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ARAŞTIRMA MAKALESİ - RESEARCH ARTICLE

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The Effect of Red Reishi Mushroom *(Ganoderma lucidum)* Extract on Carbon Tetrachloride Induced Liver Injury and Cyclooxygenase-2 Immunoreactivity

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ABSTRACT
Carbon tetrachloride (CCl_4) is a xenobiotic compound with toxicological action. It is absorbed by gastrointestinal system, respiratory system, and skin. Studies have reported that many countries have used *Ganoderma lucidum* (GL, Reishi Mushroom) as a medicinal mushroom against liver diseases induced by hepatotoxic agents such as CCl_4 for more than thousands of years and is used for many diseases, including cancer since it has been thought that it increases resistance against them and treats them. In the present study, immunohistochemical localization and expression of cyclooxygenase-2 (COX-2) by administrating carbon tetrachloride and *Ganoderma lucidum* in adult rats were examined. In the study, 32 adult Spraque-Dawley male rats that were 8-10 weeks old were used. Rats were divided into 4 groups as control, CCI_4, *Ganoderma lucidum* (GL), and CCI_4+GL. As a result of the experimental applications, the liver tissue was found to be normal in the control and GL groups, and multifocal necrosis areas, hepatocellular degeneration, cell infiltration, sinusoidal dilatation, and congestion were observed in the central and portal areas in CCI_4 group. In the CCI_4+GL group, decreases were observed in lesion severity and density. COX-2 immunoreactivity was detected as more common in hepatocyte cytoplasm in the area from the central vena to the Kiernan space, while it was observed as sporadic in the hepatocyte nucleus. While CCI_4 caused a decrease in total antioxidant level (TAS) in blood plasma samples, it caused an increase in total oxidant level (TOS), Aspartate aminotransferase (AST), and alanine aminotransferase (ALT) levels. It is seen that *Ganoderma lucidum*, which has an important place in alternative and folk medicine, reduces oxidative stress with its hepatoprotective effect and inhibits the inflammatory response in the liver.

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Kırmızı Reishi Mantarı *(Ganoderma lucidum)* Extratının Karbon Tetraklorür ile İndüklenen Karaciğer Hasarı ve Siklooksijenaz-2 İmmunoreaktivitesi Üzerine Etkisi

ÖZET
Karbon tetraklorür (CCl_4) toksik etkiye sahip ksenobiyotik bir bileşiktir. CCI_4, gastrointestinal sistem, solunum ve deri tarafından emilir. Yapılan çalışmalarda *Ganoderma lucidumun* (GL, Reishi Mantarı), CCl_4 gibi hepatotoksik ajanların neden olduğu karaciğer hastalıklarına karşı, birçok ülkede binlerce yılı aşkın süredir tıbbi mantar olarak kullanılmakta olduğu ve kansere varıncaya kadar birçok hastalığa karşı direnç arttırdığı ve tedavi ettiği düşünülerek kullanıldığı bildirilmiştir. Çalışmada yetişkin dönemdeki sıçanlara karbon tetraklorür ve *Ganoderma lucidum* uygulaması yapılarak siklooksijenaz-2 (COX-2)'nin immunohistokimyasal lokalizasyon ve ekspresyonu incelendi. Çalışmada 8-10 haftalık 32 adet yetişkin

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Anahtar Kelimeler
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Ganoderma lucidum

Spraque-Dawley ırkı erkek sıçan kullanıldı. Sıçanlar; kontrol, CCI_4, *Ganoderma lucidum* (GL), CCI_4+GL olmak üzere 4 gruba ayrıldı. Deneysel uygulamalar sonucunda kontrol ve GL grubunda karaciğer dokusu normal, CCI_4 grubunda ise sentral ve portal alanda multifokal nekroz alanları, hepatosellüler dejenerasyon, hücre infiltrasyonu, sinuzoidal dilatasyon ve konjesyon gözlendi. CCI_4 ile birlikte GL verilen grupta ise lezyon şiddet ve yoğunluğunda azalmalar tespit edildi. COX-2 immunoreaktivitesi; sentral venadan kierman aralığına kadar olan bölgede hepatosit sitoplazmasında daha yaygın olarak saptanırken, hepatosit nükleusunda sporadik olarak tespit edildi. Kan plazma örneklerinde CCI_4, Total antioksidan seviyede (TAS) azalmaya yol açarken, Total oksidan seviye (TOS), Aspartat aminotransferaz (AST) ve Alanin aminotransferaz (ALT) düzeylerinde ise artışa sebep olduğu gözlenmiştir. Alternatif ve halk tıbbında önemli yere sahip olan *Ganoderma lucidum*'un ise oksidatif stresi hepatoprotektif etkisi ile azalttığı, karaciğerdeki enflamatuar yanıtı inhibe ettiği görülmektedir.

Histopatoloji
İmmunohistokimya

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INTRODUCTION

The liver provides many functions in the body, such as the process of metabolism (Kandimalla et al., 2016). Toxic molecules of foreign origin and xenobiotics are absorbed by the intestine and then pass through the liver and they are detoxified here (Stickel et al., 2002; Elmubarak & Özsoy, 2016). Carbon tetrachloride (CCl_4) is a xenobiotic causing chemical-related injuries both in most living beings and in humans and it has the potential to develop cellular injury (Tekeli & Bildik, 2013). It has a toxic character in the organs such as the heart, testis, brain, and especially liver and kidneys (Abraham & Wilfred, 1999; Elmubarak & Özsoy, 2016). In the metabolism of action of CCl_4, enzymes that are defined as microsomal enzymes metabolizing medicine and chemical substances, play a role. After CCl_4 enters in the liver, it is converted into two free radicals, as trichloromethyl and trichloromethyl-peroxyl, by the microsomal system that is dependent on monooxygenase P-450 (Zhao et al., 2016). The toxic character of CCl_4 emerges after it is converted to trichloromethyl (CCl_3). CCl_4 is first converted to a harmful intermediate metabolite called trichloromethyl radical (-CCl_3) through the cytochrome P-450 enzyme system, and then to trichloromethyl peroxy (-$OOCCl_3$) radical in the presence of oxygen. These reactive free radicals react with polyunsaturated fatty acids and bind either to fats or lipids or start lipid peroxidation. As a result of lipid peroxidation occurring in CCl_4 exposure, more common tissue injury occurs as a result of the septicemia of some cellular enzymes (El-haskoury et al., 2018; Li et al., 2019).

A number of studies have shown that various plant extracts can protect the liver and kidney against oxidative stress caused by CCl_4 by inhibiting lipid peroxidation and increasing antioxidant enzyme activity (Shahjahan et al., 2004; Bellassoued et al., 2018). *Ganoderma lucidum* (GL, Reishi Mushroom) is used as a medicinal mushroom in many countries for more than 2000 years and has been used by considering that it increases resistance against and treats many diseases, including cancer (Kim et al., 2016). In the recent studies conducted with *G. lucidum*, its anti-tumor, anti-inflammatory, hepatoprotective, and anti-microbial effects are reported. Additionally, the components of reishi mushrooms have an antioxidant character. Its antioxidant character is provided by terpene and polysaccharide content (Cör et al., 2018). Triterpenoids, also called as ganoderic acid, carry a carboxyl group and have immunomodulatory and antioxidant properties. Thus, they gain their hepatoprotective properties (Satria et al., 2019).

COX enzyme has two types; constitutive and inducible. COX1, that is the structural cyclooxygenase, and COX-2, the inducible cyclooxygenase, catalyze the same reactions, but differ in structure and function. There are differences in structure and shape between these two enzymes caused by two amino acid changes in the amino acid sequence (Fu et al., 1990; Hawkey, 2001). While COX-1 is present in endoplasmic reticulum (ER), COX-2 is localized in ER and nuclear membrane. Both enzymes convert the arachidonic acid to prostaglandins (Dannhardt & Kiefer, 2001; Kaya Çavuşoğlu et al., 2021). COX-1 protects the tissues generally in terms of physiological reactions, while COX-2 has a completely reverse effect (Hawkey, 2001; Smith et al., 1996). COX-2, activated by inflammatory

agents, is present in inflammatory cells, especially macrophages (Hawkey, 2001). Proinflammatory agents such as cytokines, tumour necrosis factors, growth factors, and bacterial endotoxins cause the release of COX-2. Prostaglandins (PG), which the products of COX-2 and involved in inflammatory reactions, are responsible for the major symptoms of inflammation such as swelling, redness, pain, fever, and loss of function (Smith et al., 1996; Domitrovica et al., 2011). PGs developing by the induction of COX-2 degrade the cellular apoptosis mechanism in some cancer types and provide the metastasis of cancer (Hoffmann, 2000).

While PGs are synthesized via COX-1, the ones causing inflammatory response are only synthesized via COX-2. COX-2 is involved in the regulation of most of the renal functions (perfusion, fluid use, renin production) in both natural and pathophysiological (renal failure, congestive heart failure and liver cirrhosis) cases (Gambaro, 2002).

In the present study, the effects of *Ganoderma lucidum* on general oxidant/antioxidant levels, liver injury markers, histopathological lesions and COX-2 immunoreactivity levels of the rats with carbon tetrachloride-induced liver inflammation were determined.

MATERIAL and METHOD

Study design

Ethics committee approval required for the study was obtained from Animal Experiments Local Ethics Committee of Gaziantep University (GAÜN-DAM, Decision no: 2018/22). In the study, 32 adult (8-10 weeks old) *Spraque-Dawley* male rats weighing approximately 250-300 g, obtained from Gaziantep University Experimental Animals Research Centre were used as the material. Care and material administrations of the rats were also conducted at the research units of Gaziantep University Experimental Animals Research Centre. The rats were kept in an environment at the ambient temperature of 21 °C with a 12-hour light/12-hour dark cycle and were fed with standard rat feed (containing 21% crude protein) and tap water. The subjects included 32 rats in total as 8 in each group. The rats were divided into 4 groups as control, carbon tetrachloride (CCI_4), *Ganoderma lucidum* (GL), Carbon tetrachloride (CCI_4)+*Ganoderma lucidum*. Ganoderma lucidum. The *G. lucidum* extract used in the experiment was commercially obtained from GanoTurk (Seyhan, Adana). Physiological saline at a dose of 2 ml/kg was administered to the control group via gavage for 14 days, *Ganoderma lucidum* extract at a dose of 1000 mg kg^{-1} was administered to GL group via gavage for 14 days, Carbon tetrachloride at a dose of 10 ml/kg was administered to the CCI_4 group as a single dose intraperitoneally only on the first day. After giving *G. lucidum* extract to the CCI_4+GL group via gavage for the first 3 days, CCI_4 was administered in a single dose of 10 ml/kg. Then, *G. lucidum* extract was administered only via gavage for 14 days. At the end of the experiment, the rats were dissected after they have killed via cervical dislocation under ketamine hydrochloride/ xylazine (80/10 mg kg^{-1}) anaesthesia administered intramuscularly (i.m.). All the animals were weighed at the beginning and end of the study. At the beginning of the study, the animals were randomly divided into 4 groups provided that their mean weights were close to each other. The dose of CCI_4 in the study was determined according to Karakus et al., 2011, and the dose of *G. lucidum* extract was determined based on the data of researchers working in this field (Sliva et al., 2012; Lin & Lin 2006; Zhang et al., 2002).

Histological analysis

At the end of the experiment, liver tissue of the rats that were killed and dissected via cervical dislocation under general anaesthesia was fixed in 10% buffered formalin solution (SigmaAldrich, HT501128). After fixation, routine tissue follow-up (graduated alcohols, methyl benzoate, and benzole follow-up) was performed and then the tissues were embedded in paraffin and 5 µm serial sections were taken from the blocks with a microtome on slides previously coated with chrome alum gelatin (CAG). Hematoxylin-eosine, one of the histological staining methods, was applied to the sections and the histopathological changes were examined via light microscopic study (Luna, 1968).

Immunohistochemical analysis

Liver tissues taken from the rats were fixed in 10% buffered formaldehyde solution for COX-2 immunoreactivity and then, they were blocked in paraffin after passing through graduated alcohols, methyl benzoate, and benzoles. Anti-COX2 (Cyclooxygenase 2 antibody ab15191, Abcam) primary antibody was applied to 4-5 µm sections taken from paraffin blocks for one hour at room temperature in a humid environment at a ratio of 1/100. Only PBS (phosphate buffer solution) (Invitrogen™, AM9624) was dropped on the tissue sections for the negative control group. After primary antibody incubation, Streptavidin- biotin peroxidase technique, one of the indirect methods, was used (Shu et al., 1988). Chromogen application was conducted by adding 3-Amino-9-Ethylcarbazole (AEC) (Thermo Fisher Scientific, 1122). After adding AEC solution, the sections were controlled under a light microscope and when immunoreactivity developed, the reaction was stopped with distilled water and contour staining was performed with Mayer's hematoxylin. At the end of the processes, the sections were dried, and water-based adhesive was dropped and closed by using a lamella. Random areas were selected in the preparations and

their photographs were taken after they were examined under a Zeiss Primo Star integrated camera light microscope, assessment was conducted semi-quantitatively and the density was examined according to an immunoreactive score (Zhu, 1989; Seidal et al., 2001; Nur et al., 2015). COX-2 immunoreactivity in the cells was determined by comparing them with each other according to the degree of darkness of the colours.

Biochemical analysis

Blood samples taken for plasma output were centrifuged at 3000 rpm for 10 minutes and stored at -20 °C until the time of analysis. Total oxidant level (TOS) and total antioxidant levels (TAS) (Rel Assay Diagnostics, Mega Medical Industry and Trade Limited Company, Gaziantep) were measured in the blood samples (Erel, 2004; Erel, 2005). Plasma Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) activity analyses were measured by using an autoanalyzer (HumaStar 600, Germany).

Data analysis

Statistical analysis of the data was conducted in SPSS package program (IBM SPSS Statistics 22). Kolmogorov-Smirnov normality test was employed to reveal the compatibility of the data to normal distribution. For groups showing a normal distribution, one-way analysis of variance (ANOVA), a parametric test, was used. If there was a difference between the means of the experimental groups, the "Anova-Duncan" test was applied to the group averages to determine this difference and the value of $P<0.05$ was accepted as statistically significant.

RESULTS and DISCUSSION

Biochemical findings

When the biochemical data of the study were evaluated, the values in the control and *G. lucidum* groups were normal and close to each other. No statistical difference was observed between TAS, TOS, AST, and ALT values of the control and *G. lucidum* groups. There was no statistical difference between the biochemical data obtained from CCl_4 and CCl_4+GL groups in terms of TAS, TOS, AST, and ALT levels. However, a statistically significant difference was found between these two groups and the control and *G. lucidum* groups in terms of TAS, TOS, and AST levels ($P<0.01$). When ALT level was assessed, there was a statistical difference between CCl_4 group and control and *G. lucidum* ($P<0.05$). However, different from other biochemical indicators, no statistical difference was observed between the CCl_4+GL group and control and *G. lucidum* groups ($P> 0.05$). Data of the groups obtained from the study are shown in Table 1 and Figure 1.

Table 1. TAS, TOS, AST, and ALT values of the groups and statistical significance.
Çizelge 1. Gruplara ait TAS, TOS, AST ve ALT değerleri ve istatistiki önem.

PARAMETERS	GROUPS Control (n:8) Mean±SE	*Ganoderma lucidum* (GL) (n:8) Mean ±SE	CCl_4 (n:8) Mean ±SE	CCl_4+GL (n:8) Mean ±SE	P<
TAS (mmol Trolax eqivalen/L)	1.91 ± 0.045^{a}	1.85 ± 0.042^{a}	1.43 ± 0.045^{b}	1.56 ± 0.035^{b}	**
TOS (µmol H_2O_2 equiv./L)	7.33 ± 0.20^{b}	7.40 ± 0.19^{b}	8.96 ± 0.16^{a}	8.34 ± 0.17^{a}	**
AST (U/L)	158.56 ± 3.4^{b}	165.79 ± 3.75^{b}	203.38 ± 3.7^{a}	194.07 ± 6.19^{a}	**
ALT (U/L)	65.27 ± 4.21^{b}	67.10 ± 2.54^{b}	79.37 ± 1.75^{a}	$74.14 \pm 2.32^{a,b}$	*

*: P<0.05= Statistically significant difference, **: P<0.01= Statistically significant difference, a, b: The difference between the averages of the groups having different letters on the same line is significant. n: number of the subjects in the group, Mean ± SE: Mean±Standard Error.

Histological findings

Liver tissues were embedded in paraffin blocks after routine fixation and tissue follow-up processes. After staining the 5 µm serial sections taken from these blocks via microtome with hematoxylin-eosin, they were examined under a light microscope. In the sections obtained from the groups, the central vena and Kiernan's space were normal, the remark cords were regular, the parenchymal structure was normal, and sinusoids were found between hepatocytes in the control and *G. lucidum* groups. In the portal area, the connective tissue area including the hepatic artery, portal vein, and bile duct had a normal structure (Figure 2a, 2b, 2c). In the CCl_4 group, congestion was observed in the central vein and in the vein in portal area. Multifocal necrosis areas and hepatocellular degeneration were observed between the central vein and the portal region in this group sections. In addition, cell infiltration, vacuole in hepatocyte cytoplasm, irregular remark cords, sinusoidal dilatation, and congestion were observed (Figure 2d, 2e). In the CCl_4+*Ganoderma lucidum* group, focal necrosis areas and hepatic degeneration, congestion, sinusoidal dilatation, and cell infiltration were detected (Figure 2f, 2g, 2h). The incidence of the lesions occurring in this group was detected as close to the CCl_4 group. Thus, *G. lucidum* is not sufficient on its own in terms of the treatment of degeneration in the liver caused by CCl_4 (Table 2). Table 2 shows the tissue change ratings of histopathological lesions occurring in the liver tissue and the frequency ratings were adopted from Bernet et al. (1999).

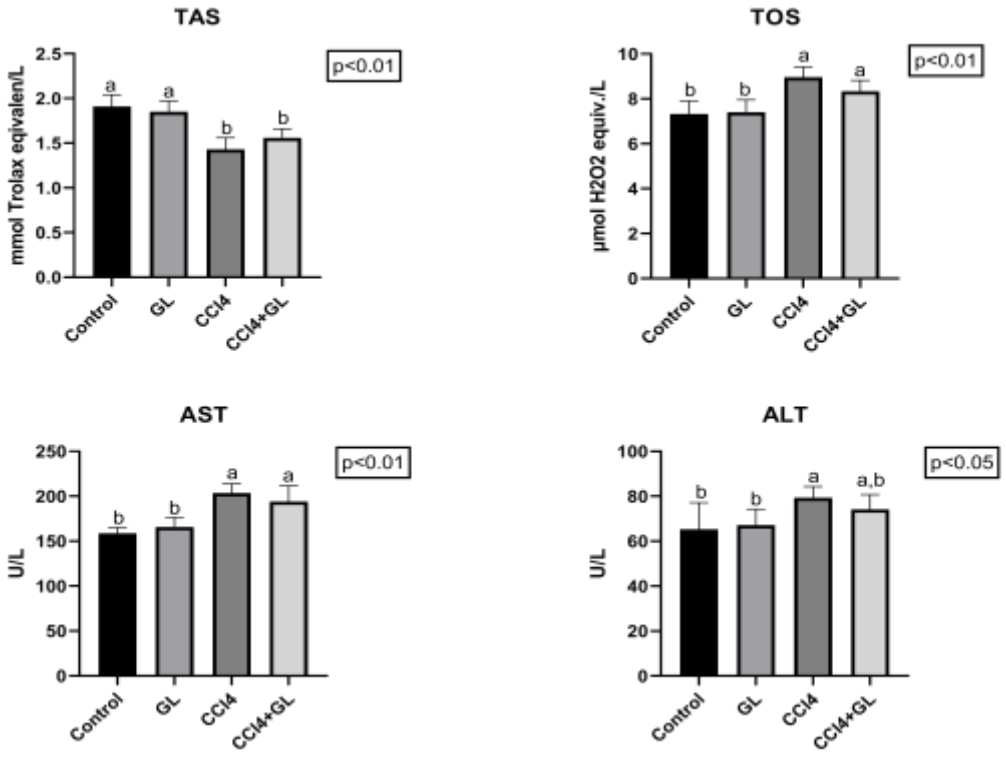
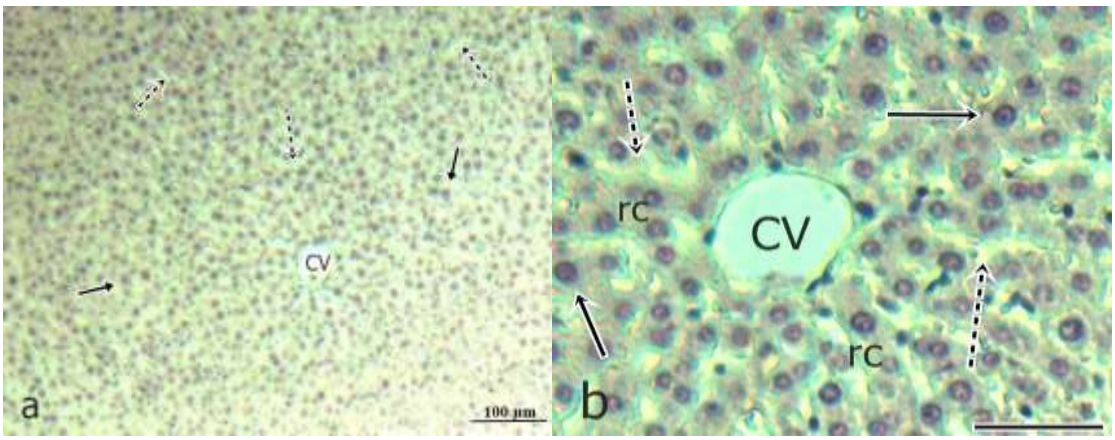


Figure 1. Graphical presentation of TAS, TOS, AST, and ALT values of the groups.
Şekil 1. Gruplara ait TAS, TOS, AST ve ALT seviyelerinin grafiksel gösterimi.

Table 2. Tissue change ratings of the histopathological lesions in the liver tissue.
Çizelge 2. Karaciğer dokusunda histopatolojik lezyonlara ait doku değişim derecelendirmeleri.

Liver lesions	**Groups** Control group	*Ganoderma lucidum* (GL)	(CCl_4)+GL	Carbon tetrachloride (CCl_4)
Degeneration in hepatocytes	-	-	++	+++
Infiltration	-	-	++	+++
Irregularity in remark cords	-	+	++	+++
Vascular degeneration	-	-	++	++
Sinusoidal dilatation	-	-	++	++
Central and portal congestion	-	-	++	++
Necrosis	-	-	++	+++
Sinusoidal congestion	-	-	++	++
Vacuolization	-	+	++	++

-: no abnormality, +: low abnormality frequency, ++: moderate abnormality frequency, +++: high abnormality frequency.



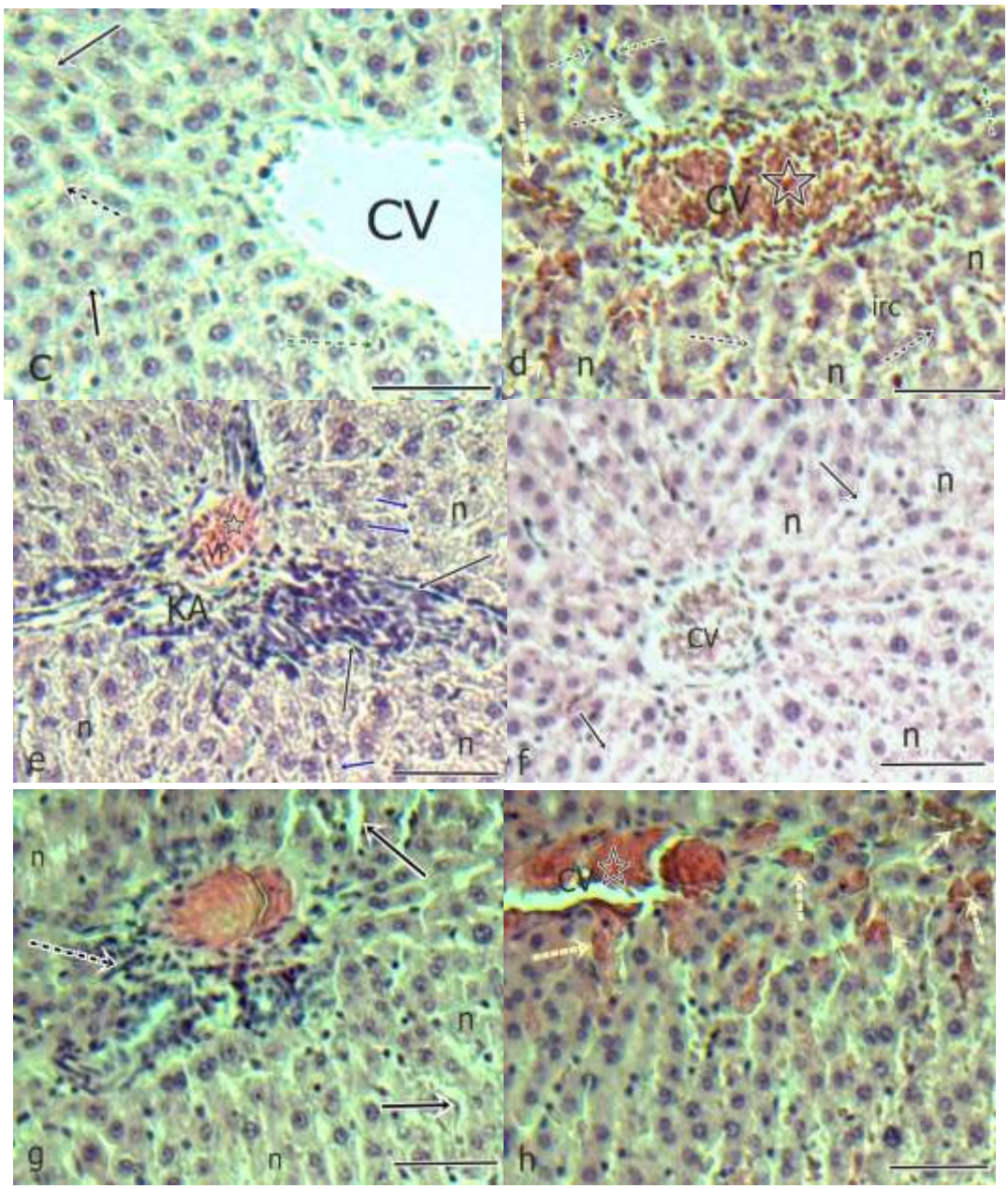


Figure 2. a, b. Liver tissue obtained from the animals in the control group. Hepatocytes and sinusoidal structure have a normal appearance (CV: central vein, arrows: hepatocyte, rc: radially sequenced remark cords, dashed arrows: sinusoid). c. Liver tissue obtained from the animals in the group to which *G. lucidum* was administered at a dose of 1000 mg kg^{-1}. Hepatocytes and sinusoidal structure have a normal appearance (CV: central vein, arrows: hepatocyte, black dashed arrows: sinusoid, green dashed arrows: kupffer cells). d, e. Liver tissue obtained from the animals in the group to which carbon tetrachloride was administered at a dose of 10 ml/kg (CV: central vein, VP: vena porta, asterisk: congestion in central and portal vein, black arrows: Cell infiltration, blue arrows: Vacuole in hepatocyte cytoplasm, black dashed arrows: Sinusoidal dilatation, green dashed arrows: kupffer cells, yellow dashed arrows: sinusoidal congestion, irc: irregular remark cords, n: Multifocal necrosis areas and hepatocellular degeneration). f, g, h. Liver tissue obtained from the animals in the group to which carbon tetrachloride at a dose of 10 ml/kg and *G. lucidum* at a dose of 1000 mg kg^{-1} are administered. vena centralis, black arrows: sinusoidal dilatation, n: Multifocal necrosis areas and hepatocellular degeneration, black dashed arrows: Cell infiltration, asterisk: congestion in central and portal vein, yellow dashed arrows: sinusoidal congestion. H&E. Bar: 100 µm.

Şekil 2. a, b. Kontrol grubundaki hayvanlardan elde edilen karaciğer dokusu. Hepatositler ve sinozoidal yapı normal görünümde (CV: vena centralis, oklar: hepatosit, rc: ışınsal dizilmiş remark kordonları, kesik çizgili oklar: sinuzoid). c. 1000 mg kg^{-1} dozunda G. lucidum uygulanan gruptaki hayvanlardan elde edilen karaciğer dokusu. Hepatositler ve sinozoidal yapı normal görünümde (CV: vena centralis, oklar: hepatosit, siyah kesik çizgili oklar: sinusoid, yeşil kesik çizgili oklar: kupffer hücreleri). d, e. 10 ml/kg dozunda karbon tetraklorür uygulanan gruptaki hayvanlardan elde edilen karaciğer dokusu (CV: vena centralis, VP: vena porta, yıldız: sentral ve portal vende konjesyon, siyah oklar: hücre infiltrasyonu, mavi oklar: hepatosit sitoplazmasında vakuol, siyah kesik çizgili oklar: sinusoidal dilatasyon, yeşil kesik çizgili oklar: kupffer hücreleri, sarı kesik çizgili oklar: sinusoidal konjesyon, irc: düzensiz remark kordonları, n: multifokal nekroz alanları ve hepatosellüler dejenerasyon). f, g, h. 10 ml/kg dozunda karbon tetraklorür ve 1000 mg kg^{-1} dozunda G. lucidum uygulanan gruptaki hayvanlardan elde edilen karaciğer dokusu (CV: vena centralis, siyah oklar: sinusoidal dilatasyon, n: multifokal nekroz alanları ve hepatosellüler dejenerasyon, siyah kesikli oklar: hücre infiltrasyonu, yıldız: sentral ve portal vende konjesyon, sarı kesik çizgili oklar: sinusoidal konjesyon. H&E. Bar: 50 µm (b, c, d ,e, f, g, h), 100 µm (a).

Immunohistochemical findings

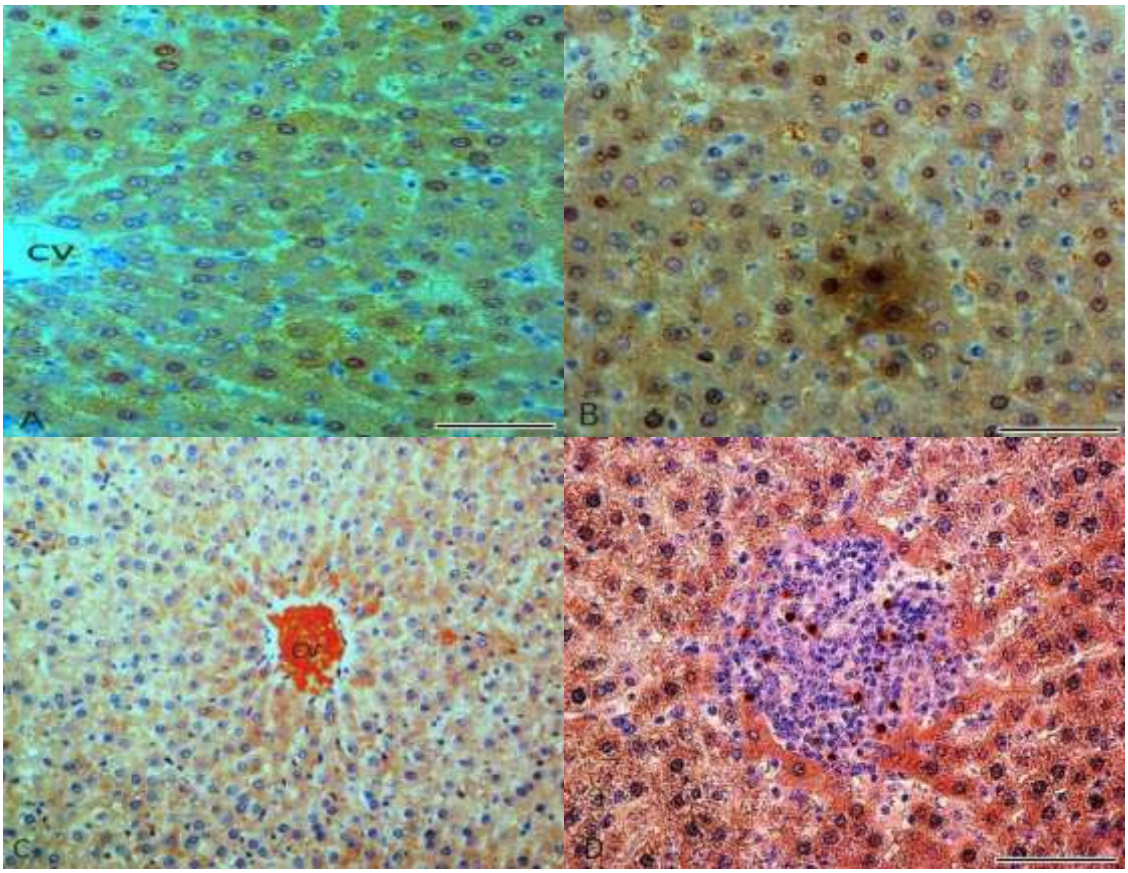
COX-2 immunoreactivity was assessed in the preparations obtained from control, *Ganoderma lucidum*, CCI_4, and CCI_4+*Ganoderma lucidum* groups. In all the groups, COX-2 immunoreactivity was commonly observed in the hepatocyte cytoplasm with moderate intensity in the region from the central vena to the Kierman's space, and sporadically in the hepatocyte nucleus (Table 3, Figure 3). COX-2 immunoreactivity was commonly observed at a higher intensity in the hepatocyte cytoplasm both in centrilobular and in perilobular area in the CCI_4 group when compared to other groups.

Carbon tetrachloride is a toxic material that is widely used by researchers to induce liver injury (El-haskoury et al., 2018; Li et al., 2019). It is known that contact with this toxic chemical induces oxidative stress with the formation of free radicals and causes tissue damage (Ganie et al., 2011). Injuries induced by the substances such as CCl_4 cause apoptosis formation in the cells. Various studies have revealed that natural antioxidants have organ-preserving potential against toxic materials such as CCl_4 (Said et al., 2018; Satria et al., 2019). In a study in which CCl_4 and locust honey there against were used, it was stated that 1 mg kg^{-1} dose of CCl_4 administration caused an increase in the

Table 3. COX-2 immunoreactivity density in the control and application groups. (+++) very dense, (++) moderately dense, (+) less dense, (-) no reaction.

Çizelge 3. Kontrol, ve uygulama gruplarında COX-2 immunoreaktivite yoğunluğu. (+++) çok yoğun, (++) orta derecede yoğun, (+) az yoğun, (-) reaksiyon yok.

Cyclooxygenase	Structure showing immune reaction	Control group	*Ganoderma lucidum* group	CCI_4 group	*Ganoderma lucidum*+CCI_4 group
COX-2	Centrilobular area	+	+	+++	+
	Hepatocyte cytoplasm	+	+	+++	++
	Hepatocyte nucleus	+	+	+	+
	Perilobular area	+	+	+++	++



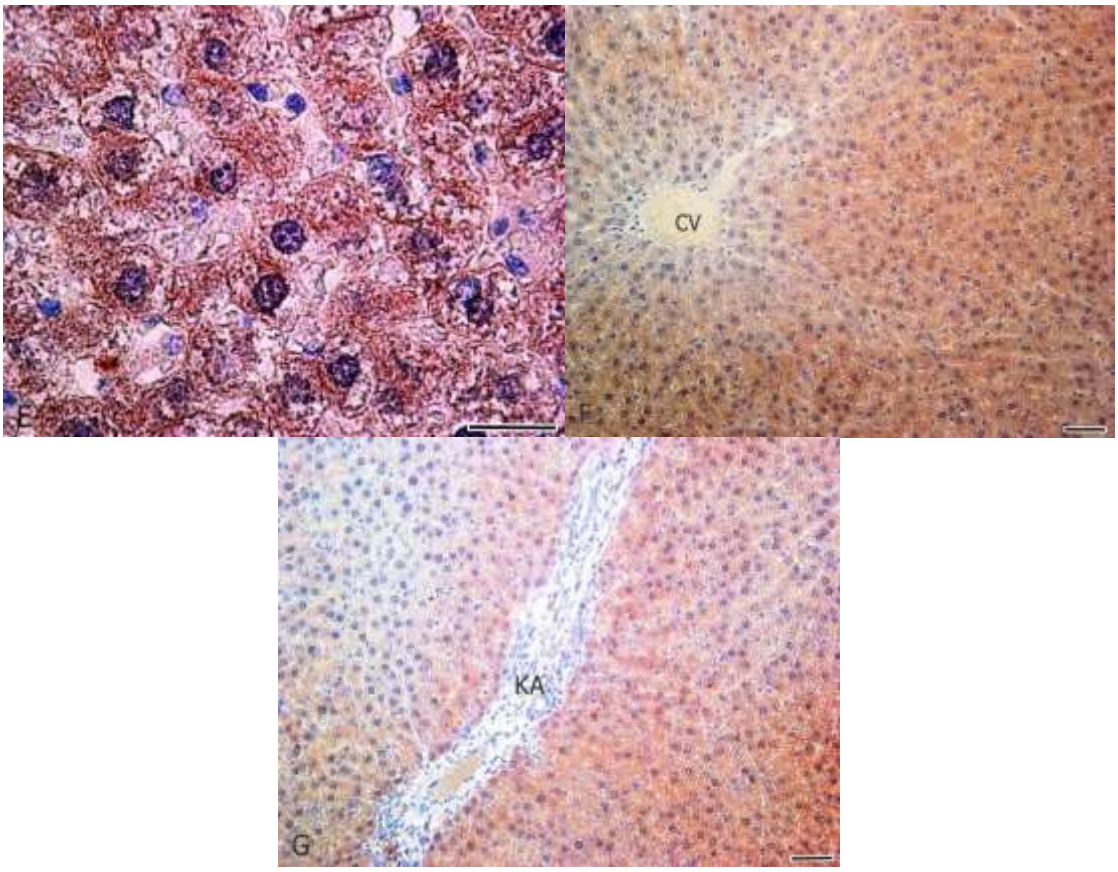


Figure 3. COX-2 immunoreactivity in the liver tissue of the rats. A, B. Control group, moderately dense immunoreactivity in hepatocyte cytoplasm and nucleus in some regions. C. *Ganoderma lucidum* group, moderately dense immunoreactivity in the hepatocyte cytoplasm around central vein. D, E. Carbon tetrachloride group, moderately dense in hepatocyte nucleus between central vena and kiernan's space, and dense immunoreactivity in the hepatocyte cytoplasm. F, G. Carbon tetrachloride+*Ganoderma lucidum* group, mildly dense around the central vein in hepatocyte cytoplasm and moderately dense immune reaction in the area up to the remaining kiernan's space. CV: central vein, KA: Kiernan's space, Bar: 50 µm.

Şekil 3. Sıçan karaciğer dokusunda COX-2 immunoreaktivitesi. A, B. Kontrol grubu, bazı bölgelerde hepatosit sitoplazması ve nükleusunda orta yoğunlukta immunoreaktivite. C. Ganoderma lucidum grubu, vena sentralis çevresindeki hepatosit sitoplazmasında orta yoğunlukta immunorektivite. D, E. Karbon tetraklorür grubu, sentral vena ve kierman aralığı arasında hepatosit nükleuslarında orta yoğunlukta, hepatosit sitoplazmasında yoğun immunoreaktivite. F, G. Karbon tetraklorür+Ganoderma lucidum grubu, hepatosit sitoplazmasında vena sentralis civarında hafif, geri kalan kierman aralığına kadarki alanda orta yoğunlukta immun reaksiyon. CV: vena sentralis, KA: kierman aralığı, Bar: 25 µm (E), 50 µm (A, B, C, D, F, G).

liver enzymes, lactic acid dehydrogenase, blood glucose, uric acid, urea, MDA, and serum kreatinine values (El-haskoury et al., 2018). In a study in which the ethanol extract of *G. lucidum* was administered, as a result of improvements in liver and kidney MDA levels, reductions were observed in the injury of these organs (Shieh et al., 2001). In another study, the curative effect of 100 mg kg^{-1} dose of *G. lucidum* administered in the liver against alcohol-induced liver cirrhosis was explained (Kwon & Kim, 2011).

Determination of alternative treatment sources against many degenerative and chronic diseases takes place among the most popular issues in the scientific world. Side effects and concerns of synthetic treatments in the society have increased the tendency to natural alternative treatment resources. The studies on the biological activities of plants and mushroom supporting this situation have increased in recent years (Ozkan et al., 2016). Liver injury after CCI_4 exposure is characterized by elevated levels of serum hepatic marker enzymes indicating cellular leak and loss of functional integrity of the hepatic membrane architecture. High levels of ALT and AST activities are sensitive indicators of liver cell injury. The increase in serum hepatic marker levels revealed that an extensive liver injury has occurred by CCI_4 due to the increased lipid peroxidation which can cause membrane injury (Pradeep et al., 2010). Many chemicals can have toxic effects on the liver. For example, liver and kidney function biomarkers of rats exposed to aluminum were adversely affected. In the group given pomegranate juice as a preservative, it was stated that serum biomarkers almost approached the values of the control group (Çiftci et al., 2022).

In recent years, *G. lucidum* has drawn attention due to its oxidative stress-reducing capability. The current results have also indicated that treatment with *G. lucidum* has decreased the effect of the CCl_4-induced hepatotoxicity as shown by the decrease in AST, ALP, and ALT liver enzymes (Lin et al., 1995; Gao et al.,

2019). Results obtained from animal experiments have shown that CCl_4 causes fibrosis and injury in the liver tissue and also increases the liver enzyme levels such as Alanine aminotransferase (ALT) and Aspartate transaminase (AST) (Gao et al., 2019). It is observed that warm water and ether extracts of orally and intraperitoneally administered *G. lucidum* have an active hepaprotective effect against CCl_4-induced liver injury and cause recovery in aspartate and alanine transaminase (AST-ALT) and lactate dehydrogenase (LDH) values that are accepted as markers for liver injury (Lin et al., 1995; Kim et al., 1999).

In the studies conducted by Koçak et al. (2019) and Atasever & Yaman (2014) for TAS, TOS, and OSI levels, they reported that a single dose of 1 ml/kg decreased the serum TAS level of CCl_4 and increased TOS and OSI levels. It is shown that the use of antioxidant legalon (silymarin) against CCI_4 reduces total oxidant and oxidative stress index levels and increases the total antioxidant level (Verma et al., 2015). In studies in which CCl_4 was administered chronically, it was noted that there was a significant increase in ALT enzyme due to the deterioration of cell membrane permeability depending on hepatocyte destruction (Abdel-Daim et al., 2016; Atasever et al., 2020). The data obtained from the current study is parallel with the findings of other researchers.

CCl_4 is widely used to cause injury in the tissues, especially in the liver (Lv et al., 2006; Lida et al., 2009; El-haskoury et al., 2018). Rats were administered with a dose of 0.2 ml/100 g of CCI_4 three times a week for eight weeks (Basu, 2003) and once a week for ten weeks (Lida et al., 2009), and then, widespread areas of necrosis were detected in the fibrous tissue in the portal region of the liver tissue. In addition, fat degeneration and inflammatory cell infiltrations have been reported in hepatocytes. In other studies, it was shown that CCI_4 caused fatty vacuoles in the hepatocyte within the liver tissue, inflammatory cell infiltrations at varying severities, and fibrosis (Atasever et al., 2020, Basu, 2003). In the present study, congestion, multifocal necrosis areas, cell infiltration, and sinusoidal dilatation around the central vein and portal region of CCI_4 were observed. This is compatible with findings of other studies in which CCI_4 was administered. Grape seed oil slightly reduced the number of fat vacuoles and partially reduced the necrosis areas, and also prevented the formation of fibrous tissues in the liver. Numerous studies have reported that grape seed oil caused histological recovery in the liver lesions caused by other toxic materials. In the studies conducted with grape seed oil against various hepatotoxins, it was thought that the hepatoprotective effects of grape seed oil were caused by antioxidant and free radical scavenger components (Khalifa et al., 2011; Al-Attar, 2015). In the current study, it is understood that *G. lucidum*, which is used against the toxic properties of CCI_4, reduced the histopathological lesions in the liver, however, it was not sufficient alone in the treatment of lesions.

Immunohistochemical findings are a current method that has been used by many researchers in order to determine and reveal the releasing tissue parts of expressed proteins (Yıldız et al., 2013; Nur et al., 2014; Nur et al., 2015). While CCl_4 increased iNOS, COX-2, TNF-α and IL-1ß mRNA, and protein expression levels in the liver tissues, it significantly decreased iNOS, COX-2, TNF-α, IL-1ß mRNA and protein expression levels that are similar to the findings in the silymarin group as a result of the inflammation inhibiting effect of the hawk tea. As a result of CCl_4 administration in the histological examination of the tissue, congestion in the central zone of the liver, haemorrhage, and necrosis in the hepatocytes were observed, and decreases in the severity of the lesions were detected in the groups to which hawk tea and silymarin were applied. Serum AST, ALT, and LDH levels that increased by the CCl_4 administration have significantly decreased as a result of hawk tea application (Zhao, 2013). In a study examining the expression of cyclooxygenases in liver tissue, COX-1 and COX-2 immunoreactivity was detected in all the groups at similar intensity and in hepatocyte cytoplasm (Nur et al., 2015). COX-2 immunoreactivity in the kidney tissue of the rats to which capsaicin was administered in puberty decreased in the macula densa, Henle's loop, distal and proximal tubules in the capsaicin group compared to the control group, while COX-1 immunoreactivity increased in the proximal tubules in the capsaicin group when compared to the control group (Yıldız et al., 2013). In a study investigating the protective effect of berberine and silymarin against CCl_4 administration, COX-2 immunoreactivity was detected in the hepatocyte cytoplasm and nucleus in the necrosis areas. Berberine and silymarin decreased the COX-2 immunoreactivity when compared to the CCl_4 group. In the histological examination of the liver, vacuole-fat formation, inflammatory cell infiltration, and extensive necrotic areas were stated in the CCl_4 group. When compared to the control group, serum AST, ALT, and alkaline phosphatase (ALP) levels increased in the CCl_4 group (Domitrovica et al., 2011). In a study that investigated the effect of L-thearinin, which is an amino acid in the tea, against CCl_4 hepatotoxicity, it was reported that L-thearinin that suppresses COX-2 and iNOX expression in the liver with increased serum TNF-α and IL-1ß in CCl_4 group, thus, inhibited the inflammatory response. L-thearinin decreased AST, ALT, and bilirubin levels and also, and there was a decrease in the severity of histopathological changes in the liver (Jiang et al., 2012).

CONCLUSION

In the present study, similar to the other studies conducted with toxic substances, it was concluded that carbon tetrachloride can cause significant biochemical and histological changes in the rats due to oxidative damage, while *G. lucidum* has a therapeutic feature by showing a reduction in the severity of the lesion. It is thought that the hepatoprotective effect of *G. lucidum* may be related to decreasing oxidative stress and inhibiting the inflammatory response in the liver due to its free radical scavenging feature. When the results of the study were assessed, *G. lucidum* showed a protective character against acute liver injury by suppressing the inflammatory response by preventing the increase of carbon tetrachloride-induced oxidant capacity and reducing the apoptotic reaction developing in hepatocytes.

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Author's Contribution

MSc. HCY, Dr. GN, Dr. HAD and Dr. IG researched literature and conceived the study. They were involved in protocol development, gaining ethical approval, experimental design. Data analysis was completed by whole authors. All authors reviewed and edited the manuscript and approved the final version of the manuscript.

Conflict of interests/Competing interests

The authors declare that there is no conflict of interest.

Ethics approval

This study was approved by Gaziantep University Animal Experiments Local Ethics Committee (permission no. 2018/22).

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Assessment of Inhibitory Ability Against Medicinally Important Enzymes with Invitro and In Silico Studies: Phenolic Content of Endemic *Centaurea cadmea* subsp. *pontica*

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ABSTRACT

Centaurea species has great potential as a traditional medicinal herb and *C. cadmea* subsp. *pontica* collected from rocky slope crevices of Küre Mountain is endemic to the flora of Türkiye. In the present work, to reveal the plant's pharmacological importance, its potency to inhibit various medicinal enzymes was investigated, supported by molecular docking studies. The half-maximal inhibitory concentration (IC_{50}) results for studied enzymes were quantified between 0.50-86.97 µg mL^{-1}, and the extract was efficient against HMG_CoA R, α-glucosidase, and α-amylase enzymes linked to diabetes and cholesterol. Nine phenolic compounds were identified in the *C. cadmea* subsp. *pontica* extract and the interactions of the most abundant phenolic compounds with the enzymes were examined with molecular docking studies. In conclusion, findings amassed from the present study inclined to support the opinion that *C. cadmea* subsp. *pontica* may be beneficial as an effective herb for formulating novel health-promoting ingredients.

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Tıbbi Açıdan Önemli Enzimlere Karşı İnhibisyon Yeteneğinin Invitro ve Insilico Çalışmalarla Değerlendirilmesi: Endemik *Centaurea cadmea* subsp. *pontica* Bitkisinin Fenolik İçeriği

ÖZET

Centaurea türleri geleneksel şifalı bitki olarak büyük bir potansiyele sahiptir. Küre Dağı'nın kayalık yamaç yarıklarından toplanan *C. cadmea* subsp. *pontica* Türkiye florasına endemiktir. Bu çalışmada, bitkinin farmakolojik önemini ortaya koymak için çeşitli tıbbi enzimleri inhibe etme pontansiyeli, moleküler yerleştirme çalışmaları ile desteklenerek araştırılmıştır. Çalışılan enzimler için yarı maksimum inhibitör konsantrasyon (IC_{50}) sonuçları 0.50-86.97 µg mL^{-1} arasında belirlendi. Bitki ekstraktı diyabet ve kolesterol ile bağlantılı olan HMG_CoA R, α-amilaz ve α -glukosidaz enzimlerine karşı etki gösterdi. *C. cadmea* subsp. *pontica* özütünde dokuz fenolik bileşik tanımlandı ve en fazla bulunan fenolik bileşiklerin enzimlerle etkileşimleri moleküler yerleştirme çalışmaları ile incelenmiştir. Sonuç olarak, mevcut çalışmadan elde edilen bulgular, bu endemik bitkinin sağlıklı yaşam kalitesinin geliştirilmesine yönelik yeni bileşenlerin formüle edilmesi için etkili bir bitki olarak yararlı olabileceğini göstermektedir.

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INTRODUCTION

The genus *Centaurea* L. belongs to the Asteraceae family, Turkiye is the major distribution center of the plants, and it is the third-largest genus in Anatolia. The taxonomically problematic *Centaurea* genus is represented by over 600 species worldwide depending on the classification used, and there are 218 species in Türkiye, and the endemism rate of the taxon has

reached 64 %. *Centaurea cadmea* subsp. *pontica* spreads to Bartın and Zonguldak provinces in the northern Anatolia region (Türkiye). The plant is a chamaephyte that grows in limestone rock cracks and on rocky slopes far away from brookside. *C. cadmea* subsp. *pontica* was described by Köse et al. (2010) and considered as the least concern (LC) category. Flowering in the plant occurs in June-September, and fruiting occurs in July-September (Köse et al., 2010; Yaman et al., 2020; Özbek, 2021; Duman et al., 2021). Plants belonging to the genus *Centaurea* were reportedly used in traditional medicine as herbal remedies for digestive, antipyretic, antidiarrheal, expectorant, and tonic effects (Bancheva et al., 2023; Astari et al., 2014). As a continuation of research on the genus and secondary metabolites, we have investigated the phenolic components of the *Centaurea cadmea* subsp. *pontica* collected from its natural habitat. Since plants contain various phytochemical ingredients, their effects on enzymes of medical importance associated with various deficiencies are being screened.

Chronic and non-communicable diseases, including cardiovascular, cancer, diabetes, and neurodegenerative diseases (Alzheimer's), have long-term adverse effects on public health and cause 63% of deaths globally. Experimental and epidemiological studies show that some phytochemical molecules play an essential role in preventing these diseases. In fact, enzyme inhibitors are used to treat some diseases such as hypertension, metabolic disorders, neurodegenerative diseases, and some cancers (Gonçalves & Romano 2017). Bioactive molecules bind to the active site of proteins and function as organic compounds that decrease the bioactivity of enzymes. Many drug molecules are used as inhibitors or activators for enzymes. These compounds affect the catalysis rate of biochemical reactions leading to conformational changes on the active surface of the target protein. Some drugs used today are inhibitors of enzymes that are associated with regulation in the ailment process. According to the currently used therapy, the "inhibition of key enzymes" approach is an effective way to regulate pathologies. For example, HMG-CoA reductase and ACE (angiotensin-converting enzyme) inhibitors regulate cholesterol and blood pressure levels, respectively. Synthetic inhibitors lead to the research for novel, safe, and effective compounds, especially from natural resources, due to their comparatively low toxicity and side effects. Investigating the new biologically active agents from natural sources is one of the study strategies for medicinal drugs due to the easy accessibility of herbal phenolic substances (Guerrero et al., 2012; Baskaran et al., 2015; Zengin et al., 2016; Gonçalves & Romano 2018; Yin et al., 2019).

Phytochemical ingredients of plants vary according to the natural environment in which they grow, locality, and altitude. Studies on the *Centaurea* genus extensively focused on phytochemical contents, antidiarrheal, antimicrobial, antioxidant, and antipyretic activities (Astari, 2013; Grafakou, 2018; Alper & Güneş, 2019; Tugay 2020). Nonetheless, *Centaurea cadmea* subsp. *pontica* has been selected to reveal its medical importance as no enzyme inhibition has been performed on this species. As a novelty, enzyme inhibition, assisted with molecular docking studies in the broad spectrum, was investigated to gain further knowledge on the endemic plant.

MATERIALS and METHODS

Plant sample and extraction method

The samples (*Centaurea cadmea* subsp. *pontica*) were collected from the rocky slopes around Ulukaya waterfall at the foothills of Küre Mountain in Ulus-Bartın province, Türkiye, in July 2020. The collected samples were left to air dry at room conditions. The voucher specimen (BofHerb_495) has been authenticated by Prof. Z. Kaya and deposited in the Herbarium of the Department of Forest Engineering, Faculty of Forestry, Bartın University.

The aerial parts of dried plant samples were grounded in a mechanic grinder, and the powdered material was extracted in methanol at ambient conditions. The admixture was sonicated for 30 min at 37°C and centrifuged at 4500 x g for 12 min. The upper solvent of the filtrated extract was evaporated at an ambient temperature of less than 55 °C. The extract was kept at -20 °C for biochemical analysis.

Determination of the total phenolic compound (TPC) and total flavonoid contents (TFC)

The analysis of TPC and TFC in the *Centaurea cadmea* subsp. *pontica* extracts were performed by the Folin-Ciocalteu reagent and aluminum chloride ($AlCI_3$) colorimetric method, respectively described by the earlier studies. The quantities of them were expressed as mg of gallic acid and quercetin per gram of the extract (Singleton & Rosi, 1965; Pękal & Pyrzynska, 2014).

Determination of individual phenolic compositions

Phenolic compound profiles of the plant extract were quantified using a reverse-phase HPLC system coupled with an SPD-M20A detector and LC 20AT pump (Shimadzu Scientific Instruments, Japan). The extract solution (20 µL) was filled to the equipment set to 1 mL min^{-1} with automatic injection. The mobile phases, solvent A (methanol) and solvent B (acetic acid, 2% v/v), were formed, and the elution was given to the reverse phase C18 analytical column (GL Sciences, 5 µm, 4.6 mm x 250 mm,) and the

temperature was kept at 25°C. The flow rate was set to 1 mL min^{-1} for gradient elution. Then, the obtained chromatographic profiles belonging to the phenolic compound in the plant extract solutions were defined and quantified with the accompaniment of the UV spectra, retention times, and chromatograms with known standard phenolic compounds. The calculated results were specified as mg g^{-1} plant extract (Elmastaş et al., 2017).

Enzymes inhibition studies

The enzyme inhibitory potency of *C. cadmea* extract was evaluated using the 96-microwell plate spectrophotometric method referred to in the following sentences (Microplate Reader, Thermo Scientific). AChE and BChE activity were evaluated using the well-known Ellman's spectrophotometric method (Ellman et al., 1961). The inhibitory assay for Angiotensin-converting enzyme (ACE) was done in Tris-HCI buffer (50 mM, pH 7.5) with a referred research (Hou et al., 2003). The reaction components of wells contain 10 µL of ACE solution, 10 µL of plant extracts, and 150 µL of substrate FAPGG solution (0.88 mM). Captopril was used as a reference compound, and the reduced absorbance due to hydrolysis of the FAPGG was read at 340 nm at 37 °C for 5 min. The α-amylase inhibition method was achieved using the modified Caraway technique (Yang et al., 2012). Briefly, 30 µL of phosphate buffer, 20 µL of extract solution, and α-amylase (30 µL) were pipetted to wells, and the plate was preincubated for 10 min at 37 °C. After the 50 µL of the starch-substrate (1 %) was pipetted to the wells, the mixture was allowed to incubate for ten min at 25°C. Then, the reaction was aborted by adding 10 ml of 25 µL of 10 % HCl and was added 100 µl of iodine/potassium iodide solution. The absorbance of the colored wells d was recorded at 630 nm. The α-glucosidase inhibitory assay was done using p-nitrophenyl α-D-glucopyranoside (2 mM) as substrate (Tao et al., 2013). In each mixture of the well microplate, 120 µL of KH_2PO4, 10 µL of test solution, and 20 µL of α-glycosidase were added and incubated for 10 min at 37 °C. After the p-NPG (50 µL) was supplemented to each well, the absorbance of released p-nitrophenyl was measured at 405 nm. The inhibitory study of pancreatic lipase was carried out using substrate pnitrophenylpalmitate (p-NPP) with cited research (El-Korany et al., 2020). The wells comprised Tris-HCI (100 mM, pH 8.2), 20 µL of lipase solution and plant extract was incubated for 10 min at 37 °C and then the absorbance of liberated p-NP was monitored at 410 nm. The urease inhibitory property of plant extracts was determined using the indophenol method to quantify the amount of ammonia formed due to the enzymatic reaction (Ikram et al., 2017). In each well, microplates briefly containing 50 µL of urea prepared in KH_2PO4 buffer (pH 8.2, 100 mM), 25 µL of jack bean urease solution, and 20 µL of extract were incubated for 15 min at 37 °C. Then, then phenol reagent and alkali reagents were added to react with the released ammonia. The absorbance of colored complex was read at 630. For the tyrosinase inhibition assay; substrate L-DOPA, mushroom tyrosinase in phosphate buffer (pH 6.8, 100 mM), and plant extract were used according to the previously described spectrophotometric method (Masuda et al., 2005). The increased absorbance of the colored mixture caused by dopachrome was recorded at 475 nm. For the HMG-CoA reductase activity assay; an HMG-CoA reductase assay kit (BioVision Inc., USA) containing HMG-CoA reductase, NADPH, HMG-CoA, and assay buffer was used, and the inhibitory ability of the plant extract was assessed by reading the absorbance at 340 nm. The inhibitory ability of plant extract was performed using *Clostridium histolyticum* collagenase with a previously designed method (Thring et al., 2009). Briefly, collagenase in tricine buffer (pH 7.5) was incubated with the extract solutions at 25 °C for 15 min and substrate FALGPA was added to wells. The reduced absorbance due to hydrolysis of the FALGPA was read at 340 nm.

Antimicrobial test

The antibacterial and antifungal properties of *C. cadmea* subsp. *pontica* were determined according to the minimal inhibitory concentration (MIC) test. Fungal strains (*Cancida utilise* and *Cancida albicans*), Gram-positive (*E. faecalis* and *S. aureus*), and Gram-negative (*K. pneumonia* and *E. coli*) were used for this test. The frozen stock was taken from cells and inoculated. Cultures were adjusted to 0.5 McFarland Units, and the extract was added as serial dilutions to a concentration of 1 mg/mL in a 96-microwell plate. Microbial cells were incubated at 37°C for 24 hours, and MIC results were evaluated by reading in the spectrophotometer.

Computational study

Molecular docking is a widely used in silico method for learning protein-ligand interactions (Meng et al., 2012). In this context, to examine in silico enzyme inhibition ability, molecular docking-based computations were executed on AChE, ACE, BChE, α-glycosidase, α-amylase, lipase, collagenase, HMG_CoA R, urease, and tyrosinase by using Autodock vina 1.2.0 (Troot & Olson, 2010; Meng et al., 2012; Eberhardt et al., 2021). The chemical structure of the plant metabolites was retrieved from PubChem. Afterward, geometry optimization and energy minimization were performed to utilize Chimera software (Pettersen et al., 2004). The Auto- DockTools 1.5.7 package (Morris & Dallakyan, 2013) was utilized to generate entry files before molecular docking. Protein structures were

withdrawn from the protein data bank (PDB) and the structure of ligands (PubChem and Drugbank). Discovery Studio (DS) 2021 was also used to prepare the targets for docking studies. Water molecules were removed using the DS 2021 program, and polar hydrogen bonds were added to the structure. The grid of the studied complexes was portrayed with literature and via the subprotocol of DS 2021. The grid box of size 126 x 126 x126 represents X, Y, and Z coordinates, with 8 comprehensive values and 0.375 A °grid point space. The results were evaluated with the DS 2021 program, and images were taken.

RESULTS

Enzyme inhibitory activity of *C. cadmea* subsp. *pontica* extract

In the current study, the inhibitory potential of the plant extract was screened against ten different medicinal enzymes to reveal the herbal medicine potential. The IC_{50} values of the plant extract were shown in Fig 1 and Table 1. In this current screening study, the extract has inhibitory potential at different concentrations and the obtained IC_{50} values were 86.97±1.93 µg/mL, 75.60±1.87 µg/mL, 24.67±1.39 µg/mL, 0.509±0.00, 82.48±1.91 µg/mL, 66.20±1.82 µg/mL, 69.15±1.84 µg/mL, 72.05±1.5 µg/mL, 26.66±1.42 µg/mL, and 46.02±1.60 µg/mL for AChE, BChE, ACE, HMG_CoA R, α-glycosidase, α-amylase, lipase, collagenase, urease, and tyrosinase, respectively. The same extract was found to be effective against HMG_CoA R, amylase, and glycosidase, which is better than the IC_{50} values of the known standards. When the result of urease was examined, the extract was obtained from *C. cadmea subsp. pontica* was found to display a remarkable effect (IC_{50}: 26.66±1.42, r2: 0.999); it is slightly weaker than the IC_{50} of the thiourea.

Table 1. IC_{50} values of *C. cadmea* subsp. *pontica* extract on studied enzymes
Çizelge 1. Çalışılan enzimler üzerine C. cadmea subsp. *pontica ekstraktının IC_{50} değerleri*

Enzymes	AChE		BChE		ACE		α-Glucosidase		α-Amylase	
Values	IC_{50}	r^2	IC_{50}	r^2	IC_{50}	r^2	IC_{50}	r^2	IC_{50}	r^2
Extract (µg mL^{-1})	86.97±1.93	0.986	75.60±1.87	0.984	24.67±1.39	0.984	69.15±1.84	0.990	82.48±1.91	0.993
Standards (µg mL^{-1})	23.36±1.36	0.985	24.84±1.39	0.992	21.36±1.33	0.988	174.3±2.24	0.993	131.2±2.11	0.995
Enzymes	**HMG_CoA R**		**Collagenase**		**Lipase**		**Tyrosinase**		**Urease**	
Values	IC_{50}	r^2	IC_{50}	r^2	IC_{50}	r^2	IC_{50}	r^2	IC_{50}	r^2
Extract (µg mL^{-1})	0.509±0.00	0.967	72.05±1.5	0.984	66.20±1.82	0.985	46.02±1.60	0.989	26.66±1.42	0.999
Standards (µg mL^{-1})	8.78±0.94	0.997	2.52±0.40	0.998	35.5±2.58	0.991	3.49±0.54	0.999	20.36±1.30	0.989

Evaluation of phenolic compounds of methanolic extracts of *C. cadmea* subsp. *pontica*

The quantification of phenolic compositions was accomplished in the obtained extract *of C. cadmea* subsp. *pontica* and the constituents were given in Table 2. Chlorogenic acid, gentisic acid, rosmarinic acid, rutin, cinnamic acid, quercetin, eugenol, apigenin, and methyl chavicol were identified as the main phytochemical compounds. Rutin was determined as the richest metabolite identified in *C. cadmea* subsp. *pontica extract*, followed by methyl chavicol, the most abundant metabolite, calculated their values as 119.49 ±1.02 and 40,83±1.74 µg g^{-1}, respectively. The total phenol and flavonoid amount of the same extract were 36.93 ± 0.51 mg g^{-1} as gallic acid equivalent (GAE), 11.66 ± 0.41 mg g^{-1} as quercetin equivalents (QE), respectively (Table 3).

Antimicrobial assessment of *C. cadmea* subsp. *pontica* plant extracts

The MIC method was used to assess the antibacterial and antifungal activity of *C. cadmea* subsp. *pontica* against different bacteria strains such as *Enterococcus faecalis*, *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumonia*, and fungal strains *Candida albicans* and *Candida utilis*. As shown in Table 4, the MIC value of the plant extract ranged from 296.6 ± 2.472 to 645 ± 2.810 for microorganisms, and the extract exhibited higher inhibitory potential against *E. coli* than other microorganisms.

Molecular docking result of phenolic compounds of *C. cadmea* subsp. *pontica*

In the present work, the enzyme inhibition potency of three secondary compounds [methyl chavicol (1), gentisic acid (2), and rutin (3)] most detected in *C. cadmea* subsp. *pontica*, according to the data obtained from the HPLC study, was assessed with the assistance of computational methods. Although many in silico analyses were carried out in the literature, in this goal was to interrelate attachment points of substances to enzymes in which the quantity of secondary metabolites varies according to the plant species and even where the plant is grown. The three target enzymes with the best interaction and binding scores are HMG_CoA R, tyrosinase, and urease.

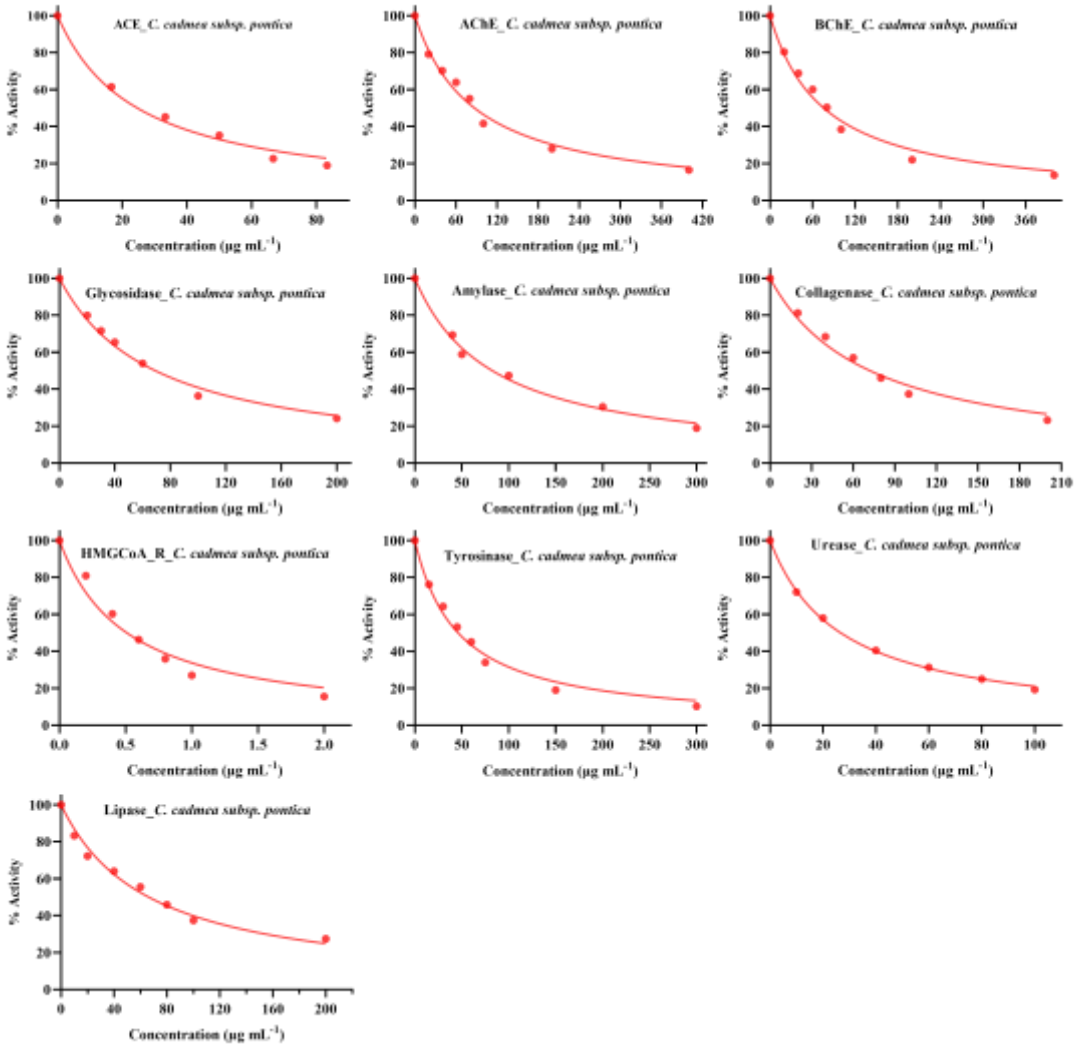

Figure 1. IC_{50} graphs for the studied enzymes of the *C. cadmea* subsp. *pontica* extracts
Şekil 1. C. cadmea subsp. *pontica ekstraktlarının incelenen enzimler için IC_{50} grafikleri*

Table 2. The main constituents of the aerial part extract of *C. cadmea* subsp. *pontica*
Çizelge 2. C. cadmea subsp. *pontica toprak üstü özütünün temel bileşenleri*

Phenolic Compounds	Retention time (min)	Amount (µg g⁻¹)
Chlorogenic acid	11.348	19.06±0.19
Gentisic acid	12.099	26.45±0.21
Rutin	26.691	119.49±1.02
Rosmarinic acid	27.226	16.88±0.18
Cinnamic acid	38.622	16.89±0.17
Quercetin	42.209	10.01±0.61
Eugenol	44.646	5.92±0.01
Apigenin	47.434	22.22±0.23
Methyl chavicol	55.471	40.83±1.74

Table 3. TPC and TFC in the plant extract
Çizelge 3. Bitki özütünde TPC ve TFC

Extracts	TPC (mg g⁻¹ DW)	TFC (mg g⁻¹ DW)
C. cadmea subsp. pontica	36.93 ± 0.51	11.66 ± 0.41

Table 4. MIC results for selected bacterial and fungal strains
Çizelge 4. Seçilen bakteri ve mantar suşları için MİK sonuçları

Microorganisms	MIC Values of *C. cadmea* subsp. *pontica* (µg mL⁻¹)
E. coli	296 ± 2.472
K. pneumoniae	645 ± 2.810
S. aureus	461 ± 2.664
E. faecalis	321 ± 2.507
C. albicans	347 ± 2.541
C. utilis	438 ± 2.641

Compound 3 exhibited the best results against target enzymes in the molecular insertion study, with a better binding tendency than control compounds. When the retrieved compounds' docking results were examined, it was concluded that the metabolites of the plant extract highly interacted with HMG_CoA R, tyrosinase, and urease, and the detailed interaction data of the selected compounds were provided in Table 5.

Table 5. The binding scores of the selected compounds (methyl chavicol_1, gentisic acid_2, and rutin_3) with the target proteins. *The known inhibitory substances are remarked for each target enzyme (BE: Binding energy).

*Çizelge 5. Seçili bileşiklerin (metil chavicol_1, gentisik asit_2 ve rutin_3) hedef proteinlere bağlanma skorları. *Bilinen inhibitör maddeler her bir hedef enzim için belirtilmiştir (BE: Bağlanma enerjisi).*

AChE	BE (kcal/mol)	HMG_CoA R	BE (kcal/mol)
1	-5.7	1	-6.2
2	-6.0	2	-7.4
3	-8.2	3	-10.3
Tacrine*	-7.6	Atorvastatin*	-7.7
BChE	**BE (kcal/mol)**	**α-Amylase**	**BE (kcal/mol)**
1	-6.0	1	-4.5
2	-6.1	2	-5.8
3	-8.3	3	-8.2
Tacrine*	-7.2	Acarbose*	-9.9
ACE	**BE (kcal/mol)**	**Collagenase**	**BE (kcal/mol)**
1	-5.2	1	-4.3
2	-5.6	2	-5.2
3	-9.5	3	-8.4
Captopril*	-4.3	EGCG*	-7.2
Urease	**BE (kcal/mol)**	**α-Glucosidase**	**BE (kcal/mol)**
1	-5.0	1	-5.2
2	-5.6	2	-6.7
3	-10.2	3	-8.8
Thiourea*	-3.5	Acarbose*	-9.5
Tyrosinase	**BE (kcal/mol)**	**Lipase**	**BE (kcal/mol)**
1	-5.6	1	-4.4
2	-6.3	2	-5.8
3	-9.7	3	-9.4
Kojic acid*	-5.5	Orlistat*	-7.2

Firstly, atorvastatin, the control compound atorvastatin for HMG-CoA R, formed a hydrogen bond with Ala110, and Ala187 residue, hydrophobic interaction with Lys108, and electrostatic interaction with Arg187 and Glu109. Compound 3 has a hydrogen bond (Thr152, Pro148, Gly149, and Gly198) and hydrophobic interaction (Phe236, Pro235, Leu239, Tyr205, Tyr307, Pro235, and Leu853) with the protein. Compound 2 has a hydrogen bond (Asn276, Gly198, and His233) and hydrophobic interaction (Pro235). Compound 1 showed only hydrophobic interaction (Pro148, Val196, Tyr307, and Val196). The interactions of the selected molecules with the control substance were shown in Fig. 2.

The control compound of tyrosinase, kojic acid, formed a hydrogen bond with Thr345 residue, hydrophobic interaction with Phe355, Ala295, Ala346, Val366 (Fig. 3). Compound 3 has a hydrogen bond (Ser291, Gln294, Asp344, Thr343, and Lys151) and hydrophobic interaction (Phe355) with the target enzyme. Second, compound 2 has a hydrogen bond (Gln37, Lys335, and Ser170) and hydrophobic interaction (Arg163 and Pro341). Compound 1 has a hydrogen bond (Thr345) and hydrophobic interaction (Phe355, Ala295, Ala346, and Val366). These locations are presented in Fig. 3.

Compound 3 displays the best numerical and visual results in possible complexes with the urease shown in Fig. 4. When the docking results are evaluated, compound 3 occurred in a hydrogen bond with Ala440, Asp633, Ala636, Asp633, and Pi-Sulfur interactions with His545 and Gly550 amino acid in the binding surface of the enzyme. Compound 2 has a hydrogen bond (Ser665, Asn668, Glu303, Asp664, and Asn668). Compound 1 has a hydrogen bond (The33) and hydrophobic interaction (Tyr32, Val36, Lys716, Phe712, and Val744). These situations are shown in Fig. 4.

DISCUSSION

Humankind has been compelled to use various natural substances by resorting to nature to manage their diseases, and the tendency to research the healing

aspects of medicinal plants has continued since ancient times. Nature has provided human beings with various opportunities, and medicinal plants have been used to cure and alleviate various diseases. Intercultural medicine essentially includes health practices, bridging indigenous medicine and modern medicine, both of which are considered complementary (Baydoun et al., 2015; Dutta et al., 2021; Tiwana et al., 2021). Interest in medicinal plants, including *Centaurea* species having several ethnopharmacological properties, is reemphasized, and they are being screened for pharmacological activities. Although various studies have been carried out on *Centaurea* species for their biological effect, the extract of *C. cadmea subsp. pontica* has not been evaluated for its potential to inhibit various enzymes and its phenolic compound content.

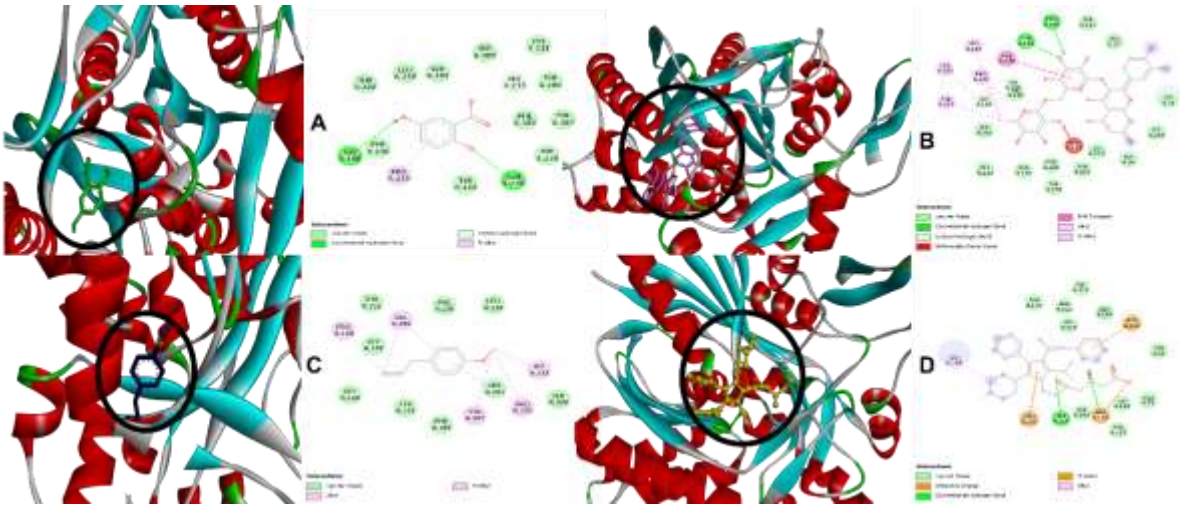

Figure 2. Demonstration of the interaction of methyl chavicol (A), gentisic acid (B), rutin (C), and atorvastatin (D) with HMG-CoA R, respectively.

Şekil 2. Metil chavicol (A), gentisik asit (B), rutin (C) ve atorvastatinin (D) sırasıyla HMG-CoA R ile etkileşiminin gösterilmesi

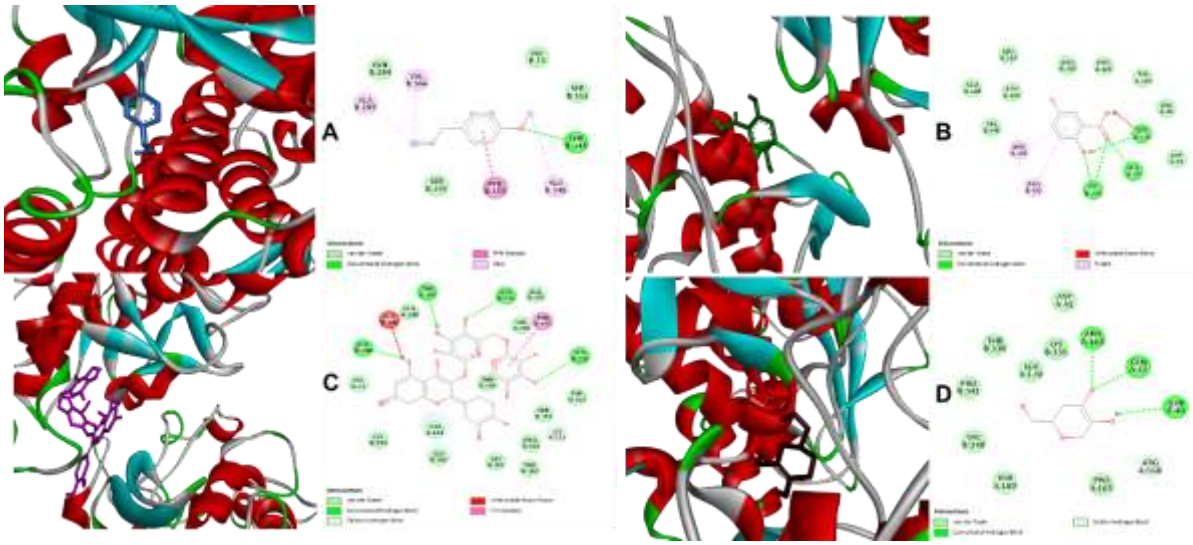

Figure 3. Demonstration of the interaction of methyl chavicol (A, blue color, stick form), gentisic acid (B, green color, stick form), rutin (C, purple color, stick form), and kojic acid (D, yellow color, stick form) with tyrosinase, respectively.

Şekil 3. Metil kavikol (A), gentisik asit (B), rutin (C) ve atorvastatinin (D) sırasıyla tirozinaz ile etkileşiminin gösterilmesi

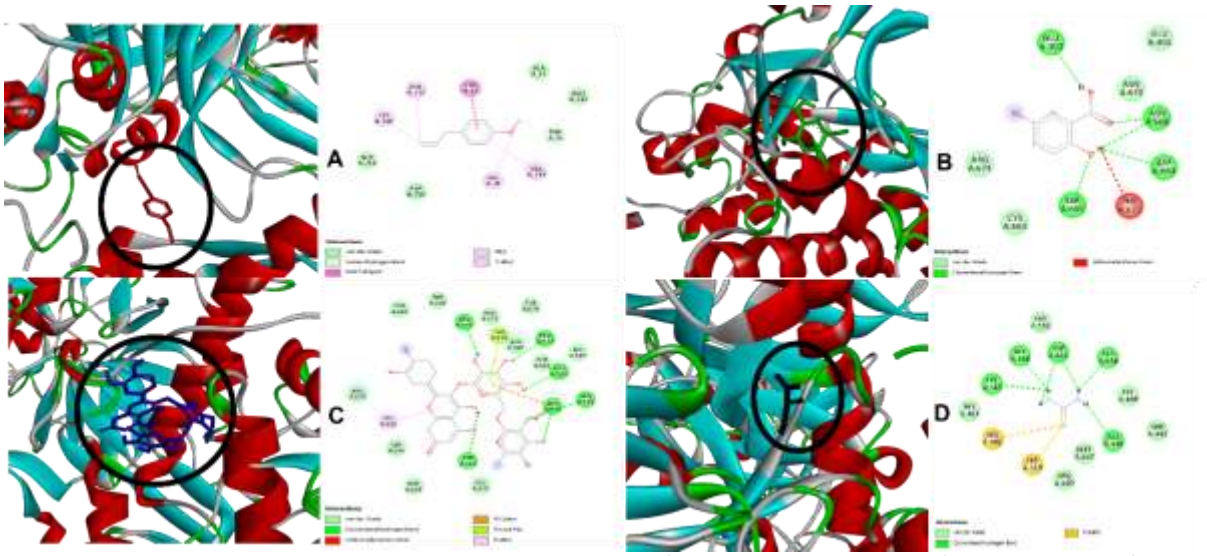

Figure 4. Demonstration of the interaction of methyl chavicol (A, red color, stick form), gentisic acid (B, green color, stick form), rutin (C, blue color, stick form), and kojic acid (D, black color, stick form) with urease, respectively.

Şekil 4. Metil chavicol (A), gentisik asit (B), rutin (C) ve atorvastatinin (D) sırasıyla üreaz ile etkileşiminin gösterilmesi

In this study, we screened the enzyme inhibition potency with molecular docking details to advance novelties related to herbal medicine. The evaluation of enzyme inhibitors such as plant-derived compounds, is one of the most important topics in pharmaceutical areas such as traditional medicine. The plant extract has lower IC_{50} values than the known standards of HMG-CoA R, α-amylase, and α-glucosidase, respectively. Although the plant extract inhibits studied enzymes (ACE, AChE, BChE, collagenase, lipase, tyrosinase), its inhibitory power is lower than known commercial inhibitors. Also, the obtained IC_{50} result of the study suggests that the plant extract may have an alternative potential to thiourea in terms of inhibiting the urease enzyme. The literature survey indicated an inadequacy of studies relevant to the enzyme inhibition effect of *C. cadmea subsp. pontica* extracts. In a study of them, Zengin (2016) reported different solvent extracts of *Centaurea* species, except *C. cadmea subsp. pontica* exhibited inhibitory activity against AChE, BChE, Try, α-Amyl, and α-Gly at the concentration of 2 mg/mL. They declared that inhibition ability changed to solvent types (ethyl acetate and chloroform) and plant taxa, and % inhibition value was between 43.94 ± 1.51 - 95.69 ± 0.06 for BChE, 60.04 ± 0.09 - 95.93 ± 0.07 for AChE, 0.91 ± 0.08 - 12.54 ± 0.08 for Try, 17.53 ± 0.08 - 59.54 ± 0.59 for α-Amyl, and 36.03 ± 0.24 - 60.31 ± 2.13 for α-Gly in chloroform extracts (Zengin et al., 2016). When the present study results are compared with the same enzymes in the aforementioned study, it is seen that *C. cadmea subsp. pontica* extracts have a higher inhibitory potential. Another study was conducted to screen for AChE inhibitory potency of *Centaurea* plants (*C. antalyensei, C. polypodiifolia,* and *C. pyrrhoblephara*) declared that methanol extracts had better ability against AChE than aqueus extracts at 2 mg mL^{-1}. Aqueous extracts of *C. antalyense* and *C. pyrrhoblephara* had no activity against AChE. The same methanolic extract, except for *C. pyrrhoblephara*, displayed inhibitory activity between 37.14 ± 8.17 - 45.50 ± 9.62 % at the concentration of 2 mg mL^{-1} (Aktumsek et al., 2013). In another work, it is stated that extracts of *C. depressa* Bieb., *C. balsamita* Lam., and *C. lycopifolia* Boiss collected from southeastern Türkiye did not show any inhibitory activity against AChE, while different solvent extracts showed moderate activity against BChE at 200 µg/mL (Boğa et al., 2016). Compared to the aforementioned studies, the current results show that *C. cadmea subsp. pontica* extracts are screened in a broader spectrum and have higher inhibitory ability. We assumed that the inhibitory ability of the plant extract could be connected to plant species and used solvents.

Secondary metabolites, including phenolic compounds, are thought to be the reason why plants have notable biological activities such as antioxidant, antimicrobial, and anti-carcinogenic. Some biological activities of plants, such as antidiabetic and anti-Alzheimer's properties, are linked with the inhibition of enzymes that play a role in the biochemical metabolic pathways. Therefore, the phenolic composition in the plant extract was investigated, as it is important to quantify their phenolic contents and to evaluate their contribution to enzyme inhibitory ability. Rutin (119.49 µg g^{-1}) is the most abundant flavonoid among the 11 metabolites calculated in the plant extract, while the amounts of chlorogenic acid, gentisic acid, and rosmarinic acid, cinnamic acid apigenin were found to be close to each other. TFC and TPC were

11.66 ± 0.41 mg g^{-1} and 36.93 ± 0.51 mg g^{-1} as QE and GAE, respectively. Different taxa of *Centaurea* have been investigated with respect to phytochemical ingredients. In one of them, Alper et al. (2021) reported that 15 phenolic compounds (caffeic acid, chlorogenic acid, cinnamic acid, p-coumaric acid, epicatechin, ellagic acid, ferulic acid, gallic acid, naringin, quercetin, rutin, vanillic acid, 3,4-dihydroxybenzoic acid, 4-hydroxybenzoic acid) were identified in the ethanolic extracts of the both *Centaurea solstitialis*. They determined the TPC and TFC in the same extract as 52.31 mg g^{-1} GAEs and 30.10 mg g^{-1} QEs, respectively (Alper et al., 2021). In the study investigating the phytochemical ingredients in *Centaurea karduchorum* from Eastern Anatolia, apigenin glucuronide, chlorogenic acid, and luteolin derivatives were quantified in plant aerial parts by LC-MS spectroscopy (Dalar et al., 2015). A study conducted to the determination of the methanol extract of *C. gigantea* declared that chlorogenic acid and flavonoids (isoorientin, 2-(4-hydroxybenzoyl)-isoorientin, isoquercitrin, orientin, and cirsiliol studied with reversed-phase HPLC analysis (Shoeb et al., 2007). Acet (2021) investigated the phenolic composition and biological activity of *C. triumfetti* and explained caffeic acid, p-coumaric acid, chlorogenic acid, t-cinnamic acid ferulic acid, and syringic acid as phenolic components. She also stated that the plant extracts had α-glucosidase and α-amylase inhibitory activity, especially in the ethyl acetate extract of the plant stem (Acet, 2021). In a previous study, chlorogenic acid, scutellarin, and syringin were isolated from the aerial parts of *Centaurea cadmea*, and the structures of these compounds were elucidated by spectroscopic methods such as NMR and LC-MS/MS (Astari et al., 2013). In similar earliear studies, researchers also investigated the metabolite components and enzyme inhibitory activities of *Centaurea* species (*C. lycopifolia, C. drabifolia,* and *C. rupestris*). They have specified caffeic acid, apigenin, chlorogenic acid, luteolin, p-coumaric acid, and quercetin as plant phenolic ingredients and their enzyme inhibitory properties on AChE, try, α-amy, and α-gly (Ćurković-Perica et al., 2014; Zengin et al., 2018). When compared with the studies exemplified in the upper lines, it is seen that the plant has some similar phenolic substances but different phenolic compositions. We could propound that the content and quantity of metabolites vary according to the plant type, tissue, extraction method, and analysis equipment.

The antibacterial activity in the plant extract may be due to the presence of phenolic and flavonoid contents. It is stated in the literature that various plants, including *Centaurea* species, exhibit antibacterial and antifungal properties in vitro conditions, and this is related to medicinally important compounds synthesized by plants. MIC values were found in the concentration range of 0.0625 to 8 mg/mL against bacterial and fungal species in various antimicrobial studies (Karamenderes et al., 2006; Köse et al., 2016; Albayrak et al., 2017; Sönmez & Çakıloğlu, 2020; Naeim et al., 2020; Reda et al., 2021). It has been reported that the aerial parts of *C. cadmea* extracted in chloroform show strong activity on *Enterococcus faecalis* (8 µg/mL) and *Bacillus cereus* (16 µg/mL) (Astari et al., 2014). These values in such a wide range are due to factors such as the plant's collection time, light exposure time, soil type, the geography where it grows, and the extraction method of metabolites. These factors affect the plant's biological properties, causing a change in secondary metabolite content. From the results obtained according to the HPLC analysis of *C. cadmea subsp. pontica*, computational chemistry estimations were done to predict the biological activities of the main compounds found in the highest amount in the plant content against target enzymes and understand the interaction mechanisms.

CONCLUSION

In the treatment of some metabolic diseases, in the context of inhibition of key enzymes that play an important role in biochemical reactions, eleven different medically important enzymes associated with common diseases in the society were chosen as the target. In summary, this present study endeavors to highlight the phenolic contents of *C. cadmea* subsp. *pontica*, which is one of the endemic medicinal *Centaure* species in folk medicine in Anatolia. In order to assert natural sources as a substitute for synthetic enzyme inhibitor substances used for therapeutic purposes, the enzyme-inhibiting potentials of these compounds should be revealed. For this reason, enzyme inhibition capacity and phenolic components *of C. cadmea* subsp. *pontica* extracts were worked for the first time in addition to molecular docking details. Accordingly, among all inhibitory potential of the extracts, it came to the forefront in terms of higher inhibitory power against HMG_CoA R, α-glucosidase, and α-amylase. We suppose that the inhibitory ability might be linked to its phenolic components, or the synergic effects supported by molecular docking studies. Compared to previous studies on *Centaurea* taxa, the high bioactivity level of the plant might be related to the climatic conditions of the plant collected region. Taken together, in vitro findings emphasize the importance *of C. cadmea* subsp. *pontica* plant. However, further experimental studies (isolation of metabolites and in vivo animal studies) could be planned in the future to better understand the detailed pharmacological effect in light of present findings.

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Author's Contributions

The contribution of the authors is equal.

Statement of Conflict of Interest

The authors have declared no conflict of interest.

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Standardization of Infusion of *Lycium barbarum* Grown by Organic Farming Methods and Enzyme Inhibitory and Antioxidant Activities

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ABSTRACT
The cultivation of medicinal and aromatic plants with conventional and organic farming techniques in order to protect biodiversity due to depleted natural resources is becoming increasingly common. The expense of organic farming techniques necessitates more careful selection of the plants to be grown. Evaluation of the bioactivity and phytochemical contents of these plants is important for the pharmaceutical and food industry. In this study, the antioxidant, antidiabetic, anticholesterolemic and antiobesity activities of the extracts obtained from the fruit, root and leaves of *Lycium barbarum* grown with organic farming techniques using infusion and decoction techniques were evaluated *in vitro*. The phytochemical contents of the extracts were investigated by spectroscopic and chromatographic techniques. In the study in which five different antioxidant activity methods were used, *L. barbarum* root decoction showed a strong antioxidant effect in almost all methods. While none of the extracts exerted an inhibitory effect on the α-glucosidase enzyme, the leaf infusion of the plant at 2 mg mL^{-1} concentration caused strong inhibitions especially on pancreatic lipase (62.16±3.33%) and pancreatic cholesterol esterase (93.98±0.54%) enzymes compared to the reference compounds. *L. barbarum* leaf infusion was standardized by RP-HPLC technique on the basis of chlorogenic acid (1.339±0.056 g $100g^{-1}$ dry extract) and quercetin-3-*O*-glucoside (1.801±0.042 g $100g^{-1}$ dry extract) as markers. The findings displayed that leaf infusions of *L. barbarum* grown with organic farming techniques could be the source of natural product development studies for hypercholesterolemia and obesity control, and the extract could be standardized using chlorogenic acid and quercetin-3-*O*-glucoside as markers.

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Organik Tarım Yöntemleri ile Yetiştirilmiş *Lycium barbarum* İnfüzyonunun Standardizasyonu ve Antioksidan ve Enzim İnhibitör Etkileri

ÖZET
Tükenen doğal kaynaklar sebebiyle biyoçeşitliliği korumak maksatlı tıbbi ve aromatik bitkilerin konvansiyonel ve organik tarım teknikleri ile yetiştirilmesi giderek yaygınlaşmaktadır. Organik tarım tekniklerinin pahalı olması yetiştirilmesi düşünülen bitkilerin daha dikkatli seçilmesini gerektir. Bu bitkilerin biyoaktivitelerinin ve fitokimyasal içeriklerinin de değerlendirilmesi ilaç ve gıda sanayi için önem arz etmektedir. Bu çalışmada, organik tarım teknikleri ile yetiştirilen *Lycium barbarum*'un meyve, kök ve yapraklarından infüzyon ve dekoksiyon teknikleri kullanılarak elde edilen ekstrelerin antioksidan, antidiyabetik, antikolesterolemik ve antiobezite aktiviteleri *in vitro* olarak değerlendirilmiştir. Ekstrelerin fitokimyasal içerikleri spektroskopik ve kromatografik tekniklerle incelenmiştir. Yaprak infüzyonunun en yüksek toplam fenol (72.53±9.13 mg GAE g^{-1} ekstre) ve toplam flavonoid (14.92±0.53 mg QE g^{-1} ekstre) içeriğine sahip olduğu bulunmuştur. Beş farklı

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antioksidan aktivite yönteminin (ABTS ve DPPH radikal süpürücü aktivite, metal bağlama kapasitesi, toplam antioksidan kapasite ve demir indirgeme gücü) kullanıldığı çalışmada, tüm bulgular değerlendirildiğinde *L. barbarum* kök dekoksiyonu, toplam antioksidan kapasite yöntemi dışındaki tüm yöntemlerde güçlü bir antioksidan etki göstermiştir. Ekstrelerin hiçbiri *α*-glukozidaz enzimi üzerinde inhibitör etki göstermezken, 2 mg mL^{-1} konsantrasyonda bitkinin yaprak infüzyonu referans bileşiklerle (orlistat ve simvastatin) karşılaştırıldığında özellikle pankreatik lipazı ve pankreatik kolesterol esteraz enzimleri üzerinde güçlü inhibisyonlara neden olmuştur. Bu sonuçlar ışığında *L. barbarum* yaprağı infüzyonu, markör olarak klorojenik asit (1.339±0.056 g $100g^{-1}$ kuru ekstre) ve kersetin-3-*O*-glukozit (1.801±0.042 g $100g^{-1}$ kuru ekstre) üzerinden RP-HPLC tekniği ile standardize edilmiştir. Bulgular, organik tarım teknikleriyle yetiştirilen *L. barbarum*'un yaprak infüzyonlarının, hiperkolesterolemi ve obezite kontrolü için doğal ürün geliştirme çalışmalarının kaynağı olabileceğini ve ekstrenin markör olarak klorojenik asit ve kersetin-3-*O*-glukozit kullanılarak standardize edilebileceğini göstermiştir.

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INTRODUCTION

The genus *Lycium* belongs to the Solanaceae family and includes about 100 species, mainly distributed in temperate and subtropical regions of America, Eurasia, South Africa and Australia. There are three known species of *Lycium*; *Lycium barbarum* L., *Lycium chinense* L. and *Lycium ruthenicum* L. (Lei et al., 2021). Goji berry is the name given to the fruits of *L. barbarum* and *L. chinense* (Kulczyński & Gramza-Michałowska, 2016). *L. barbarum* grows in northwestern and central Anatolia (TÜBİVES, 2006). Goji berry is a local food widely consumed in arid or semi-arid regions of China, Korea, Japan, Europe, North America and North America (Lei et al., 2021). Goji berry contains between 39.5% and 46.5% carbohydrates, various essential amino acids, unsaturated fatty acids, vitamin C and mineral elements. In addition, goji berry is known to contain more than 200 different compounds, including carotenoids, phenylpropanoids, flavonoids, polyphenols and polysaccharides (Tian et al., 2019). *L. barbarum* polysaccharides (LBP) are also one of the main bioactive compounds of goji berry and have recently attracted the attention of many researchers. Researchers report that goji berry and LBP supplementation are anti-tumor, hepatoprotective, neuroprotective, antidiabetic and beneficial in cardiovascular diseases and vision improvement (Zhou et al., 2022). The fruit of *L. barbarum* is used in traditional Chinese herbal medicine and as a functional food in daily life. In addition, in China, *L. barbarum*, *L. chinense* and *L. ruthenicum* species are used as a medicinal and functional food due to their anti-aging, antioxidant, antidiabetic, anticancer, cytoprotective, neuroprotective and immunomodulatory effects (Tian et al., 2019).

Diabetes is an endocrine system and metabolism disease characterized by chronic hyperglycemia, which can lead to disorders in carbohydrate, protein and fat metabolism as a result of insufficiency, absence and/or deficiency of the insulin hormone (Yalın et al., 2011). Overweight and obesity are defined by excessive accumulation of adipose tissue that impairs both physical and psychosocial health and well-being. Obesity is considered a health problem in both developed and developing countries. It is stated that both type 2 diabetes and obesity are associated with insulin resistance (Al-Goblan et al., 2014). Oxidative stress has an important role in the development of diabetes-related complications. Existing hyperglycemia in diabetic patients increases the formation of free radicals. Endogenous antioxidants are insufficient to balance toxic reactive oxygen species, and accordingly, an increase in oxidative stress occurs. Since the protective effects of antioxidants have been presented in experimental, clinical and epidemiological studies, it is thought by some researchers that antioxidants may help in the treatment of diabetes and its complications (Hamamcıoğlu, 2017). Dyslipidemia has an important place in the development of coronary artery disease, which is the number one cause of mortality in the world. In addition to dyslipidemia, diabetes and obesity are reported to cause the development of

coronary artery disease in different ways. It is stated that with the control of glycemia level and losing weight in diabetic patients, lipid parameters can also improve and the risk of developing coronary artery disease may decrease (Özdogan et al., 2015). It may be possible to treat these diseases or alleviate their complications by inhibiting key enzymes that play a role in the pathophysiology of diabetes (α-glucosidase and α-amylase enzymes), obesity (pancreatic lipase enzyme), and hyperlipidemia (pancreatic cholesterol esterase enzyme), which is the main subject of the study. Therefore, natural resources have an important role in the research of new drug molecules with antioxidant activity, effective on diabetes, obesity and lipid profile.

Medicinal and aromatic plants, which are the raw materials of herbal medicines and food supplements, are either collected from nature or cultivated. Since these plants, which are collected from nature, are the raw materials for both food and medicines, serious hazards will arise for biodiversity if they are collected uncontrollably. Cultivation of medicinal and aromatic plants with conventional or organic farming techniques is very important for both biodiversity and human health. It is necessary to examine the biological activities and phytochemical contents of these plants, whose production is very limited by organic farming techniques, and to evaluate whether they have the same or superior characteristics with the species collected from nature.

In this study, the extracts of the root, fruit and leaves of *L. barbarum* grown with organic farming were prepared by infusion technique, while the extract of the root part was prepared by decoction technique. The antioxidant (total antioxidant capacity, metal chelating capacity, ferric reducing power, 2,2-diphenyl-1-picrylhydrazil (DPPH) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonate) ($ABTS^{\cdot+}$) cation), antidiabetic (α-amylase and α-glucosidase enzymes), antiobesity (pancreatic lipase enzyme), and anticholesteremic (pancreatic cholesterol esterase enzyme) effects of the extracts were evaluated. The total phenolic and total flavonoid content of the extracts were determined by the Folin-Ciocalteu and the aluminum chloride method, respectively. Phytochemical content analysis of the extracts was achieved by Reverse Phase-HPLC (RP-HPLC) method.

MATERIAL and METHODS

Plant Material

L. barbarum grown with organic farming techniques was provided from Beyşehir Road, 3. km Akyokuş Mevki, Konya in 2021 (Certificate no: TR-OT-014-İ-197/01, Temmuz Organik Çiftliği). These species were produced in accordance with the Organic Agriculture Law and Regulation of Turkish Republic and has been certified by Nissert Ltd. Company authorized by the Ministry of Agriculture.

Chemicals

In addition to various standard compounds and high purity solvents used in the RP-HPLC study, many compounds such as enzyme substrates used in enzyme and antioxidant studies were obtained from Sigma-Aldrich.

Extraction

Extracts were prepared from the fruit and leaves of the plant by infusion method and from the roots by using both infusion and decoction methods. In the infusion method, the plant parts (10 g) were kept in hot water (200 mL) for 10 minutes in a closed flask. In the decoction method, plant parts (10 g) were boiled in cold water (200 mL) for 30 minutes. Then the extracts were filtered and this process was repeated 3 times. After combining the obtained filtrates, these extracts were frozen and dried in a lyophilizer at -80 °C.

Chemical Composition of Extracts

Total Phenol Content

After adding 100 µL of Folin-Ciocalteu reagent (10% w v-1) to the extracts prepared by infusion or decoction technique, the extracts were incubated at 25°C for 5 minutes. Then, 80 µL of sodium carbonate solution at a concentration of 7.5% was added to the mixture. The extracts were incubated for 30 minutes at room temperature in a dark place. After incubation, the absorbance of the extracts was measured at a wavelength of 735 nm using ELISA (SpectraMax i3x, Molecular Devices, USA) microplate reader. The total phenol content of the extracts was calculated as gallic acid equivalent mg (GAE) g^{-1} extract. The calibration equation was found to be $y= 6.3667x - 0.0118$, and $r^2= 0.9999$ (Zongo et al., 2010).

Total Flavonoid Content

Ethanol, 20 µL sodium acetate and 10% aluminum chloride solutions were added to the extracts, and the mixture was diluted to 1 mL with distilled water. After 30 minutes of incubation at room temperature, the absorbance of the mixture was measured at 415 nm with an ELISA microplate reader. The total flavonoid content of the extracts was expressed as quercetin equivalent mg (QE) g^{-1} extract. Calibration curve equation; $y= 2.1694x - 0.0067$, $r^2= 1$ (Kosalec et al., 2004).

Antioxidant activity

Total Antioxidant Capacity

Distilled water and 1 mL molybdate reagent were added to the extracts and the tubes were vortexed. After 90 minutes of incubation at 90°C, the tubes were

cooled in an ice bath. The absorbances of the samples were measured with an ELISA microplate reader at 695 nm and the results were expressed as ascorbic acid equivalent mg (AAE) g^{-1} extract. The calibration curve equation was found to be y= 1.8309x – 0.1606 and r^2= 0.9981 (Orhan et al., 2017).

DPPH Radical Scavenging Effect

20 µL of 1 mM DPPH (1,1-diphenyl-2-picrylhydrazil) solution was added onto the extracts in 96-well microplates. The mixture was then incubated in the dark for 30 minutes. After this process, the absorbance of the extracts and the reference compound was measured at 520 nm with an ELISA microplate reader. Ascorbic acid was used as the reference compound. In the experiment, DPPH radical scavenging activity was calculated as inhibition $\% = [(Absorbance_{control} - Absorbance_{sample})/Absorbance_{control}] \times 100$ (1). Experiments were made in triplicate repetitions (Jung et al., 2011).

Metal Chelating Capacity

2 mM 10 µL $FeCl_2$ solution was added to the extracts prepared by infusion or decoction technique, whose metal binding capacity was to be evaluated, and incubated for 5 minutes at room temperature. After this process, 5 mM ferrozine solution was added and the mixture was kept at room temperature for 10 minutes. The absorbance values of the extracts and the reference compound were then measured at a wavelength of 562 nm using an ELISA microplate reader. EDTA (Ethylene Diamine Tetra Acetic acid) was used as the reference compound. Metal chelating capacity % of the extracts was calculated as $[(Absorbance_{control} - Absorbance_{sample})/Absorbance_{control}] \times 100$ (2). Experiments were made in triplicate repetitions (Dinis et al., 1994).

Ferric Reducing Power

0.1 mol L^{-1} sodium phosphate buffer (pH= 7.2) was added to the extracts and the reference compound. After this process, 1% (w v^{-1}) 10 µL $K_3Fe(CN)_6$ solution was added and the mixture was left to incubate in an oven at 37°C. After incubation, 10% w v^{-1} trichloroacetic acid solution was added and absorbance values were calculated at 700 nm wavelength using an ELISA microplate reader. After the measurement, 0.1% (w v^{-1}) $FeCl_3$ solution was added to the mixture and the absorbance was measured again, then the difference between the two absorbance measurements was calculated. Quercetin was used as the reference compound. Experiments were made in triplicate repetitions (Orhan et al., 2017).

ABTS Radical Scavenging Activity

1 mL ABTS (7 mM) was dissolved in distilled water and 2.45 mM potassium persulfate solution. The prepared mixture was incubated for 16 hours at 20°C in the dark. pH adjusted ABTS and phosphate buffer solutions were added to the extracts prepared by infusion or decoction technique. After the samples were vortexed, their absorbance was measured at 734 nm using an ELISA microplate reader. Gallic acid was used as the reference compound. In the experiment, ABTS radical scavenging activity was calculated as inhibition $(\%) = [(Absorbance_{control} - Absorbance_{sample})/Absorbance_{control}] \times 100$ (3) (Orhan et al., 2017).

Enzyme Assays

α-Glucosidase Enzyme Inhibitory Activity

The *α*-glucosidase type IV enzyme to be used in the experiment was dissolved in a phosphate buffer solution (0.5 M, pH 6.5). The extracts were prepared at different concentrations using 80% ethanol solution. The enzyme solution and extracts were pre-incubated at 37°C for 15 minutes in a 96-well microplate. Then, 20 mM 10 µL *p*-nitrophenyl-*α*-D-glucopyranoside solution (PNG) was added to the wells. In the microplate, after incubating the mixture for 35 minutes at 37 °C, the increase in absorption at 405 nm due to hydrolysis of PNG by *α*-glucosidase was measured using an ELISA microplate reader. In the experiment, acarbose (Bayer, Turkey) was used as a reference compound. Experiments were made in triplicate repetitions (Orhan et al., 2017).

α-Amylase Enzyme Inhibitory Activity

The enzyme *α*-amylase type I-A (EC 3.2.1.1, Sigma) to be used in the experiment was dissolved in the buffer solution. 15 µL Potato starch (2.5%, w v^{-1}) prepared in phosphate buffer solution (pH 6.9) was used as substrate solution in the experiment. The extracts were prepared at different concentrations using 80% ethanol solution. After the enzyme solution was added, the mixtures were incubated at room temperature for 5 minutes, then substrate solution was added. The mixtures were then allowed to a new incubation at 37°C for 15 minutes. The mixture in the microplate to which 3,5-dinitrosalicylic acid (DNSA) color reagent solution (5.31 M sodium potassium tartrate in 96 mM DNS, 2 M NaOH) was added, was placed in an oven at 80°C. After 40 minutes, distilled water was added. The absorbances of the mixtures were measured at 540 nm using an ELISA microplate reader. Acarbose was used as reference compound. Then, the standard maltose calibration chart was prepared, the amount of maltose produced was calculated using the standard maltose calibration chart (y = 0.6762x - 0.0044 and r^2= 0.9966) and the net absorbance values were obtained. Experiments were made in triplicate repetitions (Orhan et al., 2017).

Pancreatic Lipase Enzyme Inhibitory Activity

The pancreatic lipase enzyme type II solution to be used in the experiment was prepared in 10 mM 4-morpholinepropanesulfonic acid and 1 mM EDTA buffer solution (pH 6.8). The extracts were prepared at different concentrations using 80% ethanol solution. The enzyme solution and extracts were pre-incubated for 15 minutes at 37°C in 150 µL Tris buffer (100 mM Tris-HCl and 5 mM $CaCl_2$, pH 7.0) in a 96-well microplate. After this process, 4-nitrophenyl butyrate solution, which was used as a substrate, was added to the wells. The mixture on the microplate was reincubated at 37°C for 30 minutes. The increase in absorption at 405 nm as a result of hydrolysis of 4-nitrophenylbutyrate with the pancreatic lipase enzyme was measured using an ELISA microplate reader. Orlistat was used as the reference compound. Experiments were made in triplicate repetitions (Lee et al., 2012).

Pancreatic Cholesterol Esterase Enzyme Inhibitory Activity

The porcine pancreatic cholesterol enzyme was dissolved in 100 mM buffer solution containing 100 mM NaCl (pH 7). 20 µL extracts prepared at different concentrations were added to 50 µL of phosphate buffer. After adding taurocholic acid (12 mM) and its substrate, this mixture was left to incubate at room temperature for 5 minutes. After incubation, porcine pancreatic cholesterol esterase enzyme (0.1 µg mL^{-1}) was added to the mixture and a kinetic reading was performed at 405 nm for 15 minutes using an ELISA microplate reader. Simvastatin was used as a reference compound in the experiment (Ngamukote et al., 2011).

Standardization of *L. barbarum* Leaf Infusion using RP-HPLC Method

HP Agilent 1260 series LC System and ACE 5 C18 (5 µm, 150 mm x 4.6 mm) column were used in the HPLC system used for phytochemical analysis. The device also has an HP Agilent 1260 series Autosampler unit. The column temperature was kept constant at 25°C throughout the analysis. The following standard copmoun mixtures were used for the qualitative and quantitative analysis of the phenolic compounds and flavonoids in the extract. Phenolic compound mixture; gallic acid, protocatechic acid, chlorogenic acid, vanillic acid, syringic acid, *p*-coumaric acid, ferulic acid, sinapic acid, ellagic acid, caffeic acid, *trans*-cinnamic acid, rosmarinic acid, epicatechin, catechin. The flavonoid mixture; rutin, naringenin, hesperidin, quercetin-3-*O*-glucoside, apigenin-7-*O*-glucoside, myrcetin, quercetin, luteolin, apigenin. All of the standard compounds were purchased from Sigma-Aldrich. The gradient flow was initiated with a mobile phase system containing 5% solvent A (acetonitrile: water: formic acid, 50:50:0.5) and 95% solvent B (water: formic acid, 100:0.5). Total analysis time is 55 minutes. The injection volume is 20 µl. Analyzes were carried out at 4 different wavelengths, 260, 280, 320 and 350 nm, using the DAD detector. The extract was prepared at a concentration of 10 mg mL^{-1} using 25% v v^{-1} acetonitrile solution. All sample solutions were filtered through a 0.45 µm membrane filter. Then, qualitative and quantitative analysis of the compounds in the extract was carried out (Gök et al., 2021). The calibration equations and correlation coefficients determined for chlorogenic acid (Rt= 15.36 min) and quercetin-3-*O*-glucoside (Rt= 29.52 min) were [y= 114.77x + 25.057] (r^2= 0.998) and [y= 65.002x – 40.251] (r^2= 0.998), respectively.

Statistical analysis

All analyses were repeated in triplicate and the results were averaged. All values are expressed as mean ± standard deviation (S.D.). Linear regression analyses and calculations were made using Microsoft Excel and GraphPad Instat software. A difference in $p<0.05$ values was considered statistically significant (*$p<0.05$, **$p<0.01$, ***$p<0.001$).

RESULTS and DISCUSSION

It was determined that the infusion prepared from *L. barbarum* fruits had the highest extract yield among the extracts prepared by infusion and decoction techniques from various parts of the *L. barbarum* grown with organic farming. It was found that *L. barbarum* leaf infusion had the highest total phenol (72.53±9.13 mg GAE g^{-1} extract) and total flavonoid (14.92±0.53 mg QE g^{-1} extract) content. It was determined that the extract with the lowest total phenol (9.03±0.77 mg GAE g^{-1} extract) and flavonoid (1.24±0.80 content QE g^{-1} extract) was *L. barbarum* fruit infusion (Table 1).

Five different methods were used to evaluate the antioxidant activities of the extracts prepared from various parts (root, leaves, and fruit) of *L. barbarum* using the infusion or decoction technique. In the antioxidant capacity evaluation method, which is one of these methods, *L. barbarum* fruit infusion (170.19±11.56 mg AAE g^{-1} extract) was found to be the extract with the highest total antioxidant capacity. It was concluded that the extracts obtained from the roots of the plant by both decoction and infusion techniques had the lowest total antioxidant capacity (Table 1). ABTS radical scavenging activities of all samples, except *L. barbarum* root infusion, increased in a dose-dependent manner. Especially *L. barbarum* root decoction (81.46±3.71%) showed inhibitory activity close to gallic acid (98.15±0.80%) used as reference compound. Interestingly, in the method in which DPPH radical scavenging activity was evaluated, no activity was observed at 2 mg mL^{-1}

concentrations of other extracts except *L. barbarum* fruit infusion, while *L. barbarum* root decoction and leaf infusion had the highest radical scavenging activities at both 0.5 (74.15±0.36% and 76.29±1.28%, respectively) and 1 mg mL^{-1} concentrations (79.19±3.39% and 62.84±5.81%, respectively). When the metal chelating capacity of the extracts was evaluated, it was determined that the metal chelating capacity of all extracts was the same as that of EDTA (100%), except for *L. barbarum* fruit infusion (57.13±0.75%) at a concentration of 2 mg mL^{-1}. While the absorbance values of the extracts increased depending on the concentration in the ferric reducing power method, it was found that *L. barbarum* root decoction was the most effective extract with the highest absorbance value (3.220±0.31) as in the DPHH radical scavenging method (Table 2).

Table 1. Results of the yields (w w-1), total phenol, total flavonoid contents and total antioxidant capacity of the extracts

Çizelge 1. Ekstrelerin verim (a a^{-1}), toplam fenol, toplam flavonoid içeriği ve toplam antioksidan kapasitesi sonuçları

Extracts	Yield% (w w^{-1})	Total Phenol Content mg (GAE) g^{-1} extract±SD	Total Flavonoid Content mg (QE) g^{-1} extract±SD	Total Antioxidant Capacity mg (AAE) g^{-1} extract±SD
***L. barbarum* root infusion**	7.82	40.28±3.80	4.78±1.41	98.28±5.84
***L. barbarum* fruit infusion**	27.17	9.03±0.77	1.24±0.80	170.19±11.56
***L. barbarum* leaf infusion**	11.05	72.53±9.13	14.92±0.53	121.76±11.54
***L. barbarum* root decoction**	5.96	62.48±6.12	11.85±1.38	50.39±3.01

GAE: Gallic Acid Equivalent, QE: Quercetin Equivalent, AAE: Ascorbic Acid Equivalent, SD: Standard Deviation

Table 2. ABTS, DPPH radical scavenging activity, metal chelating capacity and ferric reducing power results of the extracts

Çizelge 2. Ekstrelerin ABTS, DPPH radikal süpürücü aktivitesi, metal bağlama kapasitesi ve demir indirgeme gücü sonuçları

Samples	Concentration (mg mL^{-1})	ABTS Radical Scavenging Effect (Inhibition%±SD)	DPPH Radical Scavenging Activity (Inhibition%±SD)	Metal Chelating Capacity %±SD	Ferric Reduction Power (Absorbance±SD)
***L. barbarum* root infusion**	**0.5**	64.28±5.81***	15.26±8.33**	67.35±5.58***	0.282±0.02**
	1	28.10±6.39***	21.69±5.12**	90.92±3.72***	0.365±0.03**
	2	32.88±0.91***	-	100***	1.001±0.04***
***L. barbarum* fruit infusion**	**0.5**	14.87±0.72**	10.76±2.78*	58.44±1.81***	0.100±0.01*
	1	17.56±1.71**	17.02±1.33**	32.96±0.71***	0.185±0.01*
	2	21.52±2.59**	25.22±3.63***	57.13±0.75***	0.455±0.01**
***L. barbarum* leaf infusion**	**0.5**	25.86±0.72***	76.29±1.28***	78.01±2.46***	0.486±0.02***
	1	25.11±1.79***	62.84±5.81***	99.46±2.11***	1.096±0.00***
	2	44.84±2.16***	-	100***	2.439±0.27***
***L. barbarum* root decoction**	**0.5**	26.08±1.62***	74.15±0.36***	89.31±0.79***	0.509±0.03***
	1	30.87±0.93***	79.19±3.39***	80.85±2.78***	0.904±0.01***
	2	81.46±3.71***	-	100***	3.220±0.31***
References	**GA/AA/EDTA/QE 0.5**	99.55±1.04[a]***	89.40±0.61[b]***	100[c]***	3.930±0.00[d]***
	GA/AA/EDTA/QE 1	98.92±0.26[a]***	89.63±0.35[b]***	100[c]***	3.932±0.00[d]***
	GA/AA/EDTA/QE 2	98.15±0.80[a]***	90.48±0.59[b]***	100[c]***	3.845±0.14[d]***

-: No activity. SD: Standard Deviation. ns: Not statistically significant. *p<0.05. **p<0.01. ***p<0.001. GA: [a]Gallic acid. AA: [b]Ascorbic acid. [c]EDTA: Ethylenediamine tetraacetic acid. [d]QE: Quercetin

The inhibitory effects of extracts prepared from various parts of *L. barbarum* by infusion or decoction method on four different enzyme systems (α-glucosidase, α-amylase, pancreatic lipase and pancreatic cholesterol esterase enzyme) were evaluated. None of the extracts showed an inhibitory effect on the α-glucosidase enzyme, which was used to evaluate their antidiabetic effect potential. All of the extracts showed an extremely weak inhibitory effect (1.21±0.15-19.82±0.58%) on the α-amylase enzyme

when compared with the reference compound acarbose (98.40±0.50%). *L. barbarum* root and fruit infusion displayed no inhibitory effect on pancreatic lipase enzyme. *L. barbarum* leaf infusion at a concentration of 2 mg mL^{-1} among all extracts was determined as the extract with the highest effect with an inhibition value of 62.16±3.33%, while this inhibition value was found to be the same as the reference compound orlistat (62.80±1.76%). While all of the extracts inhibited pancreatic cholesterol esterase enzyme, *L. barbarum* leaf infusion had the highest inhibition with a value of 97.35±0.82% at a concentration of 1 mg mL^{-1}. It was determined that this value was considerably higher than that of the reference compound simvastatin (53.20±3.26%) at the same concentration. It was determined that the extract with the weakest effect on pancreatic cholesterol esterase enzyme was *L. barbarum* fruit infusion (23.29±0.50-37.35±1.30%). (Table 3).

Table 3. The Inhibitory effects of extracts on α-glucosidase, α-amylase, pancreatic lipase and pancreatic cholesterol esterase enzyme

Çizelge 3. Ekstrelerin α-glukozidaz, α-amilaz, pankreatik lipaz ve pankreatik kolesterol esteraz enzimi üzerindeki inhibitör etkileri

Samples	Concentration (mg mL^{-1})	Inhibition%±SD			
		α-Glucosidase	α-Amylase	Pancreatic Lipase	Pancreatic Cholesterol Esterase
L. barbarum root infusion	0.5	-	3.53±0.06^{ns}	-	42.47±3.93***
	1	-	3.77±2.05^{ns}	-	44.26±2.03***
	2	-	10.15±4.69*	-	45.87±1.65***
L. barbarum fruit infusion	0.5	-	1.21±0.15^{ns}	-	37.35±1.30***
	1	-	10.28±1.81*	-	23.29±0.50***
	2	-	7.77±2.65*	-	23.80±3.81***
L. barbarum leaf infusion	0.5	-	19.82±0.58**	46.01±7.26***	88.27±4.05***
	1	-	6.44±1.67*	57.91±1.67***	97.35±0.82***
	2	-	13.62±5.82**	62.16±3.33***	93.98±0.54***
L. barbarum root decoction	0.5	-	9.07±2.86*	34.05±3.97***	74.25±0.84***
	1	-	12.40±6.78**	32.22±1.78***	27.94±2.27**
	2	-	4.41±1.50*	25.20±2.08**	96.46±0.43***
References	ACA/OR/SIM 0.5	99.10±0.12^{a***}	94.86±0.60^{a***}	52.17±0.00^{b***}	47.89±5.11^{c***}
	ACA/OR/SIM 1	99.88±0.09^{a***}	98.40±0.50^{a***}	69.55±4.19^{b***}	52.25±0.12^{c***}
	ACA/OR/SIM 2	99.75±0.05^{a***}	95.25±2.60^{a***}	62.80±1.76^{b***}	53.20±3.26^{c***}

-: No activity, SD: Standard Deviation, ns: Not statistically significant *p<0.05, **p<0.01, ***p<0.001, ^{a}ACA: Acarbose, ^{b}OR: Orlistat, ^{c}SIM: Simvastatin

When the enzyme inhibitory activity findings of the extracts were evaluated, it was thought that this extract should be standardized with the RP-HPLC technique, since *L. barbarum* leaf infusion showed a strong inhibitory effect especially on pancreatic lipase and pancreatic cholesterol esterase enzymes. Leaf infusion was screened with diode array detector using RP-HPLC technique for 22 standard phenolic compounds and standardization was made by choosing chlorogenic acid (1.339±0.056 g $100g^{-1}$ dry extract) and quercetin-3-*O*-glucoside (1.801±0.042 g $100g^{-1}$ dry extract) as marker compounds in leaf infusion (Fig 1-6).

Figure 1. RP-HPLC chromatogram of chlorogenic acid

Şekil 1. Klorojenik asidin RP-HPLC kromatogramı

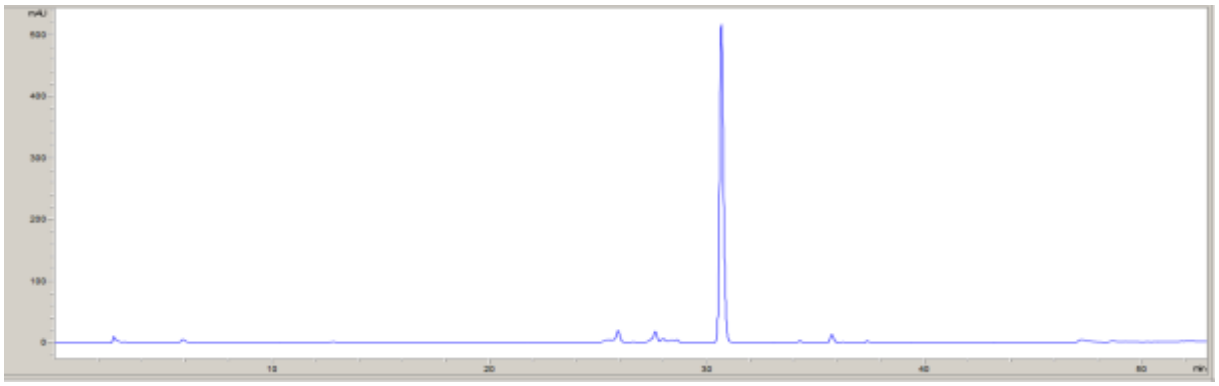

Figure 2. RP-HPLC chromatogram of quercetin-3-*O*-glucoside
Şekil 2. Kersetin-3-O-glukozitin RP-HPLC kromatogramı

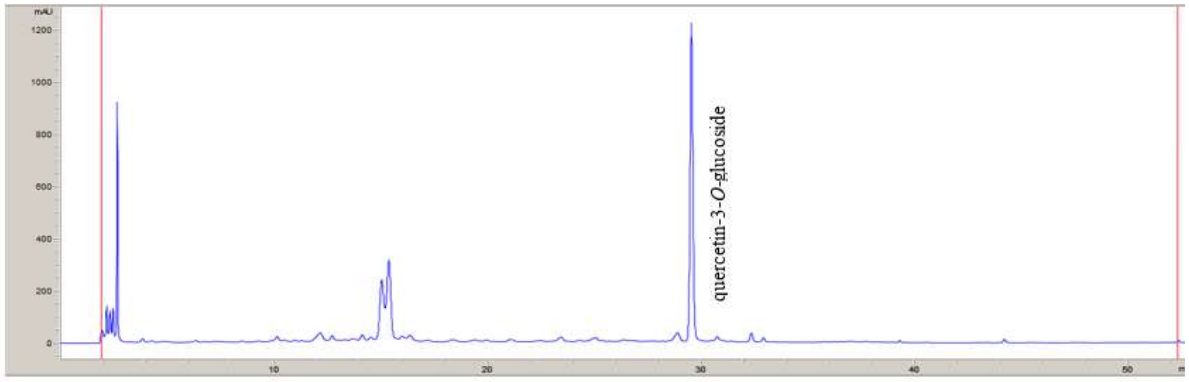

Figure 3. RP-HPLC chromatogram of *L. barbarum* leaf infusion at 260 nm
Şekil 3. L. barbarum yaprak infüzyonunun 260 nm'deki RP-HPLC kromatogramı

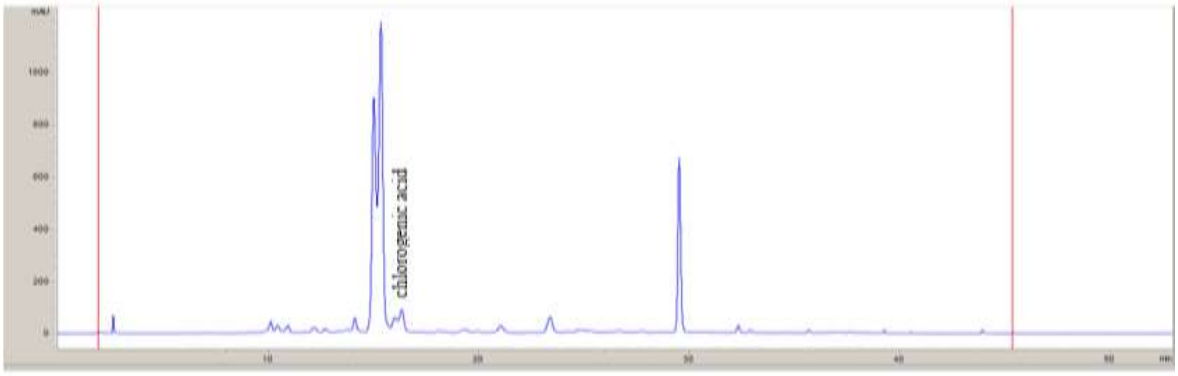

Figure 4. RP-HPLC chromatogram of *L. barbarum* leaf infusion at 320 nm
Şekil 4. L. barbarum yaprak infüzyonunun 320 nm'deki RP-HPLC kromatogramı

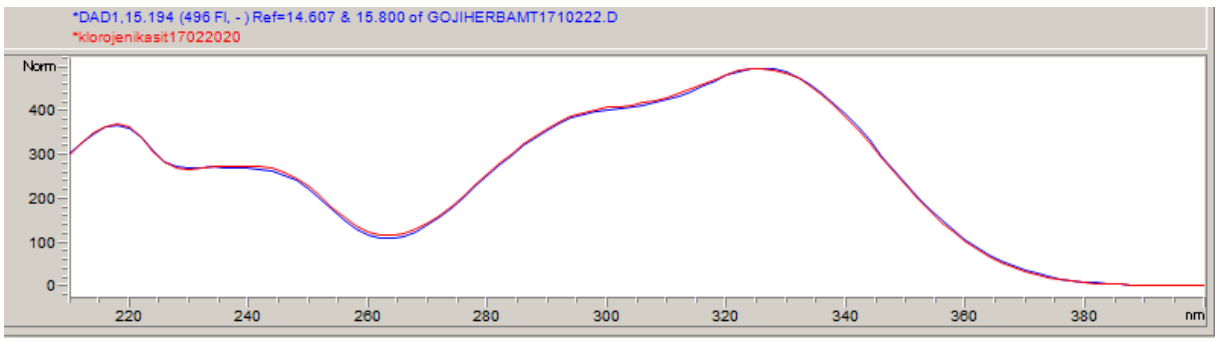

Figure 5. The overlaid UV spectra of standard chlorogenic acid and chlorogenic acid in the extract
Şekil 5. Ekstredeki klorojenik asit ve standart klorojenik asidin çakıştırılmış UV spektrumları

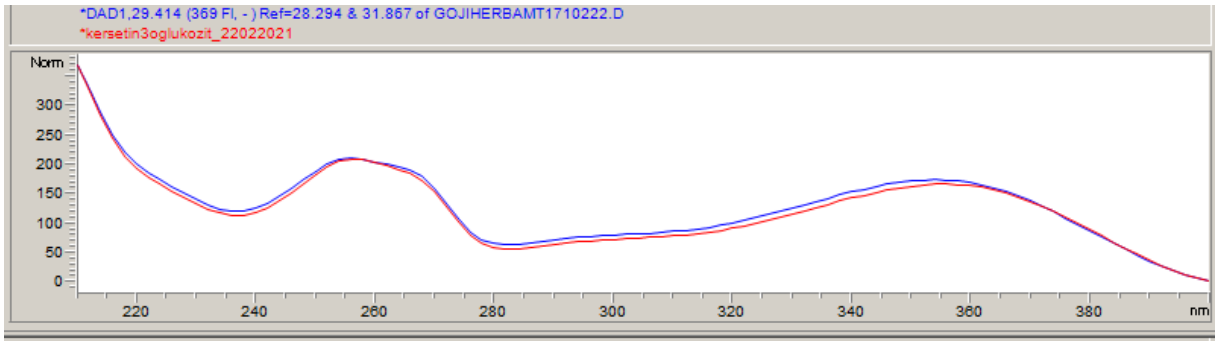

Figure 6. The overlaid UV spectra of standard quercetin-3-*O*-glucoside and quercetin-3-*O*-glucoside in the extract
Şekil 6. Ekstredeki kersetin-3-O-glukozit ve standart kersetin-3-O-glukozitin çakıştırılmış UV spektrumları

This is the first study to examine the antioxidant, antidiabetic, antiobesity and anticholesterolemic effect potential and phytochemical contents of the extracts prepared by using infusion and decoction techniques from the root, fruit and leaves of *L. barbarum* grown in organic farming techniques in Turkey.

Mocan et al. (2014) investigated the total phenol (61.59±1.68 mg GAE g^{-1} plant material) and total flavonoid (43.73±1.43 rutin mg g^{-1} plant material) content and *in vitro* antioxidant activity (DPPH radical scavenging activity and antioxidant capacity) of the 70% ethanolic extract of *L. barbarum* leaves. The DPPH radical scavenging activity of *L. barbarum* extract was calculated as 29.30±4.34 quercetin µg mg^{-1} plant material equivalent, and the antioxidant capacity was calculated as 35.72±6.29 trolox µg mg^{-1} plant material equivalent. It was determined that the DPPH radical scavenging activity of *L. barbarum* extract (29.30±4.34 quercetin µg mg^{-1} plant material equivalent) was lower than its antioxidant capacity (35.72±6.29 trolox µg mg^{-1} plant material equivalent) (Mocan et al., 2014).

Islam et al. (2017) compared the total phenol and flavonoid profiles and antioxidant capacities of extracts prepared with acetone:water:acetic acid mixture from red (*L. barbarum)* and black (*L. ruthenicum)* goji berries collected from different regions of China. The total phenol and flavonoid contents of black goji berry extracts were higher than red goji berry extracts. Likewise, considering the results of the three antioxidant activity methods (ABTS and DPPH radical scavenging activity, FRAP) used in the study, it was observed that black goji berry extracts had stronger antioxidant effects than red goji berry extracts (Islam et al., 2017).

It has been reported that the total polyphenolic content of the extracts obtained by ultrasound extraction technique using water at different temperatures from the fruits of cultivated *L. barbarum* and *L. chinensis* in Greece varies between 234.3-394.3 mg GAE L^{-1}. The antioxidant effects (DPPH and ABTS radical scavenging, peroxyl radical-induced DNA plasmid strand cleavage assay) of these extracts, which were prepared using different temperatures, were evaluated by comparing them with each other (Skenderidis et al. 2018).

Total phenol contents of the extracts prepared by using ultrasound extraction with 70% methanol from two cultivars (cv. Erma and cv. Biglifeberry) grown in Romania with organic farming were calculated as 11.6 mg GAE g^{-1} dw for cv. Erma and 15.7 mg GAE g^{-1} dw for cv. Biglifeberry. The antioxidant activity of both extracts was tested with six different methods. While cv. Biglifeberry had a high antioxidant capacity, both cultivars showed strong antioxidant activities in the CUPRAC method. Both methanol extracts moderately inhibited both α-glucosidase and α-amylase enzymes. The highest α-amylase inhibition was calculated as 0.24±0.01 acarbose mmol g^{-1} extract equivalent for cv. Biglifeberry and the highest α-glucosidase inhibition was calculated as 0.22±0.02 acarbose mmol g^{-1} extract equivalent for cv. Erma (Mocan et al., 2018).

Among the methanol extracts of red, yellow and black goji berries cultivated in Serbia, it was found that black fruits (295.7±18.8 mg GAE $100g^{-1}$) had the highest total phenol content and yellow fruits (335.5±27.1 mg hyperoside equivalent $100g^{-1}$) had the highest total flavonoid content. According to the antioxidant compound index obtained using the results of FRAP, CUPRAC, DPPH, ABTS and beta-carotene bleaching experiments, it was determined that black goji berries had the highest antioxidant activity (Ilić et al., 2020).

It was determined that the total phenol content of the methanol and digested methanol extracts prepared from the cultivated *L. ruthenicum* and *L. barbarum* fruits varied between 18.5±0.4 and 64.9±0.4 mg GAE g^{-1} of extract, and the total flavonoid content varied between 17.9±2.6 and 93.9±1.0 mg QE g^{-1} of extract. In the study, four methods (DPPH radical and nitric oxide scavenging assay, ferric reducing antioxidant power and iron chelating activities) were used to evaluate the antioxidant activity of the extracts and the results were expressed in terms of EC_{50} value, while no reference compound was used for comparison (Magalhães et al., 2022).

When the previous studies on *L. barbarum* samples grown with organic or conventional farming

techniques were evaluated, it was found that the total phenol and total flavonoid content of *L. barbarum* leaves in this study were quite high, while the total phenol and flavonoid content of the fruits were found to be quite low. It was thought that the differences in the total phenol and flavonoid results reported in previous studies and the total phenol and flavonoid content of *L. barbarum* may be caused by factors such as soil, climate factor, organic farming techniques and harvest time.

Wojdylo et al. (2018) investigated the inhibitory effects of *α*-amylase and *α*-glucosidase enzymes of 80% methanol extract obtained from the fruits of 21 different *L. barbarum* samples collected from Poland. As a result of the study, it was reported that while the inhibition rates of 21 different extracts on the *α*-glucosidase enzyme ranged from 5.7±0.3% to 15.3±2.1%, the inhibition rates of these extracts on the *α*-amylase enzyme ranged from 9.6±0.5% to 89.0±3.1%. Researchers emphasized that Goji berries can be used for medicinal or cosmetic purposes, as well as functional foods (Wojdyło et al., 2018). Nikolava et al. (2018) investigated the antidiabetic activity of Goji berry fruit extracts and their corresponding polyphenols. Researchers found a 50% inhibitory concentration on the α-glucosidase enzyme of the extract as IC_{50}=91.7 µg GAE g^{-1} fruit. As a result of the study, it was stated that some polyphenols in the examined extracts showed competitive properties against the enzyme (Nikolava et al., 2018). When we evaluated the findings in the above-mentioned two literature, it was determined that the *α*-amylase and *α*-glucosidase inhibitory activities of goji berries did not have strong inhibitory effects like the goji berries grown with organic farming used in this study. Pollini et al. (2020) examined the antioxidant capacity and total phenol content of the methanol extract of *L. barbarum* leaves collected from Italy. As a result of the study, it was determined that the total phenol content of the extract was 7.75 GAE mg g^{-1} dry leaves. The DPPH radical, ABTS radical scavenging potential and ferric reducing the power of the extract was found to be 9.39, 11.28 and 8.25 Trolox equivalent mg g^{-1} dry leaves, respectively. In the study, it was determined that the extract showed an inhibitory effect against the *α*-amylase enzyme with an IC_{50} value of 25.4 mg mL^{-1}, while this value was found to be 0.1 mg mL^{-1} for the reference compound acarbose. The findings of the enzyme inhibition assay indicated that the leaf extract of *L. barbarum* caused a concentration-dependent inhibition of the *α*-amylase enzyme (Pollini et al., 2020).

Donno et al. state that goji berry fruits contain various bioactive compounds such as various cinnamic acid and benzoic acid derivative compounds, hyperoside, catechins, various monoterpenes, organic acids, and vitamin C (Donno et al., 2015). Mocan et al. (2014) detected the presence of rutin, chlorogenic acid, gentisic acid, caffeic acid, *p*-coumaric acid, ferulic acid, quercetin-3-*O*-glucoside (25.08±0.72 µg g^{-1}), quercitrin and quercetin in 70% ethanol extracts of cultivated *L. barbarum* leaves collected from Romania by HPLC-MS technique. Chlorogenic acid (12045.96±9.25 µg g^{-1}) and rutin (5646.66±3.32 µg g^{-1}) as major phenolic compounds were detected in the extract (Mocan et al., 2014). Pollini et al. (2020) analyzed the phenolic content of the methanol extract of *L. barbarum* leaves harvested in Italy by QTOF-LC/MS method. It was stated that the most abundant phenolic acids in *L. barbarum* leaf extract were chlorogenic (358.34±0.004 µg g^{-1}) and salicylic (239.02±0.005 µg g^{-1}) acids. In this study, quercetin-3-*O*-glucoside could not be detected in the leaves (Pollini et al., 2020). In this study, it was determined that the amount of chlorogenic acid in leaf infusions was lower than the amount of chlorogenic acid found in leaf extracts of *L. barbarum* grown with organic farming in Romania, while the amount of quercetin-3-*O*-glucoside, on the contrary, was lower. In addition, *p*-coumaric acid, ferulic acid, caffeic acid, rutin, and quercetin could not be detected in the leaf samples in this study.

There are no phytochemical analysis and bioactivity studies on *L. barbarum* roots to date. And root decoction showed even higher effects on pancreatic cholesterol esterase enzyme than simvastatin at 0.5 and 2 mg mL^{-1} concentrations. While it was seen in previous studies that *L. barbarum* leaf and fruit extracts had *α*-glucosidase enzyme inhibitory activity, albeit weak, in this study infusions and decoctions obtained from all parts of the plant were found to have no *α*-glucosidase inhibitory activity. In this study, it has been reported that chlorogenic acid, which was found to be in leaf infusion by HPLC analysis, has a strong effect on pancreatic lipase enzyme, and has lipid-lowering and anticardiovascular effects *in vivo* (Miao et al., 2020; Ahmad et al., 2021). Docking studies on the pancreatic lipase enzyme of quercetin-3-*O*-glucoside, which is used for the standardization of leaf infusion, have been reported in the literature. Chen et al. (2020) revealed that quercetin 3-*O*-glucoside has a potential binding ability to pancreatic lipase enzyme with high energy scores (-30.02 kcal mol^{-1}) (Chen et al., 2020).

CONCLUSION and RECOMMENDATIONS

Supporting medicinal and aromatic plants with organic agriculture can be a solution to many problems such as protecting biodiversity and growing healthy and high-quality species. On the other hand, it is necessary to examine whether these species grown with organic agriculture have biological activities similar to or superior to those of wild species in terms of health and changes in their phytochemical contents. In this study, the phytochemical contents, antioxidant activity and effects on some metabolic enzymes (*α*-glucosidase, *α*-amylase, pancreatic lipase and

cholesterol esterase enzymes) of all parts of the *L. barbarum* plant grown with organic farming techniques in Turkey were evaluated for the first time. In terms of antioxidant activity, *L. barbarum* leaf infusion had a higher effect and this was also correlated with total phenol and flavonoid content. It has been determined that the effect of *L. barbarum* leaf infusion on pancreatic lipase enzyme, which is effective in obesity control, is close to that of orlistat. On the other hand, more *in vitro* and *in vivo* studies should be conducted on this extract so that it can be considered as a source for natural product development studies for obesity control. For the first time in this study, the determination that *L. barbarum* root decoction and leaf infusion has strong cholesterol esterase inhibitory activity showed that further antihyperlipidemic effect studies on the roots and leaves of the plant should be performed. Because of the promising antioxidant, antihyperlipidemic and antiobesity activity potential of the infusion prepared from the leaves among all plant parts tested, this extract was standardized by RP-HPLC technique using two marker compounds (chlorogenic acid and quercetin-3-*O*-glucoside). As a result, it was concluded that both roots and leaves of *L. barbarum* grown with organic farming techniques can be a good source for developing natural products.

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Contribution Rate Statement Summary of Researchers

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

Conflict of Interest Statement

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

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Değişik Pişirme Yöntemlerinin Akya (*Lichia amia*) Filetolarının Yağ Asidi Kompozisyonu Üzerine Etkileri

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ÖZET

Bu çalışmada, akya balığının (*Lichia amia*) yağ asidi kompozisyonu üzerine bitkisel yağlarda kızartma ile fırında, mikrodalgada pişirme, ızgara ve buğulama yapma gibi pişirme yöntemlerinin etkisi araştırılmıştır. Kızartma işleminin balıkların yağ asidi kompozisyonunu değiştirdiği belirlenmiştir. Ayçiçek yağı ve mısırözü yağında kızartılan filetolarda 18:2n-6 ile ∑n-6 PUFA'nın, zeytinyağı ve fındık yağında kızartılan örneklerde ise 18:1n-9 ve ∑MUFA'nın önemli düzeyde arttığı saptanmıştır ($p<0.01$). Izgara, mikrodalga ve buğulama ile pişirilen akyada ∑SFA'da bir miktar artış, ∑PUFA ve ∑n-3 PUFA'da azalma kaydedilmiştir. n-3/n-6 oranı, denenen tüm bitkisel yağlarda kontrole oranla önemli derecede azalmış ($p<0.01$); kızartma dışında pişirilen yöntemlerde ise bu oran yakın değerlerde bulunmuştur ($p>0.05$). Tüm pişirme yöntemlerinden elde edilen aterojenik indeks (Aİ) ve trombojenik indeks (Tİ) değerleri, beslenme uzmanlarının önerdiği değer olan 1.0'in altında bulunmuştur.

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Anahtar Kelimeler
Pişirme yöntemleri
Lipit kalitesi
Yağ asitleri
Akya
Bitkisel yağlar

The Effects of Different Cooking Methods on Fatty Acid Composition of Leer (*Lichia amia*) Fillets

ABSTRACT

In this study, the effects of cooking methods such as frying in vegetable oils, cooking in the oven and microwave cooking, grilling and steaming on the fatty acid composition of leer fish (*Lichia amia*) were investigated. It was determined that the frying process changed the fatty acid composition of the fish. It was determined that 18:2n-6 and ∑n-6 PUFA were significantly increased in fillets fried in sunflower oil and corn oil, and 18:1n-9 and ∑MUFA in samples fried in olive oil and hazelnut oil ($p<0.01$). A slight increase in ∑SFA and a decrease in ∑PUFA and ∑n-3 PUFA were observed in leer cooked by grilling, microwave and steaming. n-3/n-6 ratio was significantly decreased in all vegetable oils tested compared to the control.($p<0.01$); this ratio was found to be close to in methods cooked other than frying ($p>0.05$). The atherogenic index (AI) and thrombogenic index (TI) values obtained from all cooking methods were below 1.0, which is the value recommended by nutritionists.

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GİRİŞ

Balık; içerdiği vitamin, mineral, protein ve çoklu doymamış yağ asitlerinden (PUFA) dolayı güçlü bir antioksidan ve antiiflamatuvar özelliğe sahip olup insan diyetinde bulunması gerekli bir gıdadır. Önerilen günlük enerji ve besin ögeleri, güvenilir alım düzeylerine göre ortalama 100 gramlık bir porsiyon; önerilen günlük protein alımının %50'sinden fazlasını, minerallerin %10 ila %20'sini, değişen miktarlarda suda çözünen vitaminleri ve yağda çözünen A, D ve E vitaminlerinin önemli bir yüzdesini sağlar (Perea ve ark., 2008).

Deniz balıklarının yağ asidi bileşiminin yüksek seviyelerde n-3 PUFA ve D_3 vitamini içerdiği bilinmektedir. Balıketi yapısındaki yağ asitlerinden ötürü kaliteli bir diyet ürünüdür. Balık yağları genellikle; %30-40 oranında doymuş (SFA), %25-30 oranında tekli doymamış (MUFA), %25-30 oranında

ise çoklu doymamış yağ asitlerini içerir. Özellikle soğuk su balıklarında yüksek oranda bulunan EPA ve DHA'nın; plazma trigliserit düzeylerini önemli ölçüde azalttığı, düşük yoğunluklu lipoprotein kolesterol düzeylerini düşürmek gibi farklı fizyolojik işlevleri yerine getirdiği ve antitrombotik, antiinflamatuvar, antiaritmik ve vazodilatör özellikler gösterdiği bildirilmiştir (Un ve ark., 2007).

Çeşitli epidemiyolojik çalışmalar, balık tüketiminin koroner kalp hastalıklarının önlenmesinde anahtar rolünün olduğunu, kan basıncını düşürdüğünü ve kanın pıhtılaşmasını azalttığını göstermiştir (Kris-Etherton ve ark., 2003; Ruxton, 2011).

Türün yaşam alanı, beslenme şekli, besin bolluğu ve besin çeşidi, mevsim, balığın boyutu, yaş, sıcaklık, tuzluluk gibi faktörler balıkların lipit içeriği ve yağ asidi profilini etkiler (Chaouch ve ark., 2003). Balıklar; değişik bitkisel yağlarda kızartma, ızgara, buğulama ve fırında pişirme gibi farklı pişirme yöntemleri uygulanarak tüketilmektedir. Pişirme yöntemleri balık filetolarındaki yağ asidi içeriğinde önemli değişikliklere yol açmaktadır (Weber ve ark., 2008). Eikosapentaenoik asit ve DHA gibi fazla çift bağ içeren n-3 PUFA'ların, ısı uygulanarak yapılan pişirme işlemi serbest radikallerin oluşumuna neden olur (Loughrill ve ark., 2016). Sonuçta faydalı yağ asitlerinin oksidasyon ürünlerinin de tüketilme riskini beraberinde getirir (Turkkan, ve ark., 2008).

Akya (*Lichia amia*) özellikle Akdeniz bölgesinde sıklıkla tüketilen ekonomik ve besin değeri oldukça fazla olan bir deniz balığıdır. Daha önce yapılan çalışmada da bu balıkla birlikte Akdeniz'den toplanan sekiz balık türünün yağ asidi kompozisyonunu araştırılmıştır. Ancak; akyanın yağ asidi bileşimine değişik pişirme tekniklerinin etkisi ile ilgili bir çalışma yapılmamıştır. Bu çalışmanın amacı, akya filetolarının yağ asidi kompozisyonuna farklı bitkisel yağlarda kızartma, fırın ve mikrodalga fırında pişirme, ızgara ve buğulama gibi farklı pişirme tekniklerinin etkisini araştırmaktır.

MATERYAL ve METOD

Araştırmada, Mersin ili Aydıncık ilçesi Karatepe Koy'unda 2021 yılı eylül ayında olta ile yakalanan akya (*Lichia amia*) deniz balığı kullanılmıştır. Balık filetoları, sekiz farklı pişirme tekniği ile pişirilmiş, pişirilmeyen (ham, çiğ) filetolar standart olarak kullanılmıştır.

Örnekleme Yöntemi

Fındık yağı, mısır özü, ayçiçek ve zeytinyağı kızartma işlemi için kullanılmıştır. Tavaya konulan yağlar ısı yardımıyla kızgınlık derecesine ulaştıktan sonra filetoların her iki tarafı kızgın yağda üçer dakika süre ile kızartılmıştır. Farklı pişirme yöntemleriyle pişirilen balık filetolarının iç kısım sıcaklıkları ortalama 75°C olacak şekilde; fırında (180°C, 30 dakika), mikrodalgada (2450 MHz 500 W, 6 dakika), buğulamada (buğulama suyunda, 45 dakika) ve ızgarada (filetonun her iki tarafı, 8 dakika) ise kömür ateşinde pişirilmiştir.

Laboratuvar Analizleri

Örneklerin yağ asidi kompozisyonunu belirlemek amacıyla; filetolar 2:1 oranındaki kloroform – metanolde parçalandıktan sonra (Folch ve ark., 1957) lipit kısmının ayrımı için KCl kullanılmıştır. Lipitteki çözücü evaporatörle buharlaştırıldıktan sonra örneğe, asitli metanol eklenip 2 saat süreyle reflux sisteminde (85 °C'de) ısıtılmış ve böylece yağ asitlerinin metil esterlerine dönüşümü gerçekleştirilmiştir. Hekzan kullanılarak alınan metil esterleri 2 ml kalıncaya kadar evapore edilmiştir. Örnekler, Rtx-2330 (Bonded 90 % bicyanopropyl/ 10% phenylcyanopropyl polysioxane) kapiller kolon (30m x 0.25mm iç çapı x 0.20µm film kalınlığı) kullanılarak SHIMADZU GC 2010 PLUS model gaz kromatografi cihazında analiz edilmiştir. Dedektör ve enjektör sıcaklığı: 250ºC; enjeksiyon: Split-model 1/20. Gazlardan helyumun akış hızı 0.5 ml/dk; hidrojenin: 30 ml / dk ve kuru havanın 400 ml/dk. Kolon sıcaklığı 170°C'de 2 dakika tutulduktan sonra 2°C/dakika olacak şekilde 210°C'ye kadar ısıtılmış ve bu sıcaklık derecesinde 20 dakika beklenmiştir.

Numune, alete 1 mikrolitre olarak verilmiştir. Metil esterleri karışımı (Sigma-Aldrich Chemicals) kullanılarak, yağ asitleri belirlenmiştir. GC Solution (Versiyon 2.4) programı kullanılarak kromatogramlar ve toplam yağ asidi miktarları elde edilmiştir. Analizi yapılan numunenin kromatogramında oluşan pikler, standarttaki alıkonma zamanları ile karşılaştırılarak tespit edilmiştir.

İstatistik Analizler

SPSS 10.0 bilgisayar programı kullanılarak yağ asidi yüzdeleri karşılaştırılmıştır. Araştırma sonucu elde edilen tüm veriler üç tekrarın ortalaması olup, yağ asidi metil esterlerinin gaz kromatoğrafik analizlerinde, her pişirme yöntemine ait üçer numune ayrı ayrı enjekte edilmiş aynı yağ asidine ait üç değerin ortalaması alınmıştır. Yağ asidi yüzdeleri arasındaki farklılıklar tek yönlü ANOVA ile analiz edilmiştir. Farklılıklar TUKEY HSD testi ile saptanmıştır. İstatistikler farklar, $p<0.05$ düzeyinde olduğu zaman farkların önemli olduğu kabul edilmiştir.

Yağ asidi analizi ile ilgili Çizelgelerde her veri 3 tekrarın ortalamasıdır. Her tekrarda 3 enjeksiyon yapılmıştır. Her satırda aynı harflerle belirlenen veriler $P>0.05$ olasılık düzeyinde birbirinden farklı değildir.

Lipit Kalite İndeksleri

Genellikle balık lipit kalitesinin belirlenmesinde; PUFA/SFA, n-3/n-6 PUFA ile Aterojenik indeks (AI, doymuş yağ asitleri ile doymamış yağ asitleri toplamı arasındaki ilişki) ve Trombojenik indeks (TI, kan damarlarında pıhtı oluşma eğilimi) gibi parametreler kullanılmaktadır (Ulbritcth ve Southgate, 1991; Hosseini ve ark., 2014).

$$AI: \frac{12:0+4\ X\ 14:0+16:0}{\Sigma PUFA+\Sigma MUFA}$$

$$TI: \frac{14:0+16:0+18:0}{(0,5)\ X\ \Sigma MUFA+(0,5)\ X\ (N-6PUFA)+3\ X\ (N-3PUFA)+\frac{N-3}{N-6}}$$

Bu indeks, pro-trombojenik (doymuş yağ asitleri) ve anti-trombojenik yağ asitleri arasındaki ilişkiyi göstermektedir (Ulbritcth ve Southgate, 1991).

BULGULAR ve TARTIŞMA

Akya Filetolarının Kızartılmasında Kullanılan Bitkisel Yağların Yağ Asidi Kompozisyonu

Çalışmada yağda kızartma yöntemi için; ayçiçek yağı, zeytinyağı, mısırözü yağı, fındık yağı gibi farklı bitkisel yağlar kullanılmıştır. Kızartmada kullanılan yağların yağ asidi kompozisyonu Çizelge 1'de verilmiştir.

Her yağın kendine özgü bir yağ asidi kompozisyonuna sahip olduğu görülmüştür. Örneğin 16:0 ve ∑SFA en çok zeytinyağı ile mısır özü yağında, 18:1n-9 ve ∑MUFA fındık yağı ve zeytinyağında, 18:2n-6 ve ∑PUFA ile ∑n-6 PUFA mısır özü yağı ve ayçiçek yağında tespit edilmiştir (Çizelge 1).

Çizelge 1. Kızartmada kullanılan yağların yağ asidi kompozisyonu
Table 1. Fatty acid composition of oils used in frying

Yağ Asidi	Zeytin Yağı (ORT±SH)	Ayçiçek Yağı (ORT±SH)	Mısır Özü Yağı (ORT±SH)	Fındık Yağı (ORT±SH)
12:0	-	0.03±0.004	0.04±0.006	0.04±0.003
14:0	-	0.04±0.005	0.18±0.01	0.03±0.005
16:0	14.22±1.13	6.78±0.54	12.45±0.99	6.31±0.50
17:0	-	-	1.56±0.12	-
18:0	2.62±0.21	3.24±0.26	0.01±0.00	2.45±0.20
∑SFA	**16.85±1.34**	**10.10±0.81**	**14.26±1.14**	**8.83±0.70**
16:1n-7	1.15±0.09	0.15±0.01	0.08±0.01	0.20±0.02
18:1n-9	71.04±5.67	36.97±2.95	30.12±2.40	76.25±6.08
∑MUFA	**72.20±5.76**	**37.11±2.96**	**30.20±2.41**	**76.45±6.10**
18:2n-6	9.95±0.79	52.51±4.19	54.80±4.37	14.53±1.16
18:3n-6	0.28±0.02	0.23±0.02	-	0.10±0.01
18:3n-3	0.73±0.06	0.04±0.00	0.74±0.06	0.09±0.01
∑PUFA	**10.95±0.87**	**52.79±4.21**	**55.54±4.43**	**14.72±1.17**
∑n-3 PUFA	0.73±0.06	0.04±0.00	0.74±0.06	0.09±0.01
∑n-6 PUFA	10.23±0.82	52.74±4.21	54.80±4.37	14.62±1.17
n-3/n-6	0.07±0.005	0.00±0.00	0.01±0.00	0.01±0.00
∑PUFA/∑SFA	**0.64±0.05**	**5.22±0.41**	**3.89±0.31**	**1.66±0.13**
AI	0.17±0.01	0.08±0.00	0.15±0.01	0.07±0.00
TI	0.39±0.03	0.22±0.01	0.28±0.02	0.19±0.01

S.H.:Standart Hata, SFA: DoymuşYağ Asitleri, MUFA: Tekli Doymamış Yağ Asitleri, PUFA: Çoklu Doymamış Yağ Asitleri, AI: Aterojenik İndeks, TI: Trombojenik İndeks.

Değişik Bitkisel Yağlarla Kızartılan Filetoların Total Lipitteki Yağ Asidi Kompozisyonu

Bitkisel yağlarla pişirilen filetolarla, çiğ (ham) filetonun total lipitteki yağ asidi kompozisyonu Çizelge 2'de verilmiştir. Denenen yağlarda kızartılan filetolar ile çiğ filetolarda 16:0'ın SFA'lar arasında, 18:1n-9'un MUFA'lar arasında dominant yağ asitleri olduğu görülmüştür. Bitkisel yağlarda kızartılan örneklerde PUFA'lar içinde 18:2n-6, çiğ örneklerde ise % 34.33 ile 22:6n-3, yüzde dağılımda en fazla bulunmuştur. Çiğ filetolarda yağ asidi gruplarında sıralama PUFA>SFA>MUFA şeklinde olmuştur. Bu sonuçlar Akdeniz'den toplanan balıklardan elde edilen sonuçlarla uyumludur (Prato ve Biandolino, 2012; Akgül, 2019). Ayçiçek yağı ile mısır özü yağı benzer yağ asidi kompozisyonuna sahip oldukları için (Çizelge 1), bu yağlarla pişirilen filetoların yağ asidi içeriklerinin de yakın olduğu görülmüştür (Çizelge 2). Örneğin her iki yağda pişirilen örneklerde 18:2n-6 ve ∑PUFA ile ∑n-6 PUFA'ların çiğ filetolardan oldukça yüksek olduğu saptanmıştır. Bunun nedeni ayçiçek ile mısır özü yağının yüksek düzeyde 18:2n-6 içermesidir (Çizelge 1). Kızartma işlemi ile filetolara geçen linoleik asit, balığın yağ asidi kompozisyonunu değiştirmiştir.

Zeytinyağı ile fındık yağında kızartılan filetolarda ise 18:1n-9 ve dolayısıyla ∑MUFA içeriğinin hem çiğ balıklardan hem de diğer bitkisel yağlarda kızartılan örneklerden çok daha fazla olduğu tespit edilmiştir

(Çizelge 2). Bunun nedeni, zeytinyağı ile fındık yağında yüksek yüzdede bulunan 18:1n-9'un (Çizelge 1), kızartma esnasında filetolarca emilmesi ve onların yağ asidi kompozisyonunu değiştirmesidir (Çizelge2). Çiğ filetolardaki 16:0 ve ∑SFA'lar ile 20:5n-3, 22:5n-3 ve 22:6n-3 düzeylerinin değişik bitkisel yağlarda kızartılan filetolardan oldukça yüksek olduğu, oleik asit ve ∑MUFA'ların ise daha düşük olduğu görülmüştür. Bunun nedeni yukarda da belirtildiği gibi bazı yağların 18:1n-9 bakımdan zengin olmaları ve bitkisel yağlarda 20:5n-3, 22:5n-3 ve 22:6n-3 gibi n-3 yağ asitlerinin olmamasıdır.

Daha önceki çalışmalarda, kızartma işleminde kullanılan yağın yağ asidi kompozisyonunun, balıkların yağ asidi içeriğine etki ettiği belirlenmiştir (Sánchez-Muniz ve ark.1992; Little ve ark., 2000; & Başhan, 2019). Uskumrunun linoleik asit yüzdesini ayçiçek yağı 9.74, mısır özü yağı ise 13.7 kat arttırmıştır. Zeytinyağında kızartılan filetoların 18:1n-9 düzeyi % 48.18 yükselmiştir (Başhan, 2019). Zeytin yağında kızartılan lagosta (*Epinephelus coioides*) 18.1n-9 düzeyi artmış, EPA ve DHA azalma göstermiştir (Zahra Momenzadeh ve ark., 2017). Çalışmada da belirlendiği gibi belirli yağ asitleri bakımından zengin olan bitkisel yağlar, kızartma işlemi ile filetolara geçip yağ asidi kompozisyonunun değişimine neden olmuştur. Bitkisel yağların bazılarında örneğin, zeytinyağında %71.04 ile 18:1n-9; ayçiçekyağında %52.51 ve mısırözü yağında %54.80 oranı ile 18:2n-6 gibi yüksek yüzdede bulunan yağ asitleri kızartma işlemi ile balıklara geçmekte ve balıkların yağ asidi kompozisyonlarını değiştirerek n-3 PUFA'lardan EPA ve DHA yüzdelerinde önemli oranlarda azalmaya neden olmaktadır (Çizelge 1).

Gıdalardaki yağların besinsel kalitesini belirleyen başlıca parametreler; n-3/n-6 oranı, PUFA/SFA oranı, aterojenik indeks (Aİ) ile trombojenik indekslerdir (Tİ). Çalışmada çiğ akya filetosunda n-3/n-6 oranı 5.95 olarak bulunmuştur (Çizelge 2). Sağlıklı beslenme için bu indeksin 0.2 den fazla olması gerekir (Simopoulos, 2002). Çiğ akyadaki n-3/n-6 oranının, tavsiye edilen değerlerin oldukça üstünde olması, balığın önemli bir besinsel kaynak olduğunu gösterir. Farklı balık türlerinde bu oran 0.24 - 4.1 arasında belirlenmiştir (Hosseini ve ark., 2014). Mersin Karatepe Koy'undan temin edilip çalışılan sekiz balıkta n-3/n-6 oranı, 2.3-7.86 arasında saptanmıştır (Akgül, 2019). Farklı bitkisel yağlarda kızartılan akya filetolarında n-3/n-6 oranı 0.07 (mısır özü yağında kızartılmış balıklar) – 0.35 (zeytinyağında kızartılmış balıklar) arasında tespit edilmiştir (Çizelge 2). Görüldüğü gibi bitkisel yağlarda kızartılan akya filetolarında, n-3/n-6 oranı ham filetolara oranla hayli düşük bulunmuştur. Bu orana göre, denenen yağlar arasında en sağlıklı yağın zeytinyağı olduğu söylenilebilir. Mısır özü yağı ve ayçiçek yağında 18:2n-6'nın fazla düzeyde olması, bu yağlarla kızartılan balıkların n-3/n-6 oranının düşük olmasına neden olmuştur. Daha önce yapılan bir çalışmada, bu orandaki azalma en fazla ayçiçek yağında, en az zeytinyağında kızartılan örneklerde bulunmuştur (Başhan, 2019). Mısır özü yağında kızartılan Hamsi (*Engraulis encrasicholus*) balığında n-3/n-6 oranı 1.21, çiğ balıkta ise 9.93 olarak saptanmıştır (Turhan ve ark., 2011). Ayçiçek yağı, çipura balığında n-3/n-6 oranını 26.75 kat azaltmıştır (Özoğul ve ark., 2009).

Dünya Sağlık Örgütü (WHO, 2003) PUFA/SFA oranının sağlıklı bir diyet için 0.4'ten fazla olmasını önermektedir. Araştırmada çiğ akyada bu oran 1.09, değişik bitkisel yağlarda kızartılan filetolarda 0.66 – 3.63 arasında bulunmuştur. Ayçiçek yağı ve mısırözü yağında 18:2n-6 yüksek olduğu için bu yağlarda kızartılan filetolarda oran yüksek olarak saptanmıştır (Çizelge 2). Örneğin, ayçiçek yağında kızartılan sardalyada PUFA/SFA oranı kontrole oranla 3.0 kat (Gall ve ark., 1983), uskumruda ise 1.5 kat artmıştır (Başhan, 2019). Ulbricht ve Southgate 'ın (1991), kullandığı ve formülleri daha önce belirtilen indekslerden Aterojenik indeks (Aİ), çeşitli yağ asitlerinin, serum kolesterol düzeyi üzerine olan etkileriyle ilgilidir. Trombojenik indeks (Tİ) ise trombositlerin agregasyonunu uyarma potansiyelinin bir ölçüsüdür. Her iki indeksin yüksek olması, kalp-damar hastalıkları için bir risktir. Beslenme uzmanları bu indekslerin 1.0'den düşük olmasını önermektedir. Çalışmada Aİ indeksi 0.08 (fındık yağında kızartılan filetolar) – 0.53 (çiğ fileto); Tİ ise 0.20 (fındık yağında kızartılan filetolar) – 0.37 (zeytinyağında kızartılan filetolar) aralığında bulunmuştur (Çizelge 2). Fındık yağında kızartılan filetolarda her iki indeksin, çiğ ve diğer bitkisel yağlarda kızartılan filetolardan daha düşük olmasının nedeni, bu yağda kızartılan balıklarda 16:0'ın düşük; 18:1n-9 ve total MUFA'nın yüksek olmasıdır (Çizelge 2). Bu verilere göre, AI ve TI indeksi bakımından en uygun yağın fındık yağı olduğu görülür.

Kızartma Dışındaki Yöntemlerle Pişirilen Akyanın Total Lipitteki Yağ Asidi Kompozisyonu

Kızartma dışındaki teknikler olan fırında, mikrodalga fırında pişirme, ızgara ve buğulama yöntemi ile pişirilen akya filetolarında da çiğ örneklerdeki gibi SFA'lar içinde 16:0, MUFA'lardan 18:1n-9 ve PUFA'lardan 22:6n-3 dominant yağ asitleri olarak tespit edilmiştir (Çizelge 3). Bazı bireysel yağ asitleri dışında, belirtilen pişirme yöntemlerinin akya balığının yağ asidi kompozisyonunu çok önemli düzeyde değiştirmediği görülmüştür. Kontrol (çiğ) ve diğer pişirme yöntemleri ile karşılaştırıldığında buğulama yöntemi ile pişirilen filetolarda palmitik asidin bir miktar daha az yüzdede, kontrol (çiğ) ve fırında pişirilenlerde ise 22:6n-3 ve bu yağ asidine bağlı ∑PUFA ve ∑n-3 PUFA'nın ise daha fazla yüzdede oldukları belirlenmiştir. Pişirme yöntemleri arasında

hem bireysel yağ asitleri hem de yağ asidi gruplarının yüzde değerleri bakımından ham (çiğ) filetolara en yakın pişirme tekniğinin fırında pişirme olduğu söylenilebilir. Bu bulgu daha önce yapılan çalışmalarda da ortaya konmuştur. Fırınlama işleminin; PUFA içeriği ve n-3/n-6 oranı gibi parametreler dahil olmak üzere balık etinin tüm lipit özelliklerinin korunması açısından en iyi ısıl işlem olduğu birçok araştırmacı (García-Arias ve ark., 2003; Türkkan ve ark., 2008; Neff ve ark., 2014; Başhan, 2019) tarafından kabul edilmiştir.

Çizelge 2. Farklı bitkisel yağlarda kızartılan akya ile ham (çiğ) akyanın yağ asidi kompozisyonu
Table 2. Fatty acid composition of leer fried in different vegetable oils and raw (raw) leer

Yağ Asidi	Çiğ (HAM) (ORT±SH)	Ayçiçek Yağı (ORT±SH)	Zeytin Yağı (ORT±SH)	Mısır Özü Yağı (ORT±SH)	Fındık Yağı (ORT±SH)
12:0	-	0.14±0.01a	-	-	0.05±0.001b
14:0	0.96±0.08a	0.17±0.01b	0.06±0.00c	0.09±0.01c	0.07±0.01c
15:0	0.20±0.02a	0.03±0.004b	0.01±0.002c	0.02±0.003bc	0.03±0.003b
16:0	27.99±2.23a	9.30±0.74b	14.53±1.16c	12.76±1.02c	7.39±0.59b
17:0	-	-	0.19±0.02	-	-
18:0	11.21±0.89a	4.62±0.37b	3.79±0.30b	2.02±0.16c	3.00±0.24b
∑SFA	**40.38±3.22a**	**14.28±1.14b**	**18.59±1.48c**	**14.91±1.19b**	**10.54±0.84d**
16:1n-7	1.36±0.11a	0.32±0.03b	0.73±0.06c	0.11±0.01d	0.19±0.02e
18:1n-9	14.07±1.92a	35.91±2.87b	68.23±5.44c	30.67±2.45d	72.55±5.79e
20:1n-9	0.14±0.01a	0.15±0.01a	0.12±0.01a	0.09±0.01b	0.11±0.01a
∑MUFA	**15.57±2.04a**	**36.38±2.90b**	**69.08±5.51c**	**30.88±2.46d**	**72.86±5.81e**
18:2n-6	3.34±0.27a	43.85±3.50b	8.34±0.67c	50.30±4.01d	13.54±1.08e
18:3n-6	0.25±0.02a	0.15±0.01b	0.40±0.03c	0.15±0.01b	0.12±0.01b
18:3n-3	0.27±0.02a	0.27±0.02a	0.54±0.04b	0.65±0.05b	0.16±0.01c
20:2n-6	0.25±0.02a	0.16±0.01b	0.04±0.006c	0.02±0.003d	0.05±0.007c
20:3n-6	0.19±0.02a	0.01±0.001b	0.07±0.01c	0.05±0.00d	0.04±0.006d
20:4n-6	2.29±0.18a	0.64±0.05b	0.25±0.02c	0.24±0.02c	0.23±0.02c
20:5n-3	2.20±0.18a	0.31±0.03b	0.21±0.02c	0.19±0.02c	0.18±0.01c
22:5n-3	0.93±0.07a	0.14±0.01b	0.08±0.01c	0.09±0.01c	0.06±0.01c
22:6n-3	34.33±1.94a	3.81±0.30b	2.40±0.19c	2.51±0.20c	2.21±0.18c
∑PUFA	**44.05±2.72a**	**49.34±3.94b**	**12.33±0.98c**	**54.21±4.32d**	**16.60±1.32e**
∑n-3 PUFA	37.72±2.21a	4.54±0.36b	3.23±0.26c	3.44±0.27c	2.62±0.21d
∑n-6 PUFA	6.33±0.50a	44.81±3.57b	9.10±0.73c	50.77±4.05d	13.99±1.12e
n-3/n-6	5.95±0.62a	0.10±0.08b	0.35±0.052c	0.07±0.006d	0.19±0.02e
∑PUFA/∑SFA	**1.09±0.12a**	**3.45±0.42b**	**0.66±0.074c**	**3.63±0.28b**	**1.57±0.16d**
AI	0.53±0.045a	0.12±0.03b	0.18±0.07c	0.15±0.02bc	0.09±0.01b
TI	0.30±0.05a	0.26±0.032b	0.37±0.04c	0.29±0.04a	0.20±0.03d

S.H.:Standart Hata, SFA: DoymuşYağ Asitleri, MUFA: Tekli Doymamış Yağ Asitleri, PUFA: Çoklu Doymamış Yağ Asitleri, AI: Aterojenik İndeks, TI: Trombojenik İndeks.

Kızartma dışında denenen çeşitli pişirme teknikleri uskumru (Başhan, 2019), Ringa (Ilow ve Ilow, 2002) ve Deniz Levreğinin (Yanar ve ark., 2007) bireysel yağ asitleri ile yağ asitleri grubu kompozisyonu üzerinde önemli bir etki oluşturmamıştır.

Buğulama işlemi, Çipura balığında 18:1n-9 ve ∑n-3 PUFA yüzdesinde azalmaya neden olmuş, Sarı Benekli Lagos balığında ise n-3 PUFA'lardan EPA ve DHA düzeylerini değiştirmemiştir (Zahra Momenzadeh ve ark., 2017).

Çalışmada kızartma dışında uygulanan pişirme yöntemleri akya filetolarının n-3/n-6 ile PUFA/SFA oranını pek fazla etkilememiştir (Çizelge 3). Daha önce yapılan çalışmalarda, mikrodalgayla pişirilen ve buğulama yapılan dört tatlı su balığında (Neff ve ark., 2014), fırında ve mikrodalga fırınında pişirilen, buğulama, tütsüleme ve ızgara yapılan uskumruda n-3/n-6 ile PUFA/SFA oranı değişmemiştir (Başhan, 2019).

Farklı pişirme teknikleri ile pişirilen akyadaki Aİ ve Tİ değerleri, beslenme uzmanlarının önerdiği değerler arasında bulunmuştur (Çizelge 3). Çizelgede de görüldüğü gibi, ızgara, mikrodalga ve buğulama yöntemleri ile pişirilen balıkta çiğ örneklere oranla Aİ ve Tİ indekslerinin biraz daha yüksek olmasının nedeni, bu pişirme yöntemlerinde toplam ∑n-3 PUFA ile ∑PUFA yüzdelerinin daha düşük olarak saptanmış olmasıdır.

Tırsi (*Alosa immaculata*) balığında, AI indeksi ızgarada, TI ise hem ızgara hem de buğulamada artmıştır (Merdzhanova ve ark., 2016). Fırında pişirilen Hazar kutumu (*Rutilus kutum*) balığında Aİ ve Tİ değerleri kontrole yakın çıkmış, ancak mikrodalgada pişirme yönteminde artmıştır (Hosseini ve ark., 2014). Bu sonuçlar, yapılan çalışmadan elde edilenlerle uyum içindedir.

Çizelge 3. Değişik pişirme yöntemleri ile pişirilen akya filetoları ile çiğ filetoların yağ asidi kompozisyonu
Table 3. Fatty acid composition of leer fillets cooked with different cooking methods and raw fillets

Yağ Asidi	Çiğ (HAM) (ORT±SH)	Fırın (ORT±SH)	Izgara (ORT±SH)	Mikrodalga (ORT±SH)	Buğulama (ORT±SH)
12:0	-	0.13±0.01a	0.09±0.01b	0.18±0.01c	0.20±0.02c
14:0	1.13±0.09a	0.46±0.04b	0.62±0.05c	0.78±0.06d	6.43±0.51e
15:0	0.32±0.03a	0.10±0.01b	0.36±0.03a	0.47±0.04c	0.20±0.02d
16:0	26.67±1.73a	28.02±2.24a	28.24±2.25a	28.01±2.23a	24.60±1.96b
17:0	0.02±0.00a	0.08±0.01b	0.04±0.00c	0.27±0.02d	0.26±0.02d
18:0	10.39±0.67a	11.21±0.89a	12.37±0.99b	13.90±1.11b	10.06±0.80a
∑SFA	**38.54±2.52a**	**40.00±3.19a**	**41.73±3.33b**	**43.61±3.48c**	**41.76±3.33b**
16:1n-7	1.67±0.13a	1.02±0.08a	1.17±0.09b	1.08±0.09a	0.95±0.08c
18:1n-9	13.84±2.54a	11.24±0.90b	14.02±1.12a	12.92±1.03a	15.58±1.24c
20:1n-9	0.11±0.01a	0.20±0.02b	0.04±0.00c	0.08±0.01d	0.22±0.02b
∑MUFA	**15.62±2.68a**	**12.46±0.99b**	**15.23±1.22a**	**14.08±1.12c**	**16.75±1.34d**
18:2n-6	3.45±0.91a	2.19±0.17b	4.63±0.37a	2.12±0.17b	4.67±0.37a
18:3n-6	0.41±0.03a	0.34±0.03a	0.35±0.03a	0.30±0.02a	0.16±0.01b
18:3n-3	0.36±0.03a	0.35±0.03a	0.48±0.04b	0.31±0.02a	0.22±0.02c
20:2n-6	0.19±0.02a	0.31±0.02b	0.27±0.02b	0.27±0.02b	0.18±0.01a
20:3n-6	0.24±0.02a	0.17±0.01a	0.10±0.01b	0.11±0.01b	0.05±0.00c
20:4n-6	3.67±0.13a	5.04±0.40b	3.00±0.24a	3.71±0.30a	3.18±0.25a
20:5n-3	2.88±0.15a	3.22±0.26a	2.83±0.23a	2.51±0.20a	2.70±0.22a
22:5n-3	0.81±0.06a	0.87±0.07a	0.92±0.07a	1.22±0.10b	0.64±0.05c
22:6n-3	33.83±1.42a	35.04±2.80b	30.47±2.43c	31.75±2.53d	29.69±2.37e
∑PUFA	**46.85±2.78a**	**47.55±3.79a**	**43.04±3.43b**	**42.31±3.38c**	**41.49±3.31c**
∑n-3 PUFA	38.89±1.67a	39.50±3.15a	34.70±2.77b	35.79±2.86b	33.24±2.65c
∑n-6 PUFA	7.96±1.11a	8.05±0.64a	8.34±0.67a	6.52±0.52b	8.25±0.66a
n-3/n-6	4.88±0.65a	4.91±0.65a	4.16±0.55a	5.49±0.73a	4.03±0.53a
∑PUFA/∑SFA	**1.21±0.16a**	**1.18±0.15a**	**1.03±0.13a**	**0.97±0.12a**	**0.99±0.13a**
AI	0.50±0.06a	0.50±0.06a	0.52±0.06a	0.55±0.07a	0.90±0.12b
TI	0.29±0.03a	0.30±0.03a	0.34±0.04a	0.34±0.04a	0.35±0.04a

S.H.:Standart Hata, SFA: DoymuşYağ Asitleri, MUFA: Tekli Doymamış Yağ Asitleri, PUFA: Çoklu Doymamış Yağ Asitleri, AI: Aterojenik İndeks, TI: Trombojenik İndeks.

SONUÇ ve ÖNERİLER

Her bitkisel yağın kendisine özgü bir yağ asidi kompozisyonuna sahip olduğu, ayçiçek yağı ile mısır özü yağının linoleik asit (18:2n-6), zeytinyağı ile fındık yağının oleik asit (18:1n-9) bakımından zengin olduğu belirlenmiştir.

Kızartma işleminin balıkların yağ asidi kompozisyonuna önemli etki yaptığı, ayçiçek ve mısır özü yağında kızartılan filetolarda 18:2n-6 ile ∑n-6 PUFA'nın, zeytinyağı ve fındık yağında kızartılan örneklerde 18:1n-9 ve ∑MUFA'nın kontrole oranla önemli düzeyde arttığı saptanmıştır ($p<0.01$).

Izgara, mikrodalga ve buğulama ile pişirilen akyanın yağ asidi gruplarından ∑SFA'da bir miktar artış, ∑PUFA ve ∑n-3 PUFA'da azalma kaydedilmiştir.

n-3/n-6 oranı, özellikle ayçiçek ve mısırözü yağında kızartılan balıklarda önemli düzeyde azalmış ($p<0.01$); kızartma dışında pişirilen yöntemlerde ise kontrole yakın değerlerde bulunmuştur. Tüm pişirme yöntemlerinden elde edilen aterojenik (Aİ) ve trombojenik indeks (Tİ) değerleri, önerilen değer olan 1.0'in altında bulunmuştur.

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Araştırmacıların Katkı Oranı Beyan Özeti

Makaleye olan katkı oranı eşittir.

Çıkar Çatışması Beyanı

Herhangi bir çıkar çatışması beyanı yoktur.

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Investigation of Active Compounds in Propolis Structure Against Sars Cov-2 Main Protease by Molecular Docking Method: In Silico Study

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ABSTRACT

It was aimed to investigate the active ingredients limonin, quercetin and kaempferol in propolis against SARS-CoV-2 main protease(MPro) using in silico methods. Absorption, distribution, metabolism, excretion, and toxicity (ADMET) screening of ligands assists US to state their absorption properties, toxicity, and drug-likeness. Ligand molecules obtained from PubChem in smiles format were loaded on SWISSADME and PROTOX-II webservers for ADMET screening. The three compounds in propolis were obtained from the PubChem database. Compounds were located at the active site of the SARS-CoV-2 MPro receptor with PDB ID:6LU7. Molecular docking work was done with Autodock program. Molecular docking results were found as -8.7 kcal/mol in limonin, -7.5 kcal/mol in quercetin and -7.7 kcal/mol in kaempferol. In silico ADMET estimation showed they have a potential for antiviral therapy. In conclusion, we thought that propolis active components limonin, quercetin and kaempferol have the potential to be a SARS CoV-2 MPro inhibitor.

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Propolisin Aktif Bileşiklerinin Sars Cov-2 Ana Proteaz Yapısında Moleküler Yerleştirme Yöntemiyle Araştırılması: In Silico Çalışması

ÖZET

Propolisin aktif bileşikleri olan limonin, quercetin ve kaempferol'ü SARS-CoV-2 ana proteaza (MPro) karşı in silico yöntemlerle araştırması amaçlandı. Ligandların absorpsiyon, dağılım, metabolizma, atılım ve toksisite (ADMET) taraması, absorpsiyon özelliklerini, toksisitesini ve ilaca benzerliğini belirtmesine yardımcı olur. PubChem'den smiless formatında elde edilen ligand molekülleri, ADMET taraması için SWISSADME ve PROTOX-II web sunucularına yüklendi. Propolisteki üç bileşik, PubChem veritabanından elde edildi. Bileşikler, PDB ID:6LU7 ile SARS-CoV-2 MPro reseptörünün aktif bölgesine yerleştirildi. Autodock programı ile moleküler yerleştirme çalışması yapıldı. Moleküler yerleştirme sonuçları limoninde -8,7 kcal/mol, quercetin'de -7,5 kcal/mol ve kaempferol'de -7,7 kcal/mol olarak bulundu. In silico ADMET tahmini, antiviral tedavi potansiyeline sahip olduklarını gösterdi. Sonuç olarak, propolis aktif bileşenleri limonin, quercetin ve kaempferol'ün SARS CoV-2 MPro inhibitörü olma potansiyeline sahip olabileceği düşünülmektedir.

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INTRODUCTION

Coronaviruses (CoVs) are the etiological cause of serious infections in the respiratory tract as well as the digestive tract in both animals and humans. Previous

reviews of CoVs have indicated that from mammals to reptiles, and birds, a wide range of species have been affected by these viruses (Malik et al, 2020). COVID-19 was accepted as a pandemic disease by the WHO on January 30, 2020 (Rodriguez et al., 2020). Although various measures and effective treatment methods have been adopted by countries to reduce the course of the disease, prevention management strategies were limited for eradication. The SARS coronavirus main protease (Mpro) of the coronavirus consists of glycoprotein and it is required for virus replication (Hofmann et al., 2004).

The chemical composition of propolis differs depending on its source, and more than 300 components have been identified in raw propolis (Gulcin et al., 2010).

Many researchers report that propolis extract is effective in the prevention of viral infection on plants (such as cucumber mosaic, tobacco mottle, tobacco gangrene), animals (HSV-1, varicella-zoster, and influenza), and humans (human immunodeficiency-HIV, herpes simplex virus type 1 and 2, adenovirus type 2, pharyngitis virus, and poliovirus type 2 (Marcucci, 1995). Studies show that propolis has the potential to be used as an antiviral drug. (Silici et al., 2005). Propolis has a lethal effect against the influenza virus (type A) in vitro, while aqueous propolis extract greatly reduces the effect of the smallpox virus within 15 minutes (Hegazi et al., 2000).

The process of revealing the in silico structures of receptor-ligand complexes with various software is called molecular docking. The receptors consist of proteins, while the ligands may consist of another protein or small molecule. In drug discovery studies, the virtual screening process with the molecular docking method is becoming more and more important. Such a virtual scan is usually performed in three steps. First, the molecular insertion program predicts the optimal structure for the complex of a target protein and a compound from the screening libraries. Second, complexes are scored according to their binding energy strength. Finally, classification is made according to the placement scores, and the best grades are selected from the virtual scan results (Onodera et al., 2007).

The aim of this study was to investigate the propolis bioactive components limonin, quercetin and kaempferol compounds in SARS CoV-2 Mpro structure by molecular docking method and to conduct drug similarity studies of limonin, quercetin and kaempferol.

MATERIALS and METHODS

ADMET and toxicity prediction

The ADMET (absorption, distribution, metabolism, excretion and toxicity) screening helps determine the toxicity and drug-likeness of compounds. Ligand molecules and selected propolis active ligands (limonin, quercetin and kaempferol) obtained in smile format from PubChem (https://pubchem.ncbi.nlm.nih.gov) were uploaded to the SWISSADME and PROTOX-II web servers for ADMET screening. Investigating the pharmacokinetics and ADME properties of a molecule or compound is done on a server called SWISSADME. Lipophilicity, water solubility, drug similarity, pharmacokinetic properties of the molecule, blood-brain barrier (BBB) and intestinal permeability were estimated through this server. (Table 1). The analysis was carried out for each physicochemical property (Absorption, Distribution, Metabolism, Excretion, and Toxicity) by submitting a SMILE format of the query compounds taken from the PubChem database. PROTOX-II is a Rodent oral toxicity server that predicts LD50 value and toxicity class of query molecule. Toxicity values on the PROTOX-II web server are as follows: Class I: fatal if swallowed (LD50 ≤ 5 mg/kg), Class II: fatal if swallowed (5 mg/kg < LD50 ≤ 50 mg/kg), Class III: toxic if swallowed (50 mg/kg < LD50 ≤ 300 mg/kg), Class IV: harmful if swallowed (300 mg/kg < LD50 ≤ 2000 mg/kg), Class V: may be harmful if swallowed (2000 mg/kg < LD50 ≤ 5000 mg/kg) and Class VI: non-toxic (LD50 > 5000 mg/kg). (Banerjee P et al., 2018).

Table 1. Drug likeness rules and their properties
Çizelge 1. İlaç benzerlik kuralları ve özellikleri

Name of rule	Property	Rules
Lipinski's rule	Molecular weight	≤ 500
	Lipophilicity (logP)	≤ 5
	Hydrogen bond acceptor	≤ 10
	Hydrogen bond donors	≤ 5
Ghose's rule	Lipophilicity (logP)	− 5.6<logP< − 0.4
	Molecular weight	160 < MW <480
	Molar refractivity	40 < MR < 130
	Total number of atoms	20 < atoms < 70
Veber's rule	No. of rotatable bonds	≥ 10
	TPSA	≤ 140
	Hydrogen bond donor	≤ 12
	Hydrogen bond acceptor	≤ 12

Molecular Docking Method

Ligand System

Limonin, quercetin and kaempferol in propolis used in this study were taken from the PubChem database (https://pubchem.ncbi.nlm.nih.gov). 3D structures of compounds were obtained in SDF format from PubChem. Compounds in SDF format were converted to PDB format from the Open Babel GUI program.

Protein Preparation

3D structure of the SARS-CoV-2 Mpro (PDB ID: 6LU7) was retrieved from the Protein Data Bank (PDB) (http://www.rcsb.org/pdb/). The resolution of the PDB ID: 6LU7 protein is 2.16 Å. Firstly, ligands and water molecules in the 6LU7 protein structure were removed from the receptor, after that, polar hydrogen and a

charge (colman charge) were added together with the receptor in the protein structure. All preparatory processes were carried out using AutoDock 4 software (Morris et al., 2009).

Validation Method

The N3 inhibitor (N-[(5-methylisoxazol-3-yl)carbonyl]alanyl-1-valyl-N~1~-((1R, 2Z)-4-(benzyloxy)-4-oxo-1-{[(3R)-2-oxopyrrolidin-3-yl] methyl } but-2-enyl)-1-leucinamide) was deconstructed using AutoDock 4 (Jin et al., 2020). N3 inhibitor, the natural ligand of SARS CoV-2 Mpro, was superimposed on the protein structure according to the insertion procedure. Also, the root mean square deviation (RMSD) value was checked using PyMOL software to validate. If the RMSD value is less than 2.0 Å, it indicates that the method is valid. (Bell & Zhang., 2019).

Molecular Docking

It was carried out by applying all the parameters valid for the simulation of molecular docking. SARS-CoV-2 Mpro structure active region coordinates and grid box dimensions were determined in Discovery Studio program. The active site coordinates of SARS-CoV-2 Mpro are x=-9.732, y=11.403 and z=68.925. Grid box sizes are 64 Å, 60 Å and 60 Å, respectively. 100 replicates were made for each active compound to ensure the accuracy of the binding energy and amino acid interactions. Molecular docking was done with AutoDock 4 (Laskowski, 1995).

RESULTS and DISCUSSION

ADMET and toxicity prediction

The SWISSADME analysis and toxicity estimation results are shown in Table 2. Limonin, quercetin, and kaempferol showed good human intestinal solubility (HIA), and the selected propolis active compounds all belong to the same class (Class-IV) in acute rat toxicity (LD50). These phytochemicals are inactive for cytotoxicity and hepatic toxicity.

The LD50 values of propolis active compounds are limonin: 244mg/kg, quercetin:159 mg/kg, and kaempferol:3919 mg/kg.

Drug likeness prediction

When both limonin, quercetin, and kaempferol molecules are evaluated based on the Lipinski, Ghose, and Veber rules, it has been observed that the molecules are compatible with these rules, that is, these molecules are within the limits that can be considered as drugs.

The radar image obtained from the SwissADME web server in Figure 1 indicates substances that can be considered drug-like in a pink area, based on 6 different physicochemical parameters. These parameters are lipophilic (LIPO), molecular size (SIZE), polarity (POLAR), solubility (INSOLU), flexibility (FLEX), and saturation (INSATU). The areas where these parameters are restricted specify certain value ranges for the candidate molecule.

Figure 1. The radar image of limonin, quercetin, and kaempferol molecule.
Şekil 1. Limonin, kersetin ve kaempferol molekülünün radar görüntüsü.

Firstly, when the radar images of limonin, quercetin, and kaempferol molecules are evaluated, it is seen that the only limonin is in the pink area in 6 different parameters, while the quercetin and kaempferol only deviate in terms of saturation.

Tophological Polar Surface Area (TPSA) is defined as the sum of areas on all polar atoms or molecules of a molecule, including primarily nitrogen and oxygen, and later hydrogen atoms. It is mostly used as an indicator for molecular transport through biological barriers, such as the blood-brain barrier (BBB), in the body. If this value is more than 140 Å2, molecular transport through cell membranes would be difficult. It has been shown that the TPSA values for candidate molecules targeted at central nervous systems should be less than 60-70 Å2 to overcome BBB. The TPSA values of limonin, quercetin, and kaempferol molecules were evaluated as 104.57Å2, 131.36Å2, and 111.13 Å2, respectively. Since the TPSA values obtained for these three molecules are greater than 60-70 Å2, they do not have the ability to cross the BBB (Figure 2, Table 2).

Figure 2. Boiled-Egg image of limonin, quercetin, and kaempferol molecule.
Şekil 2. Limonin, kuersetin ve kaempferol molekülünün haşlanmış yumurta görüntüsü.

Table 2. The results of the ADMET test with SwissADME
Çizelge 2. SwissADME ile ADMET testinin bulguları

Property	Limonin	Quercetin	Kaempferol
Molecular weight	470.51 g/mol	302.24 g/mol	286.24 g/mol
TPSA	104.57 Å²	131.36 Å²	111.13 Å²
iLOGP	2.87	1.63	1.7
XLOGP3	1.77	1.54	1.90
WLOGP	2.81	1.99	2.28
MLOGP	1.45	-0.56	-0.03
Silicos- IT LogP	3.83	1.54	2.03
Consensus Log P	2.55	1.23	1.58
ESOL Log S	-3.92	-3.16	-3.31
ESOL class	Soluble	Soluble	Soluble
Ali LogS	-3.40	3.60	-5.00
Ali class	Soluble	Soluble	Moderately soluble
Silicos- IT LogSw	-3.58	-3.91	-3.86
Silicos-IT class	Soluble	Soluble	Soluble
GI absorption	High	High	High
BBB perme- ant	Yes	Yes	Yes
Log Kp, cm/s (Skin penetration)	-4.87 cm/s	-4.74 cm/s	-5.93 cm/s
Lipinski violations	Yes; 0 violation	Yes; 0 violation	Yes; 0 violation
Ghose violations	Yes	Yes	Yes
Veber violations	Yes	Yes	Yes
Egan violations	Yes	Yes	Yes
Muegge violations	Yes	Yes	Yes
Bioavailability score	0.55	0.55	0.55
PAINS alerts	0 alert	0 alert	0 alert
Brenk alerts	0 alert	0 alert	0 alert

Molecular weight is important to determining whether specific molecules can penetrate into particular types of barriers in the human body since large molecules can not pass through highly selective barriers. Since the molecular weight of limonin, quercetin and kaempferol is <500 g/mol, this value is within the limits of the molecule being a drug (Table 2).

Candidate drug molecules must have optimal hydrophilicity and lipophilicity (ClogP) values. CLogP values were calculated as 2.55, 1.23, and 1.58 for limonin, quercetin, and kaempferol, respectively (Table 2).

Validation Results

Revalidation was performed with the ligand N3 inhibitor to determine the strength of binding affinity. The result of the verification was shown in the Figure 3. The RMSD value of the ligand was found 1.5 Å and the binding energy was -6.9 kcal/mol.

Molecular Docking Results

The binding energies of propolis bioactive compounds after the insertion process are shown in Table 3. RMSD, and theoretically inhibitory concentration (Table 4) were calculated by molecular docking method in SARS-CoV-2 Mpro structure (PDB ID: 6LU7) for the active compounds N3 inhibitor, limonin, quercetin, and kaempferol compounds in propolis. Autodock vina results from the Molecular docking model were extracted with the 3D BIOVIA Discovery Studio 2020 program (Figure 4). In addition, the binding site estimates and bond structures of the bioactive compounds in the propolis structure in the SARS CoV-2 Mpro structure were determined (Figure 5-7).

The binding interactions of N3 inhibitor, which is an inhibitor of SARS CoV-2 Mpro receptor, and propolis active compounds were compared. According to the results, the molecular docking scores of the bioactive components limonin, quercetin, and kaempferol were determined as <-6.5 kcal/mol. Docking scores indicate good binding in the SARS CoV-2 Mpro structure. Since molecular docking study result was below 2 Å, it showed that docking study was accurate and successful.

The binding energy of N3 inhibitor was -6.9 kcal/mol, limonin -8.7 kcal/mol, quercetin -7.5 kcal/mol, and kaempferol -7.7 kcal/mol in SARS CoV-2 Mpro (PDB ID: 6LU7) structure and all results showed high binding energy. When we compared the binding energy of the N3 inhibitor with the binding energy of the active components of propolis, we saw that the N3 inhibitor had low binding affinity. Similar results were obtained when compared with other studies. In addition, inhibitor concentrations were found to be 41 µM in limonin, 85µM in quercetin, and 115 µM in kaempferol. ADMET results have shown that three compounds can meet the characteristics of being a drug.

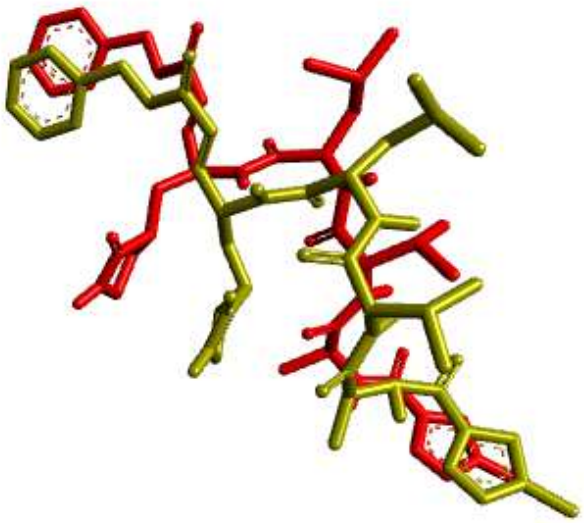
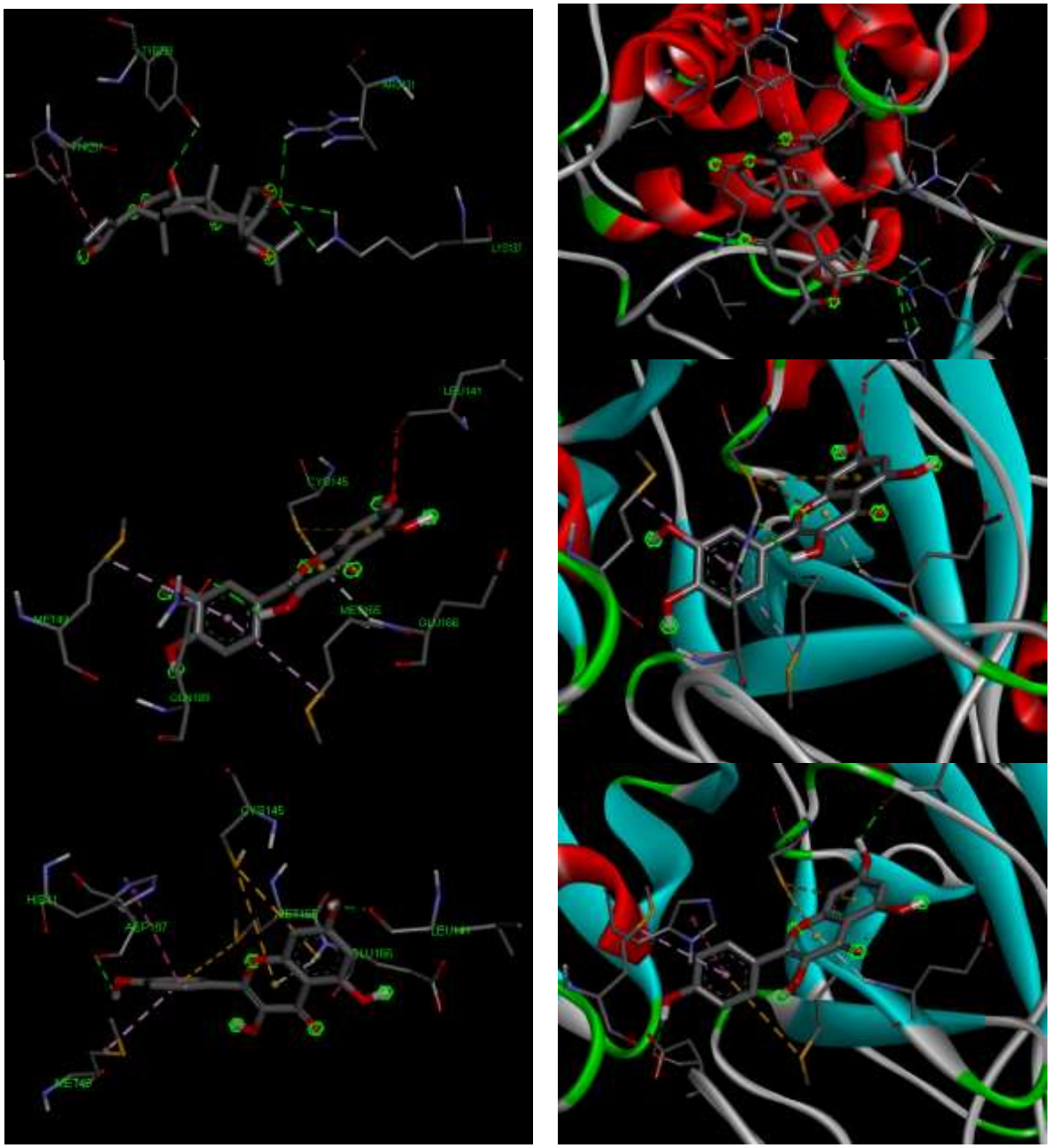


Figure 3. SARS-CoV-2 MPro receptor state before validation (red), state of the receptor after insertion (yellow), inhibitor model

Şekil 3. Doğrulama öncesi SARS-CoV-2 MPro reseptör durumu (kırmızı), yerleştirme sonrası reseptör durumu (sarı), inhibitör modeli

Table 3. Molecular docking results of propolis compounds in SARS CoV 2 Mpro structure
Çizelge 3. SARS CoV 2 Mpro yapısındaki propolis bileşiklerinin moleküler kenetlenme sonuçları

Analysis Program	Visualization Program	Protein	Ligand	Docking Score(kcal/mol)	Amino Acid	Residue
Autodock Vina	3 D BIOVIA Discovery Studio Visualizer	6LU7	N3 inhibitor	-6.9	VAL171, TYR199, LEU286, LEU287	ALA194, MET276,
Autodock Vina	3 D BIOVIA Discovery Studio Visualizer	6LU7	Limonin	-8.7	ARG131, TYR239, TYR237	LYS137,
Autodock Vina	3 D BIOVIA Discovery Studio Visualizer	6LU7	Quercetin	-7.5	MET49, CYS145, GLU166, GLN189	LEU141, MET165,
Autodock Vina	3 D BIOVIA Discovery Studio Visualizer	6LU7	Kaempferol	-7,7	HIS41, LEU141, MET165, ASP187	MET49, CYS145, GLU166,



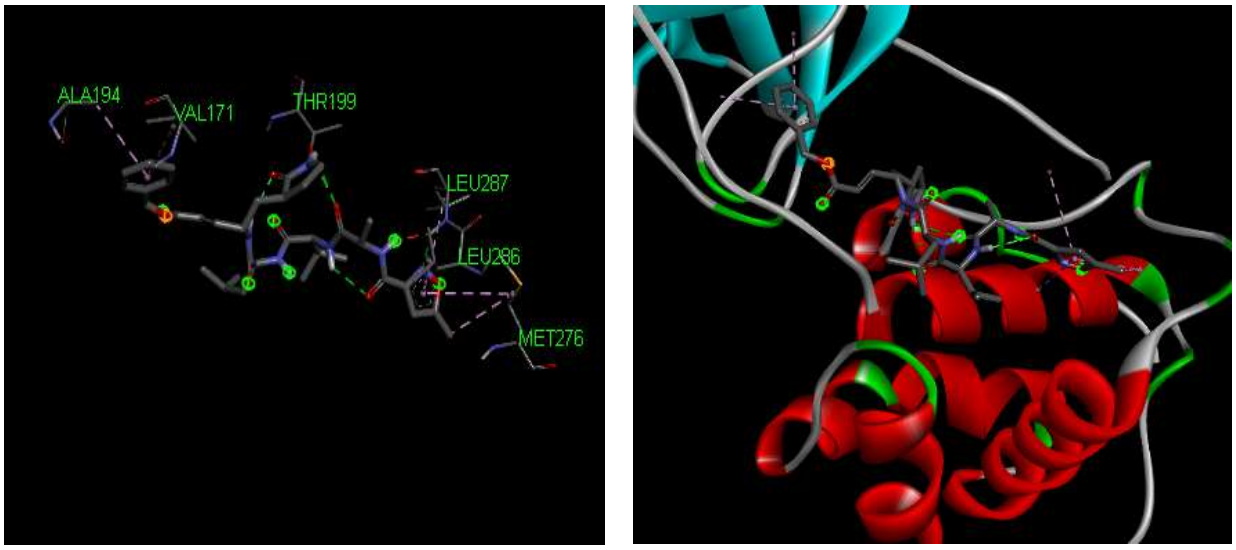


Figure 4. Molecular docking results of active compounds in the structure of SARS CoV 2 Mpro, N3 inhibitor and propolis
Şekil 4. SARS CoV 2 Mpro, N3 inhibitörü ve p[illegible]aktif bileşiklerin moleküler kenetlenme sonuçları

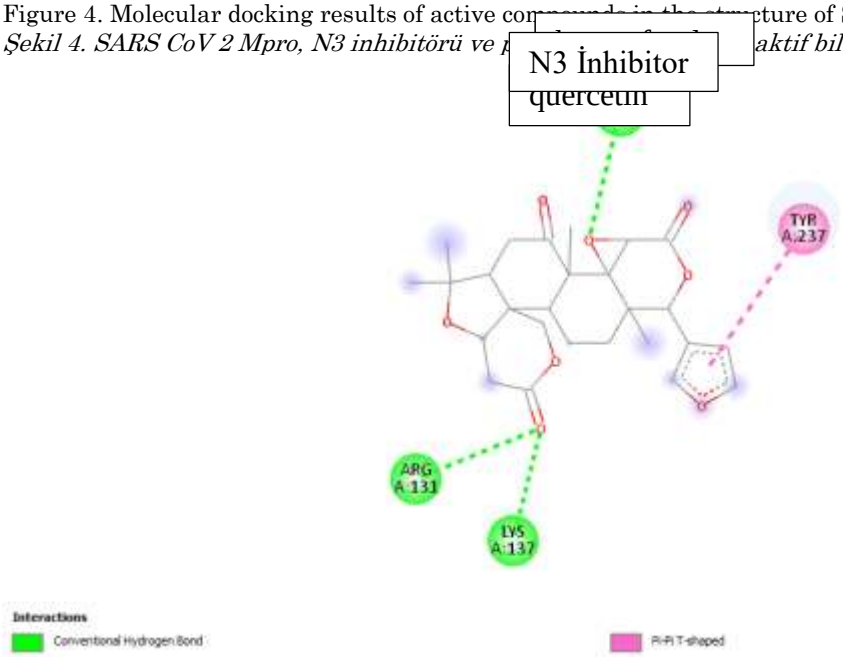


Figure 5. Bond structures in limonin SARS CoV-2 Mpro structure.
Şekil 5. Limonin SARS CoV-2 Mpro yapısındaki bağ yapıları.

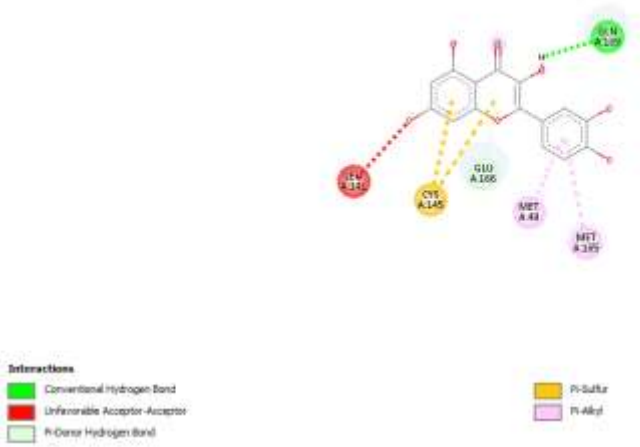


Figure 6. Bond structures in quercetin SARS CoV-2 Mpro structure.
Şekil 6. Quercetin'in SARS CoV-2 Mpro yapısındaki bağ yapıları

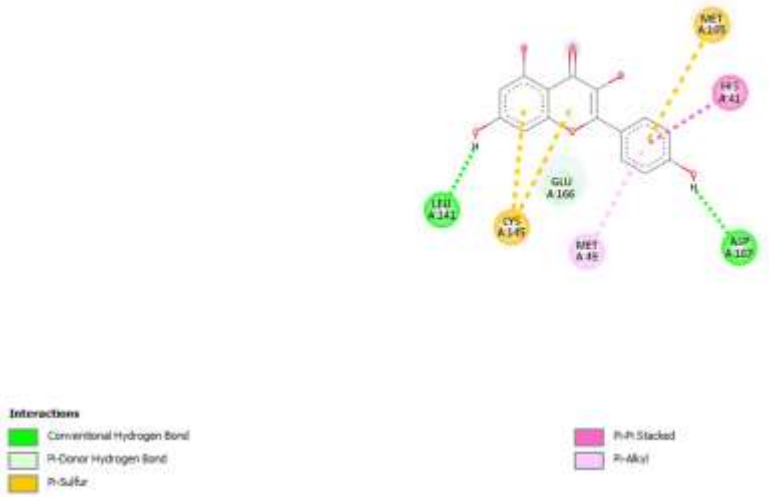


Figure 7. Bond structures in kaempferol SARS CoV-2 Mpro structure.
Şekil 7. Kaempferol'ün SARS CoV-2 Mpro yapısındaki bağ yapıları

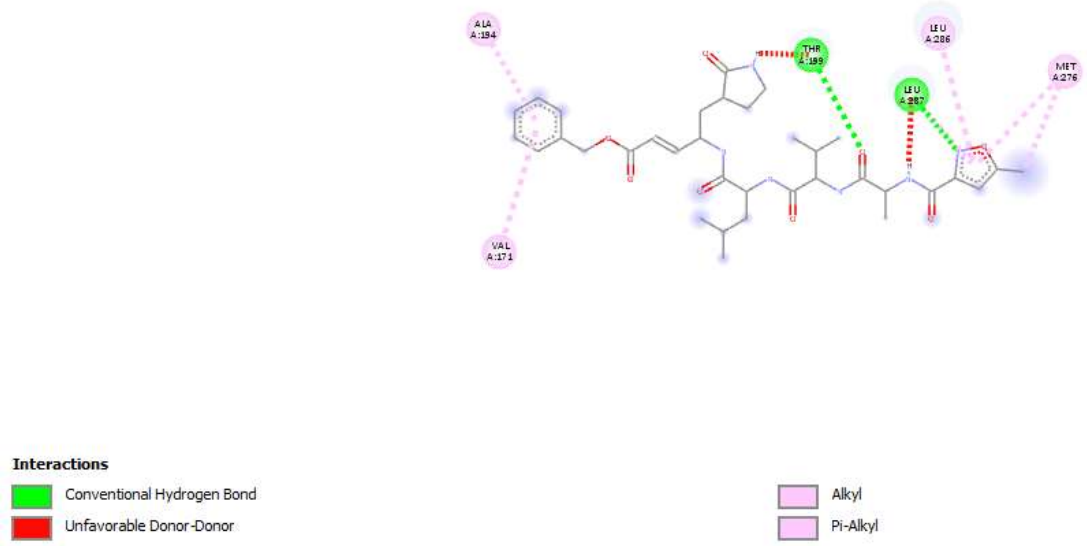


Figure 8. Bond structures in N3 inhibitor SARS CoV-2 Mpro structure.
Şekil 8. N3 inhibitörü'nün SARS CoV-2 Mpro yapısındaki bağ yapıları

Table 4. RMSD and Inhibition constant scores of limonin, quercetin, and kaempferol in SARS CoV 2 Mpro structure
Çizelge 4. SARS CoV 2 Mpro yapısında limonin, kersetin ve kaempferolün RMSD ve İnhibisyon konsantrasyonları

Analysis Program	Protein	Ligand	RMSD (Å)	Inhibition Constant
Autodock Grid	6LU7	Limonin	1.70	41 µM
Autodock Grid	6LU7	Quercetin	1.99	85 µM
Autodock Grid	6LU7	Kaempferol	1.82	115 µM

Many studies have reported that propolis and/or its components support strengthening the immune system and reducing inflammation due to their anti-inflammatory properties. These properties will help reduce the symptoms and harmful effects caused by COVID-19 (Vardeny et al., 2020).

Jin et al. found that the N3 inhibitor is promising in the SARS CoV-2 Mpro construct (Jin et al. 2020).

Vardhan et al. stated that limonin has a good binding affinity to the SARS CoV-2 Mpro (PDB ID: 6LU7) structure in their study. The binding affinity result was -8.7 kcal/mol and it was similar to this result (Vardhan et al., 2020).

In the study of Khan et al., the molecular docking score of the kaempferol compound was -6.4 kcal/mol and the inhibitory concentration was 116 micromolar in the SARS CoV-2 Mpro structure. (Khan et al., 2021). A close result was found when compared with this result. Yang et al. showed that kaempferol has a high binding

energy (-7.5 kcal/mol) at its major receptor (ACE2) for viral entry (Yang et al., 2018).

Arokiyaraj et al. determined that the binding affinities of quercetin were -6.49 kcal/mol and kaempferol was -7.76 kcal/mol in the SARS-CoV-2 Mpro structure (PDB ID: 6LU7) (Arokiyaraj et al., 2020). Their results were consistent with these findings. Luo et al. showed that 54 patients with novel coronavirus pneumonia improved their immune ability against COVID-19 after traditional Chinese medicine treatment and shortened patients' hospital stay. Compound quercetin, luteolin, kaempferol, acacetin etc., were all involved in the treatment of various disease stages on the compound level both in generality and individuality (Luo et al., 2020).

CONCLUSION

In summary, coronavirus has emerged as the deadliest disease the world has faced after the Spanish flu. It is important to find a solution to control this virus urgently. It is important to carry out studies on this virus with computer-aided drug design programs in terms of being fast and saving time. We conducted a computer-assisted drug discovery study against the protein involved in the action mechanism of SARS CoV-2. These results show that the bioactive compounds of propolis (limonin, quercetin, and kaempferol) have the ability to inhibit the target protein Mpro (PDB ID:6LU7) in SARS CoV-2 in the least energy conformation. We suggest that three compounds can prevent the coronavirus infection.

Author contributions

Concept – E.O., S.Y.; Design – İ.D., E.O.,; Supervision – E.B.K.; Resources – E.O., İ.D.; Materials – E.O.; Data Collection and/or Processing – E.O., İ.D.; Analysis and/or Interpretation – E.O., E.B.K., S.Y.; Literature Search – İ.D., E.O.; Writing – İD.,E.O.; Critical Reviews – E.B.K., İ.D.,S.Y.

Conflict of interest statement

The authors declared no conflict of interest in the manuscript.

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A Comparative Evaluation of Potential Bioactive Properties and Phenolic Profiles of Five Mediterranean Asteraceae Species

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ABSTRACT

In recent years, plants with bioactive properties as well as nutritional value have been densely researched. Asteraceae, the most species-rich family of flowering plants, includes numerous wild species most of which are consumed as bioactive compound-rich vegetables and herbal teas. In this study, radical scavenging, antibacterial, and phytotoxic activity as well as phenolic content of some Mediterranean Asteraceae species, *Calendula arvensis, Cichorium intybus* subsp. *intybus, Glebionis coronaria, Scolymus hispanicus*, and *Tragopogon porrifolius* subsp. *longirostris,* were investigated. As a result, *C. intybus, G. coronaria,* and *S. hispanicus* extracts have higher 2,2-diphenyl-1-picril-hydrazyl radical scavenging activity than that of the others ($P<0.05$). The highest superoxide radical scavenging activity and the highest gallic acid equivalent total phenolic content were detected in *G. coronaria* extract. The highest catechin equivalent total flavonoid content was found in *C. intybus* and *S. hispanicus* extracts, respectively. High-performance liquid chromatography analyses showed that the extracts contained various levels of phenolic acids and quercetin. *C. intybus*, *S. hispanicus*, and *T. porrifolius* extracts inhibited the growth of both gram-positive bacteria: *Listeria monocytogenes* and *Staphylococcus aureus* and gram-negative bacteria: *Salmonella enterica* subp. *enterica* and *Escherichia coli*. (Minimal inhibitory concentration = 4 mg mL^{-1}). All the concentrations of gallic acid (as a positive control) and 4 g L^{-1} concentrations of *C. arvensis*, *S. hispanicus* and *T. porrifolius* extracts showed severe inhibition on garden cress (*Lepidium sativum* L.) seedlings.

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Asteraceae
Phenolic content

Akdeniz Bölgesi'nden Beş Asteraceae Türünün Biyoaktif Potansiyeli ve Fenolik Profili Üzerine Karşılaştırmalı Bir Değerlendirme

ÖZET

Besin değerinin yanısıra biyoaktif özellikleri de olan bitkiler son yıllarda oldukça yoğun araştırılmaktadır. Tür çeşitliliği bakımından çiçekli bitkilerin en zengin familyası olan Asteraceae çoğu sebze ve bitki çayı olarak tüketilen ve biyoaktif bileşiklerce zengin olan birçok yabani tür içerir. Bu çalışmada, *Calendula arvensis, Cichorium intybus* subsp. *intybus, Glebionis coronaria, Scolymus hispanicus* ve *Tragopogon porrifolius* subsp. *longirostris* türlerinin radikal süpürücü, antibakteriyel ve fitotoksik aktivitesi ile fenolik içerikleri araştırıldı. Sonuç olarak *C. intybus, G. coronaria* ve *S. hispanicus* ekstraktları diğerlerinden daha yüksek 2,2-difenil-1-pikril-hidrazil radikali süpürücü aktivite gösterdi ($P<0.05$). En yüksek süperoksit radikali süpürücü aktivite ve gallik aside eşdeğer toplam fenolik madde *G. coronaria* ekstraktında gözlendi. En yüksek kateşine eşdeğer toplam flavonoid miktarı sırasıyla *C. intybus* ve *S. hispanicus* ekstraktlarında gözlendi. Yüksek Basınçlı Sıvı Kromatografi analizleri ekstraktların çeşitli düzeylerde fenolik asitler ve kuersetin içerdiğini gösterdi. *C. intybus*, *S. hispanicus* ve *T. porrifolius* ekstraktları hem gram-pozitif (*Listeria monocytogenes* ve *Staphylococcus aureus*) hem de gram-negatif (*Salmonella enterica* subp. *enterica* ve *Escherichia coli*) bakterilerin büyümesini inhibe etti (Minimal İnhibitör

Botanik

Araştırma Makalesi

Makale Tarihçesi
Geliş Tarihi : 05.07.2022
Kabul Tarihi : 10.08.2023

Anahtar Kelimeler
Antioksidan
Antibakteriyel
Fitotoksik
Asteraceae
Fenolik içeriği

Konsantrasyon = 4 mg mL^{-1}). Gallik asidin (pozitif kontrol) bütün konsantrasyonları ile *C. arvensis*, *S. hispanicus* ve *T. porrifolius*'un 4 g L^{-1} konsantrasyonları tere (*Lepidium sativum* L.) fideleri üzerinde şiddetli inhibisyon gösterdi.

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INTRODUCTION

The last few decades have been the era that plant active metabolites not only shed light on research for new medicinal, agricultural, and industrially important active compounds but also paved the way for many functional food candidates for public health (Shaikh et al., 2016). Plant extracts have been used since ancient times in curing a wide range of diseases such as diabetes, microbial infections, and stress-related disorders, and are evaluated as sources of antioxidants (Rashed et al., 2018; Petropoulos et al., 2019; Rayan et al., 2020). Infectious diseases are leading causes of death in low-income countries, also during the course of the pandemic, COVID-19 has become a significant cause of death in many countries (WHO, 2022). The unceasing production and usage of antibiotics and the rapid spread of new infections worldwide trigger the emergence of multidrug-resistant pathogens (Rashed et al., 2018). These factors have pulsed the science environment to generate new and capable antimicrobial solutions. Return to nature/naturals is the new trend to avoid different disorders among people. The need to control disease-causing microorganisms with new antimicrobials has pushed researchers towards natural products and, over the last few years, herbal sources have emerged as potential candidates (D'Amato et al., 2018).

On the other hand, phytochemicals can be utilized for biopesticide production as a replacement for synthetic chemicals in agricultural production. The term phytotoxicity is usually used for the same meaning as the term allelopathy, as a group of interactive reactions containing both stimulatory and inhibitory influences, among plants or sometimes between higher plants and microbes (Weston & Duke, 2003). Allelopathy majorly takes place in agricultural management like crop re-establishment, crop protection, and weed control. Efforts to attain eco-friendly compounds for weed control in the agroecosystems are the new trend (Scavo et al., 2020).

There is a wide range of wild species in the Mediterranean basin which have been utilized in food and medicine throughout centuries. Asteraceae family, the most species-rich family of flowering plants, includes numerous species yet to rehabilitate and are native to the Mediterranean basin. Most of these wild species are consumed for their fresh and tender leaves in salads (Petropoulos et al., 2019).

In this study, phenolic compounds in the plant extracts which have lots of bioactive properties (Zhang et al., 2019) were considered probable radical scavengers, bacterial growth inhibitors, and phytotoxic chemicals. *Calendula arvensis* (Vaill) L., *Glebionis coronaria* (L.) Spach, *Cichorium intybus* subsp. *intybus* L., *Scolymus hispanicus* L., and *Tragopogon porrifolius* subsp. *longirostris* (Sch. Bip.) Greuter, belongs to the Asteraceae family and the last three are edible and in the same tribe (Cichorieae or Lactuceae). These taxa have some bioactive properties such as antioxidative and antimicrobial. Nevertheless, current literature on their phytotoxicity or potent herbicidal activity is very limited. This study was conducted in order to contribute to the scientific knowledge about the antioxidant, antibacterial, and phytotoxic activities of the edible or traditionally used parts of *C. arvensis, C. intybus, G. coronaria, S. hispanicus,* and *T. porrifolius* grown in the specific Mediterranean regions. As far as we know, this study reports SO RSAs of these plants, also phytotoxicity of current plant extracts on cress (*Lepidium sativum* L.) seedlings and antibacterial effects of *T. porrifolius* on an organism for the first.

MATERIALS AND METHODS

Chemicals and Reagents

Methanol (extra pure), Folin-Ciocalteu phenol reagent, dimethyl sulphoxide (DMSO), and nitroblue tetrazolium (NBT) were supplied from Merck (Germany). Aluminium chloride ($AlCl_3$), sodium nitrite ($NaNO_3$), catechin, sodium carbonate (Na_2CO_3), sodium hydroxide (NaOH), L-ascorbic acid, gallic acid and DPPH (2,2-diphenyl-1-picril hydrazyl) from Sigma-Aldrich (Sigma-Aldrich Co. St. Louis). Mueller Hinton II Broth was purchased from Oxoid (Massachusetts-USA).

Plant Material

Traditionally consumed aerial parts of the plants were collected from rocky places in *Quercus coccifera* maquis at Akdeniz University Campus (Antalya/Turkey) area (altitude 10 m) on 5 May 2019. All the plant parts but *S. hispanicus* were stems with

leaves and flowers. *S. hispanicus* was stem with leaves (Figure 1, Table 1). A part of all the plant samples were dried in a cool and shady place and used for the bioactivity assays and other parts were saved as herbarium material. Voucher specimens were coded as MPL201911 (to 15) and deposited in the Medicinal Plants Laboratory of Mehmet Akif Ersoy University. Plant names were confirmed with the sources such as Flora of Turkey and the East Aegean Islands (Davis, 1985) and Plants of the World Online Powo (2022). (Table 1).

Figure 1. Plants used in the study: *Calendula arvensis, Glebionis coronaria, Cichorium intybus* subsp. *intybus*, *Scolymus hispanicus* and *Tragopogon porrifolius* subsp. *longirostris*, from left to right.
Şekil 1. Çalışmada kullanılan bitkiler: Soldan sağa, Calendula arvensis, Glebionis coronaria, Cichorium intybus subsp. intybus, Scolymus hispanicus ve Tragopogon porrifolius subsp. longirostris.

Table 1. Plants used in the study
Çizelge 1. Çalışmada kullanılan bitkiler

Plant *Bitki*	Abbreviation used in the study *Çalışmada kullanılan kısaltması*	English name *İngilizce adı*	Local name *Yerel adı*
Calendula arvensis (Vaill) L.	CA	Field marigold	Aynısafâ
Cichorium intybus subsp. *intybus* L.	CI	Chicory	Hindibâ
Glebionis coronaria (Syn. *Chrysanthemum coronarium*) (L.) Spach	GC	Garland	Sarı papatya
Scolymus hispanicus L.	SH	Golden thistle	Şevketibostan
Tragopogon porrifolius subsp. *longirostris* (Sch. Bip.) Greuter	TL	Purple salsify	Yemlik

Extraction

Air-dried and powdered plant parts were extracted three times with methanol at 40°C by using a magnetic stirrer, for three days. Afterward, the obtained methanolic extracts were filtered and evaporated to obtain crude extracts. Extract yields were calculated by the following formula:

$$Yield(\%) = \frac{\text{weight of crude extract}}{\text{weight of plant powder}} \times 100$$

Distilled water was applied to crude extracts to dissolve. Equal volumes of petroleum ether were used for partitioning each to remove chlorophylls and other lipophilic compounds. Finally, the remaining aqueous extracts were lyophilized (Harput et al., 2012). Water extracts for early seedling growth bioassay were prepared from dried and powdered plants by mixing continuously at room temperature (22°C) for 24 h. Extracts were filtered (Whatman No.1) and used as 0.5, 2, and 4 g dry matter L^{-1} concentrations for the phytotoxicity assay. Methanol extracts were prepared for HPLC analysis, DPPH and SO radical scavenging activity (RSA) tests, total flavonoid and total phenolic contents, and antibacterial activity.

Radical Scavenging Activity Assays

DPPH RSA potentials of the methanolic extracts of the plants were detected according to the method of Blois (1958). Each methanol sample or control of 200 µL was added DPPH, a stable free radical, (50 µL, 1 mM) at certain concentrations and then mixed well. The remaining DPPH had an absorbance value of 517 nm after 30 minutes. The RSA of a negative control well that contained only DPPH and solvent was taken as a comparison to the RSA of the extract. Ascorbic acid was the positive control. All assays were conducted in triplicates. RSA was asserted as percent inhibition and calculated by the following equation:

$$Inhibition\ percentage = \frac{\text{Abs (control)} - \text{Abs (sample)}}{\text{Abs (control)}} \times 100 \quad (1)$$

Inhibitory concentration (IC_{50}) values were also calculated.

Superoxide (SO) RSA was detected via the method of Kunchandy & Rao (1990). In sum, a non-enzymatic system was established to generate superoxide radical. The mixture which included 10 µL of nitroblue tetrazolium (NBT) (1 mg mL^{-1} solution in dimethyl sulphoxide, DMSO) and 30 µL of each sample was dissolved in DMSO. 100 µL of alkaline DMSO (1 mL of which contained 5 mM NaOH in 0.1 mL of water) was put to make a final volume of 140 µL and the absorbance was read at 560 nm. Ascorbic acid was the positive control. All assays were done in triplicates. RSA was asserted as percent inhibition and calculated by the following equation:

$$Inhibition\ percentage = \frac{\text{Abs (sample)} - \text{Abs (control)}}{\text{Abs (sample)}} \times 100 \quad (2)$$

Inhibitory concentration (IC_{50}) values were also calculated.

Antibacterial Activity

The Minimum Inhibitory Concentration (MIC) method using a 96-well plate by Klančnik et al., (2010) was applied to examine antibacterial activities of the extracts on the following gram (+) bacteria: *Listeria monocytogenes* (ATCC 02028) and *Staphylococcus aureus* (ATCC 25923) along with two gram (–) bacteria: *Salmonella enterica* subp. *enterica* (ATCC 700408) and *Escherichia coli* (ATCC 35150). The concentrations that entirely inhibited bacterial growth (MIC) were determined by spectrophotometric absorbance read at 600 nm in a microplate reader to detect microbial growth. Bacterial strain starters were grown overnight, and shaken at 37°C. The inoculum density was regulated to catch 0.5 on the McFarland scale with an interval of 0.080-0.100 optical density at 600 nm. 20 mg mL^{-1} of extract was prepared with DMSO and each well was added 160 µL extract which is serially diluted with Mueller Hinton II Broth. The highest final concentration of the extract was 4 mg mL^{-1}. MIC values were determined via incubation of the bacteria in 96-well microplates for 24 and 48 h at 37°C.

Phytotoxicity Assay: Early Seedling Growth Bioassay

Tests were conducted using 70 mm glass Petri dishes and two layers of filter paper. All materials but seeds were sterilized. Undamaged and almost identically sized cress seeds, (Paşa Seed Company, Balıkesir/Turkey, Cultivar name: Zeybek©) a crop that is sometimes considered a weed (Kadioğlu & Yanar, 2004), were pregerminated in distilled water for 24 h at 25°C at an incubator. 25 pregerminated seeds were laid on the filter paper in each dish which contained 4 mL of filter-sterilized plant extract and gallic acid at 0.5, 2, and 4 g L^{-1} concentrations. Each condition was tested in triplicates. All dishes were incubated in the dark, at 25°C, for 72 h. Blank (distilled water) experiments accompanied every treatment. Seeds were treated with one of the phenolic compounds, responsible for the phytotoxic activity, with gallic acid, also, in this experiment. Seedling (root and shoot) lengths were measured by using a ruler. In each case, the mean number of germinated seeds (N) and mean lengths of roots and shoots (L) were taken.

$$\text{Relative seed germination: } RSG = \frac{N}{\text{Nb}} \times 100 \quad (3)$$

where Nb is the mean number of germinated seeds in the blank.

$$\text{Relative root and shoot growth: } RRG = \frac{L}{\text{Lb}} \times 100 \quad (4)$$

where Lb is the mean length of the seedlings in blank and percent germination indices are calculated as follows:

$$GI = \frac{RSG \times RRG}{100} \quad (5)$$

Phytotoxicity ratings of tested extracts were determined according to the germination index scale proposed by Trautmann & Krasny, (1998) as; GI<40%: severe inhibition, 40%<GI<60%: strong inhibition, 60%<GI<80%: mild inhibition, GI>80%: no inhibition (Pinho et al., 2017).

Total Phenolic and Total Flavonoid Contents

The method of Singleton & Rossi, (1965), and the method of Zhishen et al., (1999)— with minor modifications— were used to detect total phenolic content (TPC) and total flavonoid content (TFC) of the extracts, respectively. For TPC, 10 µL of sample or standard (10-500 µg mL^{-1} gallic acid) plus 150 µL of diluted Folin-Ciocalteu reagent (1:4 reagent/water) and 50 µL of saturated sodium carbonate (7.5%) were added to each well of a 96-well plate and incubated for 2 h at room temperature. The absorbance was read at 725 nm. TPC was expressed as gallic acid equivalent (GAE). For TFC, 10 µL 5% sodium nitrite was added to the 10 µL sample or standard. After 5 min., 10 µL 10% aluminum chloride, 150 µL 1 M sodium hydroxide, and 50 µL water were added. The plate was mixed well. Then the absorbance was read at 510 nm. Methanol was used as the control. TFC was expressed as catechin equivalent (2-250 µg CE mL^{-1}).

HPLC Analysis

HPLC analysis of phenolic compounds (benzoic acid, caffeic acid, ferulic acid, p-coumaric acid, vanillic acid, and quercetin) were done at Scientific and Technology Application and Research Center of Mehmet Akif Ersoy University. System: Shimadzu Prominence, CBM: 20ACBM, Detector: DAD (SPD-M20A), Pomp: LC20 AT, Column Oven: CTO-10ASVp, Autosampler: SIL 20ACHT, Computer Programme: LC Solution, Mobile Phase A: 3% Formic acid, Mobile Phase B: Methanol. The elution gradient was applied at a flow rate of 1 mL min^{-1}. was: 95%A/5%B for 3 min., 80%A/20%B for 2 min., 60%A/40%B for 10 min.,

50%A/50%B for 10 min., 100%B for 10 min. until the end of the run. 10 µL methanol samples were injected into the column (Caponio et al., 1999). Benzoic acid LOD: 0.03 ppm, 270 nm, RT: 34 min., caffeic acid LOD: 0.01 ppm, 280 nm, RT: 22.7 min., p-coumaric acid LOD: 0.01 ppm, 320 nm, RT: 26.1 min., ferulic acid LOD: 0.01 ppm, 320 nm, RT: 30.1 min., quercetin LOD: 0.01 ppm, 360 nm, RT: 70.4 min., vanillic acid LOD: 0.11 ppm, 320 nm, RT: 19.2 min.

Statistical Analysis

One-way analysis of variance followed by Tukey HSD test was used to evaluate differences among groups in DPPH and SO RSA, TPC, and TFC tests. Non-normal data which also weren't suitable for transformation were treated with the Kruskal-Wallis test. The significance level was set at $P<0.05$. IBM SPSS Statistics software version 22 was used for statistical analysis.

RESULTS AND DISCUSSION

Radical Scavenging Activity and Phenolic Contents

Radical scavenging activities of the plant extracts (yields as %; 18.33, 10.25, 31.51, 16.28 and 17.10 for CA, GC, CI, SH and TL, respectively) were evaluated by DPPH and SO RSA. DPPH radical is a rare type of stable organic nitrogen radical that has a deep purple color. DPPH RSA assay is based on the reducing ability of antioxidants towards DPPH. Antioxidant ability can be seen in the decrease of its absorbance (Brand-Williams et al., 1995). On the other hand, the nitroblue tetrazolium (NBT) assay tests whether the extracts scavenge SO anions. Alkaline DMSO, used as an SO generating system, reacts with NBT to give colored diformazan. Following the DPPH RSA of ascorbic acid which was the positive control, activities of SH, GC, and CI extracts (IC_{50} values; 124.28, 136.87, and 140.20 µg mL^{-1}, respectively) were higher than the others ($P<0.05$). The lowest IC_{50} means the highest RSA. It shows the extract is effective in lower concentrations (a concentration in which the extract can scavenge 50% of the radical). The highest SO RSA was detected in GC extract. The IC_{50} values of DPPH and SO RSA were shown in Table 2. The highest gallic acid equivalent TPC was determined in GC extract (114.25 µg GAE mL^{-1}) followed by CI and TL extracts (82.92 and 76.51 GAE mL^{-1}, respectively).

Table 2. DPPH and SO radical scavenging activity (IC_{50} values) and phenolic profiles of the plant extracts
Çizelge 2. Bitki ekstraktlarının DPPH ve SO radikali süpürücü aktiviteleri (IC_{50} değerleri) ve fenolik profili

	Antioxidant activity[m] *Antioksidan aktivite[m]*				HPLC analysis (µg mL^{-1}) *YBSK analizi (µg mL^{-1})*					
PE BE	DPPH RSA (µg mL^{-1})	SO RSA (µg mL^{-1})	TPC (µg GAE mL^{-1}) *TFİ(µg GAE mL^{-1})*	TFC (µgCE mL^{-1}) *TFLİ (µg KE mL^{-1})*	Vanillic acid	Caffeic acid	p-Coumaric acid	Ferulic acid	Benzoic acid	Quercetin
CA	274.97±3.94a	59.60±7.65abc	66.97±2.44c	22.16±5.01b	2.221	27.00	0.016	0.058	21.30	4.044
GC	136.85±2.41c	36.91±13.10a	114.25±8.24a	30.50±4.66b	0.314	3.787	0.467	0.020	0.49	0.515
CI	140.23±4.66c	70.94±2.13bc	82.92±4.88b	54.16±8.47a	2.138	9.238	0.004	0.107	0.22	10.08
SH	124.28±0.06c	63.46±5.05abc	30.97±0.79d	48.50±8.78a	0.914	11.36	0.372	0.011	nd	1.237
TL	182.17±14.72b	90.35±25.01c	76.51±4.98b	27.50±3.43b	0.702	4.950	0.099	0.059	0.006	7.142
AA	15.89±2.10d	49.08±5.30ab								

m: Means of three replicates±SD, different letters show significant differences ($P<0.05$), PE: Plant extract, RSA: radical scavenging activity, TPC: total phenolic content, TFC: Total flavonoid content, GAE: gallic acid equivalent, CE: catechin equivalent, nd: not determined, AA:Ascorbic acid

m: Üç tekrarın ortalaması±SS, farklı harfler istatistiksel olarak önemli farklılıkları gösterir ($P<0.05$), BE: Bitki ekstraktı, RSA: radikal süpürücü aktivite, TFİ: toplam fenolik içeriği, TFLİ: toplam flavonoid içeriği, GAE: gallik aside eşdeğer, KE: kateşine eşdeğer, nd: belirlenemedi, AA: askorbik asit

The highest catechin equivalent TFC was found in CI and SH extracts, (54.16 and 48.50 µg CE mL^{-1}, respectively) (Table 2). The highest quercetin content was also found besides vanillic and ferulic acids in CI extract. Higher both TFC (catechin equivalent) and quercetin show that *C. intybus* is rich in flavonoids. Moreover, flavonoids are chemosystematic markers in the Cichorieae tribe (Sareedenchai & Zidorn, 2010). *C. intybus* contains several important metabolites such as 3-o-caffeoylquinic acid (Shilpa and Lakshmi 2019), hydroxycinnamic acids, including chlorogenic and cichoric acid (Sinkovič et al., 2015), and flavonoids (Dalar & Konczak, 2014; Papetti et al., 2017) that are responsible for bioactivity.

Antibacterial Activity

CI, SH, and TL extracts inhibited growth of both gram (-) (*E. coli* and *S. enterica*) and gram (+) (*S. aureus* and *L. monocytogenes*) bacteria (Minimal inhibitory concentration (MIC) = 4 mg mL^{-1}) in this study (Figures 2, 3 and 4). CA and GC extracts were weak to inhibit bacterial growth in 4 mg mL^{-1} concentrations, although they are rich in phenolic substances (Table 2). There are some reports on the antibacterial activity of both methanol and other solvent extracts such as chloroform or petroleum ether of *C. arvensis* and *G. coronaria*. For example, Tosun et al., (2012) found that essential oil and methanolic extract of *C. arvensis* showed moderate antibacterial activities against *S. aureus*. It contains sesquiterpene and flavonol glycosides, triterpene saponins and alcohols, flavonoids, carotenoids, xanthophylls, phenolic acids and is antibacterial on *Bacillus subtilis*, *E. coli*, and *S. aureus* (Kemper, 1999). The minimum concentration of inhibition for leaf extract of *C. arvensis* was determined as 2 µg mL^{-1} in chloroform against *K.*

pneumoniae and *E. coli* and in petroleum ether against *E.* coli (Jamal et al., 2014). *G. coronaria* has also an inhibitory effect on bacterial growth. For example, non-polar extract of *G. coronaria* has an antibacterial effect on *Staphylococcus mutans* at IC_{50} of less than 20 ppm concentration, besides a moderate value of DPPH RSA (EC_{50}=587 ppm) (Rayan et al., 2020). Antibacterial effects of *G. coronaria* essential oil were demonstrated against the gram-positive strains while it failed to inhibit gram-negative bacterial growth (Bardaweel et al., 2015). Caffeoylquinic acids are reported as the major component of *G.* coronaria (Lai et al., 2007; Wan et al., 2017).

As seen in the literature, *C. arvensis*, *G. coronaria*, *C. intybus* and *S. hispanicus* have certain DPPH RSAs. Besides its RSA activity, *C. intybus* has antibacterial activity on both our tested gram (+) and gram (-) bacteria, as well. There are some studies exhibiting the antibacterial activity of chicory. Its root and aerial extracts showed antibacterial activity with the disc diffusion method on both gram (-) and gram (+) bacteria. The ethyl acetate extract was the most active (Petrovic et al., 2004). Shaikh et al., (2016) determined potential activities of *C. intybus* on *Pseudomonas aeruginosa*, *B. subtilis, S. aureus, S. epidermidis*. Its seed extracts showed to have MIC values below 0.1 mg mL^{-1} against the pathogenic microorganisms tested, including *S. aureus*, *P. aeruginosa*, and *E.* coli. Ethyl acetate and ethanol extract were found considerably responsive to *S. aureus* and *P. aeruginosa*. In another study, *C. intybus* MIC was 16 to 256 µg mL^{-1} against *E. coli* CCM 3988, *S. enterica* CCM 3807, *Yersinia enterocolitica* CCM 5671, and three gram (+)

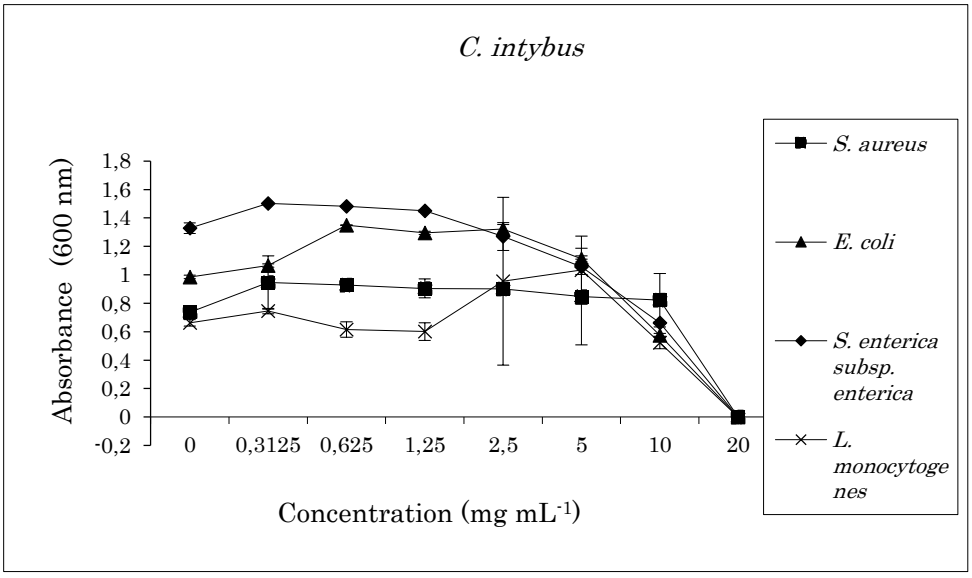

Figure 2. MIC graphic of *Cichorium intybus* subsp. *intybus* extract.
Şekil 2. Cichorium intybus subsp. intybus ekstraktının MİK grafiği

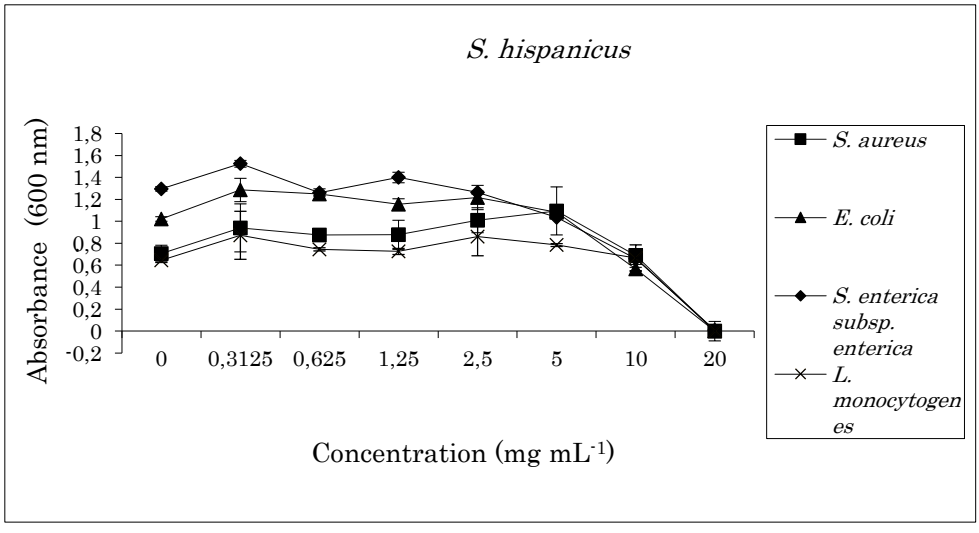

Figure 3. MIC graphic of *Scolymus hispanicus* extract
Şekil 3. Scolymus hispanicus ekstraktının MİK grafiği

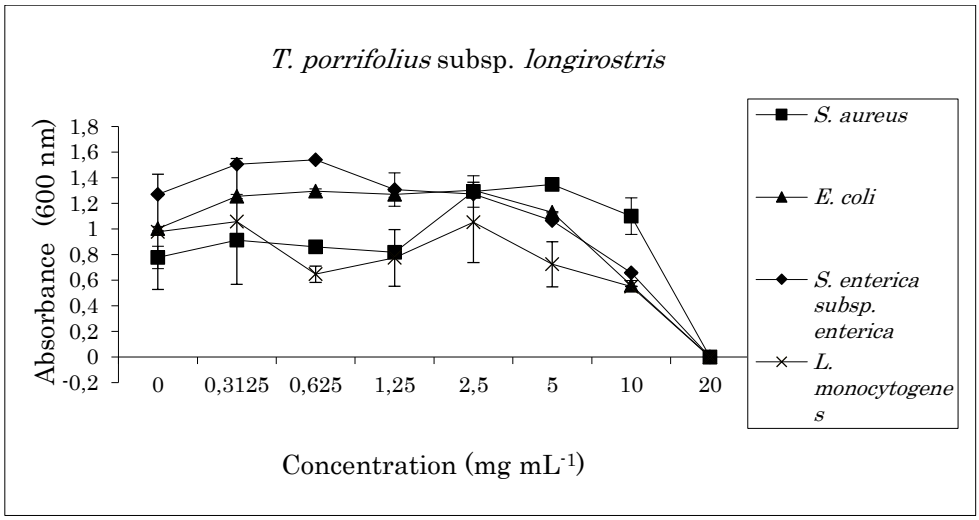

Figure 4. MIC graphic of *Tragopogon porrifolius* subsp. *longirostris* extract
Şekil 4. Tragopogon porrifolius subsp. longirostris ekstraktının MİK grafiği

bacteria: *Bacillus thuringiensis* CCM 19, *L. monocytogenes* CCM 4699, *S. aureus* CCM 2461 (Rashed et al., 2018). The whole plant of *C. intybus* is active against *Enterococcus faecalis*, with MIC values of 13.01 µg mL^{-1} and 6.50 µg mL^{-1}, respectively (Gür et al., 2017). Antifungal activities of *C. intybus* have also been reported (Abbas et al., 2014; Rehman et al., 2014; Shaikh et al., 2016; Gür et al., 2017). HPLC/DAD/MS analysis identified some hydroxycinnamic acids and flavonoids (quercetin, kaempferol, luteolin, and apigenin glycosides) in chicory (Heimler et al., 2009) and chicoric acid is its main component (Heimler et al., 2009; Tardugno et al., 2018). Although folkloric use of chicory has a rich historical background just a few of its constituents have been studied in terms of pharmacological potential. Data on the toxicity of *C. intybus* is currently limited (Mahadeva Rao et al., 2020).

One of our tested plants that has antibacterial activity is *S. hispanicus.* Marmouzi et al., (2017) reported that its roots showed the most promising antibacterial activity on *E. coli* CIP 53126 at a MIC value of 1.56 mg mL^{-1}. Furthermore, flowers exhibited the highest activity on *S. aureus* CIP 483 (3.12 mg mL^{-1}) and *B. subtilis* CIP 5262 (1.56 mg mL^{-1}) while its leaves displayed better activity (MIC=1.56 mg mL^{-1}) against *S. enterica* CIP 8039 and *P. aeruginosa* CIP 82118. On the other hand, lipophilic metabolites (at 100 µM concentration) (Kandil et al., 2020) of pollen extracts of *S. hispanicus* have DPPH RSA as IC_{50}=0.78 mg mL^{-1} (Bakour et al., 2020).

Tested plants which belong to the tribe Cichorieae, have antibacterial effects with MIC at 4 mg mL^{-1} concentration, all of our tested plants have RSA and phenolic content at different levels. All the plants we studied were collected from the same area and at the almost same time on the same day. Most of the tested plants are edible both in Turkey and European countries and have some medicinal properties (Lai et al., 2007; Marmouzi et al., 2017; Tardugno et al., 2018; Abdalla & Zidorn, 2020). In studies conducted on medicinal plants, it's pointed out that the geographical origin of the variety and the harvest season have influences on the chemical composition (Dehkordi et al., 2010). On the other hand, different functional groups of the compounds may cause different bioactive effects.

Phytotoxic Activity

The phytotoxic effects of the extracts on seedling growth of cress seeds were expressed by germination indices. Germination indices as a percentage of cress seeds treated with plant extracts are given in Table 3. Kruskal Wallis test showed that germination indices of plant extracts are significantly different ($P<0.05$). According to the germination index scale of Trautmann & Krasny, (1998) all the concentrations of gallic acid and 4 g L^{-1} concentrations of CA, SH, and, TL extracts showed severe inhibition. Strong inhibition was observed in the 4 g L^{-1} concentrations of CI and GC extracts and 2 g L^{-1} concentrations of CI, SH, and TL. 2 g L^{-1} concentrations of CA and GC extracts and 0.5 g L^{-1} concentrations of CA and SH extracts showed mild inhibition while 0.5 g L^{-1} concentrations of CI, GC, and TL extracts showed no inhibition (Table 3). The germination index can be greater than 100%, in cases that the extract enhances the germination and/or the radicle growth rather than impairing it (Trautmann & Krasny, 1998; Begum et al., 2019). Seedling growth enhanced by GC extract at 0.5 g L^{-1} concentration was observed. Similarly, Chon et al., (2003) found that the root length of alfalfa was scaled up by 13–33% when treated with extracts of *Bidens frondosa*, *Breea segeta, Chrysanthemum indicum,* and *Youngia sonchifolia*, at concentrations below 20 g dry matter L^{-1}.

Table 3. Germination indices of cress seeds treated with the plant extracts.
Çizelge 3. Bitki ekstraktları uygulanan tere tohumlarının çimlenme indisleri.

Plant extract	Germination Index (%)* *Çimlenme İndisi (%)**		
Bitki ekstraktı	0.5 g L^{-1}	2 g L^{-1}	4 g L^{-1}
CA	76.42±3.75efgh	60.04±3.40cdef	6.45±1.05ab
GC	111.38±6.04h	60.50±3.58cdefg	40.38±3.20c
CI	87.52±4.40gh	49.89±3.52cde	43.70±2.70c
SH	64.09±3.54defg	45.76±2.69cde	9.14±1.27b
TL	92.65±4.21fgh	43.69±2.28cd	18.16±2.78b
GA	38.10±3.00c	9.63±1.30b	2.69±0.20a

*: Means±SE, Different letters indicates significant differences (P<0.05) from Kruskal-Wallis test Stepwise Step-down multiple comparisons.
GA:Gallic acid
**:ortalama±SH, Farklı harfler Kruskal-Wallis, ardışık basamaklı çoklu karşılaştırma testine göre istatistiksel olarak önemli farklılıkları gösterir (P<0.05), GA: gallik asit*

HPLC analysis (Table 2) showed that well ahead the highest caffeic, vanillic and benzoic acids were found in CA extract which has shown the most severe inhibition following gallic acid at 4 g L^{-1} concentration. This suggests causative phytotoxic chemicals could be caffeic, vanillic, and benzoic acids in this plant. Activities of caffeic acid (Batish et al., 2008; Li et al., 2017), and benzoic acid (Zhu et al., 2017) are known. Some studies report the phytotoxicity of *C. arvensis* extracts. For example, Ullah et al., (2012) found that the methanolic extract of *C. arvensis* has toxic potential against *Lemna minor* at 1000 µg mL^{-1} (1 mg mL^{-1}). Not only phenolic compounds but also essential oils can cause phytotoxicity. For example, *C. intybus* oil showed significant phytotoxic activity (61.12% inhibition against *Lemna minor* at a high dose such as 1000 µg mL^{-1}) (Shah et al., 2012). *C. arvensis* extract showed 20%, 50%, and 60% inhibitions on maize, sunflower, and wheat seeds respectively (Khan et al., 2012). Methanol extracts of *C. arvensis* flowers showed 19% DPPH radical inhibition at 250 µg mL^{-1} concentration (Ercetin et al., 2012). On the other hand, about 45% DPPH radical inhibition at 250 µg mL^{-1} concentration of CA extract was found in our study. Possible reasons for this difference can be explained by localities, parts of the plants used, and methodological details. Faustino et al., (2018) identified *C. arvensis* as smart food or natural medicine because of its phenolic acids, flavonoids, and saponins in its aerial parts, detected by UHPLC-MS/MS.

The highest p-coumaric acid was found in GC and SH extracts, respectively. SH extract showed severe inhibition like CA extract at the highest concentration (4 g L^{-1}). Catechin equivalent TFC and the highest DPPH RSA were also found in SH extract (Table 2). *S. hispanicus* is rich in phenolics and flavonoids and has antioxidant (Tabaraki et al., 2013), and phytotoxic (Qasem, 2017) activity. It showed severe toxicity on cress seeds at 4 g L^{-1} concentration in our study. No data was found on the phytotoxic activity of *T. longirostris* despite containing flavonoids, terpenoids, bibenzyl derivatives, benzyl phtalides, stilbenes, dihydro isocoumarin derivatives, phenylmethane derivatives, hydroxy phenylacetic acid derivatives, esters of phenylpropanoic acids, phenylpropane derivatives, spermine derivatives, and coumarin derivatives (Abdalla & Zidorn, 2020). Among the plants we focus on, exhibiting inhibition, there are reports about their phytotoxic impacts on *Lemna minor* (Ullah et al., 2012) or some other seeds or seedlings of plants like sunflower (Ercetin et al., 2012). However, no study has been found about the inhibitory effects of these plant extracts on cress seedlings.

General knowledge about secondary metabolites from commercially interesting Cichorieae genera, including *C. intybus, S. hispanicus,* and *T. porrifolius* taxa, is satisfying. However, most of the other genera of this tribe had not been studied phytochemically at all according to Zidorn (2008). A total of 135 various flavonoid compounds were detected in 354 taxa of 299 species, including many cultivars of common vegetables like chicory and lettuce of the Cichorieae (Lactuceae) tribe (Sareedenchai & Zidorn, 2010). The highest TFC was detected in CI and SH extracts (Table 2), both belonging to the tribe Cichorieae. DPPH RSA of GC extract was not significantly different from that of CI and SH. TPC of GC was also statistically higher than the others (P<0.05). GC extract positively affected the seedling growth of cress seeds contrarily to the others at 0.5 and 2 g L^{-1} concentrations. Probably because of methodological differences or because different compounds are responsible for the phytotoxic activity. *G. coronaria* was reported to have phytotoxic activity, in the literature. Its extract inhibited root and shoot elongations of *Echinochloa crus-galli*, one of the worst weeds, 21%, and 6.3%, respectively at 1000 µg mL^{-1} concentration (Abdelgaleil et al., 2020), and it has a high DPPH RSA and allelopathic activity on the seed germination and seedling growth of two annual weeds (*Sinapis arvensis* and *Phalaris canariensis*) and two crops (*Triticum durum* and *Zea mays*) (Hosni et al., 2013). Caffeoylquinic acids are the major components

of *G. coronaria* (Wan et al., 2017). *C. intybus* root extracts have also a potential for use as bioherbicides (Wang et al., 2011). *S. hispanicus* has phytotoxic activity, too (Zidorn, 2008).

Chon et al., (2005) informed of the most prominent phytotoxic compounds via HPLC as coumarin, *trans*-cinnamic acid, *o*-coumaric acid, *p*-coumaric acid, and chlorogenic acid. Pinho et al., (2017) states that increasing -OH and -OCH_3 groups in the molecule seem to reduce phytotoxicity. Lipophilic phenolics appear to be the most causative chemicals for phytotoxicity. They tested the phytotoxicity of gallic acid, protocatechuic acid, cinnamic acid, syringic acid, 3,4,5-trimethoxybenzoic acid, 4-hydroxybenzoic acid, vanillic acid, p-coumaric acid, caffeic acid, veratric acid, and phenol and they found that cinnamic acid is the most phytotoxic because of its hydrophobicity. Further possible phytotoxic properties of hydrophobic fractions of these plant extracts should be investigated. Phenolic acids are often assumed as phytochemicals in the literature and maybe they are the most widely investigated compound type among the proposed ones (Abdelgaleil et al., 2020).

CONCLUSION

Asteraceae species we studied exhibit phytotoxicity changing from none to severe and including phenolics at various levels. We concluded that these plants have different bioactive properties depending on their phenolic variation. Furthermore, this study is the first to report in the literature on the SO RSA of phytotoxicity of *T. porrifolius* as far as is known. Assaying the bioactivity of polar and apolar fractions of plant extracts will amplify results as well as assaying plant parts separately. The phytotoxicity of these extracts at different concentrations on different weed seeds should be researched as well. Field trials will also contribute to the topic besides laboratory experiments. Briefly, the plants we studied, must be taken into account since they not only bear the potential of phytotoxicity but also are antibacterial agents.

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Contribution of the Authors as Summary

Authors declares the contribution of the authors is equal.

Statement of Conflict of Interest

Authors have declared no conflict of interest.

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Quantification of Some Phenolic Compounds in *Consolida thirkeana* (Boiss.) Bornm. by HPLC and Validation of Method

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ABSTRACT
Consolida species have traditional uses in the treatment of various diseases, especially skin diseases. There is also traditional use of some *Consolida* species in Turkey. Phenolic compounds have significant pharmacological effects, therefore it is important to know their amount in plants. *Consolida thirkeana* is endemic to Turkey and known as "boz mahmuz" and no study had conducted in terms of phenolic compounds. Therefore, some phenolic amount, which has been done for the first time for *C. thikeana*, was analyzed. In this study, *C. thirkeana* was analyzed quantitatively for caffeic acid, chlorogenic acid, hyperosit, and rutin by using HPLC and the method was validated (linearity, precision, accuracy, recovery, limits of detection (LOD), and limits of quantification (LOQ)). While chlorogenic acid (0.098%), caffeic acid (0.107%), rutin (0.078%), and hyperoside (0.134%) were detected in the aerial part, only rutin (0.007%) was detected in the root. As a result of this study, this endemic species was evaluated in terms of some phenolic compounds. It is thought that phenolic compounds can be determined on other *Consolida* species with this method.

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C*onsolida thirkeana* (Boiss.) Bornm.'daki Bazı Fenolik Bileşiklerin YPSK ile Miktarı Tayini ve Yöntemin Validasyonu

ÖZET
Consolida türleri başta deri hastalıkları olmak üzere çeşitli hastalıkların tedavisinde geleneksel kullanıma sahiptir. Türkiye'de de bazı *Consolida* türlerinin geleneksel kullanımı mevcuttur. Fenolik bileşiklerin önemli farmakolojik etkileri bulunmaktadır, bu nedenle bitkilerde miktarlarının bilinmesi önemli bir yere sahiptir. *Consolida thirkeana*, Türkiye'de "boz mahmuz" olarak bilinen endemik bir türdür ve fenolik bileşikler açısından herhangi bir çalışmaya rastlanmamıştır. Bu nedenle *C. thikeana* için bazı fenolik bileşiklerin miktar tayini yapılmıştır. Bu çalışmada, *C. thirkeana*, YPSK kullanılarak kafeik asit, klorojenik asit, hiperosit ve rutin açısından kantitatif olarak analiz edilmiş ve yöntemin validasyonu (doğrusallık, kesinlik, doğruluk, geri kazanım, tespit limitleri ve ölçüm limitleri) gerçekleştirilmiştir. Toprak üstü kısımlarında, klorojenik asit (%0,098), kafeik asit (%0,107), rutin (%0,078) ve hiperosit (%0,134) tespit edilirken, toprak altı kısmı sadece rutin (%0,007) tespit edilmiştir. Bu çalışma sonucunda bu endemik tür bazı fenolik bileşikler açısından değerlendirilmiştir. Bu yöntem kullanılarak diğer *Consolida* türleri üzerinde fenolik bileşiklerin tayininin yapılabileceği düşünülmektedir.

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INTRODUCTION

Consolida (DC.) S.F. Gray is a genus with approximately 59 species found in Ranunculaceae family, and distributed in Europe, northern Africa, and

western Asia. Anatolia, which is an important distribution area for *Consolida*, is also considered as the center of diversity in the Mediterranean (Munz, 1967; Ertuğrul et al., 2010; Jabbour and Renner, 2011; Yin et al., 2020). The general classification of *Consolida* was previously included in *Delphinium* L., then recognized as a separate genus (Suzgec et al., 2009; Ertuğrul et al., 2010; Jabbour & Renner, 2011; Pakravan et al., 2018; Yin et al., 2020). *Consolida thirkeana* (Boiss.) Bornm. is Turkish endemic species and called "boz mahmuz" (Güner et al., 2012; Hürkul, 2021; Tok & Yayla, 2022). *Delphinium thirkeanum* Boiss. is accepted as a synonym (Güner et al., 2012; Hürkul, 2021). Laciniate linear leaves, pale lilac flowers, and sessile follicles are characteristic of *C. thirkeana* (Güner et al., 2012; Hürkul, 2021).

Consolida species have been used for hundreds of years in the treatment of various diseases such as traumatic injury, rheumatism, sciatica, stomach-ache, intestinal worms, insomnia, lack of appetite, scabies, and other skin diseases. In Turkey, *Consolida* species are also used for body lice (Ulubelen et al., 1996; Baytop, 1999; Bitiş et al., 2006; Kostic et al., 2013; Yin et al., 2020). In the studies on the chemical components of *Consolida* species, 143 different compounds (alkaloids, flavonoids, and phenolic compounds) have been isolated. It has many biological activities because of the compounds it contains (Şener et al., 2007; Suzgec et al., 2009; Mericli et al., 2012; Rocchetti et al., 2020). It is known that flavonoid glycosides isolated from *Consolida* species have cytotoxic, anti-tyrosinase, anti-leishmaniasis, and anti-trypanosomatid activities (Díaz et al., 2008; Marin et al., 2009; Marín et al., 2017; Zengin et al., 2019).

Bioactive compounds such as flavonoids, and phenolic compounds are secondary metabolites produced under stress conditions. These phytochemical contents of the plants may change according to various stress conditions, climatic conditions, harvesting time of the plants and parts of the plant (Van Vuuren et al., 2007; Çiçek Polat et al., 2019; Kubes et al., 2018; Ouerfelli et al., 2021). One of the most important groups of bioactive compounds is phenolic compounds. These compounds have important pharmacological effects. Studies have found that phenolic compounds, such as chlorogenic acid, caffeic acid, rutin, and hyperoside, have significant antioxidant, anti-inflammatory, anticancer, and antimicrobial effects (Magnani et al., 2014; Gullón et al., 2017; Raza et al., 2017; Naveed et al., 2018; Birková et al., 2020; Bender & Atalay, 2021; Satari et al., 2021; Wang et al., 2021; Şeker Karatoprak et al., 2022).

High performance liquid chromatography (HPLC) is one of the most useful and easy methods used for the analysis of active compounds from plant samples. In HPLC analyses, it is important to find the appropriate solvent system for chromatographic separation of analytes. Therefore, validation of the method used for analysis is also important (Mendoza et al., 2011; Çiçek Polat & Coskun, 2016; Kendir et al., 2021). In the aim of this study, using high performance liquid chromatography, methanolic extracts of aerial part, and root of *C. thirkeana* were analysed quantitatively for caffeic acid, chlorogenic acid, hyperoside, and rutin. The reason why these four phenolic compounds were chosen for quantification was that they have proven important biological activities. Separate extracts were prepared to determine the compound profile in the aerial part and root. The linearity, precision, accuracy, recovery, limits of detection (LOD), and limits of quantification (LOQ) of the method were displayed thus demonstrating validation procedure.

MATERIALS and METHODS

Plant material

Specimens of *C. thirkeana* were collected at Ayaş, Ankara, Turkey, during the flowering period (Date:12.07.2020). The voucher sample was deposited in Herbarium of Ankara University, Faculty of Pharmacy (No: AEF 30483). (AEF: Ankara Üniversitesi Eczacılık Fakültesi Herbaryumu). Other samples collected from the same habitat on the same day were reserved for extract preparation.

Sample preparation

The aerial parts were separated from roots, and they were separately air dried in the shade. Methanol was used in the extraction process. Dried aerial parts and roots were separately powdered and extracted with methanol (Sigma-Aldrich) (24 h). Finally, the samples were extracted using an ultrasonic bath (25°C, 60 min.) (ISOLAB 621.05.010). After filtered, the extracts were concentrated with an evaporator (Heidolph WB2000) (Acıkara et al. 2019). Dry extract was dissolved in methanol (4 mg mL^{-1}). The samples prepared for analysis were stored in the refrigerator (+4°C) during the analysis.

High performance liquid chromatography (HPLC) analysis

For HPLC analysis, a liquid chromatographic system device (Agilent 1100 Series) (automatic injector, pump, thermostated column, and DAD) was used. Phenolic compounds (caffeic acid, chlorogenic acid, hyperoside, and rutin) were quantified in HPLC using a Waters Spherisorb C18 column (25 cm × 4.6 mm, 5µm) maintained at 40 °C. The mobile phase consisted of 0.01% formic acid (A) and acetonitrile (B) delivered at a flow rate of 1 mL/min. Detection of all samples was performed at a wavelength of 254 nm.

Method validation

The method was validated (ICH 2005; Çiçek Polat &

Coskun, 2016). Each compound's stock reference solutions (caffeic acid, chlorogenic acid, hyperoside, and rutin) were made by dissolving 1 mg in 2 mL methanol (500 µg mL^{-1}). For the calibration curve, different concentrations of reference solution were injected in triplicate. Carrying on intra-day and inter-day variation, the precision of method was carried out, and differences were expressed by relative standard deviation (RSD). LOD is signal/noise value is 3:1, while LOQ is signal/noise value is 10:1. For LOD and LOQ, 10 injections of standards were made and averaged. For the recovery assay, 3 different known concentrations of standards were spiked into the sample solution. The mixtures were examined using the same method that was used to analyse the samples for standards.

Statistical analysis

All analyses were executed in triplicates and the mean values were calculated. All the data presented as the mean ± standard deviation (SD), relative standard deviation (RSD), linear regression analysis and calculations were performed using Microsoft Excel program.

RESULTS and DISCUSSION

In this study, aerial part, and root of *C. thirkeana* were analyzed quantitatively for caffeic acid, chlorogenic acid, hyperoside, and rutin by using HPLC. Methanol was used in the extraction process (Acıkara et al., 2019; Okur et al., 2020; Ayla et al., 2019). Yields of aerial part and root extracts are 15.85% and 5.84%, respectively. While chlorogenic acid (0.098%), caffeic acid (0.107%), rutin (0.078%) and hyperoside (0.134%) were detected in the aerial part, only rutin (0.007%) was detected in the root (Table 1, Figure 1, Figure 2).

A liquid chromatographic system device was used for HPLC analysis, and the method was validated. Within the ranges of 5 to 100 µg mL^{-1}, 5 to 100 µg mL^{-1}, 10 to 100 µg mL^{-1}, and 5 to 100 µg mL^{-1}, the calibration plots for caffeic acid, chlorogenic acid, hyperoside, and rutin were linear. The LOD and LOQ values for these phenolics were determined (Table 2).

Table 1. Contents of chlorogenic acid, caffeic acid, rutin and hyperoside in *C. thirkeana* methanol extracts (n=3).
Çizelge 1. C. thirkeana methanol ekstrelerinde kolorojenik asit, kafeik asit, rutin ve hiperosit içerikleri (n=3).

	Caffeic acid (% ± SD*)	Chlorogenic acid (% ± SD*)	Hyperoside (% ± SD*)	Rutin (% ± SD*)
Aerial part	0.107 ± 0.002	0.098 ± 0.001	0.134 ± 0.001	0.078 ± 0.003
Root	ND**	ND**	ND**	0.007 ± 0.001

*SD: Standard Deviation; **ND: Not Detected

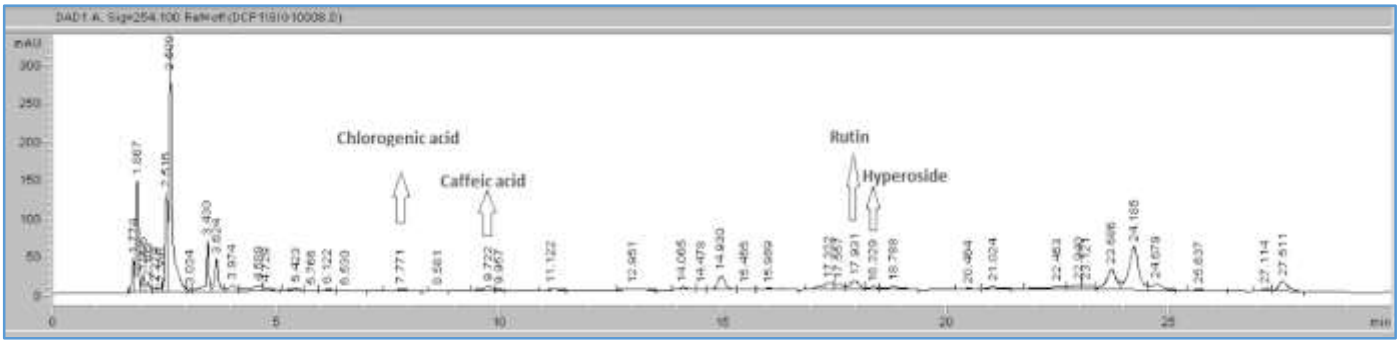

Figure 1. HPLC chromatogram of aerial part (*C. thirkeana*)
Şekil 1. Toprak üstü kısmının YPSK kromatogramı (*C. thirkeana*)

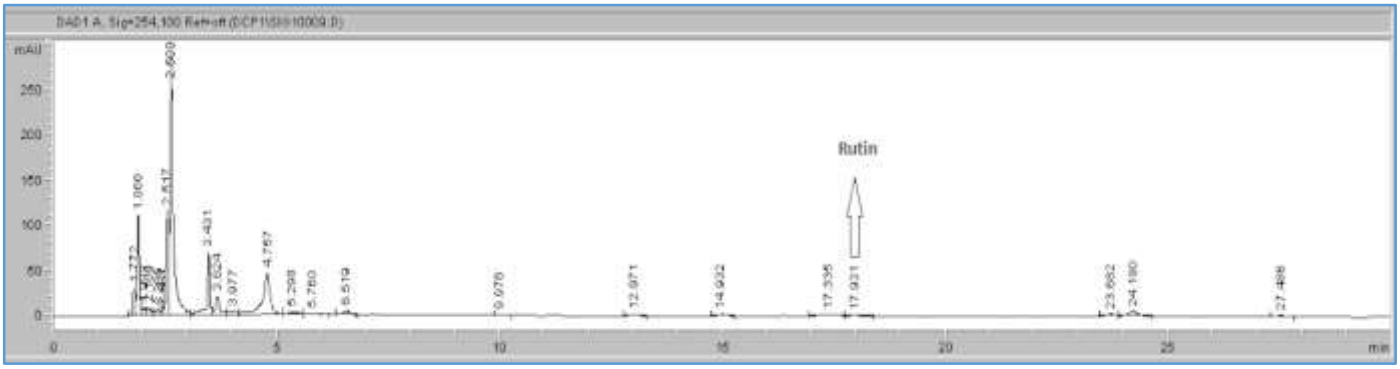

Figure 2. HPLC chromatogram of root (*C. thirkeana*)
Şekil 2. Toprak altı kısmının YPSK kromatogramı (*C. thirkeana*)

Intra-day and inter-day variations were used to determine the method's precision. The result showed that relative standard deviation (RSD) values were always less than 3% (Table 3).

For a recovery assay, 3 different known concentrations of standards were spiked into the sample solution. The mean extraction recovery of caffeic acid, chlorogenic acid, hyperoside, and rutin was in the range of 96.124-101.270%, 97.837-101.881%, 97.879-101.103, and 97.289-101.778, respectively (Table 4).

Table 2. Calibration values for standards.
Çizelge 2. Standartlar için validasyon değerleri

Standards	Calibration range (μg mL^{-1})	Linear Equation	Correlation factor (r^2±SD*)	RT** (min)	LOD (μg mL^{-1})	LOQ (μg mL^{-1})
Caffeic acid	5-100	y=65.998x-244.18	0.985 ± 0.005	9.5	0.371	1.237
Chlorogenic acid	5-100	y=11.965x-9.2492	0.99 ± 0.008	7.6	0.777	2.590
Hyperoside	10-100	y=14.1x-36.65	0.996 ± 0.004	18.4	0.348	1.160
Rutin	5-100	y=23.625x+9.898	0.995 ± 0.006	17.9	0.036	0.123

*SD: Standard Deviation; **ND: Not Detected

Table 3. Intra-day and inter-day precision data of the method.
Çizelge 3. Metodun gün içi ve günler arası kesinlik verileri.

Standards	Amount (μg mL^{-1})	Intra-day precision (RSD*%)	Inter-day precision (RSD*%)
Caffeic acid	5	1.105	0.846
	10	0.709	0.342
	25	0.315	1.255
	50	2.486	1.133
	100	0.730	2.558
Chlorogenic acid	5	1.347	1.579
	10	1.291	2.784
	25	2.913	1.357
	50	1.060	0.580
	100	0.831	2.932
Hyperoside	10	2.744	2.607
	25	2.145	0.820
	50	2.859	0.711
	100	0.415	1.090
Rutin	5	2.735	0.502
	10	1.806	1.116
	25	0.595	0.361
	50	1.354	2.659
	100	1.423	1.717

*RSD: Relative Standard Deviation

Table 4. Recovery assay's statistical data of the method (n=3).
Çizelge 4. Methot geri kazanım testinin istatistiksel verileri (n=3).

Standards	Concentration in sample (μg mL^{-1})	Amount spiked (μg mL^{-1})	Mean amount found in mixture (μg mL^{-1})	Mean recovery (%)	RSD*
Caffeic acid	0.006	0.003	0.0045	96.124	0.204
		0.006	0.006	96.739	1.200
		0.012	0.009	101.270	0.331
Chlorogenic acid	0.003	0.0015	0.0025	101.881	1.570
		0.003	0.003	97.837	2.359
		0.006	0.0045	98.854	2.694
Hyperoside	0.005	0.0025	0.00225	98.937	1.620
		0.005	0.005	101.103	1.636
		0.01	0.0075	97.879	1.502
Rutin	0.003	0.0015	0.0025	101.778	2.726
		0.003	0.003	99.369	1.355
		0.006	0.0045	97.289	2.519

*SD: Standard Deviation, *RSD: Relative Standard Deviation

Some phytochemical studies have been carried out on *Consolida* genera and there are many alkaloids isolation studies on *Consolida* species grown in Turkey. In the study of Ulubelen et al. (1996), consolidine (a new norditerpenoid alkaloid), pubescenine, gigactonine, desolline and ajaconine alkaloids were isolated from the aerial parts of *C. oliveriana* (DC.) Schrödinger. Bitiş et al. (2006) isolated delphatine, delcaroline, browniine, which are very toxic alkaloids and hetisine, dehydronapelline, 12-epidehydronapelline alkaloids from aerial parts of *C. olopetala* (Boiss.) Hayek. Especially Mericli et al. have many isolation studies on *Consolida* species. Mericli et al. (1999) isolated hetisine, hetisinone and ajadelphinine alkaloids from aerial parts of *C. stenocarpa* (Davis & Hossain) Davis. Mericli et al. (2001) isolated delcosine, delsoline, gigactonine, lycoctonine, takaosamine, atisine and hetisinone diterpenoid alkaloids from aerial parts of *C. regalis* S.F.Gray subsp. paniculata (Host) Soo var. paniculata. Mericli et al. (2012) isolated methyllycaconitine and leucanthumsine alkaloids from aerial part of *C. thirkeana* (Boiss.) Bornm. and they also isolated browniine, gigactonine and neolinine alkaloids from aerial parts of *C. sulphurea* (Boiss. & Hausskn.) P.H. Davis.

In terms of phenolic compounds, 93 phenolic acids were detected in the study on some *Consolida* species (*C. glandulosa* (Boiss. & A. Huet) Bornm, *C. hellospontica* (Boiss.) Chater, *C. raveyi* (Boiss.) Schrödinger, *C. regalis* (Boiss.) Schrödinger, *C. staminosa* P.H. Davis & Sorger and *C. stenocarpa* (Davis & Hossain) Davis) which grown in Turkey. However, in this study, information about these species was not given separately (Rocchetti et al., 2020). *p* -hydroxybenzoic, caffeic, ferulic and *p* - coumaric acids have been detected in aerial parts of *C. armeniaca* (Stapf ex Huth)F.C.Schrad and protocatechuic, vanillic, cinnamic, chlorogenic, galllic, sinapic and benzoic acids, kaempferol, quercetin, and hyperoside have been detected in *C. orientalis* (J.Gay) Schrödinger (Yin et al., 2020). Phenolic compounds have also been isolated in other species belonging to the Ranunculacea family. *p* -Hydroxy benzoic, caffeic, *p* - coumaric, chlorogenic and trans-aconitic acids were isolated from *Delphinium formosum* Boiss.&A.Huet (Dürüst et al., 2001). Caffeic, ferulic, isoferulic, fukinolic, cimicifugic A, and cimicifugic B acids were isolated from *Actaea racemosa* L. (Li et al., 2003). Considering the studies conducted, data could not be reached in terms of quantification. In addition to the detection of bioactive compounds, the determination of their amounts is also important.

This study was conducted for the first time for *C. thirkeana*. According to the results, chlorogenic acid, caffeic acid, rutin, and hyperoside were detected in *C. thirkeana*. At the root, only rutin was found. When the amount of rutin was compared, it was found that it was higher in the aerial part. Studies on *Consolida* species have generally been studied with aerial parts and bioactive compounds have been isolated. This is due to the presence of more compounds in the aerial parts.

CONCLUSION

The method used in this study for the HPLC determination of chlorogenic acid, caffeic acid, rutin, and hyperoside in *C. thirkeana* extracts is rapid, simple, effective, and reliable. The linearity, intra-day and inter-day precision, recovery, LOD, and LOQ of the method were all validated. At the end of this study, *C. thirkeana* was evaluated in terms of these phenolic compounds. Elaborated studies are needed to determine the other major compounds and it is thought that with this method, phenolic compounds can be determined on other *Consolida* species as well. Thus, comparisons can be made between species in terms of the amount of phenolic compounds.

Author's Contributions

The contribution of authors is equal.

Statement of Conflictof Interest

Authors have declared no conflict of interest.

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Total Phenolic, Total Flavonoid Contents and Antioxidant Potential of The Wild Edible Mushroom *Clitocybe odora*

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ABSTRACT

The nutritional value of the edible fungus *Clitocybe odora* (Bull.) P. Kumm was evaluated by measuring its total phenolic, total antioxidant, total oxidant, and total flavonoid contents. In this case, a soxhlet was used to extract the methanol from the mushroom. The investigation involved the utilisation of Rel Assay kits to ascertain the total antioxidant status and total oxidant status. The DPPH (2,2-Diphenyl-1-picrylhydrazyl) test was used to measure the ability to quench free radicals. Folin-Ciocalteu reagent was used to measure total phenolic content. Aluminum chloride analysis was used to determine the total flavonoid content. As a result of the study, the total antioxidant status of *C. odora* was determined to be 6.801±0.243 mmol L^{-1}, the total oxidant status was 5.748±0.137 µmol L^{-1}, and the oxidative stress index was 0.085±0.003. The extract has a scavenging activity of 73.38±1.60 percent against DPPH free radicals at a concentration of 2 mg mL^{-1}. Total phenolic content was determined as 82.646±1.623 mg g^{-1} and total flavanoid content as 117.753±3.491 mg g^{-1}. This led to the conclusion that the mushroom had significant antioxidant potential.

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Yenilebilir Doğal Mantar *Clitocybe odora*'nın Toplam Fenolik, Toplam Flavonoid İçeriği ve Antioksidan Potansiyeli

ÖZET

Yenilebilir mantar *Clitocybe odora* (Bull.) P. Kumm'nın toplam fenolik, toplam antioksidan, toplam oksidan ve toplam flavonoid içerikleri ölçülerek değerlendirildi. Bu kapsamda, mantardan metanol özütü elde etmek için soxhlet cihazı kullanıldı. Toplam antioksidan durumunu (TAS) ve toplam oksidan durumunu (TOS) belirlemek için Rel Assay kitleri kullanıldı. DPPH (2,2-Diphenyl-1-picrylhydrazyl) testi, serbest radikal süpürme yeteneğini ölçmek için kullanıldı. Toplam fenolik içeriği ölçmek için Folin-Ciocalteu reaktifi kullanıldı. Toplam flavonoid içeriğini belirlemek için alüminyum klorür analizi kullanıldı. Çalışma sonucunda *C. odora*'nın total antioksidan durumu 6.801±0.243 mmol L^{-1}, total oksidan durumu 5.748±0.137 µmol L^{-1} ve oksidatif stres indeksi 0.085±0.003 olarak belirlendi. Mantar özütünün 2 mg mL^{-1}'lik konsantrasyonda DPPH serbest radikallerine karşı 73.38±1.60'lik bir süpürme aktivitesine sahip olduğu belirlendi. Toplam fenolik madde içeriği 82.646±1.623 mg g^{-1} ve toplam flavaoid içeriği 117.753±3.491 mg g^{-1} olarak belirlendi. Bu sonuçlar mantarın önemli bir antioksidan potansiyelinin olduğunu gösterdi.

Botanik

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INTRODUCTION

Mushrooms have been used for many purposes in different communities around the world (Eralan et al., 2021). They are distributed in different ecosystems (Akata et al., 2018). In religious rituals, natural products that are of significant importance are utilized

as a source of sustenance or for medicinal purposes (Sevindik and Bal, 2021). They are invariable elements of diets in many countries (Gürgen et al., 2021). They are natural products with high nutritional properties such as protein, carbohydrates, vitamins, essential amino acids and nutritional elements (Torres-Gómez et al., 2022; Bal et al., 2023). In addition to nutritional properties, they are very useful sources from a medical point of view (Baba et al., 2020). Numerous studies have reported the diverse activities of fungi, such as antimicrobial, anticancer, antiproliferative, hepatoprotective, antioxidant, and DNA-protective properties (Canli et al., 2017; Oloke et al., 2017; Bal et al., 2019; Atila et al., 2021; Majeed et al., 2021; Bal et al., 2022; Peng et al., 2022; Sevindik et al., 2023).

Clitocybe odora (Bull.) P. Kumm (Agaricales) are mushrooms that are abundant in conifer-dominated forests and broad-leaved forests. It can spread intensively from late summer to late spring. It is an edible type of mushroom. But when consumed heavily, it causes gastrointestinal syndrome (Walther et al., 2005; Sahin et al., 2021). Antioxidant activity studies on members of the genus *Clitocybe* in the literature are shown in Table 1.

Çizelge 1. *Clitocybe* türlerinin Antioksidan aktiviteleri
Table 1. Antioxidant activities of Clitocybe species

Species	Extract	Country	References
Clitocybe alexandri	Methanol, ethanol	Portugal	Vaz et al., 2010
Clitocybe maxima	Hot water, Methanol, ethanol, aqueous	China, Taiwan	Tsai et al., 2009; Liu et al., 2012; Hu et al., 2017
Clitocybe geotropa (Current name: *Infundibulicybe geotropa*)	Ethanol, Metanol, acetone	Turkey, Serbia	Kosanić et al., 2020; Sevindik et al., 2020
Clitocybe odora	Ethanol, water	Portugal, Nigeria, Serbia	Egwim et al., 2011; Vaz et al., 2011; Dimitrijevic et al., 2015
Clitocybe squamulosa	aqueous	China	Yuan et al., 2022
Clitocybe brunneocaperata	methanol	India	Debnath et al., 2020
Clitocybe nebularis	Acetone, ethanol, methanol, distilled water	Serbia	Dimitrijevic et al., 2019; Kosanić et al., 2020
Clitocybe nuda	Water	Slovakia	Strapáč et al., 2019
Clitocybe gibba	Methanol	Korea	Kim et al., 2012

Clitocybe species have been shown to exhibit antioxidant activity in a variety of published research (Table 1). Research shows that antioxidant properties may be found in *Clitocybe* species from all over the world. We analyzed *C. odora* for its total phenolic and flavonoid content, as well as its antioxidant and oxidant potential and DPPH activity. In this study, total antioxidant and total oxidant status of *C. odora* was determined for the first time using Rel Assay kits. In addition, the suppression ratio (OSI) with antioxidant compounds included in the oxidant compounds was determined for the first time.

MATERIAL and METHOD

C. odora samples (MS-352) used in the study were collected from a fir forest in Kocaeli province. After collecting the fruiting bodies of mushroom samples, they were extracted with methanol (MeOH) for about six hours at 50 °C using a soxhlet extractor. Solvents of the resulting extracts were made using a rotary evaporator (Heidolph Laborota 4000 Rotary Evaporator).

Total Phenolic and Flavonoid Tests

Dilution with distilled water brought the volume of the MeOH extracts to 0.1 mL. After that, we added 1 mL of Folin-Ciocalteu reagent (1:9, v/v) and gave it a good whirl. 0.75 mL of a 1% Na_2CO_3 solution was added to the mixture. Following a 2-hour incubation period at room temperature, the absorbance was measured at 760 nm. From the gallic acid standard solution calibration curve, we were able to determine the total phenolic content (TPC) in mg GAE (Gallic Acid Equivalent) g^{-1} (Yurmutas et al., 2009).

Aluminium chloride analysis was used to determine the total flavonoid content (TFC) of the mushroom (Chang et al., 2002). Combined the Quercetin (0.5 mL), mushroom sample (0.5 mL), MeOH (4.3 mL), 10% Al $(NO_3)_3$, and NH_4CH_3COO (1 M) to make the final solution. Finally, a 40-minute incubation period was performed. The absorbance was checked at 415 nm. Flavonoids were expressed as mg QE (Quercetin

Equivalent) g^{-1}. In order to ensure accuracy, we triple-checked all of the results. Standard deviations were computed using the averages of the data sets used in the investigation.

Antioxidant activity tests

The TAS, TOS, and OSI values of *C. odora* were determined using Rel Assay kits (Mega Tıp/Türkiye). Trolox was utilized as a calibrator in TAS tests. The results were presented as mmol Trolox equiv./L (Erel, 2004). In order to determine the TOS values, hydrogen peroxide (H_2O_2) was utilized as a calibrator and the resulting outcomes were presented as µmol H_2O_2 equivalent L^{-1}, as reported by Erel in 2005. OSI values were determined by dividing the TOS values from the TAS values and taking the percentage (Sevindik et al., 2017).

Samples of *C. odora* were tested for their MeOH extract's ability to scavenge free radicals using 1-diphenyl-2-picrylhydrazyl (DPPH). The mushroom extracts were dissolved in 10% DMSO to make stock solutions with concentrations of 0.25, 0.50, 1, and 2 mg mL^{-1}. To 160 mL of %0.039 DPPH, 50 mL of the produced solution was added. After that, we let it sit in the dark and at room temperature for 30 minutes. The 517 nm absorbance was then measured (Shimada et al., 1992). Each extract had its unique series of procedures. The antioxidant ascorbic acid served as a standard.

DPPH free radical scavenging percentages (1);

The scavenging activity was calculated according to the formula (%)= [(ADPPH-ASample)/(ADPPH)]x100 (1)

Statistical Analysis

The analysis of all assays was performed in triplicate. The data were recorded as means ± standard deviations and analyzed in a completely randomized by using Statistical Package for Social Sciences (SPSS version 22.0). Statistically significant differences ($p<0.05$) among means of experimental results were analyzed by ANOVA and tests of significance were carried out using Duncan's multiple range tests.

RESULTS and DISCUSSION

Antioxidant activity

Organisms are constantly under stress due to environmental factors. These organisms produce oxidising free radicals as a result of their metabolic activities in response to environmental influences (Mohammed et al., 2019). As the levels of these compounds increase, the antioxidant defence system in living organisms is activated. The antioxidant defence system suppresses oxidant compounds. However, in cases where the antioxidant defence system is insufficient, oxidative stress occurs (Korkmaz et al., 2018). The occurrence of significant health conditions such as cancer, cardiovascular diseases, Alzheimer's, and Parkinson's can be attributed to oxidative stress in humans (Saridoğan et al., 2021). Supplementation with exogenous antioxidants can be utilized to reinforce the inadequate antioxidant defence system, which falls short in reducing the effects of oxidative stress. In this context, it is highly important to evaluate the potential use of mushrooms as a supplementary antioxidant (Unal et al., 2022). In this study, the MeOH extract of *C. odora* was evaluated for its DPPH free radical scavenging activity at concentrations of 0.25, 0.50, 1, and 2 mg mL^{-1}. The results obtained have been presented in Table 2.

Çizelge 2. *Clitocybe odora*'nın DPPH Aktivitesi
Table 2 DPPH Activity of Clitocybe odora

Mushroom and Control (%)	0.25 mg mL^{-1}	0.50 mg mL^{-1}	1 mg mL^{-1}	2 mg mL^{-1}
Clitocybe odora	37.49±1.30^{a}	52.94±1.46^{b}	66.29±1.23^{c}	73.38±1.60^{d}
Ascorbic acid	76.16±2.12^{a}	90.56±0.30^{b}	93.29±0.31^{c}	96.49±0.54^{d}

*Means followed by different letter(s) differ significantly at $p < 0.05$ (Duncan's multiple range test)

The study found that higher concentrations of mushroom extract resulted in greater DPPH activity. Activity was measured to be 73.38±1.60 at a 2 mg mL^{-1} concentration. The ascorbic acid control showed 96.49±0.54 activity at 2 mg mL^{-1}. Also, the DPPH activity of different concentrations of the sample was found statistically different ($p < 0.05$). The mushroom extract was found to be less active than the reference standard. Multiple research conducted in many countries have indicated that *C. odora* possesses antioxidant activity (Egwim et al., 2011; Vaz et al., 2011; Dimitrijevic et al., 2015). This study shows that the MeOH extract of *C. odora* has potent free radical scavenging action against DPPH radicals.

In this study, TAS, TOS and OSI values of *C. odora* were determined for the first time. The results obtained are shown in Table 3.

TAS value represents the antioxidant-effective compounds detected in the mushroom (Sevindik, 2020). TAS value of *C. odora* was calculated to be 6.801±0.243 in this investigation. Previously, different wild mushrooms *Gyrodon lividus* (Bull.) Sacc. (TAS:2.077, TOS:13.465, OSI:0.651), *Hohenbuehelia myxotricha* (Lév.) Singer (TAS:4.549, TOS:2.623, OSI:0.058), *Ramaria stricta* (Pers.) Quél. (TAS:4.223, TOS:8.201, OSI: 0.194), *Laetiporus sulphureus* (Bull.)

Çizelge 3. *Clitocybe odora*'nın TAS, TOS, OSI, TPC ve TFC değerleri
Table 3 TAS, TOS, OSI, TPC and TFC values of Clitocybe odora

	TAS mmol L^{-1}	TOS µmol L^{-1}	OSI	TPC mg g^{-1}	TFC mg g^{-1}
Clitocybe odora	6.801±0.243^{c}	5.748±0.137^{b}	0.085±0.003^{a}	82.646±1.623^{d}	117.753±3.491^{e}

*Means followed by different letter(s) differ significantly at p < 0,05 (Duncan's multiple range test)

Murrill (TAS:2.195, TOS:1.303, OSI:0.059), *Tricholoma virgatum* (Fr.) P. Kumm (TAS:3.754, TOS:8.362, OSI:0.223), *Suillus granulatus* (TAS:3.143, TOS:18.933, OSI:0.603), *Helvella leucopus* Pers (TAS:2.181, TOS:14.389, OSI:0.661) and *Cerioporus varius* (Pers.) Zmitr. & Kovalenko (TAS:2.312, TOS:14.358, OSI:0.627) have been reported (Bal, 2018; Sevindik et al., 2018; Sevindik, 2019; Krupodorova and Sevindik, 2020; Mushtaq et al., 2020; Selamoğlu et al., 2020; Sevindik and Akata, 2020; Krupodorova et al., 2022). TAS values for *G. lividus, H. myxotricha, R. stricta, L. sulphureus, T. virgatum, S. granulosus, H. leucopus*, and *C. varius* were found to be lower than those for *C. odora* in this research. To combat free radical damage, mushrooms make a plethora of chemicals that act as antioxidants (Sevindik, 2020). We can observe that *C. odora* has a strong antioxidant capacity in this setting.

The total oxidant potential (TOS) is a measure of all chemicals present in fungus that have oxidizing effects (Sevindik, 2020). *C. odora* had a greater TOS value than *H. myxotricha* and *L. sulphureus*, but a lower TOS value than *G. lividus, R. stricta, T. virgatum, S. granulatus, H. leucopus*, and *C. varius*. We found that the mushrooms utilized in this investigation had decreased oxidant levels. The level of suppression of oxidant chemicals generated in mushrooms by antioxidant compounds is represented by the OSI value (Sevindik, 2020). This study showed that the OSI for the *C. odora* we utilized was lower than that of *G. lividus, R. stricta, T. virgatum, S. granulosus*, *H. leucopus*, and *C. varius*, and higher than that of *H. myxotricha* and *L. sulphureus*. From these findings, it is clear that the *C. odora* we utilized in this study significantly mitigates the harmful effects of oxidant chemicals.

Total Phenolic and Flavonoid Values

Antioxidant actions are linked to total phenolic content, as is well known (Alispahić et al., 2015). Different types of wild mushrooms have been shown to have varying amounts of total phenolic contents, according to numerous research (Wong et al., 2013; Salachna et al., 2021; Bristy et al., 2022). The total phenolic content of the ethanol extract of *C. odora*, previously collected from Serbia, was reported as 38.112 mg g^{-1} (Dimitrijevic et al., 2015). *C. odora* used in this study was determined as 82.646±1.623 mg g^{-1}. It is speculated that the solvent and the site where the fungus is gathered are the primary contributors to this variation. In contrast, the *C. odora* we employed in this research has the potential to be a significant source due to its high phenolic content. It is generally agreed that flavonoids play a crucial role in protecting human health and vigor through their powerful antioxidant impact (Gąsecka et al., 2016; Shi et al., 2019). The flavonoid content of *C. brunneocaperata* has been reported in the past to be 13 g g^{-1} (Debnath et al., 2020). Using a different species, *C. odora*, we were able to determined its total phenolic content to be 117.753±3.491 mg g^{-1}. Mushrooms, in this regard, are considered a potential resource for the extraction of flavonoids. In addition, the TAS, TOS, OSI, TPC and TFC values of sample were found statistically different (p < 0,05).

CONCLUSION

C. odora, a wild edible mushroom, has its antioxidant, oxidant, phenolic, flavonoid, and oxidative stress index levels analyzed in this article. The results indicated that the mushroom had significant anti-oxidant potential. Moreover, both the phenolic and flavonoid content levels were discovered to be rather high. Mushrooms, it is believed, can serve as a natural supply of antioxidants in this setting.

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Author's Contributions

The contribution of the authors is equal.

CONFLICT of INTEREST

The authors report no declarations of interest.

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Palynology of Taxa of *Crepis* L. Genus Growing in Çanakkale, Türkiye

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ABSTRACT

This study investigated the pollen morphology of the taxa belonging to the genus *Crepis* L. collected from various localities in Çanakkale in 2015. Wodehouse (1935) and Acetolysis (Erdtman, 1960) methods were used in the research. Pollen morphologies of 3 species and two subspecies belonging to the *Crepis* L. genus were defined according to light microscopy (LM) and scanning electron microscopy (SEM) examinations. In LM, pollens are monad, radially symmetrical and isopolar. Pollens are suboblate and oblate spheroidal, Amb shape is inter-hexagonal-subtriangular. The pollen of the genus is tricolporate. The view from the equator is circular and oval. In the examinations made with LM, the ornamentation is echinulophate. Pollen has 15 lacunae as 3 poral, 6 abporal, 6 paraboral. Ornamentation is echinulophate; the tectum surface is microperforate. SEM micrographs of *C. smyrnaea* and *C. commutata* pollen show depressions at the corners of the polar field. The collapses are located on the paraboral lacunae and are distinctly lacuna-like. As a result, in this study, palynological features of *C. smyrnaea* DC., *C. micrantha* Czerep., *C. commutata* (Spreng.), *C. reuterana* Boiss. & Heldr. subsp. *reuterana* taxa were studied for the first time. *C. smyrnaea* DC. ex Froel. was collected for the first time in Çanakkale within the scope of this study, according to the type records in Flora of Turkey.

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Çanakkale Bölgesinde Yetişen *Crepis* L. Cinsine Ait Taksonların Palinolojisi

ÖZET

Bu çalışmada 2015 yılında Çanakkale ilinde çeşitli lokalitelerden toplanılan *Crepis* L. cinsine ait taksonların polen morfolojisi araştırılmıştır. Araştırmada Wodehouse (1935) ve Asetoliz (Erdtman, 1960) yöntemleri kullanılmıştır. *Crepis* L. cinsine ait 3 tür ve 2 alt türün polen morfolojileri ışık mikroskobu (LM) ve taramalı elektron mikroskobu (SEM) incelemelerine göre tanımlanmıştır. LM incelemelerinde polenler monad, radyal simetrili ve izopolardır. Polenler suboblat ve oblat sferoidal, Amb şekli interhegzagonal-subtriangular biçimdedir. Cinsin polenleri trikolporattır. Ekvatordan görünüş sirkular, ovaldir. LM ile yapılan incelemelerde ornamentasyon ekinülofattır. Polenler 3 poral, 6 abporal, 6 paraboral olmak üzere 15 lakunaya sahiptir. Ornamentasyon ekinülofat, tektum yüzeyi mikroperforat bir yapıdadır. *C. smyrnaea* ve *C. commutata* polenlerinin SEM mikrograflarında polar alanın köşelerinde çöküntüler mevcuttur. Çöküntüler paraboral lakunalar üstünde bulunmakta ve belirgin olarak lakuna görünümündedir Sonuç olarak bu çalışmada *C. smyrnaea* DC., *C. micrantha* Czerep., *C. commutata* (Spreng.), *C. reuterana* Boiss. & Heldr. subsp. reuterana taksonlarının palinolojik özellikleri ilk defa çalışılmıştır. *C. smyrnaea* DC. ex Froel. Flora of Turkey'deki tip kayıtlarına göre Çanakkale'de ilk defa bu çalışma kapsamında toplanmıştır.

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INTRODUCTION

The Asteraceae family spreads all over the world except Antarctica. With more than 1100 genera and more than 20.000 species, it ranks first in diversity among flowering plants. Among the species belonging to the family, there are annual and perennial ones. The most characteristic feature of this family is that its flower shows a flower state structure called a capitulum (Yıldız & Aktoklu, 2010). Plants are primarily found in mountain vegetation, open grasslands, and open forest areas. They are less common in humid tropical forests (Kadereit & Jeffrey, 2007).

Crepis L., the genus number 129 in the Lactuceae tribus, is in the Asteraceae family. There are 40 taxa of the genus *Crepis* registered in the 5th volume of Flora of Turkey. The genus *Crepis* was written by Lamond (Davis, 1975). In the List of Plants of Turkey, *C. foetida* L. subsp. *commutata* (Spreng.) Babcock has been upgraded from subspecies to *C. commutata* (Spreng.) Greuter (Güner et al., 2012).

The Asteraceae family was divided into nine pollen types, and the *Crepis* genus was studied within the Helianthus type (Erdtman et al., 1961). İnceoğlu and Karamustafa (1977) described light microscopy measurements of various genera in their pollen morphology studies for the Compositae family and evaluated the genus within the Lacunae. On the other hand, Enke (2009) reclassified *Crepis* (Cichorieae, Compositae) phylogenetics according to achene, pappus feathers, and pollen morphology characteristics. Wang et al. (2009) defined pollen morphological features of four *Crepis* species by light (LM) and scanning electron microscopy (SEM).

Peng et al. (2013) reported the pollen characteristics of three species of the *Crepis* genus with SEM images in their research belonging to the Asteraceae family. Yildirim et al. (2011) stated the morphological features, pollen, and seed SEM images of the *C. gemicii* species grown in Van province in Eastern Anatolia. However, researchers were not specified measurement values related to the pollen characteristics. Qureshi et al. (2002) defined the pollen morphology of *C. flexuosa*, *C. multicaulis*, *C. sancta*, and *C. thomsonii* species belonging to the *Crepis* genus in Pakistan by light microscopy.

This study aims to examine in detail the pollen morphology of the taxa of the *Crepis* genus growing in the Çanakkale province belonging to the Asteraceae family. The results obtained are intended to contribute to the genus systematic studies and the pollen atlas that can be created for Turkey.

MATERIAL and METHOD

Sampling Method

Specimens of flowering plants belonging to *Crepis* were collected during field studies in Çanakkale in 2015 (Table 1). The samples were dried and turned into a herbarium sample. Pollen slides were prepared from dried samples for microscope examination. Identification of plant samples was made by Prof. Dr. Bayram YILDIZ (Retired lecturer) and Assoc. Dr. Gül TARIMCILAR (Uludağ University, Faculty of Arts and Sciences, Department of Biology). Plant herbarium samples are kept in the Palynology Laboratory of the Biology Department of the Faculty of Science of ÇOMÜ and the IZEF Herbarium of the Faculty of Pharmacy of Ege University.

Laboratory Analysis

Preparations of pollen were prepared by Wodehouse (1935) and Acetolysis (Erdtman, 1960) methods for LM analysis. Measurements of pollen were made with a Leica 2500 DM light microscope. Pollen photos were taken with a Cence 2.0 MP camera connected to a Leıca 2500 DM microscope. Immersion oil, ocular 10X, and objective 100X were used for light microscope measurements. Each taxa in the preparations under the light microscope was measured on an average of 50 samples, excluding spins. According to the method of Wodehouse and Erdtman, P (polar axis), E (equatorial axis), P/E ratio, Meso (mesocolpium), Amb (the length of diameter in the polar view of the pollen), Icrl (inter colpus ridge length), Icsn (inter colpus spinules number), Ilgl (interlacunal gaps length), Ilgw (interlacunal gaps width), Alw (Abporal lacuna width), All (Abporal lacuna length), Pw (pore width), Pl (pore length), Plw (poral lacuna width), Pll (poral lacuna length), Pasn (number of spinules in the polar area), Pad (polar area diameter), Slle (spinule length equatorial view), Sllp (spinule length polar view) An (Aperture number) was measured.

For electron microscopy studies, according to the acetolysis method (Erdtman, 1960), pollen was placed on the stup with double-sided adhesive tape by taking it from the tube with the help of a clean, thin needle. Stups are plated with gold. JEOL SM 7100F brand SEM from Çanakkale Onsekiz Mart University Science and Technology Application and Research Center (ÇOBİLTUM) was used for electron microscope examinations. Surface ornamentations, polar and equatorial images, and spin properties of pollen were examined in detail, and photographs were taken. Some morphological features of pollen in SEM images; SLAL (spinule length at abporal lacuna), SBWAL (spinule base width at abporal lacuna), DBSICR (the distance between spinules at inter colpus ridge), SLICR

(spinule length at inter colpus ridge), SBWICR (spinule base width at inter colpus ridge), SLP (spinule length at polar area), SBWPA (spinule base width at polar area), ILGW (interlacunal gaps width) were measured using the freely available software ImageJ 1.36b (Wayne Rasband, NIH, MD, USA).

Wodehouse (1935), Erdtman (1943, 1960, 1969), Skvarla & Turner (1966, 1971), Moore & Webb (1983), Faegri & Iverson (1992), Punt et al. (2007), Türkmen et al. (2010), Pınar et al. (2016) sources were used in pollen terminology. Pollen slides are in the Palynology Laboratory of Çanakkale Onsekiz Mart University.

Table1. The places where the taxa belonging to the genus *Crepis* were collected, the dates they were collected, the collectors and the descriptors.

Tablo 1. Crepis L. cinslerine ait taksonların toplandığı yerler, toplandığı tarihler, toplayan kişiler ve teşhis edenler

TAXA	Location	Collected Date	Collectors	Descriptors
C. foetida L. subsp. *rhoeadifolia* (M.Bieb.)	Çanakkale Science High School, Çınarlı Village, Çanakkale, 35447402 D. 4435386 K., 98 m.	09.06.2015	Hanife AKYALÇIN Sunay ALTAN	Bayram YILDIZ İZMİR Gül TARIMCILAR BURSA
C. commutata (Spreng.) Greuter	Dümrek Village,Çanakkale, 35445310 D. 4426339 K.,81m.	11.06.2015	Hanife AKYALÇIN Sunay ALTAN	Bayram YILDIZ İZMİR Gül TARIMCILAR BURSA
C. smyrnaea D.C	İntepe Village, Çanakkale, 35441869 D. 4427628 K.,92m.	09.05.2015	Hanife AKYALÇIN Sunay ALTAN	Gül TARIMCILAR BURSA
C.micrantha Czerep.	Çanakkale Science High School, Çınarlı Village, Çanakkale, 35447402 D. 4435386 K.,98 m.	08.06.2015	Hanife AKYALÇIN Sunay ALTAN	Bayram YILDIZ İZMİR
C. reuteriana Boiss. & Heldr. subsp. *reuteriana*	Between İntepe and Gökçalı Village Çanakkale, 35441869 D. 4427628 K.,92m.	11.06.2015	Hanife AKYALÇIN Sunay ALTAN	Bayram YILDIZ İZMİR

Statistical Analysis

The mean (M), standard deviation (S) and min-max values of the measurements of the light microscope images and the min-max values of the electron microscope images were made in the IBM SPSS Statistics 22 program.

RESULTS and DISCUSSION

In light microscopy observations of five taxa of the genus *Crepis,* pollens are radially symmetrical, monad, and isopolar. In the pollen slides prepared according to the Wodehouse method, all the taxa are oblata spheroidae shape pollens. In slides made according to the Erdtman method, *C. smyrnaea, C. reuterana* subsp. *reuteriana* are suboblate shape, *C. foetida* subsp. *rhoeadifolia*, *C. commutata, C. micrantha* are oblatae spheriodal shape pollen. Equatorial axis values of pollens are between 19-30 µ (W) and 20-29 µ (E), and polar axis values are between 16-27 µ (W) and 17-28 µ (E) (Table 2). Pollen has a tricolporate aperture. Amb shape in pollen grains is inter-hexagonal-subtriangular (Figure 2).

The view from the equator is circular and oval. In the examinations made with LM, ornamentation is echinulophate. Pollen grains have 15 lacunae (3 poral, 6 abporal, 6 paraboral). The pollen slides examined have an operculum on the pore, and the pores are elliptical-circular. The pore length is in the range of 4.10-6.04 µ (W), 4.17-5.98 µ (E), and the pore width is in the range of 4.76-6.96 µ (W), 4.86-7.06 µ (E). The average spinule number in the polar area is 3.27-7.52 in the examinations made with LM in the Wodehouse and Erdtman method. The ornamentation is echinulophate (Table 2, Figure 2).

In SEM micrographs (Table 3, Figures 2, 3 and 4), the ends of the spinules have obtus-acute endings. Spinules are upright or curved in different directions. Ornamentation is echinulophate, tectum surface is microperforate. In the taxa belonging to this genus, collapses were observed in the polar area. The collapses, which are very prominent in the polar area of the pollens of *C. smyrnaea* and *C. commutata*, seem to be separate lacuna at the polar area border of the paraboral lacunae (Figure 2, 3).

C. commutata by Güner et al., (2012) *C. foetida* subsp. *commutata* has been upgraded from subspecies to *C. commutata*.

Except for *C. micrantha*, no significant variations were observed in pollen shape, sexin, aperture, abporal lacuna, inter lacunal gaps, spinule, mesocolpium, aperture features, and polar area features in the pollen of the genus *Crepis* according to light microscopy measurements (Table 2).

Table 2. Pollen morphological data of *Crepis* taxa in light microscopy analyses.
Tablo 2. Işık mikroskobu analizlerinde Crepis taksonlarının polen morfolojik verileri.

				POLAR AXES						EQUATORIAL AXES											MEAN OF MEASUREMENTS							
TAXA	Methods	P/E	Pollen Shape	Mean	Std. Deviation	Min-Max	Mean	Std. Deviation	Min-Max	Meso	Nexine	Sexine	Exine	Amb	Icrl	Icsn	Ilgl	Ilgw	Alw	All	Pw	Pl	Plw	Pll	Pasn	Pad	Slle	Sllp	An
C. foetida* subsp. *rhoeadifolia	E	0.88	oblatae spheroidae	24.38	1.24	23-28	27.7	0.99	25-29	14.8	1.24	2.68	4.14	25.78	10.31	5.67	2.92	1.78	6.08	6.90	7.06	5.98	8.22	7.04	5.57	7.59	2.00	2.00	3
	W	0.88	oblatae spheroidae	25.74	0.82	24-27	29.25	0.65	28-30	16.68	0.87	2.83	3.66	24.53	10.09	5.60	2.97	1.86	6.46	6.12	6.96	6.04	9.00	8.13	5.96	9.52	1.93	2.00	3
C. commutata	E	0.88	oblatae spheroidae	23.04	1.11	20-25	26.19	1.31	24-29	13.73	1.06	2.71	3.86	23.64	9.35	4.41	2.78	1.39	5.58	5.76	6.36	5.18	7.69	6.27	4.63	7.90	1.57	1.52	3
	W	0.90	oblatae spheroidae	25.14	0.84	24-27	27.96	0.69	27-29	15.23	1.00	2.85	3.80	25.47	9.43	5.08	2.96	1.85	6.12	6.02	6.75	5.80	8.69	7.73	5.81	9.19	1.84	1.89	3
C. smyrnaea	E	0.87	suboblatae	24.88	0.85	23-27	28.58	1.16	25-30	15.29	1.07	2.33	3.22	25.11	10.88	6.61	2.59	1.58	6.15	5.94	5.25	4.74	6.79	5.79	7.52	9.19	1.51	1.57	3
	W	0.88	oblatae spheroidae	25.28	1.06	23-27	28.56	1.17	25-30	15.72	1.02	2.61	3.59	25.53	10.31	5.52	2.79	1.67	5.45	6.25	5.10	4.29	7.01	6.16	5.28	8.43	1.76	1.87	3
C. micrantha	E	0.89	oblatae spheroidae	18.67	0.84	17-20	21.00	0.95	20-23	12.02	0.52	1.82	2.32	18.04	8.34	4.36	2.01	1.13	4.73	5.16	4.86	4.17	6.09	5.30	3.50	5.75	1.17	1.26	3
	W	0.91	oblatae spheroidae	18.70	1.09	16-20	20.42	0.92	19-23	11.71	0.51	1.94	2.52	19.18	7.53	4.14	2.05	1.28	4.67	5.55	4.76	4.10	6.56	5.89	3.27	5.13	1.20	1.24	3
C. reuteriana* subsp. *reuteriana	E	0.86	suboblatae	22.74	1.29	20-25	26.46	1.65	21-29	14.71	1.09	2.16	3.46	23.98	10.09	5.60	2.33	1.27	5.57	6.19	62017	5.23	7.46	6.44	4.34	5.96	1.23	1.16	3
	W	0.89	oblatae spheroidae	24.88	0.74	23-26	27.78	0.73	26-29	15.26	1.00	2.82	3.83	24.87	10.02	5.45	2.86	1.80	5.62	6.30	6.14	5.32	8.10	7.24	5.17	7.20	1.90	1.87	3

Meso, mesocolpium; Amb, the length of diameter in the polar view of the pollen; Icrl, inter colpus ridge length; Icsn, inter colpus spinules number; Ilgl, interlacunal gaps length; Ilgw, interlacunal gaps width; Alw, Abporal lacuna width; All, Abporal lacuna length; Pw, pore width; Pl, pore length; Plw, poral lacuna width; Pll, poral lacuna length; Pasn, number of spinules in polar area; Pad, polar area diameter; Slle, spinule length equatorial view; Sllp, spinule length polar view; An, Aperture number, all measurements in µm.

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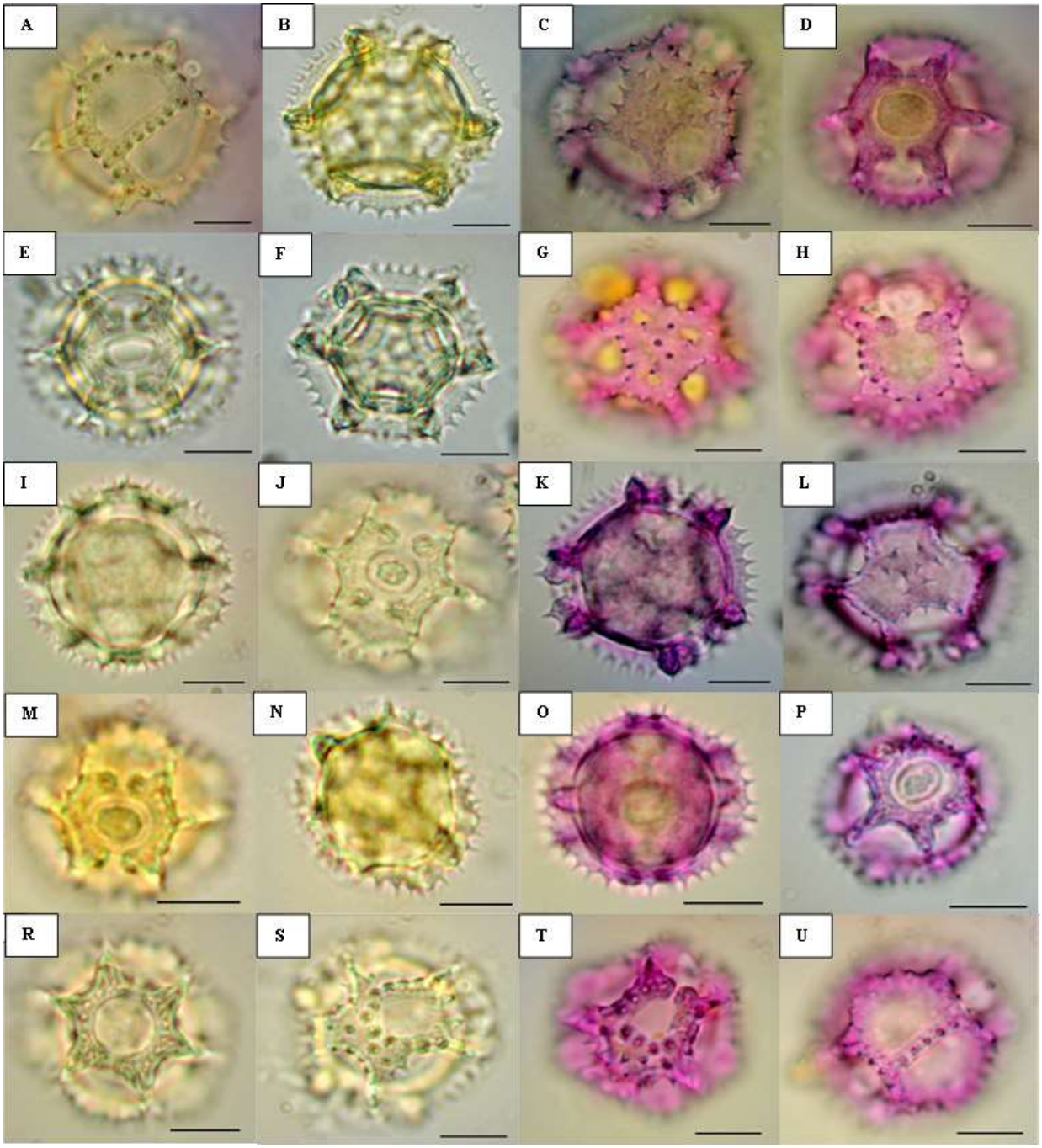

Figure 1. Pollen morphology of Crepis by light microscopy (LM). A-B (E), C-D (W) C. foetida subsp. rhoeadifolia; E-F (E), G-H C. commutata; I-J (E), K-L (W) C. smyrnaea; M-N (E), O-P (W), C. micrantha; R-S (E), T-U (W) C. reuteriana subsp. reuteriana. Erdtman (E), Wodehouse (W). Scale bar 10 µ.

Şekil 1. Erdtman ve Wodehouse yöntemine göre ışık mikroskobu resimleri. A-B (E), C-D (W) C. foetida subsp. rhoeadifolia; E-F (E), G-H C. commutata; I-J (E), K-L (W) C. smyrnaea; M-N (E), O-P (W), C. micrantha; R-S (E), T-U (W) C. reuteriana subsp. reuteriana. Erdtman (E), Wodehouse (W). Ölçek çubuğu 10 µ.

Polar and equatorial axis dimensions were smaller and significantly different between the taxa whose *C. micrantha* pollens were studied, compared to the Erdtman and Wodehouse methods—the investigated *C. reuteriana* subsp. *Reuteriana,* while the P/E ratios have a wide range in the Erdtman method, the range is very narrow according to the Wodehouse method (Table 2).

In SEM micrographs, the ends of the spinules have obtus-acute endings. Spinules are upright or curved in different directions. Ornamentation is echinulophate, and the tectum surface is microperforate. There are

apparent collapses in the polar area. Pollen morphological studies of the genus *Crepis* are limited. *C. foetida* subsp. *rhoeadifolia* was also studied by İnceoğlu and Karamustafa (1977), and light microscope measurements of the taxa were given. *C. foetida* subsp. *rhoeadifolia* pollen grain is 27.7 X 31.6 (E), 24 X 27.6 µ (W), triporate, oblate spheroid, amb inter-hexagonal. Pollen grains have been described as lacunae. In this study, pollens were observed as 24.38 X 27.70 µ (E), 25.74 X 29.25 µ (W), tricolporate, oblate spheroidal, amb subtriangular-subhexagonal (Table 2,3 and Figure 1). There is no significant difference between the results obtained and the results of the study conducted by İnceoğlu and Karamustafa (1977), except for the number of apertures. The aperture of *C. foetida* subsp *rhoeadifolia* is tricolparate, not triporate, as stated by İnceoğlu and Karamustafa (1977).

Table 3. Ornamentation and spine measurements of *Crepis* species in SEM analyses
Tablo 3. SEM analizlerinde Crepis türlerinin ornamentasyon ve spin ölçümleri

	DBSPL	SBWPL	SLPL	DBSAL	SLAL	SBWAL	DBSİCR	SLİCR	SBWİCR	SLP	SBWPA	İLGW
	Max-Min	Max-Min	Max-Min	Max-Min	Max-Min	Max-Min	Max-Min	Max-Min	Max-Min	Max-Min	Max-Min	Max-Min
C. foetida subsp. *rhoeadifolia*	1.79-0.66	1.82-1.23	2.00-1.24	1.54-0.62	2.25-0.96	1.74-1.12	1.69-0.54	1.84-0.91	1.69-0.95	2.01-1.04	1.84-0.95	0.99-0.58
C. commutata	—	—	—	—	1.60-0.92	1.25-0.82	0.87-0.71	2.02-0.93	1.56-0.86	1.41-1.14	1.38-1.14	1.19-0.43
C. smyrnaea	—	—	—	—	1.57-1.01	1.24-1.03	—	1.69-1.12	1.88-0.84	1.87-0.84	1.36-0.82	0.68-0.38
C. micrantha	—	—	—	—	1.52-1.07	1.48-0.95	0.46-0.35	1.51-1.02	1.17-0.90	—	—	0.69-0.54
C. reuteriana subsp. *reuteriana*	—	—	—	—	2.08-1.46	2.08-1.20	0.97-0.31	1.92-1.27	1.65-1.24	1.75-0.95	1.61-1.22	—

DBSPL, the distance between spinüls at paraboral lacuna; SBWPL, spinüle base width at paraboral lacuna; SLPL, spinüle length at paraboral lacuna; DBSAL, the distance between spinüls at abporal lacuna; SLAL, spinüle length at abporal lacuna; SBWAL, spinüle base width at abporal lacuna; DBSİCR, distance between spinüls at inter colpus ridge; SLİCR, spinüle length at inter colpus ridge; SBWİCR, spinüle base width at inter colpus ridge; SLP, spinüle length at polar area; SBWPA, spinüle base width at polar area; İLGW, interlacunal gaps width; –, unmeasured, all measurements in µ.

Qureshi et al. (2002) examined the four species of *Crepis* in Pakistan. Qureshi et al. (2002) are similar to the results in this study, but according to their measurement results, the pollen is more prominent in size than the pollen examined in this study.

Wang et al. (2009) reported that *Crepis* pollen has small polar areas with 1-10 spines. This study observed that the polar areas are small, and the number of spinules is low (Figures 1, 2, 3 and 4). In addition, there are visible collapsed areas in the polar regions of the pollen in this study. However, this is not reported by Wang et al. (2009).

In the study of Enke (2009), pollen of *C. foetida* was specified as echinolophate between 26-32 µ. In this study, *C. foetida* subsp. *rhoeadifolia* pollen is echinulophate with a size of 23-30 µ. The results obtained in this study regarding pollen size and ornamentation characteristics are in harmony with the results of Enke (2009). Unlike the polar region of *Crepis foetida*, depressive areas are seen in the polar region of the pollen of *C. foetida* subsp. *rhoeadifolia* (Figures 1 and 2).

The results of this research are similar to the results of the study conducted by Osman (2006). In his study, Osman (2006) evaluated the *Crepis* genus within the Launaea pollen type. Palynological features observed in Launaea pollen type and morphological features of *Crepis* pollens in this study are similar. To summarise, both Launaea type and *Crepis* pollens have 15 lacunae (3 poral, 6 abporal, 6 paraboral). Osman (2006) examined *Crepis* species within the pollen species of Launaea and expressed the polar axis length in the range of 32-44 µ. However, the polar-equatorial axis dimensions of the pollens measured in this study are shorter than the lengths specified in the pollen type of Launaea, and the pollens are small.

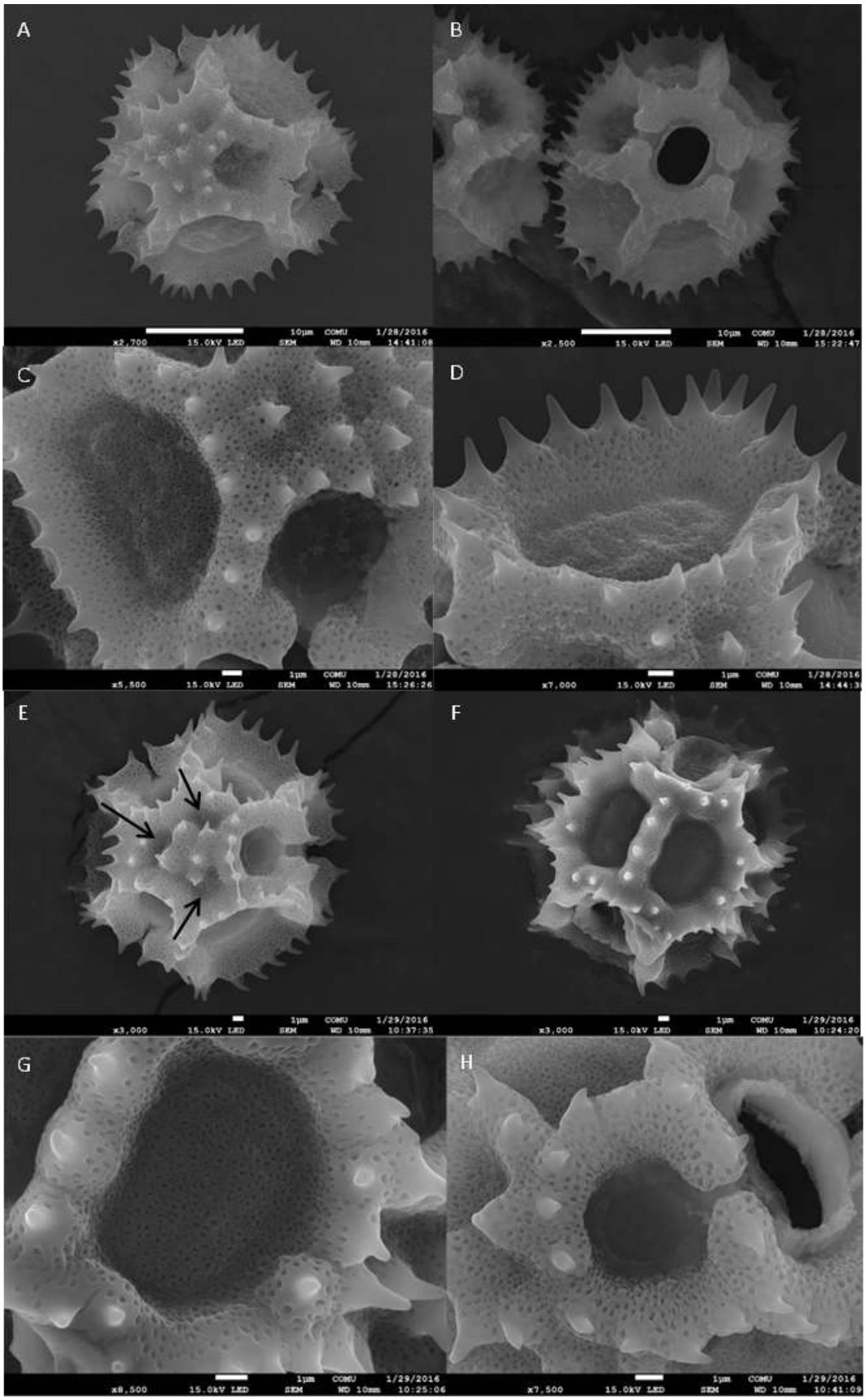

Figure 2: Pollen morphology of Crepis by SEM. A-D C. foetida subsp. rhoeadifolia; E-H C. Commutata
Şekil 2. SEM ile Crepis polen morfolojisi. A-D C. foetida subsp. rhoeadifolia; E-H C. Commutata

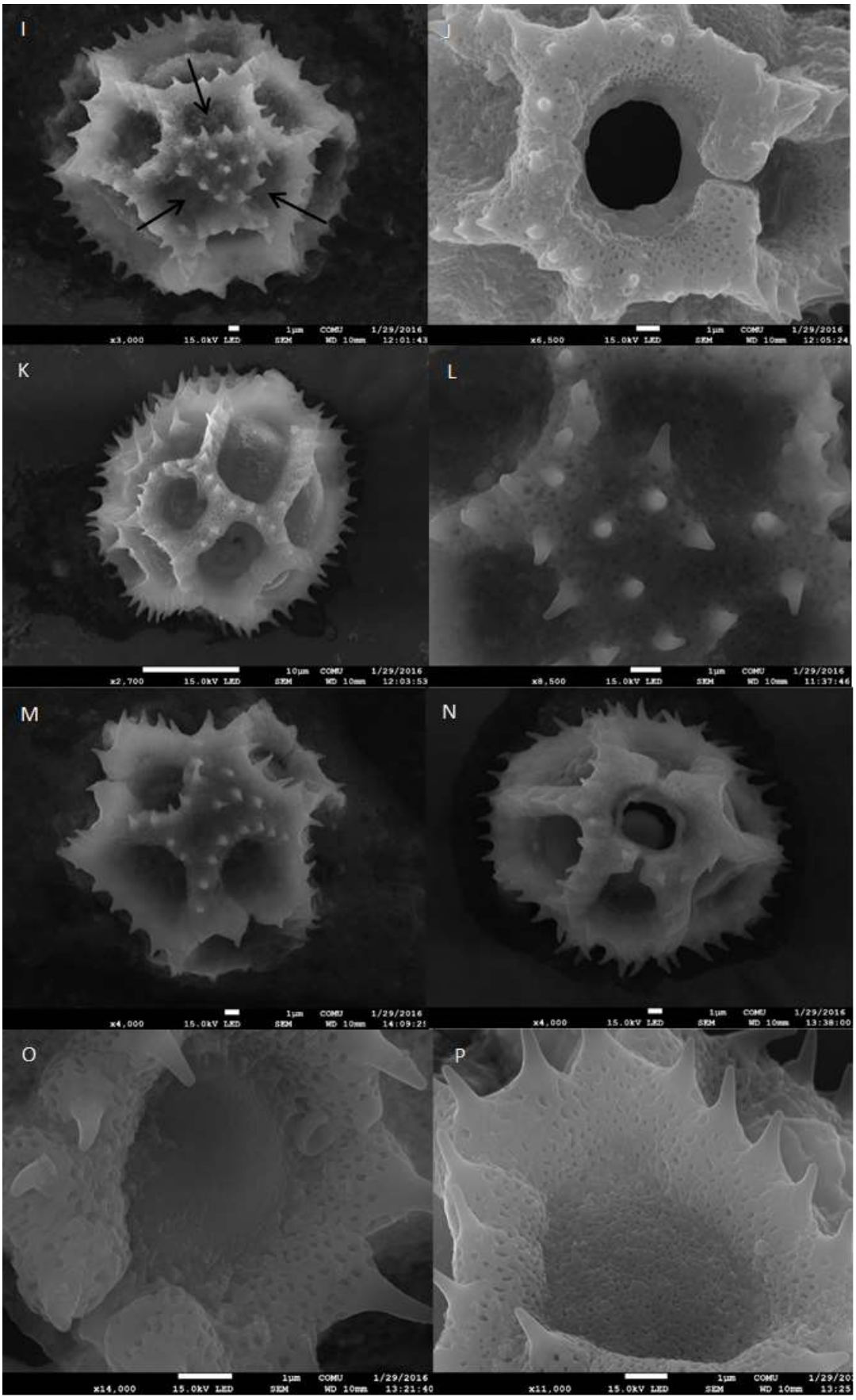

Figure 3: Pollen morphology of Crepis by SEM. I-L C. smyrnaea; M-P C. micrantha
Şekil 3: SEM ile Crepis polen morfolojisi. I-L C. smyrnaea; M-P C. micrantha

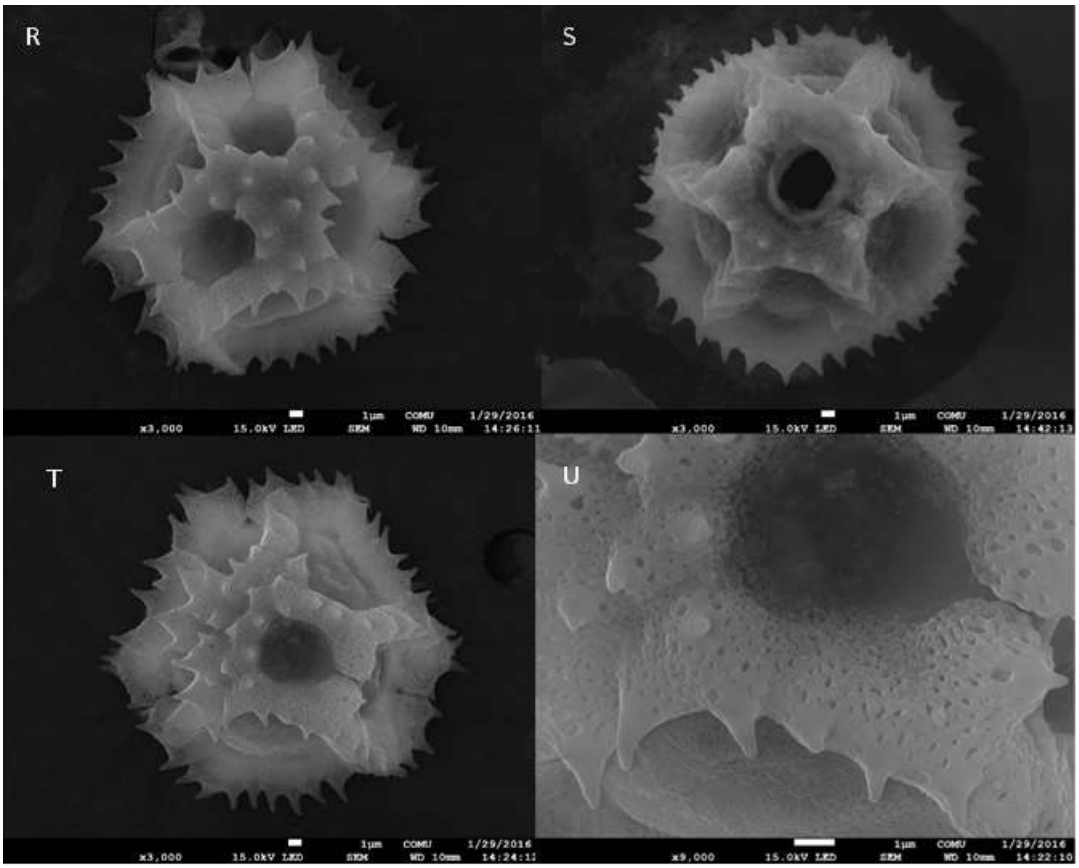

Figure 4: Pollen morphology of Crepis by SEM. R-U C. reuteriana subsp. reuteriana
Şekil 4: SEM ile Crepis polen morfolojisi. R-U C. reuteriana subsp. reuteriana

Peng et al. (2013) defined the morphological characteristics of the *Crepis* genus pollen in their study. They show similarities with the results of this study, except for the pollen sizes.

Dauti et al.(2018) compared the pollen morphology of *C. albenica* with the pollen morphology of four *Crepis* species. As a result, he stated that the pollen of *C. albenica* is larger than the other species. The taxa of *Crepis* examined in this study are similar to other taxa except for *C. albenica.*

Qiu et al. (2020) described a new species from the genus *Crepis* with morphological and molecular data. The results of this study show similarities, except for pollen sizes.

CONCLUSION

This study revealed pollen morphological characteristics of *Crepis* taxa for the first time, except for light microscopy measurements of the *C. foetida* subsp. *rhoeadifolia* taxa. The pollens of the genus *Crepis* examined within the scope of this research are compatible with the results of research in other countries regarding general pollen morphological characteristics. The most important difference of this study from other studies is that the pollen sizes are smaller, and *C. micrantha* has the smallest pollen among the taxa studied. The second significant difference is the presence of depressions in the polar areas of the studied taxa. However, the depressions are very prominent in the polar areas of *C. commutata* and *C. smyrnea* pollens. These depressions are so obvious that they appear as separate lacuna at the polar area border of paraboral lacunae. According to the type records of the Flora of Turkey (Davis, 1975), *C. smyrnaea* was collected for the first time in Çanakkale within the scope of this study. It is thought that it would be helpful to carry out pollen morphology studies, including all taxa of the genus *Crepis* and to evaluate the results of the genus by systematists. We believe the data obtained from this study will be an essential source for the pollen atlas of Turkish plants to be prepared in the future.

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of Sunay ALTAN's master's thesis. Some information about the study was presented as a poster at the 14th International Palynology Congress (IPC) and the 10th International Paleobotanical Organization Conference (IOPC) held in Salvador, Brazil, on October 23-28, 2016.

Author Contributions

Hanife Akyalçın: Project management, laboratory analysis, article writing and editing. Sunay Altan: Laboratory analysis, article writing and editing.

Conflicts of Interest

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

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Anatomical Characterization, Antimicrobial and Antimutagenic Properties of *Turgenia latifolia* (L.) Hoffm. (Apiaceae)

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ABSTRACT

The anatomical definition, antimicrobial, and antimutagenic activity of *Turgenia latifolia* (L.) Hoffm. (Apiaceae) were investigated in this research. The root cortex region of the plant is quite narrow and the cambium has 3-4 layers. Epidermis contains non-glandular trichrome in stem and leaf. Numerous secretory channels and collenchyma cells were reported in the stem cortex. Cells of palisade parenchyma were present on both sides of the leaf. Cells of spongy parenchyma were reduced to a thin layer in the center of the mesophyll and had 1–2 layers. Stomata were anomocytic. The antibacterial and antifungal activities of the methanolic leaf extract of *T. latifolia* were investigated against some selected pathogenic gram (+) (*Micrococcus luteus*, *Bacillus cereus*, *Staphylococcus aureus*, *Staphylococcus epidermis*), gram (-) bacteria (*Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella typhi*, *Proteus vulgaris*, *Enterobacter aerogenes*) and yeast (*Candida albicans*). The extract exhibited varying degrees of inhibitory effects on the growth of the different pathogenic strains. In addition, methanol leaf extract of *T. latifolia* (TLm) was analyzed for mutagenic activity. The results showed that TLm exhibited antimutagenic activity at concentrations of 10, 25 and 50 µL.

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Turgenia latifolia (L.) Hoffm'un (Apiaceae) Anatomik, Antimikrobiyal ve Antimutajenik Özellikleri

ÖZET

Bu araştırmada, *Turgenia latifolia* (L.) Hoffm'un anatomik, özellikleri, antimikrobiyal ve antimutajenik aktiviteleri araştırılmıştır.Bitkinin kök korteks bölgesi oldukça daralmış olup kambiyum 3-4 katmanlıdır. Gövde ve yaprak, epidermis örtü tüylerine sahiptir. Gövde korteksinde çok sayıda salgı kanalı ve kollenkima hücresi belirlenmiştir. Yaprağın her iki yüzeyi palizat parankiması hücreleri içerir. Sünger parankiması hücreleri, mezofilin merkezinde ince bir tabaka halinde olup ve 1-2 sıralıdır. Stomalar anomositiktir. *T. latifolia*'nın metanol yaprak ekstraktının antibakteriyal ve antifungal aktivitesi, Gram (+) (*Micrococcus luteus*, *Bacillus cereus*, *Staphylococcus aureus*, *Staphylococcus epidermis*) ve Gram (-) (*Klebsiella pneumonia*, *Pseudumonas aeroginosa*, *Escherichia coli*, *Salmonella typhi*, *Proteus vulgaris*, *Enterobacter aerogenes*) bakterilerine ve mayaya (*Candida albicans*) karşı araştırıldı. Ekstrakt, farklı patojenik suşların gelişmesi üzerinde değişen derecelerde inhibisyon etkisi göstermiştir. Ek olarak, *T. latifolia*'nın metanol yaprak ekstresinin (TLm) mutajenik aktivitesi analiz edildi. Sonuçlar, TLm'nin 10, 25 ve 50 uL'lik konsantrasyonlarının antimutajenik aktiviteye sahip olduğunu gösterdi

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INTRODUCTION

Apiaceae (Umbelliferae) is an economically important family and is a large family that includes 300–462 genera and 2,500–3,750 species around the world (Cronquist & Takhtadzhian, 1981; Heywood, 1993; Pimenov & Leonov 1993). The Apiaceae family is the eighth largest family in Türkiye and 451 species are represented (Davis, 1972). Of the 101 genera belonging to the family, 53 contain only one species. Four genera and 53 species were identified in recent studies about the flora in Türkiye. The endemism rate in Türkiye is 33%. 37 of 159 endemic species are endangered. There is a species belonging to the genus *Turgenia* identified in Türkiye (Güner et al., 2012). The Apiaceae family is the largest family utilized in conventional treatment in Türkiye. *Turgenia latifolia* (L.) Hoffm., known as Karaheci or Pıtrakin Turkish, is also distributed in Europe (Menemen, 2012), North Africa, Southwest Asia, Turkmenistan, Pakistan, and Kashmir (Cullen, 1972). The taxon is used in Iran to treat urinary tract problems (Mosaddegh et al., 2012). The species is used for rheumatism in Türkiye (Bulut et al., 2014).

Metcalfe and Chalk (1950) and Watson and Dallwitz (1992) clarified the anatomical characteristic features of the Apiaceae family. Members of the Apiaceae family, which are used as medicine (especially for antimicrobial properties), food and spice, landscape and ornamental plants, and animal feed due to the alkaloids and resins they contain, are among plant groups with economic value in the world (Shahsavari et al., 2008; Enez et al., 2016). Nowadays, herbs and herbal medicine raw materials constitute 25% of prescription medicines (Farnsworth et al., 1985). The inadequacy of synthetic drugs against increasing diseases in recent years and the detection of side effects have increased the necessity of using natural products. For this purpose, many plants were investigated in terms of microbiological pharmacological aspects and even in terms of plant defense mechanisms for biological warfare in recent years (Vanderbank, 1949).

Studies about the vegetative anatomy of *Turgenia* and its antimicrobial, antimutagenic and mutagenic features were not found in the literature. In this study, the aim was to investigate the *T. latifolia* species in Türkiye in detail in terms of anatomical, antimicrobial and mutagenic features. These findings will be presented for the first time and will serve as a resource for future studies about the species.

MATERIAL and METHOD

The samples of *T. latifolia*, selected as research subject, were collected from Kırşehir (Türkiye) in 2019. They were collected from the coordinates N 39°10′0.86″, E 34°26′15″ in May–July and converted to alcohol and herbarium samples. Species were identified according to Davis (1972).

Anatomical method

Sections were made into a permanent preparation according to the glycerin gelatin method (Vardar 1998). The cell types obtained from the root, stem and leaf sections of the species were determined by using Upright Microscope Eclipse Ni-U imaging system and photographed. Cell measurements were made from transverse and superficial sections of the taxa. Stomata and epidermis cell numbers per mm^2 were counted on the lower and upper surfaces of leaves of the same age and the stomata index was calculated (Meidner & Mansfield, 1968). Analysis of anatomical studies was made using 20 plant specimens. An average of 25 measurements was taken from tissues such as epidermis, periderm, parenchyma, collenchyma, and sclerenchyma (Table 1).

Antimicrobial method

Fresh leaves (100 g) were ejected with 1 L of methanol using a Soxhlet (ISOPAD, Heidelberg, Germany) device for 72 h at a temperature not exceeding the welding point of the solvent. The extract was filtered using Whatman filter paper (No. 1), and then concentrated in a vacuum at 60°C using a rotary evaporator (Buchi Labortechnik AG, Flawil, Switzerland). Leaf extracts were then freeze-dried and kept in the dark at + 4°C until experiments. Pathogenic bacterial cultures (*Staphylococcus aureus* ATCC25923, *Escherichia coli* ATCC1280, *Salmonella typhi* H NCTC901.8394, *Staphylococcus epidermidis* ATCC12228, *Micrococcus luteus* ATCC9341, *Bacillus cereus* RSKK863, *Enterobacter aerogenes, Klebsiella pneumonia* ATCC 27853, *Proteus vulgaris* RSKK 96026, *Pseudomonas aeruginosa* ATCC27853) and yeast (*Candida albicans* Y-1200-NIH) were used. Methanolic leaf extracts of *T. latifolia*, were analyzed for their antimicrobial activity using the well-diffusion method against pathogenic gram-positive bacteria (*S. aureus*, *S. epidermidis*, *M. luteus*, *B. cereus*), gram-negative bacteria (*S. typhi*, *E. coli*, *K. pneumonia*, *P. vulgaris*, *P. aeruginosa*, *E. aerogenes*), and one yeast (*C. albicans*) (Öğütcü et al., 2017; Rubab et al., 2021). Methanol was employed as resolvent for the extract and for control. In methanol, the organisms tested did not show antimicrobial activity. Then 1% (v/v) of 24-hour broth culture (selected pathogenic bacteria and yeast) containing 10^6 CFU/mL was placed in a sterile petri dish. Mueller-Hinton Agar (MHA) (15 mL) kept at 45°C was then poured into the Petri dishes and

allowed to solidify. Then, wells of 6 mm diameter were - carefully punched by employing a sterile cork borer and were entirely filled (20 µL) with extract. The plates were incubated for 24 h at 37 ^{0}C in the incubator. At the end of incubation, the average value obtained for two wells was employed to calculate the growth inhibition zone for each pathogenic bacteria and yeast (pathogenic bacteria and yeast were tested for resistance to four antibiotics (Kanamycin, sulfamethoxazole, ampicillin, amoxicillin) and one anticandidal (Nystatin) agent) (Nartop et al., 2020).

Table 1. Anatomical measurements of *T. latifolia*
Çizelge 1. T. latifolia'nın anatomik ölçüm sonuçları

Plant organ	Characteristic	Width (µm) Mean ± SE	Length (µm) Mean ± SE
Root	Periderm cells	23.75±1.88	40.89±1.80
	Diameter of trachea	25.73±1.05	
	Phloem cells	9.23±0.58	
	Cambium cells	4.12±0.36	
Stem	Epidermis	16.27±1.52	22.61±1.39
	Cortex cells	41.35±4.36	
	Cuticle	10.48±0,65	
	Sclerenchyma	9.25±1.00	
	Collenchyma	11.36±0.92	
	Diameter of trachea	21.46±1.17	
	Phloem	7.28±0.78	
	Pith cells	73.66±6.87	
Leaf	Upper epidermis cells	10.84±0.83	22.07±1.31
	Lower epidermis cells	16.35±1.29	27.92±1.79
	Lower epidermis cuticle	8.87±0.23	
	Upper epidermis cuticle	5.50±0.29	
	Spongy parenchyma cells	21.52±1.78	
	Palisade parenchyma cells	9.02±0.42	35.08±2.70
	Collenchyma	18.73±1.47	
	Diameter secretory channels	51.84±4.58	
	Trachea	18.73±1.47	

SE, Standard error.

Determination of Antimutagenic Activity: Micronuclei Test

The antimutagenic activity of methanol leaf extract from *T. latifolia* (TLm) was studied against sodium azide (NaN_3) in human lymphocyte cells with the micronuclei (MN) test. Eight culture media were created in the study. The 1st culture medium was negative control (pure water), 2nd culture medium was positive control (5 µM NaN_3), and the 3rd culture medium was only TLm (25 µL). TLm was added to other tubes (4-8) along with NaN_3 (5 µM) at concentrations of 10, 25, 50, 75 and 100 µL, respectively. The concentrations used in the study were determined based on preliminary studies.

For the determination of micronuclei, the procedure previously described by Fenech (2000) was used. Forty-four after the initiation of incubation at 37 °C, cytochalasin-B was added to each tube at a final concentration of 3 µg/mL to prevent cytoplasmic division. After 72 h, cells were removed from the incubator. Samples were centrifuged at 1000 rpm for 10 min. After removing the supernatant, a hypotonic solution (6 mL -0.075 M KCl) was added and samples were replaced in the incubator (7 min.). Cells were then immediately centrifuged and fixed three times with cold methanol/glacial acetic acid (3:1). The fixed cells were dropped onto slides and allowed to dry at room temperature (72 h). The preparations were stained with 6% Giemsa (Merck, Darmstadt, Germany) for 10 min. For MN analysis, bi-nucleated cells were evaluated under a light microscope (magnification 1000x) and scored (Nartop et al., 2020).

Statistical analysis

In this study, three replicates of all experiment groups were used for the reliability of data. The data in each experiment group were analyzed with SPSS 18.0 version using a one-way analysis of variance. Significance was determined by Duncan's test. In statistical calculations, the significance level was taken as $p<0.05$.

RESULTS and DISCUSSION

Anatomical results

Periderm was 5-8 layers on the outer surface of the root. The cortex had very few layers and parenchymatic cells were crushed in some parts (Table 1). Phloem elements were located in the large region of

the root, and were polygonal shaped with 10–15 layers. Cambium cells had 3–4 layers and were undulating. Xylem covered a large area, composed of trachea and sclerenchyma cells. Trachea differed in size (Figure 1 A, B).

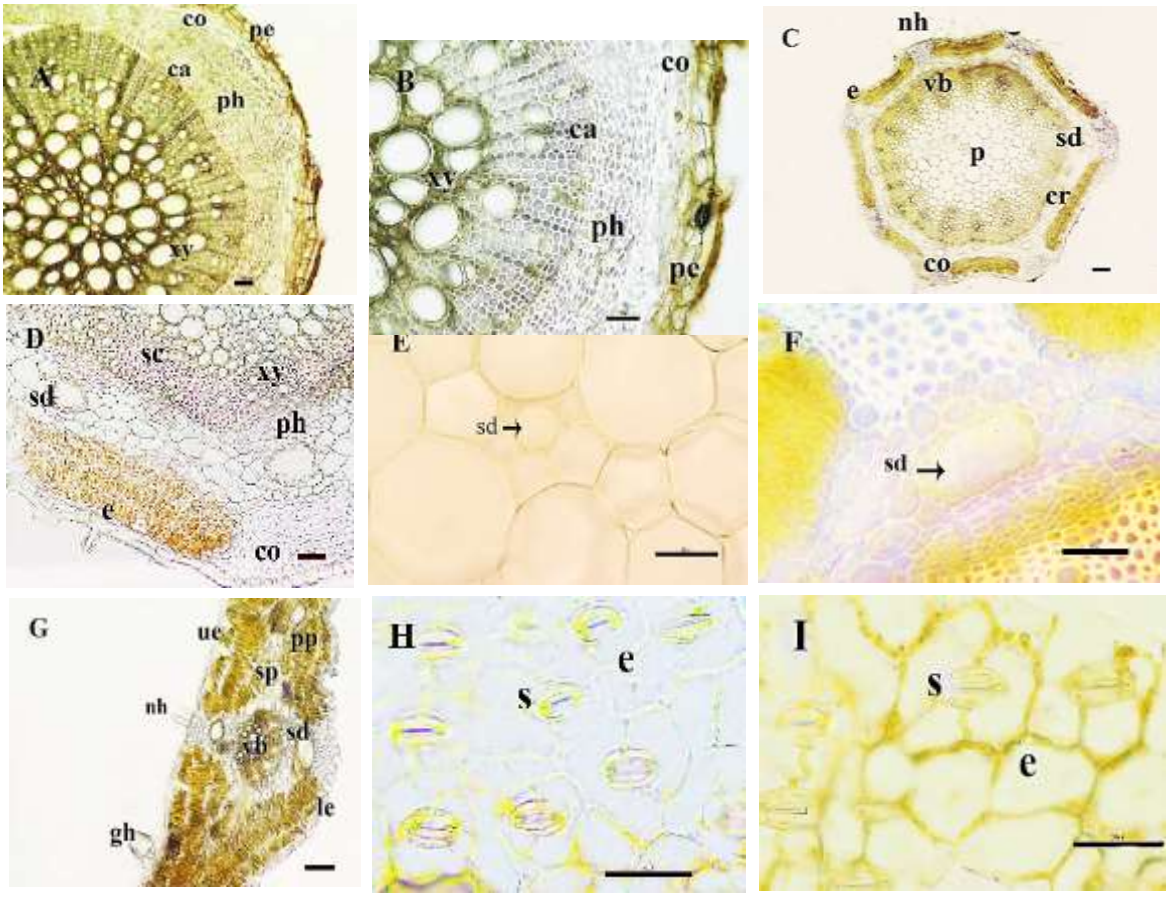

Figure 1. *T. latifolia* A, B root transverse section; C, D, E, F stem transverse section; G leaf transverse section; H leaf lower superficial section; I upper leaf superficial section. ca, cambium; cr, cortex; co, collenchyma; e, epidermis; gh, glandular hair; le, lower epidermis; nh, non-glandular hair; p, pith; pe, periderm; ph, phloem; pp, palisade parenchyma; xy, xylem; s, stomata; sc, sclerenchyma; sd, secretory duct; sp, spongy parenchyma; ue, upper epidermis; vb, vascular bundle (Scale 50 µm)

Şekil 1. T. latifolia A, B kök enine kesiti; C, D, E, F gövde enine kesiti; G yaprak enine kesiti; H yaprak alt yüzeysel kesiti; I yaprak üst yüzeysel kesiti. ca, kambiyum; cr, korteks; co, kollenkima; e, epidermis; gh, salgı tüyü; le, alt yüzey epidermis; nh, örtü tüyü; p, öz; pe, peridermis; ph, floem; pp, palizat parankiması; xy, ksilem; s, stoma; sc, sklerenkima; sd, salgı kanalı; sp, sünger parankiması; ue, üst epidermis; vb, vasküler demet (Skala 50 µm)

According to the stem transverse section of *T. latifolia*, the stem was wavy shaped. Epidermis cells were circular and rectangular and covered with cuticles. In addition, non-glandular hairs were observed on the epidermis (Table 1). There was collenchyma in groups in the cortex consisting of polygonal cells (Figure 1 C). Cortex parenchyma cells were polygonal and cylindrical shaped, with 2-3 layers. In the cortex, 14–16 secretory channels were seen (Figure 1 D, E). Vascular bundles also continued in the intervascular space. Cambium cells were distinguishable and had 1–2 layers. Sclerenchyma cells were located around the bundles. The large pith region was composed of parenchymal cells. As in the cortex, secretory canals were observed in the pith region (Figure 1 F).

According to the leaf transverse section, epidermis cells on the upper and lower surfaces had one order. Epidermis cells were generally oval and rectangular in shapes. Non-glandular and glandular hairs were observed on the epidermis. The cuticle was observed on the lower and upper epidermis. One vascular bundle was observed in the midrib region. The leaf was in isolateral type. On the upper and lower surfaces, there were 2–3 layers of thin, long, cylindrical palisade parenchyma containing plenty of chloroplasts. Spongy parenchyma had 1–2 layers. A secretory channel was

observed on the leaf. Stomata were present on the abaxial and adaxial surface (Figure 1 G, H, I). The number of upper surface stomata cells was 79 and was 160 for epidermis. Again, the number of lower surface stoma cells was 40 and was 133 for epidermis. The stomata index on the leaf upper surface was 33.05. The stomata index on the leaf lower surface was 22.98.

Antimicrobial activity

The antibacterial and antifungal activity of methanolic leaf extracts from *T. latifolia* were investigated against some microorganisms causing disease including gram-positive and gram-negative bacteria and yeast. These extracts demonstrated changing inhibitive efficacies (19 mm-30 mm). (19 mm-30 mm) when a dose of 20 mg/ml was used with the tested pathogenic species. The antibacterial activity of *T. latifolia* was compared with five commercial antibiotics and methanol leaf extracts that were as effective as the afore mentioned antibiotics (Figure 2, Table 2).

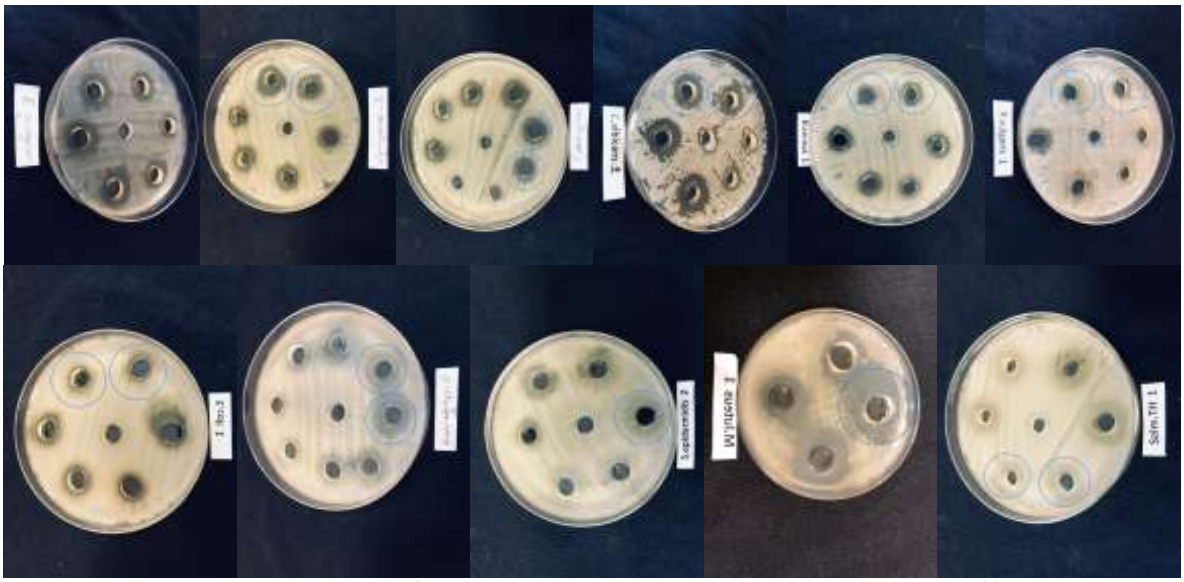

Figure 2. Photographs of inhibition zones (mm) of some pathogenic Gram (+) and Gram (-) bacteria and yeast.
Şekil 2. Bazı patojenik Gram (+) ve Gram (-) bakteri ve mayaların inhibisyon bölgelerinin (mm) fotoğrafları.

Table 2. Antimicrobial activities of leaf extract of *T. latifolia*
Çizelge 2. T. latifolia'nın yaprak ekstraktının antimikrobiyal aktiviteleri

Microorganism		Leaf extract	AMP 10*	SXT 25	AMC 30	K 30	NYS 100
Gram (+)	*M. luteus*	30	22	21	25	23	N
	S. epidermis	19	26	25	27	25	N
	S. aureus	20	30	24	30	25	N
	B. cereus	21	23	25	20	28	N
Gram (-)	*E. aerogenes*	22	21	19	20	24	N
	P. aeruginosa	21	8	18	15	14	N
	K. pneumonia	20	21	20	21	23	N
	S. typhi H	24	11	17	19	20	N
	E. coli	20	10	18	14	25	N
	P. vulgaris	21	17	19	20	21	N
Yeast	*C. albicans*	30	N	N	N	N	20

*Standard reagents (Diameter of zone inhibition (mm). SXT25, Sulfamethoxazole; AMP10, Ampicillin; NYS100, Nystatin; K30, Kanamycin; AMC30, Amoxycillin; N, Not tried.

Antimutagenic activity

We analyzed MN after treatment with different concentrations of TLm (10, 25, 50, 75 and 100 µL) against sodium azide (NaN_3). When the results in Table 3 are evaluated, TLm exhibited antimutagenic activity at concentrations of 10, 25 and 50 µL and was particularly effective at a concentration of 50 µL. However, increasing concentrations of TLm (75 and 100 µL) increased the mutagenic effect of NaN_3 in a dose-dependent manner.

In addition, we investigated the antimutagenic effects of leaf extracts of *T. latifolia* against mutagenicity of NaN_3. As seen in Table 3, leaf extracts of *T. latifolia* exhibited antimutagenic activity against the mutagenicity of NaN_3 at certain concentrations (10, 25 and 50 µL).

Table 3. Effects of NaN_3 and TLm on MN in human peripheral lymphocytes

Çizelge 3. NaN_3 ve TLm'nin insan periferik lenfositlerinde MN üzerindeki etkileri

Test Item	Concentration	MN numbers ± SE
Control		1.76 ± 0.62[a]
NaN_3	5 µM	3.20 ± 0.38[de]
TLm	25 µL	1.92 ± 0.65[a]
NaN_3+ TLm	5 µM + 10 µL	3.94 ± 0.83[cd]
NaN_3+ TLm	5 µM + 25 µL	2.80 ± 0.67[c]
NaN_3+ TLm	5 µM + 50 µL	2.36 ± 0.62[b]
NaN_3+ TLm	5 µM + 75 µL	3.35 ± 0.92[e]
NaN_3+ TLm	5 µM + 100 µL	3.68 ± 0.72[e]

[a-e]Mean ± SE, Values within each column not sharing a common superscript are significantly different ($p<0.05$) as determined by Duncan test.

In this research, the anatomical, antimicrobial, mutagenic and antimutagenic properties of the *T. latifolia* taxon, which is naturally found in Kırşehir (Türkiye) and surroundings, were investigated. No anatomical information on *T. latifolia* was available in the literature except for general taxonomic properties. The *T. latifolia* taxon had a secondary root structure. In roots, the cortex was narrowed and the central cylinder was filled with xylem elements. The tracheas in the center had larger diameters. Numerous secretory channels were observed in the stem cortex of *T. latifolia*. In *Scandix iberica* Bieb. species belonging to Apiaceae family, secretory cells in stem cortex were also mentioned (Demirpolat et al., 2019). The presence and location of collenchyma on the stem of Apiaceae famlya has taxonomic value. Stesevic et al. (2016) stated that the presence of collenchyma varies in some species belonging to the family. Collenchyma in the stem of *T. latifolia* is located in clusters just below the epidermis. In an anatomical study conducted with three genera belonging to the family, the differences of collenchyma cells between the genera are mentioned and their distinctive features are emphasized (Idman et al., 2019). In our study, secretory cells were also seen in the pith region. The Apiaceae species have special fragrances as they contain secretory cavities, which are schizogenous oil channels containing resin, oil, or mucilage. They were found in roots, petioles, stems, leaves, and fruits (Metcalfe & Chalk, 1979; Plunkett et al., 2014). The nature of the secretory elements and their contents is taxonomically important (Metcalfe, 1944). In our study, secretory canals were found in the stem, cortex and pith of the species. The number of secretory channels in the cortex especially may be characteristic for the species. Sclerenchyma cells were seen between vascular bundles based on the stem cross-section. Esau (1977) reported that in members of the Apiaceae family, the stem inter fascicular cambium sometimes produces more sclerenchyma cells towards the xylem side. The epidermis cells of the leaf of the species consisted of oval- and rectangular-shaped cells. Anisocytic stomata were scattered on both the lower and upper surfaces. Two to three epidermis cells formed around the stoma cells. The leaf was isolateral type. Metcalfe and Chalk (1972) reported that the number of epidermis cells with stomata in members of the Apiacaeae family, which mostly had isolateral leaf structure, could be very variable.

Methanol leaf extract of *T. latifolia* was examined against ten bacterial and one fungal pathogenic strain. It was concluded that this extract was more effective against gram (-) bacteria than gram (+) bacteria (Table 2). The possible reason for this may be the presence of an external impermeable membrane, fine peptidoglycan monolayer, presence of periplasmic cavity and cell wall in the composition of gram (-) bacteria (Afzal et al., 2017). *M. luteus* is considered a profiteering pathogen that may be responsible for hospital infections. It can also cause skin disease and septic shock in immunocompromised patients. This extract showed higher inhibitory activity (30 mm) against *M. luteus* than all standard antibiotics tested (Table 2) (Gül et al., 2020).

Leaf extract of *T. latifolia* exhibited higher activity (21mm) than standard antibiotic AMP10 (20mm) against *B. cereus* (Table 2). *Salmonella serovars* cause many different clinical symptoms, ranging from asymptomatic infection to severe typhoid-like syndromes in infants or in some high-sensitivity animals (Karadeniz et al., 2019; Karakılıç et al., 2020). Leaf extract of *T. latifolia* showed higher inhibition activity (24mm) than all standard antibiotics against this important pathogen of *S. typhi* (Table 2).

Leaf extract showed significantly higher inhibition activity (21 mm) than all canonical antibiotics against *P. aeruginosa* (Table 2). The genus *Pseudomonas* is widespread in nature, and causes opportunistic, and nosocomial infections. *P. aeruginosa* is a leading cause of nosocomial infections, may improve resistance to various antibiotics and cause high mortality and morbidity because of infections (Pollack, 1995; Hanberger et al., 1999). It is responsible for 10–25% of nosocomial infections (Günseren et al., 1999). As this bacteria is generally resistant to multiple antibiotics, it also causes trouble for treatment. *P. aeruginosa* septicemia occurs particularly in debilitated and immunocompromised patients and the mortality rate is 10–20% (Nadaroglu et al., 2020).

In *K. pneumonia*, leaf extract showed similar degree (20 mm) of inhibition activity as the antibiotic SXT25 (Table 2). Also, this extract had higher inhibitory effect than standard antibiotics SXT25 (18 mm), AMP10 (10 mm) and AMC 30 (14 mm) against *E. coli* (20mm) (Table 2). The extract showed higher inhibition activity (21 mm) against *P. vulgaris* than the canonical

antibiotics AMP10 (17 mm) SXT25 (19 mm) and AMC 30 (20 mm). It had the same inhibitory activity as the K30 (21 mm) standard antibiotic (Table 2). *P. vulgaris* is easily isolated in long-term care facilities and hospitals and patients with underlying diseases or patients with weak immune systems. Patients with recurrent infections, those with structural abnormalities of the urinary tract, those with urethral instrumentation, and those with hospital infections have increased intensity of infections caused by *Proteus* spp. and other microorganisms. In the case of *E. aerogenes*, this extract (22 mm) showed high inhibition activity compared to the standard antibiotics, except for K30 (24 mm) (Table 2).

Systemic fungal infections, including *C. albicans*, have emerged as significant causes of morbidity and mortality in immunosuppressed patients (Cancer chemotherapy, tissue or organ transplantation, AIDS) (Sarı et al., 2013; Nartop et al. 2014). Leaf extract of *T. latifolia* showed higher inhibitory effect (30 mm) than the commercial antifungal (20 mm) (Table 2). Also, other reports indicated that total plant extract of *T. latifolia* exhibited anticandidal effect against *C. albicans,* but stimulating effects for other *Candida* species (Sardari et al., 1998).

Bazzaz and Haririzadeh (2003) found that total plant extract of *T. latifolia* did not have any effect on *C. albicans*, *E.* coli and *S. typhi*, but its effect was insignificant against *B. subtilis*, *Morganella morgani*, *P. aeruginosa* and *S. aureus*. However, in our study, methanol leaf extracts of *T. latifolia* showed higher inhibitory activity than standard antibiotics and anticandidal agents against *P. aeruginosa*, *S. typhi*, *E. coli* (except K30) and *C. albicans* (Table 2).

This study is the first record of antimicrobial activity of methanolic leaf extract of *T. latifolia* and high antimicrobial activity was observed against the bacteria and yeast tested.

To our knowledge, there is no previous studies about the antimutagenic and mutagenic activities of *T. latifolia*. However, some species belonging to the Apiaceae family were reported to have antimutagenic effects, mutagenic effects, cytotoxic activities, and apoptosis properties (Abdelwahed et al., 2008). The antimutagenic effect of *T. latifolia* leaf methanol extracts at certain concentrations can be explained by the polyphenols (for example, flavonoids and tannins) and sesquiterpene coumarins. Actually, in previous studies, it was determined that some species belonging to the Apiaceae family contained polyphenols and sesquiterpene coumarin compounds These compounds are known to exhibit antimutagenic activity (Edenharder & Tang, 1997; Besaratinia & Pfeifer, 2004; Abdelwahed et.al., 2008; Kasaian & Mohammadi, 2018). Moreover, it was stated that polyphenolic compounds, especially flavonoids, can induce DNA damage depending on concentration and time, and that their genotoxic effects may be due to their prooxidant activities (Rusak et al., 2010; Ceker et al., 2019). In this study, the increased MN frequency at high concentrations of TLm (75 and 100 µL) may be related to the prooxidant activities of phenolic compounds. In the future, analysis of these compounds will help to clarify the cause of the biological activities of *T. latifolia*.

CONCLUSION

The root of *T. latifolia* is thickened and secondary. The shape of the stem is undulate. The stem has collenchyma and sclerenchyma tissue, non-glandular and glandular hair. Anisocytic stomata are distributed on both surfaces of the isolate leaf. A large number of secretory ducts form in the root, stem and leaf.

Microbial pathogens are a universal health concern and therefore new or alternative antibiotics are ingreat demand to combat infections and infections caused by resistant pathogens. Natural products are still recognized as unique resources.

Due to antimutagenic and antimicrobial properties, *T. latifolia* might be important as a medicinal herb. Because *T. latifolia* has the potential to be used as a new antimicrobial agent, it is thought that this study will help with the treatment of many diseases and guide future studies. Therefore, this study may provide a different perspective for pharmacological studies about the treatment of bacterial and fungal infections.

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Researchers' Contribution Rate Statement

The authors contributed equally to the article.

Conflicts of Interest Statement

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

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A New Taxon of Stachys (Lamiaceae) from Bingöl - Türkiye

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ABSTRACT
Stachys woronowii (Schisk. ex Gross.) R.R.Mill subsp. *bingolensis*, which has been collected from Genç district (Bingöl-Türkiye), has been defined as a new subspecies on behalf of the scientific world. This subspecies; differs from typical subspecies in plant height, verticillate number and spacing, and seed characteristics. *Stachys woronowii* (Schisk. ex Gross.) R.R.Mill subsp. *bingolensis* grows on steppe slopes in the oak forest openings. Description, photographs and general ecological preferences of the newly identified taxon are given.

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Bingöl (Türkiye)'den Yeni Bir Stachys (Lamiaceae) Taksonu

ÖZET
Genç (Bingöl-Türkiye) ilçesinden toplanan *Stachys woronowii* (Schisk. ex Gross.) R.R.Mill subsp. *bingolensis*, bilim dünyası adına yeni bir alttür olarak tanımlandı. Bu alt tür; bitki boyu, vertisillat sayısı ve aralığı ve tohum özellikleri bakımından tip alt türden farklıdır. *Stachys woronowii* subsp. *bingolensis* meşe açıklıklarındaki step yamaçlarda yetişir. Yeni olarak tanımlanan alttürün betimlemesi, ekolojik tercihleri ve fotoğrafları verildi.

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INTRODUCTION

The genus *Stachys* is one of the largest genera of the Lamiaceae family with 362 species in the world (POWO, 2023). Although the distribution of some taxa of this genus is in Europe, North and South America; the majority of members of the genus are native to the warm temperature regions of the Mediterranean and southwestern Asia. The genus members consisting of annual, perennial grasses and subshrubs form taxa; they grow in different habitats such as steppe, forest openings, rocky places, limestone, fallow fields, meadows and stream banks (Bhattacharjee, 1980).

In Türkiye, the genus *Stachys* is represented by 95 species (122 taxa), 65 of which are endemic, and the distribution of a significant part of these taxa is in the Mediterranean floristic region of Türkiye (Güner, 2022).

The province of Bingöl is located in the transition zone from the Eastern Anatolia Region of Türkiye to the Southeastern Anatolia Region. In the northern areas of Bingöl, the winter season is much more continental and cold, and the vegetation period is shorter; the south of this province is the areas that extend towards the Syrian deserts and have more summer drought. Bingöl is in a transition zone between these two contrasting climates. In the last 10 years, several plant taxa new for the scientific world (Behçet et al., 2017; Behçet & Yapar, 2020, 2021; Hamzaoğlu et al., 2020;

İlçim & Behçet, 2016; Doğan et al., 2015; Sinan et al., 2021) were published from Bingöl. In addition, new distribution records for Türkiye (Pınar et al., 2018; Behçet & Altınsoy, 2023; Behçet & Cengiz 2023a and 2023b) and some interesting taxa (Behçet & Yapar, 2019; Doğan & Behçet 2021) were determined.

In May 2023, the second author collected some interesting *Stacyhs* samples (Figure 1) from Genç district, which is located within the provincial borders of Bingöl and located in the transition zone between the Eastern Anatolia region and the Southeastern Anatolia region. Although these collected annual *Stachys* samples are similar to *S. woronowii* (Schisk. ex Gross.) R.R.Mill (Syn*: Sideritis woronowii* Schischk. ex Grossh., *Sideritis balansae* Boiss. and *Stachys pseudosideritis* R.Bhattacharjee & Hub.-Mor.) it was different from it with some features (Verticillaster numbers, longer lower internodes, longer plant height and robust structure and nutlet sizes and structure). *S. worowii* is an annual species (Figure 2) known to spread from Iran, Lebanon-Syria, Transcaucasus and Türkiye. *Stachys* specimens collected from Bingöl were compared with the *S. woronowii* based on the descriptions in the flora works of Türkiye (Bhattacharjee 1982), Russia (Knorrring 1977) and Iran (Rechinger 1982), and holotype images from herbarium G and the photograph taken by L. Behçet in 2023 among the rocks in the northwest of Elazığ (Figure 2); *Stachys woronowii* subsp. *bingolensis* has been identified as a new subspecies.

Figure 1. Habit of *Stachys woronowii* subsp. *bingolensis*
Şekil 1. Stachys woronowii subsp. *bingolensis'in habitusu*

MATERIALS and METHODS

Specimens belonging to *S. woronowii* subsp. *bingolensis* defined were collected from Genç district of Bingöl Province in Türkiye. In addition to the relevant literature (Bhattacharjee, 1982; Knorrring, 1977; Rechinger, 1982), samples from the herbaria BIN, GAZI, ANK, L, Max Nydegger and G were also used in the identification and evaluation of the specimens belonging to the subspecies.

RESULTS and DISCUSSION

Stachys woronowii (Schischk. ex Grossh.) R.R.Mill. subsp. *bingolensis* Behçet and Çetin, *subsp. nov.*

Type: Türkiye. B8 Bingöl: Genç district, west of Servi

town, around Harmancik village, 38° 33'541" K - 40° 15'595" D, openings of oak communities, cliffs-steppe areas, 1038 m a.s.l., 17.05.2023, *A. Çetin* 2711 (holo. BIN, iso. ANK, BIN) (Figures 1,3,4).

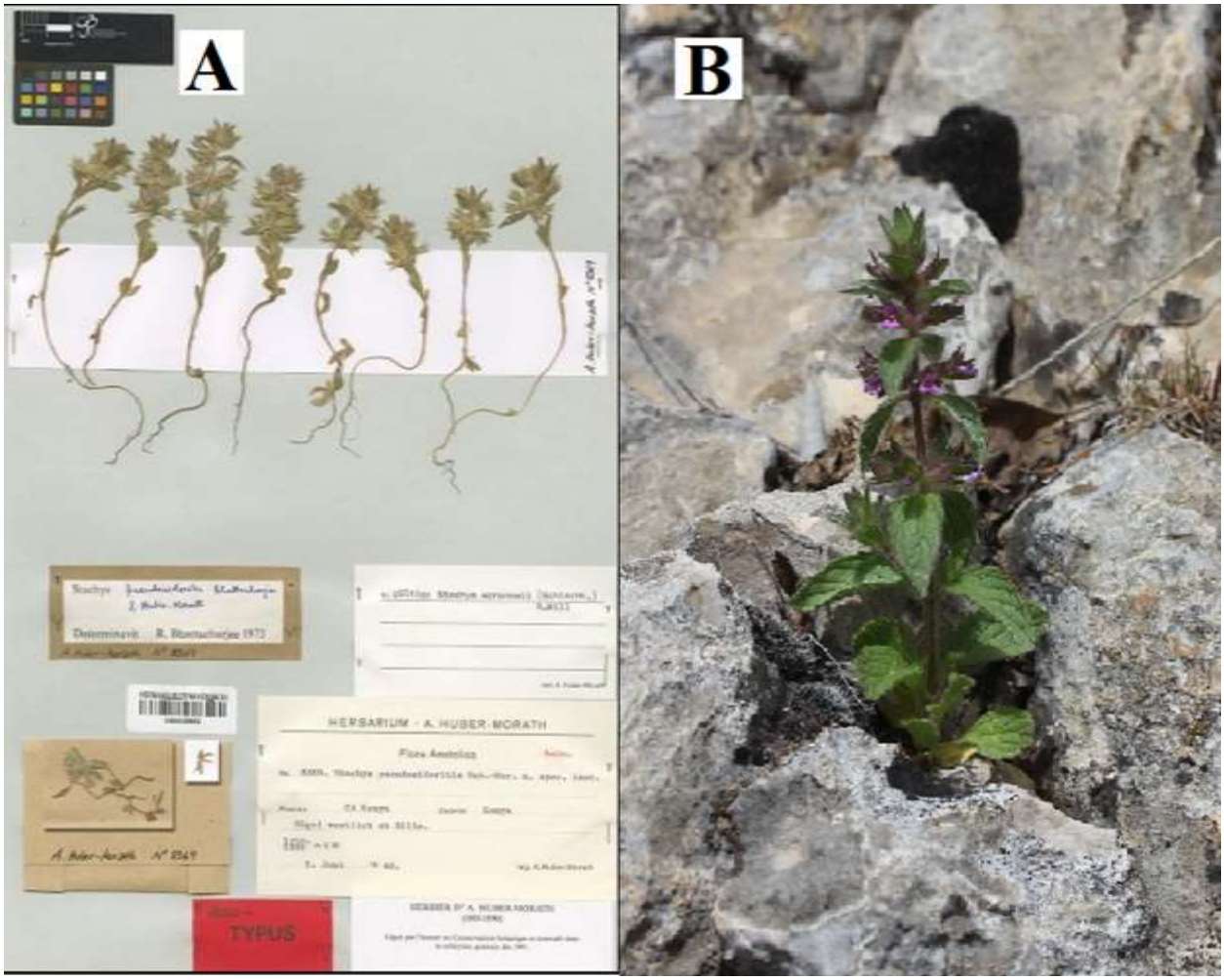

Figure 2. A. The image of *Stachys woronowii* holotype in Geneva Herbarium (G) (from JSTOR 2023), B. View of *Stachys woronowii* (subsp. *woronowii)* in its original habitat (Photo by L. Behçet)

Şekil 2. A. Cenevre Herbaryumundaki (G) Stachys woronowii holotipi'nin görünümü, B. Stachys woronowii (subsp. woronowii)'nin orjinal habitatındaki görünümü (fotoğraf L. Behçet)

Diagnosis: *Stachys woronowii* subsp. *bingolensis* differs from *Stachys woronowii* (subsp. *woronowii*)mainly because it has a plant height of 32 cm (not 2-24 cm); Verticillasters number up to 11 (not 2-8); lower verticillasters distance from each other to 37 mm (not 5-20 mm); nutlets 2 × 1.6 mm, smoth (not 1-1.5 × 1.2-1.5 mm and rugose).

Description: Annual herb, pale green, robust, divaricately branched or simple, 12-32 cm, densely glandular patent-pilose. Lower cauline and median leaves broadly obovate to elliptic, 20-35 × 8-16 mm, serrate, apex obtuse to broadly acute, base attenuate into 2-5 mm petiole. Floral leaves short-petioled to sessile, oblong to obovate, 15-25 × 5-10 mm. Vertici1lasters 8-11, lower ones distinctly remote, congested above, spicate, 5-37 mm apart, lower internodes far longer than the calyx length; (4-)6-flowered, Bracteoles linear to linear-lanceolate, herbaceous, softly spinescent, 6-7 mm, pilose. Pedicels 2-4 mm. Calyx deflexed, bilabiate, subcampanulate, 9-12 mm, base gibbous, constricted in upper part; calyx-teeth as long as or slightly shorter than tube, erect or spreading, tube strongly nerved, patent pilose; teeth unequal, erect, ovate-lanceolate mucronate, upper and lower teeth 3.5-4 and 5-5.5 mm respectively; abruptly attenuate to 1-1.5 mm mucro. Corolla pink, 8-10 mm, almost included. Stamens little exserted from corolla tube. Nutlets obovoid, 2 × 1.6 mm, smoth. Fl. 4-5; Fr.5-6. Oak forest scrub clearings, rocky limestone ridges, slopes, 1000-1100 m.

Ecology: *Stachys woronowii* subsp. *bingolensis* is a local endemic taxon which is distributed around Harmancık village within the borders of Genç district in the south and southwest of Bingöl Province in eastern Türkiye (Figure 4).

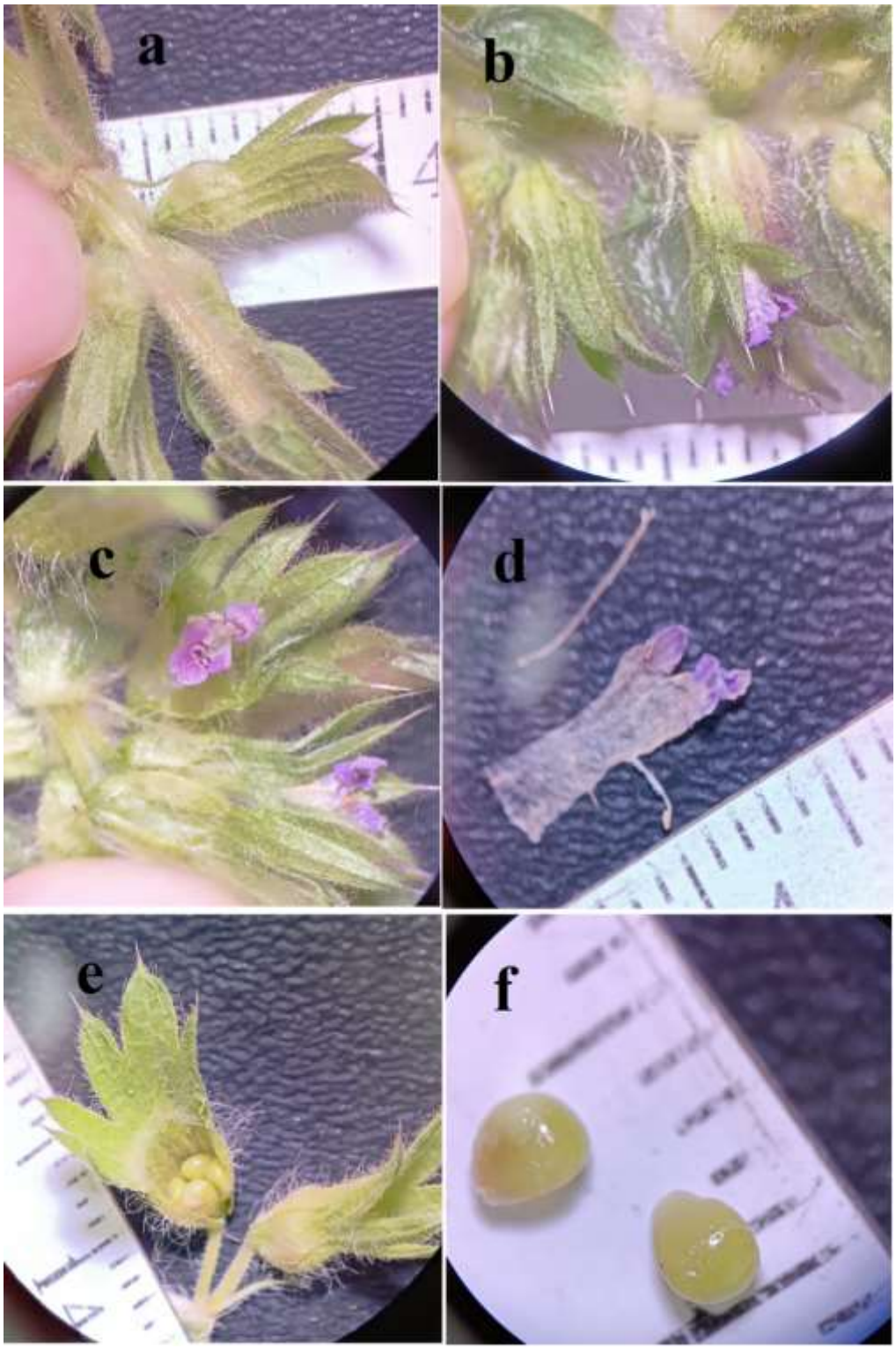

Figure 3. Stachys woronowii subsp. *bingolensis.* (a) Calyx, (b) Image of calyces in verticillasters, (c) Image of corolla shorter than calyx, (d) Style and dissected corolla, (e) The appearance of nutlets inside the calyx, (f) Nutlet structure.

Şekil 3. Stachys woronowii subsp. bingolensis. (a) Kaliks (b) Vertisillattaki kaliks görüntüsü (c) Kaliksten kısa olan korolla görünümü (d) Sitilus (boyuncuk) ve korollanın boyuna kesit görünümü(e) kaliks içindeki nutletlerin görünümü (f) Nutlet yapı ve boyut görünümü.

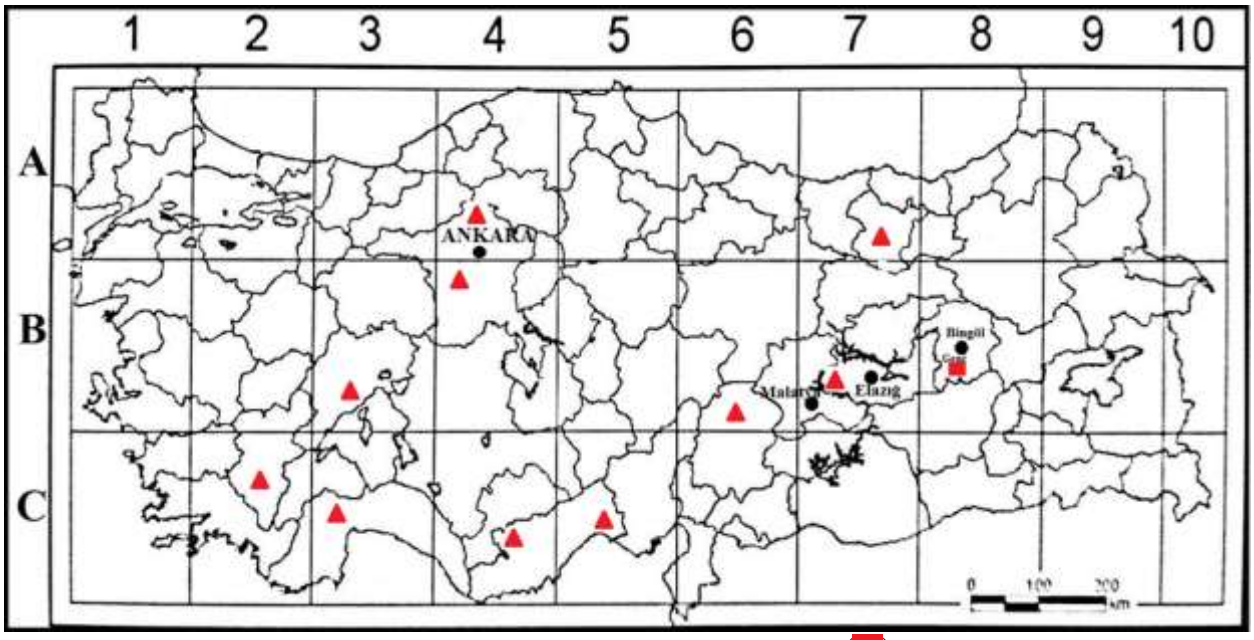

Figure 4. Distribution map of *Stachys woronowii* subsp. *bingolensis* (■) and *Stachys woronowii* subsp. *woronowii* (▲) in Türkiye

Şekil 4. Stachys woronowii subsp. bingolensiss (■) ve subsp. woronowii (▲)'nin Türkiye'deki dağılış haritası

It prefers rocky slopes in oak clearings and grows at altitudes of 1000-1100 m. Other important taxa accompanying the subspecies in the area where it is distributed are: *Adiantum capillus-veneris* L., *Antitoxicum tmoleum* (Boiss.) Pobed. *Bromus scoparius* L. *B. tectorum* L., *Campanula reuteriana* Boiss. & Balansa, *Cota coelopoda* (Boiss.) Boiss., *Crepis foetida* subsp. *rhoeadifolia* (M.Bieb.) Čelak., *Crepis alpina* L., *Dactylis glomerata* subsp. *hispanica* (Roth) Nyman, *Marrubium parviflorum* Fisch. & C.A.Mey. *Nepeta italica* L. *Polypogon viridis* (Gouan) Breistr., *Stachys annua* (L.) L., *Umbilicus luteus* (Huds.) Webb & Berthel, *Veronica orientalis* Mill. subsp. *orientalis, V. triloba* Opiz, *Vulpia ciliata* Dumort subsp. *ciliata*

In the 7th volume of the Flora of Turkey and the East Aegean Islands; *Stachys woronowii* is reported to be close to *Stachy obscura* Boiss. & Ball. in Sect Sideritopsis Bhattacharlee and differs from it with attenuate-based obovate leaves, linear-lanceolate bracts, short included corollas and deflexed calyx (Bhattacharjee 1980). *Stachys woronowii*, which grows on rocky slopes, plains and forest-bush environments; it is distributed in Iran, Lebanon-Syria, Transcaucasus and Türkiye. Specimens collected from Genç district are close to annual *Stacys woronowii* with their features (especially with its deflexed calyx structure) (Figure 2, 3).

Although there are many similarities between *Stachys woronowii* subsp. *woronowii* and *Stachys woronowii* subsp. *bingolensis*, there are also some important morphological differences, the most important of which are the high number of verticillasters (8-11), lower verticillasters being distinctly distant (to 37 mm), lower internodes far longer than the calyx length, nutlets larger and their surfaces are smooth (Table 1).

Table 1. Diagnostic characters between *Stachys woronowii* subsp. *bingolensis* and *Stachys woronowii* (subsp. *woronowii*).

Çizelge 1. *Stachys woronowii* subsp. *bingolensis* ve *Stachys woronowii* (subsp. *woronowii*) arasındaki ayırt edici karakterler

Characters / Karakterler	Stachys woronowii subsp. bingolensis	Stachys woronowii (subsp. woronowii)
Habit/Bitki	12-32 cm long, robust	2-24 cm long, slender
Verticillasters/Vertisillatlar	8-11, lower verticillasters distinctly remote, up to 3.7 cm apart and far longer than the calyx length, upper verticillasters approximate, 0.3-10 mm apart	2-8, verticillasters usually congested, 0.5-2 cm apart, usually the internodes shorter than calyx;
Nutlets/ Nutletler	2 × 1.6 mm, smooth	1-1.5 × 1-1.5 mm, rugose

While the distribution of *Stachys woronowii* subsp. *woronowii* is not found in the areas further east of Türkiye's A7 and B7 squares; distribution of *S. woronowii* subsp. *bingolensis* in B8 square was determined (Figure 4).

The number of taxa increased to 121 with this new subspecies of the genus *Stachys*, which is represented by 93 species and 120 taxa (endemism rate 53.3%) in Türkiye.

Additional Specimens: *Stacyhs woronowii* (subsp. *woronowii*): Türkiye, B7 Elazığ: northwest of Baskil district, east of İçlikaval village, rocky-steppe areas to the north of the irrigation dam, 1500-1600 m, 05.05.2022, *L.Behçet* 20701(BIN); ibid.,14.07.2022, *L.Behçet* 20830 (BIN); ibid, the rocky slopes between the villages of Odabaşı and Tavşanuşağı, 1400-1500 m, 12.05.2023 *L.Behçet* 20929 (BIN and Figure 2B); Türkiye, C4 Konya: Hügel westlich ob Sille, 1250-1300 m, 5.June 1948, *A.Huber.-Morath* 8369 (G; E−Photo!) (Holotypus); Türkiye, Tunceli: 7 km SW of Pertek, near the bridge the over, in the road Murat, in the road Elazığ-Erzurum), stony, glassy slope near the river, 1200 m, 28.05.1959 *E.HENNIPMAN, P.NIJHOFF, C.SWENNEN, AS.TULP, WJM.VADER, WJJO. DE WILDE* 1551 (L; E−Photo!); Türkiye, B6 Maraş: Elbistan-Darende 30. km, serpentine rocky-steppe areas, 1600 m, 09.07.1981, Herbarium Max Nydegger Flora Anatolica 16752 (E−Photo! Although it is identified as *Stachys pseudosideritis* Bhattacharjee & Hub.-Mor., which is the synonym of *Stachys woronowii*; it resembles *Stachys ramosissima* Montbret & Aucher ex Benth. in that the specimen's corolla length significantly exceeds the calyx length and the calyces are erect (not deflexed).)

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Author's Contributions

The contribution of the authors is equal.

Statement of Conflict of Interest

Authors have declared no conflict of interest.

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Taxonomic Evaluations of the Achene Fatty Acid Composition of Three Morphologically Similar *Tripleurospermum* (Asteraceae) Species

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ABSTRACT

Tripleurospermum tempskyanum, *T. disciforme* and *T. decipiens* (Asteraceae) are species with similar morphological characteristics that can therefore be easily confused with one another. In this study, achene fatty acid content of *T. tempskyanum* and *T. disciforme* from Türkiye was analysed using gas chromatography (GC) for the first time. The fatty acid data for these species together with the fatty acid data in the literature of *T. decipiens* were evaluated in terms of taxonomy using cluster analysis and principal components analysis. Eleven fatty acids were determined in the achenes of *T. tempskyanum* and *T. disciforme*. Additionally, linoleic acid (C18:2n6c), palmitic acid (C16) and α-linolenic acid (C18:3n3c) were detected as major fatty acids. The results of cluster analysis and principal components analysis indicated that achene fatty acids may be used as a chemotaxonomic marker to support the morphological separation of these species.

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Morfolojik Olarak Birbirine Benzeyen Üç *Tripleurospermum* (Asteraceae) Türünün Aken Yağ Asidi İçeriğinin Taksonomik Değerlendirmeleri

ÖZET

Tripleurospermum tempskyanum, *T. disciforme* ve *T. decipiens* (Asteraceae) morfolojik özellikleri bakımından birbirlerine benzeyen türlerdir ve bu yüzden birbirleri ile karıştırılabilirler. Bu çalışmada, Türkiye'den *T. tempskyanum* ve *T. disciforme*'nin aken yağ asidi içeriği ilk kez gaz kromatografisi (GC) kullanılarak analiz edildi. *T. decipiens*'in literatürdeki yağ asidi verileri ile birlikte bu türlerin yağ asidi verileri, kümeleme analizi ve temel bileşenler analizi kullanılarak taksonomik açıdan değerlendirildi. *T. tempskyanum* ve *T. disciforme*'nin akenlerinde 11 adet yağ asidi tespit edildi. Ayrıca, sırasıyla linoleik asit (C18:2n6c), palmitik asit (C16) ve α-linolenik asit (C18:3n3c)'lerin, major yağ asitleri olduğu belirlendi. Kümeleme analizi ve temel bileşenler analiz sonuçları, yağ asidi içeriğinin, bu türlerin morfolojik ayrımını desteklemek için kemotaksonomik bir belirteç olarak kullanılabileceğini gösterdi.

Botanik

Araştırma Makalesi

Makale Tarihçesi
Geliş Tarihi : 18.05.2022
Kabul Tarihi : 15.05.2023

Anahtar Kelimeler
Yağ asidi
Kemotaksonomi
Çok değişkenli analizler
Tripleurospermum

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INTRODUCTION

Tripleurospermum Sch.Bip., with c. 41 species, is one of the small genera of Asteraceae (Oberprieler et al., 2007). Most *Tripleurospermum* species are distributed in Türkiye with c. 33 taxa, the main center of its diversity (Inceer, 2021a; Inceer & Ozcan, 2021; Teksen et al., 2022). The endemism rate in the genus is above fifty percent with 17 endemic taxa in Türkiye.

Tripleurospermum tempskyanum (Freyn & Sint.) Hayek, *T. disciforme* (C.A.Mey.) Sch.Bip. and *T. decipiens* (Fisch. & Mey.) Bornm. are similar to one another in terms of their morphological traits and can therefore be easily confused with one another (Inceer, 2021b, Figure 1). *T. tempskyanum* is separated from *T. disciforme* by possessing non-mucilaginous achenes and from *T. decipiens* by having perennial habits and a hemispherical receptacle (Inceer, 2021b). *T. disciforme* differs from *T. decipiens* by possessing

ecoranate achenes and an ovoid-oblong receptacle (Enayet Hossain, 1975). *T. disciforme* and *T. decipiens* share similar anatomical traits in the leaves and achenes, while *T. tempskyanum* differs from these species to some degree in terms of various anatomical traits of the leaves and achenes (Inceer & Ozcan, 2021).

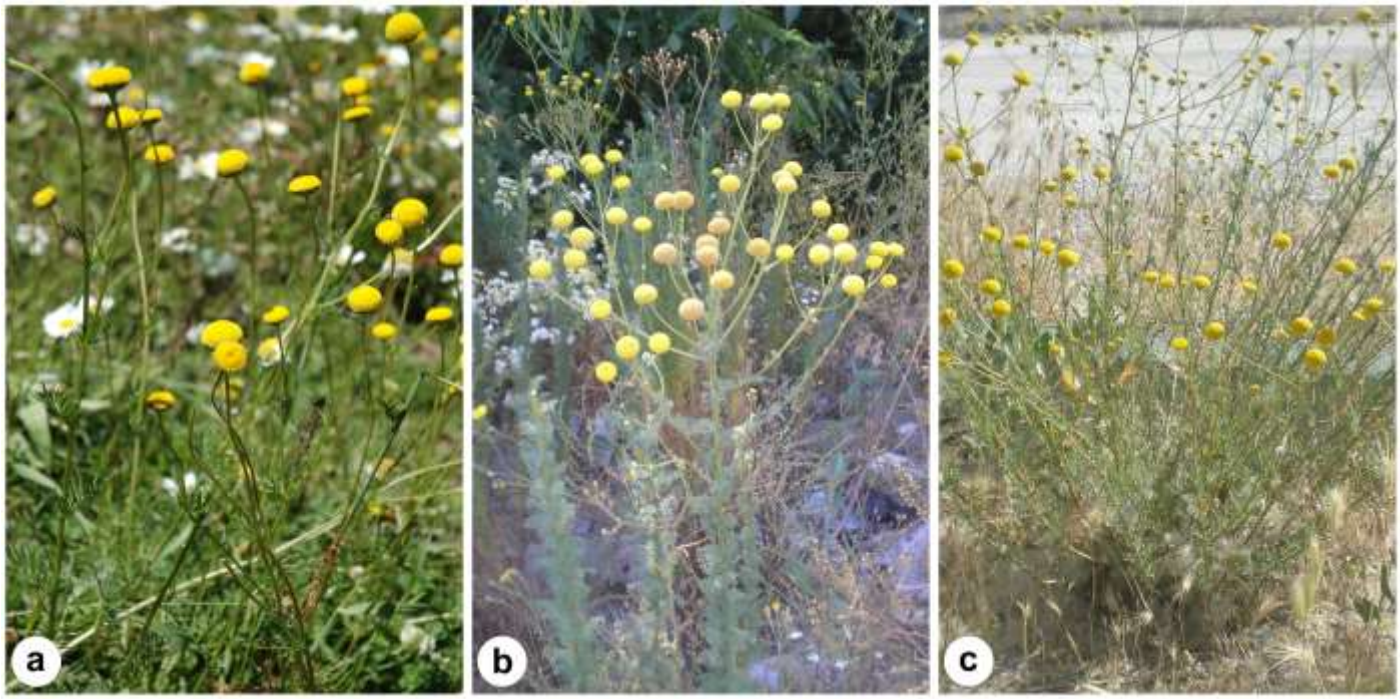

Figure 1. Habit of the studied species; a: *T. tempskyanum*, b: *T. disciforme*, c: *T. decipiens*
Şekil 1. Çalışılan türlerinin genel görünüşü; a: *T. tempskyanum*, b: *T. disciforme*, c: *T. decipiens*

In Türkiye, *T. tempskyanum* mainly grows in wet environments in Uludağ National Park in the province of Bursa (Inceer, 2021b). *T. disciforme* grows in damp places, such as meadows, fields and river beds (Enayet Hossain, 1975), while *T. decipiens* grows on steppe, rocky slopes and cultivated and fallow fields (Enayet Hossain, 1975).

Fatty acids (FAs) are found in all organs of plants. Additionally, FAs in the seeds can be good chemotaxonomic markers in certain plant groups, such as *Achemilla* L., *Carex* L., *Micromeria* Bentham, *Satureja* L. and *Thymus* L. (Marin et al., 1991; Ayaz et al., 1999; Ayaz & Olgun, 2000). Likewise, the FAs in the fruit (achene/cypsela) are of chemotaxonomic significance in some members of Asteraceae, such as *Centaurea*, L. *Matricaria* L., and *Tripleurospermum* (Ayaz et al., 2016; 2017; Janaćković et al., 2017). The achene FA profile of *T. decipiens* was previously reported by Ayaz et al. (2016). However, no FA analysis of the achenes in *T. tempskyanum* and *T. disciforme* has been conducted to date. The aims of the present work are to fill the gaps in the existing literature and to investigate FA variation among *T. tempskyanum*, *T. disciforme* and *T. decipiens* using cluster analysis and principal components analysis.

MATERIALS and METHODS

Plant Material

Achenes from five specimens of *T. tempskyanum* and *T. disciforme* were collected from the Turkish provinces of Bursa and Izmir, respectively. The locality and voucher details are given in Table 1. The vouchers are deposited in the KTUB herbarium.

Table 1. Collection data of *T. tempskyanum* and *T. disciforme*
Çizelge 1 T. tempskyanum ve T. disciforme'nin koleksiyon verileri

Species	Locality	Voucher
T. tempskyanum	A2 Bursa: Uludağ National Park, near hotels, meadows, damp places, 1690 m a.s.l., 27.6. 2007, N40°07′0.4″, E29°06′45.3″	Inceer 354
T. disciforme	B2 Izmir: Boz Dağ, Gölcük plateau, near Gölcük lake, meadows, roadsides, 1057 m a.s.l., 06.7.2008, N38°19′14.26″, E28°01′25.07″	Inceer 593

Fatty Acid Analysis

Total lipids of mature achenes were extracted as described by Folch et al. (1957), with some minor modifications. Pulverized achene samples (0.5 g) were

extracted using chloroform/methanol (2:1, v/v) in triplicate at 4°C for 18 h. The extracted lipids in the chloroform phase were separated by partitioning with one-fourth sodium chloride solution (0.9%, in water, w/v). These were then collected and evaporated using a rotary evaporator (Laborata 4003, Heidolph Instruments, Schwabach, Germany).

Analysis of FA methyl ester (FAME) extracted with *n*-hexane was carried out using an IUPAC (1989) accredited analysis method. A Perkin Elmer Auto System XL gas chromatography (GC) equipped with a flame-ionization detector was used for the FAME analysis. The column characteristics, injector and detector temperatures of the GC running conditions were selected as described elsewhere by Ayaz et al. (2016). The gas carrier was again helium. The FAs in the lipid samples were identified and quantified by comparison with the retention times of a standard FAME, as used earlier by Ayaz et al. (2016).

Multivariate Analyses

The data (12 quantitative characters) for the achene FAs of *T. tempskyanum* and *T. disciforme,* together with additional data for the achene FAs of *T. decipiens* (Ayaz et al., 2016), were evaluated using clustering analysis (UPGMA, dissimilarity, and standardized variable) and principal components analysis (PCA). These multivariate analyses were performed on Statistica version 12 software.

RESULTS and DISCUSSION

Achene Fatty Acids

The results of achene FA analysis are summarized in Table 2. This shows that the FA profiles varied considerably between *T. tempskyanum* and *T. disciforme*. The major FA in these species is linoleic acid, with values of 47.62% and 43.96%, respectively. Table 2 also shows total FA contents (the sum of the individual FAs quantified) in *T. tempskyanum* and *T. disciforme*, 33.43% and 26.13% for SFAs, 4.77% and 10.09% for MUFAs and 61.81% and 63.80% for PUFAs, respectively. These results indicate that PUFA levels are higher than SFAs and MUFAs levels in these species. Similar results have been reported for other species of *Tripleurospermum* (Ayaz et al., 2016). As already noted by Shorland (1963) and Janaćković et al. (2017), Asteraceae species possess rich linoleic acid contents in the achenes.

The present results show that the levels of unsaturated FAs are higher than those of SFAs in both *T. tempskyanum* and *T. disciforme* (Table 2). The concentrations of total unsaturated FAs in *T. tempskyanum* and *T. disciforme* are 66.58% and 73.89%, respectively. These results agree with previous report for a number of other species of the genera *Tripleurospermum*, *Achillea* L., *Anthemis* L., *Matricaria* and *Tanacetum* L. (Ayaz et al., 2016). The present results indicate that palmitic acid is the major SFA in both *T. tempskyanum* and *T. disciforme* (Table 2). As seen in Table 2, palmitic acid levels in *T. tempskyanum* are higher than those of *T. disciforme*. This finding is in agreement with the previous report for *Tripleurospermum* and other Anthemideae (Asteraceae) genera, such as *Anthemis* and *Matricaria* (Ayaz et al., 2016).

The results of this work also show that levels of α-linolenic and oleic acids in both species are higher than those of other FAs, after linoleic and palmitic acids. The results also indicate that these FA levels are higher in *T. disciforme* than in *T. tempskyanum*.

Table 2. Fatty acid profiles in the achenes of *T. tempskyanum* and *T. disciforme* (mean value ± standard deviation, %)
Çizelge 2 T. tempskyanum ve T. disciforme'nin yağ asidi profilleri (ortalama değer ± standart sapma, %)

Fatty acid	*T. tempskyanum*	*T. disciforme*
Capric acid (C10:0)	0.25±0.1	0.93±0.30
Myristic acid (C14:0)	0.41±0.15	0.58±0.06
Palmitic acid (C16:0)	23.13±0.38	19.90±0.47
Stearic acid (C18:0)	3.46±0.36	3.12±0.29
Arachidic acid (C20:0)	1.09±0.08	0.44±0.14
Behenic acid (C22:0)	2.35±0.20	-
Lignoseric acid (C24:0)	2.74±0.21	1.16±0.15
Palmitoleic acid (C16:1)	1.28±0.06	1.50±0.19
Oleic acid (C18:1n9c)	3.49±0.34	8.59±0.47
Linoleic acid (C18:2n6c)	47.62±0.53	43.96±0.69
α-Linolenic acid (C18:3n3c)	14.19±0.29	17.82±0.25
Arachidonic acid (C20:4n6c)	-	2.02±0.02
∑ SFA	33.43±0.46	26.13±0.47
∑ MUFA	4.77±0.29	10.09±0.56
∑ PUFA	61.81±0.65	63.80±0.72
PUFA/SFA	1.85	2.44
n-6/*n*-3	3.36±0.07	2.47±0.05

Eleven FAs were identified and quantified from the achenes of both species in the present study (Table 2).

However, the achenes of *T. disciforme* contain no behenic acid, and the achenes of *T. tempskyanum* contain no arachidonic acid. Similarly, *T. decipiens* has no behenic acid in its achenes (Ayaz et al., 2016). Capric acid is present as a minor FA (0.25%) in *T. tempskyanum*, whereas arachidic acid is found as a minor FA (0.44%) in *T. disciforme*. Similar results have been reported for various other species of *Tripleurospermum*, *Achillea* and *Matricaria* (Ayaz et al., 2016).

The accumulation of PUFAs with linoleic and α-linolenic acids is known to increase the meat quality of animals grazed on pastures, grasslands or meadows. In addition, linoleic and α-linolenic acids are not synthesized by herbivores and other consumers (Wee et al., 2017). The results of the FA analysis show a high accumulation of PUFA in the achenes of both *T. tempskyanum* and *T. disciforme* and that these species may be a rich PUFA source for domestic animals.

Taxonomic Implications

The results of the cluster analysis for *T. tempskyanum*, *T. disciforme* and *T. decipiens* are presented in Figure 2, which shows that these species are connected with each other at several levels depending on their FA profiles. In the dendrogram (Figure 2), *T. tempskyanum* is linked to *T. disciforme* in the same group at a low level, and these species exhibit a high level of similarity in terms of the FA profiles. On the other hand, *T. decipiens* is linked to these species at a high level in another group, and this species is thus similar to *T. tempskyanum* and *T. disciforme* at a low level in terms of the FA profiles. These findings indicate that the FA profiles of the achenes are useful for the delimitation of these species.

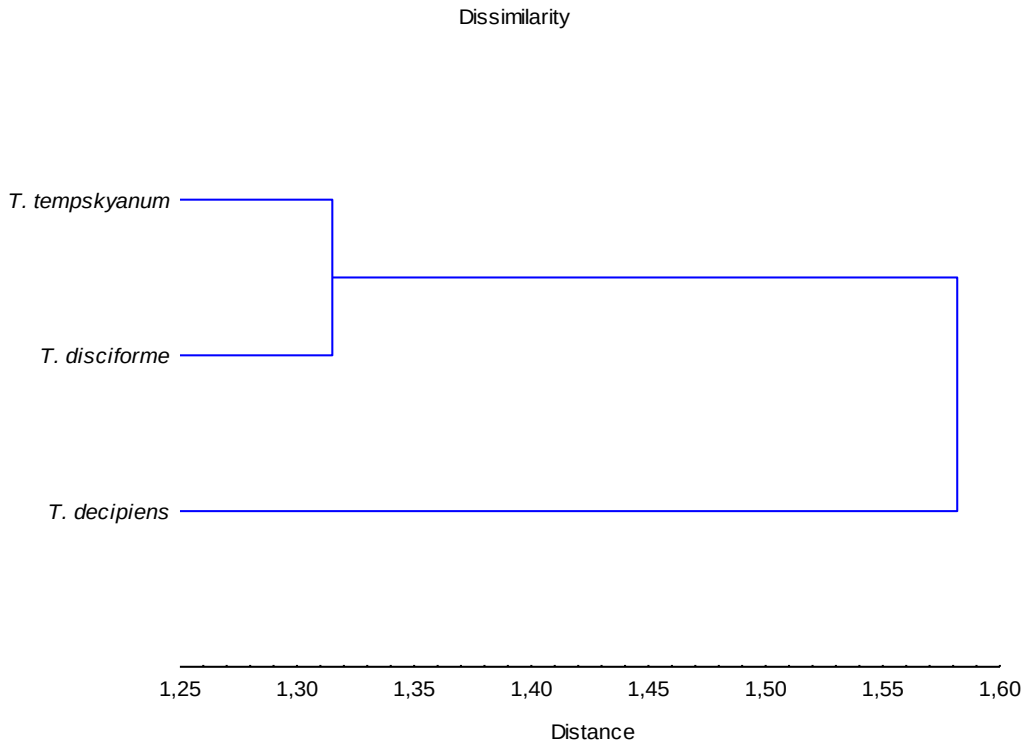

Figure 2. UPGMA clustering of *T. tempskyanum*, *T. disciforme* and *T. decipiens* on the basis of achene fatty acids
Şekil 2. T. tempskyanum, T. disciforme and T. decipiens'in aken yağ asitlerine dayalı UPGMA kümelenmesi

The PCA results showed that two PC factors accounted for 100% of the total variance (Figure 3). PC1 with an 8.14 eigenvalue describes approximately 68% of the variance. Its loadings indicate that it receives high contributions from stearic (-0.99), oleic (0.96), palmitic (-0.93), lignoseric (-0.92) and arachidonic (0.90) acid variables (Figure 4). PC2 with a 3.85 eigenvalue explains 32% of the difference in the data set, showing a high positive loading for capric (0.84) and myristic (0.80) acids (Figure 4). The results indicate that stearic, oleic, palmitic, lignoseric, arachidonic, capric and myristic acids explain most of the total variation among the species.

CONCLUSION

The species *T. tempskyanum*, *T. disciforme* and *T. decipiens* have similar morphological traits. It is therefore difficult to separate these species on the basis of morphological characters. The multivariate analysis results show that the achene FAs may be used as a chemotaxonomic marker to support the morphological separation of these species.

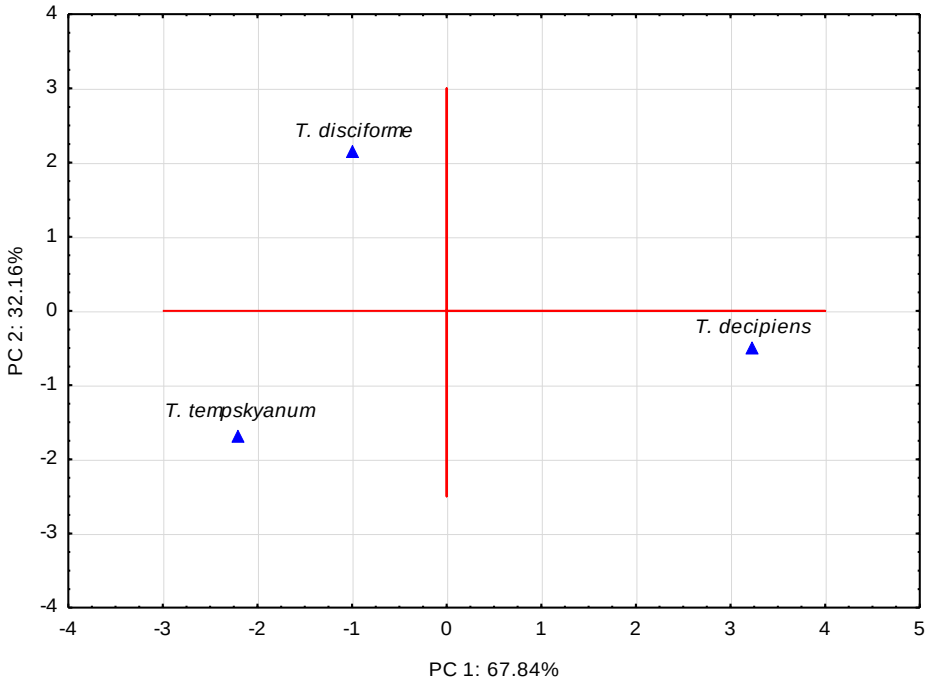

Figure 3. Results from principal components analysis of *T. tempskyanum*, *T. disciforme* and *T. decipiens* based on achene fatty acids

Şekil 3. T. tempskyanum, T. disciforme and T. decipiens'in aken yağ asitlerine dayalı temel bileşenler analiz sonuçları

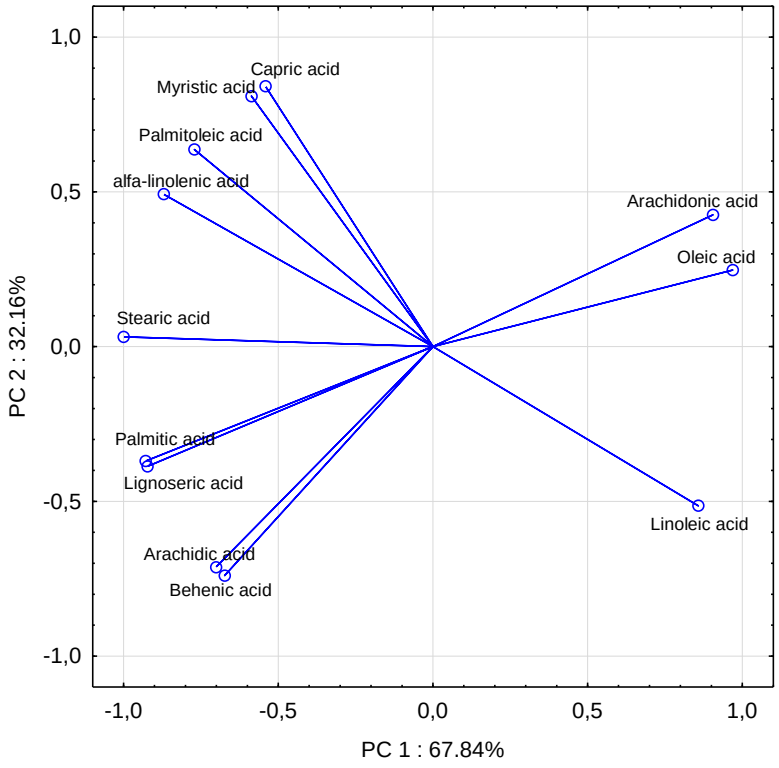

Figure 4. Results from principal components analysis of achene fatty acid composition in *T. tempskyanum*, *T. disciforme* and *T. decipiens*

Şekil 4. T. tempskyanum, T. disciforme and T. decipiens'de yağ asidi içeriklerinin temel bileşenler analiz sonuçları

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

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Rifampisin Dirençli *Mycobacterium tuberculosis* Kompleks Suşları Üzerine Benzimidazolyum Tuzlarının Antimikobakteriyel Etkinliğinin Araştırılması

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ÖZET

Çalışmada, Sağlık Bakanlığı Adana Bölge Tüberküloz Laboratuvarı'na gönderilen klinik örneklerden izole edilen rifampisin dirençli tüberküloz suşları ile referans suş *M. tuberculosis* H37Rv üzerine benzimidazol çekirdeği içeren 3 farklı bileşiğin ((S1): 1-(N-metilftalimid)-3-benzilbenzimidazolyum bromür, (S2): 1-(N-metilftalimid)-3-(4-metilbenzil) benzimidazolyum bromür, (S3): 1-(N-metilftalimid)-3-(naftalen-1-ilmetil) benzimidazolyum bromür) antimikobakteriyel aktivitesinin tespiti amaçlandı. Benzimidazol türevi bileşiklerinin rifampisin dirençli 35 klinik *M. tuberculosis* ve H37Rv suşlarında antimikobakteriyel aktiviteleri in vitro şartlarda BACTEC MGIT 960 sistemi kullanılarak test edildi. Ayrıca, antimikobakteriyel etkili bileşiklerin olası etkileşimleri moleküler doking ile incelendi. Çalışma sonucunda sadece S2 bileşiğinin yalnız *M. tuberculosis* H37Rv suşuna karşı antimikobakteriyel aktivite gösterdiği, rifampisin dirençli *M. tuberculosis* suşlarına karşı aktivitesinin olmadığı belirlendi. S1 ve S3 bileşiklerinin ise hem klinik hem de referans suşa karşı antimikobakteriyel aktivitesi tespit edilemedi. Moleküler doking sonuçları S2'nin InhA ile bağlandığını ve onu inhibe ederek antimikobakteriyel etkisini gösterebileceği ortaya çıkardı. Sonuç olarak S2 bileşiğinin tüberküloz tedavisinde yeni ajan olarak sunulabilir ancak daha kapsamlı çalışmaların yapılmasına da ihtiyaç duyulmaktadır.

Mikrobiyoloji

Araştırma Makalesi

Makale Tarihçesi
Geliş Tarihi : 08.04.2023
Kabul Tarihi : 25.08.2023

Anahtar Kelimeler
Tüberküloz
Rifampisin
Moleküler doking
Benzimidazol

Investigation of the Antimicobacterial Activity of Benzimidazolium Salts on Rifampicin Resistant *Mycobacterium tuberculosis* Complex Strains

ABSTRACT

In this study, it was aimed to determine the antimycobacterial activity of 3 different compounds ((S1): 1-(N-methylphthalimide)-3-benzylbenzimidazolium bromide, (S2): 1-(N-methylphthalimide)-3-(4-methylbenzyl) benzimidazolium bromide, (S3): 1-(N-methylphthalimide)-3-(naphthalen-1-ylmethyl) benzimidazolium bromide) containing benzimidazole nuclei on *M. tuberculosis* reference strain H37Rv and rifampicin resistant tuberculosis strains isolated from clinical samples sent to the Ministry of Health Adana Regional Tuberculosis Laboratory. Using the BACTEC MGIT 960 system, the antimycobacterial activity of benzimidazole derivative compounds were examined in vitro against *M. tuberculosis* H37Rv and 35 clinical strains of *M. tuberculosis* that were resistant to rifampicin. Furthermore, molecular docking was used to look into the possible interactions of the antimycobacterial compounds. According to the results of the study, compound S2 showed antimycobacterial activity against the *M. tuberculosis* H37Rv strain but it didn't have any effect on rifampicin-resistant *M. tuberculosis* strains. Compounds S1 and S3 didn't exhibit any antimycobacterial activity against both clinical and reference strains. The results of the molecular docking analysis revealed that S2 could bind to InhA and thus could exert

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its antimycobacterial activity by inhibiting it. As a result, the S2 compound can be suggested as a new agent for the treatment of tuberculosis, however, more comprehensive studies are needed.

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GİRİŞ

Yaklaşık 30 yıllık küresel halk sağlığı çabalarına rağmen, Tüberküloz (TB), dünya çapında bulaşıcı bir patojen tarafından önde gelen ölüm nedeni olmaya devam etmektedir (WHO, 2018). *Mycobacterium tuberculosis*'in neden olduğu TB, yılda yaklaşık 10 milyon kişiyi etkilemektedir.

TB tedavisinde ilaçların önerilen kombinasyonu, birinci basamak rifampisin, izoniazid, etambutol ve pirazinamid dahil olmak üzere 6 aylık standart bir rejimi içerir (Khan ve ark., 2021). Rifampisin, TB için standart altı aylık birinci basamak tedavi rejiminin temel ilacıdır ve 50 yılı aşkın bir süredir etkili TB tedavisinin temel taşı olmuştur (Schwœbel ve ark., 2020; Malenfant ve ark., 2021) . Rifampisin dirençli TB (RD-TB), dünya çapında insan sağlığı için artan bir endişe olmuştur ve TB'nin kontrolü için ciddi bir tehdit oluşturmaktadır (Prasad ve ark., 2018).

İlaca dirençli TB'nin mevcut tedavisi oldukça zor ve karmaşıktır. Hasta uyumunu iyileştirmek, nüks riskini ve ilaç direnci evrimini azaltmak amacıyla daha kısa rejimleri değerlendirmek için çok fazla araştırma yapılmalıdır (Gill ve ark., 2022). Bu bağlamda dirençli TB gelişimini yavaşlatmak, dirençli varyantların bulaşmasını sınırlamak, aynı zamanda tedavi sonucunu iyileştirmek için yeni tedavi rejimlerine, ilaçlara ve teşhislere acilen ihtiyaç vardır (Gygli ve ark., 2017).

Benzimidazol çekirdeği, biyolojik aktivite çalışmalarında önemli bir yere sahiptir (Yoon ve ark., 2013). Yapılan çalışmalarda, benzimidazol iskelesinin in vitro antimikobakteriyel aktivite gösterdiği tespit edilmiştir. Bu da onu yeni antimikobakteriyel ilaçlarının keşfi için umut verici bir başlangıç noktası haline getirmektedir. Yakın zamanda, benzimidazol türevlerinin TB'a karşı kullanılması ile ilgili nispeten iyi sonuçlar elde edilmiş farklı çalışmalar rapor edilmiştir (Yoon ve ark., 2013; Gong ve ark., 2014).

Bu çalışmada, 3 farklı benzimidazolyum tuzu hem klinik RD-TB suşlarına hem de duyarlı refarans *M. tuberculosis* suşuna karşı in vitro şartlarda antimikobakteriyel aktivitesinin potansiyel etkinliğini belirlemek amacıyla test edilmiştir.

MATERYAL ve METOD

Çalışmada, 01.10.2021-01.06.2022 tarihleri arasında Sağlık Bakanlığı Adana Bölge Tüberküloz Laboratuvarı'na pulmoner tüberküloz şüphesi ile gönderilen hastalara ait klinik materyallerden izole edilen ve rifampisin direnci tespit edilen 35 klinik *M. tuberculosis* suşu ile *M. tuberculosis* H37Rv referans suşuna karşı üç farklı benzimidazolyum tuzunun antimikobakteriyel aktivitesi araştırılmıştır. Çalışma için Çukurova Üniversitesi Girişimsel Olmayan Klink Araştırmalar Etik Kurulu tarafından (Karar No:55, Sayı: 114, 10.09.2021) onay alınmıştır.

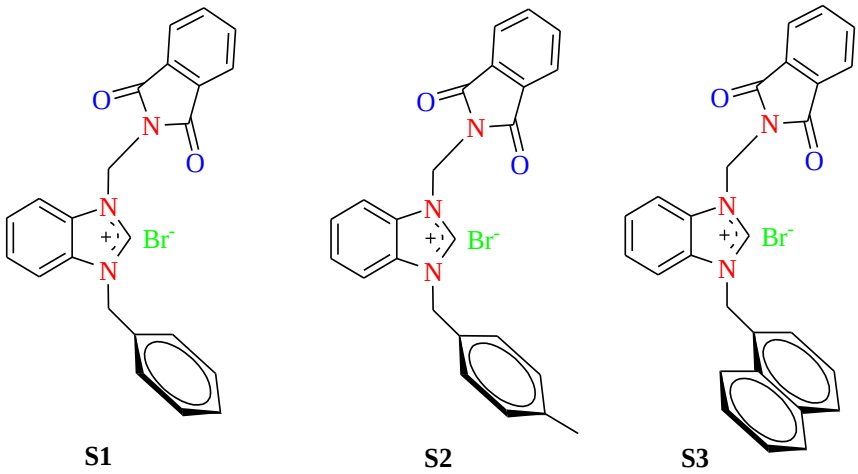
Benzimidazolyum Tuzlarının Sentezi

Benzimidazolyum tuzları, Süleyman Demirel Üniversitesi Araştırma Laboratuvarı'nda sentezlenmiştir. Benzimidazolyum tuzlarının sentezi için, 1.5 mmol oranında potasyum hidroksit, 40 ml etil alkol içindeki 1 mmol benzimidazol çözeltisine yavaş yavaş ilave edildi. Hazırlanan reaksiyon karışımı bir saat oda sıcaklığında karıştırıldıktan sonra ortama 1 mmol alkil halojenür ilave edildi ve 6 saat reflux yapıldı. Daha sonra oluşan potasyum klorür süzülerek uzaklaştırıldı. Ürün, etil alkol içinde kristallendirilerek saflaştırıldı. Sentezlenen N-alkilbenzimidazolün dimetilformamid (DMF) çözeltisine yavaş yavaş farklı bir alkil halojenür ilave edildi ve 80 °C'de 24 saat karıştırıldı. Reaksiyon bittikten sonra ortamdaki DMF vakum uygulanarak uzaklaştırıldı. Bu yöntemle, farklı benzimidazolyum tuzları sentezlendi ve ürünler etil alkol-dietil eter karışımında kristallendirilerek saflaştırıldı (Akkoç ve ark., 2016a; Akkoç ve ark., 2016b). Bu çalışma için hazırlanan benzimidazolyum tuzlarının açık yapıları aşağıda verilmiştir (Şekil 1).

Benzimidazol türevi bileşiklerin antimikobakteriyel aktivitesinin incelenmesi

Çalışmada kullanılan her bir benzimidazol türevi bileşik dimetilsülfoksit içinde çözdürülerek stok solüsyonları hazırlandı. MGIT tüplerine 800 µl OADC (MGIT GrowthSupplement, BD, ABD) ve 500 µl bakteri inoküle edildi. Üreme kontrol (GC) tüpü 1:100 oranında dilüe edilmiş bakteri inokülümü hazırlandı. Test edilen ilaç konsantrasyonları, 0.25 µg/ml, 0.5 µg/ml, 1µg/ml, 2 µg/ml ve 4 µg/ml olmak üzere 5 farklı dilüsyonda değerlendirildi. Minimum inhibisyon konsantrasyon (MIC) değerleri, her bir örnek için benzimidazol türevi içermeyen, sadece 1.0 µg/ml

rifampisin içeren GC kontrol tüpleri ile kıyaslanarak sonuçlar BD EpiCenter veritabanında 18-24 saatte bir kontrol edilerek analiz edildi (Juárez ve ark., 2020).



Şekil 1. Sentezlenen benzimidazolyum tuzlarının açık yapıları
Figure1. Open structures of synthesized benzimidazolium salts

(S1): 1-(N-metilftalimid)-3-benzil benzimidazolyum bromür
1-Benzilbenzimidazol (1.2 g, 1 mmol)'un DMF deki çözeltisine N-(bromometil)ftalimid (1.29 g, 1 mmol) ilave edildi ve reaksiyon 24 saat 80°C de sürdürülerek bileşik S1 hazırlandı (Akkoç ve ark., 2016).
(S2): 1-(N-metilftalimid)-3-(4-metilbenzil)benzimidazolyum bromür
2-((1H-benzo[d]imidazol-1-il)metil)izoindolin-1,3-dion (1 g, 1 mmol)'un DMF deki çözeltisine 4-metilbenzil bromür (0.558 g, 1 mmol) ilave edildi ve reaksiyon 24 saat 80°C de sürdürülerek bileşik S2 hazırlandı (Akkoç ve ark., 2016b).
(S3): 1-(N-metilftalimid)-3-(naftalen-1-ilmetil)benzimidazolyum bromür
1-(Naftalen-1-ilmetil)benzimidazol (1 g, 1 mmol)'un DMF deki çözeltisine N-(bromometil)ftalimid (0.929 g, 1 mmol) ilave edildi ve reaksiyon 24 saat 80°C de sürdürülerek bileşik S3 hazırlandı (Akkoç ve ark., 2016b

Moleküler Doking

TB'a karşı ilaç tasarlanmasında önemli bir hedef olan InhA enzimin üç boyutlu (3B) yapısı protein veri bankası (PDB)'den elde edildi. Kullanılan protein yapısı içerisinde etkili bir ligant içermektedir (PDB kodu: 6R9W) (Kamsri ve ark., 2020). Moleküler doking işlemi AutoDock Vina kullanılarak yapıldı (Trott ve ark., 2010). Araştırılan bileşikler ise ChemDraw programı ile çizildi (Cousins ve ark., 2022).

Doking işlemi başlamadan önce grid kutusu bağlı ligandı kapsayacak şekilde belirlendi. Sonra protein yapısı içindeki su silinerek, polar hidrojen eklenerek ve Gasteiger yükü belirlenerek işleme hazırlandı. Benzer şekilde bileşikler polar hidrojen eklenerek ve Gasteiger yükü eklenerek işleme hazırlandı. Sonra ilgili parametreleri kullanılarak AutoDock Vina komutu çalıştırıldı. Doking sonuçları Biovia Discovery Studio programı kullanılarak görüntülendi ve analiz edildi (Khawbung ve ark., 2021).

BULGULAR ve TARTIŞMA

Çalışmada, rifampisin dirençli *M. tuberculosis* klinik suşlarına karşı S1, S2 ve S3 bileşiklerinin antimikobakteriyel aktivitesi tespit edilmedi. Buna karşılık *M. tuberculosis* H37Rv referans suşunda S2 bileşiğinin (S2:1-(N-ftalimidometil)-3-(4-metilbenzil)-1H-benzo[d]imidazol-3-yum bromür)), 2 µg/ml konsantrasyonda antimikobakteriyel etki gösterdiği tespit edildi. S1 ve S3 bileşikleri ise *M. tuberculosis* H37Rv referans suşunda herhangi bir antimikobakteriyel aktivite göstermedi.

Günümüzde TB tedavisine yönelik yeni ajanların geliştirilmesi için çalışmalar yapılmakta ve giderek artmaktadır. Son zamanlarda, antimikobakteriyel aktivite çalışmaları için farklı fonksiyonel gruplara sahip yeni benzimidazol bileşikleri sentezlenmekte ve etkinliği araştırılmaktadır. Çalışmamızda olduğu gibi benzimidazol halkası içeren yeni bileşiklerin antimikobakteriyel etkinliği literatürlerde de mevcuttur. Çalışmalarda antimikobakteriyel aktiviteleri tespit edilen bileşiklerin 1, 2, 5 ve 6 pozisyonunda değişen bir dizi ile ikame edilen (halojenler, nitro, amino, metil gibi fonksiyonel gruplardan, triflorometil, hidroksil, alkoksi, sülfonil veya N-sülfonamid sübstitüe edilmiş aril/heteroaril sübstitüentleri gibi) umut verici antimikobakteriyel adaylar olduğu bildirilmiştir (Keri ve ark., 2016). Çalışmamızda ise antimikobakteriyel aktivite gösteren S2 bileşiği 4-metilbenzil grubu içermektedir.

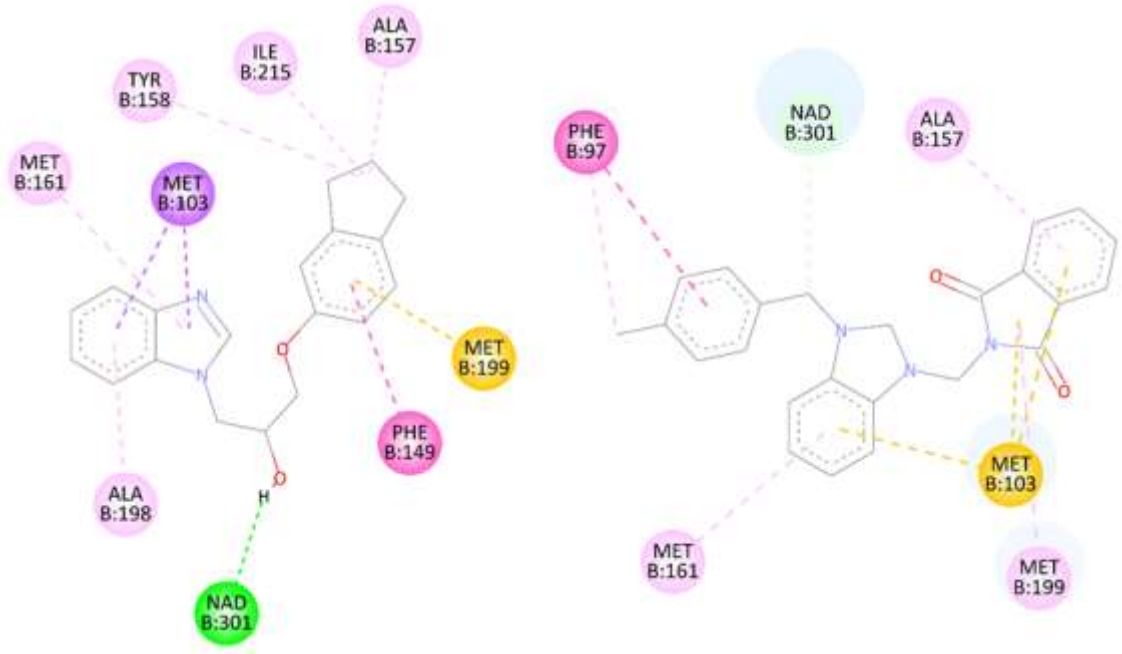
Sangani ve arkadaşları, kinolin ve metil grubuna eklenen eter bağlantılı aril halkasında flor grubuna sahip benzimidazol bileşiklerinin *M. tuberculosis* H37Rv'ye karşı daha yüksek aktivite sergilediğini tespit etmişlerdir (Sangan ve ark., 2013). Farklı

fonksiyonel gruba sahip (E)-N'-(4-ariloksibenziliden)-1H-benzimidazol-2-karbohidrazid türevlerinin *M. tuberculosis* H37Rv suşuna karşı MIC değerlerinin 1.5-25 µg/mL konsantrasyonlarında iyi bir antimikobakteriyel aktivitesi gösterdiğini bildirmişlerdir (Siddiki ve ark., 2014). Malasa ve arkadaşları yeni benzimidazol-2-il kinazolin türevlerini, umut verici antimikrobiyal ajanlar olarak tanımlamışlardır (Malasala ve ark., 2021). *M. tuberculosis* H37Rv'ye karşı 2,6-diarilpiperidin-4-on benzimidazol türevlerinin, standart rifampisin ilacına kıyasla %200 antimikobakteriyel aktivite sergilediğini tespit etmişlerdir (Aridoss ve ark., 2008). *M. tuberculosis* H37Rv suşu ve çoklu ilaca dirençli TB klinik suşlara karşı benzoimidazol-5-karbohidrazid türevlerinin potansiyel in vitro antimikobakteriyel aktiviteleri araştırılmış ve bileşiklerin orta düzeyde bir antimikobakteriyel aktivite sergilediğini bildirmişlerdir (Camacho ve ark., 2011).

Antimikobakteriyel etkinliği bulunan S2 bileşiğinin, InhA enzimi üzerindeki olası etkisini aydınlatmak amacıyla moleküler doking yapılmıştır. Moleküler doking işleminde kullanılan PDB yapıda bağlı bulunan ligandın InhA enzimiyle olan bağlanma noktaları ve bağlanma enerjisi doking işlemini valide etmek için belirlenmiştir. Ligandın InhA enzimiyle etkileşimi iyi olması ile birlikte elde edilen etkileşim noktalar bundan önce yapılmış deneysel çalışmalarla uyumlu olduğu ortaya çıkmıştır. Moleküler doking işlemi sonucunda bağlı ligandın NAD301, Met103, Phe149, Ala157, Tyr158, Met161, Ala198, Met199 ve Ile215 noktalarında InhA ile bağlandığı ortaya çıkmıştır (Şekil 2, Çizelge 1).

Çizelge 1. Standart ligandın ve S2 kodlu bileşiğin InhA (6R9W) ile etkileşim noktaları
Table 1 The interaction points of the standard ligand and the S2-encoded compound with InhA (6R9W)

Bileşikler	Bağlanma Enerji (kcal/mol)	Hidrojen Bağı	Diğer Etkileşim Noktalar				
			Karbon-hidrojen bağı	Pi-sigma	Pi-kükürt	Pi-pi	Alkil/Pi-alkil
Ligant	8.5	NAD301	-	Met103 (2)	Met199	Phe149	Ala157, Tyr158, Met161, Ala198, Ile215
S2	7.5	-	NAD301	-	Met103 (3)	Phe97	Phe97, Ala157, Met161, Met199



Ligand S2

Şekil 2. Standart ligandın ve S2 kodlu bileşiğin InhA (6R9W) ile etkileşimlerin 2 boyutlu gösterimi
Figure2. 2D representation of the interactions of the standard ligand and the S2-encoded compound with InhA (6R9W).

Standart ligandı içeren InhA enzimin X-ışını kristalografisi ile belirlendiğinde Met103, Phe149,

Ala157, Tyr158, Ala198, Met199 ve Ile215 noktalarda bir bağlanma olduğu tespit edilmiştir (Kamsri ve ark., 2020). X-ışını kristalografisi çalışmasında belirlenen yapının içerisindeki bileşiğin InhA ile NAD301 ve Met 161 dâhil burada tespit edilen bağlanma noktaların çoğunluğuyla bağlandığı açıklanmıştır (Rozwarski ve ark., 1999). Farklı bir çalışmada, birkaç ortak bağlanma noktaların belirlenmesi ile birlikte NAD ile hidrojen bağı etkileşimi tespit edilmiştir (Sullivan ve ark., 2006). Deneysel bir çalışmada triklosan'ın InhA ile burada belirlenen noktaların çoğunluğuyla etkileşimi olduğu ve NAD ile de hidrojen bağı oluşturduğu açıklanmıştır (Kuo ve ar., 2003) ve yapılan diğer çalışmalarda da benzer sonuçlar elde edilmiştir (Manjunatha ve ark., 2015). Bir inhibitörün InhA enzimiyle Phe97 amino asidiyle hidrofobik bir etkileşiminin olduğu yapılan deneysel çalışmada gözlemlenmiştir (Martínez-Hoyos ve ark., 2016). Bu modelleme çalışmasında belirlenen bağlanma noktalarının hepsi daha önceki yapılan deneysel çalışmalarda da tespit edilmiştir. Dolayısıyla moleküler doking çalışmanın sonuçları literatürdeki sonuçlara uygun oldukları ortaya çıkmıştır. Ayrıca, S2'nin etkileşimleri standart ligandın InhA ile olan etkileşimlerine benzer olduğu görülmüştür. Bu bağlamda NAD301, Met103, Ala157, Met161 ve Met199 noktalarındaki etkileşimlerin iki bileşikte de görülmüştür. Bununla birlikte S2'nin standart liganda göre daha az etkileştiği ortaya çıkmıştır. S2'nin bağlanma enerjisi standart liganttan daha yüksek olduğundan dolayı InhA enzimine bağlanma afinitesinin daha az olması beklenir (Tablo 1). Kısacası, moleküler doking çalışma sonuçları S2'nin InhA enzimiyle bağlanabildiği görülmüştür. Dolayısıyla da antimikobakteriyel etkisini InhA enzimini inhibe ederek gösterebildiği ortaya çıkmıştır.

SONUÇ ve ÖNERİLER

TB, küresel halk sağlığını tehdit eden salgın hastalıklardan biridir. TB tedavisi, *M. tuberculosis*'in ilaca dirençli varyantlarının ortaya çıkmasıyla daha zor hale gelmiştir (Khawbung ve ark., 2021). İlaca dirençli TB vakalarının arttığı bildirilmekte ve TB tedavisine yönelik yeni ajanların geliştirilmesi gerekmektedir.

Çalışmada olduğu gibi diğer yapılan çalışmalarda da yapısal olarak ortak basit benzimidazol halkasına sahip, fonksiyonel grupları farklı türevlerdeki bileşiklerin TB tedavisinde etkili olabileceği açıkça gösterilmiştir. Ancak bu bileşiklerin yeni TB ajanı olarak sunulması için makrofaj içi basillere karşı etkinliğinin araştırılması ve kapsamlı in vivo çalışmaların yapılmasına da ihtiyaç duyulmaktadır.

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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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Alıç Anacının Yenidünyada Verim ve Meyve Kalitesi Üzerine Etkileri

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ÖZET

Çalışmanın amacı, alıç anacının, Hafif Çukurgöbek (HÇG) yenidünya çeşidinin verim ve meyve kalitesi üzerine etkilerinin belirlenmesidir. Çalışmada, yenidünya çöğür anacı da kontrol olarak kullanılmıştır. Çalışmada yer alan çeşit/anaç kombinasyonlarının meyve pomolojik analizleri ve verim özellikleri belirlenmiştir. Meyve kalitesini belirlemek amacıyla her çeşit/anaç kombinasyonundan tesadüfi olarak 50 meyve alınmış ve 5 tekerrürlü (10 meyve/tekerrür) olarak fiziksel ölçüm ve kimyasal analizler yapılmıştır. Alıç anacı, yenidünya çöğür anacına göre meyve deriminde (17 Mayıs) iki gün erkencilik sağlamıştır. Meyve ağırlığı ve meyve boyutları, alıç anacında sırasıyla, 16.89 g, 32.13 mm ve 34.62 mm; yenidünya çöğür anacında 18.32 g, 31.72 mm ve 33.67 mm olarak belirlenmiştir. Tohum sayısı ve tohum ağırlığı, alıç anacında (sırasıyla 3.40 adet ve 4.40 g) yenidünya çöğür anacından (sırasıyla 4.02 adet ve 5.25 g) daha düşük bulunmuştur. Et/tohum oranı bakımından alıç anacından (2.84) yenidünya çöğür anacına (2.50) göre daha yüksek değer elde edilmiştir. Suda çözünebilir kuru madde değeri, alıç anacı üzerinde yetiştirilen meyvelerde %9.40 yenidünya çöğür anacı üzerinde yetiştirilen meyvelerde %11.30 olarak belirlenmiştir. Titre edilebilir asit değeri, alıç anacında %0.34, yenidünya anacında %0.44 olarak hesaplanmıştır. Anaçların, meyve kabuk ve et renklerine etkisi genellikle benzer olmuştur. Sadece meyve kabuk rengi L ve a*(kırmızı-yeşil renk) yenidünya anacı üzerinde yetiştirilen meyvelerde alıç anacına göre daha yüksek bulunmuştur. Birim gövde kesit alanına düşen verim ve birim alana verim bakımından alıç anacı (sırasıyla, 1.48 g $mm^{2\,-1}$ ve 1141 kg da^{-1}), yenidünya çöğür anacından (sırasıyla, 0.79 g $mm^{2\,-1}$ ve 1051 kg da^{-1}) daha yüksek değerler vermiştir. Ancak, daha kesin yargıya varılabilmesi için çalışmanın devam ettirilmesi gerekmektedir.

Bahçe Bitkileri

Araştırma Makalesi

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Anahtar Kelimeler
Alıç
Bodur anaç
Meyve kalitesi
Sık dikim
Verim

Effects of Hawthorn Rootstock on Yield and Fruit Quality in Loquat

ABSTRACT

The aim of the study is to determine the effects of hawthorn rootstocks on yield and fruit quality of the Hafif Çukurgöbek (HÇG) loquat cultivar. In the study, loquat seedling rootstock was also used as a control. Fruit pomological analysis and yield characteristics of the cultivar/rootstock combinations in the study were determined. In order to determine the fruit quality, 50 fruits were randomly picked from each cultivar/rootstock combination, and physical measurements and chemical analyses were carried out with five replicates. Plants grafted on hawthorn rootstock provide two days of earliness on the fruit harvest date. Hawthorn rootstock provided two days of earliness in fruit harvest (May 17) compared to loquat seedling rootstock. Fruit weight and fruit dimensions were 16.89 g, 32.13 mm and 34.62 mm, respectively, on quince rootstock while it was determined as 18.32 g, 31.72 mm and 33.67 mm in loquat seedling rootstock. Seed number and seed weight were lower in hawthorn rootstock (3.40 and 4.40 g, respectively) than in loquat seedling rootstock (4.02 and 5.25 g, respectively). In terms of flesh/seed ratio, a higher value was obtained from hawthorn rootstock (2.84) than from loquat seedling rootstock (2.50). The total soluble solid value was determined as 9.40% in fruits grown on hawthorn rootstock and 11.30% in fruits grown on loquat

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seedling rootstock. Titratable acidity was calculated as 0.34% in hawthorn rootstock and 0.44% in loquat rootstock. The effect of rootstocks on fruit skin and flesh color was generally similar. The fruit skin color L and a* (red-green color) were found to be higher in fruits grown on loquat rootstock than on hawthorn rootstock. Hawthorn rootstock gave higher values (1.48 g mm^{2} $^{-1}$ and 1141 kg da^{-1}, respectively) than loquat seedling rootstock (0.79 g mm^{2} $^{-1}$ and 1051 kg da^{-1}, respectively) in terms of yield per unit trunk cross-sectional area and yield per unit area. However, the study needs to be continued in order to reach a more definite conclusion.

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GİRİŞ

Meyvecilikte kullanılan anaçların, üzerine aşılı çeşitlere büyüme, gelişme, verimlilik yanında abiyotik stres koşulları ve olumsuz toprak koşullarına dayanım, meyve kalitesini doğrudan ilgilendiren irilik, renk, sertlik, besin elementi içeriği, kuru madde ve asit kapsamı gibi önemli etkileri bulunmaktadır (Bolat & İkinci 2019). Diğer meyve türlerinde olduğu gibi yenidünya yetiştiriciliğinde de bahçe tesisinde bodur anaç üzerine aşılı çeşitler tercih edilmelidir. Çünkü bodur anaçlar ile kurulan bahçelerde, budama, hastalık ve zararlılarla mücadele, hasat gibi kültürel işlemler daha kolay ve başarılı yapılabildiği gibi, bu gibi işlemler için gereken işçilik ve maliyet azalmaktadır. Bu nedenle, yirminci yüzyılın ikinci yarısından itibaren meyve yetiştiriciliğinde kullanılan bodur anaçlar sayesinde ağaçlar arası dikim mesafeleri azalmış, birim alana düşen ürün miktarı ve elde edilen gelirde önemli artışlar meydana gelmiştir (Bolat & İkinci 2019).

Bugün hemen tüm yumuşak ve sert çekirdekli meyvelerde tür içi veya türler arası melezlemelerle farklı gelişme kuvvetine sahip birçok klonal anaç elde edilmiştir. Meyvecilikte bodur ve yarı bodur anaç kullanımıyla daha küçük hacimli ağaçlar elde edilmektedir. Bu anaçlarla kurulacak bahçelerde daha sık dikim yapılarak, birim alandaki ağaç sayısında çöğür anaçlara göre 15 - 20 kata ulaşan artışlar sağlanmaktadır. Bu sayede de birim alana yapılan masraf azalırken, aynı alandan elde edilen ürün ve gelir artış göstermektedir (Rom & Carlson,1987; Öz ve ark., 1995; Akça, 2000; Gerçekçioğlu ve ark., 2009).

Yenidünyalar, çöğür anacı üzerine aşılandığında 5-10 m boyunda düzgün gövdeli, sık görünümlü, yayvanla yuvarlak arasında taçlanan, birim alana dikilebilecek ağaç sayısını sınırlayan ve merdivenle meyve hasadını zorunlu kılan büyük taçlı ağaçlar oluşturmaktadır (Polat, 2018). Büyük taç yapan ağaçların çoğu gençlik kısırlığı göstermekte ve meyve verimine geç başlamaktadır (Janick, 2011). Bu nedenle, yenidünyada büyüme kuvvetini azaltan zayıf ya da bodur anaçların kullanılması büyük önem taşımaktadır. Nitekim son yıllarda özellikle de örtüaltı yetiştiricilikte sık dikim ile ilgili farkındalığın artmasıyla bodur anaç üzerine aşılı yenidünya fidanlarına olan talep artış göstermiştir. Ancak, fidanlıklarda bodur anaçlara aşılı yenidünya fidanı üretimi olmadığından, yetiştiricilerin bu talebi karşılanamamakta ve yenidünya çöğür anacına aşılı fidanlar ile dikimler yapılmaktadır.

Yenidünya yetiştiricilerinin bodur fidan talebinin karşılanarak sık dikim yetiştiriciliğin geliştirilmesinin yanısıra, bodur anaçların meyve verim ve kalitesine etkilerinin de araştırmalar ile belirlenerek yetiştiricilerin bilgisine sunulması gerekmektedir. Yenidünyalarda bodur anaç olarak kullanılabilme potansiyeli bulunan ve araştırılması gereken türlerden biri de alıçtır. Nitekim, bazı bodur ayva anaçlarının, Hafif Çukurgöbek yenidünya çeşidine etkisini araştıran Akkuş (2020), bodur anaç olarak ayva klon anaçlarının yanı sıra kurağa da dayanıklı olan alıç anacının da çalışılmasının büyük yararı olduğunu belirtmiştir.

Alıç, Rosaceae familyasının *Crataegus* cinsi altında yer almaktadır (Ağaoğlu ve ark., 1995). Alıç bitkileri kuraklığa oldukça dayanıklıdır ve su tasarrufu sağlayan bahçecilik uygulamaları için önerilmektedir (Nas ve ark., 2012). Ayvadan daha dayanıklı olan alıçlar, muşmula, ayva ve armut için anaç olarak kullanılabilmektedir (Phipps ve ark., 2003). Nitekim İran'ın bazı bölgelerinde çevresel strese dayanıklı ve bodur anaç olarak alıç çöğürleri kullanılmaktadır (Lombard & Westwood, 1987; Qurunfleh, 1993; Hummer & Janick, 2009). Türkiye'nin farklı bölgelerinde doğal olarak yetişen alıçlar, çevirme aşılarıyla armut ve bazen de elmaya dönüştürülmektedir. Alıç, derinliği az, kurak, kumlu ve taşlı topraklarda, yetiştirilecek armutlar için iyi bir anaç özelliği taşımaktadır. Alıç anacına aşılanan armutlar fazla büyümemekte ve bodur kalmaktadır (Özbek, 1978). Diğer taraftan, pek yaygın olmamakla beraber, alıcın ayva için de anaç olarak kullanıldığı bildirilmektedir (Ghasemi ve ark., 2013; Gharaghani ve ark., 2016; Valipour ve ark., 2018; Tataria ve ark., 2020). Kurak koşullara dayanıklı olması ve bodur büyüme göstermesi nedeniyle, küresel ısınmaya bağlı

kurak iklim şartlarında yetiştiriciliği sürdürülebilir kılması bakımından alıç anacının diğer yumuşak çekirdekli meyve türlerinin yanısıra yenidünyalarda da anaç olarak kullanılması büyük önem taşımaktadır.

Bazı kaynaklarda (Demir, 1987; Polat, 1995; Polat & Kaska, 1992), alıçların, yenidünyalar için anaç olarak kullanılabileceği belirtilmekle birlikte, bu türün yenidünyalarda meyve verim ve kalitesine etkilerinin incelendiği herhangi bir araştırmaya rastlanmamıştır. Yenidünyalarda, anaç olarak alıcın kullanımına ilişkin sadece üç kaynağa (Jamil ve ark., 2012; Polat, 2020, 2021) ulaşılabilmiş ancak bunların da alıç anacına aşılanan yenidünya çeşitlerindeki aşı başarısının belirlenmesi ile ilgili olduğu görülmüştür. Bu nedenle, alıçların yenidünyalarda anaç olarak kullanılabilme imkânlarının araştırılması ve bu anacın, üzerine aşılı yenidünya çeşitlerinde meyve kalite özellikleri üzerine etkilerinin belirlenmesi büyük önem taşımaktadır. Nitekim bu çalışmada, alıç anacı üzerine aşılı Hafif Çukurgöbek yenidünya çeşidinin, meyve kalite özellikleri ile vejetatif büyüme ve meyve verimi üzerine etkileri belirlenmiştir. Literatürde, yenidünyaların meyve verim ve kalitesi ile vejetatif büyümeleri üzerine alıç anacının etkilerine ilişkin çalışmaların bulunmaması, bu araştırmanın verilerine alanındaki ilk veriler olma özelliği kazandırmakta ve literatüre katkı bakımından da önemini ortaya koymaktadır.

MATERYAL ve METOD

Bu çalışma, 2019-2020 vejetasyon periyodunda, Hatay Mustafa Kemal Üniversitesi, Ziraat Fakültesi, Bahçe Bitkileri Bölümü'ne ait araştırma alanındaki yenidünya parselinde yürütülmüştür. Araştırma alanı, 36°12′ doğu ve 36°52′ kuzey enlem derecelerinde olup denizden yüksekliği 80 metredir.

Materyal

Araştırmada, yenidünya ve alıç çöğür anaçlarına aşılı 10'ar adet 2 yaşındaki Hafif Çukurgöbek (HÇG) yenidünya çeşidine ait ağaçlar kullanılmıştır (Şekil 1). Deneme materyali ağaçlar, Goble terbiye sistemi ile şekillendirilmiş olup damla sulama sistemiyle sulanmıştır. Ayrıca yıllık teknik ve kültürel bakım işlemleri düzenli olarak yapılmıştır. Denemenin yürütüldüğü bahçede, dikim aralığı, anacı alıç olanlarda 1.0 x 0.5 m, anacı yenidünya çöğürü olanlarda 1x1 metredir.

Araştırma alanın toprak ve iklim özellikleri ile kullanılan anaçlar ve yenidünya çeşidine ait bazı bilgiler aşağıda verilmiştir.

Şekil 1. Alıç(a) ve yenidünya çöğür(b) anaçları üzerine aşılı HÇG yenidünya çeşidi
Figure 1. HÇG loquat cultivar grafted on hawthorn(a) and loquat seedling(b) rootstocks.

Araştırma Yerinin Özellikleri

Araştırma yerinin iklim özellikleri

Deneme parselinin bulunduğu bölgenin (Antakya/Hatay) iklimi, Akdeniz iklimi etkisi altında olup kışları ılık ve yağışlı, yazları ise sıcak ve kurak geçmektedir. Deneme parselinin bulunduğu bölgede, her iki yılda, en yüksek ortalama sıcaklık, ağustos ayında (sırasıyla, 28°C ve 29°C); en düşük ortalama sıcaklık ise ocak ayında (sırasıyla, 8.1°C ve 8.3°C) gerçekleşmiştir. En yüksek sıcaklık, 2019 yılında mayıs ayında 42°C, 2020 yılında eylül ayında 47°C olarak; En düşük sıcaklıklar ise 2019 yılında ocak ayında 1°C, 2020 yılında şubat ayında -2.5°C olarak gerçekleşmiştir (Anonim, 2020).

2019 yılında en fazla yağış 334.5 mm $m^{2\ -1}$ ile aralık ayında, en az yağış 0.3 mm $m^{2\ -1}$ ile mayıs ayında gerçekleşmiştir. 2020 yılında en fazla yağış 164.1 mm

$m^{2\ -1}$ ile ocak ayında meydana gelirken, temmuz, eylül ve ekim aylarında hiç yağış olmamıştır. 2019 yılında toplam yağış miktarı 1289.8 mm $m^{2\ -1}$ olarak gerçekleşirken, 2020 yılında ise toplam yağış miktarı 669.5 mm $m^{2\ -1}$ olmuştur (Anonim, 2020).

Denemenin yürütüldüğü 2019-2020 vegetasyon periyodunda çiçeklenme sonu ve meyve tutumu döneminde, sıcaklığın -2.5°C'ye düştüğü 2020 yılı şubat ayında, meydana gelen don olayında küçük meyvelerde zararlanmalar görülmüştür. Ardından hasat olumundan yaklaşık bir hafta önce, mayıs ayında gerçekleşen 42°C derecelik yüksek sıcaklıktan dolayı meyvelerde güneş yanıklıklarına bağlı zararlanmalar ve kayıplar yaşanmıştır.

Araştırma yerinin toprak özellikleri

Araştırmanın yapıldığı bahçenin 20 cm derinliğinden alınan toprak örneklerinde yapılan analizlere göre deneme alanı toprağı, alkali özellikte (pH:7.76), çok az kireçli (%2.4), orta tuzlu (EC microsiemens:446) ve kumlu tınlı (Kum:%57.37; Kil: %17.3; Silt: %25.32), organik madde bakımından yetersiz (%1.44) ve K (73.81 ppm), P (9.78 ppm), Fe (6.43 ppm), Cu (1.13 ppm), Mn (4.69 ppm), Zn (1.4 ppm) içeriğine sahip yapıdadır.

Denemede Kullanılan Anaçların ve Çeşidin Genel Özellikleri

Alıç; Rosaceae familyası, Maloidae alt familyası ve *Crataegus* cinsi altında yer almaktadır. Alıç, kışın yaprağını döken, ender olarak da yarı herdem yeşil, genelde dikenli çalı ya da ağaççık formunda bulunan odunsu bir bitki türüdür (Davis, 1972). Kurağa dayanıklı ve oldukça yavaş büyüyen bir bitki olması nedeniyle başta armut olmak üzere bazı meyve türlerinde bodurlaştırıcı anaç olarak kullanılmaktadır.

Yenidünya çöğürü; Herdem yeşil, subtropik iklim koşullarında kuvvetli büyüyen ve üzerindeki kalemi de 8-10 metre büyüten bir anaçtır (Polat, 2019).

Hafif Çukurgöbek; Türkiye'de selekte edilmiş erkenci bir çeşit olup, orta irilikte, pembe portakal renkli, gösterişli, çok lezzetli, tatlı meyvelere sahiptir. Taşınmaya ve karaleke hastalığına dayanıklı, kendine verimli bir çeşittir. 15-20 yaşlı bir bahçenin dekara verimi 1000-1200 kg'dır (Demir, 1987; Polat, 2019).

Yöntem

Deneme, tesadüf parselleri deneme desenine göre 5 tekerrürlü olarak kurulmuş, her tekerrürde 2 ağaç kullanılmıştır.

Fenolojik Gözlemler

Çalışmada yer alan anaç/çeşit kombinasyonlarının, çiçeklenme dönemleri Polat 2018 ve Akkuş (2020)'ye göre incelenmiştir.

Meyve Kalite Analizleri

Çeşide has büyüklük ve rengini alarak derim olgunluğuna erişmiş (Ferreres ve ark., 2009) meyvelerden her anaç için tesadüfi olarak 50 adet meyve alınmış ve her tekerrürde 10 meyve olacak şekilde, 5 tekerrürlü olarak Polat ve ark. (2004 ve 2005) ile Akkuş (2020)'a göre aşağıdaki ölçüm ve analizler yapılmıştır.

Meyve ağırlığı (g): Meyve örnekleri 0.01 g hassas terazide tek tek tartılarak belirlenmiştir.

Meyve eni (mm): Meyveler ekvatoral bölgeden dijital kumpas ile ölçülerek hesaplanmıştır.

Meyve boyu (mm): Meyvenin sap kısmı ile kaliks kısmı arasındaki mesafenin dijital kumpas ile ölçülmesiyle saptanmıştır.

Tohum ağırlığı (g): Her meyvede bulunan tohumlar meyve etinden ayrılarak tartılmıştır.

Tohum sayısı (adet): Her meyvede bulunan tohumların sayılmasıyla belirlenmiştir.

Et/tohum oranı: Meyve eti ağırlığının tohum ağırlığına bölünmesiyle belirlenmiştir.

Meyve kabuk ve et rengi: Meyve kabuk ve etinde renk ölçümleri C.I.E. L* a* b* metoduna göre "Minolta CR-300" renk ölçüm cihazıyla meyvenin orta kısmındaki iki bölgeden yapılmıştır.

Suda çözünebilir kuru madde oranı (SÇKM) (%): Meyve etlerinin sıkılmasıyla elde edilen meyve suyundan bir damla alınarak el refraktometresi ile % olarak belirlenmiştir.

Titre edilebilir toplam asitlik (%): Meyve etlerinin parçalanmasıyla elde edilen meyve suları süzülerek 5 ml'lik meyve suyu örneği alınmış ve saf su ile 100 ml'ye tamamlanarak seyreltilmiştir. Seyreltilen meyve suyu örneklerinin pH'sı 8.10'a ulaşıncaya kadar 0.1 N NaOH çözeltisi ile titre edilmiş ve sonuçlar malik asit cinsinden hesaplanmıştır (Karaçalı, 1990).

$$TA = \frac{V \times N \times F \times meg \times 100}{A}$$

TA: Asitlik, g 100 mL^{-1}

V: Titrasyonda harcanan NaOH çözeltisinin hacmi (ml)

N: NaOH normalitesi

F: NaOH çözeltisinin faktörü

A: titrasyon için alınan örneğin hacmi (ml)

Meg: Malik asit equvalent ağırlığı, 0.067

pH: Meyvelerin sıkılmasıyla elde edilen meyve suyuna ait pH değerleri dijital pH metre okumaları ile saptanmıştır.

Verim Özellikleri

Bitkilerin vejetatif büyümeleri ile verimliliğin birlikte değerlendirilebilmesi için aşağıdaki özellikler ölçülmüştür.

Bitki başına verim (g bitki^{-1}): Her bitkinin hasat edilen meyveleri 5 g hassas 30 kg kapasiteli terazide tartılarak belirlenmiştir.

Gövde kesit alanına düşen verim (g mm^{2} $^{-1}$): Bitkilerin aşı noktasının 5 cm üzerinden ölçülen gövde birim kesit alanına düşen verim değerleri hesaplanmıştır.

Birim alana verim (kg da^{-1}): Denemede bitki başına elde edilen verim, anaçların dikim aralığı da dikkate alınarak dekara verime dönüştürülerek hesaplanmıştır.

İstatistik Analizler

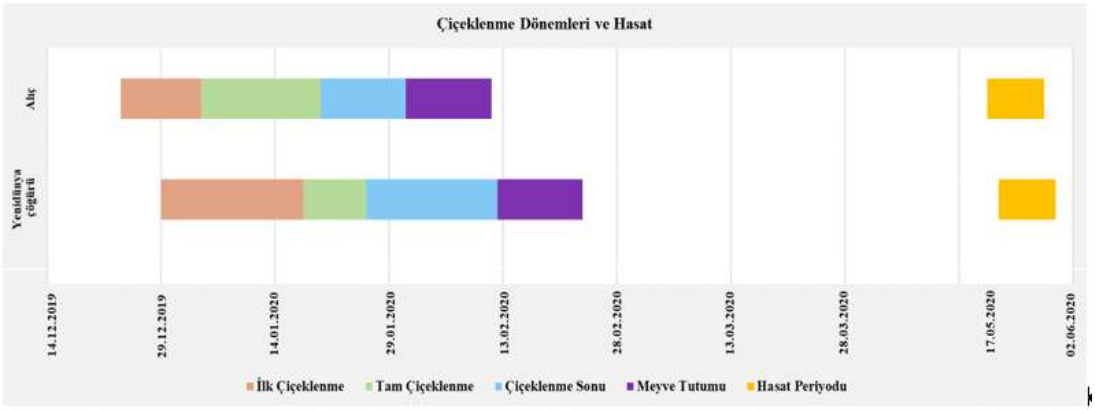
Verilerin varyans analizleri, SPSS paket programında "Tesadüf Parselleri Deneme Desenine" göre yapılmış ve ortalamalar arası farklılıklar LSD testi ile belirlenmiştir.

BULGULAR ve TARTIŞMA

Anaçların, HÇG yenidünya çeşidinin fenolojik özellikleri, meyve kalitesi, vejetatif büyüme ve verimi üzerine etkilerine ilişkin elde edilen bulgular, aşağıda ayrı başlıklar altında incelenmiştir.

Fenolojik Gözlemlere Ait Bulgular

Çiçeklenme başlangıcı, alıç anacına aşılı bitkilerde, yenidünya çöğür anacından 24 gün önce gerçekleşmiştir. Tüm çiçeklenme dönemleri ve meyve tutumu, alıç anacı üzerindeki bitkilerde, yenidünya çöğür anacından daha erken meydana gelmiştir. Alıç anacına aşılı olan bitkiler, yenidünya anacına aşılı olanlara göre meyve deriminde iki gün erkencilik sağlamıştır (Şekil 2).



Şekil 2. Farklı anaçlar üzerine aşılı HÇG yenidünya çeşidinin çiçeklenme ve meyve derim periyotları.
Figure 2. Flowering and fruit harvesting periods of HÇG loquat cultivar grafted on different rootstocks.

Meyve Kalite Kriterleri İle İlgili Bulgular

Denemedeki anaçların, HÇG yenidünya çeşidinin meyve kalite kriterlerine etkileri ile ilgili veriler Çizelge 1 ve Şekil 3'de verilmiştir.

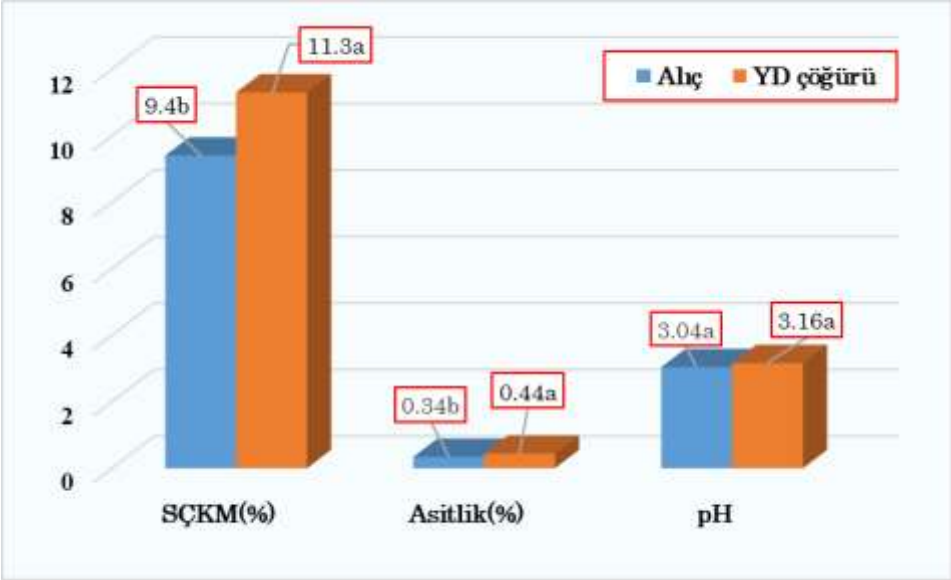
Çizelge 1. Farklı anaçların Hafif Çukurgöbek yenidünya çeşidinin meyve kalite özellikleri üzerine etkileri
Table 1. The effects of different rootstocks on fruit quality characteristics of loquat cv. Hafif Çukurgöbek.

Anaçlar *Rootstock*	Meyve ağırlığı (g) *Fruit weight (g)*	Meyve eni (mm) *Fruit width (mm)*	Meyve boyu (mm) *Fruit length (mm)*	Tohum ağırlığı (g) *Seed weight (g)*	Tohum Sayısı (adet) *Seed number per fruit*	Et/Tohum oranı *Flesh/seed ratio*
Alıç *(Hawthorn)*	16.89±0.31	32.13±0.38	34.62±0.49	4.40±0.27	3.40±0.25	2.84±0.17
YD çöğürü *Loquat seedling*	18.32±1.30	31.72±0.46	33.67±1.09	5.25±0.35	4.02±0.30	2.50±0.21
$LSD_{\%5}$	ÖD^{x}	ÖD	ÖD	ÖD	ÖD	ÖD

^{x}: Önemli değil, ^{x}:*Not significance*

Çalışmada, meyve ağırlığı, yenidünya çöğür anacına aşılı bitkilerde 18.32 g ve alıç anacına aşılı bitkilerde 16.89 g olarak belirlenmiştir. Önceki çalışmalarda, yenidünya çöğür anacına aşılı olan HÇG yenidünya çeşidinin meyve ağırlığı 20.45 g - 39.70 g arasında belirlenmiştir (Paydaş ve ark.,1992; Polat ve ark., 2004, 2005 ve 2010; Polat ve Çalışkan, 2011). Bu farkın, ağaç yaşı, bahçenin kurulu olduğu bölgenin toprak yapısı, o yılki iklim koşulları, meyve tutum oranı ve bitki besleme durumunun farklılık göstermesinden kaynaklanmış olabileceği düşünülmektedir. Akkuş ve Polat'ın (2022) yapmış olduğu çalışmada, Quince-A, Quince-C ve BA-29 anaçlarına aşılı HÇG çeşidinin meyve ağırlıklarını

sırasıyla 18.80 g, 23.33 g ve 20.22 g olarak belirlerken, Sezer ve Polat (2022) aynı çeşidin ortalama meyve ağırlıklarını sırasıyla 23.58 g, 22.08 g ve 24.81 g olarak belirlemiştir. Genel olarak çalışmadan elde edilen meyve ağırlığı değerleri, Akkuş ve Polat (2021) ile Sezer ve Polat'ın (2022) değerlerinden daha düşük bulunmaktadır. Bu farklılıkların denemede yer alan anacı alıç olan bitkilerin, ayva anaçlarına aşılı bitkilerden 2 yaş, yenidünya çöğür anacına aşılı bitkilerin de bir yaş daha küçük olmasından kaynaklı olabileceği düşünülmektedir.



Şekil 3. Anaçların HÇG yenidünya çeşidinin SÇKM, asitlik ve pH özellikleri üzerine etkileri.
Figure 3. The effects of rootstocks on TSS, acidity and pH properties of HÇG loquat cultivar.

Meyve eni ve boyu değerleri alıç anacında sırasıyla 32.13 mm ve 34.62 mm, yenidünya çöğür anacında sırasıyla 31.72 mm ve 33.67 mm olarak ölçülmüştür. Akkuş ve Polat (2021) ile Sezer ve Polat'ın yapmış olduğu çalışmalarda, ayva klon anaçları üzerinde yetiştirilen HÇG meyvelerinin meyve en ve boy değerleri, bu çalışmada elde edilen değerlerden kısmen yüksek bulunmuştur.

Çalışmada, tohum sayısı ve tohum ağırlığı, alıç anacında (sırasıyla 3.40 adet ve 4.40 g) yenidünya çöğür anacından (sırasıyla 4.02 adet ve 5.25 g) daha düşük bulunmuştur. Et/tohum oranı bakımından alıç anacından (2.84) çöğür anacına (2.50) göre daha yüksek değer elde edilmiştir. Ayva klon anaçları üzerinde yetiştirilen HÇG meyvelerinin tohum sayısı ve tohum ağırlığı, Akkuş ve Polat (2021) tarafından sırasıyla 3.34-3.82 adet ve 4.50-5.71 g; Sezer ve Polat (2022) tarafından ise sırasıyla 4.04-4.16 adet ve 5.59-6.04 g olarak belirlenmiştir. Et/tohum oranı ise her iki çalışmada da bu çalışmada belirlenen değerden daha yüksek bulunmuştur. Görüldüğü üzere, alıç anaçlarına aşılı bitkilerin meyvelerinin içerdiği tohum sayısı ve ağırlığı, gerek ayva anaçları gerek yenidünya çöğür anacına aşılı bitkilerinin değerlerinden daha düşüktür.

Alıç anacı üzerine aşılı bitkilerin meyve ağırlığı değeri, yenidünya çöğür anacı üzerinde yetiştirilenlere göre kısmen daha düşük olmasına karşın meyve boyutları daha yüksek bulunmuştur. Ayrıca, alıç anacı üzerindeki meyvelerin gerek tohum ağırlığı, gerek tohum sayısı, çöğür anacına göre daha düşük bir değere sahipken; et/tohum oranı yenidünya anacına göre daha yüksek bulunmuştur. Her üç özellik de yenidünya yetiştiriciliğinde meyve kalitesi bakımından büyük önem taşıyan ve tüketici tercihlerini etkileyen çok önemli özelliklerdir. Yenidünya meyvelerinde tohum sayısının ve iriliğinin az olması ve meyve eti/tohum oranının da yüksek olması istenir. Çalışmanın bulguları, alıç anacının, belirtilen meyve kalite özelliklerine etkisinin tüketici tercihleri açısından olumlu yönde olduğunu göstermektedir. Ancak, bu bulgular, alıç anacına ilişkin henüz ilk veriler olduğu için, bu aşamada genelleştirilmesinin doğru olmayacağı düşünülmektedir.

Nitekim, Polat (2007) ile Polat ve Çalışkan'ın (2011) yaptığı çalışmalarda yenidünya çöğür anacına aşılı HÇG çeşidinde et/tohum oranı 3.85-8.92 değerleri arasında bulunmuştur. Çalışmada, yenidünya çöğür anacından elde edilen et/tohum oranının oldukça düşük olduğu gözlemlenmiştir. Bunun, denemedeki bitkilerin henüz çok genç yaşta olmalarından kaynaklanmış olabileceği düşünülmektedir.

Çalışmada, suda çözünebilir kuru madde alıç anacı üzerinde yetiştirilen meyvelerde %9.40 yenidünya çöğür anacı üzerinde yetiştirilen meyvelerde %11.30 olarak belirlenmiştir. Paydaş ve ark. (1992), Polat ve ark. (2004, 2005 ve 2010), Durgac ve ark. (2006) ile Polat ve Çalışkan (2011), yenidünya çöğür anacı

üzerinde yetiştirilen meyvelerde SÇKM'yi %8.05-12.80 arasında ölçmüşlerdir. Ayva klon anaçları üzerinde yetiştirilen HÇG meyvelerinin SÇKM içeriği, Akkuş ve Polat (2021) tarafından %10.32-12.36; Sezer ve Polat (2022) tarafından ise %9.24-10.80 arasında belirlenmiştir. Önceki çalışmalarla kıyaslandığında elde ettiğimiz SÇKM değerlerinin benzerlik gösterdiği görülmektedir.

Titre edilebilir asit değeri, alıç anacında %0.34, yenidünya anacında %0.44 olarak hesaplanmıştır. Ayva klon anaçları üzerinde yetiştirilen HÇG meyvelerinin titre edilebilir asit içeriği, %0.34-0.81 arasında (Akkuş ve Polat, 2021; Sezer ve Polat, 2022) belirlenmiştir. Yenidünya çöğür anacı üzerinde yetiştirilen HÇG meyvelerinde ise titre edilebilir asit değeri %0.40-0.92 arasında (Polat ve Çalışkan, 2011; Tepe, 2013) bulunmuştur. Bu araştırmanın bulguları, önceki çalışmalarda belirlenen bulgular ile uyumlu bulunmaktadır.

Çalışmada, pH değerleri alıç anacında 3.04, yenidünya çöğür anacında 3.16 olarak belirlenmiş olup, bu bulgular, Polat ve Çalışkan'ın (2011) yenidünya çöğür anacında (3.26); Akkuş ve Polat (2021) ile Sezer ve Polat'ın (2022) ayva anaçlarında belirledikleri pH değerleri (sırasıyla,3.42-3.79 ve 3.34-3.52) ile benzer bulunmuştur.

Anaçların HÇG yenidünya çeşidinin meyve kabuk ve et renkleri üzerine etkileri Çizelge 2'de verilmiştir.

Çizelge 2. Anaçların HÇG yenidünya çeşidinin meyve kabuk ve et renkleri üzerine etkileri.
Table 2. The effects of rootstocks on fruit skin and flesh colors of HÇG loquat cultivar.

Anaçlar *Rootstock*	Meyve kabuk rengi *Fruit skin color*			Meyve et rengi Fruit flesh color		
	L	a	b	L	a	b
Alıç *(Hawthorn)*	63.32±0.97b[x]	10.90±0.65b	57.64±1.77a	68.74±0.75a	8.96±0.46a	53.32±0.46a
YD çöğürü *Loquat seedling*	67.86±0.59a	13.13±0.44a	55.94±0.73a	69.37±0.39a	9.55±0.45a	55.39±0,80a
LSD%5	*	*	ÖD[y]	ÖD	ÖD	ÖD

[x]:Aynı sütunda farklı harfler LSD testine göre % 5 düzeyinde önemli farklılığı göstermektedir, [y]: Önemli değil
[x]:Different letters within columns are indicate significant difference by LSD's test at P < 0.05. [y]:Not significance

Meyve kabuk ve et renk değerleri incelendiğinde; meyve kabuk rengi L ve a* dışındaki tüm parametreler bakımından anaçlar arasında önemli bir farklılık olmadığı görülmektedir. Meyve kabuk rengi L(parlaklık) ve a*(kırmızı-yeşil renk) yenidünya anacı üzerinde yetiştirilen meyvelerde alıç anacına göre daha yüksek bulunmuş ve anaçlar arasındaki farklılıklar istatistiksel olarak önemli çıkmıştır (Çizelge 2). Bu bulgular, Sezer ve Polat'ın (2022) ayva anacı üzerinde yetiştirilen meyvelerde belirlediği renk değerlerine benzerlik göstermektedir. Literatürde, yenidünyalarda alıç anacının kullanımına ilişkin yayınlanmış kaynaklara ulaşılamadığından bu çalışmanın verilerinin karşılaştırılması mümkün olamamıştır. Buna karşın, alıç anacının ayva için anaç olarak kullanıldığı bir araştırma sonucuna ulaşılabilmiştir. Dört ayva klon anacı (BA-29, A, B ve C) ile ayva ve alıç çöğürlerinin üzerine aşılı İsfahan ayva çeşidinin meyve kalite özelliklerine etkisinin incelendiği bir çalışmada (Tatari ve ark., 2020), alıç, BA-29 ve QA anaçlarının çöğür anacına göre daha yüksek meyve ağırlığı, toplam çözünür katı madde/toplam asitlik ve meyve sertliği değerlerine sahip oldukları belirlenmiştir.

Vejetatif Büyüme ile İlgili Bulgular

Yıllık sürgün uzunluğu, kalem ve anaç çapı bakımından yenidünya çöğür anacı (sırasıyla, 65.02 cm, 41.09 mm ve 43.18 cm) alıç anacına (11.78 cm, 5.45 mm, ve 15.36 mm) göre önemli düzeyde daha yüksek değerler vermiştir. İncelenen öteki bütün parametreler bakımından da yenidünya çöğür anacının daha kuvvetli vejetatif büyüme gösterdiği belirlenmiştir. Elde edilen bu ilk verilere göre alıç anacı, yenidünya çöğür anacına göre üzerine aşılı kalemde yaklaşık %60 bodurluk sağlamaktadır. İran'da yapılan bazı çalışmalarda da alıç anacının, üzerine aşılı farklı ayva ve armut çeşitlerinde önemli düzeyde bodurluk sağladığı belirlenmiştir (Abdollahi ve ark. 2012; Ghasemi ve ark. 2013; Rahmati ve ark, 2015; Abdollahi ve ark. 2018; Tataria ve ark., 2020).

Alıç anacının, üzerindeki kalemi bodurlaştırıcı etkisi, yenidünyalarda sık dikim yetiştiricilik açısından büyük önem taşımakta ve alıcın yenidünyalarda bodur anaç olarak kullanılabileceğini göstermektedir.

Verim ile İlgili Bulgular

Denemedeki anaçların, HÇG yenidünya çeşidinin verim parametreleri üzerine etkilerine ilişkin veriler Çizelge 3' te verilmiştir.

Bitki başına verim bakımından yenidünya çöğür anacı (1051.74 g /bitki^{-1}) alıç anacına (570.89 g bitki^{-1}) göre daha yüksek bir değere sahip olmasına rağmen gerek birim gövde kesit alanına düşen verim, gerek birim alana verim bakımından alıç anacı (sırasıyla, 1.48 g mm^{2} $^{-1}$ ve 1141 kg da^{-1}), yenidünya çöğür anacından (sırasıyla, 0.79 g mm^{2} $^{-1}$ ve 1051 kg da^{-1}) daha yüksek değerler vermiştir. Bitki başına verim ve birim gövde kesit alanına düşen verim bakımından anaçlar

arasındaki farklılık istatistiksel olarak önemli bulunurken, birim alana verim bakımından anaçlar arasındaki farklılık istatistiksel olarak önemsiz bulunmuştur. Bu bulgular, alıç anacının yenidünya yetiştiriciliğinde, bodur anaç olarak sık dikime uygun bir anaç olduğunu ve yenidünya çöğür anacına göre önemli verim artışı sağlayacağını göstermektedir. Ancak, elde edilen bu veriler alanındaki ilk veriler olması nedeniyle, kesin bir yargıya varmak için araştırmanın sürdürülmesi gerekmektedir.

Çizelge 3. Anaçların HÇG yenidünya çeşidinin meyve verim unsurları üzerine etkileri.
Table 3. The effects of rootstocks on fruit yield components of HÇG loquat variety.

Anaçlar *(Rootstock)*	Bitki başına verim (g) *Yield (g plant-1)*	Birim gövde kesit alanına düşen verim (g mm^{2-1}) *Yield per unit trunk cross sectional area (g mm-2)*	Birim alana verim (kg da^{-1}) *Yield (kg da-1)*
Alıç *(Hawthorn)*	570.89±29.43 b[x]	1.48±0.15a	1141±30.15a
YD çöğürü *(Loquat seedling)*	1051.74±32.86 a	0.79±0.11b	1051±33.12a
$LSD_{\%5}$	*	*	ÖD[y]

[x]:Aynı sütunda farklı harfler LSD testine göre % 5 düzeyinde önemli farklılığı göstermektedir, [y]: Önemli değil.
[x]:Different letters within columns are indicate significant difference by LSD's test at P < 0.05. [y]:Not significance.

Yenidünyalarda bodur anaç olarak kullanılabilen ayva anaçları ile yapılan önceki çalışmalarda (Akkuş ve Polat, 2021; Sezer ve Polat, 2022) bitki başına verim, 279.35 g bitki^{-1}-955.67 g bitki^{-1}; birim gövde kesit alanına verim, 0.69 g mm^{2-1} 1.02 g mm^{2-1}; birim alana verim ise 558 kg da^{-1} 1911 kg da^{-1} olarak belirlenmiştir. Yenidünyalarda alıç anacının kullanımına ilişkin yayınlanmış kaynaklara ulaşılamadığından mevcut verilerin karşılaştırılması mümkün olmamakla birlikte, ayva için anaç olarak alıcın kullanıldığı bir araştırma sonucuna ulaşılabilmiştir. BA-29, A, B ve C ayva klon anaçları ile ayva ve alıç çöğürlerinin üzerine aşılı İsfahan ayva çeşidinin bazı kalitatif ve kantitatif özelliklerine etkisinin incelendiği bir çalışmada, birim gövde kesit alanına düşen verim bakımından en düşük değer (0.22 kg cm^{2-1}) ayva çöğüründen, en yüksek değer ise alıç anacından (0.35 kg cm^{2-1}) elde edilmiştir. Elde edilen sonuçlara göre, İsfahan ayva çeşidi ile bahçe tesisinde bodur anaç olarak alıç önerilmiştir (Tatari ve ark., 2020).

Alıç anacı üzerine aşılı HÇG yenidünya çeşidinin bitki taç yapısı ve yaşı diğer anaçlardan küçük olmasına rağmen, bitki başına verim ve birim alana verim bakımından Quince-A anacından fazla ürün vermesi ve gövde kesit alanına düşen verim bakımından da Quince-A, Quince-C ve BA-29 ayva anaçlarından daha yüksek değere sahip olması, alıç anacının yenidünyalarda anaç olarak kullanılabilmesi açısından, çalışmanın en dikkat çekici ve önemli bir bulgusu olduğu söylenebilir. Ancak bu anaca ilişkin verilerin diğer anaçlarla tam kıyaslanabilmesi için çalışmaların sürdürülmesi ve öteki anaçlarla kıyaslamalı istatistiksel analizlerin yapılarak değerlendirilmesi gerekmektedir.

SONUÇ ve ÖNERİLER

Bu çalışmadan elde edilen bulgular, alıç anacının yenidünyalarda kullanımı açısından ilk bulgular olması bakımından oldukça önemli ve değerlidir. Bu bulgular, anacı alıç olan bodur fidanlarla sık dikim yapılarak birim alandan daha fazla ürün alma olanaklarının mümkün olduğunu göstermektedir. Çalışmanın bulguları genel olarak değerlendirildiğinde; alıç anacının yenidünya çöğür anacına göre daha üstün sonuçlar verdiği görülmektedir. Özellikle alıç anacının kalemin vejetatif büyümesini baskılayarak birim gövde kesit alanına düşen verim bakımından önemli verim artışı sağlaması dikkat çekici bulunmuştur. Henüz iki yaşındaki ağaçlarda görülen bu önemli verim artışının, ileri yaşlarda çok daha yüksek değerlere ulaşacağı beklenmektedir. Ancak bu tür çalışmalarda daha kesin sonuçların elde edilebilmesi için çalışmaların bir süre daha devam ettirilmesi gerekmektedir. Ayrıca, ileriki çalışmalarda yenidünya/alıç kombinasyonunda aşı uyuşmazlığına ilişkin hususların araştırılması da yararlı olacaktır.

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Determination of the Effects of Different Irrigation Strategies on Leaf Osmotic Potential and K and Ca Ion Concentrations in Red Pepper with Furrow and Drip Irrigation Methods

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ABSTRACT

A study was managed to identify the water stress effect on marketable yield, osmatic potential, and potassium (K) and calcium (Ca) ions for drip and furrow irrigated processing red pepper in the 2010 and 2011 growing seasons in Tarsus, Turkey. The treatments for drip irrigation; comprise full irrigation ($D_{FI1.0}$), deficit irrigation $D_{DI0.75}$, $D_{PRD0.5}$, $D_{FPRD0.5}$, and $D_{DI0.5}$; for furrow irrigation; full irrigation ($F_{FI1.0}$), fix alternative furrow ($F_{AF0.5}$) and PRD furrow ($F_{PRD0.5}$). $F_{AF0.5}$ and $F_{PRD0.5}$ received 50 % of the water applied to $F_{FI1.0}$. In $F_{AF0.5}$ the same furrows were irrigated while in $F_{PRD0.5}$ irrigated alternately. Irrigation methods and irrigation levels had a remarkable effect on the total yield of red pepper in both experimental years. Drip irrigation treatments manufactured higher red pepper yields than the furrow irrigation treatments. The maximum yield in the drip irrigation system was acquired from the $D_{FI1.0}$ treatment followed by $D_{DI0.75}$, $D_{DI0.5}$, and $D_{FPRD0.5}$ treatments. Though $D_{PRD0.5}$, $D_{FPRD0.5}$, and $D_{DI0.5}$ applied the same amount of water, $D_{PRD0.5}$ resulted in a higher yield. In furrow treatments, $F_{FI1.0}$ resulted in the highest yield followed by $F_{PRD0.5}$ and $F_{AF0.5}$. Water use efficiency (WUE) diminished with increasing the water amount for drip and furrow irrigation methods. While lower osmotic potential values were measured in full irrigation treatments in furrow and drip irrigation plots, higher osmotic potential values were determined in treatments where water stress was determined in both years. In both drip and furrow irrigation, the lowest Ca (%) values were obtained in full irrigation, while the highest Ca values were obtained in limited irrigation with water stress in the 2010 and 2011 years. K ion values were generally similar in the first and fourth pepper harvests in drip and furrow irrigation.

Irrigation

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Karık ve Damla Sulama Yöntemleriyle Kırmızı Biberde Farklı Sulama Stratejilerinin Yaprak Osmotik Potansiyeli ile K ve Ca İyon Konsantrasyonları Üzerine Etkilerinin Belirlenmesi

ÖZET

Tarsus, Türkiye'de 2010 ve 2011 yetiştirme sezonlarında damla ve karık sulama ile kırmızı biberin pazarlanabilir verim, osmatik potansiyel ve potasyum (K) ve kalsiyum (Ca) iyonları üzerindeki su stresi etkisini belirlemek için bir çalışma gerçekleştirildi. Damla sulama konuları; tam sulama ($D_{FI1.0}$), kısınıtlı sulama $D_{DI0.75}$, $D_{PRD0.5}$, $D_{FPRD0.5}$ ve $D_{DI0.5}$'ten oluşurken; karık sulama konuları; tam sulama ($F_{FI1.0}$), sabit alternatif karık ($F_{AF0.5}$) ve PRD karık ($F_{PRD0.5}$)'tan oluşmaktadır. $F_{AF0.5}$ ve $F_{PRD0.5}$, $F_{FI1.0}$ 'a uygulanan suyun %50'sini almıştır. $F_{AF0.5}$' konusunda aynı karıklar, $F_{PRD0.5}$'te dönüşümlü olarak karıklar sulanmıştır. Sulama yöntemleri ve sulama seviyeleri, her iki deneme yılında da toplam kırmızı biber verimi üzerinde dikkate değer bir etkiye sahip olmuştur. Damla sulama konuları karık sulama konularına göre daha yüksek kırmızı biber verimi sağlamıştır. Damla sulama sisteminde en yüksek verim $D_{FI1.0}$ konusundan alınırken, ardından $D_{DI0.75}$, $D_{DI0.5}$ ve $D_{FPRD0.5}$ konuları izlemiştir. $D_{PRD0.5}$, $D_{FPRD0.5}$ ve $D_{DI0.5}$ konularına aynı miktarda sulama suyu miktarı uygulansa da, $D_{PRD0.5}$ konusunda daha yüksek verimle

Sulama

Araştırma Makalesi

Makale Tarihçesi
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Anahtar Kelimeler
Biber
Karık
Damla Sulama
Ozmotik Potansiyel
Kısmi Kök Kurutma

sonuçlanmıştır. Karık uygulamalarında $F_{FI1.0}$ en yüksek verimle sonuçlanırken, bu konuyu $F_{PRD0.5}$ ve $F_{AF0.5}$ konualrı izlemiştir. Damla ve karık sulama yöntemlerinde su miktarı arttıkça su kullanım etkinliği (WUE) azalmıştır. Karık ve damla sulama parsellerinde yer alan tam sulama konusunda daha düşük ozmotik potansiyel değerleri ölçülürken, her iki yılda da su stresinin belirlendiği uygulamalarda daha yüksek ozmotik potansiyel değerleri belirlenmiştir. 2010 ve 2011 yıllarında hem damla hem de karık sulamada en düşük Ca (%) değerleri tam sulamada elde edilirken, en yüksek Ca değerleri su stresi yaşanan kısıntılı sulama konularında elde edilmiştir. Damla ve karık sulamada birinci ve dördüncü biber hasadında K iyon değerleri genel olarak benzer olmuştur.

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INTRODUCTION

Pepper is an important vegetable that is grown in most countries around the world, and covers 1.93 million hectares (Penella & Calatayud, 2018). Pepper is consumed as a spice and only its fruit and only its fruit is used in cooking. According to data from 2017, the world's pepper production was 34 million tons (Penella & Calatayud, 2018). Sweet and paste pepper (*Capsicum annum L.)* is one of the most important vegetable products, especially in the Mediterranean region, where high efficiency and good fruit quality can be obtained in greenhouse conditions compared to open field conditions (Serret et al., 2018). Water resources are limited in the Mediterranean region, and there is increasing pressure on the use of these resources by other sectors such as domestic, urban, and industrial sectors, as well as irrigation. The agricultural sector is the largest consumer of water resources in the world, and it is important to use water efficiently (Sezen et al., 2017). The drip irrigation system can be used to apply irrigation water more efficiently and uniformly, resulting in significant savings in irrigation water usage, and maximizing water usage efficiency. Traditional irrigation scheduling is mainly based on monitoring the water content of the soil in the plant root zone. However, the internal water condition of the plant, which is located at the midpoint of the soil, plant, water, and atmospheric system, to both climate and soil water changes. Many published studies have shown that partial root-zone drying (PRD) increases the physiological mechanisms of osmotic pressure, stomatal conductivity, and enzyme activities in plants. The PRD system puts the plant in a continuous water retention state, drying out part of the plant root system while the remaining roots are well watered and develop better (Mousavi et al., 2010). Liu et al., (2006) reported that the mechanism of the PRD irrigation strategy increases the abscisic acid (ABA) and provides better yields than the products that are properly irrigated condition. Dodd (2009) compared the impacts of PRD and RDI (regular deficit irrigation) on the yield of varied crop species have indicated that dissimilar PRD and RDI plants were more subject to the potential decrease in yield. This risk could be reduced by near monitoring plant water status to prevent the development of heavy water stress that could notably diminish yield. The benefits of the PRD in comparison to RDI are also based on the increasing root growth and development and better control of vegetative vigor and assimilate partitioning (Mingo et al., 2004; Costa et al., 2007). This study aims to compare the effects of full, deficit irrigation (DI) regimes and partial root-zone drying (PRD) strategy under drip and furrow irrigation conditions through periodic measurements of K and Ca ion concentrations and leaf osmotic potential in red pepper (*Capsicum annuum* L.) leaves in Mediterranean climate conditions. In addition, the effects on marketable fruit yield, WUE, and IWUE of red pepper under furrow and drip irrigation conditions were compared under deficit irrigation, partial root-zone drying (PRD), and full irrigation conditions.

MATERIAL and METHOD

Experimental site, soil description, and climate

The field experiment was managed for 2 years (2010 - 2011) at the Tarsus Soil and Water Resources Unit of Alata Horticultural Research Institution (37°01′N and 35°01′E and altitude 60.0m above sea level), in Topcu, Tarsus, Turkey. The soil of the experimental field is clay loam clay texture with a relatively high water-holding capacity. The available soil water holding capacity of the experimental site was determined 120 mm in a 0.60 m soil profile. The soil water content at field capacity ranged from 0.29 to 0.37 g g^{-1} and wilting point ranged from 0.15 to 0.23 g g^{-1} on a dry weight basis for the experimental area. In the effective root zone, soil water contents at the field capacity and

permanent wilting point are 284 and 164 mm, respectively. The dry soil bulk densities varied from 1.44 to 1.45 g cm^{-3} throughout the 60 cm soil profile.

The experimental area has a well-specified typical Mediterranean-type climate. Average the average yearly rainfall is 616 mm, and 54% of whole falls during the winter months. Considering long-term climatic information (1952-2007), the yearly evaporation, yearly average temperature, and yearly relative humidity of the region were 1490 mm, 17.8 ºC, and 70.66%, respectively. The seasonal (April to October) mean rainfall during the 2010 and 2011 growing seasons are 165 and 172 mm, respectively.

Experimental design and treatments

The experiment was set out in a completely randomized block design with four replications for drip and furrow irrigation experiments. In drip irrigation, the first treatment ($D_{FI1.0}$) which is considered full irrigation, is the required amount of water to complete field capacity in the upper 60 cm soil depth when 25% of the soil available water is used. Also, drip treatments comprised deficit irrigation ($D_{DI0.75}$, $D_{DI0.5}$) and partial root drying ($D_{PRD0.5}$ and $D_{FPRD0.5}$). $D_{DI0.75}$ and $D_{DI0.5}$ received 75 and 50% of $D_{FI1.0}$, $D_{FPRD0.5}$ received 50% of $D_{FI1.0}$ irrigation permanently from one side of the crop row; $D_{PRD0.5}$ received 50% of $D_{FI1.0}$ water alternately from the lateral.

In furrow treatments, the ($F_{FI1.0}$) treatment which is considered as a full irrigation, is the required amount of water to complete to field capacity in the 60 cm soil depth when 40% of the soil available water is used. Deficit irrigation treatments comprised fixed alternate furrow ($F_{AF0.5}$) and PRD furrow ($F_{PRD0.5}$) in the furrow irrigation. $F_{AF0.5}$ and $F_{PRD0.5}$ received 50% of the water applied to $F_{FI1.0}$. In $F_{AF0.5}$ the same furrows were irrigated while $F_{PRD0.5}$ irrigated alternate furrows.

In drip-irrigated plots, laterals were settled in each plant row for $D_{FI1.0}$, $D_{DI0.75}$, and $D_{DI0.5}$ treatments, and inline emitters with a discharge rate of 2.3 L h^{-1}were spaced at 20 cm intervals on the lateral line (Sezen et al., 2006). In the $D_{PRD0.5}$ treatment plots, two drip laterals were settled out 20 cm away from the plant row. In the $D_{FPRD0.5}$ treatment plots, a single lateral was placed 20 cm away from the plant row. The system was operated at 100 kPa during the growing season. The control unit of the system occurs of a pump, gravel, and disk filters, a flow meter, control valves, a fertilizer tank, and pressure gauges.

The amount of irrigation water was computed based on the pre-irrigation soil water (Wi) in the measured soil profile according to the following equation (Eq. (1)):

$$I = (W_{FC} - W_i).\gamma \,.\, D.\, A$$

where I is the amount of irrigation water (m^3); WFC is the soil water at field capacity; γ is the soil bulk density (g cm^{-3}); D is the soil depth (0.6 m), and A is the area of the plot (m^2).The seasonal ET value was calculated using a water balance method expressed by Allen et al. (1998). WUE and IWUE values were calculated as the marketable yield of red pepper divided by seasonal ET and total irrigation during the all-growing season in 2010 and 2011 (Sezen et al., 2019).

Agronomic practices

Seedlings of Capsicum annuum (Karaisalı), a variety widely used in the region, were gently transplanted into the plots on 15 April 2010 and 19 April 2011, respectively in the first and second year of the study. Karaisali pepper is a local cultivar that originated from Adana city. The cultivar has capita-type pepper fruit. The fruit has a flabby and cylindrical form with a deep green color; the ripe fruit has red color. Thick flesh, good aroma, and sweet taste properties are appropriate for fresh table consumption in immature and mature stages in the local region. This cultivar is also extensively used for pepper paste manufacture in the area by the local people. The plants were grown in seven 70 cm spaced rows in each plot with 25 cm spacing in each row. Each plot had sizes of 10m long and 7 plant rows (4.9 m) in width with 280 plants per plot (5.7 plants m^{-2}).

All treatment plots received the same amount of total fertilizer. Preplant manure was applied at a rate of 20-30 tons per hectare in a manner that soil organic matter content was provided over 2%. The following fertilizer program was applied in the experiment at planting: 200 kg ha^{-1} N, 100 kg ha^{-1} P_2O_5, 250 kg ha^{-1} K_2O, 100 kg ha^{-1} CaO, and 50 kg ha^{-1} MgO. The total amount of P_2O_5 fertilizer and the remaining amount of fertilizer was applied through fertigation and began 3 weeks after transplanting. In each application, 1/6th of the above-mentioned fertilizer was applied for 3 weeks until the production of green fruit pepper. Also, microelements (Mn, Zn, Cu, B, and Mo) were applied at a rate of 5 kg ha^{-1}. Microelements were applied at 3 different times starting 3 weeks after transplanting, second during the green fruit formation phase, and third application at the red pepper formation phase (Salk et al., 2008).

The harvest area in each sub-plot was 28.0 m^2 (five rows, each 8 m in length). Mature red peppers were harvested six times in 2010 and seven times in 2011. The first collection was performed on August 02, 2010 (109 DAT: days after transplanting) and the final collection was performed on November 04, 2010 (203 DAT); the corresponding figures for the second year were July 19, 2011 (91 DAT) and November 03, 2011 (198 DAT), respectively.

Measurement of Leaf Osmotic Potential

As a physiological response when plants are exposed to water stress cells undergo osmotic regulation. In this case, the production of water-soluble organic

substances usually sugars and amino acids is increased or there may be an accumulation of ions such as K and Ca. This osmotic regulation, which also ensures the preservation of turgor in the plant, was measured in the leaves of red pepper plants grown on different irrigation treatments in drip and furrow irrigation experiments. Thus, the effect of water stress on plants that applied the PRD irrigation strategy with the classical drip and furrow methods with limited irrigation was presented comparatively. In both drip and furrow irrigation, leaf samples were taken before irrigation applications and the dates are indicated in the text. For this purpose, mature leaf samples were taken periodically during the experiment and the "osmotic potential" values were measured with the help of the "osmometer" device (Turner & Buirchell, 2007).

Determination of K and Ca Ion Concentrations in Leaves

In drip and furrow irrigation plots, osmotic regulation occurs with the accumulation of K and Ca elements as a reaction of plants to water stress, especially in deficit irrigation treatments. For this purpose, the concentrations of K and Ca elements at different irrigation levels were determined in leaf tissues. Dried and ground leaf samples were burned at 550 °C and dissolved in 3.3% (v/v) HCl and elemental concentrations were read in an atomic absorption spectrometer (Varian 220 FS) in emission mode (Dasgan et al., 2009).

Treatments were compared using Duncan's multiple test. Additionally, the values in the charts are given as mean ± Standard errors.

RESULTS and DISCUSSION

Irrigation, seasonal ET, Marketable Yield, WUE, and IWUE

To accomplish a uniform plant stand, a total of 55 mm in 2010 and 20 mm in 2011 of irrigation water was implemented same to all treatment plots. In drip irrigation plots, the first treatment irrigation was implemented on June 07, 2010, and May 18, 2011. The last irrigation practice was provided on October 25, 2010, and October 21, 2011. The sum of total irrigation water applied altered from 385 mm to 715 mm in 2010 and 395 mm to 770 mm according to the treatments in drip irrigation plots Table 1). Irrigation frequencies in the drip irrigation treatments ranged from 4 to 9 days in 2010 and 4 to 8 days in 2011. Drip irrigation treatments were irrigated 22 times in the first year and 25 times in the second year.

In the furrow plots, the first treatment irrigation was implemented on June 11, 2010, and June 07, 2011. The last irrigation practice was implemented on October 28, 2010, and October 21, 2011. The sum of irrigation water applied ranged from 429 mm to 823 mm in 2010 and 452 mm to 884 mm depending on the treatment (Table 1). Irrigation frequencies ranged from 6 to 13 days in 2010 and from 6 to 12 days in 2011 in the furrow treatments. Furrow irrigation treatments were irrigated 16 and 18 times in the 2010 and 2011 seasons, respectively.

Seasonal evapotranspiration (ET) by red pepper ranged from 515 mm in $D_{PRD0.5}$ to 809 mm in $D_{FI1.0}$ treatment in 2010; and 539 mm in $D_{PRD0.5}$ to 824 mm in $D_{FI1.0}$ treatment in 2011 (Table 1). The ET values raised with the increasing amount of irrigation under furrow and drip irrigation treatments. PRD treatments in the drip irrigation systems ($D_{FPRD0.5}$, $D_{PRD0.5}$) showed slightly lower ET than $D_{DI0.5}$ treatment even though receiving the same amount of water.

WUE and IWUE values were notably impacted by irrigation treatments and irrigation methods (Table 1). WUE values varied from 5.46 kg m^{-3} in $D_{FI1.0}$ to 7.14 kg m^{-3} in the $D_{PRD0.5}$ in 2010 and varied from 5.79 kg m^{-3} in $D_{FI1.0}$ to 7.48 kg m^{-3} in the $D_{PRD0.5}$ in 2011 in drip treatments. $D_{PRD0.5}$ resulted in the highest WUE values in drip treatments. In furrow treatments, WUE values varied from 3.84 to 5.80 kg m^{-3} in 2010; 4.23 to 4.82 kg m^{-3} in 2011. IWUE values ranged from 6.18 kg m^{-3} in $D_{FI1.0}$ to 9.55 kg m-3 in $D_{PRD0.5}$ treatment in the 2010 and varied from 6.21 kg m^{-3} in $D_{FI1.0}$ to 10.20 kg m^{-3} in the $D_{PRD0.5}$ in the 2011 season.

Leaf Osmotic Potential

As a physiological response when plants are exposed to water stress cells undergo osmotic regulation. This osmotic regulation, which maintains the turgor state of the plant, was measured in the leaves of red pepper plants grown under different irrigation treatments in drip and furrow irrigation plots. For this purpose, the mature leaf samples that have completed their development periodically during the experiment were taken and the "osmotic potential" values were measured with the "osmometer" device in the laboratory. The first osmometer measurement was started on July 02, 2010, and the last measurement was made on September 29, 2010. The temporal variation of leaf osmotic potential in drip and furrow irrigation is given in Figures 1 and 2.

While lower osmotic potential values were measured in $D_{FI1.0}$ treatments, higher osmotic potential values were determined in $D_{DI0.5}$ and $D_{FPRD0.5}$ treatments, where water stress was determined in Figure 1. While lower osmotic potential values were measured at the beginning of the experiment, the osmatic potential values increased towards the end of the season.

While lower osmotic potential values were measured for $F_{FI1.0}$, increased water stress resulted in higher osmotic potential values, especially for $F_{AF0.5}$ treatment in Figure 2. Leaf osmotic potential values resulted in higher values under increasing stress

conditions. Mullet ve Whitsitt, (1996) indicated that the mechanism of tolerance to water stress has been reported as "osmotic regulation" and protection of membranes in the cell.

Table 1. Yield, irrigation, ET, WUE, and IWUE values of red pepper in-furrow and drip irrigation treatments (2010, 2011)

Çizelge 1. Karık ve damla sulama uygulamalarında kırmızı biberde verim, sulama, ET, WUE ve IWUE değerleri (2010, 2011)

Years	Irrigation methods	Treatments	Seasonal irrigation (mm)	ET (mm) **	Yield (kg ha^{-1}) **	WUE (kg m^{-3}) **	IWUE (kg m^{-3}) **
2010	Furrow	$F_{PRD0.5}$	439	602 ± 17.15 b	34940 ± 64.42 a	5.80 ± 0.18 a	7.95 ± 0.10 a
		$F_{AF0.5}$	439	631 ± 11.21 b	31720 ± 127.80 b	5.03 ± 0.15 ab	7.22 ± 0.18 a
		$F_{FI1.0}$	823	928 ± 12.28 a	35590 ± 156.84 a	3.84 ± 0.20 b	4.32 ± 0.19 b
	Drip	$D_{PRD0.5}$	385	515 ± 9.40 d	36750 ± 147.20 c	7.14 ± 0.18 a	9.55 ± 0.18 a
		$D_{FPRD0.5}$	385	558 ± 5.85 c	34160 ± 91.29 d	6.12 ± 0.20 b	8.87 ± 0.09 a
		$D_{FI1.0}$	715	809 ± 11.56 a	44170 ± 148.55 a	5.46 ± 0.16 b	6.18 ± 0.13 c
		$D_{DI0.75}$	561	707 ± 14.49 b	40830 ± 147.20 b	5.78 ± 0.18 b	7.28 ± 0.10 b
		$D_{DI0.5}$	385	572 ± 9.31 c	34920 ± 147.20 d	6.10 ± 0.20 b	9.07 ± 0.18 a
2011	Furrow	$F_{PRD0.5}$	452	638 ± 10.23 c	30740 ± 91.29 b	4.82 ± 0.20 ns	6.80 ± 0.18 a
		$F_{AF0.5}$	452	663 ± 14.93 b	29320 ± 380.09 b	4.42 ± 0.15 ns	6.49 ± 0.12 a
		$F_{FI1.0}$	884	980 ± 9.97 a	41500 ± 204.12 a	4.23 ± 0.20 ns	4.69 ± 0.14 b
	Drip	$D_{PRD0.5}$	395	539 ± 7.14 d	40330 ± 204.12 b	7.48 ± 0.19 a	10.20 ± 0.21a
		$D_{FPRD0.5}$	395	572 ± 11.87 c	33760 ± 132.92 d	5.90 ± 0.20 b	8.55 ± 0.19 bc
		$D_{FI1.0}$	770	824 ± 7.63 a	47790 ± 204.12 a	5.79 ± 0.18 b	6.21 ± 0.18 d
		$D_{DI0.75}$	595	752 ± 11.35 b	47170 ± 142.83 a	6.27 ± 0.16 b	7.93 ± 0.14 c
		$D_{DI0.5}$	395	592 ± 16.05 c	35970 ± 203.47 c	6.08 ± 0.18 b	9.11 ± 0.13 b

Letters indicate significant differences at *P < 0.05 and **P < 0.01

Figure 2 showed that while lower osmotic potential values were measured for $F_{FI1.0}$, increased water stress resulted in higher osmotic potential values, especially for $F_{AF0.5}$ treatments. The temporal variation of leaf osmotic potential values for 2011 in drip and furrow irrigation treatments are given in Figures 3 and 4. The first osmometer measurement was started on June 07, 2011, and the last measurement was made on October 04, 2010.

In 2011, while the lowest osmotic potential values were measured for $D_{FI1.0}$ in drip irrigation, the highest values were determined for $D_{DI0.5}$ and $D_{FPRD0.5}$ treatments. While lower osmotic potential values were measured at the beginning of the experiment, osmotic potential values increased towards the end of the season in 2011 (Figure 3).

In 2011, while the lowest osmotic potential values were measured for $F_{FI1.0}$ in furrow irrigation, it resulted in the highest osmotic potential values for $F_{AF0.5}$. While lower osmotic potential values were measured at the beginning of the experiment in furrow irrigation treatments, the osmatic potential values increased towards the end of the season in 2011 (Figure 4). Physiological, biochemical, and molecular biological levels of the plant were investigated in PRD applications in different plants and, for example, potato crops showed positive physiological regulation due to osmotic adaptation (Su et al., 2020). Water stress occurs from the osmotic salinity of soil and water. (Lian et al., 2004). Under stress conditions, absisic acid (ABA) is produced and osmotic adjustment processes carry it to the leaves via the xylem (Schachtman & Goodger, 2008). The osmotic concentration in the leaves of plants is related to its osmotic potential. After the salt of the water decreases, the osmotic potential in the leaf is irrigated conditions (Saleh, 2012). Conversely, Alvares et al., (2012) reported that no osmotic adjustment was observed in plants submitted to water stress. The osmatic potential responds not only to water stress but also to other factors including cultivar, environment, soil type, and the relationships between canopy and root system, i.e. the resistance to water movement. Therefore, the water potential thresholds to schedule irrigation are site-specific (Garcia-Tejera et al., 2021).

K and Ca Ion Concentrations in Leaves

K and Ca ion concentrations in pepper were determined on the leaf samples taken in the first (August 02, 2010, and July 19, 2011) and fourth harvest (September 13, 2010 and September 05, 2011) periods in the 2010 and 2011 experimental years for furrow and drip irrigation (Figures 5-8).

In drip irrigation, the Ca ion values of 2010 were determined to be higher both in the first harvest and in the fourth harvest compared to the 2011 season. The highest Ca (%) values were determined for $D_{PRD0.5}$ in both trial years and the lowest Ca (%) values for $D_{FI1.0}$

treatment (Figure 5). Similar to drip irrigation, it was determined that Ca ion values in furrow irrigation in 2010 were higher both in the first harvest and in the fourth harvest compared to the 2011 season. In both experimental years, the highest Ca (%) values were determined for $F_{AF0.5}$ treatment, and the lowest Ca (%) values were determined for $F_{FI1.0}$ treatment (Figure 6). In drip irrigation, the K ion values of both years were generally similar in the first and fourth harvests. In the 2010 and 2011 experimental years, the highest K (%) values were found in $D_{FPRD0.5}$, $D_{DI0.5}$, and the lowest K (%) values were in $D_{PRD0.5}$ (Figure 7). In the furrow irrigation treatments, the K ion values of both years were generally similar in the first and fourth harvests.

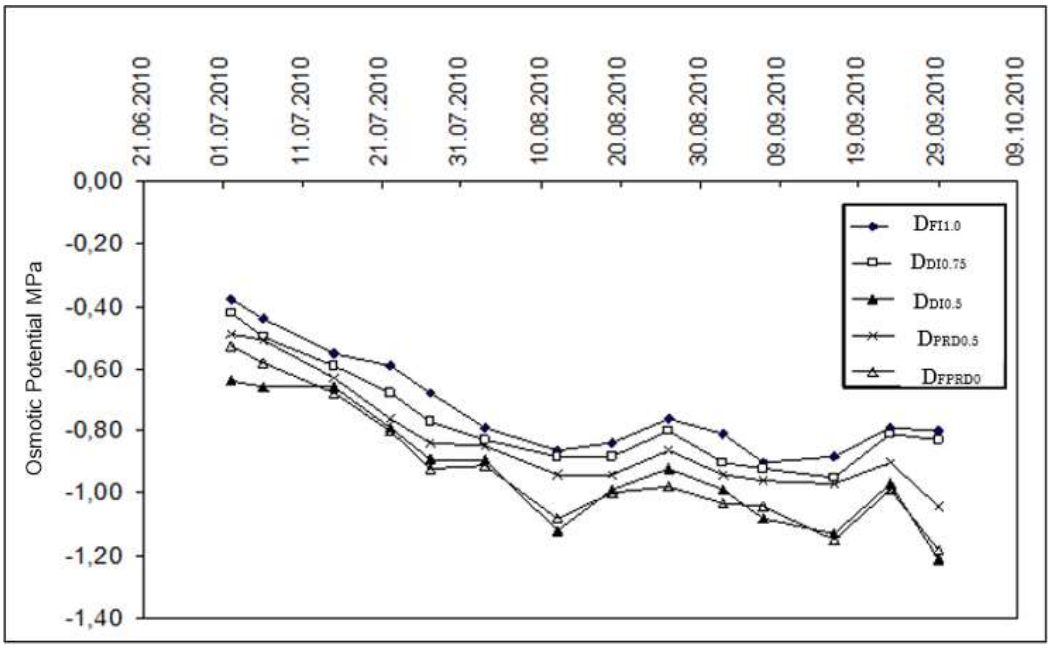

Figure 1. Temporal change of leaf osmotic potential in drip irrigation treatments (2010)
Şekil 1. Damla sulama konularında yaprak ozmotik potansiyelinin değişimi (2010)

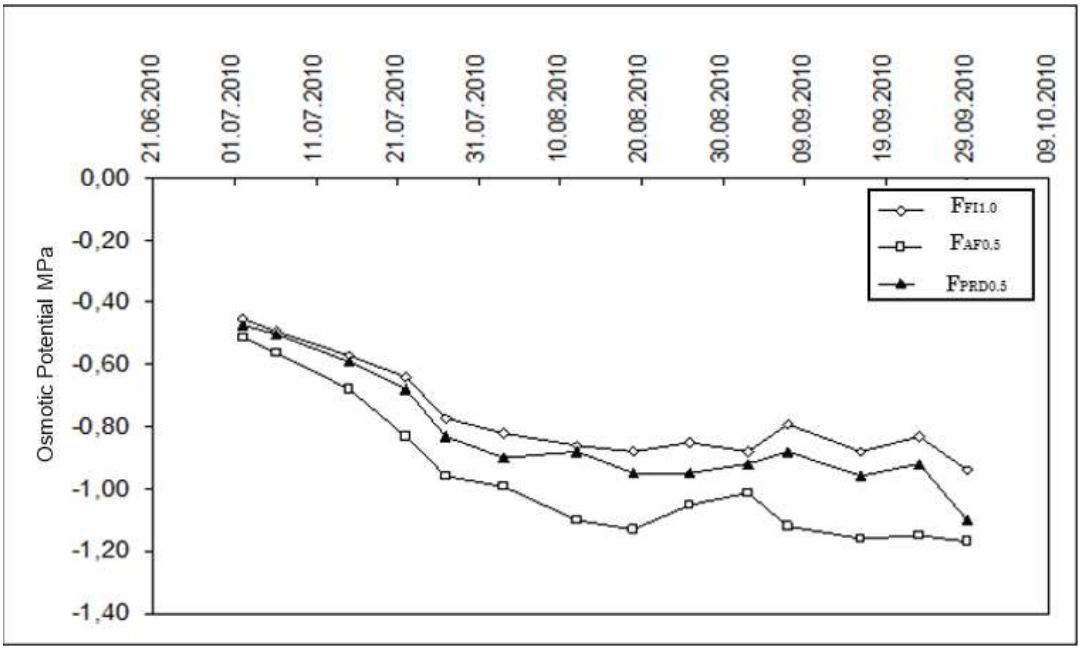

Figure 2. Temporal change of leaf osmotic potential in furrow irrigation treatments (2010)
Şekil 2. Karık sulama konularında yaprak ozmotik potansiyelinin değişimi (2010)

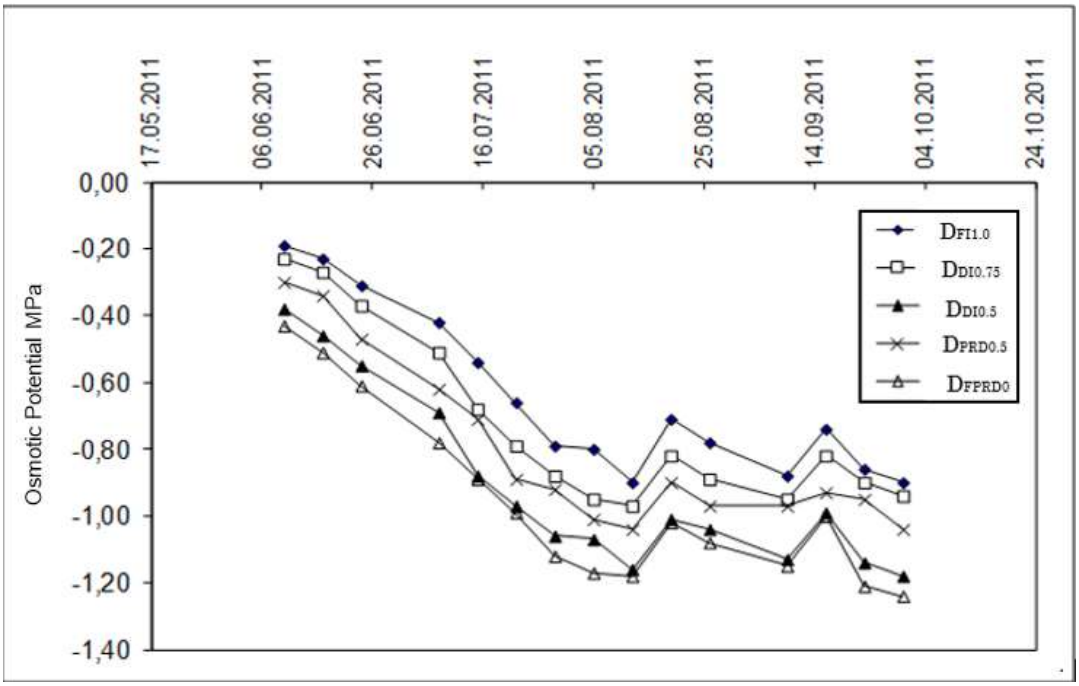

Figure 3. Temporal change of leaf osmotic potential in drip irrigation treatments (2011)
Şekil 3. Damla sulama konularında yaprak ozmotik potansiyelinin değişimi (2011)

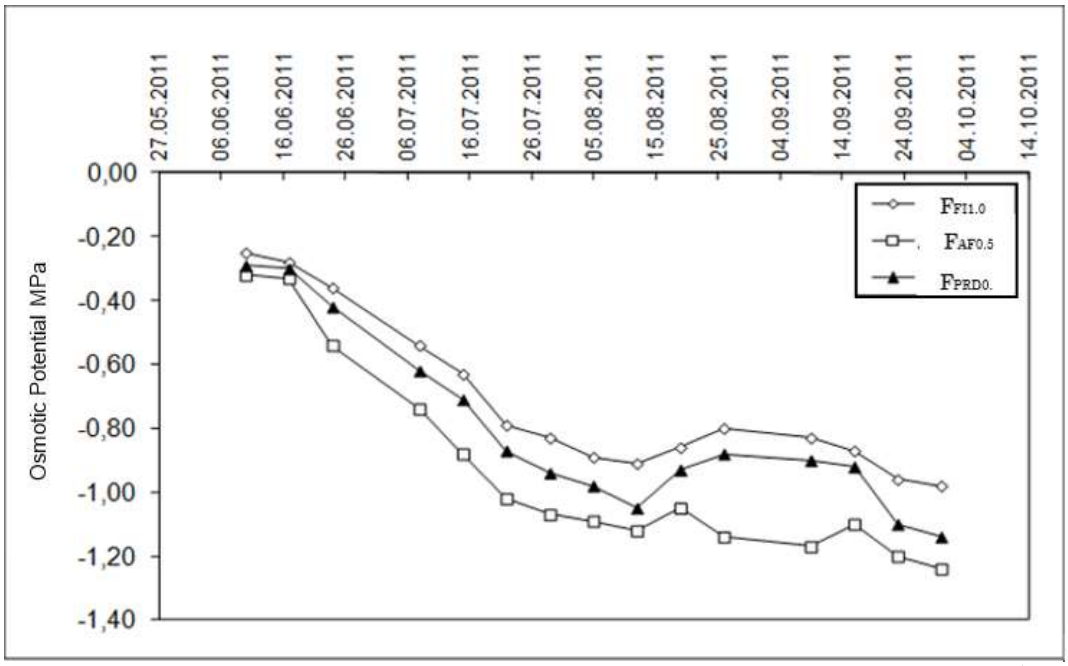

Figure 4. Temporal change of leaf osmotic potential in furrow irrigation treatments (2011)
Şekil 4. Karık sulama konularında yaprak ozmotik potansiyelinin değişimi (2011)

In the 2010 and 2011 growing seasons, K (%) values varied between 2.1-2.5 % and 2.3-2.8%, respectively (Figure 8). Potassium (K) ion consentrations plays an important role in protein synthesis, photosynthesis, stomatal regulation, sugar transport, enzyme activity, and improving yield and quality (White and Karley, 2010; Marschner, 2012; Oosterhuis et al., 2014). Potassium (K) values, which is the most abundant inorganic cation that provides plant growth, tend to increase from the first harvest to the fourth harvest in drip and furrow irrigation treatments in 2010 and 2011 growing seasons. Similar many studies were obtained by Shabala and Cuin, (2008); Walker et al., (2000); Amjad et al., (2014)

CONCLUSION

In this study, the impacts of irrigation methods, irrigation water amount and irrigation strategies (deficit and PRD) are notably significant for acquire higher marketable yields of red pepper under the Mediterranean climatic conditions in Turkey.

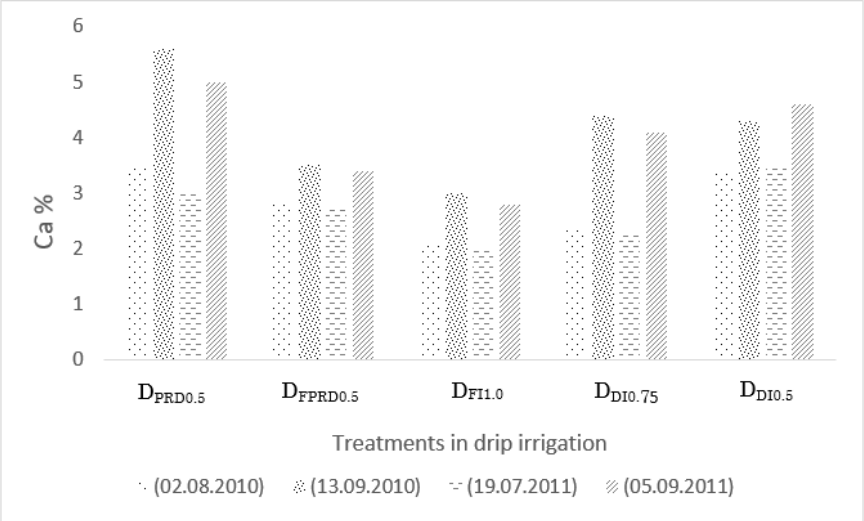

Figure 5. Changes of Ca ion concentrations in leaves in drip irrigation treatments (2010-2011)
Şekil 5. Damla sulama konularındaki yapraklardaki Ca iyon konsantrasyonlarının değişimleri (2010-2011)

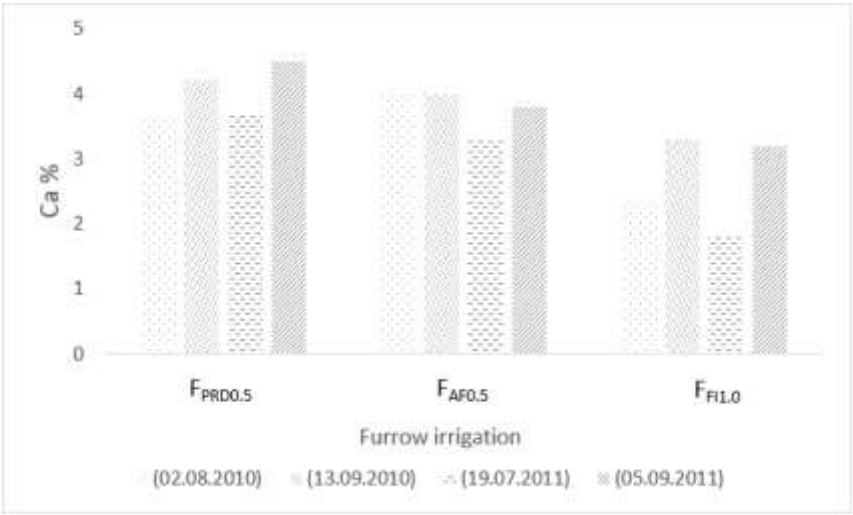

Figure 6. Changes of Ca ion concentrations in leaves in furrow irrigation treatments (2010-2011)
Şekil 6. Karık sulama konularında yapraklardaki Ca iyon konsantrasyonundaki değişimleri (2010-2011)

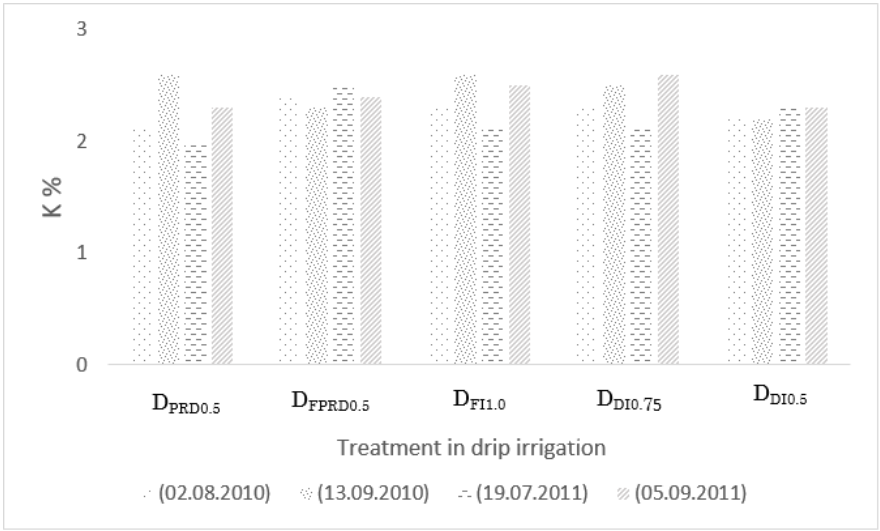

Figure 7. Changes of K ion concentrations in leaves in drip irrigation treatments (2010-2011)
Şekil 7. Damla sulama konularında yapraklardaki K iyon konsantrasyonlarının değişimleri (2010-2011)

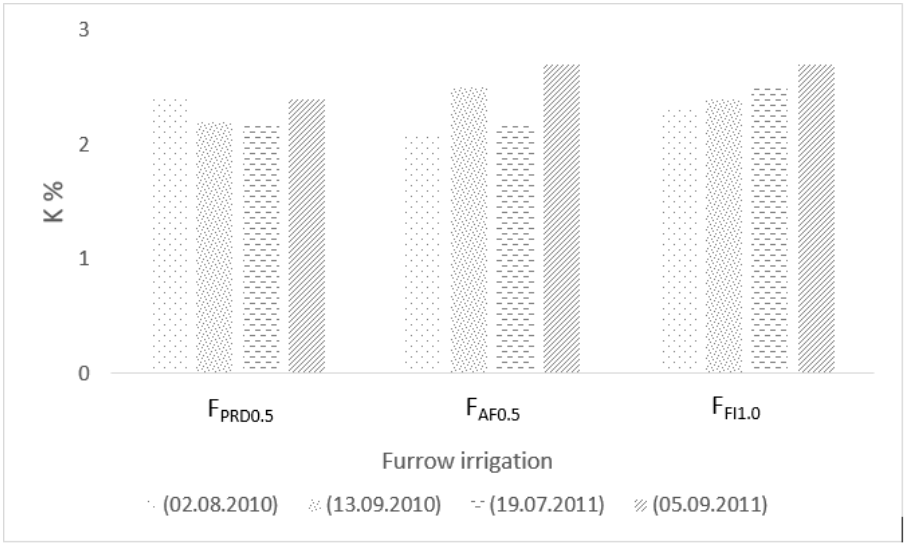

Figure 8. Changes of K ion concentrations in leaves in furrow irrigation treatments (2010-2011)
Şekil 8. Karık sulama konularında yapraklardaki K iyon konsantrasyonundaki değişimleri (2010-2011)

This results revealed that irrigation methods and irrigation levels had a prominent impact on marketable yield of red pepper. The highest yield was obtained from the $D_{FI1.0}$ treatment in drip irrigation which had the highest ET in drip irrigation in both years. Furthermore, the $D_{FI1.0}$ treatment provided in greater quality red pepper yield compared to other deficit irrigation treatments, since the higher irrigation level absolutely impacted yield quality parameters (data not shown). In furrow irrigation, the highest marketable red pepper yield was acquired from the $F_{FI1.0}$ treatment which had the highest ET value. Regarding the rising water shortage conditions in the Mediterranean region, $D_{DI0.75}$ and $F_{PRD0.5}$ reveal to be well alternative to full irrigation for high marketable yields and further high water use efficiency. Different water deficit levels at all growth stages of red pepper, especially PRD treatments, indicated that the ET values of deficit irrigation treatments were significantly reduced compared to full irrigation applications in furrow and drip irrigation methods. The results showed that WUE and IWUE values diminished with enhancement irrigation level in furrow and drip irrigation methods. In order to ensure sustainable agriculture, especially efficient water management strategies should be established in arid and semi-arid regions with insufficient water resources. The findings in this study indicated that $D_{FI1.0}$ and $F_{FI1.0}$ applications, which are irrigated by using 25% of the available water in drip irrigation and 40% of the available water in furrow irrigation and completed to field capacity at 60 cm soil depth are recommended to obtain higher marketable yield and quality of red pepper in the Mediterranean region. While lower osmotic potential values were measured in full irrigation treatments in furrow and drip irrigation plots, higher osmotic potential values were determined in treatments where water stress was determined in both years. In both drip and furrow irrigation, the lowest Ca (%) values were obtained in full irrigation, while the highest Ca values were obtained in limited irrigation with water stress in 2010 and 2011 years. K ion values were generally similar in the first and fourth pepper harvests in drip and furrow irrigation in 2010 and 2011 seasons.

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Author's Contributions

The contribution of the authors is equal.

Statement of Conflict of Interest

The authors have declared no conflict of interest.

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Türkiye Latridiidae (Coleoptera) Faunası İçin Yeni Kayıtlar

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ÖZET
Balıkesir İli meşe ve kayın alanlarında bulunan Latridiidae familyasına bağlı türleri saptamak amacıyla pencere ve çukur tuzak kullanılarak yürütülen bu çalışma Nisan-Aralık ayları arasında 2012-2014 yılında gerçekleştirilmiştir. Çalışmanın sonucunda, Latridiidae (Coleoptera) familyasına bağlı dokuz cinse ait toplam 22 tür tespit edilmiştir. Bu türlerden, *Cartodere nodifer* (Westwood, 1839), *Corticaria longicollis* (Zetterstedt, 1838), *C. obscura* C.N.F Brisout de Barneville, 1863, *Corticarina minuta* (Fabricius, 1792), *Enicmus fungicola* (C. G. Thomson, 1868) ve *E. testaceus* (Stephens, 1830) olmak üzere altı tür Türkiye için yeni kayıt niteliğindedir. İlk kez Türkiye'den lokalite kaydı verilen 13 tür ise *Metophthalmus hungaricus* Reitter, 1884, *Enicmus transversus* (Oliver, 1790), *E. brevicornis* (Mannerheim, 1844), *Latridius minutus* (Linnaeus, 1767), *Corticarina curta* (Wollaston, 1854), *Cortinicara gibbosa* (Herbst, 1793), *Melanophthalma rhenana* Rücker & Johnson, 2007, *M. taurica* (Mannerheim, 1844), *M. fuscipennis* (Mannerheim, 1844), *Corticaria serrata* (Paykull, 1798), *C. elongata* (Gyllenhal, 1827), *C. pubescens* (Gyllenhal, 1827) ve *Migneauxia crassiuscula* (Aubé, 1850)' dır. *E. rugosus* (Herbst, 1793) ve *M. distinguenda* (Comolli, 1837) türleri Marmara Bölgesi, *Cartodere apfelbecki* (Reitter, 1901) türü ise Balıkesir İli lokal faunası için yeni kayıttır. Ek olarak, kaydedilen türlerin zoocoğrafik dağılışları da değerlendirilmiştir.

Entomoloji

Araştırma Makalesi

Makale Tarihçesi
Geliş Tarihi : 26.01.2023
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Anahtar Kelimeler
Coleoptera
Latridiidae
New records
Balıkesir
Türkiye

New Records for The Latridiidae (Coleoptera) Fauna of Turkey

ABSTRACT
This study, which was carried out using window and pitfall traps, was conducted between April-December in 2012-2014 to identify the species connected to the Latridiidae (Coleoptera) family found in oak and beech areas in Balıkesir. As a result of the study, a total of 22 species belonging to nine genera of the family Latridiidae were determined. Six of these species, *Cartodere nodifer* (Westwood, 1839), *Corticaria longicollis* (Zetterstedt, 1838), *C. obscura* C.N.F Brisout de Barneville, 1863, *Corticarina minuta* (Fabricius, 1792), *Enicmus fungicola* (C. G. Thomson, 1868) and *Enicmus testaceus* (Stephens, 1830) are new records for Turkey. The 13 species whose locality was recorded for the first time from Turkey are *Metophthalmus hungaricus* Reitter, 1884, *E. transversus* (Oliver, 1790), *Enicmus brevicornis* (Mannerheim, 1844), *Latridius minutus* (Linnaeus, 1767), *Corticarina curta* (Wollaston, 1854), *Cortinicara gibbosa* (Herbst, 1793), *Melanophthalma rhenana* Rücker & Johnson, 2007, *M. taurica* (Mannerheim, 1844), *M. fuscipennis* (Mannerheim, 1844), *Corticaria serrata* (Paykull, 1798), *Corticaria elongata* (Gyllenhal, 1827), *C. pubescens* (Gyllenhal, 1827) and *Migneauxia crassiuscula* (Aubé, 1850). *E. rugosus* (Herbst, 1793) and *M. distinguenda* (Comolli, 1837) species are new records for the Marmara Region, while Cartodere apfelbecki (Reitter, 1901) species are new records for the local fauna of Balikesir Province. In addition, the zoogeographic distribution of the recorded species was also evaluated.

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INTRODUCTION

Despite their wide geographical distribution, long history among taxonomists, their place in the ecosystem's nutrient cycle, their decomposing role with fungi, and the fact that some 30 species are listed as storage pests in many places, there is very little information on the number of species, their local and regional zoogeographic distribution and biology of the Latridiidae (Coleoptera) family, which we can call 'mold beetles' in Turkish.

Latridiidae family, which occurs in all regions except the north and south Polar Regions, is represented by 31 genera and 839 species depending on two subfamilies in the world. While 235 species belonging to 17 genera belonging to the Latridiidae family have been recorded in the Western Palearctic Region, 182 species belonging to 17 genera are known in Europe. In Turkey, 57 species belonging to 12 genera were recorded (Rücker, 2018, 2020, 2021).

Most of the species of the family Latridiidae do not show strong diagnostic morphological features, with the exception of their yellow, yellow-brown, or dark brown to black exterior, small size, and the 3-3-3 tarsal formula. Despite the increasing number of studies, very few species have been revised, and the abundance of synonyms causes specimens to be misidentified or to remain under old names in many collections and to receive little attention from systematists (Hartley et al., 2007; Trikhleb & Simutnik, 2008; Trikhleb, 2009; Rücker et al., 2009; Lord et al., 2010; Quiroz-Gamboa & Serna, 2011; López, 2014; Otero & Rücker, 2017; Rücker, 2018, 2020, 2021). Despite many studies in the world in recent years, there are almost no studies on the Latridiidae fauna of Turkey.

The aim of this study was to determine the species belonging to the family Latridiidae in oak and beech areas of Balıkesir Province and to contribute to the knowledge of the distribution, biodiversity, and local and regional zoogeographic distribution of these species.

MATERIAL and METHODS

The material of the study consisted of Latridiidae species caught between April-December in 2012-2014 using the window and pitfall trap method in old, hollow oak (*Quercus frainetto* Ten., *Q. cerris* L., *Q. infectoria* Olivier., *Q. petraea* Lieble., *Q. pubescens* Willd., *Q. frainetto* Ten.× *Q. petraea* Lieble., *Q. cerris* L.× *Q. pubescens* Willd., *Quercus* spp. L. (dead tree) and beech (*Fagus orientalis* Lipsky., *F. sylvatica* L., *Fagus* spp. L. (dead tree)) trees in 11 different localities in broad-leaved forests of Balıkesir Province. Considering the width of the sampling areas, the number of traps set was determined between five and 10 for each trapping method. The map of the oak and beech areas where window and pitfall traps were set is shown in Figure 1, while coordinates, altitude and biotope information are given in Table 1.

Figure1. The Map of the study fields (Google Earth pro 2022).
Şekil 1. Çalışma alanlarının genel görünümü (Google Earth pro 2022).

Table 1 The Information belonging to study fields
Çizelge 1. Çalışma Alanlarına Ait Bilgiler

No	Localty	Coordinate	Altitude	Trap	Biotope
1	Erdek District, Göletaltı	40°28'16"N 27°53'52"E	290-345 m	W, P	*Quercus petraea*, *Q. frainetto* × Q.petraea
2	Erdek District, Kurtboğazı	40°27'45"N 27°49'25"E	601-612 m	W, P	*Fagus orientalis, F. sylvatica*
3	Gönen District, Şarkoluk Store	40°08'54"N 27°29'35"E	406-508m	W, P	*Quercus frainetto, Q. cerris*
4	Gönen District, Porta Hill	40°07'37"N 27°25'44"E	730-776 m	W, P	*Fagus orientalis, Fagus* spp.
5	Susurluk District, Bağıran Stream	39°51'41"N 28°18'13"E	794-812 m	W, P	*Fagus orientalis, F. sylvatica*
6	Susurluk District, Darıalan	39°52'06"N 28°16'35"E	570-798 m	W, P	*Q. petraea, Quercus* spp.
7	Balya District, Ilıca Quarter, Hisaralan	39°54'19"N 27°50'43"E	311-325 m	W, P	*Quercus frainetto, Q. cerris*
8	Karesi District, Bakacak Quarter, Koruluk	39°40'52"N 27°43'31"E	432-495 m	W, P	*Quercus infectoria, Q. frainetto, Q. cerris, Quercus* spp., *Q. cerris* × *Q.pubescens, Q. pubescens*
9	Savaştepe District, Mancılık	39°21'24"N 27°48'45"E	782-832 m	W, P	*Quercus cerris, Q. frainetto*
10	Bigadiç District, Ulus Mountain	39°19'26"N 27°23'41"E	1612-1632 m	W, P	*Fagus orientalis, Fagus* spp.
11	Bigadiç District, Davutlar Village	39°29'15"N 28°19'21"E	666-719 m	W, P	*Quercus cerris*

Field Studies

In Turkey and other countries, studies using window and pitfall traps are among the techniques used to identify species that belong to the Latridiidae family. (Sama et al., 2011; Jansson et al., 2011; Platia et al., 2014; Rücker, 2018). The window trap (W), as shown in Figure 2A, consisted of a 30 x 60 cm wide transparent plastic plate with a tray underneath. These traps were placed in the trunks of oak and beech trees (<1 m) beside or in front of a hollow entrance, at a height of 1.5-2.5 m above the ground. The tray was half filled with trapping liquid (1:1 mixture of ethylene glycol and water with a little detergent to reduce surface tension). Pitfall trap (P) consisted of 250 ml plastic containers with an upper diameter of 6.5 cm. These traps were placed at the base of the cavities in the trunk of old hollow oak and beech trees so that the mouth of the trap was flush with the soil level, and the trap was half filled with trapping liquid and camouflaged with stones and plant parts (Figure 2B). The material collected from the traps was taken regularly at three-week intervals, labeled and brought to the laboratory and the trap liquid was completed. The material collected from the traps was regularly taken at three-week intervals, labeled, brought to the laboratory and the decreasing trap fluid was completed.

Figure 2. The trapping methods. A. Window trap; B.. Pitfall trap.
Şekil 2. Tuzak yöntemleri. A. Pencere Tuzak, B. Çukur Tuzak.

Laboratory analysis

The samples brought to the laboratory were washed with water using a 0.1 mm wire strainer to remove the trap liquid. The cleaned samples were taken into a 30 x 50 cm rectangular white container with water and the Latridiidae species were separated from the other insects caught under a white light table lamp and taken into 2 ml Ependorf tubes containing 70% ethyl alcohol. The separated specimens were grouped into upper and lower taxa under Nikon model SMZ 1500 and Olympus model SZX10 stereo microscope and species level distinctions, aedeagus removal, and identification procedures were performed. The material referred to in this study is stored in Balikesir University, Faculty of Arts and Sciences, Department of Biology, Zoological Museum, Balikesir, Turkey.

RESULTS

Among the 8537 individuals of the Latriididae family recorded in the study, nine of the 22 species belong to Latridiinae and 13 belong to the Corticariinae subfamily.

Familya Latridiidae

Altfamilya Latridiinae Erichson, 1842

Genus *Metophthalmus* Motschulsky, 1850

Metophthalmus hungaricus Reitter, 1884

Material examined: Balıkesir, Savaştepe, Mancılık, 39°21'25"N 27°48'43"E, 791 m, *Q. frainetto*, 06.VIII.2012, 4P, 1♀. Totally 1 ex.

Distribution in the world: Europe: Bulgaria, Greece, Hungary, Italy, Romania, Sicily, and Ukraine; **Asia:** Turkey (Rücker, 2018, 2021, Anonymous, 2023).

Distribution in Turkey: Regions of Turkey entering the Asian continent (Rücker, 2018, 2021). This is the first time a locality record is given with this study.

Genus *Latridius* Herbst, 1793

Latridius minutus (Linnaeus, 1767)

Material examined: This species was identified in traps set on each date. Totally 141♀♀ 23♂♂, 164 exs.

Distribution in the world: Europe: Albania, Austria, Azores, Baliear Islands, Belarus, Belgium, Bosnia and Herzegovina, Britain, Bulgaria, Croatia, Czech Republic, Denmark, Estonia, Feroe Islands, Finland, France, Georgia, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Liechtenstein, Lithuania, Luxembourg, Macedonia, Malta, Moldova, Netherlands, Norway, Poland, Portugal, Romania, Russia: Central European Territories, Russia: Eastern European Territories, Russia: Northern European Territories, Russia: Southern European Territories, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey, Ukraine, and Yugoslavia; **Asia:** Mongolia, Russia: East Siberia, Russia: Far East, Turkey, and Russia: West Siberia; **North Africa:** Canary Islands, Madeira Archipelago, and North Africa; **Afrotropics Region; Australia Region; Nearctic Region:** North America; **Neotropical Region:** South America; **Near East**; **Near Region**; **East Region:** (Johnson, 2007; Rücker, 2018, 2021; Anonymous, 2023).

Distribution in Turkey: Turkey (Asia and Europe) (Johnson, 2007; Anonymous, 2023). This is the first time a locality record is given with this study.

Genus *Enicmus* (C.G. Thomson, 1859)

Enicmus transversus (Oliver, 1790)

Material examined: This species was identified in traps set on each date. Totally 252♀♀ 24♂♂, 276 exs.

Distribution in the world: Europe: Armenia, Austria, Azerbaijan, Balear Adası, Belarus, Belgium, Bosnia and Herzegovina, Britain, Bulgaria, Croatia, Czech Republic, Denmark, Estonia, Finland, France, Georgia, Germany, Greece, Hungary, Ireland, Italy, Latvia, Liechtenstein, Lithuania, Luxembourg, Macedonia, Malta, Moldova, Netherlands, Norway, Poland, Portugal, Romania, Russia: Central European Terrtories, Russia: Eastern European Territories, Ruissia: Northern European Territories, Russia: Southern European Territories, Slovakia, Slovenia, Spain, Sweden, Switzerland, Ukraine, and Yugoslavia; **Asia:** Afghanistan, Cyprus, Israel, Jordan, Nepal, Russia: West Siberia, Syria, and Turkey; **North Africa:** Algeria, Canary Islands, Egypt, Madeira Archipelago, Morocco, and Tunisia; **Afrotropics Region**; **East Palearctic**; **Near East**; **East Region** (Johnson, 2007; Rücker, 2018; Anonymous, 2023).

Distribution in Turkey: Regions of Turkey entering the Asian continent (Rücker, 2018; Anonymous, 2023). This is the first time a locality record is given with this study.

Enicmus rugosus (Herbst, 1793)

Material examined: This species was identified in traps set on each date. Totally 389♀♀ 70♂♂, 459 exs.

Distribution in the world: Europe: Austria, Belarus, Belgium, Bosnia and Herzegovina, Britain, Bulgaria, Croatia, Czech Republic, Denmark, Estonia, Finland, France, Georgia, Germany, Greece,Hungary, Italy, Latvia, Lithuania, Netherlands, Norway, Poland, Portugal, Romania, Russia: Central European Territories, Russia: Eastern European Territories, Russia: Northern European Territories, Serbia and Montenegro, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey, Ukraine and Yugoslavia; **Asia:** China, Iran, Mongolia, Russia: Eastern Siberia, and Baikal Region, Russia: Far East, Russia: West Siberia, and Turkey; **North Africa:** Morocco, and Tunisia; **East Palearctic:** (Johnson, 2007; Rücker, 2018, 2021; Anonymous, 2023).

Distribution in Turkey: İzmir (Karşıyaka-Yamanlar

Mountain) (Johnson, 2007; Tezcan et al., 2010; Rücker, 2018, 2021). This species is reported for the first time from Marmara Region.

Enicmus brevicornis (Mannerheim, 1844)

Material examined: This species was identified in traps set on each date. Totally 656♀♀ 149♂♂, 805 exs.

Distribution in the world: Europe: Austria, Belgium, Bosnia and Herzegovina, Britain, Croatia, Czech Republic, Estonia, Finland, France, Georgia, Germany, Greece, Hungary, Italy, Latvia, Lithuania, Netherlands, Norway, Poland, Romania, Russia: Central European Territories, Russia: Southern European Territories, Slovakia, Spain, Sweden, Switzerland, Ukraine, and Yugoslavia; **Asia:** Iran, and Turkey; **North Africa:** Algeria, Morocco, and Tunisia; **East Palearctic** (Johnson, 2007; Rücker, 2018, 2021; Anonymous, 2023).

Distribution in Turkey: Regions of Turkey entering the Asian continent (Johnson, 2007; Rücker, 2018, 2021). This is the first time a locality record is given with this study.

Enicmus fungicola (C. G. Thomson, 1868)

Material examined: This species was identified in traps set on each date. Totally, 79♀♀ 5♂, 84 exs.

Distribution in the world: Europe: Austria, Belarus, Belgium, Britain, Bulgaria, Croatia, Czech Republic, Denmark, Estonia, Finland, France, Germany, Hungary, Italy, Latvia, Lithuania, Netherlands, Norway, Poland, Romania, Russia: Central European Territories, Russia: Northwest European Territories, Slovakia, Slovenya, Sweden, Switzerland, Ukraine, and Yugoslavia (Johnson, 2007; Rücker, 2018; Anonymous, 2023).

Distribution in Turkey: This species is the first record for the fauna of Turkey.

Enicmus testaceus (Stephens, 1830)

Material examined: This species was identified in traps set on each date. Totally 98♀♀ 1♂, 99 exs.

Distribution in the world: Europe: Austria, Belarus, Belgium, Britain, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece,Hungary, Ireland, Italy, Latvia, Lithuania, Netherlands, Norway, Poland, Romania,Russia: Northern European Territories, Serbia and Montenegro, Slovakia, Spain, Sweden, Switzerland, and Yugoslavia; **Asia:** Iran; **North Africa:** Algeria, and Canary Islands (Johnson, 2007; Rücker, 2018, 2021; Anonymous, 2023).

Distribution in Turkey: This species is the first record for the fauna of Turkey.

Genus ***Cartodere*** (C.G.Thomson, 1859)

Cartodere (Cartodere) apfelbecki (Reitter, 1901)

Material examined: Balıkesir, Erdek, Göletaltı, 40°28'15"N 27°53'52"E, 345 m, *Q. petraea,* 16.VII.2012, 2W, 1♀; Susurluk, Darıalan, 39°52'03"N 28°16'39"E, 572 m, *Q. petraea,* 27.VII.2012, 10W, 1♀ 1♂; Karesi, Bakacak, Koru, 39°40'53"N 27°43'33"E, 485 m, *Q. frainetto,* 02.VIII.2012, 7W, 1♀; Gönen, Porta Hill,, 40°07'36"N 27°25'47"E, 759 m, *Fagus* spp., 10.VIII.2012, 6P, 2♀♀; Erdek, Göletaltı, 40°28'15"N 27°53'52"E, 345 m, *Q. petraea,* 20.IX.2012, 2P, 1♀; Susurluk, Darıalan, 39°52'04"N 28°16'37"E, 582 m, *Q. petraea,* 26.IX.2012, 2P, 1♀; Susurluk, Bağıran Stream, 39°51'40"N 28°18'14"E, 806 m, *F. orientalis,* 26.IX.2012, 8P, 1♀; Susurluk, Darıalan, 39°52'04"N 28°16'37"E, 582 m, *Q. petraea,* 23.V.2013, 2P, 1♀; Erdek, Kurtboğazı, 40°27'46"N 27°49'25"E, 604 m, *F. orientalis,* 24.V.2013, 3W, 1♀; Erdek, Kurtboğazı, 40°27'46"N 27°49'26"E, 601 m, *F. sylvatica,* 24.V.2013, 4W, 1♂; Gönen, Porta Hill,, 40°07'36"N 27°25'48"E, 735 m, *F. orientalis,* 24.V.2013, 1W, 1♀; Gönen, Porta Hill,, 40°07'38"N 27°25'44"E, 730 m, *F. orientalis,* 24.V.2013, 4W, 1♀; Savaştepe, Mancılık 39°21'26"N 27°48'47"E, 832 m, *Q. cerris,* 11.VI.2013, 6P, 1♀; Susurluk, Bağıran Stream, 39°51'40"N 28°18'14"E, 806 m, *F. orientalis,* 19.VI.2013, 8P, 1♀; Erdek, Göletaltı, 40°28'15"N 27°53'53"E, 334 m, *Q. petraea,* 26.VII.2013,4W, 1♀; Erdek, Kurtboğazı, 40°27'46"N 27°49'26"E, 601 m, *F. sylvatica,* 26.VII.2013, 4W, 1♀; Susurluk, Darıalan, 39°52'04"N 28°16'36"E, 578 m, *Q. petraea,* 16.VIII.2013, 4W, 1♀; Susurluk, Bağıran Stream, 39°51'41"N 28°18'13"E, 807 m, *F. sylvatica,* 16.VIII.2013, 5W, 1♀; Erdek, Kurtboğazı, 40°27'45"N 27°49'26"E, 606 m, *F. orientalis,* 27.IX.2013, 8P, 1♀; Erdek, Kurtboğazı, 40°27'46"N 27°49'23"E, 608 m, *F. sylvatica,* 01.XI.2013, 10P, 1♀; Karesi, Bakacak, Koru, 39°40'55"N 27°43'39"E, 455 m, *Quercus* spp., 7.XI.2013, 11W, 1♀; Erdek, Kurtboğazı, 40°27'46"N 27°49'23"E, 608 m, *F. sylvatica,* 28.XI.2013, 10P, 1♀; Erdek, Göletaltı, 40°28'11"N 27°53'51"E, 290 m, *Q. petraea,* 26.VI.2014, 1W,1♀; Erdek, Kurtboğazı, 40°27'46"N 27°49'23"E, 612 m, *F. orientalis,* 26.VI.2014, 1P, 1♂; Gönen, Porta Hill,, 40°07'37"N 27°25'44"E, 730 m, *F. orientalis*, 26.VI.2014, 5P, 1♀; Totally 24♀♀ 3♂♂, 27 exs.

Distribution in the world: Europe: Bulgaria, Hungary, Serbia, and Turkey; **Asia:** Turkey (Johnson, 2007; Rücker, 2018, 2021; Anonymous, 2023).

Distribution in Turkey: Anatolia, and İstanbul (Johnson, 2007, Rücker, 2018, 2021; Anonymous, 2023). This species is reported for the first time from Balıkesir Province.

Note: The main distribution area is Turkey, that is, Anatolia, and Istanbul. Probably migrated to Bulgaria, Serbia, and Hungary from Istanbul (Rücker, 2018).

Cartodere (Aridius) nodifer (Westwood, 1839)

Material examined: Balıkesir, Karesi, Bakacak, Koru, 39°40'51"N 27°43'29"E, 478 m, *Q. infectoria*, 18.V.2012, 3W, 1♀; Balya, Ilıca Village, Hisaralan, 39°54'24"N 27°50'37"E, 317 m, *Q. frainetto,* 31.VII.2012, 1P, 1♀; Balya, Ilıca Village, Hisaralan, 39°54'25"N 27°50'39"E,

325 m, *Q. frainetto,* 31.VII.2012, 3P, 1♀; Karesi, Bakacak, Koru, 39°40'51"N 27°43'29"E, 478 m, *Q. infectoria,* 02.VIII.2012, 3W, 1♀; Savaştepe, Mancılık 39°21'24"N 27°48'45"E, 825 m, *Q. cerris,* 06.VIII.2012, 5W, 1♀; Gönen, Porta Hill,, 40°07'37"N 27°25'44"E, 730 m, *F. orientalis,* 10.VIII.2012, 5P, 1♀; Susurluk, Bağıran Stream, 39°51'40"N 28°18'13"E, 795 m, *F. orientalis,* 31.X.2012, 9P, 1♀; Bigadiç, Davutlar Village, 39°29'12"N 28°19'17"E, 685 m, *Q. cerris,* 16.V.2013, 10W, 1♀; Savaştepe, Mancılık 39°21'25"N 27°48'43"E, 802 m, *Q. frainetto,* 21.V.2013, 9W, 1♀1♂; Susurluk, Darıalan, 39°52'04"N 28°16'38"E, 584 m, *Q. petraea,* 23.V.2013, 3W, 1♀; Erdek, Göletaltı, 40°28'15"N 27°53'53"E, 334 m, *Q. petraea,* 24.V.2013, 4W, 1♀; Gönen, Şarkoluk Store, 40°08'53"N 27°29'44"E, 439 m, *Q. frainetto,* 24.V.2013, 2W, 1♀; Gönen, Şarkoluk Store, 40°08'56"N 27°29'38"E, 425 m, *Q. frainetto,* 24.V.2013, 3W, 1♀; Savaştepe, Mancılık 39°21'26"N 27°48'41"E, 782 m, *Q. cerris,* 11.VI.2013, 2W, 1♀; Gönen, Porta Hill,, 40°07'37"N 27°25'44"E, 730 m, *F. orientalis,* 21.VI.2013, 5P, 1♀; Karesi, Bakacak, Koru, 39°40'55"N 27°43'39"E, 455 m, *Quercus* spp., 28.VI.2013, 11W, 1♀; Bigadiç, Davutlar Village, 39°29'15"N 28°19'21"E, 706 m, *Q. cerris,* 11.VII.2013, 5P, 1♀; Erdek, Göletaltı, 40°28'15"N 27°53'54"E, 322 m, *Q. frainetto* × *Q. petraea,* 26.VII.2013, 6W, 1♀; Erdek, Kurtboğazı, 40°27'45"N 27°49'26"E, 606 m, *F. orientalis*, 26.VII.2013, 8P, 1♂; Bigadiç, Davutlar Village, 39°29'12"N 28°19'17"E, 685 m, *Q. cerris,* 13.VIII.2013, 10W, 2♀♀; Susurluk, Darıalan, 39°52'06"N 28°16'36"E, 565 m, *Q. petraea,* 16.VIII.2013, 6P, 1♀; Gönen, Şarkoluk Store, 40°08'51"N 27°29'43"E, 508 m, *Q. cerris,* 21.VIII.2013, 8P, 1♀; Karesi, Bakacak, Koru, 39°40'55"N 27°43'35"E, 495 m, *Q. cerris,* 06.IX.2013, 9W, 1♀; Bigadiç, Davutlar Village, 39°29'17"N 28°19'20"E, 699 m, *Q. cerris,* 10.IX.2013, 8W, 1♀; Savaştepe, Mancılık 39°21'26"N 27°48'47"E, 832 m, *Q. cerris,* 13.IX.2013, 6P, 1♀; Erdek, Kurtboğazı, 40°27'46"N 27°49'22"E, 611 m, *F. sylvatica,* 27.IX.2013, 7P, 1♀; Erdek, Kurtboğazı, 40°27'46"N 27°49'23"E, 608 m, *F. sylvatica,* 27.IX.2013, 10W, 1♀; Balya, Ilıca Village, Hisaralan, 39°54'22"N 27°50'39"E, 321 m, *Q. frainetto,* 03.X.2013, 2W, 1♀; Susurluk, Bağıran Stream, 39°51'40"N 28°18'13"E, 795 m, *F. orientalis,* 30.X.2013, 9P, 1♀; Gönen, Şarkoluk Store, 40°08'53"N 27°29'33"E, 406 m, *Q. cerris,* 01.XI.2013, 7P, 1♀; Bigadiç, Ulus Mountain, 39°19'25"N 27°23'41"E, 1.617 m, *F. orientalis,* 05.XI.2013,2P, 1♀; Erdek, Kurtboğazı, 40°27'45"N 27°49'26"E, 606 m, *F. orientalis,* 28.XI.2013, 8W, 2♀♀; Gönen, Şarkoluk Store, 40°08'53"N 27°29'33"E, 406 m, *Q. cerris,* 28.XI.2013, 7P, 1♀; Erdek, Kurtboğazı, 40°27'46"N 27°49'23"E, 608 m, *F. sylvatica,* 16.V.2014, 10P, 1♀; Susurluk, Bağıran Stream, 39°51'36"N 28°18'15"E, 795 m, *F. orientalis,* 30.V.2014, 2P, 1♀; Bigadiç, Davutlar Village, 39°29'15"N 28°19'24"E, 666 m, *Q. cerris,* 19.VI.2014, 2P, 1♀; Bigadiç, Ulus Mountain, 39°19'21"N 27°23'41"E, 1.617 m, *F. orientalis*, 21.VIII.2014, 7P, 1♀; Totally 38♀♀ 2♂♂, 40 exs.

Distribution in the world: Europe: Albania, Austria, Azores, Baliear Islands, Belarus, Belgium, Bosnia and Herzegovina, Britain, Bulgaria, Croatia, Czech Republic, Denmark, Estonia, Faroe Islands, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Liechtenstein, Lithuania, Luxembourg, Macedonia, Malta, Moldova, Netherlands, Norway, Poland, Portugal, Romania, Russia: Central European Territories, Russia: Eastern European Territories, Russia: Northern European Territories, Russia: Northwest European Territories, Russia: Southern European Territories, Slovakia, Slovenia, Spain, Sweden, Switzerland, and Ukraine; **Asia:** Cyprus, and Japan; **North Africa:** Canary Islands, Madeira Archipelago, and Morocco; **Afrotropics Region; Australia Region; Neotropical Region:** South America; **Nearctic Region:** North America, and South Greenland; **East Palearctic Bölge**; **Near East**; **Near Region**; **East Region** (Johnson, 2007; Rücker, 2018, 2021; Anonymous, 2023). **COS**

Distribution in Turkey: This species is the first record for the fauna of Turkey.

Altfamilya Corticariinae Curtis, 1829

Genus *Corticarina* Reitter, 1880

Corticarina curta (Wollaston, 1854)

Material examined: Balıkesir, Susurluk, Darıalan, 39°52'04"N 28°16'38"E, 584 m, *Q. petraea,* 22.VI.2012, 3W, 1♀; Susurluk, Darıalan, 39°51'48"N 28°17'55"E, 750 m, *Q. petraea,* 27.VII.2012, 15P, 1♀; Karesi, Bakacak, Koru, 39°40'55"N 27°43'35"E, 495 m, *Q. cerris,* 02.VIII.2012, 9W, 1♀; Karesi, Bakacak, Koru, 39°40'57"N 27°43'34"E, 432 m, Q. pubescens, 02.VIII.2012, 16P, 1♀; Gönen, Şarkoluk Store, 40°08'55"N 27°29'35"E, 443 m, *Q. frainetto,* 27.IX.2012, 6W, 1♀; Bigadiç, Davutlar Village, 39°29'18"N 28°19'22"E, 719 m, *Q. cerris,* 19.X.2012, 6W, 1♀; Karesi, Bakacak, Koru, 39°40'51"N 27°43'30"E, 493 m, *Q. cerris,* 04.XII.2012, 4P, 1♀; Susurluk, Darıalan, 39°52'04"N 28°16'37"E, 582 m, *Q. petraea,* 23.V.2013, 2W, 1♀; Susurluk, Bağıran Stream, 39°51'40"N 28°18'12"E, 803 m, *F. orientalis,* 23.V.2013, 7W, 1♀; Erdek, Kurtboğazı, 40°27'46"N 27°49'23"E, 608 m, *F. sylvatica,* 24.V.2013, 10W, 2♀♀; Gönen, Porta Hill,, 40°07'36"N 27°25'47"E, 776 m, *F. orientalis,* 24.V.2013, 9W, 2♀♀ 1♂; Susurluk, Bağıran Stream, 39°51'41"N 28°18'13"E, 807 m, *F. sylvatica,* 19.VI.2013, 5W, 1♀; Susurluk, Darıalan, 39°52'04"N 28°16'37"E, 582 m, *Q. petraea,* 19.VI.2013, 2W, 1♀; Erdek, Kurtboğazı, 40°27'46"N 27°49'25"E, 604 m, *F. orientalis,* 21.VI.2013, 3W, 1♀; Gönen, Şarkoluk Store, 40°08'55"N 27°29'37"E, 425 m, *Q. frainetto,* 21.VI.2013, 1W, 2♀♀; Susurluk, Bağıran Stream, 39°51'39"N 28°18'11"E, 794 m, *F. orientalis,* 23.VII.2013, 1W, 2♀♀; Gönen, Porta Hill,, 40°07'36"N

27°25'48"E, 735 m, *F. orientalis,* 26.VII.2013, 1W, 1♀; Erdek, Göletaltı, 40°28'15"N 27°53'53"E, 334 m, *Q. petraea,* 26.VII.2013, 4P, 1♀ 1♂; Gönen, Şarkoluk Store, 40°08'56"N 27°29'38"E, 425 m, *Q. frainetto,* 21.VIII.2013, 3W, 2♀♀; Bigadiç, Davutlar Village, 39°29'17"N 28°19'20"E, 699 m, *Q. cerris,* 08.V.2014, 8W, 1♀; Gönen, Şarkoluk Store, 40°08'53"N 27°29'44"E, 439 m, *Q. frainetto,* 16.V.2014, 2W, 1♀; Gönen, Şarkoluk Store, 40°08'56"N 27°29'38"E, 425 m, *Q. frainetto,* 16.V.2014, 3W, 1♀; Totally 27♀♀ 2♂♂, 29 exs.

Distribution in the world: Europe: Austria, Azores, Belgium, Bosnia and Herzegovina, Britain, Bulgaria, Croatia, France, Georgia, Greece, Hungary, Italy, Macedonia, Malta, Poland, Portugal, Romania, Russia: Southern European Territories, Serbia and Montenegro, Slovenia, Spain, Switzerland, Turkey, Ukraine, Vóreion Aiyáion (North Aegean Island), and Yugoslavia; **Asia:** Cyprus, Turkey, and Uzbekistan; **North Africa:** Algeria, Canary Islands, Egypt, Madeira Archipelago, Morocco, and Tunisia; **Nearctic Region:** Alaska, America, and Canada; **Neotropical Region** (Johnson, 2007; Rücker, 2018, 2021; Anonymous, 2023). **COS**

Distribution in Turkey: Turkey (Asia, and Europe) (Johnson, 2007; Rücker, 2018, 2021; Anonymous, 2023). This is the first time a locality record is given with this study.

Corticarina minuta (Fabricius, 1792)

Material examined: Balıkesir, Karesi, Bakacak, Koru, 39°40'58"N 27°43'36"E, 453 m, *Q.infectoria*, 17.07.2012, 15W, 1♀; Erdek, Kurtboğazı, 40°27'46"N 27°49'23"E, 608 m, *F. sylvatica,* 15.VIII.2012, 10P, 1♀; Bigadiç, Ulus Mountain, 39°19'20"N 27°23'43"E, 1.615 m, *F. orientalis,* 19.X.2012, 6W, 1♀; Gönen, Porta Hill,, 40°07'37"N 27°25'44"E, 730 m, *F. orientalis,* 23.X.2012, 5W,1♀; Gönen, Porta Hill,, 40°07'36"N 27°25'47"E, 768 m, *F. orientalis,* 23.X.2012, 7W,1♀; Savaştepe, Mancılık 39°21'24"N 27°48'45"E, 825 m, *Q. cerris,* 21.V.2013, 5W, 1♀; Bigadiç, Ulus Mountain, 39°19'24"N 27°23'42"E, 1.619 m, *F. orientalis,* 30.V.2013, 1W, 1♀; Karesi, Bakacak, Koru, 39°40'49"N 27°43'28"E, 485 m, *Q. frainetto,* 03.VI.2013, 2W, 1♀; Karesi, Bakacak, Koru, 39°40'55"N 27°43'35"E, 495 m, *Q. cerris,* 03.VI.2013, 9W, 1♀; Karesi, Bakacak, Koru, 39°40'53"N 27°43'40"E, 461 m, *Q. cerris,* 03.VI.2013, 14P, 1♀; Balya, Ilıca Village, Hisaralan, 39°54'22"N 27°50'39"E, 321 m, *Q. frainetto,* 07.VI.2013, 2W, 1♀; Susurluk, Bağıran Stream, 39°51'41"N 28°18'13"E, 807 m, *F. sylvatica,* 19.VI.2013, 5W, 1♀; Erdek, Göletaltı, 40°28'11"N 27°53'51"E, 290 m, *Q. petraea*, 21.VI.2013, 1W, 1♀; Gönen, Şarkoluk Store, 40°08'54"N 27°29'35"E, 451 m, *Q. cerris,* 21.VI.2013, 5P, 1♀; Gönen, Şarkoluk Store, 40°08'56"N 27°29'38"E, 425 m, *Q. frainetto,* 21.VIII.2013, 3W, 1♀; Gönen, Şarkoluk Store, 40°08'55"N 27°29'35"E, 443 m, *Q. frainetto,* 21.VIII.2013, 6W, 1♀; Bigadiç, Davutlar Village, 39°29'17"N 28°19'20"E, 699 m, *Q. cerris,* 10.IX.2013, 8W, 1♂; Erdek, Kurtboğazı, 40°27'46"N 27°49'23"E, 608 m, *F. sylvatica,* 27.IX.2013, 10W, 1♀; Bigadiç, Ulus Mountain, 39°19'24"N 27°23'47"E, 1.632 m, *F. orientalis,* 08.X.2013, 9W, 1♀; Savaştepe, Mancılık 39°21'26"N 27°48'41"E, 782 m, *Q. cerris,* 07.XI.2013, 2W, 1♀; Bigadiç, Ulus Mountain, 39°19'23"N 27°23'46"E, 1.608 m, *F. orientalis,* 08.V.2014, 8W, 1♀; Savaştepe, Mancılık 39°21'26"N 27°48'41"E, 782 m, *Q. cerris,* 19.VI.2014, 2W, 1♀; Savaştepe, Mancılık 39°21'25"N 27°48'47"E, 830 m, *Q. cerris,* 19.VI.2014, 3W, 1♀; Totally 22♀♀ 1♂, 23 exs.

Distribution in the world: Europe: Austria, Belarus, Belgium, Britain, Croatia, Czech Republic, Denmark, Estonia, Finland, France, Georgia, Germany, Hungary, Ireland, Italy, Latvia, Lithuania, Netherlands, Norway, Poland, Portugal, Romania, Russia: Central European Territories, Russia: Eastern European Territories, Russia: Northern European Territories, Russia: Southern European Territories, Slovakia, Slovenya, Spain, Sweden, Switzerland, and Ukraine; **Asia:** Canary Islands, and Russia; **North Africa:** Afghanistan, China, Kazakhstan, Mongolia, Russia: East Siberia, Russia: Far East, and Russia: West Siberia; **Nearctic Region:** Alaska, America and Canada; **Neotropical Region**; **Near Region** (Johnson, 2007; Rücker, 2018, 2021; Anonymous, 2023). **COS**

Distribution in Turkey: This species is the first record for the fauna of Turkey.

Genus *Corticaria* Marsham, 1802

Corticaria pubescens (Gyllenhal, 1827)

Material examined: This species was identified in traps set on each date. Totally 76♀♀ 2♂♂, 78 exs.

Distribution in the world: Europe: Austria, Belarus, Croatia, Czech Republic, Denmark, Estonia, Finland, France, Georgia, Germany, Hungary, Italy, Latvia, Lithuania, Norway, Poland, Portugal, Romania, Russia: Central European Territories, Russia: Eastern European Territories, Russia: Northern European Territories, Russia: Southern European Territories, Slovakia,, Spain, Sweden, Switzerland, and Ukraine; **Asia:** Russia: East Siberia, Russia: Far East, Russia: West Siberia, and Turkey; **North Africa:** Canary Islands, Madeira Archipelago, Morocco, and Tunisia; **Afrotropics Region**; **Australia Region; Nearctic Region:** USA, Alaska, and North America: Canada; **Near East**; **Near Region** (Johnson, 2007; Rücker, 2018, 2021; Anonymous, 2023).

Distribution in Turkey: Regions of Turkey entering the Asian continent (Johnson, 2007). This is the first time a locality record is given with this study.

Corticaria serrata (Paykull, 1798)

Material examined: This species was identified in traps set on each date. Totally 69♀♀ 6♂♂, 75 exs.

Distribution in the world: Europe: Austria, Azerbaijan,

Azores, Belarus, Belgium, Bosnia and Herzegovina, Britain, Bulgaria, Croatia, Czech Republic, Denmark, Estonia, Finland, France, Georgia, Germany, Greece, Hungary, Ireland, Italy, Latvia, Liechtenstein, Lithuania, Luxembourg, Netherlands, Norway, Poland, Romania, Russia: Central European Territories, Russia: Northern European Territories, Russia: Southern European Territories, Slovakia, Slovenia, Spain, Sweden, Switzerland, Ukraine, and Yugoslavia; **Asia:** Afghanistan, Cyprus, Israel, Japan, Kazakhstan, Lebanon, and Turkey; **North Africa:** Algeria, Canary Islands, Madeira Archipelago, Morocco, and Tunisia; **East Palearctic**; **Nearctic Region:** Canada: Alberta, British Columbia, Labrador, Monitoba, Newfoundland, Northwest Territories, Nova Scotia, Ontario, Saskatchewan, USA: Alaska, Arizona, Montana, Wyoming; **Neotropical Region:** Chile; **Near Region:** (Johnson, 2007; Rücker, 2018, 2021; Anonymous, 2023). **COS**

Distribution in Turkey: Regions of Turkey entering the Asian continent (Johnson, 2007; Rücker, 2018, 2021). This is the first time a locality record is given with this study.

Corticaria elongata (Gyllenhal, 1827)

Material examined: This species was identified in traps set on each date. Totally 82♀♀ 4♂♂, 86 exs.

Distribution in the world: Europe: Albania, Armenia, Austria, Azerbaijan, Azores, Belarus, Belgium, Bosnia and Herzegovina, Britain, Bulgaria, Croatia, Czech Republic, Denmark, Estonia, Finland, France, Georgia, Germany, Greece,Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Macedonia, Moldova, Netherlands, Norway, Poland, Portugal, Romania, Russia: Central European Territories, Russia: Northern European Territories, Russia: Southern European Territories, Serbia and Montenegro, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey, Ukraine, and Yugoslavia; **Asia:** Afghanistan, Cyprus, India, Japan, Nepal, Pakistan, Russia: Far East, Russia: West Siberia, Saudi Arabia, and Turkey; **North Africa:** Azores Archipelago, Morocco, and Tunisia; **Etiyopya Bölgesi:** Democratic Republic of the Congo; **Afrotropics Region**; **Australia Region; Neotropical Region:** Argentina, Peru: Panguana; **Nearctic Region:** Canada, and USA; **Near East**; **Near Region**; **East Region:** (Johnson, 2007; Rücker, 2018, 2021; Anonymous, 2023). **COS**

Distribution in Turkey: Turkey (Asia, and Europe) (Johnson, 2007; Rücker, 2018, 2021). This is the first time a locality record is given with this study.

Corticaria obscura C.N.F Brisout de Barneville, 1863

Material examined: Balıkesir, Erdek, Kurtboğazı, 40°27'46"N 27°49'23"E, 608 m, *F. sylvatica*, 15.VIII.2012, 10W, 1 ♂; Erdek, Kurtboğazı, 40°27'46"N 27°49'25"E, 604 m, *F. orientalis*, 27.IX.2013, 3W, 1♀; Gönen, Porta Hill,, 40°07'36"N 27°25'47"E, 776 m, *F. orientalis*, 27.IX.2013, 3W, 1♀; Totally 2♀♀ 1♂, 3 exs.

Distribution in the world: Europe: Austria, Azerbaijan, Belgium, Croatia, Czech Republic, France, Germany, Greece, Hungary, Italy, Netherlands, Poland, Romania, Slovakia, Slovenia, Spain, Switzerland, Ukraine, and Yugoslavia; **North Africa:** Algeria, and Tunisia; **East Palearctic:** (Johnson, 2007; Rücker, 2018, 2021; Anonymous, 2023).

Distribution in Turkey: This species is the first record for the fauna of Turkey.

Corticaria longicollis (Zetterstedt, 1838)

Material examined: This species was identified in traps set on each date. Totally 53♀♀ 4♂♂, 57 exs.

Distribution in the world: Europe: Austria, Belarus, Belgium, Bosnia and Herzegovina, Britain, Croatia, Czech Republic, Denmark, Estonia, Finland, France, Georgia, Germany, Hungary, Italy, Latvia, Liechtenstein, Lithuania, Netherlands, Norway, Poland, Portugal, Romania, Russia: Central European Territories, Russia: Northern European Territories, Russia: Southern European Territories, Slovakia, Spain, Sweden, Switzerland, and Ukraine (Johnson, 2007; Rücker, 2018; Anonymous, 2023).

Distribution in Turkey: This species is the first record for the fauna of Turkey.

Genus *Cortinicara* C. Johnson, 1978

Cortinicara gibbosa (Herbst, 1793)

Material examined: Balıkesir, Erdek, Göletaltı, 40°28'15"N 27°53'54"E, 322 m, *Q. frainetto* × *Q. petraea,* 16.VII.2012, 6P, 1♀; Erdek, Kurtboğazı, 40°27'46"N 27°49'26"E, 601 m, *F. sylvatica,* 16.VII.2012, 4P, 1♀; Karesi, Bakacak, Koru, 39°40'54"N 27°43'34"E, 483 m, *Quercus* spp., 17.VII.2012, 8P, 1♀; Susurluk, Darıalan, 39°52'05"N 28°16'36"E, 563 m, *Q. petraea,* 27.VII.2012, 8P, 1♀; Balya, Ilıca Village, Hisaralan, 39°54'25"N 27°50'39"E, 325 m, *Q. frainetto,* 31.VII.2012, 3W, 1♀; Gönen, Şarkoluk Store, 40°08'51"N 27°29'43"E, 508 m, *Q. cerris,* 10.VIII.2012, 8P, 1♀; Erdek, Göletaltı, 40°28'11"N 27°53'51"E, 290 m, *Q. petraea,* 15.VIII.2012, 1W, 1♀ 2♂♂; Erdek, Göletaltı, 40°28'15"N 27°53'52"E, 345 m, *Q. petraea,* 14.X.2012, 2P, 1♂; Bigadiç, Davutlar Village, 39°29'19"N 28°19'17"E, 702 m, *Q. cerris,* 19.X.2012, 7W, 2♀♀; Karesi, Bakacak, Koru, 39°40'51"N 27°43'30"E, 493 m, *Q. cerris,* 04.XII.2012, 4W, 1♀; Bigadiç, Davutlar Village, 39°29'15"N 28°19'23"E, 678 m, *Q. cerris,* 16.V.2013, 3P, 1♀; Susurluk, Darıalan, 39°52'03"N 28°16'39"E, 572 m, *Q. petraea,* 23.V.2013, 10W, 2♀♀; Susurluk, Bağıran Stream, 39°51'36"N 28°18'15"E, 795 m, *F. orientalis,* 23.V.2013, 2W, 1♀; Susurluk, Bağıran Stream, 39°51'40"N 28°18'12"E, 803 m, *F. orientalis,* 23.V.2013, 7W, 1♀ 1♂; Gönen, Şarkoluk Store, 40°08'53"N 27°29'44"E, 439 m, *Q. frainetto,* 24.V.2013, 2W, 4♀♀; Gönen, Porta Hill,, 40°07'36"N

27°25'47"E, 776 m, *F. orientalis,* 24.V.2013, 9W, 1♀; Balya, Ilıca Village, Hisaralan, 39°54'25"N 27°50'39"E, 325 m, *Q. frainetto,* 07.VI.2013, 3W, 1♀; Bigadiç, Davutlar Village, 39°29'15"N 28°19'24"E, 666 m, *Q. cerris,* 11.VI.2013, 2P, 1♀; Karesi, Bakacak, Koru, 39°40'52"N 27°43'32"E, 490 m, *Q. frainetto,* 28.VI.2013, 6W, 1♀; Savaştepe, Mancılık 39°21'26"N 27°48'41"E, 782 m, *Q. cerris,* 11.VII.2013, 2W, 1♀; Susurluk, Bağıran Stream, 39°51'40"N 28°18'14"E, 806 m, *F. orientalis,* 23.VII.2013, 8P, 1♂; Susurluk, Darıalan, 39°52'03"N 28°16'39"E, 572 m, *Q. petraea,* 23.VII.2013, 10W, 2♀♀; Erdek, Göletaltı, 40°28'15"N 27°53'54"E, 322 m, *Q. frainetto* × *Q. petraea,* 26.VII.2013, 6W, 1♀; Karesi, Bakacak, Koru, 39°40'52"N 27°43'32"E, 490 m, *Q. frainetto,* 05.VIII.2013, 6W, 4♀♀ 2♂♂; Karesi, Bakacak, Koru, 39°40'53"N 27°43'33"E, 485 m, *Q. frainetto,* 05.VIII.2013, 7W, 4♀♀ 1♂; Bigadiç, Ulus Mountain, 39°19'23"N 27°23'39"E, 1.616 m, *F. orientalis,* 13.VIII.2013, 4W, 1♀; Bigadiç, Ulus Mountain, 39°19'23"N 27°23'46"E, 1.608 m, *F. orientalis,* 13.VIII.2013, 8P, 1♀; Bigadiç, Davutlar Village, 39°29'15"N 28°19'24"E, 666 m, *Q. cerris,* 13.VIII.2013, 2P, 1♀; Erdek, Kurtboğazı, 40°27'45"N 27°49'25"E, 607 m, *F. sylvatica,* 27.IX.2013, 5W, 1♀; Balya, Ilıca Village, Hisaralan, 39°54'25"N 27°50'39"E, 325 m, *Q. frainetto,* 03.X.2013, 3P, 1♀; Gönen, Şarkoluk Store, 40°08'53"N 27°29'44"E, 439 m, *Q. frainetto,* 01.XI.2013, 2W, 1♀; Erdek, Göletaltı, 40°28'16"N 27°53'52"E, 315 m, *Q. frainetto* × *Q. petraea,* 26.VI.2014, 5W, 1♂; Erdek, Kurtboğazı, 40°27'45"N 27°49'25"E, 607 m, *F. sylvatica,* 26.VI.2014, 5W, 1♀; Bigadiç, Ulus Mountain, 39°19'23"N 27°23'39"E, 1.616 m, *F. orientalis,* 17.VII.2014, 4P, 1♀; Totally 43♀♀ 9♂♂, 52 exs.

Distribution in the world: Europe: Austria, Azerbaijan, Azores, Belarus, Belgium, Bosnia and Herzegovina, Britain, Bulgaria, Croatia, Cyclades Islands, Czech Republic, Denmark, Estonia, Finland, France, Franz Josef Land, Georgia, Germany, Greece, Hungary, Iceland, Ireland, Italy, Kaliningrad Region, Latvia, Liechtenstein, Lithuania, Luxembourg, Macedonia, Malta, Moldova, Netherlands, Norway, Novaya Zemlya, Poland, Portugal, Romania, Russia: Central European Territories, Russia: Eastern European Territories, Russia: Northern European Territories, Russia: Northwest European Territories, Russia: Southern European Territories, Slovakia, Slovenia, Spain, Sweden, Switzerland, The Twelve Islands, Turkey, Ukraine, and Yugoslavia; **Asia:** Afghanistan, Bhutan, China, Cyprus, Democratic People's Republic of Korea, India, Indonesia, Japan, Mongolia, Nepal, Pakistan, Russia: East Siberia, Russia: Far East, Russia: West Siberia, Svalbard and Jan Mayen Islands, Taiwan, Turkey, and Vóreion Aiyáion (North Aegean Island); **North Africa:** Canary Islands, Egypt, Madeira Archipelago, and Selvagens Islands; **Etiyopya Bölgesi**; **Afrotropics Region**; **Australia Region**; **East Palearctic**; **Nearctic Region**; **Neotropical Region**; **East Region**; **Near East**; **Near Region** (Johnson, 2007; Rücker, 2018, 2021; Anonymous, 2023). **COS**

Distribution in Turkey: Turkey (Asia, and Europe) (Johnson, 2007; Anonymous, 2023). This is the first time a locality record is given with this study.

Note: *Cortinicara* sp. is represented by only one species in the Western Palearctic Region. The center of diversity of *Cortinicara* sp. is the Oriental and Australian Regions (Rücker, 2018).

Genus *Melanophthalma* Motschulsky, 1866

Melanophthalma (Cortilena) fuscipennis (Mannerheim, 1844)

Material examined: This species was identified in traps set on each date. Totally 194♀♀ 31♂♂, 225 exs.

Distribution in the world: Europe: Austria, Croatia, France, Georgia, Germany, Greece, Hungary, Italy, Portugal, Romania, Russia: Southern European Territories, Slovenia, Spain, Switzerland, and Turkey; **Asia:** Cyprus, and Turkey; **North Africa:** Canary Islands, Egypt, Madeira Archipelago, Morocco, and Tunisia (Johnson, 2007; Rücker, 2018, 2021; Anonymous, 2023).

Distribution in Turkey: Turkey (Asia and Europe) (Johnson, 2007; Rücker, 2018). This is the first time a locality record is given with this study.

Melanophthalma (Melanophthalma) taurica (Mannerheim, 1844)

Material examined: This species was detected in traps set on each date during spring, summer and fall seasons. Totally 132♀♀ 13♂♂, 145 exs.

Distribution in the world: Europe: Azerbaijan, Russia: Southern European Territories, and Ukraine; **Asia:** Afghanistan, Iran, Kyrgyzstan, Tajikistan, Turkey, and Turkmenistan (Johnson, 2007; Rücker, 2018, 2021; Anonymous, 2023).

Distribution in Turkey: Regions of Turkey entering the Asian continent (Johnson, 2007; Rücker, 2018, 2021). This is the first time a locality record is given with this study.

Melanophthalma (Melanophthalma) distinguenda (Comolli, 1837)

Material examined: This species has been intensely determined in traps set up at all times. Totally 1283♀♀ 287♂♂, 1570 exs.

Distribution in the world: Europe: Austria, Belarus, Belgium, Britain, Croatia, Czech Republic, Denmark, France, Germany, Greece, Hungary, Italy, Lithuania, Malta, Netherlands, Poland, Portugal, Romania, Sweden, Switzerland, and Ukraine; **Asia:** Iraq, and Turkey; **North Africa:** Madeira Archipelago; **Nearctic Region:** (Johnson, 2007; Rücker, 2018, 2021; Anonymous, 2023).

Distribution in Turkey: Regions of Turkey entering the Asian continent, and İzmir (Johnson, 2007; Tezcan ve ark., 2010; Rücker, 2018). This species is reported for the first time from Marmara Region.

Melanophthalma (Melanophthalma) rhenana Rücker & Johnson, 2007

Material examined: This species has been intensely determined in traps set up at all times. Totally 3092♀♀ 1066♂♂, 4158 exs.

Distribution in the world: Europe: Germany: Rhineland-Palatinate: Neuwied, Baden, Saxony, Italy: Sardinia, and Sicily; **Asia:** Iran, and Turkey (Rücker, 2018, 2021; Anonymous, 2023).

Distribution in Turkey: Regions of Turkey entering the Asian continent (Rücker, 2018, 2021). This is the first time a locality record is given with this study.

Note: The main distribution region of this species is thought to be Central Asia. It probably spread through Turkey to Italy and Germany.

Genus *Migneauxia* Jacquelin du Val, 1859

Migneauxia crassiuscula (Aubé, 1850)

Material examined: This species was identified in traps set on each date. Totally 68♀♀ 13♂♂, 81 exs.

Distribution in the world: Europe: Azerbaijan, Bulgaria, Croatia, France, Georgia, Greece, Hungary, Italy, Spain, and Ukraine; **Asia:** Afghanistan, Cyprus, Iraq, Israel, Jordan,Turkey, and Uzbekistan (Johnson, 2007; Rücker, 2018, 2021; Anonymous, 2023).

Distribution in Turkey: Regions of Turkey entering the Asian continent (Johnson, 2007; Rücker, 2018, 2021). This is the first time a locality record is given with this study.

DISCUSSION and CONCLUSIONS

In the oak and beech areas of Balıkesir province, 22 species belonging to nine genera belonging to two subfamilies were identified from 8537 specimens of Latriididae family by using window and trap trapping methods.

When we examine the geographical distribution of the genera of the family Latridiidae in the world; it is seen that the species belonging to the genera *Cartodere*, *Corticaria*, *Melanophthalma*, and *Migneauxia* are cosmopolitan. Species belonging to the genus *Enicmus* are distributed in the Holarctic, Oriental, Australian, and Neotropical regions, species belonging to the genus *Latridius* are distributed in the Holarctic, and Oriental regions, species belonging to the genus *Metophthalmus* are distributed in the Holarctic, Neotropical, and Afrotropical regions, species belonging to the genus *Corticarina* are distributed in the Holarctic, Neotropical, Afrotropical, and Oriental regions, and species belonging to the genus *Cortinicara* are distributed in the Holarctic, and Indo-Australian regions (López Fernández, 2014; Rücker, 2018, 2020, 2021).

It is possible to divide the Palaearctic Region into nine sub-regions, namely Siberia, Far East, Central Asia, Middle East, North Africa, Southern Europe, Northern Europe, Western Europe and Eastern Europe (Demir, 2019). When the distribution status of the 22 species in the subregions of the Palaearctic Region was evaluated, it was determined that 16 species were found in Siberia, 16 in the Far East, 16 in Central Asia, 16 in the Middle East, 11 in North Africa, 16 in Southern Europe, 13 in Northern Europe, 15 in Western Europe and 21 in Eastern Europe. *Enicmus transversus*, *Cartodere nodifer* and *Cortitinicara gibbossa* species have the widest distribution in all subregions of the Palaearctic Region. These species are followed by *Latridius minutus*, *Enicmus rugosus*, *E. brevicornis*, *Corticaria pubescens*, *C. serrata* and *C. elongata*.

When the status of the 22 species belonging to the Latridiidae family in the fauna of Turkey is evaluated, it is seen that six species (*Cartodere nodifer*, *Corticaria longicollis*, *C. obscura*, *Corticarina minuta*, *Enicmus fungicola* and *E. testaceus*) are reported from Turkey for the first time. 13 species (*Metophthalmus hungaricus*, *Enicmus transversus*, *E. brevicornis*, *Latridius minutus*, *Corticarina curta*, *Cortinicara gibbosa*, *Melanophthalma rhenana*, *M. taurica*, *M. fuscipennis*, *Corticaria serrata*, *C. elongata*, *C. pubescens* and *Migneauxia crassiuscula*) have been previously recorded from Turkey without giving the locality name, and with this study, the locality is recorded for the first time. *Enicmus rugosus* and *Melanophthalma distinguenda* were also detected for the first time in the Marmara Region. *Cartodere apfelbecki* is a new record for the local fauna of Balıkesir Province.

According to these data, the number of species belonging to Latridiidae family in Turkey has increased from 57 to 63. In addition to providing important information about the Latridiidae fauna of Turkey, this study is important in terms of contributing to the zoogeographic distribution of this family due to the many cosmopolitan species.

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Contribution of the Authors as Summary

The authors report that the data for this study were collected by Dr. Aylin TUVEN, and the experiment of the study was executed by Dr. Aylin TUVEN under the supervision of Ass. Prof. Dr. Sakin Vural VARLI, declare that the text of the article was written by Aylin Tüven under the supervision of Ass. Prof. Dr. Sakin Vural VARLI.

Statement of Conflict of Interest

The authors have declared no conflict of interest.

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An Investigation on The Side Effects of Some Pesticides Against The Predatory Insect *Exochomus nigromaculatus* (Coleoptera: Coccinellidae) Under Laboratory Conditions

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ABSTRACT
Several harmful pest species can cause damage to apricot trees. Chemical control is the often preferred method in controlling these pests. The use of pesticides has generally resulted in pesticide resistance and elimination of natural enemies. *Exochomus nigromaculatus* is a predatory insect of globose scale and other scale insects. Inappropriate use of pesticides has been linked with adverse effects on non-target organisms (e.g., reduction of beneficial species populations and increase of pest populations). This study evaluated the side effects of five insecticides (Acetamiprid, deltamethrin, spirotetramat, sulfoxaflor, dimethoate) and a plant extract (orange oil) on immature stages of *E. nigromaculatus* using dry film method. Acetamiprid and deltamethrin caused the highest mortality rate (97.3%) besides standard toxic dimethoate. Sulfoxaflor accounted for approximately 70% mortality to *E. nigromaculatus*. In contrast, spirotetramat and orange oil caused less than 20% mortality to the predator. As a result of the dry film method applied against the pre-adult stage of *E. nigromaculatus;* dimethoate was classified as harmful (class 4), acetamiprid and deltamethrin were classified as moderately harmful (class 3), sulfoxaflor were classified as less harmful, (class 2) while spirotetramat and orange oil were classified as harmless. Thus, it was concluded that spirotetramat and orange oil did not have a negative effect on the predatory insect, *E. nigromaculatus* and could be used safely in IPM programs.

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Kayısı Bahçelerinde Kullanılan Bazı Pestisitlerin Laboratuvar Koşullarında *Exochomus nigromaculatus* (*Coleoptera*:Coccinellidae)'a Karşı Yan Etkilerinin Araştırılması

ÖZET
Kayısı ağaçlarında çeşitli böcek türleri zarara neden olmaktadır. Bu zararlılarla mücadelede kimyasal mücadele sıklıkla tercih edilen yöntemdir. Pestisitlerin uygunsuz kullanımı, hedef olmayan organizmalar üzerinde de olumsuz etkilerle sonuçlanmaktadır (örneğin, faydalı tür popülasyonlarının azalması ve haşere popülasyonlarının artması). *Exochomus nigromaculatus*, erik koşnili ve diğer koşnillerin avcı böceğidir. Bu çalışmada, beş insektisit (Acetamiprid, deltamethrin, spirotetramat, sulfoxaflor, dimethoate) ve bir bitki ekstraktının (portakal yağı) *E. nigromaculatus*'un kuru film yöntemi kullanılarak larva dönemlerine yan etkisi değerlendirilmiştir. Acetamiprid ve deltamethrin en yüksek ölüm oranına (%97.3) neden olmuştur. Sulfoxaflor, *E. nigromaculatus* için yaklaşık %70 ölüme neden olurken, spirotetramat ve portakal yağı avcılarda %20'den az ölüme neden olmuştur. *E. nigromaculatus*'un ergin öncesi evresine karşı uygulanan kuru film yöntemi sonucunda; dimethoate zararlı (sınıf 4), acetamiprid ve deltamethrin orta derecede zararlı (sınıf 3), sulfoxaflor az zararlı (sınıf 2), spirotetramat ve portakal yağı zararsız olarak sınıflandırılmıştır. Böylece, spirotetramat ve portakal yağının avcı böcek *E. nigromaculatus*'a olumsuz bir etkisinin bulunmadığı ve IPM programlarında güvenle kullanabileceği sonucuna varılmıştır.

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INTRODUCTION

Apricot is a stone fruit tree that has spread from Central Asia to a wide geography including countries neighboring the Mediterranean (Anonymous 2019). Among the world's apricot-producing countries, Turkey ranks first with a share of 23.1% (Anonymous 2020). In the year 2020, 863 thousand tons of apricot were produced in Turkey; 53% of this production was from Malatya, 12% from Mersin and 7% from Elazig (Anonymous 2021). Apricot grown in Malatya is important for both domestic consumption as well as for the country's economy as it is an important agricultural export product.

Various diseases and pests impact apricot cultivation in Malatya. The peach twig borer and flat-headed root-borer are the main pests of apricot (Anonymous 2017). Apart from these, for years, several other harmful insect species directly affect apricot production and cause significant input costs for crop production in the world and in Turkey (Viggiani 1989; Cravedi and Molinari 1995; Ulusoy et al. 2001; Öztürk et al. 2004; Anonymous 2008; Uygun et al. 2013; Öztürk and Ulusoy 2014; Anonymous 2017). Many researchers have stated that *Sphaerolecanium prunastri* (Boyer de Fonscolombe) (Hemiptera: Coccidae) is a harmful insect pest species of stone fruits in Turkey (Bodenheimer 1953; Lodos 1982; Ülgentürk et al. 2001; Özgen and Bolu 2009; Apak 2021). The pest overwinters as first and second instar larvae in apricot trees in Malatya province. This pest sucks plant sap from the trunks of trees, especially from one or two-year branches, causing intense fumagin formation in the trees; this affects the quality and quantity of fruits produced and weakens the trees. Continued introduction and feeding of species belonging to the Scolytidae family kill weakened trees (Anonymous 2008; Uygun et al. 2013). Reportedly, this pest is found on other plants belonging to the Rosaceae family in all geographical regions of Türkiye (Bodenheimer 1953; Soydanbay 1976; Öncüer 1977; Ülgentürk et al. 2001; Özgen and Bolu 2009; Yiğit and Tunaz 2015).

Several natural enemies including predators and parasitoids from different groups have been recorded against the globose scale in Turkey. Among them, *Discodes aeneus* and *Coccophaegus* sp. are important parasitoids whereas *Exochomus quadripustulatus* and *E. nigromaculatus* have been identified as predators (Özgen and Bolu 2009; Yiğit and Tunaz 2015; Keçeci et al. 2020). A recent study that detected intense infestation with the globose scale pests of apricot orchards in Kale, Kuluncak and Akçadağ districts of Malatya province, found high rates of parasitoid *D. aeneus*, and predatory insects *E. quadripustulatus* and *E. nigromaculatus* which are the natural enemy of globose scale, in an apricot orchard in Kale district in which chemical pesticides are not utilized. When chemical pesticides were unused in the orchard, the pest were suppressed in a short period of two years by these beneficial organisms (Keçeci et al. 2020). It is reported that the application of broad-spectrum synthetic insecticides leads to the resurgence of harmful insects due to their toxic effects on beneficial insects, and has negative impacts on their success (Karacaoğlu et al. 2020; Bibi et al. 2021).

Although *E. nigromaculatus* is naturally found in the orchards, pesticide use reduces its populations hence an increase in the harmful insect population. In order to carry out sustainable agriculture, the application of pesticides with less adverse effects on beneficial insects and their application at appropriate doses are important within the scope of integrated control. In literature, there have been many studies that have sought to analyze the impact of side effects of pesticides on commercially available and widely used benefits (Hassan et al. 1985; Dalcı et al. 2009; Portakaldalı and Satar 2015a, b; Kaya and Keçeci 2021). However, the most challenging situation is the lack of knowledge of predator or parasitoid which are naturally occurring and suppressing the pest population. The aim of this laboratory study was to determine the side effects of acetamiprid, deltamethrin, spirotetramat, sulfoxaflor, dimethoate and orange oil on immature stages of *E. nigromaculatus*.

METHODS

Branches infested with globose scales as well as larvae of its predator were brought to and cultured in the laboratory after surveys carried out in Malatya Apricot orchards. *Exochomus nigromaculatus* was mass-reared in plexiglass cages 30 × 30 × 50 cm (LWH) with the two sides covered by netting to allow ventilation. Citrus mealybug (*Planococcus citri*) (Hemiptera: Pseudococcidae) with potato sprouts were offered to predators as prey. The dry film method was applied against the pre-adult stages of *E. nigromaculatus*, which is one of the plant protection products that farmers mainly use in their orchards. IOBC methods were taken into account in determining the effects of these chemicals (Hassan et al. 1985; Candolfi et al. 2000). In the experiments, the pesticides were sprayed onto glass plates of 12 cm diameter with the help of a

spray tower, and a thin film layer of chemical-infused liquid was formed with a thickness of 2±0.2 mg/cm2 on surface of the glass. Then, the treated surfaces of these plates were placed upside down and 2 cm high, 13 cm diameter, 5 cm diameter five-chamber cells made of fiber class were used as the test unit. Tap water was used to prepare the pesticide solutions for the experiment. For the control group only tap water was applied. The experiment was carried out in a randomized plot design with six groups (four pesticides, a standard toxic compound and water as control) with 4 replications. Ten 2nd stage larvae were used, one per cell in each replicate. These larvae were kept in these cells until they reached the adult stage (Karacaoğlu and Satar 2010; Satar et al. 2012). Food was provided to the larvae in the cells every day and the number of live-dead individuals were monitored and recorded.

Ten adult individuals that newly emerged were collected and placed into a 0.5 liter plastic rearing cage to mate. The number of eggs laid per female for 10 days after the adult individuals were placed in the cage was recorded daily to evaluate the effect on reproduction. From here, the effect on reproduction was calculated using the formula (R) =(1-(Rt/Rc))*100 (R: Reduction in fecundity, R_t: Egg number in the insecticide treatment, R_c: Egg number in the control treatment) (Anonymous 2010). Fecundity was used only as a quality criterion, while toxicity was determined only by considering mortality rates in pre-adult stages. Fecundity was not evaluated for characters with a corrected mortality rate of less than 50% compared to the control in the treated unit. The experiments were carried out in the climate rooms of the Plant Protection Department in Malatya Turgut Özal University. Conditions in the room were 25±1 °C temperature, 65±5% relative humidity and 14/10 hours of light/dark conditions. The active substance, trade names, formulation and dose values of the plant protection products included in the trial are given in Table 1.

Table 1. The active substance, trade names, formulation and dose values of the plant protection products used in the experiments against the pre-adult stages of *Exochomus nigromaculatus*

Çizelge 1. Exochomus nigromaculatus'un ergin öncesi dönemlerine karşı yapılan denemede kullanılan bitki koruma ürünlerinin etken maddesi, ticari adları, formülasyonu ve dozları

Active ingredient and formulation*	Trade name	Chemical group	Applied rate
Acetamiprid, (20 g/L, SP)	Mospilan 20	Neonicotinoids	40 gr /100 L
Deltamethrin (25 g/L, EC)	Decis EC 2.5	Pyrethroids	30 ml /100 L
Spirotetramat (100 g/L, SC)	Movento SC 100	Tetronic and Tetramic Acid Derivatives	100 g /100 L
Sulfoxaflor (240 g/L, SC)	Breaker 240	Sulfoximines	40 ml/100 L
Orange oil (60 g/L, EC)	Orange oil	Unknown	200 g /100 L
Dimethoate** (400 g/L, EC)	Poligor	Organophosphates	150 ml/100 L

* EC, emulsion concentrate; SC, suspension concentrate; SP, water soluble powder

** Dimethoate is included as a standart toxic.

Table 2. Classification of plant protection products according to their toxicity (IOBC)

Çizelge 2. Bitki koruma ürünlerinin toksisitelerine göre sınıflandırılması (IOBC)

Effect (%)	IOBC value	Class
0-30	1	Harmless
31-79	2	Slightly harmful
80-98	3	Moderately harmful
99-100	4	Harmful

Mortality data obtained from daily counting of the live and dead individuals were calculated and corrected using the Abbott formula (Abbott 1925). Arc-sin square root transformation was applied to the percent mortality values obtained and analysis of variance was applied to the transformed data (SPSS package statistical program, version 13.0) The differences between the applications were separated using Tukey Test (Efe et al. 2000). The plant protection products were classified according to the toxic categories developed by the IOBC (Table 2) (Hassan et al. 1985), taking into account the mortality rate (Abbott 1925).

RESULTS

The side effects of some plant protection products used in apricot orchards were assessed using the dry film method on the pre-adult stages of *E. nigromaculatus* and mortality data and IOBC classification are given in Table 3.

Acetamiprid and deltamethrin caused 97.3% mortality of predator larvae and were found to be as harmful as in standard toxic dimethoate. However, another active ingredient, sulfoxaflor, was produced 72.5% mortality on *E. nigromaculatus* immature stages and was classified as slightly harmful. Spirotetramat and orange oil presented 10.3% and 16.2% activity, respectively, and were found to be harmless and grouped in IOBC category 1. Moreover, the effect of these active ingredients on egg reproduction was accepted as zero, whereas spirotetramat 10.5% effective followed by orange oil with 12.2%.

Table 3. Classification of the effects of some plant protection products on the pre-adult stages of *Exochomus nigromaculatus* according to Abbott and IOBC

Çizelge 3. Exochomus nigromaculatus'un larva dönemlerine bazı bitki koruma ürünlerinin etkilerinin sınıflandırılması (Abbott ve IOBC)

Treatments	N*	Mortality rates of immature stages (%)	% Effect (Corrected using Abbott)	IOBC category	Reduction on reproduction capacity
Control	40	7.5±2.50a	-	-	-
Acetamiprid	40	97.5±2.50c	97.3	3	-**
Deltamethrin	40	97.5±2.50c	97.3	3	-
Spirotetramat	40	17.5±2.50a	10.3	1	10.5
Orange oil	40	22.5±4.78a	16.2	1	12.2
Sulfoxaflor	40	72.5±4.78b	70.3	2	-
Dimethoate	40	100.0± 0.00c	100.0	4	-

*Shows the number of replicates.

** Since more than 50% mortality was observed in these characters, the effect on reproduction was not evaluated. Means with the same letter in the same column were not statistically significant according to the Tukey test (P>0.05). SD= 6.21, F: 84,070 Sig: 0.000

Based on the data obtained from the side effect experiments on *E. nigromaculatus*, dimethoate, acetamiprid and deltamethrin were produced similar effect, and clustered in the same statistical group whereas sulfoxaflor was in a different group. Spirotetramat and orange oil were found to be harmless after applications. Thus, the both were statistically included in the same group as the control.

DISCUSSION

The aim of the present study was to determine the side effects of five insecticides and a plant extract to *E. nigromaculatus.* As a result of the study, acetamiprid and deltamethrin were harmful to the larva of *E. nigromaculatus*. Despite the Class II IOBC categories (slightly harmful), sulfoxaflor caused approximately 70% mortality to the predator. Spirotetramat and orange oil were harmless with 10.3 and 16.2 mortality, respectively.

Currently, no studies have determined the side effects of pesticides against *Exochomus* species. Erkılıç et al. (1994) assessed the effects of buprofezin and summer white oil against *Chilocorus bipustulatus* in their study. They stated that these active substances were harmless or less harmful to the adults of the insect. In the current study, orange oil was similarly harmless against the immature stages of *E. nigromaculatus*, which belongs to the same family as *C. bipustulatus*. Başpınar et al. (2002) tested the effects of the recommended doses of deltamethrin, azadirachtin, azadirachtin water extract, summer white oil and chlorfenapyr against adults and larvae of *Cryptolaemus montrouzieri* using spraying and dry film method. According to their results, deltamethrin was highly toxic against adults and larvae of *C.montrouzieri* with the dry film method. In another study, deltamethrin was also found highly toxic on *Discodes aeneus* Dalman (Hymenoptera: Encyrtidae), the parasitoid of globose scale found in apricot orchards (Karacaoğlu et al. 2020). We report that deltamethrin was moderately toxic to the pre-adult stages of *E. nigromaculatus*.

Brück et al. (2009) stated that spirotetramat is harmless against *Stethorus* spp, *Coccinella* spp. and *Chilocorus nigritus*. Planes et al. (2013) investigated the effects of spirotetramat, chlorpyriphos and pyriproxyfen against *Cryptolaemus montrouzieri* larvae and adults, and reported spirotetramat as harmless to *C. montrouzieri* larvae and that adults continued to reproduce after exposure. Likewise, we have determined that the active substance of spirotetramat gave similar results against the pre-adult stages of *E. nigromaculatus* belonging to the same family. Karacaoğlu et al. (2013) evaluated the effects of spirotetramat and dimethoate on the immature stages of *Chilocorus bipustulatus, Anagyrus pseudococci,* and *Amblyseius swirskii* and found that the mortality rates of *C. bipustulatus* after exposure to these chemicals were produced 11.11% and 100% mortality, respectively. Similarly, in the current study, dimethoate caused 100% mortality while spirotetramat exhibited 10.50%. Satar et al. (2018) examined the side effects of sulfoxaflor against *C. bipustulatus* (L.) (Coleoptera: Coccinellidae) under laboratory conditions and classified it according to IOBC standards. They stated that this active compound caused 71.16% mortality effect against *C. bipustulatus*. Likewise, sulfoxaflor had a similar effect on the pre-adult stages of *E. nigromaculatus* in this study. Kahraman and Öztop (2019) assessed the side effects of dimethoate, which is the active ingredient of buprofezin pesticide used in citrus orchards against *C. bipustulatus* and found that this compound was 100% effective, thus was classified as harmful according to

the IOBC categories. Dimethoate was also 100% against *E. nigromaculatus* in the present study.

Bibi et al. (2021) tested the effects of orange oil against *Cryptolaemus montrouzieri* and *Chrysoperla carnea* under laboratory conditions. They reported that orange oil caused 25.9% *C. montrouzieri* larva mortality. In this study, a mortality rate of 22.5% was determined for orange oil against the second stages of *E. nigromaculatus*.

CONCLUSIONS

In conclusion, it was determined that the presence of plant protection chemical products particularly acetamiprid and deltamethrin have a negative effect on a beneficial organism *E. nigromaculatus* by causing 97.2% insect mortality. This shows that these two insecticides are not compatible for use in IPM programs. It should be emphasized that inappropriate insecticide selections easily disrupt the natural balance and can cause pest population to rise. Incorrect practices cause damage to the agricultural ecosystem as well as economic losses for the producer. Currently, spirotetramat, a temporarily recommended pesticide against the harmful mealy plum aphid (*Hyalopterus pruni*) in apricots, had low side effects against *E. nigromaculatus* and therefore it is compatible with use in IPM programs.

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Author's Contributions

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

Statement of Conflict of Interest

Authors have declared no conflict of interest.

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Edremit Körfezi Zeytinliklerinde *Dasineura oleae* Angelini'nin (Diptera: Cecidomyiidae) Bulaşma ve Parazitlenme Oranları ile Parazitoitlerinin Belirlenmesi

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ÖZET

Bu çalışmanın ilk amacı, 2019–2021 yıllarında Balıkesir ilinin Edremit Körfez Bölgesindeki zeytinliklerde *Dasineura oleae* Angelini (Diptera: Cecidomyiidae) ve parazitoitlerinin tespiti ve tanımlanmasıdır. Diğer amaç ise, *D. oleae*'nin yaprak ve sürgünlerdeki bulaşma oranı ile bazı iklim faktörleri arasındaki ilişkileri araştırmaktır. Çalışmanın 2020–2021 yıllarında bölgeyi temsil edebilecek özellikte birbirine sınır olan üç ilçenin (Burhaniye, Edremit ve Havran), üç farklı rakımdaki (69, 163 ve 321 m) toplam dokuz bahçesinde *D. oleae*'nin bulaşma ve parazitlenme oranları hesaplanmıştır. Rakım, günlük ortalama nispi nem ve hava sıcaklığı değerlerinin *D. oleae*'nin bulaşma oranı üzerinde etkili olduğu belirlenmiştir. Günlük yağışların bulaşmada etkisiz olduğu, aylık toplam yağış miktarının ise etkili olduğu saptanmıştır. Yaprak ve sürgünlerdeki bulaşma oranları 2020 yılında 2021'e göre (%4.71–16.93) daha yüksek bulunmuştur. Çalışmanın 2020–2021 yılı verilerine göre; sürgünlerdeki bulaşma, yapraklara göre %33.10 daha yüksek bulunmuştur. Ayrıca, Burhaniye ve Havran'a kıyasla Edremit'teki zeytinliklerde bulaşma (%3.30–13.69) daha düşük olmuştur. Yaprak ve sürgünlerdeki parazitlenmeler en düşük Burhaniye'de (%27.79, %21.84), en yüksek ise Havran'da (%47.39, %30.28) tespit edilmiştir. Çalışma süresince, *D. oleae*'yi parazitleyen Hymenoptera takımından dört familyaya bağlı 10 farklı tür belirlenmiştir. *Platygaster oleae* Szelenyi en fazla (%30.21), *Torymus phillyreae* Ruschka ise en az (%1.27) rastlanan tür olmuştur.

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Anahtar Kelimeler
Dasineura oleae
Zeytin bahçeleri
Bulaşma oranı
Parazitlenme oranı
Parazitoit

Determination of Infestation and Parasitization Rates, and Parasitoids of *Dasineura oleae* Angelini (Diptera: Cecidomyiidae) in Olive Orchards in Edremit Bay

ABSTRACT

The first objective of this study was to detect and identify *Dasineura oleae* Angelini (Diptera: Cecidomyiidae) and parasitoids in olive orchards in the Edremit Bay Region of Balıkesir province in 2019–2021. The second objective of the study was to investigate the relationship between the infestation rates of *D. oleae* on leaves and shoots, and climatic factors. The infestation and parasitization rates of *D. oleae* were determined in nine orchards, with three orchards at different altitudes (69, 163, and 321 m) in three districts that border each other (Burhaniye, Edremit, and Havran), which can represent the region, in 2020–2021. It was determined that orchard altitude, daily average relative humidity and air temperature affected the infestation rates of *D. oleae*. Although daily precipitation had no effect on infestation rates, monthly total precipitation was determined to be effective. The infestation rates on leaves and shoots were found to be (4.71–16.93%) higher in 2020 than in 2021. According to the data from the 2020–2021 years, the infestation rate on shoots was 33.10% higher than that on the leaves. In addition, the infestation rate in olive orchards in Edremit was (3.30–13.69%) lower than that in Burhaniye and Havran. The parasitization rates on leaves and shoots were lowest in Burhaniye (27.79%, 21.84%) and highest in Havran (47.39%, 30.28%). During the study, 10 different species

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belonging to four families from the order Hymenoptera parasitized *D. oleae* larvae were determined. *Platygaster oleae* Szelenyi was the most common (30.21%) species recorded in the study region, while *Torymus phillyreae* Ruschka was the least common (1.27%).

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GİRİŞ

Akdeniz ikliminin hüküm sürdüğü bölgelerin vaz geçilmez bitkisi olan zeytin (*Olea europaea* L.) meyvesi, yağı, sabunu ve odunu ile insanlığa hizmet etmektedir. Türkiye'de bu iklime sahip bölgelerde zahmetsizce yetişen zeytin ağacı, ülke ekonomisi için oldukça önemli bir gelir kaynağıdır. Türk zeytinciliğinin önemli merkezlerinden biri olan Balıkesir ili, 2022 yılı verilerine göre 11 402 687 adet ile Türkiye'nin meyve veren zeytin ağacı sayısının %6.8'ini, ilin Edremit Körfez Bölgesi ise 9 697 909 adedini içermektedir. Çalışmanın yapıldığı Edremit, Havran ve Burhaniye ilçeleri ise 6 362 909 adet meyve veren ağaç sayısı ile bölgenin zeytin ağacı varlığının %65.6'sını kapsamaktadır (Anonim, 2022). Zeytin üreten Akdeniz Bölgesi'ndeki birçok yerde olduğu gibi, Türkiye'deki Balıkesir ilinin Edremit Körfez Bölgesi için de oldukça önemli gelir kaynağı olan zeytin ağacının birçok hastalık ve zararlı türü bulunmaktadır. Bu zararlılardan Zeytin sineği ve Zeytin güvesi vb. türler, birinci derecede ekonomik öneme sahip olup her yıl düzenli olarak kontrol edilmesi gereken zararlı türlerdir (Kaplan, 2019; Kaplan & Alaserhat, 2020a; Kaplan & Bayram, 2021). Bir kısmı da ekonomik önemde olmayan, doğada bulunan parazitoit ve predatörleri ile denge halinde yaşayan böceklerdir. Bu şekilde olup ikinci derece zararlılar olarak adlandırılan böceklerden biri de Zeytin yaprak siğili olarak bilinen *Dasineura oleae* Angelini (Diptera: Cecidomyiidae)'dir (Skuhrava & Skuhravy, 1997; Darvas ve ark., 2000; Kaplan, 2019). Ancak, zeytin Akdeniz Bölgesi'nin en önemli ürünlerinden birisi olmasına rağmen bu ikinci derece zararlıları hakkında çok az şey bilinmektedir. Akdeniz Bölgesi ülkelerinde Zeytin yaprak siğili ile ilgili sınırlı sayıda çalışma bulunmaktadır. İtalya'nın orta kısımlarındaki, zeytin bahçelerinde yapılan bir çalışmada, *D. oleae* gal sayısının ortalama %23–71 arasında değiştiği, bazı bahçelerde hiç parazitlenme görülmezken bazılarında ise bu oranın %43.7'ye kadar ulaştığı bildirilmiştir (Picchi ve ark., 2022). Yunanistan'da ise *D. oleae* bulaşma oranının %70–90 arasında olduğu, ağaçlar arasında bulaşma düzeyinin farklılık gösterdiği ve iç kesimlerde bulaşma oranının azaldığı saptanmıştır (Simoglou ve ark., 2012). Filistin'de, *Platygaster oleae* Szelenyi (Hymenoptera: Platygastridae) ve *Zeytinus hatayensis* Doğanlar (Hymenoptera: Eulophidae)'in, *D. oleae*'nin yerel iki parazitoiti olduğu parazitleme oranının %82.7'ye kadar ulaştığı, *Z. hatayensis*'in ise daha düşük oranlarda bulunduğu (%38.4) bildirilmiştir (Batta & Doğanlar, 2020). *Dasineura oleae*'nin doğal düşmanları olan bazı parazitoitlerin etkisine rağmen, son zamanlarda beklenmedik *D. oleae* salgınları, bilim adamlarını bu zararlının bitki fizyolojisi ve biyokimyası üzerindeki etkisini araştırmaya yöneltmiştir (Caselli ve ark., 2021). Salgın dönemlerinde *D. oleae*, genel olarak zeytin ağaçlarının öncelikle yapraklarında olmak üzere sürgün gibi diğer vejetatif organlarında gal (ur veya şişkinlik) oluşturarak verimde azalmaya neden olmaktadır. *Dasineura oleae* erginlerinin mart ayından mayıs ayı başlarına kadar doğada görüldükleri ve genç zeytin sürgünlerindeki yapraklarda epidermisin altına yumurta bıraktıkları gözlenmiştir. Yumurtadan çıkan ilk dönem larvalar zeytin yapraklarıyla beslenirken dokuları uyararak, doku anormallikleri ve şişkinliklere yol açmaktadırlar (Doğanlar ve ark., 2011; Batta, 2019). Zararlı tarafından yapraklarda gal oluşturulduğunda; bitkinin fotosentez oluşturma özelliğinin azaldığı, meyve verim miktarı ve kalitesinin düştüğü bildirilmektedir (Gonzáles ve ark., 2005; Huang ve ark., 2014). Caselli ve ark. (2021) yaptıkları çalışmada, *D. oleae* tarafından yapraklarda net-fotosentez ve stoma iletkenliğinin önemli ölçüde azaldığını göstermişlerdir. Bu konulardaki çalışmalar ile arazi çalışmalarının az olması nedeniyle, *D. oleae*'nin zeytin ağacı sağlığına ve dolayısıyla meyve üretimine verdiği zararın değerlendirilebilmesi için acilen daha ileri araştırmaların yapılması gerekmektedir. *Dasineura oleae*'nin yılda bir döl, ancak uygun iklim koşullarında iki döl de verebildiği bildirilmiştir (Darvas ve ark., 2000; Batta, 2019). Tüm Akdeniz Bölgesi ülkelerinin zeytinliklerinde var olduğu uzun yıllardan beri bilinen Zeytin yaprak siğili'nin İsrail (Gerson & Harpaz, 1968), Ürdün (Al-Tamimi, 1997), Suriye (Darvas ve ark., 2000) ve Yunanistan'da (Skuhrava & Skuhravy, 1997; Simoglou ve ark., 2012; Perdikis ve ark., 2015) bulunduğu bildirilmiştir. *Dasineura oleae*'nin Türkiye'deki varlığı değişik kaynaklara atfen Skuhrava ve ark. (2005) tarafından verilmiş ve sadece Hatay Bölgesi zeytinliklerinde Doğanlar ve ark. (2011) tarafından bu türle ilgili bazı çalışmalar yapılmıştır. Şu ana kadar

Türkiye'de bu konuda başka bir çalışma bulunmamaktadır. Son yıllarda yapılan çalışmalarda, Zeytin yaprak siğili'nin özellikle sahil kesimlerinde yüksek popülasyonlar oluşturduğu rapor edilmektedir (Doğanlar ve ark., 2011; Caselli ve ark., 2022). Tondini & Petacchi (2019), İtalya'nın Toscana Bölgesi'nin güney-batısında 2013 yılından beri *D. oleae*'nin salgın yaptığı ve bu salgının büyük oranda *D. oleae* parazitoitlerinin eksikliğinden kaynaklandığı ve bunun diğer zararlılara karşı uygulanan kimyasal mücadele uygulamalarından kaynaklanmış olabileceği bildirilmiştir.

Kuzey Ege Bölgesi'nin önemli zeytin ağacı varlığına sahip Balıkesir ilinin Edremit Körfez Bölgesi'nde bulunan üç ilçesindeki (Burhaniye, Edremit ve Havran) zeytin bahçelerinde bulunan ancak bu güne kadar üzerinde yeterince çalışma bulunmayan *D. oleae*'nin bazı özelliklerini belirlemek amacıyla ele alınan bu çalışmada; 2019–2021 yılları arasında örneklenen bahçelerde ağaçların yaprak ve sürgünlerindeki *D. oleae*'nin bulaşma oranları tespit edilerek, meteorolojik veriler (hava sıcaklığı, nispi nem, yağış) ve rakım arasındaki ilişkiler incelenmiş ve *D. oleae*'nin parazitlenme oranı ile parazitoitleri belirlenmiştir.

MATERYAL ve METOD

Çalışma ilk olarak 2019 yılında Balıkesir ili Edremit Körfezi'nde birbirine sınır ilçeler olan Burhaniye, Edremit ve Havran'ın zeytin bahçelerindeki *D. oleae* ve parazitoitlerinin tespiti için başlatılmış ve üç yıl sürmüştür. Çalışmanın 2020–2021 yıllarında adı geçen üç ilçede, bölgeyi temsil edebilecek özellikte ve farklı yüksekliklerde üçer bahçeden toplam dokuz bahçede *D. oleae*'nin bulaşma ve parazitlenme oranlarının tespiti için çalışılmıştır.

Deneme zeytin bahçelerinin özellikleri: Ayvalık (Edremit) çeşidi zeytin yetiştirilen bahçelerin deniz seviyesine göre yüksekliklerinin ortalamaları alınarak, her ilçedeki üçer bahçe, düşük rakım (69±8.29 m), orta rakım (163±11.59 m) ve yüksek rakım (321±9.21 m) olarak üç gruba ayrılmıştır. Bahçe büyüklükleri sırasıyla Burhaniye'de 26.07, 2.08, 8.65 dekar, Edremit'te 10.88, 8.62, 7.04 dekar ve Havran'da ise 21.80, 1.27, 7.93 dekar şeklindedir. Burhaniye ve Havran'daki deneme bahçelerinde çalışma süresi boyunca zararlılara karşı kimyasal mücadele uygulanmazken, Edremit'teki bahçelerde insektisit uygulamaları yapılmıştır. Edremit'teki üç bahçede 2020–2021 yılları nisan ayında Zeytin pamuklu biti [*Euphyllura* spp. (Hemiptera: Psyllidae)] ve Zeytin güvesi [*Prays oleae* Bern. (Lepidoptera: Hyponomeutidae)] için 125 g l^{-1} Beta-cyfluthrin, haziran ayı ortasında Zeytin sineği [*Bactrocera oleae* (Rossi) (Diptera: Tephritidae)] ve Zeytin güvesi için 25 g l^{-1} Deltamethrin ve temmuz sonunda yine Zeytin sineği için Alphacypermethrin 100 g l^{-1} (EC) uygulanmıştır. Edremit'teki zeytin bahçelerinde zararlılara karşı, bahsi geçen kimyasal mücadele dışında, genelde kültürel önlemlere dikkat edilmiş ve ağaçlara bordo bulamacı uygulanmıştır. Havran ve Burhaniye'deki bahçelerde ise yabancı otun fazla olduğu ve ağaçlara bordo bulamacı uygulanmadığı görülmüştür.

İklim verileri: *Dasineura oleae*'nin yaprak ve sürgünlerdeki bulaşma oranlarının meteorolojik faktörlere göre analizini yapmak için gerekli olan veriler, T.C. Çevre, Şehircilik ve İklim Değişikliği Bakanlığı Meteoroloji Genel Müdürlüğünden sağlanmıştır.

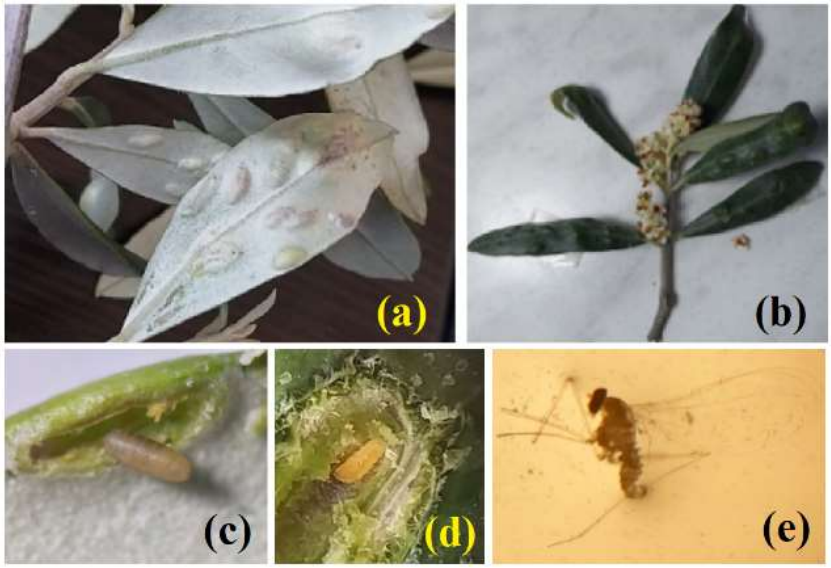
Dasineura oleae Angelini ve parazitoitlerinin elde edilmesi ve tanısı

Dasineura oleae ve parazitoitlerinin teşhisi için kullanılan laboratuvar materyalleri: içi su dolu 50 ml'lik cam kavanozlar (*D. oleae* gal sayımları yapıldıktan sonra içerisine yaprak ve sürgünlerin konulması için), plastik kavanozlar (ters çevirerek cam kavanozların üstünü kapatmak için), plastik kavanozların üzerine geçirilen siyah kılıflar (sistemin ışık almaması için), plastik kavanozlara açılan deliklere yerleştirilen Ø25x150 mm ebatlarında cam tüpler (*D. oleae* erginleri ve parazitoitlerin ışığa yönelme davranışlarından faydalanmak için), içerisinde %96'lik etil alkol bulunan eppendorf tüpleri (elde edilen *D. oleae* erginleri ve parazitoitlerinin muhafazası ve teşhise gönderilmesi için) ve Olympus SZ51 stereo mikroskop (örneklenen yaprak ve sürgünlerdeki galler ile parazitoit çıkışı olan delikli gallerin belirlenmesi için) kullanılmıştır.

Dasineura oleae'nin tanımlanması için; 2019 yılı nisan başı – mayıs sonu arasında 7 gün aralıklarla üç ilçedeki 51 köyde toplam 173 adet zeytin bahçesine rastgele girilerek içlerinde Zeytin gal sineği (*D. oleae*) olduğu düşünülen urlu (şişkin) yaprak ve sürgünler rasgele toplanmıştır (Şekil 1a, b). *Dasineura oleae* erginlerinin ışığa yönelme davranışlarından faydalanarak urlardan çıkması sağlanmış (Şekil 1e) ve taksonomik çalışma için %96 etil alkolde muhafaza edilmiştir. Elde edilen *D. oleae* ve parazitoitlerinin erginleri teşhis için Prof. Dr. Mikdat Doğanlar'a (Biyolojik Mücadele Araştırma Enstitüsü Yüreğir-Adana) gönderilmiştir.

Parazitoitlerin belirlenmesi için; yukarıdaki uygulamaya benzer şekilde 2019–2021 yıllarında üç yıl boyunca nisan başı – mayıs sonu arasında 7 gün aralıklarla üç ilçedeki 54 köyün toplam 530 adet zeytin bahçesine rastgele girilerek, bu kez urlar üzerinde olası parazitoit çıkış deliği bulunan yaprak ve sürgünler incelenmiştir. Parazitoitlerin de ışığa yönelme davranışlarından faydalanarak parazitoit çıkışları sağlanmıştır (Şekil 1c, d, e ve Şekil 2). Çıkışı sağlanan parazitoitler de içinde %96'lik etil alkol bulunan eppendorf tüplerine alınmış ve adı geçen

uzmana teşhis için gönderilmiştir. Teşhis sonuçları yayınlanmıştır (Doğanlar ve ark., 2020). Teşhis edilen türlerin örnekleri, Balıkesir Üniversitesi Edremit Meslek Yüksekokulu (Edremit-Balıkesir) Entomoloji Laboratuvarında saklanmaktadır.



Şekil 1. *Dasineura oleae* Angelini'nin (a) yaprak ve (b) sürgündeki galleri, (c) pupa, (d) larva ve e) ergini (dişi)
Figure 1. (a) galls on leaves and (b) shoots, (c) pupae, (d) larvae and e) adult (female) of Dasineura oleae Angelini

Şekil 2. *Dasineura oleae* Angelini'nin parazitoitlerinin elde edilmesi
Figure 2. Obtaining the parasitoids of Dasineura oleae Angelini

Dasineura oleae Angelini'nin bulaşma oranı ile parazitlenme oranlarının belirlenmesi ve İstatistiksel analizler

Dasineura oleae'nin bölgedeki zeytin bahçelerinde bulaşma ve parazitlenme oranları ile parazitoitlerinin belirlenmesi amacıyla 2020–2021 yıllarında çalışılmıştır. Bu çalışmada nisan ayı başından aralık ayı sonuna kadar 15 gün aralıklarla arazi çıkışları yapılmıştır. Her üç ilçede farklı üç rakımda olmak üzere belirlenen dokuz zeytin bahçesinin her birinde tüm bahçeyi temsil edecek şekilde rastgele beş adet ağaç seçilmiş, her ağaçtan rastgele 20 adet yaprak ve 2 adet sürgün olmak üzere toplam 100 adet yaprak ve 10 adet sürgün alınmıştır. Örnek alınacak sürgünlerdeki yapraklar, yaprak sapından makas ile kesilmiş, 20–25 cm'lik sürgünler ise budama makası ile kesilerek alınmıştır. Yaprak ve sürgünlerin ağaçların güney-kuzey yönlerinden alınmasına özen gösterilmiştir. Alınan yaprak ve sürgünler önce kâğıt torbalara yerleştirildikten sonra naylon poşetler içerisine konularak buz kutusu içerisinde laboratuvara götürülmüştür. Laboratuvarda zararlı ile bulaşık yaprak ve sürgünler üzerinde bulunan *D. oleae* gal sayısı ile parazitoit çıkış deliği bulunan galler mikroskop altında sayılarak kaydedilmiştir.

İki yıl boyunca, 318 yaprak ve 277 adet veri ise sürgün için incelenmiş ve analiz için SPSS 24 istatistik programına girilmiştir. Bu veriler kullanılarak *D. oleae*'nin yaprak ve sürgünlerdeki bulaşma ve parazitlenme oranlarını belirlemede aşağıdaki formüller kullanılmıştır (Tondini & Petacchi, 2019).

$$Yapraklardaki\ bulaşma\ oranı\ (\%) = \left(\frac{gal\ görülen\ yaprak\ sayısı}{toplam\ yaprak\ sayısı}\right) x\ 100 \quad (1)$$

$$Sürgünlerdeki\ bulaşma\ oranı\ (\%) = \left(\frac{gal\ görülen\ sürgün\ sayısı}{toplam\ sürgün\ sayısı}\right) x\ 100 \quad (2)$$

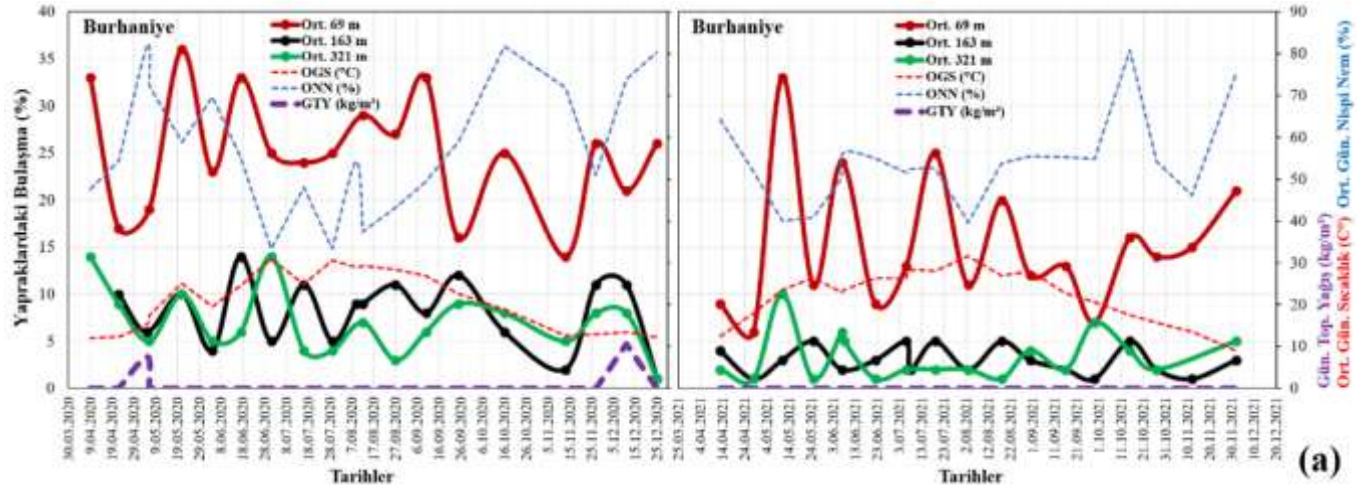
$$Parazitlenme\ oranı\ (\%) = \left(\frac{parazitlenmiş\ gal\ sayısı}{analiz\ edilen\ toplam\ gal\ sayısı}\right) x\ 100 \quad (3)$$

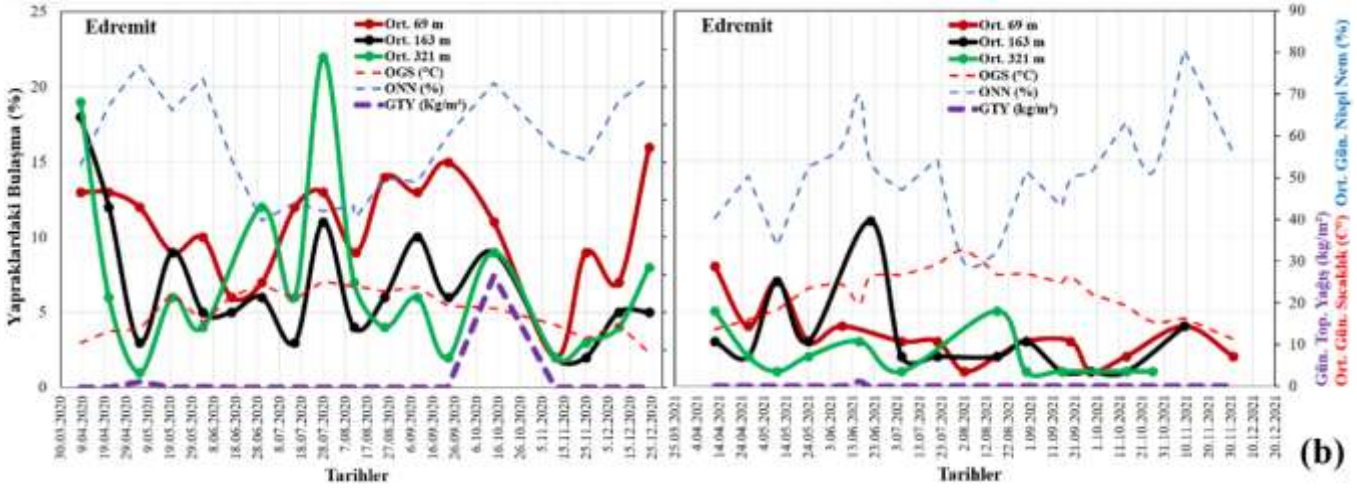
Bütün veriler normallik ve varyansın homojenliği açısından test edilmiştir. Pearson korelasyon analizi, ortalamaların karşılaştırılması için tek yönlü ANOVA ile Tukey HSD çoklu karşılaştırma testleri ve Stepwise metodu ile çoklu regresyon analizleri SPSS programıyla yapılmıştır. Ayrıca aynı programla, yaprak ile sürgünler ve 2020 ile 2021 yılı verilerinin karşılaştırılması için t-testi yapılmıştır. Burhaniye, Edremit, Havran ilçelerinin bağımsız olarak her biri ve üç ilçe birlikte (Edremit Körfezi) olmak üzere *D. oleae*'nin iki yıllık yaprak ve sürgünlere bulaşma oranları için normallik testleri yapılmıştır. Çarpıklık ve basıklık değerleri -2 ile +2 aralığında olduğundan verilerin normal dağıldığı varsayılmıştır (George & Mallery, 2003; George, 2011).

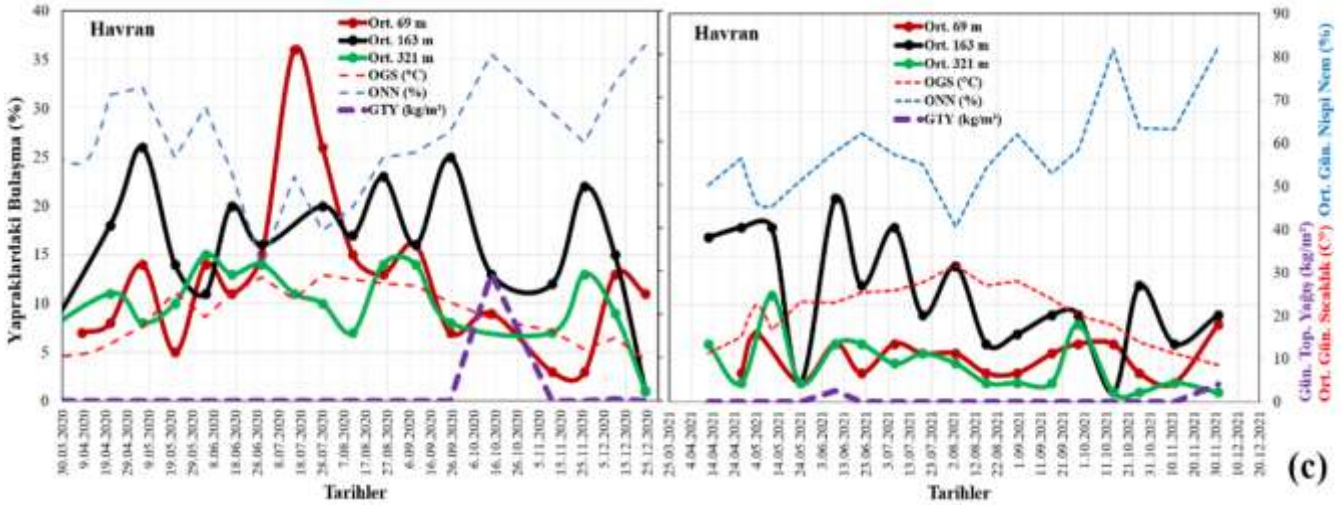
BULGULAR ve TARTIŞMA

Dasineura oleae Angelini'nin yaprak ve sürgünlerdeki bulaşma oranları

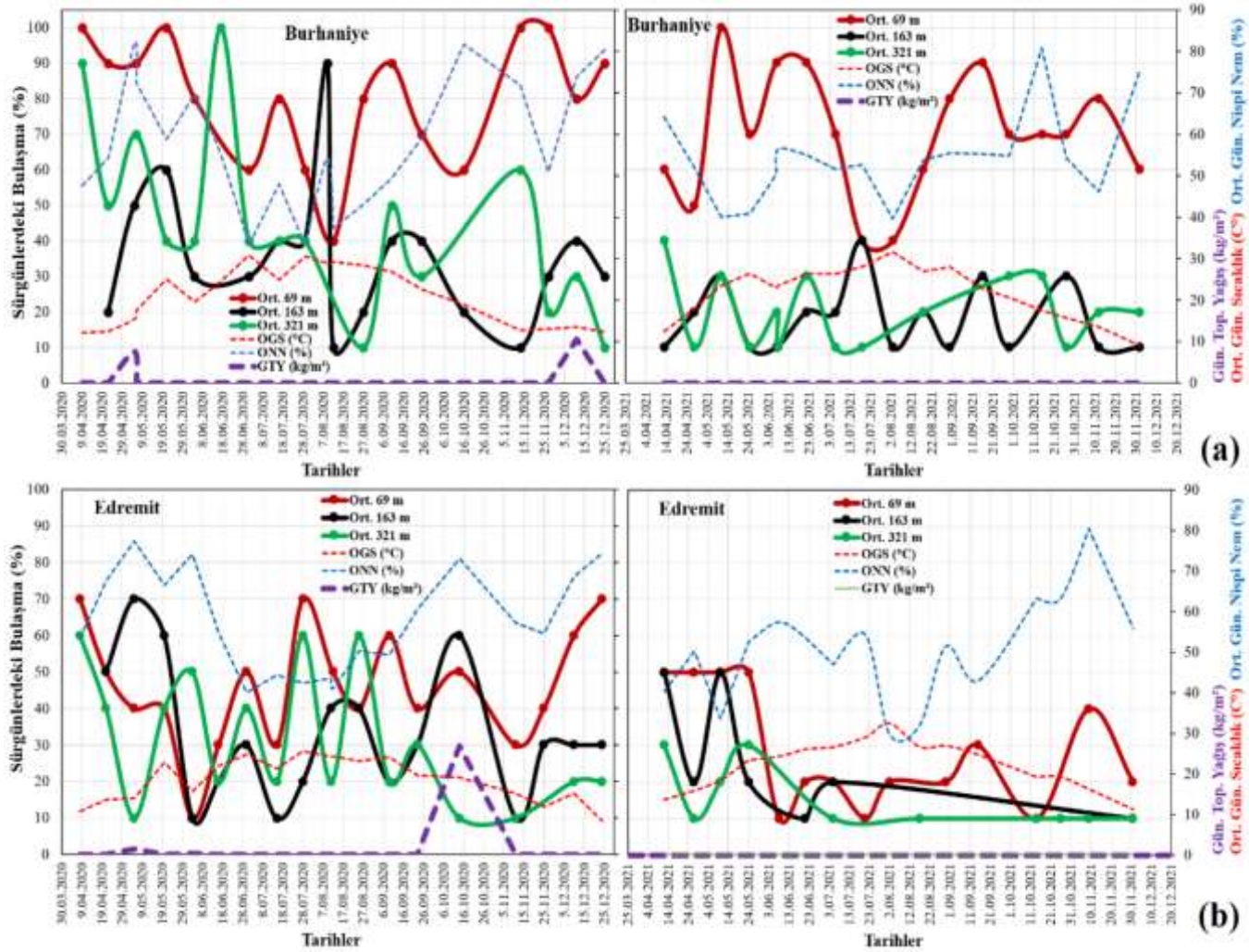
Literatürde bulunan çalışmalarda; genellikle sadece zeytin ağacından rastgele alınan yaprak ve sürgünler esas alınarak üzerlerindeki *D. oleae*'nin bulaşma oranının belirlendiği görülmüştür (Tondini & Petacchi, 2019; Batta & Doğanlar, 2020; Caselli ve ark., 2022; Picchi ve ark., 2022). Bu araştırmada ise dallardan rastgele kesilen genç sürgünlerin üzerinde bulunan yapraklar ile çiçekli ve meyveli dalların yaprakları ayrı ayrı değerlendirilmiştir. Burhaniye, Edremit ve Havran'daki zeytinliklerde *D. oleae*'nin yaprak ve sürgünlerdeki yıllık (nisan–aralık) bulaşma oranları; ortalama günlük sıcaklık (OGS), günlük nispi nem (%ONN), günlük toplam yağış (GTY) ve bahçe rakımı gibi faktörlerle ilişkili olarak Şekil 3 ve Şekil 4'de elde edilmiştir. Elde edilen verilere göre 69 m rakımdaki zeytin bahçelerinde bulaşma oranı daha yüksek, 321 m rakımlarda ise daha düşük olarak saptanmıştır (Şekil 3 ve 4)





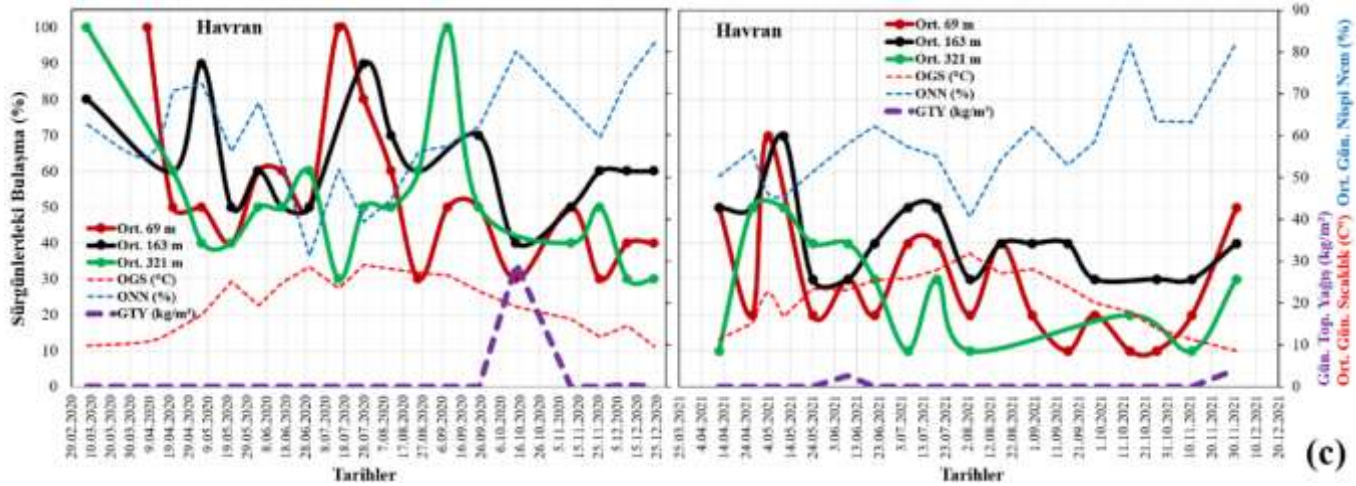


Şekil 3. *Dasineura oleae* Angelini'nin yapraklardaki bulaşma oranları; (a) Burhaniye (b) Edremit (c) Havran
Figure 3. The infestation rates of Dasineura oleae Angelini on leaves; (a) Burhaniye (b) Edremit (c) Havran



Öncelikle Burhaniye, Edremit ve Havran'dan elde edilen iki yılın verileri birlikte analiz edilmiştir. Bulaşma oranlarının bahsedilen faktörler için korelasyon ve çoklu regresyon analizleri yapılmış, sonuçlar sırasıyla Çizelge 1 ve Çizelge 2'de verilmiştir. Bu analiz sonucunda, *D. oleae*'nin yapraklardaki bulaşma oranına, rakım, günlük nispi nem ve sıcaklığın etkisinin önemli olduğu görülmüştür (Çizelge 1 ve 2). Ancak, yapraklardaki bulaşma oranının, yağış miktarı ile doğrudan bir ilişkisinin bulunmadığı belirlenmiştir. *Dasineura oleae*'nin sürgünlerdeki bulaşma oranının rakım ile ilişkili

olduğu görülmüş (Çizelge 1 ve 2), diğer değişkenlerle ise bir ilişkisi saptanmamıştır. Edremit Körfezi'ndeki zeytin bahçelerinde *D. oleae*'nin yaprak ve sürgünlerdeki bulaşma oranlarının bahçe rakımı ve günlük nispi nem miktarına göre ters (–) oranda değiştiği Çizelge 1 ve Şekil 5(a) ve (b)'de görülmektedir. ANOVA sonuçlarına göre (Post-Hoc, Tukey HSD testi), 2020 yılında 163 ve 321 m rakımdaki bahçelerin yapraklarındaki bulaşma oranları (Şekil 5a) arasında anlamlı farklar yoktur. 2021 yılında Burhaniye ve Havran bahçelerinin sürgünlerindeki bulaşma oranları (Şekil 6b) arasında da anlamlı farklar yoktur. Benzer şekilde, Şekil 5 ve 6'daki grafiklerde verilen aynı harfin takip ettiği her bir sütundaki ortalamalar arasında anlamlı bir fark yoktur ($P \geq 0.05$).



Şekil 4. *Dasineura oleae* Angelini'nin sürgünlerdeki bulaşma oranları; (a) Burhaniye (b) Edremit (c) Havran
Figure 4. The infestation rates of Dasineura oleae Angelini on shoots; (a) Burhaniye (b) Edremit (c) Havran

Çizelge 1. Edremit Körfezi'nde (üç ilçede) *Dasineura oleae* Angelini'nin yaprak ve sürgünlerdeki bulaşma oranları ile meteorolojik faktörler ve rakımlar arasındaki korelasyonlar (2020–2021)
Table 1. Correlations among the infestation rates of Dasineura oleae Angelini on leaves and shoots, meteorological factors and altitudes in Edremit Bay (in three districts) (2020–2021)

Zeytin ağacı	Korelasyon Değerleri	Ort. Günlük Sıcaklık (°C) (OGS)	Ort. Günlük Nispi nem (%ONN)	Günlük Top. Yağış (kg m^{-2}) (GTY)	Ort. Bahçe Rakımı (m) (RKM)
Yapraklardaki Bulaşma (%)	r-değeri	0.135*	-0.144**	0.043	-0.361**
	P-değeri	0.016	0.010	0.443	0.001
	N	318	318	318	318
Sürgünlerdeki Bulaşma (%)	r-değeri	-0.047	-0.010	-0.009	-0.298**
	P-değeri	0.434	0.873	0.879	0.001
	N	277	277	277	277

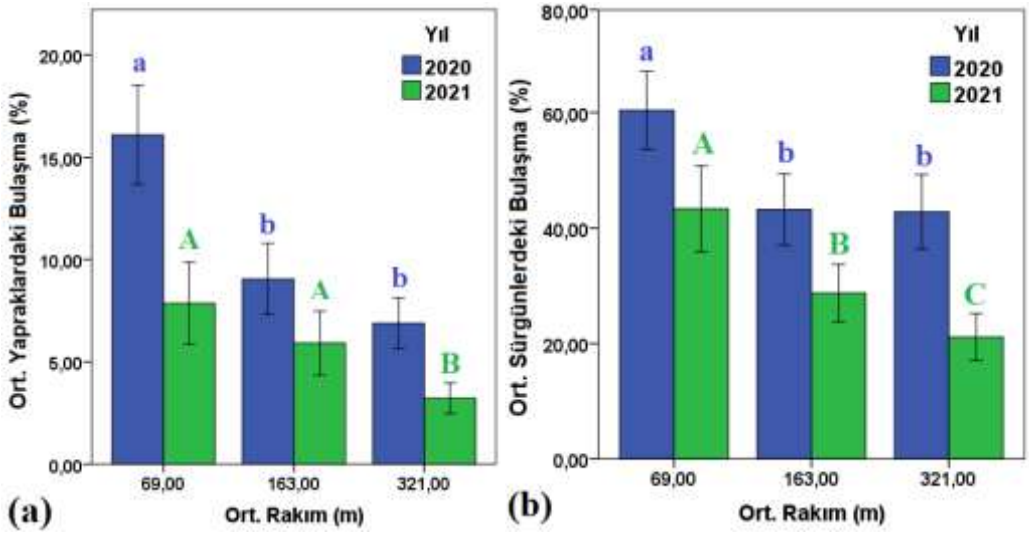
**Pearson korelasyonu 0.01 düzeyinde anlamlıdır (2-yönlü). *Pearson korelasyonu 0.05 düzeyinde anlamlıdır (2-yönlü).

Çizelge 2. Edremit Körfezi'nde (üç ilçede) *Dasineura oleae* Angelini'nin yaprak ve sürgünlerdeki bulaşma oranları ile meteorolojik faktörler ve rakımlar için regresyon sonuçları (2020–2021)
Table 2. Regression results for infestation rates of Dasineura oleae Angelini on leaves and shoots, meteorological factors and altitudes in Edremit Bay (in three districts) (2020–2021)

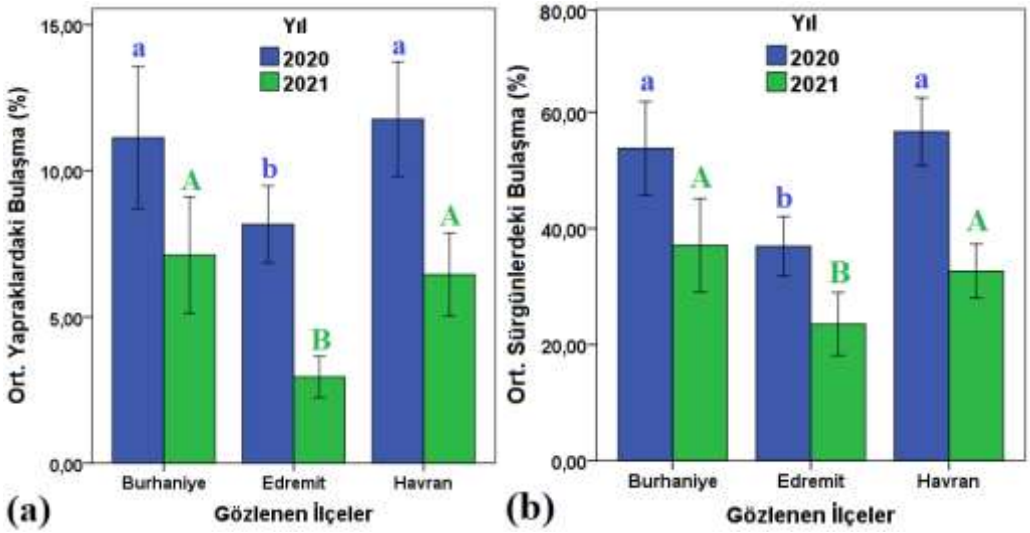
Zeytin ağacı	Regresyon denklemi *	df	F-değeri	*P*-değeri
Yapraklardaki Bulaşma (%)	YBO = 16.840 – 0.025RKM – 0.065ONN	2, 315	26.850	0.0001
Sürgünlerdeki Bulaşma (%)	SBO = 54.003 – 0.070RKM	1, 275	26.806	0.0001

*Elde edilen regresyon denklemleri $P<0.05$ düzeyinde anlamlıdır.

Yapılan t-testine göre sürgünlerdeki (X=%41.44±24.62, N=277) bulaşma ortalamalarının yapraktakine (X=%8.34±7.38, N=318) göre çok daha yüksek (%33.1) olduğu tespit edilmiştir (t[593]=22.82, $P<0.05$) (Şekil 5 ve 6). Bunun, *D. oleae* erginlerinin yumurta bırakmak için daha çok genç, taze sürgün ve yaprakları tercih etmesinden kaynaklandığı düşünülmektedir. Çalışma süresince *D. oleae* popülasyonunun denize yakın, düşük rakımlı zeytin bahçelerinde ve bahçe içerisinde ise kenar sıralarda daha yoğun olduğu gözlenmiştir. Görüldüğü gibi *D. oleae*'nin zeytin ağaçlarına bulaşma oranı öncelikle bahçe rakımı ve bölgenin iklim koşulları ile oldukça ilgilidir. Literatürde de benzer şekilde; Filistin'de



Şekil 5. *Dasineura oleae* Angelini'nin (a) yaprak ve (b) sürgünlerdeki bulaşma oranlarına bahçe rakımının etkisi.
Figure 5. Effect of orchard altitude on infestation rates of Dasineura oleae Angelini on (a) leaves and (b) shoots



Şekil 6. *Dasineura oleae* Angelini'nin (a) yaprak ve (b) sürgünlerdeki bulaşma oranlarının ilçelere göre değişimi.
Figure 6. Variation of infestation rates of Dasineura oleae Angelini on (a) leaves and (b) shoots depending on districts

yapılan çalışmalarda *D. oleae'* nin bulaşma oranının düşük rakımlı bahçelerde, yüksek rakımlılara göre daha yüksek olduğu belirtilmiştir (Batta, 2019; Batta & Doğanlar, 2020). Al-Tamimi (1997) tarafından da *D. oleae* larvalarında ana ölüm nedenlerinin, iklim koşulları (sıcaklık ve bağıl nem) ve özellikle endoparazitoit *P. oleae* gibi doğal düşmanlar dan kaynaklandığı bildirilmiştir. Benzer şekilde, *D. oleae*'nin denize yakın kesimlerde yüksek popülasyonlar oluşturduğu (Doğanlar ve ark., 2011), İtalya'nın bazı bölgelerinde salgın yaptığı ve bu salgının büyük oranda parazitoit azlığından kaynaklandığı bildirilmiştir (Tondini & Petacchi, 2019). Bir başka önemli veri ise Çizelge 3'de görüldüğü gibi iki çalışma yılının ortalamalarına göre; Burhaniye ve Havran'daki zeytinliklerin yaprak ve sürgünlerindeki bulaşma oranları arasındaki fark önemsiz bulunmakla birlikte bunların Edremit'teki bulaşma oranı ile aralarındaki farklar önemli bulunmuştur (P<0.05). Edremit'teki popülasyonun Burhaniye ve Havran'dan %3.30–13.69 daha düşük bulunmasının nedeninin bahçelerin üç farklı zamanda diğer zeytin zararlılarına karşı ilaçlanmış olmasından kaynaklanmış olabileceği kanısına varılmıştır.

Edremit Körfezindeki örnekleme yapılan zeytin bahçelerinde yaprak (%AYBO) ve sürgünlerdeki (%ASBO) aylık olarak tespit edilen ortalama *D. oleae* bulaşma oranlarının; nisan–aralık arasındaki aylara (AYNO), ortalama aylık sıcaklığa (OAS), nispi nem miktarına (%AONN) ve yağış miktarına (AOYA) göre analizleri yapılmıştır (Çizelge 4 ve 5).

Çizelge 4'de görüldüğü gibi yapraklarda (%AYBO) ve sürgünlerdeki aylık ortalama (%ASBO) bulaşma oranları ile aylar (AYNO) ve aylık ortalama yağış

miktarı (AOYA) arasında anlamlı negatif (-) korelasyon saptanmıştır (P<0.05). Benzer şekilde, zeytin bahçelerinin önemli bir zararlı türü olan Zeytin pamuklubiti'nin de popülasyon yoğunluğu ve zararının aylık yağış miktarıyla negatif ilişkili olduğu bildirilmiştir (Kaplan & Alaserhat, 2020b). Yapraklardaki aylık ortalama bulaşma oranının sadece aylık ortalama yağışa bağlı, aylık ortalama sürgünlerdeki bulaşma oranının ise sadece aylara (zamana) bağlı regresyon denklemleri elde edilebilmiştir (Çizelge 5).

Çizelge 3. İlçelerdeki zeytin bahçelerinde *Dasineura oleae* Angelini'nin ortalama bulaşma oranları (2020–2021)
Table 3. Average infestation rates of Dasineura oleae Angelini on olive orchards in the districts (2020–2021)

İlçe	Yapraklardaki bulaşma (%)	Sürgünlerdeki bulaşma(%)
Burhaniye	9.36 ± 8.97a	45.61 ± 29.21a
Edremit	5.95 ± 4.68b	31.92 ± 18.37b
Havran	9.25 ± 6.91a	45.41 ± 22.04a
Körfez Bölgesi ortalaması	8.34 ± 7.38	41.44 ± 24.62

Aynı sütunda gösterilen farklı harfler (a, b) istatistiksel olarak anlamlı farklılıkları göstermektedir (P<0.05)

Çizelge 4. Edremit Körfezinde (üç ilçede) *Dasineura oleae* Angelini'nin yaprak ve sürgünlerdeki aylık bulaşma oranları ile aylık meteorolojik faktörler arasındaki korelasyonlar (2020–2021)
Table 4. Correlations among the monthly infestation rates of Dasineura oleae Angelini on leaves and shoots, and monthly meteorological factors in Edremit Bay (in three districts) (2020–2021)

Zeytin ağacı	Korelasyon Değerleri	Aylara göre (AYNO)	Aylık ort. Sıcaklık (°C) (OAS)	Aylık ort. Nispi Nem (%AONN)	Aylık ort. Yağış (kg m^{-2}) (AOYA)
Yapraklardaki Bulaşma (%)	r-değeri	-0.282*	0.177	-0.136	-0.286*
	P-değeri	0.039	0.199	0.328	0.036
	N	54	54	54	54
Sürgünlerdeki Bulaşma (%)	r-değeri	-0.322*	-0.029	-0.070	-0.309*
	P-değeri	0.018	0.834	0.614	0.023
	N	54	54	54	54

*Pearson korelasyonu 0.05 düzeyinde anlamlıdır (2-yönlü)

Çizelge 5. Edremit Körfezinde (üç ilçede) *Dasineura oleae* Angelini'nin yaprak ve sürgünlerdeki aylık bulaşma oranları ile aylık meteorolojik faktörler için çoklu regresyon sonuçları (2020–2021)
Table 5. Regression results for the monthly infestation rates of Dasineura oleae Angelini on leaves and shoots, and monthly meteorological factors in Edremit Bay (in three districts) (2020–2021)

Zeytin ağacı	Regresyon denklemi *	df	F-değeri	P-değeri
Aylık Ort. Yapraklardaki Bulaşma (%)	AYBO = 9.507 – 0.899AOYA	1, 52	4.645	0.0360
Aylık Ort. Sürgünlerdeki Bulaşma (%)	ASBO = 53.994 – 1.838AYNO	1, 52	5.997	0.0180

*Elde edilen regresyon denklemleri P<0.05 düzeyinde anlamlıdır.

Şekil 7(a) ve (b)'de görüldüğü gibi 2021 yılında bulaşma oranları 2020 yılına göre oldukça düşük bulunmuştur. Bu durum meteorolojik faktörler açısından değerlendirildiğinde, çalışmanın yapıldığı nisan–aralık ayları arasında 2021 (21.25±6.26 °C) yılı günlük sıcaklık ortalaması 2020'ye (20.11±6.60 °C) göre 1.15 °C daha yüksek, günlük nem ortalamaları ise 2021 (%54.51±11.33) yılında 2020'ye (%61.69±11.33) göre %7.18 düşük olmuştur. Günlük yağış ortalamaları ise 2021 (1.68±5.91 kg m^{-2}) yılında 2020'ye (0.82±3.30 kg m^{-2}) göre 0.86 kg m^{-2} daha yüksek gerçekleşmiştir. Sonuç olarak, 2021 yılında 2020'ye göre günlük sıcaklık ve yağış değerlerinin daha yüksek, nispi nem değerlerinin ise düşük olması, *D. oleae* varlığını olumsuz yönde etkilemiştir. Şekil 7(a) ve (b)'de görüldüğü gibi özellikle ağustos–aralık aralığında bulaşmanın düşme eğiliminde olduğu belirlenmiştir. Nisan–temmuz arasında ise bulaşmanın en yüksek seviyede olduğu görülmektedir. Örneğin; yapraklarda en yüksek bulaşma Burhaniye'de 21.05.2020'de (%36), Edremit'te 28.07.2020'de (%22) ve Havran'da ise 15.07.2020'de (%36) tespit edilmiştir. Sürgünlerde en yüksek bulaşma Burhaniye'de 9.04.2020'de (%100), Edremit'te 8.04.2020'de (%70) ve Havran'da ise 8.04.2020'de (%100) tespit edilmiştir.

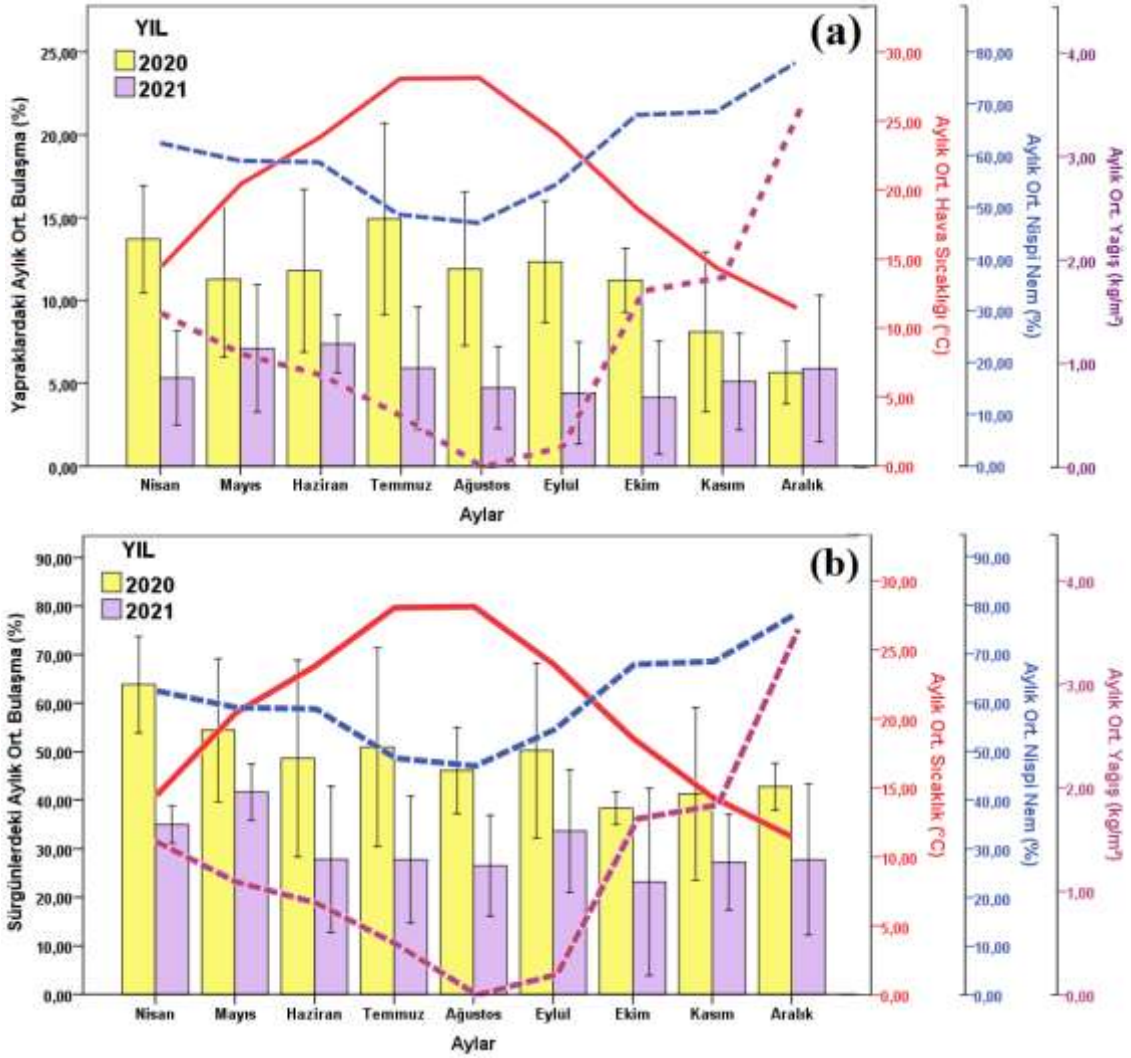
Dasineura oleae Angelini'nin parazitlenme oranı

Her iki çalışma yılına ait (2020–2021) yaprak ve sürgün gallerindeki parazitlenme oranları Çizelge 6'da verilmiştir.

Şekil 8(a) ve (b)'de görüldüğü gibi parazitlenme oranı açısından ilçeler arasındaki farklar önemli bulunmuştur (P<0.05). Ancak, ANOVA (ortalamaların karşılaştırılması), korelasyon ve regresyon

analizlerine göre; hava sıcaklığı, nispi nem, yağış miktarı ve ortalama bahçe rakımı gibi değişkenler ile yaprak ve sürgünlerdeki parazitlenme oranları arasındaki farklar önemli bulunmamıştır (Şekil 9). ANOVA sonuçlarına göre (Post-Hoc, Tukey HSD testi), Şekil 8 ve 9'da a, b veya A, B gibi farklı harflere sahip grafikteki değerler arasında önemli ölçüde anlamlı farklar belirlenmiştir (P<0.05).

Çalışma yıllarına göre yaprak ve sürgünlerdeki bulaşma ile parazitlenme oranları arasındaki farkları ortaya koymak amacıyla, verilere t-testi uygulanmıştır. Elde edilen sonuçlar Çizelge 7'de verilmiştir. Sonuçta 2020 yılında *D. oleae* ile yaprak ve sürgünlerdeki bulaşma oranları 2021 yılına göre anlamlı olarak %4.71–16.93 daha yüksek bulunmuştur. Ancak yaprak ve sürgünlerdeki parazitlenme oranlarının analizi sonucunda, 2020 ve 2021 yılları arasında istatistiksel olarak anlamlı bir fark görülmemiştir (Çizelge 7).



Şekil 7. *Dasineura oleae* Angelini'nin (a) yaprak ve (b) sürgünlerdeki aylık bulaşma oranlarının meteorolojik faktörlere göre değişimi.

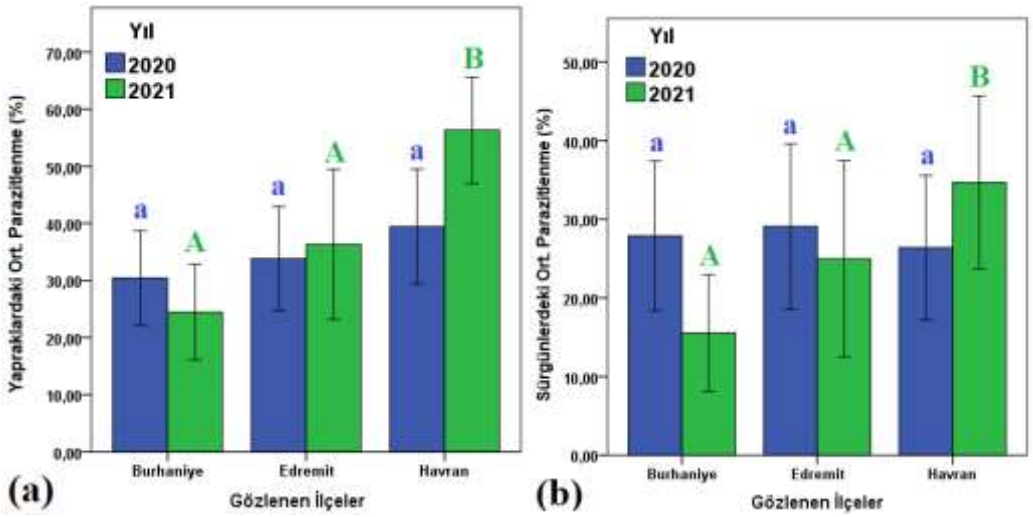
Figure 7. Variation of monthly infestation rates of Dasineura oleae Angelini on leaves and shoots depending on the meteorological factors.

Çizelge 6. *Dasineura oleae* Angelini'nin ilçelerde ortalama parazitlenme oranları (2020–2021)

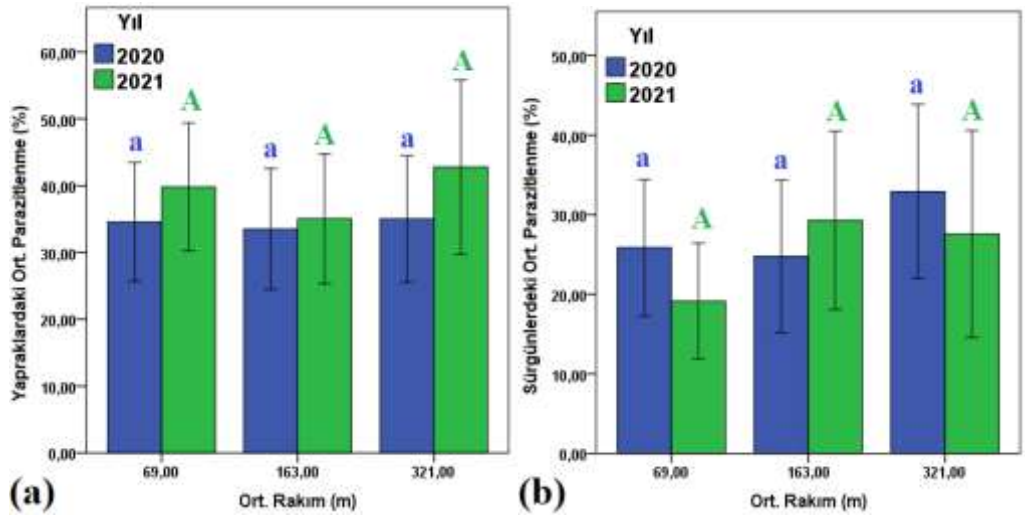
Table 6. Average parasitization rates of Dasineura oleae Angelini in districts (2020–2021)

İlçe	Yapraklardaki parazitlenme (%)	Sürgünlerdeki parazitlenme (%)
Burhaniye	27.79 ± 2.96a	21.84 ± 3.07a
Edremit	34.86 ± 3.81a	27.55 ± 4.01a
Havran	47.39 ± 3.53b	30.28 ± 3.55a
Körfez Bölgesi ortalaması	36.49 ± 2.01	26.48 ± 2.04

Aynı sütunda gösterilen farklı harfler (a, b) istatistiksel olarak anlamlı farklılıkları göstermektedir (P<0.05)



Şekil 8. *Dasineura oleae* Angelini'nin parazitlenme oranlarının ilçelere göre değişimi.
Figure 8. The variation of the parasitization rates of Dasineura oleae Angelini depending on the districts.



Şekil 9. *Dasineura oleae* Angelini'nin parazitlenme oranlarının bahçe rakımlarına göre değişimi.
Figure 9. Variation of parasitization rates of Dasineura oleae Angelini depending on the orchard altitudes

Dasineura oleae Angelini'nin parazitoitleri

Çalışma sonucunda, Balıkesir ilinin Edremit Körfez Bölgesi'ndeki Burhaniye, Edremit ve Havran ve ilçelerinde *D. oleae*'yi parazitleyen Hymeoptera takımının, 4 familyasına ait 10 tür belirlenmiştir. Eulophidae familyasına bağlı 7 türden, *Zeytinus* cinsine ait 3 tür; *Z. balikesirensis* Doğanlar ve Sakin, *Z. edremitensis* Doğanlar ve Sakin ve *Z. marmarae* Doğanlar ve Sakin türleri bilim dünyası için ilk kez tespit edilmiş ve 2020 yılında tanımlanmıştır (Doğanlar ve ark., 2020). Platygastridae, Pteromalidae ve Torymidae familyalarından ise sadece birer tür elde edilmiştir (Çizelge 8).

Dasineura oleae'nin doğal düşmanları konusunda Türkiye'de sadece Hatay'da çalışma yapılmış olup, bu çalışmada zeytin alanlarında iki farklı gal sineği, *D. oleae* ve *Lasioptera oleicola* Skuhravá, 2011 belirlenmiştir. Bölgede her iki gal sineğinden Hymenoptera takımından 5 familyaya bağlı 12 tür larva veya pupa parazitoiti olarak tespit edilmiştir (Doğanlar, 2011; Doğanlar ve ark., 2011).

Bu türlerden; *Platygaster oleae*, *Torymus phillyreae* ve *Mesopolobus mediterraneus* bu çalışmada da elde edilmiştir (Çizelge 8). Ayrıca yine aynı bölgede *D. oleae* gallerinden yeni bir parazitoit tür, *Quadrastichus dasineurae* n. sp. (Hymenoptera: Eulophidae) ilk kez tanılanmıştır (Doğanlar ve ark., 2009). *Dasineura oleae*'nin parazitoitleri ile ilgili Orta Doğu'da yapılan çalışmalarda; Filistin'deki zeytinliklerde *D. oleae*'nin iki yerli parazitoiti; *P. oleae* ve *Z. hatayensis* (Batta & Doğanlar, 2020); Suriye'nin Sahil Bölgesi'nde ektoparazitoit *Eupelmus urozonus* Dalm (Hymenoptera: Eupelmidae) ve *Z. Hatayensis* ile endoparazitoit *Platygaster demades* Walker, 1835 (Hymenoptera: Platygastridae)'in varlığı bildirilmiştir (Ramadhane ve ark., 2017). Ürdün'de ise *P. oleae* ve *Aprostocetus* sp. ile birlikte tanısı yapılamamış iki parazitoit türünün daha *D. oleae*'yi parazitlediği

belirlenmiştir (Al-Tamimi, 1997). İtalya'da yapılan çalışmalarda ise *D. oleae*'yi parazitleyen *P. demades*, *P. oleae*, *M. mediterraneus* ve *M. aspilus* (Walker 1835) belirlenmiştir (Tondini & Petacchi, 2019; Magagnoli ve ark., 2022; Picchi ve ark., 2022). Çizelge 9'da görüldüğü gibi çalışmada 629 adet parazitoit incelenmiştir. *Platygaster oleae*, *D. oleae* gallerinden en fazla elde edilen tür olmuş (%30.21), en az elde edilen tür ise *T. phillyreae* (%1.27) bulunmuştur. Ayrıca *Zeytinus* cinsine dâhil olan türler, *Aprostocetus* türlerine göre daha az tespit edilmiştir.

Çizelge 7. *Dasineura oleae* Angelini bulaşma ve parazitlenme oranlarının yıllara göre değişimi (t-testi)
Table 7. The variation of infestation and parasitization rates of Dasineura oleae Angelini depending on the study years (t-test results)

Değişken	Yıl	N	X	SS	T-testi		
					t	df	*P*-değeri
Yapraklardaki Bulaşma (%)	2020	176	10.44	7.92	6.17	311.8	0.0001
	2021	142	5.73	5.67			
Sürgünlerdeki Bulaşma (%)	2020	153	49.02	24.40	6.04	275.0	0.0001
	2021	124	32.09	21.57			
Yapraklardaki Parazitlenme (%)	2020	176	34.36	34.98	-1.18	316.0	0.2390
	2021	142	39.13	36.93			
Sürgünlerdeki Parazitlenme (%)	2020	153	27.80	34.67	0.72	275.0	0.4710
	2021	124	24.84	33.20			

N: Veri sayısını, X: ortalama değeri, SS: standart sapmayı, df: serbestlik derecesini, *P*: anlamlılık seviyesini gösterir. t: verilerin varyasyona göre farklarının boyutu hakkında bilgi verir (1.96'dan yüksek veya -1.96'dan düşük olması testin anlamlı olduğu anlamına gelir).

Çizelge 8. *Dasineura oleae* Angelini'nin Edremit Körfezi'nde (üç ilçede) belirlenen parazitoit türleri
Table 8. The identified parasitoid species of Dasineura oleae Angelini in Edremit Bay (in three districts)

Takım	Familya	Tür
Hymenoptera	Eulophidae	*Aprostocetus arenarius* (Erdös, 1954)
		Aprostocetus flavifrons (Walker, 1849)
		Aprostocetus humilis (Graham, 1961)
		Aprostocetus ligus (Walker, 1839)
		Zeytinus balikesirensis (Doğanlar ve Sakin, 2020)
		Zeytinus edremitensis (Doğanlar ve Sakin, 2020)
		Zeytinus marmarae (Doğanlar ve Sakin, 2020)
	Platygastridae	*Platygaster oleae* (Szelenyi, 1940)
	Pteromalidae	*Mesopolobus mediterraneus* (Mayr, 1903)
	Torymidae	*Torymus phillyreae* (Ruschka, 1921)

Çizelge 9. *Dasineura oleae* Angelini'nin Edremit Körfezinde (üç ilçede) belirlenen parazitoit tür ve oranları
Table 9. Determined parasitoid species and rates of Dasineura oleae Angelini in Edremit Bay (in three districts)

Tür	Birey sayısı (adet)	Bulunma oranı (%)
A. arenarius	39	6.20
A. flavifrons	60	9.54
A. humilis	63	10.02
A. ligus	77	12.24
Z. balikesirensis	39	6.20
Z. edremitensis	41	6.52
Z. marmarae	37	5.88
P. oleae	190	30.21
M. mediterraneus	75	11.92
T. phillyreae	8	1.27
Toplam	629	100.00

Hatay ili zeytinliklerinde *D. oleae* gallerinden birçok parazitoit tür elde edilmesine karşın; *E. urozonus*, *P. oleae* ve *Q. dasineurae* en fazla belirlenen türler olmuş ve bunların diğer parazitoitlerle birlikte gal popülasyonunu ciddi oranda azalttıkları bildirilmiştir (Doğanlar ve ark., 2009). Filistin'deki çalışmada ise *P. oleae*'nin, *D. oleae*'nin en etkili parazitoiti olduğu, her rakımda olmak üzere bölgedeki zeytinliklerin hepsinde bulunduğu, parazitleme oranının %82.7'ye kadar çıktığı belirtilmiştir (Batta & Doğanlar, 2020).

İtalya'da *D. oleae*'nin Pteromalidae ve Platygastridae familyalarına ait ikişer tür parazitoiti bulunmasına rağmen, yalnızca *P. oleae*'nin, gal sineğinin spesifik parazitoiti olduğu, diğerlerinin tüm Cecidomyiidae familyası türlerini parazitleyebildiği bildirilmiştir (Picchi ve ark., 2022).

SONUÇ ve ÖNERİLER

Dasineura oleae, Türkiye genelinde olduğu gibi Edremit Bölgesi'nde de her zaman parazitoitleri tarafından baskı altında tutulmuş, şu ana kadar zararlı konuma ulaşmamış ve bu nedenle pek dikkate alınmamıştır. Ancak son yıllarda özellikle Kuzey Ege Bölgesi'nin sahil kesimlerinde bulunan zeytinliklerde, *D. oleae*'nin yaprak deformasyonlarına neden olduğu ve sürgün uçlarını kıvırarak şekil bozuklarına yol açtığı gözlenmiş ve bazı üreticilerin kimyasal mücadele uyguladıkları bilgisine ulaşılmıştır. Bu çalışmada, denize yakın, fazla rüzgâr almayan bahçelerde *D. oleae* popülasyonun daha yüksek olduğu, sahilden uzaklaştıkça iç kesimdeki bahçelerde popülasyonun oldukça düştüğü görülmüş, hatta bazı bahçelerde hiç *D. oleae*'ye rastlanmamıştır. Bu çalışmada *D. oleae*'nin bulaşma oranının, örneklenen zeytinliklerin deniz seviyesine olan yüksekliğine bağlı olarak değiştiği tespit edilmiştir. Çünkü bulaşma oranlarının orta ve yüksek rakımdaki zeytinliklerdekilere (163 m ve 321 m) göre düşük rakımlardaki (69 m rakım) zeytinliklerde daha yüksek olduğu bulunmuştur. Bu ilişkinin, düşük rakımlarda bulunan zeytin bahçelerindeki sıcaklık ve nispi nemin *D. oleae* gelişimi için daha uygun olmasından kaynaklanabileceği sonucuna varılabilir. Ayrıca *D. oleae'nin* yüksek rakımlarda bulunan zeytin bahçelerinde yılda bir nesil üretirken düşük rakımdakilerde birden fazla nesil üretebildiği düşünülmektedir. Bu çalışmada, Edremit Körfezi'ndeki zeytin bahçelerinde *D. oleae*'nin birçok parazitoit türü tarafından parazitlenmesi, parazitlenmenin yapraklarda %47.39'lara kadar çıkması *D. oleae*'nin yaprak başına bulaşma oranının oldukça düşük (2020'de %10.44, 2021'de %5.73) olarak gözlenmesi ümit var bir durum olarak görülmektedir. Ancak taze sürgünlerde parazitlenmenin %21.84'de kalması ve *D. oleae*'nin sürgün başına bulaşma oranlarının 2020'de %49.02 ve 2021'de %32.09 olarak tespit edilmesi ciddiye alınması gereken bir durumdur. Bariz bir şekilde sürgünlerdeki genç yapraklarda *D. oleae*'nin bulaşma oranı diğer yapraklara göre daha fazladır. Bunun nedeni ise *D. oleae* yumurtalarını yalnızca yeni gelişmiş taze yapraklar üzerine bırakması olabilir. Akdeniz Bölgesi'ndeki zeytinliklerde olduğu gibi şuan itibariyle *D. oleae*, Balıkesir ili Edremit Körfezi'ndeki zeytin ağaçlarında da halen ekonomik bir zararlı konumunda değildir. Şimdilik, bu zararlıya karşı biyolojik mücadele çalışmalarının desteklenmesi dışında ilaveten bir mücadelenin yapılması gereksiz gibi görülmektedir. Çünkü diğer ekonomik düzeydeki zeytin zararlarına karşı Edremit'teki zeytinliklerde hâlihazırda yapılan kimyasal mücadele uygulamaları sonucunda, *D. oleae*'nin bulaşma oranı Burhaniye ve Havran'a kıyasla daha az iken, parazitlenme açısından da diğer iki ilçeye oranla önemli bir değişim görülmemiştir (Şekil 5 ve 7, Çizelge 6). Yani *D. oleae*'nin bölgedeki zeytinliklere bulaşma oranının birçok faktöre bağlı olduğu ispatlanmasına rağmen, zeytinde bulunan ekonomik zararlılara karşı yapılan kimyasal mücadelenin de *D. oleae*'nin gal oluşumunu da azaltarak etkilediği düşünülmektedir. Bunun için bölgede *D. oleae* ile ilgili daha kapsamlı çalışmaların yapılması gerekliliği ortaya çıkmaktadır.

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Araştırmacıların Katkı Oranı Beyan Özeti

Makale yazarının bu çalışmaya katkısının %100 olduğunu beyan ederim.

Çıkar Çatışması Beyanı

Makale yazarı olarak herhangi bir çıkar çatışması olmadığını beyan ederim.

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Detection of Root-Knot Nematode Species and Races in Kahramanmaraş Province, Türkiye

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ABSTRACT
Root-knot nematodes (*Meloidogyne* spp.) are organisms that spread over large areas and cause economic damage to vegetables. In this study, root-knot nematode populations obtained from vegetable growing areas of Kahramanmaraş province were identified. Overall, 132 root samples were taken from the vegetable crop fields. Root-knot nematode was detected in 25 of the collected samples and their diagnosis was determined based on biochemical (esterase isoenzyme phenotype), perineal pattern, and molecular methods. The race determination of root-knot nematodes was made according to the North Carolina Differential Host Test. Results showed that while *Meloidogyne incognita* was detected in Andırın, Onikişubat, Dulkadiroğlu, Türkoğlu, and Ekinözü districts of Kahramanmaraş, both *M. incognita* and *M. javanica* were found in Beyoğlu of Türkoğlu. This is the first report of *M. javanica* infection in Kahramanmaraş. Races of root-knot nematodes were determined as *M. incognita* race 1, race 2 and *M. javanica* race 2.

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Kahramanmaraş İlindeki Kök-Ur Nematodu Tür ve Irklarının Belirlenmesi, Türkiye

ÖZET
Bitki paraziti kök-ur nematodları (*Meloidogyne* spp.) geniş alanlara yayılan ve sebzelerde ekonomik zararlara neden olan canlılardır. Bu çalışmada, Kahramanmaraş ilindeki sebze yetiştirme alanlarından elde edilen kök-ur nematodlarının teşhisi yapılmıştır. Genel olarak, sebze alanlarından 132 adet kök örneği alınmıştır. Toplanan örneklerden 25 adetinde kök-ur nematodu tespit edilmiş ve teşhisleri biyokimyasal (esteraz izoenzim fenotipi), perineal kesit ve moleküler yöntemlere göre belirlenmiştir. Kök-ur nematodlarının ırk tespiti Kuzey Karolina Konukçu Testi'ne göre yapılmıştır. Çalışma sonunda, Kahramanmaraş'ın Andırın, Onikişubat, Dulkadiroğlu, Türkoğlu, Ekinözü ilçelerinde *Meloidogyne incognita*, Türkoğlu Beyoğlu'nda ise hem *M. incognita* hem de *M. javanica* tespit edilmiştir. Kahramanmaraş ilinde *M. javanica* ilk kez bulunmuştur. Kök-ur nematodlarının ırkları *M. incognita* ırk 1, ırk 2 ve *M. javanica* ırk 2 olarak belirlenmiştir.

Bitki Koruma

Araştırma Makalesi

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Geliş Tarihi : 13.05.2023
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Anahtar Kelimeler
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Teşhis,
Meloidogyne spp.,
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Irk

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INTRODUCTION
Among several biotic stresses, root-knot nematodes (RKN) are a major threat to vegetable production. Tomato, pepper and eggplant are some of their important host plants. Eggplant and tomato are known to be extremely susceptible to root-knot nematodes infection, causing severe damage that leads to great losses (Gowda et al. 2019; Tapia-Vázquez et al. 2022; Shaaban et al. 2023). Generally, root-knot nematodes are easily spread through vegetable areas (Collange et al. 2011; Rao et al. 2015). Second-stage juveniles (J2) cause the main damage to root cells of plants. Continuously sucking plant tissue through their styles gives rise to plants nutrition and element deficits.

Thus, it causes adverse effects on plant growth. RKN infections characteristically lead to the formation of galls on roots.

Plants that are infected by RKN lead to crop reduction, stunted growth, yellowing, and wilting, and become more sensitive towards bacteria and fungi in plants (Wang et al. 2013). The abundance of these organisms can affect the survival of the plants. Thus when they are present in high amounts of plant roots, they may dry and eventually the plant can die (Thorne, 1961).

There are some ways of controlling root-knot nematodes, including soil solarization, crop rotation, chemical control, plant resistance, and using other plant extracts or essential oils as nematicides (Young, 1992; Roberts, 1992; Sijmons et al.1994; Tzortzakakis et al. 1999; Tytgat et al. 2000). In order to efficiently control the root-knot nematodes, diagnosis of species and detection of race methods have great importance. RKN species are mostly identified based on morphologic characters (female perineal patterns) (Eisenback & Triantaphyllou, 1991), isozyme phenotypes (Esbenshade & Triantaphyllou, 1990) and molecular techniques (Powers & Harris, 1993; Powers et al. 1997; Zijlstra et al. 1995; Zijlstra et al. 2000; Adam et al. 2007).

Biochemical and molecular techniques currently improve the accuracy of RKN species identification (Resquin-Romero et al. 2023). Gerič Stare et al. (2018, 2019) reported that the best way to distinguish root-knot nematode is to use the esterase enzyme phenotype. Esterase phenotyping has been proven to be species-specific in many cases for *Meloidogyne* species (Carneiro et al. 2001; 2008). Nevertheless, using this technique is limited to the stage of adult females, but females are not always suitable for analysis due to the state of root decomposition (Salgado et al. 2015). Specific sequence characterized amplified region (SCAR) markers have been successfully developed to diagnose the dominant tropical RKNs associated with important crops such as tomato, coffee, guava, and grapevine; these nematodes include *Meloidogyne javanica*, *M. arenaria* (Zijlstra et al. 2000), *M. incognita* (Randig et al. 2002), *M. paranaensis*, *M. exigua* (Randing et al. 2002), *M. enterolobii* (Tigano et al. 2010), *M. arabicida*, *M. izalcoensis* (Correa et al. 2013) and *M. ethiopica* (Correa et al. 2014). Because of providing a faster diagnosis possibility, molecular techniques are used in the identification of root-knot nematodes. Proper and precise analysis of plant parasitic nematodes is critical for crop protection (Devran & Söğüt, 2011; Adzitey et al. 2013).

This study aimed to diagnose root-knot nematode species and races collected from vegetable growing areas of Kahramanmaraş using perineal patterns, biochemical (esterase isozyme phenotypes), and molecular techniques.

MATERIAL and METHOD

Collection of galled root samples

Surveys were conducted in August and September of 2014 and 2015 in 11 districts (Afşin, Andırın, Çağlayancerit, Dulkadiroglu, Elbistan, Ekinözü, Göksun, Nurhak, Onikisubat, Pazarcık and Türkoğlu) of Kahramanmaraş province. Root-knot nematode was found in only 5 districts (Andırın, Dulkadiroğlu, Ekinözü, Onikişubat and Türkoğlu) of the 11 districts surveyed. Overall 132 roots of eggplant (*Solanum melongena*), tomato (*Lycopersicon esculentum*) and pepper (*Capsicum annuum*) were checked and the 25 samples having galls were collected, placed into polyethylene bags labelled with necessary information including coordinates and, stored at refrigerator at +4 °C until use.

Pure cultures of nematodes

In order to make an accurate diagnosis pure cultures are needed. Egg masses of 25 populations were picked up from roots with the help of a needle for pure culture. Tomato seedlings (Falcon) were transplanted into 250-mL plastic pots filled with sterilized sandy loam soil and sand (ratio of 2:1) at the fourth true leaf stage and inoculated with a single egg mass of each population. Female and J2 were obtained 65 days after inoculation.

Species identification

Identification of nematodes was done with biochemical (esterase isozyme phenotypes), perineal pattern, and molecular methods.

Identification of species with the biochemical method

The females obtained pure cultures were crushed and homogenized individually in 5 µl of extraction buffer and 5 µl deionized water in an eppendorf tube. Electrophoresis process was carried out in a discontinuous buffer system with 8% acrylamide running gel, pH 8.8, and 4% acrylamide stacking gel, pH 6.8 in a Bio-Rad mini-PROTEIN II (Bio-Rad, Singapore).

Then, the homogenized sample was loaded carefully into the well in the stacking gel. The first and last wells were loaded with sample of *Meloidogyne javanica* as a reference. The voltage was maintained at 80 volts for the first 13 minutes and increased to 200 volts for the remaining 45 minutes of running period. Following electrophoresis, the gels were removed and put into a staining solution for 45 minutes in the dark. After this period samples were washed with deionized water and esterase phenotype bands were compared to diagnosis according to Esbenshade & Triantaphyllou (1985).

Morphological characterization

Roots were washed with tap water, and cleared from residues and each female separated with help of a

scalpel. Adult females were extracted from each root system under a light microscope (EUROMEX PB 416) and were placed into %45 lactic acid for 20-25 min. Perineal patterns of females were cut and mounted in a glycerol. The perineal pattern method was completed as described by Hunt & Handoo (2009).

Molecular characterization

DNA extraction: DNA was extracted from egg masses of each nematode population using DNeasy Tissue Blood Kit (QIAGEN, Hilden, Germany) according to instructions of the manufacturer. DNA samples were kept at -20 ºC until used.

PCR conditions: DNA amplifications were conducted with species-specific primers listed in Table 1. PCR amplifications were performed in a DNA thermal cycler with the following thermal cycling program: initial cycle, 2 min at 95°C; next 40 cycles, 1 min at 94°C; 1 min at 64°C, and 1.5 min at 72 °C for primers Fjav/Rjav and 2 min at 95 °C; next 40 cycles, 1 min at 94 °C; 1 min at 54 °C, and 1.5 min at 72 °C for primers Finc/Rinc and 2 min at 95 °C; next 40 cycles, 1 min at 94 °C; 1 min at 64 °C, and 1.5 min at 72 °C for primers Inc-K14-F/Inc-K14-R. PCR amplification reactions were performed in a total volume of 40 µL containing 4 µl of 10x PCR Buffer, 1 µl of 250 µM deoxynucleoside triphosphates (dNTPs) (Favorgen Biotech Corp., Taiwan), 1µl 20 picomoles of each primer, 2 mM MgCI2, 20 ng of template DNA and 1 Unit Taq DNA Polymerase (Vivantis). PCR was run using a Techne PHC-3 (Techne, Cambridge, UK) thermal cycler. Amplified PCR products were run on 1.5% electrophoresis gel (Bio-Rad, Hercules, CA) and analyzed under UV light.

Table 1. Primers used for molecular identification of Meloidogyne incognita and M. javanica
Çizelge 1. Meloidogyne incognita ve M. javanica'nın moleküler tespiti için kullanılan primerler

Name of primer	*Species*	*Primer sequences (5'-3')*	*DNA Fragment (bp)*	*References*
Finc	*M. incognita*	CTCTGCCCAATGAGCTGTCC	1200	Zijlstra et al. (2000)
Rinc		CTCTGCCCTCACATTAAG		
İnc-K14F	*M. incognita*	CCCGCTACACCCTCAACTTC	399	Randig et al. (2002)
İnc-K14R		GGGATGTGTAAATGCTCCTG		
Fjav	*M. javanica*	GGTGCGCGATTGAACTGAGC	670	Zijlstra et al. (2000)
Rjav		CAGGCCCTTCAGTGGAACTATAC		

Race determination

The North Carolina Differential Host Test was conducted in the growth chambers. Experiment conditions were 25±1 ºC and %60±10 humidity with 16 h light. Four-leaf stage seedlings of tobacco (NC 95), cotton (Delta Pine 16), tomato (Rutgers), peanut (Florunner) and pepper (California wonder) plants were transplanted into 250 ml plastic pots filled with sterilized sandy soil (%80 sand, %20 peat). The trial was designed as a randomized plots design, with four replications. RKN egg masses were obtained from pure cultures and were incubated for two days at 28 ºC based on the modified Baermann-funnel method (Hooper 1986) and then J2 counted under stereo microscope. Seedlings were inoculated with approximately 1000 J2. They were watered as needed and fertilized during the trial. Sixty-five days after nematode inoculation, plants were harvested and egg masses on roots were assessed using 0-5 scale galling index (Hartman & Sasser, 1985).

RESULTS

Species identification of 25 populations obtained from vegetable areas infested with RKN in Kahramanmaraş province and its districts were made using the perineal pattern, biochemical and molecular methods. Two species were identified *M. incognita* (23 populations) and *M. javanica* (2 populations). While *Meloidogyne incognita* was found in eggplant (11 populations), pepper (4 populations), and tomato (10 populations) grown in Andırın, Dulkadiroğlu, Ekinözü, Onikişubat and Türkoğlu districts, *M. javanica* was detected in tomato and eggplant in a location (Beyoğlu) in Türkoğlu district (Table 2). The morphological species diagnosis indicated that the perineal shape of *M. incognita* was oval-round, the dorsal arch was angular and high, and the striae were wavy. The perineal pattern of *M. javanica* was typical with a rounded low dorsal arch, smooth striae, and clear parallel lateral lines (Figure 1). According to the esterase isozyme phenotypes diagnostic results, three different esterase phenotypes were detected as J3, I1 and I2 in this study (Figure 2). Two population exhibited *M. javanica*-specific phenotype J3 as used reference samples. Phenotypes I1 and I2 are species-specific for *M. incognita*. Phenotype I2 was the most prevalent esterase phenotypes and were detected in 18 populations, while phenotype I1 was in 5 populations.

Species-specific SCAR primer pairs were used for the identification of *Meloidogyne incognita* and *M. javanica*. Three pairs of primers were tested on DNA samples obtained from egg masses and resulted in consistent amplifications. *Meloidogyne incognita* species primer pairs Finc/Rinc and inc-K14-F/ inc-K14-R produced a single band of 1200 bp and a single band of 399 bp for 23 populations, respectively (Figures 3 and 4). DNA samples of these populations were no amplifications when Fjav/Rjav primer set was used.

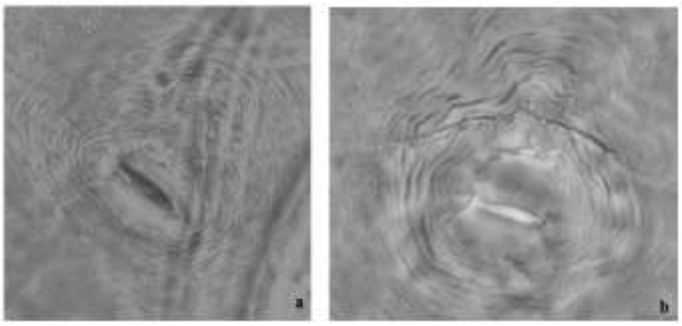

Figure 1. Perineal patterns of *Meloidogyne incognita* (a) and *Meloidogyne javanica* (b).

Şekil 1. Meloidogyne incognita (a) ve Meloidogyne javanica (b) anal kesitleri

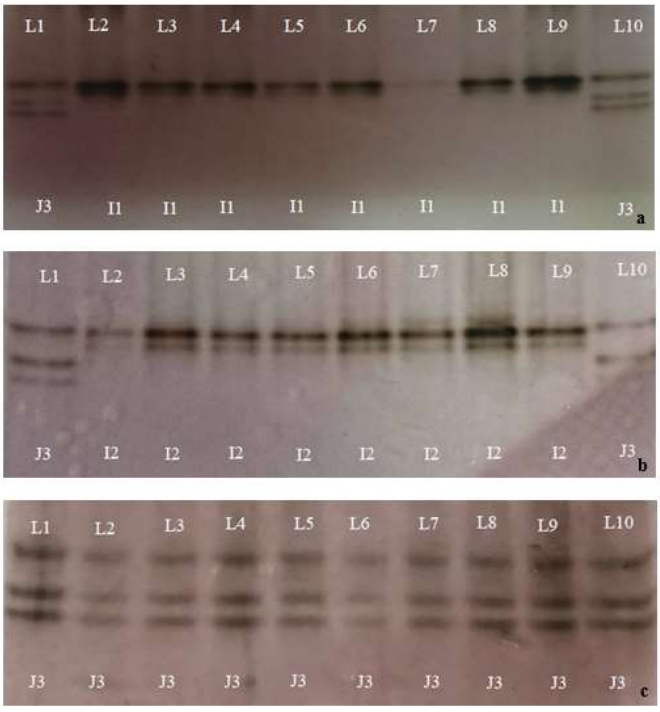

Figure 2. Esterase phenotypes results in *Meloidogyne* species a) *Meloidogyne incognita* (Est: I1) (Sample no: 56 B) b) *Meloidogyne incognita* (Est: I2) (Sample no: 49 P) c) *Meloidogyne javanica* (Est: J3) (Sample no: 53 D), Positive control (L1 and L2)

Şekil 2. Meloidogyne türlerinin esteraz fenotip sonuçları a) Meloidogyne incognita (Est: I1) (Örnek no: 56 B) b) Meloidogyne incognita (Est: I2) (Örnek no: 49 P) c) Meloidogyne javanica (Est: J3) (Örnek no: 53 D), Pozitif kontrol (L1 and L2).

Meloidogyne javanica species-specific primers Fjav/Rjav produced a band of 670 bp for two populations (Figure 5). However, the DNA of these populations was not amplified products when Finc/Rinc and inc-K14-F/ inc-K14-R primer sets were used.

In this study, race identification of root-knot nematodes was made according to the North Carolina Differential Host Test. As a result of the experiment, *M. incognita* race 1 egg mass was not found in tobacco, cotton and peanut plants but found in pepper and tomato plants. For *M. incognita* race 2 egg mass was not found in cotton and peanut plants, egg mass was observed in tobacco, pepper and tomato plants. For *M. javanica* race 2 egg mass was not found in tobacco, cotton, peanut plants, egg mass was observed in pepper and tomato plants. The races of the species were determined as *Meloidogyne incognita* race 1 (Andırın/çiçek, Döngele, Merkez; Onikisubat/Aksu; Türkoğlu/Beyoğlu), *M. incognita* race 2 (Türkoğlu/Beyoğlu and Yenipınar) and *M. javanica* race 2 (Türkoğlu/Beyoğlu) (Table 2).

DISCUSSION and CONCLUSION

Root-knot nematode populations obtained on tomato, pepper, and eggplant plants in Kahramanmaraş and its districts were diagnosed based on perineal pattern, biochemical and molecular methods, and *Meloidogyne incognita* and *M. javanica* were determined in the study. In previous studies, it was stated that the most important and common species were *M. incognita* and *M. javanica* in Türkiye (Söğüt & Elekçioğlu, 2000; Özarslandan & Elekçioğlu, 2010). In the Eastern Mediterranean Region, *M. incognita* was found as the dominant species previously

(Söğüt & Elekçioğlu, 2000; Özarslandan & Elekçioğlu, 2010; Gürkan et al. 2019, Aslan & Elekçioğlu, 2022). Similarly, in our data, *Meloidogyne incognita* was found to be the most common RKN in Kahramanmaraş. The study by Çetintaş & Çakmak (2016), diagnosis of *Meloidogyne incognita* was made according to esterase phenotypes and perineal pattern methods and while only *Meloidogyne incognita* has been reported in Kahramanmaraş (Pazarcık, Türkoğlu, and Centre) in their study, *M. incognita* and *M. javanica* were detected in Türkoğlu/Beyoğlu in this current study. Previous studies in the Mediterranean region, Gürkan et al. (2019) the species identification study of root-knot nematodes in vegetable fields (tomato, pepper, eggplant, bean and okra) of Gaziantep and Osmaniye provinces based on biochemical and perineal methods. They identified *M. incognita*, *M. javanica* and *M. arenaria* in Gaziantep and *M. incognita*, *M. javanica*, *M. arenaria* and *M. luci* in Osmaniye. In the study of Aslan & Elekçioğlu, 2022, the diagnosis of root-knot nematodes in the greenhouse vegetable areas of the Eastern Mediterranean Region was made according to biochemical and molecular (SCAR primers) methods. *M. incognita* and *M. javanica* were detected in Mersin, Adana, and Hatay provinces.

In this study, *M. incognita* race 1 was detected in Türkoğlu, Onikişubat, and Andırın, while *M. incognita* race 2 and *M. javanica* race 2 were detected in Türkoğlu. At the end of the study, *M. incognita* race 1 and race 2, *M. javanica* race 2 in tomato and eggplant

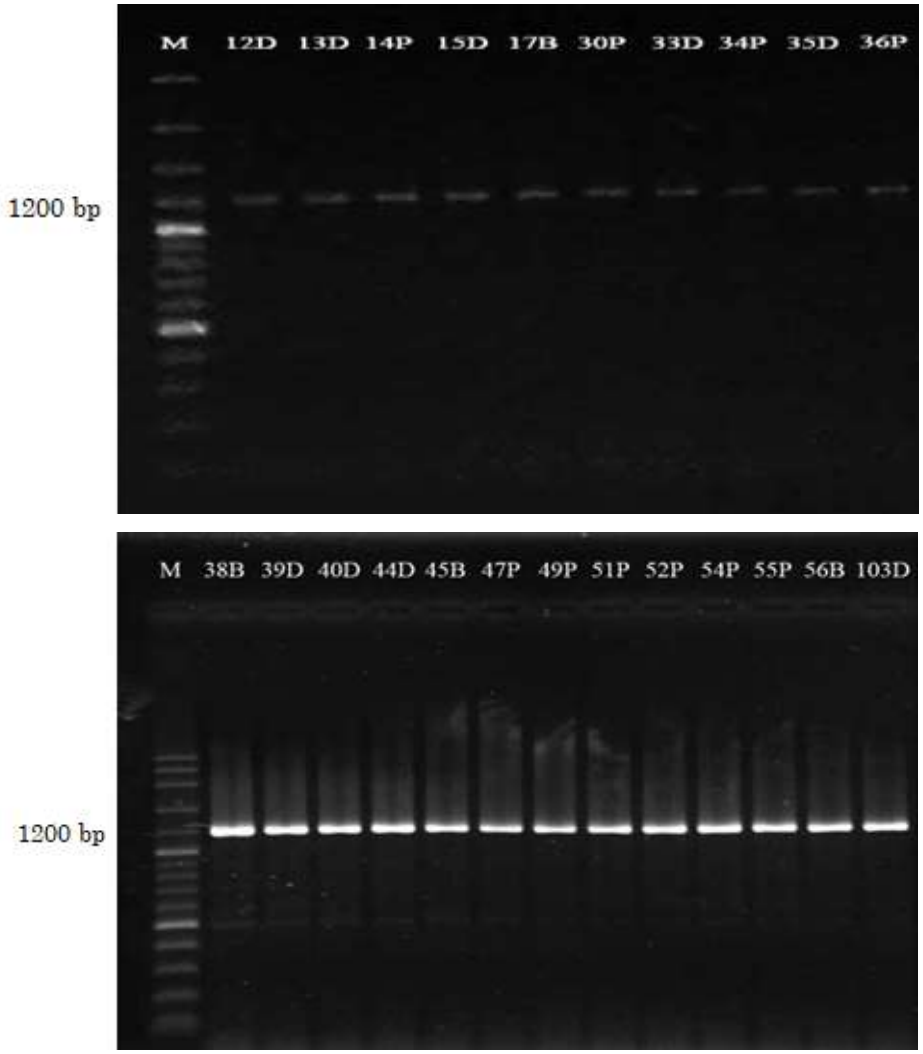

Figure 3. Amplification products with the Meloidogyne incognita Finc/Rinc SCAR primers. M: 100 bp DNA Ladder (Vivantis), Samples (12D-103D).

Şekil 3. Meloidogyne incognita Finc/Rinc SCAR primerleri ile amplifikasyon ürünleri. M: 100 bp DNA Ladder (Vivantis), Örnekler (12D-103D).

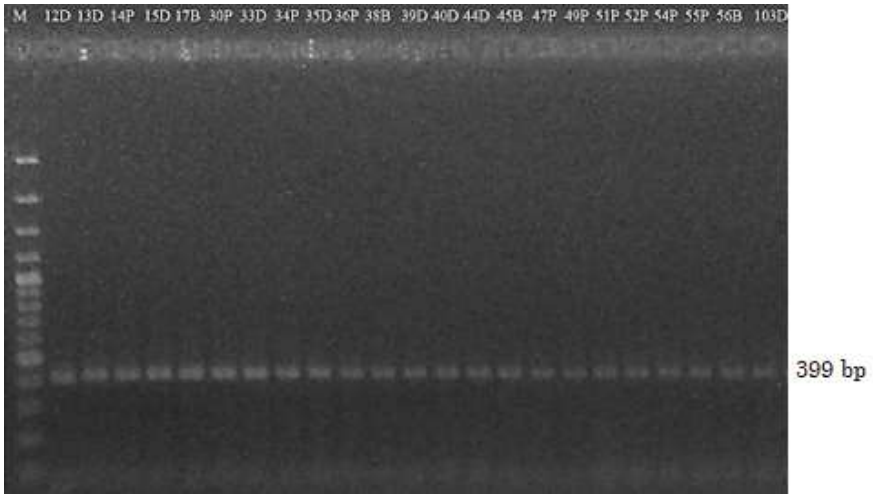

Figure 4. Amplification products with the Meloidogyne incognita Inc-K14-F/Inc-K14-R SCAR primers. M: 100 bp DNA Ladder (Vivantis), Samples (12D-103D).

Şekil 4. Meloidogyne incognita Inc-K14-F/Inc-K14-R SCAR primerleri ile amplifikasyon ürünleri. M: 100 bp DNA Ladder (Vivantis), Örnekler (12D-103D).

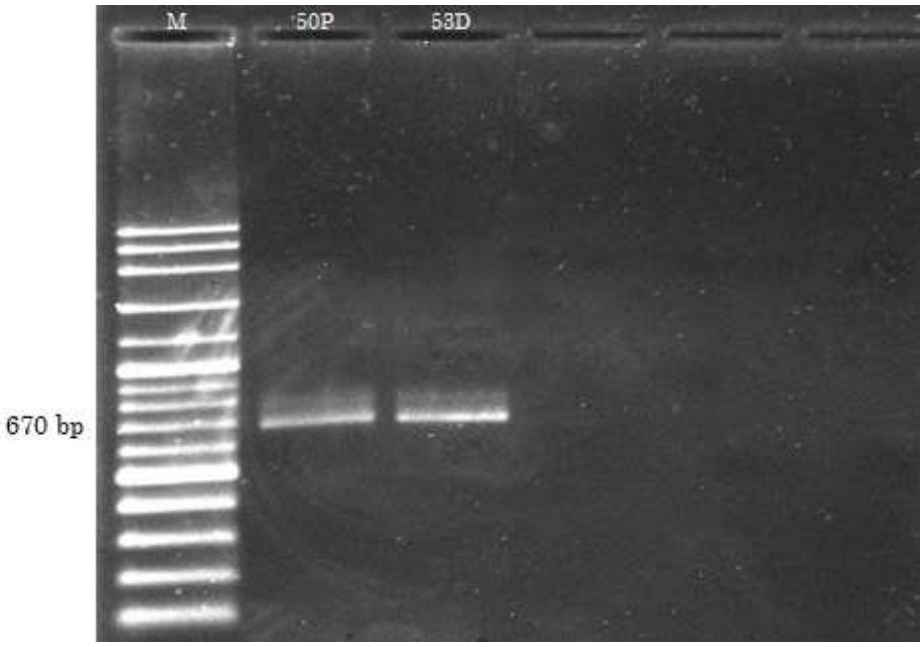

Figure 5. Amplification products with the Meloidogyne javanica Fjav/Rjav SCAR primers. M: 100 bp DNA Ladder (Vivantis), Samples (50P and 53D).

Şekil 5. Meloidogyne javanica Fjav/Rjav SCAR primerleri ile amplifikasyon ürünleri. M: 100 bp DNA Ladder (Vivantis), Örnekler (50P ve 53D).

Table 2. Species and race determination in Kahramanmaraş and its districts

Çizelge 2. Kahramanmaraş ve ilçelerindeki tür ve ırk tespiti

Sample Code	*Host plant*	*District/Location*	*Latitude*	*Longitude*	*Altitude (m)*	*Species identification**	*Race identification*
12D	Tomato	Türkoğlu/Aydın Kavak	37º24'37"	36º47'25"	696	*M. incognita* (Est I1)	Race 1
13D	Tomato	Türkoğlu/Aydın Kavak	37º25'12"	36º48'13"	682	*M. incognita* (Est I1)	Race 1
14P	Eggplant	Türkoğlu/Yenipınar	37º25'28"	36º48'29"	726	*M. incognita* (Est: I2)	Race 2
15D	Tomato	Türkoğlu/Yenipınar	37º25'28"	36º48'29"	727	*M. incognita* (Est: I2)	Race 2
17B	Pepper	Onikişubat/Aksu	37º32'08"	36º55'01"	490	*M. incognita* (Est: I2)	Race 1
30P	Eggplant	Dulkadiroğlu/Çiğli	37º29'272"	37º03'986"	689	*M. incognita* (Est: I2)	Race 1
33D	Tomato	Andırın/Döngele	37º33'847"	36º38'447"	609	*M. incognita* (Est: I2)	Race 1
34P	Eggplant	Andırın/Döngele	37º33'663"	36º38'442"	652	*M. incognita* (Est: I2)	Race 1
35D	Tomato	Andırın/Döngele	37º33'479"	36º38'236"	671	*M. incognita* (Est: I2)	Race 1
36P	Eggplant	Andırın/Döngele	37º33'434"	36º38'480"	691	*M. incognita* (Est: I2)	Race 1
38B	Pepper	Andırın/Durdular	37º33'352"	36º38'042"	704	*M. incognita* (Est: I2)	Race 1
39D	Tomato	Andırın/Centre	37º34'217"	36º21'523"	995	*M. incognita* (Est: I1)	Race 1
40D	Tomato	Andırın/Çiçekli	37º34'704"	36º20'168"	1102	*M. incognita* (Est: I2)	Race 1
44D	Tomato	Andırın/Centre	37º31'760"	36º22'240"	644	*M. incognita* (Est: I2)	Race 1
45B	Pepper	Türkoğlu/Beyoğlu	37º17'213"	36º09'630"	503	*M. incognita* (Est: I2)	Race 1
47P	Eggplant	Türkoğlu/Beyoğlu	37º17'129"	36º47'101"	506	*M. incognita* (Est: I1)	Race 1
49P	Eggplant	Türkoğlu/Beyoğlu	37º17'157"	36º47'084"	509	*M. incognita* (Est: I2)	Race 1
50P	Eggplant	Türkoğlu/Beyoğlu	37º17'254"	36º47'124"	512	*M. javanica* (Est: J3)	Race 2
51P	Eggplant	Türkoğlu/Beyoğlu	37º17'259"	36º47'120"	511	*M. incognita* (Est: I2)	Race 1
52P	Eggplant	Türkoğlu/Beyoğlu	37º17'271"	36º47'104"	515	*M. incognita* (Est: I2)	Race 1
53D	Tomato	Türkoğlu/Beyoğlu	37º17'442"	36º47'337"	503	*M. javanica* (Est: J3)	Race 2
54P	Eggplant	Türkoğlu/Beyoğlu	37º17'453"	36º47'337"	504	*M. incognita* (Est: I2)	Race 2
55P	Eggplant	Türkoğlu/Beyoğlu	37º17'440"	36º47'332"	507	*M. incognita* (Est: I2)	Race 2
56B	Pepper	Türkoğlu/Beyoğlu	37º17'427"	36º47'333"	505	*M. incognita* (Est: I1)	Race 2
103D	Tomato	Ekinözü/Centre	38º02'831"	11º33'7"	1251	*M. incognita* (Est: I2)	Race 1

*Est: Esterase phenotypes

plant, *M. incognita* race 1 and race 2 in pepper plant were determined. Race determination for the populations obtained from Kahramanmaraş was made for the first time in located in the Eastern Mediterranean Region. In previous studies of race detection in our country, *M. incognita* race 1, race 2,

race 4, race 5, race 6 and *M. javanica* race 1 and race 5 were reported (Söğüt & Elekçioğlu, 2000; Mennan & Ecevit, 2001; Devran & Söğüt, 2011; Kaçar, 2011). In the study of Çetintaş & Çakmak, (2016), only the species were identified in Türkoğlu/Aydın kavak and Dulkadiroğlu/Çiğli, while in this study, the race of *M. incognita* was determined according to the North Carolina Differential Host Test (race 1). Gurkan et al. (2019) detected race 1, race 2, race 3 of *M. incognita*, race 3 of *M. javanica* and race 1 and race 3 of *M. arenaria* from 20 populations examined in the Mediterranean regio.

This study showed that precise diagnostic of root-knot nematode relies not only on morphological features but also on other techniques including molecular and biochemical methods. Identification studies are mostly time-consuming and need much professional skills (Blok et al. 2002). Furthermore, perineal patterns of some species are closely similar to each other which makes the morphological identifications incomplete. Nevertheless, it is needed for the confirmation of the other identification steps. Thus, controlling the root-knot nematodes requires rapid and precise analysis tools. In this study, species of *Meloidogyne incognita* and *M. javanica* that are very important parasites of vegetables and lead to high crop losses in Kahramanmaraş province were determined by applying SCAR primers.

Meloidogyne spp. is one of the key plant parasitic nematode groups becoming a growing concern for vegetable producers. Control of parasitic nematodes depends on detection ability and accurate diagnosis of nematode species to apply suitable and sustainable management methods. This is the first report of *M. javanica* on tomato and eggplant in Kahramanmaraş province. These findings show a potential risk of nematode presence and possible crop losses in eggplant and tomato growing areas in this region. Setting up cultural control techniques will be needed to reduce infestation around these areas. It is suggested that more effective control management tactics such as the use of resistant varieties and crop rotation should be applied. In addition, an integrated nematode management approach involving the combination of two or more suitable approaches using locally available resources in an integrated form can be necessary to deal with the threat of *Meloidogyne* spp. in vegetable areas.

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Author's Contributions

The contribution of the authors is equal.

Statement of Conflict of Interest

The authors declare no conflict of interest.

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Geliştirilmiş Anadolu Adaçayı (Salvia fruticosa MILL.) Klonlarının Verim ve Kalite Özelliklerinin Belirlenmesi

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ÖZET

Anadolu adaçayı *(Salvia fruticosa* MILL.) gıda, kozmetik ilaç gibi birçok sektörde yaygın olarak kullanılmaktadır. Türkiye doğal florasında yayılış gösteren bu türün ticari değeri, diğer *Salvia* türlerine göre oldukça yüksektir. Ticarete konu olan ürünün büyük çoğunluğu doğadan toplanmakta ve standart bir özelliğe sahip değildir. Doğadan toplanan türlerde verim ve kaliteyi etkileyen birçok faktör bulunmaktadır. Standart özelliklere sahip bir ürünün elde edilmesi ıslah çalışmaları ile mümkündür. Bu çalışma, klonal olarak geliştirilen 6 adet C-klonu ile 1 doğal populasyonun verim ve kalite özelliklerini belirlemek amacıyla yürütülmüştür. C-klonlarında 2 yıl üst üste bitki boyu, dal sayısı, taze herba ve yaprak verimi, kuru herba ve yaprak verimi gibi verim kriterleri yanında uçucu yağ oranı ve bileşenleri belirlenmiştir. Uçucu yağ oranları, hidrodistilasyon yöntemiyle klevenger düzeneğinde belirlenmiştir. Uçucu yağların bileşen oranları ise GC-MS/FID cihazı ile kapiler kolon kullanılarak analiz edilmiştir. İki yıllık değerlendirmede, ilk yıl klonlar arasında verim kriterleri açısından bir farklılık görülmezken ikinci yıl farklılık görülmüştür. Kuru herba verimi 1100.00-4280.00 g bitki^{-1} arasında değişim gösterirken kuru yaprak verimi 123.33-576.67 g bitki^{-1} arasında değişim göstermiştir. Uçucu yağ oranı %2.00-2.33 arasında tespit edilmiştir. Analizler sonucunda, 1,8 cineol, camphor, ß-pinene ve ß-caryophyllene uçucu yağda bulunan ana bileşenler olarak belirlenmiştir. Çalışma sonucunda, geliştirilmiş Anadolu adaçayı klonlarının verim ve kalite özellikleri bakımından öne çıkan iki genotip (Fk2-9 ve Fk4-9) ticari çeşit olarak önerilmiştir.

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Anahtar Kelimeler
Salvia triloba
Clone selection
Breeding
Essential oil
1,8 cineoles

Determination of Yield and Quality Characteristics of Improved Anatolian Sage (Salvia fruticosa MILL.) Clones

ABSTRACT

Salvia fruticosa MILL. is widely used in many sectors such as food and pharmaceuticals. The commercial value of this species, which is widespread in the natural flora of Turkey, is quite high compared to other *Salvia* species. The majority of the product that is subject to trade is collected from nature and does not have a standard feature. There are many factors that affect yield and quality in species collected from nature. Obtaining a product with standard features is possible with breeding studies. This study was carried out to determine the yield and quality characteristics of 6 clonally developed C-clones. In C-clones, yield criteria such as plant height, the number of branches, fresh herb and leaf yield, dry herb and leaf yield, as well as essential oil content and components were determined for 2 consecutive years. Essential oil ratios were determined by hydrodistillation method in the Klevenger apparatus. Component ratios of essential oils were analyzed by GC-MS/FID. Dry herb yield varied between 1100.00-4280.00 g plant^{-1}, while dry leaf yield varied between 123.33-576.67 g plant^{-1}. The essential oil ratio was determined between 2.00 and 2.33%. As a result of the analysis, 1,8 cineol, camphor, ß-pinene and ß-caryophyllene were determined as the

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main components in the essential oil. As a result of the study, two genotypes that stand out in terms of yield and quality characteristics of improved Anatolian sage clones were registered as commercial varieties.

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GİRİŞ

Lamiaceae familyasına ait S. fruticosa türün tarımı son yıllarda ivme kazanmıştır. Bu tür, sıklıkla Anadolu adaçayı olarak bilinirken, adaçayı, boz çalba veya elma çalbası olarak da bilinmektedir. Yaprakları çay olarak tüketilir ve yapraklarından elde edilen uçucu yağa 'elma yağı' denir. Solunum yolu enfeksiyonlarına, sinir hastalıklarına ve ishale iyi geldiği bilinen Anadolu adaçayının aynı zamanda ağrı kesici özelliği olduğu bildirilmektedir (Baytop, 1999).

Son yıllarda doğal ürünlere olan talebin artmasıyla birlikte tıbbi aromatik bitkilerin pazar hacmi artmıştır. Türkiye'de bu bitkiler daha çok doğadan toplanmaktadır. Doğadan toplanan ürünlerde kalite standardizasyonu anlamında zorluklarla karşılaşılabilmektedir. Bununla birlikte, doğal floranın korunması için, bu bitkilerin doğadan toplanması denetlenmeli, üreticiler desteklenmeli ve standartlara uymayan ürünler pazarlanmamalıdır (Metin ve ark. 2012; Erol, 2015).

Günümüzde yeterli miktarda standart ve kaliteli ürün temini doğal bitkilerin toplanmasıyla mümkün olamamakta, bu bitkilerin düzenli olarak kültürünün yapılabilmesi için seleksiyon ve ıslah çalışmalarıyla istenilen niteliklere ulaştırılması gerekmektedir (Bayram ve ark., 2010; Leontaritou ve ark., 2020). Standart ham maddeyi elde etmenin başlıca yolu ise ilgili türde uygun çeşit geliştirmektir. Bitkilerde verim ve uçucu yağ miktarı, genetik faktörler başta olmak üzere, bitki tür ve çeşidi, bitki yaşı, yetiştirildiği ekolojik koşullar, hasat edildiği zaman, maruz kaldığı abiyotik stres koşulları gibi birçok faktöre göre değişim gösterebilmektedir (Saharkhix ve ark., 2009; Karık & Sağlam, 2017; Elmas ve ark., 2019). Tıbbi ve aromatik bitkilerden elde edilen doğal ürünlerde oluşan açığın giderilmesi için yürütülen ıslah çalışmaları ile gerek herba verimi gerekse uçucu yağ oranı açısından yeni çeşitler geliştirilmiştir (Bayram, 2001; Telci & Şahbaz, 2005; Telci ve ark., 2006; Arslan ve ark., 2014, Lal ve ark., 2021). Ancak, farklı ekolojilerde yöreye uygun çeşit geliştirme çalışmaları ve uçucu yağ ana bileşen oranları değişim konusunda değerlendirmeler kısıtlı düzeydedir. Islahta belirleyici olan, üretici ve piyasa talebi doğrultusunda araştırma yapmaktır (Franzel ve ark., 1996). Piyasa taleplerinin uzun vadede doğadan toplanarak karşılanması mümkün değildir. Ayrıca, dünya pazarları, ilaç sanayii; etken madde miktarı ve kalitesi yüksek, "standart" ürün talep etmektedir. Bu kapsamda yüksek verimli ve piyasanın talep ettiği kalite standartlarına uygun çeşit geliştirme çalışmaları önem kazanmıştır. Bugüne kadar yapılan pek çok çalışma tohumdan gelen genotipler üzerinde yürütülmüştür. Bu çalışma ile klonal olarak geliştirilen 6 adet C-klonunun (çeşit adaylarının) bazı morfolojik özellikleri, taze-kuru herba ve yaprak verim değerleri, uçucu yağ oranı ve bileşenleri ortaya konulmuş, verim açısından bazı klonların tescil edilmesi önerilmiştir.

MATERYAL ve METOD

Materyal olarak, 'Antalya Florasında Yetişen Bazı Adaçayı (*Salvia* spp.) Türlerinde Seleksiyon Islahı' ve 'Antalya Florasında Bulunan Anadolu Adaçayı (*Salvia fruticosa* MILL.) Populasyonlarında Seleksiyon Islahı ile Üstün Özelliklere Sahip Genotiplerin Belirlenmesi' isimli projelerde, 15 farklı popülasyondan 1250 klon üzerinde yapılan çalışmalardan geliştirilen, 6 adet C-klonu kullanılmıştır. Buna ek olarak, Antalya-Gelidonya populasyonu tohumlarından elde edilen fideler kontrol amaçlı çalışmada yer almıştır. Materyal bilgileri Çizelge 1'de verilmiştir. Standart üretimde daha elverişli olmasından dolayı, bu çalışmada klonal materyal kullanılmıştır.

Çalışma Batı Akdeniz Tarımsal Araştırma Enstitüsü Aksu yerleşkesinde yürütülmüştür. Deneme alanının bulunduğu Antalya ilinde yazlar sıcak ve kurak, kışlar ılık ve yağışlıdır. Antalya Meteoroloji Bölge Müdürlüğü kayıtlarına göre denemenin yürütüldüğü yıllara ait ortalama yağış, sıcaklık ve oransal nem değerlerinin, uzun yıllar ortalamalarına yakın değerler olduğu belirlenmiştir. Araştırmanın yürütüldüğü 24 ay boyunca ortalama sıcaklık 19 °C, ortalama yağış 60 mm ve ortalama nispi nem %73 olarak kaydedilmiştir (Çizelge 2).

Çalışma, tesadüf blokları deneme desenine göre 4 tekerrürlü olarak kurulmuştur. Deneme alanında toprak hazırlığı yapılmış alana, damlama sulama sistemi kurulmuş ve malç serilmiştir. Deneme alanına 28 Mart 2017 tarihinde, seçilen C-klonları sıra arası 70 cm, sıra üzeri mesafe 40 cm ve her sırada 10 bitki olacak şekilde 4 sıra halinde dikilmiştir. Yetişme sürecince, tüm kültürel uygulamalar (gübreleme, sulama, yabancı otların temizlenmesi, ilaçlama, hasat, vb. işlemler) yapılmıştır. Kültürel işlemlere ilişkin görüntüler Şekil 1'de verilmiştir. Dikimden bir yıl sonra 2018 yılı mart ayında, çiçeklenme ile birlikte ilk

yıl hasadı yapılmıştır. İkinci yıl hasat, ilk yıl olduğu gibi, çiçeklenmenin olduğu mart ayında gerçekleşmiştir. İkinci yılda klonlardan Fk5-7 ve Fd4-13 hastalık (*Fusarium* ssp.) nedeniyle önemli kayıplar yaşamış ve deneme dışı bırakılmıştır.

Çizelge 1. Araştırma kapsamında kullanılan bitkisel materyal bilgileri
Table 1. Herbal material information used within the scope of the research

Materyal	Lokasyon	Yükseklik (m)	Kordinat
Fk3-16	Kemer	31	36 34 59 K 30 34 33 D
Fk4-9	Kemer	137	36 32 06 K 30 32 35 D
Fk4-14	Kemer	137	36 32 06 K 30 32 35 D
Fk5-7	Kemer	20	36 40 96 K 30 33 43 D
Fd2-9	Demre	68	40 10 49 K 35 75 57 D
Fd4-13	Demre	5	40 10 18 K 35 75 49 D
Standard	Kumluca	57	36 14 11 K 30 24 41 D

Çizelge 2. Denemenin yürütüldüğü yıllara ait iklimsel veriler
Table 2. Climatic data of the years of the experiment

Yıllar	2017									2018		
Aylar	Haziran	Temmuz	Ağust.	Eylül	Ekim	Kasım	Aralık	Ocak	Şubat	Mart	Nisan	Mayıs
Yağış (mm)	0.0	0.0	0.0	0.0	29.0	48.0	74.0	93.0	91.0	94.0	2.0	19.0
Ort. nispi nem (%)	66.4	62.0	72.3	72.4	64.9	74.0	81.8	72.2	83.0	78.9	68.7	66.2
Ortalama sıcaklık (°C)	25.8	29.4	27.9	25.2	19.7	14.4	12.0	10.8	12.8	15.0	18.5	23.2
En yüksek sıcaklık (°C)	44.5	44.8	40.3	36.9	19.7	32.2	25.9	20.9	21.2	25.8	35.2	35.6
En düşük sıcaklık (°C)	15.5	18.3	19.0	14.7	19.7	3.1	0.8	1.7	3.4	6.8	6.7	11.9
YILLAR	2018									2019		
AYLAR	Haz.	Tem.	Ağust.	Eylül	Ekim	Kasım	Aralık	Ocak	Şubat	Mart	Nisan	Mayıs
Yağış (mm)	65.0	18.0	0.0	13.0	24.0	57.0	156.0	300.0	127.0	72.0	149.0	7.0
Ort. nispi nem (%)	72.8	65.8	71.2	65.1	67.3	72.5	78.0	85.1	80.1	76.7	75.6	71.9
Ortalama sıcaklık (°C)	25.5	28.5	28.0	25.9	20.4	15.7	11.5	9.6	11.4	13.4	15.8	21.3
En yüksek sıcaklık (°C)	38.0	43.3	40.8	40.7	35.5	31.5	21.6	17.6	20.6	27.4	27.6	36.3
En düşük sıcaklık (°C)	16.3	18.2	17.2	15.2	7.2	7.2	0.0	0.8	3.6	2.5	5.6	9.9

Örnekleme Yöntemi

Hasattan önce, toprak yüzeyinden bitkinin en uç noktasına kadar olan yükseklik bitki boyu (cm) olarak belirlenmiştir. Dal sayısı (adet) ise, biçimden önce bitkide oluşan ana dallar sayılarak tespit edilmiştir. Yeşil herba verimi (g bitki^{-1}), bitkiler, toprak seviyesinden 10 cm yükseklikte biçilip tartılarak, bitki başına yeşil herba verimi g/bitki olarak belirlenmiştir. Yeşil yaprak verimi (g bitki^{-1}) ise, taze herbadan alınan 500 g'lık örneklerde yaprak-sap ayrımı yapılarak % yaprak oranı belirlenmiş ve bundan yararlanılarak bitki başına yeşil yaprak verimi hesaplanmıştır. Kuru herba ve kuru yaprak verimleri, taze herbadan alınan 500 g'lık örnek 35 °C de kurutularak % nem kayıpları hesaplanarak belirlenmiştir (Uysal, 2015).

Laboratuvar analizleri

Uçucu yağ oranı (%), hidrodistilasyon yöntemiyle klevenger düzeneğinde belirlenmiştir. 20 g örnek üzerine 200 ml saf su ilave edilerek 3 saat süreyle distilasyon gerçekleştirilmiş ve uçucu yağ oranı (ml 100 g^{-1} kuru örnek, %) hesaplanmıştır (Anonim 2011). Adaçayı örneklerinde elde edilen uçucu yağların bileşen oranları, GC-MS/FID (Gaz kromatografisi (Agilent 7890A)-kütle detektör (Agilent 5975C)/alev iyonizasyon dedektörü) cihazı ile kapiler kolon (HP Innowax Capillary; 60.0 m x 0.25 mm x 0.25 µm) kullanılarak gerçekleştirilmiştir. Bu amaçla öncelikle örnekler 1:100 oranında n-hekzan ile seyreltilmiştir. Analizde taşıyıcı gaz olarak 0.8 ml dakika^{-1} akış hızında yüksek saflıkta helyum kullanılmıştır. Örnekler cihaza 1 µl olarak 40:1 split oranı ile enjekte edilmiştir. Enjeksiyon bloğu sıcaklığı 250°C, kolon sıcaklık programı 60°C (10 dakika), 60°C'den 220°C'ye 4°C/dakika ve 220°C (10 dakika) olacak şekilde ayarlanmıştır. Bu sıcaklık programı doğrultusunda toplam analiz süresi 60 dakika olmuştur. Kütle detektörü için tarama aralığı (m/z) 35-450 atomik kütle ünitesi ve elektron bombardımanı iyonizasyonu 70 eV kullanılmıştır. Uçucu yağın bileşenlerinin

teşhisinde MS dedektör verileri esas alınmış bu amaçla Wiley7n ve Oil Adams kütüphanelerinden yararlanılmıştır. Ayrıca bileşen tanımlamada C8-40 alkan serisi verileri ile birlikte bileşenleri alıkonma indisi verileri de dikkate alınmıştır. Sonuçların bileşen oranları FID dedektör kullanılarak belirlenmiştir.

Şekil 1. Deneme alanına ve kültürel işlemlere ilişkin görüntüler
Figure 1. Images of the trial area and cultural operations

İstatistik Analizler

İstatistik analizler için iki yıl süresince ölçülen ve gözlenen bileşen verilerinin aşağıdaki karma (mixed) model kullanılmıştır. Her yıl ayrı ayrı değerlendirilmiştir.

$$y_{ijk} = \mu + B_i + C_j + e_{ijk} \quad (1)$$

Modelde;
y_{ijk} = *i*. . blokta, *j*. klonda, *k*. gözlemi,
B_i = *i*. bloğun sabit (fixed) etkisini (*i*=1, 2…4),
C_j = *j*. klonun (genotip) rastlantısal etkisini (l= 1, 2 7),
e_{ijk} = deneysel hatayı göstermektedir.

Verilerde sıra dışı gözlemler olup olmadığı denetlenmiş, normal dağılım göstermeyen dal sayısı, kuru herba, taze yaprak ve kuru yaprak özelliklerine karekök dönüşümü uygulanmış, diğer özelliklerin normal dağıldığı görülerek istatistik analizler yürütülmüştür. Her bir özellik için yapılan varyans analizi sonucunda farklılık gösteren faktörler için ise duyarlılığı daha yüksek olan Student-Newman-Keuls (SNK) çoklu karşılaştırma testi uygulanmıştır. Verilerin analizinde SAS 9.0 istatistik paket programı kullanılmıştır (SAS Institute Inc. 2002).

BULGULAR ve TARTIŞMA

Anadolu adaçayı klonlarından elde edilen ve piyasada sıklıkla kullanılan kuru yaprak miktarı 2018 yılı genel ortalaması 326.82 g bitki^{-1}, 2019 yılı genel ortalaması 667.83 bitki^{-1} olmuştur (Çizelge 3). İkinci yıl kuru yaprak verimi iki kattan fazla artış göstermiştir. Adaçayı tarımında önemli parametrelerden olan uçucu yağ oranı 2018 yılı genel ortalaması %2.06, 2019 yılı genel ortalaması ise %2.17 olarak tespit edilmiştir. Buradan anlaşıldığı üzere genel ortalamalar açısında klonlar arasından uçucu yağ oranı açısından büyük bir değişimin olmadığı görülmektedir. Araştırmada incelenen diğer parametreler olan bitki boyu, dal sayısı, taze herba verimi, kuru herba verimi, taze yaprak verimi 2018 yılı genel ortalamaları sırasıyla 139.08 cm, 13.63 adet bitki^{-1}, 3537.34 g bitki^{-1}, 818.45 g bitki^{-1} ve 1365.24 g bitki^{-1} olarak belirlenmiştir. Aynı özelliklere ait 2019 yılı genel ortalamaları ise sırasıyla 107.31 cm, 23.05 adet bitki^{-1}, 5576.71 g bitki^{-1}, 2120.46 g bitki^{-1} ve 2409.17 g bitki^{-1} bulunmuştur. Bitki boyu ortalamaları yıllar itibari ile farklılık göstermekte ve bitki boyu ikinci yıl düşmektedir. Bu durum ilk yıl dikimde 5 aylık fidelerin kullanılması ve fidelerin belirli bir boyda olması ile açıklanabilir. Dal sayısı ortalamasında ikinci yıl bir artış söz konusudur. Bu durum ise, ilk yıl hasadından sonra apikal dormansinin kırılması ile birlikte yan dallanmanın teşvik edilmesi ile açıklanabilir. İkinci yıl verim değerleri, dallanma artışına paralel olarak artış göstermiştir.

Özelliklerde 2018 yılına ait varyans analizi Çizelge 4'verilmiştir. C-klonları arasında tüm özellikler açısından istatiksel anlamda bir farklılık görülmemiştir. Bitki boyu ölçümleri 82.33-196.33 cm arasında değişim gösterirken, bitki dal sayısı ise 7.00-19.66 adet arasında değişim göstermiştir. Önemli verim değerlerinden olan taze herba verim değeri ilk yıl en yüksek 6273.33 g bitki^{-1} olurken, sanayide asıl kullanılan kısım olan kuru yaprak verimi en yüksek 576.67 g bitki^{-1} olmuştur. Uçucu yağ oranları ise %2.00-2.50 arasında değişim göstermiştir.

Özelliklerde 2019 yılına ait varyans analizi Çizelge 5'verilmiştir. Uçucu yağ dışında klonlar arasındaki farklılıklar istatistik olarak önemli bulunmuştur.

Çizelge 3. Yıllara göre verim özelliklerine ilişkin temel parametreler
Table 3. Basic parameters of yield characteristics by years

Parametreler	Yıl	Ortlama±SH	Standart sapma	En düşük	En yüksek
Bitki boyu (cm)	2018	139.08±5.11	27.08	82.33	196.33
	2019	107.31±3.63	16.22	78.67	141.67
Dal sayısı (adet bitki^{-1})	2018	13.63±0.61	3.22	7.00	19.66
	2019	23.05±1.56	7.00	12.33	41.33
Taze herba verimi (g bitki^{-1})	2018	3537.34±272.99	1444.54	1340.00	6273.33
	2019	5576.71±389.82	1743.35	2420.00	8660.00
Kuru herba verimi (g bitki^{-1})	2018	818.45±66.89	353.97	283.00	1423.33
	2019	2120.46±180.02	805.10	1100.00	4280.00
Taze yaprak verimi (g bitki^{-1})	2018	1365.24±114.45	605.59	433.33	2760.00
	2019	2409.17±155.85	697.00	1360.00	4120.00
Kuru Yaprak Verimi (g bitki^{-1})	2018	326.82±21.39	113.23	123.33	576.67
	2019	667.83±36.44	162.98	380.00	930.00
Uçucu Yağ Oranı (%)	2018	2.06±0.03	0.15	1.67	2.33
	2019	2.17±0.14	0.15	2.00	2.33

Çizelge 4. Özelliklerde 2018 yılına ait varyans analizi
Table 4. Analysis of variance in properties for 2018

Parametreler	Varyasyon kaynağı	Serbestlik derecesi	Kareler ortalaması	F değeri	P değeri
Bitki boyu (cm)	Blok	3	534.57	0.74	0.5433
	Klon	6	858.16	1.18	0.3582
	Hata	18	724.84		
Dal sayısı (adet bitki^{-1})	Blok	3	0.56	3.29	0.0446
	Klon	6	0.12	0.70	0.6548
	Hata	18	0.17		
Taze herba verimi (g bitki^{-1})	Blok	3	4062174.00	1.75	0.1924
	Klon	6	402790.00	0.17	0.9806
	Hata	18	2318738.00		
Kuru herba verimi (g bitki^{-1})	Blok	3	38.12	0.72	0.5505
	Klon	6	5.05	0.10	0.9959
	Hata	18	52.64		
Taze yaprak verimi (g bitki^{-1})	Blok	3	182.99	3.08	0.0536
	Klon	6	23.06	0.39	0.8767
	Hata	18	59.34		
Kuru yaprak verimi (g bitki^{-1})	Blok	3	7758.28	0.50	0.6900
	Klon	6	6841.15	0.44	0.8446
	Hata	18	15658.00		
Uçucu yağ oranı (%)	Blok	3	0.02	1.20	0.3381
	Klon	6	0.06	1.49	0.2387
	Hata	18	0.02		

Bitki boyu uzunlukları 95.58-124.30 cm arasında değişim gösterirken en yüksek bitki boyu standart olarak kullanılan populasyondan elde edilmiştir. Bitki dal sayısı ise 17.20-31.50 adet bitki-1 arasında değişim göstermiştir. Fd2-9 numaralı klon en fazla dal sayısına sahip klon olmuştur. Bu klonu, 24.9 adet bitki-1 ile Fk4-15 numaralı klon takip etmiştir (Çizelge 6). Araştırmadan elde edilen bitki boyu ölçümleri, Karık (2015) ile uyum içerisindeyken, Mossi ve ark., (2011) ve Elmas ve ark. 2019'dan daha yüksektir. Bitki dal sayısı açısından diğer araştırmalar ile farklılık göstermekle birlikte ilk yıl elde edilen maksimum dal sayısı (19.66 adet bitki-1) Elmas ve ark. 2019 ile benzerlik gösterirken, ikinci yıl elde edilen değer (41.33 adet bitki-1) ise Mossi ve ark., 2011 ile benzerlik göstermektedir.

Yapılan istatistik analizi sonucunda, C-klonları arasında taze herba verimi açısından önemli bir farkın olduğu belirlenmiştir (Çizelge 5). Taze herba verimi 3835.00-7796.00 g bitki^{-1} arasında değişim gösterirken, kuru herba verim değeri 1581.30-3041.20 g bitki^{-1} arasında değişim göstermiştir. En yüksek taze herba verim değeri, Tohumluk Tescil ve Sertifikasyon Merkez Müdürlüğü'ne (TTSM) sunulan Fk4-9 numaralı klondan elde edilmiştir. Bu klonu 6573 g bitki^{-1} ile Fd2-9 numaralı klon izlemiştir. Klonlardan elde edilen kuru yaprak verimi, 525.80-830.00 g bitki^{-1} arasında değişim göstermiştir. En yüksek kuru yaprak

verim değeri, TTSM'ye tescile sunulan bir diğer çeşit adayı Fd2-9 numaralı klondan elde edilmiştir. Fk4-9 numaralı klon herba verimi açısından ön plana çıkarken Fd2-9 numaralı klon yaprak verimi açısından ön plana çıkmaktadır (Çizelge 6). Bu durum, Fd2-9 klonundaki bitkilerin internot aralıklarının dar olması ve bitki başına düşen yaprak sayısının fazla olması ile açıklanabilir. Anadolu adaçayının verimi üzerine birçok araştırıcı çalışmalar yürütmüştür (Turhan, 2020; Yılmaz, 2019; Elmas ve ark. (2019), Karık & Sağlam, 2017; Uysal, 2015; Karık, 2015; Mossi ve ark., 2011; Bayram ve ark. 1999). Çalışmadan elde edilen verim değerleri bu araştırmalardan elde edilen çalışmalardan oldukça yüksektir. Yılmaz (2019)'da 6 kat, Bayram ve ark. (1999)'dan 8 kat fazla yeşil herba verimi elde edilmiştir. Elde edilen yeşil herba verim değeri Karık (2015) ile benzerlik göstermekle birlikte, elde edilen kuru yaprak verimi Karık (2015)'den 2 kat daha fazladır. Bu durum farklı morfolojik bitkisel özellikler (sık yaprak dizilişi, kalın gövde sapı vb.) ile açıklanabileceği gibi, geliştirilmiş adaçayı klonlarının üstünlüğü ile de açıklanabilir. Çizelge 5. incelendiğinde, klonlar arasında uçucu yağ oranı açısından önemli bir farkın olmadığı görülmektedir. Oysa verim ve kalite açısından öne çıkan klonlar özelliklere göre Çizelge 6'da görülmektedir.

Çizelge 5. Özelliklerde 2019 yılına ait varyans analizi
Table 5. Analysis of variance for 2019 in features

Parametreler	Varyasyon kaynağı	Serbestlik derecesi	Kareler ortalaması	F değeri	P değeri
Bitki boyu (cm)	Blok	3	134.53	1.50	0.2652
	Klon	4	879.15	9.79	0.0009
	Hata	12	89.84		
Dal sayısı (adet bitki^{-1})	Blok	3	0.30	1.00	0.4258
	Klon	4	1.30	4.34	0.0212
	Hata	12	0.30		
Taze herba verimi (g bitki^{-1})	Blok	3	833448.00	0.73	0.5530
	Klon	4	10391853.00	9.12	0.0013
	Hata	12	1139842.00		
Kuru herba verimi (g bitki^{-1})	Blok	3	3.62	0.09	0.9656
	Klon	4	202.18	4.87	0.0144
	Hata	12	41.50		
Taze yaprak verimi (g bitki^{-1})	Blok	3	24.92	0.89	0.4733
	Klon	4	122.03	4.37	0.0208
	Hata	12	27.94		
Kuru yaprak verimi (g bitki^{-1})	Blok	3	1212.39	0.08	0.9671
	Klon	4	82268.00	5.74	0.0081
	Hata	12	14332.00		
Uçucu yağ oranı (%)	Blok	3	0.005	0.17	0.9168
	Klon	4	0.015	0.50	0.7365
	Hata	12	0.029		

Çizelge 6. Özelliklerin SNK çoklu karşılaştırma testi sonuçları (2019)
Table 6. SNK multiple comparison test results of features (2019)

Örnekler	Bitki boyu (cm)	Dal sayısı (adet bitki^{-1})	Taze herba Verimi (g bitki^{-1})	Kuru herba Verimi (g bitki^{-1})	Taze yaprak verimi (g bitki^{-1})	kuru yaprak verimi (g bitki^{-1})
Standart	124.3±7.5 a	17.2±2.6 b	4419±363 bc	1652±167 ab	1872±73 ab	525.8±50.7 c
Fk4-9	119.4±1.8 a	19.7±0.6 ab	7796±324 a	3041±405 a	2834±272 a	812.5±51.0 ab
Fd2-9	107.6±4.0 ab	31.5±3.5 a	6573±393 ab	2568±363 ab	2610±80 a	830.0±26.4 a
Fk3-16	95.6±3.0 b	20.8±3.2 ab	3835±498 c	1581±203 b	1771±175 ab	610.8±83.3 abc
Fk4-15	89.8 ±6.2 b	24.9±2.2 ab	5258±841 bc	1631±208 b	2855±509 a	560.0±42.4 bc

Günümüzde, tıbbi aromatik bitkiler için en yüksek uçucu yağ oranı tespiti yeterli olmamakta, uçucu yağda bulunan bileşenlerin değişimlerini ortaya koymak önem arz etmektedir. Bu nedenle mevcut çalışmada her iki yılda hasat ile birlikte klonların uçucu yağ bileşenleri belirlenmiştir. Elde edilen sonuçlar çizelge 7'de verilmiştir. Yapılan analizler sonucunda farklı klon ve yıllarda 16-21 arası bileşen belirlenmiştir. Tüm klonlarda 1,8 sineol, beta-pinene, beta-Caryophyllene, alpha.-pinene ve Camphor ana bileşen olarak tespit edilmiştir. Anadolu adaçayı uçucu yağ bileşenleri içerisinde 1,8 sineol önemli bir yer tutmaktadır. İlk yıl ve ikinci yıl belirlenen uçucu yağdaki 1,8-sineol oranları Çizelge 7'de verilmiştir.

Çizelge 7. Klonların 2018 ve 2019 yıllarına ilişkin uçucu yağ bileşenleri (%)
Table 7. Essential oil components of clones for 2018 and 2019 (%)

Bileşenler (%)	Fk4-9		Fk4-14		Fk3-16		Fk5-7		Fd2-9		Fd4-13		Standart	
	2018	2019	2018	2019	2018	2019	2018	2019	2018	2019	2018	2019	2018	2019
α-pinen	4.55	4.79	4.78	4.79	4.96	4.56	3.90	-	4.98	4.54	4.29	-	5.39	4.79
Kamfen	2.25	2.74	0.43	0.20	0.50	-	2.31	-	0.49	-	0.82	-	5.34	2.50
ßpinen	12.13	9.90	17.18	14.02	15.92	13.98	6.04	-	16.57	13.73	12.48	-	7.76	8.22
ß-Mirsn	4.18	4.60	6.56	5.13	6.62	5.16	12.94	-	6.43	5.56	3.90	-	4.14	6.00
Limonen	0.96	1.27	0.89	0.97	0.91	0.97	0.98	-	0.88	0.97	2.04	-	1.40	1.37
1,8-sineol	48.84	53.01	48.16	58.37	51.00	57.35	43.22	-	51.14	56.31	62.62	-	48.14	53.68
γ-terpinene	0.68	0.72	0.82	0.70	0.80	0.69	0.82	-	0.74	0.81	0.41	-	0.56	0.80
alpha.-Thujone	2.16	1.50	-	-	-	-	0.80	-	-	-	-	-	2.08	1.23
beta.-thujone	2.66	1.68	0.57	0.68	0.51	0.66	1.76	-	0.56	0.75	1.57	-	1.54	1.13
Sabinene hydrate	0.65	0.32	0.58	0.56	0.53	0.54	0.29	-	0.59	0.80	0.69	-	0.46	1.86
Camphor	4.02	5.73	0.80	1.22	0.75	1.14	4.56	-	0.64	3.13	2.26	-	8.33	3.67
Bornyl acetate	-	-	-	-	-	-	-	-	-	-	-	-	-	-
beta-Caryophyllene	9.01	6.59	9.83	6.85	8.85	6.47	12.76	-	8.73	4.92	5.32	-	10.28	7.86
delta-terpineol	0.31	0.28	0.96	1.07	0.90	1.10	4.15	-	0.95	0.73	-	-	0.15	0.31
alpha-Humulene	0.93	1.21	0.52	-	1.03	-		-	0.46	-	1.23	-	1.38	1.66
alpha-Terpineol	0.91	-	0.45	4.39	0.43	3.62	1.40	-	0.45	4.25	0.44	-	1.33	-
Borneol	-	0.83	-	-	0.41		-	-	0.30	-	-	-	-	0.68
Viridiflorol	3.03	1.59	2.92	-	2.16		1.43	-	2.15	2.07	0.86	-	0.77	1.27
Spathulenol	0.57	-	1.38	-	0.40		1.09	-	-	-	-	-	-	-
Aromadendrene	1.12	0.59	-	0.67	-	0.62	1.57	-	0.88	1.01	0.95	-	-	0.53
Terpinolene	1.02	-	3.16	-	3.31	-	-	-	3.06	-	0.13	-	0.27	1.34
Terpinen-4-ol	-	-	-	-	-		-		-	0.15	-		-	-
Caryophyllene oxide	-	0.89	-	0.21	-		-	-	-	0.92	-	-	-	0.80
alpha.-thujene	-	0.24	-	-	-		-	-	-	-	-	-	-	0.16
1-Octen-3-ol	-	-	-	0.18	-		-		-	-	-		-	0.14

Çizelge incelendiğinde, en yüksek 1,8-sineol oranı (%62.62) çalışmanın ilk yılı, Fd4-13 numaralı klonda bulunmuştur. En düşük 1,8 sineol oranı ise (%43.22), yine çalışmanın ilk yılı Fk5-7'den elde edilmiştir. Çalışmanın ikinci yılında 1,8 sineol oranları %53.01-58.37 arasında değişim göstermiştir. En yüksek 1,8-sineol oranı Fk4-14'den elde edilmiştir.

Anadolu adaçayı uçucu yağ bileşenleri içerisinde önemli bileşenlerden bir diğeri ise beta-pinendir. Klonlar arası beta-pinene oranı %6.04-17.18 arasında değişim göstermiştir. En yüksek beta-pinene oranı Fk4-14'de tespit edilmiştir. En düşük beta-pinene oranı ise Fk5-7'den elde edilmiştir. Ana bileşenlerden beta-Caryophyllene, alpha-Binene ve Camphor ise 2018 yılında sırası ile %5.32-12.76, %3.90-5.39 ve %0.64-8.33 arasında tespit edilirken, 2019 yılında %7.86-4.92, %4.54-4.79 ve %0.64-5.73 arasında tespit edilmiştir. Leontaritou ve ark., (2020), Yunanistan'da Nisan-Mayıs aylarında yaptıkları çalışmada *α*-pinene oranını %4.19, *β*-caryophellene oranını %9.74 olarak belirlemiştir. Sarrou ve ark., (2016) ise nisan-ekim ayları arası yaptığı çalışmada maksimum *β*-pinene (%14.07) ve ß-caryophyllene (%7.17) oranları nisan ayında tespit edilmişlerdir. Çalışmalardan elde edilen sonuçlar arasındaki farklılık temel olarak genetik farklılık ile açıklanabilir. Diğer yandan sıcaklık ve ışığın da etkili olduğu (Kargiolaki ve ark., 1994), ışık ve sıcaklığın ise yöresel olarak farklılık gösterebileceği dikkate alınmalıdır. Ayrıca bitkilerde bulunan uçucu yağ oranı ve bileşenleri, toplanan veya hasat edilen bitki kısımlarına (Bellomaria ve ark., 1992), bitkinin farklı gelişme dönemlerine (Porres-Martínez ve ark., 2014, Kara, 2020), hasat sonrası kurutma sıcaklığına (Venskutonis, 1997, Aydın ve ark., 2019) ve depolama koşullarına (Dinçer ve ark., 2012) göre değişiklik gösterebilir.

Bazı sektörler tarafından istenmeyen bileşenler arasında yer alan alfa ve ß-thujone oranları (Hold ve ark., 2000) klonlara göre farklılık göstermektedir. Fk4-14, Fk3-16, Fd2-9 ve Fd4-13'de alfa-thujone rastlanmazken, ß-thujone eser miktarda rastlanmıştır (Çizelge 7). Uçucu yağdaki bileşenler faydalı olabildiği kadar doza bağlı olarak birçok toksik etkiye de sahip olabilmektedir. Özellikle camphor ve thujone bileşenlerinin toksikolojisi üzerine birçok çalışmalar yapılmıştır. Nikolić ve ark., (2015), yaptığı bir çalışmada camphorun mutajenik etkisi olduğunu ortaya koymuştur. Ayrıca, Narayan & Singh (2012) camphorun özellikle küçük çocuklara toksik etki yapabileceğini bildirirken, Chen ve ark., (2013), düşüklere sebep olabileceğini bildirmiştir. Yine Lachenmeier ve ark., (2006) thujonun merkezi sinir sitemine etki ettiğini ifade ederken Olsen (2000), thujone'nun toksik olduğunu ama merkezi sinir sistemine etki etmek yerine beyni uyarı etkisi olduğunu bildirmiştir. Çalışmada ele alınan geliştirilmiş klonların düşük camphor ve thujone oranları dikkat çekicidir.

Çalışmada ayrıca, morfolojik özellikler, verim değerleri ve uçucu yağ oranları arasındaki etkileşim değerlendirilmiştir. Elde edilen sonuçlara göre 2018 yılında, uçucu yağ oranı ile bitki boyu ve dal sayısı arasında düşük seviyeden negatif korelasyon görülmüştür. Bu sonuç, Elmas ve ark. (2019)'nın yaptığı çalışma ile uyum içerisindedir. Diğer tüm parametreler arasındaki etkileşim pozitif yönde olmuştur. En yüksek pozitif korelasyon taze herba verimi ile taze yaprak verimi arasında tespit edilmiştir (Çizelge 8). Bunu taze herba verimi ile kuru herba verimi arasındaki pozitif korelasyon takip etmiştir.

2019 yılında ise, en yüksek negatif korelasyon uçucu yağ oranı ile dal sayısı arasında tespit edilmiştir. En yüksek pozitif korelasyon ise, taze herba verimi ile kuru yaprak verimi arasındabelirlenmiştir (Çizelge 9). Uçucu yağ oranı ile bitki boyu ve dal sayısı arasında tespit edilen negatif korelasyon, yetersiz ışıklanma ile açıklanabilir. Bitki boyu ve dallanma arttıkça bitkinin iç kısımlarına güneş ışığının yeterince ulaşmaması beklenen bir durumdur. Nitekim Kargiolaki ve ark., (1994), ışık yoğunluğu azaldıkça, uçucu yağ oranının azaldığını bildirmişlerdir.

Çizelge 8. Özellikler arasındaki korelasyon katsayısı (2018 yılı)
Table 8. Correlation coefficient between features (year 2018)

Parametreler	Bitki boyu*	Dal sayısı	Taze herba verimi	Kuru herba verimi	Taze yaprak verimi	Kuru yaprak verimi
Dal sayısı	0.04					
	0.83					
Taze herba verimi	0.44	0.58				
	0.01	0.0012				
Kuru herba	0.47	0.56	0.88			
verimi	0.0108	0.0019	<.0001			
Taze yaprak	0.32	0.55	0.94	0.72		
verimi	0.0986	0.0024	<.0001	<.0001		
Kuru yaprak	0.45	0.42	0.85	0.72	0.82	
verimi	0.0149	0.0280	<.0001	<.0001	<.0001	
Uçucu yağ oranı	-0.05	-0.03	0.03	0.05	0.08	0.08
	0.7986	0.8842	0.8848	0.7791	0.6856	0.6635

*Üstteki değer korelasyon katsayısını, alttaki değer olasılığı (istatistik anlamlılık) göstermektedir

Çizelge 9. Özellikler arasındaki korelasyon katsayısı (2019 yılı)
Table 9. Correlation coefficient between features (year 2019)

Parametreler	Bitki boyu *	Dal sayısı	Taze herba verimi	Kuru herba verimi	Taze yaprak verimi	Kuru yaprak verimi
Dal sayısı	-0.14					
	0.5467					
Taze herba verimi	0.32	0.22				
	0.1687	0.3569				
Kuru herba verimi	0.36	0.17	0.87			
	0.1187	0.4684	<.0001			
Taze yaprak verimi	-0.11	0.26	0.76	0.53		
	0.6202	0.2661	0.0001	0.0152		
Kuru yaprak verimi	0.10	0.38	0.72	0.74	0.52	
	0.6867	0.1021	0.0003	0.0002	0.0190	
Uçucu yağ oranı	-0.23	-0.28	0.05	-0.11	0.16	0.00
	0.3224	0.2271	0.8348	0.6418	0.5082	0.9846

*Üstteki değer korelasyon katsayısını, alttaki değer olasılığı (istatistik anlamlılık) göstermektedir

SONUÇ ve ÖNERİLER

Bu çalışma, klonal olarak geliştirilen C-klonlarının (çeşit adaylarının) bazı morfolojik özellikleri, verim değerleri, uçucu yağ oranı ve bileşenleri belirlemek amacıyla yürütülmüştür. Çalışmada, taze herba verim değeri açısından Fk4-9 numaralı klon ve kuru yaprak verim değeri açısından Fd2-9 numaralı klon öne çıkmıştır. Çalışmada ele alınan geliştirilmiş klonların yüksek 1,8 sineol, düşük camphor ve thujone oranlarına sahip olduğu tespit edilmiştir. Anadolu adaçayı farklı amaçlar için kullanılmakta ve her sektörün talep ettiği kalite kriterleri değişiklik göstermektedir. Sonuç olarak; çay tüketimi için dallı bitki parçaları kullanıldığı için herba verim değeri en yüksek ve thujone oranı düşük olan klon (Fk4-9) çaylık çeşit olarak 'TURGUT' ticari ismi ile tescil ettirilmiştir. Öte yandan sanayide kullanım için, kuru yaprak verimi ve 1,8 sineol oranı yüksek olan klon (Fd2-9) yağlık çeşit olarak 'UYSAL' ticari ismi ile tescili gerçekleştirilmiştir. İleride yapılacak olan çalışmalarda, verimin yanı sıra, bileşenler arası korelasyonun göz önünde tutulması, piyasanın talep ettiği, amaca uygun, hastalıklara dayanıklı standart çeşit geliştirilmesi önem arz etmektedir.

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Araştırmacıların Katkı Oranı Beyan Özeti

Yazarlar makaleye eşit oranda katkı sağlamış olduklarını beyan eder.

Çıkar Çatışması Beyanı

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Assessment of the Relationship Between Humic Acid Contents and Trace Elements of Some Agricultural Soils in Diyarbakır Region by Multivariate Statistical Methods

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ABSTRACT

There are important relationships between humic acid (HA) and the bioavailability, reactions and mobility of trace elements in the soil. For this reason, soils are tried to be improved chemically, biologically and physically with HA applications. In this study, the relationship of humic acid contents of 118 agricultural soil samples from Diyarbakır region with some trace elements (Al, As, Ba, Be, Cd, Fe, Mn, Pb, Sb, Sn, Se, V and P) was evaluated by multivariate statistical analysis. After the soil samples were solubilized by the microwave wet digestion method, the element contents were determined with the ICP OES (Inductively Coupled Plasma Optical Emission Spectrometer) device. SRM NIST 2586 was used as SRM (Standard Reference Material) for the accuracy of the method. Recovery values were found between 91.6% and 105.9% as a result of the analysis. Humic acid was extracted from soils by the International Society for Humic Substances (IHSS) method and determined using a shaker and centrifuge device. For the accuracy of the method, it was tested with Humic Acid Sodium Salt (HA-Na). Pearson correlation and partial correlation analysis were applied to the obtained data set. In addition, multivariate statistical analyses such as multiple regression HCA (Hierarchical Cluster Analysis) and PCA (Principal Component Analysis) were applied. Multiple regression analysis was performed according to the Step-wise method. Manganese and P ($p< 0.01$) were significant when HA was taken as the dependent variable. According to the Pearson correlation coefficient, the correlation between HA and As ($r = -0.282^{**}$) in soil was negative and significant, while Fe ($r = 0.185^{*}$), Mn ($r = 0.273^{**}$)), Sn ($r = 0.242^{*}$), Se ($r = 0.325^{**}$) and P ($r = 0.315^{**}$) were determined as positive and significant. In clustering and PCA analysis, HA, P Mn and Fe were found to be in the same group. The analyses have shown that HA has a positive effect on the plant nutrients in the soil.

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Diyarbakır Yöresindeki Bazı Tarım Topraklarının Hümik Asit İçerikleri ile Eser Elementler Arasındaki İlişkinin Çok Değişkenli İstatistiksel Yöntemlerle Değerlendirilmesi

ÖZET

Hümik asit (HA) ile topraktaki iz elementlerin biyoyarayışlılığı, reaksiyonları ve hareketliliği arasında önemli ilişkiler bulunmaktadır. Bu nedenle topraklar, HA uygulamalarıyla kimyasal, biyolojik ve fiziksel yönden iyileştirilmeye çalışılmaktadır. Bu çalışmada, Diyarbakır yöresinden 118 adet tarımsal toprak örneklerinin hümik asit içeriklerinin bazı iz elementlerle (Al, As, Ba, Be, Cd, Fe, Mn, Pb, Sb, Sn, Se, V ve P) olan ilişkisi çok değişkenli istatistiksel analizlerle değerlendirilmiştir. Toprak örnekleri mikrodalga yaş yakma yöntemiyle çözünürleştirildikten sonra element içerikleri ICP OES (Inductively Coupled Plasma Optical

Toprak Bilimi

Araştırma Makalesi

Makale Tarihçesi
Geliş Tarihi : 02.01.2023
Kabul Tarihi : 01.06.2023

Anahtar Kelimeler
İz element
Hümik asit

Emission Spectrometer) cihazı ile belirlenmiştir. Yöntemin doğruluğu için SRM (Standard Reference Material) olarak SRM NIST 2586 kullanılmıştır. Yapılan analiz sonucunda geri kazanım değerleri %91.6 ile %105.9 arasında bulunmuştur. Hümik asit topraklardan International Society for Humic Substances (IHSS) yöntemiyle ekstrakte edilmiş, çalkalayıcı ve santifrüj cihazı kullanılarak belirlenmiştir. Yöntemin doğruluğu için Hümik Asit Sodyum Tuzu (HA-Na) ile test edilmiştir. Elde edilen veri setine Pearson korelasyonu ve kısmi korelasyon analizi uygulanmıştır. Ayrıca çoklu regresyon, HCA (Hiyerarşik Küme Analizi) ve PCA (Principal Component Analysis) gibi çok değişkenli istatistiksel analizler uygulanmıştır. Çoklu regresyon analizi Step-wise yöntemine göre yapılmıştır. Manganese ve P ($p< 0.01$), HA bağımlı değişken olarak alındığında önemli bulunmuştur. Pearson korelasyon katsayısına göre toprakta HA ile As ($r = -0.282^{**}$) arasındaki ilişki negatif ve anlamlı iken, Fe ($r = 0.185^{*}$), Mn ($r =0.273^{**}$)), Sn ($r = 0.242^{*}$), Se ($r = 0.325^{**}$) ve P ($r = 0.315^{**}$) gibi diğer elementlerle pozitif ve anlamlı olarak belirlenmiştir. Kümeleme ve PCA analizinde HA, P Mn ve Fe'nin aynı grupta olduğu saptanmıştır. Yapılan analizler HA'in toprağın bitki besin elementleri lehinde pozitif etki yaptığını göstermiştir.

Korelasyon
Regresyon
ICP OES

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INTRODUCTION

Soil and water are the most important natural resources for living things and all kinds of life forms and essential food fiber and sheltering production necessary for the continuity of human life (Nebel, 1990; Çobanoğlu, 2001). It is very important to know the nature of the soil, and the interactions of the components in the soil with each other. Understanding the relationship between organic and inorganic substances that make up the main body of the soil is necessary for economically feasible, environmentally friendly and sustainable utilization of soil. Humic acids, an economical form of humus, which is the main component of soil organic matter, can support traditional fertilisation methods with significant influences towards enhancing chemical, physical and biological properties of soil, which have resulted in increasing consumption in the intensive agricultural systems. The addition of humic acids to the soil can stimulate the growth of plants beyond the sustaining mineral nutrients. These advantages of humic acids let it common usage all over the world, due to their readiy accessibility and relatively low cost, in poor organic matter-containing soils (Conte et al., 2005; Tarhan, 2011; Prado et al., 2016; Yang & Hodson, 2019).

Iron, aluminium and manganese oxides; organic matter; phosphates, carbonates and sulphides are important components of soil. The deficiency of certain elements especially those of cationic micro elements are commonly observed in the organic matter scarcity in the soils and plant responds to this stress differently. Some heavy metals mainly essential and beneficial plant nutrients play an important role in the nutrition of plants, animals, and humans, but their excessive concentrations can be toxic. The nutritional functions of humic acids appear especially in the absorption of plant nutrients in nutrient-deficient growth environments. Humic acids adsorb some pesticides applied to the soil, especially herbicides and buffer their toxicity to untargeted organisms and, prevent them from mixing into the ground waters. In addition, it has functions in limiting the mobility and availability of some toxic heavy metals as well as harmful radioactive metals to translocate to the plants (Bozkurt, 2005; Eren, 2020).

Potential toxic metals (As, Ba, Be, Se, V, Sn, Sb, Cd, Pb and Al) are the leading factors that cause pollution as a result of agricultural activities and create greater danger over time. These metals, which cause significant pollution in the soil, not only negatively affect productivity in vegetative production, but also threaten human and animal health by entering the food chain (Dağhan, 2011; Eren & Mert, 2017; Eren, 2019).

As the significance considered, the relationships of humic substances and trace elements in agricultural soils have little attention in the current scientific (Donisa at al., 2003). The aim of this study is to

evaluate the relationship of some trace elements with humic acid with some multivariate statistical methods.

MATERIAL and METHOD

Study Area and Samples

Diyarbakır City is located in North Mesopotamia and in the South-eastern Anatolia Region of Turkey. The total area of Diyarbakır is 15.355 km², of which about 2000 km² is classified as urban. It has the largest urban settlement in Tigris Basin. The continental climate of the area is called as a subtropical plateau climate.

A total of 118 soil samples were collected from agricultural soils according to the sampling criteria in different areas and seasons (May, June and July) shown on the map (Fig 1). The samples were stored at ambient temperature condition in sealed plastic bag to preserve the original quality of the soil. The soil samples were dried in an oven at 80 °C for 12 h. and samples were crushed using a rotary mill at 18 000 rpm and then were packaged in the glass bottles.

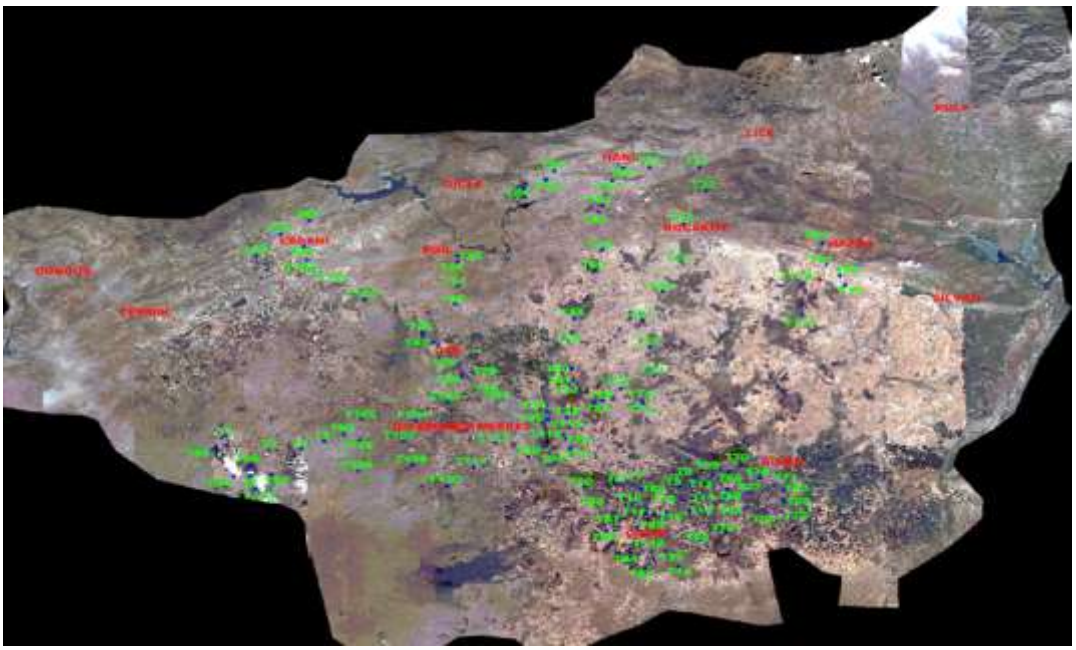

Figure 1. Map of Soil Samples Collected (37.064°, 39.068° latitude, 38.858°, 41.685° longitude)
Şekil 1. *Toplanan Toprak Örneklerinin Haritası (37.064°, 39.068° enlem, 38.858°, 41.685° boylam)*

Reagents and Digestion Procedure

Analytical grade (E. Merck, Darmstadt, Germany) nitric acid (HNO_3, 65%), HCl (37%), HF (40%) and hydrogen peroxide (H_2O_2, 30%) were used for the digestion of soil samples by means of a Milestone Start D microwave digestion system (MW). The procedures were as follows: 0.3 g of soil samples or reference material (SRM 2586 NIST Gaithersburg, MD 20899 SC), the acid mixture (3 mL HNO_3 + 9 mL HCl + 1 mL H_2O_2+ 1 mL HF) were placed in a pressure-resistant PTFE vessels, and was added to each sample and hold on until gas exhausted. The protocol of the MW digestion is given in Table 1. After the digestion procedure, the digests were filtered through Whatman 42 filter paper and diluted to 50 mL with deionized water.

The instrumental operating protocols are given in Table 2 and The Detection and Quantification Limits of Elements, Analytical Wavelengths and Accuracy Assessment of Analysis CRM SOİL-A and SRM 2586 by ICP OES showed in Table 3.

Table 1. Microwave digestion procedure for soil samples
Çizelge 1. *Toprak numuneleri için mikrodalga yakma prosedürü*

Step	*T (min)*	*T (°C)*	*Power (W)*
1	15	150	1200
2	20	150	1200

Accuracy and Precision of Analytical Method

The ICP OES (Thermo ICAP 6300) were calibrated with a multi-element Standard solutions (High-purity, ICV-4, 1408726, Charleston). As, Se and Sb elements were analyzed in hydride system and working standard solutions (10, 20, 40, 60 and 100 µg L^{-1}) were prepared by adding of ascorbic acid and 1.0 % KI and then they were diluted with 10 % HCl as the samples. The quantification (LOQ) and detection limits (LOD) for each metal were calculated as follows: 10 independent analyses of a blank solution spiked with the metal at a level of lower concentration of the analytical curve were performed. The quantification (LOQ) and detection limits (LOD) were calculated from the standard deviation (α) of all measurements

determinations (LOD = 3 x (α) and LOQ = 10 x (α). The certified standard reference materials (SRM 2586 NIST Gaithersburg, MD 20899 SC) and (CRM-SOIL-A, Lot:1309920 Charleston) were used to assess the accuracy and precision of the method.

Table 2. Instrumental Operating Conditions Using ICP-OES
Çizelge 2. ICP OES İçin Cihaz Çalışma Koşulları

Parameters	*Working conditions*	*Hydride System conditions*
Power	1150 W	1350 W
Rotation speed of pump for Flush	100 rpm	50 rpm
Rotation speed of pump for analysis	50 rpm	30 rpm
Pump rest time	5 sec.	5 sec.
Purge gas	Argon	Argon
Plasma gas	Argon	Argon
Plasma flow	12 L min^{-1}	16 L min^{-1}
Auxiliary flow	0.5 L min^{-1}	0.5 L min^{-1}
Nebulizer flow	0.6 L min^{-1}	0.3 L min^{-1}
Plasma viewing	Radial, Axial	Axial
Source equilibrium delay	20 sec.	20 sec.

Table 3. The Detection and Quantification Limits of Elements, Analytical Wavelengths and Accuracy Assessment of Analysis CRM SOİL-A and SRM 2586 by ICP OES
Çizelge 3. *Elementlerin Tayin ve Dedeksiyon sınırları, Dalgaboyları ve CRM SOİL-A ile SRM 2586 Referans Maddelerinin ICP OES ile Analizlerinin Sonuçları*

			CRM-SOİL-A			*SRM - 2586*		
Chemical Elements (λ nm)	*LOD (µg g^{-1})*	*LOQ (µg g^{-1})*	*Found values (µg g^{-1})*	*Certified values (µg g^{-1})*	*Recovery (%)*	*Found values (µg g^{-1})*	*Certified values (µg g^{-1})*	*Recovery (%)*
Al (396.152)	0.0716	0.2389	495±5.500	500±3.000	99	63526±540	66520±760	95
As (189.042)	0.0016	0.0050	0.19±0.002	0.20±0.010	95	8.2±2.200	8.7±1.50	94
Ba (493.409)	0.0015	0.0051	4.85±0.060	5.00±0.050	97	402±24.0	413±18.0	97
Be (234.861)	0.0005	0.0017	-	-	-	1.32	1.4	94
Cd (214.438)	0.0005	0.0018	0.0029±0.00025	0.003	97	2.60±0.620	2.71±0.54	96
Fe (259.940)	0.0190	0.0634	203.6±2.500	200±1.00	102	50315±962	51610±890	97
Mn (257.610)	0.0017	0.0057	0.098±0.002	0.100±0.001	98	1024±32.0	1000±18.0	102
Pb (261.418)	0.0227	0.0759	0.38±0.030	0.40±0.020	95	412±13.0	432±17.0	95
Sb (206.833)	0.0067	0.0224	0.031±0.003	0.030±0.001	103	-	-	-
Sn (189.918)	0.0037	0.0123	-	-	-	-	-	-
Se (196.090)	0.0030	0.0100	0.0095±0.0008	0.010±0.001	95	0.55±0.090	0.6	92
V (292.402)	0.0077	0.0259	0.98±0.050	0.100±0.005	98	152±12.0	160	95
P (177.495)	0.1682	0.5606	10.6±0.080	10.0±0.100	106	1029±96.0	1001±77.0	103

Isolation of Humic Acid From Soil

The humic acid (HA) coverage of soil (T) samples were studied using extraction techniques reported by the International Society for Humic Substances (IHSS) (Schnitzer & Khan 1972; Stevenson, 1982). 1 g of Humic acid sodium salt was weighed into the centrifuge tube and 50 ml of 0.5 N NaOH was added. 200 rpm for 20 hours at 25 °C in a shaker and agitated. After the agitation was completed, the extract separated by decantation was centrifuged at 6000 rpm for 30 minutes and all the suspended solid part was separated from the solution phase. This process was continued until the solution turned into a light tea color. All the extracts were then combined and the alkaline solution completely separated from the solid phase was acidified with 6 M HCl to a pH between 1.0 and 2.0 (pH meter, Inolab 720). Humic acid precipitated because its solubility was very low between pH 1.0 and 2.0. After the solution was kept in the refrigerator for 24 hours, the humic acids became visible and dark colored humic acids were obtained. Humic acid was dried in an oven at 65 °C for 24 hours and its amount was calculated according to the formula below.

The same procedures were applied to 118 soil samples, the amount of soil was taken as 50 g.

% Humic Acid = (m/n).100

m: sample weighed after oven (g)

n: ODW of the sample taken (Oven dry weight value, g)

Statistical Analysis

The data were subjected to multivariate statistical analysis procedures by using SPSS 21.0 statistical package programs. In order to reveal the coherence between the measured parameters and differentiate the origin of metals Pearson correlation, multiple regression, cluster analysis (CA) and principal component analysis (PCA) were performed after normalizing the data set (Lu et al. 2012; Zhang, 2006). The Kaiser-Meyer-Olkin (KMO) coefficient above 0.60 showed that our data set was suitable for PCA. The Barlett Test of Sphericity significance value was found to be 0.01. PCA results were interpreted according to hypothetical chemical element sources (Peris et al., 2008; Yuan et al., 2013). Hierarchical CA was performed according to Ward method (Cai et al., 2012; Chen et al., 2012; Franco-Uria et al., 2009; Mico et al., 2006; Xia et al., 2011). The results are summarized in a dendrogram. As the K-S (Kolmogorov Smirnov) normality test revealed that the data-set can be considered normally distributed ($p>0.05$) unless otherwise the related data set were transformed by log transformation. Differences between applications were considered significant if $p<0.05$ and when the analysis results were below the LOD, they were accepted as the half of the LOD.

RESULTS and DISCUSSION

In this study, a total of 118 soil samples collected from agricultural lands in different regions of Diyarbakır determined the relationships between humic acid (HA) and trace elements (Al, Fe, Ba, P, Sn, Cd, Pb, V, Sb, As, Mn, Be, Se). Statistical analysis such as Pearson correlation multiple regression analysis, HCA (Hierarchical clustering analysis, Fig. 5-6) and PCA (Principle component analysis, Fig. 3) were applied to results and The amount of humic acid obtained from soil samples showed in Table 4.

Pearson correlation was applied to results and the relationship in between HA and elements evaluated by coefficient showed a correlation of elements and humic acid with each other. Pearson correlation coefficient indicated that As showed a negative correlation with HA, while Fe, Mn, Sn and Se showed a positive correlation with humic acid and all variables stuations were showed in Table 6.

Partial correlation coefficients showed variables situations when HA stabilized and all coefficients were showed in Table 7.

The descriptive statistics for the total trace element concentration of experimental soils showed in Table 5.

The results of HA were not showed normal distribution therefore log transformation applied to results and results were showed normalized distribution (Fig. 2).

Principal component Analysis (PCA) was applied to data-sets before this KMO (Kaiser-Mayer-Olkin) coefficient and Barlett globality test were determined. These coefficients showed data suitable for component analysis. KMO coefficient of 0.624 was found and Barlett globality test was significant at $p\leq 0.01$. When scree plot Eigenvalue bigger than 1 value, it were found as 5 components, P1: Mn, Fe, Pb, Cd, Sb, P2: HA and P, P3: V, Al, As, P4: Be, Se, P5: Ba and Se (Fig.3)

Table 4. The amount of humic acid obtained from soil samples
Çizelge 4. *Toprak örneklerinden elde edilen hümik asit miktarı*

Samples	*HA (%)*	*Samples*	*HA (%)*	*Samples*	*HA (%)*	*Samples*	*HA (%)*	*Samples*	*HA (%)*
T1	0.360	**T25**	0.102	**T49**	0.390	**T73**	0.171	**T97**	0.092
T2	1.020	**T26**	0.142	**T50**	0.142	**T74**	0.152	**T98**	0.040
T3	0.133	**T27**	0.096	**T51**	0.440	**T75**	0.095	**T99**	0.085
T4	0.112	**T28**	0.170	**T52**	0.106	**T76**	0.113	**T100**	0.076
T5	2.340	**T29**	0.455	**T53**	0.134	**T77**	0.106	**T101**	0.070
T6	0.133	**T30**	0.146	**T54**	0.200	**T78**	0.130	**T102**	0.087
T7	0.390	**T31**	0.126	**T55**	0.230	**T79**	0.103	**T103**	0.230
T8	0.146	**T32**	0.211	**T56**	0.080	**T80**	0.133	**T104**	0.158
T9	0.076	**T33**	0.170	**T57**	0.076	**T81**	0.120	**T105**	0.177
T10	0.115	**T34**	0.130	**T58**	0.122	**T82**	0.132	**T106**	0.172
T11	0.230	**T35**	0.144	**T59**	0.151	**T83**	0.105	**T107**	0.166
T12	0.112	**T36**	0.090	**T60**	0.441	**T84**	0.156	**T108**	0.082
T13	0.121	**T37**	0.070	**T61**	0.092	**T85**	0.142	**T109**	0.360
T14	0.155	**T38**	0.141	**T62**	0.181	**T86**	0.130	**T110**	0.933
T15	0.074	**T39**	0.221	**T63**	0.133	**T87**	0.095	**T111**	0.110
T16	0.142	**T40**	0.154	**T64**	0.132	**T88**	0.097	**T112**	0.126
T17	0.190	**T41**	0.126	**T65**	0.232	**T89**	0.113	**T113**	0.172
T18	0.112	**T42**	0.110	**T66**	0.174	**T90**	0.220	**T114**	0.131
T19	0.121	**T43**	0.087	**T67**	0.190	**T91**	0.116	**T115**	1.230
T20	0.100	**T44**	0.121	**T68**	0.240	**T92**	0.076	**T116**	0.910
T21	0.133	**T45**	0.235	**T69**	0.122	**T93**	0.152	**T117**	0.210
T22	0.164	**T46**	0.090	**T70**	0.151	**T94**	1.920	**T118**	0.111
T23	0.100	**T47**	0.086	**T71**	0.177	**T95**	0.104		
T24	0.122	**T48**	0.132	**T72**	0.160	**T96**	1.080		

*:significant (P<0.05), **:significant (P<0.01)

Table 5. Descriptive statistics for total trace element concentration of experimental soils
Çizelge 5. *Toprakların toplam iz elementi içeriğine ait tanımlayıcı analizler merkezi eğilim ve dağılım ölçüleri*

Elements	*N*	*Mean*	*Median*	*Standard deviationnn*	*standard error*	*Distorti*	*Stickiness*	*Min.*	*Max.*	*CV (%)*
Al	118	45967.9	44811.5	16117.9	1483.7	-0.401	0.129	1522	81696	35.06
As	118	6.77	7.29	4.42	0.406	-0.083	-0.839	0.0008	16.5	65.24
Ba	117	209.9	203	75.03	6.93	1.023	3.072	63	555	35.74
Be	116	0.746	0.74	0.371	0.034	1.734	8.641	0.0003	2.88	49.74
Cd	118	0.968	0.945	0.487	0.044	-0.161	-0.311	0.00025	2	50.27
Fe	118	46587.7	45236	14651.3	1348.7	0.470	0.327	13367	86647	31.44
Mn	118	1249.6	1126.5	529.4	27.5	1.352	1.474	419	3006	42.36
Pb	118	40.5	40.25	11.3	1.03	0.511	0.778	15	74.8	27.85
Sb	118	4.43	4.37	1.81	0.16	0.767	0.680	1.54	9.92	40.79
Sn	118	0.495	0.435	0.248	0.02	1.484	3.029	0.08	1.55	50.10
Se	115	0.856	0.83	0.464	0.04	1.011	2.741	0.093	2.8	54.20
V	89	134.1	133	51.8	5.49	-0.041	-0.226	5.83	252	38.62
P	118	860.2	787	304.6	14.44	1.779	4.578	200	2154	35.40
HA (%)	118	0.221	0.133	0.316	0.029	4.643	24.471	0.040	2.340	143.63

Tablo 6. Humik asit ve toplam iz element konsantrasyonları arasındaki Pearson korelasyon katsayısı
Çizelge 6.*Pearson correlation coefficients between humic acid and total trace element concentrations*

	Al	*As*	*Ba*	*Be*	*Cd*	*Fe*	*Mn*	*Pb*	*Sb*	*Sn*	*Se*	*V*	*P*	*HA*
Al														
As	-0.178													
Ba	0.398**	-0.411**												
Be	-0.035	-0.060	-0.043											
Cd	0.020	-0.030	0.005	-0.027										
Fe	0.623**	-0.405**	0.428**	0.054	0.032									
Mn	0.374**	-0.595**	0.471**	0.021	0.104	0.734**								
Pb	0.105	0.008	0.129	0.057	0.338**	0.245**	0.225*							
Sb	0.181*	-0.311**	0.082	0.231*	0.352**	0.441**	0.515**	0.252**						
Sn	0.112	-0.279**	0.193*	-0.220*	-0.175	0.250**	0.208*	0.023	0.066					
Se	0.135	-0.066	0.025	0.179	-0.007	0.447**	0.368**	0.140	0.299**	0.224*				
V	-0.451**	-0.399**	0.100	0.029	0.043	-0.088	0.248*	-0.237*	-0.005	0.299**	0.103			
P	-0.081	0.143	-0.071	-0.113	0.101	-0.035	-0.042	-0.125	-0.046	0.161	0.138	0.111		
HA	-0.004	-0.282**	0.002	-0.098	0.082	0.185*	0.273**	0.011	0.087	0.242**	0.325**	0.040	0.315	

Table 7. The Partial correlation relationship in between elements and stabilized humic acid in soil samples
Çizelge 7.*Toprak örneklerinde elementler ve stabilize hümik asit arasındaki kısmi korelasyon ilişkisi*

	Al	*As*	*Ba*	*Be*	*Cd*	*Fe*	*Mn*	*Pb*	*Sb*	*Sn*	*Se*	*V*	*P*
Al													
As	0.020												
Ba	0.268*	-0.324**											
Be	-0.010	-0.160	0.005										
Cd	0.423**	-0.045	0.135	-0.246*									
Fe	0.636**	-0.221*	0.310**	0.223*	0.555**								
Mn	0.283**	-0.479**	0.361**	0.204	0.424**	0.663**							
Pb	0.265*	-0.131	0.249*	-0.075	0.465**	0.509**	0.507**						
Sb	0.188	-0.141	-0.037	0.289**	0.244*	0.521**	0.579**	0.300**					
Sn	-0.002	-0.141	0.120	-0.270*	0.169	0.070	0.109	0.118	-0.015				
Se	0.094	0.068	-0.127	0.257*	-0.098	0.323**	0.216	0.150	0.276*	0.293**			
V	-0.567**	-0.310**	0.005	0.145	-0.124	-0.273*	0.119	-0.206	-0.097	0.225*	0.006		
P	-0.138	0.379**	-0.173	-0.054	-0.055	-0.318**	-0.338**	-0.303**	-0.157	-0.014	-0.051	-0.084	

When we performed multiple regression analysis with Stepwise Method, it was possible to develop two models with P and Mn at significance level $p<0.01$ when the dependent variable was HA. In the regression model, $R^2 = 0.407$, $F = 23.69$ were found. The model equation is shown below; HA= -0.294+0.000201*Mn+0.000282*P. The R^2, determination coefficient, F is Fisher coefficient, N is

the sample number. As the HA independent variable, The HA can explain a significant portion of variation (R^2) in the dependent variable, as shown in Fig 4 for As, Mn, P, and Se in the regression models.

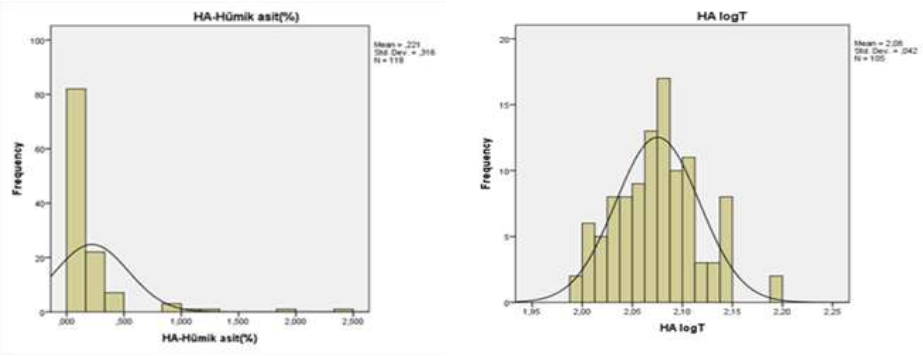

Figure 2. Histograms with normal distribution curve for the original and log-transformed data set
Şekil 2. *Orijinal ve log dönüşümü ile normalleştirilmiş veri setinin histogram ve normal dağılım eğrisi*

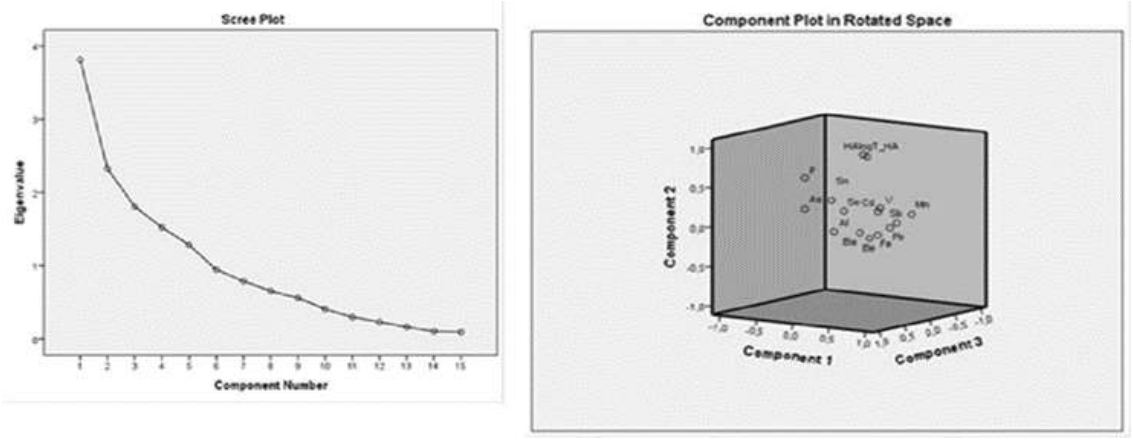

Figure 3. Scree plot and component graphs
Şekil 3. *Scree plot ve bileşen grafikleri*

Statistical data of HA and some elements

According to the results of the statistical analysis data, the relationship between humic acid and phosphorus element was better than other elements. A significant portion of soil P is bound to soil organic matter (Stevenson, 1994) and soil organic matter can only accumulate in recalcitrant fractions such as HA, fulvic acids and humin (Usta, 1995). According to the results, minimum and maximum values of P in soil were between 200- 2154 mg kg^{-1}, and the CV values were found as 35.40%. In the factor (PCA) analysis, HA and P were in the same principal component (N:118). This indicates above mentioned co-occurrence and co-accumulation fact of P and soil organic matter. According to Pearson correlation, P was found to show a significant positive correlation with HA (r = 0.315**). In the multiple regression analysis, a significant regression model was observed with P and Mn (P <0.01) when HA was the dependent variable with Step-wise regression procedures. The hierarchical cluster and factor analysis results pointed out similar coherence between P and HA.

The descriptive statistics describing the nature and occurrence of some elements and humic acid were calculated and briefly given below.

Aluminium: The mean, minimum and maximum values of Al were 45967.9, 1522, and 81696 mg kg^{-1}, respectively. The coefficient of variation (CV) was found to be 35.06%. In factor analysis, Al, V, and As were in the same in the same principal component (N: 118), in addition, Al, Ba, Cd, Mn, and Fe were also in close relation in the CA (Cluster analysis). The PCA and correlation analysis were relived a significant correlation coefficients Al-V (0.81), As-Fe (0.85), As-Pb (0.56), Cd-Sn (0.71), Fe-Mn (0.70), Fe-V (0.59) are reported in the literature (Rodriguez et al., 2015). The PCA and CA analysis aggregate Fe and Mn always in the same group due to the accumulation of these elements in the soil formation processes. A significant correlation was found between Fe and humic acid (r = 0.383*) according to Gürel et al. (2015). It can be stated that the soil organic matter and Fe concentrations of soils developing from any parent material is largely dependent on the weathering levels controlled by mainly water and thermal regime of any specific

location (Weil and Brady, 2016; Usta, 1995). In general, the more weathering causes the higher organic matter, including HA and sesquioxides (mainly Fe, Al oxi(hyd)oxides in the soil) accumulation.

Regression graphs of some elements showing significant correlation with humic acid showed in Fig.4.

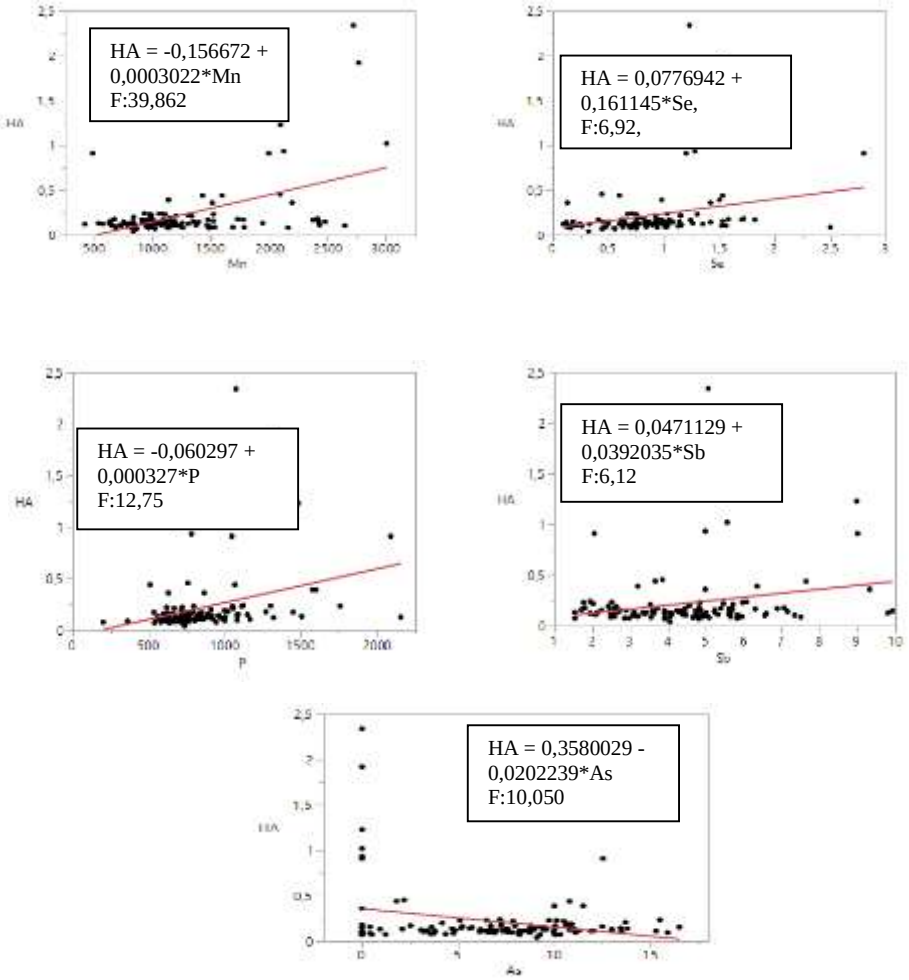

Figure 4. Regression graphs of some elements showing significant correlation with humic acid
Şekil 4. *Hümik asit ile önemli korelasyon gösteren bazı elementlerin regresyon grafikleri*

Cadmium: The mean, minimum and maximum values of Cd were found as 0.968, <0.0005, and 2 mg kg^{-1}, respectively. The CV value was 50.25%. In factor analysis, Cd, Mn, Fe, Pb, and Sb were the same factor and in cluster analysis Cd, Mn and Fe were in the same group (N: 118). This can be explained as Fe and Mn oxides have excessive adsorption capacity for Cd. (Alloway, 1996).

Humic Acid: The mean, minimum and maximum values of HA were determined as 0.221, 0.040, and 2.340 (%), respectively. According to Şahin (2012), HA reported a HA abundance of 29.3% in Chernozem soil while Gürel et al. (2015) found HA content between %0.35 and %2.09 in the Blacksea region's soils, moreover according to Tokay and Yaşar (2008) in Küçükkuyu and Burhaniye soils HA were found %0.38 and %1.23 respectively. CV value of humic acids in the soils was found as 143.63% and the range and variation of the results were too large, the data were far from normal distribution thus log transformation was applied to normalize the data. In factor and HCA analysis HA and P were the same group (N: 118).

Hierarchical clustering analysis was performed and the summarized version was given in the form of a dendrogram. When all variables are taken into account, the clustered version of the samples and the clustered version of the variables on the basis of the samples are given in Figure 5 and Figure 6.

CONCLUSIONS

Heavy metals were found to be above the permissible threshold values in some soils, but generally, the soils of the region were found to be in safe range for the respective trace elements. Both Pearson correlation analysis and multiple regression, PCA and CA analyses showed that the elements useful to the soil (P, Fe, Mn and Se) were more attached to the soils with

HA, whereas toxic elements such as As and Cd were less bound. Naturally, this is an important condition for the healthy growth of plants. Therefore, it has been concluded that HA can be a good regulator for agricultural soils and can be used for this purpose.

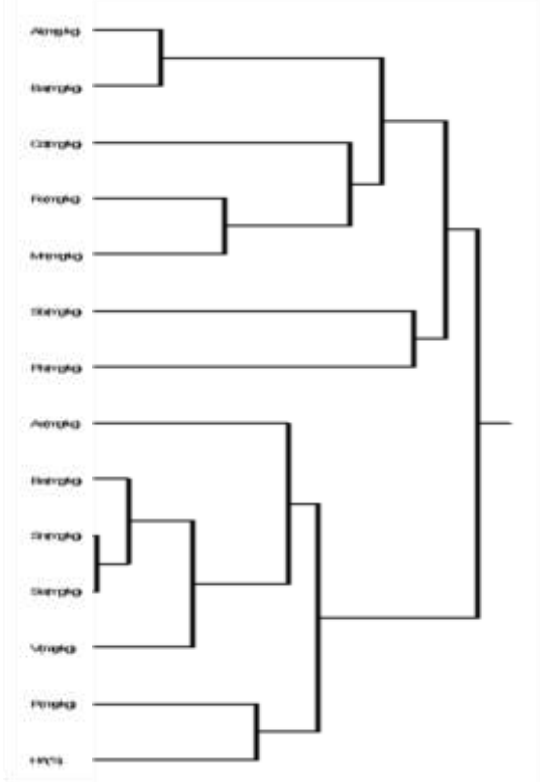

Fig. 5. Dendrogram of clustering analysis of variables

Şekil 5. *Değişkenlerin kümeleme analizinin dendrogramı*

Except for Karacadağ region, all agricultural soils in Diyarbakır province were found to be poor in terms of humic acid (Table4, Fig 1). Thus, it is advisable for producers to improve their soils with HA fertilizers.

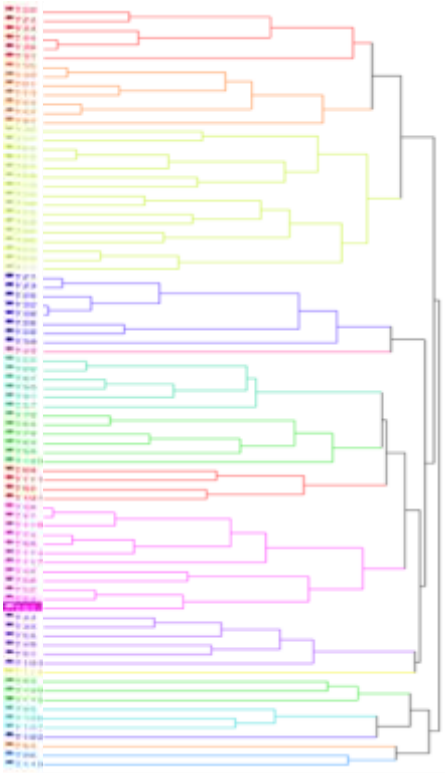

Fig. 6. Dendrogram of clustering analysis of all samples

Şekil 6. *Tüm örneklerin kümeleme analizinin dendrogramı*

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Contribution rate statement summary of researchers

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

Conflict of interest statement

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

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Antifungal Activity, Total Phenolic Content and Antioxidant Activity Properties of Some Spices Extracts as Alternative Natural Antimicrobial Agents

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ABSTRACT
In this study, extracts were obtained from rosemary, anise, cinnamon, ginger, peppermint, turmeric, fennel, clove, laurel leaves and thyme. The total phenolic content amount, antioxidant activity value and antifungal properties of these extracts were aimed to determine the extracts. Among the extracts, clove, cinnamon, turmeric and ginger were superior in terms of total phenolic content values, clove, cinnamon, turmeric, ginger, laurel leaves and rosemary extracts were superior in terms of antioxidant activity. The highest inhibition zone diameters among mold strains were determined by the use of extracts of cinnamon, turmeric, ginger, clove and laurel leaves against *Aspergillus oryzae*, *Penicillium digitatum* and *Aspergillus niger* strains. The results suggested the potential use of cinnamon and clove extracts as natural agents.

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Alternatif Doğal Antimikrobiyal Ajan Olarak Bazı Tıbbi Bitki Ekstraktlarının Antifungal Aktivitesi, Toplam Fenolik Madde İçeriği ve Antioksidan Aktivite Özellikleri

ÖZET
Bu çalışmada biberiye, anason, tarçın, zencefil, nane, zerdeçal, rezene, karanfil, defne yaprağı ve kekik' ten ekstrakt elde edilmiştir. Bu ekstraktların toplam fenolik madde miktarı, antioksidan aktivite ve antifungal özelliklerinin belirlenmesi amaçlanmıştır. Ekstraktlardan karanfil, tarçın, zerdeçal ve zencefil'in toplam fenolik madde miktarı daha yüksek iken antioksidan aktivite değerleri açısından karanfil, tarçın, zerdeçal, zencefil, defne yaprağı ve biberiye ekstraktları daha üstün olmuştur. Küf suşlarına karşı en yüksek inhibisyon zon çapları, tarçın, zerdeçal, zencefil, karanfil ve defne yaprağı ekstraktlarının *Aspergillus oryzae*, *Penicillium digitatum* ve *Aspergillus niger* suşlarına karşı belirlenmiştir. Tüm sonuçlar değerlendirildiğinde, tarçın ve karanfil ekstraktlarının doğal koruyucu ajan olarak potansiyel kullanımının yüksek olduğu tespit edilmiştir.

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INTRODUCTION

Antioxidant and antimicrobial substances have essential effects in preventing spoilage and increasing food's shelf life, quality, and safety in the food industry. Synthetic agents have toxicity, high costs, and less effect than natural agents (Ansari et al., 2013). Also, synthetic agents cause different negative health effects such as diabetes, allergenic reactions, asthma, hyperkinesis, cancer and cardiovascular diseases (Tewari et al., 2020).

Natural preservatives can be obtained from different sources as spices, animals and microbial (Ribes et al., 2018). Spice sources have important antifungal effects thanks to secondary metabolite components such as phenolic compounds, essential oils, flavonoids and alkaloids (Ciocan & Bara, 2007). Bioactive compounds having antimicrobial effects derived from spices are eugenol in cloves, thymol in thyme, carvacrol in oregano, vanillin in vanilla, allicin in garlic, cinnamic aldehyde in cinnamon and allyl isothiocyanate in mustard (Lopez-Malo et al., 2005).

The dominant active ingredient of anise is anethole;

eugenol and (E)-cinnamyl acetate of cinnamon; curcumin of curcuma; eugenol of ginger; transanethol of fennel; eugenol of clove and laurel leaves; bornyl acetate of rosemary; catechin of mint and carvacrol of thymeThese spices can be used as anticancer, antidepressant, antiviral, nematocidal, mutagenic, antispasmodic, antifungal, antibacterial and anti-inflammatory agents thanks to this bioactive component (Luigia & Giuseppe, 2005; Salehi Surmaghi, 2006; Fecka & Turek, 2007; Al-Bayati, 2008; Shojaii & Abdolahi Fard, 2011; Ribeiro-Santos et al., 2015; Koldaş et al., 2015; Kumar et al., 2011; Kumar et al., 2019; El-Saber Batiha et al., 2020). The spice extraction process ensures a great advantage that the removal of unwanted components as well as the preservation of bioactive components. For this reason, the evaluation of spice sources in extract form is safer, more effective and easier than in whole spice form (Karakaş, 2003).

Spice extracts are the new alternative to protect against the harmful effects of synthetic antioxidants and chemical preservatives and to prevent spoilage in the food industry. The total phenolic content of spice extracts is directly proportional to antioxidant activity and antifungal activity capacity. In addition to their antifungal activities, bioactive compounds including phenols, alcohols, aldehydes, ketones, ethers and hydrocarbons increase the storage stability of food products thanks to their antimicrobial and antioxidant properties (Coşkun, 2021).

The antifungal activity of bioactive components occurs through two different mechanisms of action. One of these is that the components cause retraction of the mycelium cytoplasm with an attack on the cell wall, eventually causing cell membrane disruption, resulting in the death of the hyphae. Another mechanism is the death of the microorganism as a result of the intervention of bioactive components in the enzymatic reactions of cell wall synthesis, which affect the morphogenesis and growth of bacteria or molds (Carmo et al., 2008).

Considering the above-mentioned negative aspects of chemical preservatives, natural preservatives will have a great place in the food industry. For this reason, this study investigated the total phenolic content, antioxidant activity and antifungal activity (inhibition zone diameter, minimum inhibitory concentration and minimum fungicidal concentration) properties of spice extracts as natural preservatives and aimed to determine the spice extracts with the best properties that can be an alternative to chemical preservative food additives.

MATERIAL and METHOD

Materials

Rosemary, anise, cinnamon, ginger, peppermint, turmeric, fennel, clove, laurel leaves and thyme used in the study were obtained from a local market in Konya, Turkey. *Aspergillus flavus*, *Aspergillus oryzae*, *Aspergillus niger*, *Penicillium digitatum* and *Penicillium camemberti* were obtained from Tokat Gaziosmanpaşa University Plant Protection Department and Yıldız Technical University Food Engineering Department.

Methods

Preparation of ethanolic extracts

Spice extract production was carried out by modifying the method of Grigoras et al. (2013). The raw materials were ground into powder in a laboratory grinder (Sinbo SCM2934, Turkey). Firstly, 100 g of sample with 600 mL solvent were mixed and subjected to agitation in a shaking water bath (Daihan Wisebath WSB-30, Gangwon, South Korea) for 3 h at 170 rpm at 25±1°C for ethanolic extraction and was filtered with whatman filter paper no: 1. Then, agitated with 400 mL of ethanol for 6 h and kept at room temperature for 12 h and the filtration process was repeated at the end of the period. Evaporation was carried out using a rotary evaporator (Büchi R20, Switzerland) at 40°C. Extracts were stored at 4°C until analysis.

Total phenolic content

The total phenolic content was performed colorimetrically as a method described by Maurya and Singh (2010) with slight modifications. For analysis, extracts of rosemary, anise, cinnamon, ginger, mint, turmeric, fennel, laurel and thyme were diluted 4000-fold and clove extract was diluted 7000-fold with pure ethanol. Briefly, 500 µL of extract samples are added 2.5 mL Folin -Ciolcalteu reagent (1/10, v/v in water) and 2 mL of sodium carbonate solution (7.5%, w/v, in water). The mixture was incubated for 60 min at room temperature (25±1°C) in darkness. After incubation, the absorbance was read against pure ethanol (≥ 99.5 %) at 760 nm with a UV-vis spectrophotometer (Hitachi-U1800, Japan). The total phenolic content value of each extract was expressed as milligram gallic acid equivalents per gram of extract (mg EAG g^{-1} extract).

Antioxidant activity

The antioxidant activity analyses of extract samples were estimated according to the DPPH (2-2-Diphenyl-2-picrylhydrazyl) methods described by Ahmad et al. (2013). The 4000-fold diluted extract samples (1 mL) were mixed with solution of DPPH (2 mL) and kept for 30 mins at room temperature (25±1°C). After incubation, The absorbance value was measured against pure ethanol (≥ 99.5 %) at 517 nm with a UV-vis spectrophotometer (Hitachi-U1800, Japan). The percentages inhibition of the DPPH radical were

calculated using the following Eq. (1).

% İnhibition=[(Abscontrol - Abssample)/Abscontrol] × 100(1)

Antifungal assay

Fungal cultures

Aspergillus flavus, *Aspergillus oryzae*, *Aspergillus niger*, *Penicillium digitatum* and *Penicillium camemberti* cultures were used as test micro-organisms. Molds were subcultured on Potato Dextrose Agar (PDA) plates and incubated at 26.5°C for 7 days. Then, the spores were suspended by adding 10 mL of 0.01% Tween 80 (Sawai and Yoshikawa, 2004). The concentration of spores was adjusted to equal the 0.5 McFarland standard with absorbance 0.400-0.450 (1-5 × 106 CFU mL^{-1}) at 400 nm wavelength in a UV-spectrophotometer (Hitachi-U1800, Japan) (Kızılkeçili, 2007).

Disc diffusion methods

The antifungal activity was analyzed with agar disc diffusion method. Firstly, 0.1 mL of mold suspension adjusted according to 0.5 McFarland standard were inoculated over agar with a sterile pipette (Research Plus, Eppendorf, Germany) and spread uniformly using a glass spreader. Firstly, stock ethanol solutions of concentration 100 and 200 mg mL^{-1} of extract samples were prepared from each extract. Then, on the surface of plate were placed 4 discs, and 20 µL from 100 mg mL^{-1} extract samples, 200 mg mL-1 extract samples, negative controls (ethanol) and positive controls (2 mg mL^{-1} calcium propionate) were impregnated on the disk. The plates were incubated at 25°C for 72 h. After incubation, observations were recorded as the diameter of growth inhibition around the discs and were expressed in millimeters.

Minimum inhibition concentration (MIC) and minimum fungicidal concentration (MFC)

The MIC values of the extract samples were determined by the agar dilution method. Stock solutions of dissolved extract in ethanol were added to sterile melted PDA at 50°C to give a final concentrations of 20, 10, 5, 2.5, 1.25, 0.625, 0.312, 0.156 and 0.078 mg mL^{-1} with PDA at 50°C. The resultant dilutions were poured into petri plate in the amount of 12-15 mL and waited for 10 min. Then, 0.1 mL mold suspension prepared separately for *Aspergillus flavus*, *Aspergillus oryzae*, *Aspergillus niger*, *Penicillium digitatum* and *Penicillium camemberti* was transferred in petri dishes and was spread homogeneously on the petri plate with a glass spreader. Finally, the petri dishes were incubated at 25°C for 48-72 h and the lowest concentration value at which no growth was determined as the MIC value (mg mL^{-1}).

Minimum fungicidal concentration was defined as the lowest concentration with no visible growth indicating 99.9% killing of the original inoculum. For MFC, the samples in the petri plate with no growth were transferred onto fresh PDA medium by streaking with a loop. Then, these plates were incubated at 25°C for 48-72 h, and the lowest concentrations without visible growth were recorded as MFCs.

Statistical analysis

For the statistical analysis, the JMP statistical program, version 5.01 (SAS Institute Inc., Cary, NC, USA) was used. The average values of the main variation sources were compared at $p < 0.05$ significance levels.

RESULTS and DISCUSSION

When the spice extraction in the literature is examined; water, ethanol and methanol are used as solvents in the extraction process and stated that the extract yield is higher with the ethanolic extraction process and antimicrobial properties than other solvents. Most importantly, ethanol is preferred among other chemical solvents as methanol due to its low toxicity (Ballesteros et al., 2014). Considering these data, ethanol was preferred as a solvent in the extraction process of spices used in the study.

Total phenolic content

The total phenolic content values of the extract samples obtained from spice sources are given in Table 1. The total phenolic content values of extract samples varied between 62.22 mg GAE g^{-1} and 461.38 mg GAE g^{-1}. When the results were examined, the highest total phenolic content value was determined with clove (461.38 mg GAE g^{-1}). Clove was followed by cinnamon (172.53 mg GAE g^{-1}), turmeric (173.53 mg GAE g^{-1}) and ginger (181.75 mg GAE g^{-1}), which gave statistically similar values. In a study investigating the amount and quality of the curcumin component in turmeric ethanolic extract, Himesh et al. (2011) reported that turmeric contains many phytochemical components, including curcumin, demetoxy curcumin, bisdemethoxycurcumin, zingiberene, curcum-enol, curcumol, eugenol, tetrahydrocurcumin, turmerine, turmerone and turmerin-onols, and besides, curcumin is found hydrophobic in nature and freely soluble in dimethylsulfoxide, acetone, ethanol and oils. Therefore, they applied ethanolic extraction to obtain a high percentage of curcumin component, which constitutes an important proportion of turmeric extract components. The lowest total phenolic content amount among the extract samples was obtained from anise with 62.22 mg GAE g^{-1}. The solubility of phenolic compounds is affected by solvent type, the polymerization degree of phenolics, phenolics-other ingredients interaction and insoluble complex formation (Falleh et al., 2008). Considering all these

different values, the total phenolic content values can effect significantly with different intrinsic and extrinsic factors, including the composition and amount of phenolics, spice genetics and varieties, soil and growing conditions, maturity and harvest conditions (Jeffery, 2003). The difference in total phenolic content amount may be due to the various efficiencies of extraction solvents to dissolve endogenous compounds (Anwar et al., 2009). Chirinos et al. (2007) and Chandra et al. (2014) reported that various extraction factors such as extraction time, sample, solvent ratio, temperature, pH, solvent type and extraction method, as well as crop growing factors such as soil, irrigation and climatic conditions, were effective on total phenolic content.

Table 1. Total phenolic content and antioxidant activity values of extract samples[1]
Çizelge 1 Ekstrakt örneklerine ait toplam fenolik madde ve antioksidan aktivite sonuçları[1]

Extract type *Ekstrakt Türü*	**Total Phenolic Content** *Toplam Fenolik Madde Miktarı* **(mg GAE g^{-1})**	**Antioxidant Activity** *Antioksidan Aktivite* **(%)**
Anise	62.22±1.10^{f}	48.70±1.62^{f}
Cinnamon	172.53±2.43^{b}	90.37±0.22^{ab}
Turmeric	173.53±4.64^{b}	89.18±0.15^{ab}
Ginger	181.75±10.61^{b}	87.10±0.29^{bc}
Fennel	82.45±1.68^{e}	39.44±0.15^{g}
Clove	461.38±3.04^{a}	91.83±0.07^{a}
Laurel leaves	124.78±0.59^{c}	89.13±0.52^{ab}
Rosemary	94.72±1.55^{d}	84.65±0.81^{c}
Peppermint	91.43±1.32^{de}	74.04±1.84^{d}
Thyme	77.20±2.46^{ef}	64.78±0.37^{e}

[1] Means followed by the different letters within a column are significantly different.

Antioxidant activity

The antioxidant activity values of the extract samples are given in Table 2. The antioxidant activity values of the extract samples were found to be between 39.44% and 91.83%. The highest antioxidant activity was found in clove extract with 91.83%, followed by cinnamon (90.37%), turmeric (89.18%), laurel leaves (89.13%), ginger (87.10%), rosemary (84.65%), peppermint (74.04%), thyme (64.78%), anise (48.70%) and fennel (39.44%) extracts. The relationship between antioxidant activity and total phenolic content may depend on many factors. Antioxidant activity potential has affected both synergy and structures of phenolic substances. Because the antioxidant activity level of the extract is not only dependent on the concentration of phenolic compounds but also on the structure and interaction between these compounds. This situation can explain the difference in antioxidant activities in similar total phenolic component concentrations in the sample (Piluzza & Bullitta, 2011). Contrary to all these, the total phenolic content value shows a generally compatible change with antioxidant activity of extracts. Considering the results obtained in our study, the high antioxidant activity values were determined in the extracts with high total phenolic content amount. As stated by Amer and Aly (2019), many factors lead to differences in antioxidant activity of extract despite using the same solvent. Because raw material chemical nature, particle size, storage time, the extraction conditions and interfering substances presence can significantly affect the activity of solvent (Naczk & Shahidi, 2004). A polar solvent such as ethanol can reveal more polar components together with non-polar components, and therefore antioxidant activity is obtained higher than other solvents (Liu et al., 2007). According to Silva et al. (2007), the antioxidant activity value of extracts can be increased with usage of suitable solvents by further recovery of phenolic compounds. Also, Ghasemzadeh et al. (2011) concluded that the extracts with high polarity solvents (methanol) have more effective radical scavengers compared to less polar solvents (acetone and chloroform), and this could be explained by the antioxidants found in ginger varieties or active compounds with different polarities. Chun et al. (2005) stated that the hydrophilic and hydrophobic ratio of phenolic has an important function in different antioxidant activity values of the extract samples.

Antifungal Activity

Inhibition zone diameter

The antifungal activity of anise, cinnamon, turmeric, ginger, fennel, clove, laurel leaves, rosemary, peppermint and thyme against *Aspergillus oryzae*, *Penicillium digitatum*, *Aspergillus flavus*, *Penicillum camemberti* and *Aspergillus niger* strains were determined with inhibition zone diameter using disc diffusion methods and values shown in Table 2. Phenolic compounds in extracts lead to damage to the cell walls, causing cell deformation, increasing cellular permeability and causing cellular contents to flow out and so, cell death occurs with the release of cellular contents out of the cell (Sharayei et al., 2020). Extract

samples were tested at 100 and 200 mg mL^{-1} concentrations. The negative and positive control group used ethanol and 2 mg mL^{-1} calcium propionate, respectively. The extract samples represented relatively high antifungal activity against the *Aspergillus oryzae* at 100 mg mL^{-1} and 200 mg mL^{-1} with percentage of mycelium growth inhibition varied between 14.7-39.2 mm and 15.90-55.20 mm. The highest inhibition zone diameter values against *Aspergillus oryzae* were obtained with cinnamon (39.2 mm) and clove (36.7 mm) extracts at 100 mg mL^{-1} concentration ($p<0.05$). This is followed by anise (21.4 mm), peppermint (18.8 mm), turmeric (18.0 mm), laurel leaves (16.7 mm), rosemary (16.6 mm), ginger (16.4 mm), fennel (15.4 mm) and thyme (14.7 mm). The high antifungal activity of cinnamon is associated with the presence of many bioactive components such as cinnamaldehyde, eugenol and cinnamic acid (Gill & Holly, 2004). When the antifungal activities of the extracts at 200 mg mL^{-1} concentration against *Aspergillus oryzae* were evaluated, anise, cinnamon, turmeric, ginger, fennel, clove, laurel leaves, rosemary, peppermint and thyme was determined an increase as 6.0, 16.0, 6.0, 3.6, 4.5, 14.1, 5.9, 5.6 and 1.2 units respectively, compared with the zone diameters at 100 mg mL^{-1} concentration.

All extract samples showed inhibition zone diameter against *Penicillium digitatum* as seen in Table 2 and the ethanolic extract of each of the ten spices formed a larger inhibition zone diameter in the discs compared to 2 mg mL^{-1} calcium propionate used as the positive control group (7.6 mm). The spice extract samples demonstrated more effective antifungal effect than calcium propionate used as an antimicrobial agent. The inhibition zone diameter values against *Penicillium digitatum* increased from 13.0-42.1 mm (100 mg mL^{-1}) to 16.8-57.4 mm (200 mg mL^{-1}) with increased concentration. The highest antifungal effect against *Penicillium digitatum* found with cinnamon extract with 42.1 mm in 100 mg mL^{-1} discs and 57.4 mm in 200 mg mL^{-1} discs, following clove extract with an inhibition zone of 41.0 mm in 100 mg mL^{-1} discs and 55.2 mm in 200 mg mL^{-1} discs. The high antifungal activity of cinnamon extract against *Penicillium digitatum* may be caused by cinnamaldehyde, an important bioactive component in cinnamon bark, and some studies have shown that cinnamaldehyde kills 80% of mold and bacteria (McCann, 2003). In addition, the presence of flavonoids, alkaloids, tannins, saponins, terpenes, steroids and essential oil in cinnamon extract may be responsible collectively or individually for the antifungal activity (Mahmoud, 2012). Fennel extract at 100 mg mL^{-1} showed the lowest antifungal activity against *Penicillium digitatum* with a 13.0 mm inhibition zone diameter value. The highest antifungal activity against *Aspergillus flavus* was recorded with cinnamon extract (44.2 mm) in disc containing 100 mg ml^{-1}. As seen in Table 2, increasing concentration has increased the inhibition zone diameters against *Aspergillus flavus*.

The inhibition zone diameter of 200 mg mL^{-1} extracts against *Aspergillus flavus* showed the highest effect with cinnamon and clove extracts at 50.2 mm and 44.5 mm. Inhibition zone diameters of the extract samples on *Penicillum camemberti* strain varied between 12.4-42.1 mm at 100 mg mL^{-1} concentration and between 16.5-54.7 mm at 200 mg mL^{-1} concentration. The highest inhibition zone diameter (54.7 and 51.7 mm) were observed by the concentration of 200 mg mL^{-1} of the cinnamon and clove extract samples and other extract samples were demonstrated similar values. The inhibition zone diameter values of anise, cinnamon, turmeric, ginger, fennel, clove, laurel leaves, rosemary, peppermint and thyme extracts increased at 4.1, 12.1, 2.9, 3.9, 3.7, 12.6, 2.7, 1.2, 2.2 and 2.4 units, respectively with the increase of the extract concentration from 100 mg mL^{-1} to 200 mg mL^{-1}. This high increase in cinnamon and clove extracts may be due to the high total phenolic content.

When the antifungal activity of the extract samples against *Aspergillus niger* was examined, the inhibition zone diameter of extracts prepared at 100 mg mL^{-1} concentration were determined 10.1-40.6 mm while 13.5-45.5 mm at 200 mg mL^{-1} concentration. The highest inhibition zone diameter at both 100 mg mL^{-1} and 200 mg mL^{-1} concentrations was found with the use of cinnamon, followed by clove, turmeric, laurel leaves and thyme were found to have statistically similar effects. The highest increase in the inhibition zone diameter with usage of high concentration was obtained with clove extract (9.7 units). Antifungal activity mechanisms of extracts are associated with low water-soluble properties and also with the easy incorporation of high hydrophobicity compounds into the plasma membranes and membranes of intracellular organelles (especially mitochondria) (Jing et al., 2014). These compounds can change the lipid membrane composition, such as lowering the levels of ergosterol, which is the main component of the cell membrane (Kedia et al., 2015). The decrease or absence of ergosterol can lead to maintaining cell function and integrity, membrane binding enzyme activity, cell viability and cellular transport systems, changes in cell permeability, disruption of cell organelles and cell death (Kiran et al., 2016). According to El Khoury et al. (2017), the antimicrobial activity mechanism of extracts occurs in three different ways. The first of these, enzymes responsible for intracellular functions change with the presence of -OH groups and form hydrogen bonds. The second of these explain by the loss of rigidity and integrity of the hyphae cell wall due to the interaction of these compounds with the membrane enzymes of the mold strains, while the third mechanism is expressed by

rupture of the cytoplasmic membrane, changes in the permeability of the cell membranes and granulation of the cytoplasm. Also, da Cruz Cabral et al. (2013) stated that some hydrophobic compounds in the extracts may cause to cross the cell membrane and change permeability of cations such as H+ - K+, thus changing the flow of protons and the pH of the cells, affecting chemical composition and activities. In addition to, the high phenolic compound levels can cause macromolecules loss from cell by changing of the mold cell permeability, and the deformation of structure and functionality by interacting with the membrane proteins (Fung et al., 1977).

In a study investigated compounds against *Aspergillus flavus* and *Aspergillus niger*, Kim et al. (2006) associated with targeting the mitochondrial oxidative stress defense system of compounds as action mechanism on growth inhibition of mold strain. Similar to our study results, Gupta et al. (2008) stated that the high antimicrobial activity of cinnamon may be due to cytoplasmic granulation, cytoplasmic membrane rupture and inactivation or inhibition of intracellular enzymes. Also, cinnamon have rich highly electro-negative cinnamaldehyde content (50.5%), and these electro-negative compounds affect biological processes containing electron transfer and also can be inhibit the growth of microorganisms as a result of the reaction with nitrogen-containing components (proteins and nucleic acids). In literature has been suggested that exposure of microorganisms to antifungal components may cause disruption of membrane integrity and function, which may slowly lead to loss of cell homeostasis, leakage of intracellular components, and ultimately cell death (Hammer & Carson, 2011).

Table 2. Inhibition zone diameter values against different molds of extract samples (mm)[1]
Çizelge 2 Ekstrakt örneklerinin farklı küf suşlarına karşı inhibisyon zon çapı değerleri (mm)[1]

Extract type *Ekstrakt Türü*	*Aspergillus oryzae*		*Penicillium digitatum*		*Aspergillus flavus*		*Penicillum camemberti*		*Aspergillus niger*	
	100 mg mL^{-1}	200 mg mL^{-1}	100 mg mL^{-1}	200 mg mL^{-1}	100 mg mL^{-1}	200 mg mL^{-1}	100 mg mL^{-1}	200 mg mL^{-1}	100 mg mL^{-1}	200 mg mL^{-1}
Anise	21.4±0.57^{b}	27.4±1.27^{b}	15.9±0.71^{cd}	18.3±0.42^{de}	18.6±1.56^{c}	20.8±1.56^{b}	12.4±1.27^{c}	16.5±0.71^{b}	10.1±0.14^{ef}	16.2±0.71^{de}
Cinnamon	39.2±1.13^{a}	55.2±1.41^{a}	42.1±1.13^{a}	57.4±0.85^{a}	44.2±1.84^{a}	50.2±1.84^{a}	39.6±1.70^{a}	51.7±2.69^{a}	40.6±1.70^{a}	45.5±1.27^{a}
Turmeric	18.0±0.57^{bc}	24.0±0.66^{bc}	18.1±0.99^{bc}	20.6±0.57^{cd}	17.3±1.56^{c}	21.1±0.99^{b}	17.8±1.70^{b}	20.7±2.12^{b}	15.0±0.99^{cd}	21.3±0.57^{c}
Ginger	16.4±0.71^{c}	20.0±0.85^{cd}	18.4±0.57^{bc}	22.0±0.71^{bc}	15.3±0.42^{cd}	17.8±0.85^{bc}	16.9±1.56^{bc}	20.8±1.70^{b}	12.9±0.57^{de}	15.3±1.41^{de}
Fennel	15.4±0.57^{c}	19.9±0.85^{cd}	13.0±0.42^{d}	17.1±0.42^{e}	12.0±0.28^{d}	14.3±0.99^{c}	13.0±0.57^{bc}	16.7±1.84^{b}	10.6±0.57^{e}	13.5±0.99^{e}
Clove	36.7±2.26^{a}	50.80±2.40^{a}	41.0±0.57^{a}	55.2±0.85^{a}	37.7±2.26^{b}	44.5±1.98^{a}	42.1±1.13^{a}	54.7±2.12^{a}	27.1±0.42^{b}	36.8±1.56^{b}
Laurel leaves	16.7±0.28^{c}	22.6±1.70^{bc}	20.7±0.57^{b}	23.5±0.57^{b}	16.4±1.41^{cd}	22.9±1.56^{b}	16.4±0.71^{bc}	19.1±0.99^{b}	18.4±0.71^{c}	21.6±1.84^{c}
Rosemary	16.6±1.84^{c}	22.7±2.40^{bc}	14.3±0.71^{d}	16.8±0.14^{e}	16.0±0.42^{cd}	17.7±1.98^{bc}	15.6±1.84^{bc}	16.8±1.56^{b}	10.3±1.27^{e}	16.9±1.56^{cde}
Peppermint	18.8±1.41^{bc}	24.4±1.13^{bc}	19.0±0.85^{b}	21.8±0.71^{bc}	17.0±0.99^{cd}	18.6±1.13^{bc}	16.4±1.70^{bc}	18.6±1.84^{b}	10.4±1.56^{e}	16.4±0.57^{de}
Thyme	14.7±0.71^{c}	15.9±0.71^{d}	15.7±0.57^{cd}	18.3±0.57^{de}	14.8±0.42^{cd}	17.7±1.98^{bc}	16.0±0.28^{bc}	18.4±1.56^{b}	17.4±1.41^{c}	19.8±1.27^{cd}
Negative control[2]	-		-		-		-		-	
Positive control[3]	6.8±0.80		7.6±0.00		6.4±0.40		-		6.0±0.00	

[1]Means followed by the different letters within a column are significantly different. [2]Negative control: Ethanol, [3]Positive control: 2 mg mL^{-1} calcium propionate.

Minimum inhibition concentration (MIC) and minimum fungicidal concentration (MFC)

MIC and MFC were used to evaluate the fungicidal potential of cinnamon, turmeric, ginger, clove and laurel leaves extract against *Aspergillus oryzae*, *Penicillum camemberti*, *Penicillium digitatum*, *Aspergillus niger* and *Aspergillus flavus*. The results obtained were summarized in Table 3. The lowest MIC value on *Aspergillus oryzae* strain was obtained at 0.312 mg mL^{-1} with cinnamon and clove extracts, in contrast, turmeric extract was found to have the weakest antifungal activity with high MIC value. Similarly, the lowest MIC value against *Penicillum camemberti* strain was obtained with cinnamon and clove extracts, while the MIC value of turmeric and ginger extracts was found to be higher than 20 mg mL^{-1}. On the other hand, the MIC values for *Penicillium digitatum* strain was determined as 0.312 mg mL^{-1} for cinnamon, 0.625 mg mL^{-1} for clove, 5 mg mL^{-1} for laurel leaves, 10 mg mL^{-1} for turmeric and >20 mg mL^{-1} for ginger, respectively, and so *Penicillium digitatum* strain was observed to be more resistant to ginger extract. This result was consistent with the investigation conducted by Vieira et al. (2022), wherein evaluated the antifungal effects of green tea, rosemary, cinnamon, anise, clove, curcumin and lemon balm extracts against *Aspergillus* spp. and *Penicillium* spp.

As demonstrated by the study, extracts demonstrated antifungal activity against fungi species activity with MIC values in the range of 0.55-2.18 mg mL^{-1}. The obtained results agree with El-Fallal et al. (2019) who found that clove and cinnamon were recommended as the best anti-fungal spices that exhibited antifungal activity with a minimal concentration of 0.05 g L^{-1}. As seen in the literature, the use of spices in extract form can show an inhibition effect against fungus even at very low concentrations. According to Table 3, the MIC values of all extract samples except for turmeric extract against *Aspergillus niger* strain were obtained below 20 mg mL^{-1} concentration. Turmeric (>20 mg mL^{-1}) was found as the most resistant extract of *Aspergillus niger* strain, following ginger with 10 mg mL^{-1}, laurel leaves with 5 mg mL^{-1}, clove with 0.625 mg mL^{-1} and cinnamon with 0.078 mg mL^{-1}. The MIC values of turmeric and ginger extract samples against the *Aspergillus flavus* strain were obtained as >20 mg mL^{-1}, and hence low antifungal activity on mold strain. The lowest MIC value on *Aspergillus flavus* was determined with the use of cinnamon extract with 0.312 mg mL^{-1}. As a result, the highest inhibition properties were provided on *Aspergillus niger* for cinnamon (0.078 mg mL^{-1}), on *Penicillium digitatum* for turmeric (10 mg mL^{-1}), on *Aspergillus oryzae* for ginger (5 mg mL^{-1}), on *Aspergillus oryzae* and *Penicillum camemberti* for clove (0.312 mg mL^{-1}) and *Penicillium digitatum*, *Aspergillus niger* and *Aspergillus flavus* (5 mg mL^{-1}) for laurel leaves.

Table 3. Minimum inhibition concentration values of extract samples (mg mL^{-1})[1]
Çizelge 3 Ekstrakt örneklerine ait minimum inhibisyon konsantrasyon sonuçları (mg mL^{-1})[1]

Extract type *Ekstrakt Türü*	***Aspergillus oryzae***	***Penicillum camemberti***	***Penicillium digitatum***	***Aspergillus niger***	***Aspergillus flavus***
Cinnamon	0.312	0.312	0.312	0.078	0.312
Turmeric	>20	>20	10	>20	>20
Ginger	5	>20	>20	10	>20
Clove	0.312	0.312	0.625	0.625	0.625
Laurel leaves	10	20	5	5	5

[1]Means followed by the different letters within a column are significantly different.

The MFC values of extract samples were given in Table 4. Extract samples demonstrated fungicidal effect in response to *Aspergillus oryzae*, *Penicillum camemberti*, *Penicillium digitatum*, *Aspergillus niger* and *Aspergillus flavus* with concentration between 0.312 mg mL^{-1} - >20 mg mL^{-1}. The cinnamon extract exhibited stronger fungicidal activity against *Aspergillus oryzae* (0.625 mg mL^{-1}), *Penicillum camemberti* (0.312 mg mL^{-1}), *Penicillium digitatum* (0.625 mg mL^{-1}), *Aspergillus niger* (0.312 mg mL^{-1}) and *Aspergillus flavus* (0.625 mg mL^{-1}). Birhanu et al. (2014) reported that *Cinnamomum zeylanicum* extract has potential to inhibit microorganism growth at a very low concentration compared to other extract samples and stated that the MIC values against *Aspergillus* sp. of *Cinnamomum zeylanicum* extract specified as 30%, while as 20% againts *Penicillium* sp. The second greatest effect against mold strains was obtained with clove extracts. The effect can be attributed to the eugenol component in clove. Eugenol-induced inhibition may be due to caused by the permeability of cell membranes (Li et al., 2021), disruption of the cytoplasmic membrane, impaired proton motive force, increased permeability of the phospholipid bilayer (Zhang et al., 2019), electron flow, active transport, and coagulation of cell contents (Davidson et al., 2012). The antifungal activity presented by clove extract may be attributed to the demonstration of components in great amounts such as eugenol, eugenyl acetate, beta-caryophyllene, 2-heptanone (Chaieb et al., 2007), acetyl-eugenol, alpha-humulene, methyl salicylate, iso-eugenol, methyl eugenol (Yang et al., 2003). Turmeric extract showed a fungicidal effect with 20 mg mL^{-1} on only the *Penicillium digitatum* and *Aspergillus flavus* strains. On the other hand, the MFC value of ginger and laurel leaves extract was found to be greater than 20 mg mL^{-1} for all mold strains. According to the MIC and MFC values of cinnamon extract, *Aspergillus oryzae*, *Penicillum camemberti*, *Penicillium digitatum*, *Aspergillus niger* and *Aspergillus flavus* can be shown as the first extract having high sensitivity. For this reason, cinnamon is the most successful extract in preventing mold development. This high antifungal activity of *Cinnamomum* extract can be associated with cinnamaldehyde, eugenol, cinnamic acid and organic acids (Birhanu et al., 2014). *Aspergillus* and *Penicillium* species demonstrated the highest resistance against ginger and laurel leaves extract. The extract samples used in our study were not equally effective against all mold strains may be related to having different phenolic compositions and amounts of extracts. To Moreno et al. (2006), the antimicrobial effect of various phenolic complexes is associated with the inactivation of different cellular enzymes based on the penetration rate of substances into the cell and changes in membrane permeability, and this change in cell membrane permeability has been cited as the main factor in the antimicrobial effect of a particular compound. Also, phenolic compounds can completely disrupt cell membranes, affect cell integrity and cause eventual cell death.

Table 4. Minimum fungicidal concentration values of extract samples (mg mL^{-1})[1]
Çizelge 4 Ekstrakt örneklerine ait minimum fungisidal konsantrasyon sonuçları (mg mL^{-1})[1]

Extract type *Ekstrakt Türü*	***Aspergillus oryzae***	***Penicillum camemberti***	***Penicillium digitatum***	***Aspergillus niger***	***Aspergillus flavus***
Cinnamon	0.625	0.312	0.625	0.312	0.625
Turmeric	>20	>20	20	>20	20
Ginger	>20	>20	>20	>20	>20
Clove	5	10	10	2.5	5
Laurel leaves	>20	>20	>20	>20	>20

[1]Means followed by the different letters within a column are significantly different.

CONCLUSION

This study contributes to the current knowledge of the antifungal activity of different spice extracts. Rosemary, anise, cinnamon, ginger, peppermint, turmeric, fennel, clove, laurel leaves and thyme extracts were evaluated in terms of the total phenolic content amount, antioxidant activity value and antifungal properties. The five extract samples with high inhibition zone diameters against mold strains were selected and MIC and MFC values were determined. Clove extract had the highest total phenolic content amount compared to others. The current findings showed that ten selected spice extract samples had promising antifungal activity against tested all mold strains. According to results, the highest inhibition zone diameter was obtained with anise, turmeric, fennel, rosemary and peppermint extracts against *Aspergillus oryzae*; with cinnamon, ginger, clove and laurel leaves extracts against *Penicillium digitatum* and with thyme against *Aspergillus niger*. The MIC and MFC analysis carried out in this study revealed that lower concentration of cinnamon and clove were more effective against *Aspergillus* and *Pencillium* spp. according to turmeric, ginger and laurel leaves. As a result, cinnamon and clove extracts can be recommended as functional ingredients to improve cereal products without adverse effects on product quality.

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Author contribution

Mine Aslan: Investigation, Resources, Formal analysis, Writing – original draft. Nilgün Ertaş: Methodology, Project administration, Funding acquisition, Conceptualization, Supervision, Writing-review & editing. M. Kürşat Demir: Project administration, Supervision, Writing-review & editing.

Declaration of competing interest

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

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Determination of Changes in Physicochemical and Microbiological Properties of Tomato Paste Exposed to Different Gases of Cold Plasma Technique

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ABSTRACT
This study aimed to reveal the effect of cold plasma application using different gases and mixtures on some physicochemical and microbiological properties of tomato paste. For this purpose, applications were performed in different gases and times, and the effect of each application was examined separately. As a result of the study, the pH values of the samples varied between 3.77 and 4.87, and the aw values between 0.718 and 0.819. When the color values were examined, it was determined that the L* value varied between 22.42 – 32.48, the a* value varied between 23.59 – 30.18 and the b* value varied between 12.16 – 19.52 (P<0.05). In addition to this, when the samples were evaluated microbiologically, TMAB counts varied between 3.02 – 5.42 log cfu/g, TPAB values ranged between 1.80 – 3.37 log cfu/g, total mold counts were between 3.08 – 5.67 log cfu/g, total yeast counts were between 3.13-5.42 and osmophilic yeast counts were between 1.74 – 3.49 log cfu/g. Lycopene values of samples in the study were in the range of 15.30 – 23.42 mg/100g DM. When the data obtained from the study are evaluated as a whole, it is thought that cold plasma application has positive effects on the shelf life and quality of tomato paste. In the research where two different gases and mixtures of these gases were used, oxygen gas application showed the most effect on the physicochemical and microbiological properties of the samples (P<0.05). Mixture and argon gas applications followed this effect, and prolonging the application period also increased the effect. When the data obtained from the study are evaluated as a whole, it has been revealed that cold plasma application delays the mold problem, which is one of the most critical problems in industrially produced tomato paste, extends the shelf life, and slows down the negative changes in physicochemical quality values due to storage.

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Farklı Dozlarda Soğuk Plazma Tekniğine Maruz Bırakılan Domates Salçasının Fizikokimyasal ve Mikrobiyolojik Özelliklerindeki Değişikliklerin Belirlenmesi

ÖZET
Bu çalışmanın amacı, farklı gaz ve karışımları kullanılan soğuk plazma uygulamasının domates salçasının bazı fizikokimyasal ve mikrobiyolojik özelliklerine etkisinin ortaya koyulmasıdır. Bu amaçla farklı gaz ve sürelerde uygulamalar yapılmış ve her uygulamanın etkisi ayrı ayrı incelenmiştir. Çalışma sonucunda numunelerin pH değerleri 3,77 ile 4,87 arasında, a_w değerleri ise 0,718 ile 0,819 arasında değişmiştir (P<0.05). Renk değerleri incelendiğinde L* değerinin 22.42 – 32.48 arasında, a* değerinin 23.59 – 30.18 arasında ve b* değerinin 12.16 – 19.52 arasında değiştiği tespit edilmiştir (P<0.05). Ayrıca numuneler mikrobiyolojik olarak değerlendirildiğinde depolama süresince tüm örneklerde sayıların arttığı (P>0.05), ancak CP uygulamasının bu artışı ciddi anlamda yavaşlattığı belirlenmiştir.Örneklerin TMAB sayıları 3.02 – 5.42 log kob/g, TPAB değerleri 1.80 – 3.37 log kob/g, toplam küf sayısı 3.08 – 5.67 log kob/g, toplam maya sayısı 3.13 – 5.42 log kob/g ve ozmofilik maya sayıları 1.74 – 3.49 log kob/g arası arasında değişmektedir. Çalışmadaki

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örneklerin likopen değerleri 15.30 – 23.42 mg/100g DM aralığındaydı. İki farklı gaz ve bu gazların karışımlarının kullanıldığı araştırmada örneklerin fizikokimyasal ve mikrobiyolojik özellikleri üzerinde en fazla etkiyi oksijen gazı uygulaması göstermiştir (P<0.05). Bu etkiyi karışım ve argon gazı uygulamaları takip etmiş olup uygulama süresinin uzaması etkiyi de artırmıştır. Çalışmadan elde edilen veriler bir bütün olarak değerlendirildiğinde, soğuk plazma uygulamasının endüstriyel olarak üretilen salçalarda en önemli problemlerden birisi olan küflenme sorunu geciktirerek raf ömrü uzattığı ve depolamaya bağlı fizikokimyasal kalite değerlerinde meydana gelen olumsuz değişimleri yavaşlattığı ortaya konulmuştur.

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INTRODUCTION

Tomato is one of the agricultural products with high biological activity because it contains components such as lycopene, ß-carotene, flavonoid and ascorbic acid (Himashree et al., 2022). The antioxidant (Briones-Labarca et al., 2019) and anti-inflammatory properties of these components and the use of tomatoes in the production of products with commercial value such as tomato paste, ketchup and fruit juice increase their importance (Uribe-Wandurraga et al., 2021; Aygel & Aslan, 2023). Tomato paste; is an indispensable component of the cuisines of many countries, it has its unique aroma and taste, tomatoes are obtained by subjecting them to different production processes after being harvested from the field. Since tomato is a perishable product, chemical preservatives must be used in tomato paste (Jafari et al., 2021).

Especially molds are effective in spoiling tomato paste and shortening its shelf life. But today, consumers prefer minimally processed and additive-free products. For this reason, studies on minimal treatments as alternatives to traditional heat treatment methods and chemical preservatives have increased. Today, new technologies are being developed in food production in order to, save time and energy and increase the shelf life of food (Ablay et al., 2020). Atmospheric cold plasma (CP) is one of the minimal processes applied to foods. CP has been already effective in maintaining microbial inactivation in previous studies (Herceg et al., 2016). The aim of this study should be written in detail since the optimization procedure has not been applied in this study. CP has also proved its high inactivation efficiency resulting in the detoxification of mycotoxins produced by fungi (Waghmare, 2021). CP is an emerging non-thermal decontamination technique for microorganisms in food products (Gao et al., 2021; Mao et al., 2021; Wan et al., 2021).

Plasma is accepted as the fourth state of matter (Mir et al., 2016; Varilla et al., 2020; Saremnezhad et al., 2021) produced by energizing gas in an electromagnetic field and consists of reactive species, positive and negative ions, and UV photons (Heo et al., 2021). Plasma is defined as the use of ionized gas in cold sterilization (Fernández et al., 2013). On the other hand, CP is the process that occurs as a result of applying an electric current or electromagnetic radiation to some gases at room temperature under vacuum (Yüksel & Karagözlü, 2017). CP application is effective in the decontamination of vegetative Gram-negative and Gram-positive bacteria, yeasts, viruses, and endospores (Rod et al., 2012; Yüksel & Karagözlü, 2017; Albayrak & Kılıç, 2020).

The general belief about the effect of CP application on microorganisms is related to reactive oxygen which occurs during the process and affects the cell nucleus resulting in DNA damage (Albayrak and Kılıç, 2020). Based on the gas type used, examples of reactive oxygen species (ROS) (Sruthi et al., 2022) species commonly associated with antimicrobial activity and inactivation steps include alkoxyl (RO•), peroxyl (ROO•), hydroperoxyl (HO_2•), superoxide anion (O_2•-), singlet oxygen ($1O_2$), hydroxyl radical (•OH), carbonate anion radical (CO_3•-), hydrogen peroxide (H_2O_2), and ozone (O_3). Similarly, examples of reactive nitrogen species (RNS) include nitrogen dioxide radical (•NO_2), nitric oxide (NO•), alkylperoxynitrite (ROONO), peroxynitrous acid (OONOH), and peroxynitrite (ONOO-) (Misra & Jo, 2017).

Although similar studies were carried out on sun-dried tomatoes before (Molina Hernandez et al., 2022), it was important for the planning of this study that no research was conducted to prevent mold growth, which is the most significant critical problem of industrially produced tomato pastes (especially those with low brix degrees). This study aimed to investigate the effect of CP application, which is described as a minimal process, on the microbiological quality and some physicochemical properties of tomato paste. In this context, two different gases and their mixtures were

applied to tomato pastes at two different effect times, and it was aimed to reveal the changes in the quality values of the samples by determining the changes that occurred during storage.

MATERIAL ve METHOD

Tomato Paste

The tomato paste (Brix: 28,14°, Total acidity: 1.71g/100g, Ash insoluble in 10% HCl: 0.19%) used in the research was obtained from the local market in Afyonkarahisar – Turkey. The tomato paste sample used in the research was produced from tomatoes collected in the summer of 2022. When provided, tomato paste is in the original tin can package used by the manufacturer. Before the application, approximately 15 g of tomato paste was spread on sterile Petri dishes to form a thin film in a biological safety cabinet.

The reason for using industrial products in the research was that the quality of industrial pastes (especially low brix degree) deteriorates quickly after opening, especially molding, in terms of microbiology.

Cold Plasma Application

The cold plasma application was modified from the method of Yong et al. (2017) (Fig 1). The gases (Habaş, Turkiye) used in CP application were Argon and Oxygen. The gases were purchased from Afyonkarahisar province (Kocasaban Gazları Corp., Afyonkarahisar, Turkey). The gases used in the system were mixed in certain proportions after that given to the system.

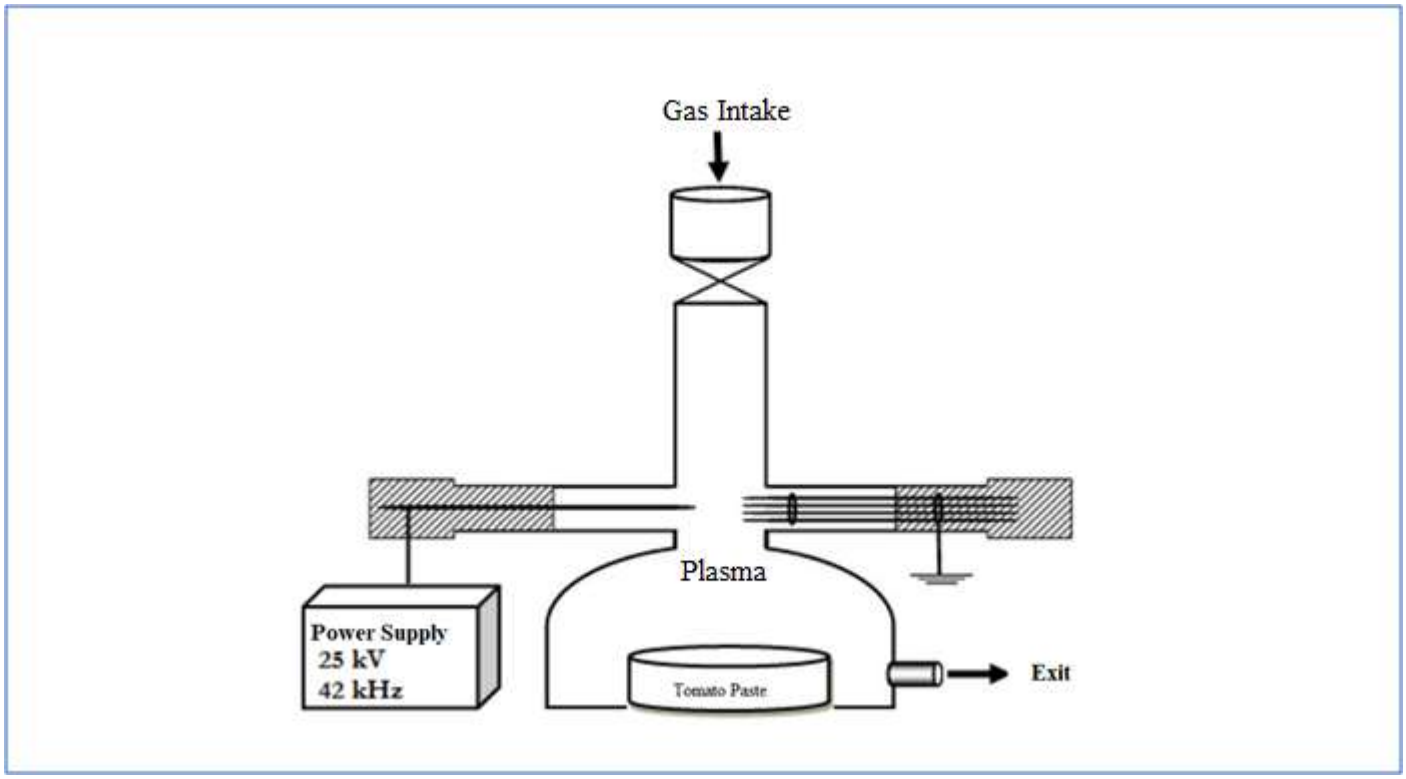

Fig 1. Application of Cold Plasma (Gök et al., 2019)
Şekil 1. Soğuk Plazma Uygulaması (Gök ve ark., 2019)

To maintain the plasma a power supply was used which has a 25 kV potential and 42 kHz frequency. The power supply was designed by a company (Diker Electronics) operating in Afyonkarahisar. The operation was carried out in continuous mode. The media in which the CP application was performed was a semicircular glass chamber with a 28 mm radius. This part is fixed to a plate made of stainless steel by a plastic ring with a diameter of 46 mm.

In order to produce the plasma form, a total of seven 1 mm thick tungsten steel electrodes (Astor, Türkiye) were used. One of these electrodes was placed horizontally in front of the other six electrodes in order to create plasma between the anode-cathode tips. The temperature of the tomato paste samples during the application was measured consistently using an infrared thermometer (Coleman-Parmer, Vernon Hills, IL).

In the research, only oxygen gas plasma was applied to FO1, and FO2 coded samples, only argon gas plasma was applied to FA1, and FA2 coded samples, and 50% oxygen + 50% argon was applied to MOA1 and MOA2 coded samples. Applied gases, mixing ratios, and flow rates are shown in Table 1. The exposure times of the samples to the gases were determined as 20 and 30 minutes. Each application was performed in 3

parallels, separately and on different days.

The processing time was determined due to the preliminary trials made before. Since the further extension of the application period causes severe quality losses in the product, it was kept at these levels. After the procedure, the samples were stored in a closed, sterile glass petri dish under aerobic conditions in the refrigerator at 4°C.

Table 1. Samples and coding used in the research
Çizelge 1. Araştırmada kullanılan örnekler ve kodlamaları

Samples	Processing/Time	Code
1	Control	C
2	%100 O_2/20 min.	FO1
3	% 100 O_2/30 min.	FO2
4	%100 Ar/20 min.	FA1
5	%100 Ar/30 min.	FA2
6	%50 O_2 and %50 Ar/20 min.	MOA1
7	%50 O_2 and %50 Ar/30 min.	MOA2

Microbiological Analysis

Preparation of samples for microbiological analysis

After the tomato paste samples were exposed to CP, 10 g of each sample was weighed on a precision balance (Laboratory Balances, Radwag PS R2.H, Poland) and put into another sterile stomacher bag. Sterile Ringer's solution with a volume of 90 mL (Merck, 115525, Germany) was added to it and homogenized for 2 minutes in a stomacher (BagMixer® 400 P-080921247). Then, the mixture was diluted with Ringer's solution by preparing serial dilutions at the desired ratios. The same procedures were applied in the control group. The control group consisted of untreated samples (Anonymous 2001).

Total mesophilic aerobic bacteria count (TMAB)

Plate Count Agar (Oxoid, CM0325) was used for total aerobic mesophilic bacteria count. It was incubated at 30 °C for 72 hours under aerobic conditions, and the total number of aerobic mesophilic bacteria was determined by counting the colonies that developed at the end of the incubation (ISO 2013a; ISO 2013b).

Total yeast count

Total yeast count was determined on Yeast Exctract Agar (Oxoid, CM0019) after incubation in aerobic conditions for 5-7 days at 30 °C. At the end of the incubation, the colony-forming units were counted and the results were calculated as viable cfu/mL (ISO 2008).

Total mold count

Total mold count was determined on Malt Exctract Agar (Merck, 1.05398) after incubation in aerobic conditions for 5-7 days at 30 °C. At the end of the incubation, the colony-forming units were counted and the results were calculated as viable cfu/mL (ISO 2008).

Osmophilic yeast count

Total mold count was determined on DG18 medium modified with gliserol (Merck, 1.00465) after incubation in aerobic conditions for 5-7 days at 30 °C. At the end of the incubation, the colony-forming units were counted and the results were calculated as viable cfu/mL (ISO 2008).

Physicochemical Analysis

The color values, pH and water activity (aw) of tomato paste samples were examined.

pH value

A 10 g tomato paste sample was mixed with 10 mL distilled water and homogenized (Daihan Wisestir, HS-30T, South Korea). To measure the pH values of the prepared mixtures a pH meter (HANNA, HI 2215 pH/ORP meter) was used (AOAC 2016).

Activity of water (a_w) value

To determine the aw values of the samples a water activity analyzer (Novasina LabTouch-aw, Lachen, Switzerland) was used (AOAC 2016).

Color analysis

Color values of tomato paste samples were determined using a colorimeter (Konica Minolta Chroma Meter CR-400, Osaka). The brightness (L*), redness (a*) and yellowness (b*) values of the samples were measured according to Akarca (Akarca, 2013). The Brown Index value was calculated according to the formulas below (Kurtuldu & Özcan, 2018).

$$x = [a + \frac{1,75 * L}{5,645 * L} + (a - (3,012 * b)]$$

$$BI = [100 * \frac{x - 0,31}{0,17}]$$

Lycopene Analysis

Lycopene analysis was conducted by using HPLC according to the method of Demiray et al. (2013).

Statistical Analysis

The results obtained in the study were made in two parallels and SPSS software program V 23.0.0 was used for the variance analysis. A significant difference was determined by Duncan's multiple range tests (*P<0.05) (Atik & Gümüş, 2021).

RESULTS and DISCUSSION

TAMB counts of the samples increased during storage (P<0.05). When the TMAB results were examined, it was determined that CP application provided 1 log reduction. An increase in TMAB values was observed in the 14-day shelf life. It was determined that the rate of increase was less in the samples treated with CP. Changes in TMAB count of samples are given Table 2.

Although TPAB numbers increased during 14 days of storage in all samples (P<0.05), CP application was effective in TPAB values of tomato paste samples (P<0.05). As a result of the study, a reduction of 2 logs was achieved. The maximum microbial decrease was achieved in the MOA2 sample. An increase was observed in the TPAB counts of the samples during the storage period. While this increase was not significant for control and FO1, it was significant for other CP applications (P<0.05). The TPAB counts of samples are given in Table 3.

Table 2. Changes in TMAB count of tomato paste samples during storage (log cfu/g)
Çizelge 2. Domates salçası örneklerinin depolama süresince TMAB sayısındaki değişiklikler (log kob/g)

Sample	TMAB Count		
	Storage Days		
	0	7	14
C	4.30 ± 0.13^{Ca}	4.79 ± 0.02^{bA}	5.42 ± 0.03^{aA}
FO1	3.23 ± 0.12^{Bd}	3.15 ± 0.04^{bCD}	3.66 ± 0.02^{aE}
FO2	3.16 ± 0.01^{Bd}	3.02 ± 0.01^{cC}	3.35 ± 0.03^{aF}
FA1	3.36 ± 0.10^{Bcd}	3.11 ± 0.03^{cDE}	4.21 ± 0.05^{aB}
FA2	3.51 ± 0.15^{Cc}	3.60 ± 0.01^{bB}	4.01 ± 0.02^{aC}
MOA1	3.98 ± 0.01^{Ab}	3.22 ± 0.02^{bF}	3.90 ± 0.04^{aD}
MOA2	3.90 ± 0.14^{Ab}	3.04 ± 0.02^{cEF}	3.75 ± 0.04^{bE}

a(→)c: Values with the same capital letters in the same rows for each analysis differ significantly (P<0.05)
A(↓)F: Values with the same capital letters in the same column for each analysis differ significantly (P<0.05) TMAB: Total Mesophilic Aerobic Bacteria

Table 3. Changes in TPAB count of tomato paste samples during storage (log cfu/g)
Çizelge 3. Domates salçası örneklerinin depolama süresince TPAB sayısındaki değişiklikler (log kob/g)

Sample	TPAB Count		
	Storage Days		
	0	7	14
C	2.82 ± 0.38^{aA}	3.37 ± 0.01^{aA}	3.24 ± 0.05^{aA}
FO1	2.31 ± 0.09^{aAB}	2.44 ± 0.04^{aC}	2.65 ± 0.09^{aBC}
FO2	2.00 ± 0.02^{bAB}	2.10 ± 0.02^{bD}	2.40 ± 0.04^{aC}
FA1	2.12 ± 0.03^{bAB}	2.75 ± 0.08^{aB}	3.11 ± 0.19^{aAB}
FA2	1.96 ± 0.10^{bB}	2.72 ± 0.04^{aB}	2.97 ± 0.10^{aAB}
MOA1	1.85 ± 0.19^{bB}	2.76 ± 0.02^{aB}	2.93 ± 0.12^{aAB}
MOA2	1.80 ± 0.05^{bB}	2.51 ± 0.05^{aC}	2.96 ± 0.18^{aAB}

a(→)b: Values with the same capital letters in the same rows for each analysis differ significantly (P<0.05)
A(↓)D: Values with the same capital letters in the same column for each analysis differ significantly (P<0.05) TPAB: Total Psychrophilic Aerobic Bacteria

Mold, yeast, and osmophilic yeast results are given in Table 4, 5, and 6, respectively. Yeast, mold, and osmophilic yeast counts increased during storage in all samples (P<0.05). A 1 log reduction was achieved in the count of mold, which is an important deterioration factor in tomato paste, depending on the gas composition applied and the processing time. During the storage period, especially on the 14th day, the total mold rate increased significantly (P<0.05).

CP application was effective on total yeast count (P<0.05). No significant difference was detected between applied gas concentrations and treatment times (P>0.05). A 0.82 log reduction was achieved with CP application. During the 14-day storage period, while the total count of yeasts increased by 2 log in the control sample, the increase remained below 1 log in the samples treated with CP. In this sense, CP application was effective in the increase rate of microorganisms.

Table 4. Changes in mold count of tomato paste samples during storage (log cfu/g)
Çizelge 4. Domates salçası örneklerinin depolama süresince küf sayısındaki değişiklikler (log kob/g)

Sample	Total Mold Count		
	Storage Days		
	0	7	14
C	4.19±0.12^{bA}	4.25±0.06^{bA}	5.67±0.03^{aA}
FO1	3.34±0.14^{bCDE}	3.34±0.07^{bD}	4.66±0.03^{aB}
FO2	3.08±0.08^{bE}	3.17±0.11^{bD}	4.53±0.02^{aC}
FA1	3.65±0.03^{cBC}	3.99±0.00^{bB}	4.20±0.04^{aD}
FA2	3.27±0.18^{bDE}	3.79±0.04^{aC}	4.14±0.02^{aDE}
MOA1	3.75±0.02^{bB}	3.74±0.03^{bC}	4.16±0.03^{aDE}
MOA2	3.47±0.02^{cBCD}	3.72±0.01^{bC}	4.10±0.02^{aE}

a(→)c: Values with the same capital letters in the same rows for each analysis differ significantly (P<0.05)
A(↓)E: Values with the same capital letters in the same column for each analysis differ significantly (P<0.05)

Table 5. Changes in yeast count of tomato paste samples during storage (log cfu/g)
Çizelge 5. Domates salçası örneklerinin depolama süresince maya sayısındaki değişiklikler (log kob/g)

Sample	Total Yeast Count		
	Storage Days		
	0	7	14
C	3.95±0.10^{bA}	5.23±0.11^{aA}	5.42±0.00^{aA}
FO1	3.21±0.13^{aB}	3.32±0.06^{aD}	3.52±0.01^{aDE}
FO2	3.13±0.08^{bB}	3.25±0.05^{bD}	3.48±0.01^{aE}
FA1	3.41±0.06^{bB}	3.50±0.01^{abBC}	3.60±0.05^{aBC}
FA2	3.21±0.10^{bB}	3.35±0.04^{abCD}	3.56±0.10^{aCD}
MOA1	3.41±0.03^{bB}	3.58±0.02^{aB}	3.66±0.05^{aB}
MOA2	3.39±0.07^{aB}	3.50±0.11^{aBC}	3.58±0.04^{aC}

a(→)b: Values with the same capital letters in the same rows for each analysis differ significantly (P<0.05)
A(↓)E: Values with the same capital letters in the same column for each analysis differ significantly (P<0.05)

Table 6. Changes in osmophilic yeast count of tomato paste samples during storage (log cfu/g)
Çizelge 6. Domates salçası örneklerinin depolama süresince ozmofilik maya sayısındaki değişiklikler (log kob/g)

Sample	Osmophilic Yeast Count		
	Storage Days		
	0	7	14
C	3.14±0.05^{bA}	3.37±0.02^{aA}	3.49±0.02^{aA}
FO1	2.05±0.04^{aC}	2.16±0.13^{aB}	2.34±0.01^{aB}
FO2	1.74±0.04^{cE}	2.07±0.05^{bB}	2.30±0.03^{aB}
FA1	2.21±0.02^{bB}	2.28±0.01^{aBB}	2.34±0.03^{aB}
FA2	2.05±0.01^{bC}	2.19±0.06^{aBB}	2.31±0.01^{aB}
MOA1	2.00±0.02^{bCD}	2.16±0.04^{aBB}	2.30±0.09^{aB}
MOA2	1.91±0.02^{bD}	2.13±0.03^{aB}	2.23±0.06^{aB}

a(→)b: Values with the same capital letters in the same rows for each analysis differ significantly (P<0.05)
A(↓)E: Values with the same capital letters in the same column for each analysis differ significantly (P<0.05)

Similarly, approximately 1.4 log reduction was achieved in the count of osmophilic yeasts, depending on the gas applied and the application time. An increase in the count of osmophilic yeasts was observed during the storage period.

The disruption of the cell structure and the resulting microbial inactivation depend on the exposure time to the cold plasma application and the type of gas used for disinfection (Ganesan et al., 2021). Reactive species such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) are significant in the inactivation mechanism of pathogens (Asl et al., 2022). ROS produced during plasma production cause strong oxidative stress, and cells are damaged by enzyme inactivation, lipid peroxidation, and DNA cleavage. In addition, RNS is highly toxic and can cause cell death by damaging DNA (Heo et al., 2021). During the plasma application, the oxygen in the air plasma causes the formation of peroxide and the formation of lethal species such as O*, O2, and O3. In addition, the

accumulation of charged particles on the outer cell membrane, and electrostatic forces could cause subsequent rupture of the cell membrane and subsequent cell death (Devi et al., 2017). DNA damage caused by UV radiation produced during plasma is also thought to be effective in microbial inactivation (Liao et al., 2017).

Akarca et al. (2023) reported that CP application significantly reduced mold growth in kosher cheese. In addition, Molina-Hernandez et al. (2022) reported that CP application significantly slowed spore germination on the growth of mold spores in sun-dried tomatoes. Similarly, Ulbin-Figlewicz et al. (2015) stated that CP application using helium gas reduced the number of yeast and mold on the meat surface by 2 logs. Devi et al. (2017) revealed that CP application to peanuts largely stopped the growth of two important molds, A. flavus and A. parasiticus, in aflatoxin production.

Although the pH values of all samples decreased during storage ($P<0.05$), in general, CP application decreased the pH value (Table 7). The increase in H ions during CP application is thought to be effective in this decrease in pH value. Reactive species produced by plasma, mainly with acidic properties such as nitric acid (HNO3) and nitrous acid (HNO2), are responsible for the pH decrease (Wang et al., 2022). The pH of tomato paste samples decreased during storage. The highest pH decrease was observed in the MOA2 sample.

Table 7. pH changes of tomato paste samples during storage
Çizelge 7. Domates salçası örneklerinin depolama süresince pH değişiklikleri

Sample	pH		
	Storage Time (Day)		
	0	7	14
C	4.39±0.07^{aB}	4.06±0.01^{bF}	3.95±0.06^{bA}
FO1	4.13±0.08^{bC}	4.34±0.02^{aD}	3.87±0.01^{cAB}
FO2	3.99±0.02^{bCD}	4.17±0.05^{aE}	3.84±0.01^{cAB}
FA1	4.57±0.04^{aA}	4.47±0.02^{aC}	3.91±0.09^{bAB}
FA2	3.90±0.02^{bD}	4.33±0.03^{aD}	3.78±0.04^{bAB}
MOA1	4.52±0.03^{bAB}	4.72±0.03^{aB}	3.77±0.05^{cB}
MOA2	3.89±0.04^{bD}	4.87±0.04^{aA}	3.79±0.07^{bAB}

a(→)b: Values with the same capital letters in the same rows for each analysis differ significantly ($P<0.05$)
A(↓)F: Values with the same capital letters in the same column for each analysis differ significantly ($P<0.05$)

The a_w value of tomato paste samples (Table 8) decreased both with CP application and during storage ($P<0.05$). This decrease in aw is thought to be caused by the drying on the surface due to the flow rate of the gas applied during the process. Lee et al. (2020) applied cold plasma to red pepper flakes and reported a similar decrease in a_w. The decrease in a_w could be attributed to the ability of O_2 and Ar gases used in plasma application to take up free water molecules on the tomato paste surface.

Table 8. a_w changes of tomato paste samples during storage
Çizelge 8. Domates salçası örneklerinin depolama süresince a_w değişiklikleri

Sample	a_w		
	Storage Time (Day)		
	0	7	14
C	0.819±0.03^{aA}	0.797±0.03^{bA}	0.788±0.04^{bA}
FO1	0.808±0.02^{aB}	0.740±0.02^{bC}	0.739±0.04^{bB}
FO2	0.798±0.01^{aC}	0.739±0.02^{bC}	0.731±0.02^{cBC}
FA1	0.801±0.02^{aC}	0.742±0.08^{bBC}	0.734±0.03^{bBC}
FA2	0.733±0.01^{aE}	0.735±0.03^{aC}	0.718±0.03^{bD}
MOA1	0.808±0.03^{aB}	0.748±0.02^{bB}	0.727±0.04^{cCD}
MOA2	0.741±0.01^{aD}	0.739±0.01^{aC}	0.734±0.01^{aBC}

a(→)c: Values with the same capital letters in the same rows for each analysis differ significantly ($P<0.05$)
A(↓)E: Values with the same capital letters in the same column for each analysis differ significantly ($P<0.05$)

Since the plasma treatment is applied only to the product's surface, the chemical reaction takes place on the product's surface. The presence of a chemical reaction can be traced from the change in color, texture, or aroma (Bermúdez-Aguirre et al., 2013). Both CP application and storage process caused an increase in L* value (Table 9) ($P<0.05$). The highest L^* value was determined in the MOA2 sample. In non-thermal applications such as CP, the free radicals formed due to the low-temperature processing of the product and the liberation of intracellular compounds during the application were effective in increasing the product's brightness (Mehta et al., 2019).

Table 9. Changes in L*values of tomato paste samples during storage
Çizelge 9. Domates salçası örneklerinin depolama süresince L değerlerindeki değişiklikler*

Sample	*L** Value		
	Storage Days		
	0	7	14
C	22.24±0.72^{bC}	25.80±1.29^{bB}	30.99±0.04^{aD}
FO1	24.82±1.12^{bBC}	30.22±1.17^{aA}	30.41±0.02^{aE}
FO2	24.32±0.02^{cBC}	28.92±0.36^{bA}	30.12±0.15^{aF}
FA1	29.67±0.07^{bA}	29.99±0.12^{bA}	30.79±0.09^{aD}
FA2	29.31±0.02^{cA}	30.74±0.09^{bA}	31.67±0.07^{aB}
MOA1	29.64±0.48^{bA}	30.59±0.08^{aA}	31.37±0.05^{aC}
MOA2	28.76±1.97^{bAB}	29.94±0.17^{aBA}	32.48±0.03^{aA}

a(→)c: Values with the same capital letters in the same rows for each analysis differ significantly (P<0.05)
A(↓)F: Values with the same capital letters in the same column for each analysis differ significantly (P<0.05)

CP application and storage time caused a decrease in *a** value (Table 10), but this decrease was not statistically significant (P>0.05). The lowest *a** value was measured in the MOA1 sample. These results are in accordance with Jayasena et al. (2015). Bermúdez-Aguirre et al. (2013) applied atmospheric cold plasma to fresh tomatoes and stated that the *a** value increased after 7 and 10 minutes of application, but this increase was not statistically significant. Similarly, *b** values (Table 11) of tomato paste samples decreased with CP application. Jiang et al., (2017), and Khani et al., (2017) reported that cold plasma application to tomatoes did not cause a significant change in color. It is thought that there is a decrease in *a** and *b** values due to the loss of phenolic components (Mehta et al., 2019). The changes in these color parameters could be a result of oxidation of both pigments and lipids (Olatunde et al., 2019).

Table 10. Changes in a*values of tomato paste samples during storage
Çizelge 10. Domates salçası örneklerinin depolama süresince a değerlerindeki değişiklikler*

Sample	*a** Value		
	Storage Days		
	0	7	14
C	28.28±0.21^{aAB}	28.18±0.07^{aA}	27.29±0.02^{bA}
FO1	28.45±0.22^{aAB}	27.81±0.74^{aAB}	27.36±0.29^{aA}
FO2	30.18±1.99^{aA}	27.57±1.97^{aAB}	26.61±0.27^{aB}
FA1	26.55±0.81^{aB}	26.18±0.05^{aAB}	25.34±0.04^{aC}
FA2	29.36±0.02^{aAB}	25.99±0.87^{bAB}	23.87±0.05^{bD}
MOA1	26.56±0.04^{aB}	25.12±0.03^{bAB}	23.59±0.04^{cD}
MOA2	26.41±0.54^{aB}	25.05±0.02^{abB}	23.81±0.13^{bD}

a(→)c: Values with the same capital letters in the same rows for each analysis differ significantly (P<0.05)
A(↓)D: Values with the same capital letters in the same column for each analysis differ significantly (P<0.05)

The lycopene results of the samples are given in Table 12. A decrease in lycopene values was observed after 14 days of storage. However, this change is not statistically significant (P > 0.05). It should be noted that only for FO1 the change between day 7 and day 14 of storage was significant (P < 0.05). Cold plasma application caused a significant change in the lycopene values of the samples (P < 0.05). A decrease in lycopene values was observed. In particular, a decrease occurred in the samples that applied 100% oxygen (FO1 and FO2), and the decrease in lycopene values was more remarkable as the application time increased.

The BI indexes of the samples decreased during storage (P<0.05). At the end of storage, the highest decrease was detected in the FO2-coded sample with a value of -3284.84, while the least decrease was detected in the MOA2-coded samples with a value of 4792.79 (Table 12)

The main reason for the decrease in the amount of lycopene is that it has a highly unsaturated structure and as a result, oxidation occurs by photooxidation or auto-oxidation. The colorless end products formed from these oxidative reactions may cause the red color to bleach or lighten (Jayathunge et al., 2019). The leading causes of lycopene degradation at elevated temperatures are isomerization and oxidation. In addition, environmental factors such as weather, light, and temperature can change the effect of these two processes on the lycopene content of tomato products (Jabbari et al., 2018). In cold plasma application, the mechanism responsible for the change in the amount of lycopene is oxidation. It should be stated that the decrease in the lycopene value, especially in the samples using 100% O_2, is due to the increase in oxidation.

Table 11. Changes in b*values of tomato paste samples during storage
Çizelge 11. Domates salçası örneklerinin depolama süresince b değerlerindeki değişiklikler*

Sample	*b** Value		
	Storage Days		
	0	7	14
C	15.93±0.17^{bA}	16.98±0.22^{aBA}	17.94±0.35^{aAB}
FO1	14.48±0.03^{cB}	15.61±0.56^{bAB}	16.93±0.17^{aAB}
FO2	14.24±0.02^{aB}	15.60±1.29^{aAB}	19.52±2.45^{aA}
FA1	13.34±0.04^{bC}	16.39±0.35^{aBAB}	17.20±1.38^{aAB}
FA2	12.16±0.03^{cE}	14.71±0.70^{bB}	16.26±0.07^{aABC}
MOA1	12.65±0.09^{bD}	12.59±0.35^{bC}	14.47±0.05^{aBC}
MOA2	12.20±0.10^{cE}	12.40±0.06^{bC}	13.11±0.02^{aC}

a(→)c: Values with the same capital letters in the same rows for each analysis differ significantly (P<0.05)
A(↓)E: Values with the same capital letters in the same column for each analysis differ significantly (P<0.05)

Table 12. Changes in Brown Index(BI) values of tomato paste samples during storage
Çizelge 12. Domates salçası örneklerinin depolama süresince Esmerleşme İndeksindeki (Eİ) değişiklikler

Sample	BI Index		
	Storage Days		
	0	7	14
C	5046.38±775.35^{aC}	3071.35±646.86^{aC}	314.546±852.02^{bAB}
FO1	7815.44±315.92^{aBC}	5054.52±180.26^{bB}	2195.24±60.74^{cAB}
FO2	10275.96±3260.81^{aAB}	4795.77±45.35^{abB}	-3284.84±5697.94^{bB}
FA1	7599.95±1447.88^{aBC}	1763.75±162.56^{abD}	-653.72±3511.83^{bAB}
FA2	13005.38±29.37^{aA}	4519.69±1263.77^{bB}	-717.67±79.67^{cAB}
MOA1	8834.24±192.23^{aB}	7255.28±37.78^{bA}	2118.48±54.52^{cAB}
MOA2	9455.06±923.49^{aB}	7500.71±100.43^{bA}	4792.79±195.35^{cA}

a(→)c: Values with the same capital letters in the same rows for each analysis differ significantly (P<0.05)
A(↓)C: Values with the same capital letters in the same column for each analysis differ significantly (P<0.05)

Table 13. Changes in lycopene values of tomato paste samples during storage
Çizelge 13. Domates salçası örneklerinin depolama süresince likopen değerlerindeki değişiklikler

Sample	Lycopene mg/100g DM		
	Storage Time (Day)		
	0	7	14
C	23.42±0.26^{aA}	23.20±0.12^{aA}	22.67±0.09^{aA}
FO1	19.58±0.11^{aE}	19.27±0.06^{aF}	18.91±0.04^{bD}
FO2	15.96±0.09^{aF}	15.82±0.10^{abG}	15.30±0.16^{bE}
FA1	22.03±0.10^{aB}	21.96±0.08^{abB}	21.60±0.07^{bB}
FA2	21.75±0.13^{aB}	21.63±0.09^{abC}	21.22±0.11^{bB}
MOA1	20.61±0.12^{aC}	20.44±0.11^{aD}	19.89±0.23^{aC}
MOA2	20.05±0.07^{aD}	19.83±0.06^{abE}	19.21±0.22^{bD}

a-b: Values with the same capital letters in the same rows for each analysis differ significantly (P<0.05)
A(↓)F: Values with the same capital letters in the same column for each analysis differ significantly (P<0.05)

In the study where two different gases and mixtures of these gases were used, oxygen gas application showed the most effect on the physicochemical and microbiological properties of the samples compared to the control sample. Mixture and argon gas applications followed this effect, and prolonging the application period also increased the effect.

CONCLUSION

Tomato paste, one of the indispensable components of many cuisines in the world, is a product especially sensitive to mold growth. In this study, the effect of CP application, which is a minimal treatment, on the microbiological quality of tomato paste was investigated. CP application caused a decrease in pH and aw values of tomato paste. While *L** value increased, *a** and *b** values decreased in the samples. For microbiological evaluation, TMAB, TPAB, total mold, total yeast and osmophilic yeast values were examined. A decrease of 2 log in TPAB value and 1 log

in other microbiological criteria was achieved. The most effective application was determined as the application coded MOA2.

Different brix ranges are produced by companies in the production of industrial tomato paste. Especially a low brix degree is preferred, especially for lower-cost products. As a result, especially after the product is opened, high water activity leads to rapid molding, significantly shortening its shelf life.

Parallel to the results obtained from similar studies on the subject, it was demonstrated in this study that CP application could be used effectively to prevent or at least delay the mold problem, which is one of the biggest problems in tomato paste production. Further studies on the subject should be planned to reveal the positive/negative biochemical and chemical effects of CP application on the product in more detail.

Credit Contribution of the Authors

Azize Atik: Conceptualization (Equal), Writing – Original Draft Preparation (Lead).

İlker Atik: Conceptualization (Equal), Writing – Review & Editing (Lead).

Gökhan Akarca: Methodology (Lead), Formal analysis (Lead).

Ayşe Janseli Denizkara: Investigation (Lead).

Declaration of competing interest

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

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Van İlindeki Süt Sığırcılığı İşletmelerinin Sosyo Demografik ve İşletmecilik Özelliklerinin Karşılaştırılması

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ÖZET

Bu araştırmada, Van İli Damızlık Sığır Yetiştiricileri Birliğine (VDSYB) üye olan ve olmayan süt sığırcılığı işletmelerinin sosyo-demografik ve işletmecilik yapılarının karşılaştırılması amaçlanmıştır. Araştırmanın materyalini, Van İli Damızlık Sığır Yetiştiricileri Birliğine üye olan 89 ve üye olmayan 89 işletmeden toplanan anket verileri oluşturmuştur. İşletmelerin sosyo demografik ve işletmecilik yapılarının karşılaştırılmasında parametrik ve non parametrik testler kullanılmıştır. İşletmelerin sosyo demografik özellikleri; yaş, eğitim durumu ve deneyim bakımından önemli farklılıkların olmadığı belirlenmiştir. Ancak işletmecilik yapıları bakımından; traktöre sahip olma, inek sayısı, süt verimi, sağım öncesi meme temizliği yapma, işletmede kayıt tutma, tarımsal desteklerden faydalanma, silaj ve hayvan hayat sigortası yaptırma değişkenleri bakımından birliğe üye olan ve olmayan işletmeler arsında önemli farklılıkların olduğu tespit edilmiştir. Bu sonuçlara göre, VDSYB'ne üye olan süt sığırcılığı işletmelerinin göreceli olarak daha bilinçli ve daha iyi üretim olanaklarına sahip olduğu söylenebilir. Bu sonuçlar tarımsal örgütlerin üyelerine ve ortaklarına doğrudan veya dolaylı olarak önemli katkılar sağladığını göstermiştir.

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Van

Comparison of Socio Demographic and Management Characteristics of Dairy Cattle Enterprises in Van Province

ABSTRACT

In this study, it was aimed to compare the socio-demographic and management structures of dairy cattle enterprises that are and are not members of the Cattle Breeders' Association of Van Province. The material of the study consisted of survey data collected from 89 member and 89 non-member dairy cattle enterprises of Van Province Cattle Breeders' Association. Parametric and non-parametric tests were used to compare the socio-demographic and management structures of the enterprises. It was determined that there were no significant differences in terms of socio-demographic characteristics of the enterprises; age, education level and experience. However, in terms of management structures; it was determined that there were significant differences between the enterprises that were members and non-members of the union in terms of the variables of having a tractor, number of cows, milk yield, udder cleaning before milking, keeping records in the enterprise, benefiting from agricultural supports, silage and animal life insurance. According to these results, it can be said that dairy cattle enterprises that are members of Cattle Breeders' Association are relatively more conscious and have better production opportunities.

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GİRİŞ

Tarımsal üretimin ana bileşenlerinden bir olan hayvansal üretim faaliyeti, hayvansal kaynaklı gıda maddelerinin üretim yeri olması, aile işgcüne istihdam sağlaması, kırsal alandan kentsel alana göçü azaltılması gibi önemli sosyal ve ekonomik fonksiyonlara sahiptir (Sayın, 2001; Yıldırım & Şahin, 2003; Aksoy ve ark., 2012; Bakır & Kibar, 2019; Özdemir ve ark., 2021; Terin ve ark., 2022). Bu nedenle hayvancılık bütün dünyada özellikle de gelişmiş ülkelerde önemli bir endüstri haline gelmiş ve ekonominin ayrılmaz bir parçası durumuna gelmiştir (Ergün & Bayram, 2021).

Hayvansal kaynaklı gıda maddelerinin başında et ve süt ürünleri gelmekte olup, Türkiye'de süt ve kırmızı et üretiminin büyük bir bölümü büyükbaş hayvancılıktan sağlanmaktadır. Nitekim 2021 yılı itibariyle Türkiye'de üretilen toplam süt miktarının %92.1'i (21,37 milyon ton) ve kırmızı et üretiminin de %74.8'i (1,46 milyon ton) sığırdan elde edilmiştir (HAYGEM, 2023). Türkiye'de hayvancılığın gelişmesi için günümüze kadar birçok ıslah ve destekleme politikası uygulanmış ve uygulanmaya devam etmektedir. Özellikle 2000'li yıllardan sonra hayvancılık desteklerinde önemli artışlar sağlanarak, hayvansal üretimin geliştirilmesine çalışılmıştır. Bu çalışmaların bir sonucu olarak toplam tarımsal üretim değeri içerisinde hayvansal üretimin payı 2003'te %25 iken, 2017'de %34'e ve 2020 yılında %55'e yükselmiştir (TÜİK, 2023a). Bu önemli artışlara karşın halen hayvansal üretiminde başta verim düşüklüğü ve kayıt dışılık olmak üzere işletmelerin küçük ölçekli ve örgütsüz olması temel yapısal sorunlar olarak karşımıza çıkmaktadır.

Türkiye'de büyükbaş hayvancılık yapan işletmelerinin %44.5'i 1-4 baş arasında büyükbaş hayvana sahip olup, bu işletmelerin sahip olduğu büyükbaş hayvan varlığının, toplam büyükbaş hayvan varlığı içindeki payı %7.9'dur (TÜİK, 2023b). Bu sonuçlara göre Türkiye'de büyükbaş hayvancılık yapan işletmelerin yaklaşık olarak yarısının küçük aile işletmesi olduğu söylenebilir. İşletmelerin küçük ölçekli olması, bu işletmelerin girdi tedariğinde ve ürün satışlarında rekabet gücüne sahip olamamasına ve dolayısıyla işletmelerin yeter gelir elde edememesine neden olmaktadır. Bu nedenle, küçük ölçekli işletmelerin ekonomik yönden güçlü, sürdürülebilir ve sahip oldukları kaynakları daha etkin ve verimli kullanabilmeleri için örgütlü olması gerekmektedir.

Damızlık Sığır Yetiştiricileri Birlikleri (DSYB) çiftçi çıkarlarını karşılıklı yardımlaşma yoluyla koruyan ekonomik örgütlerdir (Yercan, 2007; Aksoy ve ark., 2014; Tapkı ve ark., 2018; Demirbük ve Kızılaslan, 2020). Tarım ve Orman Bakanlığı tarafından çıkarılan yönetmeliklerle kurulan ve temel amaçları; hayvan yetiştiricilerini kendi aralarında örgütlendirip, rekabet edebilirliğini arttıran, üstün verimli ırkların yetiştirilmesine olanak sağlayan ve üyelerin eğitim ve ihtiyaçlarını temin ve tedarik eden kuruluşlardır (Anonim, 2001).

Literatürde DSYB'ne üye olan ve olmayan sığırcılık işletmeleri çeşitli açılardan karşılaştırılmış olup bazılarına bu kısımda yer verilmiştir. Savran (2003) tarafından Çanakkale'de yapılan çalışmada DSYB'ne üye olan ve olmayan süt sığırı işletmeleri kullandıkları üretim teknikleri ve sosyal özellikleri bakımından karşılaştırmış ve iki grup arasında sosyal yönde belirgin farklılıkların olmadığını tespit etmiştir. Amasya'da yapılan çalışmada DSYB'ne üye olan işletmelerin arazi varlığının üye olmayan işletmelere göre 2.2 kat, yem bitkisi ekim alanının 2.5 kat daha fazla olduğu belirlenmiştir (Özüdoğru, 2010). Bhuayn (2012) tarafından Kuzeydoğu Amerika'da yapılan çalışmada, süt kooperatifine üye olan işletmelerin ortalama inek sayısının, toplam süt üretiminin, mera alanlarının ve deneyim sürelerinin üye olmayan işletmelere göre daha fazla olduğu tespit edilmiştir. Erzurum'da yapılan çalışmada DSYB'ne üye olan işletmelerdeki yetiştiricilerin daha genç ve hayvan başına süt verimlerinin daha yüksek olduğu tespit edilmiştir (Aksoy ve ark., 2014). Akkurt & Köknaroğlu (2016) tarafından Isparta'da yapılan çalışmada DSYB'ne üye olan işletmelerin hayvan varlığı, ortalama aylık geliri ve günlük süt üretiminin birliğe üye olmayan işletmelere göre daha fazla olduğu belirlenmiştir. Chagwiza ve ark. (2016) tarafından Etiyopya'da yapılan çalışmada, kooperatife üye olan işletmelerin ailedeki birey sayısı, süt sığırcılığından elde edilen gelir, günlük süt üretimi ve inek başına süt veriminin üye olmayan işletmelere göre daha fazla olduğu, ancak üye olan ve olmayan işletmeler arasında deneyim, arazi varlığı ve ortalama süt satış fiyatı bakımından önemli bir farkın olmadığı belirlenmiştir. Santosh (2017) tarafından yapılan çalışmada kooperatife üye işletmelerin süt sığırcılığı bilgi düzeyi, yeniliklerin adaptasyonu, süt üretimi ve hayvan varlığının üye olmayan işletmelere göre daha fazla olduğu tespit edilmiştir. Fikadu ve ark. (2019) tarafından Etiyopya'da yapılan çalışmada kooperatife ortak olan işletme sahiplerinin kooperatife üye olmayan işletme sahiplerine göre; daha yaşlı, daha eğitimli, daha fazla hayvana sahip olduğu ve yayım servislerini daha sık ziyaret ettikleri tespit edilmiştir.

Van'da konu ile ilgili daha önce bir araştırma yapılmamış olması, çalışmanın yapılması için motivasyon kaynağı oluşturmuştur. Araştırmanın temel amacı, Van İli Damızlık Sığır Yetiştiricileri Birliği'ne üye olan ve olmayan işletmelerin sosyo-demografik ve işletme yapıları arasında fark olup olmadığını ve bu özelliklerin Birliğe üye olup olmama ile arasındaki ilişkiyi tespit etmektir.

MATERYAL ve METOD

Materyal

Araştırmanın materyalini, Van ili Damızlık Sığır Yetiştiricileri Birliği'ne üye (89) ve üye olmayan (89) toplam 178 süt sığırcılığı işletmesinden toplanan anket verileri oluşturmuştur. Anket yapılacak birlik üyesi işletme sayısının belirlenmesinde oransal örnek hacmi formülü kullanılmıştır (Newbold, 1995; Miran, 2002). Anketler 2019 yılı Haziran-Eylül ayları arasında gerçekleştirilmiştir.

$$n = \frac{N p(1-p)}{(N-1)\sigma_{p_x}^2 + p\,(1-p)} \qquad (1)$$

Yöntem

Anketlerden elde edilen sosyo-demografik veriler frekans, çapraz tablolar ve ortalamalar verilerek ile analiz edilmiştir. Birliğe üye olan ve olmayan işletmelerin sahip oldukları sosyo-demografik ve işletme yapılarına ait özellikler arasında fark olup olmadığının belirlenmesi için, verilerin normal dağılım gösterdiği değişkenlerde t-testi, verilerin normal dağılım göstermediği değişkenlerde ise Mann-Whitney U testi kullanılmıştır. Yanı sıra, işletmelerin Damızlık Sığır Yetiştiricileri Birliği'ne üyelik durumu ile sosyo-demografik ve işletmecilik özellikleri arasındaki ilişkilerin belirlenmesinde Ki kare testi kullanılmıştır. Elde dilen verilerin analizi SPSS 22 istatistik paket programı ile yapılmıştır.

BULGULAR ve TARTIŞMA

Araştırmada ortalama yetiştirici yaşı DSYB'ne üye işletmelerde 47.65 yıl ve üye olmayan işletmelerde 47.01 yıl olarak tespit edilmiştir (Çizelge 1). Antalya'da yapılan çalışmada DSYB'ne üye olan ve olmayan işletmelerde çiftlerin ortalama yaşı sırası ile 45.5 yıl ve 49.7 yıl (Kızılay & Akçaöz, 2008), Amasya'da yapılan çalışmada 44.0 yıl ve 48.0 yıl (Özüdoğru, 2010) ve Erzurum'da yapılan çalışmada 39.9 yıl ve 46.3 yıl (Aksoy ve ark., 2014) olarak tespit edilmiştir. Araştırmadan elde edilen sonuçların literatürle benzerlik gösterdiği görülmektedir. Damızlık Sığır Yetiştiricileri Birliği'ne üye olan ve olmayan işletmelerde üreticilerin ortalama yaşları arasındaki farkın önemli olmadığı tespit edilmiştir (P>0.05).

Çizelge 1. Yetiştiricilerin yaş gruplarına göre dağılımı
Table 1. Distribution of breeders by age groups

Yaş grupları	Üyelik Durumu					
	Üye		Değil		Toplam	
	Frekans	%	Frekans	%	Frekans	%
20-40	32	35.96	27	30.34	59	33.15
41-60	41	46.07	48	53.93	89	50.00
61 ve üzeri	16	17.98	14	15.73	30	16.85
Toplam	89	100.00	89	100.00	178	100.00
Ortalama	47.65		47.01		47.33	

X^2=1.11 p=0.575

Araştırmada, DSYB'ne üye yetiştiricilerin %67.42'sinin, üye olmayan yetiştiricilerin ise %62.92'sinin ilkokul mezunu oldukları belirlenmiştir (Çizelge 2). Türkiye'nin farklı illerinde yapılan çalışmalarda Damızlık Sığır Yetiştiricileri Birliği'ne üye olan yetiştiricilerin ilkokul mezunu olma oranları Uşak'ta %58.0 (Köse, 2006), Sivas'ta %49.0 (Baş Hozman, 2014), Iğdır'da %49.4 (Yeşil, 2015) ve Hatay'da %66.6 (Tapkı ve ark., 2020) olarak tespit edilmiştir. Araştırmada elde edilen sonuçlarla literatür arasında fark olduğu ve araştırma bölgesindeki yetiştiricilerin eğitim düzeylerinin daha düşük olduğu söylenebilir. Khi kare testine göre DSYB'ne üye olma ile eğitim düzeyi arasında önemli bir ilişki yoktur (P>0.05). Gül (2014) tarafından Amasya'da ve Akkurt & Köknaroğlu (2016) tarafından Isparta'da yapılan çalışmalarda da DSYB'ne üye olma ile eğitim düzeyi arasındaki ilişki istatistiki olarak önemli bulunmamıştır.

Çizelge 2. İşletmelerde yetiştiricilerin eğitim durumu
Table 2. Educational status of the breeders in the enterprises

Eğitim Düzeyi	Üyelik Durumu					
	Üye		Değil		Toplam	
	Frekans	%	Frekans	%	Frekans	%
Okur-yazar olmayan	2	2.25	3	3.37	5	2.81
Okur-yazar	6	6.74	5	5.62	11	6.18
İlkokul	60	67.42	56	62.92	116	65.17
Ortaokul	13	14.61	13	14.61	26	14.61
Lise	8	8.99	12	13.48	20	11.24
Toplam	89	100.00	89	100.00	178	100.00

X^2=1.23 p=0.873

Araştırmada Damızlık Sığır Yetiştiricileri Birliği'ne üye işletmelerde ortalama süt sığırcılığı deneyimi 25.63 yıl iken, birliğe üye olmayan işletmelerde 26.20 yıl olarak belirlenmiştir. Birliğe üye olan ve olmayan işletmelerde ortalama deneyim süresi arasında fark olup olmadığı Mann-Whitney U testi ile test edilmiş ve aradaki farkın önemli olmadığı belirlenmiştir (P>0.05).

Yetiştiricilerin mesleki bilgilerini ve becerilerini arttırabilmeleri için kurs, seminer ve çiftçi toplantıları gibi tarımsal yayım faaliyetlerine katılmaları oldukça önemlidir. Araştırmada, DSYB'ne üye yetiştiricilerin %32.58'inin, üye olmayan yetiştiricilerin ise %11.24'ünün süt sığırcılığı ile ilgili kurs, seminer ve çiftçi toplantılarına katıldıkları belirlenmiştir (Çizelge 3). Amasya'da yapılan çalışmada DSYB'ne üye çiftçilerin tamamının, üye olmayan çiftçilerin ise %17.4'ünün (Gül, 2014), Ankara'da yapılan çalışmada birliğe üye işletmelerin %9.60'ının (Özyılmaz, 2010) ve Sivas'ta yapılan çalışmada DSYB'ne üye çiftçilerin %14.2'sinin (Baş Hozman, 2014) hayvancılıkla ilgili herhangi bir kursa katıldıkları tespit edilmiştir. DSYB üyelik durumu ile süt sığırcılığı ile ilgili kurs ve seminere katılma arasındaki ilişki %5 düzeyinde istatistiki olarak önemlidir (P<0.05). Bu sonuca göre, DSYB'ne üye olan yetiştiricilerin daha fazla oranda süt sığırcılığı ile ilgili kurs ve seminere katıldıkları ve sonucun beklentilerle uyumlu olduğu söylenebilir.

Çizelge 3. İşletmelerde yetiştiricilerin süt sığırcılığı ile ilgili kursa katılım durumu
Table 3. Participation of the breeders in the dairy cattle course in the enterprises

Kursa katılma	Üyelik Durumu					
	Üye		Değil		Toplam	
	Frekans	%	Frekans	%	Frekans	%
Katıldım	29	32.58	10	11.24	39	21.91
Katılmadım	60	67.42	79	88.76	139	78.09
Toplam	89	100.00	89	100.00	178	100.00

X^2=11.854 p=0.01

Araştırmada, DSYB'ne üye üreticilerin %55.06'sının, üye olmayan üreticilerin ise %67.42'sinin tarım dışı gelire sahip olduğu belirlenmiştir. Bu sonuca göre DSYB'ne üye olmayan işletmelerin daha fazla oranda tarım dışı gelire sahip oldukları söylenebilir. Erzurum'da DSYB'ne üye olmayan işletmelerin daha fazla oranda tarım dışı gelire sahip oldukları belirlenmiştir (Aksoy ve ark., 2014). DSYB'ne üyelik durumu ile tarım dışı gelire sahip olma arasındaki ilişki istatistiki olarak %10 düzeyinde önemlidir (P<0.10). Elde edilen sonuca göre, DSYB'ne üye olmayan çiftçilerin daha fazla tarım dışı gelire sahip oldukları söylenebilir.

Çizelge 4. İşletmelerde yetiştiricilerin tarım dışı gelire sahip olma durumu
Table 4. The non-agricultural income of the breeders in the enterprises

Tarım dışı gelir	Üyelik Durumu					
	Üye		Değil		Toplam	
	Frekans	%	Frekans	%	Frekans	%
Var	49	55.06	60	67.42	109	61.24
Yok	40	44.94	29	32.58	69	38.76
Toplam	89	100.00	89	100.00	178	100.00

X^2=2.864 p=0.06

Traktör, tarımsal işletmelerde kullanılan en önemli mekanizasyon aracı olup, işletmelerin büyüklüğü ve sermayesi hakkında önemli ipuçları vermektedir. Çalışmada DSYB'ne üye işletmelerin %70.79'unun traktöre sahip olduğu, üye olmayan işletmelerde ise üreticilerin %47.19'unun traktöre sahip olduğu belirlenmiştir (Çizelge 5). Amasya'da DSYB'ne üye olan işletmelerin %90.0'nında, üye olmayan işletmelerin ise %72.0'sinde (Özüdoğru, 2010) ve Kahramanmaraş'ta DSYB'ne üye işletmelerin %54.0'ünde, üye olmayan işletmelerin %51.0'inde traktör olduğu tespit edilmiştir (Kaygısız ve ark., 2010). DSYB'ne üyelik durumu ile traktöre sahip olma arasındaki ilişki %5 düzeyinde önemlidir (P<0.05). Bu sonuca göre, DSYB'ne üye olan çiftçilerin traktöre sahip olma oranının daha fazla olduğu söylenebilir.

Toprak (arazi), tarımsal üretimde en önemli üretim faktörlerinden biridir. Süt sığırcılığı işletmelerinin hayvan beslenmesinde ihtiyaç duydukları kaba ve kesif yemlerin üretimini gerçekleştirebilmek için araziye ihtiyaçları vardır. Toplam işlenen arazi büyüklüğü DSYB'ne üye işletmelerde ortalama 124.12 dekar iken, üye olmayan işletmelerde ortalama 121.73 dekar olarak belirlenmiştir. Birliğe üye işletmeler ile üye olmayan işletmelerin işledikleri ortalama arazi miktarı arasındaki fark istatistiki olarak önemli bulunmamıştır (P>0.05).

Çizelge 5. İşletmelerde yetiştiricilerin traktöre sahip olma durumu
Table 5. Ownership of tractors by the breeders in the enterprises

Traktöre sahip olma	Üyelik Durumu					
	Üye		Değil		Toplam	
	Frekans	%	Frekans	%	Frekans	%
Evet	63	70.79	42	47.19	105	58.99
Hayır	26	29.21	47	52.81	73	41.01
Toplam	89	100.00	89	100.00	178	100.00

X^2=10.241 p=0.02

Çizelge 6. İşletmelerde işletme başına ortalama arazi varlığı (da)
Table 6. Average land holding per enterprise in the analyzed enterprises (da)

Arazi mülkiyeti	Üyelik Durumu					
	Üye		Değil		Toplam	
	Ortalama	%	Ortalama	%	Ortalama	%
Mülk arazi (da)	76.00	61.23	89.65	73.65	82.83	67.38
Kiralanan arazi (da)	37.56	30.26	29.09	23.90	33.33	27.11
Ortak arazi (da)	10.56	8.51	2.99	2.45	6.78	5.52
Toplam İşlenen arazisi	124.12	100.00	121.73	100.00	122.93	100.00

Araştırmada DSYB'ne üye olan işletmelerde ortalama inek sayısı 9.72 baş iken, üye olmayan işletmelerde 6.93 baş olarak belirlenmiştir (Çizelge 7). Mann Whitney U testine göre, DSYB'ne üye ve üye olmayan işletmelerin sahip oldukları ortalama inek sayıları arasında fark %1 düzeyinde önemlidir (P<0.01). Elde edilen sonuca göre DSYB'ne üye işletmelerin daha fazla sayıda ineğe sahip oldukları söylenebilir. Özüdoğru & Tatlıdil (2012) tarafından Amasya'da, Akkurt & Köknaroğlu (2016) tarafından Isparta'da yapılan çalışmalarda da DSYB'ne üye olan işletmelerde inek varlığı, üye olmayan işletmelere göre daha fazla bulunmuştur.

Çizelge 7. İşletmelerde işletme başına ortalama inek sayısı
Table 7. Average number of cows per enterprise in the enterprises

İnek sayısı	Üyelik Durumu					
	Üye		Değil		Toplam	
	Ortalama	%	Ortalama	%	Ortalama	%
Kültür	8.47	87.14	4.93	71.14	6.70	80.43
Kültür-melez	0.93	9.57	1.00	14.43	0.97	11.65
Yerli	0.31	3.19	0.96	13.85	0.63	7.52
Toplam inek*	9.72	100.00	6.93	100.00	8.33	100.00

* Mann-Whitney U testine göre p<0.01 düzeyinde önemlidir

Araştırmada DSYB'ne üye olan işletmelerde kültür ırkı ineklerde günlük ortalama süt verimi 13.62 kg, iken, üye olmayan işletmelerde 10.88 kg olarak hesaplanmıştır (Çizelge 8). Bu sonuçlara göre, DSYB'ne üye olan işletmelerde süt veriminin daha yüksek olduğu söylenebilir. Nitekim yapılan test sonuçlarına göre de gruplar arasındaki fark %1 düzeyinde önemlidir (P<0.01). Aksoy ve ark. (2014) tarafından Erzurum'da, Özüdoğru & Tatlıdil (2012) tarafından Amasya'da, Gençdal ve ark. (2016) tarafından Van'da ve Chagwiza ve ark. (2016) tarafından Etiyopya'da yapılan çalışmalarda da DSYB'ne veya Kooperatife üye olan işletmelerin süt verimlerinin üye olmayan işletmelere göre daha yüksek olduğu tespit edilmiştir.

Araştırmada DSYB'ne üye olan işletmelerde kültür ırkı ineklerde laktasyon süresi 6.47 ay, kültür-melez ırklarda 5.67 ay ve yerli ırklarda 5.43 ay iken, üye olmayan işletmelerde sırası ile 6.68 ay, 6.05 ay ve 4.92 ay olarak belirlenmiştir (Çizelge 9). DSYB'ne üye olan ve olmayan işletmelerde ırklara göre laktasyon süreleri arasında fark olup olmadığı test edilmiş ve her üç ırk grubunda da fark istatistiki olarak anlamlı değildir (P>0.05). Aksoy ve ark. (2014) tarafından Erzurum'da, yapılan çalışmada da DSYB üye olan (6.2 ay) ve olmayan işletmelerde (6.1 ay) laktasyon süresi bakımından fark olmadığı tespit edilmiştir.

Süt ineklerinde memede oluşan enfeksiyonların %90'nı meme başından girdiği için sağım öncesi ve sağım sonrası meme temizliği çok önemlidir (Baştan & Salar, 2012). Araştırmada DSYB'ne üye olan işletmelerde sağım öncesi ve sonrası meme temizliği yapma oranı %97.75, üye olmayan işletmelerde %88.76'dır (Çizelge 10). Gül (2014) tarafından yapılan

çalışmada DSYB'ne üye işletmelerin %96.7'sinde, üye olmayan işletmelerin ise %87.8'inde meme temizliği yapıldığını belirtmiştir. İşletmelerin meme temizliği yapması ile birliğe üye olup olmaması arasındaki ilişki %5 düzeyinde önemlidir (P<0.05). Elde edilen sonuca göre DSYB'ne üye işletmelerin daha fazla oranda meme temizliği yaptıkları söylenebilir.

Çizelge 8. İşletmelerde ortalama günlük süt verimi (kg)
Table 8. Average daily milk yield in the enterprises (kg)

Irklar	Üyelik Durumu									
	Üye					Değil				
	N	Min	Mak.	Ort.	Std. S.	N	Min	Mak.	Ort.	Std. S.
Kültür*	74	4	20	13.62	3.49	57	5	20	10.88	2.87
Kültür-Melez	15	2	10	5.67	2.16	20	3	10	6.10	2.07
Yerli	7	2	6	3.57	1.72	24	2	7	3.92	1.53

* Mann-Whitney U testine göre p<0.01 düzeyinde önemlidir.

Çizelge 9. İşletmelerde ırklara göre ortalama laktasyon süresi (ay)
Table 9. Average lactation period by breeds in the enterprises (months)

Irklar	Üyelik Durumu					
	Üye		Değil		Toplam	
	Ortalama	Std. S	Ortalama	Std. S	Ortalama	Std. S
Kültür	6.47	1.29	6.68	1.24	6.56	1.27
Kültür-melez	5.67	1.60	6.05	1.43	5.89	1.49
Yerli	5.43	1.13	4.92	1.53	5.03	1.45

Çizelge 10. İşletmelerde sağım öncesi ve sonrası meme temizliği yapma durumu
Table 10. The status of udder cleaning before and after milking in the enterprises

Temizlik	Üyelik Durumu					
	Üye		Değil		Toplam	
	Frekans	%	Frekans	%	Frekans	%
Yapıyor	87	97.75	79	88.76	166	93.26
Yapmıyor	2	2.25	10	11.24	12	6.74
Toplam	89	100.00	89	100.00	178	100.00

X^2=5.719 p=0.032

Süt sığırcılığı işletmelerinde kayıt tutmak sadece yetiştiricilerin hayvanları hakkında bilgi sahibi olmasına fayda sağlamaz, yanı sıra işletmede üretim planlarının ve maliyet hesaplarının doğru bir şekilde yapılarak verimli ve karlı bir üretimin yapılabilmesine olanak sağlar. Genel olarak eğitimli, yeniliklere açık ve modern üretim yapan işletmelerde kayıt tutma oranlarının daha yüksek olduğu söylenebilir. Araştırmada DSYB'ne üye olan işletmelerde kayıt tutma oranı %39.33 iken, üye olmayan işletmelerde bu oran %16.85 olarak belirlenmiştir. Bu sonuçlara göre hem DSYB'ne üye hem de DSYB'ne üye olmayan işletmelerde kayıt tutma oranı oldukça düşüktür. Amasya'da yapılan çalışmada DSYB'ne üye işletmelerin %71.61'inde, üye olmayan işletmelerin ise %39.79'unda (Özüdoğru, 2010) ve Bingöl'de yapılan çalışmada DSYB'ne üye işletmelerin %37.2'sinde kayıt tutulduğu belirlenmiştir (Daş ve ark., 2014). İşletmelerin kayıt tutması ile DSYB'ne üye olup olmaması arasındaki ilişki %1 düzeyinde önemlidir (P<0.01). Birliğe üye olan işletmelerin üyeliğin gerektirdiği bazı verilerin kaydını tutması gerektiği için sonucun beklentilere uygun olduğu söylenebilir. Gül (2014) tarafından Amasya'da, Gençdal ve ark. (2016) tarafından Van'da yapılan çalışmalarda da benzer sonuçlar bulunmuştur.

Hayvansal üretim faaliyetinde kaba ve kesif yem maliyetleri oldukça önemli bir yere sahiptir. Bu nedenle süt sığırcılığında yem maliyetlerinin düşürülmesi ve süt veriminin arttırılmasında silaj kullanımı oldukça önemlidir (Boyar & Yumak, 2000). Araştırmada DSYB'ne üye işletmelerin %35.96'sının, üye olmayan işletmelerin ise %15.73'ünün silaj yaptıkları belirlenmiştir. Gül (2014) tarafından Amasya'da yapılan çalışmada Birlik üyesi işletmelerin %85.2'sinin, Birlik üyesi olmayan işletmelerin %52.8'inin, Kaygısız & Tümer (2009) tarafından Kahramanmaraş'ta yapılan çalışmada DSYB'ne üye işletmelerin %69'unun, üye olmayan işletmelerin %8'inin silaj yaptıkları, Yeşil (2015) tarafından Iğdır'da ve Önal & Özder (2008) tarafından Edirne'de yapılan çalışmalarda ise DSYB'ne üye işletmelerin sırasıyla %70.1'inin ve %95.6'sının silaj yaptıkları belirlenmiştir. Bu sonuçlara göre, Van ilinde silaj

yapımı literatürdeki çalışmalara göre düşüktür. Silaj yapma durumu ile DSYB'ne üye olup olmama arasındaki ilişki istatistiki olarak %1 düzeyinde önemlidir (P<0.01). Bu sonuca göre, DSYB'ne üye işletmelerin üye olmayan işletmelere göre daha yüksek oranda silaj yaptıkları söylenebilir.

Çizelge 11. İşletmelerde kayıt tutma durumu
Table 11. Record keeping status in the enterprises

Kayıt tutma	Üyelik Durumu					
	Üye		Değil		Toplam	
	Frekans	%	Frekans	%	Frekans	%
Evet	35	39.33	15	16.85	50	28.09
Hayır	54	60.67	74	83.15	128	71.91
Toplam	89	100.00	89	100.00	178	100.00

X^2=11.125 p=0.001

Çizelge 12. İşletmelerde silaj yapma durumu
Table 12. Status of silage making in the enterprises

Silaj yapma	Üyelik Durumu					
	Üye		Değil		Toplam	
	Frekans	%	Frekans	%	Frekans	%
Evet	32	35.96	14	15.73	46	25.84
Hayır	57	64.04	75	84.27	132	74.16
Toplam	89	100.00	89	100.00	178	100.00

X^2=9.498 p=0.003

Araştırmada işletmelerin %15.73'ü hayvan hayat sigortası yaptırırken, bu oran birlik üyesi işletmelerde %21.35 ve üye olmayan işletmelerde %10.11'dir (Çizelge 13). Bu sonuçlara göre işletmelerin büyük bir kısmının hayvanlarını sigorta yaptırmadığı ve sigorta alışkanlığının henüz yeteri kadar gelişmediği söylenebilir. Amasya'da yapılan çalışmada DSYB'ne üye işletmelerin %31.7'sinin, üye olmayan işletmelerin ise %7.8'inin hayvan sigortası yaptırdıkları tespit edilmiştir (Gül, 2014). Doğu Akdeniz Bölgesinde süt sığırcılığı işletmeleri ile yapılan çalışmada ise DSYB'ne üye işletmelerin sadece %6.0'sının hayvan hayat sigortası yaptırdıkları belirlenmiştir (Özer, 2019). İşletmelerin sigorta yaptırması ile DSYB'ne üye olup olmama arasındaki ilişki istatistiki olarak %5 düzeyinde anlamlı bulunmuştur (P<0.05). Birliğe üye olan işletmelerin sigorta yaptırma konusunda daha bilinçli ve istekli olduğu söylenebilir. Yılmaz (2008) tarafından Osmaniye'de, Gül (2014) tarafından Amasya'da ve Yaylak ve ark. (2016) tarafından İzmir'de yapılan çalışmalarda da benzer sonuçlar bulunmuştur.

Çizelge 13. İşletmelerde hayvan sigortası yaptırma durumu
Table 13. The status of animal insurance in the enterprises

Sigorta yaptırma	Üyelik Durumu					
	Üye		Değil		Toplam	
	Frekans	%	Frekans	%	Frekans	%
Evet	19	21.35	9	10.11	28	15.73
Hayır	70	78.65	80	89.89	150	84.27
Toplam	89	100.00	89	100.00	178	100.00

X^2=4.24 p=0.04

Araştırmada işletmelerin %89.89'u tarımsal desteklerden faydalanırken, bu oran birlik üyesi işletmelerde %95.51 ve birlik üyesi olmayan işletmelerde %84.27'dir (Çizelge 14). Tarımsal desteklerden faydalanma ile Birliğe üye olup olmama arasındaki ilişki %5 düzeyinde istatistiki olarak anlamlı bulunmuştur (P<0.05). Bu sonuçlara göre DSYB'ne üye olan işletmeler tarımsal desteklerden daha fazla oranda faydalanmaktadır. Sonuçlar beklentilerle uyuşmaktadır. Erzurum'da yapılan çalışmada da DSYB'ne üye olan işletmelerin üye olmayan işletmelere göre daha fazla sayıda tarımsal destekten faydalandıkları vurgulanmıştır (Aksoy ve ark., 2014).

Süt sığırcılığında en önemli iş yüklerinden biri de süt sağımıdır. Türkiye'de küçük aile işletmelerinde sağım genelde el ile yapılırken, büyük işletmelerde ağırlıklı olarak makineli sağım veya sağım ünitesi şeklinde yapılmaktadır. Araştırmada DSYB'ne üye olan işletmelerde makineli sağım %39.33 iken, üye olmayan işletmelerde %29.21 olarak tespit edilmiştir (Çizelge

15). Türkiye'de çeşitli illerde yapılan çalışmalarda DSYB'ne üye işletmelerde makineli sağım oranı, Edirne'de %100 (Önal & Özder, 2008), Amasya'da %85.0 (Gül, 2014), Sivas'ta %60.9 (Baş Hozman, 2014), Iğdır'da %92.8 (Yeşil, 2015) ve Bingöl'de %18.2 (Daş ve ark., 2014) olarak belirlenmiştir. Bu sonuçlara göre, Bingöl'de yapılan çalışma dışında diğer illerde yapılan çalışmalarda makineli sağım oranı araştırma sonuçlarına göre oldukça yüksektir. Sağım şekli ile DSYB'ne üye olup olmama arasındaki ilişki istatistiki olarak anlamlı değildir (P>0.05). Erzurum'da yapılan çalışmada benzer sonuç (Aksoy ve ark., 2014) bulunmuş iken, Gül (2014) tarafından Amasya'da yapılan çalışmada makine ile sağım yapan çiftçilerin Birliğe daha fazla oranda üye oldukları tespit edilmiştir.

Çizelge 14. İşletmelerin tarımsal desteklerden faydalanma durumu
Table 14. Utilization of agricultural subsidies by the enterprises

Destekten faydalanma	Üyelik Durumu					
	Üye		Değil		Toplam	
	Frekans	%	Frekans	%	Frekans	%
Evet	85	95.51	75	84.27	160	89.89
Hayır	4	4.49	14	15.73	18	10.11
Toplam	89	100.00	89	100.00	178	100.00

X^2=6.18 p=0.013

Çizelge 15. İşletmelerde süt sağım şekli
Table 15. Milking method in the enterprises

Sağım şekli	Üyelik Durumu					
	Üye		Değil		Toplam	
	Frekans	%	Frekans	%	Frekans	%
Elle sağım	54	60.67	63	70.79	117	65.73
Makinalı sağım	35	39.33	26	29.21	61	34.27
Toplam	89	100.00	89	100.00	178	100.00

X^2=2.02 p=0.103

SONUÇ ve ÖNERİLER

Araştırmada, Van İli Damızlık Sığır Yetiştiricileri Birliği'ne (VDSYB) üye olan ve olmayan süt sığırcılığı işletmelerinin sosyo-demografik ve işletmecilik özellikleri karşılaştırılmış ve bu özelliklerin Birliğe üye olup olmama arasındaki ilişki belirlenmeye çalışılmıştır.

Araştırmada, DSYB'ne üye ve üye olmayan işletmeler arasında ortalama işletmeci yaşı, eğitim düzeyi, ortalama süt sığırcılığı deneyim süresi, ortalama işlenen arazi miktarı, laktasyon süresi ve sağım şekli bakımından istatistiki olarak anlamlı bir farkın olmadığı belirlenmiştir. Buna karşın, süt sığırcılığı ile ilgili kursa katılma, tarım dışı gelire sahip olma, traktöre sahip olma, ortalama inek sayısı, inek başına süt verimi, sağım öncesi ve sonrası meme temizliği yapma, işletmede kayıt tutma, silaj yapma, hayvan hayat sigortası yaptırma ve tarımsal desteklerden faydalanma durumları bakımından istatistiki olarak önemli bir farkın olduğu tespit edilmiştir.

Araştırmada bilinçli, göreli olarak modern ve iyi üretim imkânlarına sahip süt sığırcılığı işletmelerinin daha yüksek oranda DSYB'ne üye oldukları söylenebilir. Araştırma bölgesinde süt sığırcılığı faaliyetinin gelişmesi ve sürdürülebilir olması açısından, yetiştiricilerin Damızlık Sığır Yetiştiricileri Birliği gibi hayvancılık örgütlerine üyeliğinin teşvik edilmesi önem arz etmektedir.

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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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Bilimsel Makalelerde Raporlanması Önerilen Çıkarımsal İstatistikler

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ÖZET

Bilimsel araştırmaların planlanması yürütülmesi ve sonuçlandırılmasında yaygın olarak çıkarımsal istatistik tekniklerden yararlanılmakta ve istatistiksel bulgular raporlanmaktadır. Bu çalışmada, araştırmalarda p-değerinin yanında raporlanması gereken diğer istatistikler irdelenmiştir. Araştırmalarda en sık kullanılan çıkarımsal istatistik yöntemi "Yokluk Hipotezi Anlamlılık Testi"dir. Bu yöntemin son çıkarımsal istatistiği ise p- değeridir. İstatistik testler sonucu elde edilen bu değer gerçek değeriyle ve üç ondalık basamakla rapor edilmelidir. P-değerinin tek başına sunumundan kaçınılmalıdır. Ayrıca ifade ettiğinden daha fazla anlam yüklenilmemelidir. Bu değer test istatistiği (t, z, χ^2, F vb.) ile birlikte yazılmalıdır. Ayrıca araştırma makalelerinde örneklem büyüklüğü, mutlaka belirtilmelidir. Bunun yanında, testin gücü, güven aralığı ve etki büyüklüğü istatistiklerine yer verilmesinde yarar vardır. Çünkü p-istatistiği ve testin gücü örneklem büyüklüğünden önemli ölçüde etkilenir. Örneklemin büyük olması, p- değerinin küçük, testin gücünün yüksek olmasına neden olmaktadır. Etki büyüklüğü ise örneklem büyüklüğünden etkilenmemektedir. Bu nedenle istatistiksel yorumlar özellikle etki büyüklüğü ve güven aralığı kullanılarak yapılmalıdır.

Çıkarımsal İstatistikler

Teknik Not

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Anahtar Kelimeler
Çıkarımsal istatistik
P-değeri
Güven aralığı
İstatistiksel güç
Etki büyüklüğü

Inferential Statistics Suggested to Report in Scientific Articles

ABSTRACT

Inferential statistical techniques are widely used in the planning, execution and conclusion of scientific research and statistical findings are reported. In this study, other statistics that should be reported in addition to the p-value in studies were examined. The most frequently used inferential statistical method in research is the "Null Hypothesis Significance Test". The final inferential statistic of this method is the p-value. This value obtained as a result of statistical tests should be reported with its true value and three decimals in scientific works. P-value should not be given more meaning than it expresses and should be reported together with the test statistic (t, z, χ^2, F etc.). In addition, the sample size should be specified in research articles. In addition, it would be beneficial to include the power of the test, confidence interval and effect size statistics. Because the p-value and the power of the test are significantly affected by the sample size. The large sample size causes the p-value to be small and the power of the test to be high. The effect size is not affected by the sample size. Therefore, statistical inference should be inferred using, especially effect size and confidence interval.

Inferential Statistics

Technical Note

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GİRİŞ

Bilimsel çalışmalarda istatistiksel düşünme ve istatistiksel analiz önemli bir yere sahiptir. Ekonomi, tarım, mühendislik, sağlık, eğitim, vd. tüm alanlarda yapılan bilimsel çalışmalarda istatistiksel analiz tekniklerinden yararlanılmaktadır. Bilimsel çalışma

sürecinin; hipotez kurma, değişken belirleme, örnekleme, ölçme, veri toplama, veri analizi ve yorumlama vd. tüm aşamalarında istatistik teknikler yoğunlukla kullanılmaktadır. Bu teknikler içinde yaygın kullanılan yöntem "Yokluk Hipotezi Anlamlılık Testi, (YHAT)" yöntemidir (Yıldırım & Yıldırım, 2011; Erkuş 2017; Akbulut, 2022). YHAT kısaca "Hipotez Testi" (HT) olarak ifade edilebilir. YHAT'nin son çıkarımsal istatistiği "p-değeridir". Leek & Peng (2015)'in ifadesi ile "p-değeri bilimsel araştırma ve veri analizi sürecinde buzdağının görülen ucudur". Araştırmaların çoğunda bulgular p-değerine odaklanarak çoğu kez de p-değeri tek başına yorumlanarak sunulmaktadır. P-değerinin tanımı (Cohen, 1994; Yıldırım & Yıldırım, 2011; Aschwanden, 2016) yorumlanması (Cohen, 1994; Cohen, 2011; Kılıç, 2011; Işık 2014; Lu & Belitskaya–Levy, 2015; Halsey et al., 2015) ve önemi (Hojat & Xu, 2004;. Dahiru, 2008; Lu & Belitskaya–Levy, 2015; Halsey et al., 2015) üzerinde son yıllarda yoğun değerlendirme ve tartışmalar yapılmaktadır. P-değerinin hatalı yorumlanması konusunda (Nuzzo, 2014; Lu & Belitskaya–Levy, 2015; Greenland et al., 2016; O'Leary, 2021) ve kötüye kullanımı (Vidgen & Yasseri, 2016) konusunda da makaleler yayınlanmıştır. Bu bağlamda yayınlanan makalelerin genel sonucu p-değerinin hatalı yorumundan kaçınılması ve p-değeri ile birlikte diğer çıkarımsal istatistik ölçülerin raporlanması şeklindedir (Goddman, 2008; Wasserstein & Lazar 2016; Mark et al., 2016; Wasserstein, et al., 2019; Gao, 2020; Akbulut, 2022). Bu bağlamda önerilen istatistik ölçüler etki büyüklüğü (EB), güven aralığı (GA) ve testin gücü (TG) ölçüleridir (Whitley & Ball, 2002; APA 2010; Kul, 2014; Işık, 2014; Greenland et al., 2016; Solla et al., 2018; Balkin & Lenz, 2021).

Bu makalede p-değeri ile birlikte sunulması gereken EB, GA ve TG çıkarımsal ölçüleri ele alınmış ve bu ölçülerin hesaplanması ve raporlanması sayısal örnekler ile açıklanmıştır.

α DEĞERİ ve P-DEĞERİ

İstatistiksel analizlerde α değeri, araştırmanın planlanması, örneklem büyüklüğü ve beklenen gücün hesaplanması aşamasında karar verilen I. Tip hata olasılığıdır. I. Tip hata gerçekte yokluk/sıfır hipotezi doğru olduğu halde, test sonucunda karşıt hipotezin kabul edilmesi olasılığıdır. Bu olasılık genellikle 0.05 veya 0.01 olarak alınır.

İstatistiksel anlamlılığı ifade eden p-değeri ise, örneklem verilere YHAT veya diğer istatistiksel testlerin uygulanmasının bir sonucudur. Bilimsel makalelerde istatistiksel anlamlılık p-değeri "P" veya "p" ile sembolize edilmektedir. Kısaca ifade etmek gerekirse α, veri analizi öncesinde karar verilen olasılık değeri, p ise istatistiksel analiz ile hesaplanan olasılık değeridir. Bilimsel araştırmaların raporlanmasında en yaygın kullanılan α ve p olasılıklarının önemi, anlamı, doğru raporlanması ve doğru yorumlanması konusunda Akbulut (2022) tarafından kapsamlı bir çalışma yapılmıştır. Ayrıca bu konuda Türkçe literatürde başka çalışmalar da mevcuttur (Kılıç, 2011; Yıldırım & Yıldırım 2011; Işık, 2014; Kul, 2014; Erkuş 2017; Ünalan, 2021).

Anlamlılık durumunu ifade eden p-değeri gerçek değeri ile ve üç ondalıkla (p=0.028 veya p=0.002 gibi) yazılmalıdır. İstatistik analizlerde söz konusu olasılık 0'dan büyük 1'den küçük ($0<p<1$) bir değer alır ve hiçbir zaman sıfır olamaz. Ancak bazı istatistiksel analiz yazılımları (SPSS gibi) çok küçük p-değerini üç ondalıkla yuvarlayarak p=0.000 şeklinde sunmaktadır. Bu durumda $p<0.001$ şeklinde yazılmalıdır. Bulguların yorumlanmasında p değerine gereğinden fazla anlam yüklenmemeli ve p tek başına verilmemelidir. P değeri, örneklem büyüklüğü (n) veya serbestlik derecesi ve test istatistiği ile birlikte (z, t, χ^2, *F*, gibi) rapor edilmelidir.

ETKİ BÜYÜKLÜĞÜ

Etli büyüklüğü (EB), örneklem verilerinden hesaplanan istatistiklerin yokluk/sıfır hipotezi ile tanımlanan değerden sapma düzeyini gösteren istatistiksel bir ölçüdür. (Cohen, 1994; Sullivan & Feinn 2012; Özsoy & Özsoy, 2013, Çapık, 2014; Ünalan, 2021). Bu ölçü bir fark veya bir katsayıdır. En basit tanımıyla EB, yeni yöntemin eskisine kıyasla ne büyüklükte bir fark oluşturduğunu ifade eder (Kılıç, 2014). Kalaycıoğlu & Akhanlı (2020) EB'yi sağlık alanı için gruplar arasında klinik (diğer alanlarda teknik, ekonomik) anlamlı olan en küçük fark olarak tanımlamıştır. Bu fark değeri standardize edilerek, örneğin ortalamalar arası fark ortak standart sapmaya oranlanarak, karşılaştırılabilir bir şekle dönüştürülür (Yıldırım & Yıldırım, 2011; Çapık, 2014; Ünalan, 2021).

EB hesaplama yöntemleri araştırma tasarımı ve kullanılan istatistik test yöntemine göre farklılık gösterir. Araştırmanın tasarımı aşamasında örneklem büyüklüğünün belirlenmesinde EB ihtiyaç duyulan bir ölçüdür. EB hedef populasyondan ön çalışma örneklemi kullanılarak belirlenebilir. Eğer ön çalışma ile yeterli güvenirlikte etki büyüklüğünün belirlenememesi durumunda EB için düzenlenmiş standart değerlerden yararlanılarak örneklem büyüklüğü belirlenir. Çizelge 1'de yaygın kullanılan bazı istatistik testler için standart EB sınıflarının (küçük, orta, büyük) sınırları verilmiştir. Örneklem büyüklüğünü hesaplamada bu sınıfların genellikle alt sınırları kullanılır.

EB belirlenirken küçük etkilerin ortaya çıkartılmasının çok güç olması nedeniyle daha büyük örneklem gerektirdiği unutulmamalıdır.

Aslında EB kavramı günlük hayatta yaygın olarak

kullanılmaktadır. Örneğin "Yeni diyet haftada 1500 gr. zayıflama garantisi vermektedir, binada ısı yalıtımı en az %40 yakıt tasarrufu sağlamaktadır, ilave yemleme koyunlarda ikizlik oranını %25 artırmaktadır" gibi ifadeler ile ifade edilen EB'dir.

EB'nin rapor edilmesi ve yorumlanması, beş önemli nedene dayandırılmaktadır. Bunlar;

Çizelge 1. İstatistiksel testlere göre etki büyüklüğü sınıfları ve sınır değerleri
Table 1. Effect size classes and its limit values according to statistical tests

İstatistiksel testin amacı	İstatistiksel test	Etki büyüklüğü ve sembolü	Etki büyüklüğü sınıfları		
			Küçük	Orta	Büyük
Gruplar arası fark; d ailesi etki büyüklüğü					
İki oran farkı	Ki-Kare	Odds Oranı	1.68-3.46	3.47-6.70	≥6.71
İki ortalama farkı	t Test,	Cohen **d**, veya Hedge **g**	0.20-0.49	0.50-0.79	≥0.80
Oranlar arasındaki fark	Z test veya Ki-Kare	Cohen'in **g** istatistiği	0.05-0.14	0.15-0.19	≥0.20
Değişkenler arası ilişki; r ailesi etki büyüklüğü					
İki sürekli değişken arası ilişki	Korelasyon	Perarson'un **r** değeri	0.10-0.29	0.30-0.49	≥0.50
Çapraz tablo analizi	Ki-Kare	Cramer'in **V, ϕ**	0.10-0.29	0.30-0.49	≥0.50
Sürekli ve sıralı değişkenler arası ilişki	Pearson veya Spearman Rank korelasyonu	**r ve ρ**	0.10-0.29	0.30-0.49	≥0.50
İkiden fazla bağımsız grup veya bağımlı grupların arasındaki fark	ANOVA veya tekrarlı ölçümlerde ANOVA	η^2	0.01-0.05	0.06-0.13	≥0.14
		f	0.10-0.24	0.25-0.39	≥0.40
Fonksiyonel bağıntı analizi	Çoklu Regresyon	R^2	0.02-0.12	0.13-0.25	≥0.26

Kaynak: Özçomak ve Çebi (2017); Akbulut, (2021); Balkin ve Lenz, (2021)

i) Araştırma bulgularının pratik olarak "düşük" "orta" ve "yüksek" şeklinde değerlendirilmesini sağlar ve kullanım açısından yorumlanmasını kolaylaştırır (Hojat & Xu, 2004).

ii) Aynı konuda farklı çalışmaların rapor edilen sonuçlarının karşılaştırılmasına imkân sağlar.

iii) Daha sonra aynı kapsamda yapılacak araştırmalarda raporlanan EB değerleri dikkate alınarak gerekli ve yeteri örneklem büyüklüğünün belirlenmesini sağlar (Téllez et al., (2015).

iv) Klinik, teknik veya ekonomik yüksek EB ile sonuçlanan çalışmaların istatistiksel anlamsız bulunması durumunda, araştırmanın daha büyük örneklem ile tekrarlanarak gerçek anlamlı etkinin belirlenmesine ışık tutar.

v) EB istatistiksel anlamlılık ve diğer çıkarımsal istatistiklere göre iki noktada avantaja sahiptir. Bunlar a) Diğer çıkarımsal istatistikler örneklem büyüklüğünden etkilenirken EB örneklem büyüklüğünden etkilenmez. b) EB skalası serbest bir indekse sahiptir (Hojat & Xu, 2004; Nelson et al., 2015).

vi) Verilerin ön görülen dağılıma uygunluğu (Örneğin normal dağılım) varsayımı altında istatistiksel anlamlılık, güven aralığı ve istatistiksel güç için doğru tahminler yapılabilir. Dağılım geçerli değil ise tahminler de hatalı olacaktır. Hâlbuki EB teorik dağılımlardan bağımsız bir ölçüdür.

Bu nedenlerle araştırma raporlarının metot bölümünde ön görülen ve kullanılan EB, bulgular kısmında ise gözlenen EB rapor edilmelidir.

EB ve TG istatistiklerinin araştırma makalelerde raporlanması gerekliliği belirtilmesine rağmen Türkçe literatürde bu ölçülerin raporlanması henüz istenilen düzeyde yaygınlaşmamıştır. Eğitim bilimleri alanında Türkiye kaynaklı yayımlanan ve SSCI'da taranan bilimsel dergilerdeki makalelerin sadece %7.2'sinde EB'nin raporlandığı bildirilmektedir (Özsoy & Özsoy. 2013). Türkçe literatürde hipotez testlerinin yaygın kullanıldığı tarım bilimleri ve mühendislik alanındaki çalışmalarda p-değeri sıklıkla raporlanırken EB'yi raporlayan çalışmaya rastlanılmamıştır. Halbuki Sullivan & Feinn (2012). Cohen'e atfen EB'nin önemini "Bir araştırma sorusunun birincil ürünü p değerleri değil, bir veya daha fazla EB ölçüsüdür" şeklinde özgün bir ifade ile vurgulamıştır. Kılıç (2014) istatistiksel anlamlılıkla birlikte EB ve EB'nin güven

sınırlarının raporlanmasını önermektedir. Aynı şekilde Téllez et al., (2015) APA editörlerine atfen istatistiksel anlamlılık testlerinin bazı noktalardaki zayıflığı nedeniyle, EB ve EB'nin güven aralığının raporlanmasına dikkat çekmişlerdir.

GÜVEN ARALIĞI

Güven aralığı (GA) belirli bir olasılıkla populasyon parametrelerinin hangi değerler arasında (alt ve üst sınırlar) bulunabileceğini ifade eder. GA'nın bu değerler arasında bulunma olasılığı güven katsayısı 1-α ile ifade edilir. Bu değerin yüzle çarpımına ise 100(1-α) güven düzeyi denir. GA'nın genel hesaplama formülü,

$$GA = \text{istatistik} \pm (\text{standart hata}) x \left(\text{dağılımın} \frac{\alpha}{2} \text{olasılığındaki değeri}\right)$$

şeklindedir. GA'nın doğru tahmini için örneklemenin rastgele yapılması, örnekleme giren birimlerin birbirinden bağımsız olması gerekir. Değişkenin normal dağılıma uyum göstermemesi durumunda uygun tekniklerle GA tahmini yapılmakla birlikte (Cebeci, 2020), uygulamada daha çok değişkenin normal dağılım göstermesi şartı altında yapılır.

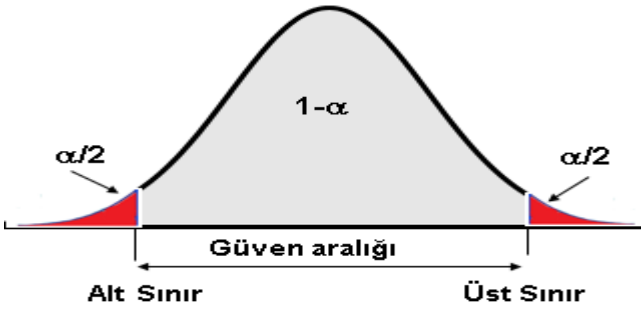
Örneğin varyansı σ^2 =100 olan bir populasyondan alınan 25 birimlik bir örneklemin ortalaması 40 ise, %95 güvenle ile (α=0.05) bu populasyonun ortalamasına ait güven aralığı hesaplanmak istensin. Bu örnek için, standart hata, $\sigma_{\bar{x}} = \sqrt{100/25}$=2'dir. Ayrıca $\alpha/2$=0.025 olup Z dağılımının sağ kuyruğunda $Z_{0.025}$ olasılığı için kesme değeri 1.96'dır. Buradan, $\bar{X} \mp \sigma_{\bar{x}} Z_{\alpha/2}$ formülü ile söz konusu populasyonun ortalaması %95 güvenle (40±2*1.96) 36.08 ile 43.92 arasında olacaktır.

Bu sonuç şu şekilde de yorumlanabilir: Bu populasyondan 100 kez örneklem alınması halinde bunların %95'inde $\bar{X}$, 36.08 ile 43.92 arasında değer alacaktır. Bu tanımı genelleştirmek gerekirse, güven aralığı tahmini, tekrarlanan örneklemeler yapıldığında, ön görülen güven düzeyinde populasyon ortalamasının hangi değerler arasında olabileceğinin bir tahminini sunar.

Uygulamada populasyon varyansı σ^2 genellikle bilinmez ve örneklem de çoğu kez sınırlı (n<30) büyüklüktedir. Bu durumlarda GA tahmini için örneklem varyansı S^2 kullanılır.

Örneklem varyansının (S^2) kullanılması durumunda yukarıdaki eşitlik $\bar{X} \mp s_{\bar{x}} t_{\alpha/2(n-1)}$ şekline dönüşür (Walpole, 1969). Burada örneklem büyüklüğüne bağlı t dağılımına ait kritik değerin ve örneklem ortalamasının standart hatası $s_{\bar{x}} = \sqrt{S^2/n}$ istatistiğinin kullanıldığına dikkat edilmelidir.

GA belirli bir güven düzeyinde populasyon parametresinin içinde bulunabileceği alt ve üst sınırları belirlenmiş sayılar kümesidir. Güven aralığını ve güven sınırlarını Şekil 1'deki gibi göstermek mümkündür.



Şekil 1: Normal dağılım varsayımı altında güven aralığı ve güven sınırları
Figure 1: Confidence interval and confidence limits under the assumption of normal distribution

GA belirlenen bir güven düzeyinde populasyon parametresinin alabileceği değerleri belirleyen örneklemden populasyona doğru yapılan bir tahmindir. Güven sınırları veya güven aralığı uygulamada genellikle %95 veya %90 güven düzeylerinde bildirilir. (Yıldırım & Yıldırım 2011). Güven aralığını yine %5'lik anlamlılık düzeyinde (α=0.05) reddedilemeyecek yokluk/sıfır hipotezi değerler kümesi olarak tanımlamak mümkündür. Yani yokluk hipotezi anlamlılık testi ile ulaşılan sonuçlara güven aralığı ile de ulaşılabilir (Yıldırım & Yıldırım 2011). Aynı durum oran testleri ve ilişki katsayılarının testi için de geçerlidir.

Dahiru (2008) p-değerinin doğru yorumlanması ve p-değerini destekleyecek diğer ölçüt olarak güven aralığının (GA) p-değeri ile birlikte sunumunun faydalı olacağına dikkat çekmektedir. Güven aralığını hesaplamanın sağladığı avantajlar Dahiru (2008) ve Yıldırım & Yıldırım (2011) tarafından, aşağıdaki gibi ifade edilmiştir.

i- Çalışma öncesi yokluk hipotezi kurulmasını gerektirmez. Güven aralıkları hipotez testinin anlamlı veya anlamsız sonuç vermesinden daha çok bilgilendiricidir.

ii- Araştırmalardan elde edilen ortalama, oran vb. ölçülerin mutlak olarak anlaşılmasını önler. Güven aralığının genişliği, tahminin güvenilirliği veya kesinliği örneklemden elde edilen değerlerin bu ölçü için hesaplanacak değerlerden biri olduğunun anlaşılmasını

kolaylaştırır.

iii- Güven aralıkları, istatistiksel anlamlılığın aksine, bir bulgunun önemli olup olmadığının yorumlanmasını (örneğin, klinik önem) daha kolay hale getirir.

iv- İstatistiksel anlamlılık testlerinin I. tip hataya karşı önlem almadığı halde, güven aralığı için böyle bir durum söz konusu değildir.

v- Güven aralığının genişliği, çalışmanın yenilenmesi durumunda benzer bulgulara ulaşabilme ihtimali hakkında bilgi verir.

vı- Güven aralıkları bilgisi meta analiz çalışmalarında farklı çalışmalardan elde edilen bilgilerin birleştirilmesini kolaylaştırır.

Bunlara ilave olarak güven aralıkları kullanılarak benzerlik veya farklılıklar karşılaştırmalı olarak daha objektif değerlendirilebilir. Bu bağlamda karşılaştırılan gruplara ait istatistiklerin tartışılmasında daha aydınlatıcı olduğu gibi kolaylık sağlar. Ayrıca GA istatistiği, araştırma sonuçlarının literatür bildirimleri ile karşılaştırılmasında benzerlik ve farklılıkları belirlemede daha tutarlı yorum yapmaya imkân verir. Yani; ortalama, oran vb. nokta tahminlerine ait bulgular literatür ile karşılaştırmalı olarak tartışılırken tarım bilimlerinde genellikle "…bildirimleri ile benzer, …bildirimlerinden küçük veya daha az, …bildirimlerinden daha yüksek, büyük" gibi göreceli ifadeler kullanılmaktadır. Güven sınırları tahmini yapıldığında güven aralığındaki bildirimler benzer, alt sınırdan küçükler daha düşük, üst sınırdan büyükler daha yüksek olarak yorumlanabilir. Böylece güven sınırları kullanılarak yapılacak tartışma ve yorumlar ile daha objektif çıkarımlar yapılabilecektir.

Ancak aynı α hatası düzeyi veya 1-α güven düzeyinde örneklem büyüklüğü arttıkça güven aralığı daralır. Yani parametre için birbirine daha yakın sınırlar tahmin edilir. Yani güven aralığı p-değerinde olduğu gibi örneklem büyüklüğü arttıkça küçülür. Bu nedenle aynı değişken için farklı çalışmalarda yapılan güven aralığı tahminlerinin karşılaştırılmasında araştırmaların örneklem büyüklükleri de dikkate alınarak yorumlanmalıdır.

İSTATİSTİKSEL GÜÇ, TESTİN GÜCÜ

YHAT ile çözümlenen araştırmalarda rapor edilmesi gereken bir diğer çıkarımsal istatistik testin gücü (TG) istatistiğidir. TG; 1-β olup burada β, istatistiksel analizlerdeki II. Tip hata düzeyidir. II. Tip hata gerçekte karşıt hipotez doğru olduğu halde, test sonucunda yokluk hipotezinin kabul edilmesi olasılığıdır. II. Tip hatanın β genellikle en fazla 0.20 olması istenir. Bu durumda yapılacak YHAT sürecinde hedeflenen testin gücü en az 0.80 olacaktır.

TG, testin gerçekte yanlış olan yokluk hipotezinin ret edilme olasılığıdır. Diğer bir ifade ile testin gücü bir testin gerçekte var olan fark veya etkiyi bulabilme olasılığıdır (Kalaycıoğlu ve Akhanlı, 2019; Ünalan, 2021). TG tıpkı p-değeri gibi şartlı bir olasılıktır. Olasılık terminolojisi ile

$TG=1-\beta = P\ (H_0\ Ret \mid H_0\ Yanlış)$ şeklinde ifade edilir (Sun et al., 2011).

Bir çalışmada TG arttıkça hem doğru etkiyi bulma şansı hem de yokluk hipotezini reddetme şansı artmaktadır. Diğer taraftan bir araştırmada TG yetersiz ise hem doğru etkiyi bulma şansı hem de yokluk hipotezini reddetme şansı azalır.

TG, anlamlılık düzeyi α, örneklem büyüklüğü n ve EB'nin bir fonksiyonudur. Yani hipotez testi sürecinde TG= f(α, n, EB) şeklinde ifade edilebilir (Özçomak & Çebi, 2017; Keskin, 2020). Bunların dışında TG, istatistiksel testin çeşidi, araştırmadaki grup sayısı, gruplardaki gözlem sayısının farklı olması, örneklem hatası, üzerinde çalışılan değişkenin varyasyonu ve testin yönü (tek yanlı veya iki yanlı) tarafından da etkilenir (Keskin, 2020). TG, anlamlılık seviyesi α, EB ve örneklem büyüklüğü ile doğru orantılı, standart sapma ile ters orantılı değişir. Yani α, EB ve örneklem büyüklüğü arttıkça TG artarken, değişkenlik ölçüsü standart sapma arttıkça TG azalır. Ayrıca TG, tek yönlü testlerde iki yönlü testlere göre daha yüksektir.

TG, teorik güç (prospective power, a priori power,), deneysel güç (retrospective power, observed power, post hoc power, archived power) ve karşılaştırmalı güç (compromise power) olmak üzere üç çeşittir (O'Kafee, 2007; Özçomak & Çebi, 2017; Keskin, 2020). Ancak karşılaştırmalı güç diğer güç analizlerine göre daha az bilinen ve uygulanan bir güç türüdür (Özçomak & Çebi, 2017).

Teorik güç; araştırmanın planlanması aşamasında örneklem büyüklüğü belirlenirken öngörülen α (genellikle 0.05), EB ve diğer etkenler dikkate alınarak örneklem büyüklüğünü hesaplamada kullanılır. Bu aşamada kullanılan teorik güç genellikle 0.80 olarak alınır. Bununla birlikte II. Tip hatayı daha küçük (β=0.10) alarak daha büyük bir örneklem ile daha güvenilir sonuçlar elde edebilmek için testin gücü 0.90 olarak da alınabilmektedir (Kalaycıoğlu & Akhanlı, 2020; Ünalan, 2021).

Keskin, (2020) teorik güç analizinin yapılmasını güç analizi için ideal kabul etmektedir. Teorik güç analizini araştırmanın tasarımı aşamasında başlangıçta yapmak oldukça yararlıdır. Araştırmalar planlanırken %80 güç, öngörülen anlamlılık düzeyi α ve klinik, teknik veya ekonomik farklılık veya ilişkiyi (EB) test edebilecek yeterli ve gerekli EB'nin belirlenmesi, araştırma tasarımının doğru yapılmasına imkân verir. Araştırmanın başında yeterli güç sağlamayan çalışmalar öngörülen araştırma tasarımı yerine yeni bir tasarım yapılabilmektedir (Lewis, 2006). Teorik güç analizinin yapılması, bir çalışmada II. Tip hata yapma

olasılığının belirlenmesi için de kullanılmaktadır (Gürkan 2007).

Bir diğer güç analizi yöntemi karşılaştırmalı güç analizidir. Karşılaştırmalı güç analizinin yapılması yaygın değildir. Ancak TG türlerinden biri olarak ifade edilmektedir. (Özçomak & Çebi 2017; Keskin 2020). Karşılaştırmalı güç analizi ölçüsü üzerine örneklem büyüklüğü, EB, α ve ß/α şeklinde ifade edilen hata oranlarının bir sonucudur (Özçomak & Çebi 2017). Yani karşılaştırmalı güç değerinin belirlenmesinde teorik güç parametrelerine ilave olarak hata oranı parametresi ß/α da dikkate alınmaktadır. Rutin hata düzeyleri α=0.05, ß=0.20 alındığında, hatalar oranı ß/α = 4 standart değer olarak kullanılmaktadır.

Araştırma raporunda teorik güç değeri ve karşılaştırmalı güç kriterleri araştırmanın metot bölümünde istatistiksel analizler alt başlığında α olasılığı, EB ile birlikte belirtilir.

Deneysel güç veya gözlenen güç, YHAT analizlerinden sonra yapılan güç analizidir. Bazı yazarlar bu güç analizinin yapılmasını doğru bulmamaktadır (Plate, et al., 2018). Fakat bazen istatistiksel olarak anlamlı sonuçlar elde edilemeyen araştırmalarda, deneysel güç analizi yapmak yararlı olmaktadır (Özçomak & Çebi 2017). Çünkü istatistiksel olarak anlamlı olmayan sonuçlar yetersiz örneklem büyüklüğünden kaynaklanabilmektedir. Bu durumda var olan etkiyi kullanarak, çalışma yeterli örneklem büyüklüğüne ulaştırılarak anlamlı sonuçlar bulunulabilir (Keskin, 2020). Ancak Sun et al. (2011) istatistiksel olarak anlamlı bulunmayan testler için güç analizi yapmanın bulgulara bir katkısının olmadığını bildirmişlerdir. Bu yazarlar, istatistiksel anlamlı sonuçlar bulunmayan durumlarda deneysel güç yerine GA ve gözlenen EB'nin raporlanmasını önermişlerdir. Ayrıca istatistiksel anlamlı bulunmayan ancak yüksek güç ile sonuçlanan araştırmalarda yokluk hipotezinin ret edilmesinde tereddüt edilmemelidir. Diğer yandan istatistiksel anlamlı bulunan sonuçlar için yokluk hipotezinin hangi güç ile ret edildiğinin bilinmesi araştırma bulgularına önemli katkı sağlamaktadır (Işık, 2014; Keskin, 2020). Bazı araştırmacılar, istatistiksel olarak anlamlı sonuçların örneklemin rastlantısal bir sonucu olabileceği ihtimalini dikkate alarak, istatistiksel anlamlı sonuçlar için özellikle küçük örneklemler ile gerçekleştirilen çalışmalarda deneysel güç analizinin gerekli olduğunu belirtmişlerdir (Ertürk, 2005). Keza APA yazım standardını esas alan dergilerde ve son yıllarda özellikle sağlık ve psikoloji alanında yayınlanan makalelerde deneysel güç analizi bulgularının da rapor edilmesi istenmektedir. Deneysel güç araştırmanın bulgular bölümünde ve genellikle veri analiz tablolarında sunulur.

Son yıllarda Türkçe literatürde yayınlanmış araştırmaların gücünü inceleyen araştırmalar yaygınlık kazanmıştır (Çapık,2014; Özçomak & Çebi 2017; Şevgin & Çetin 2017). Bu kapsamda analitik istatistik yöntemlerinin (test) uygulandığı yayınlanmış araştırmalar incelenerek Çapık (2014) hemşirelik araştırmalarında, Özçomak & Çebi (2017) işletme-iktisat alanlarında Şevgin & Çetin (2017) ise eğitim araştırmalarında güç ve etki büyüklüğünü hesaplayarak bulguları yorumlamışlardır. Çapık (2014) 61 araştırmada sonuçları verilen 725 test sonucunu incelemiş ve bu makalelerin sadece birinde istatistiksel gücün hesaplandığı ve makale metninde rapor edildiğini bildirilmiştir. Şevgin & Çetin (2017) 25 makalenin sadece birinde güç analizinin rapor edildiğini, Özçomak & Çebi (2017) inceledikleri 95 makalenin hiçbirinde güç hesaplaması yapılmadığı ve rapor edilmediğini tespit etmişlerdir. Işık (2014) ise psikoloji alanında yayın yapan Türk Psikoloji Dergisinde 1995-2013 yılları arasını 5'er yıllık periyotlarla EB raporlama oranı bakımından incelemiştir. Yazar yıllar itibariyle başlangıçta %1 düzeyinde olan oranın son 5 yılda %18'e ulaştığını tespit etmiştir. Bu araştırmaların genel sonucu olarak, analitik istatistiksel metotların yer aldığı makalelerde güç analizinin öneminin yeterince kavranmadığını vurgulamışlardır. Araştırıcılar istatistik analiz yapılan çalışmalarda EB ve güç analizi sonuçlarının rapor edilmesi gerektiğine dikkat çekmişlerdir.

Anlamlılık Testi, EB, TG ilişkisini şekilsel olarak aşağıdaki gibi göstermek mümkündür (Şekil 2).

HİPOTEZ TESTİ VE GÜVEN SINIRLARI İÇİN SAYISAL BAZI ÖRNEKLER (1)

Bağımsız İki Grup Ortalamasının Karşılaştırılması (Sayısal Örnek 1)

A ve B gibi iki bağımsız grupta ölçülen sistolik kan basıncı değerlerine ait veriler analiz edilmiştir. Ön analizde verilerin normal dağılımlı ve varyansların homojen olduğu tespit edilmiştir. Araştırmanın tasarımı ve ön analiz bulguları doğrultusunda, veriler bağımsız gruplarda t testi ile analiz edilmiştir. İki yönlü test için bulgular aşağıdaki çizelgede özetlenmiştir (Çizelge 2). Çizelge 2'de görüldüğü gibi tanımlayıcı istatistiklerin yanında sadece p değeri değil, GA, EB ve TG çıkarımsal istatistikleri de verilmiştir. Bu istatistiklerden yararlanarak araştırma bulgularının yorumlanması ve tartışılması daha fazla ve daha doğru bilgi elde edilmesine imkân verecektir.

Bu analiz sonuçları şu şekilde yorumlanabilir: Gruplar arasında 11.9 birimlik fark tespit edilmiştir. Bu fark istatistiksel olarak anlamlı ($t_{(30)}$=3.12; p=0.004) bulunmuştur. Testin gücü 0.87 ve etki büyüklüğü d=1.12 olarak her iki ölçünün yüksek düzeyde olduğu

[1] : Örnek çalışmalar sanal olup veriler simülasyonla üretilmiştir.

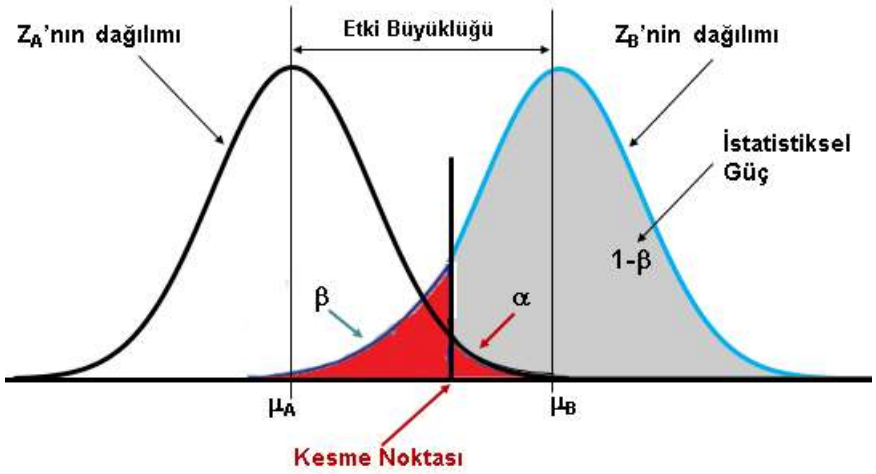
tespit edilmiştir.

Çizelge 2'de özetlenen bulgular şu şekilde hesaplanmıştır: Ortalamalar arası fark, Δ= 122.8 – 110.9 =11.9 olarak hesaplanır.

GA, EB ve daha ileri testler için ortak standart sapma (S_o) ve ortak standart hata ($S_{\bar{x}o}$) gereklidir. S_o= n_A ve n_B eşit olduğu durumda

$$S_o = \frac{S_A + S_B}{2} \quad (1)$$

şeklindedir. Buradan $S_o = \frac{9.0+12.3}{2}$=10.65'dir.



Şekil 2. Anlamlılık testi, etki büyüklüğü ve istatistiksel güç
Figure 2. Significance test, effect size and statistical power

Çizelge 2. Sayısal örnek 1 için tanımlayıcı ve çıkarımsal istatistikler
Table 2. Descriptive and inferential statistics for the numerical example 1

Grup	n	Ortalama	Standart sapma	Fark, GA, EB ve GA(EB)	İstatistik anlamlılık ve güç	
A	16	122,8	9,0	Δ=11.9; GA= [4.1-19.7]	$t_{(30)}$=3.12;	p=0.004;
B	16	110,9	12,3	d=1.12; $GA_{(EB)}$= 0.34-1.90	Güç=0.87	

GA: Güven aralığı, EB: Etki büyüklüğü

Örneklem büyüklükleri n_A=n_B eşit olduğu durumda ortak standart hata;

$$S_{\bar{x}o} = \sqrt{\frac{S_A^2}{n_A} + \frac{S_B^2}{n_B}} \quad (2)$$

eşitliği kullanılarak $S_{\bar{x}o} = \sqrt{\frac{9^2}{16} + \frac{12.3^2}{16}} = 3.81$ olarak hesaplanır.

GA genel eşitliği ise;

$$GA = \text{İstatistik} \pm \text{standart hata} \left(\text{dağılımın} \frac{\alpha}{2} \text{ olasılığındaki değeri}\right) \quad (3)$$

şeklinde olup, bu örnek için t dağılımının kritik tablo değeri yani $t_{(30)0.025}$ =2.042'dir.

Ortalama fark 11.9 ve standart hata 3.81 değerleri Eşitlik 3'te yerlerine yazılarak,

GA=11.9±3.81 x 2.042 eşitliğinde gerekli hesaplamalar yapıldığında bu ortalama fark için alt sınır 4.1, üst sınır ise 19.7 olarak bulunur.

EB olarak Cohen'in d istatistiği d =Δ/S_o eşitliğinden yararlanılarak 11.9/10.65= 1.12 olarak hesaplanır.

Etki büyüklüğünün standart hatası ise,

$$Sh_{(EB)} = \sqrt{\left(\frac{(n_1+n_2)}{(n_1 \cdot n_2)}\right) + d^2/(2(n_1+n_2))} \quad (4)$$

şeklindedir. (G*Power Manuel, 2017). Bu eşitliğe göre etki büyüklüğünün standart hatası

$$Sh_{(EB)} = \sqrt{\left(\frac{16+16}{16.16}\right) + 1.12^2/(2(16+16))} = 0.38\text{'dir.}$$

Etki büyüklüğü için %95 olasılıkla güven sınırları Z dağılımından yararlanarak hesaplanabileceği gibi, bu örnek için t dağılımının $t_{(30)0.025}$ =2.042 değeri kullanılarak Eşitlik 3'e göre $GA_{(EB)}$=1.12±0.38 x 2.042= 0.34 ile 1.90 aralığında olabileceği bulunur.

YHAT için t dağılımına göre test istatistiği,

$$t = \frac{\bar{X}_A - \bar{X}_B}{S_{\bar{x}o}} \quad (5)$$

olup, bu eşitliğe göre t = $\frac{122.8-110.9}{3.81} = \frac{11.9}{3.81}$= 3.12'dir. Bu test istatistiğinin t dağılımının iki tarafındaki olasılık değeri, yani $t_{(30)}$; 3.12' için olasılık değeri p= 0.004'dür.

Bu örnek için iki yönlü test yapılması durumuna göre α=0.05 ve EB 1.12 alındığında TG= 0.87 yani %87' olarak hesaplanır. Güç değeri hesaplama işlemleri hacimli olduğu için makale boyutu dikkate alınarak GPower 3.1 programı ile hesaplanmıştır (Faul ve ark., 2007).

Bu bulgular kısaca özetlenecek olursa; Çizelge 2'de verilen bulgular değerlendirildiğinde A ve B gruplarının ortalamaları arasındaki fark 11.9 olup, bu

fark %95 güvenle 4.1 ile 19.7 arasında değişebilmektedir. Bu fark ve %87 güç düzeyinde istatistiksel olarak anlamlıdır ($t_{(30)}$=3.12; p=0.004).

İkiden Fazla Bağımsız Grup Ortalamasının Karşılaştırılması (ANOVA) (Sayısal Örnek 2)

İncelenen bir X değişkenin dağılımı normal dağılıma uygun ve varyansları homojen olan A, B ve C gibi üç bağımsız grupta X değişkenine ANOVA testi uygulanmıştır. Elde edilen analiz bulguları Çizelge 3 ve Çizelge 4'de sunulmuştur. Bu çizelgelerdeki çıkarımsal istatistiklerin hesaplanması ve yorumlanması aşağıda detaylı olarak açıklanmıştır.

Bu örnekte (Örnek 2) araştırma tasarımı verilerine herhangi bir istatistiksel yazılım kullanılarak (SPSS, MINITAB vb.) ANOVA uygulandığında Çizelge 3 ve Çizelge 4'deki özet istatistikler elde edilebilir. Burada Çizelgelerde sunulan istatistiklerin nasıl hesaplandığı ve nasıl yorumlanması gerektiği örneklendirilmiştir.

GA değerleri şu şekilde hesaplanmıştır: Örneğin A metodunun ortalaması 122.8, standart sapması 9.0 ve standart hatası ise $Sx=9/\sqrt{16}=2.25'$ dir. Ayrıca α=0.05 için 15 serbestlik dereceli ve çift taraflı t dağılımının tablo değeri, $t_{(15)\ 0.025}$ = 2.131'dir. Bu ön hesaplamalar Eşitlik 3'te yerlerine yazılarak A grubu için GA'nın alt ve üst sınırları; 122.8±2.25 x 2.131 = 118.0 ile 127.6 olarak hesaplanır. Diğer gruplar için güven aralıkları benzer şekilde bulunmuş ve Çizelge 3'te sunulmuştur.

Çizelge 3. Sayısal örnek 2 için tanımlayıcı istatistikler
Table 3. Descriptive statistics for numerical example 2

				%95 Güven Aralığı	
	n	Ortalama	Standart sapma	Alt sınır	Üst sınır
A	16	122.8 [a]	9.0	118.0	127.6
B	16	110.9 [b]	12.3	104.4	117.5
C	16	110.8 [b]	8.2	106.4	115.2
Genel	48	114.8	11.3	111.6	118.1

a, b: Farklı harfle gösterilen ortalamalar farklıdır (p<0.01).

Çizelge 4. Örnek 2 için ANOVA bulguları ve bazı çıkarımsal istatistikler
Table 4. ANOVA findings and some inferential statistics for the example 2

	Sd	(#)Kareler toplamı	Kareler ortalaması	F	Önemlilik	EB	Güç
Gruplar Arası	2	1529.2	764.6	7.639	0.001	f = 0.50 η^2=0.25	0.858
Gruplar İçi	45	4503.9	100.1				
Toplam	47	6033.1					

(#) Analizlerin raporlanmasında genellikle yazılmaz.

Bu çalışma için tek yönlü ANOVA ile veriler değerlendirildiğinde (Çizelge 4) p=0.001 hata ile en az iki ortalama arasında fark olduğu bulgusuna ulaşılmıştır. Çizelge 4 izlendiğinde istatistiksel anlamlılık p değerinin yanında EB ve güç istatistikleri de verilmiştir. Bu istatistikler analiz hakkında daha açıklayıcı bilgiler sunmaktadır.

Farklı grupları belirlemek için yapılan LSD (Least Significant Difference) çoklu karşılaştırma testi sonucuna göre A grubu, B ve C gruplarından farklı (p<0.01) B ve C grupları ise benzerdir. Grupların güven aralıkları incelendiğinde bu sonucu görmek mümkündür. Örneğin A grubunun güven sınırları, B ve C gruplarının güven sınırları ile çakışmayıp, sayı doğrusunda daha büyük değer almaktadır. B ve C gruplarının ise güven aralıkları ise çakışmaktadır. Yani sınırlar ayrıktır.

Bu analize ait etki büyüklüğü EB, η^2 (eta kare) Eşitlik (6) yardımı ile aşağıdaki gibi hesaplanır:

$$\eta^2 = \frac{Gruplar\ arası\ kareler\ toplamı}{Genel\ kareler\ toplamı} \qquad (6)$$

$\eta^2 = \frac{1529.2}{6033.1} = 0.25$ olarak hesaplanır.

ANOVA için etki büyüklüğü " f " Eşitlik 7 kullanılarak hesaplanır.

$$f = \sqrt{\frac{\sum(\bar{x}_i - \bar{\bar{x}})^2}{k(S_o^2)}} \qquad (7)$$

Eşitlik 7'de; $\bar{x}_i$: i. grup ortalamasını, $\bar{\bar{x}}$: genel ortalamayı, k: grup sayısını, S_o: genel (ortak) standart sapmayı göstermektedir.

Eşitlik 7 kullanıldığında ANOVA için etki büyüklüğü

$f = \sqrt{\frac{(122.8-114.8)^2+(110.9-114.8)^2+(110.8-114.8)^2}{3\ (11.3)^2}} = 0.50$ olur.

G*Power yazılımı ile bu etki büyüklüğünün 0.05 hata ile 0.858 güce sahip olduğu hesaplanmıştır. Yani bu analiz gerek f istatistiği gerekse η^2 istatistiği bakımından büyük etkiye (sırasıyla; 0.50>0.40 ve 0.25> 0.14) sahiptir. Ayrıca bu testin gerçekleşen gücü α=0.05 hata düzeyinde yaklaşık olarak %86'dır.

SONUÇ ve ÖNERİLER

Sonuç olarak istatistiksel analiz uygulanan araştırmalar rapor edilirken, aşağıdaki hususlara dikkat edilmelidir.

a) İstatistiksel sonuçlar sunulurken anlamlılık düzeyi "p" ile birlikle test istatistiği değeri de (t, z, χ^2, *F*, vb.) yazılmalıdır. Anlamlılık düzeyi p-değeri, tercihen üç ondalıkla, örneğin p=0.018 gibi, yazılmalıdır.

b) P-değerinin belirlenen α değerinden (genellikle 0.05) küçük çıkması durumunda karşıt/alternatif hipoteze çok yüksek bir anlam yüklenmemelidir. Bu sonucunda hatalı olabileceği dikkate alınmalıdır. Ayrıca istatistiksel olarak anlamlı bulunan etki, fark veya ilişki için ekonomik, teknik, klinik anlamlılığı belirlemek için etki büyüklüğü de hesaplanmalıdır. Ayrıca test sonucu çıkan anlamlılık düzeyini daha güvenilir hale getirmek için gözlenen güç değeri de hesaplanmalıdır.

c) P-değerinin α değerinden büyük çıkması durumunda da sonucun anlamlı olabileceği veya anlamsız çıkmasının istatistik test metodu veya örneklem büyüklüğünden kaynaklanıp kaynaklanmadığı da irdelenmelidir. Örneklem büyüklüğünün yetersiz olup olmadığını belirlemek için de gözlenen güç hesaplanmalıdır.

d) Araştırmanın metot bölümünde öngörülen istatistik test, bu teste bağlı etki büyüklüğü, α ve güç değeri (1-β) rapor edilmelidir. Bu varsayımlar altında belirlenen örneklem büyüklüğü "n" de belirtilmedir.

e) Araştırma bulguları rapor edilirken test istatistiği, n ve p'nin yanında EB ile gözlenen güç değeri (TG) de rapor edilmelidir.

f) Ortalama, oran veya katsayıların yorumları; nokta tahminlerinden çok güven aralığı değerleri ve güven sınırları dikkate alınarak yapılmalıdır. Bunun içinde tanımlayıcı istatistiklerin verildiği tablolara güven sınırları değerleri de eklenmelidir.

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