle upon Tyne, UK) gel analysis software.

Sensory analysis

Sensory tests were conducted with a panel of ten panelists at Ordu University Faculty of Agriculture. The cheeses were graded on the basis of six criteria: (i) color and appearance; (ii) taste and flavor; (iii) body and structure, (iv) odor, (v) saltiness, and (vi) LP/cheese harmony. Using a hedonic scale, the panel was asked to grade the samples with a score of 1 to 5: 1 is being unacceptable and 5 being very good. The panel was informed about the cheese samples prior to the analysis and the cheese samples were served as 15–20 g portions with water.

Statistical analysis

All measurements were performed in duplicate. Statistical analyses of the data were carried out using Minitab 17.1.0 statistical software (State College, PA). Two-way analysis of variance (two-way ANOVA) was performed for the determination of statistical differences between cheese types and ripening periods; then, differences found statistically significant were subjected to Tukey test ($\alpha = 0.05$). For statistical evaluation of the sensory data, Kruskal-Wallis non-parametric test was applied using median values and pairwise differences, if any, were denoted.

RESULTS and DISCUSSION

Gross composition

The dry matter contents of cheeses were between 41.33 and 46.24% during the storage period of 90 days (Table 1).

Except for T2, there was no significant change observed in dry matter contents of cheese samples during storage ($P>0.05$). However, significant differences were determined for “dry matter” and “salt in dry matter” considering storage time x cheese type interaction ($P<0.05$). All cheese types fulfilled the minimum dry matter requirement of for fresh and ripened White cheese by Turkish Food Codex (TFC, 2015). These values are little under the values of six different types of spicy White cheeses (approximately 45-50% dry matter content) reported by Tarakçı and Deveci (2019). The fat in dry matter ratios ranged between 30.24 and 41.02%. The cheese samples can be classified as semi-fat cheese (25%≤fat in dry matter<45%) according to TFC (2015). Considering salt contents, the initial salt ratios of cheeses were quite high but they were stabilized with the extended time of storage. The salt in dry matter concentrations ranged between 7.44% and 13.95% (Table 1). The high concentrations of salt determined on the 1st day are likely due to residual salt from dry-salting application. These salt concentrations exceed the maximum salt level in dry matter of 6.5% (TFC, 2015). On the other hand, the salt content of the 100 traditional Van Herby cheese collected from the city center were found to be between 5.85% and 11.70% with an average value of 8.64% (Ekici et al., 2019). Considering that raw milk is utilized in traditional Herby cheese production, the high salt content of Herby cheeses could be attributed to addition of extra salt to avoid microbial spoilage in the study by Ekici et al. (2019). No significant effect of garden cress concentration was determined on the protein and fat contents of cheese samples ($P>0.05$) but salt ($P=0.008$). However, storage time was found to be significantly effective on all components ($P<0.05$).

pH and titratable acidity

Reduction in pH or increase in titratable acidity is usually a result of lactic acid accumulation due to microbial activity however; additional ingredients may also affect the titratable acidity level of cheeses. The pH values of cheese samples ranged between 5.05 and 5.58 during storage time (Table 2). Addition of garden cress increased the initial pH values of cheeses. On the other hand, the pH values of cheeses with garden cress reduced during storage while pH of the control cheese did not change that much. Confirming these findings, increase in titratable acidity of the control cheese is lower than the garden cress added cheeses (Table 2).

Table 1. Changes in gross composition of cheese samples during storage

	Cheese types *Peynir çeşitleri*	Storage time (days) *Depolama süresi (gün)*			
		1st	30th	60th	90th
Dry matter (%) *Kuru madde (%)*	C	44.67±0.94	45.00±0.47^{A}	44.50±0.71	43.83±0.24^{AB}
	T1	46.24±0.35	44.50±0.71^{A}	45.50±1.65	42.33±0.94^{B}
	T2	45.50±0.71^{a}	41.33±0.47^{Bb}	42.50±0.24^{b}	42.50±0.24^{ABb}
	T3	44.17±0.71	44.83±0.24^{A}	44.17±0.24	44.33±0.47^{A}
	T4	43.67±1.41	44.33±0.94^{A}	43.00±0.47	43.67±0.00^{AB}
Fat (%) *Yağ (%)*	C	14.00±0.00	16.50±0.00	16.75±0.35	13.25±2.47
	T1	14.75±0.35^{b}	15.75±0.35^{ab}	16.00±0.00^{a}	13.25±0.35^{c}
	T2	14.50±0.71	16.00±1.41	15.50±0.71	13.50±0.71
	T3	15.25±1.06	16.50±0.71	16.00±1.41	14.50±0.00
	T4	13.25±0.35^{c}	18.25±0.35^{a}	16.00±0.00^{b}	14.00±0.71^{c}
Protein (%) *Protein (%)*	C	21.14±0.51^{A}	15.94±1.54	17.03±0.00	20.95±1.58
	T1	19.68±0.00^{AB}	16.67±2.57	14.22±0.64	20.49±0.40
	T2	17.31±0.74^{B}	15.76±2.31	15.31±1.16	20.21±1.05
	T3	19.87±1.80^{AB}	17.94±0.26	16.03±1.93	20.39±0.79
	T4	19.87±0.26^{AB}	17.40±1.03	15.40±0.26	19.65±1.85
Salt (%) *Tuz (%)*	C	6.23±0.29^{Aa}	3.48±0.12^{b}	3.83±0.46^{b}	3.48±0.12^{b}
	T1	5.15±0.00^{ABa}	3.69±0.00^{c}	4.15±0.00^{b}	3.60±0.04^{d}
	T2	4.07±1.12^{B}	3.54±0.12	3.28±0.41	3.74±0.00
	T3	4.88±0.29^{ABa}	3.45±0.00^{b}	3.63±0.08^{b}	3.48±0.12^{b}
	T4	3.25±0.12^{Bb}	3.77±0.21^{ab}	3.80±0.08^{a}	3.51±0.08^{ab}
Fat in dry matter (%) *Kuru maddede yağ (%)*	C	31.35±0.66	36.67±0.38	37.65±1.39	30.24±5.81
	T1	31.89±0.53^{ab}	35.40±1.36^{a}	35.19±1.28^{ab}	31.30±0.14^{b}
	T2	31.86±1.06	38.73±3.86	36.47±1.46	31.76±1.49
	T3	34.55±2.96	36.67±1.57	36.22±3.01	32.71±0.35
	T4	30.37±1.79^{b}	41.02±0.29^{a}	37.21±0.41^{a}	32.06±1.62^{b}
Salt in dry matter (%) *Kuru maddede tuz (%)*	C	13.95±0.35^{Aa}	7.74±0.36^{b}	8.62±1.16^{b}	7.94±0.33^{b}
	T1	11.13±0.08^{ABa}	8.28±0.13^{b}	9.14±0.33^{b}	8.50±0.29^{b}
	T2	8.96±2.59^{B}	8.56±0.20	7.71±1.02	8.81±0.05
	T3	11.06±0.48^{ABa}	7.67±0.00^{b}	8.21±0.14^{b}	7.85±0.36^{b}
	T4	7.44±0.04^{B}	8.49±0.69	8.84±0.10	8.04±0.19

C: Control cheese without LS. The other cheeses contain garden cress with the following concentrations: T1: 0.6%, T2: 1.2%, T3: 1.8%, and T4: 2.4%. Different capital letters in the same column indicate significant differences between cheese types and different small letters in the same row indicate significant differences between storage days ($P < 0.05$).

Table 2. Changes in the pH and titratable acidity levels of White cheese samples during storage
Çizelge 2. Depolama süresince Beyaz peynir pH ve titre edilebilir asitlik değerlerinde meydana gelen değişimler

	Cheese types *Peynir çeşitleri*	Storage time (days) *Depolama süresi (gün)*			
		1st	30th	60th	90th
pH	C	5.25±0.01^{B}	5.32±0.00^{A}	5.26±0.01^{A}	5.29±0.11
	T1	5.58±0.01^{Aa}	5.28±0.01^{Bb}	5.20±0.02^{Abc}	5.15±0.03^{c}
	T2	5.37±0.16^{ABc}	5.21±0.01^{Cbc}	5.08±0.00^{CDb}	5.14±0.02^{a}
	T3	5.39±0.01^{ABa}	5.29±0.01^{Bb}	5.13±0.04^{BCc}	5.21±0.02^{bc}
	T4	5.37±0.07^{ABa}	5.35±0.00^{Aa}	5.05±0.01^{Db}	5.12±0.06^{b}
Titratable acidity (%) Titre edilebilir asitlik (%)	C	1.28±0.21^{a}	1.33±0.09^{a}	1.44±0.05^{a}	1.73±0.04^{Ca}
	T1	1.02±0.01^{c}	1.34±0.06^{b}	1.40±0.01^{b}	2.15±0.01^{ABa}
	T2	1.11±0.13^{a}	1.45±0.04^{a}	1.64±0.10^{a}	2.02±0.02^{ABa}
	T3	1.25±0.14^{c}	1.42±0.03^{bc}	1.69±0.13^{ab}	1.92±0.01^{BCa}
	T4	1.19±0.12^{c}	1.41±0.10^{bc}	1.73±0.09^{b}	2.24±0.12^{Aa}

C: Control cheese without LS. The other cheeses contain garden cress with the following concentrations: T1: 0.6%, T2: 1.2%, T3: 1.8%, and T4: 2.4%. Different capital letters in the same column indicate significant differences between cheese types and different small letters in the same row indicate significant differences between storage days ($P<0.05$).

Furthermore, titratable acidity of all cheeses increased during storage period. Both pH and titratable acidity was significantly affected by storage time and cheese type and their interaction ($P<0.05$).

Similarly, the addition of spices reduced the pH of Sürk cheese (Masatcıoğlu and Avşar, 2005), Cottage cheese (Regu et al., 2016) and Ras cheese (Hamad et al., 2020); and increased the titratable acidity of Mozzarella (Akarca et al., 2016) during storage. On the other hand, Gezmiş and Tarakçı (2020) determined that the control cheese without any spices had the highest titratable acidity among all spice added the Circassian cheeses.

WSN and TCA-SN

Proteolysis is a useful indicator of ripeness in cheese. The TCA-SN includes nitrogen fractions from 2—20 amino acids together with free ones while WSN is nitrogen fractions soluble in water and referred as the ripening index of cheese (Sousa and Malcata, 1997). Both WSN and TCA-SN rates increased throughout the ripening period and with increasing concentrations of LS, and their interactions ($P<0.05$) (Table 3). Complying with the current results, Sürk, a traditional Turkish cheese containing a mixture of spices, was shown to have higher WSN values compared to the control cheese without any spices during one month of storage (Masatcıoğlu and Avşar, 2005). Furthermore, Hamad et al. (2018) and Aktypis et al. (2018) found that the increasing concentrations of herbs increased the WSN values of cheeses. On the contrary, Mozzarella cheese was found to have lower ripening index when spices were included (Akarca et al., 2016). Addition of black cumin gave similar WSN and TCA-SN values while addition of red- and isot-pepper caused a reduction. On the other hand, mint and thyme were found to increase WSN and TCA-SN values compared to control cheese (Tarakçı and Deveci, 2019).

Table 3. Changes in the WSN/TN and TCA-SN/TN levels of White cheeses during storage
Çizelge 3. Depolama süresince Beyaz peynirlere ait WSN/TN ve TCA-SN/TN değerlerine ait değişimler

	Cheese types *Peynir çeşitleri*	Storage time (days) *Depolama süresi (gün)* 1st	30th	60th	90th
WSN/TN (%)	C	6.15±0.32^{Bc}	8.10±0.30^{Dc}	14.31±0.31^{Bb}	19.01±1.16^{Ba}
	T1	6.65±0.00^{Bd}	12.55±0.00^{Cc}	16.79±0.10^{Ab}	20.85±0.51^{ABa}
	T2	7.53±0.05^{Ad}	15.85±0.12^{Ac}	16.50±0.11^{Ab}	21.71±0.01^{ABa}
	T3	6.51±0.03^{Bc}	14.00±0.52^{Bb}	16.84±0.46^{Ab}	21.33±1.48^{ABa}
	T4	6.62±0.10^{Bd}	14.47±0.19^{Bc}	16.81±0.00^{Ab}	23.27±0.35^{Aa}
TCA-SN/TN (%)	C	4.20±0.44^{Ac}	5.55±0.13^{Bb}	5.51±0.23^{Cb}	8.07±0.21^{Ba}
	T1	2.81±0.17^{Bc}	6.31±0.03^{ABb}	6.98±0.14^{Bb}	11.03±0.81^{ABa}
	T2	3.29±0.21^{Abc}	6.75±0.38^{Ab}	7.63±0.27^{Bb}	11.28±0.31^{ABa}
	T3	3.02±0.09^{Bc}	6.29±0.17^{ABb}	9.17±0.29^{Aa}	11.13±1.32^{ABa}
	T4	3.26±0.24^{ABc}	6.49±0.09^{Ab}	9.43±0.44^{Ab}	12.84±1.50^{Aa}

C: Control cheese without LS. The other cheeses contain garden cress with the following concentrations: T1: 0.6%, T2: 1.2%, T3: 1.8%, and T4: 2.4%. WSN: Water-soluble nitrogen, TCA-SN: Trichloroacetic acid-soluble nitrogen TN: Total nitrogen. Different capital letters in the same column indicate significant differences between cheese types and different small letters in the same row indicate significant differences between storage days ($P<0.05$).

Urea-PAGE

In all cheese samples, α-casein degradation was more intense compared to ß-casein (Figure 2) and similar results were also obtained by (Tarakçı and Deveci, 2019). Considering ß-casein fraction, the addition of garden cress increased the degradation rate, especially in T2. On the other hand, considering the degradation rates of α-casein, it is seen that there is no significant difference between cheeses. Spices are usually more effective on α-casein of White cheese as determined by Tarakçı and Deveci (2019). In a study by Shori et al. (2020), extended ripening of Cheddar cheese enhanced the degradation of α_s- and ß-caseins more in *Allium sativum* added cheese compared to plain cheddar. These results suggest that garden cress may possess a unique proteolytic activity which is more specific to ß-casein.

Sensory analysis

Sensory scores for each period are shown in Figure 3. Sensory results indicate that the scores for cheese samples are very close to each other for all the criterion tested. Also, no significant differences were determined between neither cheese types nor storage periods according to the Kruskal-Wallis test applied ($P>0.05$). The structural properties of cheese samples were less appreciated when high concentrations of garden cress were included probably due to more fragile structure they gained. Similarly, saltiness level was most admired for control, T2, and T1 cheeses. The color and appearance score was lowered with the garden cress addition since the inclusion of spices/herbs/plants is not a tradition for consumption of White cheese. On the other hand, T3 and T4 cheeses were more preferred in terms of taste and flavor, odor, and LS/cheese harmony however the difference between samples was not significant ($P>0.05$).

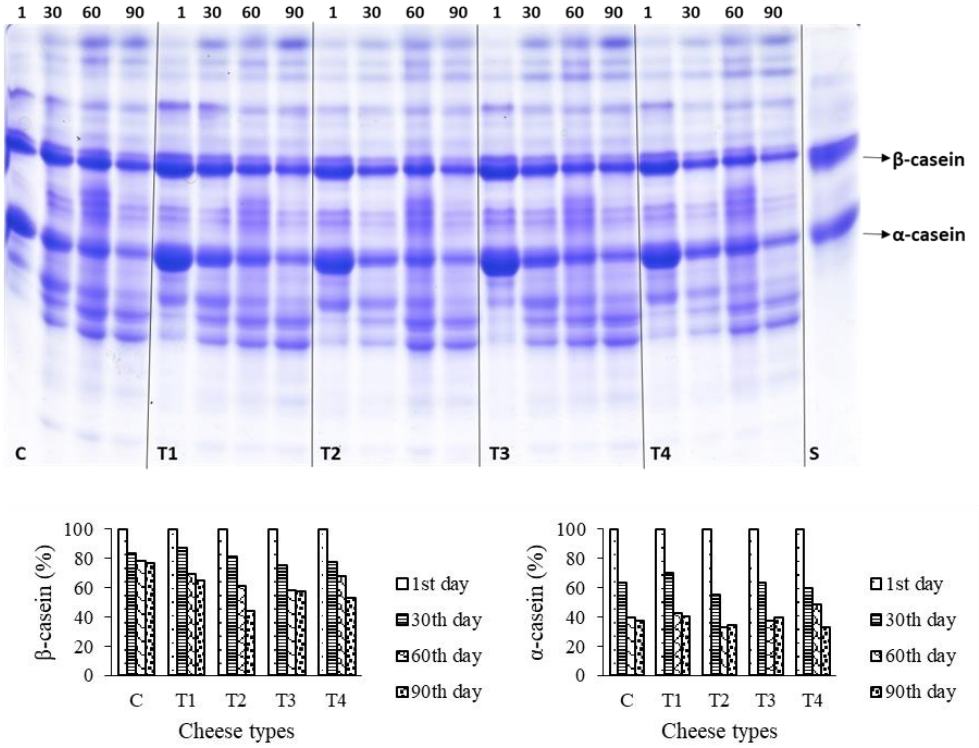

Figure 2. Electrophoretogram of cheese samples and graphs of changes in the levels of ß-casein (left) and α-casein (right) during storage

Şekil 2. Peynir örneklerine ilişkin elektroforetogramlar ve depolama süresince ß-Kazein (sol) ve α-Kazein (sağ) seviyelerindeki değişimlere ait grafikler

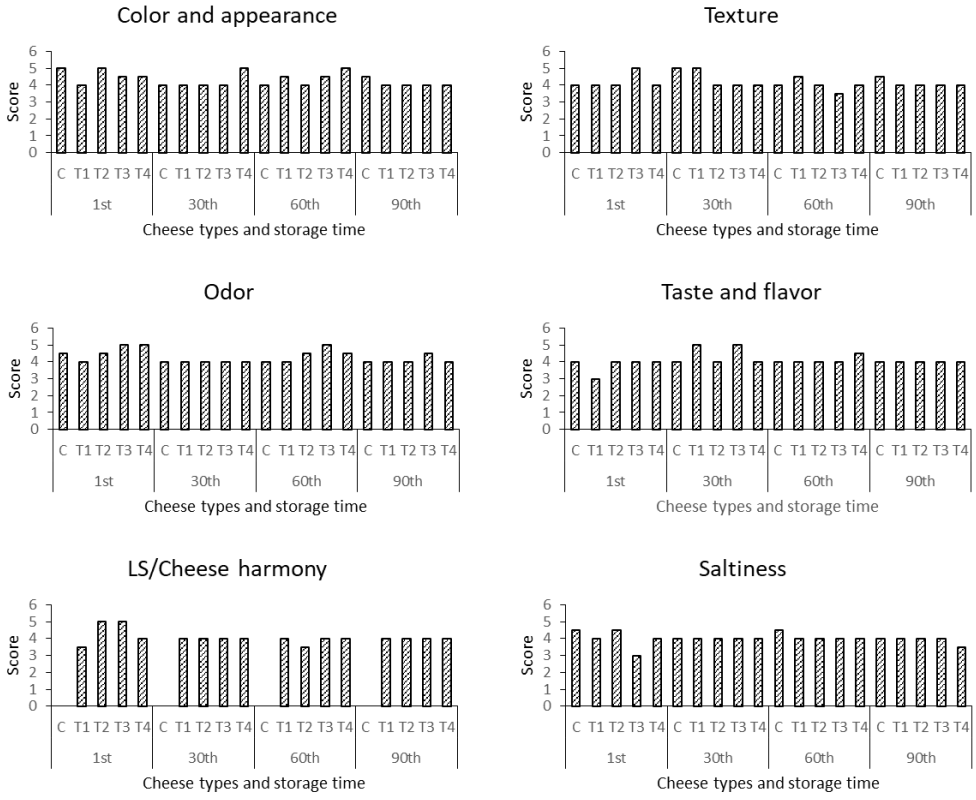

Figure 3. Sensory scores of cheese samples during storage.

Şekil 3. Depolama süresince peynirlerin duyusal skorları.

CONCLUSIONS

The incorporation of garden cress contributed to both WSN and TCA-SN levels of White cheeses, while the increase in the latter was more apparent. Considering casein fractionations, garden cress, regardless of its concentration, boosted the degradation of ß-casein. No statistical difference has been determined between cheese types regarding sensory properties. Overall, the results indicate that White cheese could be used as a suitable conveyor for garden cress and contribute to the ripening level, specifically by ß-casein degradation. Further comprehensive studies are required to get a clear picture of the effect of garden cress on the casein fractionation

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Author Contribution Statement

ZT and ÖFÇ have designed the study. ÖK and SK executed the experiment and collected the data under the guidance of ÖFÇ. ÖFÇ wrote the article and ZT reviewed the article critically.

Conflict of Interest Statement

All authors declare that they have no conflicts of interest..

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Çimlendirilmiş Çavdar (*Secale cereale*) ve Kavuzsuz Yulafın (*Avena sativa*) Bisküvi Üretiminde Kullanımı

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ÖZET

Bu çalışmada, çimlendirilmiş çavdar (*Secale cereale*) ve kavuzsuz yulaf (*Avena sativa*) tanelerinin bisküvi üretiminde kullanımı araştırılmıştır. Bu amaçla, 3 gün süre ile çimlendirme prosesi uygulanan çavdar ve yulaf taneleri %10 nem içeriğine kadar kurutulup ve ardından öğütülerek un haline getirilmiş, bu unlarda bisküvi formülasyonuna farklı oranlarda (%0, 10, 20 ve 30) buğday unu ikamesi olarak kullanılmıştır. Örneklerin; fiziksel (renk, kalınlık, çap, yayılma oranı, sertlik), kimyasal (kül, nem, ham yağ, ham protein, toplam fenolik madde ve fitik asit) ve duyusal özellikleri incelenmiştir. Çimlendirilmiş çavdar ve yulaf unu ikamesi ile bisküvilerin renk özelliklerinde *a** ve *b** değerleri artış gösterirken, *L** değeri azalmıştır. Çimlendirilmiş çavdar ve yulaf unu ikamesi örneklerin sertliğini, çapını ve yayılma oranını artırıcı, kalınlığını ise azaltıcı bir etki göstermiştir. İkame oranındaki artışla beraber; kül, nem, ham yağ, ham protein, enerji ve fitik asit içeriklerinde de bir artış meydana geldiği tespit edilmiştir. İkame oranı %0'dan %30'a yükseldiğinde, örneklerin toplam fenolik madde içerikleri de 568.00 mgGAE kg^{-1}'dan 656.25 mgGAE kg^{-1}'a yükselmiştir. Duyusal analizde en yüksek beğeniyi %10 ve 20 oranlarında çimlendirilmiş çavdar unu ikamesine sahip örnekler almıştır. Bu araştırmanın sonuçlarına göre, çimlendirilmiş kavuzsuz yulaf ve çavdardan elde edilen unların bisküvi üretiminde kullanımı, besinsel özelliklerin iyileştirilmesi bakımından önerilebilir.

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Araştırma Makalesi

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Anahtar Kelimeler
Çavdar
Yulaf
Çimlendirme
Bisküvi
Fonksiyonel özellik

Utilization of Germinated Rye (*Secale cereale*) and Hull-less Oat (*Avena sativa*) in Biscuit Production

ABSTRACT

In this study, the utilization of germinated rye (*Secale cereale*) and hull-less oat (*Avena sativa*) grains in biscuit production were investigated. For this purpose, rye and oat grains, which were germinated for 3 days, were dried to 10% moisture content and then ground into flour, and these flours were substituted with wheat flour at different rates (0, 10, 20 and 30%) in the biscuit formulation. Physical (color, diameter, thickness, spreading rate, hardness), chemical (ash, moisture, crude oil, crude protein, total phenolic substance and phytic acid) and sensory properties of samples were investigated. With the substitution of germinated rye and hull-less oat flour, the *a** and *b** values of the biscuits increased, while the *L** value decreased. The substitution of germinated rye and oat flour increased the hardness, diameter and spread ratio of the samples, but decreased the thickness. As the substitution ratio increased; ash, moisture, crude fat, crude protein, energy and phytic acid contents were increased. When the substitution ratio increased from 0% to 30%, the total phenolic content of the samples also increased from 568.00 mgGAE kg^{-1} to 656.25 mgGAE kg^{-1}. In the sensory analysis, 10 and 20% substituted germinated rye flour biscuits gained the highest appreciation. According to the results of this study, the utilization of germinated hull-less oat and rye flours in the biscuit production can be recommended from the point of nutritional

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GİRİŞ

Geniş üretim alanı, enerji verici, doyurucu, biyolojik değeri yüksek protein içeriğine sahip ve kolay muhafaza edilebilir gıdalar olan tahıllar, beslenmede hayati önem taşırlar. Son yıllarda, tüketici istekleri doğrultusunda tam tahılların üretimi ve tüketimi giderek artmaktadır (Collar, 2008). Dünya genelinde de temel gıda maddesi olarak kabul edilen tahıl ürünleri, zenginleştirilerek çeşitlendirilme potansiyeline sahiptirler (Dal, 2012). Gıda zenginleştirmedeki en önemli amaçlar; vitamin ve mineral madde eksikliklerini gidermek ve gıdaları eser miktarda bulunan bileşenlerce takviye etmektir (Kahraman, 2011). Tahıl ürünleri arasında en çok zenginleştirme çalışmalarının yapıldığı ürünlerden birisi de bisküvidir. Bisküvi, gıda tüketicileri tarafından çokça tüketilen, kolay temin edilebilen, bayatlamadan uzun süre muhafaza edilebilen, bir çok farklı çeşitte üretimi mümkün olan bir tahıl ürünüdür (Demir, 2015).

Çavdar (Secale cereale), Doğu ve Kuzey Avrupa'da, özellikle Almanya, Polonya, Rusya ve İskandinav ülkeleri için önemli bir gıda kaynağıdır (Bushuk, 2001; Katina ve ark., 2007). Buğdaydan daha ince, uzun ve kavuzsuz olan çavdarın ülkemizde pek çok yabani ve kültür formu bulunmaktadır. Çavdarın besinsel lif içeriğinin %14 olduğu rapor edilmiştir (Mankan, 2008). Literatürde çavdarın; %86.6 kuru madde, kuru maddede ise %11.5-14.0 protein, %1.7 yağ, %60 nişasta ve selüloz içeriğine sahip olduğu bildirilmiştir (Gökgöl, 1969; Baytop, 1999). Çavdar taneleri, tannin ve ferulik asit gibi antioksidan özellikteki fenolik bileşikler ve oldukça yüksek miktarda folat (yaklaşık 72-143 µg $100g^{-1}$) içermektedir (Heinonen ve ark., 2001; Kariluoto, 2008). Yapısında bulunan lignanlar, besinsel lif içeriği ve hemiselülozik bileşenler sayesinde çavdarın, kalp damar hastalıklarının ve bazı kanser türlerinin önlenmesinde etkili olduğu bildirilmiştir (Mankan, 2008).

Poaceae familyasına ait olan yulaf (Avena sativa), boyu 60-100 cm arasında değişen, nemli bölgelerde yetiştirilen, tek yıllık bir bitkidir (Turan, 2014). Yulaf tanesinin yaklaşık %25-30'unu kavuz fraksiyonu oluşturur (Butt ve ark., 2008; Liu, 2010). Yulaf hem insan hem de hayvan besini olarak kullanılabilen bir tahıldır (Butt ve ark., 2008). Genotip ve çevresel büyüme koşullarına bağlı olarak, kavuzu alınmış tanede %12-20, tam tanede ise %9-15 protein içeriğine sahip yulaf, en yüksek protein içeriğine sahip tahıl olarak kabul görmektedir (Peterson, 1992). Yulaf çeşitlerinin yağ içeriği ise %4-11 arasında değişim göstermektedir (Holland ve ark., 2001). Bileşimindeki çözünür besinsel lifi, doymamış yağ asitleri, ß-glukan ve antioksidan özellikteki tokol, sterol ile fenolik bileşikler sayesinde yulaf, antioksidan, anti-enflamatuar, hipoalerjenik ve antikarsinojenik özelliklere sahip bir tahıldır (Chen ve ark., 2015; Bei ve ark, 2017).

Çimlendirme, yüzyıllardır tahıllarda çeşitli amaçlarla uygulanan basit bir prosestir. Tahıllarda çimlendirme; tohum yapısını iyileştirmek, besin içeriğini arttırmak, anti-besinsel özellikteki bileşiklerin içeriğini azaltmak ve taneye yeni bir tat kazandırmak amacıyla uygulanabilir (Kaukovirta-Norja ve ark., 2004). Çimlenme prosesi, taneye su alımı ile başlar ve genellikle tanede kökün ortaya çıkmasıyla son bulur (Bewley ve Black, 1994). Çimlendirme ile besinsel özellikleri geliştirilen, ardından tüketime sunulan tahıllara; pirinç, arpa, çavdar ve yulaf örnek gösterilebilir (Xu ve ark., 2005; Khattak ve ark., 2007; Marton ve ark., 2010; Okur ve Madenci, 2019).

Bu çalışmada, bisküvinin besinsel içeriğini arttırmak ve duyusal özelliklerini geliştirmek amacıyla, fonksiyonel özellikte yeni bir ürün formülasyonu geliştirilmesi hedeflenmiştir. Bu araştırmada, çimlendirilmiş çavdar (Secale cereale) ve kavuzsuz yulaftan (Avena sativa) elde edilen unlar, bisküvi üretiminde %0, 10, 20, ve 30 ikame oranlarında, buğday unu yerine kullanılmıştır. Üretilen bisküvilerde fiziksel (renk, tekstür, çap, kalınlık ve yayılma oranı), kimyasal (kül, nem, ham yağ, ham protein, karbonhidrat, enerji, toplam fenolik madde miktarı, fitik asit tayini) ve duyusal analizler gerçekleştirilerek, ürünlerin kalite ve besinsel özelliklerinin değerlendirilmesi amaçlanmıştır.

MATERYAL ve METOT

Materyal:

Araştırmada kullanılan çavdar (Secale cereale, Aslım-95 çeşidi) Konya yerel buğday pazarından, kavuzsuz yulaf (Avena sativa, Katmerli çeşidi) ise Bahri Dağdaş Uluslararası Tarımsal Araştırma Enstitüsünden 2021 yılında temin edilmiştir. Araştırmada kullanılan bisküvilik buğday unu, şeker, shortening, fruktoz şurubu, tuz, süt tozu ve sodyum bikarbonat ise Konya piyasasından temin edilmiştir.

Metot:

Çavdar ve yulafın çimlendirilmesi

Çavdar ve kavuzsuz yulaf örnekleri, temin edildikten sonra, oda sıcaklığındaki musluk suyuyla toz, kir ve yabancı maddeler uzaklaştırılıp berrak yıkama suyu elde edilinceye kadar yıkanmıştır. Örnekler %2.5'luk NaOCl çözeltisinde 10 dk bekletilerek dezenfekte edilmiş, ardından süzülerek saf suyla tekrar yıkanmıştır. Yıkama işlemi sonrasında örnekler ön denemeler neticesinde belirlenen sürelerde (çavdar 3 saat, yulaf 1 gün) saf suda bekletilmiştir.

Suda bekletme sürelerinin sonunda taneler, tel ızgaralarda steril pamuk ve tülbent üzerine serilerek kontrollü çimlendirme kabini (Nüve TK 120 model, Ankara, Türkiye) içerisinde 20±2 °C' de çimlenmeye bırakılmıştır. Tanelerde nemlenmeyi sağlamak amacıyla örnekler 12 saatte bir saf sudan geçirilerek pamuk ve tülbentler yenilenmiştir. Çavdar ve yulaf örnekleri 3 gün çimlendirmenin ardından su içeriği %10'un altına düşene kadar etüvde (Nüve KD 200 model, Ankara, Türkiye) 45 °C' de kurutulmuştur. Kurutulan örnekler tam tane olarak, laboratuvar tipi bir öğütücü ile (Alveo, Türkiye) 500 µm'lik elek altına geçecek şekilde öğütülmüştür. Örnekler analiz edilene kadar steril ve hava almayan kilitli poşetlerde +4 °C'de muhafaza edilmiştir.

Bisküvi üretimi

AACC Standart (10-54.01) üretim metodu modifiye edilmiş ve bisküvi üretiminde kullanılmıştır. Kontrol örneğinin üretiminde; 100 g buğday unu, 42 g pudra şekeri, 40 g shortening, 1.5 g fruktoz şurubu, 1.25 g tuz, 1 g süt tozu, 1.5 g sodyum bikarbonat ve su (~15 ml) kullanılmıştır. Bisküvi formülasyonunda, un esasına göre %10, 20 ve 30 oranlarında, çimlendirilmiş çavdar ve çimlendirilmiş kavuzsuz yulaf unları, ayrı ayrı olmak üzere buğday ununa ikame edilmiştir. Tüm bileşenler mikserde (Kenwood KMX, Kenwood Ltd., İngiltere) 8 dakika süre ile yoğrulmuştur. Yoğrulan hamurdan eşit çap (55.0 mm) ve kalınlıkta (5.0 mm) parçalar kesilerek alüminyum tepsilere yerleştirilmiş ve 205±2 °C'deki fırında (Vestel SF8401, Türkiye) 16 dakika pişirilmiştir.

Fiziksel ve kimyasal analizler

Çimlendirilmiş çavdar, çimlendirilmiş kavuzsuz yulaf, buğday unu ve bisküvi örneklerinin renk değerleri Hunter Lab Color Quest II Minolta CR-400 (Konica Minolta Sensing, Inc., Osaka, Japan) cihazı ile ölçülmüştür. Ölçümlerdeki L* değeri siyah (0) - beyaz (100), a* değeri kırmızı (+) - yeşil (-) ve b* değeri ise sarı (+) - mavi (-) renk değerlerinin göstergesidir (Francis, 1998).

Bisküvi örneklerinin sertlik ölçümleri, bisküvi örnekleri fırından çıkarıldıktan 2 saat sonra, tekstür analiz cihazı ve 3 noktalı kırma probu (TA-XT2i, Stable Micro Systems Ltd., Surrey, UK) kullanılarak, 3 mm sn^{-1} ölçüm hızı ve 5 mm'lik bir mesafe uygulanarak gerçekleştirilmiştir (Adeola ve Ohizua, 2018).

Bisküvi örneklerinin kalınlık ve çap değerleri AACC Standart metoduna (10-50.05) göre ölçülmüştür. Yayılma oranı ise ölçülen çap değerlerinin (mm), kalınlık değerlerine (mm) oranlanmasıyla hesaplanmıştır (AACC, 1990).

Denemelerde kullanılan çimlendirilmiş çavdar, çimlendirilmiş kavuzsuz yulaf, buğday unu ve bisküvi örneklerinin nem (AACC 44-19.01), kül (AACC 08-01.01), ham yağ (AACC 30-25.01) ve ham protein (AACC 46-12.01) içeriklerinin tayininde AACC standart metotları kullanılmıştır (AACC, 1990). Tüm örneklerin karbonhidrat değerleri Karaağaoğlu ve ark. (2008)'na göre, (% Karbonhidrat = 100 – (% nem + % protein + % ham yağ + % kül)) formülü kullanılarak belirlenmiştir. Enerji değerleri ise (Enerji (kkal $100g^{-1}$) = 4 (% CHO + % Protein) + 9 (% Yağ)) formülüne göre hesaplanmıştır (Karaağaoğlu ve ark., 2008).

Örneklerin toplam fenolik madde içeriğinin tayininde, spektrometrik Folin-Ciocaltaeu metodu kullanılmıştır. Ekstraksiyon amacıyla, örneklerden 4 g tartılmış ve örnekler 20 ml asitlendirilmiş metanol (HCl/metanol/su, 1:80:10, v/v) ile 2 saat süresince 24±1 °C'de çalkalanmıştır. Süre sonunda tüplerdeki karışım, santrifüj edilmiş (3000 rpm, 10 dakika) ve supernatant elde edilmiştir. Ekstraksiyondan sonraki analiz aşamasında, örneklerden elde edilen supernatant (0.1 ml), Folin-Ciocaltaeu reaktifi (0.5 ml, %10'luk, v/v, suda) ve sodyum karbonat çözeltisi (1.5 ml, %20'lik, g/v, suda) karıştırılarak, 2 saat oda sıcaklığında (24±1 °C) karanlıkta inkübe edilmiştir. İnkübasyon süresinin sonunda, çözeltilerin absorbansları 760 nm dalga boyuna ayarlanmış spektrometrede (Libra S60, Biochrom Ltd., Cambridge, England) okunmuştur. Okunan absorbans değerlerinden, örneklerin toplam fenolik madde miktarları gallik asite (mgGAE kg^{-1}) eşdeğer olacak şekilde hesaplanmıştır (Slinkard ve Singelton, 1977, Gamez-Meza ve ark., 1999). Tüm örneklerin fitik asit içerikleri, Haug ve Lantzsch (1983)'e göre kolorimetrik olarak tespit edilmiştir. 0.3 g örnek, 50 ml 0.2 N hidroklorik asit çözeltisi ile ekstraksiyona tabi tutulmuştur. Elde edilen ekstrakttan 0.5 ml alınmış, üzerine 1 ml amonyum demir (III) sülfat çözeltisi eklenmiş ve tüpler 30 dk kaynar su banyosunda tutulmuştur. Daha sonra tüpler buz banyosunda 15 dk bekletilmiştir. Ardından örneklerin üzerine 2 ml 2,2'-bipiridin çözeltisi eklenmiş ve absorbans değerleri UV/görünür bölge spektrometresi (Biochrom Libra S22, Cambridge, Birleşik Krallık) ile 519 nm dalgaboyunda okunmuştur. Sonuçlar, kuru madde esasına göre mg $100g^{-1}$ olarak verilmiştir.

Duyusal analiz

Bisküvi örnekleri, Necmettin Erbakan Üniversitesi Gıda Mühendisliği Bölümü lisans üstü öğrencileri ve öğretim elemanlarından oluşan 10 kişilik bir grup ile duyusal analize tabi tutulmuştur. Panelistler değerlendirmeden önce analiz hakkında bilgilendirilmiş, ardından örnekler standart şekilde ışıklandırılmış bir ortamda bireysel olarak değerlendirilmiştir. Bisküviler; koku, renk, görünüş, tat, gevreklik ve genel beğeni özellikleri bakımından, 5'lik hedonik skala (5: çok iyi, 3: kabul edilebilir, 1: kötü) ile değerlendirilmiştir.

İstatistik Analizler

İki tekerrürlü olarak yürütülen denemelerden elde edilen veriler JMP istatistik programı, 14.0.1 versiyonu (SAS Institute Inc., Cary, NC, ABD) kullanılarak varyans analizine tabi tutulmuştur. Farklılıkları istatistiki olarak önemli bulunan ana varyasyon kaynaklarının ortalamaları ise Student's T testi ile karşılaştırılmıştır. Hammaddelerde tek yollu ANOVA, bisküvi örneklerinde ise çift yollu ANOVA testi ile kıyaslama yapılmıştır (Düzgüneş ve ark., 1987).

BULGULAR ve TARTIŞMA

Analitik Sonuçlar

Bisküvi üretimlerinde formülasyondaki buğday unu, çimlendirilmiş çavdar unu ve çimlendirilmiş yulaf unu örneklerine ait analitik analiz sonuçları Çizelge 1'de gösterilmektedir.

Çizelge 1. Hammaddelere ait analitik analiz sonuçları[1]
Table 1. Analytical analysis results of raw materials[1]

Özellik		BU[2]	ÇÇU[3]	ÇYU[4]
Renk özellikleri (Color properties)	L*	94.44 ± 0.06[a]	83.92 ± 0.01[c]	85.26 ± 0.01[b]
	a*	-0.54 ± 0.01[c]	1.07 ± 0.01[a]	-0.02 ± 0.02[b]
	b*	10.49 ± 0.04[c]	11.67 ± 0.01[b]	15.03 ± 0.69[a]
Kimyasal özellikler (Chemical properties)	Nem (Moisture) (%)	9.34 ± 0.11[a]	6.56 ± 0.14[b]	5.88 ± 0.26[b]
	Kül (Ash) (%)	0.70 ± 0.02[c]	1.60 ± 0.01[b]	1.86 ± 0.05[a]
	Ham protein[5] (Crude protein) (%)	10.57 ± 0.06[b]	10.06 ± 0.13[c]	19.38 ± 0.06[a]
	Ham yağ (Crude fat) (%)	1.17 ± 0.14[b]	1.22 ± 0.15[b]	5.76 ± 0.98[a]
	TFMM[6] (mgGAE kg^{-1})	693.00 ± 37.90[c]	1051.00 ± 11.23[a]	891.00 ± 15.36[b]
	Fitik asit (Phytic acid) (mg $100g^{-1}$)	184.80 ± 4.04[b]	502.30 ± 40.79[a]	456.81 ± 69.14[a]

[1]Aynı sütunda farklı harfle işaretlenmiş ortalamalar istatistiki olarak birbirinden farklıdır ($p<0.05$) ve kimyasal analiz sonuçlarında kuru madde üzerinden hesaplama yapılmıştır. Analiz sonuçları tek yollu ANOVA testi ile karşılaştırılmıştır; [2]Buğday unu; [3]Çimlendirilmiş çavdar unu; [4]Çimlendirilmiş kavuzsuz yulaf unu; [5]BU için N x 5.70, ÇÇU ve ÇYU için N x 6.25 faktörü kullanılmıştır; [6]TFMM: Toplam fenolik madde miktarı.

[1]Values within a row with different superscripts differ significantly at $p<0.05$ and the chemical analysis results were calculated on dry matter. Analysis results were compared with the one-way ANOVA test; [2]Wheat flour; [3]Germinated rye flour; [4]Germinated hull-less oat flour; [5]Factor of N x 5.70 was used for BU, and factor of N x 6.25 for ÇÇU and ÇYU; [6]TFMM: Total phenolic content.

Hammaddelerin renk değerleri arasında, buğday ununun L*, çimlendirilmiş çavdar ununun a* ve çimlendirilmiş yulaf ununun b* değerleri, diğer örneklerden istatistiksel olarak önemli derecede daha yüksek bulunmuştur ($p<0.05$). Çizelge 1'de belirtilen sonuçlara göre, çimlendirilmiş çavdar ve yulaf unu örneklerinin buğday ununa göre daha koyu renkli olduğu söylenebilir. Literatürde çimlendirilmiş çavdar unu örneğinin L* değerinin 80.98, a* değerinin 1.75 ve b* değerinin ise 12.47 olduğu bildirilmiştir. Ayrıca çimlendirme ile çavdar örneğinin L* değerinde azalış, a* ve b* değerlerinde ise artış olduğu belirtilmiştir (Tok, 2017). Çavdar örneğine benzer şekilde, yulafta da çimlendirme ile örneklerin L* değerinin azaldığı, a* ve b* değerlerinin ise arttığı bildirilmiştir. Tian ve ark. (2010) yulaf tanesinde çimlendirme ile daha fazla protein ve nişasta hidrolizatının oluşabileceğini bildirmişlerdir. Örneklere uygulanan kurutma işlemi sırasında, tane içeriğindeki nişasta ve protein hidrolizatlarında Maillard reaksiyonu gerçekleşebileceği, dolayısıyla örneklerin parlaklık değerlerinde azalma görülebileceğini belirtmişlerdir. Bir çalışmada, çimlenmeyle tohumlarda meydana gelen esmerleşmenin bir diğer nedeninin ise enzimatik esmerleşmeyi katalize eden oksidatif enzimlerin, çimlenme sırasında aktif hale gelmesi olduğu öne sürülmüştür (Bhatty, 1996).

Hammaddeler arasında, buğday ununun nem değeri, çimlendirilmiş yulaf ununun ise kül, ham protein ve ham yağ değerleri, diğer örneklerden istatistiksel olarak önemli derecede yüksek bulunmuştur ($p<0.05$). En düşük kül içeriğine sahip örneğin buğday unu, en düşük ham protein içeriğine sahip örneğin ise çimlendirilmiş çavdar unu olduğu tespit edilmiştir ($p<0.05$). Çimlendirilmiş çavdar unu-çimlendirilmiş yulaf unu örneklerinin nem değerleri ve buğday unu-çimlendirilmiş yulaf unu örneklerinin ham yağ değerleri arasında ise istatistiksel olarak önemli bir farklılığın olmadığı tespit edilmiştir ($p>0.05$). Hammaddeler arasında, toplam fenolik madde miktarı (TFMM) en yüksek olan örnek çimlendirilmiş çavdar unu (1051.00 mgGAE kg^{-1}) olarak bulunurken, en düşük TFMM değerine sahip örneğin buğday unu olduğu (693.00 mgGAE kg^{-1}) görülmüştür ($p<0.05$). Çimlendirilmiş çavdar ve yulaf unlarının fitik asit değerleri arasında istatistiksel olarak önemli bir farklılık olmadığı tespit edilirken ($p>0.05$), bu örneklerin fitik asit içeriklerinin buğday unundan daha yüksek olduğu görülmüştür ($p<0.05$).

Tok (2017) bir çalışmasında, çimlendirilmiş çavdar ununun nem değerinin %8.72, kül içeriğinin %1.75 ve protein içeriğinin ise %12.21 olduğunu tespit etmiştir. Bu çalışmada, çimlendirme ile çavdar örneğinin nem ve protein içeriklerinde artış olduğu, kül içeriğinde ise bir azalış gözlendiği bildirilmiştir. Literatürde bildirilen başka bir çalışmada, üç gün çimlendirmeyle yulafın yağ içeriğinin %5.16'dan %3.15'e, buğdayın yağ içeriğinin ise %1.7'den %0.8'e düştüğü tespit edilmiştir. Aynı çalışmada, çimlendirme ile yulafın protein ve kül içeriğinde de bir azalma gözlendiği belirtilmiş, fakat yine de çimlendirilmiş yulafın kül içeriğinin buğdaydan yüksek olduğu bildirilmiştir (Kaur ve Gill, 2020).

Literatürde, çimlendirilmiş çavdar ununun toplam fenolik madde miktarının 2771 mgGAE kg^{-1}, fitik asit içeriğinin ise 399 mg $100g^{-1}$ olarak tespit edildiği bildirilmiştir (Tok, 2017). Başka bir çalışmada, ham ve çimlendirilmiş çavdar örneğinin toplam fenolik asit içeriklerinin sırasıyla 300±13 mg $100g^{-1}$ ve 421±20 mg $100g^{-1}$ olduğu bildirilmiştir (Katina ve ark., 2007). Kaur ve Gill (2020) 72 saat çimlendirmeyle yulaf tanelerinin toplam fenolik madde miktarında iki kat artış olduğunu tespit emişlerdir. Benzer şekilde, Tian ve ark. (2010) da çimlendirmenin yulaf tanelerinin toplam fenolik madde miktarı üzerinde pozitif bir etkisinin olduğunu bildirmişlerdir. Fenolik madde içeriğindeki bu artışın sebebinin, hidrolitik enzim aktivitesi ile bağlı fenolik bileşiklerin serbest hale gelmesi ve çimlenme sonrasında tanelerdeki fenolik bileşiklerin daha iyi ekstrakte edilebilir bir forma dönüşmesinden kaynaklanıyor olabileceği belirtilmiştir (Kaur ve Gill, 2020). Yulaf tanelerinde çimlenme sırasında fitat içeriğinin %0.35'ten %0.11'e düştüğü, bu düşüşün çimlenme boyunca fitaz aktivitesinde meydana gelen artıştan kaynaklandığı bildirilmiş ve tanelerde 3 günlük kısa bir çimlenme sürecinde bile fitat içeriğinin %15-35 oranında azaltılabileceği belirtilmiştir (Kaukovirta-Norja ve ark., 2004; Tian ve ark., 2010).

Bisküvilere Ait Fiziksel Analiz Sonuçları

Çimlendirilmiş çavdar ve yulaf unu ikamesi ile üretilen bisküvi örneklerinin fiziksel özelliklerine ait çoklu karşılaştırma testi sonuçları Çizelge 2'de özetlenmiştir.

Çizelge 2. Bisküvilerin fiziksel özellikleri üzerine çimlendirilmiş çavdar ve kavuzsuz yulaf unlarının etkisi[1]
Table 2. Effect of germinated rye and hull-less oat flours on the physical properties of biscuits[1]

Faktör (Factor)	L*	a*	b*	Sertlik (Hardness) (g)	Çap (Diameter) (mm)	Kalınlık (Thickness) (mm)	Yayılma oranı (Spread ratio)
Un çeşidi (Flour variety)							
ÇÇU	71.43 ± 0.01^{b}	2.53 ± 0.01^{a}	24.94 ± 0.02^{a}	4827.69 ± 99.0^{a}	59.40 ± 0.11^{b}	7.85 ± 0.11^{a}	7.57 ± 0.09^{b}
ÇYU	73.90 ± 0.03^{a}	0.07 ± 0.01^{b}	25.10 ± 0.20 ^{a}	3668.97 ± 100.6^{b}	60.44 ± 0.16^{a}	7.66 ± 0.09^{b}	7.90 ± 0.09^{a}
Oran (Ratio)							
0	79.36 ± 0.01^{a}	-0.72 ± 0.01^{d}	22.20 ± 0.04^{d}	3039.24 ± 73.74^{d}	57.90 ± 0.14^{d}	8.05 ± 0.07^{a}	7.20 ± 0.05^{d}
10	74.58 ± 0.04^{b}	0.60 ± 0.01^{c}	24.76 ± 0.23^{c}	3869.80 ± 73.82^{c}	60.10 ± 0.14^{c}	7.80 ± 0.07^{b}	7.70 ± 0.05^{c}
20	69.98 ± 0.04^{c}	2.07 ± 0.01^{b}	25.90 ± 0.05^{b}	4721.49 ± 60.34^{b}	60.55 ± 0.07^{b}	7.65 ± 0.14^{c}	7.92 ± 0.13^{b}
30	66.73 ± 0.02^{d}	3.26 ± 0.01^{a}	27.21 ± 0.12^{a}	5362.80 ± 126.44^{a}	61.12 ± 0.18^{a}	7.52 ± 0.11^{d}	8.14 ± 0.13^{a}

[1]Aynı sütunda farklı harfle işaretlenmiş ortalamalar istatistiki olarak birbirinden farklıdır ($p<0.05$). Analiz sonuçları çift yollu ANOVA testi ile karşılaştırılmıştır.
[1]Values within a column with different superscripts differ significantly at $p<0.05$. Analysis results were compared with the two-way ANOVA test.

Un çeşidi bakımından, çimlendirilmiş çavdar ve yulaf unu ikameli bisküvi örneklerinin L* ve a* değerleri arasında istatistiksel olarak önemli farklılıklar olduğu tespit edilirken ($p<0.05$), örneklerin b* değerleri arasında önemli bir farklılık bulunmamıştır ($p>0.05$). Bisküvilerde çimlendirilmiş yulaf veya çavdar unu ikame oranı arttıkça, örneklerin L* değeri düşüş, a* ve b* değerleri ise artış göstermiştir ($p<0.05$). Literatürde, çimlendirilmiş çavdar unu ikameli bisküvi örneklerinin L* değerlerinin 60.73-67.39, a* değerlerinin 4.93-7.80 ve b* değerlerinin 24.97-26.97 arasında değişim gösterdiği bildirilmiştir. Bu çalışmada çimlendirilmiş çavdar unu oranı arttıkça, bisküvi örneklerinin L* değerlerinde azalma, a* değerlerinde ise artış gözlendiği belirtilmiştir (Tok, 2017). Tian ve ark. (2010) bir çalışmalarında, çimlendirme ile yulaf örneğinde fazla miktarda nişasta ve protein hidrolizatlarının oluştuğunu, kurutma ile bu hidrolizatlarda Maillard reaksiyonu meydana gelebileceğini ve bu durumun örneklerin L* değerinde bir düşüşe sebep olabileceğini belirtmişlerdir. Bu hipotezden yola çıkılarak, çimlendirilmiş çavdar ve yulaf unu ikameli bisküvi örneklerindeki renk değişimlerinin, pişirme sırasında uygulanan sıcaklıkla hammaddelerde meydana gelebilecek Maillard reaksiyonundan kaynaklandığı söylenebilir. Hammadde sonuçları da göz önüne alındığında, bisküvi örneklerinin renk değerlerinde meydana gelen değişimler beklenen bir sonuçtur.

Örneklerin sertlik değerleri değerlendirildiğinde, çimlendirilmiş çavdar unu ikameli bisküvi örneklerinin, çimlendirilmiş yulaf unu ikamelilere kıyasla daha sert karakterde olduğu tespit edilmiştir ($p<0.05$). Ayrıca her iki un çeşidinde de ikame oranındaki artış, örneklerin sertlik değerlerinde istatistiksel olarak önemli bir artışa neden olmuştur ($p<0.05$). Bisküvide sertlik, ürünün deformasyona gösterdiği dirençtir. Fırın ürünlerinin sertlik, dayanıklılık vb. özellikleri tekstürel açıdan oldukça önemli parametrelerdir (Ahlborn ve ark., 2005). Literatürde çimlendirilmiş çavdar unu ikameli bisküvi örneklerinin sertlik değerlerinin 3316.09-4241.43 g arasında değişim gösterdiği ve ikame oranındaki artışın sertlik değerinde de bir artışa neden olduğu bildirilmiştir (Tok, 2017). Nandeesh ve ark. (2011) bisküviye buğday kepeği ilavesinin, Baumgartner ve ark. (2018) ise yulaf kepeği ilavesinin örneklerin sertliğini artırdığını bildirmişlerdir. Sertlik değerlerindeki bu artışın, formülasyona eklenen materyallerin besinsel lif içeriklerinden kaynaklandığı belirtilmiştir. Literatürdeki çalışmalara benzer şekilde, çimlendirilmiş çavdar ve yulaf ikameli bisküvi örneklerinin sertlik değerlerindeki artışın muhtemel nedeninin, ikame maddesi olarak kullanılan tahılların tam tane şeklinde kullanılması, dolayısıyla formülasyondaki kepek fraksiyonu ve besinsel lif içeriğinin artmasından kaynaklandığı söylenebilir.

Çimlendirilmiş yulaf unu ikameli bisküvi örneklerinin çap ve yayılma oranı değerleri, çimlendirilmiş çavdar unu ikameli örneklere kıyasla daha yüksek bulunurken, kalınlık değeri için tam tersi bir durum söz konusudur. Bisküvi formülasyonunda çimlendirilmiş çavdar ve yulaf unu ikame oranlarındaki artış, örneklerin çap ve yayılma oranı değerlerini artırıcı bir etki gösterirken, kalınlığın ise azalmasına neden olmuştur ($p<0.05$). Kalınlık, çap ve yayılma oranı değerleri, bisküvinin teknolojik kalitesi bakımından önemli parametrelerdir. Genellikle bisküvide son ürünün kalınlığının düşük, çapın geniş ve yayılma oranının yüksek olması istenir (Kissell ve ark., 1971). Bu bilgi ışığında, bisküvi formülasyonundaki çimlendirilmiş çavdar ve yulaf unu ikamesinin, bisküvinin teknolojik kalitesini artırdığı sonucuna varılabilir. Ayrıca bisküvinin teknolojik özellikleri üzerinde, çimlendirilmiş yulaf ununun çavdar ununda daha pozitif bir etki sağladığı söylenebilir.

Bisküvilere Ait Kimyasal Analiz Sonuçları

Bisküvilerin kimyasal özellikleri üzerine çimlendirilmiş çavdar ve yulaf unlarının etkisi Çizelge 3'te özetlenmiştir. Un çeşidi açısından, çimlendirilmiş çavdar ve yulaf unu ikameli bisküvi örneklerinin nem ve kül değerleri arasında istatistiksel olarak önemli bir farklılık bulunmamıştır ($p>0.05$). Fakat sonuçlar ikame oranı açısından değerlendirildiğinde, ikame oranındaki artışın örneklerin nem değerlerinin önemli derecede azalmasına neden olduğu görülmüştür ($p<0.05$). En düşük kül değeri kontrol örneğinde bulunurken ($p<0.05$), %10, 20 ve 30 ikame oranlarına sahip örneklerin kül değerleri arasında istatistiksel olarak önemli farklılıklar bulunmadığı tespit edilmiştir ($p>0.05$). Bisküvi genel olarak %1-5 arasında düşük nem içeriğine sahip bir ürün olup, nem değerinin artması mikrobiyal gelişmeyi ve bozulmayı artıracağından, ürün kalitesi için istenmeyen bir durumdur (Can, 2015; Ayo ve ark., 2018). Ülkemizde sade tip bisküviler için TSE'nin belirlediği nem miktarı en fazla %6 olup, düşük nem içeriğinin ürünün raf ömrünü etkileyen önemli bir faktör olduğu belirtilmiştir (TSE, 1991). Dolayısıyla bisküvi formülasyonundaki çimlendirilmiş çavdar ve yulaf unu ikamesinin, son üründe pozitif bir etki oluşturduğu söylenebilir. Literatürde çimlendirilmiş çavdar unu ikamesi ile bisküvi örneklerinin kül miktarında artış olduğu bildirilmiştir (Tok, 2017). Literatürde bildirilen başka çalışmalarda ise çimlendirme ile meydana gelen kuru madde kaybı sonucu, tanede kül içeriğinin artış gösterdiği

belirtilmiştir (Dilber ve ark., 2003; Bibi ve ark., 2008). Çimlendirilmiş çavdar ve yulaf unu ikamesi ile bisküvilerde daha yüksek kül içeriğinin bulunmasının, buğday ununun kullanılan diğer un çeşitlerinden daha düşük kül içeriğine sahip olmasından kaynaklandığı söylenebilir. Diğer bir yaklaşım ise çavdar ve yulafta çimlendirme ile kül içeriğinde oransal bir artış meydana gelmesi ve bu artışın son ürünün kül içeriğini artırıcı bir etkide bulunmasıdır.

Çizelge 3. Bisküvilerin kimyasal özellikleri üzerine çimlendirilmiş çavdar ve kavuzsuz yulaf unlarının etkisi[1]
Table 3. Effect of germinated rye and hull-less oat flours on the chemical properties of biscuits[1]

Bileşen/Faktör (Component/Factor)	Un çeşidi (Flour variety)		Oran (Ratio)			
	ÇÇU	ÇYU	0	10	20	30
Nem (Moisture) (%)	4.60 ± 0.07^{a}	4.86 ± 0.18^{a}	5.66 ± 0.14^{a}	4.70 ± 0.04^{b}	4.40 ± 0.13^{c}	4.14 ± 0.20^{d}
Kül (Ash) (%)	1.63 ± 0.01^{a}	1.66 ± 0.02^{a}	1.58 ± 0.01^{b}	1.66 ± 0.01^{a}	1.66 ± 0.02^{a}	1.68 ± 0.02^{a}
Ham yağ (Crude fat) (%)	15.72 ± 0.12^{b}	16.66 ± 0.31^{a}	15.61 ± 0.23^{c}	16.01 ± 0.33b^{c}	16.50 ± 0.28^{ab}	16.64 ± 0.02^{a}
Ham protein (Crude protein) (%)	6.99 ± 0.01^{b}	7.91 ± 0.01^{a}	7.09 ± 0.01^{c}	7.48 ± 0.01^{b}	7.55 ± 0.01^{ab}	7.68 ± 0.01^{a}
Karbonhidrat (Carbohydrate) (%)	71.06 ± 0.15^{a}	68.92 ± 0.42^{b}	70.06 ± 0.23^{a}	70.14 ± 0.09^{a}	69.89 ± 0.43^{a}	69.86± 0.26^{a}
Enerji (Energy) (kkal 100g^{-1})	453.65 ± 0.41^{b}	457.27 ± 1.63^{a}	449.09 ± 0.62^{c}	454.61 ± 1.83^{b}	458.22 ± 0.87^{a}	459.93 ± 0.76^{a}
TFMM[2] (mgGAE kg^{-1})	621.75 ± 44.47^{a}	605.25 ± 31.38^{b}	568.00 ± 2.31^{d}	600.50 ± 7.72^{c}	629.25 ± 13.07^{b}	656.25 ± 17.67^{a}
Fitik asit (Phytic acid) (mg 100g^{-1})	194.27 ± 25.43^{a}	193.72 ± 25.93^{a}	164.40 ± 1.14^{d}	184.16 ± 1.43^{c}	203.05 ± 3.03^{b}	224.37 ± 2.20^{a}

[1]Aynı sütunda farklı harfle işaretlenmiş ortalamalar istatistiki olarak birbirinden farklıdır ($p<0.05$). Analiz sonuçları çift yollu ANOVA testi ile karşılaştırılmıştır; [2]TFMM: Toplam Fenolik Madde Miktarı
[1]Values within a row with different superscripts differ significantly at $p<0.05$. Analysis results were compared with the two-way ANOVA test; [2]Total phenolic content.

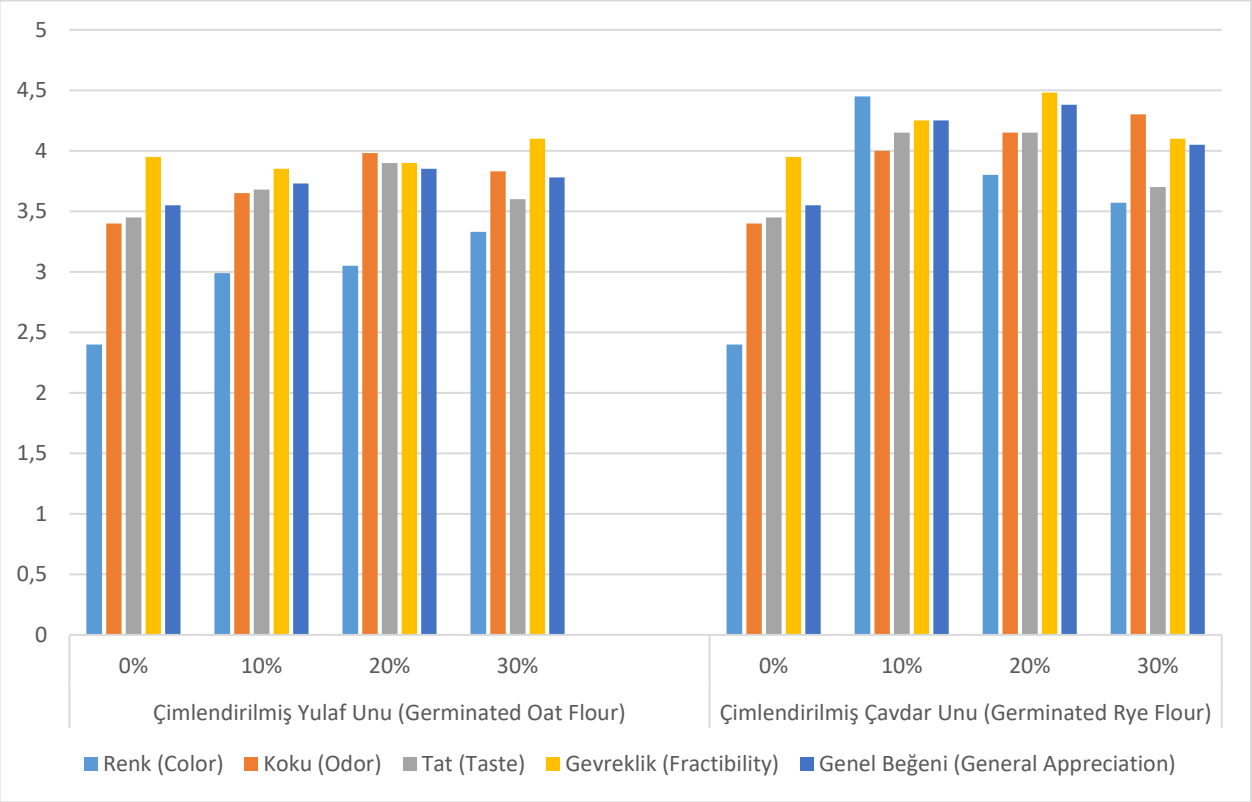
Bisküvi örneklerinin kimyasal özelliklerine ait çoklu karşılaştırma testi sonuçlarına bakıldığında, çimlendirilmiş yulaf unu ikameli örneklerin ham yağ ve ham protein içeriklerinin, çimlendirilmiş çavdar unu ikameli örneklerden istatistiksel olarak önemli derecede yüksek olduğu görülmektedir ($p<0.05$). Ayrıca ikame orandaki artış ile birlikte, bisküvi örneklerinin ham yağ ve ham protein içeriklerinde istatistiksel olarak önemli bir artış olduğu tespit edilmiştir ($p<0.05$). En yüksek protein içeriğine (%11-20) sahip tahıl çeşidi yulaftır. Ayrıca yulaf, yüksek yağ içeriğiyle de diğer tahıllardan ayrılmaktadır (Aydın, 2009). Literatürde, çimlendirilmiş yulafta protein içeriğinin %19.76'dan %21.29'a yükseldiği ve çimlendirme ile tanede çözünür protein içeriğinin arttığı bildirilmiştir (Wu, 1983; Tian ve ark., 2010). Başka bir çalışmada, erişte formülasyonunda yulaf unu ikamesi ile örneklerin protein içeriğinin arttığı bildirilmiştir (Aydın ve Göçmen, 2011). Örneklerin ham yağ ve ham protein içeriklerindeki artışın muhtemel sebebi, hammadde olarak kullanılan un çeşitleri arasındaki kimyasal kompozisyon farklılıklarıdır. Ayrıca biksüvi örneklerinin ham yağ ve ham protein içeriklerindeki artışta, hammaddelerde uygulanan çimlendirme prosesinin etkisinin olduğu da söylenebilir.

Çimlendirilmiş çavdar unu ikamesi ile üretilen bisküvi örneklerinin karbonhidrat içeriklerinin yulaf unu ile üretilenlere kıyasla daha yüksek olduğu tespit edilmiştir. Karbonhidrat değerlerinin aksine, çimlendirilmiş yulaf unu ikameli ürünlerin enerji değerlerinin daha yüksek olduğu görülmüştür ($p<0.05$). Farklı ikame oranlarına sahip bisküvi örneklerinin karbonhidrat değerleri arasında istatistiksel olarak önemli farklılıklar bulunmamıştır ($p>0.05$). Fakat ikame oranındaki artış ile örneklerin enerji değerlerinde de bir artış olduğu görülmüştür. Örneklerin karbonhidrat ve enerji değerlerindeki farklılıkların kimyasal kompozisyondaki farklılıklardan kaynaklandığı söylenebilir.

Örneklerin toplam fenolik madde miktarı arasında hem un çeşidi hem de ikame oranı açısından istatistiksel olarak önemli farklılıklar bulunmuştur ($p<0.05$). Çimlendirilmiş çavdar unu ikameli örneklerin toplam fenolik madde miktarı yulaf unu ikamelilere kıyasla daha yüksek bulunurken, ikame oranındaki artış örneklerin toplam fenolik madde miktarında istatistiksel olarak önemli bir artış sağlamıştır ($p<0.05$). Birçok bitkide önemli miktarda bulunan ve antioksidan aktivite gösteren bileşikler olan fenolik maddeler, tahıllarda özellikle tanenin dış kısımlarına yakın kepek tabakalarında yoğunlaşmaktadır (Beta ve ark., 2005). Dolayısıyla tam taneli tahıllardaki fenolik bileşik içeriği antioksidan aktiviteye önemli katkı sağlamaktadır. Tahılların ortalama antioksidan kapasitesi birçok meyve ve sebzeye eş görülmekte, fakat proses koşulları, mevsim vb. faktörlere bağlı olarak tahıllardaki antioksidan kapasitenin değişiklik gösterebileceği belirtilmektedir (Güvendi, 2011). Literatürde çimlendirme uygulamasının çeşitli tahıl ve baklagillerde toplam fenolik madde miktarında artışa neden olduğu bildirilmiştir (Yang ve ark., 2001; Domínguez-Arispuro ve ark., 2018; Kim ve ark., 2018, Kılınçer ve Demir, 2019;). Tian ve ark. (2010) bir çalışmalarında, çimlendirme ile yulaf tanelerinin

toplam fenolik madde içeriğinin %0.19'dan %0.42'ye yükseldiğini bildirmişlerdir. Başka bir çalışmada ise bisküvi ve ekmekte çimlendirilmiş çavdar unu ikamesiyle, örneklerin toplam fenolik madde miktarının kontrol örneğine göre istatistiksel olarak önemli düzeyde daha yüksek bulunduğu tespit edilmiştir (Tok, 2017). Bu bilgiler ışığında, ÇÇU ve ÇYU ikemeli örneklerin yüksek toplam fenolik madde içeriklerinin, ikame edilen hammaddelerin tam tane şeklinde kullanılmasından ve/veya hammaddelerde çimlendirme uygunlanmasından kaynaklanıyor olabileceği söylenebilir.

Bisküvi örneklerinin fitik asit değerleri arasında, un çeşidi bakımından istatistik olarak önemli bir farklılık bulunmamıştır ($p>0.05$). Fakat çimlendirilmiş çavdar ve yulaf ununun ikame oranındaki artış örneklerin fitik asit içeriklerinin önemli düzeyde artmasına neden olmuştur ($p<0.05$). Tahılların doğal yapısında bulunan fitik asit, bazı minerallerle kompleks oluşturarak bu minerallerin biyoyararlılığının düşmesine neden olmaktadır. Dolayısıyla fitik asit ürünün besinsel kalitesi üzerinde negatif etkiye sahip, anti-besinsel bir öge olarak kabul edilmektedir (Özkaya, 2004). Fakat aynı zamanda fitik asitin antioksidan özelliğinin olduğu da bilinmektedir (Graf ve ark., 1987). Tahıllarda, fitatın büyük bir kısmı aleuron tabakasında, çok az bir kısmı ise embriyoda bulunmaktadır. Bu nedenle undaki kepek miktarı arttıkça son üründeki fitik asit miktarının da artış gösterdiği bildirilmiştir (Özkaya, 2004). Bisküvi örneklerinde çimlendirilmiş çavdar ve yulaf unu kullanımı ile fitik asit içeriğinde artış meydana gelmesinin muhtemel sebebinin, çavdar ve yulafın tam tane halinde öğütülmesi ve ikame edilen bu unlarda kepek ile aleuron fraksiyonlarının da bulunması olduğu söylenebilir.



Şekil 1. Bisküvi örneklerine ait duyusal analiz sonuçları
Figure1. Sensory analysis results of biscuit samples

Duyusal Analiz Sonuçları

Bisküvi örneklerinin bazı duyusal özelliklerine ait veriler Şekil 1'de özetlenmiştir. Duyusal analiz sonuçlarına göre, genel olarak çimlendirilmiş çavdar ve yulaf unu ikamesi bisküvilerin duyusal özelliklerini geliştirici etkide bulunmuş, çimlendirilmiş çavdar ve yulaf unu ikamesine sahip örnekler panelisler tarafından daha çok beğeni kazanmıştır. Çimlendirilmiş çavdar unu ikamesi yapılan örneklerin ise duyusal değerlendirmede, çimlendirilmiş yulaf unu ikameli örneklere kıyasla daha yüksek puanlara sahip olduğu tespit edilmiştir. En iyi renk (4.45 puan) ve görünüş (4.15 puan) değerlerinin %10 ÇÇU ikameli örnekte, en iyi tat (4.15 puan), gevreklik (4.48 puan) ve genel beğeni (4.38 puan) değerlerinin %20 ÇÇU ikameli örnekte, en iyi koku (4.30 puan) değerinin ise %30 ÇÇU ikameli örnekte olduğu tespit edilmiştir. Tüm kriterler ve örnekler birlikte değerlendirildiğinde; en çok beğeni kazanan örneklerin %10 ve %20 çavdar

unu ikameli örnekler olduğu, en az beğeni kazanan örneğin ise %0 ikame oranına sahip kontrol bisküvi örneği olduğu görülmüştür. Yapılan bir çalışmada, üniversite öğrencileri tarafından en çok tecih edilen bisküvi çeşitlerinin %17.3 oranında kepekli-yulaflı ürünler, %17.0 oranında ise sade bisküviler olduğu sonucu elde edilmiştir (Karaağaoğlu ve ark., 1993). Dolayısıyla, duyusal değerlendirme verileri de göz önüne alınarak, çimlendirilmiş çavdar ve yulaf unu ikamesinin bisküvilerin albenisini artırdığı sonucuna varılabilir.

SONUÇ ve ÖNERİLER

Çimlendirilmiş çavdar ve yulaf unu ikamesiyle bisküvi örneklerinin L* değeri azalırken, a* ve b* değerleri artış göstermiştir. İkame oranındaki artış ile bisküvilerin sertliği, çapları ve yayılma oranları artmış, kalınlıkları ise azalmıştır. Artan oranda çimlendirilmiş çavdar ve yulaf unu ikamesi örneklerin kül, ham yağ, ham protein, enerji, toplam feolik madde ve fitik asit içeriklerini artırıcı bir etki gösterirken, nem içeriğini ise azaltıcı bir etki göstermiştir. Duyusal değerlendirmede ise %10 ve %20 ikame oranına sahip çimlendirilmiş çavdar unu ikameli örnekler, kontrol örneğinden dahi yüksek puanlar elde ederek en çok beğenilen örnekler olmuştur.

Sonuç olarak; bisküvi formülasyonuna çimlendirilmiş çavdar ve kavuzsuz yulaf ununun dahil edilmesi, son ürünün besinsel içeriğini artırmış, teknolojik özelliklerini geliştirmiş ve duyusal olarak ürünün daha albenili olmasını sağlamıştır. Dolayısıyla, çimlendirilmiş çavdar ve yulafın, bisküvi formülasyonunda kaliteyi artırıcı hammaddeler olarak kullanımı önerilmektedir.

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Çıkar Çatışması Beyanı

Makale yazarları aralarında herhangi bir çıkar çatışması olmadığını beyan ederler.

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Tüketicilerin Mahreç İşaretli Gümüşhane Ev Tipi Dut Ürünleri Satın Alma Motivasyonu: Gümüşhane Örneği

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ÖZET

Bu çalışmanın amacı, Gümüşhane'de ikamet eden tüketicilerin geleneksel yöntemlerle işlenmiş mahreç işaretli Gümüşhane ev tipi dut ürünlerini satın alması üzerinde etkili olan motivasyon faktörlerini ortaya koymak ve her bir kitle için müşteri odaklı pazarlama stratejilerini belirlemektir. Çalışmada kullanılan veriler, Basit Tesadüfi Örnekleme Yöntemi ile 2017 yılında Gümüşhane İlinde bu ürünleri tüketen 300 hane halkı ile yapılan anket çalışmasından elde edilmiştir. Elde edilen veriler, tüketim motivasyon faktörlerini belirlemek için Temel Bileşenler Analizi ve homojen tüketici gruplarını oluşturmak için de K-Ortalamalar Küme Analizinde kullanılmıştır. Araştırma sonuçları; mahreç işaretli Gümüşhane ev tipi dut ürünlerini yoğun düzeyde tüketen kullanıcıların satın alma memnuniyeti üzerinde kültürel entegrasyon ve sosyal çevrenin etkisi altında yüksek besin değerli beğenmeli mamullere ulaşımı temel alan hedonik ve faydacı motivasyon faktörlerinin önemine vurgu yapmıştır. Benzer şekilde, ılımlı düzeyde tüketen tüketicilerin tüketim motivasyonu üzerinde pozitif sağlık güdüsü altında yüksek besin değerli doğal yerel ürünlere doğrudan pazarlama yaklaşımı ile bölgesel kalkınmaya katkı sunan faydacı ve hedonik motivasyon faktörleri etkili olmuştur. Diğer taraftan düşük düzeyde dut ürünleri tüketen kullanıcılar, sosyal entegrasyon altında tamamlayıcılık niteliği arz eden beğenmeli doğal yerel ürünlere ulaşım için doğrudan pazarlama yaklaşımıyla bölgesel kalkınmaya katkı sunmak için hedonik motif faktörleri üzerine odaklanmışlardır. Sonuç olarak, her bir tüketici kitlesi için hedonik ve faydacı motivasyon odaklı pazarlama stratejilerinin uygulanması hem tüketicilerin tüketim memnuniyetlerini hem de arz edenlerin kazançlarını maksimum kılabilir.

Tarım Ekonomisi

Araştırma Makalesi

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Anahtar Kelimeler
Dut ürünleri
Kümeleme Analizi
Mahreç işareti
Motivasyon
Temel Bileşenler Analizi

Consumers' Purchase Motivations Towards Gümüşhane Homemade Type Mulberry Products With Protected Geographical Indication (PGI): Case of Gümüşhane

ABSTRACT

The aim of the study was to explate the motivation factors influesing on purchse of Gümüşhane homemade type mulberry products with Protect ed Geographical Indication (PGI) processed by traditional methods of the consumers residing in Gümüşhane, and to determine the customer-oriented marketing strategies for each consumer mass. The data of the study were obtained from the survey conducted through the simple random sampling method in 2017 with 300 households consumed these products in Gümüşhane. The data were used in Principal Component Analysis (PCA) to determine the motivation factors affecting their consumption preferences, and in K-means Cluster Analysis to create homogeneous consumer segments. The results of the study highlighted that utilitarian and hedonic motivation factors based on accessing to shopping products with high nutritional value under cultural entegration and social enviromental

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impacts were of a higher effect on consumption satisfaction of heavy users puchasing Gumushane homemade type mulberry products with PGI. Similarly, utilitarian and hedonic motivation factors contributing to regional development with a direct marketing approach to natural local products with high nutritional value under positive health motive were effective on moderate users' consumption motivation. On the other hand, light users focused on hedonic motivation factors contributing to regional development with a direct marketing approach for accessing to shopping natural local products with complementary attribute under social integration. Consequently, the implementation of hedonic and utilitarian motivation-oriented marketing strategies for each consumer mass could maximize both consumers' consumption satisfaction and food supplers' benefit.

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GİRİŞ

Tüketicilerin gıda ürünleri seçimi ve satın alma kararları, insan sağlığı ve çevre üzerinde pozitif etki yaratabilecek mamullerin içsel ve dışsal nitelikleri ile toplumların psikografik özellikleri arasındaki etkileşimlerin bir sonucu olarak ortaya çıkmaktadır (Xie ve ark., 2021). Dolayısıyla gıda endüstrisinde veya geleneksel yöntemlerle uygulanabilir çevre dostu teknolojik gelişmelere bağlı olarak teşekkül eden gıda tüketim olgusu, insanların gıdalara yönelik inanç ve kişilik oluşumlarıyla satın alma davranışlarında hızlı bir değişime neden olan kompleks bir fenomendir. Tüketicilerin gıda seçimi ve tüketiminde karmaşık olguların ana güdü kaynağı motivasyon, hedefe yönelik tüketim davranışlarını başlatan, harekete geçiren ve sürdüren fizyolojik ihtiyaç ve istekler ile açıklanabilmektedir. İçsel güdüler ve kültürel alışkanlıkları yansıtan fizyolojik ve psikolojik ihtiyaçlar ile sosyal, kültürel ve çevresel normları dikkate alan dışsal faktörler arasındaki etkileşimler sonucunda, satın alma tutum ve davranışı şekillenmektedir. Bu yüzden tüketicilerin satın alma motivasyonları, içsel ve dışsal ürün nitelikleri ile onların iletişim içerisinde bulundukları çevre faktörlerinin etkisi altında faydacı ve hedonik motivasyon güdülerine göre analiz edilmektedir (Lee ve ark., 2018; Picot ve ark., 2021; Martinez ve ark., 2021).

Faydacı motivasyon, ihtiyaçlardan kaynaklanan ve ihtiyacı giderme konusunda daha fonksiyonel, verimli, etkin ve rasyonel yarar sağlayan bilişsel satın alma motiflerini kapsar. Bu yüzden faydacı motivasyonlar; duyusal çekicilik, gıda güvenliği, etik değerler, besin değeri, ürün içeriği ve kalitesi, zaman ve para tasarrufu, uygunluk ve ulaşılabilirlik, yarar ve dayanıklılık güdülerini içeren fonksiyonel ihtiyaçların tatminine yönelik çabaları kapsar (Hlee ve ark., 2019; Cavalla ve ark., 2020; Picot ve ark., 2021). Hedonik motivasyon ise amaca dayalı fonksiyonel bir görevi sonlandırmaktan çok sübjektif ve duygusal alana hitap eden ruh hali, hırs ve tutku, bireysellik, keşfetme, estetik, değişiklik ve eğlence arayışı, sevgi, konfor, prestij ile mamul imajı ve markası, fiyatı, çekicilik, aşinalık ve alışkanlıklarını kapsayan duygusal tatmin deneyimleri üzerine odaklanır (Graham ve Abrahamse, 2017; Lee ve ark., 2018; Cavalla ve ark., 2020; Li ve ark., 2020; Martinez ve ark., 2021).

Tüketicilerin gıda ürünleri tüketim tercihlerinde faydacı ve hedonik motivasyon faktörlerinin toplumların bulundukları sosyoekonomik koşullar ve psikografik niteliklerine farklılık arz ettiğini, fakat tüketici davranışlarının değişimi üzerinde psikografik niteliklerin demografik ve ekonomik faktörlere göre çok daha fazla etkili olduğuna işaret eden çok sayıda tüketim araştırmaları mevcuttur. Örneğin, Honkanen ve Frewer (2009) tarafından yapılmış bir araştırmada, duyusal çekiciliğin Avrupa ülkelerinde en etkili faktör olduğu ve bu faktörü fiyat, sağlık ve etik endişelerinin takip edildiği bildirilirken; Pettinger ve ark. (2004) tarafından yürütülen bir araştırmada ise en etkili motivasyon kaynaklarının sağlık, duyusal çekicilik, uygunluk ve fiyat motivasyonları olduğu rapor edilmiştir. Benzer şekilde Xie ve ark. (2021), gıda ürünlerinin seçim motivasyonunda sağlık, duyusal çekicilik, mamul temin maliyeti, besin değeri ve kalitesi, uygunluk, etik endişeler ve alışkanlık faktörlerinin en etkili göstergeler olduğuna işaret etmiştir.

Diğer taraftan Marty ve ark. (2021), İngiltere'de uygulanmış ve Fransa'ya tatbik edilen Gıda Seçim Motivasyonu Anket araştırmasında sağlık, uygunluk, duyusal çekicilik, doğal içerik, etik endişeler, kilo kontrolü, ruh hali, aşinalık ve alışkanlık, fiyat

faktörlerinin genel olarak motivasyon üzerinde belirleyici faktörler olduklarını rapor etmişlerdir. Ayrıca Røed ve ark. (2020), gıda seçim motivasyonu ve sağlıklı beslenme pratikleri ile ilgili aracılık etki modelinde sağlık, duyusal çekicilik ve ulaşım kolaylığı motivasyonlarının en fazla etkili gıda seçim faktörleri olduğunu, fakat sağlıklı beslenme ile aşinalık ve alışkanlık pratiklerinde diyet türü, dengesi ve modeli ile çevre etiği aracılık etkilerinin daha anlamlı olduğunu ortaya koymuşlardır.

Gıda seçim motivasyonu ve sağlıklı beslenme uygulamaları altında tüketim tercihlerini yapan ve satın alma motivasyon güdüleriyle hareket eden tüketiciler, günümüzde faydacı ve hedonik motivasyon odaklı tüketim yaklaşımları sergilemekte ve gıda ürünlerinin insan sağlığı ve çevre üzerindeki negatif etkilerini minimize edecek üretim modelleri ve işleme tekniklerine büyük önem atfetmektedir (Martinez ve ark., 2021). Bu yüzden, son yıllarda şiddeti giderek artan iklim değişiklikleri ve insan sağlığı üzerinde negatif etkisi olmayan üretim modelleri ve işleme teknikleriyle üretilmiş gıda ürünlerine doğru önemli bir talep değişim trendi yaşanmaktadır (Rana ve Paul, 2017; Ritchie ve ark., 2018; Sadler ve ark., 2021; Li ve ark., 2022). Dolayısıyla tüketiciler, daha doğal ve çevre dostu üretim modelleri altında geleneksel olarak işlenmiş ev tipi mamulleri daha fazla tercih etmektedirler (Andini ve Famiola, 2019; Kanematsu ve ark., 2020; Devia ve ark., 2021; Sadler ve ark., 2021).

Yaşanan bütün bu gelişmeler ışığında tüketiciler için daha sağlıklı, doğal ve çevre dostu olan Coğrafi İşaretli (Cİ) gıda ürünlerinin üretim şekli ve işleme teknikleri, pazarlama yaklaşımları yanında arz kaynakları ve orijini, ürün içeriği ve bileşenleri gibi öz faydayı koruma ve garanti altına alma bakımından büyük bir memnuniyet sağlamış ve doğrudan pazarlama yaklaşımları ile talep trendlerinde büyük bir artış sağlamıştır. Bu kapsamda Cİ gıda ürünleri; korunan bölge orijini (PDO: Menşe işareti), korunan coğrafi işaret (PGI: Mahreç işareti) ve geleneksel özellik garantili (TSG) yerel ürünler olarak gruplandırılmaktadır (TPE, 2017). Başta Avrupa Birliği (AB) olmak üzere birçok toplum kendi kökeni, kültürü ve sosyal mirasından doğmuş yöresel ürünlerini koruyarak ve gelecek nesillere aktararak hem doğal kaynaklarını muhafaza etme hem de ekonomik ve kültürel fayda temini ile rasyonel bir yaklaşım sergileme çabası içerisindedir.

Cİ ürün sayısı 2010'da 10000 ve dünya piyasasında 50 milyar $ büyüklüğe sahipken (Giovannucci ve ark., 2009), 2017 yılında AB ülkelerinde 75 milyar € piyasa değeri ile Cİ ürünlerin EU toplam tarımsal gıda ihracatının %15.5'ini oluştururken; ABD, Çin ve Singapur ile birlikte toplam tarımsal ihracatın %50'sini karşılamaktadır. Dünya Fikri Mülkiyet Hakları Örgütü, 2019 yılında dünya genelinde 65.900'den fazla tescilli Cİ ürünlerinin bulunduğunu rapor etmiştir. Dünyada Cİ ürünlerin piyasa büyüklüğü 2020 yılında ise 200 milyar $'ı aşmıştır. Diğer taraftan AB, dünya çapında Cİ ürünlerin tanıtımı için 200 milyon € destekleme fonu da oluşturulmuştur (Cassago ve ark., 2021).

Türkiye'de ise Cİ gıda ürünleri için henüz bir iç piyasa potansiyeli oluşturulamadığı ve çalışmaların 2015-2018 Ulusal Coğrafi İşaret Stratejisi ve Eylem Planı kapsamında yürütülerek, kurumsal yapı ve piyasa potansiyelinin oluşturulması hedeflenmektedir. Bu kurumsal yapıya karşılık, 2014 yılında 13 ülkeye 7 ürün ile 2.500 ton Cİ gıda ürün ihracatı gerçekleştirilmiş ve toplam tarımsal ürün ihracatı içerisindeki payının %10'lar düzeyinde olduğu bildirilmiştir (Anonim, 2014).

Türkiye'de 2021 yılında Cİ tescili bakımından gerekli şartları yerine getirebilen yaklaşık 2500 adet potansiyel ürün mevcut olup, bunlardan 707 tanesi Cİ ile tescillenmiş ve 732 ürünün ise tescil aşaması devam etmektedir (ATO, 2021). Bu nitelikleri ile Türkiye, Cİ tescili alabilecek mamul portföyü yönünden oldukça zengin bir potansiyele sahiptir. Türkiye'de Cİ ile tescillenmiş gıda ürünleri arasında tarımsal ürünlerin payı %70 ve bunlar içerisinde meyve gruplarının oranı %50'ler düzeyindedir (Baran ve Topcu, 2018).

Gümüşhane coğrafi yapısı itibariyle PGI işareti ile tescillenmiş ve dut familyasındaki meyvelere dayalı üretilen pestil ve köme üretimi için uygun koşullara sahiptir. Diğer illere kıyasla Gümüşhane ilinde çevre kirliliğinin daha az olması, mikro klima iklim yapısı yanında pestil ve köme üretiminde ihtiyaç duyulan ceviz, dut ve kuşburnu gibi meyve türlerinin yetiştirilmesi de önemli bir rekabet avantajı sağlamaktadır (Özbek, 2010; Kalkışım ve ark., 2011; Kara ve Akyüz, 2016). Dut ürünleri üretiminde büyük bir potansiyele sahip olan Gümüşhane'de ev ölçeğinde üretilen ve kış aylarının vazgeçilmez geleneksel gıdalarının başında yer alan pestil ve köme ürünleri, ticari olarak ilk kez 1974 yılında işletme boyutunda kesintisiz üretilmeye başlanmış ve bölgesel kalkınmanın ana dinamiği olarak kabul edilmiştir.

Pestil ve köme ürünleri üretimi, tarım işletmeleri olarak kabul edilen çiftlik evlerinde ev tipi üretim, imalathanelerde ise teknolojik üretim modelleri altında gerçekleştirilmektedir (Irkın, 2013). Gümüşhane ilindeki birçok pestil ve köme ürünlerinin ticari işletmelerinde üretim süreci; yerel, iç ve dış piyasa kaynaklı tedarik fonksiyonu, teknolojik işleme ve pazarlama süreci ile entegre edilmektedir. Çiftlik evlerinde ise klasik odun ateşi ve kara kazanlar ile tarımsal üretimlerinden elde ettikleri yerel tedarik girdilerine dayalı doğal ev tipi üretim modelleri, hem öz tüketim hem de küçük ölçekte doğrudan pazarlama yaklaşımları ile yakın çevredeki tüketicilere arz edilmek üzere tercih

edilmektedir (Kalkışım ve ark., 2011). Ev tipi üretim modellerinden elde edilen dut ürünlerinin piyasaya sunulan miktarı konusunda herhangi bir kayıt olmamasına rağmen, imalat tipi üretim modeli ile üretilen dut ürünlerine ilişkin 2016 yılı kayıtlarında, yıllık olarak 5000 ton pestil ve kömenin 4500 tonu yurt içi (yoğunlukla İstanbul, Ankara ve komşu iller), 150 tonu yurt dışı (yoğunlukla Almanya ve Fransa) ve kalan 350 ton ise iç tüketime arz edilmiştir (Kara ve Akyüz, 2016).

Mahreç işareti ile tescillenmiş ve geleneksel yöntemlerle ev tipi üretim modeli altında üretilen Gümüşhane pestil, köme ve bunların farklılaştırılmış türevlerinin tüketiciler tarafından yoğun bir şekilde tercih edilmesinin temel nedenleri arasında hem insan sağlığı hem de çevre üzerinde negatif etki yaratabilecek yoğun bir işlem sürecine ve kimyasal kirleticilere maruz kalmamaları ve etik değerlere uygun hareket edilmesi gelmektedir. Diğer taraftan ev tipi dut ürünlerinin duyusal kalite ve temel fayda niteliklerinin yüksek olması, bölge orijinli doğal hammaddelerin kullanılması, izlenebilirlik ve sürdürülebilirlik, bölgesel ve kırsal kalkınmaya katkıda etnosentrizm yaklaşımı, kimyasal katkı ve koruyucu maddelerin kullanılmaması gibi faktörlerin tüketicilerin motivasyonunu pozitif yönde etkilediği kabul edilmektedir (Haas ve ark., 2010; Rana ve Paul, 2017; Ritchie ve ark., 2018; Andini ve Famiola, 2019; Kanematsu ve ark., 2020; Sanchez-Bravo ve ark., 2020; Devia ve ark., 2021; Rahman ve ark., 2021). Yaşanan bu gelişmeler ve değişim süreci kapsamında, araştırmanın amacı tüketicilerin PGI tescilli Gümüşhane ev tipi dut ürünlerini satın alma motivasyon faktörlerini belirlemek ve hedef tüketici kitleleri için pazarlama stratejileri oluşturmaktır.

MATERYAL ve METOD

Materyal

Araştırmanın ana materyalini, Gümüşhane İlini temsil etme niteliği taşıyan ve örnek kitleye seçilen tüketicilerin tek yönlü kümelenmesini önlemek için bölge dört gruba (merkez ilçe, güneyde Kelkit, kuzeyde Torul ve batıda Şiran ilçeleri) ayrılarak, 2017 yılında tüketicilerle yüz yüze yapılan anket verilerinden elde edilen birincil veriler oluşturmuştur. Diğer taraftan ikincil verileri ise Gümüşhane Tarım ve Orman İl ve İlçe Müdürlükleri, çeşitli istatistik kurum ve kuruluşların (TUİK, FAO, ATO, ISO, TSE, TPE) verileri ile yerli ve yabancı bilimsel araştırma ve raporların sonuçları oluşturmuştur.

Metodlar

Örneklem büyüklüğünün belirlenmesi

Toplam popülasyonun %85'ini oluşturan araştırma bölgesi (TUİK, 2017), tüketicilerin ev tipi dut ürünleri tüketim yoğunluklarına göre güneyde Kelkit, kuzeyde Torul, batıda Şiran ve orta kesimde ise Merkez ilçeler olarak planlanmıştır (Tablo 1). Bu dört ilçede yapılan ön anket çalışması ile dut ürünlerini geleneksel yöntemlerle işleyen ve ev tipi üretimi yapan işletmelerin köme, pestil ve türev ürünlerini tüketen ve tüketmeyen hane halklarının oranları belirlenerek, Basit Tesadüfi Örnekleme Yöntemi ile örneklem hacmi, Eşitlik 1'den yararlanarak hesaplanmıştır (Karagöz, 2019; Topcu, 2019).

$$n = \frac{Z^2 * p*(1-p)}{c^2} = 288 \text{ olarak bulunmuştur.} \quad (1)$$

Burada;
n: Örnek hacmi
Z: Z değeri, (%95 güven aralığında 1.96)
p: Ev tipi dut ürünleri tüketenlerin oranı (%75)
c: Hata terimi, (0.05 = ±5)

Araştırma bölgelerinde katılımcıların eksik veya yanıltıcı bilgi verme ve anketörlerin hatalı veri kaydetme ihtimalleri dikkate alınarak, anket sayısı %10 artırılmış ve toplam anket sayısı 317 olarak hesaplanmıştır. Fakat verilerin dijital ortama aktarımı öncesinde sayım, tasnif ve veri temizlik işlemleri sonucunda hatalı anketler ayıklanmış ve her bir ilçede yapılan net ve toplam anket sayısı 300 olarak belirlenmiştir (Çizelge 1). Anket verilerinin minimum örnek hacmi olan 288 hane halkından daha fazla tüketici ile çalışılması, merkezi limit teoremi varsayımlarından dolayı ana kütle tahmincilerinin ve faktör rotasyon tekniklerinin daha etkin ve sapmasız çalışmasına neden olmaktadır (Gujarati and Porter, 2020; Karagöz, 2019).

Çizelge 1. Araştırma bölgesindeki her bir ilçenin popülasyonu ve anket sayıları
Table 1. *The number of population and questionnaire for each district in research region*

İlçeler *Districts*	Popülasyon *Population*	Anket sayısı *Survey number*	Ek anket *Additional survey*	Hatalı anket *Survey with missing value*	Net anket sayısı *Net survey number*
Merkez	44888	112	11	6	117
Kelkit	40266	101	10	6	105
Torul	12379	31	3	2	32
Şiran	17775	44	5	3	46
Toplam	**115308**	**288**	**26**	**21**	**300**

Verilerin toplanması ve organizasyonu

Atatürk Üniversite Ziraat Fakültesi Birim Etik Kurul Başkanlığı (2021/15 sayılı karar ve 24.08.2021 tarihli) onayı ile hazırlanmış anket formunda sürekli, ordinal

ve nominal verilere dayalı ev tipi Gümüşhane köme ve pestili tüketen tüketicilerin demografik ve sosyoekonomik özellikleri ve 5'li-Likert ölçeği (1: en olumsuz, 3: kararsız/nötr ve 5: en olumlu değerleme skorları) aracılığıyla ürünlerin faydacı ve hedonik motivasyon faktörlerine yönelik tüketicilerin satın alma kararı duyarlılıkları ölçülmüştür. Diğer taraftan nominal veriler kategorisinde olan tüketicilerin dut ürünleri satın alma sıklıkları dikkate alınarak, homojen tüketici gruplarını oluşmak için hedef piyasa segmentleri oluşturulmuştur. Araştırma bölgelerinde katılımcılardan elde edilen veriler, katılımcıların ikamet ettikleri konutlarda daha önceden hazırlanmış kapalı uçlu soru formlarından oluşan soru cetvelleri üzerinden kişisel görüşme (yüz-yüze) tekniği kullanılarak elde edilmiştir.

İstatistik analizler

Katılımcılardan elde edilen birincil verilerin temizliklerinin yapılması ve kodlanmasından sonra veri kayıtlarını takiben istatistiki analizin ilk aşamasında, tüketicilerin ev tipi dut ürünleri satın alma kararı üzerinde etkili olan motivasyon faktörlerini belirlemek amacı ile Temel Bileşenler Analizi (PCA) kullanılmıştır. PCA, aralarında yüksek ilişkiye sahip maddeleri birleştirerek, içsel uyum ve dışsal geçerlilik varsayımları altında yeni faktörler oluşturabilen istatistiksel bir analiz tekniğidir (Bursal, 2019; Karagöz, 2019). PCA'da takip edilen veri setinin istatistiki olarak uygunluğunun değerlendirilmesi, faktör sayısının belirlenmesi, faktörlerin rotasyonu ve isimlendirilmesi şeklinde bir hiyerarşik süreç takip edilmiştir (SPSS 20.0, 2020; Topcu, 2019; Bursal, 2019; Baran ve Topcu, 2017).

Veri setinin PCA için uygunluğunun değerlendirilmesinde, Bartlett's Sphericity testi ve Kaiser-Meyer-Olkin (KMO) istatistikleri kullanılmıştır. Bartlett's Sphericity testi, korelasyon matrisinde faktör maddeleri arasında yüksek bir korelasyonunun olup olmadığı hipotezini test eder. KMO örneklem yeterlilik istatistiği ise gözlenen ve kısmi korelasyon katsayılarının büyüklüğünü karşılaştıran bir indekstir ve bu istatistiğin 0.50'den büyük olması gerekir. Faktör sayılarının belirlenmesi için Eigenvalues (özdeğer) istatistiği ile toplam ve açıklanan varyans yüzdeleri kullanılmaktadır. Eigenvalues istatistik değerinin 1'den büyük olması durumunda faktörlerin anlamlı olduğu, fakat aksi durumda anlamsızlığı kabul edilir. Faktör rotasyonunda ise analize dahil edilen çok faktörlü yapılarda, faktörlerinin bir birleri ile ilişkili olmadığı ve ayrışma geçerliği varsayımları altında orthogonal bir yapının mevcut olduğu kabul edilir ve yaygın bir şekilde Varimax metodu kullanılır. Mevcut araştırmada, faktörler arasında orthogonal bir yapının olduğu varsayımı ile rotasyon tekniklerinden Varimax kullanılmıştır. Son olarak, her bir faktör altındaki maddelerin binişikliği/ötüşmesi ve faktör yükleri dikkate alınarak, yüksek yüklü maddelerin oluşturdukları grupların ortak özelliklerine göre faktör isimleri verilmiştir.

İstatistiki analizin ikinci aşamasında, heterojen bir yapı arz eden tüketici kitlelerinin daha homojen alt gruplara ayrılması için kümeleme analizi kullanılır. Kümeleme analizleri, hiyerarşik ve hiyerarşik olmayan kümeleme şeklinde iki grupta değerlendirilir. Bunlar içerisinde hiyerarşik olamayan K-Means Cluster Analizi en sık kullanılan kümeleme analiz tekniğidir (Karagöz, 2019; Topcu, 2019). Bu kapsamda, PCA sonuçlarından elde edilen dut ürünleri satın alma motivasyon faktörleri ve tüketicilerin satın alma sıklıkları dikkate alınarak K-Means Cluster Analizi kullanılmıştır.

BULGULAR ve TARTIŞMA

PCA Sonuçları

Tüketicilerin ev tipi Gümüşhane pestil ve köme satın alma motivasyon faktörlerini ifade eden gözlem ve kısmi korelasyon katsayılarını karşılaştıran KMO örnek yeterlilik ölçüt indeksi, 0.881 olarak bulunmuştur. Diğer taraftan tüketicilerin satın alma motivasyonu ile ilgili faktörlerin Bartlett's Sphericity test istatistiği χ^2 = 5728.53 (p=0.000) olarak hesaplanmış ve birim matris hipotezi reddedilmiştir (p<0.001). Örneklem yeterliliğini ve uyumunu değerlendiren bu istatistikler, ev tipi pestil ve köme satın alma motivasyon faktörleri ile ilgili veri setinin PCA için çok iyi bir düzeyde olduğunu göstermiştir. Tüketicilerin mahreç işaretli Gümüşhane ev tipi pestil ve köme satın alma motivasyonları üzerinde etkili olan 36 değişken, PCA ile 9 ana motivasyon faktörlerine indirgemiş ve bu faktörlerin toplam açıklama oranı ise yaklaşık %67 olarak hesaplanmıştır (Çizelge 2).

Tüketicilerin PGI tescilli Gümüşhane ev tipi dut ürünleri tüketim motivasyonu, %11.09 açıklama oranı ile tüketicilerin yüksek besin değeri altında dengeli ve sağlıklı beslenmeyi destekleyen %9.31'lik sağlık üzerindeki pozitif etki faktörleri ile toplamda %20.40'lık açıklama payı ile temsil edilmiştir. Gıda tüketim motivasyonu üzerine yapılmış çeşitli araştırmalarda, insan sağlığı ve çevre üzerinde pozitif etki yaratan etik değerler ile insan sağlığı üzerinde direkt etkili olan besin değeri algılarını kapsayan faydacı motivasyon faktörlerinin en önemli motivasyon kaynakları olduğu rapor edilmiştir (Røed ve ark., 2020; Martinez ve ark., 2021; Marty ve ark., 2021; Xie ve ark., 2021; Li ve ark., 2022).

Diğer taraftan Gümüşhane ev tipi pestil ve köme satın alma motivasyonu, bölgesel kalkınmaya katkı sağlama (%8.75) güdüsü altında etnosentrizm yaklaşımı felsefesi ile doğal yerel ürünlere ulaşım

(%8.31) amacıyla doğrudan pazarlama yaklaşımı altında kırsal bölgeye yoğun bir ilgi gösteren tüketicilerin toplam tüketim tercihi varyasyon faktörlerinin %17.16'lık açıklanma oranını temsil etmektedir. Dolayısıyla bölge orijinli ürünlerinin PGI

Çizelge 2. Ev tipi dut ürünleri tüketim tercihleri ilgili faktörler, madde yükleri ve PCA sonuçları
Table 2. *Motivation factors, items loads and PCA results related to purchasing homemade type mulberry products*

Faktör yorumları ve değişkenler *Factor interpretations and variables*	Faktör ve değişken yükleri* *Factors and variables loads*								
	F1	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**	**F9**
Yüksek besin değeri									
Protein bakımından zengin olması	**0.853**	0.096	0.098	0.201	0.051	0.148	0.074	0.052	0.035
Vitamin bakımından zengin olması	**0.833**	0.162	0.098	0.171	0.020	0.108	0.018	0.008	0.077
Mineral madde bakımından zengin olması	**0.773**	0.178	-0.004	0.151	0.054	0.188	0.037	-0.002	0.026
Yüksek enerji değerine sahip olması	**0.749**	0.059	0.150	0.026	0.102	-0.082	0.180	0.186	0.038
Diyette diğer tatlılara göre daha doğal olması	**0.685**	0.010	0.177	-0.012	0.122	-0.010	0.260	0.145	0.165
Diğerlerine göre daha yüksek besin değeri	**0.494**	-0.014	0.080	0.178	0.300	0.300	-0.086	0.200	-0.173
Pozitif sağlık etkisi									
Kanser riskini azalttığı inancı	0.053	**0.823**	0.115	0.182	0.169	0.081	0.070	-0.012	0.146
Doku/hücre yenilemede önemli rol oynaması	0.118	**0.766**	0.098	0.079	0.223	0.110	0.038	0.183	0.222
Zihinsel gelişmede etkili olması	0.145	**0.755**	0.132	0.207	0.081	0.067	0.142	-0.024	-0.061
Vücutta su dengesini korumaya yardımcı olma	0.031	**0.668**	0.106	-0.111	0.221	0.258	-0.041	0.209	0.247
Bağışıklık sistemini güçlendirdiği inancı	0.240	**0.661**	0.074	0.159	0.037	0.100	0.104	0.352	0.134
Bölgesel kalkınmaya etki									
Üreticilerin yeterli gelir sağlamasına katkı	0.115	0.089	**0.791**	0.087	0.205	0.169	0.042	0.082	0.009
Üreticiye daha fazla katma değer sağlamak	0.243	0.187	**0.776**	0.109	0.091	0.062	0.148	0.038	-0.053
Kırsal göçün engellenmesine katkı sağlamak	0.073	0.128	**0.718**	0.257	0.178	0.023	0.155	0.053	0.095
Potansiyel kıt kaynakların etkin kullanımı	0.218	0.286	**0.642**	0.173	0.058	-0.007	0.222	0.064	-0.061
Bölgesel kalkınmaya katkı sağlamak	0.175	0.101	**0.627**	0.362	0.166	0.189	-0.138	0.122	-0.008
Kırsal kalkınmaya katkı sağlamak	0.056	0.028	**0.586**	0.498	0.162	0.197	0.044	0.165	0.036
Üretim bölgesinde doğal çevre ziyareti	0.061	0.294	**0.572**	0.235	0.056	0.058	0.156	0.069	0.066
Doğal yerel ürüne ulaşım									
Yabancı orijinli ürün olmaması	0.149	0.146	0.203	**0.718**	0.081	0.028	0.180	0.079	0.168
Diğer tatlılara göre kimyasal kalıntı riski düşük	0.120	0.145	0.178	**0.659**	0.154	0.177	0.080	0.081	-0.038
Yerli kaynaklara dayalı doğal ürün olması	0.224	0.153	0.117	**0.627**	0.191	-0.004	0.295	0.108	-0.001
Kolay bulunabilir ve ulaşım kolaylığı	0.312	0.072	0.177	**0.534**	0.239	0.041	0.141	0.234	0.058
Sosyal çevre etkisi									
Referans gruplarının yönelim etkisi	0.051	0.192	0.209	0.159	**0.807**	0.118	0.161	0.077	0.058
Sosyal grupların yönelim etkisi	0.131	0.210	0.161	0.259	**0.755**	0.122	0.223	0.051	-0.043
Besleyicilikte diğer tatlılara göre daha avantajlı	0.202	0.094	0.188	0.426	**0.591**	0.032	0.106	0.200	-0.097
Diğer tatlılara göre hediyeye daha uygun olması	0.104	0.186	0.175	-0.079	**0.498**	0.199	0.094	0.385	-0.141
Beğenmeli mamul etkisi									
Diğer tatlılara göre daha ucuz olması	0.042	0.102	0.071	0.033	0.205	**0.763**	0.132	-0.131	0.238
Hazırlama ve sunumunun daha pratik olması	0.083	0.107	0.071	0.267	0.026	**0.637**	0.366	-0.037	0.152
Geleneksel ürünü tüketimden haz duyma	0.231	0.105	0.138	0.135	0.016	**0.633**	0.091	0.239	-0.022
Sosyal entegrasyon etkisi									
Sosyal toplantılar için daha uygun olması	0.103	0.085	0.117	0.054	0.142	0.194	**0.687**	0.202	-0.029
Sosyal çevrede aranan bir ürün olması	0.083	0.179	0.132	0.135	0.202	0.190	**0.628**	-0.171	0.177
Daha uzun süre muhafaza imkânı	0.129	0.098	0.139	0.172	0.143	0.392	**0.466**	0.115	0.019
Kültürel entegrasyon etkisi									
Bölge kültürünün önemli bir parçası olması	0.230	0.084	0.136	0.181	0.109	0.224	0.056	**0.707**	0.028
Bölge orijinli geleneksel bir ürün olması	0.146	0.192	0.111	0.345	0.083	-0.127	0.258	**0.616**	0.147
Tamamlayıcılık etkisi									
Diyetin önemli bir parçası olması	0.033	0.175	0.014	-0.017	0.031	0.099	0.050	-0.088	**0.833**
Çayın iyi bir tamamlayıcısı olması	0.114	0.244	0.137	0.020	0.152	0.135	0.165	0.013	**0.589**
Eigenvalue	11.118	2.612	2.215	1.802	1.397	1.326	1.227	1.120	1.028
Açıklanan varyansların payı (%)	**11.088**	**9.309**	**8.745**	**8.309**	**7.290**	**6.661**	**5.962**	**4.680**	**4.387**
Varyansların kümülatif payı (%)	11.088	20.398	29.143	37.452	44.742	51.403	57.365	62.045	66.432
KMO (Kaiser-Meyer-Olkin) istatistiği									***0.881***
Bartlett's test of Sphericity									*(Chi-square $_{df: 630}$): 5728.53 (p: 0.000)*

**Koyu renkli rakamlar, her bir değişken için en yüksek yükleri göstermektedir.*

standartlarına göre üretilmesi ve sunumu hem tüketim motivasyon güdüsü ile gıda güvencesi hem de doğrudan pazarlama yaklaşımları altında bölgesel kalkınmaya önemli bir katkı sağlayan hedonik motivasyon faktörlerine işaret etmiştir (Topcu, 2012; Sanchez-Bravo ve ark., 2020; Devia ve ark., 2021; Kanematsu ve ark., 2020; Rahman ve ark., 2021; Sadler ve ark., 2021; Skalkos ve ark., 2021).

Mahreç işaretli Gümüşhane ev tipi köme ve pestil ürünlerinin geleneksel nitelikli orijin tescil standartları altında bazı perakendecilerde sunumu ile bu ürünlere ulaşım olanaklarını temsil eden beğenmeli ürünler (%8.86), sosyal çevrelerde (%7.29) hem sosyal entegrasyon (%5.96) hem de kültürel entegrasyon (%4.68) ile sosyal toplantılarda diğer tatlı gruplarına göre daha iyi bir tamamlayıcılık niteliği (%4.39) ile tüketicilerin satın alma motivasyonunu pozitif bir şekilde etkilemiştir.

Bütün bu hedonizm yaklaşımları paradigması altında hareket eden tüketicilerin satın alma motivasyonlarını pozitif bir şekilde etkileyen mamul imajı, sosyal çevre, sosyal ve kültürel entegrasyon güdüleri ile tüketicilerin alışkanlıkları ve tercihlerini yönlendiren hedonik motivasyon konusunda yapılmış araştırmaların sonuçları, mevcut araştırma bulgularını önemli ölçüde desteklemektedir (Cavalla ve ark., 2020; Li ve ark., 2020; Devia ve ark., 2021; Martinez ve ark., 2021; Picot ve ark., 2021; Rahman ve ark., 2021).

Kümeleme Analizi Sonuçları

Gümüşhane ev tipi pestil ve köme ürünlerini yoğun düzeyde tüketen kullanıcılar, bireysel olarak yüksek besin değeri algısı ve kültürel entegrasyon etkileşimi ile sosyal çevresinin etkisi altında beğenmeli mamullere önemli bir eğilim göstermişlerdir (Çizelge 3). Bu gruptaki tüketiciler, mahreç tescilli Gümüşhane ev tipi dut ürünlerinin tüketim memnuniyetinde hem faydacı motivasyon hem de hedonik motivasyon faktörlerini dikkate alarak, satın alma kararı vermektedirler.

Çizelge 3. Her bir kümedeki tercih faktörlerinin final küme merkez skorları ve örnek sayıları
Table 3. *Final cluster center scores of preferences factors, and sample sizes in each cluster*

Motivasyon faktörleri *Motivation factors*	Kümeler** *(Clusters)*		
	Yoğun kullanıcılar* *Heavy users*	Ilımlı kullanıcılar* *Moderate users*	Düşük kullanıcılar* *Light users*
Yüksek besin değeri	**0.109**	**0.491**	-0.191
Pozitif sağlık etkisi	-0.399	**0.605**	-0.631
Bölgesel kalkınmaya etki	-0.684	**0.309**	**0.278**
Doğal yerel ürüne ulaşım	-0.501	**0.320**	**0.029**
Sosyal çevre etkisi	**0.293**	-0.095	-0.542
Beğenmeli mamul etkisi	**0.163**	-0.006	**0.192**
Sosyal entegrasyon etkisi	-0.510	-0.029	**0.581**
Kültürel entegrasyon etkisi	**0.375**	-0.081	-0.317
Tamamlayıcılık etkisi	-0.017	-0.551	**0.307**
Her kümedeki örnek sayısı (kişi)***	***91***	***73***	***136***
Her kümedeki toplam örnek oranı (%)	30	24	46

**Koyu renkler, her bir kümedeki en yüksek final küme merkez skorlarını göstermektedir.*
***p<0.001, F istatistiğine göre, final küme merkez skorları önemli bulunmuştur.*
****Toplam örnek büyüklüğü (n), 300'dür.*

Diğer taraftan Gümüşhane ev tipi dut ürünlerini ılımlı düzeyde tüketen tüketiciler; sağlık üzerinde pozitif etki sağlayacak yüksek besin değerine sahip doğal yerel ürünlere ulaşım amacıyla doğrudan pazarlama yaklaşımı altında bölgesel kalkınmada etnosentrizm felsefesini benimsemiş hedef tüketici kitlesini tanımlamaktadır (Çizelge 3). Ilımlı düzeyde Gümüşhane ev tipi dut ürünleri tüketen tüketicilerin satın alma kararları üzerinde faydacı ve hedonik motivasyon faktörleri büyük önem arz etmektedir.

Gümüşhane ev tipi pestil ve köme ürünlerini düşük seviyede tüketen tüketiciler ise diyetleri üzerinde tamamlayıcılık niteliği arz eden beğenmeli doğal yerel ürünlere doğrudan pazarlama yaklaşımı ile ulaşım hem sosyal entegrasyonu sağlama hem bölgesel kalkınmaya katkı sunma motivasyonu altında tüketim memnuniyeti sergilemişlerdir (Çizelge 3). Dolayısıyla ana popülasyonda yüksek bir tüketim grubunu temsil eden düşük düzeydeki tüketicilerin PGI tescilli Gümüşhane ev tipi dut ürünleri satın alma kararları, hedonik motivasyon faktörleri tarafından belirlenmiştir.

SONUÇ ve ÖNERİLER

Mahreç işaretli Gümüşhane ev tipi pestil ve köme ürünlerini satın alma motivasyonu üzerinde bölge orijinli doğal yerel ürünlerin yüksek besin değerine bağlı olarak pozitif sağlık etkisi ve dolaylı olarak da çevre duyarlılıkları ile etik değerlere bağlı temel fayda güdüsü altında faydacı motivasyon faktörlerini

önceleyen satın alma kararları üzerine odaklanmışlardır. Diğer taraftan tüketicilerin ilgili dut ürünlerini satın almasında, sosyal ve kültürel entegrasyona bağlı olarak sosyal çevrenin çekici gücü ile diyetlerde tamamlayıcılık etkisi olan beğenmeli mamullerden doğal yerel dut ürünlere doğrudan pazarlama yaklaşımı ile ulaşım olanakları bölgesel kalkınma çabalarını etkin kılarak etnosentrizm yaklaşımı altında hedonik motivasyon faktörleri önemli bir etkiye sahiptir.

Mahreç işaretli Gümüşhane ev tipi pestil ve köme ürünlerini yoğun bir şekilde tüketen tüketicilerin satın alma motivasyonu üzerinde kültürel entegrasyona bağlı olarak sosyal çevresinin etkisi altında yüksek besinli dut ürünlerinin arz güvencesiyle beğenmeli mamullere ulaşım yoluyla tüketim memnuniyeti amaçlanmıştır. Dolayısıyla hedef kitlenin tüketim memnuniyetini artırabilmek için farklılaştırılmış ürün imajları altında faydacı ve hedonik motivasyon faktörlerini etkin bir araç olarak kullanarak, besin değeri üzerinden mamullerin çeşitli tutundurma ve dağıtım karması ile hedef kitleye ulaştırılması yönünde pazarlama stratejileri uygulanmalıdır.

Diğer taraftan mahreç tescilli Gümüşhane ev tipi pestil ve köme ürünlerini ılımlı düzeyde tüketen tüketiciler, pozitif sağlık güdüsü ile yüksek besin değerli doğal yerel ürünlere ulaşım amacıyla doğrudan pazarlama yaklaşımını kullanarak, bölgesel kalkınmaya katkı sunmak için etnosentrizm felsefesini benimsemişlerdir. Bu yüzden ılımlı düzeyde Gümüşhane ev tipi dut ürünleri tüketen tüketicilerin satın alma tercihlerinde doğrudan pazarlama yaklaşımları altında etnosentrizm algısını ortaya çıkaran yüksek besin değerli doğal yerel ürün tasarımı, sunumu ve tutundurulmasına yönelik faydacı ve hedonik motivasyon faktörlerine dayalı pazarlama stratejileri etkin bir şekilde kullanılabilir.

Bölge orijinli Gümüşhane ev tipi pestil ve köme ürünlerini düşük düzeyde tüketen tüketicilerin sosyal entegrasyonunun çekici güdüsü ile diyetleri üzerinde tamamlayıcılık niteliği arz eden beğenmeli doğal yerel ürünlere ulaşım için doğrudan pazarlama yaklaşımı felsefesi altında bölgesel kalkınmaya katkı güdüsüyle tüketim memnuniyetlerini maksimum kılmayı hedeflemişlerdir. Dolayısıyla bu hedef tüketici kitlesi için sosyal entegrasyon ile etnosentrizm yaklaşımı ile bölgesel kalkınmaya katkı sunacak farklılaştırılmış doğal yerel ürünlerin konumlandırılması ve sunumu yanında bu mamullere talebi artıracak tutundurma ve dağıtım karmamalarına yönelik hedonik motivasyon odaklı pazarlama stratejilerine öncelik verilmelidir.

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Gelibolu Yarımadası'ndan (Kuzey Ege Denizi, Türkiye) Avlanan Palamut (*Sarda sarda* Bloch, 1793), Uskumru (*Scomber scombrus* Linnaeus, 1758) ve Kolyoz (*Scomber colias* Gmelin, 1789) Balıklarının Toplam Boy, Toplam Ağırlık, Maksimum Vücut Çevresi ve Operkulum Çevresi arasındaki İlişkiler

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ÖZET

Bu çalışma Gelibolu Yarımadası açıklarında (Kuzey Ege Denizi, Türkiye) Ocak 2017-Aralık 2017 tarihleri arasında palamut (*Sarda sarda* Bloch, 1793), uskumru (*Scomber scombrus* Linnaeus, 1758) ve kolyoz (*Scomber colias* Gmelin, 1789) türlerinin boy-ağırlık ve boy-çevre ilişkilerini ortaya çıkarmak için ticari balıkçılar tarafından avlanan öü balıkları kullanmak suretiyle gerçekleştirilmiştir. Boy-ağırlık ilişkisi palamut, uskumru ve kolyoz balıkları için, sırasıyla, W=0.0143$TL^{2.84}$ (R^2=0.89), W=0.0059$TL^{3.11}$ (R^2=0.92) ve W=0.0059$TL^{3.11}$ (R^2=0.92) olarak tahmin edildi. Yine sırasıyla bu türlerin boy-operkulum çevre ilişkileri G_{ope}=0.3988TL+0.2512 (R^2=0.78), G_{ope}=0.3528TL+0.8122 (R^2=0.84) ve G_{ope}=0.4295TL-0.5991 (R^2=0.94) olarak hesaplanırken boy-maksimum vücut çevre ilişkileri ise G_{mak}=0.5993TL-4.1237 (R^2=0.79), G_{mak}=0.4206TL+0.2732 (R^2=0.83) ve G_{mak}=0.4676TL-0.168 olarak saptanmıştır. Bu çalışma Karadeniz'i de kapsayacak şekilde tüm Akdeniz Havzası için bu türlerin boy-operkulum ve boy-maksimum vücut çevresi ile ilgili ilk bilgileri içermektedir.

Su Ürünleri

Araştırma Makalesi

Makale Tarihçesi
Geliş Tarihi : 21.12.2021
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Anahtar Kelimeler
Balık morfolojisi
Balıkçılık yönetimi
Kuzey Ege Denizi
Türkiye

Relationships between Opercular Girth and Maximum Girth, Total Weight, Total Length of Atlantic bonito (*Sarda sarda* Bloch, 1793), Atlantic mackerel (*Scomber scombrus* Linnaeus, 1758) and Atlantic chub mackerel (*Scomber colias* Gmelin, 1789) from Gallipoli Peninsula (Northern Aegean Sea, Turkey)

ABSTRACT

This study was carried out using dead fishes caught by commercial fishermen of Gallipoli Peninsula (northern Aegean Sea, Turkey)to find out length-girth and length-weight relationships of atlantic bonito (*Sarda sarda* Bloch, 1793), atlantic mackerel (*Scomber scombrus* Linnaeus, 1758) and atlantic chub mackerel (*Scomber colias* Gmelin, 1789) between January 2017 and December 2017. The length-weight relationships were estimated as W=0.0143$TL^{2.84}$ (R^2=0.89), W=0.0059$TL^{3.11}$ (R^2=0.92) and W=0.0059$TL^{3.11}$ (R^2=0.92) for *Sarda sarda*, *Scomber scombrus* and *Scomber colias*, respectively. The length-opercular girth relationships were calculated as G_{ope}=0.3988TL+0.2512 (R^2=0.78), G_{ope}=0.3528TL+0.8122 (R^2=0.84) and G_{ope}=0.4295TL-0.5991 (R^2=0.94), whereas the length-maximum girth relationships were determined to be G_{max}=0.5993TL-4.1237 (R^2=0.79), G_{max}=0.4206TL+0.2732 (R^2=0.83) and G_{max}=0.4676TL-0.168 for *Sarda sarda*, *Scomber scombrus* and *Scomber colias*, respectively. The present study includes preliminary information on length-opercular girth and length-maximum girth relationships of these fish species for the Mediterranean Basin including Black Sea.

Fisheries

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GİRİŞ

Balıklarda boy-ağırlık ve boy-çevre gibi morfometrik ilişkilere dayalı bilgiler, bir balık popülasyonunun mevcut durumunu ortaya koyması açısından önemli parametreler olarak değerlendirilmektedir. (Anderson ve Gutreuter, 1983). Balıkçılık yönetiminde, balıkların boy ve ağırlık ilişkileri (BAİ) ile ilgili veriler (a) sadece boy verilerinin mevcut olduğu durumlarda biyokütlenin tahmininde (Tobes ve ark., 2016); (b) türlerin üreme stratejileri ve beslenme koşulları hakkında bilgi edinilmesinde (Park ve Huh, 2015) ve (c) doğal popülasyonların yönetimi ve korunmasında (Hossain ve ark., 2012) kullanılmaktadır. Ayrıca, boy-ağırlık ilişkisinden elde edilen sonuçlar balıkçılık faaliyetlerini, balık stoklarını ve çevresel izleme programlarını değerlendirmek için gereklidir (Froese ve ark., 2011). Bu bilgilere ek olarak, balıkçılık faaliyetlerinin en önemli geçim kaynağı ve balık stoklarının temel besin maddesi olarak kabul edildiği bölgelerde bu çeşit çalışmalar gün geçtikçe önem kazanmaktadır (Freitas ve ark., 2014). Bundan dolayı bu çalışmalar herbir balık populasyonu için belirli aralıklarda tekrarlanmalıdır (Torres ve ark., 2012).

Benzer şekilde, boy-çevre ilişkileri (BÇİ) de (a) biyolojik değerlendirmeler (balıkların kondisyonu ve yüzme yetenekleri bakımından) (Wootton, 1998); (b) ekolojik döngüler (av-avcı ilişkisi ve trofik seviye açısından) (Stergiou ve Karpouzi, 2003); ve (c) balıkçılık faaliyetleri (bir av aracının verimliliğinin ölçülmesi gibi) için önemli unsurlardır (Kyritsi ve ark., 2018). Böylelikle, türe özgü boy-çevre ilişkisi sayesinde boy verilerinden balıkların çevre ölçümleri, daha pratik bir şekilde, hesaplanabilmektedir (Moutopoulos ve ark., 2017). Bununla beraber, dünyada BAİ'ye kıyasla BÇİ ile ilgili çalışmalar son derece azdır. Kısa bir şekilde özetlenirse, mevcut çalışmalar Malezya Yarımadası'nın batısından (Matsushita ve Ali, 1997), Kiklad Adaları'ndan (Stergiou ve Karpouzi, 2003), Póvoa de Varzim ile Santo André arasındaki alandan (Mendes ve ark., 2006), Algarve'den (Santos ve ark., 2006), Basra Körfezi'nin kuzeyinden (Daliri ve ark., 2012), Mesolonghi-Etolikon lagün sisteminden (Moutopoulos ve ark., 2017); Jeddah mercan resiflerinden (Gabr ve Mal, 2018) ve İzmir (Beğburs ve ark., 2020) ve Saros (Cengiz, 2021a; Cengiz, 2022) Körfezlerinden gelmektedir.

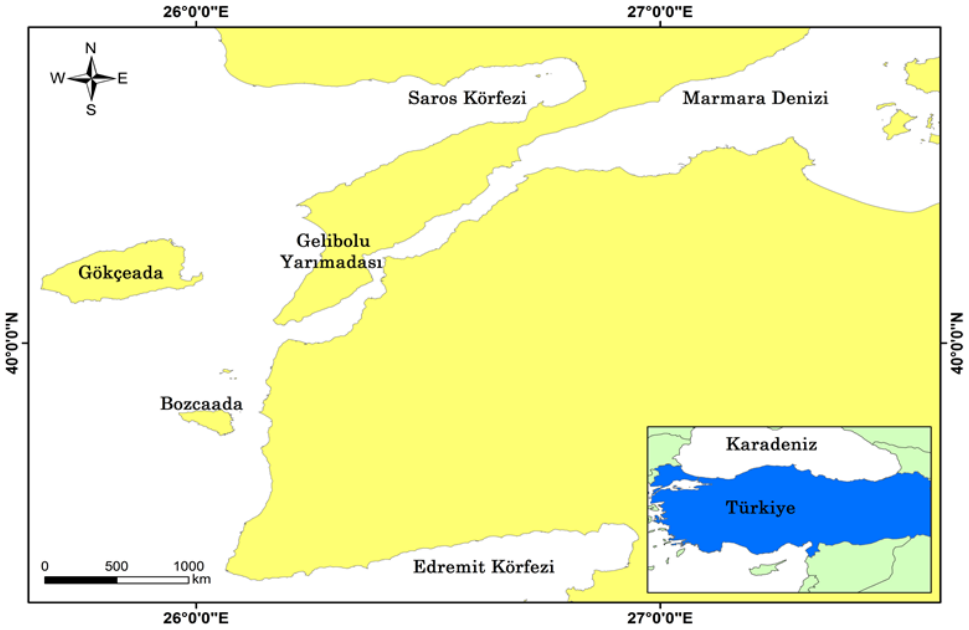
Scombridae familyası, Scombriformes takımına ait olup dünyada 54 türü içermektedir (Froese ve Pauly, 2021). Bu türlerin 10 tanesi [*Auxis rochei* (Risso, 1810), *Euthynnus alletteratus* (Rafinesque, 1810), *Katsuwonus pelamis* (Linnaeus, 1758), *Orcynopsis unicolor* (Geoffroy Saint-Hilaire, 1817), *Sarda sarda* (Bloch, 1793), *Scomber colias* (Gmelin, 1789), *Scomber scombrus* (Linnaeus, 1758), *Scomberomorus commerson* (Lacepède, 1800), *Thunnus alalunga* (Bonnaterre, 1788), *Thunnus thynnus* (Linnaeus, 1758)] Türkiye denizlerinde mevcuttur (Fricke ve ark., 2007). Başlarda *Scomber japonicus* (Houttuyn, 1782) Atlas, Hint ve Pasifik Okyanuslarında bulunan tek bir tür olarak kabul edilmişken (Collette ve Nauen, 1983) nükleer ve mitokondrial DNA'da görülen yüksek düzeyde genetik farklılıkların sonucu olarak (Scoles ve ark., 1998; Infante ve ark., 2007; Catanese ve ark., 2010) Hint ve Pasifik Okyanuslarındaki tür *Scomber japonicus* (Houttuyn, 1782), Atlas Okyanusundaki tür ise *Scomber colias* (Gmelin, 1789) olarak tanımlanmıştır (Muniz ve ark., 2018). Bundan dolayı, Atlas Okyanusu ve Akdeniz Havzasında yapılan önceki çalışmalarda söz konusu tür *Scomber japonicus* olarak ifade edilmiştir. Ticari öneme sahip olduklarından dolayı palamut (*Sarda sarda* Bloch, 1793) [Zaboukas ve Megalofonou, 2007; Valeiras ve ark., 2008; Cengiz, 2013; Čikeš Keč ve ark., 2019; Petukhova, 2020], uskumru (*Scomber scombrus* Linnaeus, 1758) [Morse, 1980; Griswold ve ark., 1992; Villamor ve ark., 2004; Costa ve ark., 2017; Attia ve Kariman, 2020] ve kolyoz (*Scomber colias* Gmelin, 1789) [Kiparissis ve ark., 2000; Carvallo ve ark., 2002; Čikeš Keč ve Zorica, 2012; Amponsah ve ark., 2016; Cengiz, 2021b] balıklarıyla ilgili dünya çapında çok sayıda çalışma yürütülmüştür.

Bu çalışmanın amaçları (a) palamut (*Sarda sarda* Bloch, 1793), uskumru (*Scomber scombrus* Linnaeus, 1758) ve kolyoz (*Scomber colias* Gmelin, 1789) balıklarının Karadeniz'i de kapsayacak şekilde tüm Akdeniz Havzası için BÇİ ile ilgili ilk verilerini ortaya çıkarmak, (b) böylelikle bu balık türlerinin BÇİ ve BAİ bilgilerini güncellemek ve (c) önceki çalışmalarla bu sonuçları kıyaslamaktır. Böylelikle mevcut çalışma, söz konusu türlerin sürdürülebilirliği için ilgili paydaşlara erken yönetim stratejilerini belirlemede yardımcı olabilir.

MATERYAL ve METOD

Türkiye'nin Kuzey Ege kıyıları Saros Körfezi, Gelibolu Yarımadası, Gökçeada ve Bozcaada Adaları ve Edremit Körfezi olarak alt bölgelere ayrılmaktadır (Cengiz, 2021c). Kuzey Ege Denizi geniş bir kıta sahanlığı, çamurlu/kumlu bir dip yapısı ve yüksek oranda nütrient konsantrasyonu ile tanınmaktadır (Maravelias ve Papaconstantinou, 2006) ve Güney Ege Denizi ile kıyaslandığında üst seviyede fitoplankton ve zooplankton miktarına sahiptir

(Theocharis ve ark., 1999). Bundan dolayı, Gelibolu Yarımadası tür kompozisyonu açısından çeşitlilik gösterdiğinden önemli bir balıkçılık alanı olarak da kabul edilmektedir (Cengiz ve ark., 2012) (Şekil 1).



Şekil 1. Türkiye'nin Kuzey Ege kıyıları ve Gelibolu Yarımadası
Figure1. The Northern Aegean coasts of Turkey and Gallipoli Peninsula

Balık örnekleri, avcılık faaliyetlerinin yasak olmadığı dönemler içinde, her ay Ocak 2017-Aralık 2017 tarihleri arasında, rastgele olacak şekilde ve ölü olarak Gelibolu Yarımadası açıklarında galsama ağı kullanan ticari balıkçılardan elde edilmiştir. Balıkların boy ölçümleri için ±1 mm hassasiyetli boy ölçüm tahtası, vücut ağırlıklarının tartımı için ±0,01 g hassasiyete sahip terazi kullanılmıştır. Türlerin boy-ağırlık ilişkisinin belirlenmesinde Le Cren (1951) tarafından önerilen $W=aL^b$ eşitliğinden faydalanılmıştır. Söz konusu eşitlikte W, türlerin toplam ağırlığını (g) ifade ederken; L, toplam boyu (cm) belirtmektedir. a ve b ise büyümeyi gösteren sabitler olup b değeri 3'ten büyük olursa türler pozitif allometrik büyüme sergilerken 3'den küçük olması durumunda negatif allometrik büyümeyi, eğer 3'e eşit ise izometrik büyümeyi işaret etmektedir (Bagenel ve Tesch, 1978). a ve b parametrelerinin sonuçlarına t-testi uygulandığında ise b değerinin izometrik büyümeden (b=3) farklı olup olmadığı ve farklı ise büyümenin pozitif veya negatif olduğu sonucuna varılmıştır (Avşar, 2005).

Balıkların operkulum ve maksimum vücut çevreleri de ±1 mm hassasiyetli bir mezura yardımıyla ölçülmüştür. Operkulum çevre uzunluğu balıkların tam operkulumun bitiminden, maksimum vücut çevre uzunluğu ise ilk sırt yüzgecinin önünden alınmıştır. Türlerin boy-çevre ilişkisinin hesaplanmasında Netter ve ark. (1988) tarafından formüle edilen $Y=a+bL$ denkleminden yararlanılmıştır. Bu denklemde, toplam boy (L) ve operculum çevre ve/veya maksimum vücut çevresi (Y) arasındaki ilişki, lineer regrasyon analizi ile tahmin edilmiştir. Bu denklemde, a (kesişen) ve b (eğim) sabitleri en küçük kareler tahmini ile bulunmuştur. Korelasyon katsayısı (r^2), bu ilişkilerin gücünü değerlendirmek için kullanılmıştır.

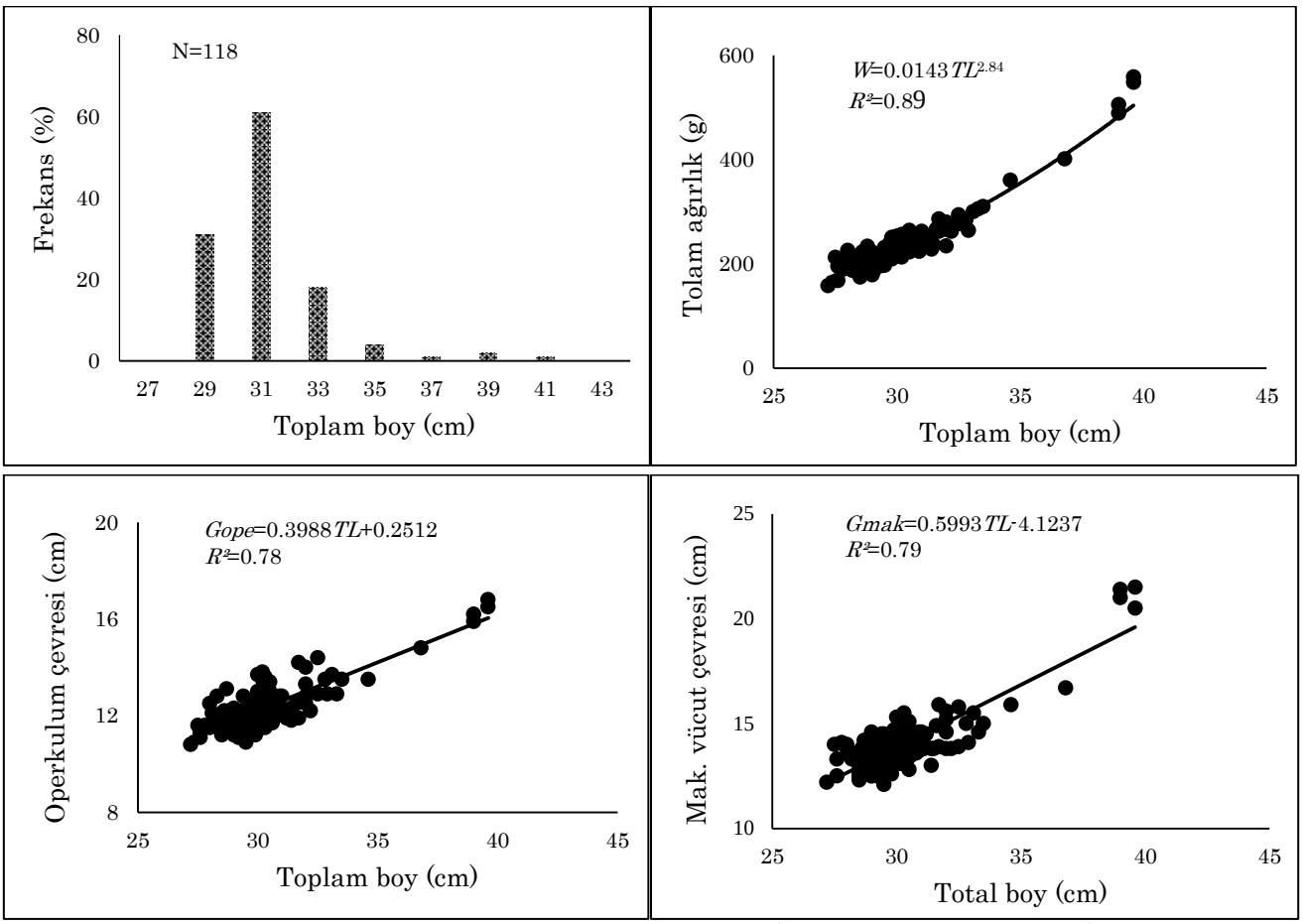
BULGULAR ve TARTIŞMA

Çalışmanın sonunda üç türe ait 368 örnek, galsama ağlarıyla avlanan ticari balıkçılar yardımıyla araştırma için kullanılmıştır.

118 tane palamut balığının ortalama boy ve ağırlık ölçümleri, sırasıyla, 30.4±0.21 (27.2-39.6) cm ve 241.15±5.85 (157.89-558.00) g olarak bulunmuştur. Boy-ağırlık ilişkisi $W=0.0143\,TL^{2.84}$ (r^2=0.89) olarak tahmin edilmiştir. B değeri ve t-testi sonuçları palamut balığının negatif allometrik büyüme gösterdiğini ortaya koymuştur. Bununla beraber, ortalama operkulum ve maksimum vücut çevreleri, sırasıyla, 12.4±0.10 (10.8-16.8) cm ve 14.1±0.15 (12.1-21.5) cm olarak hesaplanmıştır. Boy-opekulum çevre ilişkisi $G_{ope}=0.3988\,TL+0.2512$ (r^2=0.78) bulunurken boy-maksimum vücut çevresi $G_{mak}=0.5993\,TL-4.1237$ (r^2=0.79) olarak saptanmıştır (Şekil 2).

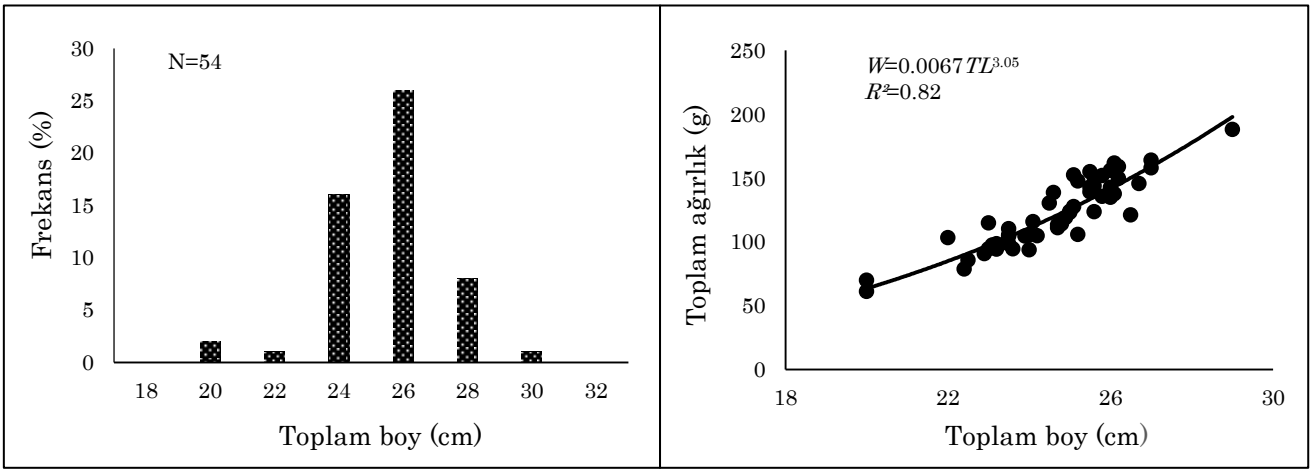
54 adet uskumru balığının ortalama boy ve ağırlık ölçümleri, sırasıyla, 24.6±0.22 (20.0-29.6) cm and 122.38±3.64 (61.00-118.00) g olarak saptanmıştır. Boy-ağırlık ilişkisi $W=0.0067\,TL^{3.05}$ (r^2=0.82) olarak hesaplanmıştır. B değeri ve t-testi sonuçları uskumru balığının izometrik büyüme gösterdiğini ortaya koymuştur. Ayrıca, ortalama operkulum ve maksimum vücut çevreleri, sırasıyla, 9.5±0.10 (8.1-11.2) cm ve 10.6±0.11 (8.8-12.2) cm olarak

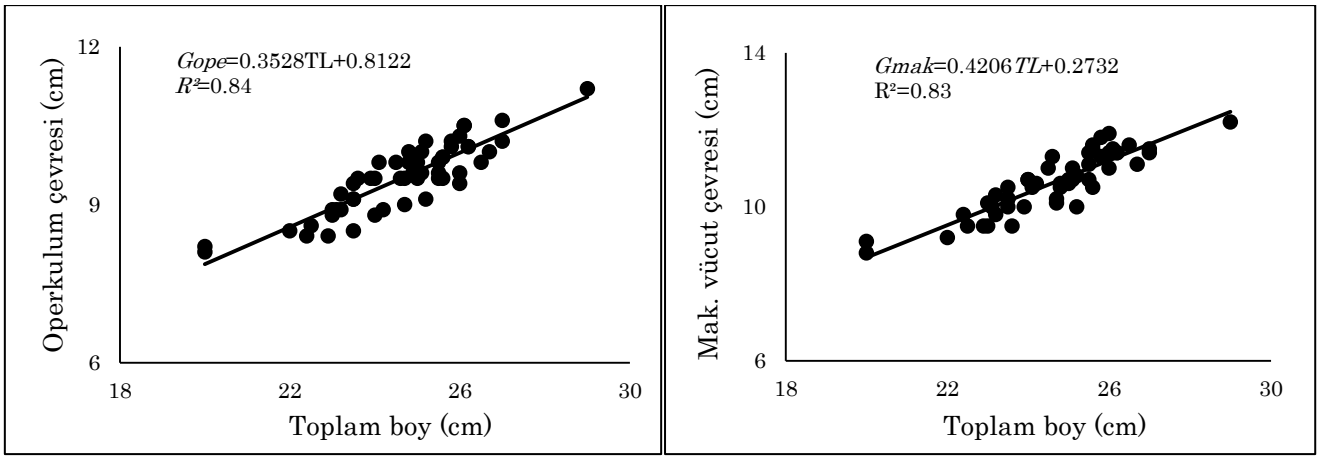
bulunmuştur. Boy-opekulum çevre ilişkisi G_{ope}=0.3528TL+0.8122 (r^2=0.84) saptanırken boy-maksimum vücut çevresi G_{mak}=0.4206TL+0.2732 (r^2=0.83) olarak hesaplanmıştır (Şekil 3).



Şekil 2. Gelibolu Yarımadas'ından yakalanan palamut balığının (*Sarda sarda* Bloch, 1793) operkulum çevresi, maksimum vücut çevresi, toplam ağırlık ve toplam boy arasındaki ilişkiler ve boy-frekans dağılımı

Figure 2. The length-frequency distribution and the relationships between opercular girth, maximum girth, total weight and total length of atlantic bonito (Sarda sarda Bloch, 1793) from the Gallipoli Peninsula



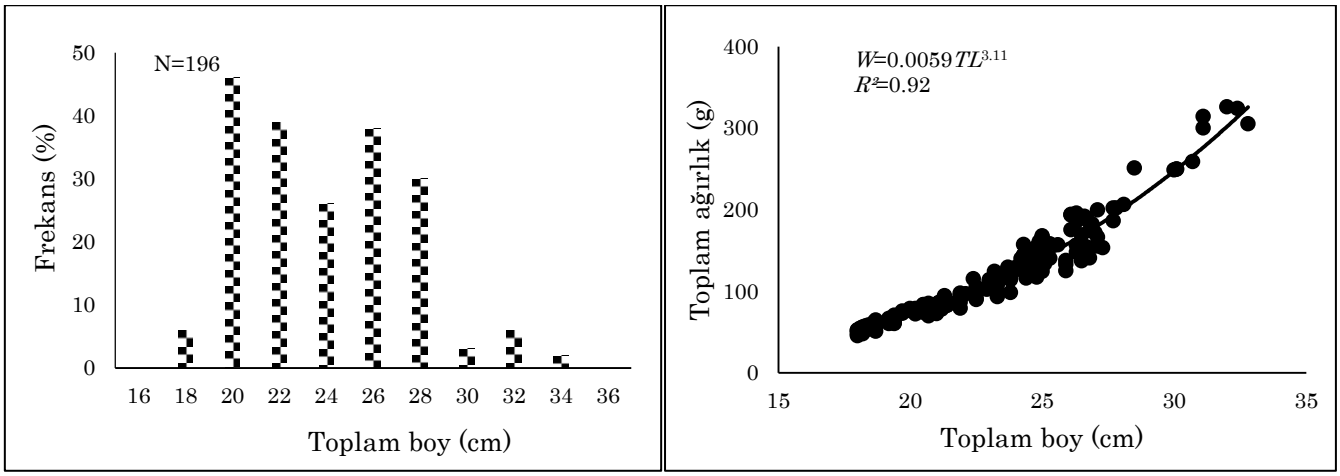


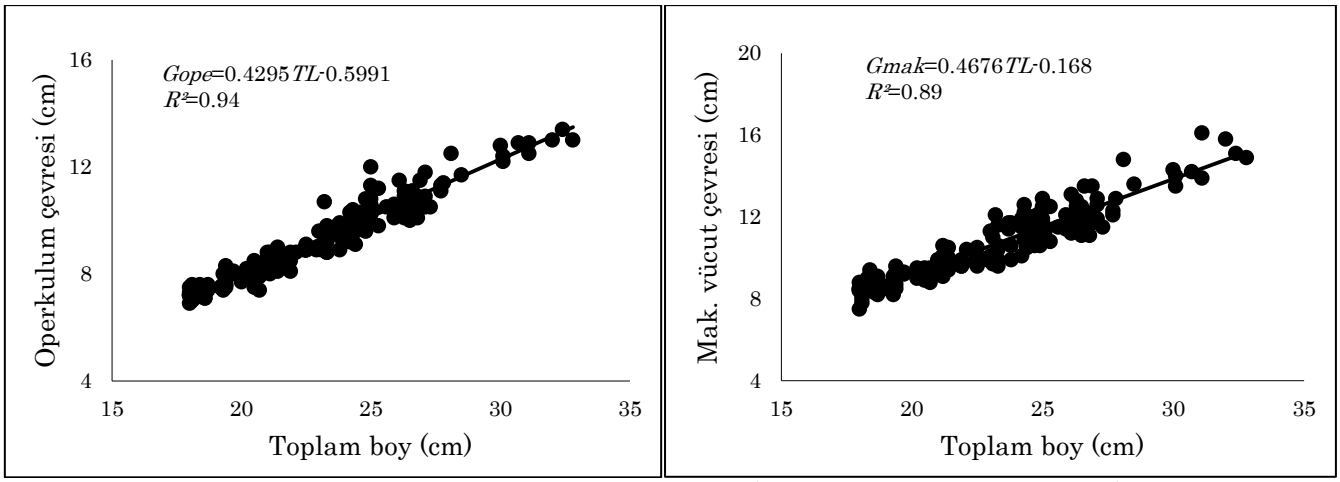
Şekil 3. Gelibolu Yarımadas'ından yakalanan uskumru balığının (*Scomber scombrus* Linnaeus, 1758) operkulum çevresi, maksimum vücut çevresi, toplam ağırlık ve toplam boy arasındaki ilişkiler ve boy-frekans dağılımı

Figure 3. The length-frequency distribution and the relationships between opercular girth, maximum girth, total weight and total length of atlantic mackerel (Scomber scombrus Linnaeus, 1758) from the Gallipoli Peninsula

196 tane kolyoz balığının ortalama boy ve ağırlık ölçümleri, sırasıyla, 22.8±0.25 (18.0-32.8) cm ve 114.75±4.15 (45.50-325.72) g olarak bulunmuştur. Boy-ağırlık ilişkisi $W=0.0059TL^{3.11}$ ($r^2=0.92$) olarak tahmin edilmiştir. *B* değeri ve *t*-testi sonuçları kolyoz balığının pozitif allometrik büyüme gösterdiğini ortaya koymuştur. İlaveten, ortalama operkulum ve maksimum vücut çevreleri, sırasıyla, 9.2±0.12 (6.9-13.4) cm and 10.5±0.14 (7.5-16.1) olarak bulunmuştur. Boy-opekulum çevre ilişkisi $G_{ope}=0.4295TL-0.5991$ ($r^2=0.94$) hesaplanırken boy-maksimum vücut çevresi $G_{mak}=0.4676TL-0.168$ ($r^2=0.89$) olarak bulunmuştur (Şekil 4).

Çizelge 1 palamut (*Sarda sarda* Bloch, 1793), uskumru (*Scomber scombrus* Linnaeus, 1758) ve kolyoz (*Scomber colias* Gmelin, 1789) balıkarının boy-ağırlık ilişkisi üzerine yapılan çalışmaları derlerken Çizelge 2 ve Çizelge 3, sırasıyla, bu türlerin boy-operkulum çevresi ve boy maksimum vücut çevresi ilişkileri üzerine yapılan araştırmaları göstermektedir. Boy-ağırlık ilişkisindeki *b* değeri 2.5 ile 3.5 (Froese, 2006) veya 2 ile 4 (Tesch, 1971) arasındaki değişimi gösterir. Bu çalışmadaki balık türlerinin *b* değerleri beklenen aralıklar içindedir. Genellikle, aynı türün *b* değerlerinde görülen farklılıklar avlanan bireylerin sayısal değerine ve bu bireylerin boy ve ağırlık aralığına (Moutopoulos ve Stergiou, 2002), örnekleme için kullanılan av aracı türüne (Kapiris ve Klaoudaos 2011) ve bu av aracının seçiciliğine (İşmen ve ark., 2007), balıkların cinsiyetine, gonadsal faaliyetine, çevresel faktörlerde görülen yıllık varyasyonlara, mevsimsel döngülere ve türlerin korunmasına yönelik alınan önlemlere gibi bağlıdır (Wootton, 1998; Cengiz ve ark., 2019).





Şekil 4. Gelibolu Yarımadas'ından yakalanan kolyoz balığının (*Scomber colias* Gmelin, 1789) operkulum çevresi, maksimum vücut çevresi, toplam ağırlık ve toplam boy arasındaki ilişkiler ve boy-frekans dağılımı

Figure 4. The length-frequency distribution and the relationships between opercular girth, maximum girth, total weight and total length of atlantic chub mackerel (Scomber colias Gmelin, 1789) from the Gallipoli Peninsula

Bu çalışmada, toplam boy ile operkulum çevresi ve maksimum vücut çevresi arasımda linnear bir ilişki vardır ve bu da Stergiou ve Karpouzi (2003), Mendes ve ark. (2006), Santos ve ark. (2006) ve Daliri ve ark. (2012) gibi diğer çalışmaların sonuçları ile uyumludur. Bununla beraber, balıkların boy-çevre ilişkisi ise boy aralığındaki değişkenliğe (Cengiz, 2021a), balıkların cinsiyetine, beslenme faaliyetlerine ve sıcaklıkta görülen varyasyonlara bağlanabilmektedir (Wootton, 1998). Buna paralel olarak, üreme sıklığı ve gonad gelişimi gibi faaliyetler boy-çevre ilişkisine etki eden diğer etmenlerdir (Santos ve ark., 2006; Kyritsi ve ark., 2018). Bu çalışma ile diğerleri arasında BAİ ve BÇİ'nde görülen olası farklılıkların sebepleri yukarıdaki bir veya birden fazla faktörden kaynaklanmış olabilir.

SONUÇ ve ÖNERİLER

Bu çalışma Karadeniz'i de kapsayacak şekilde tüm Akdeniz Havzası için palamut, uskumru ve kolyoz balıkları'nın boy-operkulum çevre ve boy-maksimum vücut çevre ilişkileri ile ilgili ilk verileri sunmaktadır. Lagner (1978) ağ seçiciliğini, herhangi bir populasyondan, belirli bir boydaki bireylerin etkin olarak avlanırken bu boydan uzaklaşan bireylerin yakalanma olasılıklarının nispi olarak azalması şeklinde tanımlamıştır. Ağ göz genişliği, ağın elastikiyeti, donam faktörü, ağ ipi bükümünün sıkılığı, kalınlığı ve esnekliği, ipin görünürlüğü, ağın kullanılma yöntemi, ve balığın vücut şekli ve davranışı bir av aracında seçiciliği etkileyen önemli faktörlerdir (Hamley, 1975; Cengiz ve ark., 2014). Bu süreçte, balıklar ağlarla karşılaştıklarında (a) operkulumlardan (b) ağ gözlerine saplanarak ve (c) dolanmak suretiyle üç farklı şekilde yakalanmaktadırlar (Baranov, 1914). Bu noktadan hareketle, balıkların operculum ve maksimum vücut çevre ölçümleri galsama ağlarının seçiciliklerinin belirlenmesinde önemli etkenlerden biri olarak kabul edilmiş (McCombie ve Berst, 1969) ve devamında Sechin (1969) ve Kawamura (1972), operkulum ve maksimum vücut çevre ölçümleri ile ilgili verileri kullanarak galsama ağlarında seçicilik değerlerini ve en uygun ağ göz açıklığını belirleyen seçicilik modelleri geliştirmişlerdir. Sonraki süreçlerde ise boy-çevre ilişkisi seçicilik çalışmalarında kullanılabilecek temel parametrelerden biri haline dönüşmüştür (Tokai ve Omoto, 1994).

Bu çalışmadan elde edilen sonuçların, özellikle, Türkiye'de ekonomik açısından son derece önemli olan bu türlerin sürdürülebilirliği için uygun ağ göz açıklığına sahip galsama ağlarının tasarımında, Sechin (1969) ve Kawamura (1972) seçicilik modelleri göz önüne alınmak suretiyle sonradan yürütülecek çalışmalarla beraber, bir referans olarak kullanılabileceği umulmaktadır. Ayrıca, bu bilgilerin tüm dünyada bu balık stoklarının sürdürülebilir kullanımına katkıda bulunacak tüm paydaşlara aktarılması son derece önemlidir.

TEŞEKKÜR

Yazarlar yardımlarından dolayı ticari balıkçılara teşekkürü borç bilir

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.

Çizelge 1. Bu çalışma ile diğerleri arasında palamut (*Sarda sarda* Bloch, 1793), uskumru (*Scomber scombrus* Linnaeus, 1758) ve kolyoz (*Scomber colias* Gmelin, 1789) balıklarının boy-ağırlık ilişkilerinin karşılaştırılması

Table 1. Comparison of length-weight relationships of atlantic bonito (Sarda sarda Bloch, 1793), atlantic mackerel (Scomber scombrus Linnaeus, 1758) and atlantic chub mackerel (Scomber colias Gmelin, 1789) between the present study and other studies

Balık Türleri	Araştırmacı(lar)	Bölge	N	Boy Aralığı	Boy-Ağırlık İlişkisi
Sarda sarda	Diouf (1980)	Senegal	372	19.0-64.0	$W=0.0094FL^{3.10}$
	Franičević ve ark. (2005)	Adriatik Sea (Hırvatistan)	665	33.0-67.0	$W=0.0085FL^{3.12}$
	Macías ve ark. (2005)	Akdeniz'in batısı (İspanya)	183	41.0-48.0	$W=0.0046FL^{2.67}$
	Ateş ve ark. (2008)	Karadeniz ve Marmara Denizi (Türkiye)	694	23.5-71.0	$W=0.0054TL^{3.21}$
	Cengiz (2013)	Gallipoli Yarımadası (Türkiye)	238	23.8-72.0	$W=0.0028TL^{3.32}$
	Bu çalışma	Gelibolu Yarımadası (Türkiye)	118	27.2-39.6	$W=0.0143TL^{2.84}$
Scomber scombrus	Sinovčić ve ark. (2004)	Adriatik Denizi (Hırvatistan)	630	17.3-41.4	$W=0.0141TL^{2.88}$
	İşmen ve ark. (2007)	Saros Körfezi (Türkiye)	100	13.6-24.0	$W=0.0028TL^{3.29}$
	Crec'hriou ve ark. (2012)	Katalan kıyıları (Fransa)	124	19.0-46.0	$W=0.0690TL^{3.04}$
	Bolognini ve ark. (2013)	Adriatik Denizi (İtalya)	835	10.0-38.5	$W=0.0020TL^{3.39}$
	Bal ve Türker (2016)	Marmara Denizi (Türkiye)	163	12.0-27.0	$W=0.0042TL^{3.27}$
	Attia ve ark. (2020)	Sina Yarımadası (Mısır)	1106	12.3-30.9	$W=0.0094TL^{3.02}$
	Bu çalışma	Gelibolu Yarımadası (Türkiye)	54	20.0-29.6	$W=0.0067TL^{3.05}$
Scomber colias	Carvalho ve ark. (2002)	Azores (Portekiz)	349	9.0-53.0	$W=0.0049TL^{3.26}$
	Moutopoulos ve Stergiou (2002)	Kiklad Adaları (Yunanistan)	46	22.9-33.0	$W=0.0008TL^{3.70}$
	Sinovčić ve ark. (2004)	Adriatik Denizi (Hırvatistan)	1607	19.6-38.8	$W=0.0066TL^{3.14}$
	Cengiz (2012)	Saros Körfezi (Türkiye)	402	13.8-31.1	$W=0.0066TL^{3.10}$
	Daley ve Leaf (2019)	Atlas Okyanusu'nun kuzeybatısı (ABD)	1136	22.4-38.6	$W=0.0258TL^{2.72}$
	Bu çalışma	Gelibolu Yarımadası (Türkiye)	196	18.0-32.8	$W=0.0059TL^{3.11}$

N: Örnek sayısı, W: Toplam Ağırlık, FL: Çatal Boy; TL: Toplam Boy

Çizelge 2. Bu çalışma ile diğerleri arasında palamut (*Sarda sarda* Bloch, 1793), uskumru (*Scomber scombrus* Linnaeus, 1758) ve kolyoz (*Scomber colias* Gmelin, 1789) balıklarının boy-operkulum çevre ilişkilerinin karşılaştırılması

Table 2. Comparison of length- opercula girth relationships of atlantic bonito (Sarda sarda Bloch, 1793), atlantic mackerel (Scomber scombrus Linnaeus, 1758) and atlantic chub mackerel (Scomber colias Gmelin, 1789) between the present study and other studies

Balık Türleri	Araştırmacı(lar)	Bölge	N	Boy Aralığı	Boy-Operkulum Çevre İlişkisi	r^2	*b'*nin SH'si
Sarda sarda	Santos ve ark. (2006)	Algarve (Güney Portekiz)	66	40.4-63.4	G_{ope}=0.1436+0.4574*FL*	0.97	0.0139
	Bu çalışma*	Gelibolu Yarımadası (Kuzey Ege Denizi, Türkiye)	118	27.2-39.6	G_{ope}=0.3988*TL*+0.2512	0.78	0.1069
Scomber scombrus	Mendes ve ark. (2006)	Póvoa do Varzim - Santo Andre (Batı Portekiz)	104	21.6-43.0	G_{ope}=0.438*TL*-1.6690	0.91	0.0130
	Santos ve ark. (2006)	Algarve (Güney Portekiz)	213	25.5-40.4	G_{ope}=2.3897+0.2778*FL*	0.81	0.0093
	Bu çalışma*	Gelibolu Yarımadası (Kuzey Ege Denizi, Türkiye)	54	20.0-29.6	G_{ope}=0.3528*TL*+0.8122	0.84	0.1495
Scomber colias	Mendes ve ark. (2006)	Póvoa do Varzim - Santo Andre (Batı Portekiz)	166	19.5-46.4	G_{ope}=0.423*TL*-0.6120	0.97	0.0060
	Santos ve ark. (2006)	Algarve (Güney Portekiz)	699	17.0-44.4	G_{ope}=1.3952+0.3229*FL*	0.91	0.0038
	Bu çalışma*	Gelibolu Yarımadası (Kuzey Ege Denizi, Türkiye)	196	18.0-32.8	G_{ope}=0.4295*TL*-0.5991	0.94	0.0392

N: Örnek sayısı, G_{ope}: Operkulum Çevresi, FL: Çatal Boy; TL: Toplam Boy, r^2: Korelasyon Katsayısı SH: Standart Hata
*Karadeniz'i de kapsayacak şekilde tüm Akdeniz Havzası için türlerin boy-operkulum çevre ilişkisi ile ilgili ilk veri

Çizelge 3. Bu çalışma ile diğerleri arasında palamut (Sarda sarda Bloch, 1793), uskumru (Scomber scombrus Linnaeus, 1758) ve kolyoz (Scomber colias Gmelin, 1789) balıklarının boy-maksimum vücut çevre ilişkilerinin karşılaştırılması

Table 3. Comparison of length- maximum girth relationships of atlantic bonito (Sarda sarda Bloch, 1793), atlantic mackerel (Scomber scombrus Linnaeus, 1758) and atlantic chub mackerel (Scomber colias Gmelin, 1789) between the present study and other studies

Balık Türleri	Araştırmacı(lar)	Bölge	N	Boy Aralığı	Boy-Mak Vücut Çevre İlişkisi	r^2	*b'*nin SH'si
Sarda sarda	Santos ve ark. (2006)	Algarve (Güney Portekiz)	66	40.4-63.4	G_{mak}=-3.9713+0.6513*FL*	0.97	0.0193
	Bu çalışma*	Gelibolu Yarımadası (Kuzey Ege Denizi, Türkiye)	118	27.2-39.6	G_{mak}=0.5993*TL*-4.1237	0.79	0.0691
Scomber scombrus	Mendes ve ark. (2006)	Póvoa do Varzim - Santo Andre (Batı Portekiz)	63	20.4-46.5	G_{mak}=0.432*TL*-0.2740	0.93	0.0150
	Santos ve ark. (2006)	Algarve (Güney Portekiz)	212	25.5-40.4	G_{mak}=-7.9541+0.6886*FL*	0.95	0.0112
	Bu çalışma*	Gelibolu Yarımadası (Kuzey Ege Denizi, Türkiye)	54	20.0-29.6	G_{mak}=0.4206*TL*+0.2732	0.83	0.1330
Scomber colias	Mendes ve ark. (2006)	Póvoa do Varzim - Santo Andre (Batı Portekiz)	166	19.5-46.4	G_{mak}=0.0443*TL*-0.562	0.96	0.0060
	Santos ve ark. (2006)	Algarve (Güney Portekiz)	699	17.0-44.4	G_{mak}=-2.9511+0.5594*FL*	0.95	0.0049
	This study*	Gelibolu Yarımadası (Kuzey Ege Denizi, Türkiye)	196	18.0-32.8	G_{mak}=0.4676*TL*-0.1680	0.89	0.0475

N: Örnek sayısı, G_{mak}: Maksimum Vücut Çevresi, FL: Çatal Boy; TL: Toplam Boy, r^2: Korelasyon Katsayısı SH: Standart Hata
*Karadeniz'i de kapsayacak şekilde tüm Akdeniz Havzası için türlerin boy-mak. vücut çevre ilişkisi ile ilgili ilk veri

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Demirköprü Baraj Gölü'nde İlk *Viviparus contectus* (*Millet, 1813*) Kaydı ve Bazı Biyometrik Parametrelerinin Değerlendirilmesi

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ÖZET

Manisa İli sınırları içerisinde yer alan Demirköprü Baraj Gölü'nde yaşayan *Viviparus contectus* (Millet, 1813) türüne ait bazı biyometrik özelliklerinin araştırılması amaçlanmıştır. Çalışma alanında toplanan, toplam 180 bireye ait genişlik (mm), yükseklik (mm), apertur yüksekliği, vücut helezon yüksekliği ve genişliği (mm), spir yüksekliği (mm) verileri dijital kumpas ile; bireylerin ağırlıkları (g) hassas terazi kullanılarak ölçülmüştür. Bireylere ait veriler sırasıyla; ortalama kabuk yüksekliği 20.23±5.16 mm, kabuk genişliği 16.11±3.33 mm, apertur yüksekliği 11.93±2.39 mm, apertur genişliği 9.71±2.11 mm, vücut helezon yüksekliği 15.71±3.87 mm, spir yüksekliği 4.49±1.73 mm ve ağırlıkları 4.50±0.93 g olarak belirlenmiştir. Temel bileşenler analizine göre, Kabuk yüksekliği (KY), Kabuk genişliği (KG), Apertur yüksekliği (AY) ve Vücut helezon yüksekliği (VHY) arasında güçlü bir ilişki olduğu belirlenmiştir. Demirköprü Baraj Gölü'nde yaşayan *V. contectus* türü bu çalışma ile bölgeden ilk defa rapor edilmiştir.

Su Ürünleri

Araştırma Makalesi

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Geliş Tarihi : 27.08.2021
Kabul Tarihi : 31.01.2022

Anahtar Kelimeler
Viviparus contectus
Demirköprü Baraj Gölü
Biyometrik

The First Record of *Viviparus contectus* (Millet, 1813) in Demirköprü Dam Lake and Evaluation of Some Biometric Parameters

ABSTRACT

The aim of this study is to investigate some biometric characteristics of *Viviparus contectus* (Millet, 1813) in Demirköprü Dam Lake, Manisa province. Measurements of 180 individuals collected in the study area were performed using calipers and precision balances, and weight (g), width (mm), height (mm), aperture length and width (mm), spiral height (mm), body whorl height data were obtained. It was determined as 20.23±5.16 mm for shell height, 16.11±3.33 mm for shell width, 11.93±2.39 mm for aperture height, 9.71±2.11 mm for aperture width, 15.71±3.87 mm for body spiral height, 4.49±1.73 mm for spiral height and 4.50±0.93 g for weight. According to principal components analysis, there is a strong relationship between Shell height (KH), Shell width (KW), Aperture height (AH) and Body helezone height (VHS). *V. contectus* species has been reported for the first time from the region with this study.

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GİRİŞ

Manisa ili Salihli ilçesinde bulunan Demirköprü Baraj Gölü, Gediz Nehri üzerinde sulama, enerji üretimi ve taşkın kontrolü amacıyla 1954 - 1960 yılları arasında inşa edilmiştir. Geniş bir alana (6950 km^2) sahip olan barajın göl hacmi 1022 hm^3'tür (Tenekecioğlu, 2011). Baraj gölü su kaynağını, küçük dereler, Demirci Çayı ile büyük oranda Gediz Nehri oluşturmaktadır (Dereli ve ark., 2018).

Türkiye topografik, hidrografik ve buna bağlı olarak klimatolojik gelişmelerin sonucu zengin bir flora ve fauna yapısına sahiptir (Altun ve ark., 2016; Hacisalihoğlu ve ark., 2017). Bu zengin yapının fauna unsurlarından biri de Mollusca şubesinin önemli bir

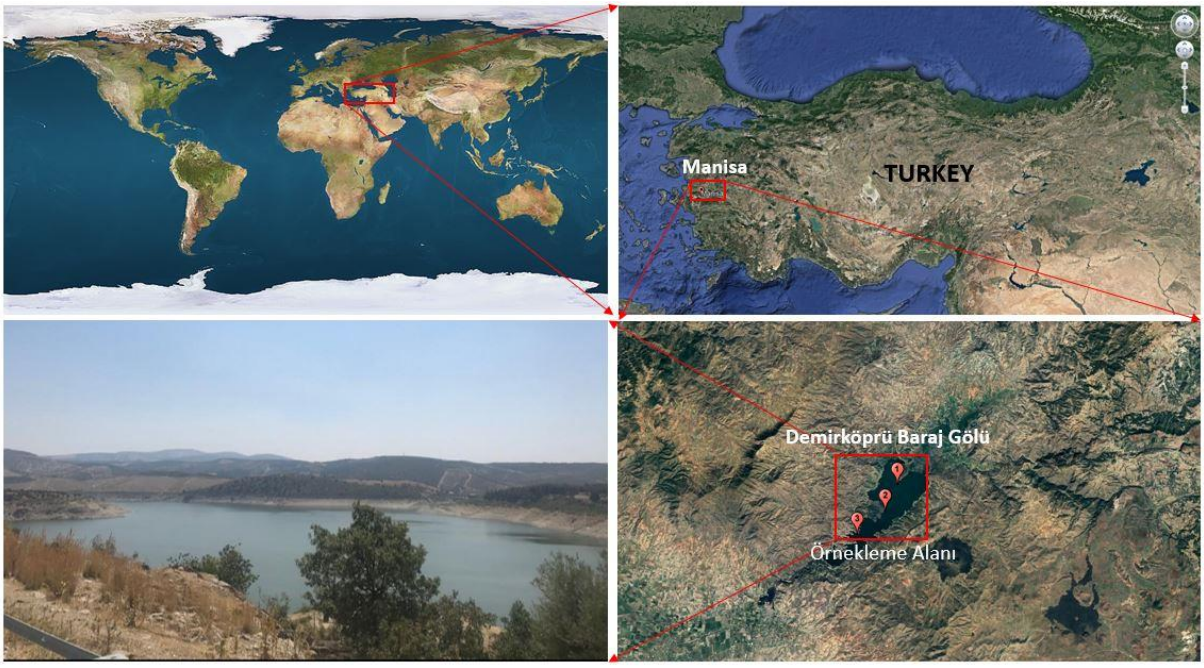
kısmını oluşturan Gastropoda sınıfında yer alan salyangozlardır. Türkiye'de bugüne kadar Gastropoda sınıfının üç alt sınıfından iki tanesi olan; 124 tür Prosobranch alt sınıfı ve 40 tür Pulmonat alt sınıfından tespit edilmiştir (Yıldırım ve ark., 2006a, 2006b; Yıldırım ve Kebapçı, 2009; Gürlek ve ark., 2019). Prosobranch'ın %63'ü endemik türlerden oluşmaktadır (Gürlek ve ark., 2019).

Viviparus cinsinin türleri genel olarak nehirler, akarsular, göletler, göller ve bataklıklar dahil olmak üzere çeşitli habitatlarda yaşar ve Kuzey'in bazı bölgelerinde, Amerika, Avustralya, Asya, Afrika ve Avrupa'da dağılım gösterirler. *Viviparus* cinsinin üyeleri genellikle detritusla beslenir, aynı zamanda suyu filtre ederler. Böylelikle su ve sedimenti temizlerler. Solungaç solunumu yaparlar ve 0 ile 20 m derinlikler arasında dağılım gösterirler. Üreme, ilkbaharda, kıyı bölgesinde dağılım gösteren bireyler ile gerçekleşir. Sonbaharda daha derin sulara ve üreme zamanı ilkbaharda daha sığ kıyı bölgelerine hareket ederler (Bugler ve ark., 2009). *Viviparus contectus* kabuk koyu yeşil renkte, yarısaydamdır ve üzerinde kahverengi spiral bantlar bulunur. Dişiler erkeklerden daha büyüktür.

Demirköprü Baraj Gölü'nde yaşayan *V. contectus* populasyonu ile ilgili ilk bilimsel çalışma özelliği taşıyan bu araştırma ile türe ait bazı biyometrik özellikleri çalışılmıştır.

MATERYAL ve METOD

Çalışmada kullanılan *V. contectus* Demirköprü Baraj Gölü'nde 3 farklı bölgeden (38.669811°-28.374477°, 38.652102°-28.365190°, 38.636297°-28.344330°, Kuzey/Doğu) (Şekil 1) Şubat-Ağustos 2021 tarihleri arasında toplanmıştır.



Şekil 1. Örnekleme alanı.
Figure1. Sampling area

Örnekler, 1 m su derinliğine kadar olan kum ve milli bölgelerden elle, metal çerçeveli kepçeler ve kürekler ile dip taranması için tırmık kullanılarak toplanmış (Şekil 2) ve strafor kutularda +4°C'de muhafaza edilmiştir (Yarsan ve ark., 2000) .

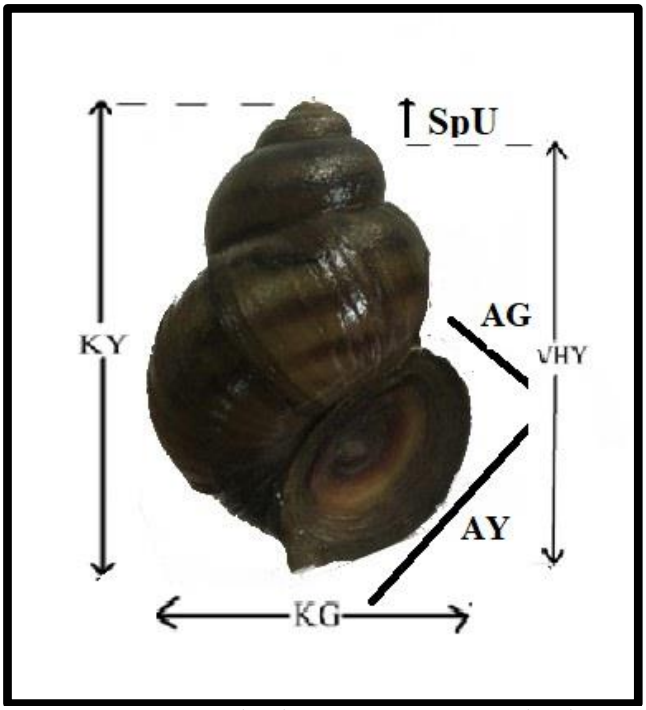
Örneklerin tür teşhisleri Glöer ve Georgıev (2014) tarafından hazırlanan teşhis anahtarlarına göre yapılmıştır. Apeksin keskin ve belirgin olması, helezonların dışbükey ve kademeli olması türün ayırt edici özellikleridir. Toplam 180 adet bireye ait biyometrik ölçüm yapılmıştır (Şekil 3).

Dijital kumpas (±0.01 mm) ile kabuk ölçümleri (kabuk yüksekliği, kabuk genişliği, apertur uzunluğu, apertur genişliği, vücut helezon yüksekliği, spir yüksekliği) (Şekil 4), hassas terazi (±0.001 g) ile ağırlık ölçümleri gerçekleştirilmiştir. Boy-ağırlık ilişkilerinin hesaplanmasında $W = a*L^b$ formülü ve bu formülün logaritmik dönüşümü yapılmış hali ($\log(W)=a+b*\log(L)$) kullanılmıştır. Biyometrik parametreler arasındaki ilişkilerin belirlenmesinde regresyon analizi ile verilerin analizi ve işlenmesinde Microsoft Excel® kullanılmıştır.

Değişkenler arasındaki ilişkilerin belirlenmesinde Statgraphics Centurion V18/19 ile bir temel bileşenler analizi (PCA) uygulanmıştır. PCA'nın kullanışlılığını doğrulamak için Kaiser-Meyer-Olkin'in örnekleme yeterliliği ölçüsü (KMO) kullanılmıştır. KMO, 0 ile 1 arasında değişir ve değişkenler birbirine çok bağımlıysa ve PCA faydalıysa 0,5'in üzerinde

Şekil 2. Arazi örneklemesi
Figure2. Field sampling.

Şekil 1. *Viviparus contectus* örnekleri
Figure1. Samples of *Viviparus contectus*



Şekil 4. Biyometrik ölçümler; Kabuk yüksekliği (KY), Kabuk genisliği (KG), Apertur yüksekliği (AY), Apertur genisliği (AG), Vücut helezon yüksekliği (VHY) ve Spir yüksekliği (SpU). (Gürlek ve ark., 2011).
Figure 4. Biometric measurements; Shell length (SL), Shell width (SW), Aperture length (AL), Aperture width (AW), Body helezone length (BHL) and Spir length (SoU) (Gürlek ve ark., 2011).

olmalıdır. Ayrıca, PCA'nın kullanışlılığını doğrulamak için Bartlett'in test ölçümü uygulanmıştır. Veriler anlamlıysa ($p < 0,001$), PCA yararlıdır ve değişkenler birbirine çok bağlıdır.

BULGULAR ve TARTIŞMA

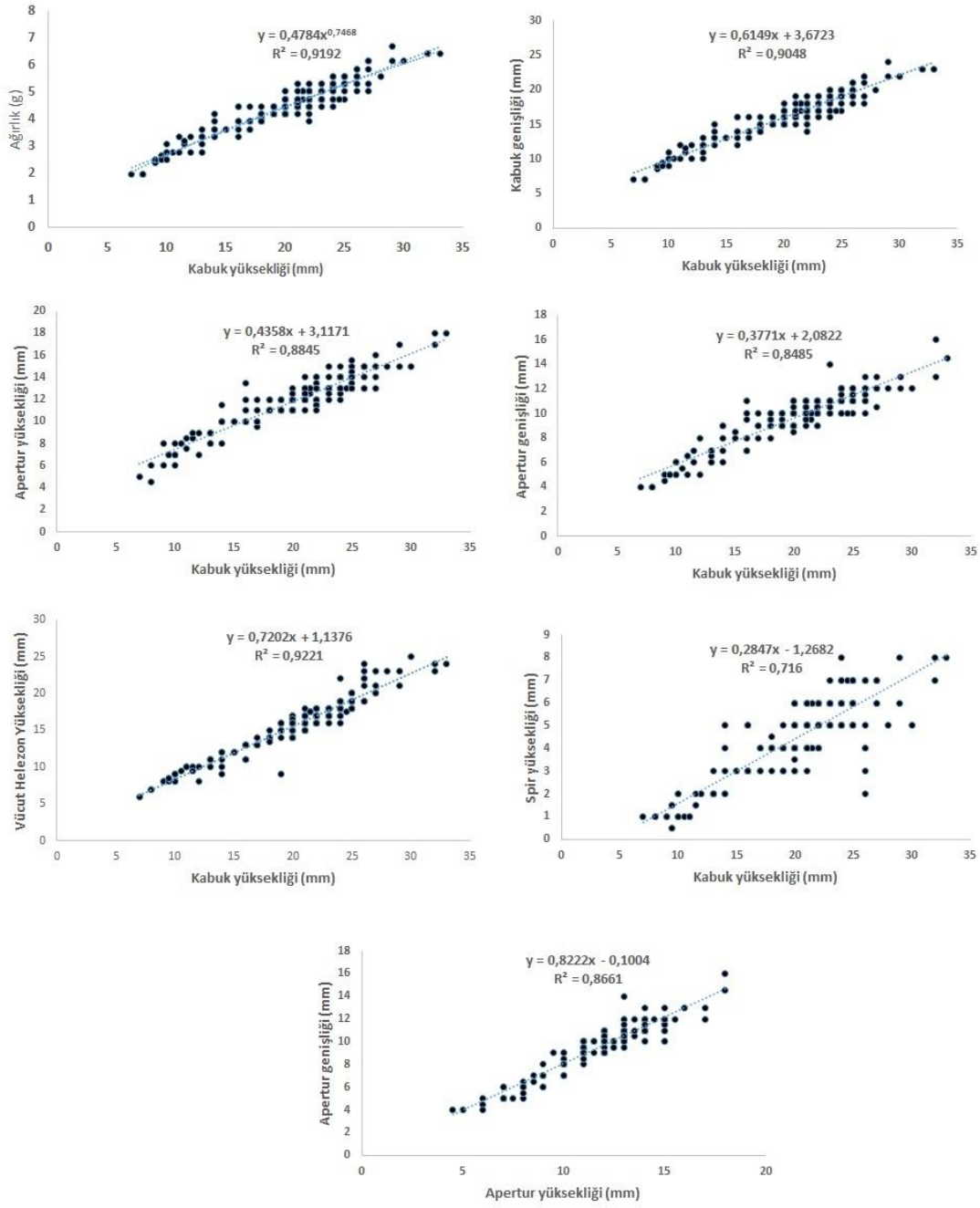
Özellikle Ağustos ayındaki saha çalışmasında baraj suyunun büyük oranda azaldığı, barajda suyun renginin koyu yeşil-kahverengiye döndüğü, baraj su toplama havzasında suyun azaldığı ve kenarlarda çok geniş kuru alanlar oluştuğu gözlenmiştir.

Türkiye, uygun evrimleşme ve yayılma merkezlerinden biri olduğu için Prosobranchia türleri açısından zengin bir faunaya sahiptir ve günümüze kadar yapılan çalışmalar, önemli sucul habitatlarda, ulaşımın elverdiği belirli alanlarda yapılmıştır (Yıldırım, 1999). Bu sebeplerden dolayı, zengin faunaya ait farklı türlerin yayılış alanları ve biyometrik özellikleri yeterince açığa çıkarılamamıştır. Bu çalışmada, *V. contectus* türü bölgeden ilk defa rapor edilmiştir.

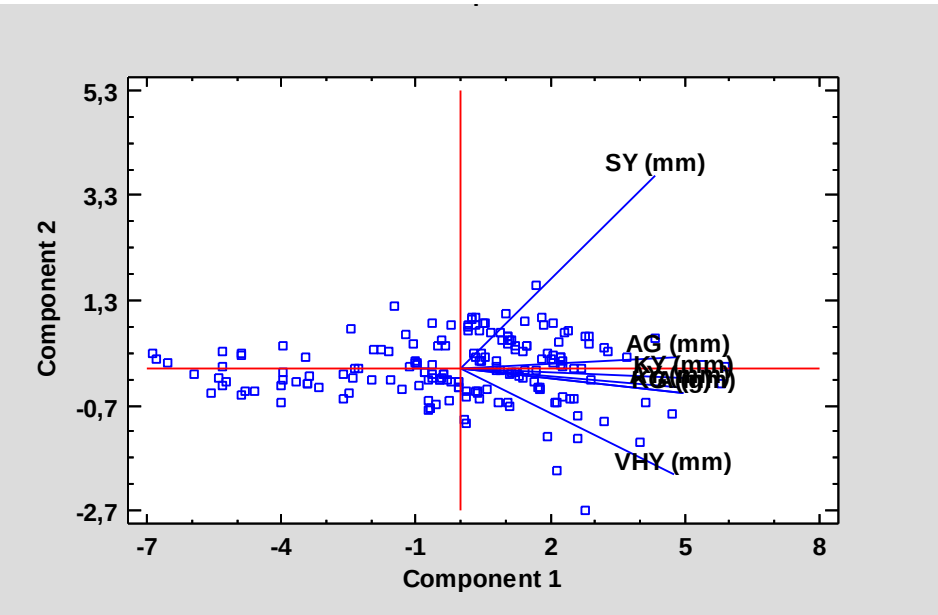
Gastropod kabuk morfolojisi ve morfometrik ilişkileri üzerine yapılan çalışmalar, kabuğun ayrıntılı tanımı, tür taksonomik tanımlaması, popülasyonlar arasındaki ayrım ve seksüel dimorfizm analizi dahil olmak üzere çeşitli amaçlarla yürütülmektedir. Morfometrik ilişkilerin kurulması, balıkçılık biyolojisi ve ekolojisi, popülasyon dinamikleri, balıkçılık değerlendirmesi ve yönetiminde kullanılan matematiksel modellerde uygulama için oldukça yararlı olan farklı morfometrik değişkenlerle ilgili dönüşüm denklemlerinin elde edilmesini sağlar (Vasconcelos ve ark., 2016). Özellikle, ağırlık-uzunluk ilişkilerinin çeşitli kullanımları vardır, yani bireysel uzunluktan ve uzunluk sınıflarından ağırlığın tahmini, yaşa göre ağırlık tahmini için boydaki büyüme denklemlerinin ağırlıktaki büyümeye dönüştürülmesi ve sonraki stok değerlendirme modellerinde kullanım, popülasyon üretimi ve biyokütlenin ve durum indekslerinin hesaplanması ve farklı habitat ve bölgelerden türler veya popülasyonlar arasında yaşam öyküsü ve morfolojik karşılaştırmalar yapılmaktadır (Ricker, 1973; Anderson ve Gutreuter, 1983; Beyer., 1991; Pauly, 1993; Richter ve ark., 2000). Demirköprü Baraj Gölü'nden elde edilen *V. contectus* popülasyonundaki bireylere ait ortalama kabuk yüksekliği 20.23±5.16 mm, kabuk genişliği 16.11±3.33 mm, apertur yüksekliği 11.93±2.39 mm, apertur genişliği 9.71±2.11 mm, vücut helezon yüksekliği 15.71±3.87 mm, spir yüksekliği 4.49±1.73 mm ve ağırlığı 4.50±0.93 g olarak belirlenmiştir. Uvaeva ve Shcherbına (2017) Ukrayna'da Tyna Nehri'ndeki yaptıkları örneklemede, 2.7-3.0 cm kabuk yüksekliğindeki bireylerin 6.0-6.9 g ve 4.0-4.4 cm kabuk yüksekliğindeki bireylerin ise 14.5-17.4 g ağırlığa sahip olduklarını bildirmişlerdir. Bu çalışmada, araştırma bölgesinde tespit edilen bireylerinin ölçülen kabuk yüksekliği ve ağırlık değeri Ukrayna'daki bireyler ile benzerlik göstermektedir (Uvaeva ve Shcherbına (2017). Uvayeva ve ark. (2021) Ukrayna'da Tyna Nehri'ndeki yaptıkları çalışmada, *V. viviparus* ve *V. contectus* türünün kabuk morfolojisinde seksüel dimorfizmin etkisini araştırmışlar ve erkek ve dişi tatlı su salyangozları (*V. viviparus* ve *V. contectus*) yaşlarına bağlı olarak kabuk morfolojisinde önemli farklılıklara sahip olduklarını göstermişlerdir. Bir ile üç yaşındaki *Viviparus* salyangozlarında seksüel dimorfizm olmadığını bildirmişlerdir. Üç yaşından sonra, olgun dişiler önemli ölçüde daha büyük kabuk genişliğine, daha yüksek vücut kıvrımına ve apertur boyutuna sahip olduklarını belirlemişlerdir. İki ile beş yaşındaki *V. viviparus*'un dişileri, erkeklerden istatistiksel olarak anlamlı daha yüksek olan ortalama kabuk genişliği ve kabuk yüksekliği ilişkisiyle erkeklerden ayırt edilebildiğini belirtmişlerdir. Yaptıkları çalışmada +1 ile +5 arasındaki yaş gruplarındaki bireylerin morfometrik ölçümlerini yapmışlar, *V. contectus* popülasyonundaki +1 yaşınadaki erkek ve dişi bireylere ait sırasıyla ortalama kabuk yüksekliği 21.1±0.2 ve 22.2±0.1 mm, kabuk genişliği 16.4±0.1 ve 18.1±0.2 mm, apertur yüksekliği 12.0±0.2 ve 13.1±0.1 mm ve apertur genişliği 8.9±0.1 ve 10.0±0.2 mm olarak bildirilmiştir. Bu çalışmada, araştırma bölgesinde tespit edilen bireylerin ölçülen kabuk değerleri +1 yaşındaki bireyler ile benzerlik göstermektedir

Bireylere ait linear regresyon analizi ile belirlenen boy-ağırlık ilişkileri Şekil 5'te verilmiştir. Elde edilen bireylere ait kabuk yüksekliği ve toplam ağırlık arasında güçlü bir ilişki olduğu [$W= 0.4784xL0.^{7468}$, korelasyon katsayısı (r^2)=0.919] belirlenmiştir. Ayrıca, kabuk yüksekliği-kabuk genişliği (r^2=0.904), kabuk yüksekliği-apertur yüksekliği (r^2=0.884), kabuk yüksekliği-apertur genişliği (r^2=0.848), kabuk yüksekliği-vücut helezon yüksekliği (r^2=0.922), kabuk yüksekliği-spir yüksekliği (r^2=0.716) ile apertur yüksekliği-apertur genişliği (r^2=0.866) arasında kuvvetli bir korelasyon olduğu tespit edilmiştir. Bartlett'in testi ve KMO, temel bileşenler analizinin (PCA) kullanışlılığını doğrulamak için kullanılmıştır. Temel bileşenler analizi, korelasyon matrisini özetlemede faydalı (KMO = 0.82) ve Bartlett'in test verileri anlamlı olduğu belirlenmiştir ($p = 0.000$; $p < .001$). Bu nedenle, temel bileşenler analizi yararlı ve değişkenler birbirleri ile ilişkili olduğu tespit edilmiştir. Kabuk yüksekliği (KY), Kabuk genisliği (KG), Apertur yüksekliği (AY) ve Vücut helezon yüksekliği (VHY) arasında güçlü bir ilişki olduğu belirlenmiştir (Şekil 6). Vasconcelos ve ark. (2016)

yaptıkları çalışmada *Hexaplex. trunculus* türünde SL ile SW, AL, AW, SpL ve SpW arasındaki ilişkilerde pozitif allometriler, büyüme sırasında, kabuk genişliği, açıklık uzunluğu ve genişliği, kule uzunluğu ve genişliğinin kabuk uzunluğundan daha hızlı arttığını göstermiştir. SL ile ShL ve TAL arasındaki ilişkilerdeki negatif allometriler, ontogenez boyunca sifonal kanalın uzunluğunun ve dolayısıyla toplam açıklığın uzunluğunun, kabuk uzunluğundan daha yavaş bir oranda arttığını ortaya koymuşlardır. Ağırlıklı değişkenler ile ilgili olarak, SL ile TWg ve TWg ile SpWg arasındaki ilişkilerdeki pozitif allometriler, büyüme sırasında hem toplam ağırlığın hem de yumuşak kısım ağırlığının (ham yenilebilir içerik) kabuk uzunluğuyla orantılı olarak arttığını göstermişler, ontogeni sırasında *H. trunculus*'un somatik büyümeye daha fazla pay ayırdığını bildirmişlerdir. *Bolinus brandaris* türünde, SL ile SW, TAL, AL, AW, SpW ve ShL arasındaki ilişkilerdeki pozitif allometriler, büyüme sırasında, kabuk genişliği, toplam açıklık uzunluğu, açıklık uzunluğu ve genişliği, helezon genişliği ve sifonal kanal uzunluğunun arttığını tespit etmişlerdir. SL ile SpL arasındaki ilişkideki izometri, ontogeni boyunca kule uzunluğu ve kabuk uzunluğunda benzer büyümeyi belirlemişlerdir.



Şekil 5. *Viviparus contectus* bireylerinin biyometrik parametreler arasındaki korelasyon.
Figure 5. Correlation between biometric parameters of Viviparus contectus individuals.



Şekil 6. *Değişkenlere [Kabuk yüksekliği (KY), Kabuk genisliği (KG), Apertur yüksekliği (AY), Apertur genisliği (AG), Vücut helezon yüksekliği (VHY) ve Spir yüksekliği (SpU)] ait temel bileşenler analizi.*
Figure 6. Principal components analysis of variables [Shell length (SL), Shell width (SW), Aperture length (AL), Aperture width (AW), Body helezone length (BHL) and Spir length (SoU)]

Uvayeva ve ark. (2021) *V. contectus* türünün +1 yaşına ait erkek ve dişi bireylerde sırasıyla KG/KY oranını 0.76±0.01 ve 0.78±0.02, VHS/KY oranını 0.49±0.02 ve 0.49±0.01, AY/KY oranını 0.61±0.02 ve 0.60±0.01, AG/KY oranını 0.50±0.01 ve 0.50±0.01 olarak bildirmişlerdir. Gastropod kabuk büyümesi, morfolojisi ve göreceli oranları, çeşitli abiyotik ve biyotik faktörlerden oldukça etkilenir. Ayrıca, morfometrik parametreler arasındaki ilişkiler, habitata bağlı olarak ve özellikle büyüme, olgunlaşma ve yumurtlama süreçlerinde meydana gelen, kabuk kalsifikasyon mekanizmalarını etkileyen ve kabukta varyasyona neden olabilecek fizyolojik koşullar nedeniyle değişebilir. Tüm bu nedenlerle, farklı popülasyonlardan ve/veya coğrafi bölgelerden türler arasındaki morfometrik ilişkilerin ve büyüme türlerinin karşılaştırılması, analiz edilen büyüklük aralıklarını dikkate almalı ve dikkatli bir şekilde yorumlanmalıdır (Vasconcelos ve ark., 2016).

SONUÇ ve ÖNERİLER

Yapılan çalışma ile Demirköprü Baraj Gölü'nde *V. contectus* türünün varlığı ilk defa bu çalışma ile ortaya konmuştur. Tatlısu salyangozlarının daha çok durgun veya az akıntılı, vejetasyonu bol sularda bulunduğu görülmüştür. Çoğu takson için, özellikle konkolojik karakterlere dayalı olarak tanımlananlar için taksonomi çok karmaşıktır ve kabuk karakterlerinin yakınlığı nedeniyle genel araştırmalarda birçok yeni tür gözden kaçabilir. Bu nedenle, Türkiye tatlısu salyangoz faunası için Demirköprü Baraj Gölü'nün malakofaunasının anlaşılması için taksonomik araştırmalar büyük önem taşımaktadır. Yapılan çalışma sonucunda, yağışların azalması ve kuraklık nedeniyle göldeki suda çekilme meydana geldiği ve ortamdaki bireylerde ölümlere neden olduğu belirlenmiştir. Bu durumun türün popülasyonlarını olumsuz etkilediği söylenebilir. Yapılan biyometrik ölçümler bireylerdeki büyüme ve gelişme durumunun oldukça iyi olduğunu göstermektedir. Ancak, göldeki suyun aşırı çekilmesi, suyun aşırı ısınması ve kirlilik gibi etmenler *V. contectus* bireylerinin yok olmasına neden olabilir. Gediz nehri giderek artan endüstrileşmeye ve kıyı yerleşimlerine bağlı olarak zehirli endüstriyel atıklar ve evsel atıklardan dolayı gün geçtikçe daha çok kirlenmekte ve tehdit altına girmektedir. Demirköprü Baraj Gölü, Gediz Nehri'nden beslenmekte ve bu durum da ortamda yaşayan canlıları olumsuz yönde etkilemektedir. Ekolojik önemi olan bu tür için Demirköprü Baraj suyunun daha dikkatli kullanılmasına, tür koruma altına alınmasına ve türün ortamdaki durumu ile ilgili daha geniş çalışmalara ihtiyaç duyulmaktadır.

Araştırmacıların Katkı Oranı Beyan Özeti

Yazarlar makaleye eşit oranda katkı sağlamış olduklarını beyan eder.

Çıkar Çatışması Beyanı

Makale yazarları aralarında herhangi bir çıkar çatışması olmadığını beyan ederler.

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Genelleştirilmiş Prokrustes Analiz Yöntemi: Duyusal Veri Üzerine Bir Uygulama

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ÖZET

Bu çalışmada, farklı besleme gruplarında yetiştirilen kuzuların et örneklerine ait duyusal özelliklerin besleme gruplarına göre değişimi Genelleştirilmiş Prokrustes Analiziyle (Generalized Procructes Analysis, GPA) değerlendirilmesi amaçlanmıştır. Bu çalışmada, farklı besleme gruplarında yetiştirilen kuzuların et örneklerine ait duyusal özelliklerin besleme gruplarına göre değişimi Genelleştirilmiş Prokrustes Analiziyle (Generalized Procructes Analysis, GPA) değerlendirilmesi amaçlanmıştır. Duyusal analizde, et örneklerinin sululuk, lezzet, koku, yumuşaklık ve genel beğeniden oluşan özellikleri, 41 yarı-eğitimli panelist tarafından 1-9 arasında değişen hedonik skala ile değerlendirilmiştir. Buna göre, GPA analizinden elde edilen ilk iki faktör, et örneklerinin duyusal özellikleri arasındaki değişkenliğin yaklaşık %76.74'ünü açıklamıştır. Toplam değişimin açıklanmasında en fazla katkıyı sağlayan ilk faktörün (%40.72) oluşumunda, yumuşaklık, sululuk ve genel beğeni özellikleri önemli rol oynamıştır. Et örneklerinin hataları genel olarak birbirine yakın bulunmuştur. Aynı zamanda, GPA'dan elde edilen uzlaşma haritasında, panelistler tarafından et örneklerinin açıkça ayırımı yapılmıştır. Panelistler arasında duyusal özellikler bakımından et örnekleri arasında bir uzlaşma sağlanmıştır. Sonuç olarak, duyusal analize katılan panelistlerin, panel davranışlarının incelenmesi ve panelistler arasındaki değişkenliğin azaltılmasında, GPA yöntemi etkili ve alternatif bir çözüm yolu sunmaktadır.

Biyoistatistik

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Anahtar Kelimeler
Prokructes analiz
Uzlaşma
Duyusal test

Generalized Procrustes Analysis Method: An Application on Sensory Data

ABSTRACT

In this study, it was aimed at the evaluation of the relationships between lambs sensory properties and meat samples using with Genaralized Procructes Analysis (GPA). In sensory analysis, the characteristics of juiciness, flavor, odour, tenderness and overall liking of the meat samples were evaluated by hedonic scale ranging from 1-9 by 41 semi-trained panelists. Accordingly, the first two factors obtained from the GPA analysis explained approximately 76.74% of the variability between the sensory properties of the meat samples. The tenderness, juiciness and overall liking characteristics played an important role in the formation of the first factor which has the most contributed (40.72%) to the explaining of the total variation. Residuals of the meat samples were generally found close to each other. At the same time, the consensus configuration map obtained from the GPA of the meat samples has clearly distinguished by the panelists. However, the majority of panellists have provided a consensus between the meat samples in terms of sensory properties. As a result, GPA method provides an effective and alternative solution for the examine of the panel behavior and reducing the variability between panelists who are involved in the sensory analysis.

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GİRİŞ -

Duyusal testler, görme, koku alma, dokunma, tat alma ve duyma duyularıyla algılanan ürünlere verilen tepkileri, ölçmek, analiz etmek ve yorumlamak için kullanılan bilimsel bir yöntem olarak geliştirilmiştir (Stone ve Sidel, 2004). Duyusal analizler, subjektif testler sınıfında yer almasına karşın, değerlendirmelerinin insan tarafından gerçekleştirilmesi, tüketici algısı için doğrudan bir ölçüm olanağı sunduğundan önemlidir. Bu nedenle, duyusal panel testler pek çok çalışmada kullanılmaktadır. Ancak duyusal testlerde önemli bir problem, farklı panelistlerin aynı gıda örneğini değerlendirirken ortaya çıkan değişkenliktir. Özellikle panelistler arasında örneği tanımlamada, bir fikir birliğinin olmaması önemli bir değişkenlik kaynağıdır. Bu durum, duyusal test sonuçlarının yorumlanmasını zorlaştırmaktadır (Wu ve ark., 2003; Tomic, 2013). Son yıllarda, duyusal testlerde farklı panelistlerden elde edilen bilgilerin, bir araya getirilmesinde Genelleştirilmiş Prokrustes Analizi (Generalized Procrustes Analysis, GPA) kullanılmaktadır. Yöntem, panelistlerin aynı örneklere verdikleri farklı skorlar arasında, üç farklı prokrustes transformasyon (translasyon, izotropik ve rotasyon) uygulayarak eşleştirme yapmaktadır. Eşleştirme sonucunda, değişkenlik azaltılmakta ve ortak panelist görüşlerinin yer aldığı bir uzlaşı matrisi elde edilmektedir. Duyusal teste yönelik yorumlamalar, bu uzlaşı matrisi üzerinden yapılmaktadır (Meullenet ve ark., 2007; Li, 2014).

Keskin ve ark. (2012) tarafından ekstansif ve yarı ekstansif koşullarda yetiştirilen koyun ve keçilere ait et örneklerini değerlendirdikleri duyusal teste ilişkin sonuçlar GPA yöntemiyle incelenmiştir. Duyusal teste katılan yarı-eğitimli 10 panelist, yetiştirme koşullarına göre oluşturulan altı et örneğini, beş farklı duyusal özellik (renk, sertlik, koku, lezzet ve genel beğeni) bakımından, hedonik skalada değerlendirmiştir. GPA sonucunda, aynı et örneklerini değerlendiren panelistler arasındaki değişimin azaltılmasında rotasyon ($p<0.05$) ve translasyon ($p<0.01$) transformasyonlarının etkisi önemli bulunmuştur. Et örnekleri ve duyusal özellikler arasındaki toplam değişimin yaklaşık %61.11'i iki faktör tarafından açıklanmıştır. Çalışma sonucunda, panelistler ekstansif koşullarda yetiştirilen koyun ve keçi etlerini tüm duyusal özellikler bakımından, yarı ekstansif koşullarda yetiştirilenlerden daha çok tercih ettiği bildirilmiştir.

Rodriques ve Teixeira (2013) tarafından yapılan çalışmada ise Terrincho kuzularında cinsiyet ve karkas ağırlıklarının, etin altı farklı duyusal özellikleri (sertlik, lezzet yoğunluğu, koku yoğunluğu, lifli ve tat düzeyleri) üzerine etkisi GPA yöntemiyle değerlendirilmiştir. Duyusal panel testi 10cm'lik yapılandırılmamış bir ölçekte, 11 eğitimli panelist tarafından gerçekleştirilmiştir. GPA sonucunda, ilk iki faktör duyusal özellikler ve et örnekleri arasındaki toplam değişimin yaklaşık %72.76'sını açıklamıştır. Panelistler, cinsiyet etkisi ayırt edememiş ancak ağır hayvanların etlerinin daha sert ve daha yoğun kokulu, hafif hayvanların etlerini ise daha lezzetli bulmuşlardır.

İki farklı domuz ırkından elde edilen etlerin değerlendirilmesi amacıyla Rodriques ve Teixeira (2014) tarafından yapılan duyusal analizde, GPA yöntemi için veri matrisi dört et örneği, dört duyusal parametre ve 10 panelistten oluşturulmuştur. Panelistler, farklı ırklara ait et örnekleri arasında anlamlı farklılıklar bulmuşlardır. Translasyon dönüşümü sonucunda, elde edilen et örneklerinin hatalarının benzer ve düşük bulurken, panelistler arasında da farklılıklar belirlenmiştir.

Bu çalışmada, farklı besleme gruplarında yetiştirilen kuzuların et örneklerine ait duyusal özelliklerinin, besleme gruplarına göre değişimi Genelleştirilmiş Prokrustes Analiziyle (Generalized Procructes Analysis, GPA) incelenmiştir.

MATERYAL ve METOD

Duyusal Verilerin Elde Edilmesi

Çalışmanın veri setini, 40 baş Norduz erkek kuzusu etinde gerçekleştirilen, duyusal panel test verileri oluşturmuştur. Kuzular 3-4 aylık yaşta sütten kesilmiştir. Deneme başlangıcından önce hayvanlar, besleme gruplarını oluşturmak üzere kura yöntemi ile 10'ar başlık dört gruba ayrılmıştır. Gruplar aşağıda tanımlanmıştır.

1. Grup (DDGS-0) :120 gün süre ile karma yem (n=10)
2. Grup (DDGS-120):120 gün süre ile mısır DDGS'li karma yem (n=10)
3. Grup (DDGS-75) :45 gün süre ile karma yem + 75 gün süre ile mısır DDGS'li karma yem (n=10)

4. Grup (DDGS-45) :75 gün süre ile karma yem + 45 gün süre ile mısır DDGS'li karma yem (n=10)

Gruplar belirlenen besi süresine ulaştıklarında (38 kg) besi sonlandırılmıştır. Kesim sonrası 24 saat soğuk hava deposunda bekletilen karkasların sol yarımından *longissimus lumborum* (LL); L1-L5) örnekleme yapılmış ve bu örnekler duyusal testte kullanılmıştır. Duyusal testte, 41 yarı-eğitimli

panelist yer almıştır. Panelistlerden, et örneklerini beş duyusal özellik (yumuşaklık, sululuk, lezzet, koku ve genel beğeni) yumuşaklık) bakımından değerlendirilmesi istenmiştir. Et örneklerinin değerlendirilmesinde hedonik skala (1= aşırı sert, aşırı kuru, aşırı kötü lezzetli, aşırı kötü; 9= aşırı yumuşak, aşırı sulu, aşırı iyi lezzetli, aşırı iyi) kullanılmıştır. Panelistlere rastgele servis edilen örneklerde, bir örnekten diğerine geçerken kullanılması için tuzsuz kraker ve su servis edilmiştir. Bu denemeye ilişkin ayrıntılı bilgiler Karaca ve ark. (2021) tarafından verilmiştir. Bununla beraber, hayvan araştırma prosedürleri, Van Yüzüncü Yıl Üniversitesi Hayvan Deneyleri Yerel Etik Kurulu'nun onayı ile gerçekleştirilmiştir (Karar No. 2018/05).

İstatistik Analizler

Çalışmada, istatistiki analizler iki aşamada gerçekleştirilmiştir. İlk aşamada, duyusal özellikler arasındaki ilişkilerin belirlenmesi amacıyla Sperman korelasyon analizi yapılmıştır. Aynı zamanda, beş duyusal özellik bakımından dört farklı besleme grubundan gelen et örneğindeki farklılıkların belirlenmesinde Genelleştirilmiş Doğrusal Karışık Model (Generalized Linear Mixed Model, GLMM) yöntemi kullanılmıştır. Sperman korelasyon analizi ve GLMM yöntemlerinin analizinde SAS v 9.4.1 (SAS Inst. Inc., Cary, NC, USA) paket programı kullanılmıştır.

İkinci aşamada ise aynı örnekleri değerlendiren panelistler arasındaki farklılıkları değerlendirmek amacıyla GPA yöntemi kullanılmıştır. GPA'da öncelikli olarak, her panelist için et örneklerine ilişkin değerlendirme skorlarının yer aldığı bireysel matrisler oluşturulmuştur. Bireysel matrislerin satırlarında dört et grubu yer alırken, sütunlarında beş duyusal özellikler yer almaktadır. Oluşturulan bireysel matrislerden ortak bir uzlaşı matrisi elde etmek ve panelistlerin değerlendirme skorları arasındaki değişkenliği azaltmak amacıyla üç farklı prokrustes transformasyon (translasyon, rotasyon ve izotropik) kullanılmaktadır.

Transformasyonlar, panelistlerin gıda ürününü değerlendirirken ortaya koydukları ölçek davranışlarına göre geliştirilmiştir. Translasyon transformasyonu, panelistlerin bir ürünü değerlendirirken ölçeğin sadece belirli kısımlarını kullanması sonucunda ortaya çıkan değişkenlik durumunda kullanılmaktadır. Örneğin, bir panelist 1-100 arasında değişen bir çizgi ölçekte, tüm ürünleri değerlendirirken sadece 5-25 aralığını, bir diğer panelist ise sadece 60-100 aralığındaki skorları kullanabilir. Bu iki uç panelist, farklı ölçekleme davranışına sahip olmasaydı, birbirleriyle tamamen anlaşabilir ve örnekleri benzer olarak algılayabilirlerdi. Bu durum, iki panelistin ürünü değerlendirirken ortalama skorlardan sapan skorları olarak ifade edilir. Translasyon işlemiyle bu skorlar düzeltiler (Dijksterhuis, 1996; Tomic, 2013; Tárrega ve Tarancón, 2014). Rotasyon transformasyonu ise aynı ürünü değerlendiren panelistler arasındaki yorumlama farklılıkları durumunda uygulanmaktadır. Bu transformasyon aynı zamanda yorum etkisi olarak da adlandırılmaktadır. Bir panelistin ürünü değerlendirirken duyusal özellikler arasında uyum sağlamak amacıyla rotasyon (döndürme) işlemi yapılır. Böylelikle, panelistin farklı eşli skorları birbirine yaklaştırılır ve aralarındaki uzaklıklar azaltılmaktadır (Dijksterhuis, 1996; Lawless ve Heymann, 2010; Tomic, 2013; Tárrega ve Tarancón, 2014). Bir diğer transformasyon isotropik ölçeklemedir. Bu transformasyon, değişim etkisi olarak da bilinmektedir. Panelistlerin kullandığı farklı puanlama aralıklarıdır. Örneğin, bir panelist örneği değerlendirirken 10 ile 95 aralığı gibi geniş bir aralığı kullanırken, bir diğer panelist 60 ile 80 gibi dar bir aralığı kullanabilir. Ölçeği kullanırken ortaya çıkan bu değişkenlik, ölçekleme davranışında istenmeyen bir durumdur. Ölçek aralığındaki bu farklılıklar, panelistin ürünü değerlendirirken bir ön yargıya sahip olmadığı varsayılarak, kontrol altına alınmaktadır (Arnold ve Williams, 1986; Dijksterhuis, 1996; Grice ve Assad, 2009).

GPA'da bireysel matrislerdeki bilgilere dayanarak uygun transformasyonun belirlenmesinde, Prokrustes transformasyonlar ve ANOVA'nın kombinasyonundan geliştirilmiş PANOVA tabloları kullanılmaktadır. Belirlenen transformasyon aracılığıyla 41 panelistin bireysel matrisleri birleştirilerek, çok boyutlu bir uzlaşı matrisi elde edilmektedir. GPA'dan elde edilen uzlaşı matrisinin boyutlarının belirlenmesinde ve önemliliklerinin test edilmesinde, permutasyon testi yapılmıştır. Veri seti şansa bağlı olarak 1000 kez permute edilmiş veride uzlaşma konfigürasyonu $R_u = 0.407$ (% 65.7) olarak elde edilmiştir. Çok boyutlu bir matris olan uzlaşı matrisinin kolay yorumlanabilmesi amacıyla Temel Bileşenler Analizi aracılığıyla uzlaşı matrisinin boyutlarının azaltılmış ve yorumlamaların tamamı, bu uzlaşı matrisi üzerinden yapılmıştır. GPA analizleri, Microsoft Excel XLSTAT Deneme Versiyonu (Addinsoft, 2019) kullanılarak gerçekleştirilmiştir.

BULGULAR

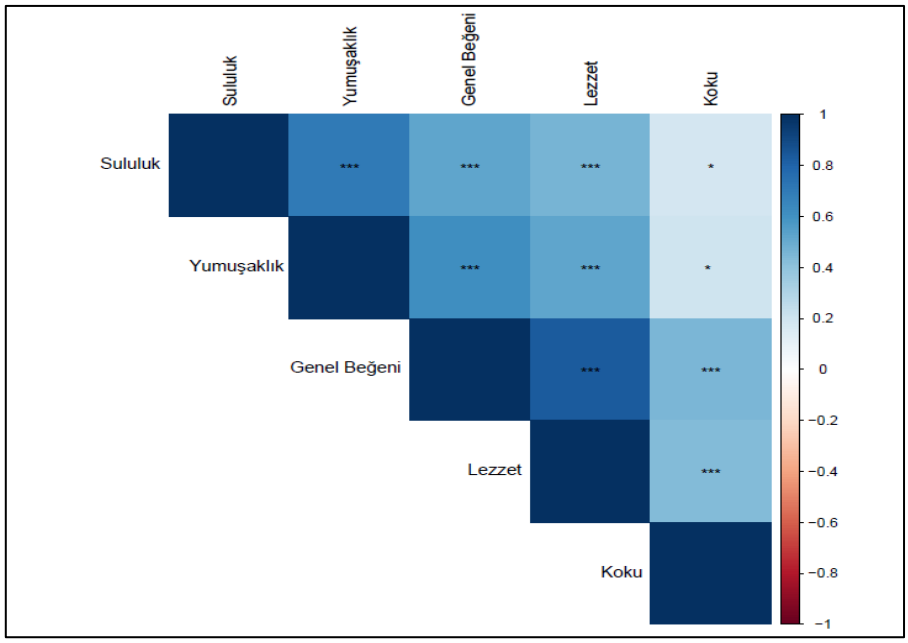
Duyusal Test Sonuçları

Duyusal teste katılan 41 yarı-eğitimli panelistin, demografik özellikleri Çizelge 1'de verilmiştir. Duyusal testte, kadın ve erkek sayısı birbirine oldukça yakındır. Teste katılan panelistlerin en küçüğü 21 ve en büyüğü 63 yaşında olup, geniş bir değişim aralığına sahiptir. Panelistlerin çoğunluğu, 25-29 (%29.27) ve 30-39 (39.02) yaş aralığındadır.

Çizelge 1. Panelistlerin demografik özellikleri
Table 1. Demographic characteristics of the panelists

Özellikler		Sayı	Yüzde (%)
Cinsiyet	Kadın	21	51.2
	Erkek	20	48.8
Yaş	<25	1	2.44
	25-29	12	29.27
	30-39	16	39.02
	40-49	9	21.95
	50-59	2	4.88
	>60	1	2.44

Et örneklerinin duyusal özellikleri arasındaki ilişkilerin verildiği korelasyon haritası Şekil 1'de verilmiştir. Korelasyon haritasındaki renk skalasında, pozitif ve negatif korelasyonlar ayrı renklerde gösterilmektedir. Bununla birlikte katsayısının büyüklüğüne göre skaladaki renkler, açıktan-koyuya doğru değişmektedir. Korelasyon haritası incelendiğinde, haritanın tamamında mavi rengin tonları görülmektedir. Renk skalasında duyusal özellikler arasında açıktan-koyu maviye doğru değişen renkler zayıf korelasyondan-güçlü korelasyona doğru bir değişimi ifade etmektedir. Örneğin koku özelliği ile sululuk ve yumuşaklık özellikleri arasındaki korelasyonlar önemli ($p<0.05$), ancak açık mavi rengin skaladaki aralığı incelendiğinde diğer özellikler göre katsayı daha düşüktür. Haritada en büyük korelasyon katsayısının, en koyu renge sahip olan lezzet ve genel beğeni özellikleri arasında olduğunu söylemek mümkündür ($r=0.830$; $p<0.001$).



Şekil 1. Duyusal özellikler arasındaki korelasyon haritası
Figure 1. Correlation map between sensory traits

Panelistlerin, beş duyusal özelliğe göre besleme grupları arasındaki farklılıkların belirlenmesinde genelleştirilmiş doğrusal karışık modele ilişkin sonuçlar Çizelge 2'de verilmiştir. Beş duyusal özellik bakımından, et örnekleri arasında istatistiki olarak önemli bir farklılık bulunmamıştır ($p>0.05$).

Çizelge 2. Et örnek gruplarının duyusal özellikleri için ortalama (standart hata) değerleri
Table 2. Mean (standart error) values for sensory traits of meat sample groups

Duyusal Özellikler	DDGS-0[1]	DDGS-120	DDGS-75	DDGS-45	p
Sululuk	1.624 (0.069)	1.689(0.067)	1.523(0.073)	1.528 (0.073)	0.269
Yumuşaklık	1.652 (0.068)	1.725 (0.066)	1.565(0.071)	1.657 (0.068)	0.440
Lezzet	1.707 (0.067)	1.703 (0.067)	1.648 (0.069)	1.711 (0.066)	0.901
Koku	1.804 (0.063)	1.796 (0.064)	1.799 (0.064)	1.819 (0.063)	0.994
Genel Beğeni	1.738 (0.066)	1.780 (0.064)	1.662 (0.068)	1.787 (0.064)	0.525

[1]DDGS-0: Karma yemle 120 gün beslenen grup; DDGS-120: DDGS ile 120 gün beslenen grup; DDGS-75: DDGS ile 75 gün beslenen grup; DDGS-45:DDGS ile 45 gün beslenen grup.

Genelleştirilmiş Prokrustes Analizine İlişkin Sonuçlar Duyusal Test

Prokrustes varyans analizi (PANOVA), GPA analizinin ilk adımıdır ve sonuçları Çizelge 3'de verilmiştir. Çizelgede, her üç transformasyonun serbestlik derecesi, kareler toplamı, kareler ortalaması, yaklaşık F değerleri ve önemlilik durumları ANOVA'dan elde edilmektedir. PANOVA sonuçlarına göre, panelistler arasındaki toplam değişimin azaltılmasında en büyük katkı translasyon transformasyonundan elde edilmiştir ($p<0.0001$). Her üç transformasyonda, en büyük hata kareler ortalaması (3.858) translasyon transformasyonundan elde edilmiştir. Dolayısıyla, duyusal teste katılan panelistlerin çoğunluğunun hedonik skalanın farklı aralıklarını kullandığını söylemek mümkündür. Translasyon transformasyonu uygulandıktan sonra hata kareler ortalaması 1.419 olarak elde edilmiştir.

Çizelge 3. PANOVA sonuçları
Table 3. PANOVA results

Varyasyon Kaynakları	Serbestlik Derecesi	Kareler Toplamı	Kareler Ortalaması	F	Pr>F
Ölçekleme sonrası hatalar	160	219.902	1.374		
Ölçekleme	40	67.076	1.677	1.220	0.195
Rotasyon sonrası hatalar	200	286.978	1.435		
Rotasyon	400	564.583	1.411	1.027	0.428
Translasyon sonrası hatalar	600	851.561	1.419		
Translasyon	200	771.512	3.858	2.807	< 0.0001
Düzeltilmiş toplam	800	1623.073	2.029		

Translasyon transformasyonundan sonra ortak panelist görüşlerinin yer aldığı uzlaşı matrisine ilişkin sonuçlar elde edilmektedir. Buna göre, Çizelge 4'de et örnek gruplarından elde edilen hata varyans bilgisi verilmiştir.

Çizelge 4. Et örneklerinden elde edilen hata varyanslarına ilişkin sonuçlar
Table 4. Residual variance for meat samples

Et örnekleri[1]	Hata varyansları
DDGS-0	57.558
DDGS-120	57.421
DDGS-75	50.444
DDGS-45	54.479

[1]DDGS-0: Karma yemle 120 gün beslenen grup; DDGS-120: DDGS ile 120 gün beslenen grup; DDGS-75: DDGS ile 75 gün beslenen grup; DDGS-45:DDGS ile 45 gün beslenen grup.

Çizelge 4 incelendiğinde, et örnek gruplarının hataları birbirine yakın bulunmuştur. Hata varyansı diğer gruplara göre yüksek olan DDGS-0 ve DDGS-120 gruplarına ait et örnekleri, panelistlerin duyusal özellikler bakımından fikir ayrılığı yaşadığı gruplardır. Hata varyansı daha düşük olan DDGS-75 grubuna ait et örnekleri ise panelistler tarafından en çok uzlaşı sağlanan grup olarak belirlenmiştir.

Uzlaşı matrisinde, boyut indirmek amacıyla uygulanan Temel Bileşenler Analizine ilişkin sonuçlar Çizelge 5'de verilmiştir.

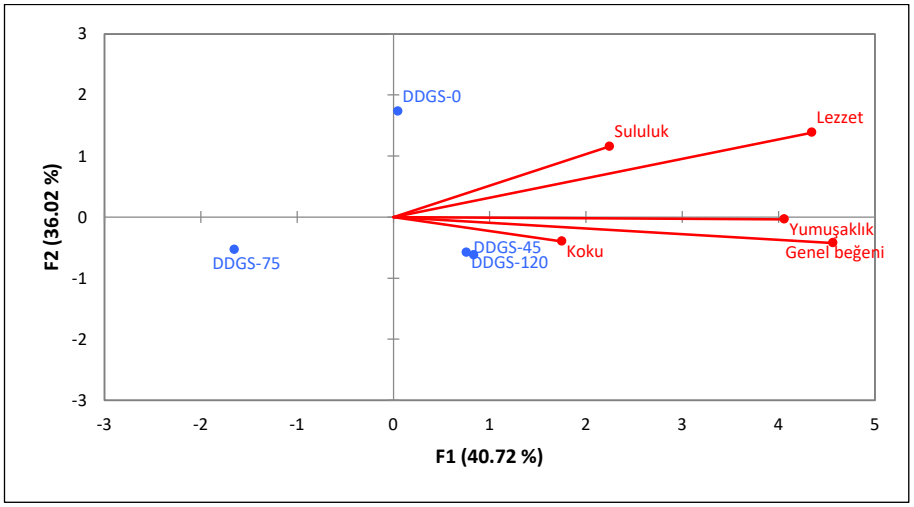
Çizelge 5'de besleme gruplarına ait et örnekleri ve duyusal özellikler arasındaki toplam varyasyonu, birinci faktör (F1) yaklaşık %40.72'sini, ikinci faktör (F2) ise %36.02'sini açıklamaktadır. Dolayısıyla, duyusal özellikler ve et örnek grupları arasındaki toplam değişkenliği, ilk iki faktör yaklaşık % 76.74'ünü açıklamaktadır. Temel bileşenler belirlendikten sonra, et örnekleri ile duyusal özellikler arasındaki ilişkilerin iki boyutlu grafiksel gösterimi Şekil 2'de verilmiştir.

Çizelge 5. Öz değerler, değişkenlik ve kümülatif değişkenliğe ilişkin sonuçlar
Table 5. Results on eigenvalues, variability, and cumulative variability

	Faktör 1(F1)	Faktör 2 (F2)
Öz değerler	1.693	1.498
Değişkenlik (%)	40.716	36.019
Kümülatif Değişkenlik(%)	40.716	76.736

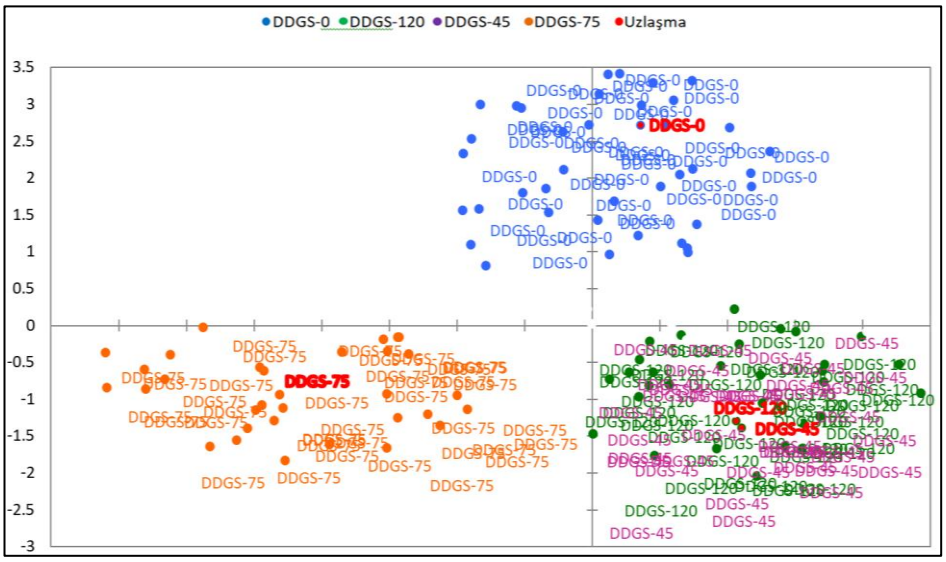
Şekil 2'de DDGS-45, DDGS-120 ve DDGS-0 aynı bölgede sınıflandırılmış ve F1'in pozitif bölgesine yerleşmiştir. Bununla birlikte DDGS-75 grubu F1'in negatif bölgesinde yer almıştır. Panelistler tarafından DDGS-75 ve DDGS-0 grubu ayrılmıştır. Bununla beraber, panelistler tarafından DDGS-45 ve DDGS-120 grupları neredeyse aynı gruplar olarak nitelendirilmiştir. İkinci boyuta göre ise DDGS-0 pozitif bölgede, DDGS-75, DDGS-45 ve DDGS-120 grupları negatif bölgeye yerleşmiştir. Duyusal özelliklerden sululuk ve lezzet F1 ve F2'nin pozitif bölgesine yerleşirken, koku, yumuşaklık ve genel beğeni F1'in pozitif bölgesine F2'nin ise negatif bölgesine yerleşmiştir. Şekil 2'ye göre tüm duyusal özellikler F1'in pozitif bölgesine yerleşmiştir. Bu bölgede bulunan duyusal özelliklerin daha yüksek puanlara sahiptirler. DDGS-75 grubunun, duyusal

özelliklerle arasında ilişki bulunmazken, DDGS-0 grubu her iki boyuttaki tüm duyusal özelliklerle pozitif ilişkili, DDGS-45 ve DDGS-120 grubu ise birinci boyut için duyusal özelliklerle pozitif, ikinci boyut için ise negatif ilişkilidir. Şekil 3'de duyusal özellikler bakımından uzlaşı sağlanan et örnek gruplarına ilişkin grafiksel gösterim verilmiştir.



Şekil 2. Duyusal özellikler ve et grupları arasındaki korelasyonların iki boyutlu grafikte gösterimi F1: GPA'dan elde edilen ilk temel bileşen; F2: GPA'dan elde edilen ikinci temel bileşen;DDGS-0: Karma yemle 120 gün beslenen grup; DDGS-120: DDGS ile 120 gün beslenen grup; DDGS-75: DDGS ile 75 gün beslenen grup; DDGS-45:DDGS ile 45 gün beslenen grup.

Figure 2. Two-dimensional graphic representation of correlations between sensory traits and meat samples F1: first principal component of GPA; F2: second principal component of GPA;DDGS-0: no DDGS included in diet for 120 days; DDGS-120: DDGS included in diet for 120 days; DDGS-75: no DDGS included in diet for 45 days + DDGS included in diet for 75 days; DDGS-45: no DDGS included in diet for 75 days + DDGS included in diet for 45 days.



Şekil 3. Et örnek grupları için uzlaşma haritası F1: GPA'dan elde edilen ilk temel bileşen; F2: GPA'dan elde edilen ikinci temel bileşen; DDGS-0: Karma yemle 120 gün beslenen grup; DDGS-120: DDGS ile 120 gün beslenen grup; DDGS-75: DDGS ile 75 gün beslenen grup; DDGS-45:DDGS ile 45 gün beslenen grup.

Figure 3. Consensus map of meat sample groups. F1: first principal component of GPA; F2: second principal component of GPA; DDGS-0: no DDGS included in diet for 120 days; DDGS-120: DDGS included in diet for 120 days; DDGS-75: no DDGS included in diet for 45 days + DDGS included in diet for 75 days; DDGS-45: no DDGS included in diet for 75 days + DDGS included in diet for 45 days.

Şekil 3'de et örnek gruplarında yer alan kırmızı renkteki grup isimleri, bu gruplarda panelistler tarafından uzlaşı sağlandığını ifade etmektedir. Buna göre, noktaların çoğunluğu ilk boyuta yakındır. Çünkü birinci faktör değişkenliğin yaklaşık %40.72'sini açıklamıştır. DDGS-120 ve DDGS-75 gruplarına ait et örneklerinde panelistler arasında uzlaşı sağlanmıştır. Çünkü, gruplar harita üzerinde farklı bölgelere yerleşmiş ve panelistler tarafından ayırt edilebilmiştir. Ancak DDGS-120 ve DDGS-45 et örnekleri ise panelistler tarafından benzer gruplar olarak nitelendirildiğinden açıkça bir ayırım yapılamamıştır.

TARTIŞMA VE SONUÇ

Bu çalışmada, dört farklı besleme grubunda yetiştirilen kuzuların et örnekleri, beş duyusal özellik bakımından GPA yöntemiyle değerlendirilmiştir. GPA analizinden önce duyusal özellikler arasındaki korelasyonlar incelenmiş, yumuşaklık-sululuk ($p<0.001$) ve lezzet-genel beğeni ($p<0.001$) özellikleri arasında önemli pozitif korelasyonlar belirlenmiştir (Şekil 1). Bununla beraber, et gruplarının duyusal özellikler bakımından farklılıkların belirlenmesinde genelleştirilmiş doğrusal karışık model sonuçları incelendiğinde tüm duyusal özellikler bakımından gruplar arasında farklılık belirlenmemiştir ($p>0.05$) (Çizelge 2). Etin başlıca duyusal özelliklerini ise görünüm (renk, yağ içeriği vb.) tekstür (yumuşaklık ve sululuk) ve lezzet olarak sıralamak mümkündür. Yapılan çalışmalar, etin duyusal özellikleri üzerinde genotip, yaş, cinsiyet, besleme, kesim öncesi (nakliye, açlık süresi vb.) ve sonrası (depolama koşulları, olgunlaştırma süresi ve pişirme şekli vb.) pek çok uygulamanın önemli etkiye sahip olabileceğini göstermiştir (Honikel, 2004; Ferguson and Warner, 2008; Guerrero et al., 2013). Söz konusu faktörler, tüketici tercihlerini önemli düzeyde etkileyebildiğinden, bu faktörlerin tüketici talepleri doğrultusunda iyileştirilmesine yönelik yeni bilgilere ihtiyaç duyulmaktadır.

Panel verilerine translasyon transformasyonu uygulandıktan sonra et örneklerine ilişkin hatalar, genel olarak birbirine yakın bulunmuştur. Ancak, duyusal özellikler bakımından panelistler arasında en çok uzlaşı sağlanan grup DDGS-75, en çok fikir ayrılığı yaşanan gruplar ise DDGS-0 ve DDGS-120 gruplardır (Çizelge 4).

Duyusal özellikler ve et grupları arasındaki ilişkilerin yer aldığı Şekil 2 incelendiğinde, DDGS-75 grubunun duyusal özelliklerle arasında ilişki bulunmamaktadır. Bu gruptaki et örneklerinin panelistler tarafından tercih edilmediğini söylemek mümkündür. En çok fikir ayrılığı yaşanan DDGS-0 ve DDGS-120 grafiğin farklı bölgelerinde yer aldığından, panelistler DDGS-0 grubuna ait et örneklerini sululuk ve lezzet bakımında tercih ederken, DDGS-120 grubuna ait et örneklerini yumuşaklık, koku ve genel beğeni özellikleri bakımından daha çok tercih etmişlerdir.

Şekil 3'de verilen uzlaşma haritasında DDGS-75, DDGS-0 ve DDGS-120 grupları harita üzerinde farklı bölgelere yerleştiğinden, panelistler bu grupları açıkça ayırt edebilmiştir. Ancak, DDGS-120 ve DDGS-45 grupları ayırt edilememiştir.

Lorenzo ve ark. (2016) tarafından ekstansif ve yarı ekstansif koşullarda yetiştirilen tay etlerinin duyusal özelliklerinin incelendiği çalışmada, veri matrisi 24 et örneği, 8 duyusal nitelik ve 8 panelist şeklinde oluşturulmuştur. Hatası düşük olan grubun (3 kg ticari yemle beslenen yarı entansif grup) diğer gruplara göre panelistler arasında en çok uzlaşma sağlanan grup olduğu belirtilmiştir. Uzlaşma haritasında et örnekleri farklı bölgelere yerleşerek, panelistler tarafından örnekler arasındaki farklılıklar açıkça ayırt edilebildiği ifade ediliştir. Benzer şekilde, Rodrigues ve Teixeira (2009) tarafından Cabrito Transmontana oğlaklarının cinsiyet ve karkas ağırlıklarının duyusal özellikler üzerine olan etkisini inceledikleri çalışmada, 6 et örneği, 8 duyusal özellik ve 11 değerlendiriciden oluşan veri matrisi GPA yöntemi kullanılarak analiz edilmiştir. 4 kg-erkek ve 8 kg-dişi grupları en düşük hataya sahip olmuş ve bu grupların et örnekleri arasında panelistler arasında en fazla uzlaşı sağlanmıştır. Oluşturulan uzlaşma haritasında birinci boyut değişkenliğin %83'ünü açıkladığından, grupların bu boyuta daha yakın konumlandığı belirtilmiş ve tüm et örnekleri harita üzerinde açıkça ayırılmıştır. Ayrıca, GPA yönteminin keçi etinin duyusal özelliklerinin değerlendirilmesinde doğru bir metot olduğu belirtilmiştir. Prokrustes analizinde, temel bileşenler analizi (PCA) kullanılarak elde edilen ve et örnekleri arasındaki toplam değişimin açıklanmasında ilk iki faktör %76.74'ünü açıklamıştır (F1 %40.72 ve F2 %36.02) (Çizelge 5). Et örneklerinin GPA yöntemiyle değerlendirilmesine ilişkin yapılan çalışmalarda toplam değişimin açıklanmasında farklı oranlar elde edilmiştir. Örneğin, Rodriques ve Teixeira (2009), GPA'dan elde edilen ilk iki faktörün toplam değişkenliğin % 93'ünü açıkladığını belirtirken, Kor ve Keskin (2011) ise ilk iki faktörün toplam değişimin %84.29'unu açıkladığını bildirmişlerdir. Panea ve ark. (2012) tarafından yapılan çalışmada ilk faktörün %50.42 ve ikinci faktör %27.31'ini açıklamıştır. Benzer şekilde Alcalde ve ark. (2014), ilk faktörün değişimin %76.91'ini, ikinci faktörün %9.56'sını açıkladığını saptamışlardır. Rodriques ve Teixeira (2014) ve GPA analizi sonucunda elde edilen üç faktörün (F1 %59.13, F2 %24.2, F3 %16.44) toplam değişkenliğin %100'ünü açıkladığını belirtmişlerdir. Bu çalışmada, GPA'dan elde edilen toplam değişimin

açıklama oranının makul düzeyde olduğunu söylemek mümkündür.

Genelleştirilmiş Prokrustes Analizinin (GPA) son adımı olan, et örneklerinin ve duyusal özellikler arasındaki ilişkilerin incelendiği iki boyutlu grafikte (Şekil 2), panelistler tarafından DDGS-75 grubu ve duyusal özellikler arasında bir ilişki bulunmamaktadır. Şekil 3'de uzlaşma haritasında da bu grup, panelistler tarafından açıkça ayrılmıştır. Bununla beraber, DDGS-0 grubu daha lezzetli ve sulu bulunurken, DDGS-45 ve DDGS-0 grupları daha yumuşak, daha az kokulu ve genel beğeni olarak da daha çok tercih edilmiştir. Panelistler tarafından, DDGS-0 grubu açıkça diğer gruplardan ayrılırken, DDGS-120 ve DDGS-45 grupları benzer bulunmuştur.

Sonuç olarak, GPA yöntemiyle, et gruplarının duyusal özellikleri bakımından panelistler arasında uzlaşma sağlanmıştır. Panelistler tarafından, et örnek gruplarının uzlaşma haritası üzerinde duyusal özellikler bakımından ayrımı yapılabilmiştir. GPA yöntemi, duyusal teste katılan panelistlerin ürünü değerlendirme hassasiyeti hakkında yararlı bilgiler sağlamaktadır. Bu anlamda, duyusal testlerin değerlendirilmesinde kullanılan istatistiki yöntemlere, alternatif bir yöntem olarak önerilmektedir.

TEŞEKKÜR

Bu çalışma, ilk yazarın Yüksek lisans tezinden özetlenmiştir. Bununla beraber, çalışmanın özeti 3. Uluslararası Tarım, Hayvancılık ve Kırsal Kalkınma Kongresinde (ISPEC) sözlü bildiri olarak sunulmuş ve kongre kitabında özeti basılmıştır (20-22 Aralık 2019/VAN, s: 42). Yazarlar, bu çalışma verilerinin kullanımına izin veren, proje yürütücüsü Doç. Dr. Serhat KARACA ve proje ekibine teşekkürü bir borç bilirler.

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Yazarlar makaleye eşit oranda katkı sağlamış olduklarını beyan eder.

Çıkar Çatışması Beyanı

Makale yazarları aralarında herhangi bir çıkar çatışması olmadığını beyan ederler.

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Süt Sığır İşletmelerinde Sığır ve Buzağı Yetiştirme Uygulamaları: Erzurum İli İspir İlçesi Örneği

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ÖZET

Bu çalışmada, Erzurum ili İspir ilçesinde faaliyet gösteren işletmelerde sığır ve buzağı yetiştirme uygulamalarını ortaya koymak amacıyla 394 işletme sahibiyle yüz yüze anket yapılmıştır. Veriler Windows IBM SPSS (SPSS, 20.0.) istatistik programında frekans analizine tabi tutularak oransal değerler elde edilmiştir. Yetiştiricilerin ineklerin kızgınlığa geldiğini böğürme davranışından anladıklarını (% 75.6) ve % 66.5'inin ineklerinin doğumdan iki ay sonraki kızgınlıkta tohumlandığını bildirmişlerdir. İşletmelerin çoğunluğunun (% 72.1) düvelerini ilk olarak 24 aylık yaşta, % 26.6'sının ise 18 aylık yaşta tohumladıkları saptanmıştır. İşletmelerin hayvanlarını iki ay kala kuruya çıkardığı (% 64.2) belirlenmiştir. Yetiştiricilerin % 72.1'inin gebe ineklere septisemi aşısı yaptırdığı tespit edilmiştir. İspir İlçesi'ndeki yetiştiricilerin yeterli veterinerlik sağlık hizmeti almadığı—saptanmıştır. İşletmelerin % 96.2'si buzağılara septisemi aşısı yaptırdığı ve yetiştiricilerin tamamının doğumdan sonra buzağılara göbek bakımı uyguladığı belirlenmiştir. İşletmelerin % 99.3'ünün buzağılara ağız sütü verdiği ve bunu annesinden emerek (% 75.4) almasına müsaade ettiği tespit edilmiştir. İşletmelerin % 81.7'sinin buzağıları 4-6 aylık yaşta sütten kestikleri belirlenmiştir. Sonuç olarak Erzurum ili İspir İlçesinde bulunan sığırcılık işletmelerinde sığır yetiştirme konusunda bazı hatalı yöntemler uygulandığı, hayvan sağlığı ve veteriner sağlık hizmeti alma konularında önemli derecede eksikliklerin olduğu tespit edilmiştir. İspir ilçesinde sığırcılığın kalkınması için ilgili kurum ve kuruluşlarla iş birliği yapılarak planlı çiftçi eğitim faaliyetlerinin yürütülmesi önerilmektedir.

Zootekni

Araştırma Makalesi

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Anahtar Kelimeler
Erzurum
İspir
Sığırcılık
Buzağı
İşletme

Cattle and Calf Raising Practices in Dairy Cattle Farms: The Case of Erzurum Province İspir County

ABSTRACT

In this study, a face-to-face survey was conducted with 394 farmers to reveal the cattle and calf raising practices performed in cattle enterprises in İspir county of Erzurum province. Proportional values were obtained by carring out frequency analysis on the obtained data in IBM SPSS statistics for Windows (SPSS 20.0.). Most of the breeders (75.6%) stated that bellowing behavior is the primary sign to determine the heat cows and 66.5% reported that their cows were inseminated in estrus two months after birth. It was also determined that in most of the enterprises (72.1%) heifers were inseminated at 24 months of age, and 26.6% at 18 months of age. It was found out that in 64.2% of the enterprises the cows were dried off two months before the birth. In 72.1% of the farms, pregnant cows were vaccinated against septicemia. In İspir county, breeders were determined to have deficiencies in receiving veterinary health services. In 96.2% of the farms, calves were vaccinated against septicemia and all of the breeders performed umbilical cord care to the calves after birth. It was also determined that in 99.3% of the farms, colostrum was fed to the calves and farmers (75.4%) allowed the calves to consume this colostrum by sucking their dams. In

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81.7% of the enterprises, calves were weaned at the age of 4-6 months. As a result, it was revealed that some deficient practices are applied in cattle and calf breeding in cattle farms in İspir county of Erzurum province, and there are serious deficiencies in animal health and receiving veterinary health services. In order to develop cattle breeding in the county and to carry out animal husbandry under more scientific conditions, it was revealed that a planned farmer training activities should be carried out in cooperation with relevant institutions and organizations.

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GİRİŞ

Hayvansal üretim içerisinde sığır yetiştiriciliği önemli bir yer tutmaktadır. Özellikle dünya nüfusunun çok hızlı bir şekilde artması beslenmeyle alakalı birçok sorunu da beraberinde getirmiştir (Özsağlıcak ve Yanar, 2021). Hayvancılığı gelişmiş olan ülkelerde toplam hayvan varlığı içerisinde sığırların payı oldukça büyük olup et ve et üretiminin büyük bir kısmı bu hayvanlardan karşılanmaktadır (Kaygısız ve ark. 2022). Türkiyede 2021 yılı istatistiklerine göre toplam hayvan varlığının (küçükbaş+büyükbaş) % 23.8'ini sığırlar oluşturmaktadır (TÜİK, 2022). Süt sığırcılığı işletmelerinde istenilen verimin elde edilmesi ve bunun devamının sağlanması için çiftlik yönetiminin geleneksel yetiştiricilik anlayışından ziyade; değişen, gelişen ve yeni teknik bilgilerin kullanılmasıyla mümkün olabilecektir. Bunun için bu tür işletmelerde sığır yetiştirme uygulamalarının modern anlamda yapılması işletmelerin başarısında ve performansında önemli rolleri bulunmaktadır

Anketler, diğer sektörlerde olduğu gibi bir yöre veya bölgenin hatta bir ülkenin hayvancılık politikalarının şekillenmesinde de oldukça büyük önem taşımaktadır. Çünkü, yapılan anketlerden alınan sonuçların değerlendirilmesi ile sorunların belirlenmesi, çözümler üretilmesi, gerekli düzenlemelerin yapılması ve hayvancılığı çok daha ileriye götürerek istenilen kalitenin yakalanması mümkün olabilmektedir.

Türkiye'de (Şeker ve ark., 2012; Özyürek ve ark., 2014; Koçyiğit ve ark., 2021) ve dünyada (Duguma ve ark., 2012) farklı bölgelerde sığır yetiştirme uygulamaları ile ilgili yapılan mevcut çalışmalarda konunun önemi vurgulanmıştır.

Türkiye İstatistik Kurumu 2021 yılı verilerine göre Erzurum ilindeki büyükbaş hayvan sayısı 860404 baş olup Türkiye sığır varlığının % 4.77'sine tekabül etmektedir. Erzurum İl'i İspir ilçesi sığır varlığı, toplamda 23102 baş olup Erzurum sığır varlığının yaklaşık %2.68'ini oluşturmaktadır (TÜİK, 2022).

Bu çalışma, İspir ilçesindeki süt sığırcılığı işletmelerinde sığır yetiştirme uygulamalarını araştırmak, hatalı veya eksik uygulamaları saptayarak yetiştiricilere çözüm önerileri sunmak amacıyla yürütülmüştür.

MATERYAL ve METOD

Bu araştırmanın yürütülmesi için Atatürk Üniversitesi Ziraat Fakültesi Etik Kurul Başkanlığından 07.01.2022 tarih ve 2022/5 sayılı kararı ile Etik kurul belgesi alınmıştır. Erzurum ili İspir ilçesinde Basit Tesadüfi Örnekleme Yöntemi ile seçilen 394 sığırcılık işletme sahibi ile yüz yüze anket yapılmıştır. Anketlerden elde edilen veriler mevcut araştırmanın materyalini oluşturmuştur. Örnek büyüklüğü hesaplanmasında Arıkan (2007) tarafından önerilen formül kullanılmıştır.

$$n = \frac{N.t^2.p.q}{(N-1).D^2+t^2.p.q} \quad (1)$$

Bu formülde;
n=Örnek sayısı,
N=Toplam işletme sayısı,
D= örnekleme hatası (%5),
t=Tablo değeri (α= 0.05 için t=1.96),
p=Hesaplanacak oran (0.5), q=1-p.

Tahmini minimum örnek büyüklüğü yukarıda verilen (1) nolu formül kullanılarak 325 olarak hesaplanmıştır. Düşme, kaybolma, zarar görme ve değerlendirilmesi mümkün olmayabilecek anketler gibi herhangi bir sorunla karşılaşma ihtimali düşünülerek anket sayısı fazla tutulmuş ve toplamda 394 adet anket değerlendirilmiştir. Elde edilen veriler Excel 2010 programına girilmiştir. Burada gerekli kodlamalar yapılarak analiz için hazır hale getirilmiştir. IBM SPSS statistics for Windows, version 20.0. (SPSS) istatistik programında frekans analizi yapılarak sayısal ve oransal değerler elde edilmiştir. Oransal değerler kullanılarak grafikler oluşturulmuştur.

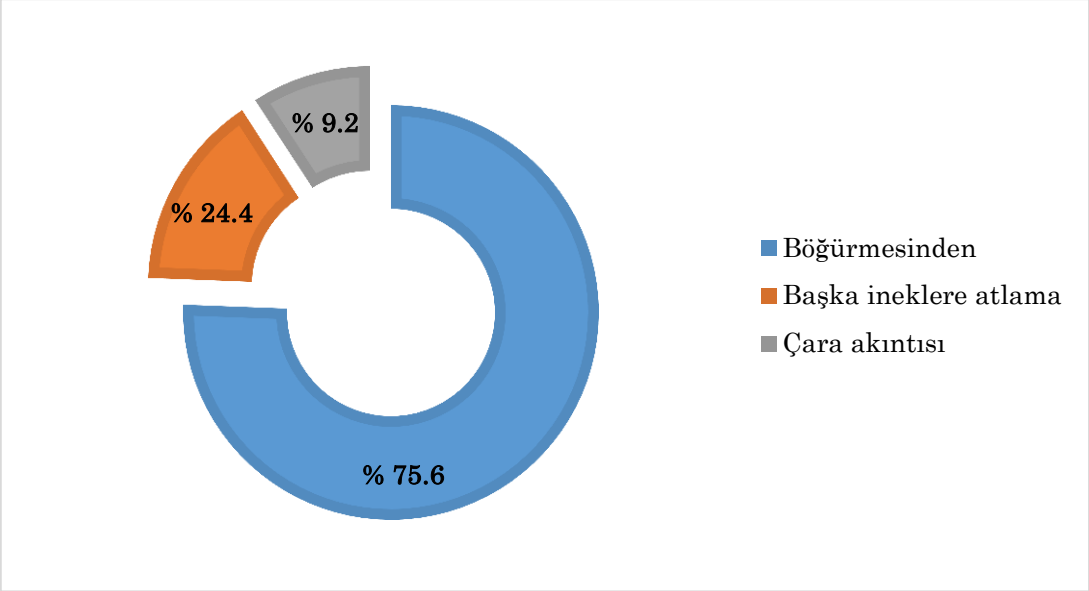
BULGULAR ve TARTIŞMA

Sığır Yetiştirme Uygulamaları

Araştırma bölgesinde yetiştirme uygulamaları kapsamında; ineklerin kızgınlığa gelme

belirtileri,doğan ineklerin doğumdan sonra ne zaman tohumlandıkları, düvelerin kaç aylık yaşta tohumlandıkları, gebe ineklere septisemi aşısı yaptırma durumları, ineklerin kuruya çıkma zamanları, işletmelerde görülen hastalıklar ve veterinerlik hizmeti alma gibi uygulamalar değerlendirilmiştir

Yetiştiricilerin yaklaşık % 75.0'i ineklerin böğürme sesinden, % 25.0'i ise ineklerin başka ineklere atlaması ve çara akıntısından hayvanların kızgınlığa geldiğini anladıklarını belirtmişlerdir (Şekil 1). Araştırmaya konu teşkil eden diğer çalışmalarda Erzurum ili Narman ilçesi'nde Diler ve ark. (2017), ineklerinin kızgınlık gösterdiğini geldiğini işletmelerin % 29.0'unun ineklerin başka hayvanlara atlamasından, %14.0'ü ise ineklerin böğürmesinden anladığını; Şeker ve ark. (2012) ineklerin kızgınlığa gelme belirtisi olarak Muş ilinde atlama ve sıçrama hareketlerinin (% 45.7), Tugay ve Bakır, (2008) Giresun yöresinde çara akıntısının (% 53.9), Koçyiğit ve ark. (2015) ise Hınıs ilçesinde hayvanın böğürmesinin (% 63.0) esas alındığını bildirmişlerdir. Bir öncelik sırasına bakılırsa sırasıyla atlama-sıçrama, böğürme ve çara akıntısından yetiştiricilerin hayvanların kızgınlığa geldiklerini anladıkları söylenebilir. Yapılan çalışma Koçyiğit ve ark. (2015) bulgularıyla uyumlu olduğu söylenebilir.



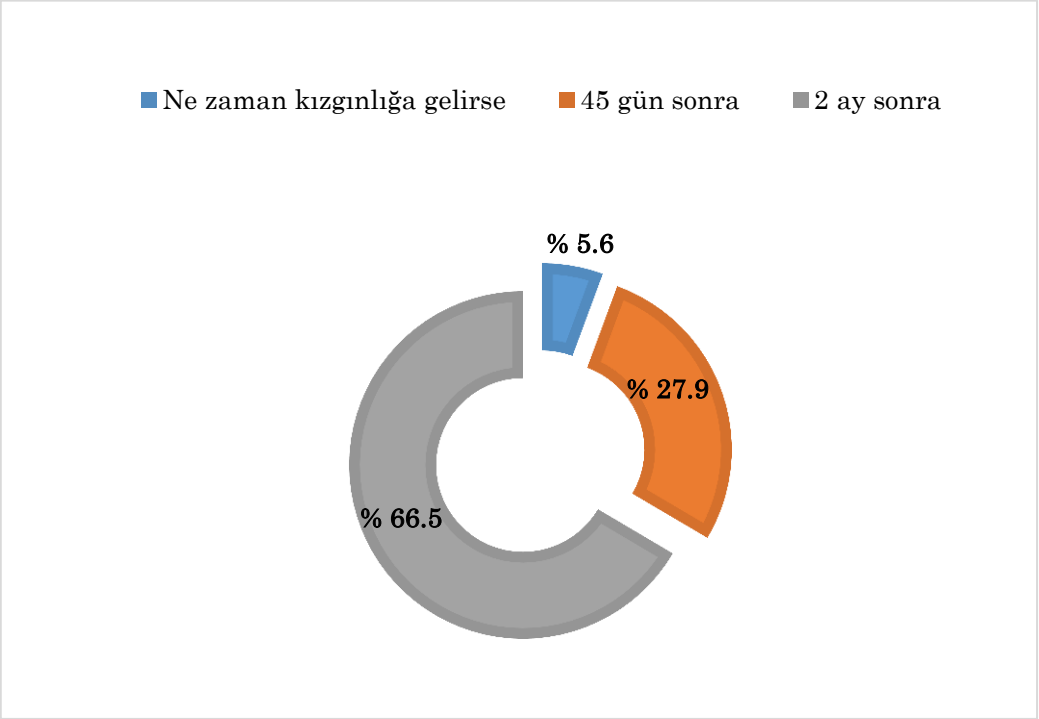
Şekil 1. İşletmenizdeki ineklerin kızgınlığını nasıl anlıyorsunuz ?
Figure 1. *How do you determine heat cows in your enterprise ?*

Yetiştiricilerin yarısından fazlasının ineklerini (% 66.5) doğumundan iki ay sonraki kızgınlıkta tohumlattıkları belirlenmiştir (Şekil 2). Genellikle yetiştiricilik açısından doğum yapmış bir ineğin doğumdan sonra yeni bir gebelik için kendini yenileyebilmesi ve bir sonraki doğuma hazırlıklı bir şekilde girebilmesi için en erken doğumdan sonraki 45-60. günlerde meydana gelen kızgınlıklarda tohumlanması gereklidir (Özhan ve ark. 2012). Konu ile ilgili yapılan araştırmalarda; Bulgular Bakır ve Kibar'ın (2019) 45 gün sonraki tohumlama değerinden düşük (%33.8), 2 ay sonraki değerinden (%30.9) ise yüksek, Kaylan ve ark.'nın (2019) 2 ay sonraki tohumlama değerinden düşük (%91), Koçyiğit ve ark. (2015)'nın 45 gün sonraki %8 ve 2 ay sonraki %10 değerinden oldukça yüksektir. Kaygısız ve ark. (2008)'nın ise inekleri "nezaman kızgınlığa gelirse" (%31) değerinden düşük, 2 ay sonraki tohumlama (% 46.0) oranından ise yüksek bulunmuştur .

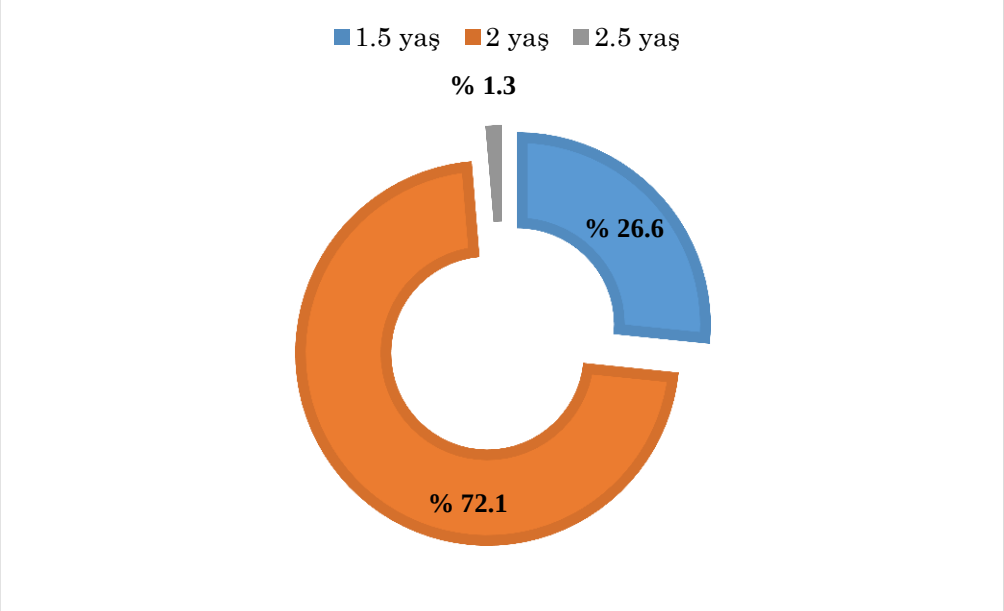
Şekil 3 incelendiğinde işletmelerin çoğunluğunun (% 72.1) düvelerini 24 aylık yaşta ve % 26.6'sı ise 18 aylık yaşta tohumladıkları belirlenmiştir. Düvelerin tohumlama yaşları olarak uygun bakım ve besleme koşullarında kondisyonlarının ve sağlık durumlarının iyi olması durumunda işletmelerdeki düvelerin 15 aylıkken tohumlanabileceği bilinmektedir. Ancak düveler gerekli canlı ağırlığa erişmemiş iseler, döl tutma ve yavrulama güçlükleri görülür (Özhan ve ark. 2012). Muş ilinde, yetiştiricilerin % 33.9'unun 18 aylıkken, %20.7'sinin ise 24 aylıkken düvelerini tohumladığı (Şeker ve ark. 2012); Erzurum ili Hınıs ilçesindeki işletmelerin büyük bir kısmı (%75.0) 24 aylık yaşta tohumlama yaptıklarını (Koçyiğit ve ark. 2015); Edirne ilinde işletmelerin ise % 61.4'ünün 15-16 aylık yaşta düvelerini tohumladıkları bildirilmektedir. (Önal ve Özder 2008). Yurt dışında yapılmış olan bir çalışmada; Sırbistan'daki süt sığırcılığı yapan işletmelerin düveleri 15-16 aylık olduklarında ilk tohumlamalarının yapıldığı ve bu düvelerin ilk buzağılama yaşlarının 24-25 ay olduğu bildirilmiştir (Bogdanovic et al. 2012).

Araştırma bölgesindeki yetiştiricilerin (% 96.2) büyük

çoğunluğunun gebe ineklere septisemi aşısı yaptırdığı tespit edilmiştir (Şekil 4). Literatür bildirişlerinde bu oranın % 10.0-% 60.0 arasında olduğu rapor edilmiştir (Koçyiğit ve ark. 2021; Demirhan ve Yenilmez, 2019; Savaş ve Yenice 2016; Ünalan ve ark. 2013). Duguma et al. (2012) sığırcılık işletmelerinde buzağı ve gebe ineklere septisemi uygulamasının düzenli olarak yapılmadığını bildirmişlerdir. Yapılan çalışma literatür bildirişlerinden yüksek bulunmuştur. Bu olumlu durum söz konusu araştırma bölgesindeki yetiştiricilerin bu uygulamayı benimsedikleri ve önem verdiklerini göstermektedir.



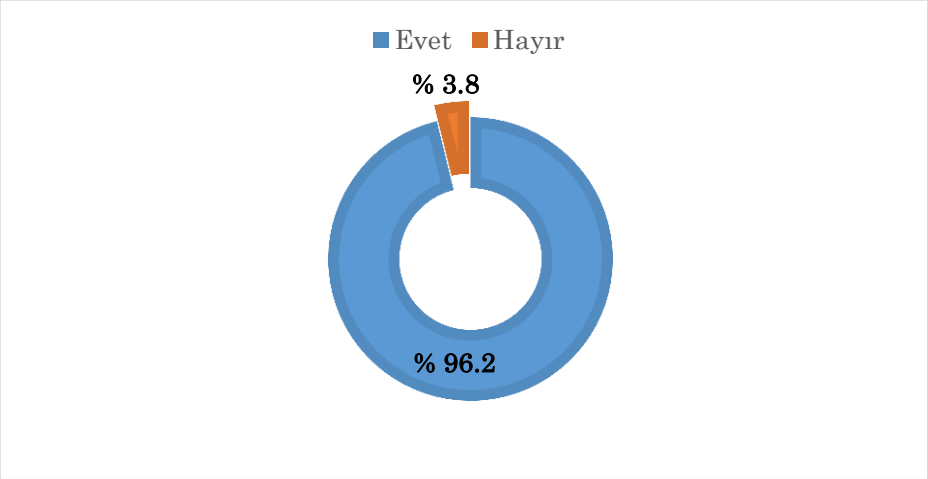
Şekil 2. İnekler doğumdan ne zaman sonra tohumlanıyor?
Figure 2. *How long after birth are cows inseminated ?*



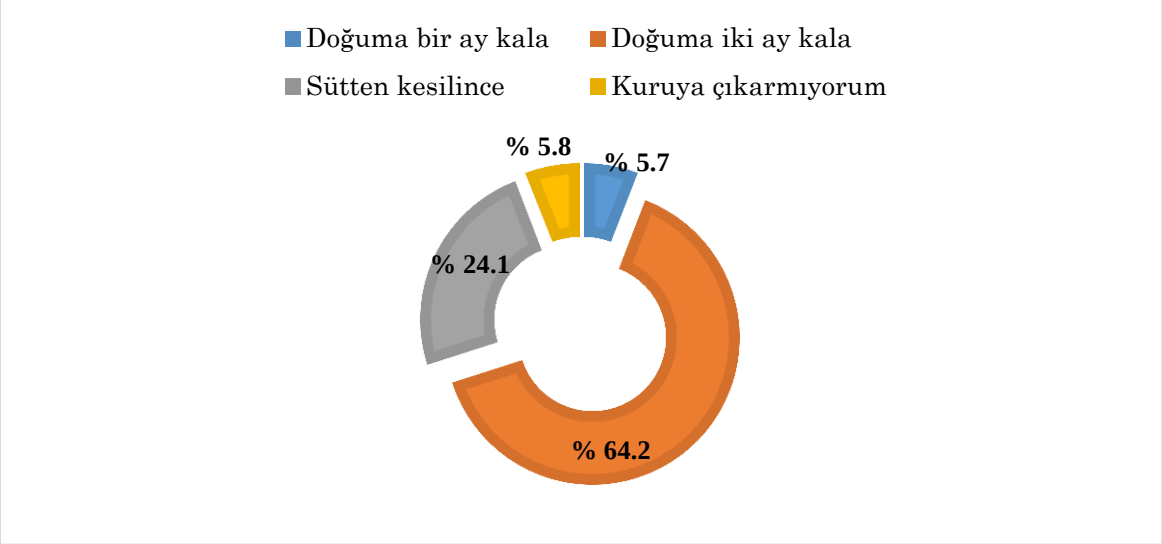
Şekil 3. Düveleri ilk ne zaman tohumlanıyor?
Figure 3. *When are the heifers first inseminated?*

Yetiştiricilerin % 64.2'si hayvanlarını doğuma iki ay kala kuruya çıkarıyor olması yetiştiricilik açısından beklenen ve istenen bir durumdur. Ancak işletmelerin yaklaşık % 36.0'lık kısmının henüz bu uygulamayı benimsemediği anlaşılmaktadır (Şekil.5). Sığır yetiştiriciliğinde teknik anlamda gebe ineklerin

kuruya çıkması normal şartlarda doğuma iki ay kala yapılmalıdır (Savaş ve Yenice 2016; Özhan ve ark. 2012). İşletmelerin yaklaşık 1/3'lik kısmının hayvanlarını kuruya çıkarma konusunda yetersiz bilgilere sahip oldukları ve bunun sonucu olarak bu uygulamalarda hatalar yapıldığı ifade edilebilir. Doğuma 2 ay kala kuruya çıkan hayvanlarda sonraki verim döneminde, sağlığında bir sorun beklenmezken, kuruya geç çıkan veya doğuma kadar sağılan ineklerin kondisyonlarının düşük olması, zayıf ve yaşama gücü düşük buzağıların elde edilmesi ve süt veriminin düşmesi gibi olumsuz durumlara neden olmaktadır. Bu nedenlerle doğuma iki ay kala ineklerin kuruya çıkarılmasının yetiştiriciye benimsetilmesinin gerekli olduğu ifade edilebilir.



Şekil 4. Gebe ineklere septisemi aşısı yapılması
Figure 4. *Vaccination status of pregnant cows for septicemia*

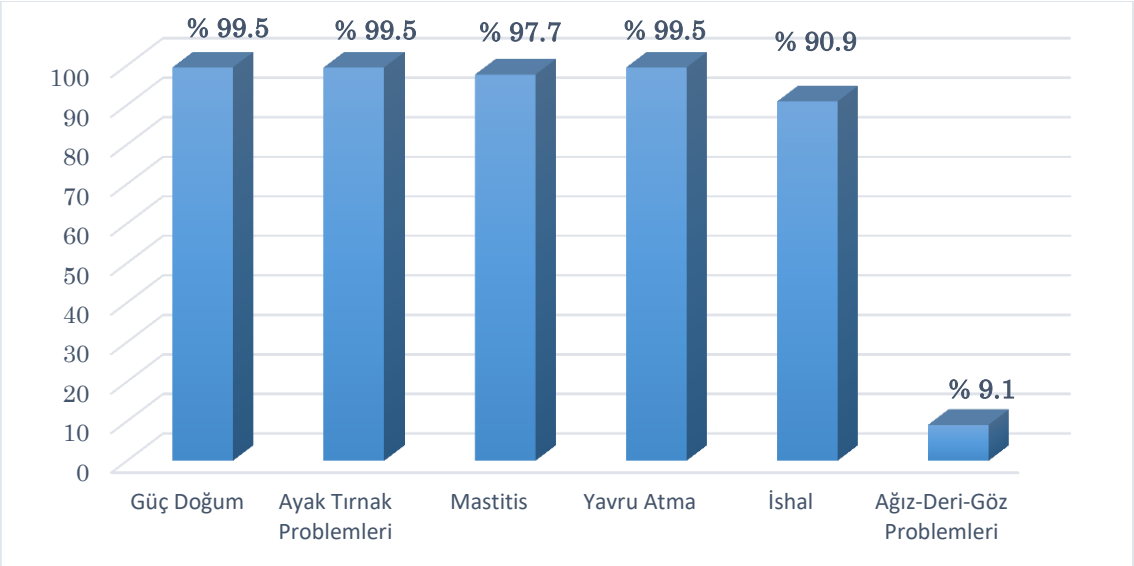


Şekil 5. İnekler ne zaman kuruya çıkıyor?
Figure 5*. When are the cows dired off?*

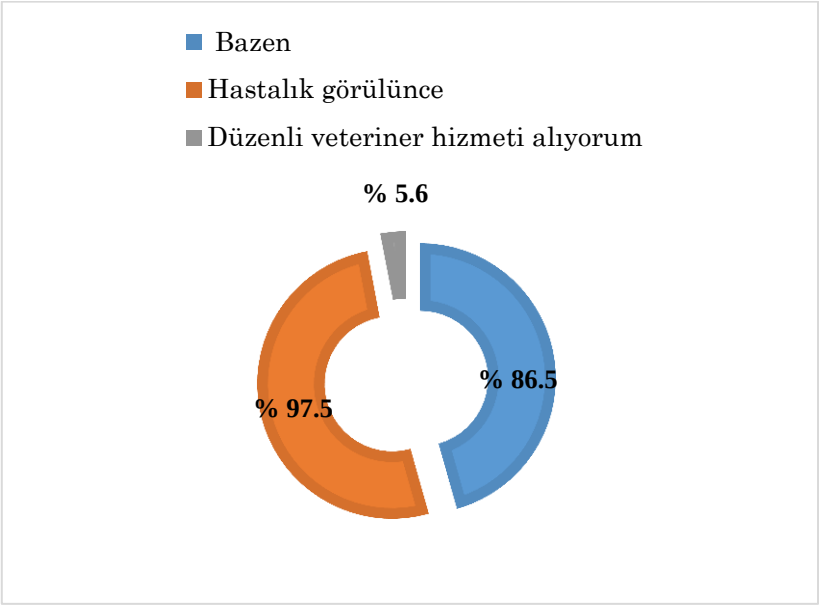
Erzurum ili İspir ilçesindeki sığırcılık işletmelerinde yetiştiricilerin ahırlarında hem birden fazla hastalıkların olması ve hem de çok yüksek oranlarda bu hastalıkların görülmesi ciddi anlamda dikkat çekilmesi gereken önemli bir durumdur (Şekil 6). Hayvanlarda canlı ağırlık kaybına, ağırlık artışında azalmaya, üretimden erken çıkmaya, laktasyon süresi ile süt veriminde azalmaya, tedavi giderlerinin artmasına ve infertilite gibi birçok sorunlara neden olan çeşitli hastalıklar işletme ekonomisini olumsuz olarak etkilemektedir (Kibar ve Bakır, 2019).

Koçyiğit ve ark. (2016) işletmelerde en çok görülen problemlerin başında %73.0 oranıyla güç doğum; Şeker ve ark. (2012) ile Öztürk, (2009) ayak ve tırnak problemleri, Tatar (2007) tarafından ise mastitis olduğu rapor edilmiştir. Güç doğumun işletmelerdeki en büyük problem olduğunu bildiren Köse (2006) % 60.0, Kaygısız ve ark. (2008) % 36.0 ve Tugay ve Bakır (2008) ise % 22.5 oranlarını rapor etmişlerdir. Diğer bir çalışmada ise en sık görülen hastalıkların %

50.0 ile şap, % 26.0 ile brusella, % 8.5 ile mastitis, olduğu bildirilmiştir. Özyürek ve ark. (2014). Yurt dışındaki çalışmalarda Duguma et al. (2012) sığırcılık işletmelerinde en çok görülen hastalıkların % 35.2 ile mastitis olduğu belirtilirken, başka bir araştırmada ise Heinrichs et al. (1987) Pensilvanya'daki süt sığırı işletmelerinde, buzağılarda görülen en yaygın sağlık probleminin % 39.2 oranıyla ishalin ve süt ırkı düvelerde ise en yaygın sağlık probleminin % 9.8 ile solunum rahatsızlıkları olduğunu tespit etmişlerdir. İşletmelerde düzenli olarak veterinerlik hizmeti alan işletme sayısı oldukça düşük (% 5.6) olup sadece hastalık görüldüğünde bu hizmeti alan işletmelerin oranının %97.5 olduğu belirlenmiştir (Şekil. 7). Ayrıca yetiştiricilerin tamamına yakını veterinerlik hizmetini Tarım il-ilçe müdürlüklerinden (% 99.7) aldıkları tespit edilmiştir.



Şekil 6. İşletmelerde yaygın görülen hastalıklar
Figure 6. *Common diseases in enterprises*



Şekil 7. Veterinerlik hizmeti alma durumu
Figure 7. *Status of receiving veterinary services*

İşletmelerin sadece hastalık görülünce veteriner hizmeti aldıkları ve bu oranları Özyürek ve ark. (2014) %73.3, Şeker ve ark. (2012) % 57.7, Akkuş (2009) % 79.0 ve Öztürk (2009) ise % 70.0 olarak bildiren yazarlardan daha yüksek oranda olduğu görülmüştür.

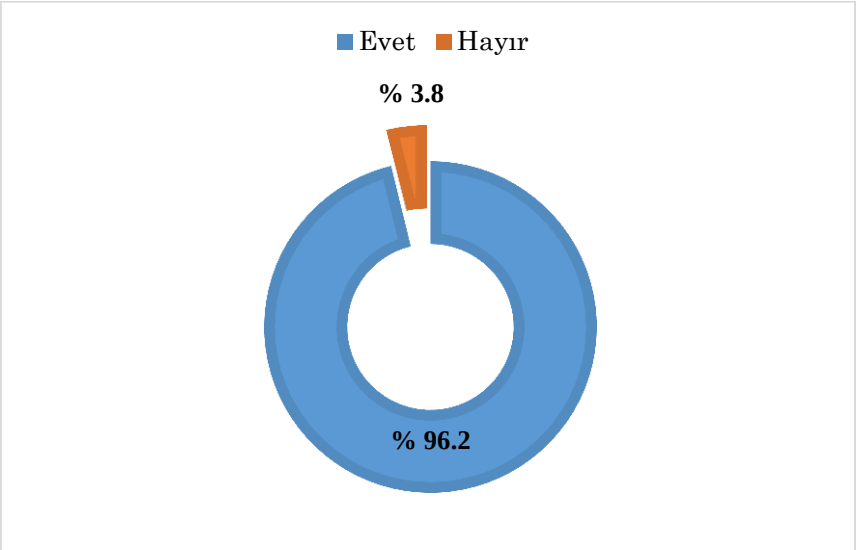
Çalışmadan farklı olarak veterinerlik hizmetini özel veteriner hekimlerden alanların oranlarının daha fazla olduğunu ifade eden literatür bildirişleride bulunmaktadır. Nitekim Tugay ve Bakır (2008) veteriner sağlık hizmetini işletmelerin % 55.3'ünü özel veteriner hekimlerden ve % 36.7'sinin ise

devletten alındığını bildirmişlerdir. Yine Şeker ve ark. (2012) sağlık hizmetini en çok serbest veteriner hekimden alan işletmelerin % 77.4 olduğunu rapor etmişlerdir. Kaygısız ve ark. (2008)'da veterinerlik hizmetini işletmelerin % 71.0'i özel veteriner hekimden ve % 29.0'unun ise devletten aldıklarını ifade etmişlerdir. Duguma et al. (2012) yaptıkları bir araştırmada Etyopya'nın Jimma şehrindeki işletmelerin, % 13.0'ünün veteriner sağlık hizmeti aldıklarını rapor etmişlerdir. Aynı araştırıcılar tarafından bu hizmeti yetiştiricilerin % 37.0'sinin yarı zamanlı olarak özel veteriner hekimlerden, % 25.9'u hem ziraat ve veteriner fakültesinden hem de özel veteriner hekimlerden, % 24.1'i veteriner fakültelerinden ve sadece % 13.0'ünün ise Tarım ve Orman Bakanlığı'ndan aldıkları ifade edilmiştir.

Buzağı Yetiştirme Uygulamaları

Anket yapılan işletmelerde buzağı yetiştirme uygulamalarında; buzağılara septisemi aşısı uygulaması, yeni doğan buzağıların anneleri ile kalma süreleri, buzağılara ağız sütü verme ve verilme süreleri, ağız sütünün verilme şekli, buzağıların sütten kesim yaşları gibi konular değerlendirilmiştir. Doğumdan sonra buzağılara göbek bakımını tüm işletmelerin (% 100) yaptığı tespit edilmiştir. Bu uygulamaların buzağıların sağlıklı şekilde doğması ve patojen mikroorganizmaların göbek bağından girişinin önlenmesi bakımından önemlidir. Göbek bakımı konusundaki çalışma bulguları Özyürek ve ark. (2014) (% 85.7) ile Ünalan ve ark. (2013)'nın (% 72.9) bildirişlerinden yüksek bulunmuştur.

Yapılan çalışmada işletmelerin tamamına yakını buzağılara septisemi aşısı (% 96.2) yaptırdığı belirlenmiştir (Şekil. 8).



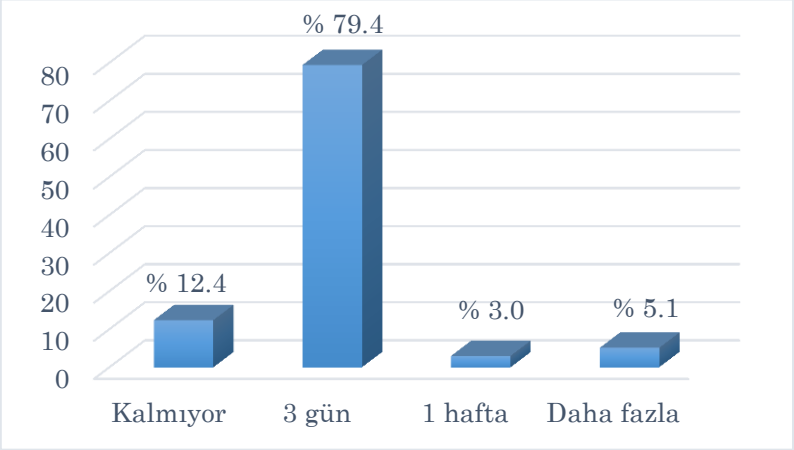
Şekil 8. Buzağılara septisemi serumu uygulaması
Figure 8. *Application status of septicemia vaccine to calves*

Buzağılara septisemi aşısı yaptırma oranı yapılan çalışmalarda %32.0-% 64.0 arasında değiştiği tespit edilmiştir (Kibar ve Bakır, 2019; Koçyiğit ve ark. 2018; Savaş ve Yenice, 2016; Koçyiğit ve ark. 2016; Ünalan ve ark. 2013). Çalışma bulguları literatür bildirişlerinden yüksek bulunmuş olup bu konuda yetiştiricilerin bilinçli olduğu ve bu uygulamadan memnun kaldıkları düşünülmektedir.

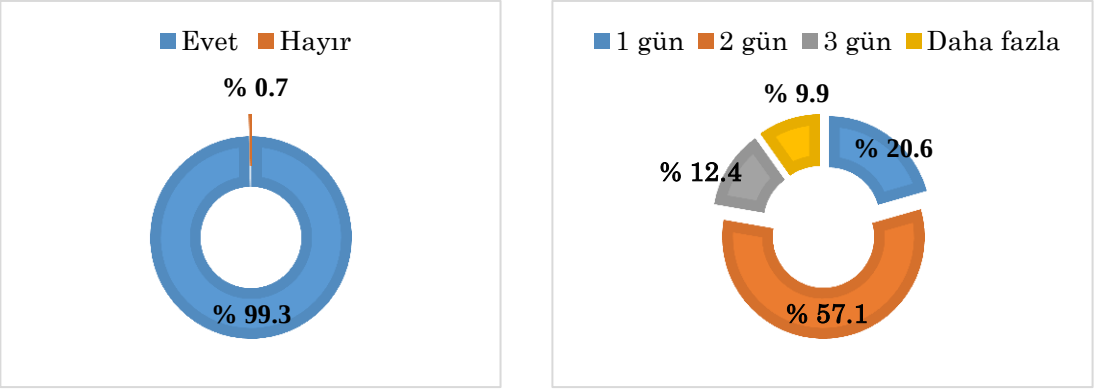
Buzağıların anneleriyle kalma süreleri bakımından (Şekil 9) genel itibariyle işletmelerin buzağıları üç gün anneleriyle birlikte (% 79.4'ü) bulundurdukları tespit edilmiştir. Çalışmada yeni doğan buzağıların analarıyla birlikte kalma süresinin yapılan çalışmalardan daha kısa olduğu söylenebilir. Nitekim Erzurum'un farklı ilçelerinde yapılmış araştırmalarda Diler ve ark. (2017) buzağıların analarıyla birlikte bir hafta kalanların % 61.0, üç gün kalanların % 26.0 ve bir haftadan daha fazla süreyle kalanların ise % 13.0 oranında olduğunu bildirmişlerdir. Koçyiğit ve ark. (2015) ise yeni doğan buzağıların %57.0'sinin analarıyla birlikte bir haftadan daha fazla, %24.0'ünün bir hafta ve %19.0'unun ise üç gün süreyle birlikte kaldıklarını ifade etmişlerdir. Yurt dışında yapılmış çalışmalarda ise Vasseur et al. (2010) işletmelerin çoğunlukla (%73.2) doğum sonrası ilk 12 saatlik dönemde, geri kalan kısmın (%32.5) ise doğumdan hemen 2 saat sonra analarından ayrıldığı bildirilmiştir. Hötzel et al. (2014) işletmelerin %71.3'ü doğumdan 12 saat sonra, %17.8'i 24-72 saat ve %10.9'ü 72 saatten fazla buzağıların anneleriyle kaldıklarını rapor etmişlerdir.

Şekil 10'da işletmelerin tamamına yakını (% 99.3) buzağılara kolostrum verdiği belirlenmiştir. Yetiştiricilerin yarıdan fazlası (% 57.1) iki gün ve % 12.4'ü üç gün boyunca doğan buzağılara kolostrumu verdikleri (Şekil 10) belirlenmiştir. Erzurum ili Narman (Diler ve ark. 2017) ve Hınıs ilçelerinde (Koçyiğit ve ark. 2015) yapılan çalışmalarda ağız

sütünü veren işletmelerin sırasıyla % 53.0 ve % 75.0 olarak, ağız sütünün verilme süresi ise 3 gün veren işletmelerin oranı sırasıyla % 16.0 ve % 21.0 olarak bildirilmiştir. Erzincan ilinde yapılan bir araştırmada kolostrumu veren işletmelerin % 98.9 olduğu ve ağız sütünü 3 gün boyunca veren işletmelerin ise % 90.8 olduğu rapor edilmiştir (Özsağlıcak ve Yanar 2021).



Şekil 9. Yeni doğan buzağıların anneleri ile kalma süreleri
Figure 9. *The time newborn calves spend with their dams*



a b

Şekil 10. Buzağılara ağız sütü (a) verme ve verilme süresi (b)
Şekil 10. *The status of feeding calves with colostrum (a) and duration of colostrum feeding (b)*

Çalışma bulguları ağız sütü verme oranı bakımından literatür bildirişlerinden yüksek olmakla birlikte 3 gün ağız sütü verilme süresi bakımından düşük düzeyde kaldığı söylenebilir. Mevcut araştırmada ağız sütü verme konusunda işletmelerin bu uygulamayı benimsedikleri, ancak verilme süresi bakımından yetersiz olduğu düşünülmektedir.

İşletmelerin yaklaşık % 75.4'ü buzağıların ağız sütünü annelerini emme yoluyla aldıklarını, % 21.3'ünün ise biberonla aldıklarını ifade etmişlerdir (Şekil 11). Yapılan araştırmalarda işletmelerin %57.0-% 92.0 oranları arasında yani genellikle ağız sütünü buzağıların annesini emerek aldığı bildirilmiştir (Kurt ve ark. 2020; Demirhan ve Yenilmez 2019; Diler ve ark. 2017; Savaş ve Yenice 2016; Koçyiğit ve ark. 2015). Ağız sütünün verilme şekli bakımından yapılmış yabancı çalışmalarda ise biberon ve kova ile vermenin yaygın olduğu görülmektedir. Hannien et al. (2007) işletmelerin %51.3'ünün biberon ile, % 36.5'inin kova ile ağız sütü verdiklerini, Vasseur et al. (2010) ise işletmelerin % 92.0'sinde sütün kovalarda, %17.7'sinde ise emzikli biberonlarda buzağılara verdiklerini rapor etmişlerdir. Başka bir çalışmada Hötzel et al. (2014) kolostromu buzağılara verilme şekli bakımından işletmelerin % 54.6'sı annelerini emerek ve % 45.4'ü ise biberonla verildiğini rapor etmişlerdir.

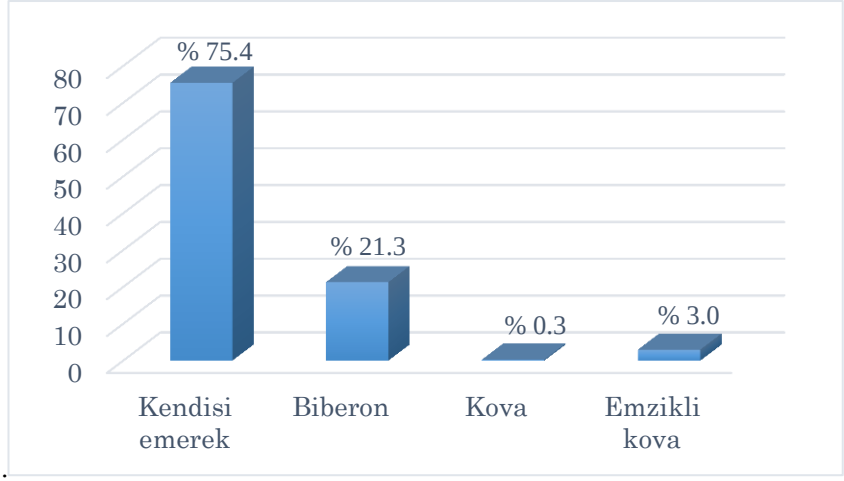
Şekil 12'de görüldüğü üzere buzağıların sütten kesilme süreleri olarak yetiştiricilerin büyük bir kısmı (% 81.7) buzağıları 1-3 ay arası sütten kestikleri, işletmelerin az bir kısmının ise (% 16.8) 4-6 ay arasında sütten kestikleri belirlenmiştir.. Sütten kesim işlemi buzağının en azından 500 g kesif yem tüketmesi ve sağlıklı olması halinde gerçekleştirilmelidir (Özhan ve ark. 2012; Tüzemen ve Yanar 2013). Araştırmaya konu teşkil eden diğer çalışmalarda genel olarak buzağıları 2-3 ay arası sütten kesen işletmelerin oranlarının % 47.5-% 91.0

arasında olduğu bildirilmiştir (Savaş ve Yenice 2016; Hozman, 2014; Tugay ve Bakır 2008; İnal, 2014, Hötzel et al. 2014).

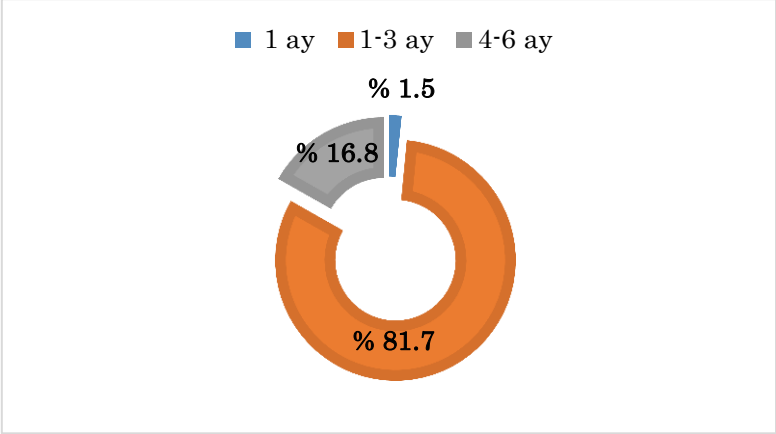
SONUÇ ve ÖNERİLER

Erzurum ili, İspir ilçesindeki Süt sığırcılığı işletmelerinin mevcut durumunu saptamak sığır yetiştiriciliği uygulamalarını incelemek amacıyla bu araştırma yapılmıştır. Yetiştiricilerin sığır ve buzağı yetiştirme uygulamalarındaki eksiklikleri ve yanlış uygulamaları şu şekilde sıralanabilir.

Yetiştiricilerin % 33.5'inin ineklerin doğumdan sonraki ilk kızgınlıkta tohumladıkları tespit edilmiştir. Özellikle doğum yapmış bir ineğin bir sonraki doğuma kendini hazırlayabilmesi için doğumdan sonraki 45-60. günlerde meydana gelen kızgınlıklarda ineklerin tohumlanması gereklidir.



Şekil 11. Ağız sütünün verilme şekli
Figure 11. *Feeding types of colostrum*



Şekil 12. Buzağıların sütten kesim yaşları
Figure 12. *Weaning ages of calves*

Düvelerin ilk tohumlama yaşları olarak işletmelerin % 72.1'i 24 aylık yaşta, % 26.6'si ise 18 aylık yaşta tohumladıkları saptanmıştır. Yetiştiricilikte uygun bakım ve besleme koşullarında kondisyonlarının ve sağlık durumlarının iyi olması durumunda ırk özelliklerine göre hayvanlar ergin canlı ağırlığının % 70-75'ine ulaştığında tohumlanabilmektedir. Modern işletmelerde düvelerin 15 aylıkken tohumlama yapılabileceği ve genellikle düvelerin 24 aylık yaşta ilk yavrularını verdikleri bilinmektedir.

İspir ilçesindeki işletmelerin % 28.0'lik kısmı gebe ineklere septisemi aşısı yaptırmaması ve ahırlarında birden fazla hastalıkların çok yüksek oranlarda görülmesi ilçedeki sığırcılık işletmelerinde hayvan sağlığında ve veterinerlik hizmetlerini (düzenli veteriner hizmeti alan işletmeler % 5.6) alma konusunda ciddi anlamda sıkıntıların olduğu saptanmıştır.

Yetiştiricilerin % 57.1'i buzağılarına iki gün kolostrum verdikleri ve işletmelerin % 75.4'ü buzağıların ağız sütünü annelerini emerek aldıkları tespit edilmiştir. Buzağılarını 1-3 aylık yaşta sütten kesen işletmelerin oranı % 81.7 olduğu belirlenmiştir. Buzağıların sağlıklı bir şekilde yetiştirilmesi ve

büyütülmesi için doğan buzağıların kolostromu ilk üç gün süreyle alması gereklidir. Buzağıların bağışıklık sisteminin gelişmesi ve zararlı patojenlere karşı kendi savunması için ağız sütü mükemmel bir gıdadır. Buzağı yetiştiriciliğinde normal sütle besleme için canlı ağırlığının % 10'u kadar süt verilmesi buzağının aşırı derece süt içmesinden kaynaklanan hastalıkların önlenmesinde hem de ekonomik öneme sahip sütün fazlaca tüketilmesinin önüne geçilmesine yardımcı olacaktır. Sütün yanında katı yemlere başlanması ve buzağı 2-3 haftalık olduğunda kaba ve kesif yem verilmesi yetiştiricilik açısından uygulanması gereklidir. Buzağının genel itibariyle günlük 500-1000 g arasında kesif yem tüketmesi ile sütten kesme işlemi uygulanabileceği bilinmektedir.

Sonuç olarak İspir ilçesindeki sığır ve buzağı yetiştirme uygulamalarında yukarda belirtilen konularda yetiştiricilerin bilinçlendirilmesi gereklidir. Bunun için birlikler,üniversiteler ve tarımla ilgili kurum ve kuruluşlarla iş birliği yaparak çiftçilere yetiştirme ve besleme konularında danışmanlık, yayım ve eğitim hizmetleri, hayvan sağlığı, suni tohumlama ve kayıtların tutulması gibi konularda eğitim faaliyetlerinin planlanarak düzenli bir şekilde bu bilgilendirilmelerin yapılması önerilmektedir.

Araştırmacıların Katkı Oranı Beyan Özeti

Yazarlar makaleye eşit oranda katkı sağlamış olduklarını beyan eder.

Çıkar Çatışması Beyanı

Makale yazarları aralarında herhangi bir çıkar çatışması olmadığını beyan ederler.

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TARSİM Büyükbaş Hayvan Hayat Sigortasına Yetiştiricilerin Yaklaşımı ve Risk Faktörlerinin Belirlenmesi

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ÖZET

Bu çalışma Türkiye'de de tarım sektörünü tehdit eden risklerin teminat altına alınması için 14.06.2005 tarihli ve 5363 Sayılı "tarım sigortaları kanunu" ile oluşturulan "tarım sigortaları havuzu (TARSİM)"nin iş ve işlemlerini yürütmek üzere kurulan "Tarım Sigortaları Havuz İşletmesi A.Ş." altında hizmet sunan büyükbaş hayvan hayat sigortası (BHHS)'nın kullanımı ve yaygınlaştırılması açısından yetiştirici görüşlerini almak, yaşanan problemleri belirlemek ve çözüm önerileri sunmak için yapılmıştır. Bu amaçla Covid-19 pandemi koşullarında 2021 yılında Basit Tesadüfi Örnekleme yöntemiyle belirlenen 173 yetiştirici ile online anket yapılmıştır. Yetiştiricilerin şahsi Whatsapp hatlarına anket soruları Damızlık Sığır Yetiştiricileri Birlikleri ile birlikte yapılmıştır. Veriler IBM SPSS İstatistik 20.0 paket programı ile değerlendirilmiştir. Değerlendirmede BHHS'nın yaygın kullanılmamasının ilk beş nedeni sırasıyla; yetiştirici gelirinin yetersiz ve düzensiz olması (%19.3), Poliçe bedelinin yüksek olması (%15.6), BHHS hakkında yeterli bilgi sahibi olunmaması (%11.0), hasar tutarının yetersiz olması (%10.7) ve sigorta poliçesi kapsamının yetersiz olması (%10.2) olarak tespit edilmiştir. Sonuç olarak; tarım sektörü ve dolayısıyla hayvancılık sektörü stratejik öneminin yanında iklim, ekonomik, sosyal, siyasal, teknolojik ve kişisel risklerden yüksek düzeyde etkilenen, kendine özgü yapısı nedeniyle üretiminde sık sık riskle karşı karşıya kalınan bir sektördür. Bununla birlikte Küresel ısınmayla meydana gelen iklim değişikliği ve kuraklık nedeniyle üretimi sık sık risk ile karşı karşıya kalması muhtemel olan bu sektör içinde, büyükbaş hayvancılığın sürdürülebilirliği için BHHS imkanından daha fazla yetiştiricinin faydalanmasını sağlamak ve teşvik etmek için sigorta poliçesinde; birim hayvan başına sigorta bedelinin makul bir seviyeye çekilmesi, sigorta poliçe bedelinde indirime gidilmesi ve gerekli diğer bazı düzenlemelerin yapılmasına ihtiyaç vardır.

Hayvan Yetiştirme

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Anahtar Kelimeler
Sığır
Hayvan hayat sigortası
Yetiştirici
Hastalık
Risk

Determination of Risk Factors and Breeders Approach for TARSIM Cattle Life Insurance

ABSTRACT

This study was carried out to obtain the opinions of breeders on the use of "cattle life insurance", to identify the problems they experienced and to offer solutions that will enable the breeders to use "cattle life insurance" widely. "Agricultural insurance pool (AIP)", which was created within the scope of the "agricultural insurance law" with the decision dated as 14.06.2005 and numbered as 5363 to guarantee the risks threatening the agricultural sector in Turkey, is managed by the agricultural insurance pool management incorporated company (AIPMIC). Similarly, "cattle life insurance (CLI)" carries out its activities within scope of AIPMIC. In this study, an online survey was conducted with 173 breeders determined by Simple Random Sampling Method in 2021 under Covid-19 pandemic conditions. Questionnaires were sent to personal

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Whatsapp lines of breeders by Cattle Breeders' Associations. The data were evaluated with IBM SPSS Statistic 20.0 package program. The top five reasons not to use widely of CLI were determined as insufficient income of grower (19.3%), high policy price (15.6%), insufficient information about CLI (11.0%), insufficient amount of damage (10.7%), and insufficient insurance policy coverage (10.2%). As a result; in addition to its strategic importance, the agricultural sector, and therefore the livestock sector, is a sector that is highly affected by climate, economic, social, political, technological and personal risks, and is frequently faced with risks in its production due to its unique structure. However, in this sector, whose production is likely to face risks due to climate change and drought caused by global warming, in the insurance policy to ensure and encourage more breeders to benefit from the BHHS facility for the sustainability of cattle breeding; there is a need to reduce the insurance cost per unit animal to a reasonable level, to reduce the insurance policy cost and to make some other necessary arrangements.

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GİRİŞ

Tarım sektörü, dünya nüfusu açısından taşıdığı kritik önemin yanı sıra ekonomik, sosyal, siyasal, teknolojik ve kişisel risklerden yüksek düzeyde etkilenen, son derece hassas bir faaliyet sahası olarak kendine özgü bir yapıya sahiptir. Tarımsal ürünlerde dışa bağımlılığın uzun vadede ülkeleri çok önemli bedeller ödemek zorunda bıraktığı bilinmektedir. Tarımsal ürünlerde dışa bağımlılığı azaltmak ya da tamamen ortadan kaldırmak miktar ve kalite olarak üretimde sürdürübelilirliği sağlamakla mümkündür. Bu açıdan tarımsal üretimin ve tarım içerisinde hayvansal üretimin sürdürülebiliğini temin etmek, tarımsal ürünlerde dışa bağımlılığı azaltmak açısından stratejik bir öneme sahiptir. Bu açıdan bakıldığında, tarımın insanlığın beslenmesindeki fonksiyonunu etkili bir şekilde yerine getirmesi tarımsal üretimi tehdit eden risklerin yönetimiyle doğrudan ilişkilidir. Bu nedenle, gelişmiş ülkeler, uyguladıkları çeşitli korumacılık politikaları, "Risk Yönetim Programları" ve bu programlar içerisinde önemli bir yere sahip olan "Tarım Sigortaları Uygulamaları" ile risk paylaşımını ve transferlerini gerçekleştirmektedirler. Türkiye'de de bu amaçla 14.06.2005 tarihli 5363 Sayılı "Tarım Sigortaları Kanunu" çıkarılmıştır. Kanun kapsamında tüm iş ve işlemler bir standarda bağlanmış ve Sigorta Havuzu kurulmuştur. Havuz ile ilgili tüm iş ve işlemler, bu Havuza katılan sigorta şirketlerinin eşit hisselerle ortak oldukları Tarım Sigortaları Havuz İşletmesi A.Ş. tarafından yürütülmektedir. Sigorta şirketleri, tarım sigortası sözleşmelerini havuz adına ve havuz tarafından belirlenen standart poliçeler üzerinden yapar ve prim ile riskin tamamını havuza devreder. Devlet, bu Kanun kapsamında yapılacak sigorta sözleşmelerine münhasır olarak çiftçi adına sigorta primine destek sağlamaktadır (TARSİM, 2021).

Dünyada tarım sigortası ilkkez İrlanda da 18. yüzyılın ikinci yarısında kooperatiflerin elinde bulundurdukları hayvanlara hayvan hayat sigortası yapılması ile gerçekleşmiştir. Daha sonra 19. ve 20. Yüzyılda Avrupa ülkelerini mütaakip ABD ve Japonya gibi devletlerde geniş kapsamlı tarım sigortaları hayata geçirilmiştir (Güngör, 2006). Türkiyede ise, Tarım Sigortaları Kanunu ile amaçlanan; tarım sigortalarının tanıtılması ve yaygınlaştırılmasının sağlanması ile üreticilerin, doğal afetlerden ve diğer oluşabilecek risklerden korunması amacına yönelik, gerekli uygulamaları hatasız ve hızlı bir şekilde yürütmektir (TARSİM, 2020).

Yıllar göre tarım sigortası türlerine ait poliçe sayısı ve bedelleri itibariyle bitkisel üretimin en yüksek paya sahiptir (Çizelge 1). Bunu büyükbaş hayvan hayat, sera ve küçükbaş hayvan hayat sigortaları izlemektedir. Son 4 yıl içerisinde bitkisel ürün sigortasının payı %6.09 oranında gerilerken, Büyükbaş hayvan hayat ve küçükbaş hayvan hayat sigortaları sırasıyla, %4.7 ve %1.6 oranında artış göstermiştir. TARSİM tarafından 2020 yılında sigortalanan büyükbaş ve küçükbaş hayvan sayıları sırasıyla, 2.899.364 baş ve 7.453.871 baş olarak gerçekleşmiştir. TÜİK'in 2020 yılı verileri itibariyle büyükbaş hayvan valığının 18.2 milyon baş, küçükbaş hayvan varlığının 51.1 milyon baş olduğu dikkate alındığda, hayvan hayat sigortası ile sigortalanan büyükbaş ve küçükbaş hayvan oranları sırasıyla;

%15.97 ve %13.77'dir.

Büyükbaş ve küçükbaş hayvanlar, çeşitli hastalıklar, doğum ve cerrahi müdahaleler, zehirli çayır otlarına ve yeme bağlı zehirlenmeler, doğal afetler, güneş çarpması, yangın ve infilak durumuna bağlı ölümler ve zorunlu kesime karşı güvence altına alınmaktadır. Yetiştirici isteğine bağlı ve ilave ödeme yapmak kaydıyla poliçe kapsamı yavru atma ve yavru kayıpları, hırsızlık ve şap hastalığı rislerini de içerecek şekilde genişletilebilmektedir (TARSİM, 2020). Tarım sektörü, nüfusun bitkisel ve hayvansal gıda ihtiyacının karşılanması, kırsal alanda önemli bir istihdam oluşturması, gıda ve gıda dışı hayvansal ürünler sanayisinin ihtiyacı olan ham madde temini başta olmak üzere ülke ekonomisine ve kırsal kalkınmaya katkı sağlayan önemli bir sektör durumundadır. İklim şartlarına bağlı olarak üretim yapılması sebebiyle tarım, dünyanın her yerinde hassas ve stratejik bir sektör olarak değerlendirilmektedir (Sevim, 2010; Yazgı ve Olhan, 2018).

Çizelge 1. Yıllar itibariyle Sektörlere Göre Poliçe Sayısı ve Sigorta Bedeli (Tarsim, 2020)
Table 1. Number and Price of Insurance Policies by Sectors by Years (Tarsim, 2020)

	2017				2020			
	Poliçe Sayısı *(Policies Number)*		Sigorta Bedeli *(Insurance Price)*		Poliçe Sayısı *(Policies Number)*		Sigorta Bedeli *(Insurance Price)*	
Sigorta Türü	Adet *(Item)*	Payı *(Percent)*	Tutarı (TL) *(Price TL)*	Payı *(Percent)*	Adet *(Item)*	Payı *(Percent)*	Tutarı (TL) *(Price TL)*	Payı *(Percent)*
Bitkisel Ürün *(Herbal Product)*	1.493.392	93.44	18.654.875.618	61.56	1.952.825	87.35	39.305.360.888	47.27
Sera *(Greenhouse)*	24.139	1.51	4.594.633.662	15.16	34.252	1.53	13.168.520.249	15.84
Büyükbaş *(Cattle)*	54.856	3.43	5.441.028.015	17.96	181.773	8.13	21.785.083.383	26.20
Küçükbaş *(Small Ruminat)*	15.441	0.97	917.105.832	3.03	57.244	2.56	6.513.908.235	7.83
Kanatlı *(Poultry)*	561	0.04	150.229.204	0.50	2.060	0.09	1.138.079.723	1.37
Su Ürünleri *(Seafood)*	77	0.00	117.094.253	0.39	125	0.01	274.101.717	0.33
Arıcılık *(Beekeeping)*	9.803	0.61	428.381.275	1.41	7.347	0.33	960.995.550	1.16
Toplam *(Total)*	1598269	100.00	30.303.347.859	100.00	2235626	100.00	83.146.049.745	100.00

Türkiye'de hayvancılık sektörü gerek kırsal alanda istihdamın sağlanmasında gerekse de ulusal beslenmenin güvence altına alınması gibi önemli stratejik fonksiyonları üstlenmiştir. Böylesi hayati öneme sahip bir sektörde üreticilerin sigorta yaptırmama nedenlerinin belirlenmesi, bu konuda gerek üreticilerin gerekse diğer sektör paydaşlarının farkındalık kazanmaları sektör için hayati öneme sahiptir (Durgut ve Dumanoğlu, 2016; Mat ve ark., 2020).

Dünya ülkelerinde olduğu gibi Türkiye'de de tarımsal alanda faaliyet gösteren yetiştiricilerin sigorta yaptırmaması için birçok sebebin varlığından söz etmek mümkündür (Nahas ve ark., 2017). Tarımsal faaliyette bulunan üreticilerin eğitim durumu, sigorta bilgi düzeyleri, işletme gelir düzeyi, sigorta kültürünün olmaması gibi faktörlerin sigorta yaptırmaları konusunda etkili olduğu düşünülmektedir (Çevrimli ve Sakarya 2019, Merritt ve ark., 2017).

Yetiştiricilerin hayvan hayat sigortası (HHS) yaptırma oranlarının artırılması için HHS konusunda sürekli bilgilendirilmeleri gerekliliği tespit edilmiş olup, HHS yaptıran üreticilerin hasar tazmini dışında diğer hususlarda pozitif yönde bir takım ek destek ve teşvik, ilave puanlama, teşvik ödeme önceliği gibi uygulamalarında poliçe ve prim üretmede sisteme olumlu katkısının olabileceği bildirilmiştir (Mat ve ark., 2020).

Yazgı ve Olhan (2018) tarafından gelir sigortası, tarım sigortasının yaygınlaştırılması açısından hayata geçirilebilecek alternatif bir model olduğu ifade edilerek, üreticiler tarafından öne sürülen eksper kaynaklı sorunların giderilmesi, sistemin işleyişine yönelik bazı konuların açıklığa kavuşturulması ve sigorta prim miktarının aktüeryal çalışmalar esas alınarak üreticilerin beklentilerini karşılayacak şekilde hesaplanması gibi düzenlemelerin tarım sigortası yaptıran üretici sayısını artıracak unsurların başında yer aldığı,

bununla birlikte sigorta primlerine verilen devlet desteğinin artırılması sigortalı olma oranını artıracağını ifade edilmiştir.

Tarımla geçimini sağlayan bireylerin yerinde kalması, tarımsal faaliyete devam etmesi ve kırsal alandan şehirlere göçün önlenmesi bakımından çiftçilerin gelir dalgalanmalarının en aza indirilmesi gerekmektedir. Bu sebeple yalnız bitkisel ürün sigortası değil büyükbaş ve küçükbaş hayvan hayat sigortalarının da yaygınlaştırılması hedeflenmelidir. Bununla birlikte tarımsal üretimle ilgili sigorta yaptırmayanların sigorta yaptırmama ve sigorta yaptıranların tekrar sigorta yaptırmama nedenleri araştırılmalıdır. Söz konusu araştırmaların sonuçlarına göre yeni politikalar oluşturulmalıdır (İkikat Tümer ve ark., 2019). Türkiyede 1994-2020 yılları arasında HHS konulu toplam 9 adet bilimsel çalışma yapıldığı bildirilmiştir (Akgün, 2021).

Yetiştiricilerin üretim faaliyetlerinde bulunurken tek başlarına risklerin üstesinden gelemeyecekleri doğaldır. Ancak yetiştiricilerin yetiştirdikleri hayvanlarda sıklıkla yaşadıkları hastalık ve doğal afet kaynaklı problemler karşısında BHHS desteği olmadan hayvansal üretimi sürdürülebilir kılmaları mümkün görünmemektedir.

Bu çalışmada, büyükbaş hayvancılık alanında BHHS kullanımı konusunda yetiştiricilerin görüşlerini ortaya koyarak, BHHS'nın yaygınlaştırılması açısından yaşanan problemlerin çözümüne yönelik önerilerde bulunmak amaçlanmıştır.

MATERYAL ve METOD

Bu çalışmada, BHHS kapsamında hayvanlarını güvence altına alan yetiştiricilerin işletmelerinde hayvancılıkla ilgili karşılaştıkları problemler ve devletten beklentilerini araştırmak için söz konusu yetiştirici görüşleri için yapılan anket çalışması materyal olarak kullanılmıştır. Anket çalışması, Covid-19 pandemi koşullarında 2021 yılında Basit Tesadüfi Örnekleme yöntemiyle belirlenen 173 yetiştirici ile çevrimiçi olarak gerçekleştirilen anket çalışması değerlendirmeye esas teşkil etmiştir. Anket çalışmasında yetiştiriciler, büyükbaş hayvan hayat sigortası yaptırıp yaptırmadığına bakılmaksızın şansa bağlı olarak tesadüfi olarak seçilmiştir.

Örnekleme Yöntemi

Çevrimiçi anket formu konusunda bilgilendirme ve anket formunun şahsi Whatsapp hatlarına gönderimi ve anket konusunda yetiştiricilerin bilgilendirmeleri konularında Damızlık Sığır Yetiştiricileri Birlikleri ile birlikte çalışılmıştır.

Anket sayısının belirlenmesinde, anket yöntemiyle veriler toplandığında tam sayım yoluyla elde edilen bilgiler daha doğru sonuçları yansıttığından; popülasyon küçükse ve istenilen bilgiye ulaşmak kolay ve ucuzsa tam sayım yapılmalıdır (Çiçek ve Erkan, 1996; Yamane, 2010). Aksi takdirde, toplam işletme sayısını gösteren N (popülasyon büyüklüğü) bilinmesine rağmen, bölgede detaylı çalışmaların yapılmadığı durumlarda ve standart sapma ve varyans değerlerinin bilinmediği durumlarda anket sayısını belirlemek için basit tesadüfü örnekleme yöntemi kullanılabilir (Yamane, 2010). Bu nedenle Eşitlik 1'de verilen örnekleme formülü kullanılmıştır. Söz konusu metod bir çok araştırmacı tarafından da kullanılmıştır (Topçu ve ark., 2012; Karadaş ve ark., 2015; Karadaş, 2018)

Araştırmanın popülasyonunu BHHS yaptırmasına bakılmaksızın, 2021 yılında 81 il'de Damızlık Sığır Yetiştiricileri Birliklerine kayıtlı 125,334 adet işletmeden örnek büyüklüğü aşağıdaki eşitlik yardımıyla belirlenmiştir (Eşitlik 1).

$$n = \frac{N.t^2.p.q}{(N-1)D^2 + t^2.p.q} \quad (1)$$

n= Örnek büyüklüğü
N= İşletme sayısı
D= Kabul edilen veya arzu edilen örnekleme hatası
t= Tablo değeri
p= Hesaplanması istenen oran
q=1-p

$$n = \frac{125334\,.\,2{,}57^2.\,0{,}5\,.0{,}5}{(125334-1).\,0{,}1^2 + 2{,}57^2.\,0{,}5\,.\,0{,}5} = 165$$

Örneklem sayısı 165 adet olarak tespit edilmiş olup, %5 fazlası alınarak 173 işletme olarak belirlenmiştir. İşletme seçimi tamamen tesadüfi olarak gerçekleştirilmiş olup, işletme seçiminde yetiştiricilerin BHHS yaptırıp yaptırmamasına bakılmamıştır. Anket çalışmasına yetiştiriciler Adana, Afyonkarahisar, Aksaray, Ankara, Antalya, Ardahan, Aydın, Balıkesir, Bingöl, Bolu, Burdur, Bursa, Çanakkale, Denizli, Diyarbakır, Elazığ, Erzurum, Eskişehir, Giresun, Hakkâri, Hatay, Isparta, İstanbul, İzmir, Kahramanmaraş, Kars,Kastamonu, Kayseri, Kırklareli, Kırşehir, Kilis, Kocaeli, Konya, Malatya, Manisa, Mersin, Muğla, Muş, Niğde, Samsun, Sinop, Şırnak, Tekirdağ ve Yozgat illeri olmak üzere toplam 44 İl'den katılım sağlanmışlardır.

İstatistik Analizler

Bilgiler Excel elektronik tablo programı yardımıyla düzenlenerek analize hazır hale getirilmiştir. Çalışmada yetiştirici görüşlerini almak amacıyla 12 sorudan oluşan çevrimiçi anket linkinin yetiştiricilerin kişisel Whatsapp hatlarına gönderimi yetiştiricilerin üyesi bulunduğu Damızlık Sığır Yetiştiricileri Birlikleri ile müşterek olarak gerçekleştirilmiştir. Anket çalışması gönüllü olan 173

işletme sahipleri ile yapılmıştır. Online anket linkinin yetiştiricilerin Whatsapp hatlarına gönderimi üyesi bulunduğu Damızlık Sığır Yetiştiricileri Birlikleri tarafından gerçekleştirilmiştir. Anket sorularına cevap veren 173 yetiştiricinin verdikleri cevaplar Excel programı yardımıyla düzenlenerek, her bir soru kategorisi için tanımlayıcı istatistiki değerlerin analizi amacıyla IBM SPSS İstatistik 20.0 paket programı kullanılarak analiz edilmiştir. Birden fazla seçeneğinin işaretlenmesine izin verilen (çoklu yanıt) soruların deskriptif analizi SPSS programı "Analyze" menüsü altında "Multiple Response" alt menüsü kullanılarak yapılmıştır.

Çalışmanın gerçekleştirilmesi için Kahramanmaraş Sütçü İmam Üniversitesi Rektörlüğü, Fen ve Mühendislik Bilimleri Etik Kurulunun 19.06.2020 Tarih ve 2020-4 Sayılı kararı ile izin alınmıştır.

BULGULAR ve TARTIŞMA

Sığırcılık işletmelerinde karlılık, buzağı verimi, çiğ süt satışı, kasaplık hayvan satışı, damızlık satış geliri ve diğer gelirlerin düzenli ve beklenen düzeyde olmasına bağlıdır.

Yetiştiricilerin yaşı ve tecrübesinin yanı sıra yetiştirilen sığırların işletme koşullarına adaptasyonu ve verim kabiliyeti ve hastalıklara karşı dayanıklı olması ile mümkündür. Bu işletmelerde özellikle hayvan başına yılda bir buzağı alınması ile birlikte süt veriminin ekonomik verim seviyesinin üzerinde olması arzu edilmektedir. Bu hedefe ulaşırken, yem, hastalıklarla mücadele ve diğer üretim girdilerine yapılacak giderlerin düşük olması istenmektedir. Bu çalışmada yetiştiricilerin sığır yetiştiriciliğinde risklerle karşı karşıya kaldıklarında devlet desteğinin varlığı önemlidir. Anket çalışmasına katılan yetiştiricilerin sahip oldukları demografik özelliklere ait tanımlayaıcı istatistikler Çizelge 2'de verilmiştir.

Yetiştiricilerin büyük çoğunluğunun 40 yaş altı (%52.0) kişilerden oluştuğu, %56.1'inin üniversite mezunu oldukları, hepsinin bir eğitim diplomasına sahip (%100) oldukları belirlenmiştir.

Çizelge 2. Yetiştiricilerin demografik bilgileri
Table 2. Demographic information of breeders

Demografik özellik *(demographic feature)*	**Tanımlama** *(Description)*	**Genel** *(General)*	
		N=173	**%100.0**
		N	**%**
Yaş *(Age)*	21-30 yaş *(age)*	27	15.6
	31-40 yaş *(age)*	63	36.4
	41-50 yaş *(age)*	55	31.8
	51 yaş ve üzeri *(51 age and older)*	28	16.2
Eğitim durumu *(Educational status)*	İlkokul *(Primary School)*	19	11.0
	Ortaokul *(Middle School)*	14	8.1
	Lise *(High School)*	43	24.9
	Üniversite *(University)*	97	56.1
Sığırcılık faaliyet süresi *(Cattle activity length)*	10 yıldan az *(less than 10 years)*	64	37.0
	11-20 yıl *(years)*	54	31.2
	21-30 yıl *(years)*	24	13.9
	30 yıldan fazla *(over 30 years)*	31	17.9
Yetiştiricinin Sosyal güvence durumu *(Social security status of the breeder)*	Bağ-Kur *(Independent Employees Institution)*	52	30.1
	Emekli Sandığı *(Pension Fund)*	12	6.9
	SSK *(Social Security Institution)*	90	52.0
	Sosyal güvence yok *(No social security)*	19	11.0
İsteğe bağlı sigorta durumu *(Optional insurance status)*	Evet *(Yes)*	136	78.6
	Hayır *(No)*	37	21.4

Büyükbaş ve küçükbaş tarım işletmelerinde HHS yaptıran kişilerle yapılan bir çalışmada işletme sahiplerinin tamamının bir eğitim diplomasına sahip oldukları ve en yüksek eğitim grubu ilkokul mezunu kişilerin (%40.2) oluşturduğu ve üniversite mezunu kişilerin oranı ise %18.6 olarak bildirilmiştir (Mat ve ark., 2020).

Yetiştiricilerin hayvancılık faaliyet süresi 10 yılın altında olanların devlet desteklerinin katkısından yararlanarak sığırcılık faaliyetine yeni başlayan kişilerden kaynaklandığını söylemek mümkündür. Yetiştiricilerin eğitim durumları incelendiğinde, büyük çoğunluğunun üniversite mezunu olmaları bu düşünceyi güçlendirmektedir (Çizelge 2).

Mat ve ark. (2020) tarafından büyükbaş ve küçükbaş tarım işletmelerinde HHS yaptıran kişilerin mesleki tecrübelerini 5 <, 5-10 yıl, 11-20 ve 21 yıl ve üzeri gruplar için sırasıyla %15.5, %20.6, %29.8 ve %34.1

olarak bildirmiş olup, en yüksek ortalamaya sahip grup 21 yaş ve üzeri dir. Bu çalışma da ise 21 yıl ve üzeri tecrübeye sahip işletme sahibi oranı %31.8 olup, benzer bulunmuştur.

Bu çalışmada sığır yetiştiricilerinin %89.0'unun bir sosyal güvenceye sahip oldukları, bunun yanında %78.6'sının daha önce isteğe bağlı olarak kendileri veya aileleri için sigorta (araç kasko, bireysel sağlık sigortası, seyahat sigortası, hırsızlığa karşı eşya sigortası vb.) yaptırdıkları belirlenmiştir. Bu durum yetiştiricilerin çoğunluğunun sigorta konusunda bilinçli olduklarını göstermektedir (Çizelge 1). Konu ile ilgili İkikat Tümer (2011) Tokat ilinde %84.0, İkikat Tümer ve ark. (2011) Erzurum ilinde %78.38 oranında çiftçilerin bir sosyal güvenceye sahip olduklarını bildirmişlerdir.

Yetiştiricilerin mevcut hayvanları hakkında hastalıklar ve diğer çevresel tehditler (yangın, hırsızlık, doğal afetler vs.) açısından risk durumlarının değerlendirilmesi istenmiş ve elde edilen cevaplar Çizelge 3'de verilmiştir.

Çizelge 3. Yetiştiricilerin mevcut hayvanlarının risk durumu hakkındaki görüşleri
Table 3. Thoughts about situation risk of animals what breeders have owned

Tanımlama *(Description)*	**Risk varlığı** *(Presence of risk)*	**Deskriptif istatistikler** *(Descriptive statistics)*	
		N	**%**
Hastalık ve çevresel tehditler (yangın, hırsızlık, doğal afetler vs.) açısından hayvanların risk durumu *(The risk status of animals in terms of diseases and environmental threats (fire, theft, natural disasters, etc.))*	Yok *(no)*	34	19.7
	Var *(Yes)*	139	80.3
	Toplam *(Total)*	173	100.0
Hayvanlar risk altında ise *(If animals are at risk)*	Az riskli *(Low risk)*	86	61.9
	Riskli *(Risky)*	41	29.5
	Oldukça fazla riskli *(Too much risk)*	12	8.6
	Toplam *(Total)*	139	100.0

Çizelge 3'e göre yetiştiricilerin %80.3'ü hayvanlar açısından hastalık ve çevresel tehditlerin var olduğunu beyan etmelerine rağmen hayvanlarının az risk altında, risk altında ve oldukça fazla risk altında olduğunu düşünen işletmelerin oranları sırasıyla, %61.9, %29.5 ve %8.6 olarak belirlenmiştir. Uğur (2010) tarafından genel olarak risk, gerçekleşme olasılığı olan fakat ne zaman gerçekleşeceği belli olmayan, istenmeyen olaylar olarak ifade edilmiştir. Üretim faaliyetleri süresince ortaya çıkabilecek riskleri belirlemek ve bu risklerin etkisini ortadan kaldırabilmek için uygulanabilecek risk önlemlerinin belirlenmesi çok önemlidir. Ortaya çıkabilecek risklerin belirlenmesi halinde süt sığırcılığı faaliyeti daha etkin olarak yürütülebilecek ve tutarlı kararların alınması şansını artıracaktır (Özsayın ve Çetin, 2004). Bu nedenle istenmeyen olayların ne zaman meydana geleceği belli olmadığından yetiştiricilerin büyük çoğunluğunun hayvanlarını az riskli bulmaları sigorta yaptırmaya bakış açılarını azda olasa yansıtmaktadır.

Yetiştiricilerin TARSİM hayvan hayat sigortasının yaygınlaştırılması açısından etkili olacağını düşündükleri konular hakkında görüşlerini önem derecelerine göre 1 ila 5 puan arasında değerlendirilmesi istenmiştir. Elde edilen sonuçlar Çizelge 4'te verilmiştir.

TARSİM dolayısıyla BHHS'nın yaygınlaşması açısından Çizelge 4 incelendiğinde; 5 puan (çok çok önemli) ile derecelendirilen konular ve oranları sırasıyla, HHS indirim tarifesinin kapsamının genişletilmesi %59.5, hayvancılıktan sağlanan gelirin yeterli ve düzenli olması %53.2, hayvan bedeli üzerinden bireysel ve müşterek sigorta oranlarında indirime gidilmesi %52.6, yetiştiricilerin ihtiyaç ve beklentileri karşılayacak şekilde sigorta kapsamının genişletilmesi %49.1, HHS hakkında yetiştiricilerin etkin şekilde bilgilendirilmesi %48.0, risk değerlendirmesi ve hasar tespitinde eksperlik hizmetinin kalitesi ve güvenilirliği %42.2, hayvan başına hasar tutarının belirlenmesi ve muafiyet kesintisi tutarı %35.8 olarak tespit edilmiştir. Yetiştiricilerin HHS hakkında etkin bilgilendirilmesi gerektiğini ifade edenlerin oranı %48.0 olarak belirlemiştir.

Konu ile ilgili olarak Kaygısız ve ark. (2017) Şanlıurfa ilinde Siyah Alaca ırkı sığırların yetiştirici şartlarında adaptasyonu hakkında yaptıkları çalışma yetiştiricilerin verim düzeyi yüksek ve ekonomik değeri olan sığırlarını sigorta yaptırma eğiliminde olduklarını bildirmişlerdir. Özsayın (2021) ise, TR22 Güney Marmara Bölgesinde yaptığı çalışmada sonuç olarak; TR22 bölgesinde büyükbaş ve küçükbaş hayvan hayat sigortası uygulamalarının başlangıcından 2019 yılına kadar hayvan hayat sigortalarında önemli ilerlemelerin olduğunu ifade etmekle birlikte, büyükbaş ve küçükbaş hayvan varlığı dikkate alındığı durumda, sigortalı hayvan

sayısı oranının düşük olduğunu ifade etmiştir. Ancak tarım sigortaları konusunda çiftçiler için gerçekleştirilecek eğitim ve bilgilendirme toplantılarına daha fazla önem verilmesinin yanısıra prim ve hasar ödemelerinde ortaya çıkan olumsuzlukların giderilmesinin bölgedeki hayvan hayat sigortası uygulamalarının yaygınlaştırılmasına katkı sağlayacağını bildirmiştir. Özeş Özgür (2019) tarafından üreticiye sağlanacak devlet desteğinin üreticiyi tarım sigortasına yönlendirmede en etkili faktör olacağını tespit etmiş olup, çeşitli eğitim programları ve kurslarla üreticinin konuyla ilgili doğru bilgilendirilmesinin üreticiyi tarım sigortası yaptırmaya yöneltecek diğer önemli bir etken olduğu ifade edilmiştir.

Çizelge 4. TARSİM hayvan hayat sigortasının yaygınlaştırılması hakkında yetiştirici görüşleri
Table 4. Breeders' opinions on the dissemination of TARSİM animal life insurance

Konular *(Topics)*	**N=173 %100**	**Her konu için 1'den 5'e kadar önemlilik** *(Significance order from 1 to 5 for each topic)*				
		Önemsiz *(Non-significant)*	**Az önemli** *(Litle significant)*	**Önemli** *(Signicant)*	**Çok önemli** *(Very significant)*	**Çok çok önemli** *(Very very significant)*
Hayvanciliktan sağlanan gelirin yeterli ve düzenli olmasi	N	28	13	24	16	92
(Being sufficient and regular of income from livestock)	%	16.2	7.5	13.9	9.2	53.2
Yetiştirici ihtiyaç ve beklentileri için sigorta kapsaminin genişletilmesi	N	13	17	32	26	85
(Expanding of insurance coverage for breeder needs and expectations)	%	7.5	9.8	18.5	15	49.1
Hayvan hayat sigortasinda indirim tarifesinin kapsaminin genişletilmesi	N	9	11	30	20	103
(Expanding the scope of the discount tariff in animal life insurance)	%	5.2	6.4	17.3	11.6	59.5
Hayvan bedeli üzerinden bireysel ve müşterek sigorta oraninda indirime gitme	N	12	14	32	24	91
(Reducing individual and joint insurance rates over animal price)	%	6.9	8.1	18.5	13.9	52.6
Risk değerlendirme ve hasar tespitinde eksperlik hizmeti kalite ve güvenilirliği	N	22	16	36	26	73
(Expertise service quality and reliability in risk assessment and damage assessment)	%	12.7	9.2	20.8	15	42.2
Hayvan başina hasar tutarinin belirlenmesi ve muafiyet kesintisi tutari	N	28	20	39	24	62
(Determination of damage amount per animal and exemption interruption amount)	%	16.2	11.6	22.5	13.9	35.8
Hayvan hayat sigortasi hakkinda yetiştiricilerin etkin bilgilendirilmesi	N	24	15	30	21	83
(Effective informing of breeders about animal life insurance)	%	13.9	8.7	17.3	12.1	48.0

Sığırcılık işletmelerinde karlılık açısından yetiştirilen sığır başına buzağı veriminin yanı sıra çiğ süt, kasaplık hayvan satışı, damızlık hayvan satışı ve diğer gelirlere bağlı bilinmektedir. Yetiştiricilerin hayvansal üretim sürecinde hayvanlarda sıklıkla karşılaştıkları hastalıklar, kazalar ve doğal afetler başta olmak üzere pek çok sorun ile karşı karşıya kalmaktadırlar. İşletmelerde üretim sürecinde yaşanan hayvan hastalıkları ve diğer problemler ile bu parametrelere ait tanımlayıcı istatistiki değerleri Çizelge 5'de verilmiştir.

Yetiştiricilerin işletmelerinde üretim sürecinde hayvanlarda yaşadıkları meme hastalığı (%68.2) ve tırnak probleminin(%49.7) diğer hastalıklara göre daha fazla oranda yaşandığını beyan etmişlerdir (Çizelge 5).

Yetiştiricilerin üretim sürecinde karşılaştıkları bulaşıcı hastalıklar ve diğer sağlık problemleri ile

bireysel mücadelenin yanı sıra hayvanlarına devlet destekli BHHS yaptırmaları riski azaltmak açısından büyük önem arz etmektedir.

BHHS yetiştiriciler tarafından yaygın kullanılmamasının sebepleri için vermiş oldukları cevaplar çoklu analiz yöntemiyle değerlendirilmiş ve Çizelge 6'da verilmiştir.

BHHS yetiştiriciler tarafından yaygın kullanılmamasının ya da tercih edilmemesinin sebepleri incelendiğinde, %19.3 oranı ile ilk sırada hayvancılıktan sağlanan gelirin yetersiz veya düzensiz olması gelmiştir. Bunu %15.6 oranı ile hayvan başına sigorta poliçe bedelinin yüksek olması, %10.7 oranı ile hayvan başına ödenen hasar tutarının yetersiz olması, %10.2 oranı ile HHS kapsamının yeterli olmaması sebeplerinin izlediği, diğer sebeplerin oranının ise toplam %33.4 olduğu tespit edilmiştir (Çizelge 6).

Çizelge 5. Yetiştiricilerin hayvanlarında karşılaştıkları problemler ve hastalıklar
Table 5. Problems and diseases faced by breeders in their animals

Hastalıklar *(Diseases)*	Tespit *(Detection)*	N=173 N	%100 %	Hastalıklar *(Diseases)*	Tespit *(Detection)*	N=173 n	%100 %
Mastitis	Var *(Yes)*	118	68.2	Zehirlenme	Var *(Yes)*	9	5.2
(Mastitis)	Yok *(no)*	55	31.8	*(Poisoning)*	Yok *(no)*	164	94.8
Tırnak problemi	Var *(Yes)*	86	49.7	Yılan sokması	Var *(Yes)*	4	2.3
(Nail problem)	Yok *(no)*	87	50.3	*(Snake bite)*	Yok *(no)*	169	97.7
Metritis	Var *(Yes)*	46	26.6	Vahşi hayvan saldırısı	Var *(Yes)*	5	2.9
(Metritis)	Yok *(no)*	127	73.4	*(Wild animal attack)*	Yok *(no)*	168	97.1
Asidoz	Var *(Yes)*	52	30.1	Şap hastalığı	Var *(Yes)*	27	15.6
(Acidosis)	Yok *(no)*	121	69.9	*(Foot and mouth disease)*	Yok *(no)*	146	84.4
Süt humması	Var *(Yes)*	18	10.4	Yavru atma	Var *(Yes)*	49	28.3
(*Milk fever)*	Yok *(no)*	155	89.6	*(Throwing offspring)*	Yok *(no)*	124	71.7
Ketosis	Var *(Yes)*	23	13.3	Yaralanma, kırık ve çıkık	Var *(Yes)*	7	4.0
(Ketosis)	Yok *(no)*	150	86.7	*(Injury, fracture, dislocation)*	Yok *(no)*	166	96.0
Yabancı cisim	Var *(Yes)*	33	19.1	Mecburi kesim	Var *(Yes)*	94	54.3
(Foreign body)	Yok *(no)*	140	80.9	*(forced slaughter)*	Yok *(no)*	79	45.7

Çizelge 6. Üreticiler için TARSİM hayvan hayat sigortasının yaygın kullanılmamasının nedenleri
Table 6. Reasons why TARSİM animal life insurance is not widely used for producers

Düşünce ve Görüşler *(Thoughts and Opinions)*	N	%
Hayvancılıktan sağlanan gelirin yetersiz veya düzensiz olması *(Insufficient or irregular being of income from livestock)*	110	19.3
Hayvan başına sigorta poliçe bedelinin yüksek olması *(Being high of insurance policy cost per animal)*	89	15.6
Hayvan hayat sigortası konusunda yeterli bilgi sahibi olunmaması *(Having insufficient knowledge about animal life insurance)*	63	11.0
Hayvan başına ödenen hasar tutarının yetersiz olması *(Being insufficient of damage amount paid per animal)*	61	10.7
Hayvan hayat sigortasının kapsamının yeterli olmaması *(Being insufficient of animal life insurance coverage)*	58	10.2
Hayvan hayat sigortasının öneminin henüz anlaşılamamış olması *(Not being yet understood of important of animal life insurance)*	54	9.5
Hasar bedelinin zamanında ve tam olarak ödeneceğine olan inancın az olması *(Being low belief in about payment as in full and on time of damage cost.)*	47	8.2
Orta ve uzun vadede hayvansal ürün piyasasında karşı duyulan güvensizlik *(Distrust in the animal product market in the medium and long term)*	40	7.0
Risk değerlendirmesi ve hasar tespitinde eksperlik hizmetinin özensiz yapılması *(Doing careless of appraisal service in risk assessment and damage assessment)*	36	6.3
Sigorta kayıtları üzerinden ilave vergi alınacağı endişesi *(Feeling concerned additional taxes paying according to insurance records)*	10	1.8
Kulak küpesi ile ilgili yaşanan sıkıntılar (küpenin düşmüş olması) *(Experienced problems related to ear earrings (earrings falling off))*	2	0.4
Ziraat bankasının sigorta acentesi olarak yaptığı hatalar *(Mistakes made by Ziraat Bank as an insurance agent)*	1	0.2
Toplam *(Total)*	571	100.0

BHHS yaygın kullanılmamasının sebepleri için yetiştiricilerin verdikleri cevapların arasında olan HHS konusunda yeterli bilgi sahibi olunmaması ve HHS öneminin henüz anlaşılamamış olması oranları sırasıyla, %11.0 ve %9.5 olarak bulunmuştur. Konu ile ilgili Mat ve ark. (2020) tarafından yapılan bir çalışmada yetiştiricilerin HHS yaptırma oranlarının artırılması için HHS konusunda sürekli bilgilendirilmeleri gerekliliği bildirilmiştir. Aynı şekilde Özsayın (2021) tarafından da tarım sigortaları konusunda çiftçilere yapılacak eğitim ve bilgilendirme çalışmalarının sigortalı hayvan sayısının artışında etkili olacağını bildirmiştir. Terin ve ark. (2016) Van ili ve ilçelerinde yaptıkları çalışmada devlet destekli tarım sigortaları uygulamalarının yavaşta olsa geliştiği ve bu gelişimin artarak devam etmesi için, sigorta prim bedellerinin bölgedeki üreticilerin tarımsal gelir düzeyleri dikkate alınarak hesaplanması ve üreticilerde sigorta bilincinin oluşturulmasına yönelik tarımsal yayım çalışmalarının yapılmasını önermişlerdir. Türkiye'de tarımsal üreticinin tarım sigortası yaptırmamasındaki en önemli sorunların, sigorta primlerinin yüksekliği, çiftçinin konuyla ilgili bilgi eksikliği, eksperlerin hasar tespitindeki davranışları, çiftçinin eğitim durumu ve sigorta şirketlerine olan güvensizliği gibi sorunlar ön plan çıkmaktadır (Özeş Özgür (2019))

Yine bu çalışmada yetiştiricilerin işletmelerinde yetiştirdikleri hayvanlarında karşılaştıkları bazı hastalık ve problemlerden kaynaklanan sığır ani ölümleri ve mecburi kesimlerinin bilgileri yetiştirici cevapları doğrultusunda Çizelge 7'de verilmiştir.

Hayvansal üretim sürecinde işletmelerin %54.3'ünün hayvanlarında ani ölüm ve mecburi kesimle karşılaştıkları beyan etmişlerdir. Ani ölüm ve mecburi kesilen hayvan sayısı < 3, 4-6, 7-9 ve > 10 baş ve daha fazla olan işletmelerin oranları sırasıyla, %36.2, %27.7, %8.5 ve %27.7 olarak belirlenmiştir (Çizelge 7).

Çizelge 7. Sürülerde ani ölüm ve mecburi kesim yaşanma durumu
Table 7. The state of being seen sudden death and compulsory slaughter in herds

Sığır Ani ölüm - Mecburi kesim durumu *(Cattle Sudden death - Forced slaughter situation)*	**Deskriptif istatistikler** *(Descriptive statistics)*	
	N=173	**% 100**
İşletmelerde ani ölüm ve mecburi kesim olma durumu *(Sudden death and forced slaughter in farms)*		
Yok *(No)*	79	45.7
Var *(Yes)*	94	54.3
Ani ölüm ve mecburi kesim var ise sığır sayıları (baş), (n=94) *(If there is sudden death and compulsory slaughter, the number of cattle (head), (n=94)*		
<3	34	36.2
4-6	26	27.7
7-9	8	8.5
>10	26	27.7

SONUÇ ve ÖNERİLER

Bu çalışmada yetiştiricilerin işletmelerinin bulunduğu bölge, il, coğrafi şartlar ve işletmelerinin varlıkları, sermaye durumları, yapılan hayvancılığın yönü, yetiştirme koşulları ve kendi tecrübelerinin etkisi altında verdikleri cevaplar değerlendirildiğinde hayvancılık sektörü, tarımın diğer faaliyet kollarında olduğu gibi iklim, ekonomik, sosyal, siyasal, teknolojik ve kişisel risklerden yüksek düzeyde etkilenen, kendine özgü yapısı nedeniyle üretiminde sık sık riskle karşı karşıya kalmaktadır. Son yıllarda küresel ısınmanın etkisiyle meydana gelen kuraklık bitkisel üretime bağlı olarak hayvansal üretimi de önemli düzeyde etkilemektedir.

Süt ve kırmızı etin önemli bir kaynağı durumundaki büyükbaş hayvancılığın sürdürülebilirliği açısından HHS uygulamasının yaygınlaşması büyük önem arz etmektedir. Bunun için HHS konusunda yayım ve eğitim çalışmaları ile bilgilendirme faaliyetlerinin yanı sıra HHS uygulamasından daha fazla yetiştiricinin faydalanmasını sağlamak için HHS sigorta kapsamının genişletilmesi, birim hayvan başına sigorta bedelinin makul bir seviyeye çekilmesi, sürü büyüklüğüne göre indirim tarifesi uygulanması ve hasar bedellerinin zamanında ödenmesinin sağlanmasının elzem olduğu belirlenmiştir. Yetiştiricilerin hayvan kayıpları ve hastalıkları nedeniyle karşılaştıkları ekonomik mağduriyetler giderilmesine yönelik tedbirlerin alınmasının yetiştiricilerin hayvan hayat sigortası yaptırma fikrine olumlu etkisi olacağı düşünülmektedir.

TEŞEKKÜR

Verilerin elde edilmesine yardımcı olan Türkiye Damızlık Sığır Yetiştiricileri Merkez Birliği (TDSYMB)'ne, İl Damızlık Sığır Yetiştiricileri Birliklerine ve katılım sağlayan yetiştiriciler ile iyi niyet dilekerini bildiren ve destek veren Tarım

Sigortaları Havuzu (TARSİM) yetkililerine de teşekkür ederiz.

Araştırmacıların Katkı Oranı Beyan Özeti

Yazarlar makaleye eşit oranda katkı sağlamış olduklarını beyan eder.

Çıkar Çatışması Beyanı

Makale yazarları aralarında herhangi bir çıkar çatışması olmadığını beyan ederler.

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Dry Period Length in Dairy Cattle: II. Influence on Calf Survival and Growth Performance

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ABSTRACT
This study was conducted to investigate the effects of dry period length (DPL) on survival rate, gender, growth performance, mortality of Holstein calves. Data belonged to 800 Holstein cows in different parities (2nd, 3rd and ≥4th) and 800 calves delivered by these cows. DPL were classified in 5 categories (≤40, 41-50, 51-60, 61-70, ≥71 days). Calves were weighed and body dimensions (body length, wither height, hearth girth) at birth and 6th months of age. There were significant relationships between DPL and calf gender (P<0.01). The cows with DPL of 51-60 days had greater female calf ratios and the cows with DPL of 61-70 days had greater male calf ratios. In the study, the relationship between calves' body weights (birth and 6th month) and DPL was found to be significant at P<0.01 level, and the relationship between body measurements (6th month body length 6th month wither height) and DPL was significant at P<0.05 level. These values the highest were observed in the calves of the cows with DPL 61-70 days. The relationship between calves survivability, mortality values and DPL was not significant (P>0.05). The results obtained from the study showed that the dry period length of the cows can be planned between 61-70 days, considering the body weight and growth performance of the calves.

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Süt Sığırlarında Kuru Dönem Uzunluğu: II. Buzağılarda Yaşama Gücü ve Büyüme Performansı Üzerinde Etkisi

ÖZET
Bu çalışma Holstein ırkı ineklerde kuru dönem uzunluğunun (KDU) buzağılarda yaşama gücü, cinsiyet, büyüme performansı, mortalite üzerindeki etkisini araştırmak amacıyla yapılmıştır. Çalışmada Holstein ırkı 800 baş inek (laktasyon sırası: 2, 3 ve ≥4) ve bu ineklerin yeni doğan buzağıları kullanılmıştır. İnekler KDU' ya göre beş kategoride KDU≤40 gün, 41-50, 51-60, 61-70, KDU≥71 gün sınıflandırılmıştır. Buzağıların doğumda ve 6 aylık yaşta canlı ağırlıkları ve bazı vücut ölçüleri alınmıştır. Çalışma sonucunda ineklerde KDU ile buzağı cinsiyeti arasındaki ilişki anlamlı (P<0.01) olmuştur. Kuru dönem uzunluğu 51-60 gün olan ineklerde dişi buzağı sayısı, 61-70 gün olan ineklerde ise erkek buzağı sayısı daha fazla olmuştur. Çalışmada buzağıların doğum ve 6. ay canlı ağırlıkları ile KDU arasındaki ilişki P<0.01 düzeyinde ve 6. ay vücut uzunluğu ve cidago yüksekliği ile KDU arasındaki ilişki P<0.05 düzeyinde anlamlı bulunmuştur. Bu değerler en yüksek 61-70 gün kuruda kalan ineklerin buzağılarında görülmüştür. Buzağıların mortalite değerleri ile KDU arasındaki ilişki anlamlı olmamıştır (P>0.05). Çalışmadan elde edilen sonuçlar, buzağıların canlı ağırlık ve büyüme performansı dikkate alındığında ineklerde kuruda kalma süresinin 61-70 gün arasında planlanabileceğini göstermiştir.

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INTRODUCTION

Dairy cows are subjected to a dry period for certain duration between two lactations to have regular milk yield in subsequent lactation (Collier et al., 2012). Optimum dry period length (DPL) is determined by taking herd size, parity and milk yield levels into consideration. Traditionally, such periods are applied as 305 days for lactation and 51-60 days for DPL (Bachman and Schairer, 2003; Grummer and Rastani, 2004). DPL are related to milk yield, milk composition, reproduction performance of the dairy cows as well as birth weight, survival rate and growth performance of the calves of these cows (Coppock et al., 1974; Kuhn et al., 2006; Pezeshki et al., 2008; Atashi et al., 2013; Hossein-Zadeh and Mohit, 2013; Rahbar et al., 2016; Metin Kıyıcı et al., 2020). Healthy calves are significant indicator of animal welfare and economic livestock farming (Lorenz et al., 2011a; McGuirk, 2008; Santman-Berends et al., 2014). Size of delivered calf is an important characteristic for ease of delivery and survival of neonatal calf (Johanson and Berger, 2003). On the other hand, epidemiological evidence suggests that small size at birth is associated with increased predisposition to metabolic diseases during adult life (Symonds et al., 2010; Vuguin, 2007). Birth weight of the calves, growth performance and survival rates are largely influenced by the animal breed, gender, age of mother, maternal ability, number of calves at birth, several genetic and environmental factors (Akbulut et al., 2001). Additionally, Atashi et al., (2013) indicated that DPL had also significant effects on calf birth weight and growth performance.

Therefore, in this study was designed to investigate the relationships between dry period length with gender, birth and 6th month growth performance and survival rates (or mortality) of Holstein calves.

MATERIALS and METHODS

Data obtained from a commercial dairy farm were used in the study. Thus, measurement of phenotypic characteristics was performed under the routine management and breeding procedure for calves at farm, no animal experiment and additional handling was involved in the study. Therefore, no ethics approval was necessary.

In the study, data obtained from cows raised in an intensive commercial dairy breeding operation (Saray Farm Dairy Operation Co.) located in Central Anatolia region of Turkey (Latitude:38°.34'66.79, Longitude: 35°.47'84.66) were used. Data belonged to 800 heads Holstein cows in different parities (2nd, 3rd and ≥4th) and 800 heads calves delivered by these cows between November 2014 and December 2015. Parities and calving body weights (kg) of multiparous cattle used in this study are provided based on DPL in Table 1.

Table 1- Parities and calving body weights (kg) of multiparous cattle based on DPL
Çizelge 1- KDU' na göre ineklerin sayısal dağılımı (baş) ve buzağılamadaki canlı ağırlık ortalamaları (kg)

Lactation Numbers *Laktasyon Sayısı*	Dry Period Length (day) *Kuru Dönem Uzunluğu (gün)*											
	≤40 (days)		41-50 (days)		51-60 (days)		61-70 (days)		≥71 (days)		General	
	n	BW (kg)	n	BW (kg)	n	BW (kg)	n	BW (kg)	n	BW (kg)	n	BW (kg)
2	30	613	47	610	106	597	33	579	15	614	231	603
3	34	609	58	627	156	613	84	623	72	609	404	616
≥4	19	617	23	616	65	600	29	604	29	617	165	611
General	83	613	128	618	327	603	146	602	116	613	800	610

BW; Body Weight (kg)

The procedure for drying off the cows was carried out by reducing the number of daily milking frequency of 3 gradually to 2 and 1 when the daily milk yield of the cattle decreased to 10 liters or below. The time between full termination of milking and parturition was monitored as dry period length. The cows with signs of parturition were taken to the individual calving pen and calves were born in these pens. Neonatal calves stayed with their mothers after the birth and consumed colostrum *ad-libitum* for three days. At the end of three days, calves were relocated into the individual pens and fed with milk until weaning (30 days) with a daily amount of 10% of their body weight. From the 7th day to weaning the calves were supplied with a concentrate mixture and alfalfa hay *ad libitum*. After weaning, animals were kept in group housing pens and received milk replacer for 40 days and fed with forage and commercial concentrate mixture *ad libitum* until the 180th day. Clean drinking water was supplied *ad libitum* at all the time. Chemical composition of the feeds used in the study (fresh milk, milk replacer, calf starter, calf grower feeds and alfalfa hay) is presented in Table 2.

To follow up growth performance of calves, body

weight and body size (body length, wither height, hearth girth) were measured right after the birth (within the first 24 hours) and at the age of 6 months. The animals were weighed to the nearest kilogram using an electronic scale (EziWeigh 5i, Tru-Test, New Zealand) placed on a concrete platform. Body measurements were taken by two person using an ordinary measuring tape and recorded in centimeters. Body length was measured as the distance from Tuber a*triculus humeri* to *Tuber ichii*; wither height was measured as the distance from the ground to the highest point of wither. Hearth girth was measured behind the front shoulder at the fourth ribs, posterior to the front leg.

Table 2. Nutritive values of fresh milk, milk replacer, concentrate mixtures and alfalfa hay used in the study
Çizelge 2. Çalışmada kullanılan taze süt, süt ikame yemi, konsantre yem ve yonca kuru otu besin değerleri

Nutrients *Besin Elementleri*	Fresh milk *Taze süt*	Milk replacer *Mama*	Calf starter feed *Buzağı başlangıç yemi*	Calf grower feed *Buzağı büyütme yemi*	Alfalfa hay *Yonca kuru otu*
Dry matter (%)	12.2	96.2	88.0	88.0	91.7
Crude protein (% of DM)	3.4	31.2	18.0	17.0	18.1
Crude fat (% of DM)	3.3	20.1	4.6	4.6	2.5
Crude ash (% of DM)	0.7	6.1	8.2	10.0	9.8
Crude cellulose (% of DM)	-	-	12.0	12.0	29.4

Statistical Analysis

Statistical analyses were performed with IBM SPSS Statistics 22.0 software (SPSS 2013). In present model, bulls effect was taken as random effect and parity (2^{nd}, 3^{rd} and $\geq 4^{th}$), calving year (2014, 2015), calving season (winter: December to February, spring: March to May, summer: June to August: and autumn: September to November) were taken as fixed effect. The relationships between DPL variable and categorical data (calf gender, survival rates (or mortality)) were tested with the use of Pearson Chi-Square Test and results were expressed in percentage (%). Mortality is the proportion of animals that die per hundred animals in an animal group (Tüzemen 2002). Mortality is calculated by the formula below;

Mortality (%) = (Number of Animals Died / Total Number of Animals) x 100

Since body length trait did not exhibit normal distribution, relevant data were subjected to non-parametric independent samples Kruskal Wallis Test and results were expressed in median (25-75 percentiles). The body weight (kg), height at withers (cm) and hearth girth (cm) traits exhibited normal distribution, so One-Way Anova Test was applied to relevant data. Significant means were compared with the use of Tukey's multiple range test.

RESULTS and DISCUSSION

Results on the effect of the different DPL on some investigated traits of calves are provided in Table 3.

In the study, the differences in survival rate were not significant (P>0.05) between the groups. The greatest survival rate (88.0%) was obtained from the calves of cows with ≤40-day dry period (mortality is %12) and the lowest survival rate (81.0%) was obtained from the calves of cows with ≥71-day dry period (mortality is %19). The annual average of the calves survival rate of the farm where the study was conducted is 94%. Calf survival characteristics; calf birth weight, gender, age of the mother, body weight of the mother, farm, calving season, calving year etc. is affected by many factors (Bilgiç ve Alıç, 2004; Koçak ve Güneş, 2005; Bayrıl ve Yılmaz, 2010). Additionalıy, Uzmay et al (2010) reported that the risk of dystocia was 1.96, 4.53 and 5.29 times higher in calves with birth weight classes 35.1-40.0, 40.1-45.0 and ≥45.1 kg, respectively. The overall mean of survival rate value reported by Karakaş (2002) as 83.7 %, Özçakır and Bakır (2003) as 96.22 %, Bayrıl ve Yılmaz (2010) as 92.1 %, Yüceer and Özbeyaz (2010) as 88.90 %, Ayaşan et al., (2016) as 83.0 % and Hızlı et al., (2017) reported as 97.12 %.

There were significant relationships between DPL and calf gender (P<0.01). The greatest number of female calves (178 – 54.4%) was obtained from the cows with DPL of 51-60 days and the lowest number of female cows (53 – 36.3%) was obtained from the cows with DPL of 61-70 days (Table 3). In the dairy cattle industries, breeders desire to have female cows to enlarge or replacement stock their herds, but beef cattle farmers desire to have male calves (Erten and Yılmaz 2012). Therefore, calf gender is an important factor in dairy cattle industries.

In this study there were significant relationships between DPL and calf birth weight (P<0.01) (Table 3). The greatest calf birth weight (42.79±4.40 kg) was obtained from the calves of cows with DPL of 61-70 days and the lowest calf birth weight (40.39±4.28 kg) was obtained from the calves of cows with DPL of ≤40 days. Atashi et al., (2013) indicated that calf birth weight did not differ for cows with DPL of 0 to 35 d, 36 to 50 d, or 51 to 60 d, but the average calf birth weight for cows with standard DPL (51 to 60 d) was less than in those with longer dry periods. Previous researchers reported no differences in calf birth weight for cows with 28-d and 49-d dry periods (Pezeshki et al., 2008), or for cows with 30-d and 60-d

Table 3- The effect of the different DPL on some investigated traits of calves
Çizelge 3- Buzağılarda incelenen bir kısım özellikler üzerinde KDU' nun etkisi

Traits* / *Özellikler*	Dry Period Length (day) / *Kuru Dönem Uzunluğu (gün)* ≤40 (n=83)	41-50 (n=128)	51-60 (n=327)	61-70 (n=146)	≥70 (n=116)	Genel	P
Rate of survival (n (%))							
Live	73 (88.0)	112 (87.5)	280 (85.6)	126 (86.3)	94 (81.0)	685 (85.6)	
Death	10 (12.0)	16 (12.5)	47 (14.4)	20 (13.7)	22 (19.0)	115 (14.4)	0.597
Total	83 (100.0)	128 (100.0)	327 (100.0)	146 (100.0)	116 (100.0)	800 (100.0)	
Gender (n (%))							
Female	42 (50.6)^{a}	61 (47.7)^{a}	178 (54.4)^{b}	53 (36.3)^{c}	55 (47.4)^{a}	389 (48.6)	
Male	41 (49.4)^{a}	67 (52.3)^{a}	149 (45.6)^{b}	93 (63.7)^{c}	61 (52.6)^{a}	411 (51.4)	0.009
Total	83 (100.0)	128 (100.0)	327 (100.0)	146(100.0)	116(100.0)	800(100.0)	
Body Weight (kg)							
Birth	40.39±4.28^{a}	40.50±4.49^{a}	40.97±3.77^{a}	42.79±4.40^{b}	41.32±3.90^{b}	41.22±4.15	0.001
6th month	184.78±20.46^{a}	188.06±31.78^{ab}	196.55±30.05^{b}	198.43±33.36^{b}	195.94±31.51^{b}	194.17±30.60	0.003
Body length (cm)							
Birth	69.0 (63.0-71.0)	70.0 (66.0-71.0)	70.0 (67.0-71.0)	70.0 (67.0-72.0)	70.0 (65.0-71.0)	---	0.067
6th month	107.0 (103.5-110.5)	108.0 (104.0-112.75)	108.0 (104.0-112.0)	109.5 (105.0-114.0)	108.0 (104.0-111.0)	---	0.047
Wither height (cm)							
Birth	71.30±6.41	72.41±5.91	72.51±5.77	73.23±5.96	72.09±6.00	72.44±5.94	0.192
6th month	106.00±4.31^{a}	105.81±5.26^{ab}	107.15±5.15^{ab}	107.20±5.65^{ab}	107.17±5.95^{b}	106.96±5.33	0.019
Hearth girth (cm)							
Birth	73.07±4.95	73.90±4.89	74.01±4.76	74.72±5.09	73.72±4.81	73.98±4.88	0.158
6th month	126.44±6.65	128.07±9.545	129.16±8.487	129.48±9.702	128.89±8.528	128.72±8.759	0.122

*Data; n (%), mean ± standard deviation or median (25-75 percentiles).
a-b: The means indicated with different superscript in the same row are significantly different

dry periods (Gulay et al., 2003). The overall mean of birth weights (41.22±4.15 kg) similar to value reported by Bush and Nicholson (1986), Başpınar et al. (1998), Johanson and Berger (2003), Uzmay et al. (2010) and greater than the values reported by Unalan (2009), Bayrıl and Yılmaz (2010) Şahiner and Demir (1998), Akbulut et al. (1993), Bardakçıoğlu (2001), Bilgiç and Alıç (2005) and Kaygısız et al. (2012). Average birth weight of Holstein-like large size breeds is commonly reported as 40-45 kg (Wattiaux 1996b). Also, in the study were determined significant ($P<0.01$) relationships between DPL with calf body weight at 6th month. The greatest calf body weight at 6th month was obtained from the calves of cows with DPL of 61-70 days (198.43±33.36 kg) and the lowest value was obtained from the calves of cows with DPL of ≤40 days (184.78±20.46 kg). These determined values were different and higher than those reported by Yanar et al (2002), Bayrıl and Yılmaz (2010), Yüceer and Özbeyaz (2010), Ayaşan et al (2016) and Aydın et al (2018).

In the study are used body length, wither height, hearth girth traits to determine growth performance of calves (Şekerden, 2010; Metin Kıyıcı and Tüzemen, 2012). The relationships between DPL and body measurements were not found to be significant ($P>0.05$). However, DPL had significant relationships with body length and wither height at 6th month ($P<0.05$). The greatest body length at 6th month was obtained from the calves of cows with DPL of 61-70 days (109.5 cm) and the lowest value was obtained from the calves of cows with DPL of ≤40 days (107.0 cm). The greatest wither height at 6th month was obtained from the calves of cows with dry period lengths of 61-70 days (107.2 cm) and the lowest value was obtained from the calves of cows with DPL of 41-50 days (106.0 cm). The 6th month body length and wither height of Holstein calves were reported as 102.53 cm and 98.31 cm by Yüceer and Özbeyaz (2010) and 134.6 cm and 94.5 cm by Doğan (2014) respectively.

CONCLUSION

Despite numerous studies about the effects of DPL on milk yield and reproduction-like traits, number of studies about the effects of DPL on growth performance and survival rate of calves is quite limited. In this study, effects of DPL on birth weight and growth performance of calves were investigated.

Calf survival characteristics; calf birth weight, gender, age of the mother, body weight of the mother, farm, calving season, calving year etc. is affected by many factors. The results obtained from the study showed that the dry period length of the cows can be planned between 61-70 days, considering the body weight and growth performance of the calves. Also in the study, it was determined that the gender of the calf was affected by the dry period length. Further research is needed for the effects of DPL on calf performance.

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Researchers Contribution Rate Declaration Summary

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

Conflicts of Interest Statement

The authors declare that they do not have any competition and any conflicts of interest.

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