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Chemical Composition and Antimicrobial Effect of Essential Oil of *Anthemis pauciloba* Boiss. var. *pauciloba* from Türkiye

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ABSTRACT

Anthemis pauciloba Boiss. var. *pauciloba* is one of four recognized varieties in Türkiye, locally known as "bol papatya." Its flowers are traditionally used as a cold infusion to treat asthma. The aim of the research was to determine the chemical composition of essential oil (EO) of *A. pauciloba* var. *pauciloba* aerial parts obtained by hydrodistillation using a Clevenger-type apparatus, examined by GC-FID, and GC-MS, simultaneously. The EO was evaluated for antibacterial and antifungal activities against microbial strains utilizing the broth-microdilution technique. *α*-Thujone (28.7%), *α*-pinene (26.7%), and *β*-thujone (9.0%) were found as the main constituents of EO. The antimicrobial activity (Minimum Inhibitory Concentration) against gram-negative, gram-positive, and yeast was observed by the essential oil. The essential oil demonstrated the highest antimicrobial activity against *Candida krusei* (MIC: 1.25 mg/mL). The antimicrobial activity of the essential oil from the aerial parts of *A. pauciloba* var. *pauciloba* was evaluated for the first time in this study.

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Keywords
Anthemis pauciloba var. *pauciloba*
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Türkiye'den *Anthemis pauciloba* Boiss. var. *pauciloba*'nın Uçucu Yağının Kimyasal Bileşimi ve Antimikrobiyal Etkisi

ÖZET

Anthemis pauciloba Boiss. var. *pauciloba*, Türkiye'de tanımlanan dört varyeteden biridir ve halk arasında "bol papatya" olarak bilinmektedir. Bitkinin çiçekleri, astım tedavisinde soğuk çay şeklinde kullanılmaktadır. Araştırmanın amacı, *A. pauciloba* var. *pauciloba*'nın toprak üstü kısımlarından Clevenger tipi aparat kullanılarak hidrodistilasyon yöntemiyle elde edilen uçucu yağın (EO) kimyasal bileşimini belirlemektir. Uçucu yağın bileşenleri, GC-FID ve GC-MS teknikleriyle eşzamanlı olarak analiz edilmiştir. Uçucu yağının mikrobiyal suşlara karşı antibakteriyel ve antifungal aktivitesini mikrodilüsyon tekniği kullanarak değerlendirmiştir. Uçucu yağın ana bileşikleri olarak *α*-tuyon (%28.7), *α*-pinen (%26.7) ve *β*-tuyon (%9.0) bulunmuştur. Gram-negatif, gram-pozitif ve mayaya karşı antimikrobiyal aktivite (Minimum İnhibitör Konsantrasyon) değerlendirilmiştir. Uçucu yağ, *Candida krusei*'ye karşı en yüksek antimikrobiyal aktiviteyi göstermiştir (MİK: 1.25 mg/mL). Bu çalışmada, *A. pauciloba* var. *pauciloba*'nın toprak üstü kısımlarından elde edilen uçucu yağın antimikrobiyal aktivitesi ilk kez değerlendirilmiştir.

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INTRODUCTION

Anthemis L. belongs to the Asteraceae family, tribe Anthemideae, and is the second largest genus in the family, with more than 210 species. It is distributed widely across Europe, Southwest Asia, and North and East Africa (Hamzaoğulu et al., 2011; Özbek et al., 2021).

Anthemis includes 51 species and 81 taxa in Türkiye (Davis, 1975; Güner et al., 2000). *Anthemis* species are known to have various biological activities, and they are commonly used in folk medicine. Essential oil from *Anthemis nobilis* flowers is commonly used for pharmaceuticals, and it is also an important source of oil in food additives, cosmetics, and aromatics. Some *Anthemis* species essential oils possess anti-ageing activity and antioxidants (Sarogluo et al., 2006). Species belonging to *Anthemis* genus are commonly referred to as "Papatya" in Türkiye. Papatya is a popular name given to plants whose flowers resemble those of German and Roman chamomile (Orlando et al., 2019).

A. pauciloba Boiss. is represented by four varieties in Türkiye, and var. *pauciloba* known local name "bol papatya", and the flowers of the plant are used as cold tea in the treatment of asthma (Melikoğlu et al., 2015; Bizim Bitkiler, 2024). *A. pauciloba* is an erect or rarely decumbent herb. Stems are simple or commonly branched near the base, (15-)30-45 cm. Leaves are variable in dissection; basal leaves are petiolate, and ± ovate in outline, and upper leaves are cuneate-spathulate. Capitula is radiate or discoid. Ligules, when present yellow (Davis, 1975).

Anthemis pauciloba var. *pauciloba* is characterized by simple stems or stems branched near the base, with basal leaves ranging from linear-oblanceolate to linear-obovate, typically bearing 3 or 7 pairs of lateral lobes. This variety has been recorded in various regions of Türkiye, particularly in mountainous and steppe ecosystems. Notable collection sites include Manisa, Isparta, Antalya, Gaziantep, Şanlıurfa, and Mardin, indicating a broad ecological distribution. The presence of this taxon across diverse habitats, including limestone rocky valleys, macchie clearings, and steppe environments, underscores its adaptability to varying climatic and edaphic conditions (Davis, 1975).

Phytochemical studies on various Anthemis species have revealed significant variations in essential oil (EO) composition due to factors such as geographic location, genetic differences, and extraction methods. For instance, the major components of *A. pauciloba var. microstephana* were identified as α-pinene (62.0%), 1,8-cineole (11.6%), and α-caryophyllene alcohol (8.0%), while *A. pauciloba var. sieheana* contained 1,8-cineole (8.27%) and ß-pinene (4.97%) (Kürkçüoğlu et al., 2009; Keskin et al., 2017). Studies on *A. pauciloba var. pauciloba* have shown variations in major constituents, with camphor (36.7%), camphene (13.9%), and α-pinene (13.6%) in one report, while another study found *α*-thujone (28.7%), *α*-pinene (26.7%), and *β*-thujone (9.0%) as dominant compounds (Kürkçüoğlu et al., 2009). Such differences highlight the influence of environmental and ecological factors on EO composition.

Among these constituents, thujone -a monoterpene ketone- is particularly notable due to its neurotoxic and bioactive properties, including potential anticancer effects (Pelkonen et al., 2013; Radulović et al., 2017). *α*-Thujone has also been reported as a primary compound in other *Anthemis* species, such as *A. carpatica* (40.2%), *A. montana*, and *A. cretica* ssp. *carpatica* (Bulatovic et al., 1997; Pavlovic et al., 2010), as well as in plants from related genera like *Artemisia* and *Salvia* (Pelkonen et al., 2013). In addition to their diverse chemical compositions, various *Anthemis* species have been reported to exhibit significant antimicrobial and anti-inflammatory properties (Radulović et al., 2017; Zámboriné et al., 2020), which further underscores their pharmacological potential. These findings emphasize the phytochemical diversity within the *Anthemis* genus and the need for further investigation into the chemical composition and biological effects of their essential oils.

The aim of this study was to determine the chemical composition of the essential oil obtained from the aerial parts of *Anthemis pauciloba* var. *pauciloba*, and to evaluate its antibacterial and antifungal activities against selected microorganisms. To the best of knowledge, this is the first study to investigate both the antimicrobial potential of this species.

MATERIALS and METHODS

Plant Material

The aerial parts of *Anthemis pauciloba* var. *pauciloba* were collected on 26 June 2014 during the flowering stage, with all specimens bearing fully developed flowers. The plant material was gathered from a stony area near Kılan village, located in Ulukışla district, Niğde province, Türkiye, at an altitude of 1390 meters. The collection was carried out by Süleyman Doğu, and a voucher specimen was deposited in the Herbarium of the Department of Biology, Necmettin Erbakan University (Herbarium number: S.D. 3560).

After collecting, the aerial parts were transported to the laboratory in paper bags, dried in a dry, shaded, well-ventilated room at ambient temperature, and subsequently ground into powder. The powdered samples were then placed in airtight zip-lock bags, the air was removed, and the bags were stored at +4 °C until further analysis.

Extraction

The essential oil was obtained by hydrodistillation using a Clevenger-type apparatus for 3h. EO of *Anthemis pauciloba* var. *pauciloba* and examined by GC-FID and GC-MS, simultaneously.

Gas chromatography (GC) and gas chromatography–mass spectrometry (GC/MS)

Anthemis pauciloba var. *pauciloba* essential oil was analysed by GC using a Hewlett-Packard 6890 (Sem Ltd., Istanbul, Turkey) system, and an HP Innowax FSC column (60 m × 0.25 mm Ø, with 0.25 µm film thickness) was used with nitrogen at 1 ml/min. The initial oven temperature was 60 °C for 10 min, and increased at 4 °C/min to 220 °C, then remained constant at 220 °C for 10 min and increased at 1 °C/min to 240 °C. Injector temperature was set at 250 °C. Percentage composition of the individual components was obtained from electronic integration using flame ionization detection (FID) at 250 °C. *n*-Alkanes were used as reference points in the calculation of relative retention indices (RRI).

GC/MS analysis was performed with a Hewlett-Packard GCD (Sem Ltd., Istanbul, Turkey), system, and Innowax FSC column (60 m × 0.25 mm, 0.25 µm film thickness) was used with helium. GC oven temperature conditions were as described above, split flow was adjusted at 50 ml/min, and the injector temperature was at 250 °C. Mass spectra were recorded at 70 eV. Mass range was from m/z 35 to 425 (Demirci et al., 2008).

Components of Essential Oil Identification

The volatile components were identified by comparing their relative retention times (RRI) to those of authentic samples or by comparing their relative retention index to a series of *n*-alkanes. For identification, an in-house (Library's Başer) and computer matching against commercial databases (Library's MassFinder software 4.0 and Wiley GC/MS Library (Wiley, NY, USA) were built up, from actual components of known essential oil was employed (Demirci et al., 2022).

Microbial Cultures

The test organisms used in the study were as follows: *Staphylococcus aureus* American Type Culture Collection (ATCC) 6538, *Salmonella Tymphirium* ATCC 14028, *Staphylococcus aureus* ATCC 700699, *Escherichia coli* Northern Regional Research Laboratory (NRRL) B-3008, *Candida albicans* ATCC 90028, and *Candida krusei* ATCC 6258.

Antimicrobial Activity

The microdilution broth susceptibility assay was tested for the antibacterial and antifungal evaluation of the EO of *A. pauciloba* var. *pauciloba* aerial parts. Stock solutions of the EO were prepared in dimethylsulfoxide (DMSO) and sterile distilled water. Overnight-grown microorganism suspensions in MHA (for bacteria) and *Candida albicans* yeast suspension in yeast medium (for fungus) were standardized to 108 CFU/mL. The wells were then filled with 100 µL of each culture suspension. The final row, which was devoid of microbes, served as a sterility control. In another row, the microbe and MHA medium were used as a growth control. The minimum inhibitory concentration (MIC, mg/mL) was obtained after a 24-hour incubation at 37°C. 20 µL of resazurin (Sigma) reagent was put on plates for visualization and incubated at 37°C for 3 hours. Ketoconazole (Fluka), itraconazole (FAGEM), Fluconazole (FAGEM), and ciprofloxacin (Merck), ampicillin (Sigma) were used as standard components (CLSI, 2006; Saltan et al., 2018). All experiments were repeated three times, and average MICs are presented in Table 2.

Statistical analysis

GraphPad Prism Software Version 9.0 was used for data analysis to evaluate differences in results between the experimental and standard groups. The findings are displayed as the average ± standard deviation (S.D.).

RESULTS and DISCUSSION

Essential Oil Yield and Composition

The present research aimed the identifying the volatile components of *A. pauciloba* var. *pauciloba* aerial parts. The essential oil was subjected to hydrodistillation to obtain it, and it was analyzed by both GC-FID and GC-MS simultaneously. The volatile components of the essential oil were listed in Table 1. The essential oil's yield was determined to be 0.15%.

A total of 85 volatile components were determined in the EO's composition of *A. pauciloba* var. *pauciloba* aerial parts, representing 94.7% of the total EO. The components of EO were grouped into six main chemical classes: oxygenated monoterpenes, monoterpene hydrocarbons, oxygenated sesquiterpenes, sesquiterpene hydrocarbons, fatty acids, and others. The essential oil of *A. pauciloba* var. *pauciloba* was defined by a high concentration of oxygenated monoterpenes (45.4%) and monoterpene hydrocarbons (30%). The essential oil was identified major

components as *α*-thujone (28.7%), *α*-pinene (26.7%) and *β*-thujone (9.0%), respectively.

Table 1. The chemical composition of the essential oil of *Anthemis pauciloba* var. *pauciloba*
Çizelge 1. Anthemis pauciloba var. pauciloba uçucu yağının kimyasal kompozisyonu

RRI[a]	KI[b]	Compound	%	Identification method
1032	1008-1039[c]	*α*-Pinene	**26.7**	t_R, MS
1035	1012-1039[c]	*α*-Thujene	0.3	t_R, MS
1076	1043-1086[c]	Camphene	0.1	t_R, MS
1118	1085-1130[c]	*β*-Pinene	1.1	t_R, MS
1132	1098-1140[c]	Sabinene	tr	t_R, MS
1135	1109-1137[c]	Thuja-2,4(10)-diene	0.4	MS
1151	1122-1169[c]	*δ*3-Carene	0.1	MS
1188	1154-1195[c]	*α*-Terpinene	0.1	t_R, MS
1213	1186-1231[c]	1,8-Cineole	0.2	t_R, MS
1224	1224[d]	*σ*-Mentha-1(7)5,8-triene	0.1	MS
1255	1222-1266[c]	*γ*-Terpinene	0.2	t_R, MS
1278	1244-1279[c]	*m*-Cymene	0.1	MS
1280	1246-1291[c]	*p*-Cymene	0.8	t_R, MS
1285	1277-1317[d]	Isoamyl isovalerate	0.1	MS
1400	1370-1414[c]	Nonanal	tr	MS
1430	1385-1441[c]	*α*-Thujone	**28.7**	MS
1451	1400-1452[c]	*β*-Thujone	**9.0**	MS
1466	1438-1480[c]	*α*-Cubebene	tr	MS
1497	1462-1522[c]	*α*-Copaene	1.7	MS
1499	1486-1500[e]	α-Campholene aldehyde	1.0	MS
1535	1496-1546[c]	*β*-Bourbonene	0.4	MS
1536	1504-1548[c]	Pinocamphone	0.2	t_R, MS
1586	1545-1590[c]	Pinocarvone	0.4	t_R, MS
1611	1564-1630[c]	Terpinen-4-ol	0.5	t_R, MS
1612	1570-1685[c]	*β*-Caryophyllene	0.4	t_R, MS
1628	1583-1668[c]	Aromadendrene	0.1	MS
1648	1597-1648[c]	Myrtenal	0.8	MS
1663	1647-1668[c]	*cis*-Verbenol	0.2	MS
1670	1643-1671[c]	*trans*-Pinocarveol	0.6	t_R, MS
1683	1665-1691[c]	*trans*-Verbenol	1.1	t_R, MS
1687	1637-1689[c]	*α*-Humulene	0.1	t_R, MS
1704	1655-1714[c]	*γ*-Muurolene	0.5	MS
1725	1696-1735[c]	Verbenone	0.3	t_R, MS
1773	1722-1774[c]	*δ*-Cadinene	0.5	MS
1776	1735-1782[c]	*γ*- Cadinene	0.2	MS
1804	1743-1808[c]	Myrtenol	0.2	MS
1830	1782-1833[d]	Tridecanal	tr	MS
1838	1789-1842[c]	*(E)-β*-Damascenone	tr	MS
1845	1805-1850[c]	*trans*-Carveol	0.4	t_R, MS
1849	1836-1837[f]	Calamenene	0.1	MS
1864	1813-1865[c]	*p*-Cymen-8-ol	0.1	MS
1929	1929[d]	2-Methyl butyl benzoate	0.1	MS
1941	1893-1941[c]	*α*-Calacorene	0.1	MS
1981	1916-1993c	Heptanoic acid	0.1	t_R, MS
2008	1936-2023[c]	Caryophyllene oxide	1.3	t_R, MS
2037	2016-2043[c]	Salvial-4(14)-en-1-one	0.3	MS
2041	1980-2060[c]	Pentadecanal	0.2	MS
2057	2014-2062[c]	Ledol	0.2	MS
2071	2003-2071[c]	Humulene epoxide II	0.2	MS
2080	2052[e]	Junenol	0.1	MS
2084	2011-2089[c]	Octanoic acid	0.1	t_R, MS
2098	2049-2104[c]	Globulol	0.2	MS
2100	2100[f]	Heneicosane	0.1	t_R, MS

2130	2130[d]	Salviadienol	0.1	MS
2131	2089-2131[d]	Hexahydrofarnesyl acetone	1.4	MS
2144	2074-2150[c]	Spathulanol	2.4	MS
2178	2134-2191[d]	*T*-Cadinol	0.2	MS
2179		*nor*-Copaonone	0.2	MS
2198	2100-2205[c]	Thymol	0.4	t_R, MS
2209	2143-2230[d]	*T*-Muurolol	0.1	MS
2210		Copaborneol	0.2	MS
2219	2142-2219[d]	Torreyol	0.1	MS
2239	2140-2246[c]	Carvacrol	0.2	t_R, MS
2247	2241-2247[d]	*trans*-*α*-Bergamotol	0.2	MS
2250	2186-2250[c]	*α*-Eudesmol	0.9	MS
2255	2180-2255[c]	*α*-Cadinol	0.3	MS
2278	2231-2278[d]	Torilenol	0.3	MS
2289		4-*oxo*-*α*-Ylangene	0.2	MS
2298	2227-2301[c]	Decanoic acid	0.1	t_R, MS
2300	2300[f]	Tricosane	0.7	t_R, MS
2312		9-Geranyl-*p*-cymene	0.9	MS
2316	2316-2320[d]	Caryophylladienol I	0.1	MS
2329		14-Acetoxy-*α*-humulene	0.1	MS
2369	2351-2402[c]	Eudesma-4(15)7-dien-1-*β*-ol	0.3	MS
2389		Caryophyllenol I	0.2	MS
2392		Caryophyllenol II	0.3	MS
2400	2339-2421[c]	Undecanoic acid	0.3	t_R, MS
2430	2334-2452[c]	Chamazulene	0.1	t_R, MS
2500	2500[f]	Pentacosane	0.1	t_R, MS
2503	2442-2524[c]	Dodecanoic acid	0.3	t_R, MS
2617	2573-2678[c]	Tridecanoic acid	0.2	t_R, MS
2670	2634-2719[c]	Tetradecanoic acid	0.5	t_R, MS
2700	2700[f]	Heptacosane	0.2	t_R, MS
2900	2900[f]	Nonacosane	tr	t_R, MS
2931	2862-2945[c]	Hexadecanoic acid	2.2	t_R, MS
		Monoterpene hydrocarbons	30	
		Oxygenated monoterpenes	45.4	
		Sesquiterpene hydrocarbons	4.1	
		Oxygenated sesquiterpenes	10	
		Fatty acid	3.8	
		Others	1.3	
		Yield (%)	**0.15**	
		Total	**94.6**	

[a]RRI: Relative retention indices calculated against *n*-alkanes; [b]KI from literature ([c, d, e, f]), [c]Babushok et al., 2011; [d]Pubchem, 2024; [e]NIST Chemistry WebBook, 2024; [f]The Pherobase, 2024; t_R: Identification based on the retention times of genuine compounds on the HP Innowax FSC column; MS: Tentative identification on the basis of computer matching of the mass spectra with those of the Wiley and MassFinder libraries and comparison with literature data. tr: Trace (<0.1 %);,%: calculated from FID data.

The oil of *A. pauciloba* var. *pauciloba* was characterized by a high number of oxygenated monoterpenes and monoterpene hydrocarbons. The major components of EO were determined as *α*-thujone, *α*-pinene, and *β*-thujone.

In an earlier study, Kürkçüoğlu et al. (2009) reported that essential oils of *A. pauciloba* var. *microstephana* and *A. pauciloba* var. *pauciloba* were obtained from the aerial parts by two techniques, hydrodistillation in a Clevenger-type apparatus and microdistillation using an Eppendorf MicroDistiller®. Major components of the oil of *A. pauciloba* var. *microstephana* were found as *α*-pinene (20.1%), *α*-caryophyllene alcohol (8.0%) and *α*-pinene (62.0%), 1,8-cineole (11.6%), respectively. Major components of the volatiles of *A. pauciloba* var. *pauciloba* were found as camphor (36.7%), camphene (13.9%), α-pinene (13.6%), guaiol (16.8%), ß-bisabolenal (8.6%), and spathulenol (7.5%), respectively (Kürkçüoğlu et al., 2009).

In another study, Keskin et al. (2017) reported that the main components of *Anthemis pauciloba* var. *sieheana* EO were 1,8-cineol (8.27 %), and *β*-pinene (4.97 %). The main constituents of *A. pauciloba* var. *sieheana*'s fatty acids

were 9,12-octadecadienoic acid methyl ester (48.46%), 9-octadecanoic acid methyl ester (16.17%), and hexadecenoic acid methyl ester (13.3%) (Keskin et al., 2017).

In the present study, the essential oil (EO) of *A. pauciloba var. pauciloba* was characterized by a high concentration of *α*-thujone (28.7%), *α*-pinene (26.7%), and *β*-thujone (9.0%) as the major components. In contrast, Kürkçüoğlu et al. (2009) reported that the predominant constituents of *A. pauciloba var. pauciloba* EO were camphor (36.7%), camphene (13.9%), and *α*-pinene (13.6%). These differences suggest that environmental factors, ecological conditions, and extraction methods may significantly influence the chemical composition of the essential oil.

Furthermore, Keskin et al. (2017) identified 1,8-cineole (8.27%) and *β*-pinene (4.97%) as the major constituents of *A. pauciloba var. sieheana* EO. The variations observed in the chemical profiles of different *A. pauciloba* varieties highlight the phytochemical diversity within the species and emphasize the potential impact of genetic and environmental factors on essential oil composition.

According to the literature, thujone is a type of monoterpene ketone that occurs naturally in different amounts within various plant species (Plkonen et al., 2013). According to the search results, *α*-thujone was determined as the main component in the EO *of A. carpatica, A. montana*, and *A. cretica* ssp. *carpatica* (Bulatovic et al., 1997; Bulatovic et al., 1998; Pavlovic et al., 2010). The essential oil of *A. carpatica* was found to contain 40.2% *α*-thujone.

Thujone is a volatile compound widely debated due to its behaviour-modulating and toxic properties (Bulatovic et al., 1997). However, a study in 2016 found that *α*-thujone stimulates an anticancer immune response. Chemotypes of *Anthemis* were identified with thujone and *cis*-epoxycimene, as the main components (Radulović et al., 2017; Zámboriné et al., 2020). Also, Thujone is a major component of EOs derived from plants like *Salvia officinalis, Salvia sclarea, Tanacetum vulgare, Artemisia absinthium,* and *Thuja occidentalis* (Pelkonen et al., 2013).

Antimicrobial Effects

The antimicrobial effects of the EO of *A. pauciloba* var. *pauciloba* aerial parts were tested against reference *S. aureus* (gram-positive bacteria), *E. coli* (gram-negative bacteria), *S. tymphirium* (gram-negative bacteria), *C. albicans* (yeast), and *C. krusei* (yeast) strains. The results of the antimicrobial effects of the EO are listed in Table 2. The EO demonstrated the highest antimicrobial activity against *C. krusei* (1.25 mg/mL). Among the tested microorganisms *C. krusei* was observed to be more sensitive to the EO. In this study, the antimicrobial activity of the essential oil of *A. pauciloba* var. *pauciloba* aerial parts was used for the first time.

Table 2. MIC values (mg/mL) of the essential oil of *Anthemis pauciloba* var. *pauciloba*
Çizelge 2. Anthemis pauciloba var. pauciloba uçucu yağının MİK değerleri (mg/mL)

	E. coli NRRL B-3008	*S. aureus* ATCC 6538	*S. Tymphirium* ATCC 14028	*S. aureus* ATCC 700699	*C. albicans* ATCC 90028	*C. krusei* ATCC 6258
EO	>10±0.00	>10±0.00	>10±0.00	>10±0.00	10±2.89	1.25±0.72
Ampicilin	0.01±0.01	0.63±0.36*	1.3±0.75*	0.02±0.01	-	-
Clarithromycin	0.02 ±0.01	0.63±0.36*	0.04±0.2	0.16±0.09	-	-
Ketoconazole	-	-	-	-	0.01±0.01	-
Itraconozole	-	-	-	-	0.04±0.02	0.01±0.01
Fluconazole	-	-	-	-	-	0.04±0.02

EO: Essential oil; *: µg/mL; -: not dedected

Although the essential oil of *A. pauciloba* var. *pauciloba* demonstrated some antifungal activity against *C. krusei* (MIC: 1.25 mg/mL), its overall antimicrobial potential against the tested microorganisms was relatively low compared to standard antifungal agents. This limited activity may be attributed to the complex chemical composition of the oil, where the presence of both active and inactive constituents may influence its bioactivity.

While it is not appropriate to directly compare the antimicrobial effects of different plant species, previous studies on *Artemisia herba-alba* have shown that essential oils rich in oxygenated monoterpenes, particularly α-thujone and ß-thujone, exhibit varying antimicrobial activities depending on their relative proportions (Mighri et al., 2010). In the present study, the antifungal effect observed against *C. krusei* suggests that *α*-thujone and *α*-pinene, as key components, may play a role in the bioactivity of *A. pauciloba* var. *pauciloba* oil. However, the significantly lower activity compared to standard agents indicates that these compounds alone may not be sufficient to achieve potent antimicrobial effects.

Further studies focusing on the isolation and testing of individual constituents and their synergistic interactions are required to clarify the specific compounds responsible for the observed antifungal activity. Additionally, investigating the effects of geographic variation and environmental factors on the chemical composition could provide deeper insights into the bioactive potential of this species.

The oils from roots and aerial parts of *Anthemis mixta* and *A. tomentosa* were evaluated for their antibacterial effect against ten bacterial species. Notably, the essential oils obtained from the aerial parts of both species were particularly effective against Gram-positive bacteria (Formisano et al., 2012). This aligns with previous findings suggesting that the lipophilic nature of essential oil components allows them to interact with the lipid bilayer of Gram-positive bacteria, increasing membrane permeability and causing cellular disruption (Burt, 2004; Bassolé & Juliani, 2012).

In comparison, the essential oil of A. pauciloba var. pauciloba in the present study exhibited limited activity against Gram-positive bacteria, except for C. krusei. This discrepancy may be attributed to differences in chemical composition between species, particularly the relative abundance of oxygenated monoterpenes such as α-thujone and α-pinene, which are known to contribute to antimicrobial activity. Furthermore, variations in extraction methods and geographic origin could also explain the observed differences in antibacterial efficacy. In another study, the essential oils of three *Anthemis* species from Türkiye were analyzed for their chemical composition and antimicrobial activity. Although the antibacterial effects reported by Kurtulmuş et al. (2009) were relatively stronger than those observed in the present study, the variability may be attributed to differences in chemical composition, likely influenced by environmental conditions.

These findings indicate that Anthemis species may possess some antimicrobial potential, though further studies are needed to clarify their efficacy. Investigations into their mode of action, synergistic effects with other antimicrobial agents, and clinical applicability could provide valuable insights for developing targeted antibacterial therapies.

CONCLUSIONS

The essential oil of *A. pauciloba* var. *pauciloba* aerial parts was identified. Also, the essential oil was found to have high antimicrobial activity against *C. krusei.* However, this is the first time that the antimicrobial activity of this essential oil has been reported.

Conflict of Interest

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

Author Contributions

Execution research project, Experimental design, Data analysis, Manuscript preparation- DK, AK, SD, BD; Experimental design, Data analysis, Manuscript preparation- DK, BD; Materials, Supervision, Writing - review & editing. DK, AK, SD, BD; Experimental design, Data analysis, Manuscript preparation. DK, BD; Materials AK, SD.

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In Vivo and *in Silico* Evaluation of the Effect of p-Acetamide and MPAEMA on the Model Organism *Galleria Mellonella* (Lepidoptera: Pyralidae)

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ABSTRACT
In this study, 2-chloro-N-(4-methoxyphenyl)acetamide (p-acetamide) and 2-(4-methoxyphenylamino)-2-oxoethyl methacrylate (MPAEMA) were resynthesized to evaluate their effect on the agricultural pest *Galleria mellonella*. The toxicities of p-acetamide and MPAEMA against the larval stage of *G. mellonella* were evaluated concurrently. The results indicate that p-acetamide has a lethal effect on insect larvae at lower doses. LC50 doses of p-acetamide and MPAEMA were 873,572 and 687,355 uM, respectively. These values represent the concentrations of the substances at which 50% of the larvae exposed to them are expected to die. The molecular docking interactions of p-acetamide and MPAEMA with the proteins superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione-S-transferase (GST) were analyzed. The binding energy between MPAEMA and glutathione peroxidase was determined to be -6.8 kcal/mol. This suggests that MPAEMA may have an inhibitory effect on glutathione peroxidase and could be further investigated for developing pesticides that target this enzyme.

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p-asetamid ve MPAEMA'nın model organizma Galleria mellonella (Lepidoptera: Pyralidae) üzerindeki etkisinin in vivo ve in silico değerlendirilmesi

ÖZET
Bu çalışmada 2-kloro-N-(4-metoksifenil)asetamid (p-asetamid) ve 2-(4-metoksifenilamino)-2-oksoetil metakrilat (MPAEMA), tarımsal zararlı *Galleria mellonella* üzerindeki etkilerini değerlendirmek amacıyla yeniden sentezlenmiştir. p-asetamid ve MPAEMA'nın *G. mellonella*'nın larva evresine karşı toksisiteleri eş zamanlı olarak değerlendirilmiştir. Sonuçlar, p-asetamidin daha düşük dozlarda böcek larvaları üzerinde öldürücü etkiye sahip olduğunu göstermektedir. p-asetamid ve MPAEMA'nın LC50 dozları sırasıyla 873.572 ve 687.355 uM'dir. Bu değerler, bu maddelere maruz kalan larvaların %50'sinin ölmesinin beklendiği madde konsantrasyonlarını temsil etmektir. p-asetamid ve MPAEMA'nın süperoksit dismutaz (SOD), katalaz (CAT), glutatyon peroksidaz (GPx) ve glutatyon-S-transferaz (GST) proteinleriyle moleküler yerleştirme etkileşimleri analiz edildi. MPAEMA ve glutatyon peroksidaz arasındaki bağlanma enerjisi -6,8 kcal/mol olarak belirlendi. Bu, MPAEMA'nın glutatyon peroksidaz üzerinde inhibitör bir etkiye sahip olabileceğini ve bu enzimi hedef alan pestisitlerin geliştirilmesi için daha fazla araştırılabileceğini düşündürmektedir.

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INTRODUCTION

The increasing global population's need for food can be met through sustainable agricultural production. Sustainability in agriculture involves selecting the right variety, appropriate fertilization, cultural practices, balanced irrigation, and addressing factors like diseases, pests, and weeds that can limit or hinder production (Zhou et al., 2025). For many years, the fight against diseases and pests that pose problems to agricultural production worldwide has relied heavily on intensive pesticide use, which directly affects quality and yield, leading to significant losses (Altıkat et al., 2009; Karimi-Maleh et al., 2024). Approximately 2.5 million tons of pesticides are used globally for combating agricultural pests and diseases, with many chemicals used having various disadvantages, such as negative effects on human health, soil, and the environment (Altıkat et al., 2009). Due to these reasons, the use of alternative control methods has become necessary.

The greater wax moth, *Galleria mellonella* (*G. mellonella*), is known to be a harmful species that can cause a decrease in productivity by settling on honeycombs in beehives (Kwadha et al., 2017). This infestation directly translates to financial losses for beekeepers, making *G. mellonella* a target for pest management strategies. However, despite its detrimental impact on apiculture, *G. mellonella* holds a paradoxical position in scientific research. It is a preferred model organism in entomological studies due to a confluence of advantageous characteristics, including its relatively simple nutritional requirements, remarkable ecological adaptability, and rapid developmental cycle. These traits make it a convenient and cost-effective subject for studying insect physiology, immunity, and even the efficacy of antimicrobial compounds (Bugyna et al., 2023). Interestingly, *G. mellonella* is a preferred species in entomological research due to its nutritional needs, ecological adaptation, and development characteristics (Çelik et al., 2024). As the negative impacts of chemical control methods (Dent & Binks, 2020) used against economically harmful insects have become apparent, biological control studies (Sefer & Büyükgüzel, 2018) have gained significance as an alternative approach (Çelik et al., 2024). In ongoing efforts to find environmentally friendly solutions, studies have been conducted to assess the lethal and repellent effects of alternative, less toxic materials on harmful insects, in addition to the use of biological control agents (Chowdhury et al., 2023). This includes exploring the potential of plant-derived compounds, essential oils, and other naturally occurring substances to disrupt insect behavior, development, or survival (Borase et al., 2024). While the primary objective of these alternative pest control strategies is to eliminate or deter target pests like *G. mellonella*, it is crucial to thoroughly evaluate their potential non-target effects. Determining the lethal concentration (LC_{50}) or lethal dose (LD_{50}) of these alternative substances is essential for understanding their direct toxicity to the target pest. However, it is equally important to assess their sublethal effects, as these can significantly impact the long-term population dynamics of the insect. These sublethal effects may manifest as alterations in crucial life-history traits, such as reduced longevity, decreased fecundity (reproductive potential), impaired development, and altered behavior (Borase et al., 2024). This highlights the complexity of using these products for pest control and underscores the need for further research to understand their full impact on insect populations.

Traditionally, various vertebrate species such as mice and rats have been used to determine the efficacy of new drugs. However, the use of mammalian models is becoming impractical due to both cost and ethical acceptance issues. Alternative models that show remarkable metabolic similarities to mammalian models are widely used as new model organisms in biological research. These alternative model systems include; Caenorhabditis elagans, *Drosophila melanogaster* and *G. mellonella* (Ménard et al., 2021). *G. mellonella* larvae are utilized as a model organism in various scientific studies due to their ability to be mass-produced in inexpensive artificial foods under controlled laboratory conditions. Besides their importance in apiculture, *G. mellonella* larvae are widely used as model organisms in studies on insect physiology and human pathogens. They play a significant role in research areas such as physiology, biochemistry, and molecular biology (Abdelaziz et al., 2024). The larvae are increasingly important as they serve as natural host insects for breeding parasitoid insects used in biological control, conducting insecticide efficacy trials, and assessing the pathogenicity of microorganisms that cause diseases in humans and other mammals (Banfi et al., 2024). Additionally, the larvae of *G. mellonella* feed on beeswax, pollen, and in some cases, honey within beehives. This feeding behavior can lead to damage to beehives and negatively affect honey production. Beekeepers have to take measures to control the population of *G. mellonella* to protect their honeybee colonies (Kwadha et al., 2017).

In the literature, there are many acrylate and amide derivatives originally synthesized and characterized. This team is also conducting monomer and polymer studies on acrylate and acrylate derivatives. In this previous studies, this team synthesized and characterized the 2-(4-methoxyphenylamino)-2-oxoethyl methacrylate (MPAEMA) monomer (Acikbas et al., 2016; Tanış et al., 2019; Temüz et al., 2024). In addition, we studied the cytotoxicity of MPAEMA by XTT cell proliferation analysis, physical, electronic, and vibration properties, and characterization of Langmuir–Blodgett thin film (Acikbas et al., 2016; Tanış et al., 2019; Temüz et al., 2024). We used the HeLa cell line to examine their cytotoxic properties, and the IC50 values for p-acetamide and MPAEMA were found to be 14.53 μg/mL and 1.8 mM, respectively (Tanış et al., 2019; Çankaya et al., 2021). In another study,

we demonstrated *in vitro* and *in silico* that p-acetamide and MPAEMA have antifungal, antibacterial, and antioxidant properties (Temüz et al., 2024). The p-acetamide molecule used in this study contains chlorine, amide, and anisole functional groups, and the MPAEMA molecule contains amide and anisole functional groups. In this study, we aimed to contribute to the literature by investigating the effects of p-acetamide and MPAEMA molecules on the agricultural pest *G. mellonella in vivo* and *in silico.*

MATERIALS and METHODS

Synthesis of p-acetamide and MPAEMA Molecules

For the synthesis of p-acetamide and MPAEMA, 4-methoxyaniline, sodium methacrylate, chloroacetyl chloride, triethylamine, TEBAC, and NaI were used as purchased from Sigma-Aldrich. This team had previously synthesized and characterized the 2-chloro-N-(4-methoxyphenyl)acetamide (p-acetamide) and 2-(4-methoxyphenylamino)-2-oxoethyl methacrylate (MPAEMA). The molecules were re-synthesized for this study (yield 80%) (Acikbas et al., 2016; Tanış et al., 2019; Temüz et al., 2024). The reaction scheme is shown in Figure 1.

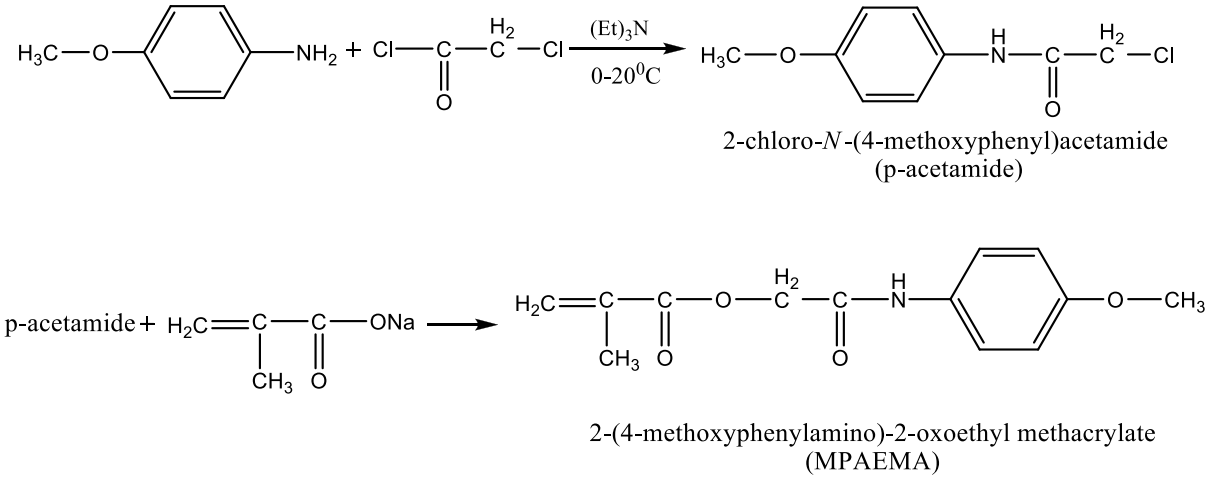


Figure 1. Synthesis of the p-acetamide and MPAEMA.
Şekil 1. p-asetamid ve MPAEMA sentezi

Rearing of Galleria mellonella Larvae

G. mellonella larvae were obtained from the stock culture maintained at the Department of Plant Protection, Faculty of Agriculture, Kırşehir Ahi Evran University. For their cultivation, the following ingredients were utilized in the artificial diet: corn flour, water, bran, milk powder, honey, glycerol, yeast, and honey nutrients. The rearing process commenced with placing wax moth eggs into 1-liter glass jars, which were filled with artificial diet to approximately one-third of their capacity. To facilitate egg laying, a paper covering was placed over the mouth of the jars, which also had holes in their covers for ventilation. The cultures were then kept in an incubator set to 26°C, with 65±5% relative humidity, and maintained in complete darkness throughout the day. Finally, the last stage larvae were harvested from these modified cultures, following the rearing technique adapted from Fracative et al. (2020), and used in subsequent experiments.

Application of p-acetamide and MPAEMA to Galleria mellonella Larvae

In this experiment, the impact of varying concentrations of specific materials on *G. mellonella* larvae was investigated. To achieve this, the larvae were subjected to different doses of the materials, specifically at concentrations of 400 µM, 800 µM, and 1200 µM. Each dose was administered in a precise volume of 2 µl. The selection of these specific concentrations was determined dynamically during the course of the study. Prior to treatment, the larvae were wiped with a sterile swab soaked in 70% alcohol to ensure cleanliness. This step was crucial for eliminating any pre-existing surface contaminants or microorganisms that could potentially confound the results of the experiment. Using a micro injector, the materials were directly injected into the left proleg of the larvae (Alvandial et al., 2016). Specifically, each 2 µl dose was directly injected into the left proleg of the larva. This specific injection site was chosen to ensure consistent material distribution and to minimize potential variations in absorption rates. A control group was maintained, receiving no treatment, while both the control and treated larvae were observed under identical conditions in an incubator set to 28±2°C, with 65±5% relative humidity, and kept in darkness. To analyze the effects of the treatments, mortality rates were recorded after a 24-hour exposure period. This specific time point was chosen to allow sufficient time for the materials to exert their

effects while minimizing the potential for confounding factors related to prolonged exposure or natural larval mortality. The mortality data collected after this 24-hour period were then subjected to statistical analysis to determine the significance of any observed differences between the treated and control groups, thereby providing insights into the impact of the materials on larval survival.

Statistical analyses

Probit analysis was subsequently performed to calculate the LC_{50} and LC_{99} doses, following the methodology established by Abbott (1925).

Molecular Docking Studies

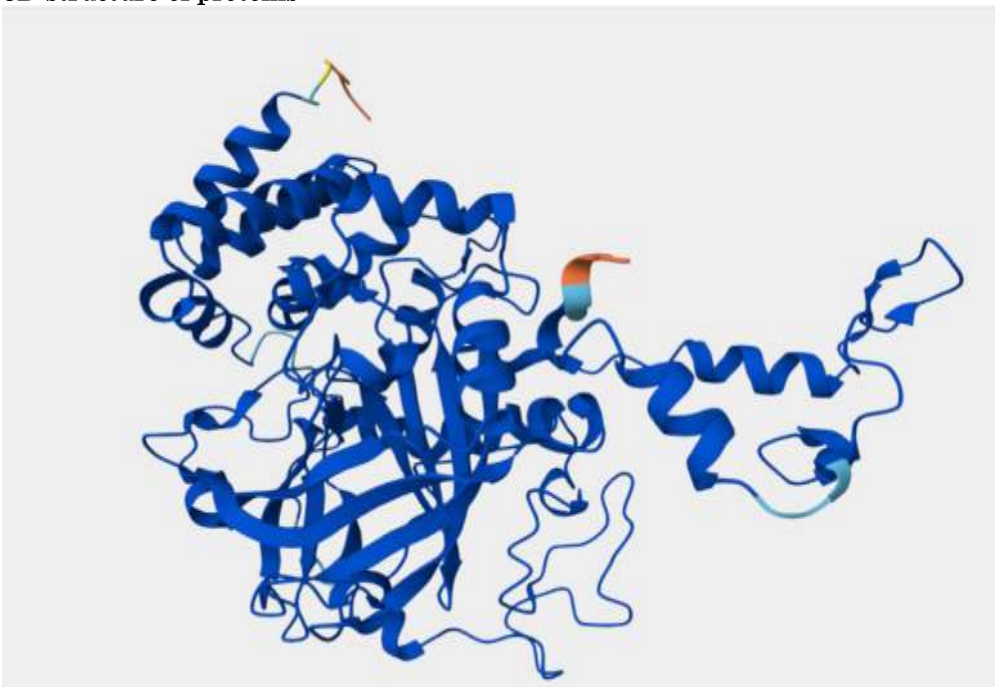
3D drawings of the synthesized p-acetamide and MPAEMA molecules were completed in GAUSSIAN programs (Temüz et al., 2024; Çoban et a.l, 2024; Çankaya et al., 2021). Insects, like vertebrates, have enzymatic and non-enzymatic defense systems. The main elements of the enzymatic system are superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione-S-transferase (GST) enzymes. Antioxidant enzymes from *G. mellonella* were chosen as target proteins. The sequences of the enzymes were obtained from UniProt (https://www.uniprot.org/) (Catalase: LOC113521268; Superoxide dismutase: LOC113520545; Glutathione peroxidase: LOC113509396; Glutathione S-transferase: LOC113515752) (Table 1). Protein structure models were built using Phyre2 and Itasser online databases (Kelly et al., 2015; Yang et al., 2015; Zheng et al., 2021). The Autodock Vina program was used for docking analysis (Trott & Olson, 2010; Yalçın et al., 2019; Çankaya & Yalçın, 2022; Çankaya et al., 2022). To detect protein-ligand interactions, the protein-ligand file (.pdbqt), which is the output of the Autodock Vina program, was selected separately for ligand and protein with Seamdock (Academic free) program, and then 2- and 3-dimensional protein-ligand interactions were observed at the amino acid level (Humphrey et al., 1996; Murail et al., 2021; Tuffery & Murail, 2020).

RESULT and DISCUSSION

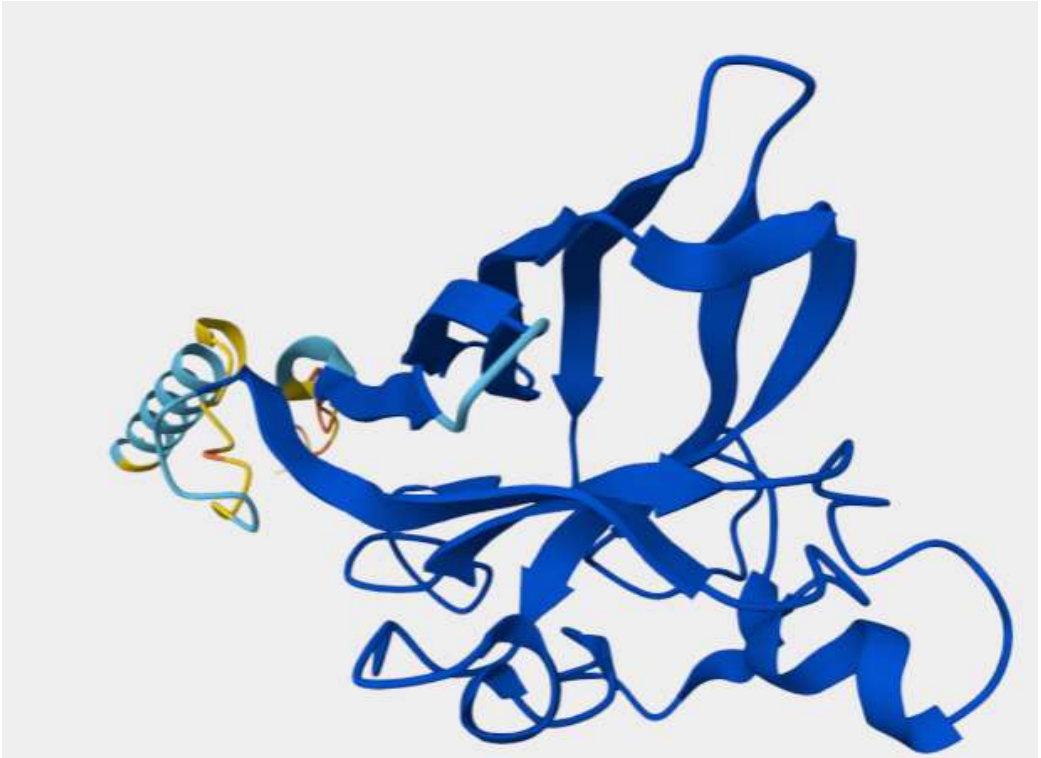
LC_{50} and LC_{99} values determined after applying the specified doses of p-acetamide and MPAEMA to the last stage larvae of the insect are presented in Table 2.

The toxicities of p-acetamide and MPAEMA against the larval stage of G. mellanolla were determined in the study. The results indicate that p-acetamide has a lethal effect on insect larvae at lower doses. Mortality of larvae increased with increasing doses. LC50 doses of p-acetamide and MPAEMA were 687,355 and 873,572 uM, respectively (Table 2).

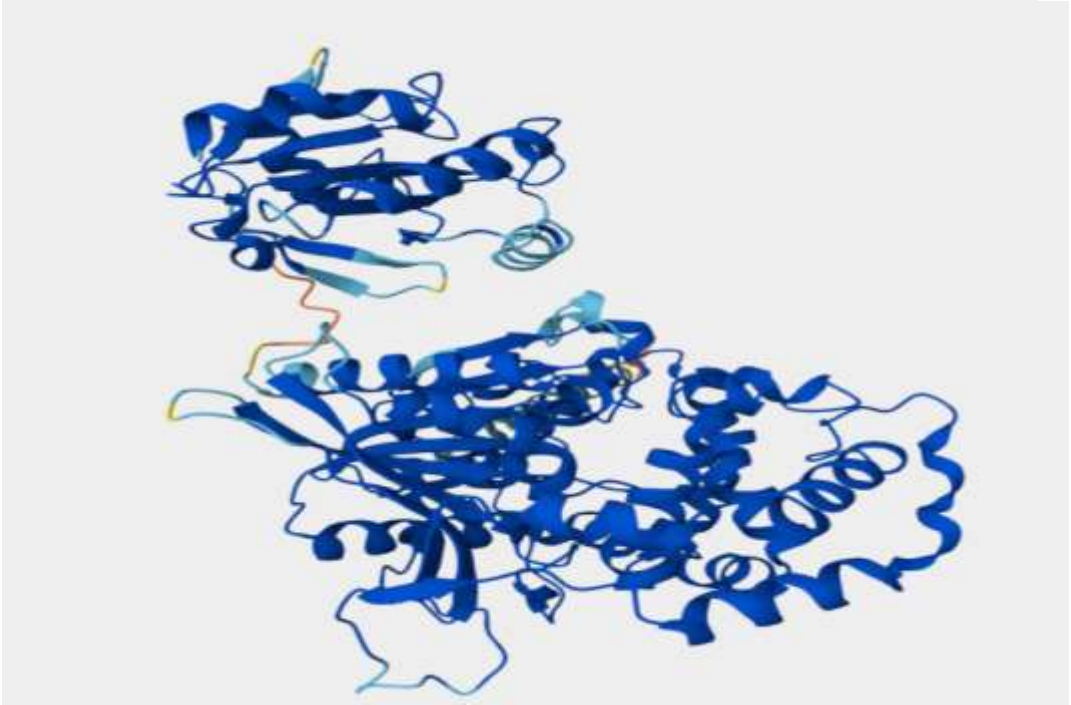
Table 1. 3D structure of proteins
Çizelge 1. Proteinlerin 3D yapıları

Proteins	**3D structure of proteins**
Catalase (LOC113521268)	

Superoxide dismutase (LOC113520545)



Glutathione peroxidase (LOC113509396)



Glutathione S-transferase (LOC113515752)

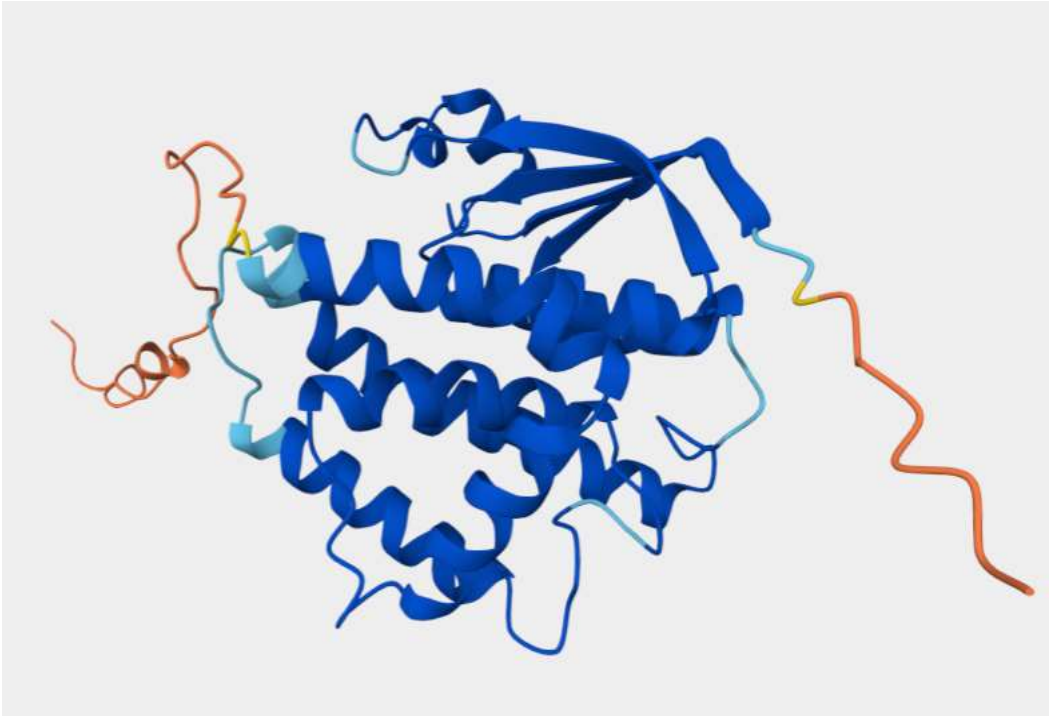


Table 2. LC50 and LC99 values of *Galleria mellanolla* larvae treated with p-acetamide and MPAEMA.
Çizelge 2. p-asetamid ve MPAEMA ile muamele edilen Galleria mellanolla larvalarının LC50 ve LC99 değerleri

p-acetamide	**Duration (24 h)**	**N**	**LC_{50} (uM)**	**LC_{99} (uM)**
		10	687,355	1565,612
MPAEMA	Duration (24 h)	**N**	**LC_{50} (uM)**	**LC_{99} (uM)**
		10	873,572	1989,332

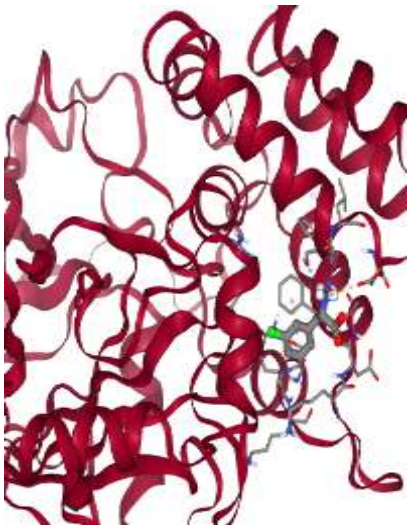
N: Number of the tested larvae.

G. mellonella larvae have become a popular non-mammalian model for studying microbial infections and testing antimicrobial drugs over the past five years. Additionally, these larvae are now being used to assess chemical toxicity, potentially offering a more accurate screening method before conducting toxicity tests on mammals. Moya-Andérico et al. (2021) studied the immediate harmful effects of various kinds of nanoparticles on *G. mellanolla* larvae, which were used as a model for nanotoxicology research. In another study, the toxicity of 19 chemicals was determined against *G. mellonella* larvae. The findings were compared to LD50 values from in vitro cell toxicity tests and in vivo acute oral LD50 values (Allegra et al., 2018). Coates et al. (2019) injected and/or force-fed larvae of *G. mellonella* with appropriate amounts of okadaic acid. They then observed the survival of the larvae and calculated the LD50 value. In this study, we used these model insect larvae for testing the toxicity of p-acetamide and MPAEMA, and we calculated the LC50 and LC99 doses.

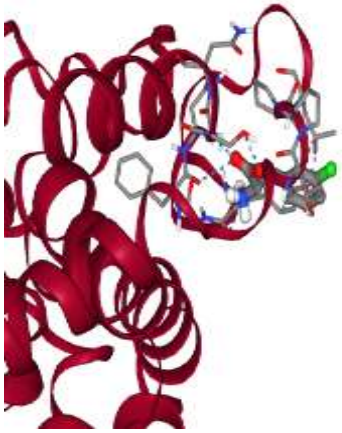
According to molecular docking results, it appears that MPAEMA binds to proteins with lower binding energy than p-acetamide (Table 3). In particular, MPAEMA appears to bind with glutathione peroxidase at -6.8 kcal/mol. Glutathione peroxidase (GSH-Px) facilitates the reduction of hydrogen peroxide and organic hydroperoxides (such as lipid and DNA hydroperoxides) using glutathione (GSH). This enzyme plays a crucial role in protecting cells from oxidative damage (Maiorino et al., 1990).

As a result, molecular docking is considered complementary to previous findings, not only at the research stage but also at an applied level. This is because this chapter reveals the effect of insecticides and their components on insect proteins and enzymes. This information is critical to understanding the potential for insects to develop resistance to these pesticides in the long term. From an environmental perspective, determining which chemical bonds in insecticides interact more strongly with amino acids in the protein of the target insect allows us to develop more specific pesticides for certain species without harming other organisms in the environment. In other words, it helps us to design chemical pesticides that are more specialized at the genetic level on target organisms (Tiwari et al., 2023; Aioub et al., 2023). In this study, the dock scores obtained with proteins confirmed the inhibitory potential of the MAPEMA against the GPx enzyme, and, consequently, their impact on insects. This study is a preliminary study in discovering new molecules to combat agricultural pests.

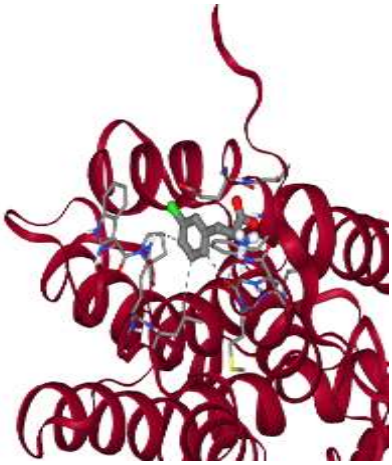
Table 3. p-acetamide and MPAEMA molecules docking analysis
Çizelge 3. p-asetamid ve MPAEMA moleküllerinin yerleştirme analizi

Ligand-Protein	Docking energy (kcal/mol)		
p-acetamide-catalase	-5,8	***hydrogen bond***	

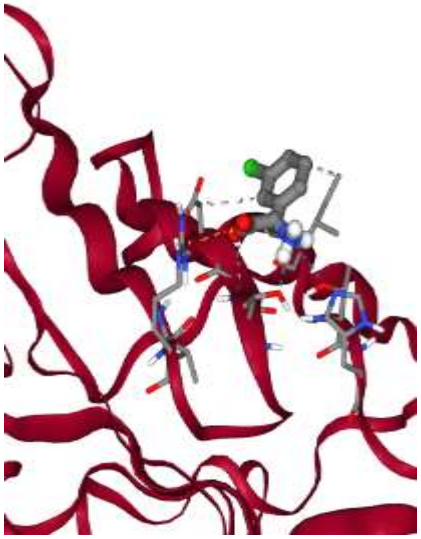
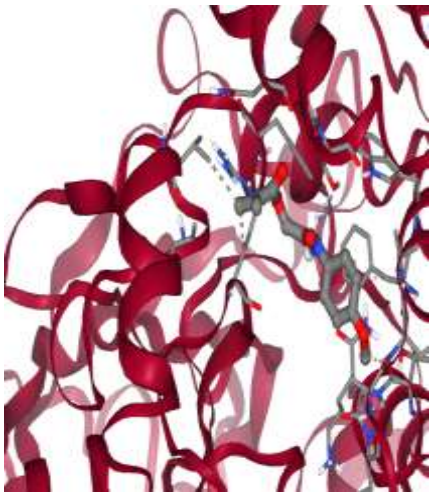
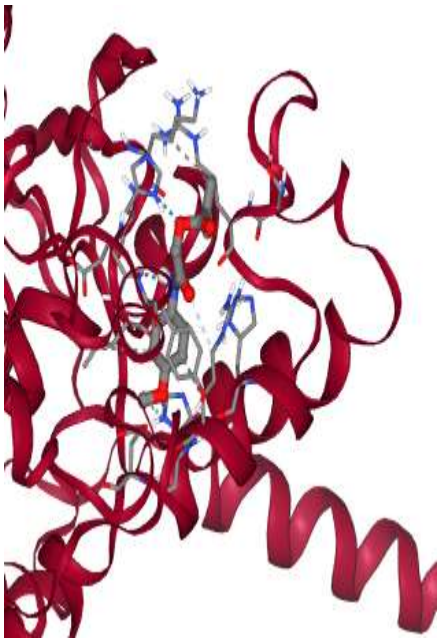
Ligand atom	Receptor
N1	S466(A) O
N1	S466(A) OG

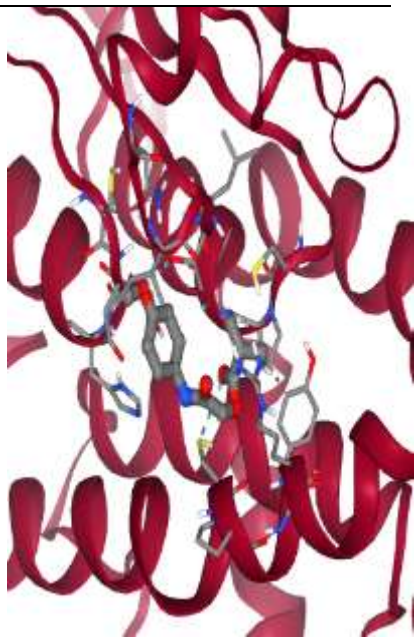
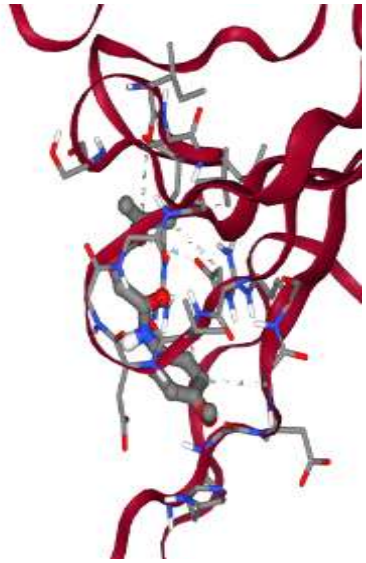
p-acetamide-glutathione peroxidase	-5	***hydrogen bond***	

Ligand atom	Receptor
N1	Y108(A) O
O2	F110(A) O
O1	S112(A) OG
O1	S112(A) OG
O2	S112(A) N

p-acetamide-glutathione S-transferase	-5	***hydrogen bond***	

Ligand atom	Receptor
N1	D36(A) O
N1	A37(A) O

p-acetamide-superocid	-4,7	*hydrogen bond* Ligand atom: N1, N1 Receptor: T34(A) OG1, T53(A) OG1	
MPAEMA-catalase	-6,1	*hydrogen bond* Ligand atom: , , OG, OG, NE Receptor: S466(A) OG, S466(A) OG, NaN() O, NaN() N, NaN() O	
MPAEMA - glutathione peroxidase	-6,8	*hydrogen bond* Ligand atom: , ND1, ND1 Receptor: E424(A) O, NaN() O, NaN() O	

MPAEMA glutathione S-transferase	**-5,1**	***hydrogen bond*** **Ligand atom** / **Receptor** C121(A) SG NH1 / NaN() O OG1 / NaN() O SG / NaN() O	
MPAEMA-superocid	**-5,2**	***hydrogen bond*** **Ligand atom** / **Receptor** E41(A) OE1 V42(A) O	

CONCLUSION

2-chloro-N-(4-methoxyphenyl)acetamide (p-acetamide) and 2-(4-methoxyphenylamino)-2-oxoethyl methacrylate (MPAEMA) molecules were resynthesized for this study to investigate the effect of the molecules on the agricultural pest *G. mellonella*. The toxicities of p-acetamide and MPAEMA against the larval stage of *G. mellonella* were evaluated simultaneously. The findings indicate that p-acetamide is lethal to insect larvae at lower doses. Additionally, the molecular docking interactions of p-acetamide and MPAEMA with the SOD, CAT, GPx, and GST proteins were analyzed. The binding energy between MPAEMA and glutathione peroxidase was found to be -6.8 kcal/mol, suggesting that MPAEMA may inhibit glutathione peroxidase and could be further explored for developing pesticides targeting this enzyme.

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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Fruit extracts of *Rosa canina* L. and *Rosa pimpinellifolia* L.: Phytochemical profiles, *in vitro* antioxidant, anti-inflammatory, xanthine oxidase inhibitory effects, and *in silico* molecular dynamics studies

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ABSTRACT

In this study, the phytochemical and biological activities of *Rosa canina* (RC) and *R. pimpinellifolia* (RP) fruit methanol extracts were investigated. HPLC analysis revealed that RP and RC extracts contained high amounts of ascorbic acid and gallic acid as the major components. In GC-MS/MS analysis, oleic acid, linoleic acid, and palmitic acid methyl ester were the most abundant compounds in both extracts. The total phenolic contents of RP and RC were 17.28±0.10 and 5.19±0,22 mg GAE/g extract, respectively. DPPH˙ scavenging activities of the extracts (13.45±0.21 µg/mL for RC and 3.41±0.05 µg/mL for RP) were observed to be higher than ascorbic acid (42.15±1.35 µg/mL). The reducing power capacities of RC and ascorbic acid were 55.57±3.23 and 87.24±2.44 µg/mL, respectively. According to the BSA denaturation assay, the anti-inflammatory effect of RP was found to be more effective at low doses with DFS and similar to the effect at high doses. RC extract showed high xanthine oxidase (XO) inhibition with an IC_{50} value of 2.28±0.25 µg/mL. The binding affinities of ascorbic and gallic acid with XO were determined as 6.20 and 6.60 kcal/mol, respectively. In addition, molecular dynamics simulations of the complexes were applied for 100 ns and observed to be stable. Binding energies were determined by performing MM/PBSA, and it was recorded that a high level of gallic acid was found at 21.50 kcal/mol. In this way, the phytochemical constituents and biological activities of two different rosehip species were compared, and ideas for their use in food, cosmetics, and medicine were presented.

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Rosa canina ve *Rosa pimpinellifolia* meyve özütleri: Fitokimyasal profilleri, *in vitro* antioksidan, anti-inflamatuar, ksantin oksidaz inhibitör etkileri ve *in silico* moleküler dinamik çalışmaları

ÖZET

Bu çalışmada, *Rosa canina* (RC) ve *R. pimpinellifolia* (RP) meyve metanol özütlerinin fitokimyasal ve biyolojik aktiviteleri araştırılmıştır. HPLC'de, RP ve RC özütleri ana bileşenler olarak büyük miktarlarda askorbik asit ve gallik asit içermektedir. GC-MS/MS'de, her özütte en yüksek miktarlarda oleik asit, linoleik asit ve palmitik asit metil esteri bulunmuştur. RP ve RC'nin toplam fenol içerikleri sırasıyla 17.28±0.10 ve 5.19±0,22 mg GAE/g özüt olarak bulunmuştur. Özütlerin DPPH˙ süpürücü aktivitesinin (RC için 13.45±0.21 µg/mL ve RP için 3.41±0.05 µg/mL), askorbik asitten (42.15±1.35 µg/mL) daha yüksek olduğu gözlenmiştir. RC ve askorbik asidin indirgeyici güç kapasiteleri sırasıyla 55,57±3,23 ve 87,24±2,44 µg/mL olarak bulundu. BSA denatürasyon deneyine göre, RP'nin anti-inflamatuar etkisinin DFS ile düşük dozlarda daha etkili olduğu ve yüksek dozlardaki etkiye benzer olduğu bulundu. RC özütü, 2,28±0,25 µg/mL'lik IC_{50} değeri ile yüksek ksantin oksidaz (XO) inhibisyonu

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Anahtar Kelimeler
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Antioksidan
Anti-inflamatuar

gösterdi. Askorbik ve gallik asidin XO ile bağlanma afiniteleri sırasıyla -6,20 ve -6,60 kcal/mol olarak belirlendi. Ayrıca, komplekslerin moleküler dinamik simülasyonları 100 ns boyunca uygulandı ve kararlı oldukları gözlendi. Bağlanma enerjileri MM/PBSA yapılarak belirlendi ve gallik asidin yüksek değerinin -21,50 kcal/mol olarak bulunduğu kaydedildi. Bu şekilde iki farklı kuşburnu türünün fitokimyasal bileşenleri ve biyolojik aktiviteleri karşılaştırılmış ve gıda, kozmetik ve ilaç gibi alanlarda kullanımına yönelik fikirler sunulmuştur.

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INTRODUCTION

In traditional medicine, plants rich in antioxidants are used as medicines to treat many diseases. Antioxidant compounds found in plants are often used in the traditional or food industry as vitamins, colorants, sweeteners, and additives in foods and beverages to extend the shelf life of products or to influence consumer behavior (Sarkar et al., 2009; Schmidt et al., 2007). Food supplements and nutraceuticals enriched with antioxidants are also necessary for a healthy life (Gostner et al., 2015). The rose hip is an indispensable and healing plant in alternative medicine. The rose hip, which can be consumed fresh or dried as a tea or jam any time of the year, also has significant health benefits. Rosehip, which has a very high nutritional value, is also rich in vitamins and minerals (Doğan et al., 2006; Güneş, 2011). It is a natural food that acts as an antioxidant and protects the body from many diseases (Shakibaei et al., 2012). Rosehip contains phenolic chemicals that are vital for humans. Since the natural antioxidants, minerals, carotenoids, bioflavonoids, tocopherol, fruit acids, vitamin C, pectin, tannin, and amino acids contained in it are good for human health, this type of fruit (Rosa spp.) has recently gained value (Çınar & Çolakoğlu, 2004; Su et al., 2007).

The plant known as rosehip is a member of the Rosaceae family's genus Rosa and subfamily Rosaideae (Keleş & Kökosmanlı, 1996; Yılmaz, 1996). The Rosaceae family is mainly found in the Northern Hemisphere, although it is widely distributed worldwide. According to Tanker et al. (1993), this nation has 35 genera and 250 species, while there are 100–120 genera and 3000–4000 species worldwide. The genus Rosa is divided into four subgenera: *Eurosa, Hesperhodos, Platyrhodon,* and *Hulthemia* (Wissemann, 2017). The Eurosa subgenus has more variants than the rest of all of these. According to Atienza et al. (2005), the subgenus consists of *Rosa banksianae* R.Br.*, R. bracteatae* Wendl.*, R. carolinae* L.*, R. chinensis* Jacq.*, R. cassiorhodon* Dumort.*, R. gallicanae* L.*, R. pimpinellifolia* L.*,* and *R. laevigatae* Michx.

Black-fruited rosehip, or *R. pimpinellifolia* L., is a small tree that resembles a shrub and reaches a maximum height of one meter. The fruits are hairless, round, and flattened laterally. It's a purple-black color. At 1200-2750 meters, *R. pimpinellifolia* L. grows on rocky, arid slopes, volcanic rocks, or limestone soils (Kutbay & Kılınç, 1996). *R. canina* L. grows 1.5–3.5 meters tall. The fruits range in shape from spherical to egg-shaped. Fruits range in size from 3 cm to 5 cm, with colors ranging from dark pink to yellowish red. In general, fall is when fruits ripen. The species *R. canina* L. is widespread throughout this nation (Kutbay & Kılınç, 1996).

The ability of the rosehip plant to thrive in different types of soil, in high and low altitudes, and in harsh continental climates has led to its spread throughout Turkey and the creation of numerous varieties (İlisulu, 1992; Yamankaradeniz, 1983). It has diuretic, antimutagenic, and antibacterial properties and treats various diseases, including rheumatic diseases, gout, stomach ulcers, sciatica, gallstone formation, biliary tract diseases, and colds. It is also known to affect hemorrhoids and diabetes. Bronchitis is treated with leaves and root parts of the plant (Orhan et al., 2009). Rosehip is a traditional remedy for treating kidney and bladder stones, diarrhea, bleeding gums, side and chest pains, and other ailments. Rosehip seeds have been found to lower triglyceride and cholesterol levels (Güneş & Şen, 2001). Digestive disorders can be avoided by eating rose hip root, fruit, and blossom (Macit & Köse, 2015). Additionally, it is highly effective in preventing colds by bolstering the immune system (Chrubasik et al., 2008; Sen & Gunes, 1996).

In this study, we compared the phytochemical contents (total phenolic, total flavonoid, GC-MS/MS, HPLC) and biological activities (antioxidant, anti-inflammatory, and xanthine oxidase) of methanol extracts from two different

rosehip species, Rosa canina (RC) In and In Rosa In *pimpinellifolia* (RP), known as natural vitamin C. In addition, the inhibitory properties of the compounds that were most pronounced in the HPLC analysis of both species and their interactions with xanthine oxidase, which plays a role in uric acid metabolism, were determined using the AutoDock program and the binding energies corresponding to the most appropriate poses were calculated using GROMACS molecular dynamics simulations and MM/PBSA methods. In this way, the unknown aspects of black and red rosehip will be revealed, and their use in food and pharmacology will be improved.

MATERIAL and METHOD

Plant Collection and Extraction

Two rosehip species, RC and RP, were collected in September 2023. RC from Yenidoğan village (Ağrı Mountains) in the Aralık district of Iğdır province (INWM00000240, diagnosed by Prof. Dr. Ahmet Zafer Tel) and RP (ARTH3561, diagnosed by Prof. Dr. Emin Eminağaoğlu) from the Ardanuç district of Artvin province in Turkey

Dried RC and RP fruits were ground into powder using a grinder. 20 g of each sample was weighed and extracted with methanol in a 750 ml Erlenmeyer flask for 3 days. The solvent in the extract was evaporated using a rotary evaporator, and a methanol crude extract was obtained.

Analysis of Phenolic Contents of Extracts by HPLC

HPLC analysis determined the phenolic contents and amounts of RC and RP fruit methanol extracts (Başar et al., 2024b). It was analyzed using 15 phenolic standards on the HPLC device. The device had a DAD sensor (300/200 nm) and an 8-micron reversed-phase hi-plex analysis column (300x7.7). The column temperature was set to 30ºC for sensitive analysis. The solvents were eluent A, 83% water (0.1 formic acid), and eluent B, 17% acetonitrile (0.1 formic acid). The flow rate of the solvent was set to 0.8 mL/min, and the injection volume to 10 µL. Sample preparation: 20 mg of the extract was weighed on a precision balance and dissolved in methanol. 1 mL of the sample was taken with an automatic pipette and filtered through a 0.45-micron filter. It was then diluted 1:1 with pure water and injected into the device. The content analysis was carried out using 20 different standard phenolic compounds (Table 1).

Analysis of the fatty acid content by GC-MS/MS

RC and RP fruit *n*-hexane extracts were analyzed on a GC-MS/MS device, as this previously published article described. This analysis used an Agilent 7000 A GC/MS Triple Quad with 7890 GC, 7693 Autosampler, and 7697A Headspace Sampler (Başar et al., 2024c). The instrument was equipped with an Agilent HP-5 (5%-phenyl)-methylpolysiloxane) (30 m x 0.25 mm x 0.25 µm) GC column. According to the previously established procedure for the analysis, the initial temperature was set at 50°C and kept constant for two minutes. It was then gradually increased to 140, 220, and 270°C until it was fixed at 270°C, and the ion temperature of the MS detector was set to 280°C. 20 mg of the sample was taken, 1 mL of MeOH was dissolved, and 1 mL of *n*-hexane was added. Then, 1 mL of KOH solution (1 M) was added and mixed with a vortex device at 2500 rpm for 30 seconds to ensure phase formation. A 0.22 µm filtered sample was taken from the upper phase (*n*-hexane phase) containing fatty acid methyl ester and analyzed with a 1 mL He gas stream by injecting 1 µL volume at a ratio of 1:10.

Total Phenol and Flavonoid Contents

The total phenol (Folin-Ciocalteu method) and total flavonoid (aluminum chloride method) contents of *R. canina* and *R. pimpinellifolia* fruit methanol extracts were determined. Gallic acid (total phenol) and quercetin (total flavonoid) were used as standards (Golmakani et al., 2014).

In TPC, 100 µL of extract (or standard gallic acid) solution (1024 µg/mL) was mixed with 500 µL of Folin-Ciocalteu reagent. After 1 minute, 1.5 mL of 20% Na_2CO_3 was added, and the mixture was left in the dark at room temperature for 2 hours. The absorbance values of the mixture solutions were measured at 760 nm. The TPC values of the samples were expressed as mg gallic acid equivalent (GAE) using the equation $y = 1.9465x + 0.0262$ ($R^2 = 0.99$) in the calibration graph of gallic acid.

In TFC, 1.5 mL methanol, 100 µL 10% aluminum chloride, 100 µL 1 M potassium acetate, and 2.8 mL deionized water were added to 500 µL extract and standard quercetin (1024 µg/mL) solutions. The mixture was left at room temperature in the dark for 30 minutes, and absorbance values were measured at 415 nm. The TFC values of the samples were expressed as mg quercetin equivalent (QE) using the equation $y = 2,2406x + 0,0245$ ($R^2 = 0.99$) in the calibration graph of quercetin.

Antioxidant Activities

The antioxidant activities of RC and RP fruit methanol extracts were determined with the $DPPH^{\cdot}$ scavenging (Blois, 1958) and reducing power (Oyaizu, 1986) activities, and also compared with standard ascorbic acid. The results were recorded as $A_{0.5}$ (reducing power), IC_{50} ($DPPH^{\cdot}$ scavenging), and expressed µg/mL. The IC_{50} value is the concentration at which 50% absorbance is effective for $DPPH^{\cdot}$ scavenging activity. The $A_{0.5}$ value is the concentration at which half of the absorbance is effective for reducing power.

The reducing capacities of RC and RP fruit methanol extracts were observed spectroscopically with the Fe3+ to Fe2+ reduction assay.[24] Briefly, 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of 1% $K_3Fe(CN)_6$ (potassium ferrisiyanür) were mixed with 1 mL of extract. The mixture was incubated in an aqueous bath at 50 °C for 20 minutes. After 2.5 mL of 10% TCA (trichloroacetic acid) was added to the mixture medium and centrifuged at 2500 x g for 10 minutes. 2.5 mL of the obtained supernatant was mixed with 0.5 mL of 0.1% $FeCl_3$ (iron (III) chloride) and 2.5 mL of ddH_2O (double deionized water). The reducing power capacities of the standards and extracts were measured at 700 nm, and changes in absorbance were followed. $A_{0.5}$ (µg/mL) values were calculated using the absorbance values.

This assay was evaluated by a 1,1-diphenyl-2-picryl-hydrazyl ($DPPH^{\cdot}$) radical scavenging assay.[26] 3 mL of the RC and RP fruit methanol extracts and solutions of standard antioxidant substances were mixed with 1 mL of 0.1 mM $DPPH^{\cdot}$ solution. Changes in absorbance at 517 nm were recorded. IC_{50} (µg/mL) values were calculated using the absorbance values.

Anti-inflammatory Activity

The anti-inflammatory effect was evaluated *in vitro* by testing its effect on bovine serum albumin (BSA) denaturation (Kandikattu et al. (2013). Briefly, 500 µL of extract or standard (Diclofenac sodium, DFS) at different concentrations (512, 256, 128, 64 µg/mL) was added tubes to 500 µL of the solution of BSA (0.2% prepared in ddH_2O). A tube with 500 µL of BSA and 500 µL of methanol was also prepared as a control. Then, the mixture was incubated at 37 °C for 15 minutes and heated at 72°Cfor 5 min. After cooling, the absorbance was measured at 660 nm in a UV-visible spectrophotometer.

$$\% \text{ Inhibition} = 100 - \frac{OD_{test} - OD_{control}}{OD_{test}} \times 100$$

Xanthine Oxidase (XO) Inhibition

50 µL of sample or allopurinol solution, 100 µL of substrate solution (0.041 mM xanthine), and 50 µL of freshly prepared enzyme solution (0.1 U/mL xanthine oxidase in phosphate buffer (pH 7.5)) were added to the 96-well microplate. The mixture was incubated at 37°C for 5 min. The reaction was then stopped by adding 100 µL of 1 M HCl. The absorbance was measured using a 292 nm UV/VIS spectrophotometer (Li et al., 2025). IC_{50} (µg/mL) values were calculated using the absorbance values.

Statistical Analysis

The arithmetic mean ± standard deviation of the mean (std) was used to express the results; n = 3. An ANOVA was employed since the data acquired using IBM SPSS 20.0 software had a normal distribution. The antioxidant activities (DPPH and reducing power) and XO inhibition between the methanol extracts and standards were then compared using the Tukey $HSD^{a,b}$ test. Additionally, Cohen's d values between the samples were determined by performing an independent t-test, which showed a significant difference since d>2.0. For every test, the findings showed significant differences between the samples ($p < 0.05$).

Molecular Docking, Molecular Dynamics (MD) Simulation, and MM/PBSA Analysis

In the molecular docking studies, the molecular structures were drawn in ChemDraw ultra-18.0, the minimum energy was adjusted using Chem3D 18.0 programs, and the molecular structure was saved in mol2 format. Xanthine oxidase [3NRZ] was selected from RSCB (Protein Data Bank). The AutoDock Vina programs were used for the active site to determine a molecule's interaction with enzymes. All data were integrated to determine the 2D and 3D interaction of the molecules with the active sites of the enzymes using the Discovery Studio (Başar & Erenler, 2024; Çolak et al., 2025; Yenigun et al., 2024).

The stability of the complexes derived from the docking was investigated using MD modeling. The GROMACS package was used to perform the MD simulations (Abraham et al., 2015). The CHARMM force field was used for the MD simulations. The unbound enzyme and the complexes were placed in a tricyclic box and solvated with TIP3P water. Na^+ and Cl^- ions were added to the system to neutralize its overall charge. Next, 50,000 steps of the steepest descent method were used to minimize the energy. NVT/NPT then set the pressure and temperature of

the system to 100 kPa and 310 K, respectively. Finally, the MD simulation was run for 100 ns (Bjelkmar et al., 2010). RMSD (Root Mean Square Deviation), Rg (Radius of Gyration), RMSF (Root Mean Square Fluctuation), and ligand hydrogen bonding diagrams were plotted using qtgrace to investigate the MD simulation results (Akkoc et al., 2023; Başar et al., 2024a). The MM/PBSA method using the tool "gmx_mmpbsa" was preferred to determine the free energy of binding of the complex formed by protein and ligand" (Valdés-Tresanco et al., 2021; Yenigun et al., 2024).

RESULTS and DISCUSSION

As a result of the extraction, 1.2 grams (yield; 6%) of RP and 1.8 grams (9%) of RC extracts were obtained. The phytochemical contents, total phenolics, total flavonoids, antioxidant, anti-inflammatory and xanthine oxidase (XO) properties of the extracts were determined. In addition, the in silico properties of the main compounds and the XO inhibitor were investigated.

HPLC Analysis

The contents of phenolic components of the rose hips were determined by HPLC analysis, and it was found that both plant species contain 15 components. While the RP contained high amounts of ascorbic acid (107.162 ng/µl), gallic acid (473.077 ng/µl), protocatechuic acid (81.522 ng/µl) and *trans*-ferulic acid (49.163 ng/µl), the RC fruit contains high amounts of ascorbic acid (43.670 ng/µl) and gallic acid (281.070 ng/µl) (Figure 1 and Table 1). Catechin and rutin were found in the RC but not in the RP, and vanillic acid, gentisic acid, neohesperidin, and coumarin were found in the RP but not in the RC.

The RC species' biochemical characteristics, collected from six distinct regions in Van, Hakkari, and Şırnak, were investigated by Encü (2015). This species' chemical composition included ascorbic acid, ellagic acid, protocatechin, rutin, quercetin, catechin, gallic acid, chlorogenic acid, caffeic acid, syringic acid, *p*-coumaric acid, ferulic acid, and phloretin. Gallic acid, chlorogenic acid, caffeic acid, ferulic acid, phloroglucinol, protocatechuic acid, *p*-coumaric acid, catechin, epicatechin, quercetin-3-glucoside, and resveratrol were detected in the HPLC analysis of the ethanol extract of RC (Fetni et al., 2020). In the HPLC analysis of the methanol extract of the root and fruit of RP, benzoic acid, caffeic acid, chlorogenic acid, protocatechualdehyde, and vanillic acid were determined as the main components (Guven et al., 2021). The content analyses vary depending on the standards used in HPLC analyses. They may also vary depending on the time of year, climate, and altitude at which the plant is harvested. However, this results are generally consistent with the literature.

Table 1. Analysis of the compounds in RP and RC methanol extracts using HPLC
Çizelge 1. RP ve RC metanol ekstraktındaki bileşiklerin HPLC ile analizi

No	Compound name	RT (min.)	LOD	LOQ	R^2	RP (ng/µL)	RC (ng/µL)
1	Ascorbic acid	3.307	1.972356	5.976836	0.99129	107.162	43.670
2	Gallic acid	4.161	2.15833	6.540393	0.99041	473.077	281.070
3	Protocatechuic acid	5.633	0.216179	0.655089	0.99989	81.522	17.079
4	Catechin	6.555	0.259225	0.785531	0.99986	ND	ND
5	Hydroxybenzoic acid	8.671	0.185641	0.562548	0.99992	ND	ND
6	Vanillic acid	9.628	0.202326	0.613109	0.99991	ND	ND
7	Gentisic acid	10.460	0.283662	0.859581	0.99983	16.511	ND
8	*p*-coumaric acid	17.214	0.136218	0.412782	0.99995	ND	ND
9	Rutin	19.288	0.237936	0.721018	0.99989	ND	ND
10	*trans*-ferulic acid	20.237	0.373309	1.131241	0.99983	49.163	9.237
11	Naringin	27.357	0.373309	1.131241	0.99972	17.684	11.986
12	*o*-Coumaric acid	28.565	0.260367	0.78899	0.99986	22.501	8.597
13	Neohesperidin	29.642	0.399215	1.209743	0.99968	ND	ND
14	Coumarin	30.696	0.048233	0.14616	0.99999	ND	ND
15	Resveratrol	32.573	0.153643	0.465586	0.99995	ND	ND
16	Quercetin	34.818	0.160099	0.485149	0.99995	9.753	6.673
17	*trans*-Cinnamic acid	35.616	0.23304	0.706183	0.99989	11.705	3.567
18	Hesperidin	36.763	0.354821	1.075215	0.99974	ND	ND
19	Alizarin	38.661	0.132739	0.402239	0.99995	ND	ND
20	Flavon	40.769	3.42617	10.38233	0.99995	ND	ND

RT: Retention time, RC: *R. canina* fruit methanol extract, RP: *R. pimpinellifolia* fruit methanol extract, ND: Not detected

GC-MS/MS Analysis

GC-MS/MS analysis determined both rosehip species' fatty acids and volatile oils content. The results showed that 21 compounds were detected in RP and seven compounds in RC (Figure 2 and Figure 3). Oleic acid methyl ester

(41.30%), linoleic acid methyl ester (32.43%), and palmitic acid methyl ester (16.47%) were detected in the highest amount in the *n*-hexane extract of RP. In comparison, oleic acid methyl ester (53.87%), palmitic acid methyl ester (22.72%), and linoleic acid methyl ester (15.46%) were detected in the *n*-hexane extract of RC (Table 2).

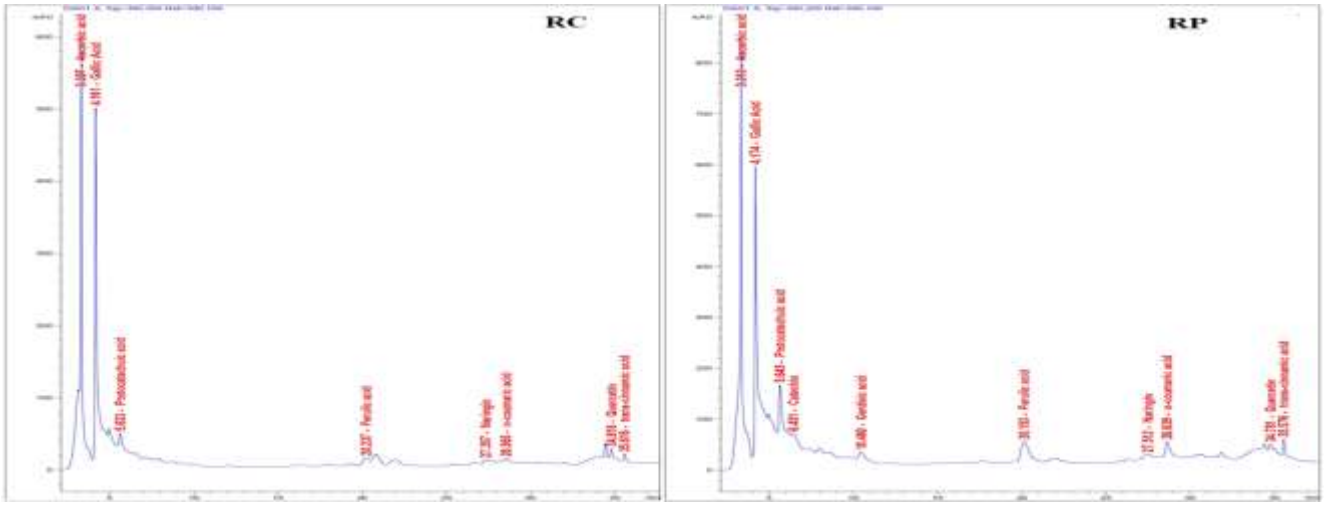

Figure 1. The HPLC chromatogram of the RC and RP
Şekil 1. RC ve RP'nin HPLC kromatogramı

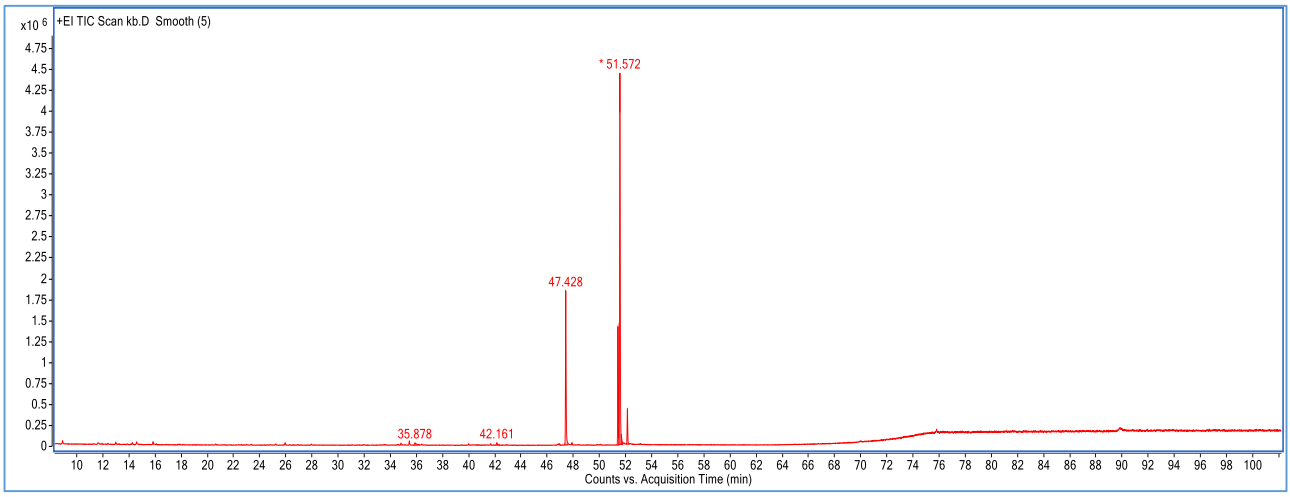

Figure 2. The GC-MS/MS chromatogram of the RP
Şekil 2. RP'nin GC-MS/MS kromatogramı

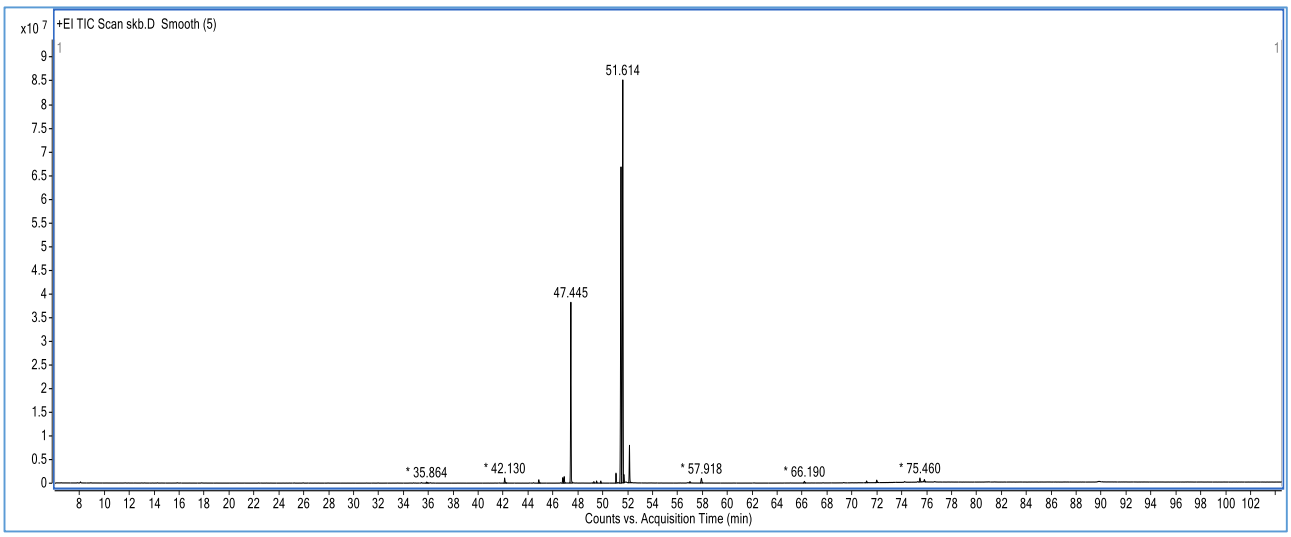

Figure 3. The GC-MS/MS chromatogram of the RC
Şekil 3. RC'nin GC-MS/MS kromatogramı

Table 2. GC-MS/MS analysis of the compounds in the RP and RC hexane extract
Çizelge 2. RP ve RC hekzan ekstraktındaki bileşiklerin GC-MS/MS analizi

No	Compound name	RT (min.)	RI	RP (%)	RC (%)
1	Lauric acid, methyl ester	35.86	1526	0.13	0.99
2	Myristic acid, methyl ester	42.13	1725	0.54	0.59
3	Pentadecanoic acid, methyl ester	44.87	1820	0.34	ND
4	7-Hexadecenoic acid, methyl ester	46.79	1900	0.53	ND
5	Palmitoleic acid, methyl ester	46.90	1899	0.62	ND
6	Palmitic acid, methyl ester	47.45	1926	16.47	22.72
7	Methyl 8-heptadecenoate	49.29	1986	0.18	ND
8	Methyl 9-heptadecenoate or 9-17:1	49.51	1989	0.18	ND
9	Heptadecanoic acid, methyl ester	49.85	2028	0.18	ND
10	γ-Linolenic acid, methyl ester	51.07	2092	0.84	ND
11	Linoleic acid, methyl ester	51.47	2092	32.43	15.46
12	Oleic acid, methyl ester	51.61	2091	41.30	53.87
13	Oleic acid, methyl ester-isomer	51.70	2091	0.62	1.43
14	Stearic acid, methyl ester	52.15	2128	3.38	4.94
15	11-Eicosenoic acid, methyl ester	57.00	2306	0.16	ND
16	Eicosanoic acid, methyl ester	57.92	2329	0.66	ND
17	Behenic acid, methyl ester	66.19	2528	0.21	ND
18	Heptacosane	71.18	2700	0.20	ND
19	Lignoceric acid methyl ester	71.99	2728	0.28	ND
20	Hexacosanol	75.46	2852	0.51	ND
21	Nonacosane	75.82	2900	0.27	ND

RT: Retention time, RI: Retention Index, RC: *R. canina*, RP: *R. pimpinellifolia,* ND: Not detected

When GC-MS analyzed the *n*-hexane extract of RP to determine the fatty acid content, linoleic acid and homo-γ-linolenic acid were determined as the main components (Guven et al., 2021). The fatty acid content of RC was reported to consist of linoleic acid, palmitic acid, and stearic acid as the main components (Ercisli et al., 2007). Therefore, this data are generally consistent with the literature.

Phytochemical Contents, Antioxidant and XO Inhibition Activities

The total phenolic content was higher in the RP than in the RC (Table 3). It is hypothesized that the reason why the total phenolic content of the RP extract is higher than that of the RC extract is due to the higher amounts of 11 compounds determined in the HPLC analysis, the presence of gentisic acid and vanillic acid only in the RP extract, and the absence of catechin and rutin in the RP extract. The $DPPH^{\cdot}$ scavenging activity was strongly influenced by ascorbic acid in both rosehip fruit extracts. However, RP also showed higher activity than other rosehip species and ascorbic acid. Although RC had a higher effect than ascorbic acid in reducing power capacity, RP had no effect (Table 3). The antioxidant activity of the extract and the standards differed significantly ($p < 0.05$) according to statistical analysis using the Tukey test. The test verified that the RC extract's activity was noticeably higher than expected ($p < 0.05$, Table 3). The methanol extracts of RC and RP had a total phenolic content of 176.48 and 225.65 mg GAE/100 g, a total flavonoid content of 0.41 and 2.02 mg QE/100 g, and a $DPPH^{\cdot}$ scavenger content of 79.16% and 87.78%, respectively (Fattahi et al., 2012).

XO is an important enzyme that catalyzes the conversion of hypoxanthine to xanthine and then to uric acid. XO also releases superoxide and hydrogen peroxide anions, which are required to catalyze the primary steps of purine metabolism. Hyperuricemia is the result of excessive uric acid synthesis, which can lead to gout. XO is an important and specific target for treating gout and hyperuricemia-related diseases, such as metabolic syndrome, diabetes, and cardiovascular disease (Singh et al., 2020). The discovery of natural substances with xanthine oxidase-inhibiting properties has increased recently. The activity of this enzyme is mediated by mechanisms that have been shown to lower uric acid levels in many natural flavonoids, phenylpropanoids, alkaloids, saponins, and polysaccharides. Flavonoids have attracted much attention due to their efficacy and safety (Xue et al., 2023).

Table 3 shows the xanthine oxidase inhibition values of the extracts and allopurinol. According to these results, no inhibitory effect of the RP extract was observed. However, it was noted that the RC extract had higher XO inhibition than the drug. This situation is thought to be because the amount of palmitic acid and oleic acid among the fatty acids it contains is higher than in the RP extract, and the catechin and rutin compounds are in the RC extract but not in the RP extract. The XO inhibition of the extract and the standards differed significantly ($p < 0.05$) according to statistical analysis using the Tukey test. The test verified that the RC extract's inhibition was noticeably higher than expected ($p < 0.05$, Table 3).

Table 3. The total phenol and flavonoid, antioxidant and enzyme inhibition activities of the RP and RC
Çizelge 3. RP ve RC'nin toplam fenol ve flavonoid, antioksidan ve enzim inhibisyon aktiviteleri

Name	Total phenol (mg GAE/g)	Total flavonoid (mg QE/g)	DPPH˙ scavenging (IC_{50} µg/mL)	Reducing power ($A_{0.5}$ µg/mL)	XO inhibition (IC_{50} µg/mL)
RP	17.28±0.10	0.03±0.00	3.41±0.05[a]	NA	NA
RC	5.19±0.22	0.13±0.05	13.54±0.53[b]	55.57±3.23[a]	2.28±0.25[a]
Ascorbic acid	NT	NT	42.15±1.35[c]	87.24±2.44[b]	NA
Allopurinol	NT	NT	NT	NT	19.95±1.441[b]
Cohen's d					
Between RP and RC	NT	NT	26.78	24.32	13.06
Between RP and standard	NT	NT	40.50	50.58	20.05
Between RC and standard	NT	NT	20.40	9.47	12.46
P value	NT	NT	0.001	0.000	0.002

GAE: Gallic acid equivalent, QE: Quercetin equivalent, RC: *R. canina* fruit methanol extract, RP: *R. pimpinellifolia* fruit methanol extract, XO: xanthine oxidase. NT: Not tested, NA: Not activity
The average values ± standard deviation of three separate samples are represented in the data. A Tukey test indicates that significant differences are shown by different letters [a-d]. ($p < 0.05$).

Anti-inflammatory Activity

Numerous studies indicate that the denaturation of proteins is one of the causes of rheumatoid arthritis. In many rheumatic diseases, the denaturation of proteins can lead to the formation of autoantigens *in vivo*. Disulfide, hydrophobic, hydrogen, and electrostatic bond changes will likely be part of the denaturation process. Several anti-inflammatory drugs have been shown to stop heat-induced protein denaturation dose-dependently (Rahman et al., 2015).

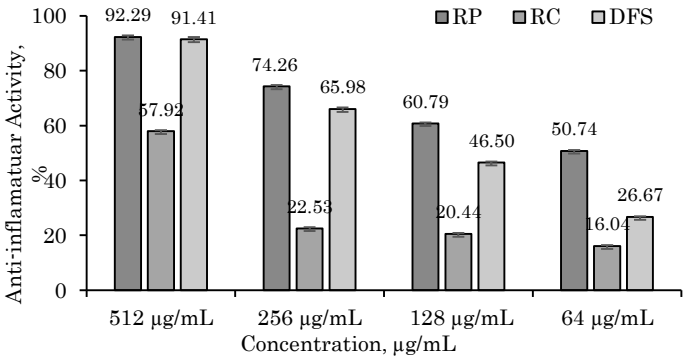

Figure 4. Anti-inflammatory activity of RP, RC, and DFS
Şekil 4. RP, RC ve DFS'nin anti-inflamatuar aktivitesi

The anti-inflammatory effect of diclofenac sodium, which was used as a standard, decreased with decreasing concentration. On the other hand, RP and RC extracts were found to denature BSA. Of these two extracts, it was observed that the RP extract denatured more than the standard, while the RC extract denatured less than the standard (Figure 4). According to this result, it is clear that RP can be used as an anti-inflammatory agent.

Molecular Docking Studies

In this study, we investigated the interactions of gallic acid and ascorbic acid, present in large amounts in both rosehip species, with xanthine oxidase using the AutoDock Vina program in a computer environment. Ascorbic acid was found to form ten hydrogen bonds that interact with XO. Four hydrogen bonds are conventional (VAL259, GLY260, SER347), while the other six are carbon-hydrogen bonds (VAL258, GLY260, ASN261, ALA346, THR262, SER347) (Figure 5 and Table 4). Gallic acid was shown to interact hydrophobically with xanthine oxidase once, forming five hydrogen bonds. Two hydrogen bonds are carbon-hydrogen bonds (GLY260, ASN351), and the other three are conventional (GLY260, SER347). The hydrophobic interaction is an amide-pi stacked interaction (GLY350) (Figure 5 and Table 4). The binding affinities of ascorbic acid and gallic acid were determined to be -6.20 kcal/mol and -6.60 kcal/mol, respectively (Table 4).

MD Simulation Studies

The investigation of the dynamic behavior of molecules and the complex systems with which they interact is of crucial importance for drug development methods. MD simulation is used as a computational method for this

purpose. Molecular docking simulations take into account the flexibility of targets, which is not the case with conventional docking techniques. Binding energy estimates can more accurately identify putative inhibitors (Liu et al., 2018).

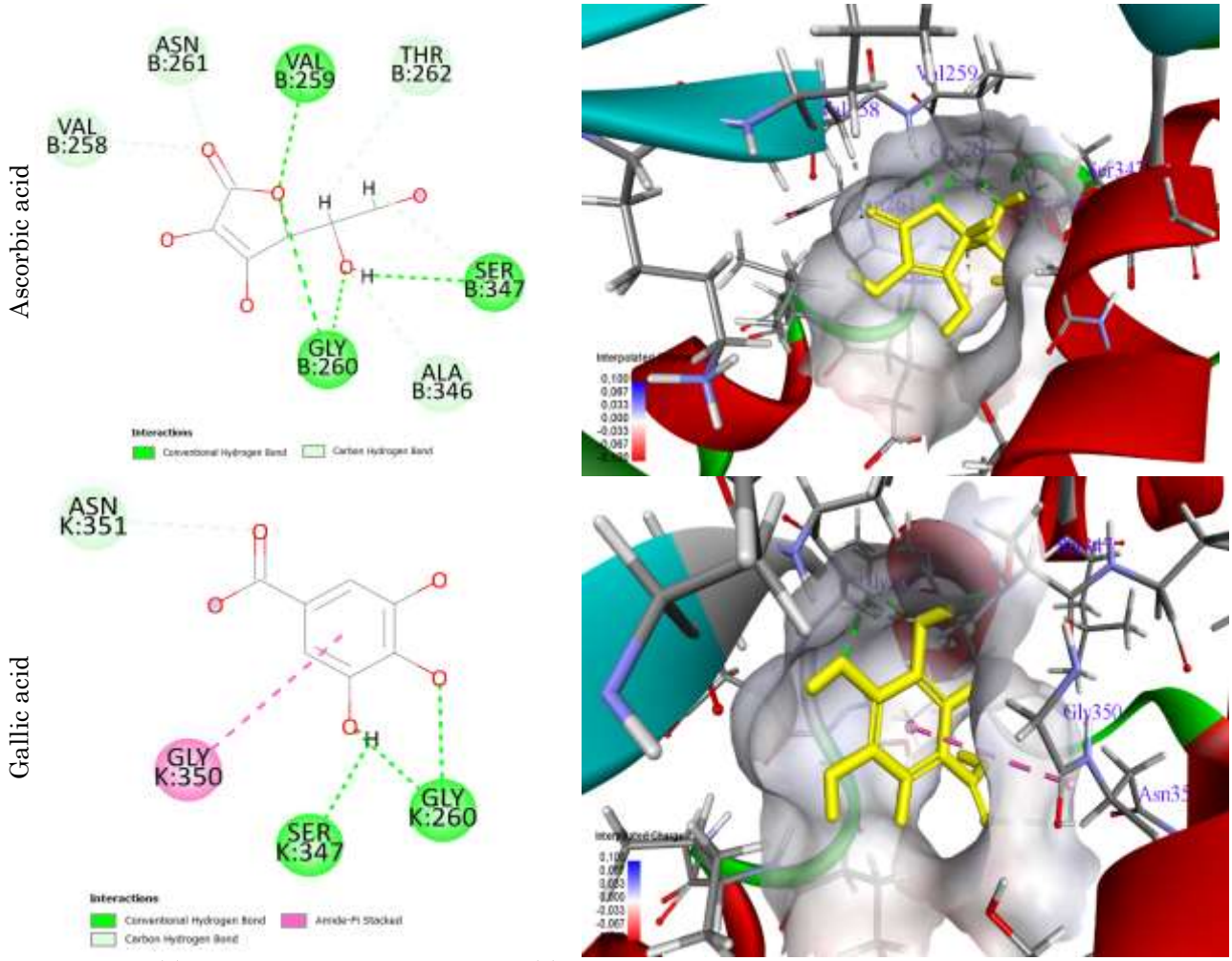

Figure 5. 2D (a), and 3D interpolated charge (b) of the interaction of compounds with XO
Şekil 5. Bileşiklerin XO ile etkileşiminin 2D (a) ve 3D interpole edilmiş yükü (b)

Table 4. XO-compounds interaction categories, species and molecular docking distance
Çizelge 4. XO-bileşiklerin etkileşim kategorileri, türleri ve moleküler yerleştirme mesafesi

Compound Name	Amino acid Names	Distance	Bond Types	Binding Affinities (kcal/mol)
Ascorbic acid	VAL259	2.28	Hydrogen Bond (Conventional)	-6.20
	GLY260	2.25	Hydrogen Bond (Conventional)	
	GLY260	2.41	Hydrogen Bond (Conventional)	
	SER347	2.14	Hydrogen Bond (Conventional)	
	VAL258	2.39	Hydrogen Bond (Carbon)	
	GLY260	2.75	Hydrogen Bond (Carbon)	
	ASN261	2.42	Hydrogen Bond (Carbon)	
	ALA346	2.73	Hydrogen Bond (Carbon)	
	THR262	2.87	Hydrogen Bond (Carbon)	
	SER347	2.63	Hydrogen Bond (Carbon)	
Gallic acid	GLY260	1.71	Hydrogen Bond (Conventional)	-6.60
	GLY260	2.31	Hydrogen Bond (Conventional)	
	SER347	1.85	Hydrogen Bond (Conventional)	
	GLY260	2.82	Hydrogen Bond (Carbon)	
	ASN351	2.89	Hydrogen Bond (Carbon)	
	GLY350	4.74	Hydrophobic (Amide-Pi Stacked)	

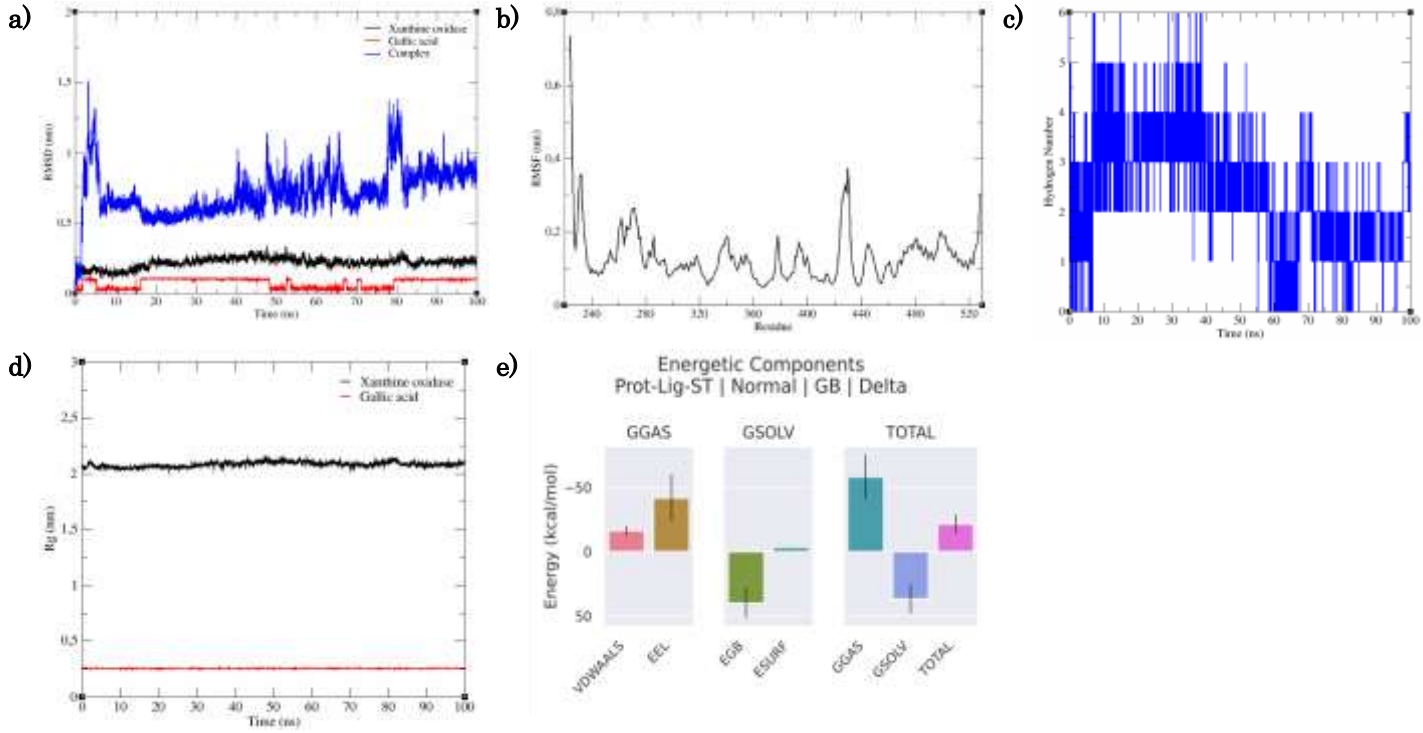

Figure 6. RMSD (a) and RMSF (b) backbone variations within MD trajectories for peptides and the complex, time-dependent H-bond interactions (c), Rg plotting (d), MM-PBSA energy (e) with gallic acid XO

Şekil 6. Peptitler için MD yörüngeleri içindeki RMSD (a) ve RMSF (b) omurga varyasyonları ve karmaşık, zamana bağlı H-bağ etkileşimleri (c), Rg çizimi (d), gallik asit XO ile MM-PBSA enerjisi (e)

The protein remained stable during the entire simulation period, as can be seen from the reduced RMSD values. RMSD mapping showed that the ascorbic acid and gallic acid complexes changed over a period of 100 ns in 9.0-9.5 nm and 0.5-0.8 nm, respectively. It was found that the complexes formed became more stable (Figures 6a and 7a). Using the Cα atoms of XO, the RMSF was calculated for all complex systems and showed that the fluctuation intensity persisted within 0.05-0.45 nm (Figures 6b and 7b). Thus, the stability of the protein structure and the presence of flexible regions required to achieve the optimal conformations were demonstrated by the RMSF plots. To determine the stability of a ligand-receptor complex, it was crucial to investigate the binding connections between proteins and ligands during MD simulations (Majewski et al., 2019). The H-bond that the ligands formed with xanthine oxidase during the 100-ns MD simulations is shown in Figures 6c and 7c. Throughout the MD simulation period, a continuous interaction of H-bonds between one and six and one and ten occurred for the complexes of gallic acid and ascorbic acid, respectively. The degree of compactness of a protein is indicated by its Rg value. The ability of a drug to alter the structure of proteins can be accurately and usefully measured using Rg. The loose molecular packing of a protein is indicated by its Rg value. The dynamics calculations for ascorbic acid, gallic acid, and XO for 100 ns were consistently around 2.10–2.20, 0.25, and 0.25 nm, respectively, as shown in Figures 6d and 7d.

MM/PBSA Analysis

MM-PBSA is a frequently used technique for determining the free energy of binding. It assumes that a ligand-protein combination with a lower predicted binding free energy is more stable and has higher ligand activity and potency. $\Delta G_{binding}$, the free energy of binding of protein-ligand complexes, was determined using MM-PBSA. The energy contributions are G_{gas} and G_{solv}, and MM-PBSA is ranked according to binding energy criteria. The GB estimates in Table 5 show that EGB and ESURF represent the polar and non-polar contributions, respectively. Although all mutants have a strong electrostatic contribution, this is balanced by a sizable positive polar contribution (EGB), which is why the van der Waals term contributes the most to the total binding free energy. As a result, the total polar contribution (EEL + EGB) is positive (Gautam et al., 2021). The $\Delta G_{binding}$ values for ascorbic acid and gallic acid are -17.37 kcal/mol and -21.50 kcal/mol for xanthine oxidase (Figures 6e and 7e). Further analysis of the MM-PBSA data revealed that the van der Waals interaction force, as opposed to the electrostatic interaction force, is important for protein-ligand binding. The output parameters of the MD simulation show a strong correlation with the docking results, indicating that the docked protein-ligand complexes remain stable during the simulation.

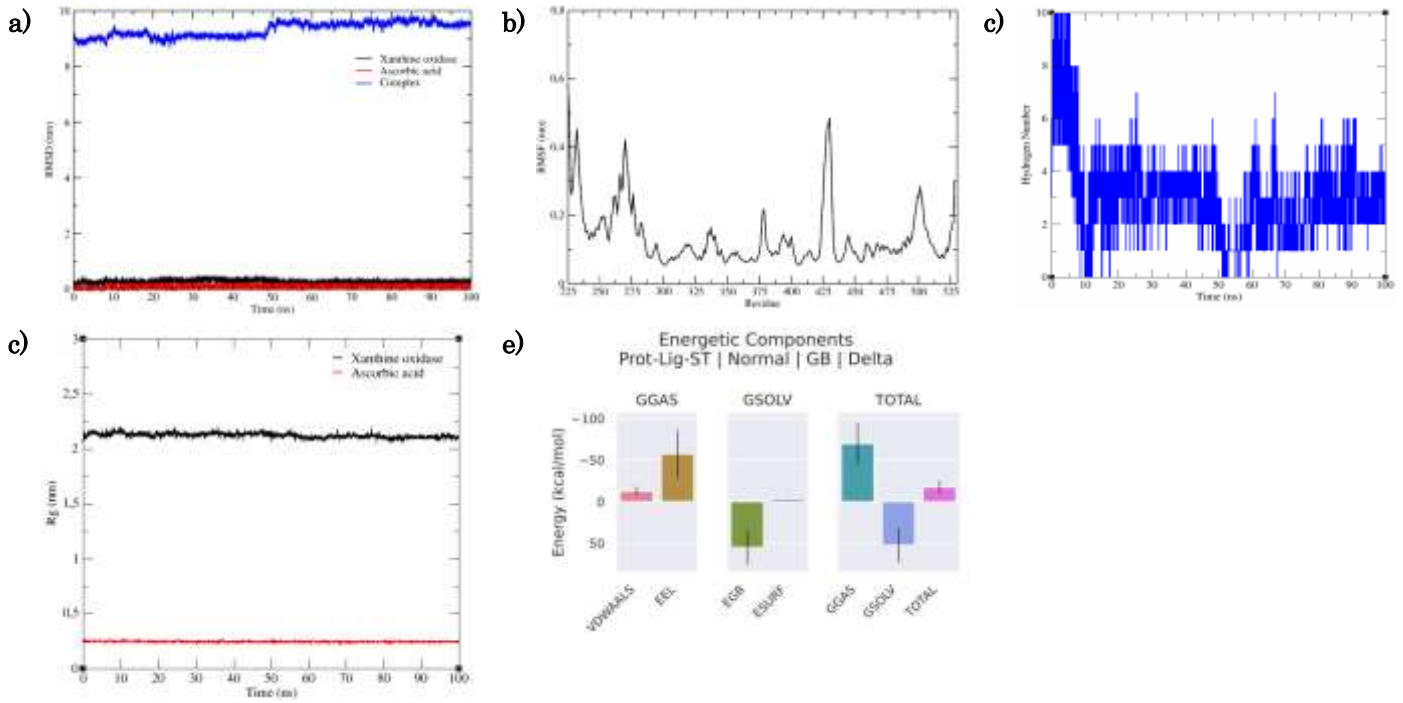

Figure 7. RMSD (a) and RMSF (b) backbone variations within MD trajectories for peptides and the complex, time-dependent H-bond interactions (c), Rg plotting (d), MM-PBSA energy (e) with ascorbic acid XO

Şekil 7. Peptitler için MD yörüngeleri içindeki RMSD (a) ve RMSF (b) omurga varyasyonları ve karmaşık, zamana bağlı H-bağ etkileşimleri (c), Rg çizimi (d), askorbik asit XO ile MM-PBSA enerjisi (e)

Table 5. Results of the energy calculation of the compound-XO complex with MM/PBSA

Çizelge 5. MM/PBSA ile bileşik-XO kompleksinin enerji hesaplamasının sonuçları

Compound-Enzyme	VDW (kcal/mol)	EEL (kcal/mol)	EGB (kcal/mol)	E_{SURF} (kcal/mol)	ΔG_{GAS} (kcal/mol)	ΔG_{SOLV} (kcal/mol)	$\Delta G_{Binding}$ (kcal/mol)
Ascorbic acid	-12.46±5.25	-57.28±28.55	55.23±20.72	-2.86±0.45	-69.74±25.31	52.38±20.79	-17.37±7.76
Gallic acid	-16.39±3.48	-41.72±18.40	39.95±11.54	-3.35±0.35	-58.11±17.29	36.61±11.38	**-21.50±7.21**

VDW: van der Waals contribution from MM, **EEL:** electrostatic energy as calculated by the MM force field, **EGB:** the electrostatic contribution to the solvation-free energy calculated by GB, **E_{surf}:** hydrophobic contribution to solvation-free energy for GB calculations, **ΔG_{gas}:** total gas phase energy (ELE + VDW + INT), **ΔG_{solv}:** sum of nonpolar and polar contributions to solvation, **$\Delta G_{Binding}$:** final estimated binding free energy calculated from the terms above (kcal/mol)

CONCLUSION

Rosehips are used to make tea, juice, jam, and marmalade. It has the highest concentration of vitamin C in all cultivated and natural plants. In addition to its diuretic, antimutagenic, and antibacterial properties, it is used to treat various diseases (rheumatic diseases, gout, stomach ulcers, sciatica, gallstone formation, biliary tract diseases, and colds). For this reason, the phytochemical analyses (total phenol, total flavonoid, GC-MS/MS, and HPLC) and the bioactivities (antioxidant activities) of the methanol extracts of the fruits of RC and RP were investigated in this study. According to the GC-MS/MS analysis results, oleic acid methyl ester, linoleic acid methyl ester, and palmitic acid methyl ester were obtained in high amounts from RP and RC. The HPLC analysis revealed that ascorbic acid, gallic acid, protocatechuic acid, and trans-ferulic acid were present in high amounts in RP, whereas RC contained high levels of ascorbic and gallic acid. The total phenolic content was higher in the fruit extract of RP, likely due to its greater phenolic compound content. The total phenolic content was higher in the fruit extract of RP. This could be due to the fact that it contains more phenolic compounds. $DPPH^{\cdot}$ scavenging activity was strongly affected by ascorbic acid in both rosehip fruit extracts. The fruit extract of RC had a higher effect than ascorbic acid in terms of reducing power. It was observed that RP and RC extracts denatured BSA in their anti-inflammatory effect. Of these two extracts, the RP extract was more denatured than the standard, while the RC extract was less denatured than the standard. RC extract also showed a higher inhibition effect than the standard in XO inhibition. In addition, the interaction of ascorbic acid and gallic acid with XO was investigated by molecular docking, and it was found that the complex formed by molecular dynamics simulation was stable at 100 ns. In MM/PBSA analysis, the binding energy of gallic acid was found to be higher than that of ascorbic acid. This result is directly proportional to the docking results. In other words, while the binding affinity of gallic acid is high in docking, the binding energy of gallic acid is high in MM/PBSA analysis. It was found that RP from both rose hips can be used as a dietary supplement and medicine due to their high phenolic compound content and high

antioxidant effect. According to these results, both types of rosehips showed a high antioxidant effect. It is assumed that this high effect is due to the phenolic compounds and fatty acid esters they contain. It is, therefore, important to use these plants in areas such as nutrition and pharmacology.

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Contribution Rate Statement Summary of Researchers

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

Conflict of Interest

The authors declare that no financial conflicts or interpersonal relationships known to them could have influenced the work published in this publication.

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Yeni Tanımlanan Bir Tür Olan *Aubrieta alshehbazii* Dönmez, Uğurlu ve M.A. Koch' un Antikarsinojenik Etkilerinin, Antioksidan Özelliklerinin ve Fitokimyasal Bileşenlerinin Tespiti

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ÖZET

Aubrieta alshehbazii, Elşahbaz obrizyası olarak bilinen endemik bir türdür. Tanımlanması yeni yapıldığından hakkında pek çalışma bulunmayan bu türün, antioksidatif, antikanserojenik etkileri ile fenolik madde içeriğinin belirlenmesi amacıyla çalışma gerçekleştirilmiştir. Antioksidatif özelliğinin belirlenmesi için DPPH radikal savıcı etkisi, total antioksidan ve oksidan kapasitesi ölçülmüştür. Antikanserojenik etki kapsamında sağlıklı HGF hücreleri ve akciğer kanser (A549) hücreleri kullanılmıştır. Bitkinin içerdiği fenolik maddeler ise LC-ESI-MS/MS ile belirlenmiştir. Analizler sonucunda bitkinin 0.1 µM ve 0.5 µM konsantrasyonda sentetik antioksidan olan BHT den istatistiksel anlamlılıkta (sırasıyla p= 0.008 ve p=0.016)) yüksek bir DPPH radikali savıcı etkiye sahip bulunmuştur. Türün total antioksidan kapasitesi standart antioksidanlardan düşüktür. Düşük konsantrasyonlarda ekstraktın A549 hücreleri üzeirne etkileri görülmezken, yüksek konsantrasyonlarda hem HGF hücreleri ve hem de A549 hücreleri üzerine sitotoksik etki gösterdiği belirlenmiştir. Fenolik içerik analizleri sonucunda yapısında en çok bulunan bileşenler, fumarik asit, kafeik asit, kuersetin, kinik asit, rosmarinik asit ve klorojenik asittir. Sonuç olarak tür radikal savıcı etkisi ile ön plana çıkmaktadır. Bu etkinin hangi bileşenden kaynaklandığının belirlenmesine yönelik çalışmalar yapılması önerilmektedir.

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Anahtar Kelimeler
Aubrieta alshehbazii
DPPH
Sitotoksik
Total antioksidan kapasite
LC-ESI-MS/MS

Determination of the Anticarcinogenic Effects, Antioxidative Features and Phytochemical Components of a Newly Identified Species *Aubrieta alshehbazii* Dönmez, Uğurlu & M.A. Koch

ABSTRACT

Aubrieta alshehbazii is an endemic species known as Elşahbaz Obrizyası. A study was conducted to determine the antioxidative, anticarcinogenic effects and phenolic substance content of this species, which is not well-studied due to its new definition. DPPH radical scavenging effect, total antioxidant and oxidant capacity were measured to determine its antioxidant properties. Healthy HGF cells and lung cancer (A549) cells were used within the scope of the anticarcinogenic effect. Phenolic substances contained in the plant were determined by LC-ESI-MS/MS. As a result of the analysis, it was found that the plant had a statistically significant (p=0.008 and p=0.016) higher DPPH radical scavenging effect than the synthetic antioxidant BHT at 0.1 µM and 0.5 µM concentrations. The total antioxidant capacity of the species is lower than standard antioxidants. While no effects of the extract on A549 cells were

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observed at low concentrations, it was determined that it had a cytotoxic effect on both HGF cells and A549 cells at high concentrations. As a result of the phenolic content analysis, the most abundant components in its structure are fumaric acid, caffeic acid, quercetin, quinic acid, rosmarinic acid and chlorogenic acid. As a result, the species stands out with its radical scavenging effect. It is recommended that studies be conducted to determine which component is responsible for this effect.

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GİRİŞ

Brassicaceae, 325 cins ve 3740 türün oluşturduğu bir familyadır. Güneybatı Asya'da, özellikle Türkiye ve İran-Turan bölgesinde oldukça fazla tür yetişmektedir (Al-Shehbaz ve ark., 2007; Karl & Koch 2013). Türkiye, Aubrieta ve Brassicaceae cinsi başta olmak üzere tür zenginliği sahip bir ülkedir. Türkiye 600'den fazla tür (bunlardan 226 sı endemik) ile Brassicaceae çeşitliliği açısından en zengin ülkeler arasındadır (Mutlu & Karakuş 2015; Yüzbaşıoğlu ve ark., 2015).

Aubrieta alshehbazii, Orta Türkiye'nin kayalık bozkırlarından toplanan yeni bir tür olarak tanımlanmaktadır. *Aubrieta alshehbazii* (*A. alshehbazii*) IUCN'nin CR tehdit kategorisine atanmıştır. Mart-Nisan aylarında çiçek açan, Mayıs-Haziran aylarında olgun meyve veren bir türdür. *A. alshehbazii* kendisine en yakın tür olan *A. pinardii* aynı bölgede yetişmektedir. Ancak *A. alshehbazii* sadece kaya üzerinde yetişirken *A. pınarii* hem toprakta hem de kaya yarıklarında bulunur. *A. alshehbazii*, 1000-1300 metre yükseklikte yetişmektedir. Bitkiler çoğunlukla kaya yarıklarıyla yetişmektedir dolayısıyla hayvanlar tarafından otlanmazlar. Ancak yakınlarda bulunan mermer ocağı türün yayılış alanı nispeten dar (yaklaşık 10 km²) olduğundan, bu durum yakın gelecekte ciddi ve büyük bir yok olma tehdidine neden olabilir (Al-Shehbaz ve ark., 2007; Koch & Kiefer 2006; Dönmez ve ark., 2017).

A. alshehbazii bitkisi morfolojik olarak incelendiğinde; 4-6 cm çiçekli saplar tüylü kaplı, 4-5 yapraklı gövde birbirine benzer, sapsız, hafifçe birbirine kenetlenmiş durumdadır. Sepal mızraksı, 7-9 × 1-2,2 mm, tüylü, dışı menekşe, içi tüysüz, iç sepal keseli, kenarlar zarsı, apeks geniş, kübik şekildedir. Petaller menekşe, 14-16 × 6-8 mm, obovat bir uzuv ve 7-10 mm bir pençe olarak farklılaşmıştır. Filamentler dar kanatlı, alt yarıda beyaz, tepeye doğru menekşedir. Meyve geniş ölçüde doğrusal, 15-19 × 3.5-4.5 mm (stil hariç), hafif kavisli, çoğunlukla düz yapıdadır. Septum; kısa saplı, tek tip tüylü, olgun valfler hafif ağsı damarlı; stil 6-8 mm dir (Al-Shehbaz ve ark., 2006; Al-Shehbaz ve ark., 2007; Dönmez ve ark., 2017).

Türkiye'de *A. alshehbazii*, morfolojik olarak *A. pinardii*'ye benzer, ancak eliptik ila obovat ve bütün yapraklar, daha kısa çiçeklenme ve sapsız yapraklar ile ayırt edilir. Cinsin birçok türü, yaprak kenarı boyunca 1-3 çift diş ile karakterize edilir. Buna göre, *A. alshehbazii*'nin tüm yaprak kenarı, *A. vulcanica* Hayek & Siehe ve *A. ekimii* gibi diğer birkaç Aubrieta türüyle birlikte yeni tür için istisnai ve çok karakteristik bir özelliktir. Ayrıca *A. alshehbazii* sapsız yapraklara sahiptir ve yaprakların tabanı gövdeye hafifçe kenetlenir. *A. alshehbazii*'nin gövdeleri zikzak görünümündeyken, diğer ilgili türler düz gövdelerle karakterizedir (Dönmez ve ark., 2017; Ancev & Goranova, 2009). Afyon ili Çay ilçesi Karakuş Dağı, Aydoğmuş-Armutlu köyleri arası, 38°23'11"K, 030°46'55"D lokasyonda 1100 m yükseklikte yetişen bir türdür (Dönmez ve ark., 2017). Bu çalışmada *A. alshehbazii* nın sulu ekstraktının, total fenolik madde miktarı, TAS, TOS ve OSI düzeyleri incelenmiştir. Ayrıca hücre kültürü ortamında belirlenen sitotoksisite düzeyleri ile bitkinin içerisinde bulunan fenolik maddelerin kalitatif/kantitatif analizi LC-ESI-MS/MS ile belirlenerek biyolojik aktivitelere sahip olma potansiyelleri değerlendirilmiştir.

MATERYAL ve METOD

Yapılan çalışmada, kullanılan bitki ekstraktlarının hazırlanması, ekstraktların içerik ve aktivite analizleri gerçekleştirilmiştir. Ekstraktların, ağız içi epitel hücrelerinden elde edilen primer bir hücre hattı olan insan gingival fibroblast (HGF) hücreleri ve küçük hücreli olmayan akciğer kanseri (A549) hücrelerine uygulanması ve laboratuvar analizlerine yer verilmiştir.

Bitki Materyali ve Ekstraksiyonu

A. alshehbazii bitkisi örnekleri 2022 yılı temmuz ayında Afyonkarahisar ili, Çay ilçesi, Karakuş Dağı (38° 35' 30" Kuzey, 31° 1' 43" Doğu) çevresinden toplanmış ve Dr. Mustafa Kargıoğlu tarafından teşhis edilmiştir. *A. alshehbazii* saf suyla yıkanıp toprak kalıntılarından arındırıldıktan sonra kurutulmuş ve blender (Waring 32BL80, Connecticut, USA) aracılığıyla öğütülerek toz haline getirilmiştir. 25 gram alınan toz formundaki bitkiye 250 mL (1:10 w/v oranında) deiyonize su eklendi. Sonra ultrasonik su banyosunda 60°C'de 2 saat tutuldu. Manyetik karıştırıcıda 700 rpm'de 60°C'de iki saat bekletildikten sonra, oda sıcaklığında 24 saat karanlık bir ortamda bekletildi. Elde edilen sıvı ekstrakt, süzgeç kâğıdı (Whatman, Grade 589/1) ve nuche erleni kullanılarak süzülerek biriktirildi. Bu yöntemle elde edilen ekstraktın çözücüsü, vakum altında rotary evaporatör ((Heidolph, 562-00000-00-0, Germany) ile uzaklaştırıldı.

DPPH Radikali Süpürücü Aktivite ile Antioksidatif Özelliklerin Belirlenmesi

Ekstraktın DPPH radikal süpürücü aktivitesi belirlemek amacıyla metanol içerisinde 10 µM DPPH çözeltisi hazırlandı. Bu stok standart kullanılarak seri dilüsyonla (0.5-10 µM aralığında) DPPH standartları hazırlandı. Çalışmada kullanılan numunelerin DPPH inhibisyon düzeylerini karşılaştırabilmek amacıyla BHT kullanıldı. Numuneler ve BHT nın farklı konsantrasyonda çözeltileri hazırlandı. 96 kuyucuklu mikrowellde kuyucuklara standart DPPH dan 200 µL hacminde ilave edildi. Kontrol olarak DPPH çözeltisi, blank olarak metanol kullanıldı. Numune kuyucuklarına ise 100 µL hacminde belirlenen konsantrasyonlardaki numuneler ilave edildi. 100 µL 10 µM konsantrasyonlu DPPH çözeltisi eklenerek 30 dakika 37 ^{0}C'de inkübasyona bırakıldı. İnkübasyon sonunda 540 nm'de (BioTek, ELx800) her bir kuyucuktaki numune ve standartların absorbans değeri ölçüldü. DPPH'a ait kalibrasyon eğrisi yardımıyla önce her bir kuyucukta bulunan indirgenmiş DPPH miktarları tespit edildi. Başlangıçta numunelerin üzerine ilave edilen 10 µM DPPH ne kadarının inhibe edildiği % olarak belirlendi (Hazman ve ark., 2021).

Ekstraktın oksidatif strese olası etkilerini belirleyebilmek amacıyla total antioksidan kapasite (TAC) ve total antioksidan kapasite (TOC) seviyeleri ticari kitler (Rell Assay, Gaziantep, Türkiye) kullanılarak analiz edildi. Numunelere ait TOC ve TAC düzeyleri oranlanarak deney gruplarına ait oksidatif stres indeksi (OSI) değerleri (OSI=TOC/TAC) belirlendi (Erel 2004; Erel 2005).

Sitotoksisite ve Antikanserojenik Etkinliğin Belirlenmesi

Çalışmada kullanılan ekstraktın sitotoksik etkileri sağlıklı bir hücre hattı HGF (insan gingival hücreleri) ve A549 (adenokarsinomik insan alveolar bazal epitel hücreleri) kullanılarak 3-[4,5-dimetilthiazol-2-yl]-2,5-difeniltetrazolium bromid (MTT) analizi ile belirlendi. Çalışmadaki hücreler yüksek glukoz Dulbecco değiştirilmiş ortam kulanılarak hazırlanan besiyerinde çoğaltıldı. Hücrelere yapılan uygulamalar flow laminer kabin içerisinde, inkübasyonlar ise uygun şartlar altında CO_2 inkübatöründe gerçekleştirildi. Hücreler 96 kuyucuklu mikroplate her bir wellde 200 µL besiyeri ve 10^4 tane hücre olacak şekilde ekildi. Hücrelerin tutunduğundan emin olduktan sonra, ekstraktlara ait önceden besiyerinde hazırlanmış stok çözeltileri yedi farklı (5, 25, 50, 100, 200, 400, 800 µg mL^{-1}) konsantrasyonda su ile çözündürülerek hücrelere uygun hacimde (50 µL) uygulandı. Kontrol kuyucuklarına ise 50 µL hacminde besiyeri eklendi. 24 saat inkübe edildi. İnkübasyon sonunda her bir kuyucuğa fosfat tampon çözeltisi içinde 5 mg mL^{-1} konsantrasyonda hazırlanmış olan MTT çözeltisi 25 µL hacminde eklendi. Hücreler 3 saat inkübe edildi. İnkübasyon sonunda formozan kristallerinin çözülmesi sağlandı. Her bir kuyucukta bulunan numunenin absorbansı 540 nm'de, ELISA mikroplate okuyucu (Biotek, ELx800) kullarak belirlendi. Herhangi bir aktif madde eklenmemiş olan (kontrol grubu) kuyucuklardaki ortalama hücre canlılığı oranı %100 kabul edilerek, her bir dozun hücre canlılığına etkisi (%) aşağıda belirtilen formül kullanılarak belirlendi (Görmez ve ark., 2024; Günay ve ark., 2016).

$$\text{Hücre Canlılığı (\%)} = [(100 \text{x} \text{Absorbans}_{\text{örnek}})/ (\text{Absorbans}_{\text{kontrol}})]$$

Total Fenolik Madde Miktarının Belirlenmesi

Ekstrakt içeriğinde bulunan toplam fenolik madde miktarı Folin-Ciocalteu's fenol yöntemi (Slinkard & Singleton, 1977) modifiye edilerek belirlendi. Analizlerde standart olarak 100-1000 µg mL^{-1} aralığında beş farklı konsantrasyonda gallik asit çözeltisi kullanıldı. Bitki ekstraktı ve standartların hazırlanan çözeltilerinden 500 µL hacminde 15 mL'lik tüplere üç tekrarlı olacak şekilde eklendi. Üzerine 700 µL deiyonize su ve 250 µL Folin-Ciocalteu reaktifi ilave edildi. Tüpler vortekle karıştırıldı. 5 dakika sonra her bir tüpe %2'lik Na_2CO_3 çözeltisinden 200 µL ilave edilerek inkübasyona bırakıldı. Numunelerin 760 nm'deki absorbansları spektrofotometrede (Shimadzu UV-VİS 1700) ölçüldü. Analizleri sonucunda elde edilen absorbanslar ve gallik asit standart eğrisinden

elde edilen doğru denklemi kullanılarak numunelerin 1 mg'ında bulunan toplam fenolik asit miktarı gallik asit eşdeğeri (µg GAE mg ekstrakt^{-1}) şeklinde hesaplandı (Balkir ve ark., 2023).

Ekstraktlarda Bulunan Bileşenlerin ve Konsantrasyonlarının Belirlenmesi

Ekstrakt bulunan bileşenlerin kalitatif ve kantitatif tayini için kullanılan LC-MS/MS cihazında ters faz UHPLC sistemi kullanılmıştır. Bu sistem bir oto örnekleyici (SIL-30AC), bir kolon fırını (CTO-10ASvp), gradient pompa sistemi (LC-30AD) ve bir degazer (DGU- 20A3R) bileşenlerinden oluşmuştur. Kromatografik ayrım 40°C'ye ayarlı kolon kullanılarak yapılmıştır. Mobil faz akış hızı 0.5mL min^{-1}, enjeksiyon hacmi sırasıyla 5 µL olarak belirlenmiştir. Kullanılan LC-MS/MS sisteminin kütle spektrometre dedeksiyonu için hem pozitif hem de negatif modda çalışan bir elektrosprey iyonlaşma kaynağı ile donanmış Shimadzu LCMS-8040 model sıralı kütle spektrometresi kullanılmıştır (Yılmaz 2020).

LC-ESI-MS/MS verileri LabSolutions yazılımı (Shimadzu) ile alınmış ve işlenmiştir. Fitokimyasalların miktar tayini için multiple reaction monitoring (MRM) modu kullanılmıştır. MRM metodu, belirli ana iyon-parçalanma iyonu geçişlerinin taranmasına dayalı olarak fitokimyasalların seçici olarak tespit edilip miktarsal tayininin yapılması için optimize edilmiştir. Uygulanan MS de; kurutucu gaz (N_2) akışı: 15 L/dk, nebülizer gaz (N_2) akışı: 3 L/dk, DL sıcaklığı: 250°C, heat block sıcaklığı: 400°C ve arayüz sıcaklığı: 350°C olarak belirlenmiştir. Ekstraktlar içerisinde miktarları belirlenen fenolik asit türlerinin miktarları mg analit g ekstrakt^{-1} şeklinde ifade edilmiştir (Yılmaz 2020).

İstatistiksel Analiz

Çalışmada kullanılan ekstraktlar üç tekrarlı (n=3) şekilde hazırlanmış, ölçülen sonuçlar ortalama ± standart sapma (mean ± SD) şeklinde ifade edilmiştir. Bu çalışmadaki verilerin istatistiki analizinde SPSS 20 paket programı kullanılmıştır. Gruplar arasındaki farklılıklar tek yönlü varyans analizi (one-way ANOVA) ile belirlenmiştir. Hangi gruplar arasında farklılığın oluştuğu ise Duncan çoklu aralık testine göre $p<0.05$ önemlilik değerinde belirlenmiştir.

BULGULAR ve TARTIŞMA

Ekstraksiyon, bitkilerden elde edilen doğal antioksidanları incelemek için ilk ve önemli adımdır. Ekstraksiyon solventinin türü ve konsantrasyonu, ekstraksiyon sıcaklığı, ekstraksiyon süresi ve ekstraksiyon pH'ı gibi birçok faktör ekstraksiyon verimliliğinde önemli rol oynar. Bunlar arasında solvent en etkili faktörlerden biridir. Gıdalardan ve tıbbi bitkilerden antioksidanların ekstraksiyonunda çok sayıda çözücü kullanılmıştır. Çözücülerin seçimi, ekstrakte edilecek antioksidan bileşiklerin kimyasal yapısına ve polaritesine dayanmaktadır. Fenoliklerin, flavanoidlerin ve antosiyaninlerin çoğu suda çözünebilen antioksidanlardır. Su, etanol, metanol, propanol, aseton ve bunların sulu karışımları gibi polar ve orta polar çözücüler ekstraksiyon için yaygın olarak kullanılmaktadır (Xu ve ark., 2017).

Serbest radikal reaksiyonu, özellikle sağlık ve gıda endüstrilerinde sorunların en önemli nedenlerinden biridir. Kanser gibi pek çok hastalık ve gıdaların oksidatif bozulmasının dahil olumsuz sonuca neden olur. Sentetik ve doğal antioksidanlar, oksidatif hasarları en aza indirmek için tıp ve gıda endüstrilerinde rutin olarak kullanılmaktadır. Ancak, çalışmalar sentetik antioksidanların genellikle olumsuz etkileri ve potansiyel toksisitelerle ilişkili olduğunu göstermiştir. Bu nedenle, bitkilerden elde edilen doğal antioksidan alternatifleri önerilir (Molole ve ark., 2022). Antioksidan-oksidan reaksiyonunun mekanizmasında hidrojen atomu transferi ve tek elektron transferi yöntemi olarak ikiye ayrılır. Hidrojen atomu transferi yöntemi, bir antioksidanın hidrojen bağışı yoluyla serbest radikalleri yakalama kapasitesini ölçmektedir. Tek elektron transferi yöntemi, bir antioksidan bileşiğin bir radikal türe karşı tek elektron transferini azaltma yeteneğine dayanır. DPPH ve TEAC hem hidrojen hem de tek elektron transferini kullanan yöntemler olarak kabul edilir, çünkü bu durumlarda radikaller, elektron indirgeme veya hidrojen transferini içeren radikal giderme yoluyla temizlenebilir (Prior ve ark., 2005). *A. alshehbazii* sulu ekstratının ve sentetik antioksidan olan BHT nin DPPH serbest radikalini yüzde inhibisyonları Şekil 1 ve Çizelge 1 de gösterilmiştir. Ekstraktın radikali giderici etkisinin düşük konsantrasyonlarda BHT den istatistiksel anlamlılıkta ($p<0.05$) oldukça yüksek olduğu görülmektedir. Yüksek konsantrasyonlarda hem ekstraktın hem de BHT'in oldukça yüksek DPPH radikali savıcı etki gösterdiği belirlenmiştir. Ayrıca radikal savıcı etkinin konsantrasyonla birlikte orantılı olarak arttığı da görülmektedir.

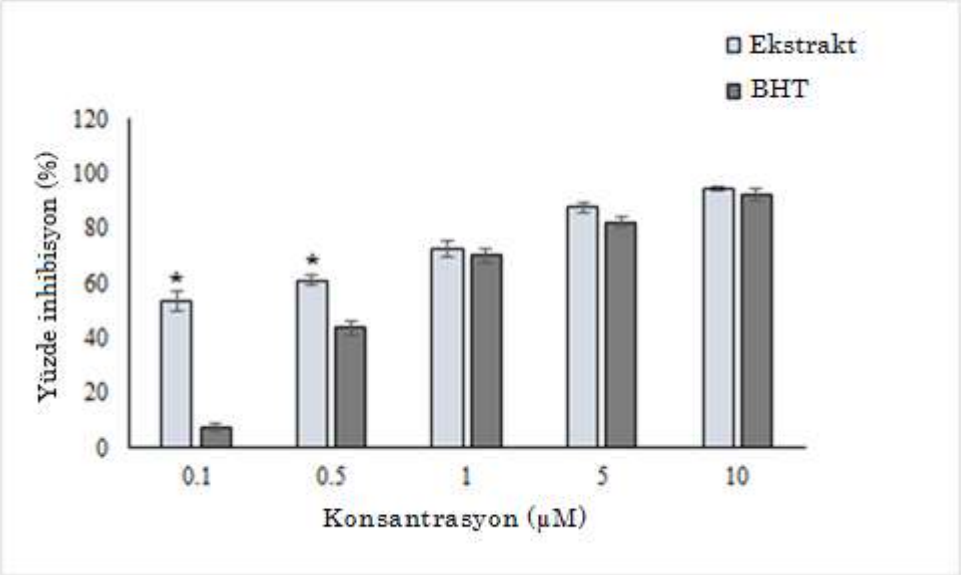
Doğal ürünlerden, elde edilen ekstraktların antioksidan kapasitelerini daha fazla değerlendirmek için Trolox eşdeğerli antioksidan kapasite, demir iyonu azaltıcı antioksidan güç, oksijen radikal absorbans kapasitesi gibi farklı değerlendirme analizleri uygulanmaktadır. Yapılan çalışmada, Trolox eşdeğer antioksidan kapasitesi analizi, ABTS radikalini temizlemeye yönelik antioksidan yeteneğini değerlendirmek için uygulanmıştır.

Çizelge 1. *Aubrieta alshehbazii* sulu ekstratı ile sentetik antioksidan BHT nin farklı konsantrasyonlarda DPPH radikalini yüzde inhibisyonu

Table 1. Percentage inhibition of DPPH radical by synthetic antioxidant BHT at different concentrations with aqueous extract of Aubrieta alshehbazii

Konsantrasyon (µM)	AAW	BHT	P Değeri
0.1	53.58±3.45[a]	7.50±1.42[b]	p=0.008
0.5	61.13±2.06[a]	43.76±2.76[b]	p=0.016
1	72.55±2.74[a]	70.14±2.49[a]	p=0.589
5	87.74±1.97[a]	82.33±1.74[a]	p=0.734
10	94.54±0.87[a]	92.33±2.36[a]	P=0.602

AAW; *Aubrieta alshehbazii* sulu ekstraktı BHT: Bütillenmiş hidroksi toluen. Veriler, ortalama±standart sapma olarak sunulmuştur. (n=3), a, b: Aynı satırdaki farklı üslere sahip ortalamalar arasındaki fark istatistiksel olarak anlamlıdır.



Şekil 1. *Aubrieta alshehbazii* sulu ekstratı ve BHT nin DPPH serbest radikalini yüzde inhibisyonu

Figure 1. Percentage inhibition of DPPH free radical by Aubrieta alshehbazii aqueous extract and BHT

Çizelge 2 de türün total antioksidan kapasitesi, oksidan kapasitesi ve oksidatif stres indeksi değerleri verilmiştir. *A. alshehbazii*'nin TAC ve TOC düzeylerinin standart maddelerden (Vitamin C ve H_2O_2) istatistiksel anlamlılıkta ($p<0.05$) düşük olduğu görülmektedir. OSI değeri ise 6.94±1.84 Arbitary Unit olarak hesaplanmıştır. ABTS radikalinin absorbansındaki azalma spektrofotometrik olarak izlenebilir. Test edilen absorbans farkı, antioksidan konsantrasyonlarına göre grafiğe geçirilir. Antioksidan kapasitesi Trolox eşdeğerleri olarak ifade edilir. ABTS antioksidanlarla hızla reaksiyona girebildiği için test, hızlılık ve basitlik avantajlarına sahiptir. Ek olarak ABTS iyonik güçten etkilenmez ve hem organik hem de sulu solventlerde çözülebilir olduğundan hem hidrofilik hem de lipofilik antioksidan aktiviteleri tespit etmek için birden fazla ortamda uygulanabilir (Awika ve ark. 2003).

Yapılan bir çalışmada kuzeybatı Anadolu endemiği *Aubrieta ekimii*'nin, toplam antioksidan aktivitesi, flavonoid ve toplam fenolik içeriği belirlenmiştir. Bitkinin özütlerinin toplam antioksidan aktivitesi 1298.51 CRE (µM bakır indirgemesine eşdeğer), ve toplam fenolik içeriği 28.24 mg mg GAE g özüt^{-1} olarak belirlenmiştir. Bir başka çalışmada ise *Aubrieta deltoidea*'nın çeşitli özütlerinin (etanol, aseton ve su) fenolik bileşikleri ve antioksidan kapasiteleri incelenmiştir. Ayrıca toplam fenolik, flavonoid ve tanen içerikleri de belirlenmiştir. *A. deltoidea* özütlerinin toplam fenolik içerilerinin 15.21±2.29 ile 58.08±8.10 mg GAE g^{-1} arasında değiştiğini belirlenmiştir (Kaska ve ark., 2017). Sunulan çalışmada literatürle uyumlu olarak total fenolik içerik 55.97±9.58 mg GAE g^{-1} bulunmuştur.

Pek çok çalışma, antioksidan aktivitelerinin toplam fenolik içerikleriyle yüksek oranda ilişkili olduğunu göstermiştir. Antioksidanlar, oksidatif zincir reaksiyonlarının başlatılmasını ve yayılmasını engelleyerek lipitlerin ve diğer moleküllerin oksidasyonunu geciktirebilen veya engelleyebilen bileşiklerdir. Fenolik bileşiklerin antioksidan aktivitesi esas olarak, serbest radikalleri nötralize etme, tekli ve üçlü oksijeni söndürme veya peroksitleri parçalamada önemli bir rol oynayabilen redoks özelliklerinden kaynaklanmaktadır (Chahardehi ve ark., 2009; Eruygur ve ark., 2024)

Çizelge 2. *Aubrieta alshehbazii* sulu ekstratı ile standart maddelerin total antioksidan statüsü, total oksidan statüsü, oksidatif stress indeksi ve total fenolik içeriği

Table 2. Total antioxidant status, total oxidant status, oxidative stress index and total phenolic content of standard substances with Aubrieta alshehbazii aqueous extract

	AAW	Standartlar
TAC (mmol Trolox Eşdeğeri L^{-1})	1.214±0.34[a]	2.198±0.33[b]
TOC (µmol H_2O_2 Eşdeğeri L^{-1})	7.827±0.88[a]	10.31±1.02[b]
OSI (Arbitary Unit)	6.94±1.84[a]	4.72±0.86[b]
Toplam fenolik içerik (mg GAE g ekstrakt^{-1})	55.97±9.58[a]	923.14±59.15[b]

TAS; Total antioksidan statüsü, TOS; Total oksidan statüsü, OSI; Oksidatif stress indeksi, AAW; *Aubrieta alshehbazii* sulu ekstraktı, GAE; Gallik asit eşdeğeri, TAS için Vitamin C, TOS için H_2O_2, Total fenolik asit içeriği için kafeik asit standart olarak kullanılmıştır. Veriler ortalama ± standart sapma olarak sunulmuştur. (n=3), a, b: Aynı satırdaki farklı üslere sahip ortalamalar arasındaki fark istatistiksel olarak anlamlıdır.

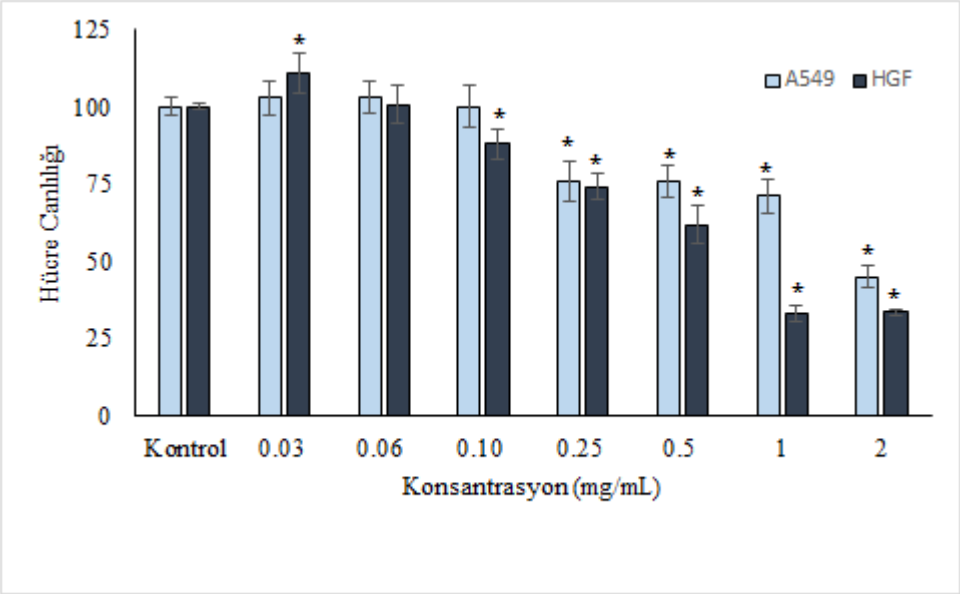
Kanser, insan vücudundaki hücrelerin sürekli olarak çoğalması ve kontrol edilememesi veya durdurulamamasıyla karakterize edilir. Sonucunda metastatik olma potansiyeline sahip kötü huylu hücrelerden oluşan tümörler oluşturmaktadır. Mevcut tedaviler arasında kemoterapi, radyoterapi ve kimyasal kökenli ilaçlar yer alıyor. Kemoterapi gibi tedaviler hastaları çok fazla zorlayabilir ve sağlıklarına daha fazla zarar verebilir. Bu nedenle kansere karşı alternatif tedavilere odaklanılmaktadır (Greenwell & Rahman 2015). Kanser tedavisi veya adjuvan tedavi için daha düşük toksisiteye ve yan etkilere sahip ve etkili bir ilaç türünün araştırılması önemlidir. Tümör oluşumu ve gelişimi birden fazla yolu, birden fazla bağlantıyı ve birden fazla hedefi içerir. Çeşitli bağlantılar arasındaki etkileşimlerin karmaşıklığı, sınırlı terapötik etkiler ve daha büyük yan etkiler gibi klinik yanıtlara yol açabilir. Son zamanlarda, kanser tedavisinde yeni bir terapötik strateji olarak doğal bileşiklerin uygulanmasına yoğun ilgi gösterilmektedir (Reyes-Farias & Carrasco-Pozo, 2019). Bitki materyalinden ekstrakte edilen metabolitler, kanser hücrelerinde apoptozu indüklemek için kullanılır. Bununla birlikte bitkiler malignite dönüşümünün ve kanser gelişiminin önlenmesinde de kritik bir rol oynar. Bu tür bitkisel ürünlerin etkilerinin anlaşılmasının artmasıyla birlikte farklı kanser türlerine karşı etkileri de tespit edilmiştir. Ancak bitkisel bileşikler ilaç olarak kabul edildiğinde, bunların güvenlik ve yan etki açısından herhangi bir sorunu olmadığı yanılgısına düşülmektedir. Sağlığa zararlı yüzlerce bitki türü bulunmaktadır. Aynı şekilde, dost bitkilerde de sitotoksisiteye neden olan birçok bileşik vardır. Testlere dayanarak antikanser bitkilerinin bile sitotoksik etkilere yol açtığı kanıtlanmıştır (Khan ve ark., 2019; Ghorani-Azam ve ark., 2018; Güçlü ve ark., 2022; Pal & Shukla, 2003).

A. alshehbazii türü ve Aubrieta cinsiyle ilgili antikanserojen aktivite çalışmalarına literatürde rastlanmamıştır. *A. alshehbazii* gibi Brassicaceae ailesine ait bazı bitkilerin antikanserojenik etkilerinin incelendiği bir çalışmada, ailenin sulforafane, erusin, indole-3-carcinol içerikleri nedeniyle prostat, meme, yumurtalık ve kolon kanserlerine karşı etkili olduğu belirlenmiştir (Mandrich & Caputo,2020).

Brassicaceae familyasına ait yenilebilir bir bitki olan *Brassica incana* Ten. ile yapılan bir çalışmada, *B. incana*'nın yapraklarından ve çiçekli tepelerinden elde edilen hidroalkolik özütlerin fenolik bileşimi ve antioksidan ve sitotoksik özellikleri incelenmiltir. Özütlerin insan kolorektal adenokarsinomu (CaCo-2) ve meme kanseri (MCF-7) hücre hatlarına karşı sitotoksisitesi, 3-(4,5-dimetiltiazol-2-il)-2,5-difeniltetrazolium bromür (MTT) testi ve laktik dehidrogenaz (LDH) salınımının belirlenmesi yoluyla değerlendirilmiştir. Ekstraktın Caco-2 hücrelerine karşı sitotoksik etkinlik gösterdiği, çiçekli üst ekstraktın en etkili olduğu belirtilmiştir (Miceli ve ark., 2020).

Sunulan çalışmadaki *Aubrieta alshehbazii* sulu ekstratının A549 ve HGF hücrelerindeki sitotoksisite düzeyleri Şekil 2 de gösterilmiştir. Yüksek doz (0.25-2 mg mL^{-1}) ekstre uygulamasının A549 kanser hücre hattında antikanserojenin etkileri görülmekle birlikte bu dozlarda HGF hücrelerinin viabilitesinde azalmada belirlenmiştir. 0.1 mg mL^{-1} ekstre uygulamasının A549 kanser hücre hattında etkili değilken, HGF hücrelerinin canlılığını azaltıcı etkisi bulunmuştur. 0.03 mg mL^{-1} ekstre uygulaması da A549 kanser hücre hattında etkili değilken, HGF hücrelerinin canlılığını artırıcı etkisi bulunmuştur.

*Aubrieta alshehbazii*nin fitokimyasal bileşimini belirlemek için LC-ESI-MS/MS analizi gerçekleştirildi. Bitki ve hastalıklar arasındaki ilişkiyi analiz etmeye yönelik araştırmalara katkı sağlayabileceğinden fenolik profile ilişkin çalışmalara ihtiyaç vardır. Fenolik içerik analizi ile bitkilerden yeni ürünlerin geliştirilmesi ve bitkilerin daha fazla kullanım alanı oluşabilir. Ayrıca bu analizler, biyolojik çeşitliliğinin korunmasına da katkı sağlayabilir. Çizelge 3 de *Aubrieta alshehbazii* sulu ekstraktının içerisinde bulunan LC-ESI-MS/MS ile belirlenen fitokimyasalların konsantrasyonları verilmiştir.



Şekil 2. *Aubrieta alshehbazii* sulu ekstratı'nın A549 ve HGF hücrelerindeki sitotoksisite düzeyleri
Figure 2. Cytotoxicity levels of Aubrieta alshehbazii sulu ekstratı in A549 and HGF cells

Çizelge 3. *Aubrieta alshehbazii* sulu ekstraktının içerisinde bulunan LC-ESI-MS/MS ile belirlenen fitokimyasalların konsantrasyonları
Table 3. Concentrations of phytochemicals determined by LC-ESI-MS/MS in the aqueous extract of Aubrieta alshehbazii

	Bitki Bileşenleri	RT[a]	M.I. (m/z)[b]	F.I. (m/z)[c]	r^{2d}	*LOD/LOQ* (µg/L)[f]	Konsantrasyon (mg analyte g ekstrakt^{-1})
1.	Kinik asit	3.0	190.8	93.0	0.996	25.7/33.3	0.248
2.	Fumarik asit	3.9	115.2	40.9	0.995	135.7/167.9	4.566
3.	Akonitik asit	4.0	172.8	129.0	0.971	16.4/31.4	N.D.
4.	Gallik asit	4.4	168.8	79.0	0.999	13.2/17.0	N.D.
5.	Epigallokateşin	6.7	304.8	219.0	0.998	237.5/265.9	N.D.
6.	Protokatekuik asit	6.8	152.8	108.0	0.957	21.9/38.6	0.116
7.	Kateşin	7.4	288.8	203.1	0.999	55.0/78.0	N.D.
8.	Gentisik asit	8.3	152.8	109.0	0.997	18.5/28.2	N.D.
9.	Klorojenik asit	8.4	353.0	85.0	0.995	13.1/17.6	0.237
10.	Protokateuik aldehit	8.5	137.2	92.0	0.996	15.4/22.2	0.042
11.	Tanik asit	9.2	182.8	78.0	0.999	15.3/22.7	N.D.
12.	Epigallokateşin gallat	9.4	457.0	305.1	0.999	61.0/86.0	N.D.
13.	Sinarin	9.8	515.0	191.0	0.999	5.8/9.4	N.D.
14.	4-OH Benzoik asit	10.5	137,2	65.0	0.999	68.4/88.1	N.D.
15.	Epikateşin	11.6	289.0	203.0	0.996	139.6/161.6	N.D.
16.	Vanilik asit	11.8	166.8	108.0	0.999	141.9/164.9	N.D.
17.	Kafeik asit	12.1	179.0	134.0	0.999	7.7/9.5	1.656
18.	Şiringik asit	12.6	196.8	166.9	0.998	82.3/104.5	N.D.
19.	Vanilin	13.9	153.1	125.0	0.996	24.5/30.4	0.061
20.	Şiringik aldehit	14.6	181.0	151.1	0.999	19.7/28.0	N.D.
21.	Daidzin	15.2	417.1	199.0	0.996	7.0/9.5	N.D.
22.	Epikateşin gallat	15.5	441.0	289.0	0.997	19.5/28.5	N.D.
23.	Piceid	17.2	391.0	135/106.9	0.999	13.8/17.8	N.D.
24.	p-Kumarik asit	17.8	163.0	93.0	0.999	25.9/34.9	N.D.
25.	Ferulik asit-D3-ISh	18.8	196.2	152.1	N.A.	N.A.	N.A.
26.	Ferulik asit	18.8	192.8	149.0	0.999	11.8/15.6	N.D.
27.	Sinapik asit	18.9	222.8	193.0	0.999	65.2/82.3	N.D.
28.	Kumarin	20.9	146.9	103.1	0.999	214.2/247.3	N.D.

29. Salisilik asit	21.8	137.2	65.0	0.999	6.0/8.3	0.021
30. Siranozid	23.7	447.0	284.0	0.997	12.1/16.0	N.D.
31. Miquelianin	24.1	477.0	150.9	0.999	10.6/14.7	N.D.
32. Rutin-D3-IS	25.5	612.2	304.1	N.A.	N.A.	N.A.
33. Rutin	25.6	608.9	301.0	0.999	15.7/22.7	0.094
34. Izokersitrin	25.6	463.0	271.0	0.998	8.7/13.5	N.D.
35. Hesperidin	25.8	611.2	449.0	0.999	19.0/26.0	0.044
36. o-Kumarik asit	26.1	162.8	93.0	0.999	31.8/40.4	N.D.
37. Genistin	26.3	431.0	239.0	0.991	14.9/21.7	N.D.
38. Rosmarinik asit	26.6	359.0	197.0	0.999	16.2/21.2	0.239
39. Ellagik asit	27.6	301.0	284.0	0.999	56.9/71.0	N.D.
40. Kozmosiin	28.2	431.0	269.0	0.998	6.3/9.2	0.047
41. Kuersitrin	29.8	447.0	301.0	0.999	4.8/6.4	0.178
42. Astragalin	30.4	447.0	255.0	0.999	6.6/8.2	0.052
43. Nikotiflorin	30.6	592.9	255.0/284.0	0.999	11.9/16.7	0.168
44. Fisetin	30.6	285.0	163.0	0.999	10.1/12.7	N.D.
45. Daidzein	34.0	253.0	223.0	0.999	9.8/11.6	N.D.
46. Kuercetin-D3-IS	35.6	304.0	275.9	N.A.	N.A.	N.A.
47. Kuersetin	35.7	301.0	272.9	0.999	15.5/19.0	0.423
48. Naringenin	35.9	270.9	119.0	0.999	2.6/3.9	N.D.
49. Hesperetin	36.7	301.0	136.0/286.0	0.999	7.1/9.1	N.D.
50. Luteolin	36.7	284.8	151.0/175.0	0.999	2.6/4.1	0.002
51. Genistein	36.9	269.0	135.0	0.999	3.7/5.3	N.D.
52. Kaempferol	37.9	285.0	239.0	0.999	10.2/15.4	N.D.
53. Apigenin	38.2	268.8	151.0/149.0	0.998	1.3/2.0	0.005
54. Amentoflavon	39.7	537.0	417.0	0.992	2.8/5.1	N.D.
55. Krizin	40.5	252.8	145.0/119.0	0.999	1.5/2.8	N.D.
56. Akasetin	40.7	283.0	239.0	0.997	1.5/2.5	0.006

[a]R.T.: Tutma süresi, [b]MI (m z^{-1}): Standart analitlerin moleküler iyonları (m z^{-1} oranı), [c]FI (m/z): Parça iyonları r^{2}[d]: Belirleme katsayısı, [e]RSD: Bağıl standart sapma, [f]LOD/LOQ (µg L^{-1}): Tespit/miktar belirleme sınırı, N.A.; Uygulanamaz, N.D.; Tespit Edilmedi

Bitkinin sulu ekstraktının içerisinde en çok bulunan bileşenler sırasıyla; fumarik asit, kafeik asit, kuersetin, kinik asit, rosmarinik asit ve klorojenik asittir. Protokatekuik asit, protokatekuik aldehit, vanilin, salisilik asit, rutin, hesperidin, kozmosiin, kuersitrin, astragalin, nikotiflorin, luteolin, apigenin ve akasetin bitkide daha az konsantrasyonda bulunan diğer fitokimyasallardır. Kafeik asit, bitkilerde ikincil metabolit olarak üretilen bir polifenoldür. Kafeik asit ve türevlerinin antioksidan (Genaro-Mattos ve ark., 2015; Tosovic, 2017), ve antikanser aktivitelerinin (Genaro-Mattos ve ark., 2015) gösterildiği pek çok çalışma bulunmaktadır. Yapılan çalışmada kafeik asitin kontrol hücreleri olan sağlıklı insan bronş epiteli hücrelerinde canlılığını azaltmadığı, küçük hücreli olmayan akciğer kanseri H1229 hücrelerinin canlılığını azalttığı belirtilmektedir. H1299 hücrelerinde, kafeik asit ve sitostatik paklitaksel ile birlikte maruz kalma, hücre proliferasyonunu tek başına paklitakselden daha fazla inhibe etmiştir (Min ve ark., 2018). Yapılan bir başka çalışmada kafeik asit uygulanmasının bir küçük hücreli dışı akciğer kanseri hücre dizisi olan A549'da anti-apoptotik proteinler survivin ve Bcl-2'nin ekspresyonunu da arttırdığı belirtilmektedir (Lin ve ark., 2012). Kafeik asit açısından zengin gıdaların tüketiminin, karsinomide ana indükleyici olan nitro bileşiklerinin (nitrozaminler ve nitrozamidler) oluşumunu önleyerek karsinojeneze karşı koruyucu bir etkiye yol açtığını göstermiştir (Damasceno ve ark., 2017). Anti-karsinojenik etkisi esas olarak antioksidan ve pro-oksidan kapasiteleri ile ilişkilidir. Kimyasal yapısında serbest fenolik hidroksillerin varlığı, OH-bağ ayrışmasının entalpisini azaltmayı ve peroksil radikalleri için H atomlarının transfer hızını ve bunların fenil halkası (katekol) üzerindeki sayılarını ve konumlarını arttırmayı mümkün kılar. Ayrıca karbon zincirinde çift bağın varlığı (doymamış yan zincir 2,3 çift bağ) fenolik radikalin stabilitesini arttırır (Son ve ark., 2002). Yapısı dolayısıyla, serbest radikallerin yok edilmesini sağlayarak reaktif oksijen türleri üretimini önlemenin yanı sıra, çeşitli kanser türlerinde bulunan kanser hücrelerinin DNA oksidasyonunun indüklenmesini sağlar (Sidoryk ve ark., 2018; Li ve ark., 2000; Silva ve ark. 2014). Kuersetin de toksik olmayan ve tümör oluşumunun çeşitli yolları üzerinde çeşitli önleyici etkilere sahip olan flavonoid bir bileşiktir. Kuersetin'in antikanser mekanizması temel olarak kanser hücresi proliferasyonunu inhibe etmek, apoptozu indüklemek, kanser hücrelerinin otofajisini indüklemek, sinyal yollarını düzenlemek, istilasını ve metastazı inhibe etmek, kemoterapi duyarlılığını arttırmak ve ilaç direncini tersine çevirmek yoluyla gerçekleşir (Tang ve ark., 2020; Neamtu ve ark., 2022)

Yapılan bir çalışmada bu ailenin, mineraller, besinler ve fitokimyasallar (örneğin selenyum, folat ve lif) açısından zengin olduğu belirlenmiştir. Özellikle, keskin ve baharatlı tatlarından sorumlu olan glukozinolat bileşikler açısından zengindir. Ailenin kemopreventif potansiyelinin muhtemelen glukozinolatlar ve bunların ikincil metabolitlerinden (izotiyosiyanatlar gibi) kaynaklandığı gösterilmiştir (Mandrich & Caputo, 2020). *Aubrieta alshehbazii* sulu ekstraktının içerisinde de flavonoid glicosidler (rutin, hesperidin, ve astragalin gibi) bulunmaktadır.

SONUÇ ve ÖNERİLER

Aubrieta alshehbazii türünün temel biyolojik aktivite analizleri ile fenolik içeriğinin belirlendiği bu çalışma, diğer çalışmalara basamak olması adına önemlidir. Türün DPPH radikalini savıcı etkisinin sentetik antioksidanlardan çok daha yüksek olduğu belirlenmiştir. Yüksek konsantrasyonda bitki ekstraktının A549 kanserli hücreler üzerine bir etkiden söz edilse bile HGF hücrelerinin canlılığını azaltıcı etkisi olması dolayısıyla antikanserojenik etkisi net olarak ortaya konulamaz. Ancak sağlık hücrelerin viabilitisinin azalmasının sebepleri daha ileri çalışmalarla araştırılabilir. Bunun dışında tür içerisinde antioksidan ve antikanserojen etkileriyle bilinen fitokimyasalların varlığı da belirlenmiştir. Bundan sonraki çalışmalar, biyolojik aktivitelerin oluşmasına sebep olan mekanizmaların ve sorumlu bileşenlerin araştırılması yönünde olabilir.

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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The Effect of Seed Sowing Density on Growth Parameters in Six Different Microgreen

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ABSTRACT
Seed sowing density is a key parameter that directly affects plant growth and the final product quality in microgreens. Proper adjustment of sowing density ensures optimal growth conditions, enhancing yield and supporting healthy and vigorous plant development. In this study, broccoli, black radish, red beet, pea, sunflower, and bean seeds were used; three different sowing densities were determined, and the seeds were sown in containers measuring 16x9x7 cm. The study investigates the effects of different seed sowing densities on plant height, hypocotyl length, stem diameter, individual plant weight, leaf area, yield, dry matter content, and chlorophyll content in microgreens. Significant changes in plant growth parameters were observed as the sowing density increased. These findings highlight the necessity of carefully optimizing sowing density in microgreens production.

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Altı Farklı Mikroyeşillikte Ekim Yoğunluğunun Büyüme Parametreleri Üzerine Etkisi

ÖZET
Mikroyeşilliklerde tohum ekim sıklığı, bitki gelişimini ve nihai ürün kalitesini doğrudan etkileyen temel bir parametredir. Tohumların ekim sıklığının doğru ayarlanması, optimal büyüme koşullarını sağlayarak verimi artırıp bitkilerin sağlıklı ve güçlü gelişimini destekler. Bu çalışmada, brokoli, siyah turp, kırmızı pancar, bezelye, ayçiçeği ve fasulye tohumları kullanılmış; üç farklı ekim sıklığı belirlenmiş ve 16x9x7 cm boyutlarındaki kaplarda tohum ekimi gerçekleştirilmiştir. Çalışma, farklı tohum ekim sıklığının mikroyeşilliklerde bitki boyu, hipokotil boyu, gövde çapı, tek bitki ağırlığı, yaprak alanı, verim, kuru madde ve krolofil içeriği üzerine etkilerini ortaya koymaktadır. Tohum ekim sıklığı arttıkça bitki büyüme parametrelerinde önemli değişiklikler olduğu gözlemlenmiştir. Bu bulgular, mikroyeşillik üretiminde tohum ekim sıklığının dikkatli bir şekilde optimize edilmesi gerektiğini vurgulamaktadır.

Bitki Fizyolojisi

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Anahtar Kelimeler
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Tohum yoğunluğu
Fiziksel parametreler

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INTRODUCTION

It is estimated that approximately 2 billion individuals worldwide suffer from hidden hunger, a chronic deficiency in essential micronutrients such as vitamins and minerals (Lowe, 2021). In today's world, healthy nutrition is prioritized for the prevention of certain diseases. As global public health awareness increases, the demand for functional foods offering various health benefits is also rising (Yaşa et al., 2023). Vitamins are crucial micronutrients for human health, and deficiencies can lead to various illnesses. Therefore, in addition to being consumed through foods, they are also made available as supplements or incorporated into functional foods. (Saman & Tomaş, 2022). It has been reported that microgreens contain significantly higher levels of functional components, such as phenolic compounds and antioxidants, compared to the amounts found in mature leaves (Gök et al., 2024).

Microgreens have garnered substantial attention for their health and beauty benefits, boasting nutritional content

up to 40 times higher than mature vegetables. Their versatility in culinary applications and positive impact on environmental sustainability and economic viability further contribute to their popularity. Grown either in soil or hydroponically, microgreens are a leading crop in controlled environment agriculture. These young greens, harvested between the sprout and baby green stages, have gained widespread appeal due to rising public interest in healthy eating. Derived from herb, vegetable, and grain seeds, microgreens typically develop a central stalk with two sets of immature true leaves, following the emergence of cotyledons (Kyriacou et al., 2020; Chandrashekharaiah, 2013).

Microgreens production involves the use of dense sowing techniques, which increase competition for resources and consequently require higher seed quantities. Variations in seed size among vegetable species necessitate the adjustment of sowing density according to the specific species. Improper sowing density, whether excessive or insufficient, can lead to negative outcomes. In this context, preliminary research to determine the optimal seed quantities for different species is crucial (Sarıyer et al., 2024). Sowing density is a significant factor influencing microgreens yield; as sowing density increases, the weight of each plant decreases due to competition among plants, while the overall yield rises with the increased number of seeds per unit area. This increase continues until the maximum production capacity is reached (Thuong et al., 2020; Palmitessa et al., 2020; Moraru et al., 2022).

This study addresses significant issues in microgreens cultivation by focusing on the effects of seed sowing density on product quality, yield, and economic profitability. The amount of seeds used during cultivation significantly influences the physical characteristics, germination rate, and growth performance of microgreens. While higher seed sowing densities may enhance yield in the short term, they can also increase production costs, thereby reducing profit margins. Therefore, determining the optimal seed sowing density for each species is crucial for improving both growth quality and economic efficiency. This research aims to identify the ideal seed sowing density for selected microgreens species to optimize growth performance, minimize production costs, and maximize profitability. By investigating the effects of sowing density on physical characteristics and yield, this study seeks to contribute to the improvement of production processes and the provision of higher-quality products to consumers.

MATERIAL and METHOD

Plant Material

This study was conducted in the climate chamber of the Department of Horticultural Sciences, Faculty of Agriculture, Çukurova University. The seeds used in the study included black radish (*Raphanus sativus niger*) and broccoli *(Brassica oleracea italica*) from the *Brassicaceae* family, red beet (*Beta vulgaris cicla*) from the *Amaranthaceae* family, pea (*Pisum sativum*) from the *Leguminosae* family, sunflower (*Helianthus annuus*) from the *Asteraceae* family, and bean (*Phaseolus vulgaris*) from *the Leguminosae* family. The seeds were not subjected to any chemical treatment or pesticide application. The black radish, broccoli, and red beet seeds were obtained from the standard varieties of Arzuman Seed Company, the pea seeds from the standard variety of Intfa Company, the sunflower seeds from the standard variety of AGR Company, and the bean seeds from the germplasm bank of the Department of Horticultural Sciences.

Plant Growing Conditions

The experiments were conducted in a climate-controlled growth chamber to determine the optimal seed sowing density for microgreens plants. Black radish, broccoli, and red beet were cultivated at 20 °C[Balik et al., 2024a], while pea, bean, and sunflower were grown at 23 °C, all under conditions of 50% relative humidity. The growth environment was supported by LED lamps designed to provide a balanced light spectrum similar to natural sunlight. These lamps ensured consistent light quality and intensity (350 µmol m−2 s−1), optimizing photosynthesis. Microgreens were subjected to 16-hour light and 8-hour dark cycles during cultivation. Transparent plastic containers measuring 16 cm × 9 cm × 7 cm (length × width × height) were used as growing trays, each filled with 250 cm^3 of peat. Experimental treatments were arranged in a completely randomized design, ensuring equal distribution across all parcels. This systematic approach aimed to enhance the accuracy and reliability of the research findings.

In this study, the selected plant species were sown at three different planting densities or seed rates based on seed size and weight (Table 1). The treatments for each plant species were as follows:

1. Treatment (Control): Seeds were sown to fully cover the surface area of the containers.
2. Treatment: Seeds were sown at 50% higher than the surface area capacity.
3. Treatment: Seeds were sown at 100% higher than the surface area capacity.

Table 1. The seed sowing density levels established for each application (g)
Çizelge 1. Her uygulama için belirlenen tohum ekim yoğunluğu seviyeleri (g)

Plant species	Treatment 1	Treatment 2	Treatment 3
Broccoli	13	19.5	26
Black radish	32	48	64
Red beet	16	24	32
Pea	100	150	200
Sunflower	28	42	56
Bean	70	105	140

Plant Nutrition

The sown seeds were irrigated with pure water until the first green cotyledon leaves appeared. Immediately after the emergence of the green cotyledons, irrigation was performed with the nutrient solution specified below. Modified Hoagland nutrient solutions at half strength were used for microgreens production. The plants were supplied with the following nutrient solution at ¼ strength (in mg/L): N (200), P (50), K (300), Ca (200), Mg (65), Fe (5.0), Mn (0.8), Cu (0.3), Zn (0.3), B (0.3), and Mo (0.05). The pH was set at 5.5, and the electrical conductivity (EC) was maintained between 1.2-1.6 dS cm-1 (for both initial and later stages) during irrigation [Balik et al., 2024b].

Plant Harvest

Microgreens were cultivated for a period of 7-15 days, depending on the growth rate of six different plant species. Harvesting was conducted when the seedlings had fully developed their first true leaf, the second leaf had begun to emerge, and the cotyledon leaves had reached the rounded margin stage.

Measurements of Plant Growth Parameters

During the harvest, measurements of plant height, hypocotyl length, stem diameter, and individual plant weight were taken for microgreens from six different plant species, with four replicates and ten plants measured per replicate under various cultivation conditions. Yield per unit area was calculated. The leaf area per plant was determined using a leaf area meter (Li-3100, LICOR, Lincoln, NE, USA), expressed in square centimeters. After harvest, the plants were weighed using a digital scale to determine the fresh leaf weight per plant in grams. Chlorophyll content in the leaves was measured using a SPAD chlorophyll meter (Minolta 502, Osaka, Japan). The fresh leaves were subsequently dried in an oven at 65 °C for 48 hours, and the dry weight per plant was recorded.

Statistical Analyses

All data were analyzed using JMP v5.0.1 statistical software, and an analysis of variance (ANOVA) was conducted. Treatment means were compared using LSD's significant difference test at $p \leq 0.05$. The significance levels for the three-way ANOVA analyzing the effects of plant species, cultivation environment, and their interactions are presented in Table 2.

RESULTS

Microgreens, a nutrient-rich source, exhibit varying growth patterns depending on the seed sowing density applied during cultivation. This study investigates the effects of three different sowing densities—Full Surface Coverage Sowing (T1), 50% above the surface area capacity (T2), and 100% above the surface area capacity (T3)—on plant height, hypocotyl length, stem diameter, single plant weight, leaf area, yield, dry matter ratio, and SPAD-chlorophyll content across six microgreens species: broccoli, black radish, red beet, pea, sunflower, and bean (Table 3).

Broccoli

In broccoli microgreens, under Full Surface Coverage Sowing (T1) conditions, plant height was measured at 6.81 cm, hypocotyl length at 5.03 cm, and stem diameter at 0.70 mm. The single plant weight was recorded at 0.081 g, leaf area at 0.64 cm², yield at 0.64 g/cm², and dry matter ratio at 6.25%. The SPAD-chlorophyll value was 42.06. In T2, plant height increased to 7.62 cm, while stem diameter decreased to 0.64 mm. The single plant weight decreased to 0.069 g, and leaf area slightly reduced to 0.62 cm²; however, yield increased to 1.00 g/cm². The chlorophyll content also showed a slight increase to 45.40. In T3, plant height further increased to 7.81 cm, maintaining the same stem diameter (0.70 mm), while the single plant weight decreased to 0.052 g. Leaf area was

reduced to 0.59 cm², yet yield reached its maximum at 1.20 g/cm², with a chlorophyll value of 43.08. Overall, despite higher sowing densities increasing yield, plant weight decreased, indicating increased competition for resources, which led to thinner stems. The chlorophyll content was highest at T2 density, while T1 exhibited the lowest value (Figure 1).

Figure 1. Image of broccoli microgreens subjected to different seed sowing density treatments
Şekil 1. Farklı tohum ekim yoğunluğu uygulamalarına maruz kalan brokoli mikrofilizlerinin görüntüsü

Black Radish

In black radish microgreens, under Full Surface Coverage Sowing (T1) conditions, plant height was measured at 12.60 cm, hypocotyl length at 8.91 cm, and stem diameter at 1.09 mm. The single plant weight was recorded at 0.26 g, leaf area at 1.41 cm², yield at 0.65 g/cm², and SPAD-chlorophyll value at 46.17. In T2 seed sowing density, plant height decreased to 10.89 cm, and stem diameter reduced to 1.01 mm. The single plant weight dropped to 0.186 g, while leaf area was measured at 1.15 cm². However, the chlorophyll value increased to 49.67. At T3 seed sowing density, plant height further declined to 10.74 cm, with stem diameter recorded at 1.05 mm and single plant weight measured at 0.178 g. Leaf area and yield slightly decreased, but the SPAD-chlorophyll value peaked at 50.76. Overall, T1 resulted in the highest plant height and weight, while T3 seed sowing density provided the highest chlorophyll content, indicating an increase in chlorophyll concentration with higher sowing densities (Figure 2).

Figure 2. Image of black radish microgreens subjected to different seed sowing density treatments
Şekil 2. Farklı tohum ekim yoğunluğu uygulamalarına maruz kalan siyah turp mikrofilizlerinin görüntüsü

Red Beet

In red beet microgreens, the Full Coverage Sowing (T1) application resulted in a plant height of 7.23 cm, a hypocotyl length of 5.17 cm, and a stem diameter of 0.74 mm. The single plant weight was measured at 0.058 g, leaf area at 0.36 cm², yield at 0.17 g/cm², and the chlorophyll content was recorded at 31.96. Under T2 seed sowing density, plant height increased to 7.86 cm, stem diameter to 0.77 mm, and single plant weight to 0.061 g. The leaf area expanded to 0.43 cm², the yield reached 0.23 g/cm², and chlorophyll content improved to 33.67. At T3 seed sowing density, plant height slightly decreased to 7.81 cm, with a stem diameter of 0.78 mm, leaf area of 0.41 cm², and chlorophyll content of 33.34. The results indicate that the T2 seed sowing density provided the best growth in

terms of plant height, leaf area, and yield, while chlorophyll content also remained highest at this density (Figure 3).

Figure 3. Image of red beet microgreens subjected to different seed sowing density treatments
Şekil 3. Farklı tohum ekim yoğunluğu uygulamalarına maruz kalan kırmızı pancar mikrofilizlerinin görüntüsü

Pea

In pea microgreens, the T1 application resulted in a plant height of 14.41 cm, a hypocotyl length of 7.10 cm, and a stem diameter of 1.76 mm. The single plant weight was recorded at 0.416 g, leaf area at 0.71 cm², and chlorophyll content at 33.24. Under T2 seed sowing density, plant height increased to 14.51 cm, stem diameter to 1.82 mm, and single plant weight to 0.433 g. Leaf area expanded to 0.85 cm², while the chlorophyll value decreased to 31.1. At T3 density, plant height slightly decreased to 14.29 cm; stem diameter was measured at 1.71 mm, and leaf area was reduced compared to T2 density. However, the chlorophyll value increased to 33.71. The data suggest that while T2 density promoted higher plant weight and leaf area, T3 density resulted in the highest chlorophyll content (Figure 4).

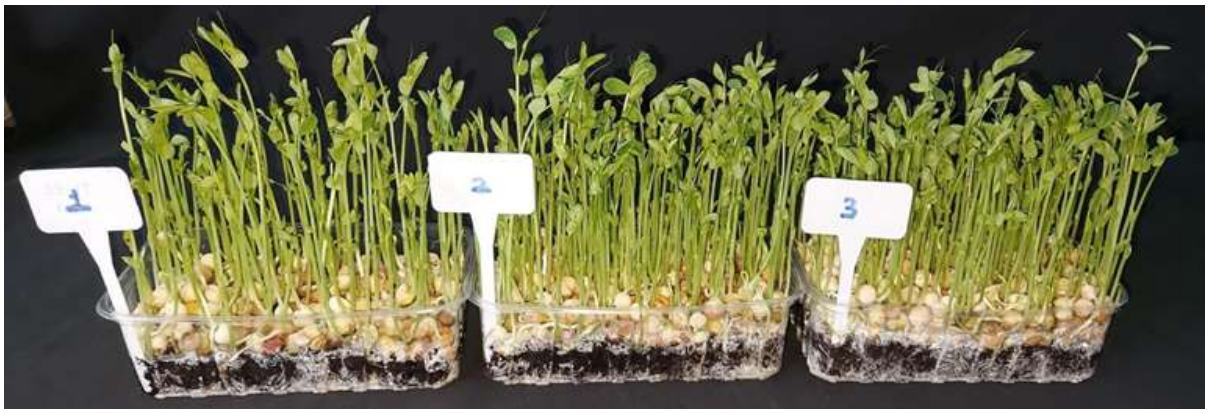

Figure 4. Image of pea microgreens subjected to different seed sowing density treatments
Şekil 4. Farklı tohum ekim yoğunluğu uygulamalarına maruz kalan bezelye mikrofilizlerinin görüntüsü

Sunflower

In sunflower microgreens, under T1 conditions, the plant height was measured at 12.08 cm, with a hypocotyl length of 8.40 cm and a stem diameter of 2.00 mm. The single plant weight was recorded at 0.884 g, leaf area at 3.39 cm², yield at 0.67 g/cm², and the chlorophyll value at 74.68. Under T2 seed sowing density, the plant height slightly decreased to 11.71 cm, while the stem diameter was measured at 1.91 mm, and the single plant weight declined to 0.821 g. Leaf area reduced to 2.93 cm²; however, yield increased to 0.81 g/cm², with a chlorophyll value rising to 77.68. At T3 seed sowing density, the plant height was recorded at 11.65 cm, with a stem diameter of 1.95 mm. Leaf area decreased to 2.59 cm², but yield increased to 0.92 g/cm², and the chlorophyll value reached 81.62. In sunflower microgreens, higher sowing densities enhanced chlorophyll content, leaf area, and yield, while plant weight was greater at lower densities (Figure 5).

Figure 5. Image of sunflower microgreens subjected to different seed sowing density treatments
Şekil 5. Farklı tohum ekim yoğunluğu uygulamalarına maruz kalan ayçiçek mikrofilizlerinin görüntüsü

Bean

In bean microgreens, the T1 application resulted in a plant height of 24.16 cm, a hypocotyl length of 20.56 cm, and a stem diameter of 2.86 mm. The single plant weight was recorded at 2.910 g, and the chlorophyll value was 35.14. Under T2 density, the plant height slightly decreased to 23.57 cm, with a stem diameter of 2.71 mm, and the single plant weight dropped to 2.790 g. The chlorophyll value remained nearly constant at 35.03. At T3 density, the plant height further decreased to 23.26 cm, the stem diameter was recorded at 2.50 mm, and the single plant weight was 2.560 g. However, the chlorophyll value increased to 36.27. Overall, higher densities in bean microgreens resulted in a slight decrease in plant height and weight, while the chlorophyll value reached its highest level at T3 density.

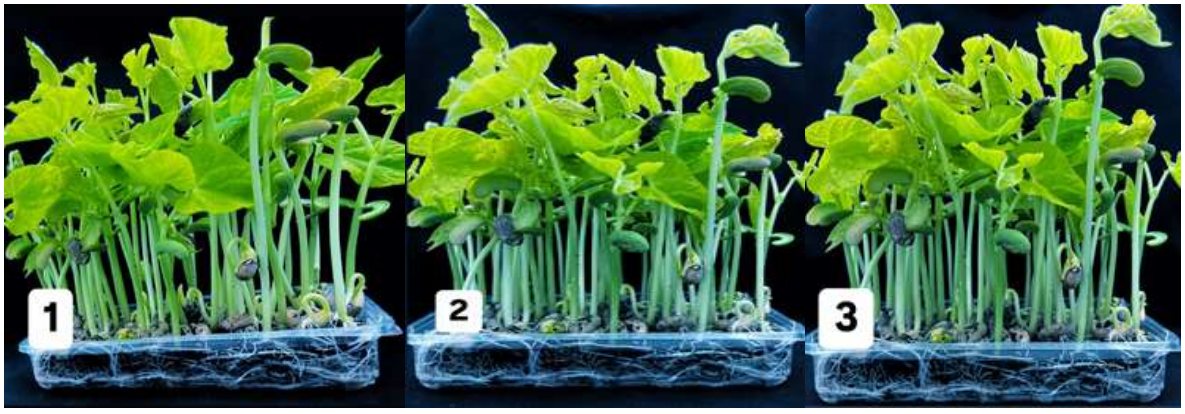

Figure 6. Image of bean microgreens subjected to different seed sowing density treatments
Şekil 6. Farklı tohum ekim yoğunluğu uygulamalarına maruz kalan fasulye mikrofilizlerinin görüntüsü

Table 2. Significance levels in a three-way ANOVA analyzing the effects of plant type, seed sowing density, and their interactions
Çizelge 2. Bitki türü, tohum ekim yoğunluğu ve bunların etkileşimlerinin etkilerini analiz eden üç yönlü ANOVA'daki anlamlılık seviyeleri

	Plant species (Ps)	Seed Sowing Density (Ssd)	Ps × Ssd
Plant length	****	*	****
Hypocotyl length	****	*	***
Stem diameter	****	****	****
Single plant weight	****	****	****
Leaf Area	****	****	****
SPAD-Chlorophyll	****	***	**
Yield	****	****	****
Dry Matter Ratio	****	**	****

*: $p > 0.05$, **: $p \leq 0.05$, ***: $p \leq 0.01$, ****: $p \leq 0.001$, ns: not significant.

Table 3. The effects of different seed sowing quantity treatments on plant height, hypocotyl length, stem diameter, single plant weight, leaf area, yield, dry matter, and SPAD-chlorophyll in microgreens

Çizelge 3. Farklı tohum ekim miktarı uygulamalarının mikrofilizlerde bitki boyu, hipokotil uzunluğu, sap çapı, tek bitki ağırlığı, yaprak alanı, verim, kuru madde ve SPAD-klorofil üzerindeki etkileri

Factor		Plant length(cm)	Hypocotyl length (cm)	Stem diameter(mm)	Single plant weight (g)	Leaf Area (cm^2/plant)	Yield (g/cm^2)	Dry Matter Ratio (%)	SPAD-Chlorophyll
Plant species									
Broccoli		7.41 e	5.70 d	0.68 f	0.067 e	0.62 e	0.95 a	5.78 d	43.51 c
Black radish		11.41 d	8.63 b	1.05 d	0.208 d	1.20 c	0.89 b	5.09 e	48.87 b
Red beet		7.63 e	5.41 d	0.76 e	0.060 e	0.40 f	0.23 e	4.16 f	32.99 e
Pea		14.41 b	7.19 c	1.76 c	0.420 c	0.78 d	0.21 e	9.54 b	32.95 e
Sunflower		11.51 c	8.38 b	1.95 b	0.840 b	2.97 b	0.80 c	7.88 c	77.99 a
Bean		23.66 a	20.03 a	2.69 a	2.753 a	15.11 a	0.71 d	11.45 a	35.48 d
P value		≤,0001*	≤,0001*	≤,0001*	≤,0001*	≤,0001*	≤,0001*	≤,0001*	≤,0001*
LSD		0.3872	0.3415	0.0427	0.0181	0.0497	4.2032	0.2850	1.8074
Seed sowing density									
T1		12.88 a	9.19 a	1.52 a	0.76 a	3.81 a	0.50 c	7.18 b	43.88 b
T2		12.69 ab	9.13 a	1.47 b	0.72 b	3.34 c	0.67 b	7.30 ab	45.56 a
T3		12.59 b	9.35 a	1.45 b	0.67 c	3.38 b	0.72 a	7.48 a	46.46 a
P value		0,1146	0,1837	≤,0001*	≤,0001*	≤,0001*	≤,0001*	0,0189*	0,0006*
LSD		0.2738	0.2415	0.0302	0.0128	0.0351	2.9721	0.2015	1.2780
Plant species×Seed sowing density.									
Broccoli	T1	6.81 h	5.03 h	0.70 kl	0.081 ı	0.64 lm	0.64 f	6.25 f	42.06 e
	T2	7.62 g	5.89 fg	0.64 l	0.069 ı	0.62 m	1.00 b	5.68 g	45.40 d
	T3	7.81 g	6.18 f	0.70 kl	0.052 ı	0.59 m	1.20 a	5.41 gh	43.08 de
Black radish	T1	12.60 d	8.91 c	1.09 h	0.26 g	1.41 g	0.65 f	4.56.ı	46.17 d
	T2	10.89 f	8.45 c	1.01 ı	0.186 h	1.15 h	1.00 b	5.07 h	49.67 c
	T3	10.74 f	8.52 c	1.05 hı	0.178 h	1.05 ı	1.03 b	5.63 g	50.76 c
Red beet	T1	7.23 gh	5.17 h	0.74 jk	0.058 ı	0.36 n	0.17 j	4.17 ıj	31.96 g
	T2	7.86 g	5.53 gh	0.77 jk	0.061 ı	0.43 n	0.23 h	4.04 j	33.67 fg
	T3	7.81 g	5.54 gh	0.78 j	0.061 ı	0.41 n	0.30 g	4.28 ıj	33.34 fg
Pea	T1	14.41 c	7.10 de	1.76 fg	0.416 f	0.71 kl	0.18 ıj	9.66 c	33.24 fg
	T2	14.51 c	6.86 e	1.82 f	0.433 f	0.85 j	0.22 h	9.34 c	31.1 g
	T3	14.29 c	7.61 d	1.71 g	0.411 f	0.78 jk	0.24 h	9.63 c	33.71 fg
Sunflower	T1	12.08 de	8.40 c	2.00 d	0.884 d	3.39 d	0.67 ef	7.55 e	74.68 b
	T2	11.71 e	8.36 c	1.91 e	0.821 e	2.93 e	0.81 d	7.91 de	77.68 b
	T3	11.65 e	8.38 c	1.95 de	0.815 e	2.59 f	0.92 c	8.19 d	81.62 a
Bean	T1	24.16 a	20.56 a	2.86 a	2.910 a	16.38 a	0.70 e	10.92 b	35.14 f
	T2	23.57 ab	19.68 b	2.71 b	2.790 b	14.06 c	0.78 d	11.74 a	35.03 fg
	T3	23.26 b	19.86 b	2.50 c	2.560 c	14.91 b	0.92 c	11.71 a	36.27 f
P value		≤,0001*	0,0016	≤,0001*	≤,0001*	≤,0001*	≤,0001*	≤,0001*	0,0436*
LSD		0.6706	0.5916	0.0740	0.0314	0.0861	7.2802	0.4936	3.1306

T1: Seeds are sown to fully cover the surface area of the containers. T2: Seeds are sown at 50% above the surface area capacity, T3: Seeds are sown at 100% above the surface area capacity.
**There are no statistical (p ‹0.05) differences between values with the same letters in the same columns.*

DISCUSSION

Although it is well known that different seed sowing density applications significantly affect yield outcomes, these effects vary depending on the species. Dubey et al. (2024) emphasized the importance of seed sowing density in the microgreens literature due to its direct impact on the growth of microgreens. In this study, it was observed that increasing seed sowing density led to a rise in the overall yield of microgreens. However, a proportional decrease in single plant weight was noted in broccoli, black radish, sunflower, and bean microgreens. In contrast, in red beet and pea microgreens, a reduction in single plant weight was observed only beyond the optimal growth level (Table 3). Choe et al. (2018) reported a linear relationship between seed sowing density and fresh weight yield. However, the findings of this study, while supporting this relationship, also demonstrate that high seed sowing density leads to a reduction in single plant weight. This outcome indicates increased resource competition among plants, where limited space and nutrient availability adversely affect the growth conditions of individual seedlings. Microgreens grown at high densities are subjected to reduced access to light, water, and nutrients per plant, resulting in a significant decline in individual development. Lee et al. (2004) reported a linear relationship between seed sowing density and yield in beet and chard microgreens. Similarly, Murphy et al. (2010) observed a comparable relationship between seed sowing density and yield in arugula (rocket). As seed sowing density increases, yield also improves; however, the associated seed costs must be carefully evaluated. Sarıyer et al. (2024) noted that densely sown okra, garden cress, and spinach microgreens exhibited higher yield values compared to sparsely sown counterparts. Consistent with these findings, this study also observed an increase in yield with higher seed density. When evaluating yield performance and the amount of seeds used, it is crucial to consider seed input to ensure an accurate yield performance analysis. A similar study conducted by Cowden et al. (2024) highlighted that the effects of increased density on biomass exhibited a similarly non-linear relationship, emphasizing that such changes in density do not always result in the expected yield improvements.

In broccoli microgreens, an increase in seed sowing density was observed to result in taller plants, while in red beet and pea microgreens, plant height increased until reaching the optimal growth level (Table 3). This phenomenon can be attributed to the closer spacing of microgreens at higher seed sowing densities, leading to competition for sunlight (Ntsoane et al., 2023). The height of microgreens plays a significant role in facilitating manual harvesting. Research indicates that taller microgreens are generally easier to harvest, as increased height simplifies the process (Palmitessa et al., 2020). Senevirathne et al. (2019) recommend an optimal harvest height of approximately 6 cm for maximum efficiency. A study conducted by Lerner et al. (2024) observed that microgreens exceeding this height exhibited a tendency to bend due to excessive elongation of the hypocotyls. This was reported as an undesirable trait from a commercial perspective, as it negatively impacts both visual appeal and post-harvest processing. In this study, we found that the height and hypocotyl length of microgreens varied depending on the species. Notably, as the height and hypocotyl length of large-seeded microgreens increased, bending and curling were observed in bean and pea microgreens. Priti et al. (2022) stated that in microgreens, seed density is directly related to seed size. This result aligns with findings by Panyapruek et al. (2016), who reported that lettuce grown at low plant density developed thicker stems. Differences in species' responses to seed sowing density likely reflect their genetic traits, physiological processes, and adaptive mechanisms to environmental factors. In densely growing species such as broccoli, this can lead to increased light competition, resulting in elongated but thinner stems. To better understand the underlying causes of such differences, in-depth investigations into genetic composition, physiological responses, and species-specific resource utilization strategies are necessary.

Overall, it was observed that an increase in seed sowing density led to a reduction or partial reduction in stem diameter across all microgreens species except for red beet (Table 3). This variation may be attributed to the stronger adaptability of red beet to high seed sowing density. Red beet appears to be a species that thrives better under dense conditions and is more resistant to competition, which may prevent a reduction in stem diameter. In contrast, for other microgreens species, high seed sowing density likely led to increased competition among plants, making access to nutrients and water more challenging and resulting in reduced stem diameter. The differing response of red beet to this condition could be explained by a combination of genetic and environmental factors. In the study by Balik et al. (2024), it was noted that certain environments were more effective in terms of plant height, hypocotyl development, stem diameter, and yield, while other environments performed less effectively. The study demonstrated that some environments yielded moderate results, whereas others were less effective in promoting plant growth due to factors such as low water retention capacity, insufficient nutrient support, and the inability to provide suitable structures for root development. The findings align with these observations, suggesting that without selecting an appropriate growing medium, seeds may encounter developmental challenges. This is further supported by the results of Ntsoane et al. (2023), which show a reduction in stem diameter with increasing seed sowing density in radish, cabbage, and arugula microgreens.

In this study, we observed that as seed sowing density increased, the leaf area of microgreens decreased in some species, while in others, it showed a partial decrease. This finding is consistent with the results of Maboko et al.

(2009), who reported that high seed sowing density in microgreens can increase the incidence of fungal diseases, thereby reducing the quality of the microgreens. Similarly, Signore et al. (2024) observed that rapini seedlings grown at the lowest seed sowing density exhibited more developed and larger true leaves compared to those grown at the highest density.

Chlorophyll pigment is essential for plants to carry out photosynthesis and significantly influences growth and yield (Hasanuzzaman & Fujita, 2022). Chlorophyll content generally increases with higher seed sowing density, aligning with the findings of Ntsoane et al. (2023). The potential contribution of increased chlorophyll content at higher seed sowing densities to photosynthetic capacity and product quality warrants further investigation. While increased chlorophyll typically supports photosynthetic efficiency, this effect may vary depending on the growing environment, genetic traits, and environmental factors. The observation of lower chlorophyll levels in some species under low seed density may indicate species-specific responses and adaptation mechanisms. To better understand these differences, more specific and controlled studies are needed.

In this study, a decrease in dry matter content was observed in broccoli microgreens, while in other species, it either increased or showed a partial increase. Several potential reasons could explain the reduction in dry matter content in broccoli microgreens. Although broccoli exhibited higher growth and yield compared to other species, this may have led to an increase in water content, consequently lowering the dry matter ratio. In microgreens, increases in yield and size are typically accompanied by a rise in water content, which suggests that while the total biomass of the plant may increase, the dry matter percentage could decrease. Additionally, the fast growth rate of broccoli may have accelerated photosynthesis and carbon accumulation, leading to higher water retention, which could negatively affect dry matter content. Changes in seed sowing density also influence dry matter content in microgreens; higher dry matter content generally extends the shelf life of microgreens (Sánchez et al., 2018; Valverde-Miranda et al., 2021). Producers should be informed not only about the variables examined in this study (growth stage, harvest height and leaf characteristics, yields) but also about additional factors such as fungal infections when selecting sowing densities (Nolan, 2018).

CONCLUSION

This study observed that as seed sowing density increased, the results varied according to the genetic differences of the species. From an economic perspective, an increase in seed sowing density in broccoli microgreens resulted in higher yields and better quality products. This suggests that producers may consider increasing sowing density during broccoli microgreens production, as denser sowing can lead to more plants and thus higher yields. However, in red beet and pea microgreens, partial positive results were observed after reaching the optimal growth level; this emphasizes the importance of determining the ideal seed sowing density for each species.

Economically, determining the appropriate seed sowing density can optimize production costs while also improving productivity and quality. Excessive seed density can increase competition between plants for nutrients and water, which may lead to lower yields. Therefore, producers, particularly for fast-growing species like broccoli, need to carefully determine the sowing density, taking into account the economic benefits of higher sowing density. These findings can help microgreens producers develop more efficient and cost-effective production strategies. However, further research is needed, particularly to conduct more in-depth analyses of the economic impact of seed sowing density on different species.

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Contribution Rate Statement Summary of Researchers

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

Conflict of Interest

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

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Hypericum calycinum L. Türü Üzerine Detaylı Anatomik Çalışma

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ÖZET

Bu çalışmada, *Hypericum* L. cinsine ait *H. calycinum* türünün toprak üstü kısımları anatomik açıdan detaylı bir şekilde incelenmiştir. Cinsin Türkiye'de 119 taksonu bulunmaktadır. Bunlardan 49'u endemiktir. Bunlardan en bilineni H. *perforatum* L. türü, yara, yanık, depresyon tedavilerinde etkili olduğu klinik çalışmalarda gösterilmiştir. Türün gövde enine kesitlerinde 4 adet gövde kanadı, kanatlarında şeffaf gland, ve öz bölgesinde de şeffaf gland'a rastlanılmış, ayrıca çalışmada diğer çalışmalardan farklı olarak gövde boyuna da kesitler alınmıştır. Yaprakların hipostomatik, stoma tiplerinin anizostik ve anomostik olduğu, mezofil tabakasının bifasiyal olduğu görülmüştür. Sepal'in üst tabakasında iki sıralı epidermis ve sıralı bir şekilde şeffaf gland rastlanılmış, Petal'in mezofil tabakasında sıralı şeffaf gland tespit edilmiştir. Anter kısmının üç teka'dan oluştuğu, ovaryum'da C tipi salgı kanalının çok yoğun bir şekilde yer aldığı kaydedilmiştir. Bu araştırmadaki amaç, bitkilerin sadece vejetatif kısımlarının değil, generatif kısımlarının da incelenip bir bütün olarak düşünülmesinin gerekliliğini, bunların sonucunda bitkiler hakkında daha doğru bilgilere ulaşılabilineceğini ve taksonomik sınıflandırma için de yardımcı olacağını vurgulamaktadır.

Botanik

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Anatomi

A Comprehensive Anatomical Study of *Hypericum calycinum* L.

ABSTRACT

In this study, the above-ground parts of *H. calycinum* species belonging to the genus *Hypericum* L. were examined in detail anatomically. There are 119 taxa of the genus in Turkey. 49 of them are endemic. The most well-known of these, *H. perforatum* L., is effective in treating wounds, burns, and depression in clinical studies. In the transverse sections of the stem of the species, 4 stem wings, glandular pockets on the wings, and glandular pockets in the pith area were found, and in our study, unlike other studies, stem longitudinal sections were also taken. It was observed that the leaves were hypostomatic, stomatal types were anisoplastic and anomositic, and the mesophyll layer was bifacial. Two rows of epidermis and an ordered transparent gland were found in the upper layer of the sepal, and an ordered transparent gland was found in the mesophyll layer of the Petal. It was noted that the anther consisted of three thecae, and the ovary had a very dense C-type glandular duct.

This research aims to emphasize that not only the vegetative parts of plants but also the generative parts should be examined and considered as a whole, as a result, more accurate information about plants can be obtained and it will be helpful for taxonomic classification.

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GİRİŞ

Hypericum L. cinsi (Hypericaceae, Guttiferae) familyasına ait olup, dünyada yaklaşık 500 takson ile temsil edilmektedir. Türkiye'de ise 119 takson bulunmaktadır. Bunlardan 49'u endemik ve endemizm oranı %41,17'dir. Cinsin en iyi bilinen türü *H.perforatum* L. 'dir. Yaprakları güneşe tutulduğunda şeffaf binbirdeliğe sahip olduğu için binbirdelikotu da denilmektedir. St.John'un askerleri kılıç yaralarını iz bırakmadan geçirdiği için kılıç otu ismi ile, ingilizce olarak da St. John's Wort olarak bilinmektedir (Yalım Kaya & Can, 2018). Ilıman, yüksek dağlarda çoğunlukla açıklık alanlarda rastlanılan ve toprak üstü kısmı tıbbi açıdan kullanılan önemli bir bitkidir (Crockett ve ark., 2011; Güner ve ark., 2012; Meseguer ve ark., 2013). Yara ve yanık tedavilerinde etkili bir etki göstermesinin yanı sıra orta şiddetli depresyon tedavisinde de kullanılmaktadır (Scotti ve ark., 2019; Wills ve ark., 2000). Üzerinde en çok çalışma yapılmış olan *H. perforatum* türü özütlerinin depresyon tedavisinde kullanılma etkinliği plasebo etkisi ile karşılaştırmalı olarak klinik açıdan incelenmiş ve plaseboya kıyasla olumlu sonuçlar verdiği görülmüştür (Linde ve ark., 2008; Bombardelli ve Morazzoni, 1995). Son yıllarda yapılan çalışmalar incelendiğinde *Hypericum* cinsi üzerinde anti-inflamatuar, anti-kanser, antioksidan aktiviteleri açısından da kullanılabilirliği gösterilmiştir (Cardile ve ark, 2023; Deng ve ark., 2022; Zhai ve ark., 2022; Rafailovska ve ark., 2023). Öztürk ve ark. (1996)'nın yapmış olduğu çalışma da *H. perforatum* ve *H. calycinum* türlerinden elde etmiş oldukları ektraktlar kullanılarak hayvan modelleri üzerinde antidepresan etkileri incelenmiş ve sonuç olarak ekstrelerin desipramin ve trimipramin içeren antidepresan ilaçlar kadar etkili oldukları, *H. calycinum*'un antidepresan etkisinin de *H. perforatum* kadar güçlü olabileceği ve depresyon tedavilerinde terapötik amaçlarla kullanılabilir potansiyele sahip olduğu rapor edilmiştir.

Bu denli öneme sahip tıbbi bitkiler dikkatleri çekmekte ve üzerlerinde yapılan çalışmalar da git gide yoğunlaşmaktadır. *Hypericum* türleri üzerine yapılmış olan çalışmalar incelendiğinde kimyasal çalışmaların anatomik çalışmalara göre daha fazla yapıldığı tespit edilmiştir (Perrore vd. 2013; Potoğlu ve Tokur 2004; Ciccarelli vd. 2001). Yapılmış olan çalışmalar incelendiğinde *Hypericum* L. taksonlarının ayırt edilmesinde gövde de yer alan kanat sayısı ve yapısı, salgı kanal tipleri, şeffaf veya siyah gland yapısının var olup olmaması çok önemli rol oynamaktadır (Cicarelli ve ark., 2001a; Cicarelli ve ark., 2001b). Bu zamana kadar yapılmış olan çalışmalar incelendiğinde bitkilerin genellikle vejetatif organlarının çalışıldığı, generatif organların anatomisi sadece Altıntaş (2015) ve Tekin (2016) tarafından çalışıldığı ve bu konudaki çalışmaların kısıtlı kaldığı net bir şekilde görülmektedir. Çalışmadaki amaç, bitkilerin sadece vejetatif organları açısından değil, generatif organlarını (çiçek)'da ele alarak bir bütün halinde görmek, farklılıklarını ortaya koymak ve bu şekilde yapılacak olan değerlendirmelerin bitki tür teşhis anahtarlarının doğruluğunun arttırılması ve türlerin deskripsiyonlarının daha doğru bir şekilde yapılması açısından katkı sunması ve sonraki çalışmalara da yön vermesi beklenilmektedir.

MATERYAL ve METOD

Çalışmanın ana materyali Batı Karadeniz bölgesi, Düzce ili, Gölyaka ilçesi, Güzeldere Köyü yol kenarı gölgelikli alanlardan 2023 yılı haziran ayında toplanmıştır (Şekil 1, 2). Taksonomik teşhisi için Davis'in yazmış olduğu Flora of Turkey kitabından yararlanılmıştır (Davis, 1978). Anatomik çalışmalar için doğadan toplanan 25 adet örneğin 20 adeti %70'lik etanol'de tespit edilmiştir. El yardımı ile gövde, yaprak, sepal, petal, anter ve ovaryum'dan 20 tekerürlü kesitler alınmış, 1:9 oranında safranin – fast green boyası ile boyandıktan sonra (Bozdağ ve ark., 2016) gliserin-jelatin ile kalıcı preparat haline getirilmiştir. Kesitlerin fotoğraflandırılması ve yapıların ölçümleri Nikon Eclipse E200 marka mikroskop, Nikon Digital Sight DS-L3 marka ölçüm cihazı ile ölçülmüştür. Salgı kanallarının tipleri Cicarelli ve ark. (2001a) 'a, stoma tipleri Prabhakar (2004) ve Barclay (2007)'a göre belirlenmiştir. Stoma indeksinin hesaplanması için SI= Stoma sayısı / Stoma sayısı +Epidermal hücre sayısı x 100 formülü'nden yararlanılmıştır (Paul ve ark., 2017).

İstatisik Analizler

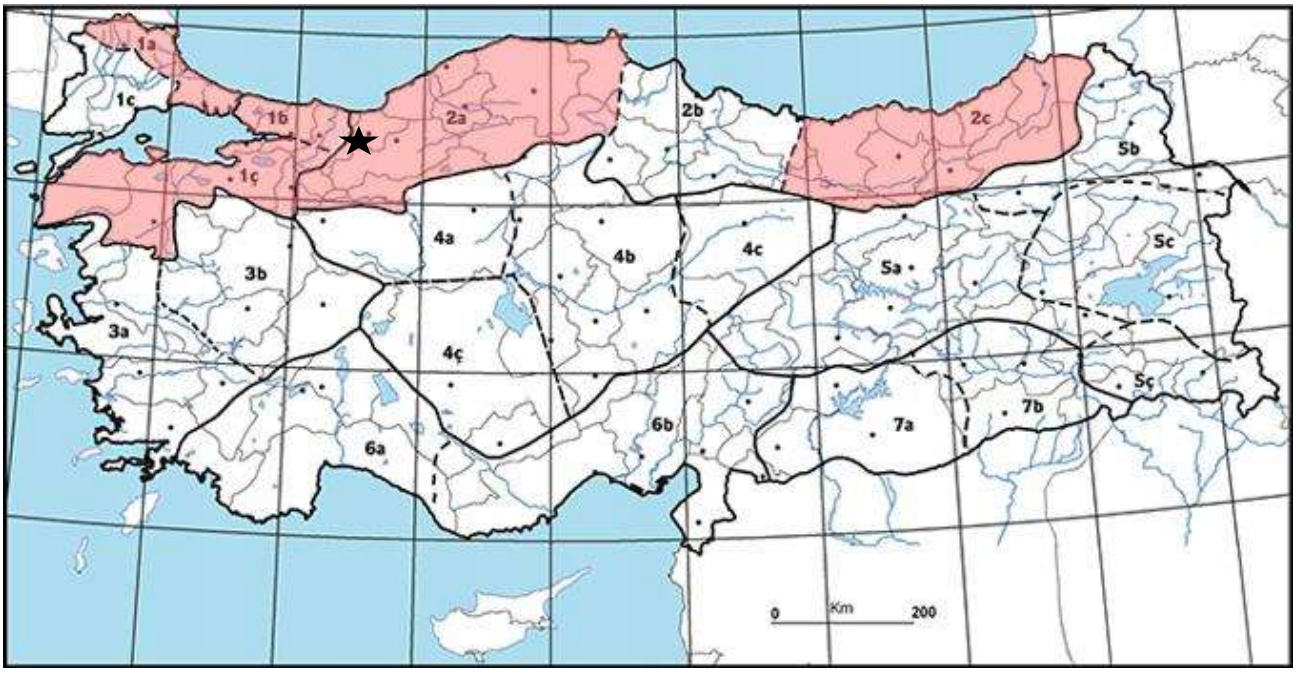
Çalışma sonucunda elde edilmiş olan verilerin ortalama ve standart sapma değerleri IBM SPSS statistics 25 paket programı kullanılarak hesaplanmıştır (SPSS, 2017). Ölçümler 20 adet bireyde vegetatif ve generaif kısımlarından alınan kesitler 25 tekrarlı yapılmış olup, $\overline{X}$: Ortalama(µm) ± Standart sapma şeklinde verilmiştir (Standart hata yapılan çalışmaların güvenirliliği açısından önem arz ettiği için ortalamanın yanında standart hata'nın verilmesi uygun görülmüştür).

BULGULAR ve TARTIŞMA

Cicarelli ve ark. (2001a)'nın yapmış oldukları çalışma da salgı yapılarını temelde 2'ye ayırmıştır bunlar; (a) şeffaf glandlar: bunlar küre veya elips biçimlidir. İki hücre katmanı ile sınırlanmış subepidermal boşluklardan oluşur. (b) 3 çeşit salgı kanalı bulunmaktadır. A Tipi: Genellikle dar çapa sahip dört köşeli hücrelerden oluşmaktadır. B Tipi: Geniş lümenlere sahip olan ve uzun, soluk görünüme sahiptir. C Tipi: Yoğun renkli ve daha fazla hücre

katmanlarıyla çevrili geniş boşluklardan oluşur. Yapılan çalışmadaki salgı yapılarının sınıflandırılmasında da bu kaynaktan yararlanılmıştır (Altınbaşak, 2015; Şengüler, 2009). Çalışmada bitkinin toprak üstü kısımlarının tamamı detaylı bir şekilde incelenmiştir.

Şekil 1. *H. calycinum* türünün doğadaki görünümü. A- Habitat, B,C- Çiçeğin yakından görünümü
Figure 1: Natural appearance of H. calycinum. A - Habitat, B, C - Close-up views of the flower



Şekil 2. *H.calycinum* türünün yayılış gösterdiği alanlar ve örneklerin toplandığı, yıldız ile işaretli lokalite (Aslan,2012).
Figure 2: The areas where the H. calycinum species is distributed and the locality marked with a star where the samples were collected (Aslan,2012).

Gövde Anatomik Yapısı

Gövde enine kesiti incelendiğinde dört adet kısa gövde kanadı ve her bir kanatta şeffaf gland'ın varlığı dikkat çekmektedir (Şekil 3 A-D). *H. tetrapterum* Fr. türünde de dört adet gövde kanadı ve kırmızımsı bir şeffaf glandın varlığı belirtilmiştir (Yağan ve ark., 2012). Altıntaş (2024) çalışmasında, *H. androsaemum* L., *H. linarioides* ssp. *linarioides* Bosse, *H. lydium* Boiss., *H. montanum* L., *H. perforatum* L. ve *H. pruinatum* Boiss. & Balansa türlerinin hepsinde iki adet kanat tespit ederken, sadece H. lydium Boiss. türünde kanatlarda salgı kanalına rastlandığını ifade etmiştir. *H. thymopsis* Boiss. türünde de iki adet kanat ve glandiferöz emergens oluşumuna dair bilgiler vermiştir (Tekin, 2017). Tuzlu habitatta yaşayan *H. salsugineum* N. Robson & Hub.-Mor. türünde ise gövde kanat sayısının iki olduğu tespit edilmiştir (Acar, 2023). Türde gövdenin dış kısmında tek sıralı epidermis tabakası yer almaktadır. Altınbaşak (2019) çalışmasında *H. spectabile* Jaub. & Spach türünde iki sıralı epidermis tabakasının varlığından, *H. thymopsis* türünü gövdesinin dış kısımda papilli yapıda belirgin uzantılara sahip tek sıralı epidermis tabakası gözlemlendiğini ifade etmiştir. Türün Epidermis tabakasının altında 9-10 sıralı korteks tabakası tespit edilmiştir. *H. orientale* L.'de 7-13 sıralı, *H. bithynicum* Boiss.'da 3-7 sıralı ve *H. origanifolium* var. *origanifolium* Wild'da 4-6 sıralı korteks gözlemlenmiştir (Altıntaş, 2015). Ayrıca incelemiş olduğumuz türün endoderma tabakası net bir şekilde tek sıralı olduğu gözlemlenmiştir. İncelenen türün floem tabakası 11-15 sıralı olup, üstünde A tipi salgı kanalı bulunmakta, ksilem tabakası geniş bir alan kaplamaktadır (Şekil 3 E). Tekin (2017) çalışmasında *H. thymopsis* türünde de floem tabakasında A tipi salgı kanallarının varlığını rapor etmiştir. *H. capitatum* var. *luteum* N. Robson, *H. microcalycinum* Boiss. & Heldr. ve *H. uniglandulosum* Hausskn. ex Bornm. taksonlarından sadece *H. capitatum* var. *luteum* N.Robson'da floemde A tipi salgı kanalı görülmediğini fakat diğerlerinde bulunduğunu ifade etmişlerdir (Gürhan & Arabacı, 2022). *H. avicullariifolium* Jaub. et Spach türünde gövde kesitinde salgı yapısının var olup olmadığı belirtilmemiş, *H. bithynicum* Boiss. türünde de salgı kanalı olduğu fakat tipi belirtilmemiştir (Türkten, 2022). Gövde de yer yer iki sıralı olmak üzere çoğunlukla tek sıralı öz ışınları bulunmaktadır. Tekin (2017) yapmış olduğu çalışma da *H. thymopsis* türünde de 1-2 sıralı öz ışınları yer aldığı belirtilmiştir. Gövdenin öz kısmında parankimatik hücreler bulunmakta olup öz kısmında sıralı bir şekilde salgı ceplerine rastlanılmıştır (Şekil 3 A,F). Gövde boyuna kesiti ilk defa çalışmada incelenmiş ve stoma hücrelerinin varlığı tespit edilmiştir (Şekil 3 G,H). Gövde enine ve boyuna kesitlerinde yer alan yapılara ilişkin ölçümler Çizelge 1 ve 2'de verilmiştir.

Yaprak Anatomik Yapısı

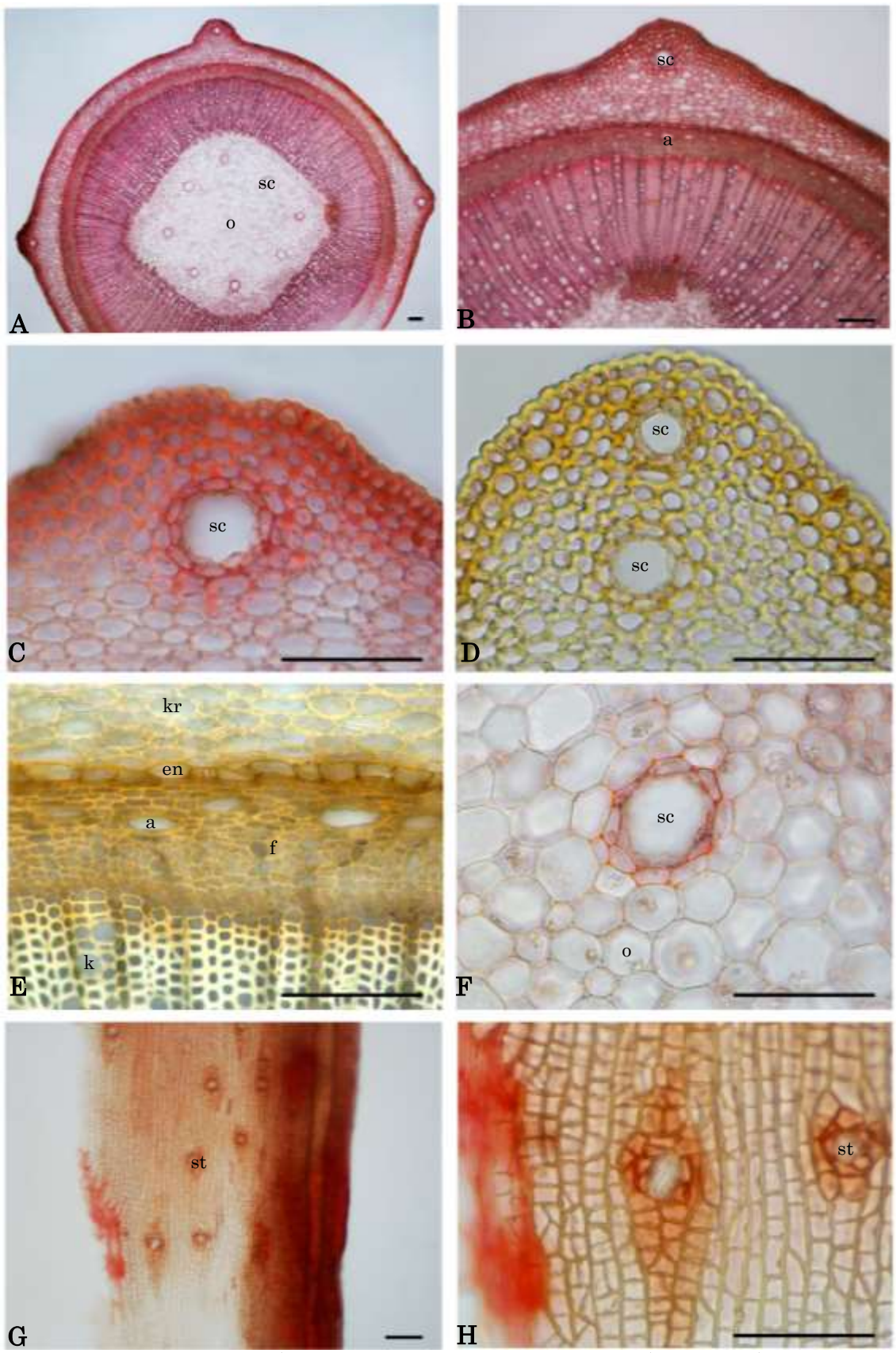
Yaprak üst yüzeyinde stoma bulunmamakta, yani yapraklar hipostomatiktir (Şekil 4 A-D). 1 mm²'ye düşen epidermis sayısı ortalama 1168 olarak belirlenmiştir. Yaprak alt yüzeyindeki stomalar anizostik ve anomostik tiplerde olup (Şekil 4 C-F), 1 mm²'deki stoma sayısı ortalama 384, epidermis sayısı ortalama 1380 ve stoma indeksi 27.82 olarak hesaplanmıştır (Çizelge 3). *H. scabrum* türü yaprağının amfistomatik olduğunu rapor etmiştir (Ergin ve ark., 2022). Acar, (2023) çalışmasında *H. salsugineum*'un yapraklarının amfistomatik olduğunu ve stoma tiplerinin anizostik olduğunu belirtmiştir. Türün yaprak enine kesitleri incelendiğinde, mezofil tabakasının bifasiyal yapıda (Şekil 5 A,C), alt ve üst yüzeylerde tek sıralı epidermis tabakası, çok az sayıda epidermis tabakasının altında hipodermis hücreleri içinde druz kristalleri gözlemlenmiştir (Şekil 5 C). Palizat parankimasının iki sıralı olduğu, mezofil tabakasında B tipi (Şekil 5 C,D), iletim demetlerinin floem tabakasının üzerinde de A tipi salgı kanallarının tespit edildiği görülmüştür (Şekil 5 B). Ayrıca alt yüzeydeki stomaların kseromorfik özellikler gösterdiği belirlenmiştir (Şekil 5 D). Yapılara ilişkin ölçümler Çizelge 4'de verilmiştir. *H. perforatum* türünün yaprak mezofilinin bifasiyal olduğunu belirtmişlerdir (Gürhan & Arabacı, 2022; Verma ve ark., 2022). Altıntaş (2024) ise *H. perforatum*'un ekvifasiyal mezofile sahip olduğunu ifade etmiştir. Ergin ve ark., (2022) *H. scabrum* L. türünün ekvifasiyal olduğunu belirtmiştir. *H. salsugineum* türünde ise yaprak mezofil tipinin ekvifasiyal olduğu ve yer yer salgı bezi varlığından bahsedilmiştir (Acar, 2023). Yaprak enine kesitinde alt yüzeyde bulunan stomaların epidermis seviyesinden altında olduğu yani kseromorfik olduğu anlaşılmaktadır (Şekil 5 D).

Sepal Anatomik Yapısı

Sepal enine kesiti incelendiğinde üst yüzeyinde iki sıralı epidermis tabakası, alt yüzeyinde ise tek sıralı epidermis yer almaktadır (Şekil 6 A). Üst yüzeydeki epidermisler, alt yüzeydekilerden daha büyük boyutlardadır (Şekil 6 A, Çizelge 5). Mezofil tabakasının tek tip olduğu ve alt epidermis'e doğru sıralı bir şekilde salgı ceplerinin bulunduğu görülmüş, iletim demetlerinin floem'lerinin üzerinde A tipi salgı kanalı tespit edilmiştir (Şekil 6 A,B, Çizelge 5). *H. orientale* türünün floeminde A tipi salgı kanalı, *H. bithynicum*'un mezofilinde siyah gland, *H. origanifolium* var. *origanifolium*'da ise B tipi salgı kanalı bulunmaktadır (Altıntaş, 2015). Tekin (2017) çalışmasında sepalin iletim demetlerinde floem üzerinde A tipi, mezofilde B tipi salgı kanalı varlığını ifade etmiştir.

Petal Anatomik Yapısı

Petalin her iki yüzeyi tek sıralı epidermisle çevrili olup, alt yüzeydeki epidermisler papillaması bir yapı göstermektedir. Üst yüzeydeki epidermisler, alt yüzeydekinin boyutlarından büyüktür (Şekil 6 C,D, Çizelge 5).



Şekil 3. *H. calycinum* türünün gövde (A-F: gövde enine, G-H: gövde boyuna) kesitleri (a: A tipi salgı kanalı, en: endoderma, f:floem, k:ksilem, kr: korteks, sc: şeffaf gland, st:stoma, o: öz). Bar:100 µm

Figure 3. Cross-sections of H. calycinum species (A-F: transverse sections, G-H: longitudinal sections). (a: type A secretory canal, en: endoderma, f: phloem, k: xylem, kr: cortex, sc: secretory pocket, st: stomata, o: pith) Bar: 100 µm.

Çizelge 1. İncelenen *H. calycinum* türünün gövde enine kesitindeki yapıların anatomik boyutları
Table 1. Anatomical dimensions of the structures in the stem cross-section of the studied H. calycinum species

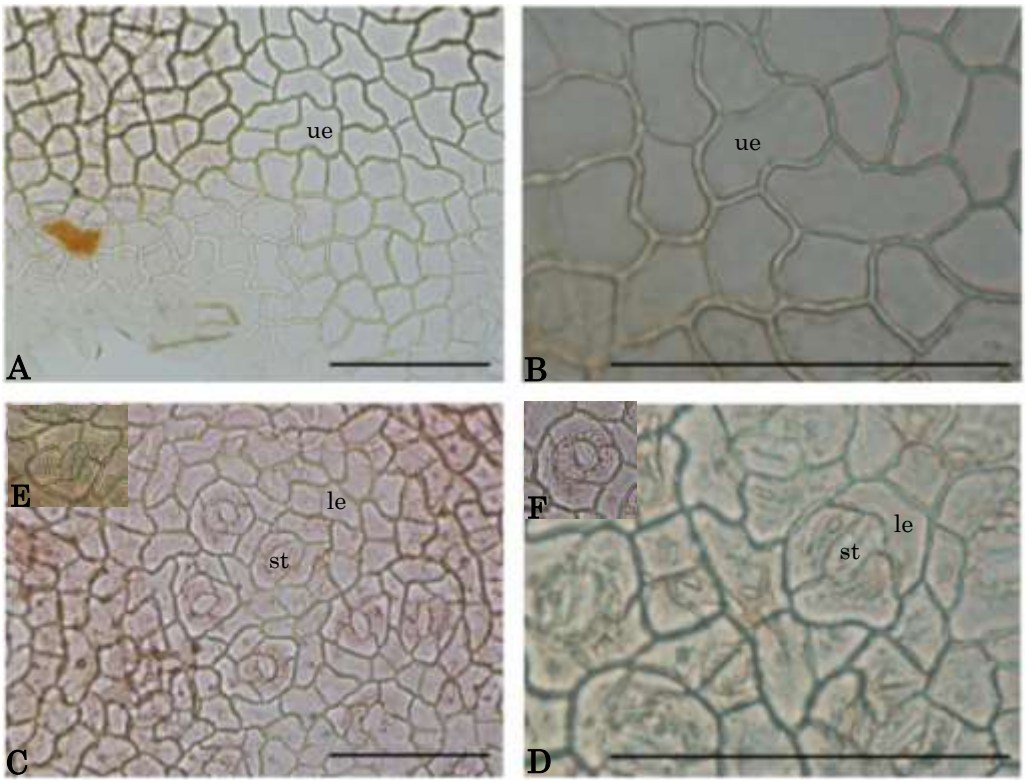
	$\bar{X}$ ±S.sapma
Epidermis en	16.90 ± 3.42
Epidermis boy	9.23 ± 1.18
Korteks en	26,95 ± 5.50
Korteks boy	12.11 ± 3.08
Endodermis en	29.36 ±4.52
Endodermis boy	9.54 ± 2.39
Öz hücreleri çap	41.71 ± 6.98
Floem en	8.33 ± 2.44
Floem boy	6.60 ± 1.25
Ksilem çap	19.73 ± 2.91
Gövde kanadındaki salgı hücresi çapı	47.22 ± 8.52
Öz bölgesindeki salgı hücresi çapı	58.04 ± 9.69

$\bar{X}$: Ortalama(µm), S.sapma :Standart sapma

Çizelge 2. İncelenen *H. calycinum* türünün gövde boyuna kesitindeki yapıların anatomik boyutları
Table 2. Anatomical dimensions of the structures in the longitudinal section of the stem of the studied H. calycinum species

	$\bar{X}$ ±S.sapma
Epidermis en	11.22 ± 1.91
Epidermis boy	23.94 ± 6.37
Stoma en	21.13 ± 3.01
Stoma boy	26.90 ± 3.85

$\bar{X}$: Ortalama(µm), S.sapma :Standart sapma



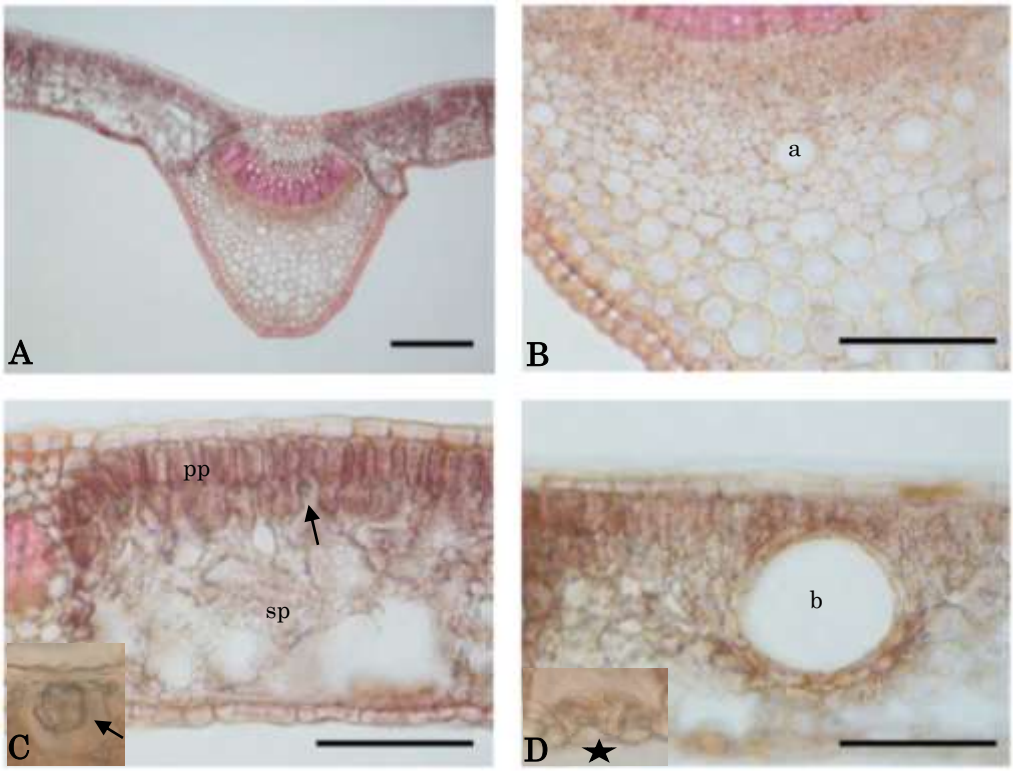
Şekil 4. *H. calycinum* türünün yaprak yüzeysel kesitleri (A,B: Yaprak üst yüzeyi, C-F: Yaprak alt yüzeyi) (ue:üst epidermis, le: alt epidermis, st:stoma). Bar:100 µm
Figure 4. Surface cross-sections of H. calycinum leaves (A, B: Upper leaf surface, C, D: Lower leaf surface) (ue: upper epidermis, le: lower epidermis, st: stoma). Bar: 100 µm.

Mezofilde sıralı bir şekilde şeffaf gland tespit edilmiştir (Şekil 6 D, Çizelge 5). *H. thymopsis* türünde ise iletim demetinde floem üzerinde A tipi, mezofilde B tipi salgı kanallarının varlığı rapor edilmiştir (Tekin, 2017).

Çizelge 3. İncelenen *H. calycinum* türünün yaprak yüzeysel anatomik boyutları
Table 3. Surface anatomical dimensions of the leaves of the studied H. calycinum species

	$\bar{X}$ ±S.sapma
Üst epidermis en	23.40 ± 4.96
Üst epidermis boy	20.53 ± 3.93
Alt epidermis en	17.85 ± 4.01
Alt epidermis boy	20.89 ± 5.82
Alt yüzey stoma en	21.85 ± 3.34
Alt yüzey stoma boy	23.04 ± 2.74

$\bar{X}$: Ortalama(µm), S.sapma :Standart sapma

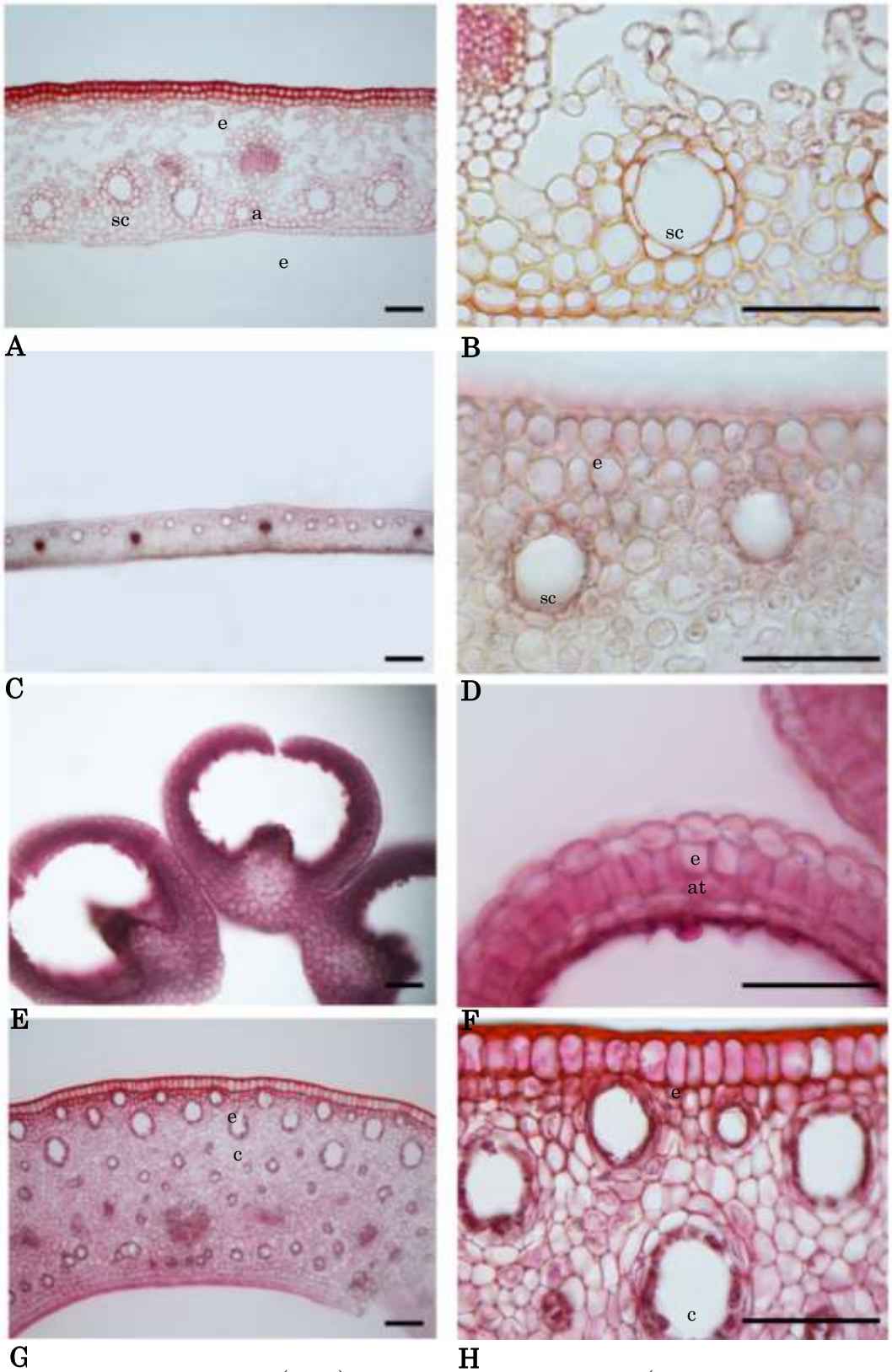


Şekil 5. *H. calycinum* türünün yaprak enine kesitleri (ok: druz kristali, yıldız: kseromorf stoma) (a: A tipi salgı kanalı, b: B tipi salgı kanalı, sp: sünger parankiması, pp: palizat parankiması). Bar:100 µm
Figure 5. Cross-sections of H. calycinum leaves (arrow: druse crystal, star: kseromorph stoma) (a: type A secretory duct, b: type B secretory duct, sp: sponge parenchyma, pp: palisate parenchyma). Bar: 100 µm.

Çizelge 4. İncelenen *H. calycinum* türünün yaprak enine anatomik boyutları
Table 4. Anatomical dimensions of the leaf cross-section of the studied H. calycinum species

	$\bar{X}$ ±S.sapma
Üst epidermis en	22.39 ± 7.06
Üst epidermis boy	10.30 ± 2.31
Alt epidermis en	16.93 ± 4.26
Alt epidermis boy	9.16 ± 2.36
Palizat en	12.17 ± 1.83
Palizat boy	26.70 ± 3.89
Sünger en	15.22 ± 4.59
Sünger boy	18.74 ± 6.79
Salgı kanal çapı	86.50 ± 7.40
Floem en	4.09 ± 0.96
Floem boy	3.92 ± 0.96
Ksilem çap	11.63 ± 2.55
Korteks çap	27.19 ± 14.22

$\bar{X}$: Ortalama(µm), S.sapma :Standart sapma



Şekil 6. *H. calycinum* türünün generatif (çiçek) organlarının enine kesitleri (A.B: Sepal, C,D: Petal, E,F: Anter, G,H: Ovaryum) (a: A tipi salgı kanalı, b: B tipi salgı kanalı, c: C tipi salgı kanalı, at: ara tabaka, e: epidermis, sc: şeffaf gland). Bar:100 µm

Figure 6. Cross-sections of the generative (flower) organs of H. calycinum (A, B: Sepal, C, D: Petal, E, F: Anther, G, H: Ovary) (a: type A secretory duct, b: type B secretory duct, c: type C secretory duct, e: epidermis, sc: secretory pocket). Bar: 100 µm.

Çizelge 5. İncelenen *H. calycinum* türünün Generatif (Çiçek) organlarının anatomik boyutları
Table 5. Anatomical dimensions of the generative (flower) organs of the studied H. calycinum species

	$\bar{X}$ ±S.sapma
Sepal üst epidermis en	22.49 ± 5.61
Sepal üst epidermis boy	14.64 ± 2.24
Sepal alt epidermis en	18.39 ± 4.77
Sepal alt epidermis boy	11.67 ± 1.91
Sepal salgı kanalı çap	66.12 ± 12.66
Sepal sünger parankiması çap	19.25 ± 5.44
Sepal floem en	6.00 ± 1.37
Sepal floem boy	4.53 ± 1.41
Sepal ksilem çap	4.61 ± 1.15
Petal üst epidermis en	23.29 ± 3.60
Petal üst epidermis boy	29.00 ± 5.82
Petal alt epidermis en	19.27 ± 4.36
Petal alt epidermis boy	16.14 ± 4.71
Petal sünger parankiması çap	16.40 ± 3.05
Petal Salgı kanalı çap	48.62 ± 11.63
Anter epidermis en	28.08 ± 4.55
Anter epidermis boy	18.18 ± 3.37
Anter polen çap	17.63 ± 1.56
Ovaryum epidermis en	13.89 ± 2.30
Ovaryum epidermis boy	27.46 ± 3.17
Ovaryum korteks çap	18.45 ± 3.50
Ovaryum salgı kanalı çap	51.38 ± 16.33

$\bar{X}$: Ortalama(µm), S.sapma :Standart sapma

Anter Anatomik Yapısı

Anter yapısına bakıldığında, 3 tekadan oluştuğu, epidermis, endotesyum ve ara tabakanın görülebildiği, tapetum'un mevcut olmadığı ve polenlerin 3 apertürlü olduğu tespit edilmiştir (Şekil 6, Çizelge 5). *H. orientale*, *H. bithynicum* ve *H. origanifolium* var. *origanifolium* taksonlarının anterlerinin 2 teka'dan oluştuğu ve konektif kısmında da druz kristalleri bulunduğunu belirtmiştir (Altıntaş, 2015).

Ovaryum Anatomik Yapısı

Ovaryumun üst yüzeyinde tek sıralı epidermis yer almakta, aksilar plasentalanma ve 5 karpelli olduğu gözlemlenmiştir. *H. orientale*, *H. bithynicum* ve *H. origanifolium* var. *origanifolium*, *H. androsaemum*, *H. linarioides* ssp. *linarioides*, *H. lydium*, *H. montanum*, *H. perforatum* ve *H. pruinatum* taksonlarının hepsinde karpel sayısının 3 olduğu görülmüştür (Altıntaş, 2015; Altıntaş, 2024). Karpel hücrelerinde yoğun bir şekilde C tipi salgı kanalları ve iletim demetlerinin floem üzerinde A tipi salgı kanalı tespit edilmiştir. Ovül tipi anatrop olarak belirlenmiştir (Şekil 6, Çizelge 5). Altıntaş (2015) çalışmış olduğu *H. orientale*, *H. bithynicum* ve *H. origanifolium* var. *origanifolium* taksonlarından sadece *H. origanifolium* var. *origanifolium*'un ovaryumunda salgı kanalına rastlanmadığını diğerlerinde ise C tipi salgı kanalına rastlandığını ifade etmiştir. *H. thymopsis* türünün ovaryumun karpel hücrelerinde tek sıralı C tipi salgı kanalı bulunduğu ifade edilmiştir (Tekin, 2017).

SONUÇ ve ÖNERİLER

Bu çalışmada, *H. calycinum* türünün anatomik özellikleri detaylı bir şekilde incelenmiştir. Bitkinin gövde kanat sayısının 4 olması, daha önemlisi gövde kanatlarında ve öz bölgesinde salgı ceplerinin varlığı, ayrıca gövdenin boyuna kesiti de incelendiğinde stoma varlığının tespit edilmesi de daha önce anatomisi çalışılmış olan diğer türlerden ayırt edilmesinde yardımcı olmaktadır (Şekil 3 A-H). Yaprakların hipostomatik (Şekil 4 A-D), mezofil tipinin bifasiyal fakat, palizat parankimasının iki sıralı olması, palizat parankimasında druz kristallerinin varlığı dikkat çekmektedir (Şekil 5 A-D). Çiçek kısımları (sepal ve petal)' nda şeffaf gland'ın varlığı, ayrıca sepal'in üst yüzeyinde iki sıralı epidermisin varlığı, Anter'in 3 teka'dan oluşması, ovaryum'da daha önce çalışılan diğer türlerde tek sıralı C tipi salgı kanalı bulunurken bu türde çok yoğun bir şekilde bulunması diğer türlerden ayıran özelliklerden olduğu görülmektedir (Şekil 6 A-H). Elde edilen bulgular, bu türlerin bitki anatomisi ve sistematiği açısından önemli veriler sağladığı görülmektedir. Gövde enine kesitinde gözlemlenen kanat sayısı ve kanat uzunluğu, kanat'ta salgı yapısının bulunması vb. açılarından türler arasında farklılıklar göstermektedir. Özellikle *H. tetrapterum* ve *H. androsaemum* türlerinde bulunan salgı cepleri, salgı kanal tipi bu gruptaki bitkilerin

anatomik özelliklerinin belirlenmesine katkı sağlamaktadır (Yağan ve ark., 2012; Altıntaş, 2024). Yaprak yapısında, hipostomatik özellikler ve stomaların çeşitliliği, *H. scabrum* türünde amfistomatik özelliklerle karşılaştırıldığında farklı morfolojik adaptasyonların varlığına işaret etmekte olduğu ifade edilmiştir (Ergin ve ark., 2022). Yaprak mezofilinin bifasiyal yapıda olması, *H. perforatum* gibi türlerde görülen ekvifasiyal özellikler ile karşılaştırıldığında, bitkilerin farklı ekolojik koşullara yanıt verme biçimlerini ortaya koymaktadır (Gürhan & Arabacı, 2022; Altıntaş, 2024). Çiçek kısımlarının da incelenmesi farklı epidermis yapılarına ve salgı yapılarının farklılıklarına dikkat çekmekte ve bunların ortaya konulması sayesinde taksonların taksonomik sınıflandırılmasına katkıda bulunmaktadır (Altıntaş, 2015).

Genel olarak, bu çalışma, *H. calycinum* türünün vegetatif organlarının yanısıra generatif organlarının anatomik farklılıklarını belirleyerek, sonraki yapılacak olan çalışmalarda taksonomik sınıflandırmanın da daha doğru bir şekilde yapılması için yardımcı olması beklenmektedir. Sonuç olarak, bitki taksonlarının özellikle de generatif kısımlarının da incelenmesi, bitki sistematiği ve bitkiler hakkında daha derin bilgi sahibi olunması açısından ve gelecekte yapılacak olan araştırmalar için de ışık tutacaktır.

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Distribution of *Crocosmia x crocosmiiflora* (Iridaceae) Outside of Parks and Graveyards in NE Anatolia (Türkiye)

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ABSTRACT
Using plants as ornamentals is one of the most important ways of introduction to the new ecosystems for alien species. *Crorocosmia x crocosmiiflora* seems to be a good example of this situation in Eastern Black Sea Region of Türkiye where it has been planted in many parks and graveyards as an ornamental plant. This hybrid taxon was observed in 37 different locations, outside of parks and graveyards in Artvin (5 records), Rize (17 records), Trabzon (8 records) and Giresun (7 records) cities, mainly on roadsides, fields, thickets, forest margins and waste areas during the field studies on Bur Cucumber. Present observations revealed that Montbretia clearly escaped from cultivation area and continue to increase its distribution range and population density/number in the North East Anatolia. Like other naturalized alien species in Türkiye, Montbretia is rapidly naturalizing in the Eastern Black Sea Region, which is the most important naturalization center of exotic plants in Türkiye, so it will become a part of the wild flora of the this region in the near future.

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KD Anadolu (Türkiye)'da *Crocosmia x crocosmiiflora* (Iridaceae)'nın Park ve Mezarlıklar Dışındaki Dağılımı

ÖZET
Bitkilerin süs bitkisi olarak kullanılması, yabancı türlerin yeni ekosistemlere girişinin en önemli yollarından biridir. *Crorocosmia x crocosmiiflora*, birçok park ve mezarlıkta süs bitkisi olarak yetiştirildiği Türkiye'nin Doğu Karadeniz Bölümü'nde bu duruma iyi bir örnek olarak görünmektedir. Bu hibrit takson, İtdolanbacı türü üzerine yapılan arazi çalışmaları sırasında, mezarlık ve park alanları dışında, Artvin (5 kayıt), Rize (17 kayıt), Trabzon (8 kayıt) ve Giresun (7 kayıt) illerinden toplam 37 lokasyonda, çoğunlukla yol kenarları, tarla kenarları, çalılıklar, orman kenarları ve boş alanlarda gözlenmiştir. Mevcut gözlemler, Afrika Yıldızı'nın kültürden açıkça kaçtığını ve Kuzey Doğu Anadolu'da yayılış alanını ve popülasyon yoğunluğu/sayısını artırmaya devam ettirdiğini ortaya koydu. Türkiye'deki yabancı türlerin öncelikli doğallaşma merkezi olan bu bölgede, diğer birçok yabancı tür gibi Afrika Yıldızı da yakın gelecekte bölgenin doğal florasının bir parçası olma yolundadır.

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INTRODUCTION
Today, 3.9% of the world's plants have naturalized outside their natural habitats as a result of human mobility and activities (van Kleunen et al., 2015). Using plant species for ornamental purposes is one of the most important reasons of naturalization. Due to heavy rainfall throughout the year, North East Anatolia is home to many of ornamentally used alien genera (i.e. Duman & Güner, 1996) and species (i.e. Terzioğlu & Coşkunçelebi, 2017;

Terzioğlu & Coşkunçelebi, 2022; Coşkunçelebi & Terzioğlu, 2022). It is reported that the total number of vascular plant taxa of Türkiye is 12354 (Terzioğlu et al., 2021) of which the 340 alien ones introduced to Türkiye (Uludağ et al., 2017). However, the compilation studies carried out within the scope of the TERIAS project (final report) show that this number is over 400 (Terzioğlu & Coşkunçelebi, 2020).

Crocosmia x crocosmiiflora (Lemoine) N.E.Br. (Montbretia, Iridaceae) called as "Mezarlık çiçeği/Graveyard's flower Turkish/English" by local people is a well-known garden hybrid flower (Nelson, 1993). Although the parent species of this horticultural originated taxon are native to South Africa, *C. x crocosmiiflora* is recorded as an introduced plant in many countries (POWO, 2024; Figure 1). In the world, it has been widely used as an ornamental plants and has become an invasive species in many countries by escaping into nature (CABI, 2024). Montbretia is firstly planted as an ornamental plant in graveyards and parks due to its attractive flowers since an unknown date in North East Anatolia. However, it is listed as an exotic plant by Ergül–Bozkurt (2017) and Terzioğlu (2022) depending on herbarium records and field observations without any further remarks. It is well known that the intentional or unintentional movement of such taxa by humans from one region to another accelerates the naturalization process of ornamental plants (Richardson, et al., 2000). Present observation during the field studies on invasive alien plant species in North East Anatolia revealed that Montbretia have many healthy populations outside of parks and graveyards such as in roadsides, fields, thickets, forest margins and waste areas and gained an invasive feature in this region. However, this hybrid is not listed in the vascular plant check list of Türkiye by Güner et al. (2012), alien flora of Türkiye by Uludağ et al. (2017) or any other relevance literatures, up to now.

In the present paper, several new localities outside of parks and graveyards of *Crocosmia x crocosmiiflora* in the Eastern Black Sea Region of Türkiye are recorded for the first time and discussed.

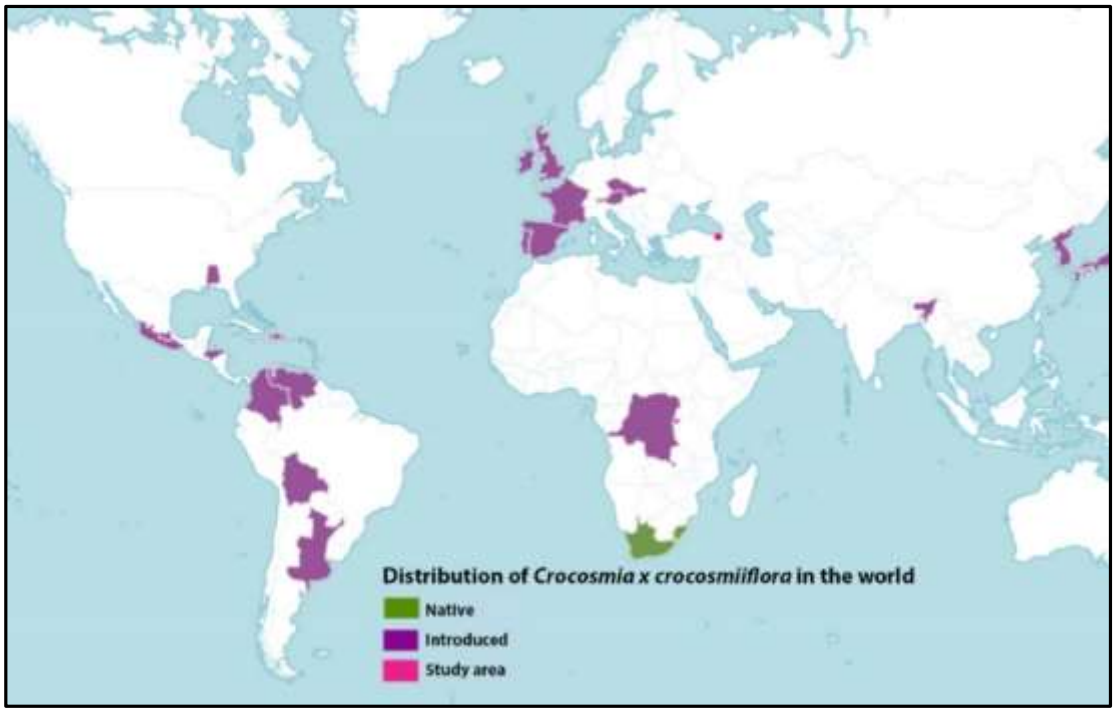

Figure 1. Distribution of *C. x crocosmiiflora* in the world (Modified from POWO, 2024).
Şekil 1. C. x crocosmiiflora'unın dünyadaki yayılışı (POWO, 2024'den uyarlanmıştır).

MATERIAL and METHODS

Crocossmia x crocosmiiflora was identified by using relevant literature (Goldblatt & Brown, 2002; Goldblatt & Manning, 2020; Żurawik et al, 2015; NRA, 2010) and samples of both KATO (21808!, 21952!, 24454!, 24455!, 24456!) and KTUB (Coşkunçelebi 1470!, 1471!) which stored in the herbarium of Forest Botany (KATO) and Biology (KTUB) at Karadeniz Technical University. As well, field observations and remarks of the present authors during the excursions on invasive plant species distributed in North East Anatolia were also used in identification. List of records belong to the Montbretia is ordered alphabetically by the names of the cities including the name of administrative area, the name of localities with GPS coordinates, altitudes and the date of collection/observation. The distribution map was prepared with the help of ArcGIS 10.5 program (Esri, 2014) based on the records mentioned in Ergül–Bozkurt (2017), Terzioğlu (2022) and observations in the current study. The hybrid taxon was named in Turkish according to guidelines of Menemen et al. (2016).

RESULTS and DISCUSSION

Crocosmia x crocosmiiflora (Lemoine) N. E. Brown, Trans. Roy. Soc. South Africa. 20: 264, 1932 (Figure 2).
Syn.: *Montbretia x crocosmiiflora* Lemoine, The Garden 18: 188 (21 August 1881) [as *M. crocosmiaeflora*]. *Tritonia x crocosmiiflora* (Lemoine) Nicholson, Illustrated Dictionary of Gardening 4: 94 (1888) [as *T. crocosmiflora*].
Type: Illustration in Morren, La Belgique Horticole 31: t. 14 (1881), neo., designated by Goldblatt et al. (2004).
The description of present alien hybrid was prepared based on fresh materials and herbarium specimens together with Goldblatt & Brown (2002) and Goldblatt & Manning (2020).
Stiff, leafy, clump–forming geophyte with underground rhizome, 35–100 cm. Rootstock a depressed-globose corm, 1.5–2.5 cm in diam., rooting from below. Stem laxly, 2–4-branched. Leaves 5–8, mostly basal, plane, narrowly lanceolate, ± 2⁄3 as long as stem, 8–20 mm wide, cauline narrower than basal. Spike lightly flexuose, mostly 6–10 flowered; bracts reddish with dry brown tips, 6–10 mm. Flowers distichous, bright orange, paler in throat, unscented; perianth tube funnel–shaped, slightly curved forward, 12 mm, tepal spreading, subequal, 15–22 mm. Filaments 15–22 mm, anthers 6–8 mm long, yellow. Style arching over stamens, dividing, branches ± 4 mm, apically bifid. Fruit an inflated globose, 3–lobed capsule, ca. 8 mm. Seeds blackish, ca. 2.5 mm diam., often aborted or ± globose, reddish brown.
Flowering period: July-September.
Habitat: Parks, graveyards, near sea coast, roadsides, thickets, wood margins and waste grounds.
Altitude: 4–577 m.
Vernacular (Turkish) name: Afrika yıldızı according to guidelines of Menemen et al. (2016).

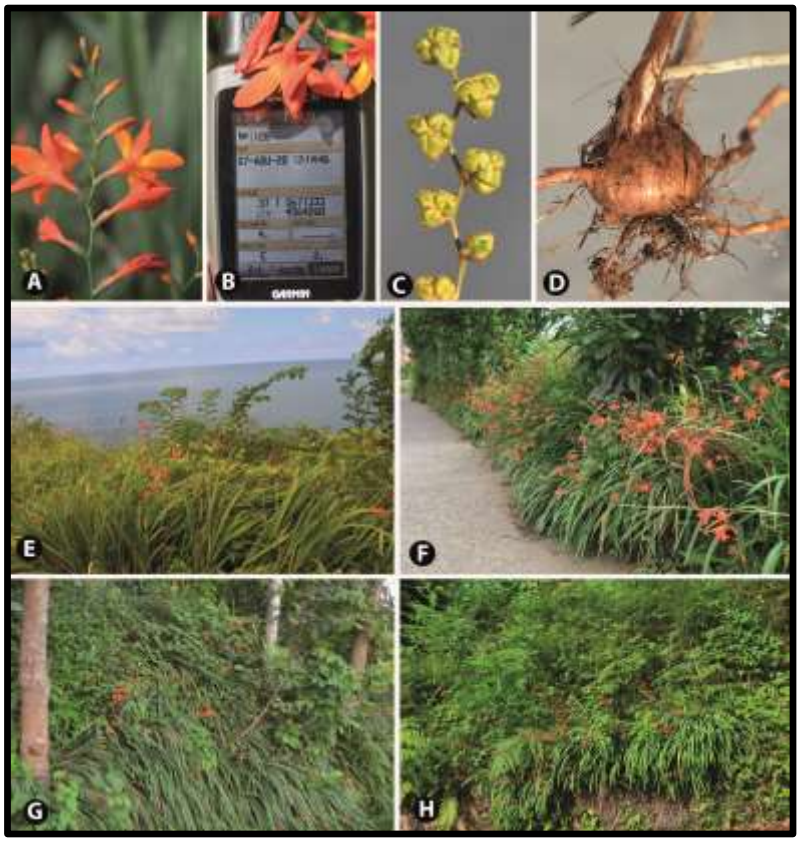

Figure 2. Some distinctive parts and habitat types of *C. x crocosmiifolia*; **A**–Inflorescence (Lightly flexuose spike), **B**–Coordinate information of the 13th record (Rize: Ardeşen, Işıklı), **C**–Fruit (Globose capsules), **D**–Rootstock (Rooted corm), **E**–**H**–Habitats (Sea coast, roadside, wood margin and waste ground, respectively).

Şekil 2. *C. x crocosmiifolia'nın bazı ayırt edici kısımları ve habitat tipleri;* ***A*** *- Çiçek kurulu (Gevşek–zigzag başak),* ***B****–13. Noktaya (Rize: Ardeşen, Işıklı) ait koordinat bilgisi,* ***C***–Meyve (*Yuvarlağımsı kapsüller),* ***D***–*Toprakaltı gövdesi (Köklenmiş kormus),* ***E***– ***H***–*Habitatlar (Sırasıyla; deniz kenarı, yol boyu, orman kenarı ve boş alan).*

The genus *Crocosmia* Planch. includes 8 species and one hybrid taxa, *C. x crocosmiifolia*, native to Sudan to S. Africa and Madagascar (POWO, 2024). It has been widely cultivated as an ornamental and has escaped from cultivation area (parks, graveyards, etc.) to field sides, disturbed sites, wasteland, along roadsides, and shrub lands

in many countries (Ensbey et al., 2014). A total of 39 locations were coordinated by GPS during the field study in Black Sea Region of Türkiye (Table 1, Figure 3). These findings confirmed that this hybrid taxon escaped from parks and graveyards to roadsides, thickets, sea coast, wood margins, field sides and waste grounds and going on to widen its distribution in Black Sea Region of Türkiye (Table 1, Figure 3). So, every new habitat it reaches is a potential expansion point for this taxon.

Table 1. Records of *Crocosmia x crocosmiiflora* in Eastern Black Sea Region of Türkiye
Çizelge 1. Türkiye'nin Doğu Karadeniz Bölümü'nde Crocosmia x crocosmiiflora kayıtları

No	Square (Davis, 1974) and Locality Information	Altitude (m)	Latitude	Longitude	Remarks
1	A8 Artvin, Hopa, Çamlı, Hopa-Sarp road, 07 August, 2020	6	41.372.486.439	41.342.479.343	NR*
2	A8 Artvin, Kemalpaşa, Sarp, 04 August, 2020	42	41.518.013.981	41.548.646.122	NR
3	A8 Artvin, Kemalpaşa, Kayaköy, 04 August, 2020	79	41.525.029.928	41.624.675.336	NR
4	A8 Artvin, Hopa, 15 June 2013	200	-	-	Ergül–Bozkurt (2017)
5	A8 Artvin, Kemalpaşa, Kayaköy, 04 August, 2020	305	41.504.271.178	41.563.190.466	NR
6	A8 Rize, Çamlıhemşin, Kavak, 18 August, 2020	344	41.052.362.452	41.037.985.002	NR
7	A8 Rize, Çamlıhemşin, Behice, Hincipici, 18 August, 2020	274	41.086.406.697	41.037.155.908	NR
8	A8 Rize, Kalkandere, Ünalan, 19 August, 2020	577	40.928.060.077	40.494.783.202	NR
9	A8 Rize, Merkez, Tekke, 19 August, 2020	201	40.954.852.400	40.532.291.834	NR
10	A8 Rize, İyidere,Hazar, 08 August, 2020	147	40.992.175.141	40.347.411.440	NR
11	A8 Rize, Çayeli, Yeşilköy, 05 August, 2020	14	41.023.629.628	40.691.158.492	NR
12	A8 Rize, Çayeli, Yaka, Hopa-Sarp road, 04 August, 2020	24	41.080.475.637	40.702.828.844	NR
13	A8 Rize, Ardeşen, Işıklı, 07 August, 2020	4	41.209.954.608	41.043.465.768	NR
14	A8 Rize, Ardeşen, Fırtına, 07 August, 2020	16	41.180.190.026	40.969.852.283	NR
15	A8 Rize, Ardeşen, Bayırcık, 07 August, 2020	322	41.125.113.012	41.087.334.392	NR
16	A8 Rize, Çayeli, Yenitepe, 07 August, 2020	186	41.046.717.364	40.726.301.393	NR
17	A8 Rize, Çayeli, Haremtepe, 07 August, 2020	231	41.043.065.475	40.728.359.355	NR
18	A8 Rize, Çayeli, Haremtepe, 07 August, 2020	235	41.041.184.589	40.730.047.097	NR
19	A8 Rize, Çayeli, Haremtepe, 07 August, 2020	247	41.040.908.754	40.730.420.575	NR
20	A8 Rize, Çayeli, Haremtepe, 07 August, 2020	269	4.103.682.587	40.736.571.024	NR
21	A8 Rize, Çayeli, Haremtepe, 07 August, 2020	371	41.035.278.658	40.732.842.619	NR
22	A8 Rize, Çayeli, Haremtepe, 07 August, 2020	413	41.032.022.422	40.732.543.101	NR
23	A8 Trabzon, Of, Sefaköy, 26 August, 2020	365	40.901.691.045	40.295.484.226	NR
24	A8 Trabzon, Sürmene, Çamburnu, Kuleli, 07 August, 2020	18	40.916.928.213	40.194.083.880	NR
25	A8 Trabzon, Sürmene, Çamburnu, Kutlular, 07 August 2020	52	40.908.066.915	40.203.363.869	NR
26	A8 Trabzon, Sürmene, Çamburnu, Kutlular, 07 August 2020	194	40.902.198.732	40.205.465.768	NR
27	A8 Trabzon, Sürmene, Çamburnu, Kutlular, 07 August 2020	189	40.902.132.803	40.204.016.136	NR
28	A8 Trabzon, Çaykara,2022	250	-	-	Terzioğlu (2022)

29	A8 Trabzon, Sürmene,16.07.2013	150	-	-	Ergül–Bozkurt (2017)
30	A8 Trabzon, Sürmene, Yeniay, Kumru, 07 August, 2020	124	40.911.733.175	40.192.090.544	NR
31	A7 Giresun, Eynesil, Ören, Camidüzü Mahallesi,11 August, 2020	289	41.032.518.202	39.148.425.712	NR
32	A7 Giresun, Eynesil, Çorapçılar, Derebaşı, 11 August, 2020	58	41.062.773.267	39.150.897.784	NR
33	A7 Giresun, Eynesil,Çorapçılar, 11 August, 2020	254	41.055.030.022	39.173.847.553	NR
34	A7 Giresun, Merkez, Yukarıalınlı, 12 August, 2020	550	40.858.664.334	38.366.928.912	NR
35	A7 Giresun, Merkez, Samanlıkkıranı, 12 August, 2020	90	40.890.750.513	38.419.732.441	NR
36	A7 Giresun, Keşap, Geçit, 12 August, 2020	731	32.289.255.469	82.097.187.668	NR
37	A7 Giresun, Tirebolu, Arageriş, Mınak, 12 August, 2020	155	40.951.717.075	38.766.344.261	NR

NR: New Record.*

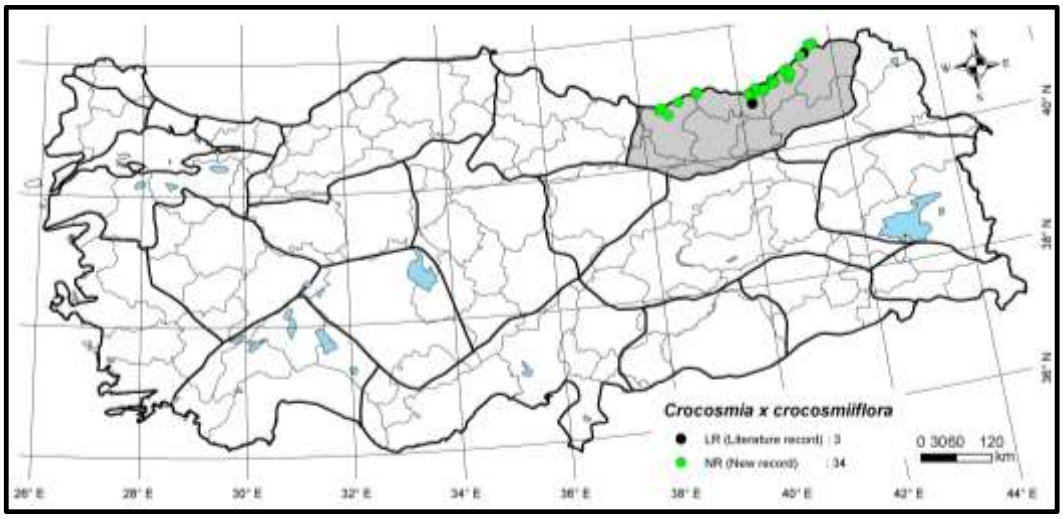

Figure 3. Distribution map of *Crocosmia x crocosmiiflora* in Eastern Black Sea Region of Türkiye.
Şekil 3. Crocosmia x crocosmiiflora'nın Türkiye'nin Doğu Karadeniz Bölümü'ndeki yayılış haritası.

Spread of alien species, especially invasive ones, considered the second most significant threat to biodiversity after habitat loss (Wilcove et al., 1998). Depending on the adaptation capacity, alien taxa spends decades in their newly introduced ecosystems, before becoming invasive according to their abilities. Thanks to their ecological adaptation capacities, they begin to change the species composition and some ecological conditions in these ecosystems (Reaser et al., 2020). *C. x crocosmiiflora* is a hybrid taxon that it is disease resistant, remarkably vigorous and easily propagated (Kumar et al., 2019). So, it remains widely cultivated today because it is so easy to grow, is undemanding of care and soil, and thrives in a wide range of habitats and climate zones (Goldblatt & Brown, 2020). Its rapid vegetative reproduction capacity, it persists in abandoned gardens and slowly spreads into meadows, roadsides and eventually into undisturbed vegetation, and it in order to escape from the parks and graveyards in Türkiye. This rapid spreading is estimated that this taxon will be a serious weed in Türkiye starting from Black Sea Region. Although Montbretia could be confused with equitant leaved *Iris* taxa distributed in this region before blooming, it is easy to distinguish from other taxa by the distinct shape and attractive orange scarlet flowers in flowering stage.

Although this hybrid taxon was carried to Türkiye as an ornamental plant, this study underlined that it has started to spread out of control outside parks and graveyards in NE Anatolia. It has also been observed that this hybrid formed dense populations over the years in this region. This is because it should be monitored regularly and if necessary physical/mechanical control should be conducted. It is reported that the most practical way of preventing and managing invasions is to provide detailed information about introduction routes and vectors. This information also is necessary both to prevent the escape of alien species to natural habitats and to prevent their further spread.

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The authors declare the contribution of the authors is equal.

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The authors have declared no conflict of interest

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Investigation of the flora, general vegetation structure, and EUNIS habitat types of some natural sites areas in Gülnar and Silifke (Mersin-Türkiye)

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ABSTRACT
The aim of this study was to determine the floristic characteristics, EUNIS habitat types, and general vegetation structure of Çağlayan, Ilısu, and Yerköprü Waterfalls, Göksu Delta, Narlıkuyu, Roman Ruins, Şeytanderesi, and Cambazlı Cistern and Akdere Tahta Port natural site areas in Gülnar and Silifke districts of Mersin (Türkiye). According to the findings, 214 different taxa belonging to 65 families and 173 genera were identified. A total of 31 taxa were identified in Çağlayan Waterfall, 32 in Ilısu Waterfall, 54 in Yerköprü Waterfall, 62 in Göksu Delta, 47 in Narlıkuyu, 26 in Roman Ruins, 63 in Akdere Tahta Port, 84 in Şeytanderesi, and Cambazlı Cistern. A total of 8 (3.66%) endemic plant taxa were identified in the research areas. According to the phytogeographic regions, 77 taxa are Mediterranean (35.32%), 11 taxa are Euro-Siberian (5.04%), 7 taxa are Irano-Turanian (3.21%), 45 taxa are widely distributed (20.64%), and 76 taxa are of unknown phytogeographic region (35.77%). In this study, 9 main habitats and 22 sub-habitat types were identified. In terms of general vegetation structure, characteristic species of *Quercetea ilicis* and *Cisto-Micromerietea* syntaxonomic classes were found in all study areas. Character species of *Quercetea pubescentis* class were observed in all areas except Göksu Delta, Şeytan Creek, and Cambazlı Cistern. In Şeytanderesi and Cambazlı Cistern, and Akdere Tahta Port, character species belonging to the *Querco-Fagetea* syntaxonomy class were found differently from the others. These results contribute to the flora and vegetation literature by determining the flora of Mersin province, determining the EUNIS habitat types of natural sites in this country, and determining the general vegetation structure of the research area.

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ÖZET
Bu araştırma Mersin (Türkiye) ili Gülnar ve Silifke ilçelerinde bulunan Çağlayan, Ilısu ve Yerköprü Şelalesi, Göksu Deltası, Narlıkuyu, Roma Kalıntıları, Şeytanderesi ve Cambazlı Sarnıcı ile Akdere Tahta Limanı doğal sit alanlarının floristik özelliklerini, EUNIS habitat tiplerini ve genel vejetasyon yapısını belirlemek amacıyla gerçekleştirilmiştir. Araştırmada toplam 65 familya ve 173 cinse ait 214 farklı takson tespit edilmiştir. Çağlayan Şelalesi'nde 31, Ilısu Şelalesi'nde 32, Yerköprü Şelalesi'nde 54, Göksu Deltasında 62, Narlıkuyuda 47, Roma Kalıntılarında 26, Akdere Tahta Limanında 63, Şeytanderesi ve Cambazlı Sarnıcında 84 takson belirlenmiştir. Bu araştırmada toplam 8 adet (%3.66) endemik bitki taksonu tespit edilmiştir. Fitocoğrafik bölgelere göre taksonların 77 taksonun Akdeniz (%35.32), 11 taksonun Avrupa-Sibirya (%5.04), 7 taksonun İran-Turan (%3.21) elementi, 45 taksonun Geniş yayılışlı (%20.64) ve 76 taksonun fitocoğrafik bölgesi belli

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olmayan (%35.77) şeklinde dağılım gösterdiği belirlenmiştir. Bu çalışmada 9 ana habitat, 22 alt habitat tipi tanımlanmıştır. Genel vejetasyon yapısı itibariyle çalışma alanlarının tamamında *Quercetea ilicis* ve *Cisto-Micromerietea* sintaksonomik sınıflarının karakter türlerine rastlanılmıştır. Göksu Deltası ile Şeytan Deresi ve Cambazlı Sarnıcı hariç diğer tüm alanlarda *Quercetea pubescentis* sınıfının karakter türleri gözlemlenmiştir. Şeytan Deresi ve Cambazlı Sarnıcı ile Akdere Tahta Limanında diğerlerinden farklı olarak *Querco-Fagetea* sintaksonomi sınıfına ait karakter türler bulunduğu belirlenmiştir. Bu sonuçlar Mersin ili florası, ülkemizdeki doğal sitlerin EUNIS habitat tiplerinin ve araştırma alanının genel vejetasyon yapısının belirlenmesi ile flora ve vejetasyon literatürüne katkı sağlamaktadır.

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INTRODUCTION

Türkiye is one of the significant plant diversity regions in the world in terms of its plant taxa and the total number of endemic plant species it contains (Avcı,1993). Due to the diverse climatic, edaphic, and geographical features among its regions, Türkiye exhibits a rich plant diversity (Davis, 1965-1985; Davis et al., 1988; Güner et al., 2000; Davis & Hedge, 1975). In the flora of Türkiye, 3,649 taxa (31.82%) out of 11,707 are endemic (Güner et al., 2012). The significance and diversity of Turkey's flora become clearer when compared to the overall number of plant species across the entire European continent. The first studies on Turkish plants began in the early 1700s, carried out by Tournefort (Baytop, 2010). The most extensive research was conducted by Davis (1965-1985; Davis et al., 1988). Subsequently, Güner et al. (2012) published a list of the plants of Türkiye, and 6 volumes of the Illustrated Flora of Turkey have been published since 2014 (Güner & Ekim, 2014; Güner et al., 2018; 2022; 2023a; 2023b; 2024).

The study areas in this research are eight natural sites in Mersin province, in the Mediterranean region of Türkiye. The definition of a Natural site is as follows: "Areas belonging to geological epochs, possessing extraordinary features due to their rarity, located on the surface, underground, or underwater, and requiring preservation." (Anonymous, 1983). Natural sites, defined as first, second, and third-degree natural sites during the dates of the conducted research, have been redefined into three categories with the regulation published in 2022 (Anonymous, 2022). According to the redefinition in Anonymous (2022), they are now categorized as 1- Areas of Absolute Protection, 2- Areas of Qualified Natural Conservation, and 3- Areas of Sustainable Conservation and Controlled Use. Areas of Absolute Protection are land, water, and marine areas where all impacts related to use and access are restricted to ensure the preservation of resource values. In these areas, human entry may be prevented, and special measures may be taken for scientific research, education, or environmental monitoring purposes. Qualified Natural Conservation Areas are land, water, and marine areas characterized by an unchanged natural structure, minimal impact from human activities, dominance of natural processes, and the preservation of traditional ways of life based on the natural environment. These areas allow residents to utilize existing resources in line with conservation goals while safeguarding the traditional lifestyles dependent on the natural habitat. On the other hand, areas of Sustainable Conservation and Controlled Use are areas where low-intensity activities, wind and solar energy facilities, tourism, settlements, and livestock are permitted while considering the region's natural structure, ecological values, and natural landscape features.

The study areas are situated in the Mediterranean phytogeographic region of Türkiye. While the Irano-Turanian phytogeographic region spans a larger area than other regions, the Mediterranean phytogeographic region has the highest concentration of endemic plant species in Turkey, with 3,321 endemic plant locations (34.3%) (Avcı, 1993; Güner et al., 2012; Şenkul & Kaya, 2017). Despite being a rich region in terms of plant diversity and endemic plants, there are many areas in the Mediterranean region where flora and vegetation studies have not been conducted. Floristic studies carried out in the natural protected areas of this region are also scarce (Tel et al., 2022a).

Identifying the floristic characteristics of the area will enhance understanding of the floristic structure flora of Türkiye and the Mediterranean region. Floristic studies have been carried out by Gemici (1992), Tezcan (1995), Düzenli et al. (1996a), Düzenli et al. (1996b), Savran et al. (1999), Ekim et al. (2000), Zeren & İspirgil (2001), Uçar

(2002), Everest & Rauss (2004), Orcan et al. (2004), Aksay (2006), Karaömerlioğlu & Düzenli (2008), Dinç (2008), Yıldızbakan et al. (2010), Yıldıztugay & Küçüködük (2010), Şirin & Ertuğrul (2015), Şen (2019), Topal et al. (2022) in the surrounding area of the study site. In addition to these, floristic and vegetation-oriented studies have been conducted in Natural and Cultural sites by Tel (2009), Tel et al. (2010), Tel & Tak (2012), Tel & İlçim (2016), Tel & Eğilmez (2015), Anonymous (2016), Ortaç (2017), Tel & Tak (2018), Tel et al. (2018), Tel et al. (2019), Ortaç & Tel (2021), Tel et al. (2021), Tel et al. (2022a; 2022b), Tel et al. (2023), Tel et al. (2024), Tel et al. (2025).

There is no study on habitat classification in the research area. Habitat classification is known to be a very important issue for the sustainability of natural resources (Moss & Roy, 1998). The European Union has established the European Nature Information System (EUNIS) (Anonymous, 2024a; Davies et al., 2004) to utilize existing natural resources better, compare different habitat types, analyze habitats in more detail, create a common classification system, and build a database. Today, species and habitats are constantly threatened by extinction due to intensive use (Arslan et al., 2012). Therefore, the EUNIS classification system records habitat data comparably and provides a reference for the conservation of natural resources. Davies et al. (2004) ranked EUNIS habitat types hierarchically. The system is currently organized into 10 main categories and their subheadings. A: Marine habitats, B: Coastal habitats, C: Inland surface waters, D: Mires, bogs and fens, E: Grasslands and lands dominated by forbs, mosses or lichens, F: Heathland, scrub and tundra, G: Woodland, forest and other wooded land, H: Inland unvegetated or sparsely vegetated habitats, I: Regularly or recently cultivated agricultural, horticultural and domestic habitats, J: Constructed, industrial and other artificial habitats (Eunis habitat type hierarchical view (Davies et al., 2004).

The European Union has established the European Nature Information System (EUNIS) (Davies et al., 2004; Anonymous, 2025) to utilize existing natural resources better, compare different habitat types, analyze habitats in more detail, create a common classification system, and build a database. Today, species and habitats are constantly threatened by extinction due to intensive use (Arslan et al., 2012). Therefore, the EUNIS classification system records habitat data comparably and provides a reference for the conservation of natural resources. Davies et al. (2004) and Anonymous (2025) ranked EUNIS habitat types hierarchically. It is necessary to precisely determine the legally binding protected habitat types in EUNIS (Arslan et al. 2012). In Türkiye, although not at the habitat type level, some species or specific plant communities are protected on-site in areas with conservation statuses such as national parks, nature conservation areas, genetic conservation forests, research forests, etc. (Arslan et al. 2012).

Most countries have not yet fully developed the EUNIS habitat classification and generally use their own habitat classification types on a country-by-country basis. Turkey is ahead of other countries in this context and has reached the stage of determining EUNIS habitat types with the National Biodiversity Inventory and Monitoring Project (Terzioğlu et al., 2015).

In this way, one or more habitat types are preserved in these areas (Arslan, 2012). The eight areas in this study are natural sites with conservation status. Studies on habitat classification in Turkey are few and include Karaömerlioğlu (2007), Karaömerlioğlu & Düzenli (2008), Arslan & Arslantürk (2009), Arslan et al. (2014), Ulu et al. (2014), Mergen & Karacaoğlu (2015), Çiftçi (2015), (Terzioğlu et al., 2015), Erdoğan (2016), Geven et al. (2016), Şahin & Karavelioğlu (2018a), Şahin & Karavelioğlu (2018b), Tug et al. (2018), Seyfe (2019), Özen & Ürker (2020), Çakmak & Aytaç (2020), Çakmak & Aytaç (2021) and Demir et al. (2022).

This study aims to identify the floristic characteristics, EUNIS habitat types, IUCN threat categories of endemic taxa, and the general vegetation structure of the natural protected areas in the Çağlayan, Ilısu, and Yerköprü Waterfalls, Göksu Delta, Narlıkuyu, Roman Ruins, Şeytanderesi, and Cambazlı Cistern, and Akdere Tahta Port, located in the Gülnar and Silifke districts of Mersin province, Turkey. In this study, the general vegetation characteristics and Eunis habitat types of the study areas were revealed. The study areas are protected areas as they have natural protected status and provide information about the general vegetation and floristic structure of the region.

The Study Area and Its General Characteristics

The study areas in this research include eight localities located in the districts of Gülnar and Silifke within the Mersin province, situated in the Mediterranean region of Turkey. These localities include Çağlayan, Ilısu, and Yer Köprü Waterfall, Göksu Delta, Narlıkuyu, Roman Ruins, Şeytanderesi, and Cambazlı Cistern, as well as Akdere Tahta Port (Table 1). All of these areas are designated as natural sites.

The Çağlayan Waterfall is located in the Gülnar district and is designated as a third-degree natural site. It covers an area of 0.8 hectares. The prominent natural landscape elements in the area include the Çağlayan waterfall, which gives the region its name, and the surroundings. Within the natural site, the waterfall occupies 0.59 hectares, 0.34 hectares of forested areas, and 0.25 hectares are designated as irrigated agricultural land. The Ilısu

Waterfall is situated in the Gülnar district of Mersin. It has been classified as a third-degree natural site and spans an area of 98.2 hectares (Anonymous, 2016). Although the area in the Gülnar district exhibits natural landscape characteristics, the presence of an energy facility and associated structures has led to changes in this natural landscape. The study area has a unique geomorphology due to the waterfalls and features of prominent hills. Limestone rocks are abundant in the hills in and around the study area. The study area has an elevation ranging from 700 to 900 meters. Yerköprü Waterfall is located at the Mersin province intersection of Mut and Gülnar districts. It spans an area of 204.6 hectares and is distinguished by mainly mountainous, rocky landscapes, with some occasional flat areas. The Mediterranean climate primarily influences the study area. Geomorphologically, it is situated within a valley with a length of 2.1 km. Of the total area of Yerköprü Waterfall, 188.35 hectares consist of bare rock and debris, 26.33 hectares are covered with scrubland, 6.3 hectares include rivers and streams, 3.36 hectares are dry farming land, and 0.68 hectares are forested. The altitude of the study area is 650 meters (Anonymous, 2016).

Table 1. General characteristics, coordinates, and protection status of the research areas (Anonymous, 2016)
Çizelge 1. Araştırma alanlarının genel özellikleri, koordinatları ve koruma statüleri (Anonim, 2016)

No	Study area	Size (hectare)	Coordinates	Conservation Status
1	Çağlayan Waterfall	0.8	36º 09' 38.95" North 33º 33' 30.27" East	III. Degree Natural Site
2	Ilısu Waterfall	98.2	36º 33' 33.81" North 33º 04' 53.78" East	III. Degree Natural Site
3	Yerköprü Waterfall	204.6	36º 32' 31.41" North 33º 10' 54.10" East	Natural Site
4	Göksu Delta	5381,48	36º 19' 06.32" North 33º 53' 17.56" East	I and III. Degree Natural Site
5	Narlıkuyu	175,07	36º 26' 27.18" North 34º 06' 52.29" East	I.and III. Degree Natural Site
6	Roma Ruins	13,99	36º 22' 33.17" North 33º 55' 47.54" East	I.Degree Natural Site
7	Şeytanderesi and Cambazlı Cistern	813,19	36º 31' 26.27" North 34º 02' 58.98" East	I. Degree Natural Site
8	Akdere Tahta Port	345,91	36° 16′ 12.10″ North 33°48' 24.29" East	I and III. Degree Natural Site

The Göksu Delta lies to the south of the Silifke district in Mersin province, where the Göksu River meets the sea between Silifke and Taşucu. As a wetland, it is under protection and holds the status of Türkiye's first Ramsar site, as well as being designated as a Special Environmental Protection Area. The total area covered by Göksu Delta is 5381.48 hectares. Within this area, 118.63 hectares are comprised of lakes, 21.73 hectares are river floodplains, 1792.61 hectares are coastal dunes, 410.18 hectares are abandoned land, 19.7 hectares are designated as settlement areas, and 3018.63 hectares are used for irrigated agriculture (Anonymous, 2016). Narlıkuyu is a natural site designated as a first and second-degree area. The site covers an area of 175.07 hectares, including the coastal strip and the offshore Dana Island. Within the area, there are numerous coves and settlements of various sizes. The Narlıkuyu location has an approximate elevation of 50 meters and is composed of alluvial materials and limestone. The Roman Ruins are located in the district of Silifke. The Natural site covers an area of 13.99 hectares, including 0.82 hectares of scrubland and 13.17 hectares of dry farming land. It holds the designation of a first-degree natural site, and its altitude is 280 meters. Şeytanderesi and Cambazlı Cistern are located in the district of Silifke. The area covers a total of 813.19 hectares, comprising 677.26 hectares of bare rock and debris, 115.42 hectares of scrubland, 16.64 hectares of dry farming land, and 3.87 hectares of irrigated agricultural land. The area exhibits natural landscape characteristics, extending along a deep valley's approximately 26 km long riverbed. Cambazlı Cistern, located next to Şeytanderesi, shares similar habitat features. Akdere Tahta Port is in Silifke. The area possesses first and third-degree natural site characteristics, covering a total of 345.91 hectares. Within this area, 310.87 hectares of scrubland, 24.28 hectares are designated as forested land, and 10.76 hectares are allocated for irrigated agriculture. The site has an approximately 200-meter coastline (Anonymous, 2016).

The Climate Characteristics of Study Areas

The climate characteristics of the Silifke district

The Mediterranean climate prevails in the Silifke district. Hot and arid conditions characterize the summer season, while the winter season is mild and rainy. The climate changes as one moves inland from sea level (Anonymous, 2023c). The average annual temperature is 18.8°C, with the highest average temperature reaching 23.4°C and the lowest at 14.9°C. August is the hottest month, while January is the coldest. The yearly average precipitation totals 611.6 mm, with the dry season lasting from March to October. December, January, and February receive the most rainfall, whereas July has the least rain. The highest precipitation, with an average of 122 mm, is observed in December (Anonymous, 2023c). The Ombro-thermic climate diagram for the Silifke district is presented in Figure 1-2.

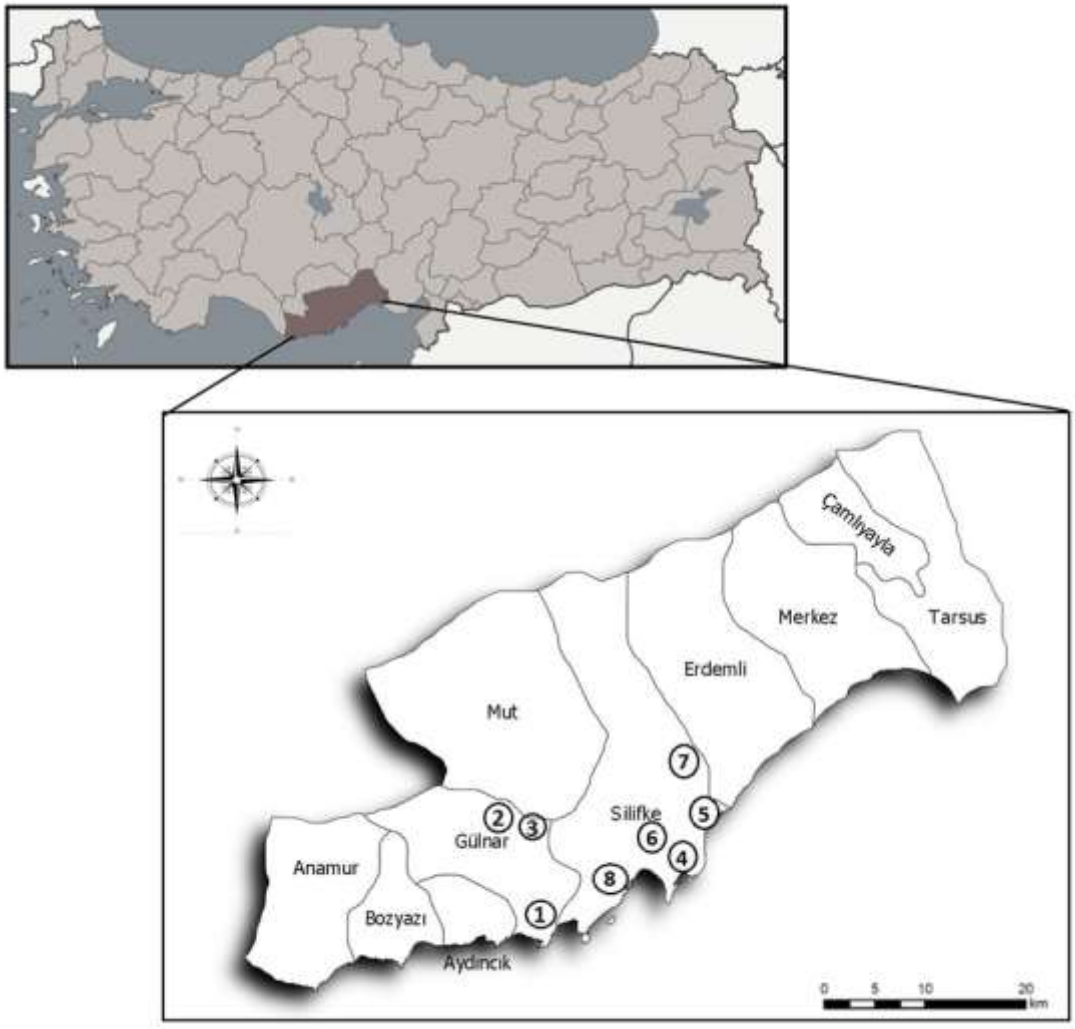

Figure 1. Study area location map 1. Çağlayan Waterfall, 2. Ilısu Waterfall, 3. Yerköprü Waterfall, 4. Göksu Delta, 5. Narlıkuyu, 6. Roma Ruins, 7. Şeytanderesi and Cambazlı Cistern, 8. Akdere Tahta Port. (Anonymous, 2023a; 2023b)

Şekil 1. Çalışma alanı konum haritası 1. Çağlayan Şelalesi, 2. Ilısu Şelalesi, 3. Yerköprü Şelalesi, 4. Göksu Deltası, 5. Narlıkuyu, 6. Roma Harabeleri, 7. Şeytanderesi ve Cambazlı Sarnıcı, 8. Akdere Tahta Limanı. (Anonim, 2023a; 2023b)

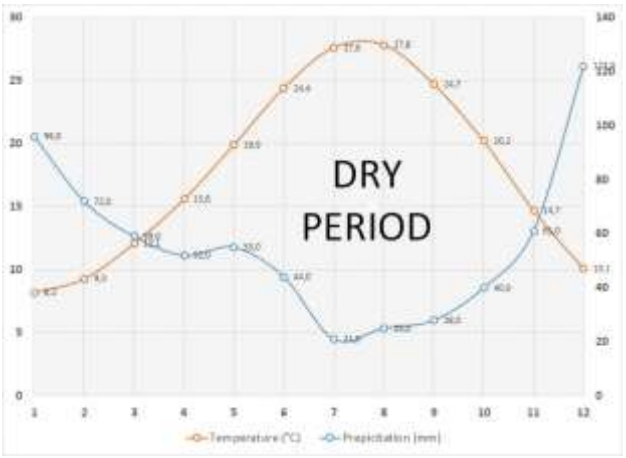

Figure 2. Ombro-thermic climate diagram of Silifke

Şekil 2. Silifke'nin ombro-termik iklim diyagramı

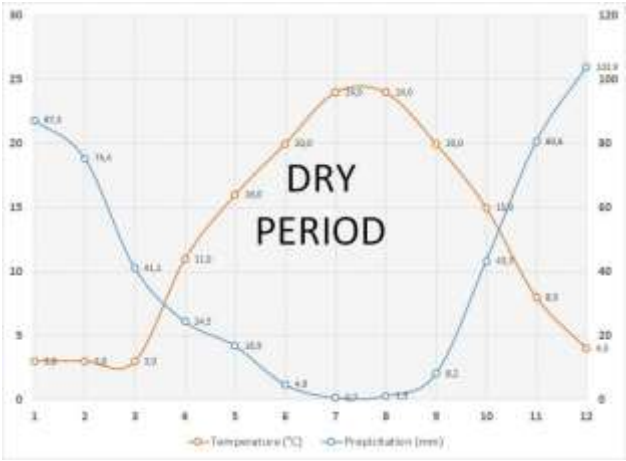

Figure 3. Ombro-thermic climate diagram of Gülnar

Şekil 3. Gülnar'ın ombro-termik iklim diyagramı

The climate characteristics of the Gülnar district

A Mediterranean climate prevails in the Gülnar district. In the higher elevations of the district, winters are cold and snowy, while summers are cool and rainy. Continental climate characteristics become more evident as we move towards the interior (Anonymous, 2023d). Throughout the year, temperatures range between -2 and 30°C, with daily average highs exceeding 26°C. The hottest month in Gülnar is July. The cold season begins at the end of November and lasts until mid-March, with January being the coldest month in Gülnar. The rainy season starts at the end of October and continues until the beginning of April (Anonymous, 2023d). The Ombro-thermic climate diagram for the Gülnar district is presented in Figure 3.

MATERIAL and METHOD

The data for this study includes plant samples gathered from the study areas between 2014 and 2016. The study areas where identified plant taxa were collected are depicted in Figure 1, and their general characteristics are provided in Table 1. Accordingly, the figures and tables refer to the following locations: (1) Çağlayan Waterfall, (2) Ilısu Waterfall, (3) Yerköprü Waterfall, (4) Göksu Delta, (5) Narlıkuyu, (6) Roman Ruins, (7) Şeytanderesi and Cambazlı Cistern, (8) Akdere Tahta Port. In the flora list (Appendix-1) and tables, the International Union for Conservation of Nature (IUCN) and the European Nature Information System (EUNIS) have been abbreviated.

Sampling Method

The study areas were in two different districts of Mersin province and were considered in the same study since their habitats and general vegetation structures were similar. The plants collected from the study area were dried, identified, and placed in the herbarium of Adıyaman University (Seçmen et al., 2000).

The Braun-Blanquet method was not used in the vegetation assessment of the study areas. As a result of the observations made and the evaluation of the plant samples collected, the general vegetation structure of the areas was determined based on observation. For Eunis habitat types, habitat types were observed in all study areas, and 1st and 2nd level habitat types were determined according to Anonymous (2024a).

Laboratory Analysis

The identification and diagnosis of the samples were based on The Flora of Turkey and the East Aegean Islands (Davis, 1965-1985; Davis et al., 1988; Güner et al., 2000; Güner & Ekim, 2014; Güner et al., 2018; 2022; 2023; 2024). Prof. Dr. Ahmet İlçim and Biologist Ergün Özuslu identified the plants that presented difficulties in diagnosis. Plant specimens are housed in the Adıyaman University Herbarium. Taxon names were assigned based on The International Plant Names Index (Anonymous, 2024b), The WFO PlantList (Anonymous, 2024c), Bizim Bitkiler (Anonymous, 2024d), and the Turkey Plant List Vascular Plants (Güner et al., 2012). The threat categories of endemic taxa were established based on Ekim et al. (2000), Güner et al. (2012), and the IUCN Red List (Anonymous, 2024e). Habitat types were determined by utilizing the EUNIS habitat type hierarchical view (Davies et al., 2004).

RESULTS and DISCUSSION

In the study areas, 214 distinct taxa (170 species, 29 subspecies, and 15 varieties) from 65 families and 173 genera were identified. Specifically, 31 taxa were identified at Çağlayan Waterfall, 32 at Ilısu Waterfall, 54 at Yer Köprü Waterfall, 62 at Göksu Delta, 47 at Narlıkuyu, 26 at Roman Ruins, and 63 at Akdere Tahta Port, while 84 taxa were determined at Şeytanderesi and Cambazlı Cistern. The distribution and percentages of identified taxa based on phytogeographic regions are presented in Table 2.

Table 2. Phytogeographic distributions and rates of taxa
Çizelge 2. Taksonların fitocoğrafi dağılımları ve oranları

Phytogeographic Region	Taxa Number	Rate (%)
Mediterranean	77	35.98
Euro-Siberian	11	5.14
Irano-Turanian	7	3.27
Widespread	45	21.02
Unknown	76	35.50

As a result of the separate evaluation of the study areas, a total of 31 taxa belonging to 23 families and 30 genera were identified at Çağlayan Waterfall. No taxa in the Natural Protected Area are considered as critical species (in

any endangered category). The surroundings of the waterfall have been converted into a garden, resulting in partial disruption of natural conditions. The area contains maquis and aquatic vegetation. The distribution of taxa across phytogeographic regions is as follows: 14 Mediterranean, 7 Widespread, 1 Irano-Turanian, and 9 taxa with unknown distribution. The taxa distribution across phytogeographic regions is as follows: 14 Mediterranean, 7 Widespread, 1 Irano-Turanian, and 9 taxa with an unidentified distribution. Regarding the number of taxa, the families are listed as follows: Fabaceae with 5, Asteraceae with 3, and Rosaceae with 2.

At Ilısu Waterfall, a total of 32 taxa belonging to 22 families and 31 genera were identified. The streambed surroundings consist of typical Mediterranean vegetation, comprising maquis elements and a mixture of *Pinus brutia* Ten. The waterfall, being relatively difficult to access and distant from residential areas, has preserved its natural state. The phytogeographic regions of the identified taxa include 13 Mediterranean, 7 Widespread, 1 Irano-Turanian, 1 Euro-Siberian, and 10 taxa with unknown distribution. The families are listed according to the number of species and subspecies as follows: Poaceae with 5, Lamiaceae with 3, and Brassicaceae with 2. No endemic taxa were found in the study area.

A total of 54 taxa from 37 families and 52 genera were identified at Yerköprü Waterfall. The research area and its surroundings are characterized by forested, shrubland, aquatic, and rocky habitats. The area hosts typical Mediterranean phytogeographic region plant species such as *Pinus brutia* Ten. Forest, *Quercus cocciferae* L., *Phillyrea latifolia* L., *Paliurus spina-christi* Mill., and *Asphodelus aestivus* Brot.. In the area, a limited amount of rocky and evergreen forest habitat, as well as streamside habitat, is observed. There are three endemic taxa evaluated as "critical species" in the area (Table 3). The dominant species in the area is *Pinus brutia* Ten. The phytogeographic regions of the identified taxa include 18 Mediterranean, 5 Widespread, 2 Irano-Turanian, 2 Euro-Siberian, and 28 taxa with unknown distribution. Families are listed in terms of the number of taxa as Asteraceae 7, Lamiaceae 3, and Fabaceae 3.

A total of 61 taxa from 30 families and 55 genera were identified at Göksu Delta. The phytogeographic regions of the identified taxa include 14 Mediterranean, 2 Euro-Siberian, 18 Widespread, and 27 taxa with unknown distribution. The Irano-Turanian element was not identified. The ranking of families based on the number of taxa is as follows: Asteraceae 8, Brassicaceae 8, Fabaceae 7, and Poaceae 4. No endemic taxa were found in the study area. There is a rare species, *Pancratium maritimum* L. in the area, and the IUCN danger category is Least Concern (LC) (Juan Vicedo, 2018) (Table 3).

In Narlıkuyu, there are 26 taxa belonging to 19 families and 25 genera. The plants are listed according to phytogeographic regions as 14 Mediterranean, 1 Euro-Siberian, and 11 taxa with unknown distribution. Widespread and Irano-Turanian element plants were not identified. Based on the number of taxa in the study area, the families are listed as Asteraceae 3, Asparagaceae 2, Fabaceae 2, and Lamiaceae 1. No endemic taxa were identified in the study area. There are two rare taxa in the area. These are *Dianthus polycladus* Boiss. and *Zygophyllum album* L.f. both taxa are in the Vulnerable (VU) category. (Table 3).

Table 3. Endemic and rare taxa found in the research area
Çizelge 3. Araştırma alanında bulunan endemik ve nadir taksonlar

Family	Taxa	Phytogeographic Region	IUCN Threat Category	Study Area
Asteraceae	*Centaurea chrysantha* Wagenitz	Mediterranean	End./EN	3
Boraginaceae	*Alkanna hispida* Hub.-Mor.	East Mediterranean	End./EN	5, 3
Lamiaceae	*Nepeta isaurica* Boiss. & Heldr. Ex Benth.	East Mediterranean.	End./LC	7
Lamiaceae	*Phlomis nissolii* L.	Irano-Turanian	End./LC	7
Lamiaceae	*Stachys rupestris* Montbret & Aucher ex Benth.	East Mediterranean	End./LC	7
Lamiaceae	*Stachys buttlerii* R.R. Mill	East Mediterranean	End./EN	7
Lamiaceae	*Sideritis rubriflora* Hub.-Mor.	Mediterranean	End./NT	8
Boraginaceae	*Paracaryum calycinum* Boiss. & Balansa	Irano-Turanian	End./LC	3
Caryophllaceae	*Dianthus polycladus* Boiss.	East Mediterranean	Rare/VU	5
Amaryllidaceae	*Pancratium maritimum* L.	Mediterranean	Rare/LC	4
Fabaceae	*Lathyrus variabilis* (Boiss. & Kotschy) Celak.	East Mediterranean	Rare/VU	7
Zygophyllaceae	*Zygophyllum album* L.f.	-	Rare/VU	5

End: Endemic, EN: Endangered, Vu: Vulnerable, NT: Near Threatened, LC: Least Concern.

At the Roman Ruins, there are 47 taxa belonging to 27 families and 45 genera. The phytogeographic regions of these taxa include 22 Mediterranean, 2 Irano-Turanian, 2 Euro-Siberian, 7 Widespread, and 14 taxa with unknown distribution. In terms of the number of taxa in the study area, the families are listed as Fabaceae 7, Asteraceae 6, and Asparagaceae 3. The only endemic taxon in the area is *Alkanna hispida* Hub.-Mor. (Table 3). It is categorized

as Endangered (EN) according to the IUCN threat category. Maquis vegetation was observed in the area.

At Şeytanderesi and Cambazlı Cistern, a total of 85 taxa belonging to 39 families and 75 genera were identified. The phytogeographic regions of these taxa include 39 Mediterranean, 16 Widespread, 4 Irano-Turanian, 4 Euro-Siberian, and 22 taxa with unknown distribution. Four endemic and one rare taxa were identified (Table 3). The families are organized in order of the number of taxa they contain, as follows: Asteraceae with 12, Lamiaceae with 11, and Fabaceae with 10. Maquis and rocky vegetation were observed in the area.

At Akdere Tahta Port, a total of 63 taxa belonging to 29 families and 59 genera were identified. The phytogeographic regions of these taxa include 29 Mediterranean, 11 Widespread, 2 Irano-Turanian, 2 Euro-Siberian, and 19 taxa with unknown distribution. In terms of the number of taxa in the study area, the families are listed as Fabaceae 11, Asteraceae 10, Lamiaceae 5, Brassicaceae 4, and Primulaceae 3. The endemic taxon *Sideritis rubriflora* Hub.-Mor. was identified in the area (Table 3). The area contains *Pinus brutia* Ten. and maquis vegetation. In this study, a total of 8 taxa (3.68%) of endemic plants were identified. The IUCN threat categories of endemic taxa were determined as follows: three taxa in Endangered (EN), one tax in Near Threatened (NT), and four taxa in Least Concern (LC). Additionally, four taxa that are not endemic but rare in the area were identified. One of these taxa was determined to be in the Endangered (EN) category, and three of them were determined to be in the "Vulnerable" (VU) category. This study identified eight endemic taxa and four rare taxa. The IUCN threat categories of endemic taxa according to the Türkiye Plant Red List (Ekim et al. 2000) and the areas where they were collected are provided in Table 3.

In examining the overall vegetation structure of the study areas, although this research does not include a detailed vegetation analysis, the taxa present in the field were identified as characteristic species of higher syntaxonomic units to outline the general vegetation structure of the area. This will aid in future, more detailed vegetation studies. In this context, at Çağlayan Waterfall, the *Cisto-Micromerietalia* Oberd (1954) alliance of the *Cisto-Micromerietea* Oberd (1954) class is defined by the characteristic species *Cistus creticus* L. The *Quercion ilicis* Br.-Bl. ex Molinier 1934 alliance within the *Quercetea ilicis* Br.-Bl. ex A. & O. Bolòs 1950 class is defined by the defining species *Quercus coccifera* L. and *Phillyrea latifolia* L.*,* whereas the *Olea-Ceratonion* Br.-Bl. Ex Guinochet et Drouineau 1944 and *Ceratonio-Pistacion lentisci* Zohary et Orshan 1959 (Synonym: *Ceratonio-Rhamnion oleoidis* Barbero et Quézel 1979) alliances are represented by the key species *Ceratonia siliqua* L.. The *Pistacio-Rhamnetalia* Rivas-Martinez 1974 order is represented by the characteristic species *Quercus coccifera* L., which is the characteristic species of the *Andrachno-Quercion cocciferae* Barbero et Quézel 1979 alliance. The characteristic species defining the Alneto-Ulmion Br.-Bl. et Tx. (1943) alliance of the Populetalia Albea Br.-Bl. ex Tchou 1948 order, within the *Querco-Fagetea* Quezel et al. 1980 class, is *Alnus glutinosa* subsp. *antitaurica* Yalt.. The *Quercetalia ilicis* order is represented by the characteristic species *Phillyrea latifolia* L. and *Laurus nobilis* L. while the *Cisto-Micromerietalia* order is represented by the characteristic species *Cistus creticus* L.*.* In the area, although the *Cisto-Micromerietea* class is defined by the characteristic species *Calicotome villosa* L. and the *Quercetea ilicis* class by *Smilax aspera* L., no characteristic species were identified for any order or alliance. The *Quercetea pubescentis* Quezel et al. 1978 class and the *Querco-Cedretalia libani* Barbero et al. 1974 order are represented by the characteristic species *Pinus brutia* Ten. in the area.

At Ilısu Waterfall, higher syntaxonomic units representing the general vegetation structure of the area have been identified. The *Cisto-Micromerietalia* order of the *Cisto-Micromerietea* class is shown by the characteristic species *Cistus creticus* L. of the *Cistion Orientale* alliance. The *Quercetalia ilicis* order of the *Quercetea ilicis* class is described by the characteristic species *Quercus coccifera* L. and *Phillyrea latifolia* L. of the *Quercion ilicis* alliance. Additionally, the *Pistacio-Rhamnetalia* order is described by the characteristic species *Quercus coccifera*, which is the characteristic species of the *Andrachno-Quercion Cocaterae* alliance. The *Quercetalia ilicis* order is described by the characteristic species *Phillyrea latifolia* L., the Cisto-Micromerietalia order is represented by the characteristic species *Cistus creticus*, and the *Querco-Cedretalia libani* order is described by the characteristic species *Pinus brutia* Ten. In the area, although the *Cisto-Micromerietea* class is defined by the characteristic species *Cistus creticus* L. and *Calicotome villosa* (Poir.) Link., and the *Quercetalia ilicis* class is represented by the characteristic species *Hedera helix* L., no characteristic species for any order or alliance was identified. The *Quercetea pubescentis* (Oberd, 1948), Doing Kraft, 1955 class, and the *Querco-Cedretalia libani* order are defined by the characteristic species *Pinus brutia* Ten. in the area.

At Yerköprü Waterfall, higher syntaxonomic units representing the overall plant composition of the region have been identified. The Cisto-Micromerietalia order of the *Cisto-Micromerietea* class is described by the characteristic species *Cistus creticus* L. of the Cistion Orientale alliance. The *Quercetalia ilicis* order of the *Quercetea ilicis*class is described by the characteristic species *Quercus coccifera*, *Jasminum fruticans* L., and *Phillyrea latifolia* of the *Quercion ilicis* alliance. The *Olea-Ceratinion* alliance is described by the characteristic species *Capparis spinosa* L. and *Olea europaea* L.. The Pistacio-Rhamnetalia order is defined by the characteristic species *Quercus coccifera*

L. and *Arbutus andrachne* L. of the *Andrachno-Quercion cocaterae* alliance, and the *Querco-Juniperion excelsae* Barbero and Quézel 1979 alliance is represented by the characteristic species *Punica granatum* L.. The *Populetalia albea* order of the *Querco-Fagetea* class is defined by the characteristic species *Salix alba* L. of the *Populion albae* Br.-Bl. ex Tchou 1949 alliance, and the *Querco-Cedretalia libani* order of the *Quercetea pubescentis* class is represented by the characteristic species *Ostrya carpinifolia* Scop. of the *Ostryo-Quercion* Quézel, Barbero & Akman 1978 alliance. The *Quercetalia ilicis* order is represented by the characteristic species *Phillyrea latifolia* L.. The *Pistacio-Rhamnetalia* order is represented by the characteristic species *Cercis siliquastrum* and *Laurus nobilis* L., the *Cisto-Micromerietalia* order is described by the characteristic species *Cistus creticus* L., and the *Querco-Cedretalia libani* order is defined by the characteristic species *Pinus brutia*. In the area, although the *Cisto-Micromerietea* class is represented by the characteristic species *Calicotome villosa* (Poir.) Link. *Cistus creticus*, and *Micromeria myrtifolia* Boiss. & Hohen, the *Quercetea ilicis* class is represented by the characteristic species *Hedera helix* L., and the *Quercetea ilicis* class is represented by the characteristic species *Geranium purpureum* Vill.., no characteristic species for any order or alliance was identified. The *Quercetea pubescentis* class and the *Querco-Cedretalia libani* order are described by the characteristic species *Pinus brutia* Ten. in the area.

In the Göksu Delta, the *Cisto-Micromerietea* class is described by the characteristic species *Sarcopoterium spinosum* of the *Cisto-Micromerietalia* order, and the *Quercetea ilicis* class is defined by the characteristic species *Myrtus communis* L. of the *Pistacio-Rhamnetalia* order. The Quercetalia ilicis order, represented by the Olea-Ceratinion alliance, is also represented by the characteristic species *Myrtus communis* L.

In Narlıkuyu, the *Cisto-Micromerietea* class is represented by the characteristic species *Sarcopoterium spinosum* of the *Cisto-Micromerietalia* order. The *Querceta ilicis* class is described by *Pistacia terebinthus* L. and *Quercus coccifera* of the Pistacio-Rhamnetalia order. The *Querco-Cedretalia libani* order, belonging to the *Quercetea pubescentis* class, is defined by *Pinus brutia*. The Cisto-Micromerietea class is described by *Calicotome villosa* L., while the *Quercetae ilicis* class is represented by the key species *Quercus coccifera, Asparagus acutifolius, Pistacia terebinthus* L., *Olea europaea* L., and *Smilax aspera* L.. The *Quercion ilicis* alliance, representing the *Quercetalia ilicis* order of the *Quercetea ilicis* class, is characterized by species such as *Phillyrea latifolia* L., *Quercus coccifera* L., *Asparagus acutifolius* L., and *Pistacia terebinthus* L.. The *Olea-Ceratinion* alliance is described by species such as *Ceratonia siliqua* L., *Capparis spinosa* L., and *Olea europaea* L.. The *Quercion calliprini* (Zohary 1962) Quézel, Barbéro and Akman 1978 alliance is defined by the key species *Pistacia terebinthus,* belonging to the same *Quercetea ilicis* class. The *Querco-Fagetea* class is represented by the key species *Smilax excelsa* L., belonging to the *Castaneo sativae Carpinion orientalis* Quézel, Barbéro and Akman 1980 alliance of the *Rhododentro-Fagetalia orientalis* Quézel, Barbéro and Akman 1980 order.

In the Roman ruins, the *Cisto-Micromerietalia* order of the *Cisto-Micromerietea* class is defined by the character species *Cistus creticus* belonging to the *Cistion Orientale* alliance. The *Quercetalia ilicis* order of the *Quercetea ilicis* class is defined by the character species *Laurus nobilis*. The *Pistacio-Rhamnetalia* order is represented by the character species *Pistacia terebinthus* and *Clematis cirrhosa* L. The *Querco-Cedretalia libani* order of the *Quercetea pubescentis* class is represented by the character species *Quercus cerris* L. The *Cisto-Micromerietea* class is represented by the key species *Micromeria myrtifolia*. The *Quercetea ilicis* class is represented by the character species *Laurus nobilis*, *Quercus coccifera*, *Asparagus acutifolius*, *Pistacia terebinthus*, *Jasminum fruticans*, and *Olea europaea*. The *Quercion ilicis* alliance, belonging to the *Quercetalia ilicis* order of the *Quercetea ilicis* class, is represented by the character species *Quercus coccifera*, *Asparagus acutifolius*, and *Pistacia terebinthus*. The *Olea-Ceratinion* alliance is represented by the character species *Ceratonia siliqua*, *Clematis cirrhosa*, and *Olea europaea*. The *Quercion Calliprini* alliance is represented by the character species *Pistacia terebinthus.* The Pistacio-Rhamnetalia order, within the *Andrachno-Quercion Cocaterae* alliance, is represented by the character species *Quercus coccifera*. It has been established that the Querco-Cedretalia Libani order, which belongs to the *Quercetea pubescentis* class, is characterized by the defining species *Quercus cerris*, also serving as the characteristic species of both the *Ostryyo-Quercion* Quézel, Barbéro and Akman 1978 and *Geranio-Cedrion* Barbéro and Akman 1978 alliances. Furthermore, it has been determined that the *Querco cerridis-Carpinetalia orientalis* Quézel, Barbéro and Akman 1980 order is represented by Quercus cerris, the defining species, which also serves as the characteristic species of the *Quercion frainetto* Horvat 1954 alliance.

In Şeytanderesi and Cambazlı Cistern, the character species representing the *Cisto-Micromerietea* class are *Micromeria myrtifolia* and *Calicotome villosa* (Poir.) Link. It has been determined that the *Cisto-Micromerietalia* order of the *Cisto-Micromerietea* class is described by the key species *Cistus creticus* L. and *Sarcopoterium spinosum* (L..) Spach.. The *Querco-Cedretalia libani* order, belonging to the *Quercetea pubescentis* class, is represented by the character species *Pinus brutia* Ten.. The Quercetalia ilicis order of the *Quercetea ilicis* class is represented by the character species *Laurus nobilis* L. and *Phillyrea latifolia* L.. The *Pistacio-Rhamnetalia* order is represented by the character species *Pistacia terebinthus* and *Myrtus communis* L. It has been determined that the *Cisto-Micromerietalia* order of the *Cisto-Micromerietea* class is represented by the character species *Cistus*

creticus L.. The *Quercetea ilicis* class is represented by the character species *Jasminum fruticans* L., *Laurus nobilis* L., *Quercus coccifera* L., *Arbutus unedo* L., *Pistacia terebinthus* L., *Olea europaea* L., and *Smilax aspera* L.. It has been observed that the *Quercetalia ilicis* order of the *Quercetea ilicis* class is represented by the character species *Arbutus unedo* L., *Quercus coccifera* L., *Phillyrea latifolia* L., and *Pistacia terebinthus* L., which are the character species of the *Quercion ilicis* alliance. The Quercion Calliprini alliance is defined by the character species *Pistacia terebinthus* L.. The *Olea-Ceratinion* alliance is represented by the character species *Olea europaea* L., *Myrtus communis* L., *Capparis spinosa* L., and *Ceratonia siliqua* L.. The *Pistacio-Rhamnetalia* order of the *Quercetea ilicis* class, within the *Andrachno-Quercion Cocaterae* alliance, is represented by the character species *Quercus coccifera* and *Arbutus andrachne*. It has been determined that the *Populetalia Albea* order of the *Querco-Fagetea* class is represented by the character species *Salix alba* L. of the Populion albea alliance.

In Akdere Tahta Port, it has been determined that the *Cisto-Micromerietalia* Oberd (1954) order of the *Cisto-Micromerietea* Oberd (1954) class is represented by the character species *Cistus creticus* L. and *Sarcopoterium spinosum* L.. Additionally, it is represented by *Pinus brutia* Ten, which belongs to the *Querco-Cedretalia libani* order of the *Quercetea pubescentis* class. The *Cisto-Micromerietalia* order of the *Cisto-Micromerietea* class is represented by the character species *Cistus creticus L.*, which belongs to the *Cistion Orientale* alliance. The Cisto-Micromerietea class is described by the character species *Cistus creticus* L., *Calicotome villosa* L., and *Micromeria myrtifolia* L.. The *Quercetea ilicis* Br.-Bl. ex A. & O. Bolòs 1950 class is represented by the character species *Phillyrea latifolia* L., *Pistacia terebinthus* L., and *Olea europaea* L.. The *Querceta pubescentis* (Oberd, 1948), Doing Kraft, 1955 class is represented by the character species *Securigera varia*(L.) Lassen *(syn. Coronilla varia* L.*)*. Lastly, the *Querco-Fagetea* class is represented by the character species *Hedera helix*. The *Quercion ilicis* Br.-Bl. ex Molinier 1934 em. Rivas-Martínez 1975 alliance of the *Quercetalia ilicis* Br.-Bl. ex Molinier 1934 em. Rivas-Martínez 1975 order, belonging to the *Quercetea ilicis* class, is represented by the character species *Phillyrea latifolia* L. and *Pistacia terebinthus* L.. The *Quercion calliprini* alliance is represented by the character species *Pistacia terebinthus* L. and *Cyclamen persicum* Mill. The *Olea-Ceratinion* alliance is represented by the character species *Olea europaea* L., *Ceratonia siliqua* L., and *Capparis spinosa* L.. Additionally, the *Ceratonio-Rhamnion Oleoides* alliance is represented by the character species *Ceratonia siliqua* L.. It has been determined that the Quercetalia ilicis order of the Quercetea ilicis class is represented by the character species *Phillyrea latifolia* L.. The *Pistacio-Rhamnetalia* order is represented by the character species *Pistacia terebinthus* L. and *Capparis spinosa* L.. The *Quercetea pubescentis* class is defined by *Coronilla varia* in the *Querco Cerridis-Carpinetalia Orientalis* order and the *Quercion anatolicae* Akman, Barbéro and Quézel 1979 alliance. Additionally, *Securigera varia* (L.) Lassen *(syn. Coronilla varia* L.*)* is represented in the *Ostriyo-Quercion* alliance of the *Querco-Cedretalia libani* order and in the *Quercus cerris* character species of the same order. Furthermore, the *Quercion frainetto* alliance of the *Querco Cerridis-Carpinetalia orientalis* order is represented by the character species *Quercus cerris* L..

When the study areas were compared among themselves in terms of syntaxonomic superunits and character species; Cistion orientale, Quercion ilicis and Querco-Cedretalia libani are similar in these three areas, and the syntaxonomic superunits in Çağlayan, Ilısu and Yerköprü waterfalls are similar to each other, It was determined that the syntaxonomic superunits of Şeytanderesi and Cambazlı Cistern, and Akdere Tahta Port were similar, and that Cistion orientale, Quercion ilicis, Olea-Ceratinion, Quercion Calliprini and Andrachno-Quercion Cocaterae were found in the study areas.

It was observed that the vegetation structure and syntaxonomic superunits of Göksu Delta, Narlıkuyu, and Roman Ruins are different. It is thought that this is due to the differences in habitat, distance between the study areas, geographical structure and altitude.

As a result of the comparison of Narlıkuyu and Roman Ruins, it was observed that these two areas were similar to each other and the common upper units in both areas were Cistion orientale, Olea-Ceratinion, and Quercion Calliprini. It is thought that this situation may be due to the same geographical structure, habitat, and climate characteristics of both study areas.

Göksu Delta, on the other hand, showed a different vegetation structure from the other areas due to its wetland characteristics.

Due to the detailed study of the vegetation structure of this study areas not being conducted with the Braun-Blanquet method (Braun-Blanquet, 1932), plant communities have not been established. Therefore, there is no opportunity for comparison with similar vegetation studies in the nearby surroundings.

As a result of the work carried out on EUNIS in Türkiye between 2011-2020, a total of 140 EUNIS habitat types were identified in the 3rd Level. In addition, it has been determined that there are 26 new habitat types that are not defined in EUNIS, without any level restrictions (Çakmak & Aytaç, 2021). Tak & Tel (2024) identified 37 habitat types belonging to the European Nature Information System (EUNIS) in Akdağ (Malatya).

Within the scope of the National Biodiversity Inventory and Monitoring Project, 10 basic habitat types were identified across the provinces where the project was completed, and a total of 257 habitat records were given from 46 different habitat types across 25 provinces (Terzioğlu et al., 2015).

The study conducted by Çakmak & Aytaç (2021) in Türkiye, it was determined that out of the 326 third-level habitat types in the EUNIS habitat classification, 138 are found in Turkey. In this study, 22 habitat types at Level 2 were identified related to 9 habitat types at Level 1 (Çakmak & Aytaç, 2021). Turkey is a country with high biological and habitat diversity (Kanca et al., 2019). Therefore, in the future, data obtained from determining all habitat types in Turkey and establishing geographic information systems can be utilized by various disciplines. Many countries have completed the EUNIS habitat classification and EUNIS habitat types of nearly 30 provinces in Turkey have been determined (Terzioğlu et al., 2015; Kanca et al., 2019). All of these provinces have inland water habitats, grasslands and non-grass herbaceous habitats, forests, agricultural areas and man-made habitats (Terzioğlu et al., 2015). This situation will ensure that Turkey's habitat richness is known, utilized properly and EUNIS habitat types are obtained for the whole of Turkey. When the literature studies are examined, it is seen that marine habitats, marshes and peatland habitats are represented at very low levels, while heathland, scrubland and tundra are highly represented (Terzioglu et al., 2015; Kanca et al., 2019; Çakmak & Aytaç, 2021). It is thought that this is due to the fact that most of the studies were conducted in terrestrial areas and the data on heathland, shrubland and tundra are high. Since terrestrial habitats are mostly in this study, the habitat types of the study are similar to the study of Terzioğlu et al. (2015).

According to Terzioglu et al. (2015), the habitats found in all study provinces in Turkey are C (Inland water habitats), E (Grasslands and non-grass herbaceous habitats), G (Forests), I (Agricultural areas) and J (Man-made habitats). Of these habitats, J (Man-made habitats) and G (Forests) are the most densely populated habitats. The same results were obtaıned in thıs study.When analyzing the EUNIS habitat types in the study area, the classification of habitat types in the research areas based on the EUNIS habitat categorization system (Davies et al., 2004; Anonymous, 2024a) revealed the presence of 22 habitat types at level 2 across 9 habitat types at level 1 (Table 4). When individually evaluated in the study areas: At Çağlayan Waterfall, 6 habitat types at level 1 and 11 habitat types at level 2. Ilısu Waterfall exhibited 5 habitat types at level 1 and 10 habitat types at level 2 Yerköprü Waterfall demonstrated 5 habitat types at level 1 and 9 habitat types at level 2. Göksu Delta displayed 7 habitat types at level 1 and 16 habitat types at level 2. Narlıkuyu showcased 5 habitat types at level 1 and 9 habitat types at level 2. Roman Ruins presented 2 habitat types at level 1 and 4 habitat types at level 2. Şeytanderesi and Cambazlı Cistern featured 4 habitat types at level 1 and 9 habitat types at level 2 Akdere Tahta Port revealed 5 habitat types at level 1 and 13 habitat types at level 2 These identifications were based on classification according to the EUNIS habitat classification system.

A total of 214 taxa (170 species, 29 subspecies and 15 varieties) from 65 families and 173 genera have been identified in this study. The phytogeographic regions are distributed as follows; 11 taxa are of the Euro-Siberian region (5.14%), 7 taxa belong to the Irano-Turanian region (3.27%), 45 taxa are of the Widespread (21.02%), 76 taxa have an unknown phytogeographic region (35.50%), and 77 taxa represent the Mediterranean element (35.98%). The prevalence of species from the Mediterranean phytogeographic region is due to the fact that the study areas are situated within the Mediterranean region. In the study areas, Asteraceae is the largest family by the number of taxa, particularly in Yerköprü Waterfall, Göksu Delta, Narlıkuyu, and Şeytanderesi and Cambazlı Cistern; Fabaceae in Çağlayan Waterfall, Roman Ruins, and Akdere Tahta Port; and Poaceae in Ilısu Waterfall. In the Flora of Turkey, Poaceae is the family with the greatest number of taxa (Davis, 1965-1985; Davis et al., 1988). Nevertheless, it has been noted that in the study areas, Asteraceae, Fabaceae, and Lamiaceae are the families with the greatest number of taxa. This result is anticipated given the study area's location in the Mediterranean region.

Eight endemic taxa were identified in the research area. The endemism rate is 3.68%. Compared to studies conducted in neighboring regions, the highest endemism rate was reported by Tel et al. (2018) with 18.60%, while the lowest endemism rate was found in the floristic study of Tel et al. (2022a) with 1.30%. The high endemism rate reported in the studies by Tel et al. (2018) can be explained by the region's distinct microclimate characteristics, elevation, and habitat diversity. On the other hand, the lowest endemism rate in the study by Tel et al. (2022a) can be attributed to the presence of wetland habitats in the area, as well as the vegetation structure consisting of dune and halophyte plants (Table 5).

When the studies conducted on the natural sites in the research area and its surroundings are compared based on the phytogeographic regions of taxa, it is observed that, except Ortaç & Tel (2021), all other areas exhibit a higher presence of Mediterranean phytogeographic region elements (Aksay, 2006; Yıldıztugay & Küçüködük, 2010; Tezcan, 1995; Tel et al., 2018; Tel et al., 2019; Tel et al., 2021; Tel et al., 2022a; 2022b; Tel et al., 2023). This result is an expected outcome due to the research areas being in the Mediterranean area (Table 5).

Table 4. EUNIS Habitat classification types and codes in the study areas
Çizelge 4. Çalışma alanlarındaki EUNIS Habitat sınıflandırma tipleri ve kodları

Study area	EUNIS Classification Code	Habitat Name	EUNIS sub-classification code and description
Çağlayan Waterfall	C	Inland surface waters	C2: Surface running waters (Running waters, including springs, streams and temporary water courses)
	F	Heathland, scrub and tundra	F5: Maquis, arborescent matorral and thermo-Mediterranean brushes F5.2: Maquis; F5.5: Thermo-Mediterranean scrub F6.21: Eastern Quercus coccifera garrigues F9.1: Riverine scrub
	G	Woodland, forest and other wooded land	G3.75: Pinus brutia forests
	H	Inland areas with minimal or absent vegetation	H3: Inland cliffs, rock pavements and outcrops H3.4: Wet inland cliffs
	I	Regularly or recently cultivated agricultural, horticultural and domestic habitats	I1.2: Mixed farming of market gardens and horticultural crops
	J	Constructed, industrial and other artificial habitats	J1.2: Housing structures in villages and urban outskirts
Ilısu Waterfall	C	Inland surface waters	C2: Surface running waters (Running waters, including springs, streams and temporary water courses)
	F	Heathland, scrub and tundra	F5: Maquis, arborescent matorral and thermo-Mediterranean brushes F5.2: Maquis; F5.5: Thermo-Mediterranean scrub F6.21: Eastern Quercus coccifera garrigues F9.1: Riverine scrub
	G	Woodland, forest and other wooded land	G3.75: Pinus brutia forests
	H	Inland areas with minimal or absent vegetation	H3: Inland cliffs, rock pavements and outcrops H3.4: Wet inland cliffs
	J	Constructed, industrial and other artificial habitats	J1.2: Residential buildings of villages and urban peripheries
Yerköprü Waterfall	C	Inland surface waters	C2: Surface running waters (Running waters, including springs, streams and temporary water courses)
	F	Heathland, scrub and tundra	F5: Maquis, arborescent matorral and thermo-Mediterranean brushes F5.2: Maquis; F5.5: Thermo-Mediterranean scrub F6.21: Eastern Quercus coccifera garrigues F9.1: Riverine scrub
	G	Woodland, forest and other wooded land	G3.75: Pinus brutia forests
	H	Inland areas with minimal or absent vegetation	H3: Inland cliffs, rock pavements and outcrops H3.4: Wet inland cliffs
	I	Regularly or recently cultivated agricultural, horticultural and domestic habitats	I1.2: Mixed crops of market gardens and horticulture
Göksu Delta	B	Coastal habitats	B1.2: Sand beaches above the driftline B1.3: Shifting coastal dunes B1.5: Coastal dune heaths
	C	Inland surface waters	C1 Surface standing waters C2: Surface running waters C2.4: Tidal rivers, upstream from the estuary C3.1: Diverse helophyte communities
	D	Mires, bogs and fens	D2: Valley mires, poor fens and transition mires
	F	Heathland, scrub and tundra	F5: Maquis, arborescent matorral and thermo-Mediterranean brushes F5.2: Maquis; F5.5: Thermo-Mediterranean scrub F6.21: Eastern Quercus coccifera garrigues F9.1: Riverine scrub
	G	Forests, woodlands, and other wooded areas	G3.75: Pinus brutia forests
	I	Regularly or recently cultivated agricultural, horticultural and domestic habitats	I1.2: Mixed crops of market gardens and horticulture
	J	Constructed, industrial and other artificial habitats	J1.2: Residential buildings of villages and urban peripheries
Narlıkuyu	B	Coastal habitats	B1.2:Sand beaches above the driftline B1.3: Shifting coastal dunes B1.5: Coastal dune heaths

	A	Marine habitats	A1.1: High energy littoral rock
	F	Heathland, scrub and tundra	F5: Maquis, arborescent matorral and thermo-Mediterranean brushes F5.2: Maquis; F5.5: Thermo-Mediterranean scrub
	G	Woodland, forest and other wooded land	G3.75: Pinus brutia forests
	J	Constructed, industrial and other artificial habitats	J1.2: Residential buildings of villages and urban peripheries
Roma Ruins	F	Heathland, scrub and tundra	F5: Maquis, arborescent matorral and thermo-Mediterranean brushes F5.2: Maquis; F5.5: Thermo-Mediterranean scrub
	I	Regularly or recently cultivated agricultural, horticultural and domestic habitats	I1.2:Mixed crops of market gardens and horticulture
Şeytanderesi and Cambazlı Cistern	C	Inland surface waters	C2: Flowing surface waters (including springs, streams, and seasonal waterways)
	H	Inland areas with minimal or absent vegetation	H3: Inland cliffs, rock pavements and outcrops H3.5: Almost bare rock pavements, including limestone pavements
	F	Heathland, scrub and tundra	F5: Maquis, arborescent matorral and thermo-Mediterranean brushes F5.2: Maquis; F5.5: Thermo-Mediterranean scrub F6.21: Eastern Quercus coccifera garrigues F9.1: Riverine scrub
	I	Regularly or recently cultivated agricultural, horticultural and domestic habitats	I1.2: Mixed crops of market gardens and horticulture
Akdere Tahta Port	B	Coastal habitats	B1.2: Sand beaches above the driftline B1.3: Shifting coastal dunes B1.5: Coastal dune heaths B2.1: Shingle beach driftlines
	F	Heathland, scrub and tundra	F5: Maquis, arborescent matorral and thermo-Mediterranean brushes F5.2: Maquis; F5.5: Thermo-Mediterranean scrub F6.21: Eastern Quercus coccifera garrigues F9.1: Riverine scrub
	G	Forests, woodlands, and other wooded areas	G3.75: Pinus brutia forests
	H	Inland areas with minimal or absent vegetation	H3: Inland cliffs, rock pavements and outcrops H3.5: Almost bare rock pavements, including limestone pavements H3.4: Wet inland cliffs
	I	Regularly or recently cultivated agricultural, horticultural and domestic habitats	I1.2: Mixed crops of market gardens and horticulture

Table 5. Distribution rates of taxa determined in studies in and around the research area according to phytogeographic regions (%)

Çizelge 5. Araştırma alanı ve çevresinde yapılan çalışmalarda tespit edilen taksonların fitocoğrafik bölgelere göre dağılım oranları (%)

Studies	Mediterranean Element	Euro-Siberian Element	Irano-Turanian Element	Widespread and Unknown	Endemism Rate
Research Area	35.98	5.14	3.27	56.52	3.68
Tezcan (1995)	40.60	2.00	3.50	54.13	7.01
Aksay (2006)	26.48	7.33	13.90	52.31	5.29
Yıldıztugay & Küçüködük (2010)	35.10	3.10	4.90	56.90	3.70
Tel et al (2018)	23.20	6.90	10.50	59.30	18.60
Tel et al. (2019)	38.40	6.10	4.30	51.20	9.10
Tel et al. (2021)	36.80	3.90	4.50	54.80	4.50
Ortaç & Tel (2021)	14.50	6.10	29.60	49.80	4.04
Tel et al. (2022b)	48.69	1.73	6.08	43.47	2.60
Tel et al. (2022a)	34.60	4.50	5.00	55.90	1.30
Tel et al. (2023)	56.07	0.00	0.00	43.93	1.51
Tel et al. (2024)	29.32	0.00	7.01	63.15	14.03

Comparing research studies conducted in the study area and surrounding regions based on the families with the highest number of taxa, it is observed that in the works of Yıldıztugay & Küçüködük (2010), Ortaç & Tel (2021), Tel et al. (2018; 2022a) and Tezcan (1995) Asteraceae family is in the first place. In contrast, this study, as well as the works of Aksay (2006), Tel et al. (2019), Tel et al. (2021; 2022b), and Tel et al. (2023), indicated that Fabaceae

family holds the first place. It is thought to result from the fact that the Asteraceae and Fabaceae families, which contain the most taxa, also have a high ranking in terms of the total number of taxa in the Flora of Turkey.(Davis, 1965-1985; Davis et al. 1988). This is attributed to their strong generative reproductive capacity (Table 6).

Table 6. Families containing the most taxa in studies in the study area and nearby areas
Çizelge 6. Çalışma alanı ve yakın bölgelerdeki çalışmalarda en fazla takson içeren familyalar

Studies	Fabaceae	Lamiaceae	Asteraceae
Research area	26	16	28
Tezcan (1995)	25	20	39
Aksay (2006)	18	17	17
Yıldıztugay & Küçüködük (2010)	35	15	40
Tel et al (2018)	5	4	13
Tel et al. (2019)	26	13	13
Tel et al. (2021)	22	15	13
Ortaç & Tel (2021)	27	27	43
Tel et al. (2022b)	32	12	4
Tel et al. (2022a)	15	10	33
Tel et al. (2023)	12	6	9
Tel et al. (2024)	7	10	5

When the research areas were compared among themselves, it was seen that the highest number of endemic taxa was found in Şeytanderesi and Cambazlı Cistern, while there were no endemic taxa in Çağlayan Waterfall, Ilısu Waterfall, Göksu Delta and Narlıkuyu. This may be due to the different climate, altitude, soil structure and habitat of Şeytanderesi and Cambazlı Cistern. When the identified taxa in the study areas are compared according to phytogeographic regions, it is observed that elements from the Mediterranean phytogeographic area are common in all areas. This can be attributed to the fact that the study areas are situated within the Mediterranean phytogeographic region.

Regarding the general vegetation structure of the area, plant communities have not been established, as the vegetation structure of the study areas was not analyzed in detail using the Braun-Blanquet method. When compared with some phytosociological studies conducted in nearby and distant regions, it was observed that the study area is similar to the *Quercetea ilicis, Quercetea pubescentis* Doing-Kraft ex Scamoni et Passarge 1959 classes, and *Quercetalia ilicis* order in Tel et al. (2010)'s study, but no similarity was found in lower syntaxonomic units (Tel et al., 2010; Tel & Tak, 2012; Tel & Eğilmez, 2015). The vegetation structure of the study area has been determined to show similarities with the *Quercetea ilicis*, *Quercetea pubescentis* classes, *Quercetalia ilicis*, and *Querco-Cedretalia libani* Barbero et al. 1974 orders as identified in the study by Uçar (2002). When compared with the study by Aksay (2006), it was observed that the vegetation structure of the study area shows similarity with the *Quercetea ilicis* class, but no similarity was found in lower syntaxonomic units. Additionally, in the study by Tel & Tak (2021), while there is similarity with the *Quercetea pubescentis* class, no similarity was found in the lower subunits. The reason for this could be the differences in phytogeographic regions, climate, elevation, and soil structure of the study areas. The evaluation indicates similarity at the class level but differences at the order and alliance levels. This is thought to be due to the diversity of habitat types.

As a result of human activities, plant and animal species can often be adversely affected. This situation can frequently conflict with biodiversity conservation efforts. Therefore, it is essential to preserve habitat diversity, which is one of the key factors enhancing biological diversity (Arslan et al., 2012). This can only be achieved through the establishment of a habitat classification system and databases that ensure the efficient utilization of resources. The study conducted by Çakmak & Aytaç (2020) in Türkiye, it was determined that out of the 326 third-level habitat types in the EUNIS habitat classification, 138 are found in Turkey. In this study, 22 habitat types at level 2 were identified related to 9 habitat types at level 1. Turkey is a country with high biological and habitat diversity (Kanca et al., 2019). Therefore, in the future, data obtained from determining all habitat types in Turkey and establishing geographic information systems can be utilized by various disciplines.

CONCLUSION

In conclusion, 214 taxa (170 species, 29 subspecies and 15 varieties) belonging to 65 families and 173 genera were identified in this study conducted to determine the floristic and general vegetation structure of the study areas in the Mediterranean region. When the distribution of taxa according to phytogeographic regions was analyzed, it was seen that the Mediterranean phytogeographic region elements ranked first with a rate of 35.98%. 170 species, 29 subspecies and 15 varieties) In this study, 22 level 2 habitat types were identified related to 9 level 1 habitats. As a result of the classification of habitat types in the research areas according to the EUNIS habitat classification

types when individually evaluated in the study areas: At Çağlayan Waterfall, 6 level 1 habitats and 11 level 2 habitat types were identified. Ilısu Waterfall exhibited 5 level 1 habitats and 10 level 2 habitat types. Yerköprü Waterfall demonstrated 5 level 1 habitats and 9 level 2 habitat types. Göksu Delta displayed 7 level 1 habitats and 16 level 2 habitat types. Narlıkuyu showcased 5 level 1 habitats and 9 level 2 habitat types. Roman Ruins presented 2 level 1 habitats and 4 level 2habitat types. Şeytanderesi and Cambazlı Cistern featured 4 level 1 habitats and 9 level 2 habitat types. Akdere Tahta Port revealed 5 level 1 habitats and 13 level 2habitat types.

Although this study does not offer an in-depth vegetation analysis, the taxa observed in the field were recognized as characteristic species of higher syntaxonomic units to outline the general vegetation structure of the area. Accordingly, at Çağlayan Waterfall, the *Cisto-Micromerietalia* alliance of the *Cisto-Micromerietea* class is defined by the characteristic species *Cistus creticus*. The *Quercion ilicis* alliance of the *Quercetea ilicis* class is represented by the characteristic species *Quercus coccifera* and *Phillyrea latifolia*, while the *Olea-Ceratonion* and *Ceratonio-Rhamnion Oleoides* alliances are represented by the characteristic species *Ceratonia siliqua.* The *Pistacio-Rhamnetalia* order is represented by the characteristic species *Quercus coccifera*, which is the characteristic species of the *Andrachno-Quercion Cocciferae* alliance.The *Alneto-Ulmion* alliance of the *Populetalia Albea* order, belonging to the *Querco-Fagetea* class, is represented by the characteristic species *Alnus glutinosa* subsp. *antitaurica*. The *Quercetalia ilicis* order is represented by the characteristic species *Phillyrea latifolia* and *Laurus nobilis* while the *Cisto-Micromerietalia* order is represented by the characteristic species *Cistus creticus.* In the area, although the *Cisto-Micromerietea* class is represented by the characteristic species *Calicotome villosa*, and the *Quercetea ilicis* class is described by the characteristic species *Smilax aspera*, no characteristic species for any order or alliance was identified. The *Quercetea pubescentis* class and the *Querco-Cedretalia Libani* order are defined by the characteristic species *Pinus brutia* in the area.

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Contribution Rate Statement Summary of Researchers

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

Conflict of Interest

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

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Appendix 1. Taxa list in the study area
Ek 1. Çalışma alanındaki takson listesi

No	Family Name	Taxa	Phytogeographic Region	Threat Category	Study area	Plant Collection Date	Collecting Number
1	Amaranthaceae	*Chenopodium album* subsp. *album* var. *album*	-	-	4	08.05.2016	Ortaç 1144
2	Amaranthaceae	*Halimione portulacoides*	-	-	4	08.05.2016	Ortaç 1145
3	Amaranthaceae	*Salicornia europaea*	-	-	4	08.05.2016	Ortaç 1148
4	Amaranthaceae	*Salsola kali*	-	-	4	03.04.2016	Ortaç 1149
5	Amaranthaceae	*Salsola soda*	-	-	4	07.04.2016	Ortaç 1151
6	Amaryllidaceae	*Allium ampeloprasum*	Med. Elm.	-	6	23.07.2016	Ortaç 1438
7	Amaryllidaceae	*Allium neapolitanum*	Med. Elm.		8	03.04.2016	Ortaç 1439
8	Amaryllidaceae	*Pancratium maritimum*	Med. Elm.	Rare/LC	4	22.07.2016	Ortaç 1424
9	Anacardiaceae	*Pistacia terebinthus*	E. Med. Elm.	-	5,6,7,8	07.04.2016	Ortaç 1017
10	Anacardiaceae	*Pistacia palaestina*	E. Med. Elm.	-	1,3	07.04.2016	Ortaç 1017
11	Apiaceae	*Eryngium maritimum*	-	-	4	07.04.2016	Ortaç 1023
12	Apiaceae	*Turgenia latifolia*	Wide.	-	6	07.04.2016	Ortaç 1025
13	Apocynaceae	*Nerium oleander*	Med. Elm..	-	1,2,3,4	08.05.2016	Ortaç 1026
14	Apocynaceae	*Vincetoxicum canescens* subsp. *canescens*	-	-	3	22.07.2016	Ortaç 1032
15	Araceae	*Arum dioscoridis* var.*dioscoridis*	E. Med. Elm.	-	2,7,8	03.04.2016	Ortaç 1425
16	Araliaceae	*Hedera helix*	-	-	2,3,8	03.04.2016	Ortaç 1028
17	Asparagaceae	*Asparagus acutifolius*	Med. Elm.	-	4,5	24.10.2016	Ortaç 1441
18	Asparagaceae	*Drimia maritima*	-	-	3,4,5,6,7,8	23.10.2016	Ortaç 1456
19	Asparagaceae	*Prospero autumnale*	Med. Elm.	-	6,7	22.07.2016	Ortaç 1453
20	Aspleniaceae	*Asplenium ceterach*	-	-	7	03.04.2016	Ortaç 1001
21	Asteraceae	*Asteriscus spinosus*	Med. Elm.	-	1,6,8	04.05.2016	Ortaç 1065
22	Asteraceae	*Carduus pycnocephalus* subsp. *albidus*	Wide..		3,4,7,8	22.07.2016	Ortaç 1037
23	Asteraceae	*Carthamus lanatus*	Wide.	-	6,7	07.05.2016	Ortaç 1038
24	Asteraceae	*Centaurea iberica*	Wide.		4	08.05.2016	Ortaç 1040
25	Asteraceae	*Cichorium intybus*	Wide.	-	7,8	03.04.2016	Ortaç 1046
26	Asteraceae	*Centaurea chrysantha*	-	End/EN	3	22.07.2016	Ortaç 1039
27	Asteraceae	*Cota tinctoria* var. *tinctoria*	-	-	3	22.07.2016	Ortaç 1033
28	Asteraceae	*Conyza canadensis*	-	-	7	04.04.2016	Ortaç 1047
29	Asteraceae	*Crepis sancta*	Wide.		4,8	04.05.2016	Ortaç 1049
30	Asteraceae	*Crupina crupinastrum*	Wide.		8	04.05.2016	Ortaç 1050
31	Asteraceae	*Hirtellina lobelii*	E. Med. Elm.	-	7	07.05.2016	Ortaç 1075
32	Asteraceae	*Inula crithmoides*	-	-	4,8	08.05.2016	Ortaç 1055
33	Asteraceae	*Inula graveolens*	Med. Elm.	-	4	22.03.2016	Ortaç 1056
34	Asteraceae	*Inula heterolepis*	E. Med. Elm.	-	3	22.07.2016	Ortaç 1057
35	Asteraceae	*Inula viscosa*	Med. Elm.		4,5,1,2	07.04.2016	Ortaç 1058
36	Asteraceae	*Lactuca saligna*	-	-	4	05.04.2016	Ortaç 1059
37	Asteraceae	*Notobasis syriaca*	Med. Elm.	-	7	07.05.2016	Ortaç 1063
38	Asteraceae	*Phagnalon graecum*	E. Med. Elm.	-	5,6,7,8,3	22.07.2016	Ortaç 1066
39	Asteraceae	*Picnomon acarna*	Med. Elm.	-	5,6,7,8,1,3	22.07.2016	Ortaç 1067
40	Asteraceae	*Ptilostemon diacantha*	E. Med. Elm.	-	7	04.04.2016	Ortaç 1068
41	Asteraceae	*Rhagadiolus stellatus*	Med. Elm.	-	6	04.04.2016	Ortaç 1069
42	Asteraceae	*Senecio vernalis*	Wide.	-	7,8	07.04.2016	Ortaç 1070
43	Asteraceae	*Senecio vulgaris*	-	-	6	04.04.2016	Ortaç 1071
44	Asteraceae	*Sonchus oleraceus*	-	-	4	07.04.2016	Ortaç 1074
45	Asteraceae	*Tussilago farfara*	Eu.-Sib. Elm.		8	04.05.2016	Ortaç 1077
46	Asteraceae	*Xanthium orientale* subsp. *italicum*	Wide.	-	7	07.04.2016	Ortaç 1079
47	Asteraceae	*Xeranthemum annuum*	Wide..	-	7	04.04.2016	Ortaç 1080
48	Betulaceae	*Alnus glutinosa* subsp. *antitaurica*	E. Med. Elm.	-	1	07.05.2016	Ortaç 1083
49	Betulaceae	*Ostrya carpinifolia*	Med. Elm.	-	3	03.04.2016	Ortaç 1163
50	Boraginaceae	*Alkanna hispida*	E. Med. Elm.	End./EN	3,6	22.07.2016	Ortaç 1084
51	Boraginaceae	*Cynoglossum creticum*	-	-	8,1	08.05.2016	Ortaç 1090
52	Boraginaceae	*Cynoglossum montanum*	Av.-Sib. Elm.	-	7	04.04.2016	Ortaç 1091
53	Boraginaceae	*Echium parviflorum*	Akd. Elm.	-	4	07.04.2016	Ortaç 1093
54	Boraginaceae	*Onosma rascheyana*	Ir.-Tur. Elm.	-	7	03.04.2016	Ortaç 1097
55	Boraginaceae	*Paracaryum calycinum*	Ir.-Tur. Elm.	End./LC	3	22.07.2016	Ortaç 1098
56	Brassicaceae	*Arabis alpina subsp. brevifolia*	E. Med. Elm.	-	7	03.04.2016	Ortaç 1101
57	Brassicaceae	*Arabis verna*	Med. Elm.	-	7,8	04.05.2016	Ortaç 1102
58	Brassicaceae	*Biscutella didyma*	-		8	04.05.2016	Ortaç 1104
59	Brassicaceae	*Cakile maritima*	-	-	4,5	08.05.2016	Ortaç 1105

60	Brassicaceae	*Capsella bursa- pastoris*	Wide.	-	4	08.05.2016	Ortaç 1106
61	Brassicaceae	*Cardaria draba*	Wide.	-	4	05.04.2016	Ortaç 1107
62	Brassicaceae	*Conringia clavata*	Wide.		8	04.05.2016	Ortaç 1108
63	Brassicaceae	*Draba verna*	-	-	2	06.04.2016	Ortaç 1110
64	Brassicaceae	*Diplotaxis tenuifolia*	-	-	1	03.04.2016	Ortaç 1109
65	Brassicaceae	*Nasturtium officinale*	Wide.	-	1,2	08.05.2016	Ortaç 1115
66	Brassicaceae	*Raphanus raphanistrum*	Wide.	-	4,8	08.05.2016	Ortaç 1117
67	Brassicaceae	*Sinapis alba*	-	-	4	05.04.2016	Ortaç 1118
68	Brassicaceae	*Sinapis arvensis*	Wide.	-	4,7	03.04.2016	Ortaç 1119
69	Brassicaceae	*Thlaspi perfoliatum*	Wide.	-	4	05.04.2016	Ortaç 1121
70	Campanulaceae	*Campanula strigosa*	E. Med. Elm.		8	04.05.2016	Ortaç 1125
71	Campanulaceae	*Michauxia campanuloides*	E. Med. Elm.	-	3	03.04.2016	Ortaç 1127
72	Capparaceae	*Capparis spinosa*	-	-	5,7,8,3	08.05.2016	Ortaç 1129
73	Caprifoliaceae	*Valeriana dioscoridis*	E. Med. Elm..	-	3	23.10.2016	Ortaç 1416
74	Caryophyllaceae	*Dianthus polycladus*	E. Med. Elm.	*Rare/VU*	5	08.05.2016	Ortaç 1132
75	Caryophyllaceae	*Silene aegyptiaca* subsp. *aegyptiaca*	-	-	7	04.04.2016	Ortaç 1136
76	Caryophyllaceae	*Silene colorata*	-	-	5,6	08.05.2016	Ortaç 1138
77	Caryophyllaceae	*Spergularia marina*	Wide.	-	4	05.04.2016	Ortaç 1141
78	Cistaceae	*Cistus creticus*	Med. Elm.	-	6,7,8,1,2,3	08.05.2016	Ortaç 1152
79	Colchicaceae	*Colchicum cilicicum*	E. Med. Elm.	-	6,7	26.10.2016	Ortaç 1444
80	Convolvulaceae	*Calystegia sepium* subsp. *sepium*	Wide.	-	4,6,1	08.05.2016	Ortaç 1157
81	Crassulaceae	*Sedum album*	Wide.	-	7,8	03.04.2016	Ortaç 1164
82	Crassulaceae	*Umbilicus luteus*	-	-	7,3	03.04.2016	Ortaç 1165
83	Cupressaceae	*Cupressus sempervirens*	-	-	8,2,3	03.04.2016	Ortaç 1004
84	Cupressaceae	*Juniperus oxycedrus* subsp. *oxycedrus*	Wide.	-	7,2	03.04.2016	Ortaç 1007
85	Equisetaceae	*Equisetum hyemale*	-	-	2	06.04.2016	Ortaç 1002
86	Ephedraceae	*Ephedra foeminea*	-	-	7	03.04.2016	Ortaç 1008
87	Ericaceae	*Arbutus andrachne*	-	-	7,3	22.07.2016	Ortaç 1171
88	Ericaceae	*Arbutus unedo*	-	-	7	22.03.2016	Ortaç 1172
89	Euphorbiaceae	*Euphorbia helioscopia*	Wide	-	7	08.05.2016	Ortaç 1175
90	Euphorbiaceae	*Euphorbia peplus* var. *peplus*	Wide.	-	4	08.05.2016	Ortaç 1178
91	Euphorbiaceae	*Mercurialis ovata*	Eu.-Sib. Elm.	-	3	22.07.2016	Ortaç 1179
92	Fabaceae	*Anagyris foetida*	Med. Elm.	-	6,7,8	04.05.2016	Ortaç 1183
93	Fabaceae	*Anthyllis vulneraria subsp. boissieri*	Wide		8,3	22.07.2016	Ortaç 1184
94	Fabaceae	*Astragalus hamosus*	-	-	7,8	04.05.2016	Ortaç 1187
95	Fabaceae	*Calicotome villosa*	Med. Elm.	-	5,7,8,1,2,3	22.07.2016	Ortaç 1189
96	Fabaceae	*Ceratonia silique*	Med. Elm.	-	5,6,7,8,1	04.05.2016	Ortaç 1190
97	Fabaceae	*Cercis siliquastrum* subsp. *Hebecarpa*	-	-	3	22.07.2016	Ortaç 1191
98	Fabaceae	*Securigera varia (syn: Coronilla varia)*	Wide.		8	04.05.2016	Ortaç 1197
99	Fabaceae	*Hippocrepis emerus* subsp. *Emeroides*	-	-	6	03.04.2016	Ortaç 1195
100	Fabaceae	*Lathyrus annuus*	Med. Elm.	-	7	04.04.2016	Ortaç 1207
101	Fabaceae	*Lathyrus aphaca* var. *Modestus*	Med. Elm.	-	6,7	04.04.2016	Ortaç 1209
102	Fabaceae	*Lathyrus variabilis*	E. Med. Elm.	Rare/VU	7	07.05.2016	Ortaç 1210
103	Fabaceae	*Lathyrus vinealis*	Ir.-Tur. Elm.	-	6,8	04.05.2016	Ortaç 1211
104	Fabaceae	*Lotus corniculatus* var. *corniculatus*	Wide.	-	4	08.05.2016	Ortaç 1212
105	Fabaceae	*Medicago marina*	-	-	4	08.05.2016	Ortaç 1217
106	Fabaceae	*Melilotus officinalis*	Wide.	-	4	03.04.2016	Ortaç 1221
107	Fabaceae	*Ononis viscosa* subsp. *breviflora*	-	-	4	22.07.2016	Ortaç 1226
108	Fabaceae	*Pisum sativum* subsp.*elatius*	Med. Elm.	-	6	04.04.2016	Ortaç 1227
109	Fabaceae	*Onobrychis caput-galli*	Med. Elm.	-	2	06.04.2016	Ortaç 1222
110	Fabaceae	*Trifolium campestre*	Wide.	-	4	22.07.2016	Ortaç 1232
111	Fabaceae	*Trifolium purpureum* var. *Purpureum*	E. Med. Elm.	-	1	08.05.2016	Ortaç 1233
112	Fabaceae	*Trifolium repens* var. *Giganteum*	-	-	8	04.05.2016	Ortaç 1234
113	Fabaceae	*Trifolium stellatum var. Stellatum*	-	-	8,1	04.05.2016	Ortaç 1236
114	Fabaceae	*Trigonella spicata*	E. Med. Elm.	-	4	22.07.2016	Ortaç 1237
115	Fabaceae	*Vicia hybrida*	Wide.	-	6,7,8	04.05.2016	Ortaç 1241
116	Fabaceae	*Vicia sativa* subsp. *sativa*	-	-	4,7,8	22.07.2016	Ortaç 1242
117	Fabaceae	*Vicia villosa* subsp. *villosa*	Wide.	-	7,1	26.10.2016	Ortaç 1243

118	Fagaceae	*Quercus cerris*	Med. Elm.	-	6,8	04.04.2016	Ortaç 1245
119	Fagaceae	*Quercus coccifera*	Med. Elm.	-	5,6,7,1,2,3	22.07.2016	Ortaç 1246
120	Geraniaceae	*Erodium malacoides*	Med. Elm.	-	6,7,8	08.05.2016	Ortaç 1251
121	Geraniaceae	*Erodium moschatum*	Med. Elm.	-	7	07.05.2016	Ortaç 1252
122	Geraniaceae	*Geranium dissectum*	-	-	4	08.05.2016	Ortaç 1253
123	Geraniaceae	*Geranium molle*	-	-	3	22.07.2016	Ortaç 1256
124	Geraniaceae	*Geranium purpureum*	-	-	3	22.07.2016	Ortaç 1257
125	Hypericaceae	*Hypericum hircinum*	-	-	3	22.07.2016	Ortaç 1258
126	Hypericaceae	*Hypericum origanifolium*	-	-	7,3	22.07.2016	Ortaç 1259
127	Hypericaceae	*Hypericum perfoliatum*	Med. Elm.	-	2	06.04.2016	Ortaç 1260
128	Illecebraceae	*Paronoychia argentea* var. *argentea*	Med. Elm.	-	4	22.07.2016	Ortaç 1262
129	Iridaceae	*Iris albicans*	Wide.	-	7	07.05.2016	Ortaç 1430
130	Juglandaceae	*Juglans regia*	Wide.	-	1	07.05.2016	Ortaç 1263
131	Juncaceae	*Juncus inflexus*	Wide.	-	4	22.07.2016	Ortaç 1435
132	Juncaceae	*Juncus maritimus*	-	-	4	22.07.2016	Ortaç 1436
133	Lamiaceae	*Ajuga chamaepitys* subsp. *chia*	-	-	7	22.03.2016	Ortaç 1264
134	Lamiaceae	*Ballota saxatilis* subsp. *saxatilis*	E. Med. Elm.	-	5,7	23.07.2016	Ortaç 1267
135	Lamiaceae	*Lamium amplexicaule*	Eu.-Sib. Elm.	-	7	27.10.2016	Ortaç 1268
136	Lamiaceae	*Micromeria myrtifolia*	E. Med. Elm.	-	3,6,7,8	22.07.2016	Ortaç 1277
137	Lamiaceae	*Nepeta isaurica*	E. Med. Elm.	End./LC	7	23.07.2016	Ortaç 1278
138	Lamiaceae	*Nepeta nuda* subsp. *albiflora*	Wide.	-	7	23.07.2016	Ortaç 1279
139	Lamiaceae	*Origanum onites*	E. Med. Elm.		8,2,3	22.07.2016	Ortaç 1281
140	Lamiaceae	*Phlomis nissolii*	İr.-Tur. Elm.	End./LC	7	23.07.2016	Ortaç 1285
141	Lamiaceae	*Phlomis viscosa*	E. Med. Elm.	-	7,8,2	04.05.2016	Ortaç 1286
142	Lamiaceae	*Salvia viridis*	Med. Elm.	-	7,8	07.04.2016	Ortaç 1297
143	Lamiaceae	*Sideritis rubriflora*	Med. Elm.	End./ NT	8	04.05.2016	Ortaç 1300
144	Lamiaceae	*Stachys butlerii*	E. Med. Elm.	End./EN	7	23.07.2016	Ortaç 1303
145	Lamiaceae	*Stachys rupestris*	E. Med. Elm.	End./LC	7	27.10.2016	Ortaç 1307
146	Lamiaceae	*Mentha longifolia* subsp. *typhoides*	Wide.	-	1,2,3,4	22.07.2016	Ortaç 1276
147	Lauraceae	*Laurus nobilis*	Med. Elm.	-	6,7,1,3	23.10.2016	Ortaç 1312
148	Lythraceae	*Punica granatum*	-	-	3	22.07.2016	Ortaç 1323
149	Malvaceae	*Alcea digitata*	Ir.-Tur. Elm.	-	6,7	23.07.2016	Ortaç 1315
150	Malvaceae	*Malva sylvestris*	-	-	4,6	22.07.2016	Ortaç 1316
151	Moraceae	*Ficus carica* subsp. *carica*	Med. Elm.	-	1	27.10.2016	Ortaç 1317
152	Moraceae	*Ficus carica* subsp. *rupestris*	Ir.-Tur. Elm.	-	2,3	23.10.2016	Ortaç 1318
153	Myrtaceae	*Myrtus communis* subsp. *communis*	-	-	4,7	07.04.2016	Ortaç 1322
154	Oleaceae	*Jasminum fruticans*	Med. Elm.	-	6,7,3	27.10.2016	Ortaç 1327
155	Oleaceae	*Olea europaea*	-	-	5,6,7,8,3	23.10.2016	Ortaç 1329
156	Oleaceae	*Phillyrea latifolia*	Med. Elm.	-	5,7,8,1,2,3	22.07.2016	Ortaç 1330
157	Onagraceae	*Epilobium angustifolium*	-	-	2	24.07.2016	Ortaç 1331
158	Papaveraceae	*Fumaria asepala*	Ir.-Tur. Elm.	-	7,8,1	04.05.2016	Ortaç 1333
159	Papaveraceae	*Papaver rhoeas*	Wide.	-	6,7	26.10.2016	Ortaç 1336
160	Papaveraceae	*Papaver syriacum*	-	-	1	23.07.2016	Ortaç 1337
161	Pinaceae	*Pinus brutia*	E. Med. Elm.	-	5,7,8,1,2,3	07.04.2016	Ortaç 1011
162	Plantaginaceae	*Linaria chalepensis* var. *chalepensis*	E. Med. Elm.	-	6,7,8,3	23.10.2016	Ortaç 1394
163	Plantaginaceae	*Plantago coronopus* subsp. *coronopus*	Eu.-Sib. Elm.	-	4	25.10.2016	Ortaç 1339
164	Plantaginaceae	*Plantago lanceolata*	Wide.	-	7	23.07.2016	Ortaç 1340
165	Plantaginaceae	*Plantago maritima*	-	-	4	08.05.2016	Ortaç 1342
166	Plantaginaceae	*Veronica cymbalaria*	Med. Elm.	-	7,8	27.10.2016	Ortaç 1401
167	Platanaceae	*Platanus orientalis*	Wide.	-	1,2,3	27.10.2016	Ortaç 1343
168	Plumbaginaceae	*Limonium angustifolium*	Med. Elm.	-	4,8	22.07.2016	Ortaç 1344
169	Poaceae	*Arundo donax*	-	-	8,1	26.10.2016	Ortaç 1462
170	Poaceae	*Avena sterilis* subsp. *ludoviciana*	-	-	6	23.07.2016	Ortaç 1463
171	Poaceae	*Cynodon dactylon* var. *dactylon*	-	-	4,2	25.10.2016	Ortaç 1466
172	Poaceae	*Dactylis glomerata subsp. glomerata*	Eu.-Sib. Elm.	-	2	24.07.2016	Ortaç 1467
173	Poaceae	*Hordeum bulbosum*	Wide.	-	2	24.07.2016	Ortaç 1469
174	Poaceae	*Hordeum murinum* subsp. *glaucum*	-	-	4,2	25.10.2022	Ortaç 1470
175	Poaceae	*Lolium rigidum* var. *Rottbollioides*	E. Med. Elm.	-	4,2	25.10.2016	Ortaç 1471
176	Poaceae	*Phragmites australis*	Eu.-Sib. Elm.	-	4,8	08.05.2016	Ortaç 1474
177	Poaceae	*Poa bulbosa*	Wide.	-	3	23.10.2016	Ortaç 1476

178	Polygonaceae	*Polygonum maritimum*	-	-	5	22.07.2016	Ortaç 1346
179	Pteridaceae	*Adiantum capillus-veneris*	-	-	2,3	08.05.2016	Ortaç 1000
180	Primulaceae	*Anagallis arvensis* L. var. *arvensis*	-	-	4,6,7,8	22.07.2016	Ortaç 1351
181	Primulaceae	*Cyclamen persicum*	E. Med. Elm.	-	8	24.10.2016	Ortaç 1355
182	Primulaceae	*Cyclamen cilicium*	-	-	8	24.10.2016	Ortaç 1353
183	Ranunculaceae	*Adonis aestivalis* subsp. *aestivalis*	Wide.	-	6	26.10.2016	Ortaç 1356
184	Ranunculaceae	*Adonis annua*	Med. Elm.	-	7	23.07.2016	Ortaç 1357
185	Ranunculaceae	*Clematis cirrhosa*	Med. Elm.	-	6	26.10.2016	Ortaç 1361
186	Ranunculaceae	*Delphinium peregrinum*	Med. Elm.	-	7	23.07.2016	Ortaç 1362
187	Ranunculaceae	*Staphisagria macrosperma*	Med. Elm.	-	7	23.07.2016	Ortaç 1363
188	Rhamnaceae	*Paliurus spina- christi*	-	-	4,5,6,7,2,3	22.07.2016	Ortaç 1369
189	Rhamnaceae	*Rhamnus lycioides* subsp. *oleoides*	-	-	6,7	08.05.2016	Ortaç 1371
190	Rhamnaceae	*Ziziphus jujuba*	-	-	4	25.10.2016	Ortaç 1372
191	Rosaceae	*Prunus spinosa*	Eu.-Sib. Elm.	-	6,3	23.10.2016	Ortaç 1378
192	Rosaceae	*Rosa canina*	-	-	8,2	24.10.2016	Ortaç 1380
193	Rosaceae	*Rubus sanctus*	Wide.	-	1,2,3	08.05.2016	Ortaç 1381
194	Rosaceae	*Sanguisorba minor*	-	-	6,1	27.10.2016	Ortaç 1382
195	Rosaceae	*Sarcopoterium spinosum*	E. Med. Elm.	-	4,5,7,8	22.07.2016	Ortaç 1383
196	Rutaceae	*Ruta chalepensis*	-	-	6,7	24.10.2016	Ortaç 1386
197	Salicaceae	*Salix alba*	Eu.-Sib. Elm.	-	7,3	22.07.2016	Ortaç 1390
198	Scrophulariaceae	*Scrophularia rimarum*	-	-	7	26.10.2016	Ortaç 1396
199	Scrophulariaceae	*Verbascum sinuatum* subsp. *sinuatum* var. *sinuatum*	Med. Elm.	-	4	22.07.2016	Ortaç 1399
200	Scrophulariaceae	*Veronica anagallis-aguatica*	Wide.	-	4	25.10.2016	Ortaç 1400
201	Scrophulariaceae	*Scrophularia xanthoglossa*	-	-	1	23.07.2016	Ortaç 1398
202	Smilacaceae	*Smilax aspera*	-	-	5,7,1	22.07.2016	Ortaç 1454
203	Smilacaceae	*Smilax excelsa*	Eu.-Sib. Elm.	-	2	26.10.2016	Ortaç 1455
204	Styracaceae	*Styrax officinalis*	-	-	6,8,3	27.10.2016	Ortaç 1406
205	Tamaricaceae	*Tamarix smyrnensis*	-	-	4,5	22.07.2016	Ortaç 1407
206	Thymelaeaceae	*Daphne oleoides* subsp. *oleoides*	-	-	5,8	24.10.2016	Ortaç 1409
207	Thymelaeaceae	*Daphne sericea*	E. Med. Elm.		7,8,3	22.07.2016	Ortaç 1410
208	Thymelaeaceae	*Thymelaea hirsuta*	Med. Elm.	-	4	08.05.2016	Ortaç 1411
209	Urticaceae	*Parietaria judaica*	Wide.	-	4,6,3	22.07.2016	Ortaç 1414
210	Urticaceae	*Urtica dioica*	Eu.-Sib. Elm.	-	6,7,3	23.10.2016	Ortaç 1415
211	Vitaceae	*Ampelopsis orientalis*	E. Med. Elm.	-	2,3	23.10.2016	Ortaç 1420
212	Verbenaceae	*Vitex agnus-castus*	Med. Elm.	-	4,5,8	08.05.2016	Ortaç 1419
213	Xanthorrhoeaceae	*Asphodelus aestivus*	Med. Elm.	-	4,8,1,3	22.07.2016	Ortaç 1443
214	Zygophyllaceae	*Zygophyllum album*	-	Rare/VU	5	08.05.2016	Ortaç 1423

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Lectotypification of the name *Kundmannia syriaca* (Apiaceae)

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ABSTRACT
Kundmannia syriaca was recently transferred to the genus *Antalia*. During this research it became clear that the name *K. syriaca* needed to be typified. One of the two isotypes preserved in the herbarium Geneva was designated as the lectotype of *K. syriaca*.

Botany

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Kundmannia syriaca (Apiaceae) adının lektotipifikasyonu

ÖZET
Kundmannia syriaca yakın zamanda *Antalia* cinsine aktarılmıştır. Bu araştırma sırasında *K. syriaca* isminin tiplendirilmesi gerektiği ortaya çıkmıştır. Cenevre herbaryumunda muhafaza edilen iki izotipten biri *K. syriaca* adının lektotipi olarak belirlenmiştir.

Botanik

Araştırma Makalesi

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Anahtar Kelimeler
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INTRODUCTION

Kundmannia Scop. is an endemic Mediterranean monotypic genus that includes *K. sicula* (L.) DC. (Tutin, 1968; Doğru-Koca, 2024). Before Doğru-Koca (2024), the genus *Kundmannia* included not only *K. sicula* but also *K. syriaca* Boiss. and *K. anatolica* Hub.-Mor. However, in phylogenetic studies, the monophyletic clade comprising *K. syriaca* and *K. anatolica* populations occurred far from the clade of *K. sicula*. Therefore, these two species were recently transferred to a new genus, *Antalia* Doğru-Koca (Doğru-Koca, 2024).

Kundmannia sicula is distributed from the west Mediterranean to Greece (Tutin, 1968; Knees, 2003; POWO, 2023; and specimens in cited herbaria). In the protolog, the original material was collected from Sicily (Linnaeus, 1753) (Figure 1). It was recently both lectotypified and epitypified by Jury et al. (2006). While an icon was selected as the lectotype in Zanoni (1675), Davis and Sutton's specimen, collected from Sicily and kept in BM [BM001134523 (photo!)], was selected as the epitype.

The other two species *Kundmannia syriaca* (current accepted name: *Antalia syriaca* (Boiss.) Doğru-Koca) and *K. anatolica* Hub.-Mor. (current accepted name: *A. anatolica* (Hub.-Mor.) Doğru-Koca) are endemic to Türkiye. Both of them occur in the Taurus Mountains of southern Anatolia (Hedge & Lamond, 1972; Huber-Morath, 1983). *A.*

syriaca morphologically differs from *A. anatolica* by its slightly unequal rays (vs. strongly unequal rays), much broader upper stem leaves (vs. relatively less broad), and green or yellow-green petals (vs. white) (Huber-Morath, 1983; Doğru-Koca, 2024).

Antalia anatolica was recently transferred and its original materials are kept in herbaria G [holo. G00367095!] and E [iso. (photo!) E00279098]. *A. syriaca*, because of its epithet, is supposed to be distributed in Syria. However, it only occurs in Hatay-Adana, so only in Türkiye (Figure 1).

Figure 1. Distribution and type localities of the species of *Antalia*. The red circle marks the epitype locality of *Kundmannia sicula* in Sicily. Yellow and purple circles indicate the distribution of *Antalia anatolica* and *Antalia syriaca*, respectively. Black bordered circles indicate type localities.

Şekil 1. Antalia türlerinin dağılımı ve tip lokaliteleri. Kırmızı daire Kundmannia sicula'nın Sicilya'daki epitip lokalitesini göstermektedir. Sarı ve mor daireler sırasıyla Antalia anatolica ve Antalia syriaca'nın dağılımını göstermektedir. Siyah kenarlı daireler tip lokalitelerini göstermektedir.

Recently, Pimenov & Jacquemoud (2020) published a comprehensive study to reveal the nomenclatural types of the Umbelliferae kept in G-BOIS. They confirmed that there is a holotype of *Kundmannia syriaca* in the herbarium G (Figure 2A). Hence, they cited this specimen barcoded G00367018 (image available at https://www.ville-ge.ch/musinfo/bd/cjb/chg/adetail.php?id=260719&base=img&lang=en) as the holotype (Pimenov & Jacquemoud, 2020) (Figure 2B). On the "holotype", there was a label, "Montagnes d'Antioche", handwritten by Boissier and a typewritten note "Syria, Mai-Jul. 1846" (Figure 2C). "Antioche" means Antakya, which is a city in Türkiye.

On the other hand, a relevant specimen was also found in G, barcoded G00757283 (image available at https://www.ville-ge.ch/musinfo/bd/cjb/chg/adetail.php?id=537838&base=img&lang=en) collected by Boissier (Figure 2D). It had a label the same as the "holotype" of *Kundmannia syriaca* in G (Figure 2E). However, it was identified as a "holotype of *K. sicula*" by Pimenov in 2005 (Figure 2F). Then, in 2019, a scientist labeled it as *K. syriaca* (Figure 2F). On examination of these specimens, my conclusion was that there was confusion about these two herbarium sheets in G. First, the sheet G00757283 could not be a holotype of *K. sicula* because of its morphological characteristics and the location it was collected. *K. sicula* is a west Mediterranean element and its type of location is from Sicily (not Syria), as explained previously. It is absolutely another specimen of the original materials of *K. syriaca*. Secondly, if there are two original materials for a taxonomic name, they should be accepted as syntypes.

In the current study, I propose a lectotype for the name of *Kundmannia syriaca* Boiss. according to Art. 9.3 of the International Code of Nomenclature (Turland et al., 2018).

Figure 2. Original materials of *Kundmannia syriaca* Boiss. in herbarium Geneva. A. Holotype confirmation by Pimenov and Jacquemoud (2020). B. Isolectotype, barcoded G00367018. C. Close-up handwritten label of the isolectotype. D. Lectotype, barcoded G00757283. E-F. Close-up labels of the lectotype. G. Other sheet of the isolectotype. H-I. Divided root of the isolectotype sheets.

Şekil 2. Kundmannia syriaca Boiss. türünün Cenevre herbaryumundaki orjinal materyalleri. A. Pimenov ve Jacquemoud (2020) tarafından onaylanan holotip. B. İzolektotip, G00367018 barkodlu. C. İzolektotipin yakın çekim el yazısı etiketi. D. Lektotip, G00757283 barkodlu. E-F. Lektotipin yakın çekim etiketleri. G. İzolektotipin diğer herbaryum örneği. H-I. İzolektotip yapraklarının bölünmüş kökü.

MATERIALS and METHODS

Approximately 220 samples were examined on various herbaria visits to HUB, ANK, GAZI, EGE, ISTE, ISTF, P, G, UPA, ATU, and ATHU. Digital images of *Kundmannia* specimens preserved in virtual herbaria B, BM, E, G, GH, GOET, JE, K, L, LE, Linn, MW, and W were also carefully examined. Numerous Floras (Tutin, 1968; Hedge & Lamond, 1972; Knees, 2003) and databases (GBIF, 2023; POWO, 2023) were used for the morphological investigations. The Shenzhen Botanical Code (Turland et al., 2018) was also followed. Furthermore, the amendments to the articles cited here were reviewed at the Madrid Code meeting and it was concluded that they had no impact on the outcome (Turland et al., 2024).

RESULTS

Antalia syriaca (Boiss.) Doğru-Koca, Bot. J. Linn Soc. 206: 95-103 (2024).

Homotypic Synonym: *Kundmannia syriaca* Boiss., Diagn. Pl. Orient. 10: 31 (1849).

Type locality in protologue: "Hab. in fissuris rupium calcareorum ponè urbem *Antiochiam* [Antakya] sitorum medio Junio vix florere incipiens. (Boiss.)" (Boissier, 1849).

LECTOTYPE (**designated here**): (Türkiye, C6 Hatay) Montagnes d'Antioche [Antakya], Syria, Mai–Jul. 1846. *E. Boissier* (G, barcode G00757283 [!]; isolectotype: G, barcode G00367018 [!]).

DISCUSSION

The specimen barcoded G00367018, which was divided into half at the root, was pasted on two separate sheets (Figs. 1B, G-I). One of them, which Pimenov determined as a holotype, was young, so it was a morphologically poor specimen (Fig. 1A). The second half did not have a barcode (second sheet) (Fig. 1G). Both sheets should be accepted as a specimen, because they were preserved in the same folder, as exampled in ICN Art. 8 Ex. 9. Whereas the other specimen, barcoded G00757283, was a relatively mature specimen. Although it was clear from the labelling that both (G00367018 and G00757283) were gathered from the same population and at the same time, they are two different individuals. Pimenov & Jacquemoud (2020) determined one of them as the holotype belonging to *Kundmannia syriaca*, while Pimenov labelled (not published) the other one as the holotype of *K. sicula*. It is impossible for a specimen collected from Antakya to be the type species of *K. sicula*, as Pimenov had labelled, because the locality of that type of *K. sicula* is Sicily. Morphologically, both specimens were very similar to *K. syriaca*. Therefore, according to ICN Art. 9.6, these two specimens were considered as original materials, syntypes of *K. syriaca,* and one of them was selected as a lectotype according to Art. 9.3 (Turland et al., 2018).

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Bioactivity-guided isolation of inositol as acetylcholinesterase inhibitory from endemic *Campanula baskilensis* Behcet: *In vitro* bioactivity, PCA analysis, and *silico* supporting studies

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ABSTRACT

This study aimed to identify the bioactivity-guided molecule in the fractions of *Campanula baskilensis* leaf methanol: chloroform extract. The bioactivity of leaf fractions was investigated to assess and isolate bioactive molecule/molecules and structural configurations. Fractionation and isolation processes were done using advanced column chromatography techniques. *In-vitro* bioactivity tests were applied, including enzyme inhibition, antibacterial, and DNA protection activities. The isolated compound was characterized using the NMR technique. *In silico* analyses were investigated using molecular docking, molecular dynamics, and final-state free energy calculations. 14 different fractions were obtained (F1-F14) through the fractionation. F12 has the highest AChE inhibition (IC_{50}; 6.97±2.90 µg/mL), F6 has significant inhibition against carbonic anhydrase and α-amylase (IC_{50}; 5.61±0.01 and 18.82±1.48 µg/mL). F12 and F11 have the highest antibacterial activity against *E. coli* (15.40±1.10 and 13.00±0.80 mm). F12 and F5 fractions have the highest protection activity in plasmid DNA, and F6 has the highest deoxyribose protection activity. Many fractions have high and varied bioactivity due to the bioactive compound components, as in F12. Principal component analysis showed that F12 positively correlated with the high inhibition activity for several bacteria and enzymes and high DNA protection. Therefore, further fractionation was applied using Sephadex LH-20 with ethyl acetate:methanol: hexane (5:5:1) to F12. Inositol was isolated according to results from the obtained fraction; the molecule characterization was clarified using the H-NMR and C13-NMR spectra. Molecular docking results showed binding between inositol and AChE. Further, molecular dynamics results showed the stability of inositol-AChE within 100 nanoseconds, and the energy calculations (gmx-MMPBSA) showed the strength of this interaction.

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Endemik *Campanula baskilensis* Behçet bitkisinden asetilkolinesteraz inhibitörü olarak inositolün biyoaktivite rehberliğinde izolasyonu: *İn vitro* biyoaktivite, PCA analizi ve *İn silico* destekleyici çalışmalar

ÖZET

Bu çalışmada *Campanula baskilensis* yaprak metanal:kloroform ekstraktının fraksiyonlarında biyoaktivite-yönlendirmeli moleküle ulaşılması amaçlanmıştır. Biyoaktif molekül/moleküllere ve yapısal konfigürasyona ulaşmak ve izole etmek için yaprak fraksiyonlarının biyoaktivitesi araştırıldı. Fraksiyonlama ve izolasyon işlemleri gelişmiş kolon kromatografisi tekniği kullanılarak gerçekleştirildi. Fraksiyon örnekleri için enzim inhibisyonu, antibakteriyel ve DNA koruma aktiviteler dahil olmak üzere *in vitro* biyoaktivite testleri uygulandı. İzole edilen bileşik NMR tekniği kullanılarak karakterize edildi. *İn silico* analizlerle moleküler doking, moleküler dinamikler ve

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Campanula baskilensis
Biyoaktivite rehberliğinde

son durum serbest enerji hesaplamalarını araştırıldı. Fraksiyonlama işlemi sonucunda 14 farklı fraksiyon elde edildi (F1-F14). F12 AChE karşı (IC_{50}; 6.97±2.90 µg/mL), F6 karbonik anhidraz ve □-amilaza karşı (IC_{50}; 5.61±0.01 ve 18.82±1.48 µg/mL) yüksek inhibisyon aktivite gösterdi. F12 ve F11 *E. coli*'ye karşı en yüksek antibakteriyel aktivite gösterdi (15.40±1.10 ve 13.00±0.80 mm). F12 ve F5 fraksiyonları plazmit DNA'sında en yüksek koruma aktivitesine sahiptir ve F6, deoksiribozu korumak için en yüksek aktiviteye sahiptir. Birçok fraksiyonun, F12'de olduğu gibi, yüksek biyoaktif bileşikler içerdiğinden yüksek ve çeşitli bir biyoaktiviteye sahip olduğu gösterilmiştir. F12'nin test edilen çeşitli bakteri ve enzimler için yüksek inhibisyon aktivitesinin yanı sıra yüksek DNA koruması ile ana bileşen analizi pozitif korelasyon gösterdiği. Bu nedenle F12'ye Sephadex LH-20 ve etil asetat:metanol:hekzan (5:5:1) kullanılarak daha ileri fraksiyonlama ilerletildi. F12'den inositol izole edildi ve karakterizasyonu H-NMR ve C13-NMR spektrumları kullanılarak açıklandı. Moleküler doking sonuçlarına göre inositolün AChE enzimine bağlanabileceğini gösterdi. Ayrıca, moleküler dinamik sonuçları inositol-AChE'nin 100 nanosaniye içinde kararlı olduğunu gösterdi ve enerji hesaplamaları (gmx-MMPBSA) bu etkileşimin gücünü gösterdi.

izolasyon
İn vitro biyoaktivite
İn siliko çalışmalar

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INTRODUCTION

Natural products have always been a significant and successful source of drugs (Harvey, 2000). Several previous studies have emphasized that the chemical components of plants performed exceptionally well in drug discovery and development (Newman and Cragg, 2016). Identifying and isolating novel chemical components with high biological activity from natural product sources has contributed significantly to the development of pharmacology. It can be used as a model for developing a new potential drug or improving the effectiveness of previously used drugs (Nastić et al., 2018).

Natural bioactive ingredients are abundant in plants, also known as Phytochemicals. Despite its abundance of plants, only a few have been isolated and identified (Singh and Chaudhuri, 2018). The primary reason for the biosynthesis of bioactive compounds is protection or attractional in most plants. In addition, it has a biological activity reflected in multiple ways on other organisms, which could have beneficial or harmful effects on human and animal health (Bernhoft, 2010). Phytochemical benefits can act as a substrate for biochemical reactions, a cofactor or inhibitor for enzymes, remove unwanted components in the intestine, an agonist and antagonist to the cell receptor, a toxic chemical scavenger, can positively affect beneficial gut microorganisms, and can be a growth inhibitor as an antibacterial factor.

Moreover, through laboratory tests, many studies have shown the effectiveness of these materials against various diseases such as cancer, cardiovascular disease, diabetes, ulcers, inflammation, infection, neurologic disease, and many other diseases (Dillard and German, 2000). The most important groups of dietary phytochemicals are phenolics, alkaloids, nitrogen-containing compounds, organosulfur compounds, phytosterols, and carotenoids. Phenolics and carotenoids are the most studied dietary phytochemical groups related to human health and well-being (Liu, 2004).

This process necessitates studying these chemical structures and biochemical effects to achieve the researchers' requirements and/or to comprehensively understand the natural products and their effective contents to keep up with modern biological research, drug discovery, and development. The traditionally established process to isolate bioactive plant compounds begins with identifying and preparing the plant's materials, commonly by drying them and then extracting them using various chemical solvents, depending on the polarity from lowest to highest (Sarker and Nahar, 2012). A characteristic model to isolate a pure chemical compound from its natural origin is bioactivity-

guided fractionation, based on step-by-step separation of extracted components based on variances in the physical and chemical properties and estimation of the bioactivity, followed by another round of separation and assaying (Malviya and Malviya, 2017). Using numerous separation technologies for the plant parts extracts, such as column chromatography, gives a crude bioactive compound (Bucar et al., 2013).

Campanula L. is the most comprehensive type of the Campanulaceae family. It comprises 420 sub-genus, most spread in the circumboreal area, southern Asia, and northern Mexico (Alcitepe, 2011; Lammers, 2007; Yildirim, 2018). *Campanula* species cover a variety of chemical compounds; flavonoids, phenolics, anthocyanins, polyethylene's, phenylpropanoids, essential oils, acylated triterpenoids, glycosides, resins, coumarins, as well as wide range of subgroups of compounds; catechin, diosmin, quercetin, pelargonidin, delphinidin, cyanidin derivatives, fraxin, linalool, α-terpineol, lavandulyl acetate, (E, E)-allo-ocimene (allo-ocimene), ß-pinene, α-cadinene, ß-farnesene, ß-caryophyllene, myo-inositol, lipids (glycolipids and phospholipids), fatty acid (Linoleic and oleic), sterols (stigmasterol and β-sitosterol), tocopherols (α-tocopherol, β-tocopherol and γ-tocopherol) and alkaloids (Brandt et al., 2017; Dumlu et al., 2008; Hassanien et al., 2014; Ishida et al., 2008; Kim et al., 2017; Ouzounis et al., 2014; Vergauwen et al., 2000). *Campanula* secondary metabolites have numerous biological activities: antibacterial, antifungal, insecticidal, anticancer, and antioxidant potentials (Cuendet et al., 2001; Dumlu et al., 2008; Kim et al., 2006; Vincken et al., 2007). *Campanula* species have been used in traditional and oriental medicine for centuries. It has been used to reduce the risk of a wide range of respiratory diseases, including asthma, bronchitis, larynx inflammation, tonsillitis, pulmonary tuberculosis, and wart inflammation. It also has stimulating, antiallergic, antiphlogistic (anti-inflammatory drug), antioxidant, spasmolytic, antiviral, and antimicrobial properties and is used as an antiepileptic and constipation-regulating herb (Qi et al., 2020; Rameau et al., 1989).

In this previous study, *C. baskilensis* Behcet leaf part extract showed a higher biological activity than the other parts. This study aimed to achieve the active compounds through fractionation (Marah et al., 2024). For this purpose, we tested a wide range of bioactivity for each of the fractions of *C. baskilensis* including antibacterial (disc diffusion), enzyme inhibition (urease, acetylcholine esterase, butyrylcholine esterase, lipase, carbonic anhydrase, α-amylase, α-glucosidase and tyrosinase) activities and DNA damage protective capability all of the activities had been standardized and estimated using the standard materials. in addition to the spectroscopic examination H-NMR and C13-NMR to demonstrate the structure and chemical properties for the isolated molecules. Finally, we performed an *in silico* study to check the effectiveness of the isolated molecule by applying molecular docking, molecular dynamics, and energy calculations for the final state.

MATERIAL and METHOD

Plant material and chemicals

Campanula baskilensis Behcet sp. nov. is a locally endemic and distributed *Campanula* species known to be extended in the Baskil (Elazığ) district in the Eastern Anatolia territory of Turkey; 15 km south of Baskil town, 38° 27'18"N, 38° 49'41"E, southeast of Topalkem village, rocky area, 900–950 m a.s.l., gathered in July, L. Behçet 11860 (holotype: ANK, isotypes: Bingöl Univ. Herb. and Mustafa Kemal Univ. Herb.) by Prof. Dr. Lütfi Behçet, Bingol University, Faculty of Arts, Department of Biology (Behçet and İlçim, 2018). All chemicals and reagent materials used are from pioneer companies: Sigma-Aldrich, Merck, Acros, Himedia, Thermoscientific, Gelentham, Isolab, and Carlo Erba.

Fractionation and Isolation

This separation process was carried out on the sub-fractionation of the effective fractions in their antibacterial, enzyme inhibition, and DNA-related activities to reach the main pure compounds. The fractionation process was applied to the leaf part of *C. baskilensis* to obtain different fractions with different characteristics, and then isolate a pure compound from the plant. 251.00 grams of the leaf part of the plant was collected and dried in the shade at room temperature, completely ground via a laboratory blender, and ready for extraction, then the extraction done using the methanol-chloroform (MeOH: $CHCl_3$, 1:1) solvent system by applying the maceration extraction method procedure three times in a row. Then, 32.00 g of extract was obtained by removing the solvent in the evaporator. The extract was impregnated with silica gel, eluted with hexane (Hex), and loaded onto the silica column. Also, chloroform, ethyl acetate (EA), and methanol solvents were used, respectively, according to the increasing polarity of the elution. With this crude fractionation, 14 main fractions were obtained. The fraction with high activity was treated with advanced chromatography using a Sephadex (LH-20) column in the methanol-ethyl acetate-hexane (5:5:1) solvent system to obtain the main components. The total number of fractions reached 14 (F1-F14) (Figure 2). This process was carried out the same way, using the Sephadex (LH-20) column until the pure compound in the leaf part of the *C. baskilensis* plant was reached. The molecular structure was determined using 1D and 2D NMR

techniques: H-NMR, C13-NMR, Heteronuclear Single Quantum Coherence (HSQC), Heteronuclear Multiple Bond Correlation (HMBC), and Correlated Spectroscopy (COSY).

Enzyme inhibition activity

In the tests of the inhibitory ability of the *C. baskilensis* leaf fractions, we selected many enzymes whose abnormal activities may cause health problems and various human diseases. The first two enzymes associated with Alzheimer's disease, inhibition activity for the fraction's samples tested against cholinesterase's enzymes acetylcholine esterase (AChE) and butyrylcholine esterase (BChE), inhibition activity of the samples had been spectrophotometrically measured using the method by Ellman et al. (1961), based on the reaction of dithiobis(2-nitrobenzoic acid) (DTNB) reagent with thiocholine which will produce a yellow colored 5-thio-2-nitrobenzoate, which can be determined at 412 nm for both enzyme assays; After mixing 20 µL of the samples (8–1024 µg/mL), 140 µL of Na-K phosphate buffer (0.1 mM pH 8.0), 20 µL (3.3 mM) DTNB, and 20 µL of AChE or BChE (0.03 U/mL), the mixture was incubated for 15 minutes at 25 °C. After adding 10 µL of 1 mM butyrylcholine chloride or acetylcholine iodide, absorbance readings were determined at 412 nm. The results of the samples for inhibition activity were compared to galantamine as a positive control in both enzymes. The urease inhibition capacity for fractions and thiourea was determined spectrophotometrically based on a previously published method (Zhang et al., 2006), depending on the hydrolysis of urea in the presence of samples with a phenol-form), 25 µL of urease (5 U/mL, 100 mM Na-K buffer pH 8.2), 50 µL of urea (100 mM), and 10 µL fractions (8–1024 µg/mL) were mixed and incubated for 15 minutes at 30 °C. After that, 70 µL of alkaline reagent (2.5% NaOH + 4.7% NaOCl) and 45 µL of phenol (8% phenol + 0.1% sodium nitroprusside) were added, and the mixture was incubated for 50 minutes at 30 °C. The absorbance value was read at 630 nm.

The carbonic anhydrase (CA) inhibition activity for the fraction samples has been determined spectrophotometrically (Chanda et al., 2019), and acetazolamide was used as a positive control in this test. The basis of the experiment was the color shift of the enzyme-substrate *p*-nitrophenyl acetate (*p*-NPA) from transparent to yellow upon hydrolysis by the enzyme; 60 µL of the sample, 90 µL of 115 U/mL carbonic anhydrase (in 0.05 M pH 7.4 Tris-SO_4 buffer) was thoroughly mixed. The mixture was incubated for 15 minutes at 25 °C. After adding 60 µL of 10 mM *p*-NPA and 15 minutes at room temperature, the mixture's absorbance was determined at 400 nm.

The following two enzymes associated with diabetes mellitus are α-glucosidase and α-amylase. First, α-glucosidase inhibition activity was obtained using spectrophotometric methods (Mayur et al., 2010; Sancheti et al., 2010). As identical to the carbonic anhydrase inhibition test, inhibition activity determination for the fraction's samples against α-glucosidase enzyme was related to the color conversion of the enzyme-substrate *p*-nitrophenyl α-D-glucopyranoside (PNPG) from colorless to yellow-colored *p*-nitrophenol; 10 µL of the sample (8–1024 µg/mL), 25 µL of 0.2 U/mL enzyme, 25 µL of 0.5 mM PNPG, and 50 µL of 20 mM pH 6.9 phosphate buffer, 50 µL of 0.2 M $NaCO_3$ was added, and the combination was allowed to sit at 37 °C for half an hour. The absorbance of the mixture was then measured at 410 nm. α-amylase inhibition activity for the fraction samples was determined spectrophotometrically; A homogenous mixture of 82 µL of the sample (8–1024 µg/mL) and 10 µL of 1 U/mL α-amylase (20 mM PBS, pH 6.9) was made. After 10 minutes at 37 °C, 8 µL of substrate (% 1% starch) was added, and it was once more kept at 37 °C for 12 minutes. To halt the reaction, 50 µL of 10% HCl, 15 µL of iodine-KI (2.5 mM iodine (I_2) + 6.5 mM KI (ddH_2O)), and 50 µL of ddH_2O were added. The mixture was then heated to a boil for 10 minutes, and once it cooled, absorbance values were taken at 620 nm. (Ercan and El, 2016), Depending on the color conversion in the test solution caused by the bonding of iodine reagent with the disaccharides produced by hydrolysis of starch by α-amylase in an acidic medium, absorbance values were measured at 620 nm. In both assays, acarbose was used as a positive control.

The lipase inhibition capacity for the fraction samples was determined using orlistat as a positive control based on a previously published method (McDougall et al., 2009; Trentin et al., 2020). The basis of the experiment was based on the color shift of the enzyme-substrate *p*-nitrophenyl octanoate (*p*-NPO) from transparent to yellow upon hydrolyzation by the enzyme, Samples of 20 µL (8–1024 µg/mL), 200 µL Tris-HCl buffer (100 mM Tris-HCl, pH 8.2), 20 µL 1 mg/mL lipase enzyme solution, and 20 µL 5.1 mM p-nitrophenyl octanoate were mixed homogeneously then incubated at 37 °C for 30 minutes, absorbance values were determined at 410 nm.

The last enzyme, tyrosinase, participates in melanin biosynthesis, and the inhibitory activity for the samples and the standard inhibitor kojic acid has been obtained based on a previously published method (Addar et al., 2019). The principle of this assay also depended on the color conversion made by the enzyme hydrolysis ability on the substrate (L-DOPA); 10 µL of sample, 150 µL of phosphate buffer (0.1 M, pH = 6.8), and 20 µL of tyrosinase enzyme (150 U/mL, 200 µg/mL), then incubated for 10 minutes at 37 °C. Next, 20 µL of 5 mM L-DOPA was added, and a reading was taken at 475 nm.

Antibacterial Activity

C. baskilensis leaf fractions' antibacterial activity had been tested against two groups of bacteria: Three species of the gram-positive bacteria (*Staphylococcus aureus*, ATCC 25213; *Enterococcus faecalis*, ATCC 29212; and *Bacillus cereus*, CCM 99) and three species of the gram-negative bacteria (*Pseudomonas aeruginosa*, ATCC 15442; *Klebsiella pneumoniae*, ATCC 10031; and *Escherichia coli*, ATCC 25922). Tetracycline was used as a positive control in both tests.

The disc diffusion test, 1 mg/mL concentration of each fraction, was applied for the disc diffusion test based on a previously published method (Reller et al., 2009). 100 µL of 0.5 McFarland bacteria were spread on Mueller-Hinton agar placed in the petri dish, and then 40 µL of sample solution and positive control were absorbed with 6 mm discs and placed on the petri dish's surface. Results were measured in millimeters for the inhibition zones after 18 hours of incubation at 37 °C (except *B. cereus* incubated at 30 °C).

DNA damage protection potential assays

C. baskilensis leaf fractions also had been tested for DNA protective activities, as DNA protection activity; using agarose-gel electrophoresis of plasmid DNA (pBR322) based on the protocol published in past studies (Baiseitova et al., 2021; Russo et al., 2003; Sevgi et al., 2015; Tepe et al., 2011). Test done as follows: firstly, in an Eppendorf tube, we add 4 µL glycerol, 5 µL fractions solution (1 mg/mL), 3 µL of pBR322 plasmid DNA (172 ng/µL), and 1 µL of 30% H_2O_2, respectively. The previous step was followed by incubation under UV radiation for 5 minutes at 25 °C. The Final step of preparing the solution for the test mixture was to add 2 µL of color indicator (bromophenol blue) to the test tubes. After that, the test mixture was carefully loaded into the agarose gel wells (1.5 % prepared by mixing 1X Tris-Borate-EDTA buffer and 2 µL of ethidium bromide). Then, electrophoresis was applied for 120 minutes at 90 volts. Results of the % DNA protective activity for the fractions calculated using the ImageJ program for UV transilluminator on the recorded gel image.

Molecular Docking and Molecular Dynamics Evaluation

Due to the high biological activity of F12, especially in the AChE inhibition, we tested this molecule against the acetylcholinesterase crystal structure (PDB code: 1C2O). Molecular docking has been applied using the open-source software Auto Dock Vina (Trott and Olson, 2010). Using the optimized compound structures, we applied the docking with the target protein X-ray crystal structures with a high resolution from the PDB bank(Berman et al., 2000). The docking experiments maintained both the protein and compound in a flexible state. The free-binding affinities of the conformations binding to the active pockets of each protein, along with their interactions, were analyzed using DiscoveryStudio visualization software (BIOVIA, 2017).

The stability of the inositol-AChE resulting from docking was examined by GROMACS (Abraham et al., 2015). The CHARMM force field was applied to form the AChE force field, and then the complex was solvated with TIP3P water.

To balance the system's overall potential, Na^+ and Cl^- ions were included. Subsequently, the steepest descent method, which involved 50,000 steps, was employed to minimize energy consumption. After that, NVT/NPT set the system's temperature and pressure to 310 K and 100 kPa, respectively. Ultimately, the MD simulation took 100 ns to complete (Bjelkmar et al., 2010). The results of the molecular dynamics simulation were analyzed by generating ligand hydrogen bond plots using Grace, along with assessments of RMSF (root mean square fluctuation), Rg (radius of gyration), and RMSD (root mean square deviation) (Akkoc et al., 2023; Başar et al., 2024).

The Molecular Mechanics/Poisson-Boltzmann surface area (MM/PBSA) was applied to achieve the binding-free energy of the end time complex using gmx_mmpbsa software (Valdés-Tresanco et al., 2021).

Statistical Analysis

Statistical analysis was done for all test results for the *C. baskilensis* fraction samples using the SPSS 22.0 (Statistical Package for the Social Sciences) package program. The test was conducted to find out the difference between the samples and between the samples with positive controls for all assays by applying the one-way ANOVA; nonparametric using the Kruskal-Wallis 1-way ANOVA (K samples), which reports statistically significant differences when p values ≤ 0.05 and the confidence interval is 95%, and the duplicate results values results were performed as mean values±standard deviation. Using the Minitab Biplot graph property, principal component analysis (PCA) was further applied to locate the variables that better differentiate between fractions.

RESULTS and DISCUSSION

The results of enzyme inhibition capacity for the *C. baskilensis* leaf fractions were compared to the reference materials utilized to treat the health problems associated with an over-activity of enzymes under the same standard conditions for enzyme action. All the enzyme inhibition results for both fraction samples and the standard reference materials have been inserted in Table 1. The inhibition result of AChE showed F12 and F14 fractions to have the highest inhibition activity, while the F1 fraction showed the lowest inhibition activity among all fractions. Results also showed that F8, F12, and F14 have higher inhibition activity than galantamine. BChE inhibition results showed that the inhibition effects of the F4 and F10 fractions were very close to each other. At the same time, both fractions showed a maximal inhibition effect among all fractions. Also, F4 and F10 fractions proved their higher inhibition capacity than galantamine, a standard BChE inhibitor. The following two enzymes are urease and carbonic anhydrase. It is worth noting that *Campanula* and the other members of the *Campanulaceae* family have never been tested before for their inhibition activity against these two enzymes. For the urease enzyme, among all of *the C. baskilensis* fractions, the F7 fraction gave the maximal inhibitory effect; further, the F1, F2, F5, and F7 fractions' inhibitory effect was higher than thiourea. In the study conducted by Korkmaz et al. (2020), the effects of water, *n*-hexane, acetonitrile, and methanol extracts of *C. latifolia* on acetylcholinesterase inhibitory activity against galantamine were investigated. The resultant values (IC_{50}) were found to be 532.03±0.64 µg/mL for water extract, 242.55±2.43 µg/mL for methanol extract, 100.94±3.59 µg/mL for acetonitrile extract, 295.42±0.33 µg/mL for n-hexane extract, and 10.48±0.09 µg/mL for galantamine. In this study, the acetylcholinesterase inhibitory activity was found to be higher in general.

Table 1. The enzyme inhibition activities of *C. baskilensis* fractions
Çizelge 1. C. baskilensis fraksiyonlarının enzim inhibisyon aktiviteleri

Samples	Activity, IC_{50} (µg/mL)							
	AChE	BChE	Urease	CA	α-glucosidase	α-amylase	Lipase	Tyrosinase
F1	54.24±4.47^{e}	24.06±5.62^{bcd}	33.12±0.00^{ab}	181.45±1.34^{j}	99.28±2.93^{e}	73.72±2.17^{e}	98.66±1.16^{f}	36.28±4.48^{bc}
F2	31.24±0.37^{d}	4.85±1.27^{a}	34.05±3.60^{ab}	14.08±0.01^{b}	47.50±2.56^{c}	208.47±0.31^{j}	69.46±2.58^{e}	303.93±7.16^{g}
F3	32.64±3.07^{d}	81.00±0.64^{e}	42.52±0.00^{bc}	10.72±0.18^{b}	447.26±1.98^{i}	45.95±0.16^{cd}	56.29±4.04^{de}	33.91±1.90^{bc}
F4	11.32±0.80^{ab}	10.01±3.96^{ab}	70.94±3.90^{ef}	132.29±3.18^{i}	135.29±1.16^{g}	95.11±0.67^{g}	52.42±5.53^{cd}	58.98±0.87^{de}
F5	20.71±4.84^{bcd}	8.74±4.87^{ab}	21.91±2.58^{a}	78.57±2.42^{g}	38.60±1.79^{abc}	34.55±0.70^{b}	32.99±1.02^{b}	93.91±7.88^{f}
F6	30.16±3.02^{d}	16.70±5.51^{abc}	45.96±4.87^{bc}	5.61±0.01^{ab}	103.20±0.49^{ef}	18.82±1.48^{a}	53.36±0.96^{cd}	69.93±1.72^{e}
F7	25.67±3.02^{cd}	27.88±2.95^{cd}	20.96±1.23^{a}	53.28±0.75^{f}	44.05±1.75^{bc}	42.80±1.44^{bc}	69.58±5.03^{e}	36.37±5.76^{bc}
F8	7.90±0.05^{ab}	16.01±2.42^{abc}	383.75±0.00^{h}	35.22±1.14^{cd}	111.24±0.79^{f}	93.39±3.39^{g}	91.21±3.44^{f}	98.90±5.59^{f}
F9	9.23±6.96^{ab}	9.76±0.39^{ab}	47.01±1.66^{bc}	43.01±0.41^{de}	74.45±3.49^{d}	147.66±4.59^{i}	90.34±5.56^{f}	70.19±4.96^{e}
F10	14.30±4.23^{abc}	4.67±6.16^{a}	65.99±5.82^{de}	31.18±1.81^{c}	131.95±0.90^{g}	81.00±1.58^{ef}	35.83±2.96^{b}	96.28±4.54^{f}
F11	26.63±4.25^{cd}	32.91±5.21^{d}	40.24±1.12^{bc}	92.73±1.18^{h}	129.93±1.99^{g}	89.27±3.63^{fg}	92.10±4.53^{f}	24.66±3.48^{ab}
F12	6.97±2.90^{a}	33.38±2.65^{d}	86.91±4.39^{g}	54.04±0.91^{f}	36.78±0.26^{ab}	73.39±0.43^{e}	57.24±0.05^{de}	43.72±4.63^{cd}
F13	20.77±2.02^{bcd}	10.64±2.18^{ab}	84.73±7.48^{fg}	8.68±6.12^{ab}	47.31±2.46^{c}	72.08±3.34^{e}	26.07±4.23^{b}	23.15±4.34^{ab}
F14	6.06±1.29^{a}	10.59±5.04^{ab}	52.42±3.09^{cd}	47.90±3.57^{ef}	237.34±5.11^{h}	106.04±4.64^{h}	40.82±5.69^{bc}	55.64±5.24^{de}
Galantamine	8.03±0.68^{ab}	7.60±2.37^{a}	-	-	-	-	-	-
Thiourea	-	-	37.13±3.28^{b}	-	-	-	-	-
Acetazolamide	-	-	-	0.64±0.08^{a}	-	-	-	-
Acarbose	-	-	-	-	34.25±2.63^{a}	54.60±3.15^{d}	-	-
Orlistat	-	-	-	-	-	-	10.25±1.14^{a}	-
Kojic acid	-	-	-	-	-	-	-	7.85±0.65^{a}
P value	0.020	0.026	0.012	0.011	0.011	0.012	0.013	0.013

Values are mean±standard deviation; Different superscript letters ($^{a-j}$) indicate significant differences in values within the same column ($p \leq 0.05$), with a confidence interval of 95%.

On the other hand, looking at the inhibition results of the CA enzyme, F6 had a better inhibitory effect than other fractions; however, acetazolamide was the most efficient compared with the fraction samples. The following two enzymes that we tested are related to type 2 diabetes mellitus; therefore, researching compounds capable of determining their effectiveness is of excellent health importance. For the α-glucosidase inhibition activity, results determined that the F12 fraction from *C. baskilensis* leaf fractions has a higher inhibition effect compared to other fractions. In contrast, the F3 fraction has the lowest effect. Also, when the α-glucosidase inhibition level of F5 and F12 fractions was compared with other samples, it showed an inhibition effect close to the reference inhibitor molecule acarbose. The study conducted by Korkmaz et al. (2020) investigated the effects of water, acetonitrile, and methanol extracts of C. latifolia on α-glucosidase inhibitory activity against acarbose. The resultant values (IC_{50}) were found to be 193.33±4.64 µg/mL for water extract, 30.42±1.04 µg/mL for methanol extract, 214.06±2.04 µg/mL for acetonitrile extract, and 35.03±0.22 µg/mL for acarbose. In this study, α-glucosidase inhibitory activity was higher. However, in the study conducted by Zarei and Tahazadeh (2020), the α-glucosidase inhibition activity

of the methanol extract of the *C. involucrata* plant was investigated, and the IC_{50} value was found to be 20 µg/mL. It was determined that it had more effective α-glucosidase inhibition activity than this samples. On the other hand, the α-amylase inhibition test results showed that the inhibition activity of the F6 fraction of the *C. baskilensis* leaf fraction was more effective than that of other fractions. Among the *C. baskilensis* leaf fractions, the F2 fraction was found to have the lowest inhibitory activity. The activity of F3, F5, and F6 fractions showed a higher inhibition effect on α-amylase activity than acarbose. The inhibition activity results against the lipase enzyme showed that the F13 fraction was more effective than the other fractions in inhibiting the lipase enzyme activity. In the study carried out by Kim et al. (2011), α-amylase inhibition activities of water and ethanol extracts of *C. takesimana* Nakai and *Codonopsis lanceolata*, *Adenophora remotiflora*, *Asyneuma japonicum,* and *Adenophora triphylla* species belonging to the *Campanulaceae* family were compared with acarbose. In the study, 3.7±7.8% for *A. remotiflor* water extract, 3.9±7.5% for *C. takesimana* ethanol extract, 5.2±12.3% for *A. remotiflor* ethanol extract, 5.6±9.8% for *A. triphylla* ethanol extract, 14.6±7.9% for *A. japonicum* ethanol extract, and 80.15±0.23% for acarbose were found to have inhibitory effects. In this study, the inhibitory activities of fractions F3, F5, F6, and F7 gave higher effects compared to acarbose. Also, *C. takesimana, C. lanceolata, A. remotiflora, A. japonicum,* and *A. triphylla* samples were more effective compared to each other.

In contrast, the F1 fraction was found to have the lowest inhibitory activity among the fractions. Also, the results demonstrate that *C. baskilensis* leaf fractions have a lower inhibitory effect than orlistat. The last enzyme in this study was tyrosinase; the inhibitory activity of the tyrosinase enzyme was applied by comparing the *C. baskilensis* leaf fractions' activity results with the activity of the reference inhibitor material, kojic acid. As the results showed, the tyrosinase inhibition activity of the F13 fraction was found to be more effective than the other fractions, while the activity of the F2 fraction was found to have the lowest inhibitory effect; however, fractions were determined to have lower inhibition activity than kojic acid. The study conducted by Korkmaz et al. (2020) investigated the effects of water, n-hexane, acetonitrile, and methanol extracts of C. latifolia on tyrosinase inhibitory activity against kojic acid. The resultant values (IC_{50}) were found to be 248.84±5.66 µg/mL for water extract, 226.91±3.27 µg/mL for methanol extract, 53.41±0.64 µg/mL for acetonitrile extract, 189.72±6.02 µg/mL for n-hexane extract and 32.58±0.27 µg/mL for kojic acid. In this study, the tyrosinase inhibitory activity was found to be higher for F1, F3, F7, F11, F12, and F13.

Antibacterial activity

The antibacterial activity for the *C. baskilensis* leaf fractions was determined using a disc diffusion test to determine the bacterial growth inhibition as a zone in millimeters for the fractions in Table 2.

Table 2. Antibacterial activities of *C. baskilensis* fractions
Çizelge 2. C. baskilensis fraksiyonlarının antibakteriyel aktiviteleri

Antibacterial property	Sample	Gram-negative bacteria			Gram-positive bacteria		
		E. coli	*P. aeruginosa*	*K. pneumoniae*	*E. faecalis*	*B. cereus*	*S. aureus*
Inhibition zone, mm	F1	11.00±0.90	20.20±0.80	6.30±0.00	*na*	*na*	*na*
	F2	7.90±0.70	7.00±0.10	7.80±0.60	6.20±0.00	*na*	*na*
	F3	9.40±0.80	7.50±0.50	7.80±0.30	*na*	9.80±0.40	9.30±0.90
	F4	11.00±0.70	21.40±1.00	6.20±0.10	*na*	*na*	*na*
	F5	8.40±0.30	7.60±1.00	9.00±0.40	*na*	*na*	10.30±0.40
	F6	7.90±0.60	8.60±0.20	7.50±0.60	*na*	10.70±0.40	8.40±0.00
	F7	7.10±0.00	7.70±0.10	7.00±0.60	*na*	10.40±1.30	8.60±0.80
	F8	9.80±0.40	8.70±0.10	10.00±0.30	*na*	12.50±3.50	8.30±0.10
	F9	11.00±0.50	7.80±1.00	7.40±0.40	*na*	12.10±1.30	8.70±0.40
	F10	6.80±0.40	7.00±0.00	6.60±0.00	*na*	12.10±1.30	9.30±0.40
	F11	15.40±1.10	8.60±0.80	6.60±0.90	*na*	*na*	*na*
	F12	13.00±0.80	8.10±1.60	8.30±0.60	*na*	10.10±0.80	7.80±0.80
	F13	6.70±0.00	7.80±0.50	8.40±1.50	*na*	9.2±00.10	10.20±0.60
	F14	7.60±0.40	8.10±1.30	7.10±0.60	*na*	9.70±0.40	7.80±0.00
	Tetracycline	32.10±0.20	31.50±0.70	30.30±0.00	41.30±0.00	28.30±0.00	17.00±0.00

na: no activity

As the results of the disc diffusion test, firstly, for the gram-negative bacteria, against *E. coli,* F11 and F12 fractions gave the best inhibition effect among all fractions; however, tetracycline was more effective against *E. coli.* For the *P. aeruginosa, inhibition zones for F1 and F4 fractions were at* the top, yet tetracycline showed higher activity. Against *K. pneumoniae,* the most effective fractions were F8, F12, and F13, all of which had less activity than tetracycline. Also, we tested fraction samples against three gram-positive bacteria the fractions' growth inhibition activities were as follows: F2 fraction was the only fraction with an antibacterial effect against *E. faecalis,* yet its activity was lower than tetracycline activity. Against *B. cereus, the* F8 fraction showed the highest activity among the fractions. However, it was the nearest fraction in activity to tetracycline. Against *S. aureus, the* F13 fraction

was found to have the highest inhibition activity among all fractions; however, tetracycline was found to have a higher inhibition activity against *S. aureus* when compared with fraction results. In the study conducted by Sinek et al. (2012), the antibacterial activity of the essential oils of *C. glomerata* against various bacteria was investigated and the inhibition zones were found as 6 mm for *P. aeruginosa*, 10 mm for *S. aureus*, 8 mm for *E. faecalis* and 10 mm for *B. cereus*. When compared to this samples, most of them had higher effects.

DNA related activities

The DNA-related activities tests aim to investigate the activity of protecting and preventing damage to DNA from reactive oxygen species and Ultraviolet (UV) radiation. *C. baskilensis* leaf fractions were investigated to determine protective effectiveness in protecting pBR322 plasmid DNA against UV radiation and oxidative stress (Figure 1). Agarose gel electrophoresis was applied to the *C. baskilensis* leaf fractions at 1 mg/mL. As both the agarose gel image and the chart of % plasmid DNA forms clarified, the results of protection activity for fractions were as follows: F12 followed by F5 fraction have the highest protection activity for the supercoiled form of plasmid DNA, while F11 has the lowest. On the other hand, F12, F2, and F3 fractions have the highest protection activity for the open-circular form of plasmid DNA, while F7 has the lowest effect. Among all fractions, the F11 fraction barely had a protection effect in both forms of plasmid DNA.

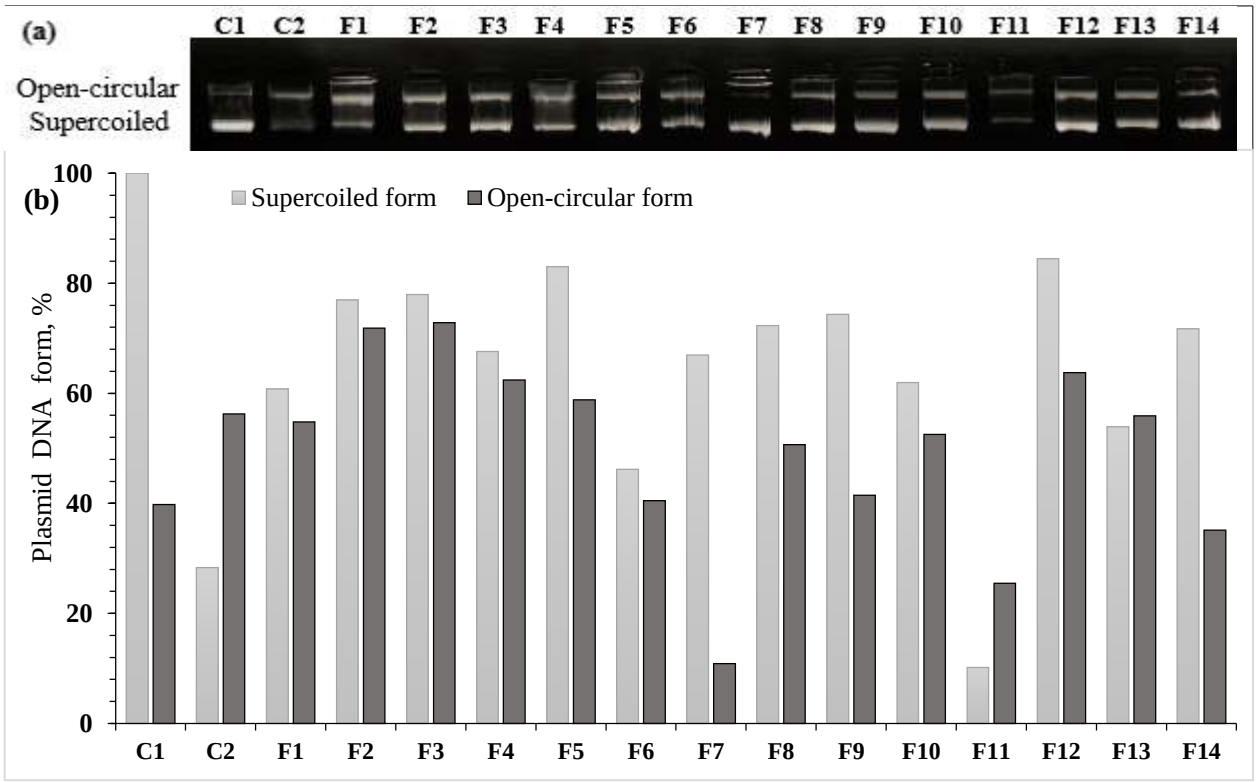

Figure 1. The results of DNA damage protection potential activities of *C. baskilensis* leaf fractions.
Şekil 1. C. baskilensis yaprak fraksiyonlarının DNA hasarı koruma potansiyel aktivitelerinin sonuçları

(a) DNA protection activity: Agarose gel electrophoresis image; lane 1: plasmid DNA as a positive control (C1), lane 2: plasmid DNA with H_2O_2 and UV as a negative control (C2) and lane 3-23: plasmid DNA + H_2O_2 + UV + different fraction samples. **(b)** Comparing chart of % density of the open-circular and supercoiled forms of plasmid DNA.
(a) *DNA koruma aktivitesi: Agaroz jel elektroforezi görüntüsü; şerit 1: pozitif kontrol olarak plazmid DNA'sı (C1), şerit 2: negatif kontrol olarak H_2O_2 ve UV içeren plazmid DNA'sı (C2) ve şerit 3-23: plazmid DNA + H_2O_2 + UV + farklı fraksiyon örnekleri.* ***(b)*** *Plazmid DNA'sının açık dairesel ve süper sarmal formlarının % yoğunluk tablosunun karşılaştırılması.*

Principal component analysis (PCA)

The investigated dataset is made easier to understand by the principal component analysis. Multivariate analysis examines how the variables relate to the experimental groups and outliers. All fraction samples with enzyme inhibitory activities ($1/IC_{50}$), antibacterial activity, and DNA protection activity were examined using Principal Component Analysis (PCA). The first two main components' eigenvalues were significant after PCA. The variance with enzyme inhibitions was 27.7% and 21.2% for the first component (PC1), the variance with antibacterial activity was 48.7% and 22.5% for the first component (PC1), and the variance with DNA protective activity was

75.3% and 24.7% for the first. F12 was positively correlated with AChE inhibition, *K. pneumonia, B. cereus*, and *S. aureus* growth inhibition, and the supercoiled form protection of plasmid DNA according to a biplot graphic (Figure 2). We conclude that the F12 component is mostly the main reason behind the high bioactivity, which encourages us to do further fractionation to reach the pure compounds.

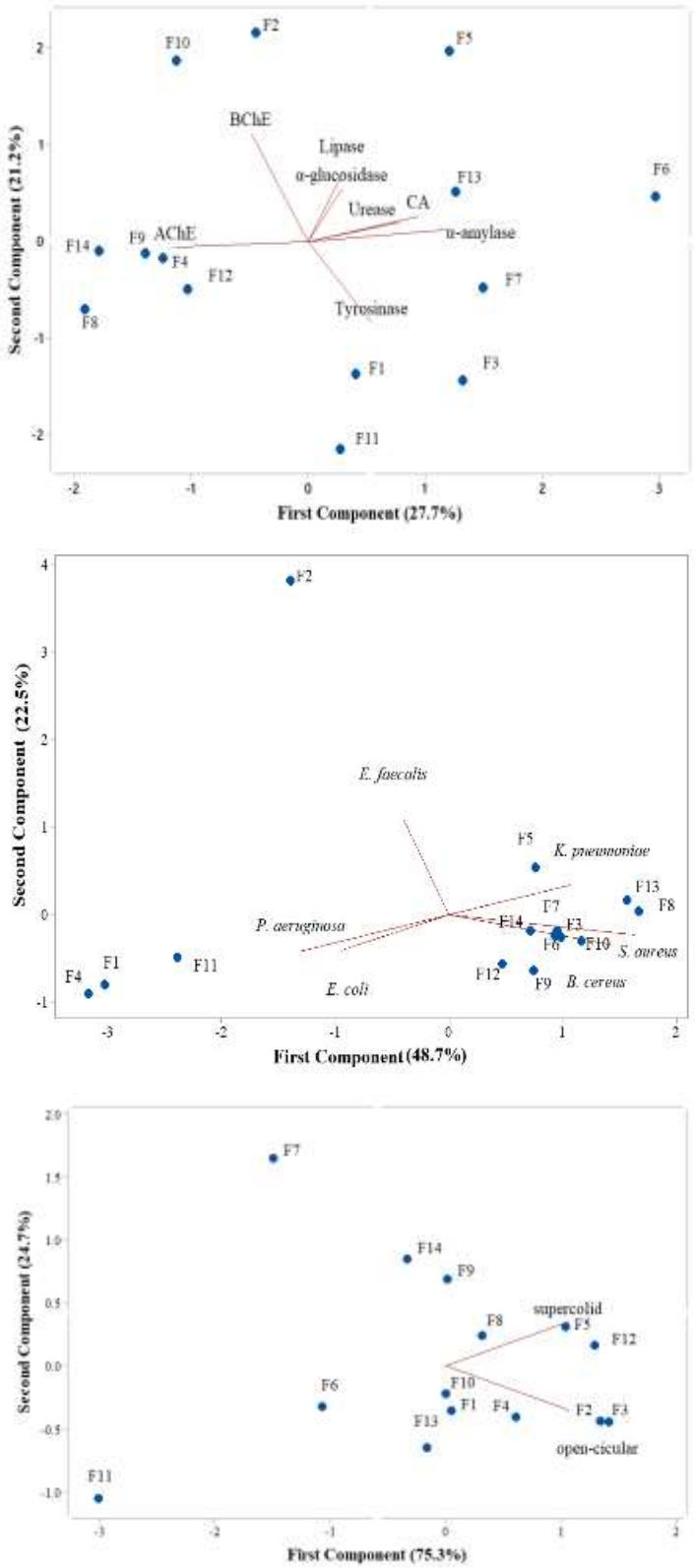

Figure 2. Biplot graph for PC1 and PC2 of fraction samples (F1-F14) with all activity results
Şekil 2. Tüm aktivite sonuçlarıyla birlikte fraksiyon örneklerinin (F1-F14) PC1 ve PC2'sine ait Biplot grafiği

Inositol isolation and characterization

C. baskilensis leaf fractions proved that it has high bioactivity in general; isolation of the leaf part and its detailed fractionation scheme are given in (Figure 3). At the end of this study, bioactive molecules had been isolated from the leaf part of *C. baskilensis*. The structures of isolated molecules were determined spectroscopically using NMR.

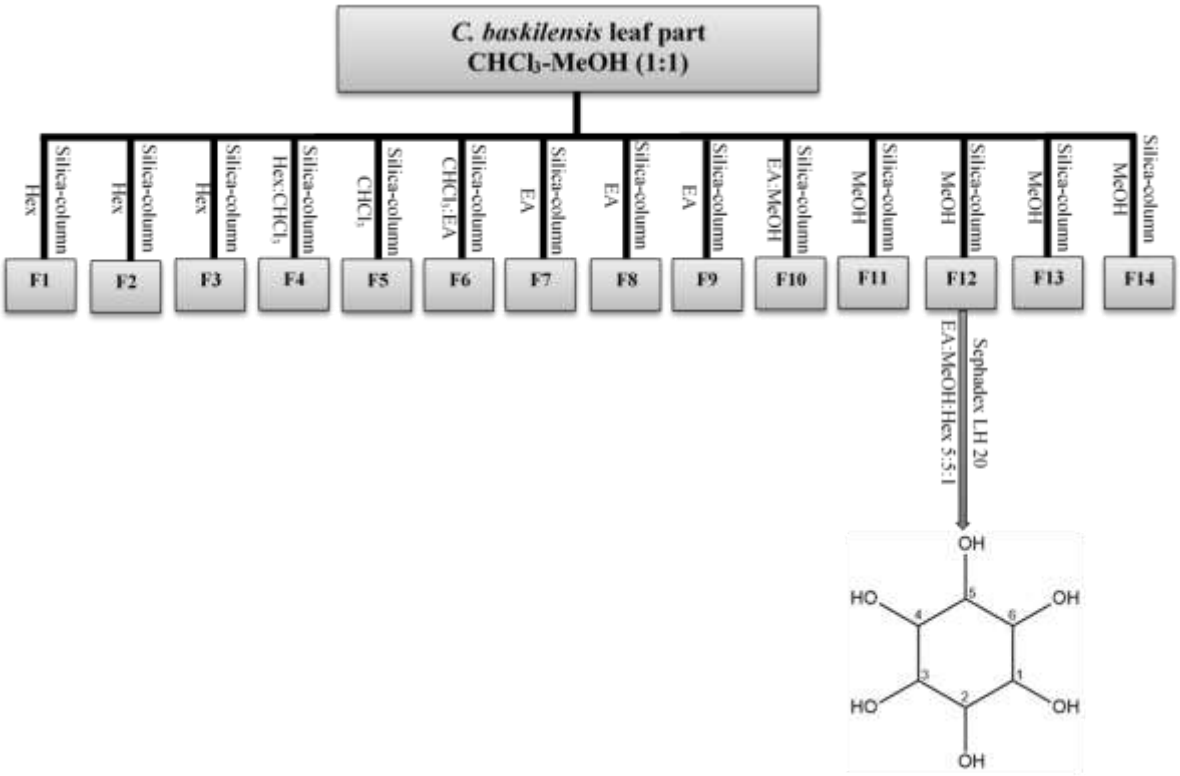

Figure 3. Fractionation scheme of *C. baskilensis* leaf
Şekil 3. C. baskilensis yaprağının fraksiyonlama şeması

Since the F12 fraction was highly active in many bioassays, the chromatographic isolation sub-fractionation process led to isolating a colorless cubic crystalline material. This isolated metabolite was dissolved with DMSO, and its structure was investigated using H-NMR spectroscopy. The structure of the isolated compound was identified through NMR spectra as a carbocyclic sugar inositol, and its structure was compatible with the literature data. H-NMR and C13-NMR spectra belonging to the inositol compound have been clarified in (Figure 4-Figure 7, Table 3) In his study by Dzhumyrko and Shinkarenko (1971), a molecular substance with an inositol structure was isolated from *C. oblongifolia* leaf ethanol extract by fractional crystallization, and the mineral was defined as L-inositol. Another study by Nikolova et al. (2019) that was related to the analysis of the metabolite profiles of *C. lanata*, one of the endemic plants of the Balkans, inositol, had been identified and isolated as a result of this study.

Table 3. H-NMR and 13C-NMR spectrum data of the inositol molecule
Çizelge 3. İnositol molekülünün H-NMR ve 13C-NMR spektrum verileri

Carbon No.	H-NMR (600 MHz, DMSO d6)	C13-NMR (150 MHz, CDCl3 d-1 d)	H-NMR (270MHz, DMSO d6)(Salazar-Pereda et al., 1997)	^{13}C13-NMR (67.8 MHz)(Salazar-Pereda et al., 1997)
1	3.10	72.32	3.10 (*ddd*)	72.01
2	3.68	73.08	3.68 (*td*)	72.79
3	3.10	72.32	3.10 (*ddd*)	72.01
4	3.31	73.21	3.32 (*ddd*)	72.91
5	2.89	75.69	2.88 (*ddd*)	75.38
6	3.31	73.21	3.32 (*ddd*)	72.91

Inositol isomers were investigated in a previous study on *Campanula* species. The *C. elator*, *C. taurica*, and *C. hohenaceri* species were extracted using hot water and then extracted using ethyl acetate. The resulting precipitate was extracted and crystallized in an ethanol-water mixture after the obtained ethyl acetate extract was

concentrated and chloroform was added. The assays conducted revealed that the crystals were meso-inositol (Dzhumyrko and Shinkarenko, 1972).

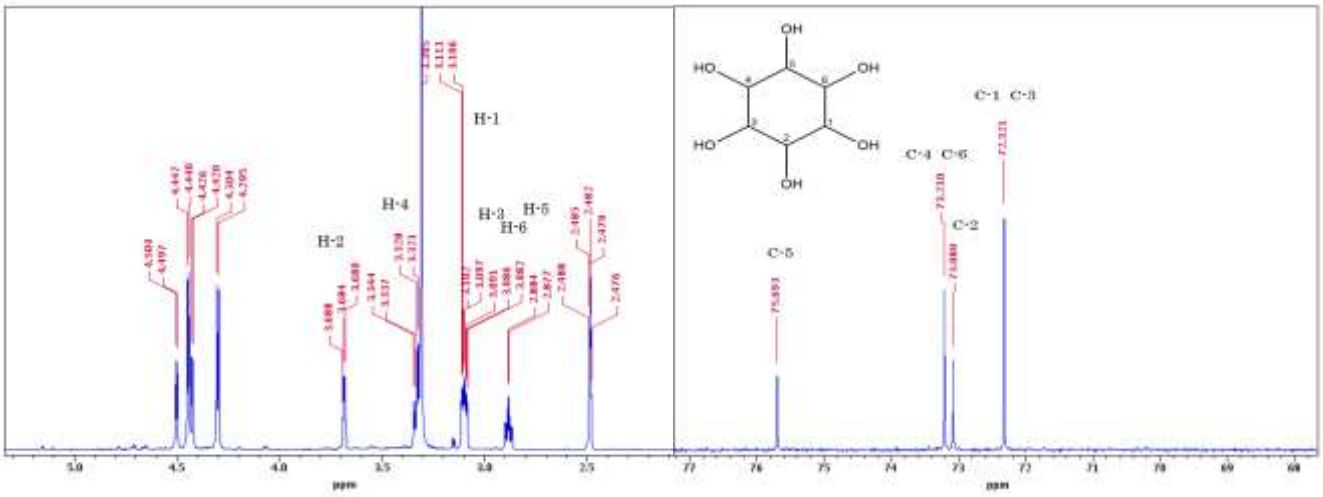

Figure 4. H-NMR and C13-NMR spectrums of the inositol molecule
Şekil 4. Inositol molekülünün H-NMR ve C13-NMR spektrumları

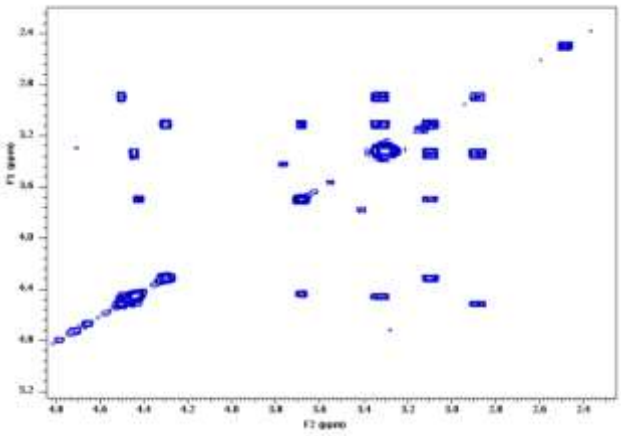

Figure 5. COSY-NMR spectrum of the inositol molecule
Şekil 5. Inositol molekülünün COSY-NMR spektrumu

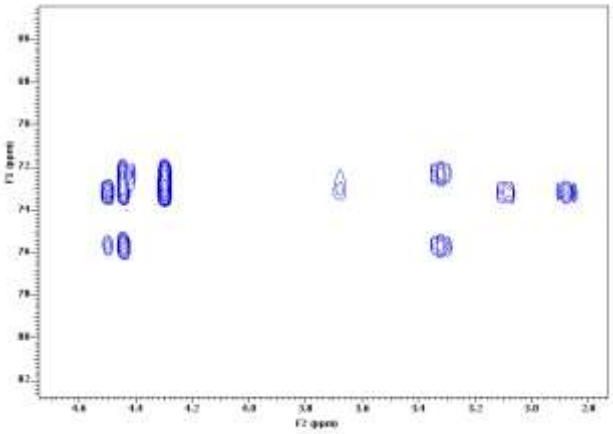

Figure 6. HMBC-NMR spectrum of the inositol molecule
Şekil 6. Inositol molekülünün HMBC-NMR spektrumu

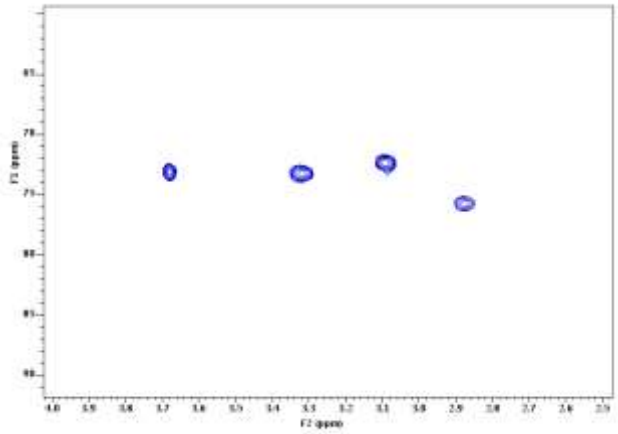

Figure 7. HSQC NMR spectrum of the inositol molecule
Şekil 7. İnositol molekülünün HSQC NMR spektrumu

Molecular Docking and Molecular Dynamics

We theoretically investigated the molecular interaction between inositol and the standard inhibitor (galantamine) and the most inhibited enzyme to sketch a potential mechanism for AChE inhibition brought on by F12 against the enzymes examined *in vitro*; also for comparison, we investigated the molecular interaction between inositol and the standard inhibitor with BChE, being from the same category and sharing a similar function. It is worth noting that the theoretical interaction results were consistent with the practical inhibition results for both enzymes. The binding energy of the enzyme-inositol interaction is -6.1 kcal/mol. Seven interactions were found by molecular docking between the isolated molecule and acetylcholine esterase: a carbon-hydrogen bond with PRO410 residue, three conventional hydrogen bonds with ASN233, two with ASN533, and the final one with GLU313 residue. Nine interactions formed between galantamine and AChE: two conventional hydrogen bonds with SER239 residue; two carbon-hydrogen bonds with SER239 and TYR124 residues, two pi-pi stacked hydrophobic interactions with TRP286 residue; a pi-pi T-shaped hydrophobic interaction with TYR72 residue; two pi-alkyl hydrophobic interactions with TYR72 and TRP286 residues. The interaction between inositol and BChE consisted of a single conventional hydrogen bond between the molecule and GLN498 residue. Four interactions formed between galantamine and BChE: a carbon-hydrogen bond with ASN83 residue, pi-pi stacked hydrophobic interaction with TRP82 residue; two pi-alkyl hydrophobic interactions with TRP82 residue (Table 4, Figure 8).

Table 4. Molecule docking scores between inositol and galantamine with AChE and BChE
Çizelge 4. AChE ve BChE ile inositol ve galantamine arasındaki molecule doking skor

Compound	Binding energy (kcal/mol)	
	AChE	BChE
Inositol	-6.1	-5.7
Galantamine	-8.3	-9.0

Considering the result of molecular docking, we tested the kinetics and stability via the molecular dynamics of each isolated compound and compared it with the reference material against AChE as the highly inhibited enzyme. Greater RMSDs suggested that the backbone had structural changes over the simulation period, whereas smaller RMSD values suggested that the protein was highly stable. Over 100 ns, the inositol-AChE complex changed by about 5 nm (Figure 9). Throughout the simulation, the root mean square fluctuation (RMSF) method is used to compute the changes in each amino acid residue with and without specific ligand molecules. Using the Cα atoms of AChE, the RMSF was computed for all complex systems and revealed that the fluctuation intensity persisted between 0.05-0.35 nm (Figure 9). To determine the stability during the MD simulations, it was essential to examine the bonding interactions between ligands and proteins (Majewski et al., 2019).

Figure 9 depicts the H-bond that formed between inositol and AChE throughout 100 ns. Throughout the MD simulation, there was continuous interaction around five bonds, which most likely contributed to the stability of this interaction. A protein's radius of gyration (Rg) indicates its degree of compactness. Rg is a practical and reliable indicator of a drug's capacity to alter protein structure. The Rg value of a protein indicates its loose molecular

packing. The dynamics computation for 100 ns showed that AChE and inositol were constant at less than 2.35 nm (Figure 9).

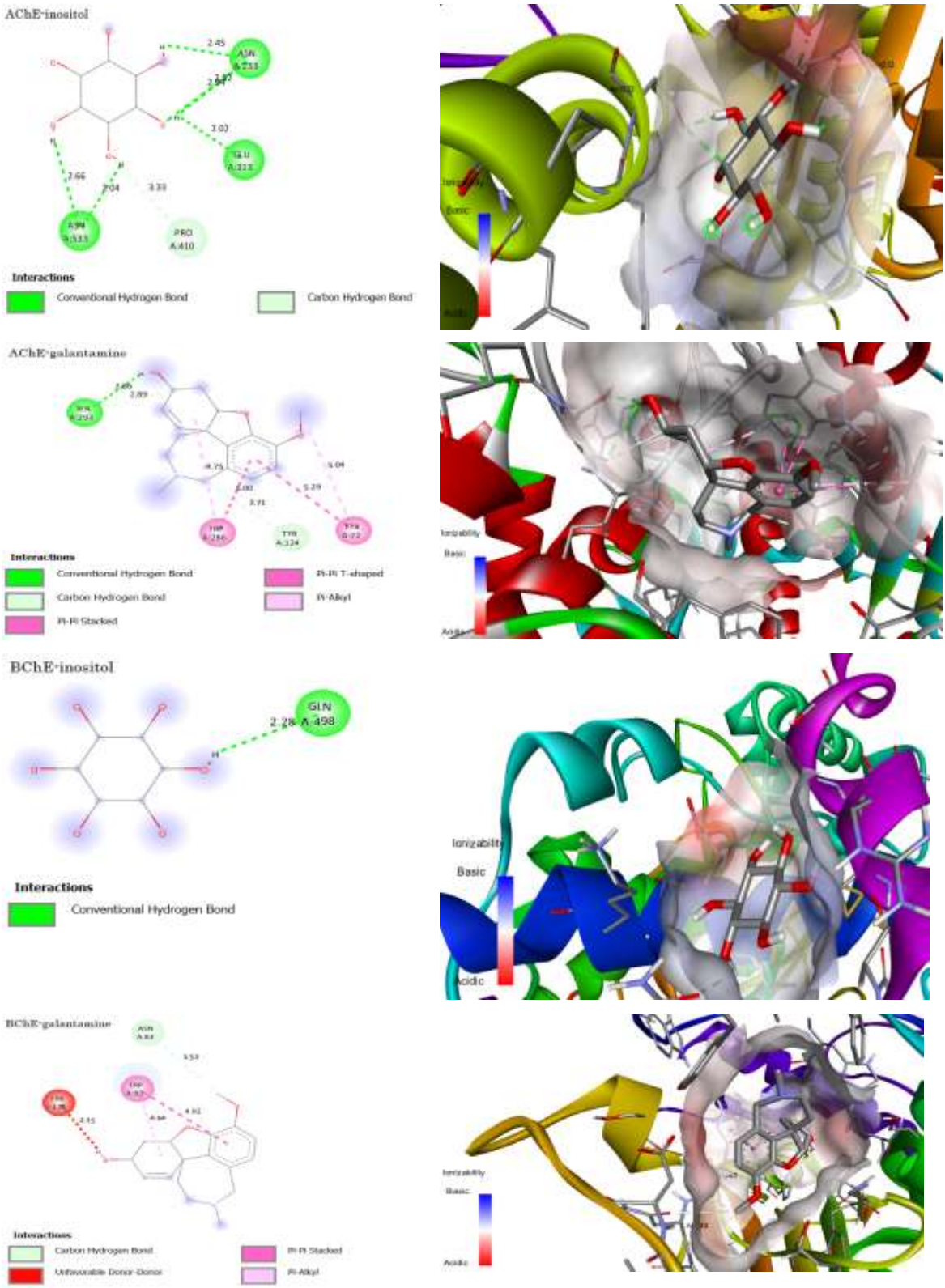

Figure 8. Molecule docking interaction between inositol and galantamine with AChE and BChE
Şekil 8. Inositol ve galantamine ile AChE ve BchE arasındaki molekül doking etkileşimi

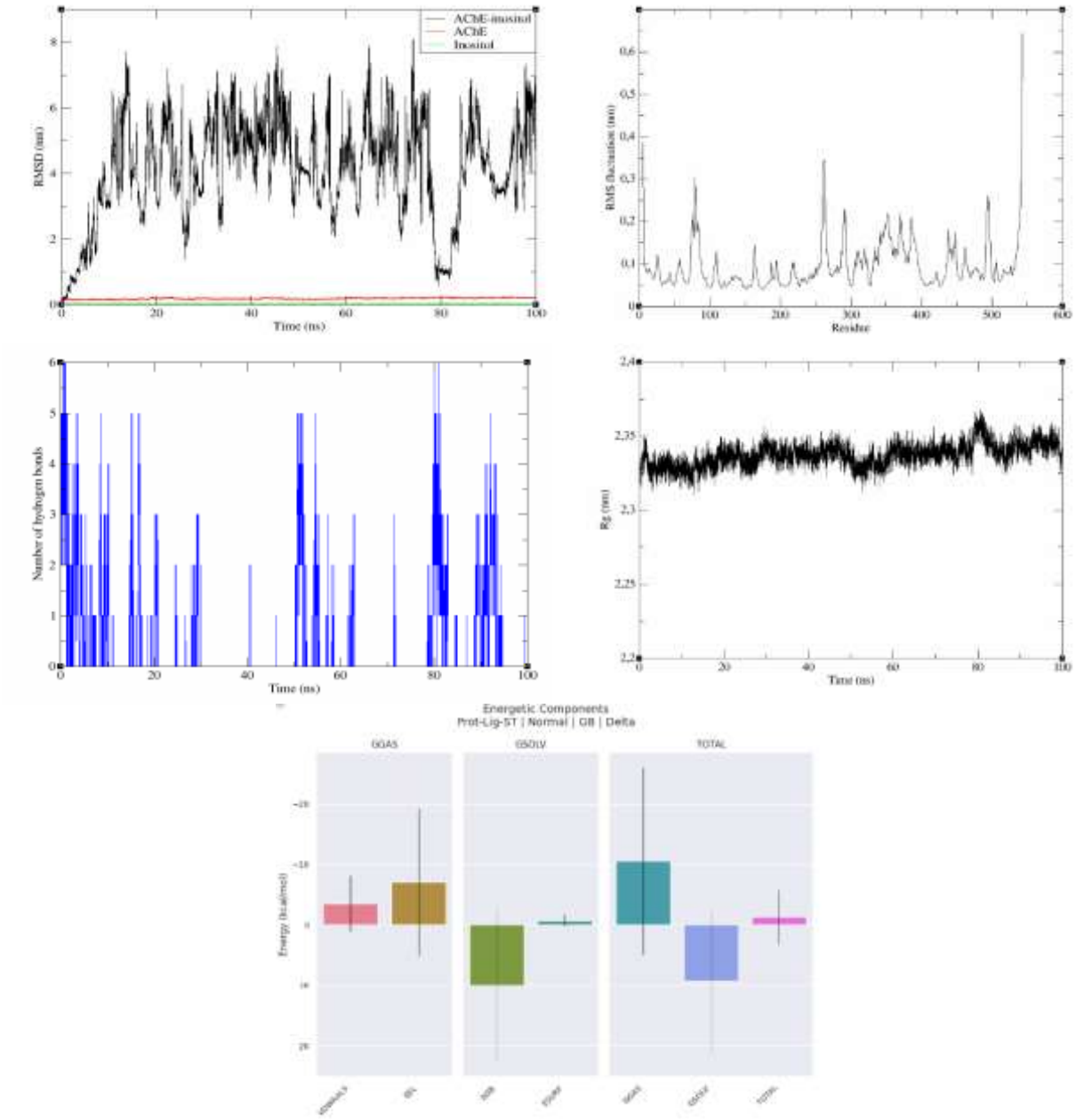

Figure 9. Molecular dynamic simulation results between inositol and AChE for 100 ns, RMSD, RMSF, time-dependent H-bond interactions, Rg plotting, Gmx_mmpbsa energy of Delta.

Şekil 9. InoSitol ve AChE arasındaki 100 ns'lik moleküler dinamik simülasyon sonuçları, RMSD, RMSF, zamana bağlı H-bağı etkileşimleri, Rg çizimi, Delta'nın Gmx_mmpbsa enerjisi.

On the other hand, the RMSD for the galantamine-AChE complex changed by about 0.5 nm. In contrast, the RMSF was computed for all complex systems and revealed that the fluctuation intensity persisted between 0.05 and 0.4 nm. H-bond that formed between galantamine and AChE throughout 100 ns was a continuous interaction around 2-3 bonds, which most likely contributed most to the stability of this interaction. Rg value of a protein indicates its loose molecular packing, for 100 ns showed that AChE and galantamine were constant between 2.3-2.35 nm (Figure 10).

The formula ΔGbinding = Gcomplex- (Greceptor+Gligand) was used to calculate the binding energy (ΔGbinding) of the protein-ligand system, where Greceptor stands for the binding energy of AChE and Gligand for the energy of the unbounded ligand. ΔG binding can also be expressed as follows: When ΔH is the binding enthalpy and −TΔS is the conformational entropy after ligand binding, the formula is ΔG binding = ΔH -TΔS. For comparing relative binding free energies for similar systems, the approximated value—the effective free energy when the entropic factor is taken out—is usually adequate. This analysis discovered that the average effective free energy of binding

was -1.32±3.79 kcal/mol for inositol and -11.92±0.02 kcal/mol for galantamine (Table 5, Figures 9 and 10). Following the docking data, MMPBSA simulations indicated a stable interaction of the inositol molecule with the AChE.

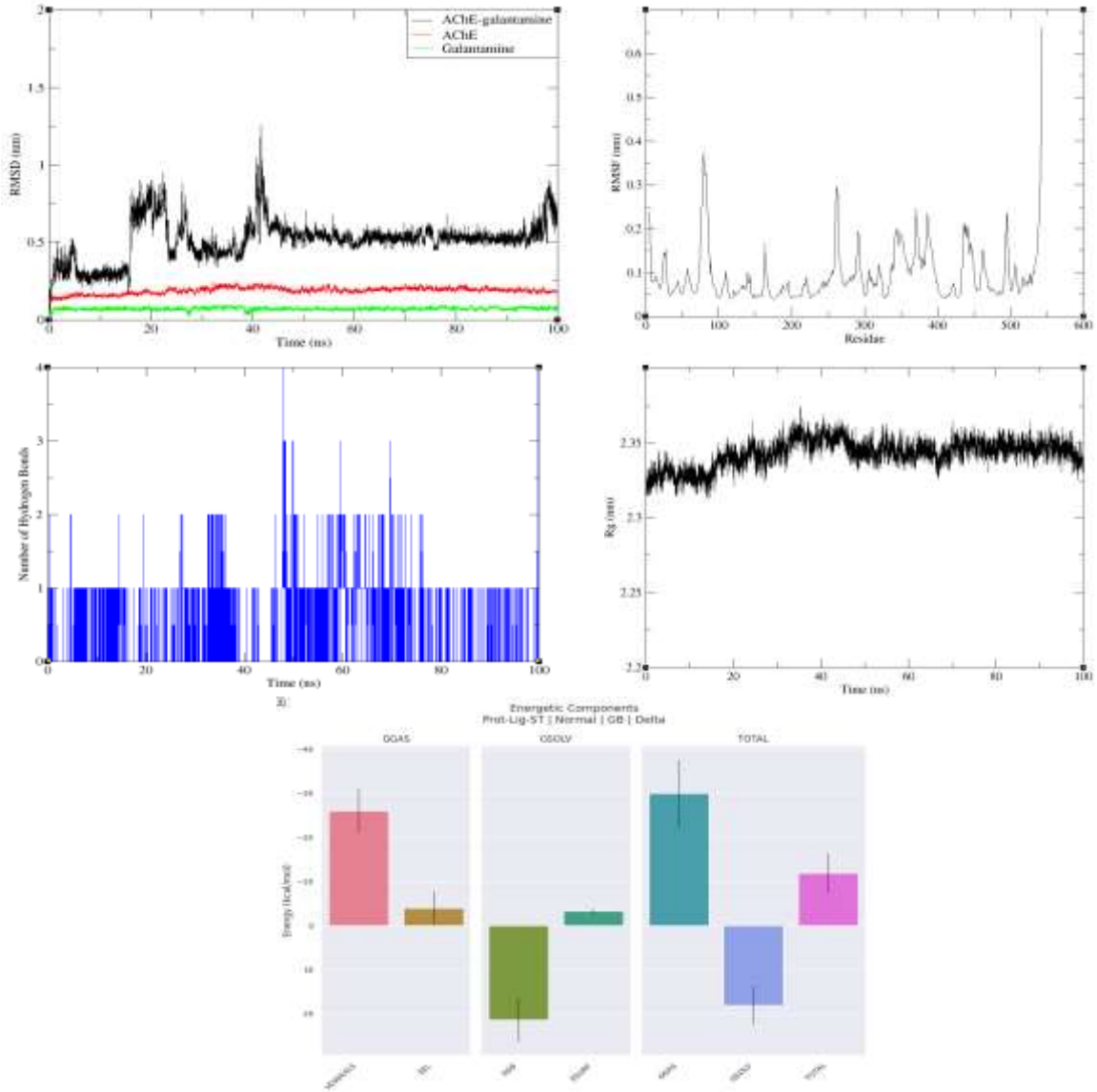

Figure 10. Molecular dynamic simulation results between galantamine and AChE for 100 ns, RMSD, RMSF, time-dependent H-bond interactions, Rg plotting, Gmx_mmpbsa energy of Delta.

Şekil 10. Galantamine ve AChE arasındaki 100 ns'lik moleküler dinamik simülasyon sonuçları, RMSD, RMSF, zamana bağlı H-bağı etkileşimleri, Rg çizimi, Delta'nın Gmx_mmpbsa enerjisi.

CONCLUSION

Isolation was applied to reach the active compounds of the *C. baskilensis* leaf part. The fractionation process led to the obtaining of 14 different fractions in the first part. Numerous *in-vitro* bioassays have been applied to investigate the bioactivity of fractions, antibacterial, enzyme inhibition, and DNA-related assays. The results of these assays proved that many of the tested fraction samples had high biological effectiveness. All obtained activity results have been compared with standard antibiotics and standard inhibition drugs. The results were compared with the studies with the same biological activity tests in the literature. The principal component analysis leads us to conclude that the F12 component is primarily responsible for the high activity examined in this study, which was the subject of the second fractionation. As a result, pure compounds were obtained when the isolation of the fractions with the highest effectiveness was applied. The structure of the isolated compound was clarified using the NMR spectroscopic technique. The isolated compound was identified as inositol. We decided to move forward with exposing the inhibitory mechanism for the isolated compound against the more inhibited enzyme by F12; we tested this isolated compound and galantamine theoretically by simulating molecular docking in a vacuum via molecular docking and also made an advanced simulation about the stability of the complex for 100 ns under the

standard conditions of the living organism. It became clear that this compound can form a relatively stable association due to its formation of many hydrogen bonds, as proven by the study of molecular dynamics and energy calculations. Ultimately, taking this path may also allow us to discover the true capabilities of the compounds that isolate and test them *in silico* and *in vitro*.

Table 5. Free energy of binding obtained using MMPBSA calculations (Complex;Receptor-Ligand)
Çizelge 5 MMPBSA hesaplamaları kullanılarak elde edilen bağlanma serbest enerjisi (Kompleks;Reseptör-Ligand)

Energy Component	Average AChE*	Average BChE*
ΔBOND	-0.00±0.00	-0.00±0.05
ΔANGLE	0.00±0.00	0.00±0.07
ΔDIHED	0.00±0.00	-0.00±0.05
ΔUB	-0.00±0.00	0.00±0.01
ΔIMP	0.00±0.00	0.00±0.00
ΔCMAP	0.00±0.00	0.00±0.00
ΔVDWAALS	-3.51±0.09	-26.00±0.01
ΔEEL	-7.05±0.24	-4.02±0.01
Δ1-4 VDW	-0.00±0.00	-0.00±0.03
Δ1-4 EEL	0.00±0.00	-0.00±0.03
ΔEGB	9.98±0.25	21.36±0.00
ΔESURF	-0.73±0.02	-3.25±0.00
ΔGGAS	-10.56±0.31	-30.03±0.02
ΔGSOLV	9.25±0.24	18.10±0.00
ΔTOTAL	-1.32±0.09	-11.92±0.02

*Values are mean±standard error, kcal/mol

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Contribution Rate Statement Summary of Researchers

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

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Determination of Genome Size Differentiation and Ploidy Levels in Some Citrus Rootstock Populations

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ABSTRACT
Determining the ploidy level of plant material used in breeding is important, especially for biotechnological applications. The genetic diversity in citrus enables the development of rootstocks and cultivars adapted to various climates and soils. Various suitable rootstocks are used in commercial citrus production.This study was conducted to determine the genome size and ploidy levels of citrus rootstocks widely used worldwide using flow cytometry. The rootstocks used in the study included Gou-Tou, C-35, Troyer citrus, Taiwanica, Citremon, Yuzu, Sunki mandarin, Flying Dragon, Yuma citrus, Macrophylla and Chinese orange. Fresh leaf tissue from each rootstock was mixed with triploid Tahitian lemon leaf tissue, used as a standard species, and cell nuclei were isolated. The cells stained with propidium iodide were analysed by flow cytometry, and histograms and cytograms were obtained. According to the results, although all species had diploid genome sizes, differences were observed between species in terms of genome volume. Yuzu seedlings were found to have the largest genome size (0.808 pg/2C), while Flying Dragon trifoliate had the smallest genome size (0.700 pg/2C).

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Bazı Turunçgil Anaç Popülasyonlarında Genom Büyüklüğü Farklılıklarının ve Ploidi Seviyelerinin Belirlenmesi

ÖZET
Bitki ıslahında kullanılan materyalin ploidi seviyesinin belirlenmesi, özellikle biyoteknoloji uygulamaları açısından önemlidir. Turunçgillerdeki genetik çeşitlilik, farklı iklim ve topraklara uyumlu anaç ve çeşitlerin geliştirilmesini sağlamaktadır. Ticari üretimde çeşitli uygun anaçlar kullanılmaktadır. Bu çalışma, dünya genelinde yaygın olarak kullanılan turunçgil anaçlarının flow sitometri ile genom büyüklüklerini ve ploidi seviyelerinin belirlenmesi amacıyla yürütülmüştür. Çalışmada Gou-Tou, C-35, Troyer Citrange, Taiwanica, Citremon, Yuzu, Sunki mandarin, Flying Dragon, Yuma Citrange, Macrophylla ve Çin portakalı anaçları kullanılmıştır. İlgili anaçlardan elde edilen taze yaprak dokuları standart tür olarak kullanılan triploid Tahiti limon yaprak dokusu ile karıştırılmış ve hücre çekirdekleri izole edilmiştir. Propidium iodid ile boyanmış hücreler flow sitometri ile okunmuş ve histogram ve sitogramlar elde edilmiştir. Elde edilen sonuçlara göre, tüm türlerin diploid genom büyüklüğüne sahip olmalarına karşın genom hacmi açısından türler arasında farklılıkların olduğu belirlenmiştir. Yuzu fidanlarının en büyük (0.808 pg/2C), Flying Dragon trifoliatın ise en küçük genom hacmine sahip olduğu (0.700 pg/2C) tespit edilmiştir.

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INTRODUCTION

Citrus, originating in southeast Asia, are commonly cultivated in Türkiye as in the rest of the world. According to the FAO Statistical Database (FAO, 2022) 4.348.742 tons of citrus fruits from different species were produced in Türkiye in 2022. Citrus fruits have a very significant share of global fruit production. Citrus species have been cultivated in subtropical and tropical regions for years. Rootstocks play a vital role in citrus cultivation by mitigating challenges such as adverse climates, subpar soil conditions, and various biotic and abiotic stresses, thus ensuring optimal production (Narukulla at al., 2023). Rootstock selection is very important for citrus cultivation. Rootstock has very large effects on tolerance to cold and diseases, adaptation to different climate and soil conditions, fruit yield and quality. As a result, the use of rootstocks for citrus species has become mandatory. An ideal citrus rootstock has high ability to adapt to different soil conditions, is compatible with citrus species and varieties, resistant to biotic and abiotic stress conditions and has high polyembryony rates. Gou-Tou, C-35, Troyer Citrange, Taiwanica, Citremon, Yuzu, Sunki mandarin, Flying Dragon, Yuma Citrange, Macrophylla and Chinese citrus rootstocks are among the rootstocks commonly used in citrus cultivation (Davies & Albrigo, 1994). As a significant proportion of citrus rootstocks have a tendency toward nucellar embryony, seeds display the same characteristics as main plants and they display performance like clonal proliferation.

Variations in genome size constitute a significant aspect in evolutionary studies and the characterization of species. In addition genome sizes are very important for identification of significant citrus genotypes (Šimoníková et al., 2022). With flow cytometry analyses, genome volumes and different ploidy structures may be determined in citrus species and varieties. For calculation of the nuclear DNA content of a species, it is necessary to determine ploidy level, chromosome counts and how big chromosomes are. Before beginning breeding studies, differences in ploidy level among individuals to be used as genotype and identification of these differences is very important in terms of breeding studies being successful. Flow cytometry is currently the most sensitive, rapid and reliable method used for determination of nuclear DNA content, and use with this aim has become more common in recent years (Tuna, 2014). Determination of ploidy level in plants with traditional methods includes counting chromosomes during mitosis division in preparations made from root tip tissues with light microscopy; however, this method is very demanding, slow and performing ploidy analysis of many plant species takes time (Nix et al., 2024). Additionally, it is not useful for determination of ploidy levels in plant species with small chromosomes and high ploidy levels, and may cause misclassification of species. As the plant sample for ploidy level determination increases, the light microscopy method may not be sufficient. As studies may not be sufficient for determination of ploidy level using root tip tissues, the flow cytometry method has become a method chosen for ploidy analyses in recent years due to being convenient, rapid, sensitive and reliable (Johnson et al., 1998; Brummer et al., 1999; Tuna, 2014). As chromosomes are found in the nuclei of cells in plants, there is a close correlation between the nuclear DNA amount and ploidy level. As a result, due to the convenience, speed and reliability of the flow cytometry method, in recent times ploidy analysis has been performed by determining the nuclear DNA content in plant cells with the flow cytometry method. Flow cytometry analysis has many advantages compared to the chromosome count method in traditional methods such as preparation of samples being easy, it is rapid and there is no need for a root tip cell in mitosis to perform analyses, just a small leaf tissue is sufficient (Nakandala et al., 2023). Flow cytometry was first developed as a method to identify the nuclear DNA content of organisms and it is a very important and easily applied method in these studies (Salameh, 2014). The flow cytometry method is a method based on analysis of light after being absorbed by cells as they pass singly through fluorescence detectors generally (Soni et al., 2024). However, in order to ensure light absorption by cells, the cell nuclei from enzymatically degraded leaf samples are freed and cell nuclei stained with fluorescent stains like DAPI or PI are used to determine nuclear DNA amounts. With the flow cytometry method, one person can analyze over 100 plants per day, it performs chromosome counts very rapidly, provides accurate and reliable results, and increases significance and this situation has led to flow cytometry currently being the most chosen method for chromosome counts. However, the disadvantage of the method is that it must be set to the plant species to be analyzed (Ellialtioglu et al., 2000). Applications where flow cytometry is commonly used in plants include determination of ploidy level, nuclear DNA content and estimation of genome volume (Palomino et al., 2003). Determining the genome size in citrus species through flow cytometry offers several advantages:

a) Utilization in plant breeding: The knowledge of citrus genome size can be employed in plant breeding. Genome size aids in understanding inter-species relationships and degrees of relatedness. Additionally, it is crucial for comprehending plant traits such as adaptability, stress tolerance, and productivity.

b) Diversity and hylogenetic analyses: Variations in genome size among different citrus species and cultivars aid in understanding species relationships and phylogenetic structures.

c) Plant biotechnology applications: Genome size information is crucial in transgenic studies and plant biotechnology applications. It can be used to evaluate the effectiveness and efficiency of genetic manipulations in target plants.

d) Conservation and conservation biology: Genome size information is essential for the conservation and preservation of rare or threatened citrus species. It can assist in managing and conserving populations of endangered species by informing conservation strategies.

e) Agricultural productivity and quality improvement: Genome size helps in identifying genetic traits that may impact agricultural productivity and quality. This information can contribute to the development of agricultural practices and processes aimed at improving productivity and quality.

In conclusion, determining genome size in citrus species through flow cytometry provides valuable information and opportunities for various fields including plant breeding, molecular studies, conservation biology and horticulture.

In this study, the nuclear DNA amounts in seedling populations of citrus genotypes were examined with the flow cytometry method and ploidy levels and genome volumes were determined. Thus, the ploidy levels among genotypes were investigated for presence of significance from a statistical viewpoint.

MATERIAL and METHOD

Plant materials

In this study citrus rootstocks's (Gou-Tou, C-35, Troyer Citrange, Taiwanica, Citremon, Yuzu, Sunki mandarin, Flying Dragon, Yuma Citrange, Macrophylla and Chinese orange rootstocks) seeds and seedlings were used during the experiment. The characteristics of citrus rootstocks used as materials in the scope of the study are specified below. Tahitian lime (*Citrus latifolia* Tan.) was used as a control in the study.

Gou Tou Sour Orange (*Citrus aurantium* L. var. Gou Tou), A rootstock of Chinese origin, apart from being resistant to tristeza disease, there is no information about soil wants or resistance to other diseases. In Florida plants grafted onto Gou Tou rootstock have larger crown structure compared to those grafted on other bitter oranges (Saunt, 2000). Gou Tou sour orange is reported to reduce yield in grapefruits (Louzada et al., 2008). Gou Tou sour orange is tolerant of *Phytophthora citrophthora* and *Phytophthora* parasitical diseases (Matheron et al., 1988).

C-35 citrange (*Poncirus trifoliata* (L.) Raf. X *Citrus sinensis*. Osb. 'Ruby'), A rootstock obtained by hybridization of Ruby Blood orange and trifoliate orange. It is tolerant of gummosis (*Pytophthora citrophthora* (Smith & Smith) Leon.) and Tristeza diseases and resistant to nematodes. Resistance to cold is equivalent or slightly better than Carrizo citrange. Trees have moderate size and those grafted on Troyer have 25 % smaller crown. Good compatibility with sandy, sandy-clayey and clayey soils; however, is more susceptible to limey soils than Carrizo citrange (Saunt, 2000).

Troyer citrange (*Poncirus trifoliata* (L.) Raf. x *Citrus sinensis* (L.) Osb.), A sweet orange and trifoliate orange hybrid. Generally, it has trifoliate properties, with more compliant characteristics in terms of environmental conditions and compatibility with varieties so it is used more often in recent years and in most cases is chosen as alternative to sour orange rootstock. Proliferation with seed and grafting is easy, growth is moderate, yield is high, maturation and fruit setting are early, effects on fruit quality are high and economic lifespan is at moderate levels (Ozcan & Ulubelde, 1984).

Macrophylla (Alemow) (*Citrus macrophylla* Wester), Important features are resistance to salinity and boron. Generally, it has good compatibility with all varieties. However, it is used as rootstock for lemon and lime mainly due to susceptibility to Tristeza and *Xyloporosis* diseases. It appears to be tolerant of Exocortis and Psorosis diseases. Varieties grafted on it grow rapidly and set early fruit. However, quality of fruit is negatively affected. It is susceptible to cold.

Flying Dragon (*Poncirus trifoliata* var. Monstrosa), The trifoliate clone Flying Dragon was found in America in 1915. This rootstock ensures tight crown formation for lime, grapefruit and tangelo, and has the effect of dwarfing mandarin and orange. It is very sensitive to calcium and chlorosis. It develops excessively slowly on mild sandy soils. It is resistant to tristeza (CTV) virus and *Phytophthora* spp. (root neck rot). It is sensitive to Exocortis. The Eureka group showed incompatibility with lemons. Due to showing dwarfing effect on all citrus types and varieties, it is appropriate for dense planting, ensuring convenient harvesting of grafted varieties. It has positive effects on fruit quality, like the trifoliate rootstock. The body having zigzag form and many thorns makes grafting difficult (Aubert & Vullin, 1998). The Flying Dragon rootstock which is resistant to cold and humid conditions and sensitive to high-lime soils, does not have a tendency to form nucellar plants at high rates (Ashkenazi et al., 1992; Ferguson & Chaparro, 2004).

Sunki mandarin (*Citrus sunki* (Hayata) hort ex. Tanaka), It is very widely used as rootstock in China. It is tolerant of Tristeza and Xyloporosis, but sensitive to Exocortis. Studies have reported Sunki is susceptible to *Phytophthora*

brown rot. This rootstock is tolerant of salt, has moderate resistance to low temperatures, and can withstand chlorosis in limey soils. It is a polyembryonic rootstock Louzada et al. (2008) reported adaptation to limey soils was good, and that it was tolerant to iron chlorosis. Fruit yield, fruit juice amounts and sugar content in fruit juice was equivalent or superior to fruit obtained from trees grafted on bitter orange (Saunt, 2000).

Yuzu (*Citrus junos* Sieb. ex Ten.), It is a common rootstock in the southern regions of China. From China, production spread to Japan and it forms an important commercial rootstock in Japan. Proliferation from seeds is easy, with slow growing features. It is a rootstock with high fruit quality and yield. It is resistant to *Phytophthora*, fungus and nematodes. It is tolerant of tristeza, dwarfing and spalling diseases. It has moderate levels of resistance to limey and salty soils. It has high resistance to low temperatures and polyembryony tendency.

Taiwanica (*Citrus taiwanica* Tan. and Shim.), It is a rootstock that is easily proliferated from seeds, and has moderate levels of tree growth, fruit quality and yield. It is resistant to *Phytophthora* disease, very susceptible to fungal disease and susceptible to nematode damage. It is tolerant of tristeza, dwarfing and spalling diseases. It has moderate levels of resistance to limey soils, with weak resistance of saline soil conditions. It has moderate resistance to low temperatures and is a rootstock with very high tendency for polyembryony.

Yuma Citrange (*P. trifoliata* × *C. sinensis*), A trifoliate orange hybrid. It matures in the months of October-November. It is a rootstock susceptible to iron deficiency. It has smaller fruits than Citrumelo. It forms trees of moderate size, with trifoliate leaves, and crown volume in global structure. It has low tendency toward polyembryony, and forms zygotic plants at high rates (Jaskani et al., 2006). It is a very suitable rootstock for grapefruit. In terms of features like susceptibility to disease and nematodes, fruit quality and adaptation to soil types, Yuma citrange is similar to Carrizo and Troyer citranges.

Chinese bitter orange (*C. myrtifolia* Rafinesque), Chinese bitter orange, susceptible to CTV, matures from January-March. Leaves are small and don't have pointed tips. Fruit are small and rounded, with variability in seed numbers from low to high, and it forms small tress. The peel of the fruit has moderate roughness, with color ranging from orange to dark orange. It originated in China.

Citremon (*Poncirus trifoliata* (L.) Raf. X *Citrus lemon* (L.) Burm), Most of these hybrids show abnormal small leaf features. They die in the germination stage or a short while after. Large leaved plants survive, fruit has many seeds and rough structure, trees show rapid development like lemon.

Sampling Method:

Two hundred seeds from the following citrus rootstocks were sown and germination rates were precisely determined during four weeks. Seedling populations comprising 100 plants from each rootstock were obtained.

Laboratory Analysis:

Seed Selection:

Seeds that exhibited no abortive properties, were plump, and did not display any fungal pathogen symptoms were selected within the scope of the study. The seeds demonstrated a high germination rate in the pre-germination test.

Planting the Selected Seeds and Determination of Germination Rates:

Seeds were provided from open pollinated mature fruits of Gou-Tou sour orange, C-35 citrange, Troyer citrange, Taiwanica, Citremon, Yuzu, Sunki mandarin, Flying Dragon trifoliate orange, Yuma Citrange, Macrophylla and Chinese orange rootstocks trees in Citrus orchards in Adana – Türkiye. The seeds were then dried in a shaded area and treated with CAPTAN'H® fungicide. 200 seeds from each genotype were sown in growing media containing of vermiculite No:3 in greenhouse. Seedlings were counted 15 days later after seed germination then seedlings with three developed leaves were transferred into the plastic pots containing peat moss. Subsequently, the plants were subjected to a series of periodic maintenance operations in order to ensure optimal growth and development. The seedlings grown well without any blemishes were used for cytometry analysis.

Isolation and Staining of Nuclei:

To release cell nuclei, approximately 50 mg of fresh tissue form each seedling leaf was mixed with Tahiti lime (*Citrus latifolia* Tan.) leaf pieces, which were used as a control, and chopped into small pieces with a sharp razor in a sterile Petri dish containing 300 µl of nuclei buffer (pH 7.4) of the following composition: 0.14 M NaCl, 0.003 M KCl, 0.012 M NaH_2PO_4, 0.002 M KH_2PO_4, 0.1 % Triton 100, 50 µg of RNase and 100 µl of dithiothreitol. For measurements of absolute DNA values, Tahiti lime leaf tissues were included as an internal standard, as previously described by (Ollitrault & Michaux-Ferriere, 1994; Ollitrault et al., 1994). Tahiti lime was described as

triploid and nuclear DNA content was found to be 1.17 pg / 2C by (Ollitrault & Michaux-Ferriere, 1994; Ollitrault et al., 1994). The suspension was filtered through a 50 µm pore nylon filter into microcentrifuge tubes. After filtration, 100 µl of propidium iodide was added for staining of the DNA. Then the suspensions were incubated for approximately 5 min at room temperature. After incubation, each sample was run on a flow cytometer (Seker et al., 2003). For estimation of DNA content of nuclei, the relative fluorescence of nuclei was measured by using a CA-III Flow Cytometer (Partec GmbH, Münster, Germany) with an Argon laser light source operating at a wavelength of 488 nm. Histograms and cytograms were evaluated on DPAC Software (Partec GmbH, Münster, Germany). From 2000 to 5000 nuclei were counted per flow cytometry measurement. The nuclear DNA contents of different seedlings were calculated by comparison of relative positions for G_{0-1} peaks corresponding to the sample nuclei and the nuclei isolated from Tahiti lime or mungbean, respectively. This permits accurate determination of the unknown DNA content (Seker et al., 2003).

Data analysis and estimation of nuclear genome size: The nuclear DNA contents of the different rootstock seedlings were calculated by comparison of the relative positions for the G_{0-1} peaks corresponding to the sample nuclei and the nuclei isolated from Tahiti lime, respectively. This permits accurate determination of the unknown DNA content. Calculation was made according to the formula:

$Q = R \times (E / S)$

where Q = unknown DNA content (pg / 2C), R = standard 2C DNA content (1.17 pg), E = sample G_{0-1} peak mean, and S = standard G_{0-1} peak mean.

Statistical Analysis

Statistical analyses were carried out with the genome results obtained from each seedling. Analysis of variance was used to determine statistical signifcant diference of genome size variation data by using SAS software. The mean separation was done using Fisher's least signifcant diference (LSD) test if the F test was signifcant at $P<0.05$.

RESULTS and DISCUSSION

Germination rates the seeds of different citrus rootstocks were given in (Table 1) and compared in (Fig. 2). According to the obtained results germination rates were differed statistically important among the rootstocks. The highest germination rate was found on Troyer citrange (16.3 %) at the end of first week. The lowest rates were found in Flying Dragon (5.0 %), Taiwanica (5.3 %) and Citremon (6.2 %). Troyer citrange seeds can be considered that having earliest tendency for germination. Seed germination continued to increase rapidly in all surveyed rootstocks. The highest germination percentage was determined on C-35 Citrange (40.5 %) whereas the lowest in Yuzu (19.0 %) 14 days after seed sowing. While the highest germination rate was found in C-35 Citrange (95.2 %), Troyer Citrange (94.5 %), Macrophylla (92.6 %) and Yuma citrange (88.9 %) rootstocks whereas the lowest rate was observed in Yuzu rootstock (68.3 %) after three weeks of sowing. All viable seeds were germinated at the end of four weeks of sowing. The highest germination percentages were obtained from C-35 citrange (98.0 %) and Troyer citrange (96.5 %) rootstocks whereas the lowest germination percentage was determined in Yuzu (74.0 %). To summarize, 1921 seedlings were obtained at the end of seed germination studies from 2200 seeds. The number of seedlings per genotype was recorded maximum in C35 citrange (196 seedlings) whereas the minimum in Yuzu (148 seedlings) followed by Flying Dragon trifoliate orange (163 seedlings). The difference for germination rates could be due to genotypic difference. A study conducted by Cimen (2020), C35 citrange had the lowest germination rate under in vitro germination conditions. Contrarily, C35 citrange had the highest germination rate in our research. The reason for the high difference between two researches could be origin of seed source and seed conservation conditions. Some of the citrus genotypes like C-35 citrange and Troyer citrange produced two or more seedlings from one single seed due to nucellar embryony (Navarro & Juarez, 2007) stated that Troyer citrange has the highest graft success if two weeks old seedlings used for shoot tip grafting. The seedlings obtained from each rootstock were uniform for further evaluation.

Flow cytometry analyses are rapid, preparation of nuclear suspensions is easy and statistical distribution of DNA content of large populations is completed rapidly. Cutting fresh leaf tissues from young seedlings with a sharp razor ensures mixing of large numbers of cell nuclei with the nuclear buffer solution. Leaves from young citrus seedlings and Tahiti lime were mixed and prepared for analysis and flow cytometry formed two large peaks showing G_{0-1} peaks for both species. Calculation of total genome in G_{0-1} peak ratio was successfully completed as published by many researchers. Results obtained from flow cytometry analyses show seedlings of the species used in this study had diploid genome. The G_{0-1} peak ratios were smaller than the Tahiti lime genome used as control species. The mean genome volumes for seedling species analyzed in this study are given in (Table 2) and genome size variations among species are given in (Fig. 1). According to the obtained results, Yuzu (0.808 pg) has the largest genome while Flying Dragon trifoliate had the smallest genome (0.700 pg). There were statistical

differences determined between the seedling populations of the eleven different rootstocks. The Yuzu rootstock is easily proliferated from seeds, has slow growth characteristic, and is a rootstock with high fruit quality and yield. Yuzu has high resistance to low temperatures and polyembryony tendencies. Though it is resistant to *Phytophthora*, fungus and nematodes and tolerant of tristeza, having dwarfing effect, it has moderate resistance to lime and salty soils so commercial use in the world has not become widespread.

Çizelge 1. Narenciye anaç tohumlarının çimlenme oranları*
*Table 1. Germination rates of citrus rootstock seeds**

Rootstock species and hybrids	Germination rates (%)				
	7 days	14 days	21 days	28 days	Total number of seedlings
Yuzu CRC	6.8 cd	19.0 e	68.3 d	74.0 d	148
Chinese sour orange	8.1 bcd	22.7 de	78.0 bcd	88.0 abc	176
Yuma Citrange SRA	14.3 abc	36.5 abc	88.9 a	91.0 abc	182
C-35 Citrange	15.0 ab	40.5 a	95.2 a	98.0 a	196
Macrophylla	12.1 abcd	30.2 abcd	92.6 a	94.5 ab	189
Troyer Citrange	16.7 a	38.0 ab	94.5 a	96.5 a	193
Gou-tou	7.5 bcd	28.8 bcde	81.0 bc	82.5 bcd	165
Citremon	6.2 d	30.1 abcd	80.7 bc	89.0 abc	178
Taiwanica	5.3 d	24.6 de	78.5 bcd	82.0 cd	164
Sunki Mandarin	9.7 abcd	25.5 cde	75.2 cd	83.5 bcd	167
Flying Dragon	5.0 d	22.1 de	74.8 cd	81.5 cd	163
Significance	**	**	**	**	

*P value= 0,0132 (P<0.05).
**The differences among the values are statistically significant.

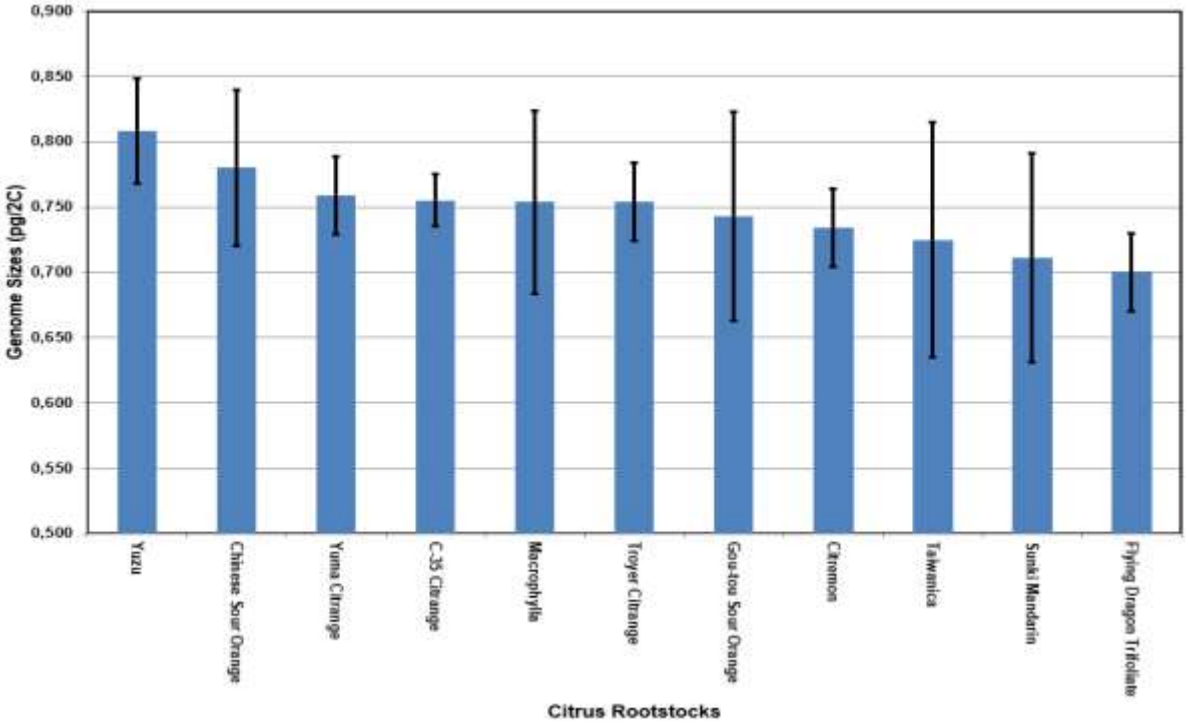
Çizelge 2. Turunçgil anaçlarının 2C DNA içeriklerinin karşılaştırılması*
*Table 2. Comparison of the 2C DNA content of the citrus rootstock species**

Rootstock species and hybrids	Ploidy level	Genome size (pg/2C) means
Yuzu CRC	diploid	0.808±0.04 a
Chinese sour orange	diploid	0.780±0.06 a
Yuma SRA	diploid	0.759±0.03 a
C-35 Citranj	diploid	0.755±0.02 a
Macrophylla	diploid	0.754±0.07 ab
Troyer Citranj	diploid	0.754±0.03 ab
Gou-tou	diploid	0.743±0.08 ab
Citremon	diploid	0.734±0.03 b
Taiwanica	diploid	0.725±0.09 b
Sunki Mandarin	diploid	0.711±0.08 b
Flying Dragon	diploid	0.700±0.03 b
Significance	-	**

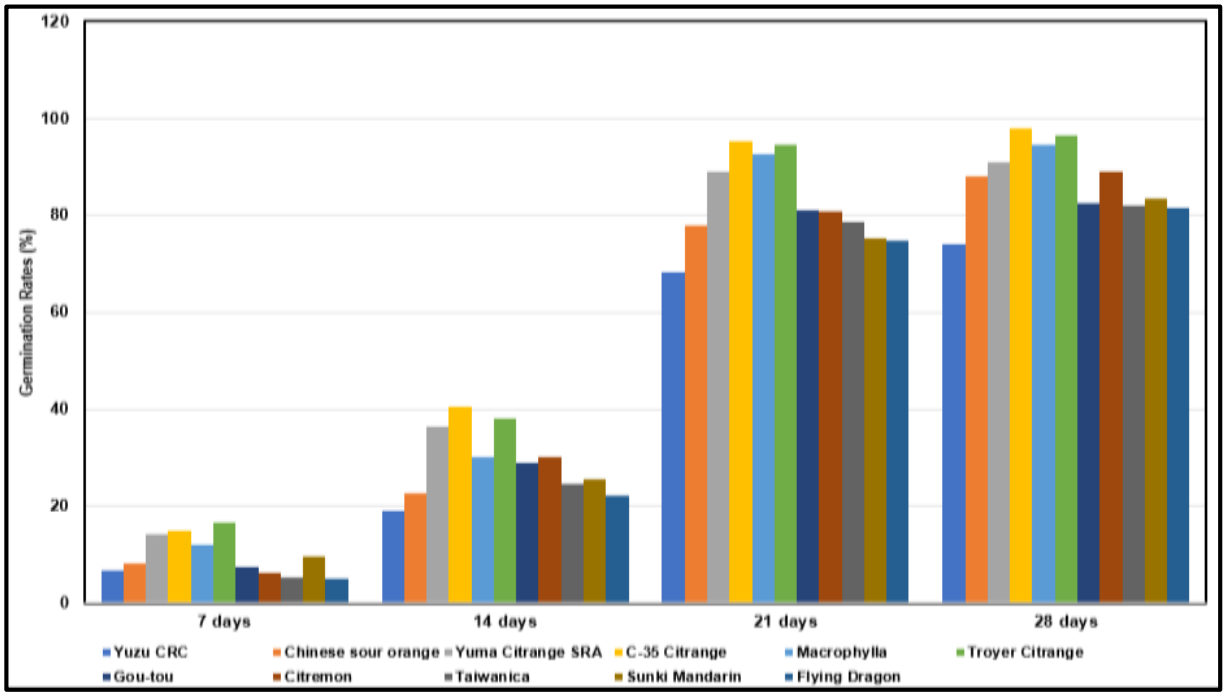
*P= 0,0421 (P<0.05).
**The differences among the values are statistically significant.

Diploidy is the most common ploidy level in Citrus and its related genera with the basic chromosome number $x = 9$. However, some polyploid genotypes were found in Citrus and related genera. Tetraploid Hong Kong wild kumquat (*Fortunella hindsii* Swing.), Triploid Tahiti lime, tetraploid strains of *Poncirus trifoliata*, allotetraploid *Clausena excavata* Burm. F., tetraploid *Clausena harmandiana* and hexaploid *Glycosmis pentaphylla* are some examples of natural polyploidy found in the germplasm of the *Aurantioideae* subfamily.

Polyembryony is commonly observed in citrus and related genus of Clausena, Fortunella and Poncirus Withnucellar embryony several embryos occur from one seed and these plants carry all the characteristics of the mother plant. As a result, seedlings obtained from species and varieties with excess polyembryony tendency have diminished genetic expansion (Polatoz, 1995). Seedlings occurring from nucellar embryos may be used as rootstock due to having similar features to the mother. Tetraploid plants can be found in zygotic citrus seedling populations and polyploidy level could be reach up 2.5 % in Rutaceae according to the previous articles (Cameron, 1968; Rom & Carlson, 1987; Seker et al., 2003).



Şekil 1. Turunçgil anaçları arasındaki genom büyüklüğü farklılıkları (çubuklar standart hatayı göstermektedir)
Figure1. Genome size variations among citrus rootstocks (bar indicates the standard error)



Şekil 2. Turunçgil anaçlarının birinci, ikinci, üçüncü ve dördüncü hafta sonundaki çimlenme oranlarının karşılaştırılması
Figure 2. Comparison of germination rates of citrus rootstocks at the end of first, second, third and fourth weeks

A study by Seker et al. (2003), determined that trifoliate seedlings had the smallest genome among citrus species. As a result, both common trifoliate orange and Flying Dragon trifoliate orange were revealed to have small genomes. The Poncirus genus was determined to have smaller genome volume than the citrus genus. Again, Sunki mandarin was found to have smaller genome volume. The study carried out by Seker et al. (2003) determined Cleopatra mandarin has smaller genome than other citrus species.

Mandarins having small genome is considered to be effective on both species having small canopy volume, small

leaves and small fruit. As a result, Sunki mandarin and Cleopatra mandarin are two valuable rootstocks especially for mandarin species and varieties. C-35, Carrizo and Troyer citrange species have larger genome compared to trifoliates. The statistical differences between them are significant. The reason for this is associated with citranges having larger genome volume than the other parent of orange Cimen (2020) demonstrated C-35 citrange seedlings were diploid with 0.794 pg / 2C relative genome sizes. This result confirmed our findings in C-35 seedlings.

CONCLUSIONS

Different ploidy levels can have a decisive impact on plant development and morphology. For instance, variations in growth rates and sizes can be observed among diploid, triploid, and tetraploid plants. This information enables plant breeders to select rootstocks with desired traits and control plant growth. Rootstocks with different ploidy levels can also affect fruit yield and quality. For example, triploid rootstocks often reduce fruit size while enhancing fruit quality. Therefore, ploidy levels can be consciously chosen to increase fruit yield and quality for specific purposes. Certain ploidy levels may confer greater resistance to diseases and pests. This trait is crucial for maintaining plant health and increasing harvest yields. For instance, some triploid rootstocks exhibit higher resistance to root rot or nematodes. Different ploidy levels can influence plant crossability and reproductive ability. Knowing the ploidy level of a particular plant species helps determine which other plant species it can cross with, thus preventing unwanted crossbreeding. When selecting plants to be cultivated in a specific area, it is important to choose plants that can adapt to the climate, soil, and other environmental factors of that region. Ploidy levels can affect a plant's adaptation ability, assisting in the selection of rootstocks suitable for a particular region.

The determination of ploidy levels is typically accomplished successfully through studies utilizing flow cytometry technique (Kaya et al., 2020). Genome size refers to the amount of DNA in an organism's non-replicated haploid set of chromosomes (Swift, 1950). In diploid (2n = 2x) organisms, genome size refers to the amount of DNA contained in haploid (n number of chromosomes) chromosomes. Genome size is expressed as the C value, which is the amount of DNA content in the genome in picograms. The 2C value is the amount of DNA in the nucleus of a somatic cell, regardless of its ploidy level (Kaya & Sakiroglu, 2012; Tuna, 2014). Significant (about 1000-fold) differences are observed between species in terms of genome size (C value). On the other hand, the genome size remains constant among different individuals of a species and therefore becomes species specific. Therefore, the C values of species are extremely important for biology, genetics, taxonomy and evolution studies (Rees & Walters, 1965; Price & Bachmann, 1975; Ohri, 1998; Ozkan et al., 2003; Ollitrault et al., 2007). Flow cytometry is the newest, fast, sensitive and economical method used to determine genome size today. Since there is a very close relationship between the C values determined by flow cytometry and the chromosome numbers of the species, this parameter is also used to determine the ploidy levels of the species (Tuna et al., 2001; Mavioğlu Kaya, 2010). This study showed that nucellar seedling populations of Gou-Tou sour orange, C-35 citrange, Troyer citrange, Taiwanica, Citremon, Yuzu, Sunki mandarin, Flying Dragon trifoliate orange, Yuma Citrange, Macrophylla and Chinese orange rootstocks had only diploid genomes. In a study conducted by Guo et al. (2008), similar to our study results, they reported that the Gou-Tou sour orange rootstocks they used as study material was diploid. There were no polyploids in the surveyed seedling population due to high tendency to nucellar embriyony. Polyploidy may have great potential for citrus rootstock breeding. Autotetraploid and allotetrapoid rootstocks may have potential especially for dwarfing of citrus trees.

Determining ploidy levels in citrus is crucial due to its significance in plant cultivation, diversification, and breeding endeavors. Knowledge of ploidy levels plays a critical role in selecting parent plants for breeding programs. For instance, differential traits may exist between diploid (2n) and triploid (3n) variations in some citrus species, influencing the selection of desired traits in new cultivars. Ploidy levels can significantly impact fruit productivity, size, quality, and resilience. Thus, determining ploidy levels is essential for achieving higher yields and superior fruit quality. Certain ploidy levels can affect a plant's resistance to diseases and pests, hence informing the selection and cultivation of resistant varieties. Ploidy levels also influence a plant's reproductive capability; for example, triploid plants are often sterile and do not produce seeds, necessitating the selection of suitable plants for seed production. Additionally, the determination of ploidy levels serves as fundamental information in genetic research, guiding plant genetics and breeding efforts. Consequently, the identification of ploidy levels in citrus species is imperative for plant cultivation, breeding programs, and genetic research, contributing to the development of more productive, resilient, and high-quality cultivars and ensuring the sustainability of agriculture.

Polyploidy, the condition where an organism has more than two complete sets of chromosomes, plays a crucial role in the development of citrus fruits. It can result in larger fruit size, improved disease resistance, and enhanced stress tolerance, which can be beneficial for both the quality and yield of citrus crops. Polyploidy can also contribute to hybrid vigor and potentially improve the organoleptic qualities of citrus fruits, such as taste, color, and texture. However, as noted in the study, specific polyploid citrus species or varieties were not determined, which suggests

that further exploration into polyploid citrus cultivars could be valuable. To better understand and potentially develop polyploid citrus varieties, several methods can be employed to induce polyploidy:

Colchicine treatment: Colchicine is one of the most commonly used chemicals to induce polyploidy. It disrupts the process of chromosome segregation during cell division, leading to chromosome doubling. This method is often applied to meristematic tissues (such as root tips or shoot tips) of citrus plants to generate tetraploid individuals from diploid ones. Oryzalin treatment: Oryzalin, another chemical agent, can also be used to induce polyploidy. It works by inhibiting microtubule formation during cell division, preventing the separation of chromosomes, thus resulting in polyploid cells. In vitro culture: Tissue culture techniques can be used in conjunction with chemical treatments to generate polyploid plants. This method allows for the controlled environment required to induce polyploidy and regenerate whole plants from the treated cells. Somatic hybridization: This method involves fusing cells from different citrus species or varieties, followed by the induction of polyploidy in the hybrid cells. Somatic hybridization can lead to the creation of interspecific or intergeneric hybrids with desired traits, including polyploidy. Genome editing techniques: Newer genome editing technologies, such as CRISPR/Cas9, could also offer a precise approach to induce or modify polyploidy in citrus fruits, though this method is still in the experimental stages for polyploid induction.

By incorporating these methods into citrus breeding programs, researchers and growers can potentially create new, more resilient citrus varieties with improved agronomic and sensory qualities. Understanding and utilizing polyploidy could open up new avenues for the citrus industry, helping to meet the increasing global demand for high-quality fruits.

This study has laid the foundation for research aimed at determining ploidy levels in citrus rootstocks. The continuation and expansion of such studies are crucial for identifying new plant species and varieties, as well as elucidating which rootstocks exhibit resilience to various stress factors.

Author Contribution Statement

These authors contributed equally to this work.

Conflict of Interest

The authors declare that they have no conflict of interest.

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Screening of Eggplant F_3 Segregating Population for Salt Tolerance

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ABSTRACT
Utilizing salt-tolerant varieties in affected lands is the most prominent environmentally friendly solution. Wild relatives of eggplant have tolerance to some abiotic stresses. The aim of the study was to assess salinity tolerance in the third filial segregating population of eggplant lines that were previously associated with salt tolerance, then they could be used in breeding programs. The 50 F_3 families resulting from crossings of the inbred line BATEMTDC47 (*Solanum melongena* L.) and *Solanum incanum* L. were screened under 150 mM NACl stress. A total of fourteen seedlings at the four-five leaves stage from each of the 50 F_3 lines, accompanied by seedlings of two parents, were examined beside, four seedlings per line served as controls. All stressed seedlings were assessed comparatively with their controls by 0-5 visual scale, on the 12th day following the final salt treatment. Additionally, malondialdehyde (MDA) and proline levels in stressed fresh leaf samples were analyzed. The most tolerant four plantlets from each line were selected and transferred to the greenhouse to generate F_4 seeds. During the greenhouse cultivation period, 13 morphological traits including plant and fruit features, such as plant height, stem diameter, anthocyanin presence, fruit color, and fruit shape etc., were studied. Following the observations, F_3 plants were self-pollinated to produce F_4 generation. Except for a few outliers, the visual scale and proline accumulations showed concurrent increases and reductions. Overall, the results also demonstrate that enhancement of salt tolerance of *Solanum melongena* can be improved using *Solanum incanum* as a donor of alleles.

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Patlıcanda F3 Kademesindeki Açılım Popülasyonunun Tuzluluk Toleransı Açısından Taranması

ÖZET
Toprak tuzluluğundan etkilenen alanlarda tuza dayanıklı çeşitlerin kullanılması çevre dostu bir çözümdür. Patlıcanın yabani akrabaları bazı abiyotik streslere karşı toleransa sahiptir. Bu çalışmanın amacı, patlıcanda türler arası melez programından geliştirilen F_3 popülasyonunun tuza tolerans durumunu belirlemektir. Arzu edilen özelliklere sahip bir kültür patlıcanı saf hattı BATEM-TDC47 (*Solanum melongena* L.) ve *Solanum incanum* L. arasındaki melezlemelerden geliştirilen 50 F_3 hattı ve ebeveynlerinden 4-5 yapraklı büyüme aşamasındaki on dörder bitki 150 mM NaCl stresi altında test edilmiştir. Her bir hat ve ebeveyn için dörder adet bitki de kontrol olarak kullanılmıştır. Son tuz uygulamasından sonraki 12. günde bitkiler 0-5 görsel skalası kullanılarak değerlendirilmiştir. Ayrıca, stres altındaki bitkilerden alınan taze yaprak örneklerinde malondialdehit (MDA) ve prolin düzeyleri analiz edilmiştir. Yapılan gözlemler sonucu her hattan tuza en dayanıklı dört bitki seçilerek F_4 jenerasyonunu üretmek için seraya transfer edilmiştir. Serada normal koşullarda yürütülen yetiştirme dönemi boyunca bitki boyu, gövde çapı, meyve rengi, meyve şekli ve antosiyanin varlığı gibi bitki ve meyve özellikleri de dâhil olmak üzere patlıcan için önemli 13 morfolojik özellik incelenmiş ve bu esnada

Bahçe Bitkileri

Araştırma Makalesi

Makale Tarihçesi
Geliş Tarihi : 25.09.2024
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Anahtar Kelimeler
Abiyotik stres
Islah
PCA
Tuz stresi
Yabani türler

F_3 bitkileri kendilenerek F_4 nesli üretilmiştir. Araştırma sonuçları değerlendirildiğinde, görsel skala ve prolin birikimlerinin bazı istisnalar dışında paralel artışlar ve azalışlar gösterdiği belirlenmiş olup, *S. melongena*' nın tuz toleransının arttırılması için *S. incanum*' un polen donörü olarak kullanılabileceği açıklanmıştır.

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INTRODUCTION

Sustainable agriculture is under threat of salinity increase in soil, this turned into a serious problem on a global scale and approximately one billion hectares' arable land has been affected by salinity (Ivushkin et al., 2019). Generally, salt stress restricts the water and nutrient uptake by roots from soil, thus plant growth reduces and eventually plants die under constant exposure to stress. The majority of plants are sensitive to salt stress, and they can't remain alive under high stress or their yield decreased (Dasgan et al., 2002). In particularly, vegetables are more sensitive to salinity compared to the other major crops (Chinnusamy et al., 2005) and they are classified as glycophytes (Hannachi and Van Labeke, 2018; Brenes et al., 2020a). Eggplant is known as moderately sensitive to salt stress however for sufficient fruit yield, salinity threshold value in soil should be under 1.5 dS m−1 (Ünlükara et al., 2010). A salt concentration in soil, equivalent to 100–200 mM NaCl causes in glycophytes to exhibit total inhibition of growth (Tang et al., 2015; Brenes et al., 2020a). Halophytes can sustain their life at level of 200 mM NaCl and above soil salinities (Himabindu et al., 2016). Salinity effects on plants may vary depend on various factors; however, tolerance to salt stress, mainly in control of genotype (Sumalan et al., 2020). Utilizing tolerant varieties on affected lands is the most prominent environmentally friendly solution. Tolerance or resistance of plants can be improved by using several techniques such as interspecific hybridization (Plazas et al., 2019; Cebeci et al., 2024), with the interspecific hybridization, useful genes from wild relatives could flow to the gene pools to improve salt tolerance levels of crops (Sekara et al., 2007).

Described as moderately sensitive to salinity (Maas, 1990; Ünlükara et al., 2010), it is urgent to improve resilient varieties to sustain of eggplant production on agricultural lands under salinization threat. As well as local genotypes are valuable gene sources to enhancement of new tolerant varieties (Ayranci and Bagci, 2020), eggplant has significant wild relatives such as S. insanum, S. incanum, S. linnaeanum and S. sisymbriifolium which are known have more tolerance to abiotic stresses and it can be crossed with many of its relatives (Plazas et al., 2019). Successful crossings of S. melongena with S. incanum, S. aethiopicum and S. torvum have been reported previously (Daunay et al., 1991, Nee, 1999, Kameswara Rao, 2011; Cebeci et al., 2024). Breeding programs for tolerance to salt stress have been newly started in eggplant (Brenes et al., 2020b; Ortega-Albero et al., 2023). Successful results have been reported for almond (Dejampour et al., 2012), cotton (Tiwari et al., 2013), rice (Kilian et al., 2021) and Egyptian clover (Dwivedi et al., 2022). In a study conducted by Ortega-Albero et al., (2023), compared the responses of cultivated eggplant, S. insanum L., and their interspecific hybrid, under 200 and 400 mM NaCl stress by determining some growth parameters and the levels of biochemical stress markers. At the end of the study researchers stated that, the hybrid S. melongena x S. insanum presented an obvious heterotic effect in terms of plant growth, and it was found as being more salt-tolerant than two parents. Phylogenetic analysis by Lester and Hasan (1991) was revealed very close relationship between S. incanum and S. melongena, thus, interspecific hybridization between them expected to exhibit better heterosis. Although several studies have identified desirable tolerance features of S. incanum L. (Gramazio et al., 2016), its potential under salt stress was reported for the first time by Cebeci et al. (2024).

Under stress conditions, each plant species can continue their growth depending on the efficiency of the stress response mechanisms. There are two main types of tolerance mechanisms in plants; one of them is protection for ion toxicity and osmotic stress and the other is osmolyte biosynthesis and protection for oxidative stress (Ortega-Albero et al., 2023). A commonly used indicator of lipid peroxidation in plant tissue that elevates in response to oxidative stress is the level of malondialdehyde (MDA). In general, salinity-sensitive plants have a greater MDA content than salinity-tolerant ones (Yasar, 2003). Proline, which is an amino acid, plays a major role to protect plants from various stresses. Higher proline accumulation in plants results higher tolerance to stress conditions. Also, it facilitates the plants to recover from stress in a quick way (Hayat et al., 2012). The success of plant breeding programs is dependent on establishing a strong modelling basis which is constitute pyramid the different traits constantly measured at different times. Breeding objectives often concern improving multiple quantitative traits simultaneously, such as yield, quality, and resistance to both biotic and abiotic stress factors. These traits are

monitored by observations or tests made on lines or segregating populations obtained at different stages of breeding programs. (Araus and Cairns, 2014; Vieira et al., 2025). Researchers employed several techniques to determine genetic diversity of a germplasm such as principal component analysis (PCA) which is a multivariate statistical method has capability to convert many similar correlated factors into fewer factors named principal components (Ziegel, 2002).

This study aimed to assess of possibilities of successful introgressions of salt tolerance traits from S. incanum to the cultivated eggplant. The plant materials identified as tolerant (50 F3 lines) will assist in further improvement of salt tolerant eggplant lines and varieties.

MATERIAL and METHOD

Plant material

This research is a part of a comprehensive project titled "Development of Tolerant Inbred Lines to Salt and Drought Stresses in Eggplant through Interspecific Hybridization". Previous studies by Cebeci et al., (2024), F_2 segregating population (256 seedlings) and parents; high yielding eggplant inbred line (BATEM-TDC47) and salt tolerant wild relative *Solanum incanum* L., were tested under salt stress. In terms of growth parameters and biochemical analysis, 50 individuals were determined as salt tolerant. In this study, these 50 F_3 lines were tested to reveal their salt tolerance potential within the scope of the mentioned project.

Stress Treatment

A total of 30 seeds per line were sown in viols (15x10) on August 10, 2022. Uniform seedlings with 2-3 true leaves were transferred to the 1 litre pots. After two weeks, seedlings with 4-5 true leaves in 1 litre pots were subjected to salt stress in 100 m^2 semi-controlled compartment conditions. During the experiment, mean temperature and humidity were recorded as 30 ^oC and 70% with HOBO data logger. For the test, 14 seedlings in the 4-5 leaves growth stage per F_3 lines and parents, a totally of 728 seedlings, were evaluated under severe salt stress conditions generated by applying 150 mM NaCl solution. The salt solution was administered in daily aliquots of 50 mM. In addition, 4 seedlings per F_3 lines and parents, 228 seedlings, were employed as controls and irrigated with similar amount of nonsaline water. On the 12^{th} day of the salt treatment on October 17, four seedlings were selected as salt tolerant, considering symptom severity using 0-5 visual damage scale (Kiran, 2015; Cebeci et al., 2024) (Table 1) and transferred to the 650 m^2 greenhouse to produce F_4 generation. During this period, some of their morphological features were noted.

MDA and Proline Determination

On the 12^{th} day after the last salt treatment, fresh leaf samples from each line and parent were collected to MDA and proline analysis. Measurement of malondialdehyde (MDA) was conducted on fresh leaf extracts by the trichloroacetic/thiobarbituric acid method as described by Hodges et al. (1999). The 50 mg ground fresh leaf samples were homogenized in 80% (v/v) methanol, and the extract was centrifuged at 3000xg for 10 minutes at +4°C. The supernatant was divided into two parts, and A and B mixtures were prepared with them. The mixtures A and B were prepared as follows: A: 1 ml extract, 1 ml 20% TCA (Trichloroacetic Acid), B: 1 ml extract + 1 ml 20% TCA (Trichloroacetic Acid) + 0.65% TBA. These samples were incubated at 95°C for 20 minutes, then cooled on ice and centrifuged at 12,000 g at 4°C for 10 minutes. After stopping the reaction on ice, the absorbance of the supernatants was measured at 532 nm. The nonspecific absorbance at 600 and 440 nm was subtracted, and malondialdehyde concentration was determined using the equations by Hodges et al. (1999) and expressed as µmol g^{-1} FW. Proline concentration was detected in fresh plant material by using the ninhydrin-acetic acid method according to Bates et al. (1973). Proline was extracted in 3% aqueous sulfosalicylic acid, the extract was mixed with acid ninhydrin solution, incubated for 1 h at 95 °C, cooled on ice, and then extracted with two volumes of toluene. The absorbance of the supernatant was read at 520 nm, using toluene as a blank. Proline concentration was expressed as µmol g^{-1} FW.

Morphological measurements

The salt-treated seedlings in pots were evaluated using 0-5 visual damage scale as presented in Table 1 (Kiran et al., 2015; Cebeci et al., 2024), and the most tolerant four seedlings were selected per F_3 lines. These selected salt-tolerant lines were transferred to planting sites in the greenhouse to produce F_4 generation following stress treatments in pots. Morphological observations were performed on plants grown under normal conditions, including some phenotypic traits. During the greenhouse period, under normal irrigation conditions, some of their fruit and plant features, such as plant height, stem diameter, fruit color, fruit shape, leaf size, anthocyanin presence, were studied (Table 1) to determine the features of salt-tolerant lines. A total of 13 phenotypic features, regarding plant and fruit characteristics derived from the International Union for the protection of new varieties of plants (UPOV, 1992), were visually observed once 50% of the plants in a genotype had started to fruit set.

Table 1. The visual rating scale for the morphologic features of eggplant used in the study*
*Çizelge 1. Denemede kullanılan morfolojik değişimleri gösteren parametreler**

No	*Features (Özellikler)*	*Ratings (Puanlama)*
1	Plant height	Short: 1; Medium:3; Long:5
2	Stem diameter	Narrow:1; Medium:3; Wide:5
3	Growth habit	Closed:1; Open:3
4	Fruit colour	White:1; Purple:3; Green:5
5	Seconder fruit colour	White:1; Purple:3; Green:5
6	Fruit colour distribution	Plain:1; Mottled:3; Netted:5
7	Fruit shape	Round:1; Oval:3; Long:5
8	Prickliness	Presence:1; Absence:3
9	Hairiness	Less:1; Medium:3; Very:5
10	Leaf size	Small:1; Medium:3; Large:5
11	Anthocyanins	Absence:1; Very:3; Less:5; Medium:7
12	Fruit curvature	Presence:1; Absence:3
13	Sepal size	Small:1; Large:3
14	Salt stress symptoms	0:no effect; 1:local yellowing and curling of leaves with slow growth; 2; necrosis and chlorosis on 25% of the leaf; 3: necrotic spots on the leaves and defoliation by 25-50%; 4: necrosis by 50-75% and death of several plants; 5: severe necrosis on leaves by 75-100% and/or predominant plant deaths

*: The first 13 observations were noted during greenhouse period and the last one was noted from seedlings in pots.
*: İlk 13 gözlem seraya aktarılmış bitkilerden, en son gözlem ise saksıdaki fidelerden alınmıştır.

Data evaluation

Scale results were used to drawn pivot charts (Figure 2) in Microsoft Office Excel program (version 2016). For the clarification of the results, correlation graphic was created using MDA, proline and 0-5 visual scale data. Whole data derived from the measured parameters in greenhouse were subjected to multivariate analysis through a Principal component analysis (PCA) which was explained which parameter is more effective in explaining the variation, before running the program all data were standardized. Additionally, whole data was used to Hierarchical clustering of the 50 F_3 eggplant lines and parents using Ward's method. Data were analyzed using the software program PAST version 4.03.

RESULTS and DISCUSSION

One of the permanent solutions to mitigate detrimental effects of salt stress is selection and breeding of tolerant varieties which can grow under abiotic stress conditions (Dasgan et al., 2002). Therefore, this study aimed to reveal salt tolerance potential of F3 segregating population. For this purpose, seedlings of 50 F3 lines were exposed to 150 mM NaCl stress in pots during the four-five leaves stage. Previous year F2 population were tested under similar conditions by Cebeci et al. (2024), in terms of acquired results, in the present study 0-5 scale and MDA, proline accumulations were considered to find differences. Visual observation of the plants after 12 days of treatment with increasing salinity proved that salt stress inhibited growth of S. incanum, BATEM TDC47 and F3 lines as compared to their non-stressed controls. Reductions in growth observed among the F3 lines highly variable. Although both species and F3 lines were affected negatively under salt application, the detrimental effects of salt stress were more obvious in BATEM TDÇ47 than in S. incanum. As other wild relatives, S. incanum has species specific vegetative developmental stages and generally wild relatives initially presents slow growth when compared to the cultivated ones, however this slow growth in the beginning mostly is an evident of strong plant which has some tolerances in the further vegetative stages.

Because of evaluation is less laborious, cheaper, and less time-consuming, screening studies for salinity tolerance are generally conducted on germination, seedling, and young plant stages (Akinci et al., 2002; Dasgan et al., 2002). The identification of salt-tolerant plants generally depends on classic phenotyping, visually assessing of symptoms and measuring of agronomic and physiologic traits. For visually assessing, mostly damage scales were effectively used by the researchers for screening in some vegetable crops like melon (Kuşvuran et al., 2007), eggplant (Kiran, 2015; Cebeci et al., 2024), tomato (Dasgan et al., 2002), basil etc. (Bekhradi et al., 2015). In this study, at the 12th day of the last salt treatment, all seedlings were evaluated considering 0-5 visual damage scale, and results for segregating population were ranked between 1.14 – 2.93. The visible symptoms of salt stress in sensitive plantlets under salt stress were presented in Figure 1.

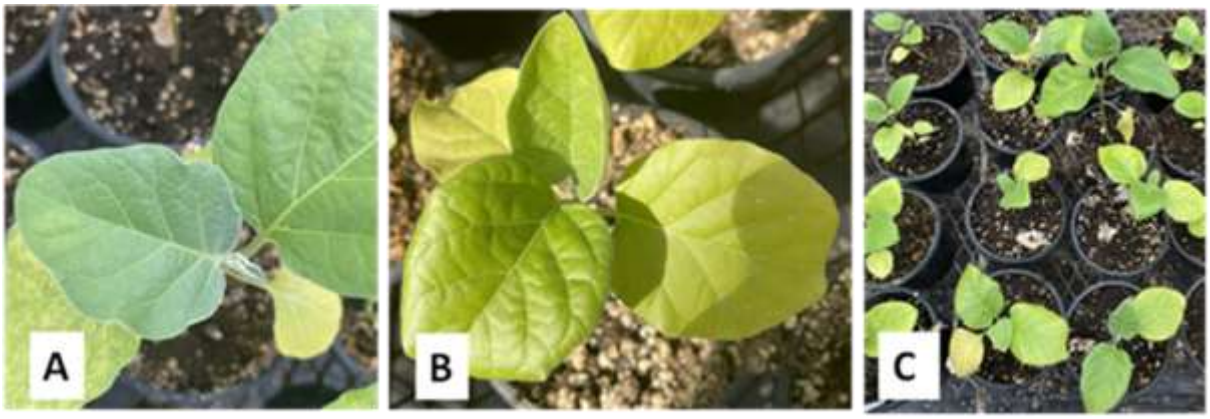

Figure 1. Responses to salt stress of F3 lines; A: putative salt tolerant, B: sensitive, C: distribution of F3 lines in terms of salt tolerance

Şekil 1. F3 hatlarının tuz stresine verdiği cevaplar; A: tuza tolerant, B: hassas, C: tuz toleranslılık bakımından F3 hatlarının dağılımı

Solanum incanum exhibited the least damage and got 1.14 point from scale evaluation and BATEM TDC47 had 1.71 scale point. Distribution of F3 lines according to their visual symptoms under salt stress were depicted in Figure 2. While the scale value of 13 F3 lines varies between 1 and 1.49, most of the lines were placed among the range of 1.5-1.99. However, 11 F3 lines had higher scale values compared to the female parent. Solanum melongena has been described as moderately sensitive or tolerant to salt stress, the response largely depends on the genotype (Plazas et al., 2019). Ortega-Albero et al. (2019) reported in their study on comparison the behavior of an eggplant cultivar (MEL), the wild relative S. insanum (INS) and their hybrid (HYB) that both parents, closely related genetically, would not differ in the type of responses to salt stress but could differ in the magnitude or efficiency of those responses. In the present study, it was seen that the female parent was moderate tolerant to salt stress. However, some of the F3 lines demonstrated sensitivity compared to the female parent. Although a study on the responses to salt stress in wheat at the seedling stage was stated that several genotypes outperformed their parents as revealing positive mid-parent transgressive segregations (Dadshani et al., 2019). In this study negative transgressive segregations were observed during evaluations of visual symptoms on eggplant seedlings. Similarly, in a study conducted on durum wheat, it was reported that no strong genetic correlation was determined on tolerance levels and the measured parameters among the segregating population (Genc et al., 2010).

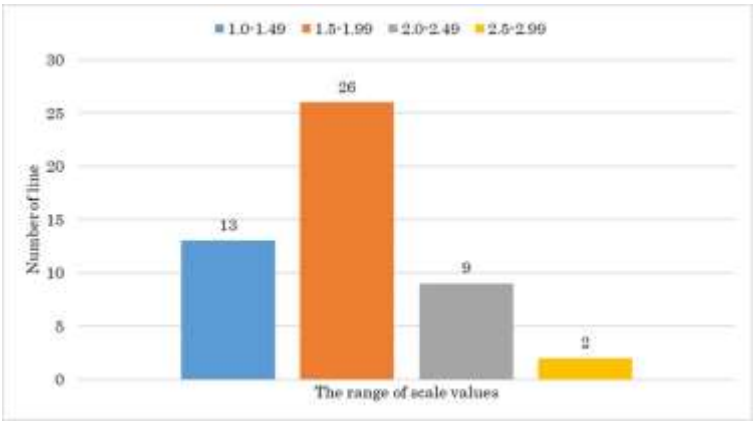

Figure 2. Distribution of F3 lines according to 0–5 rating scale generated from visual symptoms of plantlets

Şekil 2. F3 hatlarının fidelerinde oluşan görsel semptomların 0-5 görsel skalasına göre dağılımı

In a study, BC_1 population derived from backcrosses between *Lycopersicum esculentum* and *Lycopersicum pennellii* in tomato, interactions between tolerance and family were generally found insignificant and low heritability was reported (Saranga et al., 1992). This might be due to the involvement of many gene families in salt tolerance response of crops. In fact, in the mapping study conducted in cotton, 128 genes from 3 gene families associated with salt tolerance were identified. It was explained that such abnormalities in the estimated frequencies of Mendelian ratios in a segregating population were segregation distortion (SDR). In their study, SDRs were detected in salt stress-related genes segregating in the genetic map of the F_2 generation derived from interspecific crosses (Shehzad et al., 2021). Prohens et al. (2012) emphasized that distribution range and accompanying variance for some traits evaluated in the segregating generations (F2 and BC1P2) were generally

greater than those observed in the non-segregating generations (P1, P2 and F1) in eggplant. In this study, it was observed that the variance for salt stress response was quite high among the F_3 family. In a study conducted by Brenes et al. (2020) young plants of *S. melongena* and *Solanum torvum* subjected to salt stress under 0-100-200 and 300 mM NaCl doses, their results indicated that *S. torvum*, which is a common rootstock plant for eggplant was found more tolerant than *S. melongena* at high salt concentrations. Consistent with this study, wild relative *S. incanum* presented better resilience under salt stress than cultivated eggplant; additionally, performance of some F_3 lines was found similar to be *S. incanum*. Adaptation capability of the lines is important, although female parent of the study, called as a sensitive parent it is not sensitive as the regular lines, it has good adaptation skills and have fine marketable fruits, male parent of the study can be cultivated on a broad ecology from continental Africa to Southwestern Europe, China or continental America. Therefore, it is possible to develop salinity tolerant eggplant lines among these F_3s suitable for different ecologies.

After visual evaluation by 0-5 scale, leaf samples from all stressed young plants and parents were collected for MDA and proline analysis. Proline and MDA values of segregating population was ranking between 5.02- 14.32 µmol g^{-1} FW and 3.48-22.26 µmol g^{-1} FW respectively. Although increments in proline level means that plant has some tolerances (Hayat et al., 2012) to the stress conditions and increments in MDA level means sensitivity of the plants to the stressed conditions (Yaşar, 2003). Therefore, as expected, *S. incanum*'s MDA level was found among one of the lowest values (7.89 µmol g^{-1} FW) conversely, its proline amount was found as the highest (12.98 µmol g^{-1} FW) after one of the F_3 genotype (14.32 µmol g^{-1} FW) (Figure 3).

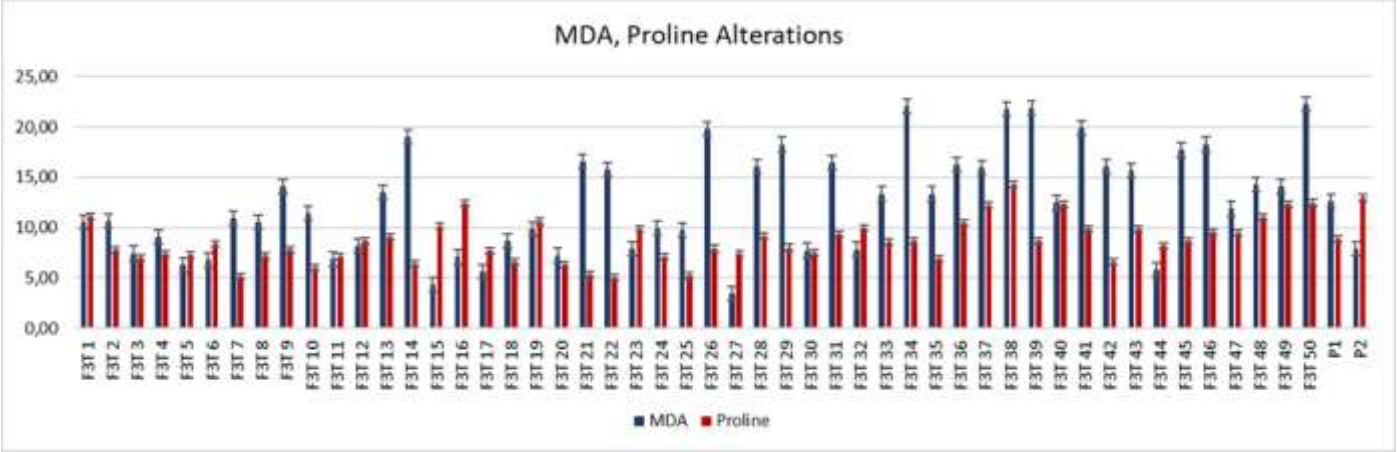

Figure 3. MDA (µmol g^{-1} FW) and proline (µmol g^{-1} FW) alterations of F_3 population, *S. incanum* L. (P2) and Batem TDC47 (P1)

Şekil 3. F_3 populasyonu, S. incanum L. (P2) and Batem TDC47 (P1)'de MDA (µmol g^{-1} FW) ve prolin (µmol g^{-1} FW) oranlarındaki değişimler

According to graphic, presented in Figure 3 generally, when MDA values increased, proline values were decreased under salt stress application. One of the significant results of the study, MDA increment in *S. incanum* (P2) lagged behind the proline increment, and individuals such as F3T1, F3T5, F3T6, F3T27 and F3T44 also presented similar results as their male parent in salt-stressed conditions (Figure 2). Different from the present study, Brenes et al. (2020b) reported that increasing of both MDA and proline in *S. torvum* higher than the cultivar eggplant in their salt tolerance study. In other study conducted by Brenes et al. (2020a), similarly, MDA and proline in *S. insanum* showed higher increases than the eggplant by the salt application.

In addition to this, a correlation graphic (Figure 4) was created using MDA, proline, and visual scale results. According to Figure 4, although there were a few outliers, the visual scale and proline accumulations showed concurrent increases and reductions. These outliers observed on visual scale results or MDA and proline analyses may be due to experimental conditions. Therefore, it was clear from the study, during the selection stage of salt-tolerant plants, it should be considered that higher proline, lower MDA amounts, and the lowest scale points.

Diversity among the populations can be calculated by many methods. Evaluation of the morphological parameters provides a basic method for quantifying genetic diversity under various conditions (Hanci and Cebeci, 2019) like salinity stress. In this study, besides fourteen morphologic parameters, MDA and proline analysis results were also used to evaluate F_3 genotypes by principal component analysis. According to results, the first principal component presented the highest variance among the other sixteen components. Additionally, the first six principal components with Eigenvalues >1 contributed 69.99% of the variation among the genotypes (Table 2).

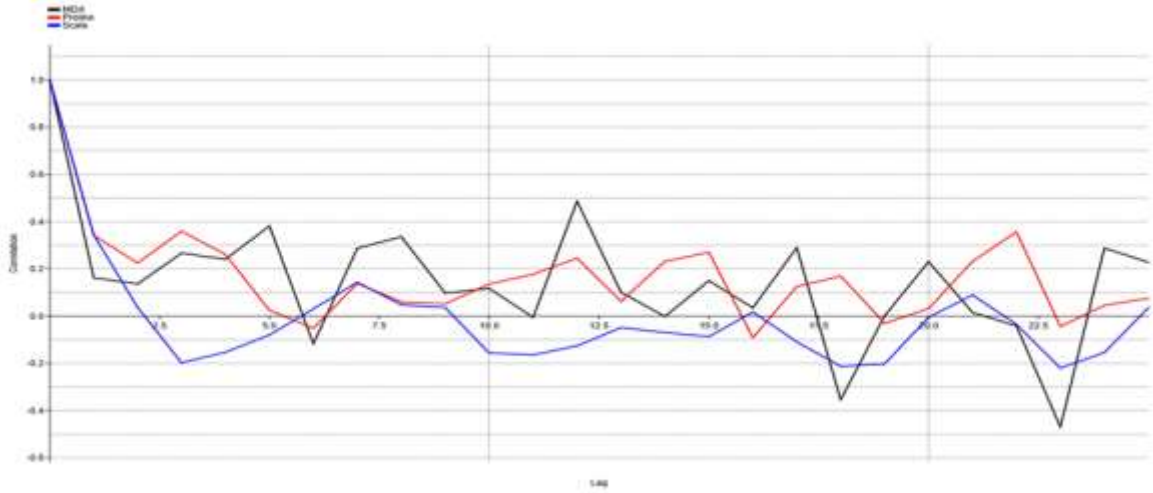

Figure 4. Correlation graphic drawn by MDA, proline and scale results
Şekil 4. MDA, prolin ve skala sonuçlarına göre çizdirilen korelasyon grafiği

Table 2. The first six Eigen values and percentage of variation for each principal component
Tablo 2. İlk 6 Eigen değeri ve herbir temel bileşenin varyasyon yüzdesi ile kümülatif varyasyon derecesi

Principal Component (Temel Bileşen)	*Eigen Values (Eigen değerleri)*	*Variance % (Varyasyon %)*	*Cumulative variance % (Kümülatif varyasyon %)*
1	3.05	19.08	19.08
2	2.06	12.90	31.99
3	1.92	11.97	43.96
4	1.72	10.78	54.74
5	1.34	8.39	63.12
6	1.09	6.87	69.99

The distributions of both genotypes (Figure 5-A) and parameters (Figure 5-B) were examined on the coordinate plane. The origin point on the coordinate plane is the area with the least variation, the close positioning of the parameters or genotypes indicates that there are many similarities between them. With respect to PCA analysis, the first six components accounted for 69.99% of the total variation for salt stressed F_3 genotypes and parents. Bi plot revealed obvious differences between male parent *S. incanum* (P2) and female parent (P2) and F_3 genotypes (Figure 5). Female parent BATEM TDC47 (P1) has good marketable capacity thus, it is understood from Figure 5, F_3 genotypes near the P1 may also have good marketable capacity. Moreover, parent *S. incanum* (P2) was distributed far from the other genotypes of the study which means that selected F_3 genotypes have tolerance to salt stress, besides have better morphological behavior than *S. incanum* (P2). Different plant species (wild relatives) and cultivars within a crop species exhibits great differences in their response to salt stress (Marschner, 1995) and genetic variations in a species considered as a beneficial tool for screening and breeding to tolerance to stress (Dasgan et al., 2002). In addition to this, bi-pilot also revealed strong correlation between MDA accumulation - anthocyanin presence and plant height – stem diameter.

Additionally, observed data was used to Hierarchical clustering of the 50 F_3 eggplant lines and parents using Ward's method. Dendrogram was divided into two main groups and the sub-groups. While group A was formed with 20 F_3 lines beside sensitive parent (P1) and tolerant parent (P2), the group B was formed 28 F_3 eggplant lines. Tolerant parent (P2) was formed as a separate subgroup under the group A.

CONCLUSION

In conclusion, the results of plant growth measurements and biochemical analysis indicate that *S. incanum* has more tolerance to salt stress than *S. melongena*. This is mostly because of its ability to accumulate higher concentrations of proline and lower concentrations of MDA. Additionally performance of F_3 population generally found between male (*S. incanum*) and female (BATEM TDC47) parents. Finally, *S. incanum* can contribute to the development of salt-tolerant eggplant pure lines and cultivars as a pollen donor in further breeding studies. Moreover, to sustain eggplant production under salt-treated lands, it could also be used as a rootstock.

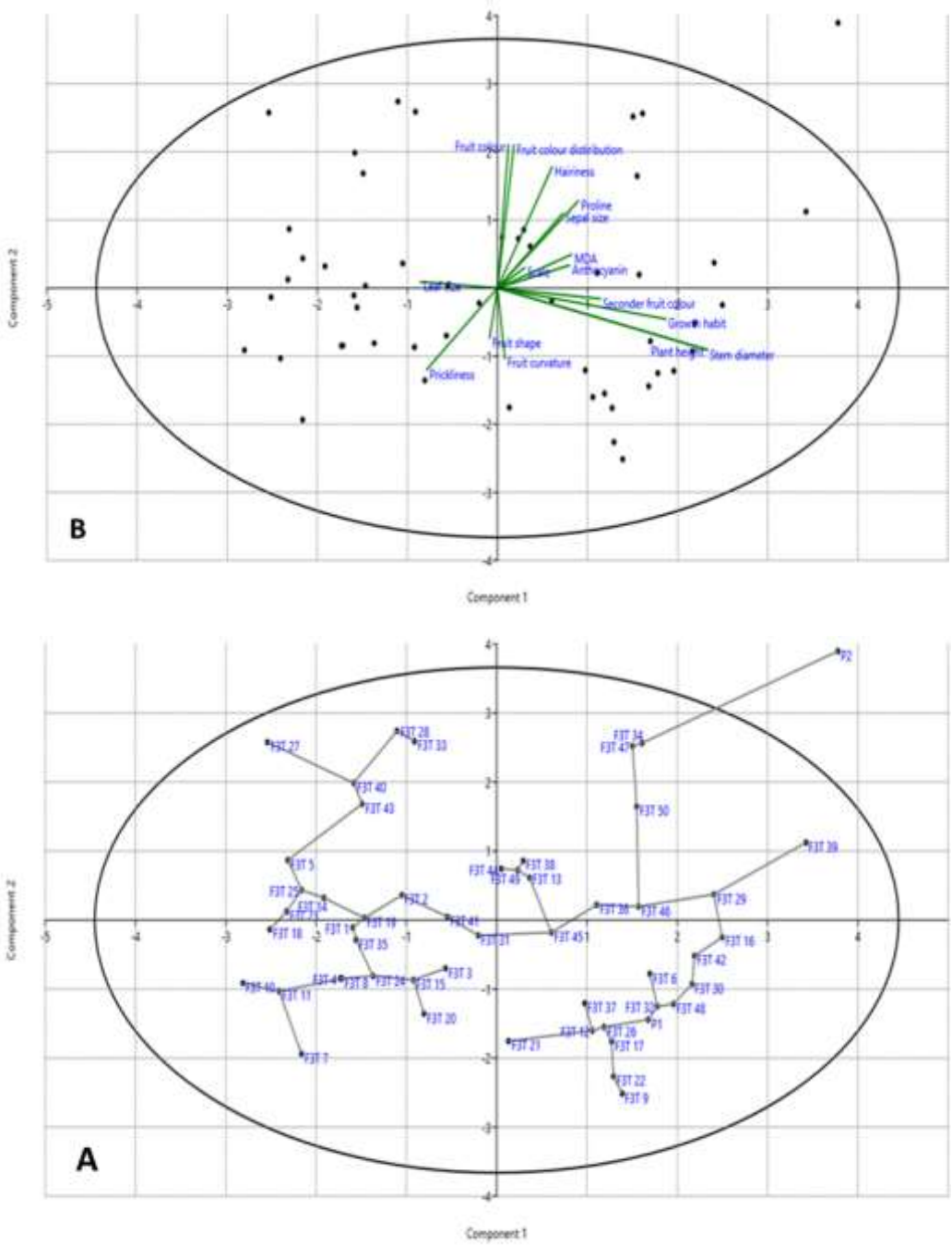

Figure 5. Distribution of F_3 genotypes, parents (A) and employed parameters (B) based on the first and second components

Şekil 5. F_3 hatları, ebeveynler (A) ve kullanılan parametrelerin (B) birinci ve ikinci temel bileşen göz önünde bulundurularak oluşturulan dağılımı

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Contribution Rate Statement Summary of Researchers

EC: Project administration, Investigation, Statistical evaluation, Writing – original draft, Writing – review & editing. HFB: Investigation, Data collection, Writing – original draft, Writing – review & editing. SK: Investigation, Data evaluation, Writing – review & editing. SSE: Data evaluation, Writing – review & editing. All authors read and confirmed the manuscript.

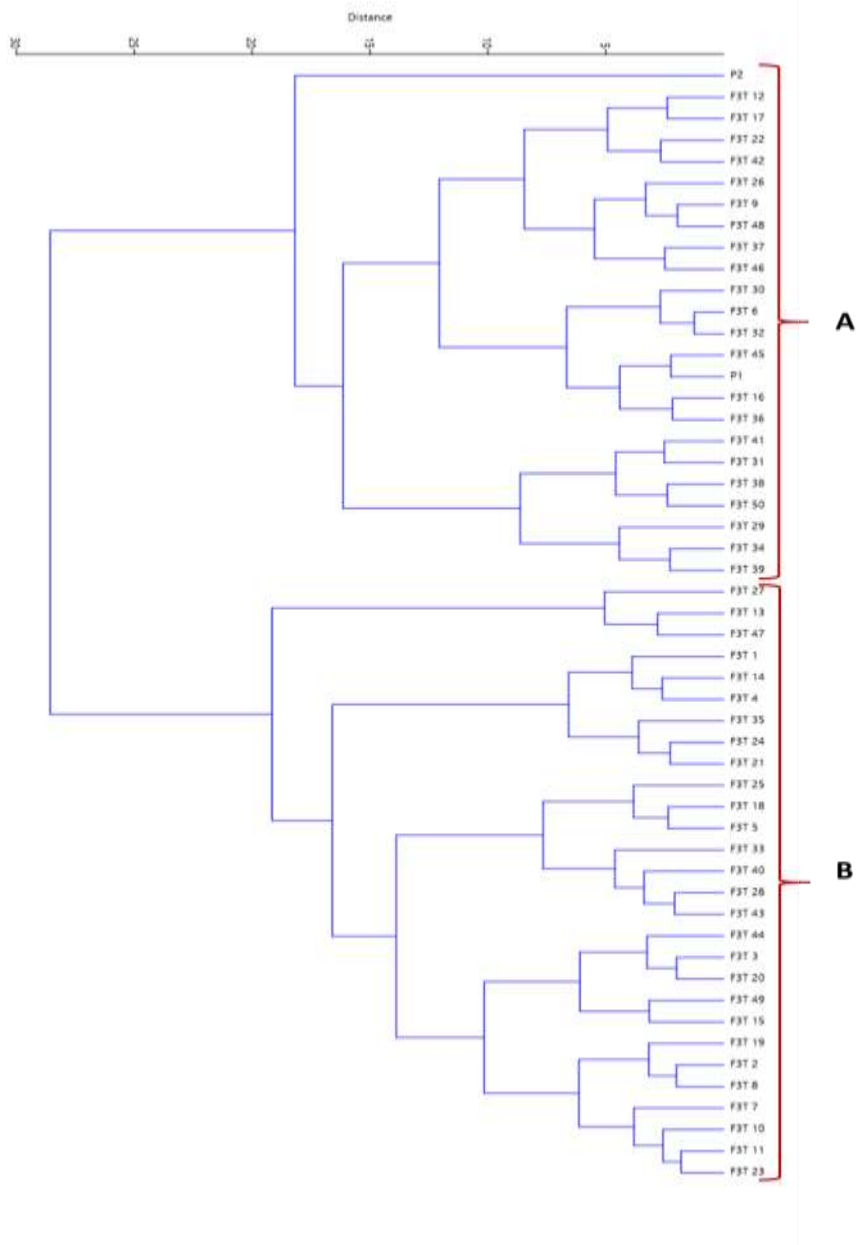

Figure 6. Hierarchical clustering of the 50 F3 eggplant lines and parents using Ward's method
Şekil 6. Ebeveynleri ile birlikte 50 F3 patlıcan hattının Ward's metodu ile hiyerarşik olarak kümelenmesi

Statement of Conflict of Interest

Authors have declared no conflict of interest.

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Identification of Chlorophyll and Color Content in Grape Leaves During Two Growth Stages (Flowering And Set)

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ABSTRACT
Chlorophyll concentration is a measure of the leaf's ability to photosynthesize. It is essential for advancing the physiological state of plants. A rapid estimate of in situ leaf chlorophyll concentration is frequently provided by SPAD meters. When utilizing SPAD meters to accurately quantify leaf chlorophyll content, it is important to consider the variations in growth conditions of vegetation species. This study aimed to calculate the chlorophyll (a*, b*) and L* (Lightness) levels from SPAD values in grape leaves for pickling during two vine different growth stages: blooming and set. In this study, 189 plants from Narince x Isabella combination, 39 plants from Narince x Kishmish Vatkana combination, and 218 plants from Narince x Regent combination were used. In addition, Narince, Künefi, Erciş, Dökülgen, Fenerit, Hatun Parmağı, Horoz Karası, Muhammedi, Karaerik, and Vakkas grape varieties from this local germplasm and Italia and Kyoho varieties known to be susceptible to powdery mildew were included. In this study, chlorophyll content has decreased statistically from flowering to setting for L*, a*, and b* concentration. Analyzing two factors (genotypes and periods) have statistical differences for both main and interactions. The study reported here indicates that a statistical separation ratio of chlorophyll a*/b* was discovered. According to the results of the study, concentrations of L*, a*, and b* decreased from flowering to berry set phases. This study points out that various developmental stages in plant species might affect the link between leaf chlorophyll content and SPAD readings.

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Üzüm Yapraklarındaki Klorofil ve Renk Içeriğinin Iki Asma Büyüme Evresinde (Çiçeklenme ve Tane Tutumu) Belirlenmesi

ÖZET
Klorofil konsantrasyonu yaprağın fotosentez yapma yeteneğinin bir ölçüsüdür. Bu bitkilerin fizyolojik durumunu ilerletmek için önemlidir. SPAD metreler tarafından sıklıkla yaprak klorofil konsantrasyonunun hızlı bir şekilde tahmin edilebilmektedir. SPAD metreler bitki türlerinin büyüme koşullarındaki değişiklikler dikkate alınarak yaprak klorofil içeriğini doğru bir şekilde ölçmek için kullanılabilirler. Bu çalışmanın amacı, salamura için üzüm yapraklarındaki SPAD değerlerinden klorofil (a*, b*) ve L* (Parlaklık) seviyelerini iki farklı asma büyüme aşaması olan çiçeklenme ve tane tutma sırasında hesaplamaktır. Bu çalışmada, Narince x Isabella kombinasyonundan 189, Narince x Kishmish Vatkana kombinasyonundan 39, Narince x Regent kombinasyonundan ise 218 bitki kullanılmıştır. Ayrıca, yerel gen kaynaklarımızdan Narince, Künefi, Erciş, Dökülgen, Fenerit, Hatun Parmağı, Horoz Karası, Muhammedi, Karaerik ve Vakkas üzüm çeşitleri ile küllemeye duyarlı olarak bilinen Italia ve Kyoho çeşitleri dahil edilmiştir. Bu çalışmada, klorofil içeriği (L*, a* ve b* konsantrasyonları için) çiçeklenmeden tane tutumuna istatistiksel olarak azalmıştır. Genotipler ve dönemler

Bahçe Bitkileri

Araştırma Makalesi

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Anahtar Kelimeler
Klorofil
Renk
SPAD
Asma
Fotosentez

hem ana faktör hemde etkileşim faktörleri için farklılıklar göstermektedir. Burada bildirilen çalışma hibrit ve kültür asması çeşitleri arasında, klorofil a*/b* oranının farklılaştırıldığını göstermektedir. Çalışmanın sonuçlarına göre, L*, a* ve b* konsantrasyonları çiçeklenmeden tane tutumu evresine doğru azalmıştır. Bu çalışma, bitki türlerindeki çeşitli gelişim evrelerinin yaprak klorofil içeriği ile SPAD okumaları arasındaki bağlantıyı etkileyebileceğini göstermektedir.

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INTRODUCTION

Photosynthesis is a vital biochemical process in plants, and the key pigment involved in this process is chlorophyll (Samdur et al. 2000). Leaf chlorophyll content can be measured in the laboratory by applying several techniques (Monje and Bugbee 1992; Richardson et al. 2002). However, many researchers have reported that extraction under laboratory conditions is time-consuming, destructive, laborious, and expensive (Monje and Bugbee 1992; Uddling et al. 2007). Researchers have found that leaf chlorophyll content and leaf color can be determined easily and quickly by some optical methods without damaging the leaf (Markwell et al. 1995; Madeira et al. 2003). Different plant species which are reported that the relative chlorophyll content of leaves was estimated with a portable SPAD-502 meter based on the spectral transmittance properties of the leaves (Campbell et al. 1990; Madeira et al. 2003; Leon et al. 2007; Anand and Byju 2008; Ruiz-Espinoza et al. 2010; Ling et al. 2011; Jiang et al. 2017). There are many studies on determining leaf chlorophyll content, and leaf chlorophyll and leaf color content have a considerable positive correlation (Zotarelli et al. 2003; Netto et al. 2005; Anand and Byju 2008; Jiang et al. 2017). Grapevine leaves are a product whose quality decreases with storage. It has also been stated that grapevine leaves have a very high nutritional value in terms of fiber sources (Mürtezaoğlu, 2006). Underhill and Critchley (1995) reported an increase in the activity of peroxidases and polyphenol oxidase enzymes, which are frequently responsible for tissue darkening, in aging leaves. Considering that aging is directly related to leaf chlorophyll content, it is important for leaf consumers and producers to know the chlorophyll content before and after harvest. After establishing a general correlation relationship for a plant species, it is possible to use a chlorophyll meter in applications where exact values are not required. For example, a rapid assessment of relative chlorophyll in grape leaves to be used for pickling would be useful for leaf producers or researchers to detect senescence, nutrient deficiencies, and decreased chlorophyll content in leaves. To this knowledge, there is a piece of limited information on the accuracy and usefulness of the SPAD-502 chlorophyll meter for estimating chlorophyll content in pickling grape leaves. Some studies have reported differences between the regression equations for chlorophyll content and SPAD index (Campbell et al. 1990; Fanizza et al. 1991), and that the difference may be due to specific leaf weight (Yamamoto et al. 2002). The mathematical correlation calculation between SPAD value and chlorophyll content may be essential to optimize the advanced interpretation with the chlorophyll meter. The research was conducted to determine chlorophyll (a* and b*) and L* (lightness) levels from SPAD values in two growth stages (flowering and set) of grape leaves for pickling.

MATERIALS AND METHOD

Plant material

The research was carried out in the grapevine seedling production greenhouse located in the Application and Research Area of the Faculty of Agriculture at Gaziosmanpaşa University ın 2024. In the study, F1 plants obtained from Narince x Isabel (NVL, 189 plants), Narince x Kişhmişh Vatkana (NKV, 39 plants), and Narince x Regent (NRG, 218 plants) hybrids were used. In addition, Narince, Künefi, Erciş, Dökülgen, Fenerit, Hatun Parmağı, Horoz Karası, Muhammedi, Karaerik, and Vakkas grape varieties from thislocal germplasm and Italia and Kyoho varieties known to be susceptible to powdery mildew were included (Fig. 1A).

Experiment Design and determination of chlorophyll (a* and b*), and L* (Lightness) values

The study was conducted using a randomized complete block design with 3 replications, with 1 shoot and 3 leaves on each shoot in each replication. Leaf samples were harvested from the 4th, 5th, and 6th leaves on the shoots, which reached 2/3 of the mature leaf size from the tip (Kılıç, 2007). Nine leaves were taken from each genotype in

the F1 plants of the combinations during the flowering (end of May) and berry set (beginning of July) periods (Fig. 1A). Chlorophyll contents were determined by measuring the leaves of each F1 individual in 3 directions (right, middle, and left lobes) with Konica Minolta (SPAD-502 Plus, serial number: 20005480) branded SPAD reader for chlorophyll (a* and b*) values, and then L* (Lightness) was determined with Konica Minolta branded color measurement device with serial number 8203581 (Fig. 1B).

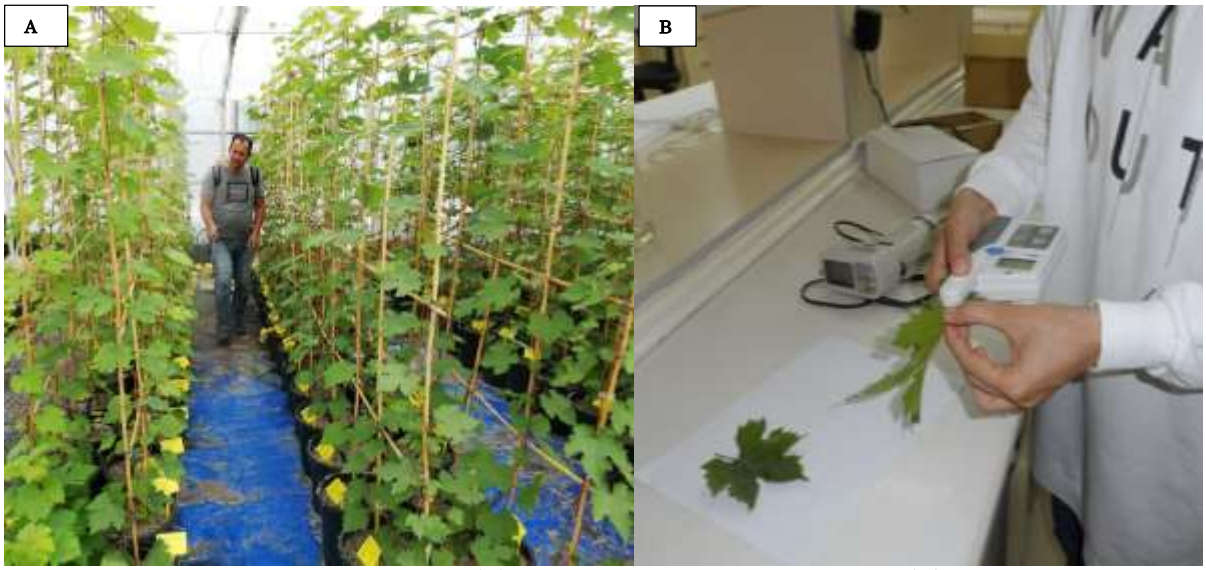

Figure 1. Images of the research area (A) and the use of the SPAD meter (B)
Şekil 1. Araştırma alanı (A) ve SPAD-502 metrenin kullanımına (B) ait görüntüler

Statistical Analysis

The Agricola package was used in R Studio to perform all descriptive analyses. Analysis of Variance (ANOVA) in R Studio was used to examine the importance of genotypes and periods, as well as how they interacted with various (L*, a*, b*, and a*/b*). The chi-square test was utilized to confirm that all the data were normal before the analysis was conducted. The primary impacts (genotypes and periods) on various (L*, a*, b*, and a*/b*) were assessed using linear models. Tukey HSD post-hoc analysis was performed using R Studio's agricolae package. The ggbiplot2 package in R Studio was used to perform Principal Component Analyses (PCAs) for the various (L*, a*, b*, and a*/b*) datasets R Core (2013).

RESULTS and DISCUSSION

Thisresults showed that the hybrids NRG had the lowest L* content (40.50 nm) while cultivars had the highest L* content (44.38 nm). The L* content was highest in the flowering (42.77 nm) period compared with the setting (40.68 nm) in leaves. Chlorophyll a* in the NRG hybrids had the highest content (-14.15 µg cm^{-2}), whereas cultivars had the lowest content of Chlorophyll a*, which is -18.72 µg cm^{-2}. The chlorophyll a* saw an increase from flowering (-14.65 µg cm^{-2}) to setting (-16.90 µg cm^{-2}). The cultivars (30.26 µg cm^{-2}) were shown to have the greatest chlorophyll b*, while NRG hybrids had little chlorophyll b* (19.22 µg cm^{-2}). The chlorophyll b* was decreased from flowering (23.84 µg cm^{-2}) to setting (20.44 µg cm^{-2}). The ratio of a*/b* was greatest in NKV hybrids with 0.8, while it was least in cultivars with 0.63 (Table 1).

Figure 2A displays four different compounds present in leaves: chlorophyll (a* and b*), L*, and a*/b*. PCA was used to analyze and display the complex relationships between genotypes and periods in leaves. To ensure a reliable and representative dataset, each data point represented the centroid of four measurements for each parameter. The Cos2 value, which illustrates the relative influence of each variable on the principal components, was used to show each variable's contribution to the primary components. The first PC in Figure 2A is responsible for 65.3% and 25.3% of the total variation in the data. Consequently, there was a negative correlation between the levels of chlorophyll a* and chlorophyll b*, L*, and a*/b*contents (Fig. 2A). Chlorophyll b* and L* were shown to have an especially strong association, as seen by the dark blue square. On the other hand, as the dark red square shows, there were strong negative associations between both chlorophyll a* and L* (as seen by the moderate red square), and a* and b* (as seen by the dark red square) (Fig. 2B). Every variable is displayed, and along PC1's vertical axis, various colored circles signifying the genotypes and periods are dispersed. The NVL hybrids are represented by the purple circle, which also contributes significantly to various chlorophyll (a* and b*) and L*

contents. In contrast, cultivars have the lowest total variance. NRG and NKV hybrids differed in their contributions and were found between NVL hybrids and cultivars (Fig. 2C). However, along the horizontal axis, flowering was more broadly distributed than the setting, suggesting that this time had a bigger impact on various chlorophyll (a* and b*) and L* contents (Fig. 2D).

Table 1. Chlorophyll (a* and b*), L*, and a*/b* contents of different Cultivars and F1 hybrids (NKV, NRG, and NVL) in flowering and set periods.

Çizelge 1. Çiçeklenme ve tane tutumu dönemlerinde farklı çeşit ve F1 hibritlerinin (NKV, NRG ve NVL) klorofil (a ve b*), L* ve a*/b* içerikleri.*

Genotypes (G)[x]	L*(nm)	a* (µg cm^{-2})	b* (µg cm^{-2})	a*/b*
Cultivars	44.38±0.32a	18.72±0.26d	30.26±0.45a	0.63±0.00d
NKV	42.17±0.21c	15.70±0.17b	21.85±0.29c	0.81±0.02a
NRG	40.50±0.08d	14.15±0.07a	19.22±0.12d	0.74±0.01b
NVL	42.82±0.09b	17.41±0.07c	24.86±0.13b	0.71±0.00c
Periods (P)[y]				
Flowering	42.77±0.08a	16.90±0.07a	23.84±0.12a	0.72±0.00b
Setting	40.68±0.01b	14.65±0.02b	20.44±0.09b	0.74±0.00a
G	< 2e-16 ***	< 2e-16 ***	<2e-16 ***	1.49e-10 ***
P	< 2e-16 ***	< 2e-16 ***	<2e-16 ***	0.00644 **
G x P	7.29e-15 ***	8.46e-08 ***	<2e-16 ***	9.78e-13 ***

x, Mean separation in Genotips; y, Mean separation in Periods; G, Genotips; P, Periods; G x P, interactions; For a given factor (different letters within a column represent significant differences (Tukey test, ***, Significant at p-value < 0.001). Data are stated as averages of the data and their standard deviations.

A study was conducted to state the total chlorophyll contents such as (a* and b*) in Solanum lycopersicum L. leaves that had interveinal chlorosis caused by continuous lighting by using the SPAD-502. According to their results, there is a positive correlation between the chlorophyll content index and the contents of chlorophyll a* and chlorophyll b* in the leaf. These findings imply that the portable chlorophyll meter SPAD-502 Plus can be a useful tool for nondestructive estimation of chlorophyll content in leaves (Shibaeva et al., 2020). In addition, many writers have described the SPAD-502 as a trustworthy instrument for determining the amount of chloride in the leaves of plants cultivated in comparable environmental conditions (Campbell et al., 1990; Yang et al., 2014). The concentration of both chlorophyll and carotene pigments decreased as a result of an unfavorable precipitation distribution throughout the May–July summer growing season (Zielewicz et al., 2020). It is seen that the chlorophyll content has decreased statistically from setting to flowering for L*, a*, and b* concentrations (Table 1, Figure 1A and B). A study was conducted to compare the methods that are frequently employed to SPAD readings and absolute leaf chlorophyll concentration. Three field datasets and one synthetic dataset were used to compare these methods. It is reported that SPAD readings obtained in the field for different vegetation types and the leaf chlorophyll concentration measured in the lab using a destructive approach are better suited for smaller data sets than the polynomial functions as the linear and exponential functions have fewer fitting parameters (Zhang et al., 2022). According to the findings from a butterhead lettuce study, butterhead lettuce leaves' chlorophyll concentration may be accurately estimated using SPAD measurements (León et al., 2007). The relationship between leaf chlorophyll concentration and SPAD readings in plant species may change as a result of varying growth vigor, leading to distinct functions for even the same species. This is comparable to the findings that showed the mathematical model utilized to calculate the concentration of chlorophyll in leaves using SPAD readings varies depending on the stage of growth of the leaves. Researchers have examined leaves at the vegetative and reproductive growth stages of the plant to determine the most appropriate function model, and they used correlation analysis to examine the link between tomato (Solanum lycopersicum) leaf chlorophyll concentration and the Minolta SPAD-502 plus chlorophyll meter. Another finding has demonstrated there is a substantial link between the SPAD value and the total chlorophyll contents (a*, b*, and total chlorophyll) in tomato leaves (Jiang et al., 2017). As a demonstration from thisstudy two factors (genotype and periods) have statically differences for both main and interactions (Table 1, Figure 2C and D). One of the most crucial chemical components of plants is the amount of chlorophyll since it directly regulates photosynthesis and biomass production (Zielewicz et al., 2020). Pheoporphyrin, a magnesium atom joined to the nitrogen atoms in the middle of the porphyrin system, is the building block of a chlorophyll molecule. All plants and green algae include chlorophyll, which comes in two types: chlorophyll a* and chlorophyll b*. In addition, plants with higher levels of chlorophyll have larger levels of total chlorophyll, and the ratio of chlorophyll a* to b* is roughly 3:1 (Rajalakshmi et al., 2015). Another study has impressed the importance of chlorophyll (a*/b*) connections and has reported testing plant leaves at various developmental stages, physiological conditions, and environmental conditions is necessary (Chen and Roca, 2018).

The chlorophyll a*/chlorophyll b* ratio is a sign of the functional balance between the effectiveness of light collection and electron transport in plants (Zhang et al., 2020). It is evident from the study presented here have a statistical differentiation ratio of chlorophyll a*/b* was found as from 0.63 to 0.81 between cultivars and hybrids (Table 1). Other studies have also reported that the feature of correlation analysis of leaf chlorophyll content might be affected by the leaf condition (Bullock and Anderson, 1998). The study presented currently has reported that the concentrations L*, a*, and b* have been negatively affected from the setting to the flowering periods (Table 1).

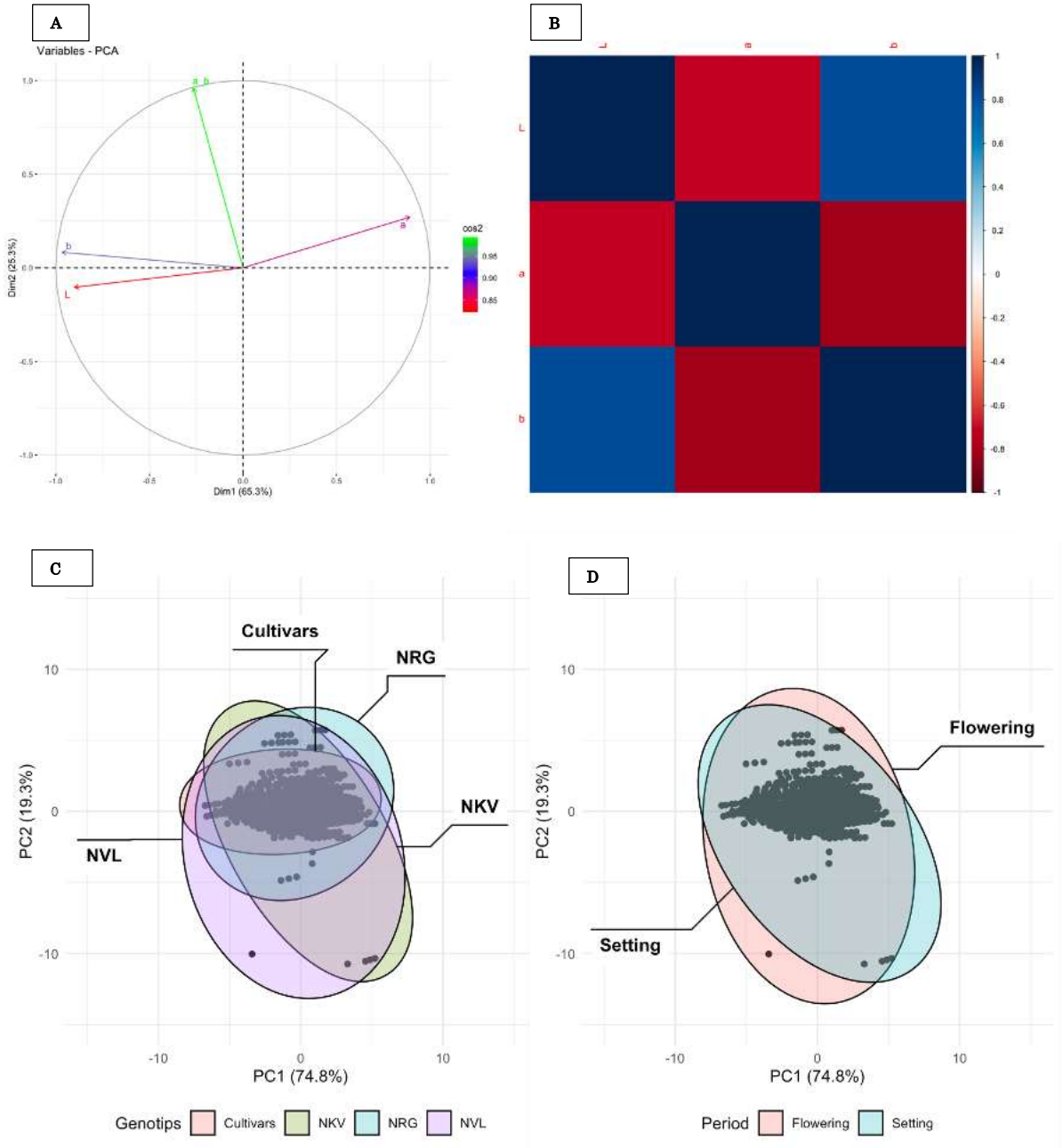

Figure 2. PCA biplot of colored by genotypes and periods. All variables (A), correlations (B), genotypes (C), and periods (D) are demonstrated.

Şekil 2. Genotiplere ve periyotlara göre renklendirilmiş PCA biplot'u. Tüm değişkenler (A), tüm değişkenlerin korelasyonu (B), genotipler (C) ve periyotlar gösterilmektedir.

CONCLUSION

The amount of chlorophyll in a leaf is a gauge of its capacity for photosynthetic activity, and it is crucial for improving plants' physiological conditions. Variations in vegetation species' growing conditions must be taken into account when using SPAD meters to measure the amount of chlorophyll in leaves precisely. As a result of this

study, chlorophyll content has statistically decreased from flowering to setting. In addition, there are statistical differences for both main and interaction factors when analyzing two factors (genotypes and times). A statistical separation ratio of chlorophyll a*/b* was also found. This study highlights that the relationship between leaf chlorophyll concentration and SPAD readings may vary depending on the developmental stage of the plant species.

Author's Contributions

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

Conflicts of Interest Statement

The author has stated that there is no conflict of interest.

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Peyzaj Bitkilerinin Polinatörler Açısından Değerlendirilmesi 'Iğdır Üniversitesi Şehit Bülent Yurtseven Kampüsü'

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ÖZET

Arılar hem tarımsal üretim hem de çevresel denge açısından önemli polinatörlerdir. Aynı zamanda arıcılık sektörü de bu canlıların sağlıklı bir şekilde varlıklarını sürdürebilmesine bağlıdır. Peyzaj bitkileri, arıların beslenme ihtiyaçlarını karşılamak ve polinasyon hizmetlerini desteklemek açısından önemli rol oynamaktadır. Özellikle şehirleşme ve zararlı tarımsal uygulamalar arıların doğal yaşam alanlarının azalmasına neden olduğundan, peyzaj düzenlemeleri büyük bir önem kazanmıştır. Bu çalışma, özellikle bal arıları ve diğer polinatörlerin Iğdır Üniversitesi Şehit Bülent Yurtseven Kampüsünde peyzaj alanlarında kullanılan süs bitkilerinden faydalanma potansiyelini incelemeyi amaçlamaktadır. 2017-2024 yılları arasında yapılan gözlemler ve çalışmalar ile literatür ışığında kampüs alanında 27 farklı familyaya ait toplam 82 takson tespit edilmiştir. Bu taksonların özellikleri detaylı bir şekilde incelenmiş, verilerin analizi için çeşitli grafikler hazırlanmıştır. Ayrıca, taksonların çiçeklenme periyotları ile Iğdır ilinin iklim verileri arasındaki ilişkiler de belirlenerek, bu ilişkilerin arıların aktif olduğu dönemlerle karşılaştırılması yapılmıştır. Elde edilen verilere göre, tespit edilen 82 taksondan 71'inin, arılar gibi polinatörlerin yararlanabileceği en az iki farklı kaynak (polen, nektar ve salgı) barındırdığı ortaya konulmuştur. Bunun yanı sıra, söz konusu bitkilerin çiçeklenme periyotları yıl boyunca arıların faaliyetlerini destekleyebilecek bir dağılım göstermektedir. Bu bulgular, özellikle kentsel peyzajda kullanılan bitki taksonlarının, arılar ve diğer polinatörler için önemli bir besin kaynağı oluşturduğunu ve bu tür bitkilerin ekosistem hizmetlerine katkı sağladığını desteklemektedir. Ayrıca bu araştırma, kentsel alanlarda yapılan peyzaj düzenlemelerinin, biyolojik çeşitliliğin korunması ve polinasyon süreçlerinin desteklenmesi açısından ne denli önemli bir rol oynadığını gözler önüne sermektedir. Sonuç olarak, bu tür bitkilerin bilinçli bir şekilde seçilmesi ve yaygınlaştırılması hem yerel ekosistemlerin sağlığı hem de tarımsal üretimin devamı için önemli bir avantaj sağlayabilir.

Botanik

Araştırma Makalesi

Makale Tarihçesi
Geliş Tarihi : 05.02.2025
Kabul Tarihi : 21.03.2025

Anahtar Kelimeler
Arılar
Iğdır
Peyzaj
Polinasyon
Süs bitkileri

Evaluation of Landscape Plants in terms of Pollinators 'Iğdır University Şehit Bülent Yurtseven Campus'

ABSTRACT

Bees are important pollinators for both agricultural production and environmental balance. At the same time, the beekeeping sector depends on the healthy survival of these creatures. Landscape plants play an important role in meeting the nutritional needs of bees and supporting pollination services. Landscaping has gained great importance, especially since urbanization and harmful agricultural practices have reduced the natural habitats of bees. This study aims to examine the potential of honeybees and other pollinators to utilize ornamental plants used in landscaping areas at Iğdır University Şehit Bülent Yurtseven Campus. A total of 82 taxa belonging to 27 different families were identified in the campus area in the light of observations

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and studies conducted between 2017-2024 and the literature. The characteristics of these taxa were examined in detail and various graphs were prepared to analyze the data. In addition, the relationships between the flowering periods of the taxa and the climatic data of Igdir province were determined and these relationships were compared with the periods when bees were active. According to the data obtained, it was revealed that 71 out of the 82 taxa identified contain at least two different resources (pollen, nectar and secretion) that can be utilized by pollinators such as bees. In addition, the flowering periods of these plants show a distribution that can support the activities of bees throughout the year. These findings support that plant taxa, especially those used in urban landscapes, provide an important food source for bees and other pollinators and contribute to ecosystem services. Furthermore, this research demonstrates the important role that landscaping in urban areas plays in conserving biodiversity and supporting pollination processes. As a result, the deliberate selection and dissemination of such plants can provide a significant advantage for both the health of local ecosystems and the continuation of agricultural production.

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GİRİŞ

Biyoçeşitlilik; genetik çeşitlilik, tür çeşitliliği ve ekosistem çeşitliliği gibi unsurları içeren geniş bir kavramdır. Ayrıca kuşlar, memeliler, böcekler, bitkiler ve mikroplar gibi farklı habitatlarda yaşayan tüm canlı organizmaların çeşitliliğini de ifade eder (Uslu & Shakouri, 2013). Biyoçeşitliliğin önemli bir parçası olan bitkiler, havayı temizlemek, toprağa organik madde kazandırmak ve erozyonu önlemek gibi kritik işlevler üstlenirken, aynı zamanda diğer canlılara barınma ve beslenme ortamı sunarak ekosistemin sürekliliğini destekler (Aslan & Uslu, 2021).

Bitkiler, insanların ve diğer canlıların yaşamını sürdürebilmesi için son derece önemli bir rol oynamaktadır. İnsanlık tarihinin ilk günlerinden itibaren bitkiler, besin kaynağı başta olmak üzere birçok alanda hayati bir öneme sahip olmuştur. Bununla birlikte, bitkilerin varlığını sürdürebilmesi için tozlaşma süreci kritik bir aşamadır. Aynı zamanda tozlaşma için su, rüzgâr gibi cansız etmenlerden ya da kelebekler, böcekler, kuşlar ve yarasalar gibi canlı tozlaşma vektörlerinden faydalanırlar. Bitkilerin büyük bir çoğunluğu, başka canlıların yardımıyla tozlaşır. Bu noktada, özellikle arılar gibi polinatörlerin rolü büyük bir öneme sahiptir. Ekolojik denge, bitkilerin ve diğer canlıların birbirine olan bağımlılığı üzerine kuruludur. Bu nedenle tozlaşma süreci, doğanın devamlılığı için vazgeçilmezdir (Silici, 2005; Özbek, 2010).

Bal arıları beslenmek ve bal özü depolamak için bitkileri ziyaret eder. Bu ziyaretler sırasında bazı bitkilerden polen, bazılarından nektar, bazılarından hem polen hem de nektar ve bazılarından da salgı toplarlar. Polen sadece çiçeklerde bulunurken, nektar bazı çiçeklerin özel kısımlarında, bazı bitkilerin gövde, dal, yaprak ve yaprak saplarında, salgılar ise sadece gövde, dal, yaprak, yaprak sapı ve meyve gibi organlarda bulunur. Doğal ortamdaki arı yuvalarından toplanan ve kovanlarla üretilen bal yanında kullanım alanlarının artmasına bağlı olarak önemi her geçen gün artan polen, propolis, arı sütü ve arı zehri gibi ürünler, ilkel toplumlardan günümüze kadar insanoğlunun ilgisini çekmiştir. Eski Mısır'da başlayan arıcılık faaliyetleri, bal arılarının yüksek adaptasyon yeteneği sayesinde günümüzde kutup bölgeleri hariç neredeyse tüm dünyaya yayılmış durumdadır. Gelişmekte olan ülkelerde kırsal kesimde yaşayan insanlar için ek gelir ve istihdam kaynağı olarak görülen arıcılık, gelişmiş ülkelerde ise temel bir meslek ve tozlaşmadaki etkinliği nedeniyle bitkisel üretim için önemli bir girdi olarak kabul edilmektedir (Fıratlı & Gençer, 1995).

Arıcılık faaliyeti kolay olmayan ama elde edilen ürünlerin ise oldukça önemli olduğu bir sektördür (Tapkı & Demirci, 2024). Arıcılık, bal arısı kolonilerinin bulundukları bölgelerde nektar akımının en yoğun olduğu dönemlerde işçi arı popülasyonunun en yüksek seviyeye çıkarılmasıyla yapılan bir tarımsal faaliyettir. Bu popülasyon bal, polen, arı sütü üretimi ve bitkilerin tozlaşması gibi çeşitli amaçlarla kullanılır (Güler, 2006).

Güzel çiçekler arasında dolaşan arılar, görsel olarak hoş bir manzara oluşturur, ancak bu durum rastlantısal değildir. Arılar ve çiçek açan bitkiler, doğada birbirine bağlı ortaklardır. Her iki taraf da birbirinin yaşamını ve

üremesini sürdürebilmesi için belirli işlevler yerine getirir. Bu da aralarında karşılıklı bir yaşam ilişkisi olduğunu gösterir (Sorkun ve ark., 2012).

Arıcıların karşılaştığı en önemli sorunlardan biri, arıların hangi bitki türlerinden daha verimli şekilde nektar ve polen sağladığının belirlenmesidir (Öder, 2006). Bu tespit, arıcılığın verimliliğini artırmak için oldukça kritiktir. Çünkü doğru bitki kaynaklarının belirlenmesi, arıların sağlıklı bir şekilde gelişebilmesini ve yüksek verim elde edilmesini sağlar. Arıcılar, bulundukları bölge veya ülkenin diğer bölgelerinde arıcılık için en uygun doğal kaynakları araştırmalıdır. Bu, arıcılıkla ilgili verimliliği sınırlayan doğal koşullara olan bağımlılığı en aza indirgemek için önemlidir. Özellikle, arıların uçuş alanı içinde yoğun olarak ziyaret ettikleri polenli bitki türleri ve alt türlerinin belirlenmesi, arıcılığın verimliliğini artırmaya yardımcı olacaktır (Tutkun, 2011).

Dünyada arıların yararlandığı bilinen bitki türlerinin yaklaşık yüzde yetmişbeşi Anadolu'da yetişmektedir. Türkiye'nin biyolojik zenginliği sadece bitki ve hayvan türleriyle sınırlı olmayıp, aynı zamanda geniş bir iklimsel çeşitliliğe de sahiptir (Genç & Dodoloğlu, 2011).

Doğu Anadolu Bölgesinde yer alan Iğdır ili zengin bir bitki örtüsüne sahiptir ve ilde Türkiye flora varlığının yaklaşık % 10'una denk gelen yaklaşık 1000 ila 1100 bitki türü bulunduğu tahmin edilmektedir. Iğdır ili, zengin bir biyoçeşitliliğe sahip olmasına ek olarak, mikro klima koşullarına sahip olması sayesinde çok sayıda tarımsal ürün yetiştirilmesi bakımından büyük bir potansiyele sahiptir. Ayrıca, Türkiye'nin en yüksek dağı olan ve endemik bitki türleri bakımından oldukça zengin olan Ağrı dağının önemli bir kısmının Iğdır ili sınırları içerisinde ve Iğdır ekolojik koşulları dahilinde bulunması ilin biyoçeşitliliği açısından da oldukça önemlidir (Türkoğlu, 2017).

Bal arılarının faydalanabileceği polen ve nektarlı bitkilerin araştırılmasına yönelik çalışmalar mevcuttur (Sorkun & Doğan, 1994; Deveci ve ark., 2015; Ulus & Özdemir, 2018; Bahadırlı & Gül, 2020). Ancak bu konuda Iğdır özelinde yapılmış ve peyzaj çalışmalarını içeren herhangi bir çalışmaya rastlanılmamıştır. Bu çalışmada; Iğdır Üniversitesi Şehit Bülent Yurtseven Kampüsünde bal arıları başta olmak üzere diğer polinatörlerin bitki taksonlarından faydalanabilme özellikleri incelenerek literatüre katkı sağlamak amaçlanmıştır. Bu çalışmanın ilerleyen dönemlerde yeşil alanlarda kullanılan bitkisel materyale dönük yapılacak diğer çalışmalara yön verebileceği düşünülmektedir. Ayrıca bu çalışmanın çevresel sürdürülebilirlik ve arıcılıkla ilgili yeni yaklaşımlar geliştirilmesine de katkı sağlayabileceği değerlendirilmektedir.

MATERYAL ve METOD

Bu çalışmanın ana materyalini 2017-2024 yılları arasında Iğdır Üniversitesi Şehit Bülent Yurtseven Kampüsünde peyzaj projesi kapsamında dikimi ve bakımı yapılan ve 7 yıl içerisinde aşamalı olarak adaptasyonu sağlanmış peyzaj bitkileri (Ağaçlar, ağaççıklar, çalılar ve sarılıcılar) oluşturmaktadır. Çalışmalar için düzenli olarak her mevsimde kampüs alanında gözlemler yapılarak spor alanları, refüjler, giriş düzenlemeleri, rekreasyon alanları, oturma-dinlenme alanlarındaki bitkiler yerinde incelenmiş ve düzenli bakımları yapılmıştır.

Iğdır'da bulunan ilk yükseköğretim kurumu, Kars Kafkas Üniversitesi'ne bağlı Iğdır Meslek Yüksekokulu olup 1995 yılında kurulmuştur. 2006 yılında Kafkas Üniversitesine bağlı olarak Iğdır Ziraat Fakültesi kurulmuş ve ardından 2008 yılında Iğdır Üniversitesi adı altında bağımsız bir üniversite kurularak diğer birimler buraya bağlanmıştır. 2014 yılında ise Iğdır Üniversitesi Şehit Bülent Yurtseven Kampüsü hizmet vermeye başlamıştır. Şehit Bülent Yurtseven Kampüsü içerisinde çok sayıda bina tesis edilmiş ve bu alanların Peyzaj Projesi tamamlanıp hizmete sunulmuştur. Iğdır Üniversitesi Şehit Bülent Yurtseven Kampüsünün 2016 yılında başlanan peyzaj projesi 2020 yılında tamamlanmıştır (Şekil 1).

Meteorolojik verilere göre Iğdır'ın Türkiye'de en az yağış alan yerlerindendir (Güner, 1993). Ayrıca yazları sıcak ve kurak kışları ise soğuk bir iklim mevcuttur (Çizelge 1). Iğdır arıcılık açısından değerlendirildiğinde ise 264 işletme yer alırken toplam 121 ton bal üretilmektedir (Anonim, 2025a).

Çizelge 1. Iğdır ili 1941-2024 arası ortalama iklim verileri (Anonim, 2025b)
Table 1. Iğdır province average climate data between 1941-2023 (Anonymous, 2025b)

IĞDIR (Aylar)	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	Yıllık
Ortalama Sıcaklık (°C)	-3.2	-0.1	6.4	13.2	17.8	22.3	26.0	25.4	20.6	13.2	6.0	-0.2	12.3
Ortalama Güneşlenme Süresi (saat)	2.5	3.9	5.3	6.1	7.4	9.5	10.1	9.6	8.5	6.0	4.1	2.4	6.3
Ortalama Yağışlı Gün Sayısı	5.88	5.98	7.21	10.49	13.63	9.90	5.40	3.71	3.82	7.59	6.11	6.01	85.7
Aylık Top. Yağış Miktarı Ort. (mm)	14.7	15.6	22.9	34.2	47.2	31.3	14.1	9.5	11.4	25.9	18.6	13.7	259.1

Bu araştırmada, Iğdır Üniversitesi Şehit Bülent Yurtseven Kampüs alanında yer alan peyzaj projesi kapsamında dikilen bitkilerin arı çekme potansiyeli açısından değerlendirilmesi hedeflenmiştir.

Şekil 1. Iğdır Üniversitesi Şehit Bülent Yurtseven Kampüsünden genel görünüm
Figure 1. General view from Iğdır University Şehit Bülent Yurtseven Campus

Bu amaçla, 2017-2025 yılları arasında bakımları yapılan ve takibi gerçekleştirilen bitkileri değerlendirmek için, üç aşamalı bir yöntem tercih edilmiştir. Birinci aşama olarak taksonların tespiti yapılarak taksonlara ait fotoğraflar çekilerek kayıt altına alınmıştır (Davis 1965-1988; Akkemik, 2014a; Akkemik, 2014b). Ikinci aşama olarak taksonların özellikleri doğrultusunda polen, nektar ve salgı üreten taksonlar ve çiçeklenme dönemleri literatüre göre belirlenmiştir (Sıralı & Deveci, 2002; Özkan ve ark., 2016; Öztürk ve ark., 2017; Sarı, 2021; Sarı, 2022; Caf ve ark., 2022; Anonim, 2025c). Üçüncü aşamada ise kampüste çiçekleriyle dikkat çeken bitkilerden bitki kartları oluşturulmuştur.

BULGULAR

Iğdır Üniversitesi Şehit Bülent Yurtseven Kampüsünde yapılan çalışma neticesinde belirlenen ağaç, ağaççık, çalı ve sarılıcıların; familyaları, bilimsel adları, bitki türleri, yaşam formları, çiçeklenme dönemleri ve taksonların arıcılık açısından önemi belirlenmiştir. Çalışma kapsamında kampüs alanında, 18'i açık tohumlu (Gymnospermae), 64'ü kapalı tohumlu (Angiospermae) olmak üzere 27 familyaya ait toplam 82 takson tespit edilmiştir (Çizelge 2).

Kampüs alanında yapılan çalışmada en fazla taksona sahip familyalar sırasıyla belirlenmiştir. Bu sıralamada *Rosaceae* familyası (13 takson), *Cupressaceae* familyası (9 takson), *Pinaceae* familyası (8 takson), *Oleaceae* familyası (8 takson), *Fabaceae* familyası (6 takson) olarak sıralanmaktadır (Şekil 2).

Çalışma alanındaki bitkiler büyüme formu açısından incelendiğinde, taksonların %52'si ağaç formunda, %4'ü ağaççık, %39'u çalı ve %5'i ise sarılıcı özellik göstermektedir (Şekil 3). Taksonların %68'i egzotik türlerden oluşurken, geri kalan %32'lik kısmı ise doğal taksonlardır (Şekil 4).

Tespit edilen taksonların arı çeken özelliklerine göre oransal dağılımları, polen ve nektar üreten takson sayısı 42, polen 10, polen ve salgı 16, polen, nektar ve salgı 13 ve hiçbiri 1 şeklinde sıralanmaktadır (Şekil 5). Belirlenen taksonlar incelendiğinde İlkbahar döneminde mayıs ayı çiçeklenmenin en yoğun olduğu dönem olarak görülmektedir (Şekil 6).

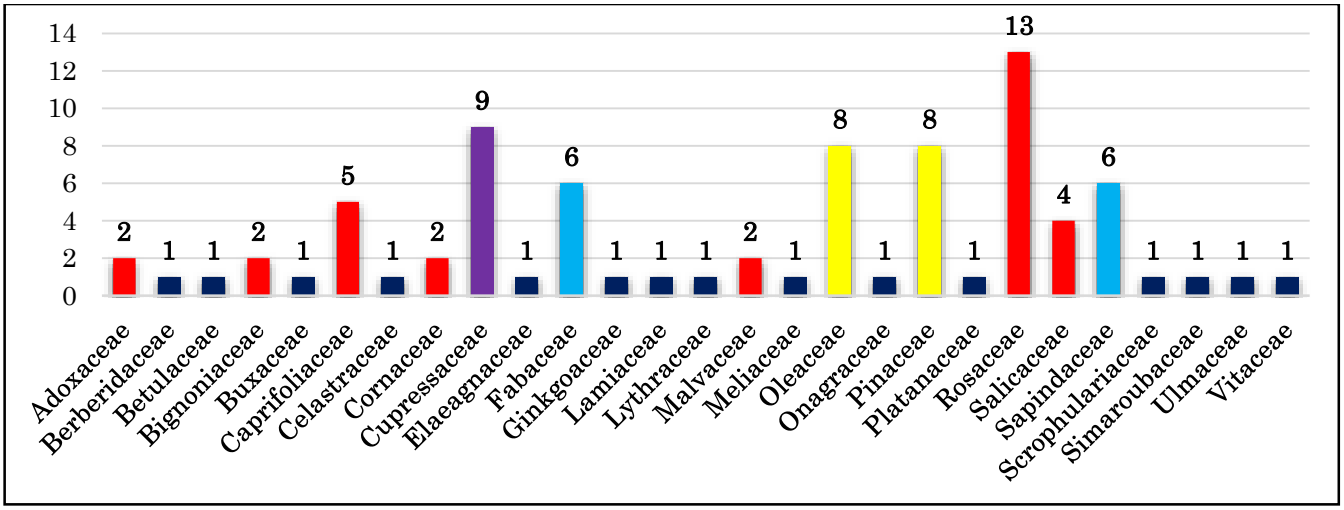
Taksonların çiçeklenme periyotları ile Iğdır ilinin ortalama iklim verileri arasındaki ilişkiler, Şekil 7'de görsel olarak gösterilmektedir. Bu ilişki, çiçeklenme dönemlerinin yerel iklim koşullarıyla olan etkileşimini ortaya koyarak, bitki türlerinin çiçeklenme zamanlaması üzerinde iklimsel faktörlerin nasıl bir rol oynadığını anlamaya yönelik önemli bir veri sağlamaktadır.

Çizelge 2. Kampüs alanında tespit edilen taksonlar ve özellikleri (familyalar harf sırasına göre yazılmıştır)
Table 2. Taxa identified in the campus area and their characteristics (written in alphabetical order of families)

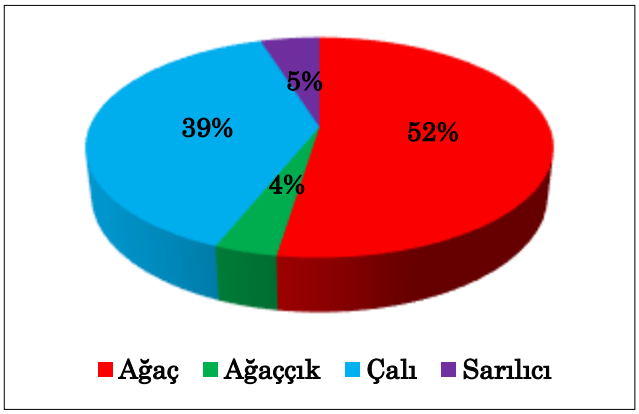
Familyası	Bilimsel adı	Bitki türü	Yaşam formu	Çiçeklenme dönemi (aylar)	Arıcılık açısından önemi
Adoxaceae	*Viburnum opulus* L.	Çalı	Doğal	5-7	PN
	Viburnum tinus L.	Çalı	Doğal	2-4	PN
Berberidaceae	*Berberis thunbergii* DC. 'Atropurpurea'	Çalı	Egzotik	4-5	PN
Betulaceae	*Betula pendula* Roth.	Ağaç	Doğal	3-4	PS
Bignoniaceae	*Campsis radicans* L.	Ağaç	Egzotik	6-9	PN
	Catalpa bignonioides Walt.	Ağaç	Egzotik	5-7	PN
Buxaceae	*Buxus sempervirens* L.	Çalı	Doğal	3-5	PN
Caprifoliaceae	*Abelia × grandiflora* (Andre) Rehd.	Çalı	Egzotik	6-9	PN
	Lonicera japonica Thunb.	Sarılıcı	Egzotik	5-7	PN
	Lonicera nitida cv. Maigrun	Çalı	Egzotik	5-7	PN
	Symphoricarpos albus (L.) S.F. Blake	Çalı	Egzotik	4-5	PN
	Weigela floribunda (Sieb. & Zucc.) K. Koch.	Çalı	Egzotik	4	PN
Celastraceae	*Euonymus japonica* Thunb. 'Aurea'	Çalı	Egzotik	5-7	PN
Cornaceae	*Cornus alba* L. 'Sibirica'	Çalı	Egzotik	5-6	PN
	Cornus stolonifera Michx. 'Flaviramea'	Çalı	Egzotik	5-6	PN
Cupressaceae	*Cupressus sempervirens* L.	Ağaç	Doğal	4-5	PS
	Cupressus arizonica Greene 'Glauca'	Ağaç	Egzotik	8-9	PS
	Cuprocyparis leylandii (A.B. Jacks. & Dallim.) Farjon	Ağaç	Egzotik	3-4	PS
	Juniperus horizontalis Moench	Çalı	Egzotik	4-5	P
	Juniperus sabina L.	Çalı	Doğal	4-5	P
	Junıperus × media Van Melle 'Mint Julep'	Çalı	Egzotik	4-5	P
	Platycladus orientalis (L.) Franco	Ağaç	Doğal	4-5	PS
	Thuja orientalis L. 'Compacta Nana'	Çalı	Egzotik	4-5	PS
	Thuja occidentalis 'Smaragd'	Ağaç	Egzotik	4-5	P
Elaeagnaceae	*Elaeagnus angustifolia* L.	Ağaç	Doğal	4-6	PN
Fabaceae	*Cercis siliquastrum* L.	Ağaç	Doğal	3-4	PNS
	Gleditsia triacanthos L.	Ağaç	Egzotik	5-7	PN
	Robinia hispida L.	Ağaç	Egzotik	4-6	PN
	Robinia pseudoacacia L.	Ağaç	Egzotik	4-6	PN
	Robinia pseudoacacia L. 'Umbraculifera'	Ağaç	Egzotik	Yok	Yok
	Wisteria sinensis DC.	Sarılıcı	Egzotik	4-7	PN
Ginkgoaceae	*Ginkgo biloba* L.	Ağaç	Egzotik	4-5	P
Lamiaceae	*Lavandula angustifolia* Mill.	Çalı	Doğal	6-8	PN
Lythraceae	*Lagerstroemia indica* L.	Ağaç	Egzotik	7-9	PN
Malvaceae	*Hibiscus syriacus* L.	Çalı	Egzotik	6-9	PN
	Tilia tomentosa Moench.	Ağaç	Doğal	6-7	PNS
Meliaceae	*Melia azedarach* L.	Ağaç	Egzotik	4-5	PN
Oleaceae	*Fraxinus angustifolia* Vahl	Ağaç	Doğal	4-5	P
	Fraxinus excelsior L.	Ağaç	Doğal	4-5	P
	Fraxinus ornus L. subsp. *ornus*	Ağaç	Doğal	4-5	P
	Forsythia intermedia Zabel.	Çalı	Egzotik	3-4	P
	Ligustrum japonicum Thunb.	Ağaççık	Egzotik	6-9	PN
	Ligustrum ovalifolium Hassk.	Çalı	Egzotik	6	PN
	Ligustrum ovalifolium Hassk. var. Aureum	Çalı	Egzotik	6	PN
	Syringa vulgaris L.	Çalı	Egzotik	4-5	PN
Onagraceae	*Gaura lindheimeri* Engelm. & A.Gray	Çalı	Egzotik	5-10	PN
Pinaceae	*Cedrus deodora* (Roxb.) Loud.	Ağaç	Egzotik	9-11	PS
	Picea abies L. Karst.	Ağaç	Egzotik	5-6	PS

	Picea orientalis L.	Ağaç	Doğal	4-5	PS
	Picea pungens Engelm.	Ağaç	Egzotik	4-5	PS
	Pinus brutia Ten.	Ağaç	Doğal	5	PS
	Pinus mugo 'Mops'	Ağaççık	Egzotik	5-6	PS
	Pinus nigra J.F. Arnold	Ağaç	Doğal	5	PS
	Pinus nigra 'Pyramidalis'	Ağaç	Doğal	5	PS
Platanaceae	*Platanus orientalis* L.	Ağaç	Doğal	3-5	PNS
Rosaceae	*Chaenomeles speciosa* (Sweet) Nakai	Çalı	Egzotik	2-6	PN
	Cotoneaster coriaceus Franch	Çalı	Egzotik	5-6	PN
	Cotoneaster horizontalis Decne	Çalı	Egzotik	5-6	PN
	Cotoneaster lacteus W.W.Sm.	Çalı	Egzotik	6-7	PN
	Malus floribunda siebold ex. Van Houtte	Ağaç	Egzotik	4-5	PNS
	Photinia × fraseri Dress 'Little Red Robin	Çalı	Egzotik	4-6	PN
	Pyracantha coccinea M.Roem.	Çalı	Doğal	4-6	PN
	Pyracantha angustifolia (Franch.) CK Schneid	Çalı	Egzotik	4-6	PN
	Prunus cerasifera Ehrh.	Ağaç	Egzotik	3-4	PNS
	Prunus cerasifera cv. 'Pissardi Nigra'	Ağaç	Egzotik	3-4	PNS
	Prunus serrulata Lindl. 'Kanzan'	Ağaç	Egzotik	4-5	PN
	Rosa meilland L.	Çalı	Doğal	4-10	P
	Spiraea x vanhouttei (Briot) Zabel.	Çalı	Egzotik	4-5	PN
Salicaceae	*Populus nigra* L.	Ağaç	Doğal	3-4	PS
	Salix babylonica L.	Ağaç	Egzotik	4-5	PNS
	Salix caprea 'Pendula'	Ağaççık	Doğal	4-5	PNS
	Salix nigra Marshall	Ağaç	Doğal	4-5	PNS
Sapindaceae	*Acer negundo* L.	Ağaç	Egzotik	3-4	PNS
	Acer negundo L. 'Flamingo'	Ağaç	Egzotik	3-4	PNS
	Acer pseudoplatanus L.	Ağaç	Doğal	3-4	PNS
	Acer saccharinum L.	Ağaç	Egzotik	3-4	PNS
	Aesculus hippocastanum L.	Ağaç	Egzotik	4-5	PN
	Koelreuteria paniculata Laxm	Ağaç	Egzotik	7-8	PN
Scrophulariaceae	*Buddleja davidii* Franch	Çalı	Egzotik	7-10	PN
Simaroubaceae	*Ailanthus altissima* (Mill.) Swingle	Ağaç	Egzotik	5-6	PS
Ulmaceae	*Ulmus mınor* Mill. 'Umbraculifera'	Ağaç	Doğal	4-5	PS
Vitaceae	*Parthenocissus quinquefolia* (L.) Planch.	Sarılıcı	Egzotik	5-6	PN

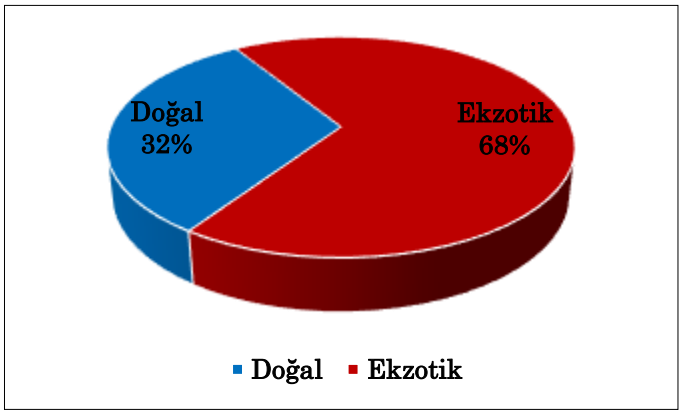
P: polen, N: nektar, S: salgı



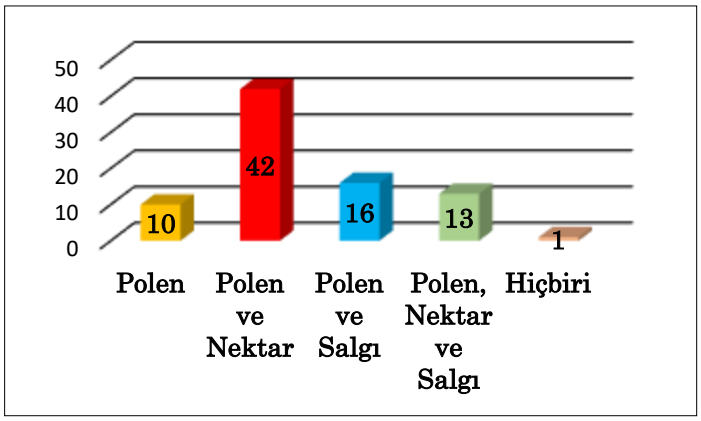
Şekil 2. Iğdır Üniversitesi Şehit Bülent Yurtseven Kampüsünde bulunan taksonların familyalara göre dağılımı
Figure 2. Distribution of taxa found in Iğdır University Şehit Bülent Yurtseven Campus according to families



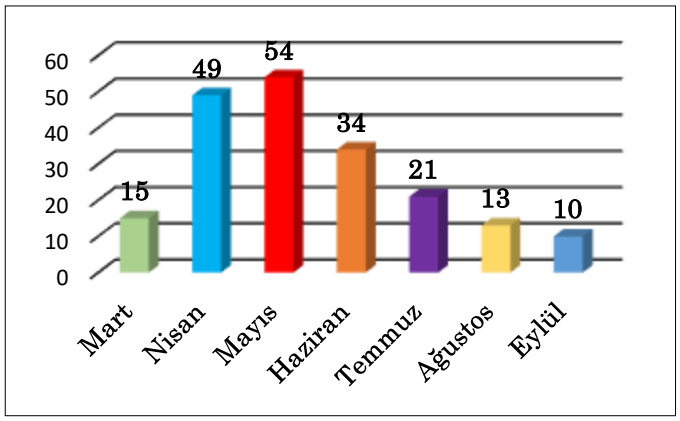
Şekil 3. Taksonların bitki türlerine göre dağılımı
Figure 3. Distribution of taxa according to plant species



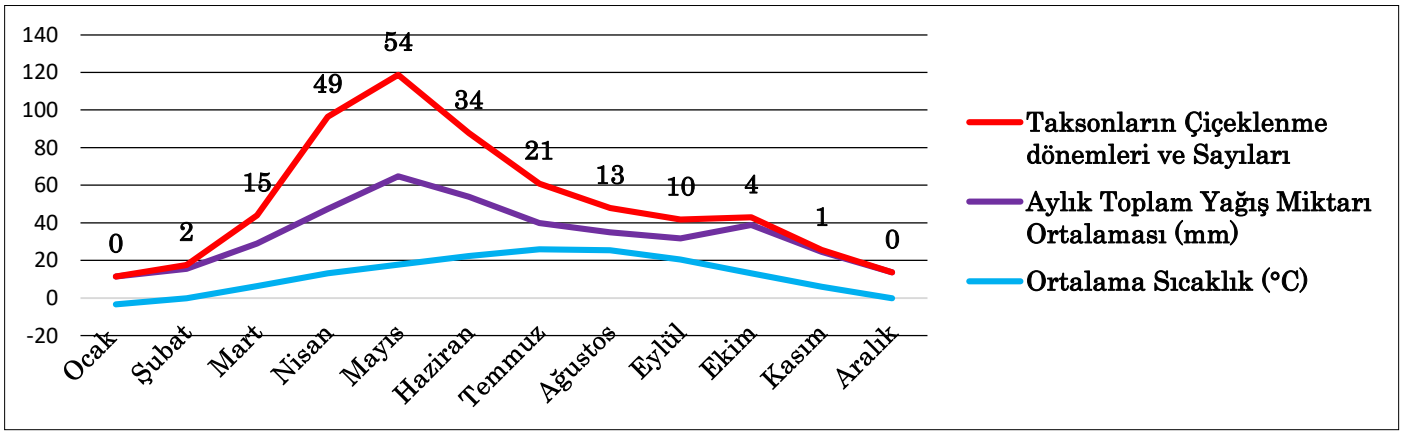
Şekil 4. Taksonların yaşam formlarına göre dağılımı
Figure 4. Distribution of taxa according to life forms



Şekil 5. Taksonların arı çeken özellikleri
Figure 5. Bee-attracting features of taxa



Şekil 6. Aylara göre açık çiçek taşıyan takson sayıları
Figure 6. Number of taxa with open flowers by month

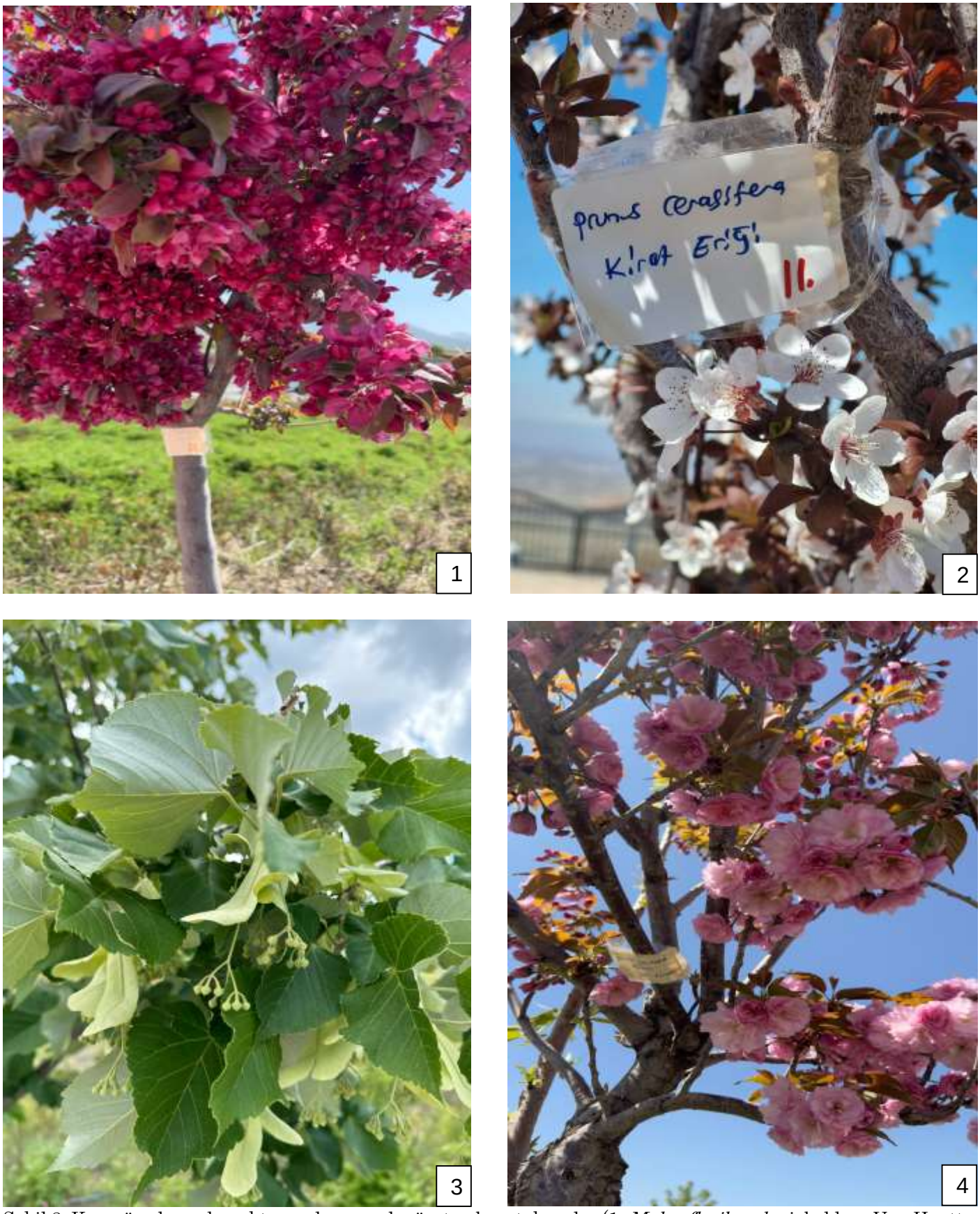


Şekil 7. Aylara göre açık çiçek taşıyan takson sayıları ile Iğdır iklimi arasındaki ilişkiler
Figure 7. Relationships between the number of taxa carrying open flowers according to months and Igdir climate

TARTIŞMA

Türkiye'de mevcut olan tüm bitkilerin 500 kadarı arıcılık için önem taşıyan, arılara nektar ve polen sunan önemli arı bitkileridir. Bu bitkilerin tamamı arıcılık için önemli olmakla birlikte, ekonomik anlamda dominant nektar ve polen verimi olan bitki sayısı 50-60 civarındadır (Sorkun, 2008; 2010).

Mach ve Potter (2018) yaptıkları bir çalışmada, 72 çiçekli odunsu bitki türü üzerinde arı ziyaretlerini incelemişlerdir. Çalışma, kent peyzajlarının, arılar ve diğer polinatör böcekler için uygun barınma alanları sunabileceğini ortaya koymaktadır. Yazarlar, kent alanlarında yer alan çiçekli odunsu bitkilerin, tozlayıcı popülasyonlarının korunmasına katkıda bulunabilecek potansiyel kaynaklar sağladığını ve bu tür bitkilerin, kentsel çevrelerde biyolojik çeşitliliğin artırılması için önemli bir rol üstlenebileceğini vurgulamışlardır.



Şekil 8. Kampüs alanında nektar, polen ve salgı üreten bazı taksonlar (1. *Malus floribunda* siebold ex. Van Houtte, 2. *Prunus cerasifera* Ehrh.,3. *Tilia tomentosa* Moench., 4. *Prunus serrulata* 'Kanzan')

Figure 8. Some taxa producing nectar, pollen and secretions in the campus area (1. Malus floribunda siebold ex. Van Houtte*, 2. Prunus cerasifera* Ehrh.*, 3. Tilia tomentosa* Moench.*, 4. Prunus serrulata* 'Kanzan'*)*

Caf ve ark. (2022) Bingöl ili açık yeşil alanlarında kullanılan peyzaj bitkilerinin arıcılık açısından önemi hakkında yaptıkları çalışmada arıcılık açısından bakıldığında 18 familya ve 33 cinse ait toplam 37 taksonun önemli bir potansiyel taşıdığı tespit etmişlerdir. Öztürk ve ark. (2017) Van ili peyzaj bitkilerinin arıcılık açısından değerlendirilmesini konu alan çalışmalarında ise toplamda 51 familyaya ait 163 takson arı bitkisi olarak belirlemişlerdir. Belirledikleri familyaların 3 tanesi Gymnospermae, 48 familya ise Angiospermae'dir. Tespit ettikleri taksonlardan 12 tanesi Gymnospermae, 151 takson ise Angiospermae şubesinde yer almaktadır. Çalışmada, en fazla takson sayısına sahip familyaların sırasıyla *Rosaceae* (19 takson), *Liliaceae* (15 takson) ve *Fabaceae* (13 takson) olduğunu göstermektedir. Taksonların nektar, polen ve salgı üretim özelliklerine göre yapılan gruplandırmalarda, sadece nektar üreten 2 takson, yalnızca polen üreten 15 takson, hem nektar hem polen üreten 92 takson, polen ve salgı üreten 37 takson ile nektar, polen ve salgıyı bir arada üreten 17 taksonun arı bitkisi olarak önemli olduğunu belirlenmişlerdir. Iğdır Üniversitesi Şehit Bülent Yurtseven Kampüsünde yapılan çalışmada ise 18'i açık tohumlu (Gymnospermae), 64'ü kapalı tohumlu (Angiospermae) olmak üzere 27 familyaya ait 82 takson tespit edilmiş ve tespit edilen taksonlardan 71 taksonun arıcılık açısından önemli bir potansiyel taşıdığı belirlenmiştir. Taksonların nektar, polen ve salgı üretim özelliklerine göre bakıldığında ise 10 taksonun sadece polen, 42 taksonun hem polen hemde nektar, 16 taksonun polen ve salgı, 13 taksonun polen, nektar ve salgı barındırdığı tespit edilmiştir.

Karaca ve ark. (2006) yaptıkları çalısmada nektar ve polen açısından arıcılık için önemli olan 23 familya ve bu familyalara ait 91 bitki türü tespit etmişlerdir. Iğdır Üniversitesi Şehit Bülent Yurtseven Kampüsünde ise 27 familyaya ait 82 takson belirlenmiştir. Özellikle *Rosaceae* familyasının, 13 taksonla en fazla çeşidi barındırması, bu bitkilerin kampüs çevresinde polinatörler için önemli bir besin kaynağı oluşturduğunu ve arıların bu bitkileri yoğun bir şekilde ziyaret ettiğini göstermektedir.

Sarı (2022), Artvin'de gerçekleştirdiği çalışmada, polen, nektar ve salgı üreten bitki taksonlarının sayısını iklim verileriyle karşılaştırarak, arıların bitkilerden en fazla yararlandığı ayın mayıs olduğunu (92 takson) ortaya koymuştur. Ancak mart ve nisan aylarında arıların faydalanabileceği takson sayısının fazla olmasına rağmen, bu dönemlerdeki iklim değerleri ortalamalarına göre uygun değer aralıklarının dışında kalması nedeniyle bu aylara ilişkin verileri grafiğinde yer vermemiştir. Günlük sıcaklık koşullarının uygun olduğu durumlarda, arıların mart ayı itibariyle örnek alanlardaki bitki taksonlarından faydalanma potansiyeline sahip olduğunu gözlemlenmiştir. Bununla birlikte, çiçekli taksonların çiçeklenme dönemleri, çevresel faktörler, mikro iklim koşulları ve bitki bakım uygulamaları gibi değişkenler tarafından etkilenerek literatürde belirtilen çiçeklenme periyotlarının öne çekilmesi ya da gecikmesi mümkün olduğu kanısına varmıştır. Bu kapsamda Iğdır Üniversitesi Şehit Bülent Yurtseven Kampüsünde yapılan çalışmada polen, nektar ve salgı üreten takson sayısı ile iklim verileri karşılaştırıldığında, arıların bitkilerden en fazla yararlandığı dönemin mayıs ayı (54 takson) olduğu gözlemlenmektedir. Günlük sıcaklık koşullarının uygun olması durumunda, arıların mart ayı itibariyle faaliyete başlayarak örnek alanlardaki bitki taksonlarından yararlanma potansiyeline sahip olduğu anlaşılmaktadır.

Karaköse ve ark. (2018) bulgularına paralel olarak, bitkilerde çiçeklenmenin zirveye ulaşması, sıcaklığın uygun seviyelere gelmesi ve yağış miktarının en düşük seviyelere inmesiyle ilişkilidir. Bu durum, sıcaklık, yağış ve çiçekli bitkiler arasında doğrudan bir bağlantı olduğunu göstermektedir. Bal arıları, 12-13°C'nin altındaki sıcaklıklarda etkin bir şekilde çalışamazlar (Korkmaz, 2015). Bu bağlamda, elde edilen verilere göre, arıların en fazla aktif olduğu dönem mayıs ile ekim ayları arasında yoğunlaşmaktadır. Ancak, Iğdır il merkezinin mikro iklim etkileri göz önünde bulundurulduğunda, arıların mart ayından itibaren faaliyete geçmesi mümkün olabilmektedir. Bu durum, bölgesel iklimsel faktörlerin, arıların biyolojik faaliyetlerini ve polinasyon süreçlerini ne denli etkileyebileceğini ortaya koymaktadır.

Lowenstein ve ark. (2019) kent ekosistemlerinde bitkiler ile tozlaşma arasındaki etkileşimi inceleyerek, tozlayıcılar tarafından tercih edilen bitki taksonlarını belirlemeye yönelik bir araştırma gerçekleştirmişlerdir. 3 yıl süren araştırma sürecinin sonunda, tozlayıcılar tarafından yüksek çekiciliğe sahip olarak tanımlanan 42 takson tespit edilmiştir. Yazarlar, bu taksonların kentlerde polinatör popülasyonunun artırılmasına katkı sağlayacağına vurgu yaparak, bu türlerin peyzajda kullanılmasını önermişlerdir. Ayrıca çalışmada genel olarak çok yıllık ve yerli bitkilerin daha fazla tozlayıcı ziyareti aldığı sonucuna varılmış ve bunun yanı sıra, çiçeklenmiş olmasına rağmen tozlayıcılar tarafından ziyaret edilmeyen 57 taksonun varlığı da gözlemlenmiştir. Bu bulgular, kent peyzajlarında tozlayıcı faaliyeti ve biyolojik çeşitliliği artırmaya yönelik stratejilerin geliştirilmesinin önemini ortaya koymaktadır.

Egzotik bitki türlerinin tozlayıcılar üzerindeki etkisi genellikle türden türe değişiklik göstermektedir. Bazı egzotik türler, tozlayıcılara besin kaynağı sağlama açısından yetersiz kalırken, diğerleri yerli türlerle karşılaştırıldığında eşdeğer veya daha üstün besin kaynakları sunabilmektedir (Salisbury ve ark., 2015).

Son yıllarda gerçekleştirilen bazı çalışmalar, egzotik bitki türlerinin yeterli besin maddelerini (örneğin, uygun miktar ve kalitede polen ve/veya nektar) sunabilmesi durumunda, polinatörlerin bu bitkileri kolaylıkla diyetlerine

dahil edebileceğini ortaya koymuştur (Somme ve ark., 2016). Bu bulgular, egzotik bitkilerin tozlayıcı ekosistemlerinde yer alırken, belirli koşullar altında potansiyel olarak besin kaynaklarını çeşitlendirebileceğini ve polinasyon süreçlerine katkı sağlayabileceğini göstermektedir. Iğdır Üniversitesi Şehit Bülent Yurtseven Kampüsünde ise 56 egzotik takson arasında hem polen hem de nektar kaynağı sağlayan 35 takson tespit edilmiştir. Öte yandan, 26 doğal takson arasında ise hem polen hem de nektar kaynağı sunan yalnızca 6 takson bulunmuştur. Bu sonuçlar, kent parklarında yer alan egzotik bitkilerin, polinasyon açısından önemli besin kaynakları sağladığını ve dolayısıyla tozlayıcı faaliyeti destekleyebilecek potansiyeli barındırdığını göstermektedir. Bu bulgular, egzotik bitkilerin kentsel peyzajlarda polinasyon hizmetlerinin sürdürülebilirliğine katkı sağlayabileceğini ortaya koymaktadır.

Fukase ve Simons (2016) yaptıkları çalışmada; yerli bitki türlerinin bulunduğu kentsel alanların, yerli olmayan bitki türlerinin bulunduğu alanlara göre polinatörlere daha kesintisiz kaynaklar sunduğunu ve bu alanların çeşitli polinatörleri çekme etkisi gösterdiğini belirtmişlerdir. Bunun yanı sıra, Landis ve ark. (2014) doğal bitki türlerinin, yetişkin kelebekler ve onların larvaları için beslenme ortamı sağladığını ifade etmişlerdir. Bu bulgular, yerli bitki örtüsünün polinatörler için daha verimli ve sürdürülebilir bir yaşam alanı sunduğunu ortaya koymaktadır.

Aslan ve Uslu (2021) tarafından yapılan bir çalışmada ise kentsel peyzajlarda polinatörleri çekerek tozlaşmaya katkı sağlayan doğal bitki örtüsündeki bitki türlerinin kullanılması, kentsel biyoçeşitliliği artırmak adına önemli bir yaklaşım olarak öne çıkmaktadır. Doğal bitki türleri, bulundukları yörenin iklimine uyum sağlamış bitkiler oldukları için, değişen çevre koşullarına yabancı (egzotik) türlere kıyasla daha iyi adapte olurlar ve genellikle daha dayanıklıdırlar. Ayrıca, doğal bitki türleri, kentsel ve kırsal alanlar arasındaki ekosistem bütünlüğünü koruyarak, daha az bakıma ihtiyaç duyarlar. Bu durum, egzotik türlerle yapılan düzenlemelere kıyasla daha düşük maliyetler anlamına gelir. Daha az bakım ile sürdürülebilirliğe katkı sağlarken aynı zamanda su, gübre ve kimyasal kullanımı açısından da tasarruf yaratır.

Kentsel peyzaj çalışmalarında sıklıkla yabancı türlerin tercih edilmesinin ardında birkaç temel neden bulunmaktadır. Bunlar arasında doğal türlerin yeterince tanınmaması, bu türlerin üretim yöntemlerinin bilinmemesi ve dolayısıyla fidanlıklarda bu bitkilerin temin edilememesi gibi engeller yer almaktadır. Bununla birlikte, doğal türlerin kentsel peyzajda yaygınlaştırılabilmesi için daha kapsamlı bir eğitim ve farkındalık çalışması gereklidir. Doğal bitki türlerinin daha az bakım gerektirmesi, kimyasal kullanımını da azaltarak, özellikle arıcılık açısından önemli bir avantaj sağlar. Kimyasal gübreler ve pestisitlerin sınırlı kullanımı, polinatörlerin özellikle arıların, sağlıklı bir şekilde beslenmesini ve gelişmesini destekler. Arılar, zararlı kimyasallara maruz kaldıklarında hastalık riski artar ve popülasyonları azalabilir. Bu nedenle, doğal bitkilerin tercih edilmesi hem arıcılığın sürdürülebilirliği hem de ekosistem dengesi için önemlidir.

Özkan ve ark. (2016) çalışmalarında, araştırma alanında yayılış gösteren bitki taksonları arasında en yüksek nektar verimine sahip olan türlerin *Tilia tomentosa*, *Robinia pseudoacacia* ve *Acer campestre* olduğunu belirtilmişlerdir. Elde edilen verilere göre, kampüs alanında *Tilia tomentosa* ve *Robinia pseudoacacia* türleri yoğun bir şekilde tercih edilmiştir. Ancak, *Acer campestre* bitkisine Iğdır Üniversitesi Şehit Bülent Yurtseven Kampüsünde yer verilmediği tespit edilmiştir. Bu bulgular, kampüs peyzajında tozlayıcılar açısından önemli potansiyel taşıyan nektar kaynaklarının kullanım tercihlerini ve bitki türlerinin kentsel alanlarda nasıl farklılıklar gösterdiğini ortaya koymaktadır.

Baydar ve Gürel (1998) Antalya'nın doğal florasında *Apis mellifera* (bal arısı) türünün polen toplama aktivitesi, polen tercihi ve farklı polen tiplerinin morfolojik ile kalite özelliklerini inceledikleri çalışmalarında, *Fabaceae* familyasına ait türlerin polenlerinin, diğer bitki familyalarına ait türlerle karşılaştırıldığında hem protein hem de mineral madde açısından önemli ölçüde daha zengin olduğunu tespit etmişlerdir. Bu bulgu, *Fabaceae* familyasının polenlerinin arılar için besin değeri açısından büyük önem taşıdığını ve polinasyon süreçlerinde bu familya üyelerinin rolünün kritik olabileceğini göstermektedir. Iğdır Üniversitesi Şehit Bülent Yurtseven Kampüsünde ise bu familyaya ait 6 takson tespit edilmiştir.

Konifer bitkilerinden çamlar (*Pinus* sp.), köknarlar (*Abies* sp.), ladinler (*Picea* sp.), serviler (*Cupressus*), ve mazılar (*Thuja* sp.) rüzgârla tozlaşan türler olduklarından, arılar bu bitkileri genellikle çok az miktarda polen toplamak amacıyla ziyaret edebilirler. Özellikle çamlar gibi bazı iğne yapraklı türlerde üretilen bal çiği nedeniyle arılar, bu ağaçları salgı balı kaynağı olarak da ziyaret edebilmektedir. Bunun yanı sıra, konifer bitkilerinin tomurcukları, reçineleri, uçucu yağları ve polenleri, arıların faydalandığı diğer kaynaklar arasında yer almakta olup, az miktarda propolis kaynağı olarak da bu bitkiler ziyaret edilebilmektedir. Kentlerde süs bitkisi olarak kullanılan çeşitli çam türlerinin, dolaylı yoldan arılara katkı sağladığı söylenebilir. Gymnosperm türleri, genel olarak arılar için pek çekici olmayıp, bu bitkiler arılar tarafından yalnızca sınırlı bir çekiciliğe sahip olarak değerlendirilmiştir. Ancak, çiçeklenme dönemlerinde bu türler, arılar için az miktarda polen kaynağı sunabilmektedir. Bu durum, gymnosperm bitkilerinin tozlayıcılar üzerindeki rolünün sınırlı olmakla birlikte, bazı koşullarda polinasyon

süreçlerine katkıda bulunabileceğini göstermektedir (Sarı, 2022). Araştırma kapsamında, Iğdır Üniversitesi Şehit Bülent Yurtseven Kampüsünde 12 konifer taksonunun bulunduğu tespit edilmiştir.

Yaygın bir dağılım gösteren pirolizidin alkaloidleri (PA), bitkilerin ikincil metabolitleri olup, çiftlik hayvanlarını ve insanları etkileyen en önemli doğal toksinler olduğu tahmin edilmektedir. Ağırlıklı olarak *Fabaceae* (*Crotalaria* cinsi), *Boraginaceae* (tüm cinsler) ve *Asteraceae* (*Senecioneae* ve *Eupatorieae* cinsleri) familyalarında bulunan PA'ların yaklaşık 6000 bitki türünde mevcut olduğu tahmin edilmekte olup, 350'nin üzerinde PA tanımlanmıştır. Bitkilerin çiçekli ve tohum kısımları en yüksek oranda toksin içermektedir (Martinello ve ark., 2014).

Bazı çiçekli bitki türlerinin polen ve nektarları, bal arıları için toksik özellikler taşıyabilmektedir. Özellikle, *Ericaeae, Ranunculaceae, Euphorbiaceae, Solanaceae, Plantaginaceae, Acanthaceae* ve *Meliaceae* gibi bitki familyalarında yer alan bazı türler, polen ve nektarlarının toksik bileşikler içermesi nedeniyle bal üretimi sırasında arılar ve insanlar için tehlike arz edebilmektedir. Bu bitkilerden elde edilen bal, zehirli özellikler gösterebilir ve bu durum, hem arıların sağlığını hem de bal tüketicilerinin güvenliğini tehdit edebilir (Hassen ve Muche, 2020). Kentsel peyzajda yaygın olarak kullanılan ve toksik özellikleri hakkında yeterli bilgi bulunmayan birçok süs bitkisi mevcuttur. Bu bitkiler, dikkatsiz kullanım durumunda yalnızca insan sağlığına değil, aynı zamanda çeşitli hayvan türlerine de zarar verebilecek potansiyele sahiptir. Bu bağlamda, özellikle bu tür taksonlara karşı daha fazla farkındalık oluşturulması, kentsel alanlarda bitki seçimi ve kullanım miktarlarının peyzaj mimarları tarafından titizlikle planlanması, olası olumsuz etkilerin önlenmesi açısından önemli bir koruma olacaktır. Iğdır Üniversitesi Şehit Bülent Yurtseven Kampüsünde bulunan *Meliaceae* familyasında yer alan *Melia azedarach* L. dikkat edilmesi gereken bir türdür.

İnsektisitler, tozlayıcı böcekler üzerinde doğrudan olumsuz etkiler yaratmaktadır. Özellikle tarım alanlarında, tarımsal zararlılara karşı uygulanan zirai mücadele ilaçları, tozlaştırıcı böcek popülasyonlarının azalmasına yol açabilmektedir (Tirado ve ark., 2013; Bağrıaçık, 2017). Bu ilaçların etkileri, tozlaştırıcıları doğrudan öldürmek veya onların davranışlarını, yaşam sürelerini ve hastalıklara yatkınlıklarını olumsuz yönde etkileyebilmek şeklinde farklılık gösterebilmektedir (Ellsworth, 2014). Dolayısıyla, tozlaşma süreçlerinin sağlıklı bir şekilde gerçekleşebilmesi adına, insektisitlerin ve diğer zararlı uygulamaların tozlaştırıcıları etkilemesini önlemek büyük önem taşımaktadır. Bu bağlamda, biyolojik mücadelenin insektisitlere alternatif olarak tercih edilmesi önerilmektedir. İlaçlamanın gerekli olduğu durumlarda ise çiçeklenme dönemine özel dikkat gösterilmeli; tozlaştırıcıların daha az aktif olduğu çiçeklenme öncesi ya da sonrası dönemlerde uygulama yapılarak, tozlaştırıcıların minimum düzeyde zarar görmesi sağlanmalıdır (Ulus & Özdemir, 2018). Bu bağlamda çalışmanın yürütüldüğü kampüs alanında süs bitkilerine düzenli bir kimyasal uygulanmadığından bu yönde bir veri takip edilememiştir.

Süs bitkisi olarak yaygın bir şekilde kullanılan *Buddleja davidii* (kelebek çalısı), özellikle kelebekleri çekmesiyle bilinse de arılar için oldukça sınırlı (%3) bir polen ve nektar kaynağı sağlamaktadır (Sorkun, 2008). Bu bulgu, kelebek çalısının arıların beslenme ihtiyaçlarını karşılamak için yeterli bir kaynak oluşturmadığını göstermektedir. Ancak peyzaj tasarımında kelebeklerin yanı sıra diğer polinatörlerin varlığını destekleyen bir rol oynayabilir.

Lonicera caprifolium (sarılıcı hanımeli), hoş kokulu çiçekleri ile dikkat çekici bir süs bitkisi olmasına rağmen, arılar için polen ve nektar kaynağı açısından oldukça sınırlıdır. Diğer taraftan *Lavandula angustifolia* (lavanta), güzel kokulu çiçekleriyle öne çıkan bir tıbbi aromatik bitki olup, arılar tarafından nektar, polen ve uçucu yağları için faydalanılan önemli bir kaynaktır. Bu özellikleri, lavantayı kentsel peyzajlarda tozlaşmayı destekleyen önemli bir bitki yapmaktadır. Ayrıca *Fraxinus* cinsi (dişbudaklar), genellikle polen kaynağı olarak öne çıkmakla birlikte, aynı zamanda propolis üretiminde de arılar için değerli bir kaynak oluşturan ağaçlardır (Sarı, 2022). Çalışma alanına bakıldığı zaman ise *Lavandula angustifolia* Mill. Kampüs alanında yoğun olarak tercih edilmiş her yıl lavanta çayı etkinliği düzenlenerek temmuz ayından itibaren hasat edilerek lavanta yağı elde edilmektedir.

Ayrıca *Fraxinus angustifolia* Vahl, *Fraxinus excelsior* L., *Fraxinus ornus* L. subsp. *ornus* kampüs alanında tespit edilen dişbudak taksonlarıdır. Bu bitkiler yalnızca peyzaj tasarımında estetik ve fonksiyonel açıdan önemli olmakla kalmaz aynı zamanda arıların, beslenme ve polinasyon süreçlerinde oynadığı role katkı sağlayarak biyolojik çeşitliliği artırmakta ve sürdürülebilir ekosistemlerin oluşumuna olanak tanımaktadır. Bu bitkilerin, kentsel peyzajlarda doğru bir şekilde yerleştirilmesi, arı popülasyonlarının desteklenmesi ve tozlaşmanın verimli bir şekilde sağlanması adına büyük önem taşımaktadır.

SONUÇ ve ÖNERİLER

Bu araştırmanın sonucunda, Iğdır Üniversitesi Şehit Bülent Yurtseven Kampüsünde 27 farklı familyaya ait toplam 82 taksonun hem kampüs alanının floristik zenginliği hem de arıların polinasyon süreçlerine sağladığı katkılar açısından önemli bir değer taşıdığı belirlenmiştir. Araştırmada incelenen 71 taksonun, arılar açısından kritik rol oynayan polen, nektar ve salgı ürünlerinden en az ikisini sunduğu gözlemlenmiştir. Dahası, bu

taksonların çiçeklenme periyotları oldukça uzun bir döneme yayılmaktadır. Mart ayından ekim ayına kadar yaklaşık 8 ay süresince çiçeklenme gösteren bitkilerin bulunması, arıların aktif olarak çalıştığı dönem boyunca sürekli olarak bitkisel kaynak bulabilmesini sağlamaktadır. Bu sonuçlar, kentsel peyzajda kullanılan bitki türlerinin, özellikle odunsu taksonların arıların polinasyon faaliyetleri açısından taşıdığı büyük potansiyeli gözler önüne sermektedir. Bu bitkiler, yalnızca estetik ve peyzaj açısından değil aynı zamanda ekosistem hizmetleri açısından da önemli bir rol üstlenmektedir. Bu türlerin, kentsel çevrelerde biyolojik çeşitliliği artırmanın yanı sıra, arıların polinasyon hizmetlerinin sürekliliğini sağlamak için de kritik bir öneme sahip olduğu görülmektedir.

Bu çalışma neticesinde aşağıdaki önerilerde bulunulmuştur;

1. Peyzaj Planlamasında Arıcılık Odaklı Bitki Seçimi: Kentsel alanlarda yapılan peyzaj düzenlemelerinde, arıların polinasyon faaliyetlerini destekleyecek bitkilerin tercih edilmesi önemlidir. Özellikle çiçeklenme dönemi farklılıkları göz önünde bulundurularak, arıların aktif dönemleri boyunca beslenebileceği bir bitki çeşitliliği oluşturulmalıdır. Ayrıca kuraklığa dayanıklı ağaç,ağaççık,çalı ve çim türleri tercih edilmeldir.
2. Çeşitliliğin Artırılması: Peyzajda kullanılan bitki türlerinin çeşitlendirilmesi önerilmektedir. Nitekim bu durum arıların farklı besin kaynaklarına erişmesini sağlamaktadır. Bu çeşitlilik, sadece arıların sağladığı polinasyon faaliyetlerini desteklemekle kalmaz, aynı zamanda biyolojik çeşitliliği artırarak ekosistemin sağlıklı bir şekilde işlemesine katkı sağlamaktadır.
3. Biyolojik çeşitlilikle İlgili Eğitim ve Bilinçlendirme: Kentsel alanlarda biyolojik çeşitlilik ve arıların bu alandaki rolleri ile ilgili eğitimlerin yaygınlaştırılması, farkındalık artırmak amacıyla eğitim programları, seminerler ve bilgilendirici araçlar (panolar, tabelalar) kullanılmalıdır. Bu yöntemler, çevre dostu davranışları teşvik ederek doğa ile bilinçli bir ilişki kurulmasına katkı sağlamaktadır.
4. Arıların Korunması İçin Yönetim Stratejileri: Peyzaj düzenlemeleri yapılırken, arıların sağlıklı bir şekilde varlıklarını sürdürebilmesi için zararlı kimyasalların kullanımının azaltılması ve doğal yaşam alanlarının korunması gerekmektedir. Arıların sağlıklı bir ekosistemde aktif olarak yer alabilmesi için bu faktörlerin göz önünde bulundurulması önemlidir.
5. Araştırmaların Sürekliliği: Peyzaj bitkilerinin arıcılık açısından daha geniş ve kapsamlı olarak değerlendirilmesi amacıyla, farklı iklim bölgelerinde ve ekosistemlerde benzer çalışmaların yapılması önerilmektedir. Bu tür araştırmalar, peyzaj bitkilerinin arıcılık üzerindeki etkilerini daha kapsamlı bir şekilde incelemeye ve etkili çözümler geliştirmeye olanak tanıyacaktır.
6. Doğal Türlerin Tercih Edilmesi: Kentsel peyzajlarda polinatörleri çekerek tozlaşmaya katkı sağlayan doğal bitki türlerinin kullanılması, kentsel biyoçeşitlilik adına önemlidir. Doğal türlerin, az bakım gerektirmesi ve ekzotik türlere kıyasla bulundukları bölgeye daha iyi adapte olmalarından dolayı bitkisel peyzaj tasarımlarında daha çok tercih edilmesi önerilmektedir.
7. Pirolizidin alkaloidleri (PA): Pirolizidin alkaloidleri üreten bitkilerin bal arıları tarafından ziyaret edilmesinin, ürünlerinde toksik bileşik riskini artırabileceği göz önünde bulundurularak, peyzaj düzenlemelerinde bu bitkilerin kullanımına dikkat edilmesi önerilmektedir. Ayrıca biyoçeşitlilik artırma amacıyla yapılan uygulamalarla birlikte, polen alerjisi, böcek fobisi gibi bireysel sağlık sorunları da dikkate alınması gereken önemli bir konu olarak ortaya çıkmaktadır. Bu bağlamda toksik etkisi bilinen türlerin bitkisel tasarımlarda kullanımının kısıtlanması önerilmektedir.

Sonuç olarak, kentsel alanlarda yapılan peyzaj düzenlemeleri ve bitki seçimi kararlarının, bal arıları başta olmak üzere polinatörlere destek verecek şekilde yapılandırılmasının, sürdürülebilir bir çevre için büyük bir katkı sağlayacağı aşikârdır. Bu çalışma, kentsel floranın estetik değerinden öte, ekolojik ve biyolojik çeşitliliği artırıcı, ekosistem hizmetlerini güçlendirici önemli işlevlere sahip olduğunu ortaya koymaktadır.

Araştırmacıların Katkı Oranı Beyan Özeti

RT Çalışma alanı ile ilgili verilerin değerlendirilmesi ve yorumlanması, literatür araştırması, saha çalışmalarının yürütülmesi ve makale yazımı, TK Makalenin yazımına katkı sağlamak ve çalışma alanına ilişkin verilerin değerlendirilmesi ve yorumlanması. Tüm yazarlar makale yazımında katkı sağlamış olduklarını beyan ederler.

Çıkar Çatışması Beyanı

Makale yazarları aralarında herhangi bir çıkar çatışması olmadığını beyan ederler.

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The Braconid Biodiversity (Hymenoptera: Ichneumonoidea: Braconidae) of Bingöl and Diyarbakır Provinces (Eastern of Türkiye)

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ABSTRACT
In this study, a total of 19 braconid species have been collected in Bingöl province and Diyarbakır around *Euphorbia macroclada* Boiss in the period between 2017 and 2023. The collected species belong to seven subfamilies: Agathidinae (one genus, one species), Braconinae (five genera, seven species), Cheloninae (one genus, five species), Euphorinae (one genus, one unidentified species), Microgastrinae (one genus, one species), Opiinae (one genus, one species), and Rogadinae (one genus, three species, with one unidentified species). All species are recorded for the first time from Bingöl and Diyarbakır (except for *Agathis anglica* Marshall (Agathidinae), *Chelonus obscuratus* Herrich-Schäffer (Cheloninae), *Bracon variator* Nees, and *Vipio mlokossewiczi* Kokujev (Braconinae)), and as a first record in association with *Euphorbia macroclada*. An unidentified *Aleiodes* sp. (Rogadinae) collected from Diyarbakır, it is likely to be a new species, however, it is recommended not to be named until more specimens are collected, as it is just a single specimen, and is missing some of its body parts (as broken antenna and some legs). A full description with illustrations is provided.

Plant Protection

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Bingöl ve Diyarbakır İllerinin (Türkiye'nin Doğusu) Braconid Biyoçeşitliliği (Hymenoptera: Ichneumonoidea: Braconidae)

ÖZET
Bu çalışmada, Bingöl ve Diyarbakır illerinde 2017-2023 yılları arasındaki dönemde *Euphorbia macroclada* Boiss çevresinde toplamda 19 braconid türü toplanmıştır. Toplanan türler yedi altfamilyaya aittir: Agathidinae (bir cins, bir tür), Braconinae (beş cins, yedi tür), Cheloninae (bir cins, beş tür), Euphorinae (bir cins, bir tanımlanamayan tür), Microgastrinae (bir cins, bir tür), Opiinae (bir cins, bir tür) ve Rogadinae (bir cins, üç tür, bir tanımlanamayan tür). Tüm türler Bingöl ve Diyarbakır için ilk kayıt niteliğindedir. *Agathis anglica* Marshall (Agathidinae), *Chelonus obscuratus* Herrich-Schäffer (Cheloninae), *Bracon variator* Nees ve *Vipio mlokossewiczi* Kokujev (Braconinae) hariç) ve *Euphorbia macroclada* ile ilişkili ilk kayıttır. Diyarbakır'dan toplanan tanımlanamayan *Aleiodes* sp. (Rogadinae) yeni bir tür olma olasılığı yüksektir, ancak daha fazla örnek toplanana kadar adının açıklanmaması önerilmektedir. Çünkü bu sadece tek bir örnektir ve vücudunun bazı kısımları (kırık anten ve bazı bacaklar gibi) eksiktir. Bu tür resimleriyle birlikte tam bir tanımlama sağlanmıştır.

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INTRODUCTION

The cosmopolitan family Braconidae (Hymenoptera: Ichneumonoidea) is one of the most species-rich families in the order Hymenoptera (Quicke & van Achterberg, 1990; Wharton, 1993; Quicke, 2015; Chen & van Achterberg, 2019), with more than 21,220 described species in more than 1100 genera (Yu et al., 2016). Braconids are often black, brown with reddish markings, and some exhibit amazing colors and patterns (Gadallah et al., 2021). Over 40 braconid subfamilies are currently recognized in the family Braconidae (Chen & van Achterberg, 2019).

Members of the family are mostly recognized by the following combination of characters: second metasomal tergite is fused with third tergite (secondarily flexible in Aphidiinae); vein 2m-cu of fore wing is absent (except in extremely few cases); vein 1RS+M of fore wing is present; vein 1r-m of hind wing is present basal to the separation of veins R1 and RS (van Achterberg, 1993).

The monophyly of Braconidae is conspicuously supported in numerous molecular studies (examples are those by van Achterberg, 1984; Quicke & van Achterberg, 1990; Sharanowski et al., 2011; Li et al., 2016; Quicke et al., 2020). Many of the braconid members are important as biological control agents against more than 120 pest species of the orders Coleoptera, Diptera, Hemiptera, and Lepidoptera, that cause serious damage to various agricultural, horticultural, and forestry plants and trees (Wharton, 1993; Austin & Dowton, 2000).

The braconid fauna of Bingöl and Diyarbakır is largely incomplete due to the paucity of faunal studies, as well as the greater taxonomic complexity of the family. Examples of the important faunistic works concerning this family in both regions are those by Ölmez & Ulusoy (2003), Güz & Kilinçer (2005), Güler & Çağatay (2007), Beyarslan et al. (2014), Çetin Erdoğan (2014), Beyarslan & Deveci (2019), Beyarslan & Şahin (2019), Beyarslan et al. (2020), Beyarslan & Çakıcı (2021).

The aim of the present work is to increase this knowledge about this family in two of the largely ignored Turkish provinces, Bingöl and Diyarbakır.

MATERIAL and METHOD

The study area

Bingöl province has interesting ecological features as it is located in a transition region between the rainy and long winter climate of Türkiye's Eastern Anatolia Region and the hot and dry Southeastern Anatolia Region (Behçet, 2025). Bingöl Province is in the Upper Euphrates section of the Eastern Anatolia Region. It is surrounded by Muş to the east, Erzurum and Erzincan to the north, Tunceli and Elazığ to the west, and Diyarbakır to the south. Bingöl Province is located between 38° 53′ 10″ N 40° 30′ 60″ E (Figure 1, Table 1).

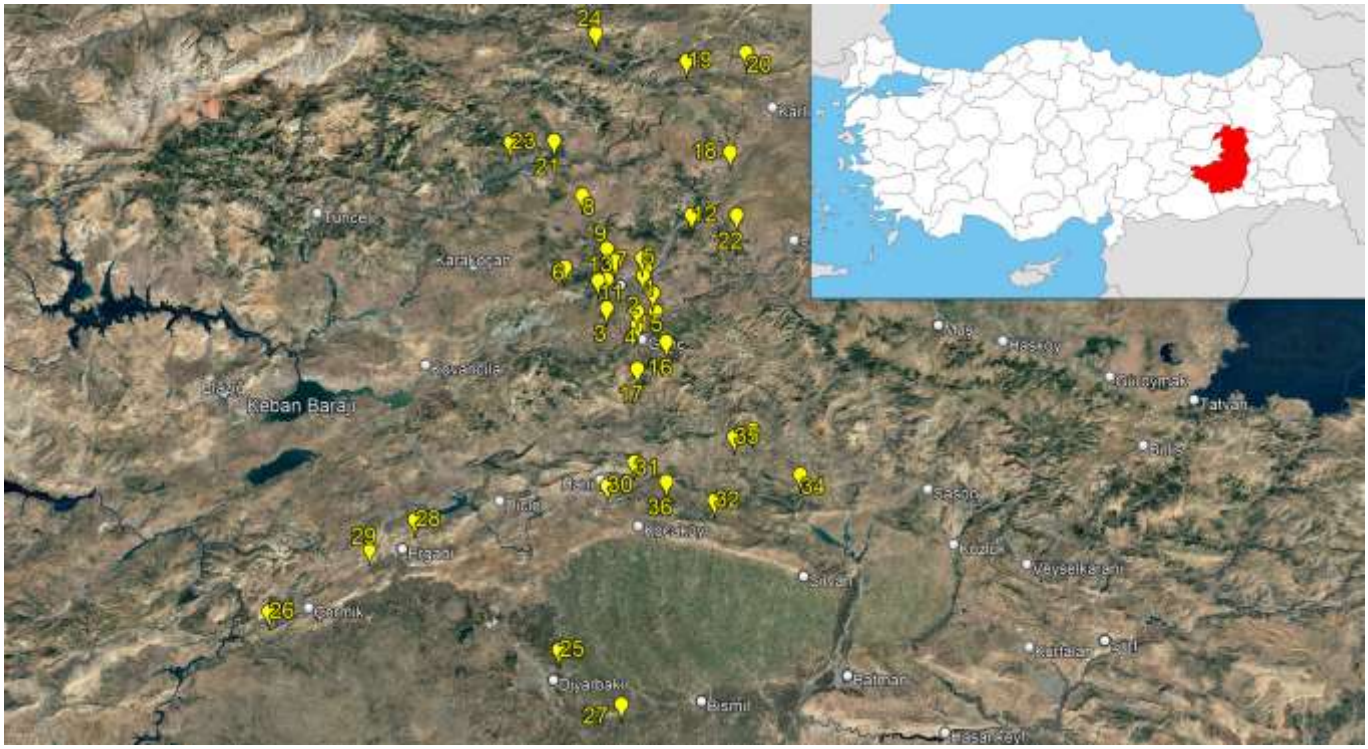

Figure 1. Sampling sites in Bingöl and Diyarbakır.
Şekil 1. Bingöl ve Diyarbakır'da örnek toplanan lokaliteler.

The province has seven districts outside the city center, namely Adaklı, Genç, Karlıova, Kiğı, Solhan, Yayladere, and Yedisu. The city center is located on a plain overlooking a branch of the Göynük River, which joins the Murat River near Genç District, in the northwest corner of the Çapakçur plain at an altitude of 1151 meters above sea level. Bingöl, on the Elazığ-Tatvan road, was previously established in the valley here, but because of the rapid development of the city after the 1950s, it was moved to the dominant plain (Anonymous 2025a).

Due to being open to the humid-cool air masses coming from the north and the altitude factor, Bingöl and its surroundings have hot summers and cold winters. According to the data of the General Directorate of Meteorology, the annual average temperature in Bingöl is 12.1 degrees. Annual precipitation is 873.7 mm, the number of days with snowfall is 24.5 days, and the number of days with frost is 94.1 days (Anonymous, 2024a).

Diyarbakır is in the central part of the Southeastern Anatolia Region. It is bordered in the east by Batman and Muş, and in the west by Şanlıurfa, Adıyaman, Malatya, to the north are Elazığ and Bingöl, and to the south are Mardin provinces. It is located on a plain at the eastern edge of the basalt plateau of Karacadağ, about 100 m above the Tigris Valley. It is characterised by a harsh continental climate. Summers are very hot, but winters are not as cold as those in the Eastern Anatolia Region. The main reason for this is that the Southeastern Taurus Mountains block the cold winds coming from the north. The city has an average annual precipitation of 496 millimeters, and 2% of this precipitation falls in the summer months. As you move towards the foothills of the mountains in the north, precipitation increases (Anonymous 2025b).

The steppe is the dominant vegetation in Diyarbakır. Herbaceous plants are more abundant in the steppe vegetation. The surrounding mountains are covered with oak forests in places. The forested areas do not even cover one-tenth of the total surface area of the province (Anonymous, 2024b).

Table 1. Localities' species are collected.
Çizelge 1. Türlerin toplandığı lokaliteler.

Number	Province	District	Locality	Altitude (m)	Cordinates	
1	Bingöl	Bingöl	Celtiksuyu	1015	38° 51′ 10″ N	40° 35′ 10″ E
2	Bingöl	Bingöl	Celtiksuyu	1045	38° 52′ 57″ N	40° 35′ 21″ E
3	Bingöl	Bingöl	Çiçekyayla	1511	38° 49′ 22″ N	40° 27′ 48″ E
4	Bingöl	Bingöl	Garip	992	38° 46′ 50″ N	40° 33′ 17″ E
5	Bingöl	Bingöl	Ekinyolu	1036	38° 54′ 00″ N	40° 34′ 17″ E
6	Bingöl	Bingöl	Kırkağıl	1731	38° 54′ 48″ N	40° 22′ 42″ E
7	Bingöl	Bingöl	Kurudere	1145	38° 54′ 37″ N	40° 28′ 33″ E
8	Bingöl	Bingöl	Sancak	1587	39° 54′ 30″ N	40° 22′ 34″ E
9	Bingöl	Bingöl	Sancaklı	1451	38° 60′ 28″ N	40° 28′ 45″ E
10	Bingöl	Bingöl	Sarıçiçek	1045	38° 53′ 43″ N	40° 35′ 56″ E
11	Bingöl	Bingöl	Üçyaka	1704	38° 50′ 37″ N	40° 27′ 11″ E
12	Bingöl	Bingöl	Yukarıağaçeli	1425	38° 58′ 41″ N	40° 42′ 36″ E
13	Bingöl	Bingöl	Yukarıpınar	1470	38° 51′ 11″ N	40° 28′ 70″ E
14	Bingöl	Genç	Ardıçdibi	1091	38° 46′ 28″ N	40° 36′ 54″ E
15	Bingöl	Genç	Derenköy	1363	38° 45′ 30″ N	40° 40′ 80″ E
16	Bingöl	Genç	Doğanca	1164	38° 42′ 51″ N	40° 32′ 44″ E
17	Bingöl	Genç	Yayla Bucağı	1345	38° 38′ 18″ N	40° 31′ 41″ E
18	Bingöl	Karlıova	Kalencik	1770	39° 90′ 14″ N	40° 45′ 70″ E
19	Bingöl	Karlıova	Kaynarpınar	1767	39° 23′ 2.8″ N	40° 45′ 42″ E
20	Bingöl	Karlıova	Viranşehir	1843	39° 22′ 41″ N	40° 57′ 56″ E
21	Bingöl	Kiğı	Demirkanat	1289	39° 13′ 30″ N	40° 19′ 55″ E
22	Bingöl	Solhan	Hazarşah	1313	38° 58′ 27″ N	40° 35′ 25″ E
23	Bingöl	Yayladere	Güneşlik	1371	39° 12′ 10″ N	40° 10′ 43″ E
24	Bingöl	Yedisu	Karapolat	1440	39° 26′ 55″ N	40° 29′ 30″ E
25	Diyarbakır	Diyarbakır	Yukarıkılıçtaşı	597	37° 56′ 49″ N	40° 14′ 54″ E
26	Diyarbakır	Çermik	Göktepe	716	38° 50′ 34″ N	39° 22′ 31″ E
27	Diyarbakır	Çınar	Yuvacık	558	37° 48′ 56″ N	40° 25′ 15″ E
28	Diyarbakır	Ergani	Yakacık	888	38° 15′ 58″ N	39° 50′ 50″ E
29	Diyarbakır	Ergani	Pınarkaya	860	38° 14′ 56″ N	39° 42′ 50″ E
30	Diyarbakır	Hani	Çardaklı	805	38° 20′ 27″ N	40° 22′ 37″ E
31	Diyarbakır	Hani	Serenköy	817	38° 24′ 10″ N	40° 30′ 14″ E
32	Diyarbakır	Hazro	Ormankaya	995	38° 17′ 55″ N	40° 46′ 50″ E
33	Diyarbakır	Kulp	Güllük	862	38° 28′ 80″ N	40° 53′ 54″ E
34	Diyarbakır	Kulp	İnkaya	789	38° 20′ 54″ N	41° 20′ 51″ E
35	Diyarbakır	Kulp	Zeyrek	864	38° 28′ 60″ N	40° 51′ 31″ E
36	Diyarbakır	Lice	Savat Bucağı	925	38° 17′ 55″ N	40° 46′ 50″ E

Collection and identification

The present study is based on 37 braconid specimens collected around *Euphorbia macroclada* in different areas of Bingöl and Diyarbakır provinces using a sweep net in the period of 2017-2023 (Figure 1, Table 1). The specimens were prepared for examination and identification. For identification to the subfamily level, we use van Achterberg's key (1993). For the generic and specific levels, we used Nixon (1986), Simbolotti & van Achterberg (1999), Sharkey et al. (2009), van Achterberg & Long (2010), van Achterberg (2011), Edmardash & Gadallah (2023), Shaw et al. (2022) [Agathidinae], Quicke (1987), Beyarslan & Fischer (1990), Tobias (1995), Beyarslan et al. (2006, 2008), Quicke et al. (2022) [Braconinae], Aydoğdu (2008), Edmardash & Gadallah (2019), Ranjith & Priyadarsanan (2023) [Cheloninae], van Achterberg & Haeselbarth (2003) [Euphoridae], Fernandez-Triana & van Achterberg (2017), Shaw et al. (2024) [Microgastrinae], van Achterberg (2023) [Opiinae]; van Achterberg & Shaw (2016), van Achterberg et al. (2020) [Rogadinae]. Distribution of species is based on Yu et al. (2016) and Gadallah et al. (2022).

List of abbreviations: AS = Abdominal sternites, 1CU1 = first abscissa of cubital vein, 2CU1 = second abscissa of cubital vein, cu-a = cubito-anal transverse vein, 1-M = first abscissa of medial vein, 1r-m = radio-medial transverse vein, 1-SR+M = first abscissa of sectio-radial vein amalgamated with median vein, 2-SC+R = second abscissa of sectio-radial vein amalgamated with radial vein, 3-SR = third abscissa of sectio-radial vein, M+CU (=M+CU1) = medial vein amalgamated with cubital vein, MOD = Maximum diameter of lateral ocellus, OOL = ocular-ocellar length, r = first abscissa of radial vein, r-m = radio-medial transverse vein, T = abdominal tergites.

RESULTS

In total, there are 19 braconid species in 11 genera and seven subfamilies: Agathidinae (one genus, one species), Braconinae (five genera, seven species), Cheloninae (one genus, five species), Euphorinae (one genus, one unidentified species), Microgastrinae (one genus, one species), Opiinae (one genus, one species), and Rogadinae (one genus, three species, of which one is unidentified). A faunistic list comprising the species, previous records from Türkiye, and extralimital distribution is provided for each species. All specimens are deposited in the Efflatoun Bey Collection, Cairo University, Faculty of Science, Entomology Department (EFC) (Cairo, Egypt) and Bingöl University, Agriculture Faculty, Department of Plant Protection (Bingöl, Türkiye).

List of species

Subfamily Agathidinae

Agathis anglica Marshall, 1885

Agathis anglica Marshall, 1885: 265, ♀, ♂.

Material examined: 1♀, Bingöl: Kurudere [38° 54′ 37″ N, 40° 28′ 33″ E], 1145 m, 7.v.2023, sweep net, leg. Emin Kaplan.

Previous records from Türkiye: Imbros & Tenedos Islands (Beyarslan et al., 2002a); Eastern Anatolia (Çetin Erdoğan, 2013), Diyarbakır, Mardin, and Şanlıurfa provinces (Çetin Erdoğan, 2014), Marmara region (Çetin Erdoğan & Beyarslan, 2001), East Black Sea region (Çetin Erdoğan & Beyarslan, 2009), Erzurum Province (Güçlü & Özbek (2002), Central Anatolia Region (Çetin Erdoğan & Beyarslan, 2016), Aegean region, Southeastern region (Çetin Erdoğan et.al, 2009)

Extralimital distribution: Egypt (Edmardash & Gadallah, 2023), Morocco, Europe, Thailand, Ukraine (Shaw et al., 2022).

Comments. *Agathis anglica* was previously recorded from Diyarbakır by Çetin Erdoğan (2013). First record for Bingöl and in association with *Euphorbia macroclada* Boiss.

Subfamily Braconinae

Bracon (*Bracon*) *intercessor* Nees, 1834

Bracon intercessor Nees, 1834: 71, ♀, ♂.

Material examined: 1♂, Bingöl: Sancak [39° 54′ 30″ N, 40° 22′ 34″ E], 1587 m, 29.v.2023, sweep net, leg. Emin Kaplan.

Previous records from Türkiye: Adana, Antalya, Burdur, Hatay, Isparta, Içel, Kahramanmaraş (Beyarslan, 1986), Adapazarı, Afyonkarahisar, Antalya, Bilecik, Bursa, Çanakkale, Edirne, Gaziantep, Istanbul, Izmit, Kırklareli, Tekirdağ (Beyarslan & İnanç, 1995), Aegean region (Beyarslan et al., 2002b), Haymana (Güler & Çağatay, 2007), East Black Sea (Beyarslan & Cetin Erdogan, 2010), Erzurum (Beyarslan, 1999; Güçlü & Özbek, 2011), Çanakkale (Beyarslan et al., 2002a), Ganos Mountains (Beyarslan et al., 2006), Izmir Province (Civelek et al., 2002), North-eastern Anatolia (Beyarslan, 2016), Northern Türkiye (Beyarslan et al., 2008), South-eastern Anatolia (Beyarslan et al., 2014), Western Black Sea region (Beyarslan et al., 2005), Bitlis (Beyarslan & Şahin, 2019).

Extralimital distribution: Afghanistan, Europe, Iran, Israel/Palestine, Kazakhstan, Russia, Syria, Turkmenistan.

Comment. This is the first record of *B. intercessor* in association with *E. macroclada* Boiss.

Bracon (*Glabrobracon*) *lividus* Telenga, 1936

Bracon lividus Telenga, 1936: 390, ♀.

Material examined: 1♀, Bingöl: Genç, Derenköy [38° 45′ 30″ N, 40° 40′ 80″ E], 1363 m, 8.v.2022, sweep net, leg. E. Kaplan; 1♀, Bingöl: Kırkağıl [38° 54′ 48″ N, 40° 22′ 42″ E], 1731 m, 27.v.2022, sweep net, leg. E. Kaplan; 1♂, Bingöl: Yukarıağaçeli [38° 58′ 41″ N, 40° 42′ 36″ E], 1425 m, 31.v.2023, sweep net, leg. E. Kaplan; 1♀, Bingöl: Karlıova, Kalencik [39° 90′ 14″ N, 40° 45′ 70″ E], 1770 m, 6.vi.2023, sweep net, leg. E. Kaplan.

Previous records from Türkiye: Bitlis (Beyarslan & Şahin, 2019), Marmara, Mediterranean regions of Türkiye (Beyarslan, 1999), North-eastern Anatolia (Beyarslan, 2016), East Black Sea region (Beyarslan & Çetin Erdoğan, 2010), Imbros & Tenedos Islands (Beyarslan et al., 2002a), South-eastern Anatolia (Beyarslan et al., 2014).

Extralimital distribution: Armenia, Cyprus, Germany, Greece, Hungary, Iran, Israel/Palestine, Russia.

Comments. This is the first record of *B. lividus* in Bingöl, and in association with *E. macroclada*.

Bracon (*Glabrobracon*) *variator* Nees, 1811

Bracon variator Nees, 1811: 7, ♀.

Material examined: 1♀, Diyarbakır: Lice, Savat Bucağı [38° 17′ 55″ N, 40° 46′ 50″ E], 925 m, 14.v.2023, sweep net, leg. E. Kaplan.

Previous records from Türkiye: Mediterranean regions of Türkiye (Beyarslan, 1986), Thrakien Gebiet (Beyarslan, 1987), Mediterranean and Maramara regions (Beyarslan, 1999), Ankara Province (Güler & Çatağay, 2001), Aegean region (Beyarslan et al., 2002b), Erzurum (Güçlü & Özbek, 2011), Imbros & Tenedos Islands (Beyarslan et al., 2002a), Western Black Sea region (Beyarslan et al., 2005), Ganos Mountain (Thrace region) (Beyarslan et al., 2006), Northern Türkiye (Beyarslan et al., 2008), East Black Sea region (Beyarslan & Çetin Erdogan, 2010), Fifteen not mentioned localities in Türkiye (Papp, 2012), South-eastern Anatolia (Beyarslan et al., 2014), North-Eastern Anatolia (Beyarslan, 2016), Bitlis Province (Beyarslan & Şahin, 2019).

Extralimital distribution: Azerbaijan, Europe, Iran, Israel/Palestine, Jordan, Kazakhstan, Syria, Turkmenistan.

Comments. *Bracon variator* was previously recorded in Bingöl (Beyarslan & Şahin, 2019), and in association with *E. macroclada*. It was reported on *Euphorbia* sp. as one of its plant associates in Bitlis Province by Beyarslan & Şahin, 2019.

Glyptomorpha (*Glyptomorpha*) *pectoralis* (Brullé, 1832)

Vipio pectoralis Brullé, 1832: 382, ♀, ♂.

Material examined: 1♂, Bingöl: Sarıçıçek [38° 53′ 43″ N, 40° 35′ 56″ E], 1045 m, 16.v.2019, sweep net, leg, E. Kaplan; 1♂, Bingöl: Yayladere, Güneşlik [39° 12′ 10″ N, 40° 10′ 43″ E], 1371 m, 13. vi.2019, sweep net, leg. E. Kaplan; 1♂, Diyarbakır, Çermik, Göktepe [38° 50′ 34″ N, 39° 22′ 31″ E], 716 m, 24.iii.2019; 1♀, Bingöl: Karlıova, Viranşehir [39° 22′ 41″ N, 40° 57′ 56″ E], 1843 m, 1.iv.2019, sweep net, leg. E. Kaplan; 1♀, Bingöl: Çiçekyayla [38° 49′ 22″ N, 40° 27′ 48″ E], 1511 m, 23.v.2019, sweep net, leg. E. Kaplan; 1♂, Diyarbakır: Kulp, İnkaya [38° 20′ 54″ N, 41° 20′ 51″ E], 789 m, 12.iv.2019, sweep net, leg. E. Kaplan; 1♂, Bingöl: Genç, Doğanca [38° 42′ 51″ N, 40° 32′ 44″ E], 1164 m, 16.v.2019, sweep net, leg. E. Kaplan.

Previous records from Türkiye: Adana, Adıyaman, Edirne, Erzurum (Beyarslan, 1991), Mediterranean and Marmara regions (Beyarslan, 1999; Beyarslan et al., 2006), North-eastern Anatolia (Beyarslan, 2016), Marmara (Beyarslan & İnanç, 1994), Aegean region (Beyarslan et al., 2002b), Western Black Sea region (Beyarslan et al., 2005), East Black Sea (Beyarslan & Cetin, 2010), South-eastern Anatolia (Beyarslan et al., 2014), Bitlis Province (Beyarslan et al., 2014; Beyarslan & Şahin, 2019).

Extralimital distribution: Afghanistan, Azerbaijan, Central Asia, China, Croatia, Europe, India, Iran, Israel/Palestine, Kazakhstan, Malaysia, North Africa, Mozambique, Pakistan, Russia, South Africa, Ukraine.

Comments. This is the first record of *G. pectoralis* in association with *E. macroclada.*

Iphiaulax (*Iphiaulax*) *impostor* (Scopoli, 1763)

Ichneumon impostor Scopoli, 1763: 287, sex undetermined.

Material examined: 1♀, Bingöl: Sancaklı [38° 60′ 28″ N, 40° 28′ 45″ E], 1451 m, 12.v.2019, sweep net, leg. E. Kaplan.

Previous records from Türkiye: Bilecik, Belgrader wald (Fahringer, 1922), Imbros & Tenedos (Beyarslan et al., 2002a), Mediterranean and Marmara regions (Beyarslan, 1999), Kars & Erzurum Provinces (Özbek et al., 2009)

Extralimital distribution: Algeria, Azerbaijan, Central Asia, China, Croatia, Europe, Iran, Israel/Palestine, Japan, Korea, Morocco, Russia, Sudan, Ukraine.

Comments. This is the first record of *I. impostor* in association with *E. macroclada*.

Pseudovipio castrator (Fabricius, 1798)

Ichneumon castrator Fabricius, 1798: 223, ♀.

Material examined: 1♂, Bingöl: Üçyaka [38° 50′ 37″ N, 40° 27′ 11″ E], 1704 m, 13. vi.2023, sweep net, leg. E. Kaplan.

Previous records from Türkiye: Adana, Adiyaman, Antalya, Erzurum, Içel, Kırklareli, Tekirdağ-Ganos (Beyarslan, 1991), Amasya (Fahringer, 1922), Artvin (Güçlü & Özbek, 2011), Holzschlägen (Fahringer, 1926), Imbros & Tenedos Islands (Beyarslan et al., 2002a), Marmara (Beyarslan & Inanç, 1994; Beyarslan, 1999), North Türkiye (Beyarslan et al., 2008), North-eastern Anatolia (Beyarslan, 2016), South-eastern Anatolia (Beyarslan et al., 2014), Western Black Sea region (Beyarslan et al., 2005).

Extralimital distribution: Algeria, Azerbaijan, Croatia, Egypt, Europe, Iran, Israel, Palestine, Russia, Sudan, Ukraine.

Comments. This is the first record of *P. castrator* in Bingöl, and in association with *E. macroclada.*

Vipio mlokossewiczi Kokujev, 1898

Vipio mlokossewiczi Kokujev, 1898: 295, ♀.

Material examined: 1♀, Bingöl: Solhan, Hazarşah [38° 58′ 27″ N, 40° 35′ 25″ E], 1313 m, 31.v.2023, sweep net, leg. E. Kaplan.

Previous records from Türkiye: Amasya-Merzifon-Tavsan, Afyon-Bolvadin-Kapaklı, Ordu-Akkus-Yakarıdüğencili, Ordu-Catalpınar (Beyarslan et al., 2008), South-eastern Anatolia (Beyarslan et al., 2014), Bingöl, Diyarbakır (Korkmaz & Kaplan, 2022).

Extralimital distribution: Afghanistan, Azerbaijan, Cyprus, Georgia, Iran, Israel/Palestine, Romania, Tajikistan, Turkmenistan, Uzbekistan.

Comments. This is the second record of *V. mlokossewiczi* from Bingöl and Diyarbakır, and the first record of it in association with *E. macroclada.* It was previously recorded from both provinces by Korkmaz & Kaplan (2022).

Subfamily Cheloninae

Chelonus (*Chelonus*) *elongatus* Szépligeti, 1898

Chelonus elongatus Szépligeti, 1898: 208, ♂.

Material examined: 1♂, Diyarbakır: Yukarıkılıçtaşı [37° 56′ 49″ N, 40° 14′ 54″ E], 597 m, 29. iii 2018, sweep net, leg. E. Kaplan.

Previous records from Türkiye: Istanbul (Aydoğdu & Beyarslan, 2007), Bitlis (Beyarslan et al., 2020).

Extralimital distribution: China, Finland, Germany, Hungary, Iran, Poland, Serbia, Switzerland.

Comments: This is the second record of *C. elongatus* for the Turkish fauna, and the first record from Bingöl in association with *E. macroclada*.

Chelonus (*Chelonus*) *inanitus* (Linnaeus, 1767)

Cynips inanita Linnaeus, 1767: 919, sex undetermined.

Material examined: 1♀, Bingöl: Sancak [39° 50′ 30″ N, 40° 22′ 34″ E], 1587 m, 29.v.2023, sweep net, leg. E. Kaplan; 1♀, Bingöl: Garip [38° 46′ 50″ N, 40° 33′ 17″ E], 992 m, 20.v.2018, sweep net, leg. E. Kaplan; 1♂, Bingöl: Celtiksuyu [38° 51′ 10″ N, 40° 35′ 10″ E], 1015 m, 23.vi.2023, sweep net, leg. E. Kaplan; 1♂, Bingöl: Karlıova, Kaynarpınar [39° 23′ 2.8″ N, 40° 45′ 42″ E], 1767 m, 1.vi.2019, sweep net, leg. E. Kaplan; 1♂, Bingöl: Ekinyolu [38° 54′ 00″ N, 40° 34′ 17″ E], 1036 m, 12.vi.2021, sweep net, leg. E. Kaplan; 1♂, Bingöl: Yedisu, Karapolat [39° 26′ 55″N, 40° 29′ 30″ E], 1440 m, 2.vi.2019, sweep net, leg. E. Kaplan.

Previous records from Türkiye: Eskişehir-Alpu-Karakütük (Aydoğdu, 2017), Iğdır-Merkez (Beyarslan et al., 2020), Imbros & Tenedos Islands (Beyarslan et al., 2002a), Erciyes-Dağı (Kohl, 1905), Eregli (Fahringer, 1922), Marmara (Aydoğdu & Beyarslan, 2002), Giresun-Dereli-Kümbet, Trabzon-Maçka-Ocaklı (Aydogdu & Beyarslan, 2011), Marmara, western and middle Black Sea region (Aydogdu & Beyarslan, 2007).

Extralimital distribution: Algeria, China, Croatia, Egypt, Europe, Iran, Israel/Palestine, Kazakhstan, Korea, Russia, USA (introduced).

Comments. This is the first record of *inanitus* in association with *E. macroclada*.

Chelonus (*Chelonus*) *obscuratus* Herrich-Schäffer, 1838

Chelonus obscuratus Herrich-Schäffer, 1838: 154, ♀, ♂.

Material examined: 1♂, Bingöl: Genç, Yayla Bucağı [38° 38′ 18″ N, 40° 31′ 41″ E], 1345 m, 15.v.2023, sweep net, leg. E. Kaplan.

Previous records from Türkiye: Adana, Antalya, Burdur, Gaziantep, Kahramanmaraş, Icel. Isparta (Beyarslan, 1985), Eskişehir-Sivrihisar-Babadat (Aydoğdu, 2017), Aydin, Denizli (Beyarslan et al., 2002), Bilecik, Kastamonu, Sinop (Aydoğdu & Beyarslan, 2007), Eastern Mediterranean region of Türkiye (Sertkaya & Bayram, 2005), Giresun (Aydoğdu & Beyarslan, 2011), Bingöl (Korkmaz & Kaplan, 2022), Van, Gaziantep (Beyarslan et al., 2020).

Extralimital distribution: China, Egypt, Europe, Israel/Palestine, Kazakhstan, Russia, Tunisia, Ukraine (Yu et al., 2016), Iran (Ameri et al., 2018).

Comments. This is the second record of *C. obscuratus* in Bingöl, and the first record in association with *E. macroclada*. As it was first recorded from Bingöl by Korkmaz & Kaplan (2022).

Chelonus (*Chelonus*) *scabrator* (Fabricius, 1793)

Ichneumon scabrator Fabricius, 1793: 174, sex undetermined.

Material examined: 1♂, Bingöl: Yukarıpınar [38° 51′ 11″ N, 40° 28′ 70″ E], 1470 m, 12. vi.2023, sweep net, leg. E. Kaplan.

Previous records from Türkiye: Bayburt-Konursu, Gümüşhane-Şiran-Karaşeyh (Aydoğdu & Beyarslan, 2011), Central Anatolia (Aydoğdu, 2017), Marmara, Western and Middle Black Sea regions (Aydoğdu & Beyarslan, 2007), Afyon, Bursa, Edirne, Istanbul, İzmit, Denizli, Muğla, Tekirdağ, Uşak (Aydogdu & Beyarslan, 2002), Ganos Mountain (Thrace region) (Beyarslan et al., 2006), Uplands of Türkiye (1000-2000m) (Lozan, 2005).

Extralimital distribution: China, Europe, Iran, Kazakhstan, Russia, Ukraine.

Comments. This is the first record of *C. scabrator* in Bingöl, and in association with *E. macroclada*.

Chelonus (*Microchelonus*) *ibericus* (Tobias, 2001)

Microchelonus (*Microchelonus*) *ibericus* Tobias, 2001:

Material examined: 1♂, Bingöl: Kiğı, Demirkanat [39° 13′ 30″ N, 40° 19′ 55″ E], 1289 m, 29.v.2021, sweep net, leg. E. Kaplan.

Previous records from Türkiye: Ankara (Papp, 2014).

Extralimital distribution: Czech Republic, Spain.

Comments. This is the first record of *C. ibericus* in Bingöl, and in association with *E. macroclada*.

Subfamily Euphorinae

Syntretus sp.

Material examined: 1♂, Bingöl: Celtiksuyu [38° 52′ 57″ N, 40° 35′ 21″ E], 1045 m, 20.v.2023, sweep net, leg. E. Kaplan.

Comments. The genus *Syntretus* is represented in Türkiye by three species: *S. daghestanicus* Tobias, 1976, *S. elegans* (Ruthe, 1856), and *S. ocularis* van Achterberg & Haeselbarth, 2003. But because of the bad condition of the specimen we have, in addition to being a male specimen, we could not identify it to the species level. This is also the first plant association for the genus.

Subfamily Microgastrinae

Microplitis tuberculifer (Wesmael, 1837)

Microgaster tuberculifer Wesmael, 1837: 43, ♂, ♀.

Material examined: 1♂, Diyarbakır: Hazro, Ormankaya [38° 17′ 55″ N, 40° 46′ 50″ E], 995 m, 15.v.2023, sweep net, leg. E. Kaplan.

Previous records in Türkiye: Mediterranean area of Türkiye (Beyarslan, 1988), Istranca Mountains (İnanç & Beyarslan, 2001), Thrace region, Gaziantep and Şanlıurfa (İnanç and Beyarslan, 1997), East Marmara region (İnanç & Beyarslan, 2001), Aegean region (Beyarslan et al., 2002b).

Extralimital distribution: Azerbaijan, China, Croatia, Europe, India, Japan, Kazakhstan, Korea, Kyrgyzstan, Mongolia, Morocco, Russia, Ukraine, Uzbekistan, Vietnam.

Comments. This is the first record of *M. tuberculifer* in Bingöl, and in association with *E. macroclada*.

Subfamily Opiinae

Biosteres (*Biosteres*) *analis* (Wesmael, 1835)

Opius analis Wesmael, 1835: 130, ♀.

Material examined: 1♀, Diyarbakır: Ergani, Yakacık [38° 15′ 58″ N, 39° 50′ 50″ E], 888 m, 24. iii.2019, sweep net, leg. E. Kaplan.

Previous records from Türkiye: Bilecik-Ayvacik (Beyarslan, 2015a).

Extralimital distribution: Europe, Russia.

Comments. This is the first record of *B. analis* in Bingöl, and in association with *E. macroclada*.

Subfamily Rogadinae

Aleiodes aestuosus (Reinhard, 1863)

Rhogas aestuosus Reinhard, 1863: 265, ♀.

Material examined: 1♀, Diyarbakır: Kulp, Zeyrek [38° 28′ 60″ N, 40° 51′ 31″ E], 864 m, 21.v.2017, sweep net, leg. E. Kaplan.

Previous records from Türkiye: no locality cited (van Achterberg et al., 2020).

Extralimital distribution: Afghanistan, Albania, Armenia, Azerbaijan, Bulgaria, Cyprus, Georgia, Greece, Iran, Iraq, Israel/Palestine, Jordan, Russia, Syria, Tunisia, Turkmenistan, Uzbekistan.

Comments. This is the first record of *A. aestuosus* in Bingöl and in association with *E. macroclada.*

Aleiodes schirjajewi (Kokujev, 1898)

Rhogas reticulator var. *schirjajewi* Kokujev, 1898: 299, ♂.

Material examined: 1♂, Diyarbakır: Hani, Serenköy [38° 24′ 10″ N, 40° 30′ 14″ E], 817 m, 14.v.2017, sweep net, leg. E. Kaplan; 1♂, Diyarbakır: Hani, Çardaklı [38° 20′ 27″ N, 40° 22′ 37″ E], 805 m, 14.v.2017, sweep net, leg. E. Kaplan; 1♀, Diyarbakır: Kulp, Güllük [38° 28′ 80″ N, 40° 53′ 54″ E], 862 m, 21.v.2017, sweep net, leg. E. Kaplan; 1♂, Bingöl: Genç, Ardıçdibi [38° 46′ 28″ N, 40° 36′ 54″ E], 1091 m, 26.v.2017, sweep net, leg. E. Kaplan; 1♂, Diyarbakır: Çınar, Yuvacık [37° 48′ 56″ N, 40° 25′ 15″ E], 558 m, 26.iv.2017.

Previous records from Türkiye: East Maramara region (Aydoğdu & Beyarslan, 2006).

Extralimital distribution: Bulgaria, Hungary, Iran, Italy, Kazakhstan, Moldova, Russia, Ukraine.

Comments. This is the second record of *A. schirjajewi* for Türkiye, it was first recorded by Beyarslan (2015) from Marmara, and the first record of it in Bingöl and in association with *E. macroclada*.

Aleiodes sp.

Figures 2 (A-C), 3(A-C), 4(A-C)

Figure 2. *Aleiodes* sp. (male). A, lateral habitus; B, frontal view of head; C, fronto-ventral view of head showing hypoclypeal depression.

Şekil 2. *Aleiodes* sp. (erkek). A, lateral habitus; B, başın önden görünümü; C, hipoklipeal çöküntüsünü gösteren başın fronto-ventral görünümü.

Colour (Figures 2A-C, 2B). Head black (except clypeus, hypoclypeal depression, and mandible reddish); eye and ocelli black, palpi reddish yellow, antenna entirely black; mesosoma dark reddish (except axillae, metanotum, basal half of propodeum, posterior area of mesopleuron, and metapleuron are black); all legs reddish brown (including coxae); metasoma reddish brown, black at apex. Fore wing (Figure 4A) hyaline with black pterostigma and veins, with vein M+CU1 yellowish at base, vein 1-SR+M mostly yellowish; hind wing hyaline, with pale brown veins (Figure 4B).

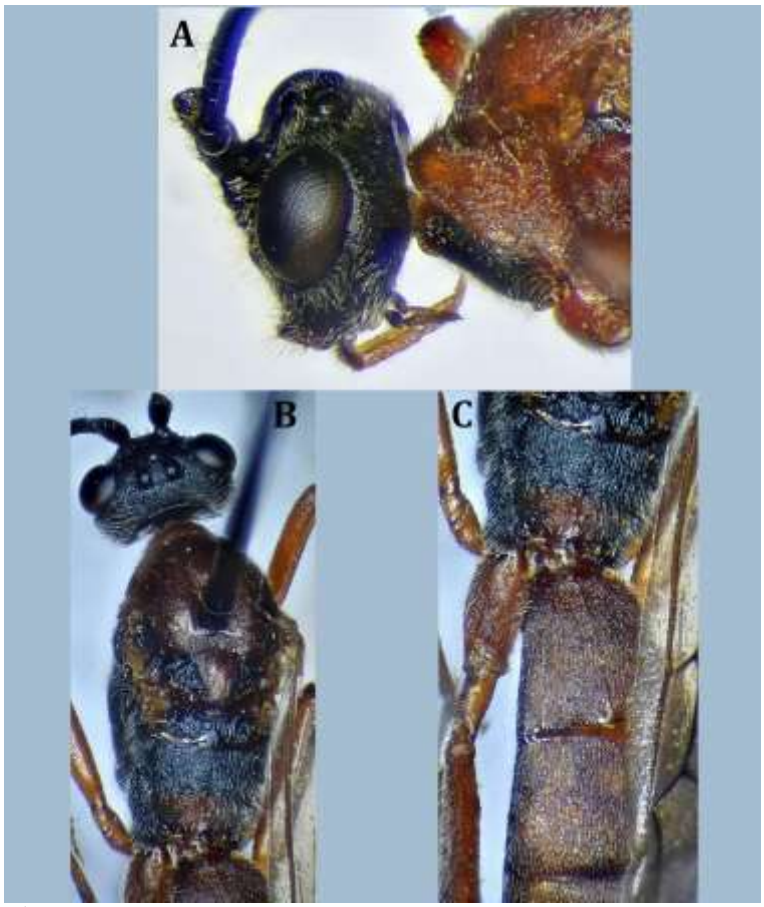

Figure 3. *Aleiodes* sp. (male). A, lateral view of head and part of mesosoma; B, dorsal view of head and mesosoma; C, dorsal view of propodeum and first two metasomal tergites.

Şekil 3. *Aleiodes* sp. (erkek). A, başın ve mesosoma kısmının lateral görünümü; B, baş ve mesosoma kısmının dorsal görünümü; C, propodeumun ve ilk iki metasomal tergitin dorsal görünümü.

Head (Figures 2B, C, 3A, B). Occipital carina complete dorsally and ventrally; antenna with basal segments of flagellum distinctly longer than wide; OOL 1.6× MOD, finely coriaceous-rugulose; vertex rugose; clypeus 5.0× wider than long, ventral margin rather thin, indistinctly protruding medially (when seen in lateral view); width of hypoclypeal depression 0.26× minimum width of face; length of eye 1.3× length of temple in dorsal view; face behind antennal toruli transversely ridged; frons behind posterior ocellus transversely rugose; clypeus located near lower level of eye; malar space 1.3× as long as basal width of mandible; mandible not massive, sickle-shaped, sharply pointed at apex.

Mesosoma (Figures 2A, 3B, C). Lateral side of pronotum rugose; mesoscutal lobes finely punctate, with smooth interspaces; scutellum finely, superficially punctate, without lateral carina; axilla and metanotum coarsely longitudinally ridged; scutellar depression relatively large, densely carinated longitudinally; propodeum longitudinally ridged, more or less smooth posteriorly, with middle longitudinal carina not reaching posterior margin, with lateral complete longitudinal carina; mesopleuron smooth anteriorly and ventrally, remaining longitudinally rugulose; metapleuron coarsely puncto-rugose. *Legs* long and slender; hind coxa large, reaching 3/4th T_1 laterally; hind femur slender, 5.7× as long as its maximum width; hind tibia distinctly long, 12.5× as long as its apical width, distinctly curved along its outer side (except at basal third), with two subequal spurs; hind basitarsus 6.0× as long as wide; inner hind tibial spur 0.3× hind basitarsus; tarsal claws without pecten. *Fore wing* (Figure 4A) densely setose along its whole length, base normally setose; vein r 0.57× vein 3-SR; vein 1CU1 oblique, 0.25× vein 2CU1; vein r-m indistinctly sclerotized, 0.5× vein 3-SR; second submarginal cell relatively long; vein cu-a

inclivous, straight; vein 1-M slightly curved to nearly straight; hind wing (Figure 3B) with marginal cell gradually widened towards apex, its apical width 2.0× as wide as its width at level of hamuli; 2-SC+R subquadrate; vein m-cu entirely absent; M+CU: 1-M= 62: 38; vein 1r-m 0.8× 1-M.

Metasoma (Figs 2A, 4C). Densely finely pubescent, with whitish suberect setae; T_1 0.8× as long as its apical width, T_1 and T_2 longitudinally rugulose, with distinct medio-longitudinal carina; medio-basal area of T_2 wide and short, smooth; T_3 longitudinally rugose, smooth apically.

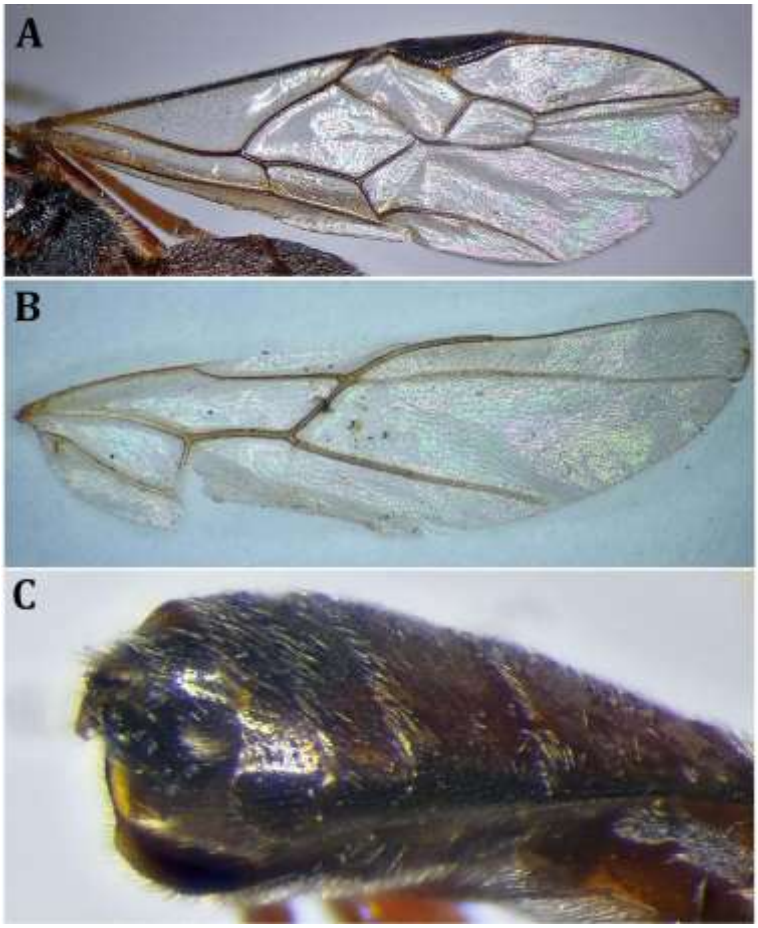

Figure 4. *Aleiodes* sp. (male). A fore wing; B, hind wing; C, lateral view of metasoma (part).
Şekil 4. *Aleiodes* sp. (erkek). A. ön kanat; B, arka kanat; C, metasoma'nın yan görünümü.

Material examined: 1♂, Diyarbakır: Ergani, Pınarkaya [38° 14′ 56″ N, 39° 42′ 50″ E], 860 m, 12.v.2017, sweep net, leg. E. Kaplan.

Remarks. Based on van Achterberg et al.'s key (2020: 19, couplet 3), our specimen agrees with *A. sibiricus* (Kokujev, 1903) in having anterior part of clypeus short and subparallel-sided, present near lower level of eye; hind femur slender; tarsal claws slender, without pecten; temple behind eye densely setose, convex and curved in dorsal view. However, it differs from *A. sibiricus* in having AS5-10 distinctly longer than broad (in *A. sibiricus*, AS5-10 as long as broad). Comparing with *A. sibiricus* description (van Achterberg et al., 2020: 227), it differs from it in the following combination of characters: frons coarsely transversely rugose (Figure 2B) (in *A. sibiricus*, frons smooth); OOL 1.6× MOD (in *A. sibiricus*, OOL 1.1× MOD); width of hypoclypeal depression 0.26× minimum width of face (in *A. sibiricus*, 0.5×); propodeum longitudinally rugose at base, nearly smooth posteriorly, with lateral protruding carina (in *A. sibiricus*, propodeum densely finely rugose, without lateral protruding carina); vein r of fore wing 0.57× vein 3-SR (in *A. sibiricus*, r 0.3× vein 3-SR); vein r-m 0.5× 3-SR (in *A. sibiricus*, r-m 0.7× 3-SR); hind femur 5.75× as long as its width (in *A. sibiricus*, hind femur 4.1× as long as wide); length of inner hind tibial spur 0.3× hind basitarsus (in *A. sibiricus*, 0.4×); T_1, T_2 and basal half of T_3 longitudinally striated, with distinct medio-longitudinal carina (in *A. sibiricus*, T_1 and T_2 densely finely rugose, but irregularly rugose on posterior quarter of T_2, both with indistinct medio-longitudinal carina, basal half of T_3 finely rugose); second submarginal cell of fore wing not short (in *A. sibiricus*, second submarginal cell of fore wing short).

Based on van Achterberg & Shaw (2016: 13, couplet 3), our specimen belongs to *Aleiodes apicalis* group for the following combination of characters: apical half of hind wing marginal cell gradually widened towards apex; metasomal T_2 with wide smooth, triangular area mediobasally; occipital carina reduced ventrally, not reaching hypostomal carina; and mesopleuron partly smooth and shiny. It is likely an undescribed species, but because the specimen is without incomplete antennae and the claws of the hind leg are missing, we have refrained from allocating a species name until more specimens are collected.

DISCUSSION

Taxonomic and faunistic knowledge of the braconid wasps in the Bingöl and Diyarbakır provinces is very poor due to the paucity of regional studies, in addition to some taxonomic complexities in the family Braconidae compared with the well-studied other Turkish provinces. The only recent studies concerning the family Braconidae in these two provinces under study were carried out by some authors (examples of those are Ölmez & Ulusoy, 2003; Çetin Erdoğan, 2013, 2014; Beyarslan, 2015a, b, 2019; Beyarslan & Devici, 2019; Beyarslan & Şahin, 2019; Beyarslan et al., 2020; Korkmaz & Kaplan, 2022).

In the present study, 19 braconid species in 11 genera and seven subfamilies (Agathidinae, Braconinae, Cheloninae, Euphorinae, Microgastrinae, Opiinae, and Rogadinae) are examined and identified using suitable, available keys, as well as by comparing with the original descriptions as closely as possible. All of the identified species are first recorded in association with *Euphorbia macroclada*, only *Bracon variator* was previously recorded in association with an unidentified *Euphorbia* sp. in Bingöl (Beyarslan & Şahin, 2019). On the other hand, all of them are recorded for the first time for Bingöl or Diyarbakır, except for four braconid species: *Agathis anglica* Marshall (Agathidinae) (Çetin Erdoğan, 2014), *Bracon variator* Nees (Beyarslan & Şahin, 2019), and *Vipio mlokossewiczi* Kokujev (Korkmaz & Kaplan, 2022) (Braconinae), and *Chelonus obscurus* Herrich-Schäffer (Cheloninae) (Korkmaz & Kaplan, 2022).

Based on van Achterberg & Shaw (2016), this single *Aleiodes* specimen is found to belong to the *Aleiodes apicalis* group for the following reasons: apical half of hind wing marginal cell gradually widened towards apex; metasomal T_2 with a wide, smooth, triangular area mediobasally; occipital carina reduced ventrally, not reaching hypostomal carina; and mesopleuron partly smooth and shiny. Despite the possibility of intraspecific variation within species of this genus, on comparing this *Aleiodes* specimen precisely with other western Palaearctic species of the *apicalis* group (based on van Achterberg et al., 2020), it does not agree with any of them, so it is likely an undescribed species. But because of the bad condition of the specimen, we have refrained from allocating a species name until more specimens are collected.

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Author's Contributions

The authors declare that the contributions of the authors are equal.

Conflict of Interest Statement

There is no conflict of interest between the authors.

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New Ant (Hymenoptera: Formicidae) Species Discovery for Türkiye: *Emeryopone loebli* (Baroni Urbani, 1975)

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ABSTRACT
Emeryopone loebli (Baroni Urbani, 1975) is recorded in Türkiye for the first time in two different localities. These records are the fourth and northernmost documentation of the species. One of these two records was recorded by citizen scientists, thus showing the importance of citizen science.

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Türkiye İçin Yeni Karınca (Hymenoptera: Formicidae) türü keşfi: *Emeryopone loebli* (Baroni Urbani, 1975)

ÖZET
Emeryopone loebli (Baroni Urbani, 1975) Türkiye'den ilk kez iki farklı lokaliteden kaydedilmiştir. Bu kayıtlar türün dördüncü ve en kuzeydeki dokümantasyonudur. Bu iki kayıttan biri vatandaş bilim insanları tarafından kaydedilmiş olup, vatandaş biliminin önemini göstermektedir.

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INTRODUCTION

The beginning of studies on the ant fauna of Türkiye dates back more than 170 years (Rigler, 1852). However, despite the contribution of studies of the following period, the most comprehensive data on the country's actual ant biodiversity and species richness originated about five decades ago. Among these studies, the first ant checklist of the country published by Kiran & Karaman (2012) reported 306 taxa (286 species and 20 subspecies). This study presented a figure indicating the number of known ant species from each province, whose analysis clearly showed the incompleteness of this knowledge, especially regarding faunal details in the eastern and southeastern parts of the country. Based on the most recent data on findings of native and foreign researchers, it is currently known that 385 taxa represent the ant fauna of Türkiye. The relatively significant increase in a short period of about ten years is remarkable, but it is clear that there is still a notable lack of data, although the deficiency in the eastern and southeastern parts of the country has decreased to some extent (Kiran & Karaman, 2021).

Therefore, during the research of the ant fauna of Kahramanmaraş and Adıyaman provinces, in order to fill the gap in the knowledge on the ant biodiversity of southeastern Türkiye, a new genus and species, *Emeryopone loebli* (Baroni Urbani, 1975), belonging to the subfamily Ponerinae Lepeletier de Saint-Fargeau, 1835, was identified. It is the very first record of this species for Türkiye, and also the very first nest-based record of the species globally.

MATERIAL and METHOD

During a field study in the Adıyaman province of Türkiye, a thorough sampling was performed from various microhabitats such as under stones, on the ground, in rock cracks, dead wood, on tree trunks and twigs, etc. Ant samples were collected using a direct sampling method via an aspirator and (for preservation before preparations and identifications in the laboratory) put in tubes containing 96% ethanol. The initial evaluation of the samples revealed the presence of *Emeryopone* Forel, 1912. The specimens, considered to be *E. loebli,* were prepared for microscopic evaluation and were identified using the original description of the species, color photos of the species, and the identification key of Varghese (2006). Digital photographs of the specimens were taken using a Nikon D800e camera attached to 3.2× and 8× microscope objectives. The Helicon Focus software (Helicon Soft Ltd., Kharkiv, Ukraine) was used to stack the photographs. The distribution map of the species was prepared with Google Earth. The material is deposited in the Entomological Museum of Trakya University (EMTU), Edirne, Türkiye.

RESULTS and DISCUSSION

Emeryopone loebli (Baroni Urbani, 1975) (Figure 1A-C)

Material: 12 workers, **TÜRKİYE-Adıyaman**-Derinsu Vill.; 37°52.094' N, 38°24.381' E; 911 m a.s.l.; 30.v.2024; leg. Karaman, C. & Aksoy, V.; EMTU, 24/0851. 2 workers, **Gaziantep**-Şahinbey; 37°00.2677' N, 37°21.1167' E; 900 m a.s.l.; 30.iv.2024; leg. Söylemez, T.B. & Özalp, K.; EMTU.

Remarks: The genus *Emeryopone* was first described by Forel in 1912 from Indonesia (Sumatra). Today, the genus is represented by five species (*E. buttelreepeni* Forel, 1912; *E. franzi* Baroni Urbani, 1975; *E. loebli*, *E. melaina* Xu, 1998; *E. narendrani* Varghese, 2006) distributed in Israel, Iran, Saudi Arabia, India, Nepal, Southern China, Indonesia, and Malaysia (Schmidt & Shattuck, 2014; Khalili-Moghadam et al., 2023). Among these five species, *Emeryopone loebli* is the only one with a distribution in the Palaearctic region (Figure 2).

Emeryopone loebli was first described from Israel by Baroni Urbani in 1975 as *Belonopelta loebli* Baroni Urbani, 1975, which is also a member of the subfamily Ponerinae. The first record of the species is based on four workers, which were collected from an arid habitat characterized by *Eucalyptus* sp. and *Opuntia* sp. (Baroni Urbani, 1975). Collingwood (1985) recorded the species, with a single worker, from Saudi Arabia, which was collected from a habitat characterized by palm. The third record of the species was from Iran by Khalili-Moghadam et al. (2023). Khalili-Moghadam et al. (2023) also recorded a single worker in a rotting oak log from a habitat characterized by oaks. In the present study, the species was recorded from a 25-30-year-old, humid oak forest in Adıyaman and from a barren land in Gaziantep. These two recent records correspond to the northernmost record of the species, showing that the species is more widespread than is known.

The petiole of the specimen on which the Iranian record of Khalili-Moghadam et al. (2023) was based is conspicuously longer not only than the specimens included in this study but also than the paratype of the species, which can be reached on www.antweb.org with the number CASENT0915184. The Iranian record is based on only one worker, making it unclear if this longer petiole corresponds to a variation in the population of the species or if the record belongs to a different species. The Turkish records are morphologically similar to the paratype of the species except for the ommatidia number. Turkish samples have four ommatidia, while it was reported in the original description of the species that the type has only one ommatidium (Baroni Urbani, 1975). The different numbers of ommatidia of the ant material obtained in this study from the type material are thought to be a variation within the species. More records and specimens are needed to make a definitive judgment about the diversity and abundance of the species in the western Palearctic region. However, it can be said from the records in this study that the species may be much more widespread in the region and is still waiting to be discovered.

The record of *E. loebli* from Gaziantep is based on a record by citizen scientists. Such a record of an *Emeryopone* specimen, which is very rare and hard to find, highlights the importance of the work done by amateurs, in addition to professionals. It is believed that citizen scientists can detect more new records, especially in the eastern and southeastern regions of Türkiye, where the ant fauna has not been extensively studied.

Figure 1. *Emeryopone loebli* worker. A. Head, B. Habitus, C. Dorsal view.
Şekil 1. Emeryopone loebli *işçisi. A. Baş, B. Habitus, C. Üstten görünüm.*

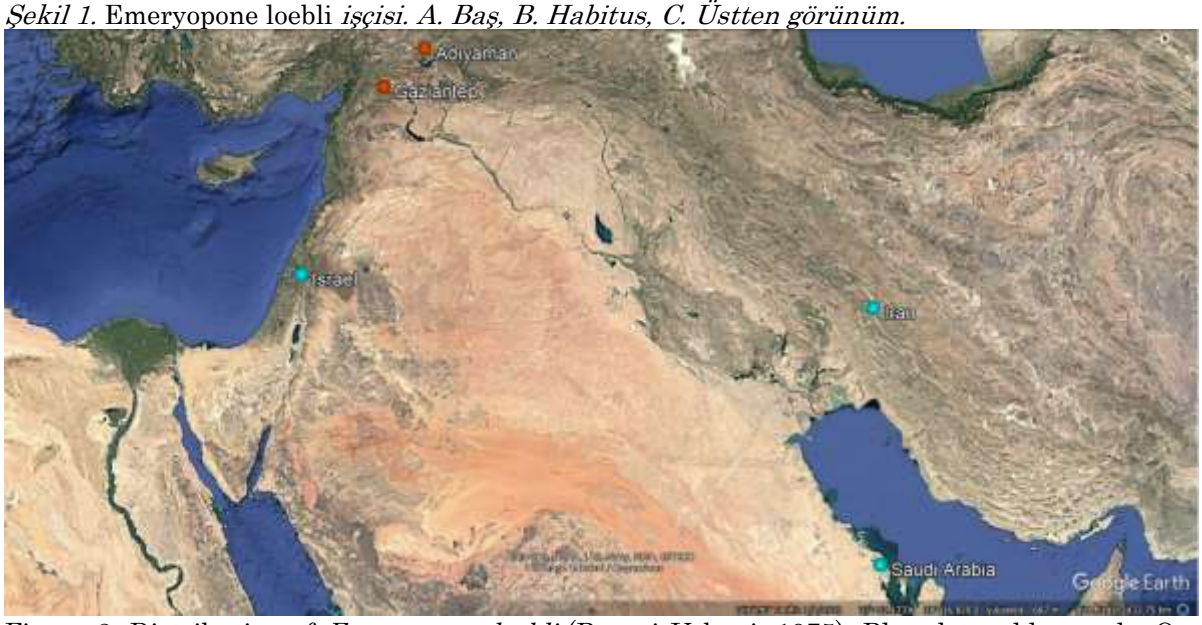

Figure 2. Distribution of *Emeryopone loebli* (Baroni Urbani, 1975). Blue dots: old records, Orange dots: recent records of the species.
Şekil 2. Emeryopone loebli *(Baroni Urbani, 1975)'nin dağılımı. Mavi noktalar: türün eski kayıtları, Turuncu noktalar: türün son kayıtları.*

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Contribution Rate Statement Summary of Researchers

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

Conflict of Interest

Authors have declared no conflict of interest.

Ethic Statement

The authors declare that they have complied with Research and Publication Ethics in their work.

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Mortality Effect of *Origanum syriacum L.* (Lamiaceae) and *Satureja montana L.* (Lamiaceae) Essential Oils on *Pieris brassica L.* (Lepidoptera: Pieridae)

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ABSTRACT

Cabbage, *Brassica oleracea* var. *capitata* L. (Brassicaceae), is one of the most widely consumed vegetables worldwide, and one of its most significant pests is *Pieris brassicae* L. (Lepidoptera: Pieridae). In recent years, the use of plant essential oils in pest control has significantly increased as an alternative control method. This study investigates the lethal effects of essential oils obtained from *Origanum syriacum* (Lamiaceae) and *Satureja montana* (Lamiaceae) on *P. brassicae* larvae. The main components of the essential oil of *O. syriacum* were identified as o-cymene (39.77%), carvacrol (28.16%), and thymol (12.78%), while the essential oil components of *S. montana* were determined as carvacrol (50.03%), o-cymene (15.12%), and γ-terpinene (13.02%). The essential oils were applied to different developmental stages (L1-L5) of *P. brassicae* using a spraying technique at various concentrations (5-10-20-50-100 µL mL^{-1}). The experiment was designed with 10 replicates, each containing 10 larvae from different developmental stages of *P. brassicae*, with water used as the control. To determine larval mortality rates, dead and living larvae were counted at 24-hour intervals for a total of 72 hours. The results showed that both *S. montana* and *O. syriacum* essential oils had a lethal effect ranging from 50% to 100%. However, *S. montana* essential oil was found to be more effective against *P. brassicae* larvae than *O. syriacum* essential oil.

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Origanum syriacum L. (Lamiaceae) ve *Satureja montana* L. (Lamiaceae) Uçucu Yağlarının *Pieris brassicae* L. (Lepidoptera: Pieridae) Üzerindeki Ölüm Etkisi

ÖZET

Lahana, *Brassica oleracea* var. *capitata* L. (Brassicaceae), tüm dünyada en fazla tüketimi yapılan sebzelerden birisi olup, en önemli zararlılarından birisi de *Pieris brassicae* L. (Lepidoptera: Pieridae)'dir. Son yıllarda zararlılarla mücadelede bitkisel uçucu yağların kullanılması alternatif mücadele yöntemi olarak önemli derecede artış göstermiştir. Bu çalışmada *Origanum syriacum* (Lamiaceae) ve *Satureja montana* (Lamiaceae) bitkilerinden elde edilen uçucu yağların *P. brassicae* larvaları üzerindeki ölüm etkisi araştırılmıştır. *O. syriacum* bitkisinin uçucu yağının ana bileşenleri o-cymene (%39.77), carvacrol (%28.16), ve thymol (%12.78) olarak belirlenmişken, *S. montana'* nın uçucu yağ bileşenleri carvacrol (%50.03), o-cymene (%15.12) ve γ-Terpinene (%13.02) olarak belirlenmiştir. Uçucu yağlar, *P. brassicae*'nin (L1-L5) dönemlerine, farklı konsantrasyonlarda (5-10-20-50-100 µL mL-1) spreyleme tekniği kullanılarak uygulanmıştır. Deneme 10 tekerrür olacak şekilde kurulmuş, her tekerrürde *P. brassicae*'nin farklı larva dönemlerinden 10'ar adet larva ile kontrol olarak su kullanılmıştır. Larvaların ölüm oranını belirlemek için toplamda 72 saate kadar 24 saatlik aralıklarla ölü-canlı sayımı yapılmıştır. Hem *S. montana* hem de *O. syriacum* uçucu yağlarının %50-%100 oranlarında ölüm etkisine sahip olduğu, ancak *S. montana* uçucu yağının *P. brassicae* larvaları üzerinde *O. syriacum* uçucu yağından daha etkili olduğu tespit edilmiştir.

Entomoloji

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Kekik
Geyik otu
Carvacrol
Bitkisel insektisit

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INTRODUCTION

Cabbage, *Brassica oleracea var. capitata* L. (Brassicaceae), is one of the most favorable vegetables in this group because it can be grown in almost any region. While Türkiye ranks 10th in world cabbage production, China is first and is followed by India, Russia, South Korea, and Japan (FAO, 2020). Türkiye produces approximately 965 tons of leaf cabbage annually (TÜİK, 2022).

Most of the common pests of cabbage belong to the order Lepidoptera. These are the tobacco caterpillar *Spodoptera litura* (F.), cabbage semi-looper *Trichoplusia ni* (Hübner), the cotton leafworm, *Spodoptera littoralis* (Boisduval), gram pod borer, *Helicoverpa armigera* Hbn. (Lepidoptera: Noctuidae); diamondback moth, *Plutella xylostella* L. (Lepidoptera: Plutellidae); cabbage leaf webber *Crocidolomia binotalis* Zell., the cabbage webworm, *Hellula undalis* (F.) (Lepidoptera: Pyralidae), cabbage butterfly *Pieris brassicae* L., small cabbage white butterfly, *Pieris rapae* L., (Lepidoptera: Pieridae), and the others as follows, cabbage aphid *Brevicoryne brassicae* L. (Hemiptera: Aphididae), painted bug *Bagrada hilaris* (Burmeister) (Hemiptera: Pentatomidae), flea beetle (Coleoptera: Chrysomelidae), *Liriomyza brassicae* Riley (Diptera: Agromyzidae) and the mustard sawfly, *Athalia lugens proxima* Klug (Tenthredinidae: Hymenoptera) (Ulusoy and Ölmez Bayhan, 2006; Kaya and Kornoşor; 2008; Ahuja et al., 2012; Diome et. al., 2019).

In regions where Brassicaceae vegetables are grown, synthetic pesticides are used intensively without tolerance to pest problems. Unfortunately, using chemicals to control pests does not always succeed. These conditions have a direct impact on human health, wildlife, the environment, and so on. For pests, it causes considerable problems (Long, 1987) and leads to the build-up of pest resistance to pesticides. These crops, which are harvested or placed on the market after intensive spraying, cause many negative effects on human beings in cases of chronic poisoning. For these reasons, the application of biological control methods, especially for vegetable pests, is being extensively studied in developed countries, and "Integrated Pest Management" is being adopted individually for each crop (Bayhan et al., 2002; Akdağcık., 2010; Paudel et al., 2022; Bayhan et al., 2020). Furthermore, the use of plant extracts and Essential oils (EOs) as, one of the alternative control options for both disease and pest control has increased significantly over recent years (Walia et al., 2017; Kaleeswaran et al., 2019; Mondédji, et al., 2021; Bahadirli, 2022; Çeliktaş et al., 2022; Erdogan Eliuz and Bahadirli, 2022).

The Lamiaceae family plays an important role in the food industry, cosmetics, medicine, pharmacology, and lately, the agrochemical industry. The genus *Origanum* L., which has 44 species and 19 hybrids, grows widely, especially in the Mediterranean basin (Duman et al., 1998; Dirmenci et al., 2018). Flora of Türkiye is very rich in terms of *Origanum* species, with 23 species including eight hybrids; furthermore, six species naturally occur in Hatay province. *Origanum syriacum* L. is a perennial herbal medicine, distributed in Lebanon, Syria, Jordan, Palestine, Palestine and Türkiye (Meyers, 2005). *O. syriacum* has been collected from flora to be used as a flavoring spice, fresh herb, folk-medicine applications, and an additive in other herbal products (Atar and Colgecen, 2020). The genus *Satureja* has 200 species that are distributed natively in the Middle East and Europe (Chorianopoulos et al., 2004; Macelli et al., 2020). According to recent studies, *Satureja* species from Türkiye has been increased to 17 and 7 of them are endemic (Dirmenci et al., 2019; Duman et al., 2023). *S. montana* is a perennial shrub, native to Albania, Austria, France, Greece, Italy, Spain and Yugoslavia (Anonymous, 2023). *S. montana* has been used in folk medicine, pharmacology, flavoring agent in foods (Redzic, 2006; Ćopra-Janićijević et al., 2020).
The objective of the study is to evaluate mortality activity of the essential oils of *O. syriacum* and *S. monatana* on the larvae of *P. brassicae* under laboratory conditions.

MATERIAL ve METHOD

Origanum syriacum plants were in full flowering period from Yayladağı-Hatay province of Türkiye in July in 2021. *O. syriacum* plant samples were air-dried at room temperature for a week. *S. montana* EO's were hydro-distilled from aerial parts of the plants in a Clevenger-type apparatus for three hours. *S. montana* essential oil was obtained from the company Dropena, Hatay, Türkiye. Both EOs were kept in amber vials at 4^0C until analysis.

Mass breeding of test insects

Pieris brassicae caterpillars were collected from cabbage fields in Adana province. Mortality activity tests were

carried out in the Vegetable and Ornamental Plant Pests Laboratory of the Plant Protection Department of Çukurova University. EOs were obtained from the Hatay Mustafa Kemal University, Department of Field Crops, Medicinal Aromatic Plants Laboratory.

Pieris brassicae were grown under laboratory conditions according to the standard methods described by (Firake et al. (2017). Infested plants and/or larvae were kept under optimal conditions (25±2°C temperature, 120 µmolm−2.s−1 light density, 70±5% relative humidity, and 16:8, light: dark period) to obtain the next-generation larvae to be used in this study. The taxonomic description of the pest was performed by the first author.

Gas Chromatography-Mass Spectrometry (GC-MS)

The essential oil composition was determined with GC-MS (Agilent 7890A-5975C) equipped with an HP5-MS 19091S-433 model column (30m X 250 µm film X 0.25 µm). Helium was a carrier gas. The detector transfer line temperature was 260 °C, and the detector ionization temperature was 250 °C. The analysis program was as follows: starting with 60 °C increased to 150 °C with 5 °C min-1, from 150 to 200 °C with 1.5°C min-1, from 200 °C to 240°C with 4 °C min-1. The EO components were defined using Wiley and NIST databases.

Mortality effects of *S. montana* and *O. syriacum* essential oils on *P. brassicae* larvae by spray assay

The EOs were evaluated against the second and/or third instar larvae of *P. brassicae* utilizing spraying. The bioassay of the plant extracts' essential oils (EOs) was carried out against the caterpillars of *P. brassicae* following a procedure similar to that described by Çeliktaş et al. (2022). Each treatment was replicated 10 times, and each replicate contained 10 larvae at a different stage and a control (water). Observations on the mortality of the larvae were made at 24-hour intervals for up to 72 hours. Mortality rates were estimated using the Abbott formula. Therefore, *S. montana* and *O. syriacum* were evaluated for their insecticidal potential against *P. brassicae* larvae (Fig 1).

Fig 1. General views after application *Origanum syriacum* essential oil to the living *Pieris brassicae* larvae on fresh cabbage leaves

Şekil 1. Origanum syriacum uçucu yağının taze lahana yaprakları üzerindeki canlı Pieris brassicae larvalarına uygulanması sonrasındaki genel görünümü

Application of Essential Oils against *P. barassicae* larvae

Essential oil solutions (5-10-20-50-100 µL mL^{-1} concentrations) were prepared with Tween 80 and applied on

caterpillars which were grown on the cabbage three times. For this purpose, square Petri dishes (diameter 100 Ø) including 1.5% prepared on water agarose and a broad cabbage leaf were placed in as infection atmosphere in it. In addition, 10 larvae, which were L1-L5 stage**,** were infected with a watercolor brush. Essential oil solutions were sprayed on Petri dishes three times with equal flow caps. The edges of Petri dishes were covered with parafilm and placed into climate cabinets (Nüve TK 120). Dead individuals were checked at 24, 48 and 72 h after oil application.

Statistical analyses

The data was analyzed by Abbott tests (Abbott, 1925), and interactions within applications and application times were determined by Analysis of Variance (ANOVA). These tests were performed by using SPSS Statistics 23.0 (IBM Corporation, New York, NY, USA).

RESULTS and DISCUSSION

In this study, the mortality effect of EOs obtained from *Satureja montana* and *Origanum syriacum* plants were tested on *Pieris brassicae* larvae. The chemical composition of *O. syriacum* and *S. montana* EOs were determined with GC/MS method (Table 1). EO analysis for *O. syriacum* has resulted in 18 components with a total of 93.47%. *S. montana* EO contained 31 components with a total of 99.68%. The main components of *O. syriacum* were *σ*-cymene (39.77%), carvacrol (28.16%), and thymol (12.78%), while in *S. montana* EO carvacrol (50.03%), o-cymene (15.12%) and *γ-Terpinene* (13.02%).

Table 1. The essential oil composition of Origanum syriacum and Satureja montana (%)
Çizelge 1. Origanum syriacum ve Satureja montana uçucu yağ bileşenleri (%)

#	RT	CAS-Number	Compound Name	*O. syriacum*	*S. montana*
1	5.89	002867-05-2	*α*-Thujene	1.02	1.55
2	6.08	000080-56-8	*α*-Pinene	*n.d.*	1.12
3	6.50	000079-92-5	Camphene	0.17	0.55
4	7.33	000127-91-3	*β*-Pinene	0.06	0.27
5	7.52	053907-72-5	1-Octen-3-ol	1.54	1.14
6	7.79	000123-35-3	*β*-Myrcene	0.47	1.76
7	8.23	000099-83-2	l-Phellandrene	0.09	0.28
8	8.41	013466-78-9	*δ*-3-Carene	0.06	0.10
9	8.63	000099-86-5	*α*-Terpinene	0.92	1.96
10	8.96	000527-84-4	*σ*-Cymene	**39.77**	**15.12**
11	9.05	000535-77-3	Cymol	0.52	0.94
12	9.12	000470-82-6	1.8-Cineole	*n.d.*	1.03
13	10.13	000099-85-4	*γ*-Terpinene	6.50	**13.02**
15	11.16	006728-26-3	2-Hexanal	0.11	0.11
16	11.64	000586-62-9	*α*-Terpinolene	*n.d.*	1.21
17	14.03	000507-70-0	Borneol	0.65	1.87
18	14.30	998016-25-4	2-Isopropylfuran	*n.d.*	0.12
19	14.46	000562-74-3	Terpineol	0.04	1.13
20	15.07	000470-08-6	*β*-Fenchyl alcohol	*n.d.*	0.25
21	18.98	000089-83-8	Thymol	**12.78**	0.11
22	19.40	003228-02-2	Carvacrol	**28.16**	**50.03**
25	21.76	005208-59-3	*β*-Bourbonene	*n.d.*	0.15
26	22.93	998193-98-7	Trans *β*-Caryophyllene	0.04	2.78
27	23.25	023986-74-5	Germacrene-D	*n.d.*	0.11
28	23.58	000489-39-4	Aromandendrene	*n.d.*	0.11
29	24.06	006753-98-6	*α*-Humulene	*n.d.*	0.84
30	24.81	000483-75-0	Naphthalene	*n.d.*	0.19
33	25.85	000495-61-4	*β*-Bisabolene	*n.d.*	0.82
34	26.04	000483-75-0	Naphthalene	*n.d.*	0.32
35	26.31	000483-76-1	*δ*-Cadinene	*n.d.*	0.35
36	28.25	000499-75-2	Caryophyllene oxide	1.13	0.34
Total				**93.47**	**99.68**

n.d.= not detected

Several studies show EO variation of *O. syriacum* according to the locality, plant parts, genetics, and ecology. *O. syriacum* samples from Egypt in different studies showed that the main components of EO carvacrol, thymol, and/or γ-terpinene (Fleisher & Fleisher, 1991; Baser et al., 2003; Viuda-Martos et al., 2010; Gendy et al., 2015). Alma et al. (2003) found EO main components of *O. syriacum* from Hatay, Türkiye as γ-terpinene (27.79%), carvacrol (26.97%), *p*-cymene (15.69%), and *β*-caryophyllene (12.59%). In addition to that, EO composition from Amman has resulted that carvacrol (41.1%), *p*-cymene (30.22%), γ-terpinene (4.27%), and *cis*-sabinene hydrate (3.22%) being the main components (Al-Kalaldeh et al., 2010). Furthermore, *p*-cymene was found as a main component of *O. syriacum* EO from Lebanon (El-Alam et al., 2019). Similar to that result, *p*-cymene, thymol, and carvacrol were found to be the most abundant components in Lebanon *O. syriacum* (Shehadeh et al., 2019). *S. montana* was found to have high genetic variability with 26 subspecies (Čopra-Janićijević et al., 2020). Compounds of *S. montana* from Bosnia-Herzegovina resulted in linalool and *α*-Terpineol being most abundant in steam distillation while linalool, cis-sabinene hydrate, and *p*-cymene were most abundant in headspace sampling (Čopra-Janićijević et al., 2020). Like this results, carvacrol was found at 45 % in *S. montana* samples from Croatia (Bezic et al., 2005). In addition, carvacrol was found at 1.1% in *S. montana* EO from Montenegro. These studies clearly show that *S. monatana* has great variation in terms of EO composition.

The efficacy of essential oils dissolved in Tween 80 on insect mortality is shown in Tables 2 and 3. The general view of these experiments after application is shown in Figure 2. The larvae at all stages (L1-L5) were randomly placed in Petri dishes, and it was determined that the highest mortality in *P. brassicae* larvae occurred in the second and third stages (L2-L3), while there was no mortality in the last two larval stages (L4-L5). Individual effects and interactions of concentrations and exposure duration are found to be significant on insect mortality by comparing them with the control during EO applications. Significant differences were obtained for inter-concentrations and an increase in exposure duration ($p<0.05$). The statistical analysis also showed that *O. syriacum* and *S. montana* EOs have different effects on *P. brassicae,* and *O. syriacum* essential oils' mortality activity results are given in Tables 2 and 3. All EO applications showed higher effects on *P. brassicae* larvae than the control. The mortality averages ranged between 0.0644 to 1.5708 in all EO applications. The mortality activity of *O. syriacum* EOs showed more variation according to different hours and inter-concentration concentrations compared to *S. montana* EOs. The higher concentrations, 50% and 100%, showed the highest mortality activity, therefore, it could be said that 50% with the same effect should be preferable. When the application hours increased, the accumulated amount, their impact was reduced to low doses. *S. montana* EOs' mortality activity is shown in Table 3. The results showed that 50 % and 100% of applications' mortality activity was not changed according to hours.

Figure 2. General view of dead Pieris barassicae larvae on cabbage leaves after applying essential oils at different concentrations

Şekil 2. Farklı konsantrasyonlardaki uçucu yağ uygulamasından sonra lahana yaprakları üzerindeki ölü Pieris barassicae larvalarının genel görünümü

In this study, which evaluated the efficacy of *O. syricaum* application on *P. brassicae* larvae in terms of concentration and time, it was found that the most effective concentrations over 24 hours were 50% and 100%, with no statistical difference between these two concentrations. In the 48-hour period, there were no statistical differences between the 20%, 50%, and 100% concentrations. However, in the 72-hour application, all concentrations were effective, and there was no statistical difference between them except the control application (Table 2).

When the effect of *S. montana* essential oil was investigated on *P. brassicae* larvae, similar results were observed of *O. syriacum* essential oil. No statistical difference was observed between the 50% and 100% concentration applications after 24, 48, and 72 hours. For this essential oil, the most effective treatment, like with *O. syriacum* essential oil, was found as a 50% concentration applied over a 24-hour period (Table 3).

Table 2. Mortality activity of different consantration of Origanium syriacum essential oil against Pieris brassicae larvae

Çizelge 2. Origanium syriacum esansiyel yağının farklı konsatrasyonlarının Pieris brassicae larvalarına karşı ölüm aktivitesi

Concentrations	Average Deaths		
	24h	48h	72h
Control	0.1893±1.00d*	0.2536±0,113c	0.2536±0,104b
% 5	0.6532±0.329c	0.8937±0.586b	1.1902±0.80a
% 10	0.8850±0.126c	1.0541±0.586b	1.3362±0.80a
% 20	1.1746±0.126b	1.3696±0.837a	1.4545±0.80a
% 50	1.4413±0.958a	1.4877±0.837a	1.5320±0.332a
% 100	1.5368±0.958a	0.0000±0.113a	0.0000±0.104a

*Results were given as averages±SE (standard error). F (2,60)= 5.00, p<0.01 Data in the same column were lettered.

Table 3. Mortality activity of different concentration of Satureja montana essential oil against Pieris brassicae larvae

Çizelge 3. Satureja montana esansiyel yağının farklı konsantrasyonlarının Pieris brassicae larvalarına karşı ölüm aktivitesi

Concentrations	Average Deaths		
	24h	48h	72h
Control	0.0644±1.00c*	0.1429±1.00c	0.1429±0.530d
% 5	0.7189±0.167b	0.8918±0.99b	1.1444±0.83c
% 10	0.7939±0.167b	0.9570±0.99b	1.2574±0.83b
% 20	0.9268±0.167b	1.1141±0.99b	1.3733±0.83b
% 50	1.2667±0.108a	1.4009±0.582a	1.5368±0.379a
% 100	1.4923±0.108a	1.5368±0.582a	0.0000±0.530a

*Results were given as averages±SE (standard error). F (2, 60)= 5.00, p<0.01 Data in the same column were lettered.

In recent years, there has been a rapid increase in bioinsecticide studies on economically important lepidopteran and other insect species. Although the mortality rates of 10 different EOs on *Cydalima perspectalis (*Walker) (Lepidoptera: Crambidae) at different larval stages. Greatly vary the highest effect on the 2nd and 5th instar larvae was obtained by using the EO of *Origanum onites*, with a mortality rate of 80.0-71.6%. The results confirmed that the EOs from *O. vulgare* can be used in the control against *C. perspectalis* (Göktürk et al., 2020). In this study, both EOs were found to be most effective during the L2-L3 stages, while, in contrast, the Göktürk et al. (2020) study found that they had no lethal effect on the L5 stage larvae. Besides, these studies, the EO of *O. syriacum* was evaluated for insecticidal activity against adults of *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) and *Rhyzopertha dominica* (F.) (Bostrychidae) by the fumigation method. The essential oil showed an excellent fumigant effect on *R. dominica* than *S. oryzae* at 48 h and 72 h. The authors concluded that *O. syriacum* EO has the potency to be a natural insecticide (Karan et al., 2018) Our results were also indicating that *O. syriacum* was effective at 50% dose EO on *P. brassicae* at 24 hours, like the Karan et al. (2018).

Satureja essential oil was assessed against a diverse group of insects from Coleoptera to Diptera, Hemiptera, Homoptera, Lepidoptera, Phthiraptera, and Thysanoptera orders, and similarly, on other arthropods, including mites and ticks, and plant pathogenic nematodes. Among the large species of *Satureja* studied, the EOs of *S. hortensis, S. montana*, and *S. thymbra* are considered the most promising species in pest management (Ebadollahi et al., 2021; Valcárcelet al., 2021). It can be seen that successful results have been obtained in various studies with the aqueous suspension of *S. montana* on a variety of organisms. *S. montana* EO showed significant larvicidal activity fourth stage larvae of the common house mosquito (*Culex pipiens* L.) (Diptera: Culicidae) (Michaelakis et al., 2007). Likewise, it was found to be a potential nematicide against *Bursaphelenchus xylophilus* Nickle, and 100% mortality was observed 24 hours after application (Barbaso et al., 2012). Complete repellency (100%) against western flower thrips (*Frankliniella occidentalis*) (Pergande) (Thysanoptera: Thripidae) treated at 2.0 % concentration was obtained after 1 hour on green bean leaves in petri dishes (Picard et al. 2012), and high larvicidal efficacy was also observed against *Culex quinquefasciatus* third instar larvae (Benelli et al., 2017). In a study of the contact assay (topical application) against the fruit fly *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae), a high level of toxicity was found in both sexes (Park et al., 2016). In the contact test against *Leptinotarsa decemlineata* Say. (Coleoptera: Chrysomelidae), a high mortality rate was observed in the first (100 %), second (97.7 %), third (95.5 %), and fourth (97.7 %) larvae and in the 96-h adult (88.8 %) at the concentration of 20 L/cm^2 after 96 h (Usanmaz-Bozhuyu, 2018). *Satureja montana* was detected as both ixodicidal agents and

insecticidal (Valcárcel et al,. 2021). In addition, *Origanum* species, with carvacrol being one of the most important compounds responsible for the larvicidal effects on *Anisakis simplex* (Nematelmintos: Anisakidae) (Lopez et al., 2019).

Beyond the essential oils tested in this study, various botanical insecticides have been tested on different lepidopteran species or *P. brassicae* larvae for pest control purposes (Ali et al., 2017; Hossain et al., 2020; Kardian, 2021). Two botanical insecticides, an aqueous extract of tobacco leaves (*Nicotiana tabacum*) and tuber roots (*Derris elliptica*), were tested for their mortality (contact and residual) and feed reduction impacts against the fall armyworm, *Spodoptera frugiperda*. These insecticides have the potential to be used by farmers in the field as they are easy to prepare and use (Kardian-Maris, 2021). In addition, the effects of several commonly used botanicals (Mahogany seed kernel, tobacco leaf, garlic, neem leaf, neem seed kernel extracts, neem oil, and control) on the main lepidopteran pests found in summer cabbage have been investigated. It has been reported that neem oil is the most effective of the other botanical insecticides in use, in terms of many of the factors which have been studied. So, botanicals could be used sensibly to improve the production of summer cabbage, which would be safe for farmers and the environment (Hossain et al. 2020). There are promising results when looking at current studies on *P. brassicae*. The efficacy of various new synthetic (thiamethoxam 25% SP, acetamiprid 20% SP, and pyriproxyfen 10.8% EC) and botanical insecticides *Aloe vera* (L.) Burm. f. leaves, grapefruit (*Citrus* × *paradisi* Macfad.) bark, spearmint (*Mentha spicata* L.) leaves, and neem (*Azadirachta indica* A. Juss.) leaves have been studied on cabbage butterflies in terms of larval feeding, behavior, development, and mortality. The neem extract (7%) combined with pyriproxyfen also caused significant larval stress. Thus, neem extracts at 7% alone can be used to control *P. brassicae* in vegetable crops to ensure a safe food supply (Ali et al., 2017).

Based on the above literature information, it is known that various plants, including neem oil, have been studied in the mentioned research, and some of these have even been converted into commercial products (Isman, 1997; Mordue et al., 1998; Ujvary, 2001). However, as in this study, it is considered important to investigate plants with high concentrations of active compounds in their essential oils, such as *S. montana* and *O. syriacum*, as potential alternatives. This research will provide a diversity of methods for pest control. It is anticipated that these studies will pave the way for future research and contribute to the creation of a database in the field.

According to this study, it was possible to observe different or similar effects in each of the organisms, even though the studies were carried out on different insects. Contrary to the study by Michaelakis et al. (2007), it was found that *S. montana* EO did not have a lethal effect on *P. brassicae* larvae during the L4 and L5 stages; rather, it was ineffective in terms of insecticidal activity. Additionally, although it was effective for a shorter duration and dose compared to the Barbaso et al. (2012) study, it did not achieve the same level of effectiveness in as little as 1 hour or at a very low dose of 2% as observed in the Picard et al. (2012) study. Furthermore, similar to the study by Lopez et al. (2019), it was determined in this study that carvacrol was the most effective component of the *S. montana* essential oil. Eroğlu et al. (2023) in study, three several doses (0.75, 1, and 1.25 mg/mL) of *N. meyeri* extract were given to the third instar larvae using the droplet feeding method. as a result of feeding the larvae with the highest dose of 1.25 mg/mL *N. meyeri* extract, the level of increase in the detoxification genes p450 and udp genes was at the highest level at the 12 hours. The results demonstrate that *N. meyeri* extract (1.25 mg/mL) was determined to be a promising botanical extract for the control of *P. brassicae* larvae. In addition to Zakaria (2016), in this study, the results showed that the yield of essential oil of *Capparis* species leaves from *Damascus* (Syria) province was 0.052 % v/w. Also, these results suggest that Caterpillars of *P. brassicae,* which attack caper it's not harmful for *Capparis* species wild growing in *Damascus*, because they are suppressed by the parasitoid, *Cotesia glomerata*.

CONCLUSION

This study demonstrated that both *Satureja montana* and *Origanum syriacum* essential oils (EOs) exhibit insecticidal activity against *Pieris brassicae* larvae, with *S. montana* EO proving to be more effective. The observed differences in efficacy can be attributed to the chemical composition of the essential oils, particularly the presence and proportions of active compounds such as carvacrol, o-cymene, thymol, and γ-terpinene. While both essential oils share carvacrol and o-cymene as major components, their varying concentrations and additional compounds contribute to their differential effectiveness. The findings of this study provide valuable insights into the potential use of plant-derived essential oils as biopesticides. The laboratory-based results indicate that these essential oils could serve as promising alternatives to synthetic insecticides, offering a more sustainable and environmentally friendly approach to pest management. However, further research is necessary to validate these findings under field conditions. Future studies should focus on optimizing application methods, determining long-term effects on pest populations, assessing the impact on non-target organisms, and evaluating the economic feasibility of large-scale application. Given the increasing concerns about pesticide resistance and environmental contamination caused by chemical insecticides, the development of botanical insecticides derived from essential oils presents a viable solution. The use of *S. montana* and *O. syriacum* essential oils could potentially be integrated into pest

management programs, reducing reliance on conventional pesticides and promoting ecological balance. Overall, this study contributes to the growing body of research on plant-based insecticides and highlights the potential of essential oils in sustainable agriculture. Further interdisciplinary collaboration between entomologists, chemists, and agronomists will be essential in translating these preliminary findings into practical applications for agricultural pest control.

Contribution Rate Statement Summary of Researchers

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

Conflict of Interest

The authors of the article declare that they have no conflict of interest.

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Exogenous Application of SiO_2 Nanoparticles Enhances Drought Stress Tolerance in Wild and Cultivated Chickpea Plants

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ABSTRACT
Drought is one of the most significant stress factors that constrain plant growth. Many studies focused on methods to enhance the plant stress tolerance against drought. Recently, the focus has been on the exogenous applications and, in particular, the nanomaterials powered by advancements in the field of nanotechnology. Silicon appears to support some plants against different stress factors, including drought. Despite this, there is a remarkable lack of studies on the use of silicon for enhancing drought tolerance in wild and cultivated chickpeas. In this study, 150 mg L^{-1} SiO_2 nanoparticle spraying was applied to two chickpea varieties, cultivated and wild, under drought stress. Changes were analyzed in morphological, physiological, and biochemical traits to find the change in plants' drought tolerance. Under drought stress, SiO_2 treatment increased antioxidant enzyme activities in both species. Similarly, nanoparticle treatment increased some growth characteristics of plants. Additionally, significant increases in leaf relative water content were detected in plants treated with SiO_2 under drought conditions. In this study, the effect of SiO_2 nanoparticle application on the stress tolerance of wild and cultivated chickpea plants has been studied. Basically, the results showed that exogenous application of SiO_2 NPs increases drought tolerance by stimulating water status and growth parameters, and by activities of antioxidant enzymes in both wild and cultivated species of chickpea.

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Dışardan Uygulanan SiO_2 Nanopartiküller Yabani ve Kültür Nohut Bitkilerinde Kuraklık Stres Toleransını Arttırmaktadır

ÖZET
Kuraklık, bitki büyümesini sınırlayan en önemli stres faktörlerinden biridir. Birçok çalışma, bitkilerin kuraklığa karşı toleransını artırma yöntemlerine odaklanmıştır. Son zamanlarda ise özellikle nanoteknoloji alanındaki ilerlemelerle güçlenen nanomalzemelerin bitkilere dıştan uygulamalarına odaklanılmıştır. Silikon, kuraklık dahil olmak üzere farklı stres faktörlerine karşı bazı bitkileri destekler gibi görünmektedir. Buna rağmen, yabani ve kültür nohutlarında kuraklık toleransını artırmak için silikon kullanımına dair kayda değer bir çalışma eksikliği vardır. Bu çalışmada, kültür ve yabani olmak üzere iki nohut türünde, kuraklık stresi altında 150 mg/L SiO_2 nanopartikül spreylemesi uygulanmıştır. Bitkilerin kuraklık toleransındaki değişimi belirlemek amacıyla morfolojik, fizyolojik ve biyokimyasal özelliklerdeki değişiklikler analiz edilmiştir. Kuraklık stresi altında, SiO_2 uygulaması her iki türde de antioksidan enzimlerin aktivitelerini artırmıştır. Benzer şekilde, nanopartikül uygulaması bitkilerin bazı büyüme özelliklerini de artırmıştır. Ayrıca, kurak koşullarda SiO_2 uygulanan bitkilerde yaprak oransal su içeriğinde önemli artışlar tespit edilmiştir. Bu çalışmada, SiO_2 nanopartikül uygulamasının yabani ve kültür nohut bitkilerinin stres toleransı üzerindeki etkisi incelenmiştir. Sonuçlar

Fitopatoloji

Araştırma Makalesi

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Anahtar Kelimeler
SiO_2 Nanopartikül
Nohut
Kuraklık Toleransı
Osmotik Stres

temel olarak, SiO_2 NP'lerin dışsal olarak uygulamasının, hem yabani hem de kültür nohut türlerinde su durumu ve büyüme parametrelerini uyararak ve antioksidan enzimlerin aktivitelerini artırarak kuraklığa toleransı artırdığını göstermiştir.

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INTRODUCTION

Chickpea (*Cicer arietinum* L.) is a legume plant that is generally grown under rain-fed conditions in arid and semi-arid regions (Millan et al., 2006). Important abiotic stress factors such as drought limit the expected yield of chickpea, and therefore, many researchers are studying chickpea breeding to manage drought-induced yield losses (Maqbool et al., 2017). Drought stress, triggered by global warming, is causing significant losses in agricultural productivity worldwide. This issue poses a major challenge to agricultural production, as it intensifies with rising temperatures, leading to reduced crop yields and threatening food security on a global scale (Chakraborty & Newton, 2011). Drought stress is one of the most significant challenges to chickpea farming in the changing climate conditions and ever-growing population of the world (Nadeem et al., 2019). It is responsible for an estimated 45-50% reduction in chickpea yields globally (Thudi et al., 2014).

From a plant perspective, drought disrupts photosynthesis due to changes caused in the ultrastructure of cell organelles. Furthermore, alterations in metabolites and enzymes essential for the synthesis and enhancement of photosynthetic products contribute to this physiological impact (Wahab et al., 2022). In the contemporary context, researchers are actively engaged in either developing new plant varieties or exploring applications that induce drought tolerance as part of efforts to address and mitigate the impact of drought (Gaur et al., 2019; Kandhol et al., 2022).

The use of nanoparticles (NPs) represents one of the most promising approaches developed so far in terms of enhancing plant tolerance against drought-caused damage (Kandhol et al., 2022). This strategy has been proven to be very effective in helping plants survive under drought conditions (Ahmed et al., 2021; Kandhol et al., 2022; Mohamed and Abdel-Hakeem, 2023). Nanoparticles have demonstrated potential to interact with plants and other organisms due to their distinctive nano-size, unique physical properties, and high surface area to volume ratio (Dura and Kepenkçi, 2022; Wang et al., 2023). Application of various types of nanoparticles on crop plants was reported to be effective in improving the plant health condition under osmotic stress conditions (Heikal et al., 2023; Mohamed and Abdel-Hakeem, 2023). Among various nanoparticles, the potential of silicon-based nanoparticles has recently been encouraged for assessment in terms of both biotic as well as abiotic stress agents (Du et al., 2022; Wang et al., 2022). Silicon NPs showed significant outcomes in improved growth, decreased water stress, and stimulation of a range of antioxidant enzymes, in addition to greater chlorophyll content and gas exchange attributes that ensured enhanced biomass and crop yield under stress interactions (Raza et al., 2023). Moreover, available empirical evidence suggests that silicon has positive impacts on plant growth. It supports plants in mitigating both biotic and abiotic stresses (Debona et al., 2017; Li et al., 2018). In this context, various studies have reported that silicon nanoparticles are capable of regulating the endogenous concentration of hormones, such as auxins, ABA, IAA, cytokinins, ethylene, gibberellins, and jasmonic acid. Altered phytohormone levels have significant impacts on plant growth, development, and productivity across various organs and tissues (Mukarram et al., 2022). In the same way, SiO_2 NPs control CAT, APX, SOD activities and the AsA-GSH cycle, thus providing enhanced efficiency to the defense system (Fatemi et al., 2021). Recent findings suggest that the use of SiO_2 NPs could exert a notable influence on the drought stress tolerance of maize seedlings. Notably, after SiO2 NPs treatment, the increase in proline levels and reduction in total soluble sugars can significantly contribute to stress tolerance. The findings underscore the potential of SiO_2 NPs in facilitating osmotic adjustment, with specific emphasis on modulating proline accumulation as opposed to the accumulation of soluble sugars. Moreover, the utilization of SiO_2 nanoparticles decreases the malondialdehyde levels when compared to untreated vegetation subjected to drought conditions (Sharf-Eldin et al., 2023).

Research examining the effect of silicon NPs on chickpea plants against abiotic stress conditions has indicated the potential applicability of these nanoparticles in cultivation. Silica nanoparticles mitigate aluminum-induced injuries in *Cicer arietinum* by inhibiting cytotoxic agents and upregulating protective genes (Chandra et al., 2020). Particularly, considering cross-pollination and genetic exchanges between wild varieties and cultivated varieties

of chickpea plants, positive effects on stress tolerance have been observed (Coyne et al., 2020). Recent experiments indicate that applying conventional Zn+Si fertilizer to the leaves can mitigate the damage caused by osmotic stress in chickpeas. This protective effect is mainly due to the activation of adaptive mechanisms, such as the increased accumulation of compatible solutes and improved scavenging of reactive oxygen species (ROS) (Mohamadzadeh et al., 2023; Zahedi et al., 2023).

Although there are several studies about the effects of silicon on the physiology and growth of cultivated chickpeas, limited information on this substance under osmotic stress situations exists in the case of the wild relative (*Cicer reticulatum* L.) of the species. This study tests exogenous application of SiO_2 nanoparticles in two lines of chickpea cultivars, the cultivated variety, *Cicer arietinum*, and the wild type, *Cicer reticulatum*-under controlled drought stress. The objective is to determine the directional changes in osmotic stress tolerance within both species as a result of the application and subsequently conduct a comparative analysis. This literature review indicates that this is the first study to investigate and compare the effects of SiO_2 nanoparticles on drought-induced stress tolerance in both wild and cultivated chickpea plants.

MATERIAL and METHOD

Plant Material and Growth Conditions

Cicer arietinum ILC-482 and *Cicer reticulatum* AWC-611 seeds were used for the experiments. In order to surface sterilize the seeds, they were initially washed with 70% ethanol and surface sterilized with 1% NaOCl and washed with sterile water five times. Chickpea seeds were placed between sterile papers in petri dishes, with 10 seeds in each dish. Once seedlings germinated and radicles elongated to about 5 cm, they were transferred to pots prepared in a hydroponic setup in oriented Hoagland solution. Plants were grown in a growth room chamber kept at a constant temperature of 25±1°C. It was set on a long day photoperiod cycle of 16:8 hours light: dark with 300 µmol $m^{-2}s^{-1}$ light density. There was a cultivation for 21 days over the environment set at a relative humidity of 65-70%.

SiO_2 NPs Treatment

In this study, commercially purchased SiO_2 NPs (Sigma Aldrich) were used, and the concentration of NPs (150 mg/L) was determined according to Yıldız (2018). After 21 days of growth, chickpea plants were sprayed with SiO_2 NPs every day for three days (three times total). Adjustments were made to ensure that each spray application delivered 20 mL of SiO_2 NP solution per plant. After a total of three spray applications, the SiO_2 NPs were uniformly distributed over the plant shoot tissues, resulting in a total deposition of 9 mg of SiO_2 NPs per plant. Untreated control groups were sprayed with pure water that did not contain nanoparticles. At this stage, the nutrient solution's surface was carefully covered to prevent the nanoparticles from mixing with the solution where the roots are located. Figure 1 presents representative images of the plants grown for the experiment.

Drought Stress Treatments

After being grown for a total of 3 weeks, SiO_2 NPs spraying was performed for three consecutive days at the same time each day. Twenty-four hours after the final spray application, the plants were transferred to a PEG 6000 solution to induce drought stress. The final concentration of PEG6000 (20%) was added to Hoagland solutions containing the roots of the plants. The concentration of PEG6000 was determined based on a study related to chickpeas by Kumar et al., (2019). Plants without PEG treatment served as drought control groups. The overall condition of the plants was monitored following PEG6000 application. When the plants in the drought groups lost turgor in their leaves, the experiment was terminated. After drought stress, plant biomass data and leaf relative water content were calculated, and plants were harvested and stored. Measurement methods are given in detail in subsequent sections. Plant leaves were rapidly harvested, ground with liquid nitrogen, and then stored at -80 °C until the day of analysis.

Determination of plant biomass

The length of the shoot was measured by using a ruler from the root collar to the tap. The fresh weights of the shoot and root were recorded, and then the samples were dried in an oven at 80°C and weighed again, with the results determined in grams.

Water status of the leaves

Harvested plant leaves were taken in equal numbers, weighed on a precision scale, and their fresh weights were recorded (FW). Subsequently, these leaves were immersed in pure water at 25°C for 4 hours to become turgid. After ensuring turgidity, excess water on the leaves was removed with the help of a tissue, and they were weighed

again to record turgid weights (TW). The leaf samples, whose weights were determined, were then dried in an oven at 65°C for 48 hours, and the dry weight was recorded as 'g' (DW). The relative water content of leaves (%) was calculated by ratioing the obtained fresh and dry weights using the following equation 1 (Smart & Bingham, 1974):

$$\textbf{Relative water content} = \frac{(\boldsymbol{FW - DW})}{(\boldsymbol{TW - DW})}\ \boldsymbol{x}\ \mathbf{100} \quad (1)$$

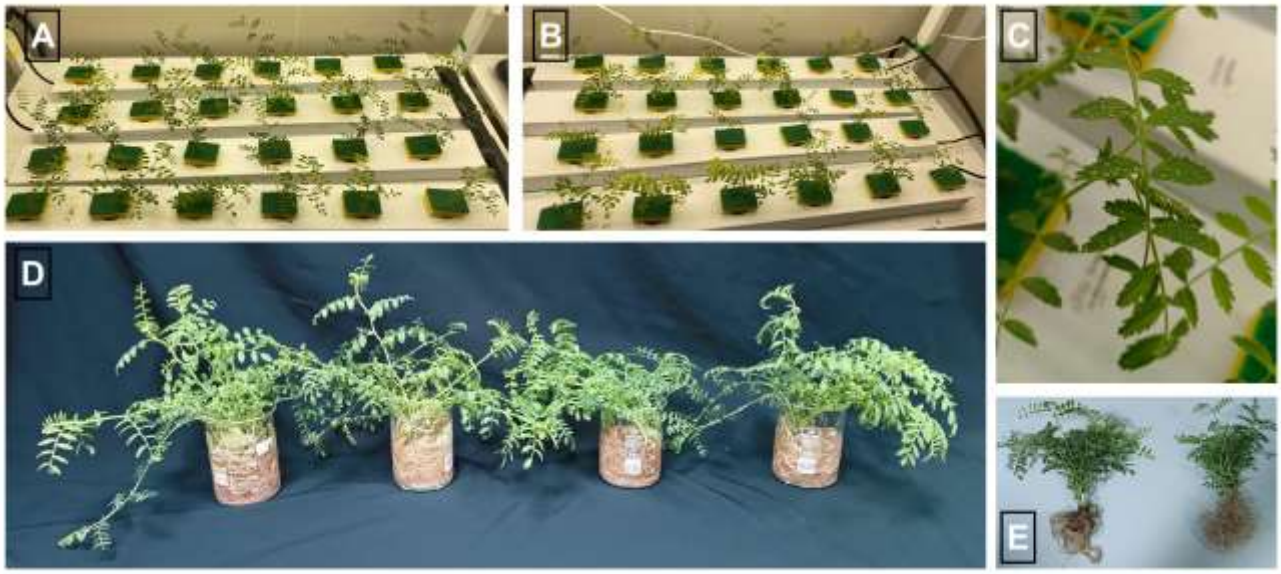

Figure 1. Growth and development of plants in a hydroponic system (A, B), close-up view of a plant leaf showing sprayed SiO_2 nanoparticle (C), Progression of plant growth for *C. reticulatum* and *C. arietinum* over time in the hydroponic setup, showing increased foliage and plant height (D). Root systems of the plants removed from the hydroponic setup (E).

Şekil 1. Hidroponik sistemde bitkilerin büyümesi ve gelişimi (A, B), SiO2 nanopartikülü püskürtülmüş bir bitki yaprağının yakın plan görüntüsü (C), hidroponik sistemde zamanla artan yaprak yoğunluğu ve bitki boyunu gösteren C. reticulatum ve C. arietinum bitkilerinin büyüme ilerlemesi (D). Hidroponik sistemden çıkarılan bitkilerin kök sistemleri (E).

Estimation of free proline content from leaves

The free proline content was analysed based on the Bates et al. (1973) method with minor modifications. The procedure involved homogenizing 0.5 g of leaf tissue in 10 mL of 3% sulfosalicylic acid, followed by centrifugation. A 2 mL filtrate was combined with 2 mL of acetic acid and 2 mL of acid-ninhydrin. The mixture was boiled at 95°C for 1h. After the boiling, the reaction was stopped by placing the tubes on ice. Finally, toluene (4 mL) was added to the mixture, and the upper phase was measured at 520 nm using a spectrometer. The results were quantified in µmol proline/g FW by constructing a standard curve.

Lipid peroxidation levels of leaves

The malondialdehyde (MDA) level was determined to assess membrane damage and lipid peroxidation resulting from drought stress and/or SiO_2 NPs treatments. After the experiment, 0.2 g of fresh leaf tissues were rapidly frozen using liquid nitrogen and ground into a fine powder. Leaf powders were mixed in a trichloroacetic acid (5%, TCA) solution. The mixture was then subjected to centrifugation for 15 minutes at 12,000 g. Following centrifugation, the supernatant was carefully combined with equal volumes of 0.5% thiobarbituric acid (TBA) and 20% TCA. The mixture was then boiled at 95 °C for 25 minutes. After the centrifugation process, the final supernatant was spectrophotometrically read at 532 and 600 nm. MDA concentrations were quantified using an extinction coefficient of 155 $mM^{-1}\,cm^{-1}$. The results were reported in nmol MDA per gram of fresh weight (Ohkawa et al., 1979).

Determination of Catalase Enzyme Activity

Catalase enzyme activity was assessed in accordance with the methodology described by Aebi (1984). Leaf tissues (one g) were mixed in 5 mL of potassium phosphate buffer (pH: 7 and 0.1 mM EDTA), with the addition of 100 mg

polyvinylpyrrolidone (PVP). The reaction was initiated by combining 2.8 mL of potassium phosphate buffer (pH: 7, without EDTA), 80 µL of H_2O_2 (0.5M), and 120 µL of enzyme extract. Catalase activity was determined by monitoring the reduction in absorbance within a 30-second interval at 240 nm. The results were quantified as H_2O_2 min^{-1} g^{-1} FW.

Statistical analysis

All statistical analyses were conducted with Minitab 18 software. The Shapiro-Wilk test was employed to test the normality and homogeneity of variance in the data. The examination of the impact of SiO_2 treatments on the studied analysis profile of chickpea plants was conducted through two-way analysis of variance (ANOVA) followed by Fisher's LSD post hoc multiple comparisons test. The data presented in this study were validated through a minimum of two independent experiments.

RESULTS and DISCUSSION

Effect of SiO_2 NPs on Chickpea Biomass

It was found that biomass parameters of *C. arietinum* were higher than those of *C. reticulatum*. PEG-induced drought stress diminished substantially both shoot and root fresh and dry weights in *C. arietinum,* while in the case of *C. reticulatum* these decreases were very slight. On the other hand, no remarkable change in shoot length as a result of drought stress was seen in either of these species. Figure 2A shows that under drought conditions, the fresh weights of plants treated with SiO_2 were significantly higher compared to those treated with H_2O, but with no major significant differences between SiO_2 and H_2O treatment under well-watered conditions in both *Cicer arietinum* and *Cicer reticulatum*. The SiO_2-treated plants under drought conditions in the case of *Cicer arietinum* show only a slightly higher shoot dry weight than the plants treated with H_2O. However, under well-watered conditions, there is no significant difference between SiO_2 and H_2O treatments. In the case of *Cicer reticulatum*, no significant differences between SiO_2 and H_2O treatments can be seen for shoot dry weight under both experimental conditions (well-watered and drought) (Figure 2B). Significantly higher values were observed in root fresh weight with the treatment of SiO_2 compared to the treatment of H_2O (Figure 2C). Both *Cicer arietinum* and *Cicer reticulatum*, under all conditions, show no significant difference in root dry weight between SiO_2 and H_2O treatments (Figure 2D). Both *Cicer arietinum* and *Cicer reticulatum*, under all conditions, do not show any significant difference in shoot lengths between SiO_2 and H_2O treatments (Figure 2E). While in the case of *Cicer arietinum*, in the SiO_2-treated plants, the relative water content is considerably higher than that of H_2O-treated plants under well-watered conditions; under drought conditions, the SiO_2 treatment is not significantly different from H_2O treatment. In contrast, in *Cicer reticulatum*, the relative water content is similar between SiO_2 and H_2O treatments when well-watered, while under drought, SiO_2-treated plants exhibit a significantly higher relative water content than H_2O-treated plants of this species (Figure 2F).

The negative effects of drought stress were more pronounced in *C. arietinum* compared to the wild type, however, foliar application of SiO_2 NPs had positive effects on growth parameters for both species under drought. Many studies have shown that drought stress disrupts the intercellular water balance, slows down cell division, reduces photosynthesis efficiency, and, as a result of all these processes, reduces growth and development (Seleiman et al., 2021). The obtained results indicated that exogenous SiO_2 NPs applications reduced these negative effects of drought, and as a result, growth parameters were stimulated in both species. Maintaining growth parameters under drought conditions is crucial for crop yield (Zhang et al., 2022). Several hypotheses have been put forward on how exogenous SiO_2 NPs applications protect plant yield under stress conditions. Among these hypotheses, it is one of the most reported results that photosynthesis rate increases with SiO_2 NPs application, and this stimulates growth parameters under stress conditions (Alharbi et al., 2022; Elshayb et al., 2021; Uddin et al., 2023; Zahedi et al., 2023). Kalal et al., (2022) showed that SiO_2 NPs treatment protected ps1 and ps2 complexes under drought conditions. Considering that these complexes are severely affected by radicals, it can be concluded that the amount of radicals decreased by SiO_2 treatment, and as a result, photosynthetic efficiency was preserved.

Several studies have shown that Si application increases water content under control and drought conditions. In these studies, it was generally stated that Si application decreases the transpiration rate and thus maintains water status, especially under stress conditions (Irfan et al., 2023). In this study, SiO_2 NPs application significantly increased leaf water content in both species. Maintaining water under drought conditions is part of the avoidance strategy and is vital for plants to tolerate drought (Seleiman et al., 2021). The contribution of SiO_2 NPs application to this situation is important for chickpea plants to tolerate drought.

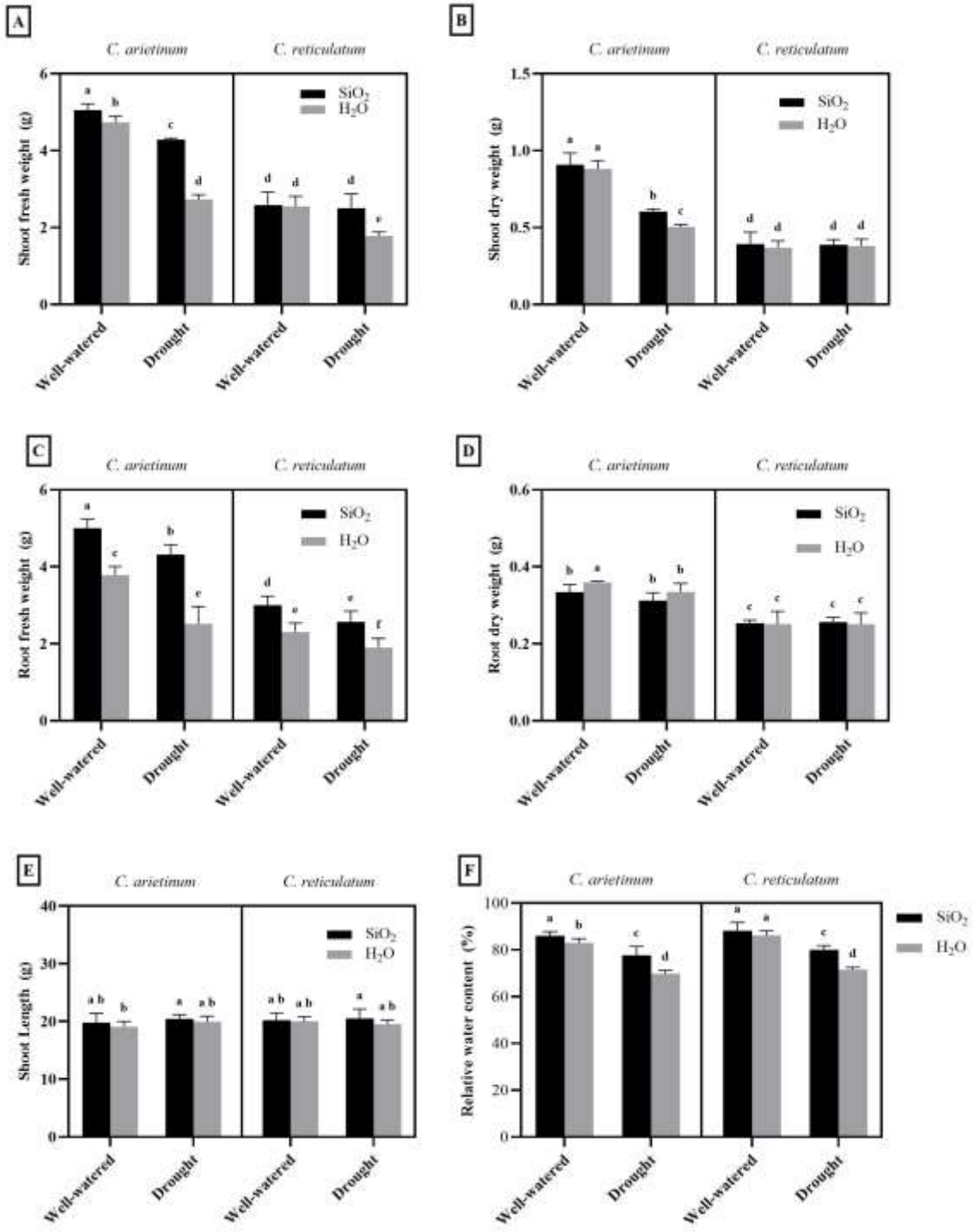

Figure 2. Effects of foliar application of SiO_2 NPs on shoot fresh weight (A), shoot dry weight (B), root fresh weight (C), root dry weight (D), shoot length (E), and relative water content (F) in *Cicer arietinum* and *Cicer reticulatum* genotypes under well-watered and drought conditions. Different letters indicate statistically significant differences ($p \leq 0.05$).

Şekil 2. İyi sulanan ve kuraklık koşulları altında Cicer arietinum ve Cicer reticulatum genotiplerinde SiO_2 NP'lerinin yapraktan uygulamasının sürgün yaş ağırlığı (A), sürgün kuru ağırlığı (B), kök yaş ağırlığı (C), kök kuru ağırlığı (D), sürgün uzunluğu (E) ve oransal su içeriği (F) üzerindeki etkileri. Farklı harfler istatistiksel farklılıkları göstermektedir ($p \leq 0.05$).

Effect of SiO_2 NPs on Lipid Peroxidation

In both species, the drought stress caused an increase in MDA content when compared with its well-watered groups. However, the increment was more pronounced in *C. arietinum* than of *C. reticulatum*. However, the SiO_2 NPs treatment decreased the MDA content in drought-conditioned plants of both species. Foliar spray of SiO_2 NPs has been reported to significantly decrease the MDA content in *Cicer arietinum* and *Cicer reticulatum* under drought conditions. Additionally, MDA levels in these samples were decreased by 34.46% and 27.33%, respectively, compared to those in the H_2O-treated group (Figure 3). These decreases in MDA levels were statistically significant ($p<0.001$). From the results presented above, it follows that SiO_2 NPs worked against oxidative stress in chickpea genotypes under drought conditions. As for well-watered plants from both chickpea genotypes, application of SiO_2 NPs resulted in no statistically significant change in MDA levels (For *Cicer arietinum*, p=0.106, and for *Cicer reticulatum*, $p = 0.189$). This implies that under well-watered conditions, foliar treatment with SiO_2 nanoparticles did not affect MDA content. It seems that the protective role of SiO_2 NPs is effective only under drought stress. This means that SiO_2 NPs are helpful in enhancing the drought tolerance capability of chickpea due to a reduction in oxidative damage, but it does not show negative effects on plants under normal watering conditions.

MDA is an end product of lipid peroxidation. Increased levels of free radicals typically lead to higher MDA production, making MDA levels a well-established marker of oxidative stress (Chauhan et al., 2022). In this study, the increase in MDA levels in both species under drought may be an indication that the plants were subjected to oxidative stress. However, the lower MDA level in wild chickpea *C.reticulatum* may be an indication that the wild species is less subjected to oxidative stress or copes better with oxidative stress under drought conditions compared to cultivated *C.arietinum*. The decrease in MDA content in both species with exogenous SiO_2 NPs application under drought conditions is important in terms of stress tolerance. MDA content is lower in plants with high antioxidant enzyme capacity under stress conditions (Feng et al., 2023). It has been shown in numerous studies that the antioxidant defense system is stimulated by SiO_2 NPs application, and the mechanism involved was described in detail by Huang et al., (2024). The fact that catalase activity was higher in the *C. reticulatum* than in the *C. arietinum* is consistent with the proposed hypothesis in the literature.

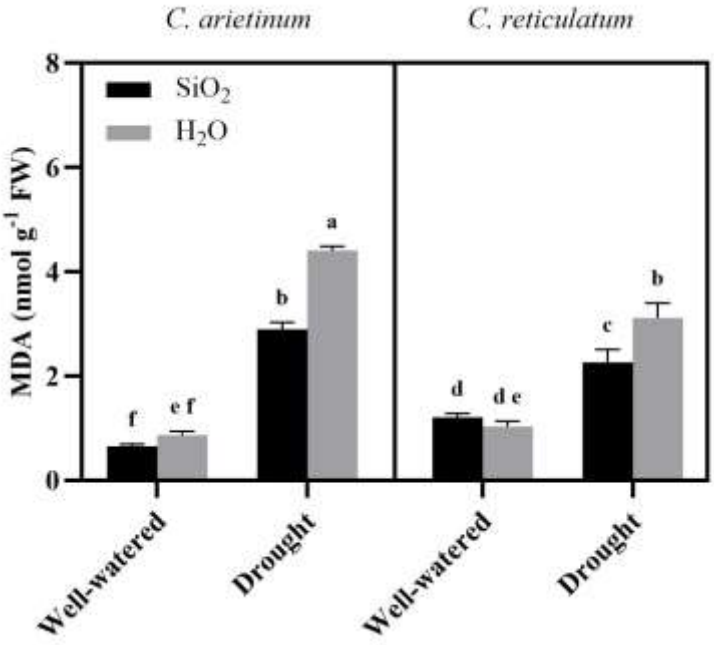

Figure 3. MDA content of all experimental groups. Different letters indicate statistically significant differences ($p\leq0.05$).

Şekil 3. Tüm deney gruplarının MDA içeriği. Farklı harfler istatistiksel farklılıkları göstermektedir ($p\leq0.05$).

Effect of SiO_2 NPs on Proline Content

Drought stress increased proline content in both species, while SiO_2 treatment lightly increased proline content in *C. arietinum* and slightly decreased it in *C. reticulatum*. More specifically, in well-watered conditions, *C. arietinum* did not show any significant differences in its proline content between SiO_2 and H_2O treatments (p=0.096).

However, under drought conditions, SiO_2-treated *C. arietinum* was found significantly higher proline content compared to plants receiving H_2O treatment (p=0.003). Similarly, in well-watered conditions, *C. reticulatum* also did not have any significant difference in proline content between SiO_2 and H_2O treatments (p=0.251). However, under drought conditions, the SiO_2 treatment had a significantly lower proline content compared to that of the H_2O treatment in *C. reticulatum* (p<0.001). In the *Cicer arietinum* group, the average proline content of plants sprayed with H_2O and SiO_2 NPs under drought conditions was determined to be 11.38 and 12.55 µmol g^{-1} FW, respectively. It was observed that SiO_2 NPs application increased proline content by 10.3% under drought conditions in this plant species. Conversely, in the *Cicer reticulatum* group, an opposite effect was observed. The average proline content of plants sprayed with H_2O and SiO_2 NPs under drought conditions was found to be 14.15 and 12.57 µmol g^{-1} FW, respectively, indicating that SiO_2 application reduced proline content by 11.17% under drought conditions in this species. Interestingly, the findings showed that under drought conditions, SiO_2 treatment had opposite effects on the proline content in *C. arietinum* and *C. reticulatum* (Figure 4).

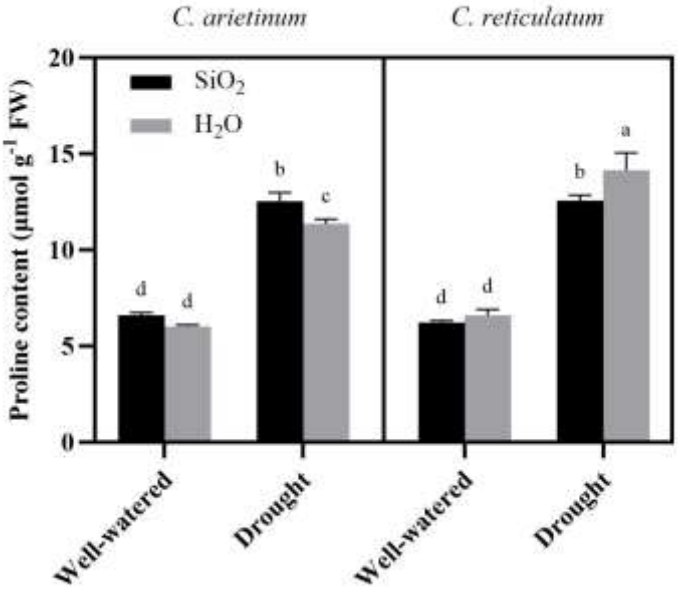

Figure 4. Effects of foliar application of SiO_2 NPs on proline content in *Cicer arietinum* and *Cicer reticulatum* genotypes under well-watered and drought conditions. Different letters indicate statistically significant differences (p≤0.05).

Şekil 4. İyi sulanan ve kurak koşullar altında Cicer arietinum ve Cicer reticulatum genotiplerinde SiO_2 NP'lerin yapraktan uygulanmasının prolin içeriğine etkileri. Farklı harfler istatistiksel farklılıkları göstermektedir (p≤0.05).

The application of SiO_2-NPs significantly increased proline accumulation in the leaves of treated *C. arietinum* (domesticated chickpea) plants, underscoring their potential role in enhancing drought tolerance. In contrast, the proline content in *C. reticulatum* (wild chickpea) groups displayed an opposite trend. This divergence suggests that wild and domesticated chickpea genotypes may employ different strategies to cope with drought stress. While *C. arietinum* appears to rely on proline accumulation as a key adaptive mechanism, *C. reticulatum* may utilize alternative physiological or biochemical pathways to mitigate drought effects. These findings may highlight the importance of considering genetic variability when developing nanoparticle-based strategies for improving drought resilience in crops. Upon reviewing the literature, it becomes apparent that there are reports showing that the amount of proline decreased (Hajizadeh et al., 2022) and increased (Abd-El-Aty et al., 2024) as a result of SiO_2 NPs application. Further research is needed to elucidate the underlying mechanisms driving these differences and to optimize nanoparticle applications for diverse plant genotypes.

Wild relatives, unlike domesticated species, were not exposed to intense anthropogenic selection pressure focused on enhancing yield-related traits under optimal and controlled conditions (Quezada-Martinez et al., 2021). As a result, they may have retained a broader range of adaptive mechanisms to cope with environmental stresses, such as drought. This genetic diversity could explain why wild species often exhibit greater resilience to drought compared to their domesticated counterparts, which have been selectively bred for high productivity, sometimes

at the expense of stress tolerance. Understanding these differences is crucial for developing drought-resistant crop varieties, as wild relatives may serve as valuable genetic resources for improving the resilience of domesticated species in the face of increasing drought conditions due to climate change (Kapazoglou et al., 2023).

Proline, a key osmoprotectant, is known to accumulate in plant tissues under stress conditions, serving as a protective mechanism against cellular damage (Dikilitas et al., 2020). In this study, the observed species-dependent increase in proline content in nanoparticle-treated plants suggests that nanoparticles may stimulate the biosynthesis of proline or enhance the plant's ability to regulate osmotic balance under stress. This aligns with previous findings that nanoparticles can modulate physiological and biochemical pathways, improving stress resilience (Mohammadi et al., 2016; Zhang et al., 2020). Additionally, the relationship between proline levels and other stress-responsive mechanisms, such as antioxidant enzyme activity and photosynthetic efficiency, warrants deeper investigation. These findings underscore the potential of nanoparticles as a novel tool for improving crop resilience, but long-term studies are essential to evaluate their ecological and physiological impacts on plants and the environment.

Effect of SiO_2 NPs on Catalase Enzyme Activity

Catalase enzyme activity increased in both species under drought conditions, the increase being more pronounced in *C. reticulatum*. Exogenous SiO_2 NPs treatments also increased catalase enzyme activity in both species. In the *Cicer arietinum* group, the average catalase (CAT) enzyme activity of plants sprayed with H_2O and SiO_2 NPs under drought conditions was determined to be 2.05 and 3.50 μmol H_2O_2 min^{-1} g^{-1} FW, respectively. It was observed that SiO_2 application increased CAT enzyme activity by 70.7% under drought conditions in this plant species ($p<0.001$). Similarly, in the *Cicer reticulatum* group, a comparable effect was observed. The average CAT enzyme activity of plants sprayed with H_2O and SiO_2 NPs under drought conditions was found to be 2.82 and 5.62 μmol H_2O_2 min^{-1} g^{-1} FW, respectively, indicating that SiO_2 application increased CAT enzyme activity by 99.3% under drought conditions in this species ($p<0.001$). *C. arietinum* in well-watered conditions, CAT activity was found significantly different between the plants treated with either SiO_2 or H_2O ($p=0.006$), while under drought conditions, SiO_2 treatment increased CAT activity significantly when compared with the treatment with H_2O ($p<0.001$). Likewise, *C. reticulatum* as expected, under well-watered conditions, no significant difference in CAT activity is found between SiO_2 and H_2O treatments ($p=0.159$). However, under drought conditions, SiO_2 treatment tends to significantly increase CAT activity compared to that by H_2O treatment at $p<0.001$ (Figure 5). We could therefore conclude here that SiO_2 treatment causes a dramatic increase in CAT activity in both crop species under drought conditions. This result seems to reveal that SiO_2 causes some sort of protection from oxidative stress damage that results from drought.

The effects of nanoparticle application on drought tolerance vary between wild and domesticated species in terms of catalase enzyme activity. Wild species tend to exhibit a greater increase in catalase enzyme activity under drought stress, indicating their enhanced capacity to manage oxidative stress more effectively. This can be attributed to their evolutionary adaptation to environmental stress conditions over time. In contrast, domesticated species may show a more limited rise in catalase activity, as they have been selectively bred for high yield under optimal conditions rather than stress tolerance. Nanoparticle applications have the potential to improve drought tolerance in domesticated species by enhancing catalase activity. However, the natural adaptation mechanisms observed in wild species could serve as a valuable genetic resource for future breeding programs. These findings highlight the importance of nanoparticle technology and the genetic diversity of wild species to develop resilience against drought stress.

Other studies have also shown that nanoparticles enhance stress tolerance, primarily by influencing antioxidant systems (Wang et al., 2018). Nanoparticles modulate the activity of key antioxidant enzymes, such as catalase, superoxide dismutase, and peroxidase, which play essential roles in mitigating oxidative damage caused by drought, salinity, and heavy metal toxicity. By boosting the efficiency of these antioxidant systems, nanoparticles help maintain cellular homeostasis and reduce the accumulation of reactive oxygen species (ROS), thereby enhancing overall stress resilience. The antimicrobial activity of metal oxide-NPs or their forms synthesized with plant extract has also been reported to be mainly against environmental, food, and plant pathogens (Şahin et al., 2021; Şahin et al., 2022; Soylu et al., 2022). These findings highlight the potential of nanoparticles as a valuable tool for improving plant stress tolerance through their interaction with antioxidant defense mechanisms.

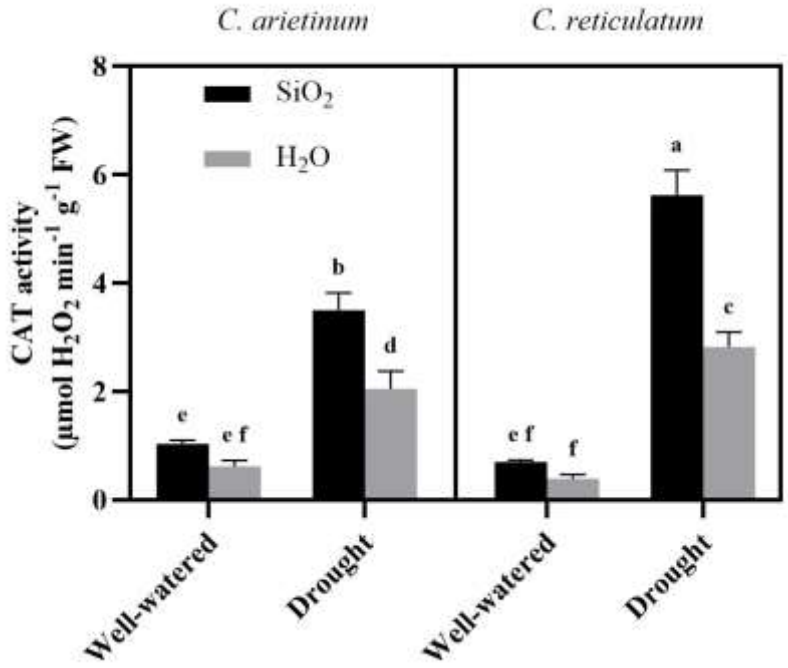

Figure 5. Effects of foliar application of SiO_2 NPs on CAT enzyme activity in *Cicer arietinum* and *Cicer reticulatum* genotypes under well-watered and drought conditions. Different letters indicate statistically significant differences ($p \leq 0.05$).

Şekil 5. Cicer arietinum ve Cicer reticulatum genotiplerinde iyi sulanmış ve kuraklık koşullarında SiO_2 NP'lerinin yaprak uygulamasının CAT enzim aktivitesi üzerindeki etkileri . Farklı harfler istatistiksel farklılıkları göstermektedir ($p \leq 0.05$).

CONCLUSIONS

Drought is considered to be the most important abiotic stress factor that reduces the yield of crops in agricultural areas. The ever-increasing human population has made it imperative to get more and more yield from agricultural areas. Therefore, the mechanisms necessary for plants in agricultural areas to cope with this stress have been intensively investigated. In order to contribute to these investigations, in the present study, the effects of exogenously applied SiO_2 NPs on two chickpea species with different drought tolerances under drought conditions were determined. In this study, exogenous SiO_2 NPs application similarly altered the parameters studied in both *C. arietinum* and *C. reticulatum*, except for proline content. Interestingly, under drought conditions, SiO_2 NPs treatment increased proline content in *C. arietinum* but decreased it in *C. reticulatum*. Under drought conditions, the increase in catalase (CAT) activity with SiO_2 application in both plant species suggests that this nanoparticle dose triggered the antioxidant defense system and induced noticeable improvements in plant resilience to drought stress. These results make it difficult to explain, but this results show that exogenous SiO_2 NPs application induces different responses depending on the tolerance level of the plant. However, the molecular response of this result needs to be studied in detail.

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Contribution Rate Statement Summary of Researchers

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

Conflicts of interest

The authors declare that there are no conflicts of interest.

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Investigation of Viral Agents in Walnut (*Juglans* spp.) Trees by High Throughput Sequencing from Niğde province, Türkiye

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ABSTRACT
In October 2023, virus-like symptomatic walnut trees in Niğde province were investigated using high-throughput sequencing (HTS) to investigate viral infections. Double-stranded RNA (dsRNA) extraction was conducted, followed by conversion of dsRNA into cDNA. PCR was then performed using barcoding with MID primers for metagenomic analyses. The PCR products from walnut samples B13, B22, B23, and B21 + B5 were sequenced using the Illumina NovaSeq 6000 sequencing platform. The sequencing data underwent bioinformatics analysis using Geneious Prime and CLC Genomic Workbench. In this comparison, more contigs were constructed in CLC Genomic Workbench, resulting in more precise outcomes with BLASTx analysis. According to the map, to reference analyses of B21+B5 HTS data, 29,574 reads matched with cherry leaf roll virus RNA1 (CLRV) RefSeq NC_015414, and 405 reads matched with CLRV RNA2 RefSeq NC_015415. However, 4 reads matched with cucumber mosaic virus (CMV) RefSeq NC_001440 from B23 HTS data. To confirm the presence of these viruses, virus-specific primers were used to test the total RNA extracted from walnuts. CLRV was detected in sample B21 but not in B5. CMV was not detected in sample B23. Improving nucleic acid extraction methods is recommended to enhance the detection of viral agents using high-throughput sequencing.

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Niğde İli Ceviz (*Juglans* spp.) Ağaçlarındaki Viral Etmenlerin Yüksek Kapasiteli Dizileme ile Araştırılması

ÖZET
Niğde ilinde, Ekim 2023'te, virüs benzeri simptomlar gösteren ceviz ağaçları, viral enfeksiyonları araştırmak için yüksek kapasiteli dizileme kullanılarak analiz edilmiştir. Çift sarmallı RNA (dsRNA) ekstraksiyonu yapılmış, ardından dsRNA'lar cDNA'ya dönüştürülmüştür. Daha sonra metagenomik analizler için MID primerleri ile barkodlama kullanılarak PCR gerçekleştirilmiştir. B13, B22, B23 ve B21+B5 ceviz örneklerinin PCR ürünleri Illumina NovaSeq 6000 dizileme platformu kullanılarak dizilenmiştir. Dizileme verisi Geneious Prime ve CLC Workbench kullanılarak biyoinformatik analize tabi tutulmuştur. Bu karşılaştırmada, CLC Workbench'te daha fazla contig oluşturulmuş ve BLASTx analizi ile daha doğru sonuçlar elde edilmiştir. B21+B5 HTS verisinin referansa göre haritalama-analizlerine göre, 29.574 okuma cherry leaf roll virüsü RNA1 (CLRV) RefSeq NC_015414 ile ve 405 okuma CLRV RNA2 RefSeq NC_015415 ile eşleşmiştir. Ancak, B23 HTS verisindeki 4 okuma cucumber mosaic virus (CMV) RefSeq NC_001440 ile eşleşmiştir. Bu virüslerin varlığını doğrulamak amacıyla, cevizlerden ekstrakte edilen total RNA'yı test etmek için virüse özgü primerler kullanılmıştır. CLRV, B21 örneğinde tespit edilmiş ancak B5'te tespit edilmemiştir. CMV, B23 örneğinde tespit edilmemiştir. Yüksek kapasiteli dizileme kullanılarak viral ajanların tespitini arttırmak için nükleik asit ekstraksiyon yöntemlerinin iyileştirilmesi önerilmektedir.

Fitopatoloji

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Anahtar Kelimeler
Ceviz
dsRNA
Niğde
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INTRODUCTION

Walnut (*Juglans regia L.*) belongs to the *Juglandaceae* family and is considered one of the highest-quality nuts in temperate regions, particularly Central Asia. Walnuts are used in various culinary applications and offer numerous nutritional benefits, including minerals, antioxidants, and vitamins that support heart and brain health (Sütyemez, 2008). Walnuts are widely distributed globally and can be cultivated in many regions, including all parts of Türkiye (Budak, 2010). In Türkiye, 360,000 tons of walnuts were produced, with significant contributions from the provinces of Kahramanmaraş, Bursa, and Bilecik (TUIK, 2023). It is reported that Türkiye will achieve self-sufficiency in walnuts from 2020 to 2045 (Güvenç and Purlu, 2022).

However, walnut trees are susceptible to fungal, bacterial, viral, and virus-like diseases. Notably, the cherry leaf roll virus (CLRV) (*Nepovirus avii*) and plum pox virus (PPV) (*Potyvirus plumpoxi*) have been identified in walnuts (Savino et al., 1977; Baumgartnerova, 1996). CLRV was reported in walnuts in the Lake Van Basin in Türkiye (Ozturk et al., 2008; Siphaioglu et al., 2011; Yegül and Baloğlu, 2019).

Plant pathogenic agents can be identified through high-throughput sequencing (HTS) technologies like Illumina sequencing combined with bioinformatics analysis. In the Illumina sequencing, a little slide is flooded with fluorescently labeled nucleotides and DNA polymerase. Also, there is one terminator for stopping the length read. When the terminator sees the fluorescently labeled nucleotides, it stops and reads the read lengths. In this working principle, a slide is used to detect the lengths. It read an average of 50-300 bp (Sharma et al., 2022).

HTS can identify all DNA and RNA viruses in a sample through a single analysis when appropriate methodology is used. This advanced technique provides an in-depth and comprehensive assessment of a plant's viral health status, allowing for a thorough understanding of any potential viral threats that may affect its overall phytosanitary condition (Kreuze et al., 2009; Moubset et al., 2022). Furthermore, the acquired sequence data can be leveraged for multiple applications, including elucidating the population structuring, ecological interactions, and evolutionary dynamics of viruses. It also aids in differentiating variants with varying impacts on disease etiology.

HTS offers flexibility in its implementation, allowing for individual, bulked, or mixed samples (Maree et al., 2018). A range of nucleic acid populations can be utilized for HTS. The primary methods have focused on double-stranded RNA (dsRNA), virus-derived small interfering RNA (siRNA), virion-associated nucleic acids (VANA), total RNA, whether or not it has undergone rRNA depletion, and polyadenylated RNA. The advantages and disadvantages of each approach for virus discovery and etiology research can vary significantly (Ma et al., 2019). Regardless of the assessment criteria, the dsRNA-based method showed a greater diversity of RNA viruses than the VANA method. Dissimilarity analyses revealed that both methods were reproducible but not necessarily convergent (Ma et al., 2019).

Although there has been no record of virus identification in walnuts by HTS, there are many examples from other perennial crops like grapevine, mulberry, and pomegranate (Maliogka et al., 2015; Caglayan et al., 2020; Gürcan et al., 2021).

In October 2023, walnut trees exhibiting virus-like symptoms were observed in Niğde province. These symptoms included line patterns, yellow discoloration between the lateral veins of the leaves, dark green patches on the leaf surface, bright yellow veins, black veins, and narrow leaves. We collected walnut leaf samples that exhibited these symptoms to explore the connection between the observed symptoms and potential viral agents. The samples were then subjected to dsRNA extraction, followed by Illumina sequencing and subsequent bioinformatics analysis.

MATERIAL and METHOD

Plant Materials

In October 2023, shoot samples were collected from five walnut trees (B5, B13, B21, B22, B23) showing virus-like disease symptoms in Ulukışla and central Niğde province. Two of these samples (B5 + B21) were analysed as a bulk sample randomly due to the sequencing quota, while the remaining samples were analysed individually. The samples were preserved using liquid nitrogen and stored at -80°C until analysis.

Double-strand RNA Isolation and Viral Nucleic Acid Enrichment

Double-stranded RNAs (dsRNA) were purified from each sample using two rounds of CF11 cellulose chromatography, following the protocol established by Marais et al. (2018). The undiluted dsRNA samples were then subjected to cDNA synthesis with Dodeca primers, followed by PCR amplification employing MID primers as described by François et al. (2018). For the cDNA amplification with the Dodeca primer, the preparation for each sample included 0.6 µL of nuclease-free water, 0.4 µL of Dodeca primer (as shown in Table 1), and 9.5 µL of dsRNA, resulting in a total volume of 10.5 µL. Subsequently, the mixture underwent a short-term denaturation at 95°C for 5 minutes.

Table 1. Primer sequences used for cDNA and PCR amplifications of walnut dsRNAs
Çizelge 1. Ceviz dsRNA'larının cDNA ve PCR amplifikasyonları için kullanılan primer dizileri

Primer	Base Sequence (5'- 3')	Walnut Sample Name
LDF_007_Dodeca	CGTGGAGACTCTGGNNNNNNNNNNT	B23
LDF_011_Dodeca	ACGCCATCACACGGNNNNNNNNNNT	B13
LDF_078_Dodeca	GTGACCGACACCGTNNNNNNNNNNT	B5+B21
LDF_084_Dodeca	TACGACCGCTGCACNNNNNNNNNNT	B22
Tag156_4_LDF_007	AAGGTAGAAGCGTGGAGACTCTGG	B23
Tag34_4_LDF_011	AATACTGTGGACGCCATCACACGG	B13
Tag590_4_LDF_078	GCAAGATGTAGTGACCGACACCGT	B5+B21
Tag684_4_LDF_084	GGCATATACCTACGACCGCTGCAC	B22

The total volume of the reaction mix for cDNA amplification was 9.5 µL, which included the following components: 2 µL of dNTP (10 mM), 2 µL of DTT (100 mM), 0.5 µL of RNase OUT (20 U/µL), 4 µL of 5X Reverse Transcriptase Buffer, and 1 µL of SuperScript Reverse Transcriptase (200 U/µL). These were combined in each tube containing Dodeca primers and dsRNA to achieve a final cDNA volume of 20 µL. The cDNA synthesis procedure involved 10 minutes at 25°C, 1 hour at 42°C, and 5 seconds at 70 °C.

The PCR reaction mix for enriching viral nucleic acids consisted of the following components: 33.50 µL of water, 5 µL of 10X Buffer, 1.25 µL of dNTP, 5 µL of Tag (MID) Primer (refer to Table 1), 0.25 µL of Dream Taq polymerase enzyme, and 5 µL of cDNA sample, totaling 50 µL. The amplification conditions were set as follows: 1 minute at 94 °C, 1 minute at 65 °C, and 45 seconds at 72 °C, followed by 40 cycles of 1 second at 94 °C, 1 second at 45 °C, and 5 minutes at 72 °C. The process concluded with a final extension of 5 minutes at 72 °C and 5 minutes at 37 °C. To visualize the PCR products, 10 µL of the reaction was loaded onto a 1.5% agarose gel for electrophoresis.

Sequencing

The PCR products obtained were sent to Genoks (Ankara, Türkiye). Library construction was carried out using the Illumina DNA Prep Kit, previously known as Nextera XT. We requested sequencing for each sample at a depth of 5 M, with a read length of 2 x 150 bp. The sequencing platform used was the Illumina Novaseq 6000.

Bioinformatics Analysis

After performing quality analyses on the FASTQ data provided by the company, we conducted a set of paired, trimming, and merging analyses using the reads. Trimming was performed using the BBDuck Adapter/Quality Trimming Version 38.37 for Geneious Prime and in CLC Genomic Workbench with the default parameters. *De novo* assembly was performed with default parameters of the SPAdes assembler in Geneious Prime 2024.0.5, while CLC's assembly system in CLC Genomic Workbench 11.0.1. Subsequently, BLASTn analyses were carried out against a custom viral database (retrieved on 19 May 2024) using the resulting contigs. BLASTx analysis was performed against NCBI nucleotide data only using contig data by CLC Genomic Workbench. After the blast analyses, map-to-reference analyses were conducted using the detected viruses as references with both software tools. The BLASTn and BLASTx analyses used contigs from de novo assembly, while map-to-reference analyses utilized read pairs obtained after trimming.

Confirmatory Analyses for Detected Viruses

To validate the viruses detected in Illumina sequencing data, total RNA extraction, cDNA synthesis, and virus PCR tests were performed on the samples using virus-specific primers.

Total RNA extraction was performed with modifications based on the protocol by MacKenzie et al. (1997). After cDNA synthesis, the total RNA extracted from the samples was subjected to PCR using virus-specific primers. The analysis included CLRV and cucumber mosaic virus (CMV) (*Cucumovirus CMV*), which were tested with virus-specific primers (Werner et al., 1997; Grieco et al., 2000) (Table 2).

Table 2. Specific primers used for virus tests of B5, B21, and B23 walnut samples
Çizelge 2. B5, B21 ve B23 ceviz numunelerinin virüs testleri için kullanılan spesifik primerler

Virus Scientific Name	Nucleotide Sequence (5'-3')	Reference
Nepovirus avii (CLRV)	F: TGGCGACCGTGTAACGGCA R: GTCGGAAAGATTACGTAAAAGG	Werner et al., 1997
Cucumovirus CMV (CMV)	F: CCATCACCTTAGCTTCCATGT R: TAACCTCCCAGTTCTCACCGT	Grieco et al., 2000

For cDNA synthesis, a mixture was prepared containing 4 µL of nuclease-free water, 1 µL of random hexamer, 4 µL of RNA, and 1 µL of dNTP (10 mM), making a total volume of 10 µL. This mixture was subjected to short-term denaturation at 65 °C for 5 minutes. Following denaturation, a second mixture was added to each sample, consisting of 3.2 µL of nuclease-free water, 0.5 µL of DTT (100 mM), 0.3 µL of RNase OUT (20 u/µL), 5 µL of 5X RT Buffer, and 1 µL of MML-V Reverse Transcriptase, bringing the final volume to 20 µL. The cDNA synthesis procedure included 10 minutes at 25 °C, 45 minutes at 42 °C, and 10 minutes at 70 °C.

PCR amplification was conducted using a mixture of 6.4 µL of nuclease-free water, 10 µL of 2X Master Mix, 0.8 µL of forward primer (10 µM), 0.8 µL of reverse primer (10 µM), and 2 µL of the cDNA sample. The amplification program comprised an initial denaturation at 94°C for 2 minutes, followed by 35 cycles of 45 seconds at 95 °C, 45 seconds at 60 °C (for CMV) or 50 °C (for CLRV), and 45 seconds at 72 °C, concluding with a final extension at 72 °C for 10 minutes.

Each PCR product was visualized using 1.2% agarose gel electrophoresis. The gels were stained with ethidium bromide and visualized using a gel imaging system.

RESULTS and DISCUSSION

In walnut samples, abnormal leaf development was observed. Yellow discolorations along the lateral veins, bright yellow lines following the veins, dark green areas between the veins, and blackening of the veins were also monitored (Figure 1).

Figure 1. Symptoms observed in walnut trees: A) Sample B5, abnormal leaf development; B) Sample B13, blackening of the main veins on the leaves; C) Sample B21, yellow discoloration between the lateral veins of the leaves; D) Sample B22, bright yellow blotches along the main and lateral veins of the leaves; E) Sample B23, dark and light green blotches on the leaves.

Şekil 1. Ceviz ağaçlarında görülen belirtiler: A) Örnek B5, anormal yaprak gelişimi; B) Örnek B13, yapraklardakianadamarların kararması; C) Örnek B21, yaprakların yan damarları arasında sarı renk değişikliği; D) Örnek B22, yaprakların ana ve yan damarları boyunca parlak sarı lekeler; E) Örnek B23, yapraklarda koyu ve açık yeşil lekeler.

The dsRNAs randomly amplified using Dodeca and Tag (MID) primers exhibited multiple bands and smears (Figure 2). The dsRNA extraction protocol eliminated DNA by applying DNase I, as the target genomes are RNAs in this method.

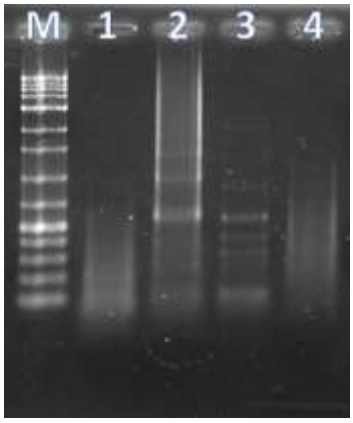

Figure 1. dsRNA products on agarose gel amplified using Dodeca and Tag (MID) primers. M: Marker; 1: B13, 2: B21+B5, 3: B22, 4: B23 walnut samples.

Şekil 2. Dodeca ve Tag primerleri kullanılarak çoğaltılmış agaroz jel üzerindeki dsRNA ürünleri. M: Markör; 1: B13, 2: B21+B5, 3: B22, 4: B23 ceviz örnekleri.

Read numbers were changed according to the samples, around 11.7-21.2M after sequencing (Table 3). Trimming in the CLC Genomic Workbench led to removing reads from 107.000 to 337.000 (Table 4). A quality score below 0.05 for CLC Genomic Workbench and sequence reads quality below Q10 for Geneious Prime was trimmed. The purpose of trimming is to eliminate adapter sequences and low-quality reads, enhancing the accuracy of results by removing low-quality data.

Table 3. Illumina data obtained after sequencing

Çizelge 3. Sekanslamadan sonra elde edilen Illumina verisi

Sample Name	Number of Reads for R1	Number of Reads for R2	Total Number of Reads (R1+R2)	Range of Sequence Length
B13	7,397,993	7,397,993	14,795,986	35-151
B22	9,514,828	9,514,828	19,029,656	35-151
B23	10,624,402	10,624,402	21,248,804	35-151
B21+B5	5,871,496	5,871,496	11,742,992	35-151

Table 4. Number of reads after trimming in the CLC Genomic Workbench and merging in Geneious Prime

Çizelge 4. CLC Genomic Workbench'te kırpma ve Geneious Prime'da birleştirme sonrasında okuma miktarı

Sample Name	Number of Total Reads	CLC Genomic Workbench	Geneious Prime	
		Number of Reads After Trimming	Number of Merged Reads	Number of Unmerged Reads
B13	14,795,986	14,678,130	4,752,456	2,194,808
B22	19,029,656	18,692,547	5,303,888	3,146,332
B23	21,248,804	21,131393	6,876,590	3,393,604
B21+B5	11,742,992	11,624,878	3,701,881	1,245,290

Merged read amounts were changed around 3.7-6.9M, unmerged reads were around 1.3-3.4M according to samples in Geneious Prime (Table 4). When the paired reads are aligned and combined, they are called "merged" and the others are called “unmerged”. Both types of files (merged and unmerged) were selected for the *de novo* assembly stage.

Comparison of Contigs After Assembly with Geneious Prime and CLC Genomic Workbench

For the B13 sample, the total number of contigs was 908 in CLC Genomic Workbench, while Geneious Prime showed only 152 contigs. In the case of the B21+B5 sample, CLC Genomic Workbench produced 3.566 contigs compared to just 69 in Geneious Prime. For the B22 sample, CLC Genomic Workbench recorded 964 contigs, whereas Geneious Prime had 73. Lastly, for the B23 sample, CLC Genomic Workbench yielded 1.910 contigs, while Geneious Prime resulted in 210. When comparing the two bioinformatics software, it is evident that despite N50

values and length of the produced contigs being higher for Geneious Prime, CLC Genomic Workbench is more effective for generating of fold higher number of contigs than Geneious Prime. In addition to this, minimum contig lengths were found to be higher in the CLC Genomic workbench than in Geneious Prime. As shown in Table 5, since N50 values were lower, CLC Genomic Workbench consistently produced more contigs than Geneious Prime. Means that the number of bases in all contigs longer than N50 will be close to the number of bases in all contigs shorter than N50. A higher number of contigs enhances the likelihood of detecting viral genomes in blast analyses (Table 5).

Table 5. *De novo* assembly results were derived from different assemblers
Çizelge 5. Farklı montajcılar kullanılarak elde edilen De novo montaj sonuçları

Sample Name	*De novo* Assembly Tools	Min. Contig Length	Max. Contig Length	Total Numbers of Contig	N50 value
B13	CLC	100	844	908	224
	Geneious (SPAdes)	74	1031	152	656
B21+B5	CLC	100	1258	3566	295
	Geneious (SPAdes)	73	2001	69	806
B22	CLC	100	2802	964	246
	Geneious (SPAdes)	81	2806	73	1038
B23	CLC	100	3018	1910	232
	Geneious (SPAdes)	74	3136	212	899

BLASTn Analysis of Contigs Using Geneious and CLC Genomic Workbench

A custom viral database of the NCBI virus RefSeq was created for both software programs to conduct blast analyses. BLASTn analysis was performed using contigs obtained from four samples (B13, B22, B23, and B21 + B5). As shown in Table 6, most contigs exhibited short-length sequence hits (less than 50 nt) to viruses in the database, and many contigs did not match any entries in the database.

For the B13 sample, Geneious Prime identified hits from 13 viruses, while CLC Genomic Workbench detected hits from 16 viruses. In the B22 sample, Geneious Prime found hits from four viruses, whereas CLC Genomic Workbench identified hits from 13 viruses. For the B23 sample, Geneious Prime matched hits from five viruses, while CLC Genomic Workbench found hits from 42 viruses. Finally, in the B21+B5 sample, Geneious Prime recorded hits from four viruses, compared to CLC Genomic Workbench, which detected hits from 33 viruses.

Table 6. Number of plant viruses revealed via BLASTn analyses in Geneious Prime and CLC Genomic Workbench
Çizelge 6. Geneious Prime ve CLC Genomic Workbench'te BLASTn analizi ile tespit edilen edilen bitki virüslerinin sayısı

Sample Name	A number of plant viruses	
	Geneious Prime	CLC Genomic Workbench
B13	13	16
B21+B5	4	13
B22	5	42
B23	4	33

BLASTx Analysis of Contigs Using CLC Genomic Workbench

BLASTx was performed against the NCBI database using the contigs from four samples: B13, B22, B23, and B21 + B5. BLASTx is useful for identifying viral protein motifs, which allows for the discovery of unknown viral agents. This method is particularly effective for detecting newly discovered viruses, as it targets proteins.

For the B13 sample, the contigs were matched to three viruses using CLC Genomic Workbench. In the case of the B23 sample, contigs were matched to one virus, while for the B21+B5 sample, the contigs were matched to two viruses (Table 7).

BLASTx analysis against NCBI was more accurate than BLASTn against the custom viral database in detecting viruses, as indicated by resulting read matches in the map-to-reference analysis.

Genome Mapping to Reference Sequences in Geneious Prime

Blueberry scorch virus, cereal yellow dwarf virus, orchid fleck virus genomic RNA, pedilanthus leaf curl virus, pepper chlorotic spot virus, potato virus Y, raspberry leaf blotch virus, red clover associated virus, spinach

amalgavirus, tomato chlorotic leaf distortion viruses were selected as reference for sample B13 to perform map to reference analysis. High Plains wheat mosaic virus, longan witches broom-associated virus, rice yellow mottle virus, and turnip vein-clearing virus were selected as references for sample B22 to perform map-to-reference analysis. CLRV RNA1, High Plains wheat mosaic virus, sunn-hemp mosaic virus, and tomato leaf curl Cebu virus were selected as references for sample B21+B5 to perform map-to-reference analysis. Only CLRV RNA 1 had a high match with the B21+B5 contigs with Geneious Prime. CLRV RNA 1 had 27.112 reads matched with the B21+B5 sample in BLASTn with Geneious Prime.

Table 7. BLASTx results from CLC Genomic Workbench for walnut samples (B13, B22, B23, B21+B5)
Çizelge 7. Ceviz numuneleri için CLC Genomic Workbench'ten elde edilen BLASTx sonuçları (B13, B22, B23, B21+B5)

Accession Number	Description (CLC Genomic Workbench B13)	Hits for Total Contig Number	Min-max read length (nt)
NP_619665	coat protein [Grapevine virus A]	1	28-28
NP_597746	Replicase [Tobacco mosaic virus]	1	32-32
YP_008492928	RNA-dependent RNA polymerase [Tomato mottle mosaic virus]	1	27-27
Accession Number	**Description (CLC Genomic Workbench B23)**	**Hits for Total Contig Number**	**Min-max read length (nt)**
NP_040777	capsid protein [Cucumber mosaic virus]	1	54-54
Accession Number	**Description (CLC Genomic Workbench B21+B5)**	**Hits for Total Contig Number**	**Min-max read length (nt)**
YP_004382746	polyprotein 1 [CLRV]	3	71-418
YP_004382747	polyprotein 2 [CLRV]	2	29-50

Genome Mapping to Reference Sequences in CLC Genomic Workbench

According to BLASTn results, bean necrotic mosaic virus, chicory yellow mottle virus satellite, eggplant latent viroid, High Plains wheat mosaic virus, prunus necrotic ringspot virus, arabis mosaic virus small satellite, CLRV RNA1, and CLRV RNA2 were selected as references for sample B21+B5 to perform map-to-reference analysis. Rice stripe virus RNA 3 was selected as a reference for sample B23 to perform map-to-reference analysis. Only CLRV RNA1 and CLRV RNA2 had a high match according to the CLC Workbench. CLRV RNA 1 had 29,574 reads matched with the B21+B5 sample in BLASTn with CLC Genomic Workbench, and CLRV RNA 2 had 405 reads matched with the B21+B5 sample in BLASTn with CLC Genomic Workbench.

Based on the results from BLASTx, CLRV RNA 1 and CLRV RNA 2 were chosen as references for sample B21+B5 for map-to-reference analysis. Grapevine virus A was selected as a reference for sample B13, while CMV RNA 3 was chosen for sample B23. Among these, only CLRV RNA 1 and CLRV RNA 2 had a high match with the B21+B5 sample in the BLASTx analysis conducted with CLC Genomic Workbench. CLRV RNA 1 had 29.574 reads matched with the B21+B5 sample in BLASTx with CLC Genomic Workbench, and CLRV RNA 2 had 405 reads matched with the B21+B5 sample in BLASTx with CLC Genomic Workbench. Additionally, CMV RNA 3 showed a minor match with the B23 sample in the same analysis. CMV RNA 3 had 4 reads that matched with B23 sample. Notably, no viral hits were identified in the BLASTx analysis for sample B22.

Confirmatory Analysis Results

According to the PCR test results, the CLRV was detected in only one sample (B21), which had a size of 431 bp (Figure 3). This sample was collected from the Ulukışla-Porsuk area, known for its extensive cherry tree cultivation in the Ulukışla district of Niğde. While CLRV primarily affects cherry trees, it can also impact other hosts. Notably, studies conducted in the Van region of Türkiye have shown that CLRV was detected in walnuts (Ozturk et al., 2008).

CONCLUSION

The primary factor contributing to the rising prevalence of diseases affecting walnut (*Juglans regia*) orchards is the absence of comprehensive agricultural insurance coverage among the majority of the orchards analyzed in this study. This lack of financial protection limits farmers' ability to implement effective disease management strategies, such as timely fungicide applications, integrated pest management (IPM) practices, and orchard sanitation measures. Furthermore, insufficient knowledge regarding walnut cultivation, including optimal pruning techniques, soil health management, and disease prevention protocols, exacerbates the susceptibility of

orchards to pathogenic infections. This knowledge gap significantly hinders early diagnosis and intervention, facilitating the rapid spread of fungal, bacterial, and viral pathogens, thereby compromising overall orchard productivity and sustainability.

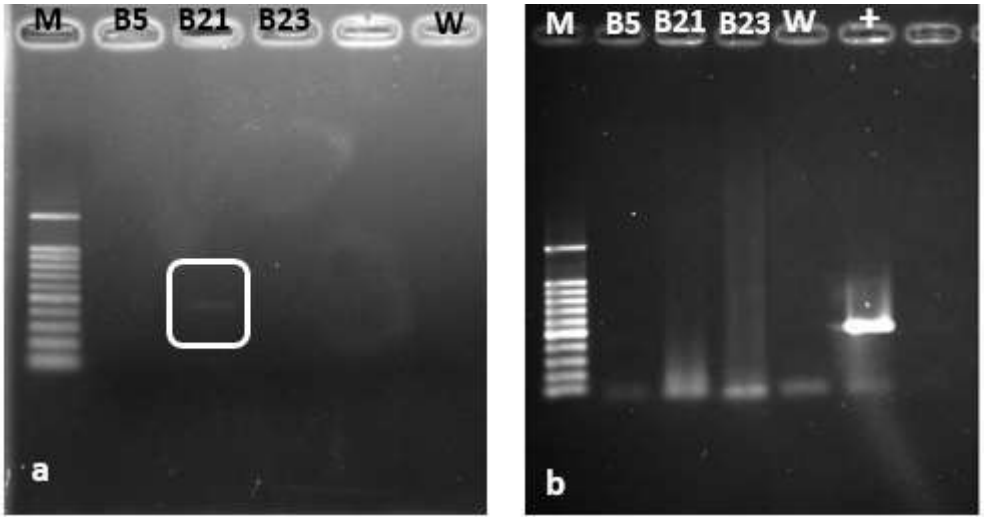

Figure 3. RT-PCR analysis of total RNA from walnut samples for CLRV (a) and CMV (b). M: 100 bp DNA Ladder (Thermo Scientific), +: CMV positive control, W non-template control.

Şekil 3. Ceviz örnekleri toplam RNA'dan CLRV (a) ve CMV (b) RT-PCR analiz sonucu. M: 100 bp DNA Ladder (Thermo Scientific), +: CMV pozitif control, W templete içermeyen kontrol.

In this study, walnut (*Juglans regia*) samples collected from the Ulukışla region in Niğde, an area predominantly known for its intensive cherry (*Prunus avium*) cultivation, were examined for the presence of viral disease symptoms. Previous studies have reported the occurrence of cherry leaf roll virus (CLRV) in walnuts from Van province (Öztürk et al., 2008; Sipahioğlu et al., 2011; Yegül and Baloğlu, 2019), highlighting its potential threat to walnut production. Given that CLRV is a polyphagous virus capable of infecting both walnut and cherry trees, the coexistence of these host species in various locations provided a suitable environment for investigating its spread and impact. Symptomatological analysis of walnut samples revealed characteristic viral disease manifestations, including irregular leaf development, chlorotic vein discoloration, and generalized foliar yellowing, all of which suggest possible CLRV infection or coinfection with other viral pathogens. These findings underscore the necessity for further molecular diagnostics to confirm viral identity and assess its epidemiological significance in walnut-growing regions.

Comparative analyses were conducted using Geneious Prime and CLC Genomics Workbench to evaluate their performance in bioinformatics workflows. CLC Genomics Workbench demonstrated superior efficacy, generating a higher number of contigs, which facilitated more accurate sequence assembly and improved reference matching. Furthermore, a comparative assessment of BLASTn and BLASTx revealed that BLASTx served as an effective validation tool for BLASTn, leading to enhanced accuracy in sequence identification. BLASTx, which focuses on protein-level homology, proved particularly advantageous for detecting novel viruses, as it enables the identification of conserved protein domains even when nucleotide-level similarity is low. The bioinformatics analysis identified a significant sequence match for cherry leaf roll virus (CLRV) in the B21+B5 sample, further substantiating the presence of viral infection.

Cherry leaf roll virus (CLRV) is primarily transmitted through vegetative propagation methods such as grafting, leading to the development of a characteristic symptom known as 'blackline' at the graft union. This necrotic reaction disrupts vascular connectivity, ultimately affecting tree vigor and productivity. Given that CLRV infection can cause substantial reductions in walnut (*Juglans regia*) yields, it is crucial for growers to implement effective disease management strategies. Since no chemical treatments are available for viral infections due to the high mutation rates and ability of plant viruses to evade host defense mechanisms, integrated management approaches are recommended. These include the use of virus-free, certified planting materials, rigorous sanitation of grafting tools, control of insect and weed vectors that may facilitate viral transmission, and systematic removal of infected trees to prevent further spread within orchards. Implementing these preventive measures is essential for maintaining the long-term sustainability of walnut production.

Author's Contribution

The authors declare that BMT: collected the samples, performed the extractions, PCR, and bioinformatics analyses, and writing the manuscript; SÖ: performed the bioinformatics analyses and editing the manuscripts; CUS: collecting the samples, preparing the samples for HTS and writing-editing the manuscripts.

Conflict of interests/Competing interests

The authors declare that there is no conflict of interest.

Ethics approval

The authors declare that there is no ethical issue.

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Determination of Plant Parasitic Nematode Fauna and Evaluation of Soil Quality in Olive Orchards of Çanakkale Province, Türkiye

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ABSTRACT
In September 2023, a total of 185 soil samples were collected from the olive orchards in Çanakkale province's Centre district and the districts of Ayvacık, Bayramiç, Biga, Ezine, and Lapseki to identify the plant-parasitic nematode communities present, determine their distribution maps, and evaluate soil quality by demonstrating the use of nematodes as bioindicators. A total of 22.257 nematode individuals were examined, and 33 genera belonging to 19 families were identified. The Rhabditida order constituted 47.62% of the population, followed by the Tylenchida order with 23.18% and the Aphelenchida order with 22.44%. The most prevalent plant-parasitic nematodes were identified as *Merlinius* spp. Siddiqi, 1970 (Tylenchida: Dolichodoridae) (10.41%), *Tylenchus* spp. Bastian, 1865 (Tylenchida: Tylenchidae) (3.47%) and *Helicotylenchus* spp. Steiner, 1945 (Tylenchida: Hoplolaimidae) (1.77%). The dominance of the p-p 3 group indicates that this group has a common life strategy among herbivorous nematodes and poses a potential threat in agricultural ecosystems. The prevalence of the c-p 2 group among free-living nematodes highlights the critical role of this group in ecosystem processes, particularly in organic matter cycling and soil health.

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Türkiye, Çanakkale İli Zeytin Bahçelerindeki Bitki Paraziti Nematod Faunasının Belirlenmesi ve Toprak Kalitesinin Değerlendirilmesi

ÖZET
Eylül ayı 2023'te Çanakkale ilinin Merkez ilçesi ile Ayvacık, Bayramiç, Biga, Ezine ve Lapseki ilçelerindeki zeytin bahçelerinden toplam 185 toprak örneği toplanmıştır. Bu örnekler bölgede bulunan bitki-paraziti nematod topluluklarını tanımlamak, dağılım haritalarını belirlemek ve nematodların biyoindikatör olarak kullanımını göstererek toprak kalitesini değerlendirmek amacıyla incelenmiştir. Toplamda 22.257 nematod bireyi incelenmiş ve 19 familyaya ait 33 cins tanımlanmıştır. Popülasyonun %47.62'sini Rhabditida takımı oluştururken bunu %23.18 ile Tylenchida ve %22.44 ile Aphelenchida takip etmiştir. En yaygın bitki-paraziti nematodlar *Merlinius* spp. Siddiqi, 1970 (Tylenchida: Dolichodoridae) (%10.41), *Tylenchus* spp. Bastian, 1865 (Tylenchida: Tylenchidae) (%3.47) ve *Helicotylenchus* spp. Steiner, 1945 (Tylenchida: Hoplolaimidae) (%1.77) olarak tespit edilmiştir. Herbivor nematodlar arasında p-p 3 grubunun baskınlığı, bu grubun ortak bir yaşam stratejisine sahip olduğunu ve tarımsal ekosistemlerde potansiyel bir tehdit oluşturabileceğini göstermektedir. Serbest yaşayan nematodlar arasında c-p 2 grubunun yaygınlığı ise bu grubun ekosistem süreçlerinde, özellikle organik madde döngüsü ve toprak sağlığında oynadığı kritik rolü vurgulamaktadır.

Bitki Koruma

Araştırma Makalesi

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INTRODUCTION

The olive tree stands out as an agricultural activity of both economic and cultural importance (Kocadağlı, 2011; Pilak & Ülger, 2021). Olive trees have a history spanning thousands of years and are an essential part of dietary habits and traditional culinary culture (Liphschitz et al., 1991). Due to their richness in phenolic compounds and vitamins, olives have become a valuable food in terms of nutrition and health (Ozturk et al., 2021). The health benefits and high nutritional value of olives, combined with olive oil production, have led to increasing global demand (Özata & Cömert, 2016). Furthermore, olive cultivation plays a significant role in sustainable agriculture, particularly in arid regions, by protecting soil health and combating desertification (Pleguezuelo et al., 2018).

Most of the global olive production takes place in regions with a Mediterranean climate (Sakar & Ünver, 2014). Türkiye, located in the Mediterranean basin, ranks among the top countries worldwide with a total production area of 889.000 hectares and a production volume of 1 million tons (FAO, 2024). In Türkiye, olives and olive oil are products with high income potential and hold a significant position in exports (Tunç & Yilmaz, 2023). Olive cultivation is concentrated mainly in the Aegean, Marmara, and Mediterranean regions of the country. Çanakkale, located in the Marmara Region, is one of Türkiye's leading provinces in terms of olive production area and volume (TUİK, 2022).

Plant-parasitic nematodes are generally cylindrical, thread-like microscopic organisms. A significant portion of important species of these nematodes belong to the order Tylenchida within the phylum Nematoda (Kepenekçi & Ökten, 1999). Approximately 4,100 species of plant-parasitic nematodes have been identified globally, many of which cause significant economic losses in agricultural production (Kornobis, 2023). These economic losses are estimated to be around 157 billion dollars annually (Chariou & Steinmetz, 2017). As one of the major pests in agricultural areas, these nematodes cause serious damage to olive trees, especially olive seedlings, posing a significant threat to olive production worldwide (Ali et al., 2014). By damaging the root systems of plants and agricultural crops, they threaten the health of the plants (Göze Özdemir, 2022). *Pratylenchus* spp. Filipjev, 1936 (Tylenchida: Pratylenchidae) and *Meloidogyne* spp. Goeldi, 1892 (Tylenchida: Meloidogynidae) are nematodes with the greatest potential to harm olive trees (Belahmar et al., 2015). Particularly, finding and implementing alternative control methods against root-knot nematodes is of great importance (Çetintaş et al., 2018).

Nematodes are widely used as effective bioindicators to monitor ecosystem health and environmental changes. They are regarded as key components of biodiversity and nutrient cycling in soil ecosystems. Therefore, the diversity of nematodes provides important information about soil health and biodiversity. The abundance of fungivorous and bacterivorous nematodes, in particular, is evaluated as a reflection of sustainable agricultural practices (Yeates & Bongers, 1999). These organisms are critical for maintaining the balance of soil microflora and fauna, and they are considered potential bioindicators for sustainable soil management practices (Moura & Franzener, 2017).

This study aims to determine the distribution maps and densities of nematodes using soil samples collected from olive orchards in Çanakkale province and its districts. By mapping the distribution of plant-parasitic nematodes commonly found in olive orchards, the areas with high concentrations of these pests will be identified. If areas with a high concentration of *Meloidogyne* spp. and *Pratylenchus* spp., which cause significant economic losses in olive trees, are identified, this will contribute to the development of effective nematode control programs in these areas.

MATERIALS and METHODS

Survey

To determine the genera and densities of plant-parasitic nematodes a total of 185 soil samples were collected between 2023 and 2024 from olive orchards in Çanakkale's the centre district as well as the districts of Ayvacık, Bayramiç, Biga, Ezine, and Lapseki. The soil samples were collected in amounts of 1 kg from a depth of 10-30 cm beneath the canopy drip line of trees, ensuring the representation of the region. The samples were placed in polyethylene bags, labeled, and stored in coolers. After the sampling process was completed the soil samples were transported to the Nematology Laboratory at Çanakkale Onsekiz Mart University's Faculty of Agriculture and stored at + 4 °C in a refrigerator until the analysis phase (Figure 1).

Nematode Extraction from Soil

The Baermann Funnel Method, which facilitates the migration of mobile nematodes from the soil medium to a water medium was used to extract nematodes from the soil (Hooper, 1986). This method was carried out using 12

cm diameter and 2 cm height plastic petri dishes with plastic sieves placed at a height of 0.5 cm. Filter paper was laid on the sieves, and 100 g of homogeneous soil samples were added. After the soil was moistened with water the petri dish was sealed and left for 48 hours. At the end of the period the water in the petri dish was transferred to 100 ml glass measuring cylinders and left for 24 hours to allow the nematodes to concentrate. The water was then carefully reduced to 10 ml, placed in 10 ml glass tubes, and stored under appropriate conditions.

Figure 1. Map of olive sampling areas from districts
Şekil 1. İlçelerden alınan zeytin örnekleme alanlarının haritası.

Light Microscopy Diagnosis at Genus Level

The water in the glass tube was diluted to 1 ml and homogenized using a vortex mixer. Subsequently a 100 µl water sample was taken with a micropipette, placed on a microscope slide and covered with a coverslip. To immobilize the nematodes and ensure accurate identification the samples were prepared on a heated plate set to a specific temperature. The counting and identification of the samples at the genus level were performed using a Leica DM 1000 light microscope and Leica Application Suite v4 software.

Nematode Communities and Analyses

Taxonomic keys were primarily used for the classification of nematodes. For the identification of plant-parasitic nematodes the book "Plant-Parasitic Nematodes: A Pictorial Key to Genera" by Mai et al. (1996) was particularly useful. The life cycle traits of nematodes were ranked from 1 to 5 based on the colonizer-persister classification proposed by Bongers (1990; 1999). The feeding types of nematodes were determined in accordance with the classification criteria presented by Yeates et al. (1993) and Du Preez et al. (2022). To evaluate the maturity of nematode community composition in the ecosystem, structure and enrichment indices were calculated (Ferris et al., 2001; Ferris & Bongers, 2009). The Nematode Indicator Joint Analysis (NINJA) software, an online tool was used to analyze the data (Sieriebriennikov et al., 2014).

NINJA is an R-based automated calculation system designed to facilitate the computation of nematode-based biological monitoring metrics. The program was developed to automate statistical and analytical processes for calculating various metrics. As a parametric tool, NINJA primarily employs ANOVA-based parametric approaches in analyses. ANOVA is a fundamental statistical method used to evaluate whether there are significant differences among sampling areas, providing results supported by mean values, standard deviations, and p-values. Additionally, it calculates the mean and standard deviation values for sampling areas and presents them to users in summary tables. By implementing these parametric analysis methods quickly and accurately, NINJA simplifies data analysis processes for users and offers additional tools for visualizing results. This system stands out as a reliable and accessible solution for nematode-based ecological studies.

RESULTS

In the present study a total of 22.257 nematode individuals were identified. The Tylenchida order comprised

23.18% of the nematode population with 5,159 individuals while the Aphelenchida order represented 22.44% with 4.994 individuals. The Dorylaimida order accounted for 2.80% with 624 individuals, and the Mononchida order made up 3.96% with 882 individuals. Lastly the Rhabditida order formed the largest portion of the population with 47.62% amounting to 10.598 individuals (Table 1).

Table 1 The prevalence rates, cp series, and feeding types of nematode communities
Çizelge 1. Nematod toplulukların bulunma oranları, cp serileri ve beslenme tipleri.

Genus Name	Order: Family	Prevalence Rate (%)	C-p Class	P-p Class	Feeding Type
Aglenchus Andrassy, 1954	Tylenchida: Tylenchidae	0.14	0	2	Herbivores
Anguina Scopoli, 1777	Tylenchida: Anguinidae	0.02	0	2	Herbivores
Aphelenchoides Fischer, 1894	Aphelenchida: Aphelenchoididae	13.96	2	0	Fungivores
Aphelenchus Bastian, 1865	Aphelenchida:Aphelenchidae	8.47	2	0	Fungivores
Belonolaimus Steiner, 1949	Tylenchida: Belonolaimidae	0.02	0	3	Herbivores
Boleodorus Thorne, 1941	Tylenchida: Tylenchidae	0.02	0	2	Herbivores
Coslenchus Siddiqi, 1978	Tylenchida: Tylenchidae	0.17	0	2	Herbivores
Discocriconemella De Grisse & Loof, 1965	Tylenchida: Criconematidae	0.02	0	3	Herbivores
Ditylenchus Filipjev, 1936	Tylenchida: Anguinidae	0.94	2	0	Fungivores
Dorylaimus Dujardin, 1845	Dorylaimida: Dorylaimidae	2.56	4	0	Omnivores
Eucephalobus Steiner, 1936	Rhabditida: Cephalobidae	46.53	2	0	Bacterivores
Filenchus Andrassy, 1954	Tylenchida: Tylenchidae	1.22	2	0	Fungivores
Gracilacus Raski, 1962	Tylenchida: Tylenchulidae	0.04	0	2	Herbivores
Heterodera Schmidt, 1871	Tylenchida: Heteroderidae	0.01	0	3	Herbivores
Helicotylenchus Steiner, 1945	Tylenchida: Hoplolaimidae	1.77	0	3	Herbivores
Hoplolaimus von Daday, 1905	Tylenchida: Hoplolaimidae	0.36	0	3	Herbivores
Malenchus Andrassy, 1968	Tylenchida: Tylenchidae	0.36	0	2	Herbivores
Meloidogyne Goeldi, 1892	Tylenchida: Meloidogynidae	0.11	0	3	Herbivores
Merlinius Siddiqi, 1970	Tylenchida: Dolichodoridae	10.41	0	3	Herbivores
Mononchus Bastian, 1865	Mononchida: Mononchoidea	3.96	4	0	Predators
Paratylenchus Micoletzky, 1922	Tylenchida: Tylenchulidae	0.49	0	2	Herbivores
Pratylenchoides Winslow, 1958	Tylenchida: Pratylenchidae	0.35	0	3	Herbivores
Pratylenchus Filipjev, 1936	Tylenchida: Pratylenchidae	1.18	0	3	Herbivores
Psilenchus de Man, 1921	Tylenchida: Psilenchidae	0.28	0	2	Herbivores
Rhabditis Dujardin, 1844	Rhabditida: Rhabditidae	1.09	1	0	Bacterivores
Rotylenchulus Linford & Oliveira, 1940	Tylenchida: Hoplolaimidae	0.02	0	3	Herbivores
Rotylenchus Filipjev, 1936	Tylenchida: Hoplolaimidae	0.08	0	3	Herbivores
Scutellonema (Steiner, 1937) Andrassy, 1958	Tylenchida: Hoplolaimidae	0.13	0	3	Herbivores
Trophurus Loof, 1956	Tylenchida Telotylenchidae	0.17	0	3	Herbivores
Tylencholaimus De Man, 1876	Dorylaimida: Tylencholaimoidea	0.16	4	0	Fungivores
Tylenchorhynchus Cobb, 1913	Tylenchida: Telotylenchidae	1.41	0	3	Herbivores
Tylenchus Bastian, 1865	Tylenchida: Tylenchidae	3.47	0	2	Herbivores
Xiphinema Cobb, 1913	Dorylaimida: Longidoridae	0.08	0	5	Herbivores

These findings indicate that the Rhabditida order was dominant in the population and the Tylenchida and Aphelenchida orders also constitute significant proportions. The Dorylaimida and Mononchida orders were represented at lower rates in the population.

Classification of Nematodes According to Their Feeding Type

When analyzing the feeding type composition of nematode communities in districts of Çanakkale, bacterivorous nematodes were found to be the most common. Bacterivorous nematodes were the dominant group in Ayvacık (46.5%), Bayramiç (53.1%), Biga (51.1%), Ezine (43.6%), Lapseki (49.4%), and the (55.7%). Fungivorous nematodes

also had significant proportions in Ayvacık (25.0%), Bayramiç (23.3%), Biga (18.8%), Ezine (24.3%), Lapseki (23.9%), and the centre district (20.5%). Herbivorous nematodes were present in all districts at rates ranging from 15.0% to 25.0%, while predator and omnivorous nematodes were found at lower levels compared to other groups. These results showed that bacterivorous and fungivorous nematodes were dominant across Çanakkale although differences in feeding types were observed among the districts (Figure 2).

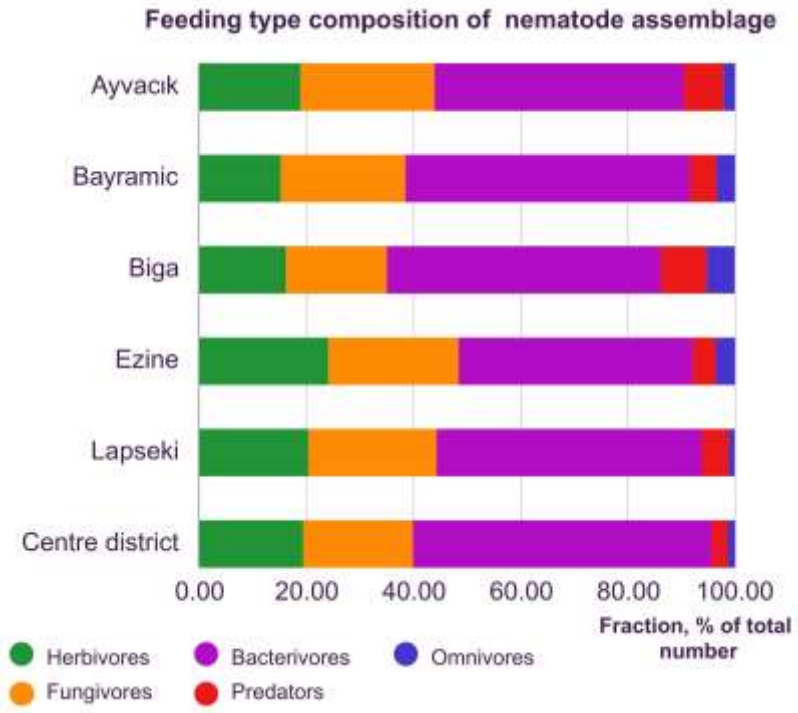

Figure 2. Distribution of nematode communities by feeding types
Şekil 2. Nematod topluluklarının beslenme tiplerine göre dağılımı.

According to the feeding type composition results of free-living nematode communities, bacterivores were the most dominant group. Bacterivores reached the highest rates in all districts, particularly in the the centre district (69.1%). In other districts, bacterivore rates were 62.6% in Bayramiç, 61.0% in Biga, 62.1% in Lapseki, 57.4% in Ayvacık, and 57.5% in Ezine. Fungivores were the second most prevalent group after bacterivores, with higher proportions in Ayvacık (30.8%) and Ezine (32.1%). Predatory and omnivorous nematodes were detected at lower rates in all districts, with predator rates ranging from 3.7% (the centre district) to 10.3% (Biga) and omnivore rates between 1.4% (Lapseki) and 6.3% (Biga). These results indicated that bacterial decomposition is dominant in soil ecosystems and that environmental conditions influence the distribution of nematode feeding types (Figure 3).

In the composition of herbivorous nematode communities ectoparasites were the dominant group in all districts. The highest rate was observed in Biga (89.3%) and the lowest in the centre district (47.3%). Epidermal/root hair feeders were detected at rates of 17.8% in Ayvacık, 35.3% in Bayramiç, 4.8% in Biga, 19.1% in Ezine, 21.1% in Lapseki, and 27.9% in the centre district. Migratory endoparasites were found at 5.9% in Biga, 8.4% in Ezine, and 8.5% in Ayvacık, with rates below 1.7% in other districts. Semi-endoparasites were prominent in Lapseki (16.3%) and the centre district (23.6%), while sedentary parasites were found in low proportions only in Ayvacık (0.7%) and Lapseki (5.3%). These results showed that ectoparasites were dominant in all regions, while other feeding groups vary depending on the district (Figure 4).

The food web analysis values for each district indicate variability in the soil ecosystems (Figure 5). Ayvacık and Ezine, with high Enrichment Index (EI) and moderate Structure Index (SI) values, possess nutrient-rich and structurally balanced ecosystems, while Biga showed the highest structural development with the highest SI value despite its low EI. Lapseki, with both a high EI and low SI, indicates a nutrient-rich but structurally weaker ecosystem. Bayramiç and the centre district with both low EI and SI values, reveal that their ecosystems were weaker in both nutrient and structural aspects (Ferris et al., 2001). These differences between the districts were clearly seen in the graph.

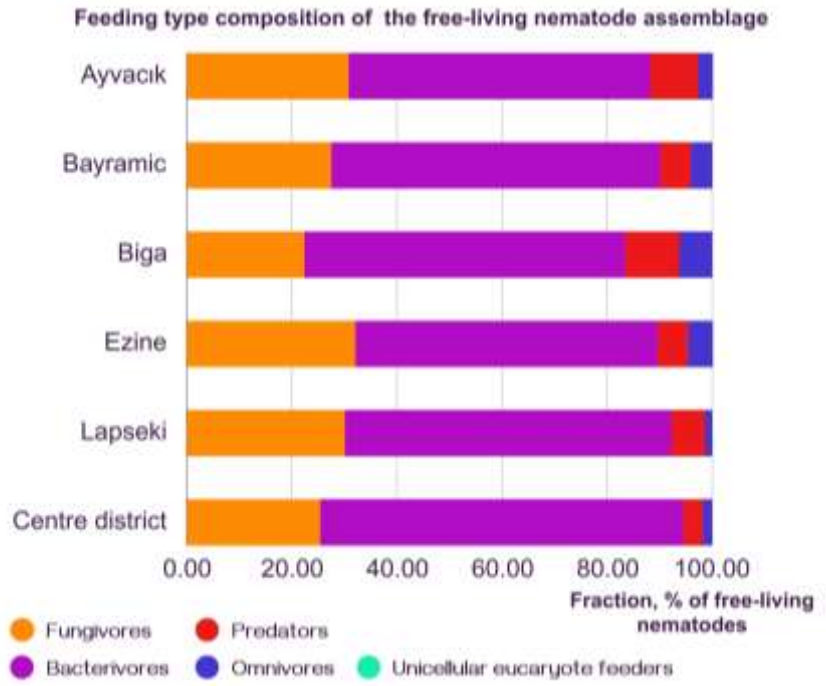

Figure 3. Distribution of free-living nematode communities based on feeding types
Şekil 3. Serbest yaşayan nematod topluluklarının beslenme tiplerine göre dağılımı.

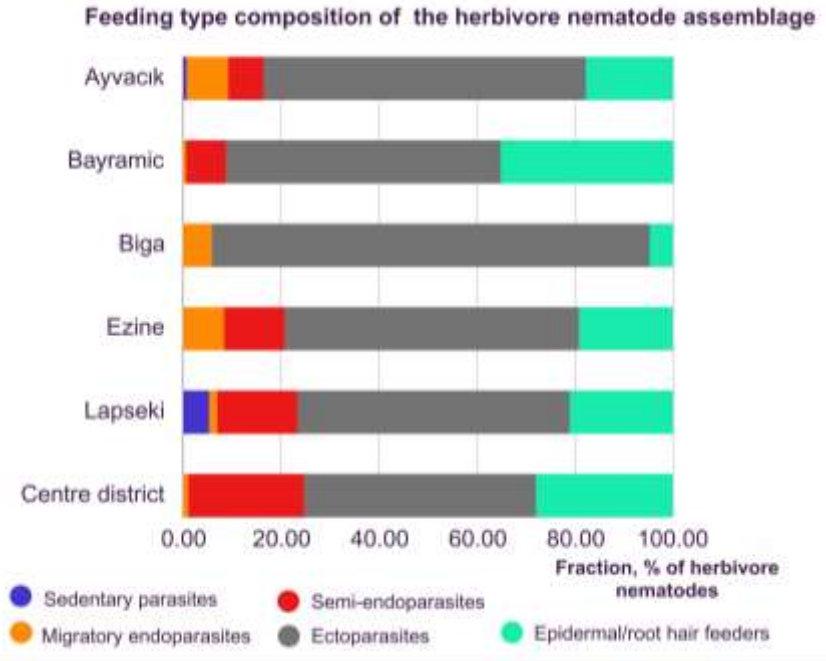

Figure 4. Distribution of plant-parasitic nematode communities by feeding types
Şekil 4. Bitki paraziti nematod topluluklarının beslenme tiplerine göre dağılımı.

Index Analysis and Classification According to C-P Series

In the colonizer-persister structure of free-living nematode communities, c-p 2 was the most common group in all districts, with the highest rate in the centre district (93.2%) and the lowest in Biga (83.5%). The c-p 4 group had the highest proportion in Biga (16.5%) and the lowest in the centre district (5.7%). The c-p 1 group reached the highest value in Lapseki (2.7%) and the lowest in Ayvacık and Bayramiç (0.9%). As a result, the c-p 2 group emerged as the most prevalent in all districts, while the c-p 4 and c-p 1 groups showed less prevalence (Figure 6).

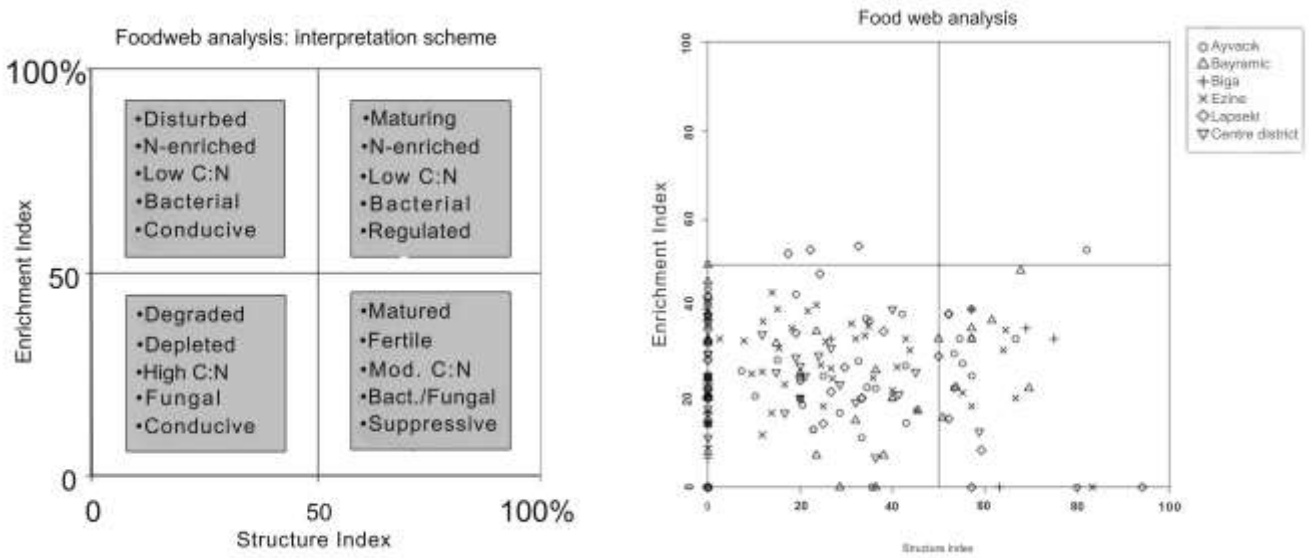

Figure 5. Food web analysis of total nematode communities across all districts
Şekil 5. Tüm ilçelerdeki toplam nematod topluluklarının besin ağı analizi.

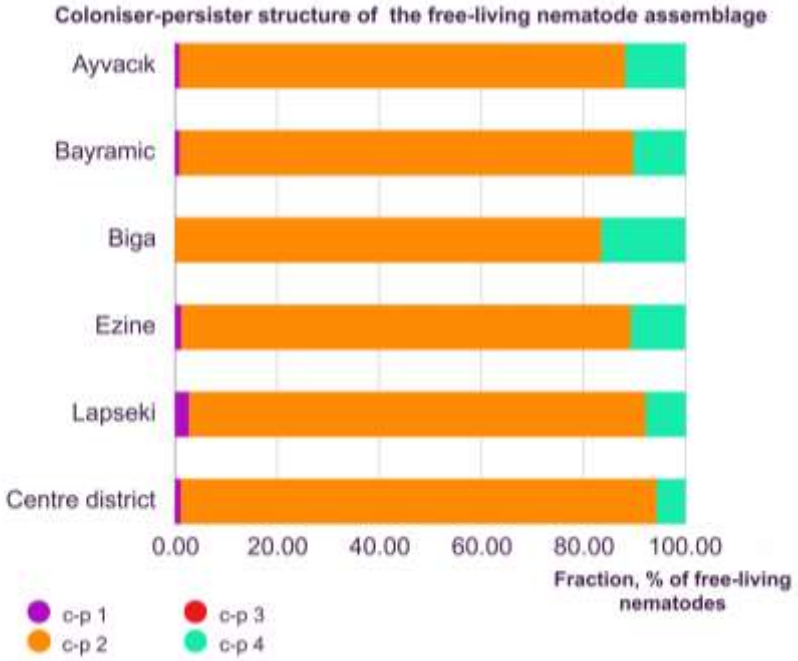

Figure 6. Distribution of cp series in free-living nematode communities
Şekil 6. Serbest yaşayan nematod toplulukların cp serilerinin dağılımı.

The results of the life strategy analysis of herbivorous nematodes indicated that the p-p 3 group was dominant in all districts, with proportions of 80.8% in Ayvacık, 61.0% in Bayramiç, 95.2% in Biga, 77.8% in Ezine, 77.2% in Lapseki, and 69.2% in the centre district. The p-p 2 group typically ranked second, with its highest rate observed in Bayramiç (39.0%). The p-p 5 group was found only in low proportions in Ezine (1.0%) and the centre district (0.3%). These findings showed that the p-p 3 group were dominant in the life strategy of herbivorous nematodes across all regions (Figure 7).

The maturity and plant-parasitic index analyses of nematodes provide important insights into the ecosystem status and the effects of agricultural activities in the studied districts. The Maturity Index (MI) values reflect the impacts of environmental degradation and enrichment (Bongers, 1990). Low MI values in Ayvacık (2.22), Bayramiç (2.20), Biga (2.35), Ezine (2.20), Lapseki (2.15), and the centre district (2.10) suggest higher environmental degradation and nutrient enrichment. The MI2-5 index follows a similar trend with low values in Ayvacık (2.23), Bayramiç (2.21), Biga (2.35), Ezine (2.22), Lapseki (2.18) and the centre district (2.12) indicating that pollution-related stress was more pronounced particularly in Lapseki and the centre district (Bongers & Korthals, 1993).

Sigma MI values also show that nematode community structures were less mature in Ayvacık (2.34), Bayramiç (2.26), Biga (2.43), Ezine (2.35), Lapseki (2.26) and the centre district (2.22). The Plant-Parasitic Index (PPI) for plant-parasitic nematodes was determined as Ayvacık (PPI: 2.80), Bayramiç (2.65), Biga (2.86), Ezine (2.80), Lapseki (2.73), and the centre district (2.68), indicating that plant health was more affected by plant-parasitic nematodes, particularly in Biga and Ayvacık.

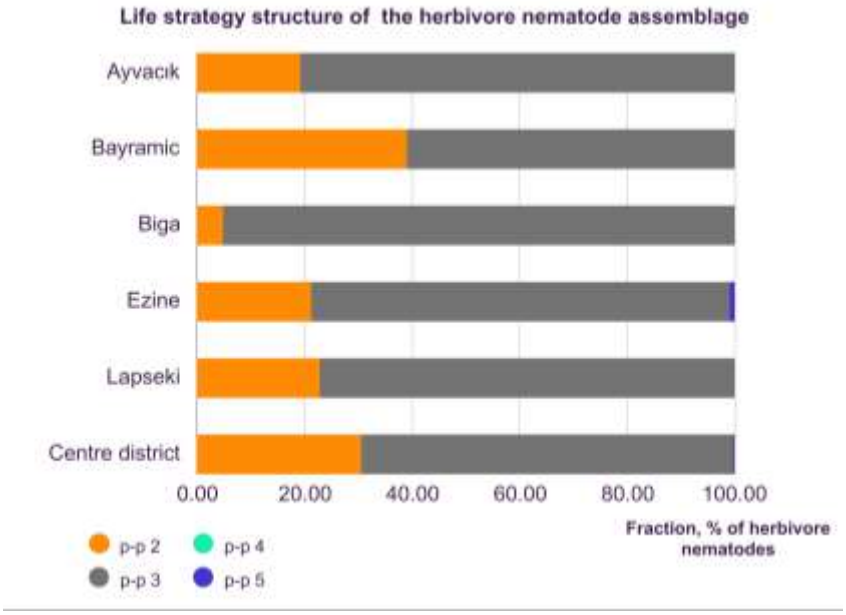

Figure 7. Distribution of pp series in plant-parasitic nematode communities
Şekil 7. Bitki paraziti nematod toplulukların pp serilerinin dağılımı.

Based on the Enrichment Index (EI) and Structure Index (SI) values, we can compare the ecological statuses of nematode communities in Ayvacık, Bayramiç, Biga, Ezine, Lapseki, and the centre district (Ferris et al., 2001). Ayvacık (EI 26.99, SI 28.51), Ezine (EI 27.68, SI 27.84), and Lapseki (EI 27.72, SI 20.41) were nutrient-rich areas, while Biga (EI 21.85, SI 36.37) stands out in terms of ecosystem complexity and biodiversity. Bayramiç (EI 23.01, SI 25.65) showed moderate levels of both nutrient richness and ecosystem complexity, whereas the centre district (EI 23.35, SI 16.90) drew attention with both the lowest EI and SI values, indicating that it has the lowest organic matter richness and ecosystem complexity, and it may be more vulnerable to environmental pressures.

The Basal Index (BI) and Channel Index (CI) values exhibited notable and similar variations. The BI values were calculated as 55.29 for Ayvacık, 60.14 for Bayramiç, 55.01 for Biga, 55.12 for Ezine, 58.55 for Lapseki, and 65.75 for the Centre district. These results indicated that the BI values in all regions were high (>50), suggesting that the soil food web was depleted or damaged. The higher BI value observed in the centre district suggested that this area was one of the most affected by soil degradation.

The CI values were determined as 92.88 in Ayvacık, 92.04 in Bayramiç, 100 in Biga, 88.84 in Ezine, 82.89 in Lapseki, and 89.26 in the centre district. It was observed that the CI values were high (>50) in all regions, indicating that decomposition processes were predominantly carried out by fungi and that complex organic matter was being broken down slowly. Particularly, the CI value of 100 in Biga indicated that fungal-dominated decomposition was at its maximum level, and the transformation of organic matter occurred more slowly in this region compared to others. These findings revealed that the biological functionality of nematode communities and their effects on ecological processes varied across different regions.

The triangular diagram reflected the ecological structure and strategies of nematode communities, balancing between enrichment, stress tolerance, and stability (de Goede et al., 1993). Most district points were close to the c-p 2 region indicating that nematode communities in the districts could quickly respond to nutrient increases, although ecosystem complexity was limited. Ayvacık, Lapseki, and Ezine were located closer to stability suggesting that the nematode communities in these areas were more resilient to environmental changes. Ayvacık and some other districts showed a balanced structure between enrichment and stress tolerance, but most points indicate that nematodes preferred more stressful and less balanced soils (Figure 8).

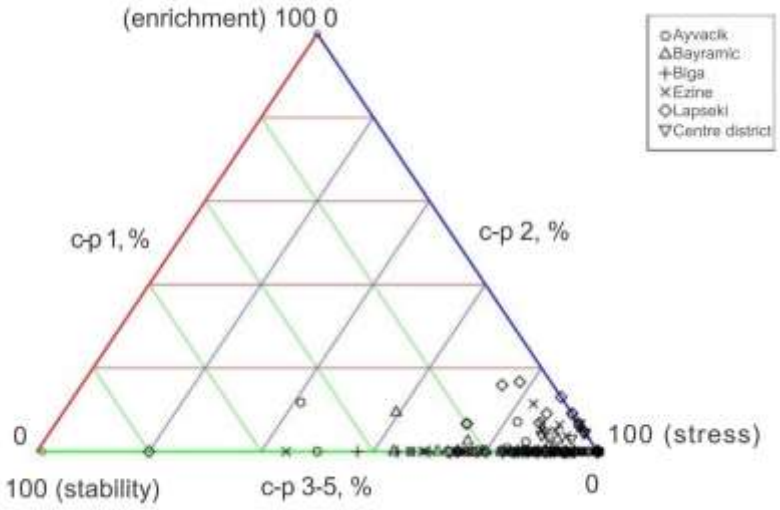

Figure 8. Triangular diagram illustrating the structure of nematode communities
Şekil 8. Nematod topluluklarının yapısını gösteren üçgen diyagram.

Metabolic Footprints

The results for the Composite footprint, Enrichment footprint, and Structure footprint values were as follows: The Composite footprint values were calculated as 29.52 for Ayvacık, 31.56 for Bayramiç, 31.39 for Biga, 60.31 for Ezine, 32.26 for Lapseki, and 32.53 for the centre district. These results indicated that Ezine had the highest value in terms of ecosystem functionality and biological activity compared to other regions. The Enrichment footprint values were determined as 2.82 in Ayvacık, 3.55 in Bayramiç, 1.29 in Biga, 8.41 in Ezine, 6.97 in Lapseki, and 4.88 in the centre district. These values suggested that Ezine and Lapseki exhibited a decomposition process more sensitive to nutrient inputs. The Structure footprint values were identified as 19.67 in Ayvacık, 21.66 in Bayramiç, 25.86 in Biga, 36.27 in Ezine, 9.61 in Lapseki, and 18.17 in the centre district. These findings indicated that Ezine and Biga had complex and stable food webs, while Lapseki likely had a less developed ecosystem structure. Overall, the variation in these metabolic footprint values across different regions revealed that the contributions of nematode communities to ecological processes depended on local conditions.

The Herbivore footprint, Fungivore footprint, Bacterivore footprint, Predator footprint, and Omnivore footprint values exhibited distinct differences. The Herbivore footprint values were determined as 2.16 in Ayvacık, 1.46 in Bayramiç, 1.11 in Biga, 6.57 in Ezine, 11.26 in Lapseki, and 3.22 in the centre district. These results showed that Lapseki and Ezine had a greater impact from plant-parasitic nematodes on the ecosystem compared to other regions. The Fungivore footprint values were calculated as 1.93 in Ayvacık, 1.68 in Bayramiç, 1.29 in Biga, 4.51 in Ezine, 2.25 in Lapseki, and 2.40 in the centre district. These values revealed that Ezine had higher biological activity in fungus-based energy transformation processes compared to other regions. The Bacterivore footprint values were identified as 5.76 for Ayvacık, 6.81 for Bayramiç, 3.12 for Biga, 13.03 for Ezine, 9.15 for Lapseki, and 8.77 for the centre district. This indicated that bacterial decomposition processes mediated by bacterivorous nematodes were dominant in Ezine. The Predator footprint values were 4.14 in Ayvacık, 3.17 in Bayramiç, 3.33 in Biga, 7.18 in Ezine, 3.27 in Lapseki, and 4.79 in the centre district. The high value in Ezine emphasized the significance of predator species in the food web of this region. The Omnivore footprint values were recorded as 15.53 for Ayvacık, 18.44 for Bayramiç, 22.54 for Biga, 29.02 for Ezine, 6.34 for Lapseki, and 13.36 for the centre district. Notably, Ezine and Biga showed high values, indicating complex food webs dominated by omnivorous nematodes. These findings demonstrated that nematode communities contributed differently to ecosystem processes across trophic levels, depending on the region.

DISCUSSION

Although Çanakkale is a significant region for olive cultivation in Türkiye, plant-parasitic nematodes cause substantial damage to olive trees (Castillo et al., 1999; Nico et al., 2002). Among them, *Pratylenchus* spp. (root lesion nematodes) and *Meloidogyne* spp. (root-knot nematodes) stand out as nematodes with the potential to cause severe damage to olive trees (Belahmar et al., 2015). In this study, a total of 33 nematode genera were identified, and key harmful nematodes, such as *Meloidogyne* and *Pratylenchus*, were found in olive plants. Similar taxa have also been reported in global studies (Belahmar et al., 2015; Hamza et al., 2015; Guesmi-Mzoughi et al., 2022). In

previous studies conducted in Türkiye, Kepenekci (2001) identified 23 different plant-parasitic nematode genera in research carried out in the Mediterranean and Black Sea regions, but *Meloidogyne* was not detected. In another study conducted in Ödemiş, Izmir, the species *Helicotylenchus multicinctus* was identified in olive orchards (Yıldız & Gözel, 2015). Literature review revealed only one study focused on determining the plant-parasitic nematode fauna in olive orchards in the Çanakkale region. This study, conducted by Öztürk (2023), identified 12 different nematode genera from 15 samples taken from olive orchards in Ayvacık, Bayramiç, Bozcaada, the centre district and Ezine. However, *Meloidogyne* and *Pratylenchus*, known to cause significant damage to olive plants, were not detected. This could be explained by the limited number of sampling sites or the inadequacy of the soil samples. Therefore, more comprehensive research covering a wider range of areas is necessary to fully determine the plant-parasitic nematode fauna in the region.

When plant-parasitic nematode groups (p-p) and free-living nematode groups (c-p) from different districts of Türkiye are evaluated, it is observed that the p-p 3 group is dominant among plant-parasitic nematodes in all regions. In agreement with this study Palomares-Rius et al. (2015), Ali et al. (2017) and Hamza et al. (2018) found that the p-p 3 group was followed by the p-p 2 group, while the p-p 5 group was present at lower rates. A similar finding was observed in Öztürk (2023) study on free-living nematodes in Çanakkale, where the c-p 2 group was the most common, with no detection of the c-p 5 group. The consistency between this study and Öztürk's study supports the widespread presence of the c-p 2 group and suggests that free-living nematodes exhibit similar strategies under Mediterranean climatic conditions. These results generally indicate that the p-p 3 group dominates among plant-parasitic nematodes, while the c-p 2 group is dominant among free-living nematodes in olive-growing Mediterranean ecosystems.

Significant differences in nematode community feeding types across districts of Çanakkale are also observed. Bacterivorous nematodes were generally the dominant group, indicating that bacterial decomposition processes play an essential role in organic matter cycling within the districts. Fungivorous nematodes were also widespread, underscoring the contribution of fungi to soil ecosystems (Ferris et al., 2001). Herbivorous nematodes showed variability among the districts, suggesting that they may have agricultural significance as plant-parasites in some areas. Predator and omnivorous nematodes were found at lower rates, suggesting that these groups play more limited roles in ecosystem balance. In a similar study conducted by Cakmak (2024) on olive trees, bacterivorous nematodes were also dominant (70.7%), and plant-parasitic nematodes (20.9%) were observed at significant rates. These findings highlight that bacterivorous and fungivorous nematodes play central roles in organic matter cycling throughout Çanakkale, although there are some differences in feeding types between districts.

Plant-Parasitic Index (PPI) values in various districts of Çanakkale generally range between 2.65 and 2.86, aligning with similar international studies conducted in the Mediterranean region, where PPI values in olive-growing areas typically range from 2.0 to 3.0. In a study by Ali et al. (2017) in Morocco, PPI values varied according to irrigation methods, with an average of 2.60. This value closely matches the PPI values in Bayramiç and the centre district, suggesting that regional differences and agricultural practices directly affect PPI values. The PPI value of 2.85 detected in Ragusa, Italy by Landi et al. (2022) is consistent with the 2.86 value observed in Biga, indicating that similar ecological conditions in olive-growing soils may contribute to higher PPI values. However, the lower PPI value of 2.03 found in Foggia suggests that this region may have different environmental conditions and soil composition (Bongers et al., 1997). In conclusion, the PPI values of this study largely correspond with those from various regions of the Mediterranean.

Metabolic footprints have provided valuable insights into ecosystem functions and services within the soil food web, highlighting the roles of nematode communities in carbon transfer and nutrient cycling. Additionally ANOVA results, with p-values ranging between 0.01 and the threshold of 0.05, confirmed statistically significant differences between regions. This finding indicates that local conditions have a strong influence on the metabolic activities and ecological roles of nematode communities. The usability of metabolic footprints as sensitive indicators of soil ecosystem functions and services supports a detailed understanding of regional ecological dynamics (Ferris, 2010).

Composite footprint values revealed the highest value in Ezine (60.31), emphasizing this region's superior ecosystem functionality and biological activity. The composite footprint serves as a comprehensive indicator measuring energy flow within the soil food web regardless of the trophic roles of nematode communities. Enrichment footprint values were particularly high in Ezine (8.41) and Lapseki (6.97), reflecting increased carbon utilization by lower trophic levels (cp-1 and cp-2) in response to nutrient inputs and dynamic resource enrichment processes. Structure footprint values were notably higher in Ezine (36.27) and Biga (25.86), indicating the presence of complex and stable food webs dominated by higher trophic groups (cp-3 to cp-5), which play a regulatory role in suppressing opportunistic organisms. Additionally herbivore footprint values were highest in Lapseki (11.26), suggesting more intense energy flow through plant-parasitic nematodes in this region. Fungivore and bacterivore footprint values showed regional variation, with Ezine standing out in fungal (4.51) and bacterial (13.03)

decomposition pathways, highlighting dominant energy transformations in these processes. Predator and omnivore footprint values were particularly prominent in Ezine and Biga, underscoring strong trophic interactions and regulatory functions in these regions. This finding suggests potential contributions to pest suppression and maintaining food web balance.

The metabolic footprint data are not only limited to understanding ecosystem functions but also provide critical information for strategies aimed at improving soil health. For instance, the high enrichment footprint values in Ezine and Lapseki emphasize the sensitivity of these regions to organic matter inputs, which could be optimized to enhance decomposition processes. Furthermore, the dominance of predator and omnivore nematodes in regions like Ezine and Biga highlights the regulatory roles of these communities in ecosystem stability. These findings support the applicability of metabolic footprints in improving agricultural lands, restoring ecosystems, and managing soil health (Ferris et al., 2012). Overall, these results demonstrate that metabolic footprints are effective tools for evaluating soil ecosystem status through a multidimensional approach.

This study provides valuable insights into the distribution of plant-parasitic nematodes in olive-growing areas of Çanakkale and their impact on plant health, offering important information about the region's ecosystem. The PPI values observed align with international studies conducted in various regions of the Mediterranean. This highlights the need for more extensive sampling and research across diverse areas to gain a more comprehensive understanding of the nematode fauna in the region. Additionally, using nematodes as bioindicators can offer valuable information about environmental conditions and soil health. It is known that different nematode groups serve as indicators of soil quality and ecosystem health. Therefore, increasing research on the use of both plant-parasitic and free-living nematodes as bioindicators to understand the ecological balance in olive-growing areas would be beneficial for preserving soil health and promoting sustainable agricultural practices. Such studies can help olive growers develop environmentally friendly farming practices and enhance the role of nematodes in ecosystems.

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Summary of Researchers' Contribution Rate Declaration

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

Conflict of Interest Statement

The authors declare no conflict of interest.

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Morphological and Molecular Characterization of *Terfezia claveryi* and its Distribution in Türkiye

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ABSTRACT

The samples were collected from Ankara, Gaziantep, Konya, Niğde, and Şanlıurfa (Türkiye), between 2020 and 2023. Morphological analyses and nrITS rDNA sequence-based phylogenetic methods were used to examine the samples. Twenty-one specimens displayed morphological features consistent with *Terfezia claveryi* Chatin at both macro and micro levels. Genetic analysis revealed over 99% sequence similarity with this species. Detailed documentation included habitat descriptions, geographical coordinates, collection dates, and photographic records of macroscopic and microscopic structures. These findings contribute valuable insights into the distribution and taxonomy of *T. claveryi* in Türkiye.

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Terfezia claveryi'nin Morfolojik ve Moleküler Karakterizasyonu ve Türkiye'deki Dağılımı

ÖZET

Örnekler, Türkiye'nin Ankara, Gaziantep, Konya, Niğde ve Şanlıurfa illerinden 2020 ile 2023 yılları arasında toplanmıştır. Örneklerin incelenmesinde morfolojik analizler ve nrITS rDNA dizisine dayalı filogenetik yöntemler kullanılmıştır. Yirmi bir örnek, makro ve mikro seviyede *Terfezia claveryi* Chatin ile uyumlu morfolojik özellikler sergilemiştir. Genetik analiz, bu türle %99'dan fazla dizi benzerliği olduğunu ortaya koymuştur. Detaylı dokümantasyon kapsamında habitat tanımları, coğrafi koordinatlar, toplama tarihleri ve makroskobik ile mikroskobik yapıların fotoğrafik kayıtları yer almıştır. Bu bulgular, Türkiye'deki *T. claveryi*'nin dağılımı ve taksonomisi hakkında değerli bilgiler sunmaktadır.

Mikoloji

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INTRODUCTION

Commonly known as the "desert truffle" the genus *Terfezia* (Tul. & C. Tul.) Tul. & C. Tul. belongs to the order *Pezizales* J. Schröt. and the family *Pezizaceae* Dumort., and typically appears during the rainy season between March and May (Alsheikh, 1994). Species within this genus are notable for producing edible subterranean fruiting bodies and are primarily associated with arid and semi-arid regions (Zambonelli et al., 2014). Despite their preference for such climates, *Terfezia* species display remarkable ecological adaptability, thriving in diverse environments, including deciduous and coniferous forests, prairies, and heathlands. These fruiting bodies are highly valued for culinary and medicinal purposes, particularly in regions such as the Middle East, North Africa, and the Mediterranean. The genus is also known for forming symbiotic mycorrhizal associations with specific plants, with a marked affinity for members of the family *Cistaceae* Juss., particularly the species of *Helianthemum* Mill. However, some species are also collected in habitats dominated by oaks and pines. The genus demonstrates significant versatility in its mycorrhizal associations, capable of forming various types such as sheeting

ectomycorrhizae, endomycorrhizae, and ectomycorrhizae (Bordallo et al., 2015; Zitouni-Haouar et al., 2018).

Terfezia members have long been valued for their dual role in culinary and medicinal applications, owing to their high nutritional content and the presence of bioactive compounds (Veeraraghavan et al., 2021). Historically, desert truffles were primarily consumed as a dietary staple in specific regions. However, their significance has only recently garnered broader attention. Today, they are highly regarded for their economic and nutritional contributions and emerging scientific research potential (Bordallo et al., 2015).

According to the Mycobank (https://www.mycobank.org/), *Terfezia* comprises approximately 55 currently recognized species. Seven species have been documented in Türkiye: *T. albida* Ant. Rodr., Muñ.-Moh. & Bordallo, *T. arenaria* (Moris) Trappe, *T. boudieri* Chatin, *T. cistophila* Ant. Rodr., Bordallo, Kaounas & Morte, *T. claveryi* Chatin, *T. leptoderma* (Tul. & C. Tul.) Tul. & C. Tul., and *T. olbiensis* (Tul. & C. Tul.) Sacc. (Akata et al., 2022). These findings highlight the diversity of *Terfezia* species in the region, contributing to understanding truffle biodiversity in Türkiye.

T. claveryi Chatin is extensively distributed across North African and Arabian Peninsula countries, including Morocco, Egypt, Syria, Iraq, Kuwait, and Iran. Its range also spans Mediterranean countries such as Spain, Portugal, Italy, France, Hungary, and Türkiye. These regions are characterized by arid and semi-arid ecosystems where *T. claveryi* primarily associates with *Helianthemum* species in alkaline soils (Marasas & Trappe, 1973; Jamali & Banihashemi, 2012; Abdelaziz, 2018; Zitouni-Haouaret al., 2018). Morphologically, this species is distinctive, producing hypogeous ascomata with a subglobose to turbinate form, weighing between 20 and 350 grams. The mature peridium varies in colour from reddish-brown to blackish-brown, while the gleba is firm and fleshy. The asci are hyaline, generally containing 7-8 spores, which may be globose, ellipsoidal, or subglobose in shape. The ascospores are globose, light brown, and exhibit a reticulate surface texture, occasionally adorned with truncated warts (Jamali & Banihashemi, 2012).

T. claveryi, a desert truffle of significant commercial value, has been traditionally utilised in Middle Eastern folk medicine to address conditions affecting the eyes and skin (Mandeel & Al-Laith, 2007). This species is renowned for its rich nutritional profile, which includes high concentrations of proteins, carbohydrates, and dietary fibre, alongside an array of bioactive compounds such as ascorbic acid, anthocyanins, phenolics, flavonoids, and carotenoids (Veeraraghavan et al., 2021). These attributes, combined with its diverse biological properties, position *T. claveryi* as a medicinally valuable species. Its biological activities encompass anticancer, antimicrobial, antidiabetic, hepatoprotective, antimutagenic, and anti-inflammatory effects, emphasising its potential as a therapeutic agent (Janakat & Nassar, 2010; Akyüz et al., 2015a; Dahham et al., 2018; Malik et al., 2018; AlAhmed & Khalil, 2019).

Economically, *T. claveryi* is highly prized for its culinary and medicinal applications, commanding significant market value. In Europe, its price ranges from 20 to 60 Euros per kilogram, while in Arab countries such as the United Arab Emirates, Qatar, Kuwait, and Saudi Arabia, prices can soar to 220 Euros per kilogram, reflecting its high demand and cultural importance (Abdelaziz, 2018). Furthermore, *T. claveryi* serves as a host for diverse mycoviruses, with recent studies identifying five distinct mycoviruses in its isolates, thereby opening avenues for exploring its role in fungal virology (Sahin et al., 2023; Akata et al., 2024a).

Akata et al. (2022) documented the presence of *T. claveryi* in 15 locations across various provinces of Türkiye, including Adana, Aksaray, Elazığ, Denizli, Diyarbakır, Karaman, Konya, Malatya, Şanlıurfa, and Yozgat, as part of the Checklist of Turkish Truffles. These reports primarily relied on morphological traits for species identification. In contrast, the present study adopts an integrated methodology that combines morphological observations with molecular techniques, offering a more precise and robust framework for identifying *T. claveryi*.

Beyond the fundamental objective of identifying this species, the study seeks to deepen scientific insights into its geographical range, contributing valuable data to expand the understanding of *T. claveryi* distribution across Türkiye.

MATERIAL and METHOD

The study employed an integrative approach that combined conventional morphological methods with advanced molecular techniques to identify and classify specimens collected from Gaziantep, Niğde, Şanlıurfa, Ankara, and Konya in Türkiye (Figure 1). Detailed analyses of macroscopic and microscopic features were conducted to characterize the samples comprehensively. Additionally, the study incorporated ribosomal DNA (rDNA) analysis, focusing on the Internal Transcribed Spacer (ITS) region, to enhance the accuracy of species identification through molecular sequencing. This multi-faceted methodology ensured a robust and reliable classification of the collected specimens.

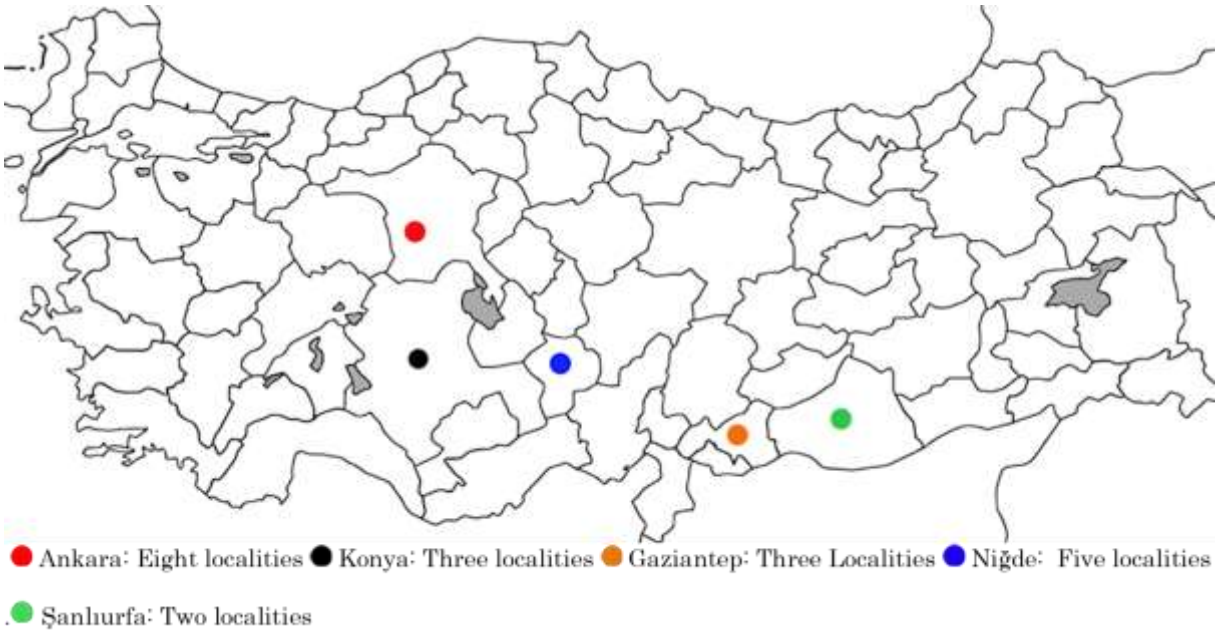

Figure 1. Distribution of *Terfezia claveryi* specimens collected during the present study.
Şekil 1. Bu çalışma sırasında toplanan Terfezia claveryi örneklerinin dağılımı.

Morphological Study

Specimens of *Terfezia claveryi* ascomata were collected during field studies conducted in 2020 and 2023, with detailed documentation of their macroscopic characteristics and ecological surroundings performed on-site. Microscopic examinations were conducted using a Euromex Oxion Trinocular light microscope (LM) and a ZEISS EVO 40XVP scanning electron microscope (SEM). The LM analyses were conducted at 100x magnification, with approximately 30 measurements taken for each feature, subsequently subjected to statistical evaluation. Chemical reagents, including Melzer's reagent, 5% KOH, and Congo red, were used during the analytical processes. For SEM imaging, sections of the gleba were mounted on stubs using double-sided adhesive tape and coated with a thin layer of gold particles to improve conductivity. Imaging was performed under a 20 kV accelerating voltage using the ZEISS EVO 40XVP SEM. The specimens were preserved and catalogued in the Fungarium of the Faculty of Science at Ankara University upon identification.

Molecular Characterization

Determination of the ITS rDNA sequences

Genomic DNA extraction was successfully performed on 21 specimens of the genus *Terfezia*. Afterwards, the nuclear ribosomal internal transcribed spacer (nrITS) rDNA regions were amplified through polymerase chain reaction (PCR). The genomic DNA isolation and ITS rDNA amplification processes adhered strictly to the methodologies outlined by Rogers and Bendich (1994) and White et. al (1990) as well as those described in subsequent studies (Akata & Erdoğdu, 2020; Akata et al., 2024b, 2024c, 2024d), ensuring both the accuracy of the procedure and the reproducibility of the findings.

Molecular Phylogeny Study

A comprehensive phylogenetic analysis of fungal samples was conducted using MEGA-X software, with nucleotide sequences derived directly from the collected specimens (Kumar et al., 2018). Comparative sequences were carefully selected from GenBank, with closely related fungal taxa forming the ingroup and more distantly related sequences designated as the outgroup, identified via NCBI BLAST searches. Sequence alignment was executed using the MUSCLE algorithm, enabling the determination of the most appropriate nucleotide substitution model (Kimura, 1980). Phylogenetic trees were subsequently constructed using the Neighbor-Joining approach, and the reliability of branching patterns was assessed through 1000 bootstrap replicates by Felsenstein (1985). The methodology for this analytical framework aligns closely with the protocols detailed by Akata et al. (2024c; 2024d).

RESULTS

Terfezia claveryi Chatin, (1892), (Figure 2-4).

Macroscopic and microscopic features

Ascomata 40-90 mm diam., initially grows underground but gradually emerges as it matures, semi-globular or pear-shaped, and sometimes lobed, or irregular, **Surface** smooth, commonly developing grooves or becoming wrinkled with age, pale yellow combined with orange-brown tones or ochre-salmon at first, reddish-brown to brownish-black at maturity, **Gleba** solid, fleshy, and succulent texture, the initial colour range of whitish to creamy or pale pinkish, transitioning to a yellowish to pinkish-salmon shade upon maturation, fertile sections are interspersed with sterile veins of a whitish-pink, occasionally spotted with patches of yellow to yellow-brownish discolorations. **Peridium** 500–600 µm thick, whitish to pale yellow, sometimes with a pinkish hue, with a slender brown region present at the surface, the outermost layer made up of hyaline and thin hyphae, innermost layer composed of thick hyphae with yellowish walls. **Asci** 60–90 x 55–65 µm, spherical to pear-shaped, non-amyloid, and 7-8 spored. **Ascospores** 16–18 (19) µm, spherical, excluding ornamentation, initially smooth and hyaline, yellowish to pale brown at maturity, with irregular and coarse reticulum and embellished with rounded warts, occasionally truncated.

Material examined: TÜRKİYE— **Gaziantep**, ***Dülük***, near *Helianthemum salicifolium,* 25 Apr. 2020, 927 m, 37° 08' N, 37° 21' E, ANK AKATA & SAHIN 008, (GenBank accession number: MZ089983.1); ***Küllü***, near *H. ledifolium*, 25 Apr. 2020, 763 m, 37° 03' N, 37° 34' E, ANK AKATA & SAHIN 009, (GenBank Accession number: MZ089984.1); 782 m, 37° 03' N, 37° 35' E, ANK AKATA & SAHIN 010, (GenBank Accession number: MZ089985.1); **Niğde**, ***Gümüşköy***, near *H. canum*, 26 Apr. 2020, 1290 m, 37° 29' N, 34° 36' E, ANK AKATA & SAHIN 011, (GenBank Accession number: MZ089986.1); ***Ulukışla***, near *H. canum,* 1 May 2020, 1380 m, 37° 32' N, 34° 30' E, ANK AKATA & SAHIN 014, (GenBank Accession number: MZ089989.1); ***Yeniköy***, near *H. canum,* 1 May 2020, 1046 m, 37° 43' N, 34° 18' E, ANK AKATA & SAHIN 015, (GenBank Accession number: MZ089990.1); ***Zengen***, near *H. canum,* 2 May 2020, 1042 m, 37° 45' N, 34° 15' E, ANK AKATA & SAHIN 016, (GenBank Accession number: MZ089991.1); ***Altay***, near *H. canum,* 2 May 2020, 1228 m, 37° 38' N, 34° 27' E, ANK AKATA & SAHIN 017, (GenBank Accession number: MZ089992.1); **Şanlıurfa**, ***Yılmaz***, near *H. ledifolium*, 28 Apr. 2020, 650 m, 37° 04' N, 37° 59' E, ANK AKATA & SAHIN 012, (GenBank Accession number: MZ089987.1); ***Uğurcuk***, near *H. ledifolium*, 28 Apr. 2020, 370 m, 37° 03' N, 37° 59' E, ANK AKATA & SAHIN 013, (GenBank Accession number: MZ089988.1); **Ankara, Polatlı, *Üçpınar***, near *H. ledifolium*, 18 Apr. 2023, 1054 m, 39° 36' N, 32° 06' E, ANK AKATA 8764, (GenBank Accession number: OR398211.1); ***Kuşçu,*** near *H. canum,* 18 Apr. 2023, 1008 m, 39° 37' N, 32° 14' E, ANK AKATA 8768,(GenBank Accession number: PP494016. 1);1028 m, 39° 35' N, 32° 15' E, 8772,(GenBank Accession number: PP494016.1); ***Beyceğiz***, near *H. ledifolium*, 18 Apr. 2023, 1000 m, 39° 39' N, 32° 08' E, ANK AKATA 8770,(GenBank Accession number: OR398223.1); ***Kargalı***, *H. canum,* 18 Apr. 2023, 1065 m, 39° 36' N, 32° 15' E, ANK AKATA 8773, (GenBank Accession number: PP494023.1); ***Hacıtuğrul***, near *H. ledifolium*, 30 Apr. 2023, 970 m, 39° 44' N, 32° 13' E, ANK AKATA 8779, (GenBank Accession number: OR394962.1); ***Macun***, near *H. ledifolium,* 30 Apr. 2023, 980 m, 39° 39' N, 32° 16' E, ANK AKATA 8780,(GenBank Accession number: PP494025.1); ***Eskipolatlı***, near *H. ledifolium,* 30 Apr. 2023, 930 m, 39° 31' N, 32° 12' E, ANK AKATA 8784,(GenBank Accession number: PP494026.1); **Konya**, ***Hadim***, near *H. ledifolium*, 11 May 2023, 1382 m, 37° 04' N, 32° 27' E, ANK AKATA 8785,(GenBank Accession number: PP494038.1); ***Sarayköy***, near *H. canum,* 11 May 2023, 1303 m, 37° 53' N, 32° 22' E, ANK AKATA 8789,(GenBank Accession number: PP494039.1); ***Karadiğin***, near *H. ledifolium*, 11 May 2023, 1179 m, 37° 45' N, 32° 22' E, ANK AKATA 8790,(GenBank Accession number: OR394967.1).

Evolutionary History of *T. claveryi* Specimens

The evolutionary relationships of this *T. claveryi* samples were assessed using nuclear ribosomal ITS (nrITS) rDNA sequences, which were generated through standard molecular protocols and archived in the NCBI GenBank database. Comprehensive details on the collection sites and corresponding GenBank accession numbers are presented in Table 1. To elucidate phylogenetic relationships, nrITS rDNA sequences from multiple species within the genus *Terfezia* were analyzed, with the nrITS rDNA sequence of *Mattirolomyces terfezioides* (Mattir.) E. Fisch. designated as the outgroup (Figure 4). Molecular phylogenetic reconstruction revealed the presence of seven distinct clades. Clade 1 exclusively included isolates of *T. claveryi*, while Clades 2 to 7 represented other *Terfezia* species. Placing *M. terfezioides* on a separate branch confirmed its role as the outgroup. BLAST analysis demonstrated over 99% similarity between the nrITS rDNA sequences of Turkish *T. claveryi* specimens and various isolates of *T. claveryi*. Phylogenetic results further substantiated the strong genetic affinity of Turkish *T. claveryi* specimens to other isolates of the same species, with high bootstrap support values underscoring the robustness of their clustering.

Figure 2. Ascomata of *Terfezia claveryi* .
Şekil 2. Terfezia claveryi 'nin askokarpları.

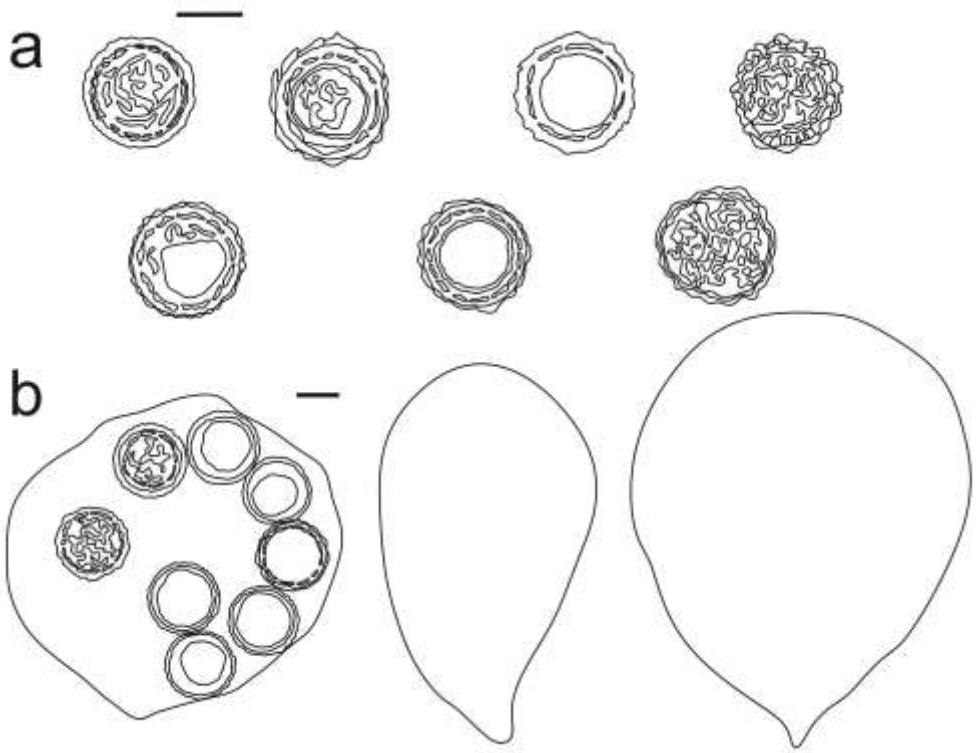

Figure 3. *Terfezia claveryi* : a. spores, b. asci (scale bars: 10 µm).
Şekil 3. Terfezia claveryi: a. sporlar, b. ascuslar (ölçek: 10 µm).

Figure 4. A single spore of *Terfezia claveryi* (SEM)
Şekil 4. Terfezia claveryi'nin sporu (SEM)

Table 1. GenBank accession numbers and localities of the *Terfezia* specimens analyzed in this study
Çizelge 1. Bu çalışmada analiz edilen Terfezia örneklerinin GenBank erişim numaraları ve lokaliteleri

Species	Specimen Voucher/Isolate/Strain	nrITS GenBank Accession Number	Geographical origin	Reference
Terfezia claveryi	ANK AKATA 8764	OR398211.1	Türkiye: Ankara	Current study
	ANK AKATA 8768	PP494016.1	Türkiye: Ankara	Current study
	ANK AKATA 8770	OR398223.1	Türkiye: Ankara	Current study
	ANK AKATA 8772	PP494020.1	Türkiye: Ankara	Current study
	ANK AKATA 8773	PP494023.1	Türkiye: Ankara	Current study
	ANK AKATA 8779	OR394962.1	Türkiye: Ankara	Current study
	ANK AKATA 8780	PP494025.1	Türkiye: Ankara	Current study
	ANK AKATA 8784	PP494026.1	Türkiye: Ankara	Current study
	ANK AKATA 8785	PP494038.1	Türkiye: Konya	Current study
	ANK AKATA 8789	PP494039.1	Türkiye: Konya	Current study
	ANK AKATA 8790	OR394967.1	Türkiye: Konya	Current study
	ANK AKATA & SAHIN 008	MZ089983.1	Türkiye: Gaziantep	Current study
	ANK AKATA & SAHIN 009	MZ089984.1	Türkiye: Gaziantep	Current study
	ANK AKATA & SAHIN 010	MZ089985.1	Türkiye: Gaziantep	Current study
	ANK AKATA & SAHIN 011	MZ089986.1	Türkiye: Nigde	Current study
	ANK AKATA & SAHIN 012	MZ089987.1	Türkiye: Sanliurfa	Current study
	ANK AKATA & SAHIN 013	MZ089988.1	Türkiye: Sanliurfa	Current study
	ANK AKATA & SAHIN 014	MZ089989.1	Türkiye: Nigde	Current study
	ANK AKATA & SAHIN 015	MZ089990.1	Türkiye: Nigde	Current study
	ANK AKATA & SAHIN 016	MZ089991.1	Türkiye: Nigde	Current study
	ANK AKATA & SAHIN 017	MZ089992.1	Türkiye: Nigde	Current study
	J029	MK910035.1	Iraq: Kahlar	Aish et al. 2020
	MZR12	MK967454.1	Iraq	-
	RA80	MH810271.1	Iraq	-
	LBMB 8	MF940187.1	Algeria: Saida	Zitouni-Haouar et al. 2018
	RUF-TC3	MK610443.1	Iran	-
	Fars Province, Iran	MN583175.1	Iran	Behzadi et al. 2021
	Fa1	GQ888693.1	Iran: Fars	Mostowfizadeh-Ghalamfarsa et al. 2010
Terfezia arenaria	MA:FU 59216	HQ698069.1	Spain: Caceres	Kovacs et al. 2011
	MA:FU 40130	HQ698066.1	Spain: Badajos	Kovacs et al. 2011

	NE_Algeria_1	KP217812.1	Algeria	Dafri & Beddiar 2017
	NE_Algeria_3	KP217814.1	Algeria	Dafri & Beddiar 2017
	NE_Algeria_5	KP217816.1	Algeria	Dafri & Beddiar 2017
	2004244	MW508705.1	Portugal: Setúbal	Santos-Silva et al. 2021
	j466	OP458227.1	Portugal: Lavre	-
	F	LT718230.1	Tunisia	Radhouani et al. 2019
	K1	LT718227.1	Tunisia	Radhouani et al. 2019
Terfezia boudieri	type 1	AF092096.1	Israel	Ferdman et al. 2005
	j371	OP458234.1	United Arab Emirates	-
	bou04	AF276672.1	Kuwait	Díez et al. 2002
	LBMB 19	MF940178.1	Algeria: Oued Daoura	Zitouni-Haouar et al. 2018
	105ET	AF387656.1	-	-
	99TC	AF387657.1	-	-
Terfezia olbiensis	AH 46143	MF940204.1	Spain: Tocon de Quentar, Granada	Zitouni-Haouar et al. 2018
	j588	OP458229.1	Greece: Attica, Artemis	-
	MA:FU 5408	HQ698102.1	Spain: Madrid, Toledo	Kovacs et al. 2011
Terfezia aff. olbiensis	MA:FU 54676	HQ698147.1	Spain: Valladolid, Castromonte, La Espina	Kovacs et al. 2011
	tO16	HM056221.1	Spain: Albacete	Bordallo et al. 2013
Terfezia albida	MUB:Fung-0029 (Type Material)	NR_137053.1	Spain: Albacete	Bordallo et al. 2013
	j574	OP458226.1	Spain: Albacete, Lezuza	-
	j113	KP728824.1	Spain: Badajoz	Bordallo et al. 2015
	j384	KP728826.1	Greece: Rafina Attica	Bordallo et al. 2015
	j479	KP728829.1	Greece: Zagora Magnesia	Bordallo et al. 2015
Terfezia cistophila	MUB Fung-j477 (Type Material)	NR_160445.1	Greece: Nea Makri Attica	Bordallo et al. 2015
	2004865	MW508653.1	Portugal: Portalegre	Santos-Silva et al. 2021
	MA:FU 65481	HQ698097.1	Spain: Toledo	Kovacs et al. 2011
	MA:FU 24971	HQ698090.1	Spain: Segovia	Kovacs et al. 2011
	MA:FU 57171	HQ698093.1	Spain: Caceres	Kovacs et al. 2011
Terfezia leptoderma	MA:FU 59232	HQ698096.1	Spain: Badajos	Kovacs et al. 2011
	MA:FU 26757	HQ698087.1	Spain: Caceres	Kovacs et al. 2011
	MA:FU 28367	HQ698088.1	Spain: Caceres	Kovacs et al. 2011
	MA:FU 41323	HQ698092.1	Spain: Madrid	Kovacs et al. 2011
Mattirolomyces terfezioides	943	MT890667.1	-	Lu et al. 2022

DISCUSSION and CONCLUSION

T. claveryi stands out as a distinctive species, characterized by its complex, reticulate spores, a peridium with shades ranging from reddish-brown to brownish-black, and gleba that exhibits reddish hues (Díez et al., 2002). While this species shares some morphological and ecological similarities with other *Terfezia* species reported in Türkiye (Díez et al., 2002; Kovács et al., 2011; Akata et al., 2022), it can be differentiated from them. For instance, *T. arenaria* and *T. boudieri* differ from *T. claveryi* based on distinct morphological characteristics. However, differentiating *T. claveryi* from species like *T. albida*, *T. cistophila*, *T. leptoderma*, and *T. olbiensis* is more challenging, as the differences in their physical features are not as pronounced (Kovács et al., 2011; Bordallo et al., 2013; Türkoğlu & Castellano, 2014; Türkoğlu et al., 2015). This complexity in distinguishing *T. claveryi* from certain other *Terfezia* species highlights the need to carefully examine and compare their specific traits.

T. boudieri typically thrives in basic soils, which are either rich in calcareous or contain gypsiferous marl, and associate with *Helianthemum* species. This species is notably characterized by its sizeable ascomata and distinct spores adorned with warts on a reticular structure (Moreno et al., 2002; Sbissi et al., 2011; Akyüz et al., 2012). Similarly, *T. claveryi* is found in comparable soil types and bears a macroscopic and mycorrhizal association resemblance to *T. boudieri*, making it challenging to distinguish between the two species based solely on their appearance (Díez et al., 2002; Moreno et al., 2002). Pay attention to the distinctive ornamentation patterns on their spores for precise identification and distinction between *T. claveryi* and *T. boudieri* (Zitouni-Haouar et al., 2018). The key distinguishing feature lies in their spore structures: Spores of *T. claveryi* are reticulate but lack warts, presenting a smoother appearance under microscopic observation. In contrast, the spores of *T. boudieri* are characterised by the presence of warts superimposed on a reticulate background (Moreno et al., 2002; Kovács et al., 2011). This subtle yet significant difference in spore ornamentation is crucial in correctly identifying and differentiating these species.

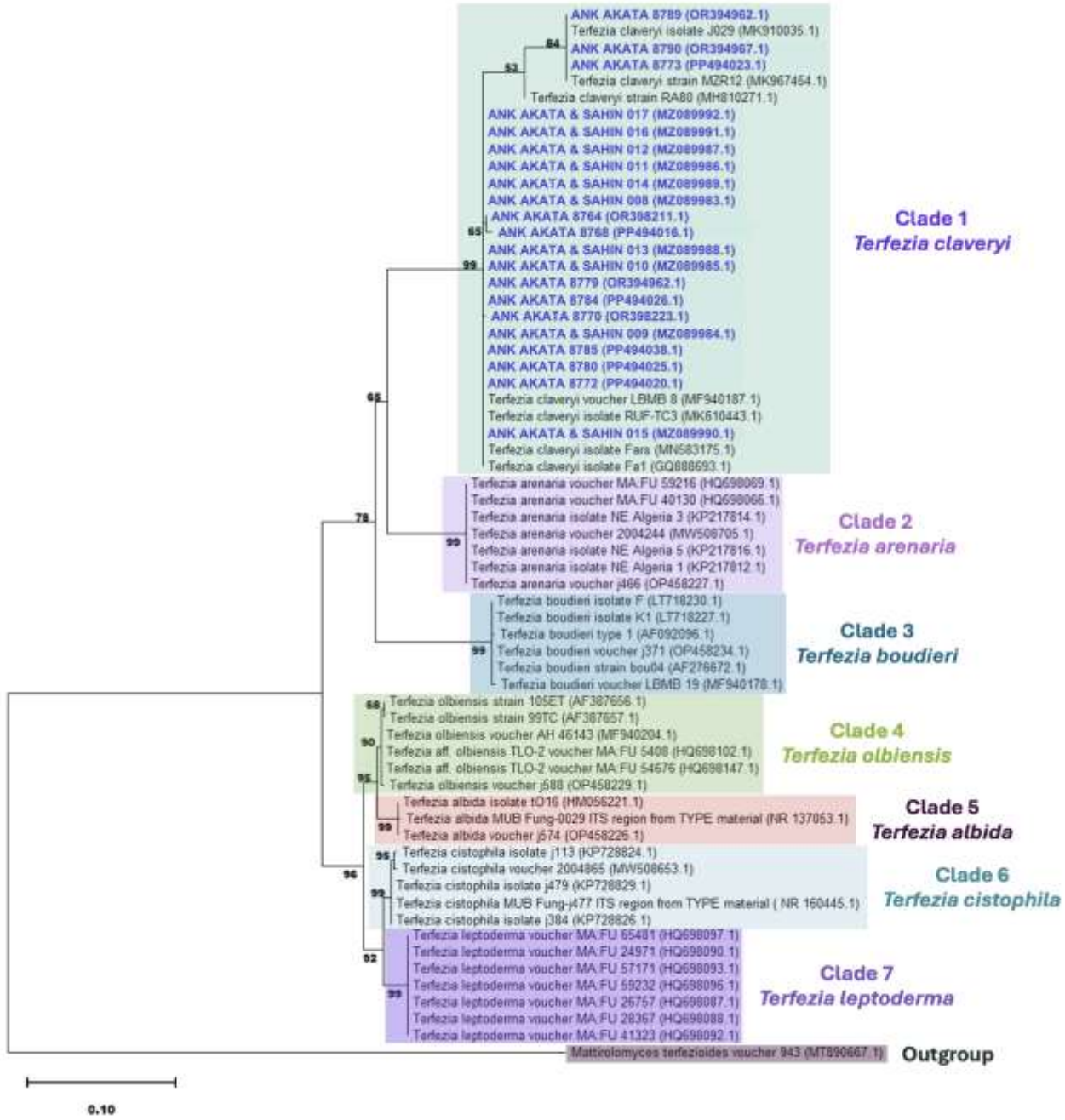

Figure 5. The evolutionary connections among 63 fungal specimens are illustrated in a phylogenetic tree created using the nrITS rDNA region and the maximum likelihood (ML) method. Bootstrap rates (≥50) are allocated to each branch to denote confidence levels. Sequences utilized for tree construction were obtained from the NCBI GenBank database. Additionally, *Mattirolomyces terfezioides* was included in the phylogenetic tree as the representative outgroup. Each sequence is accompanied by its respective GenBank accession number, and a scale bar in the lower left corner indicates a genetic distance of 0.10.

Şekil 5. nrITS rDNA bölgesi ve maksimum olabilirlik (ML) yöntemi kullanılarak oluşturulan bir filogenetik ağaçta 63 mantar örneği arasındaki evrimsel bağlantılar gösterilmektedir. Güven seviyeleri, her şubeye önyükleme oranları (≥50) atanarak belirtildi. Ağacın oluşturulması için kullanılan diziler NCBI GenBank veri tabanından elde edilmiştir. Ek olarak, Mattirolomyces terfezioides temsili dış grup olarak filogenetik ağaca dahil edilmiştir. Her diziye ilgili GenBank erişim numarası belirtilmiş ve sol alt köşedeki ölçek çubuğu 0.10'luk bir genetik mesafeyi temsil etmektedir.

T. arenaria is known for its unique peridium, which initially appears whitish with delicate pink undertones and black speckles. As this species matures, its peridium transforms, adopting a more brownish hue. A standout feature of *T. arenaria* is its robustly warty spores, which distinctly sets it apart from closely related species like *T. claveryi*. The latter is recognized for its reticulate spores, distinguishing between these species (Díez et al., 2002; Kovács et

al., 2011).

T. cistophila, known for its spiny-spored structure, is notably characterized by a peridium that turns intensely black. The gleba presents a light ochre colour, distinctively different from the outer peridium. A remarkable feature of this species is its spermatic odour, and it thrives in acidic soils and is typically found in areas where *Cistus* species are prevalent (Bordallo et al., 2015). On the other hand, *T. claveryi* is distinguishable from *T. cistophila* in several aspects. One of the most notable differences is its spore structure; *T. claveryi* produces reticulate spores devoid of spines, contrasting sharply with the spiny spores of *T. cistophila*. The peridium of *T. claveryi* ranges in colour from reddish-brown to brownish-black, and a pinkish-salmon hue characterises its gleba. It does not emit a spermatic odour (Díez et al., 2002). Furthermore, its habitat preferences are quite distinct; *T. claveryi i*s primarily found in arid and semi-arid regions, favouring calcareous, clayey, or sandy alkaline soils (Díez et al., 2002; Akyüz et al., 2015b; Türkoğlu et al., 2015). This species tends to associate with *Helianthemum* species, indicating a specific ecological relationship akin to that observed in *T. cistophila* with *Cistus* species (Díez et al., 2002; Bordallo et al., 2015).

T. albida is another species that shares its habitat preferences with *T. claveryi*, predominantly thriving in arid and semi-arid regions. This species has a particular affinity for calcareous, alkaline soils, similar to *T. claveryi*. However, a key aspect of the environmental association of *T. albida* is its tendency to grow in conjunction with *Helianthemum* species, a characteristic it shares with *T. claveryi* (Bordallo et al., 2013). Despite these similarities in habitat, *T. albida* is distinctly set apart from *T. claveryi* in several notable ways. The most striking difference lies in the colouration of its peridium, which is white, contrasting sharply with the reddish-brown to brownish-black peridium of *T. claveryi*. Additionally, the gleba of *T. albida*, presents a grayish-green coloration, offering a unique visual differentiation from the pinkish-salmon gleba of *T. claveryi* (Díez et al., 2002; Bordallo et al., 2013; Türkoğlu et al., 2015). Another distinguishing feature of *T. albida* is its spore structure. Like *T. cistophila, T. albida* produces spiny spores, a trait that diverges from the reticulate spores of *T. claveryi*. Furthermore, *T. albida* emits a spermatic odour, an attribute it shares with *T. cistophila* but not with *T. claveryi* (Díez et al., 2002; Bordallo et al., 2013; 2015)

T. olbiensis is characterized by its brown peridium and greenish-grey gleba, with its spiny spores being a notable attribute. This species often becomes a target for larvae and rabbits, likely because it emerges early in the year during humid, low-light conditions. It emits a unique scent and has a milder flavour than other *Terfezia* species (Bordallo et al., 2013). *T. olbiensis* is known to favour environments with limestone and clayey soils, commonly reported to be associated with pine and oak trees. According to research by Bordallo et al. in 2013, this species is often found in areas where the soil composition includes limestone and clay, conducive to the growth of pine and oak forests. Conversely, studies conducted by Akyüz et al., 2015b; Türkoğlu & Castellano, 2014, have documented the occurrence of this species in Türkiye, explicitly noting its association with *Helianthemum* species. The distinctions in morphology, including differences in peridium and gleba colourations and spore ornamentation, combined with their unique habitat and soil preferences and mycorrhizal partnerships, provide valuable criteria for differentiating *T. claveryi* from *T. olbiensis*.

The genetic diversity among fungal species far exceeds their morphological variation, highlighting the importance of integrating genetic data with conventional morphological techniques to improve the accuracy of species identification. Over the years, several genetic markers, including ribosomal RNA gene regions such as nrITS, nrSSU, and nrLSU, along with protein-coding gene sequences, have played a pivotal role in molecular systematics (Raja et al., 2017). The internal transcribed spacer (ITS) region is particularly valued for its high resolution in fungal molecular taxonomy. Recent advancements in high-throughput sequencing technologies and bioinformatics have further facilitated detailed genome-wide comparisons and phylogenomic analyses, which may soon surpass traditional molecular phylogenetic approaches that rely on a limited number of genetic markers (Marian et al., 2024). This study employed nuclear ITS rDNA sequences to characterize T. claveryi specimens collected from regions across Türkiye molecularly. The analysis revealed a sequence similarity exceeding 99% between these specimens and other *T. claveryi* isolates with sequence data available in the GenBank database, underscoring the efficacy of ITS-based approaches in identifying and confirming fungal taxa.

This study enhances the understanding of *Terfezia claveryi* distribution in Turkey, identifying 21 new localities through combined morphological and molecular methods (ITS rDNA). The findings improve taxonomic accuracy, provide insights into the species' ecology, and support conservation, sustainable harvesting, and commercial potential. This study underscores the value of integrating traditional and molecular approaches for studying desert truffles in arid ecosystems.

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Contribution Rate Statement Summary of Researchers

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

Conflict of Interest

The authors have declared no conflict of interest.

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Striped Hyena, *Hyaena hyaena* (Linnaeus, 1758) (Carnivora: Mammalia), Distribution, Threats and Conservation Recommendations in Batman Province

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ABSTRACT

The striped hyenas, *Hyaena hyaena* (Linnaeus, 1758), are listed as Near Threatened (NT) category globally, while they are listed as Vulnerable (VU) category in Türkiye according to the IUCN (The International Union for Conservation of Nature). In this study, which was carried out between 2020 and 2023, it is aimed to determine the presence of striped hyenas, their distribution areas, threats to the species, and present conservation recommendations in Batman Province. Direct and indirect observations were made during all four seasons in 104 locations determined by interviews with hunters, shepherds, and local people, and camera traps were set up in these locations. The presence of striped hyenas was determined in 20 different localities of Batman Province. Threats to this species were determined during the observations, and protection recommendations were presented. This study is the most comprehensive research to date on the distribution of striped hyenas in a province in Türkiye.

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Çizgili Sırtlan, *Hyaena hyaena* (Linnaeus, 1758) (Carnivora: Mammalia), Batman İlindeki Dağılışı, Türe Yönelik Tehditler ve Koruma Önerileri

ÖZET

Çizgili sırtlan, *Hyaena hyaena* (Linnaeus, 1758), IUCN (Dünya Doğa ve Doğal Yaşamı Koruma Birliği)'ne göre küresel ölçekte Tehdite Yakın (NT) kategorisinde listelenirken Türkiye'de Hassas (VU) kategorisinde listelenmektedir. 2020-2023 yılları arasında gerçekleştirilen bu çalışmada Batman ili sınırları içerisinde çizgili sırtlanın varlığının, yayılış alanlarının, türe yönelik tehditlerin belirlenmesi ve koruma önerilerinde bulunulması amaçlanmıştır. Avcılar, çobanlar ve yöre insanları ile yapılan görüşmeler sonucunda belirlenen 104 lokasyonda dört mevsim süresince belirli aralıklarla doğrudan ve dolaylı gözlemler gerçekleştirilmiş ve fotokapanlar kurulmuştur. Çalışma sonucunda Batman ilindeki 20 farklı lokalitede çizgili sırtlan varlığı tespit edilmiştir. Gözlemler süresince türe yönelik tehditler belirlenerek koruma önerileri sunulmuştur. Bu çalışma, Türkiye'de bir ildeki çizgili sırtlanların yayılışları ile ilgili şu ana kadar yapılmış en kapsamlı araştırmadır.

Zooloji

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INTRODUCTION

Türkiye serves as an ecological bridge between Europe, Africa, and Asia, and is home to three of the 36 global biodiversity hotspots (Noroozi et al., 2019), namely the Mediterranean, Caucasus, and Iran-Anatolia. The flora and fauna of Türkiye, which has a very rich biodiversity compared with its neighbours, has adapted to these three continents (Akman, 1999; Eken et al., 2006; Kaya et al., 2011; Çağatay et al., 2012; Ambarlı et al., 2016). Mammals

represent the most diverse class of animals on Earth, encompassing 6399 species across 27 orders, 167 families, and 1314 genera (Musser & Carleton, 2005). Within the Palearctic region alone, there are 843 species distributed across 13 orders and 42 families. While Europe is home to about 200 mammal species, about 170 mammal species belonging to 9 orders (Eulipotyphla, Chiroptera, Lagomorpha, Rodentia, Cetacea, Carnivora, Pinnipedia, Perissodactyla and Artiodactyla) are distributed in Türkiye (Wilson & Reeder, 2005; Eken et al., 2006; Kaya et al., 2011; Özkazanç, 2017; Selçuk and Kefelioğlu, 2020; Seyfi et al., 2021). Hyaenidae, one of the six carnivore families distributed in Türkiye, is represented by four species (*Crocuta crocuta*, *Proteles cristata*, *Hyaena brunnea,* and *Hyaena hyaena*) in the world (Albayrak et al., 1997, 2008; Leakey et al., 1999; Koepfli et al., 2006; Yıldırım, 2010; Atay et al., 2017; Kılıç, 2018). *Hyaena hyaena* (Linnaeus, 1758), listed as Near Threatened (NT), in IUCN Red List of Threatened Species, has a very wide distribution from Africa, including much of eastern and northeastern Africa, south to central Tanzania, the Middle East and the Arabian Peninsula, Türkiye, the Caucasus, Central Asia, and the Indian subcontinent (Rieger, 1981; Mills & Hofer, 1998; Wagner, 2006; Yıldırım, 2010). There are five recognized subspecies of striped hyenas (*H. h. hyaena*, *H. h. barbara*, *H. h. dubbah*, *H. h. sultana,* and *H. h. syriaca*) that exclusively inhabit semi-deserts, steppes, rocky areas, and sparsely forested valleys. Of these, only *H. h. syriaca* is distributed in Syria and the Caucasus along with Anatolia (Rieger, 1981; Mills & Hofer, 1998; Singh, 2008). Despite their wide distribution, there are very few studies on the population status and ecology of striped hyena (Abi-Said & Abi-Said, 2007; Alam et al., 2015; Wagner, 2006). The first study on their distribution in Türkiye was conducted by Kumerloeve (1967), who reported that the species was almost extinct in Türkiye. It would seem that the limited research on the distribution of the species in Türkiye is relatively contemporary, which serves to underscore the local endangered status of the species (Özkurt et al., 1998; Kasparek et al., 2004; Akay et al., 2010; Yıldırım, 2010; Kılıç, 2018; Çoğal et al., 2021). Human-induced factors are threated (Leakey et al., 1999; Wagner, 2006; Kılıç, 2018), the species is classified as "Near Threatened" (AbiSaid and Dloniak, 2015) on the IUCN red list globally, it is listed as "Vulnerable" (Jdeidi et al., 2010) in the Mediterranean Basin, including Türkiye. To date, no comprehensive research has been conducted on the local distribution of striped hyenas in Southeastern Anatolia. The aim of this study was to determine the distribution areas of striped hyenas in Batman province and to reveal the threats to the species and the measures that can be taken against these threats.

MATERIAL and METHOD

Study area

Batman province is located at 41°10′-41° 40′E and 38° 40′-37°50′ N, and has a continental climate. The annual average temperature of Batman province is 16.7 °C. The annual average rainfall is 530 mm. The altitude of the province is approximately 550 meters, and the north and northeast of the province are covered with high mountains and forested areas, but the areas in the south are flatter and steppe. The highest mountains of the province are Mereto Mountain with an altitude of 2973 meters and Sason Mountains with an altitude of 2500 meters. Dicle River, Batman, Sason, Garzan, and Pisiyar Streams are important rivers of the province.

Method

In this study, direct and indirect observations (camera traps, interviews, footprints, feces, etc.) were conducted at regular intervals during four seasons to determine the distribution areas of striped hyenas in Batman province between 2020 and 2023. Based on interviews with hunters, shepherds, local people living in rural areas, and preliminary field studies on habitat localities have been determined where the striped hyena is likely to be distributed. Spypoint Force-20 camera traps were used in this study. These camera traps are equipped with 48 low-glow LEDs and 20 megapixels resolution and have 24 24-meter flash range, 21 21-meter detection range, color by day, infrared by night photo type, up to 5 images per capture, and 0,7 second trigger speed. Fifteen camera traps were set up at 104 different localities during the fieldwork (Figure 1). Each camera trap was left in the field for at least 15 days in each season.

RESULTS

The presence of striped hyena was recorded in 20 of the 104 localities where camera traps were set up in Batman province. Of the 20 localities where the striped hyena was detected, one was an active striped hyena nest, four were interviews with local people, four were dead individuals, and eleven were detected using camera traps (Table 1). The striped hyenas in the camera trap images were examined according to the patterns on their bodies, and those with different patterns were counted as individuals (Figure 2).

Eighteen of the localities where striped hyenas were detected are located within the Tigris Valley Important Nature Area (approx. 1355 km^2), which covers the districts of Hasankeyf and Gercüş in the southern part of Batman province (approx. 1000 km^2) (Figure 3).

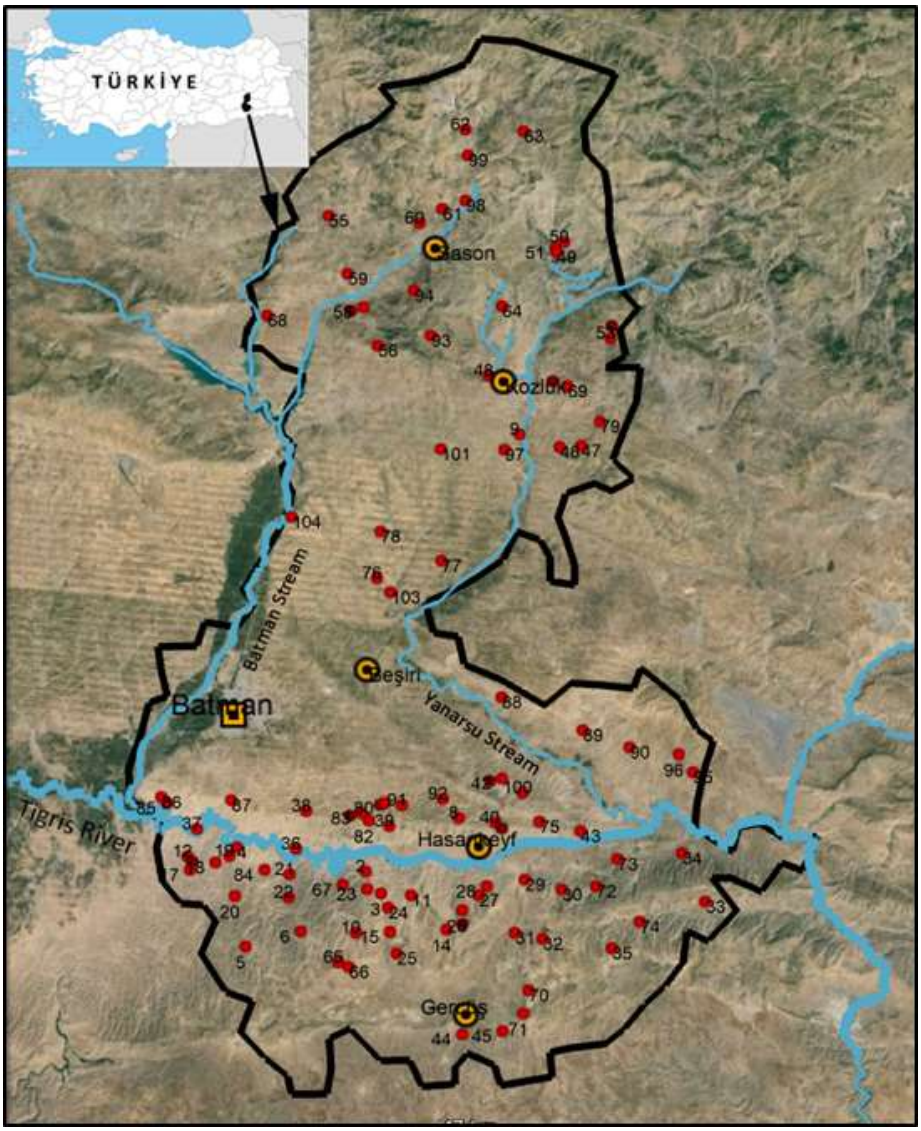

Figure 1. Observation points of the striped hyena in the Batman province.
Şekil 1. Batman ilinde çizgili sırtlanın gözlem noktaları.

Table 1. Detection method of striped hyena and detected number of individuals.
Çizelge 1. Çizgili sırtlan tespit yöntemi ve tespit edilen birey sayısı.

Detection method	Number of individuals
Nest	1
Interview	4
Dead	4
Camera trap	11
TOTAL	**20**

Striped hyenas have been found to use natural caves in valleys within the Tigris Valley Important Nature Area, which includes the Tigris River, as nests. When the hours of activity of the striped hyena were examined, it was determined that it is not active between 09.00 and 18.00, with its most intense activity occurring between 18.00 and 06.00 after dark in the evening. It is thought that striped hyenas prefer to stay in their nest due to human activities throughout the day.

It was determined that the most important factor threatening the striped hyena is road kills (three of the four individuals). In addition, direct or indirect human-induced threats, such as the decrease in their habitat due to the expansion of human settlements and agricultural areas, the decrease in the number of animals they prefer as food, the decrease in the number of other large carnivores that leave food remains, and environmental pollution.

In addition, it was determined that the striped hyena with an injured leg, which was captured by a camera trap in

its nest near Kesiktaş village, died by sticking into oil waste approximately 5,500 meters away from its nest (Figure 4).

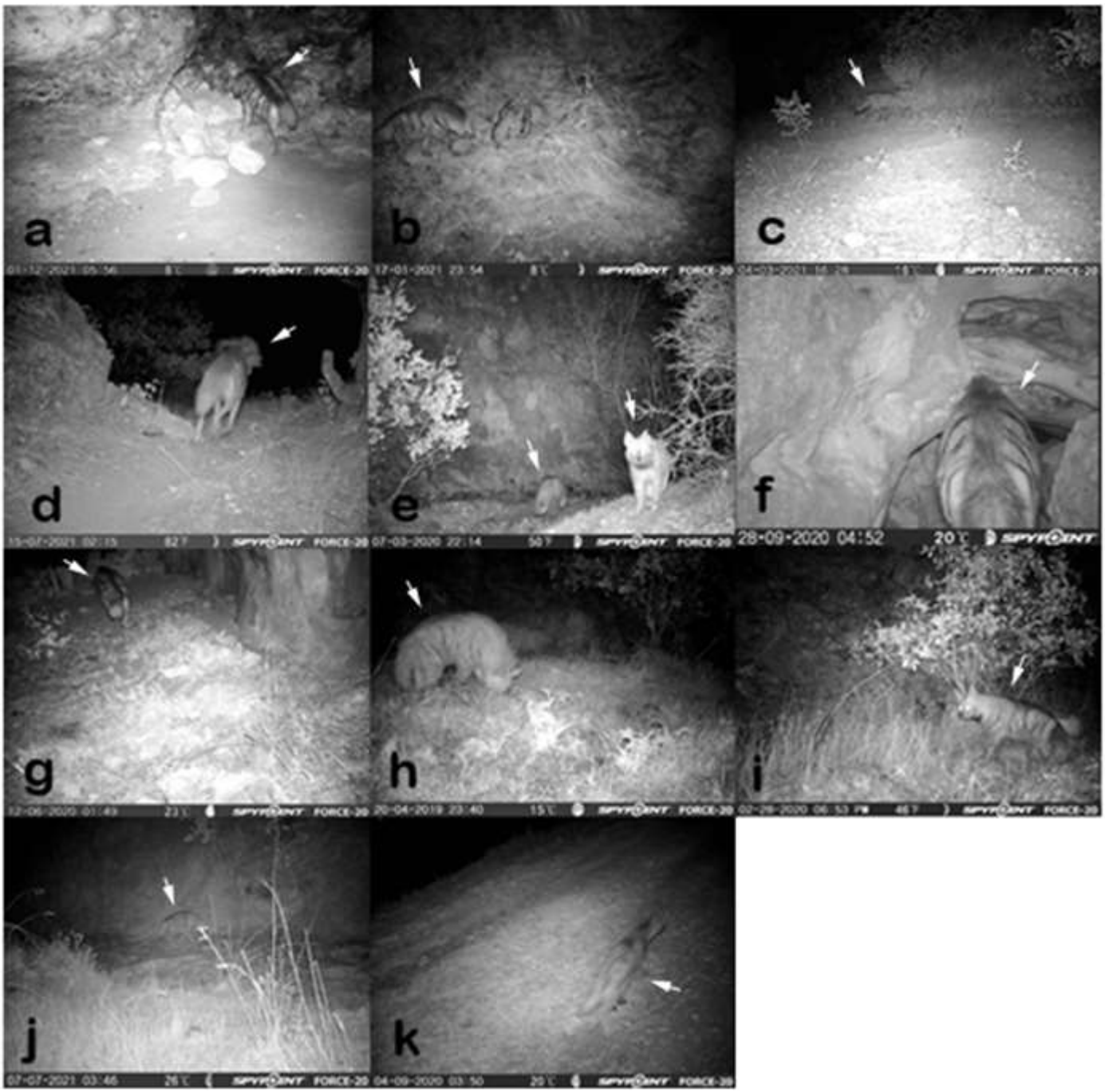

Figure 2. Camera trap images of striped hyena (The arrow indicates the striped hyena). (a.Kesikağaç village, b.Kumluca village, c.Mağaralı village, d.Kantar village, e.Kömürcü village, f.Aksu village, g.Kışlak village, h.Akarca village, i.Boğazköy village, j.Çardaklı village, k.Palamut village).

Şekil 2. Çizgili sırtlan fotokapan görüntüleri (Ok çizgili sırtlanı göstermektedir). (a.Kesikağaç köyü, b.Kumluca köyü, c.Mağaralı köyü, d.Kantar köyü, e.Kömürcü köyü, f.Aksu köyü, g.Kışlak köyü, h.Akarca köyü, i.Boğazköy köyü, j.Çardaklı köyü, k.Palamut köyü).

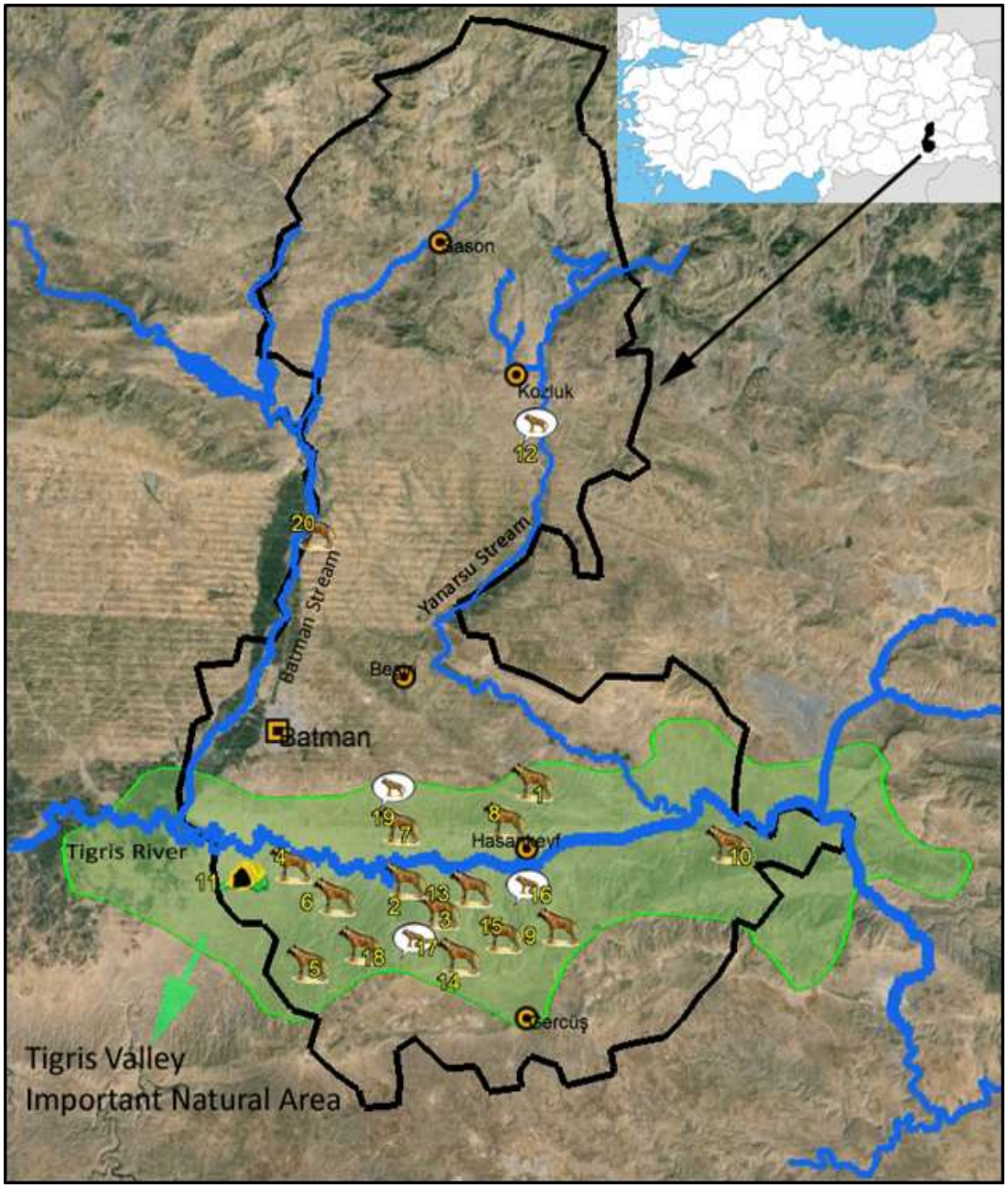

Figure 3. Localities of striped hyenas were detected (1-Kesiktaş village/Beşiri, 2-Kumluca village/Hasankeyf, 3-Mağaralı village/Gercüş, 4-Kantar village/Gercüş, 5-Kışlak village/Gercüş, 6-Akarca village/Gercüş, 7-Demirbilek village/Batman, 8-Raman Mountain/Batman, 9-Akyar village/Gercüş, 10-Palamut village/Hasankeyf, 11-Eymir village/Gercüş 12-Çayönü village/Kozluk, 13-Çardaklı village/Gercüş, 14-Boğazköy village/Gercüş, 15-Aksu village/Gercüş, 16-Karaköy village/Hasankeyf, 17-Geçitköy village/Gercüş, 18-Kömürcü village/Gercüş, 19-Yolveren village/Batman, 20.Bıçakçı village/Batman, camera-trap, nest, interview).

Şekil 3. Çizgili sırtlanların tespit edildiği lokaliteler (1-Kesiktaş köyü /Beşiri, 2-Kumluca köyü /Hasankeyf, 3-Mağaralı köyü/Gercüş, 4-Kantar köyü /Gercüş, 5-Kışlak köyü /Gercüş, 6-Akarca köyü /Gercüş, 7-Demirbilek köyü /Batman, 8-Raman dağı/Batman, 9-Akyar köyü /Gercüş, 10-Palamut köyü /Hasankeyf, 11-Eymir köyü /Gercüş 12-Çayönü köyü /Kozluk, 13-Çardaklı köyü /Gercüş, 14-Boğazköy köyü /Gercüş, 15-Aksu köyü /Gercüş, 16-Karaköy/Hasankeyf, 17-Geçitköy köyü /Gercüş, 18-Kömürcü köyü /Gercüş, 19-Yolveren köyü /Batman, 20.Bıçakçı köyü /Batman, fotokapan, yuva, anket).

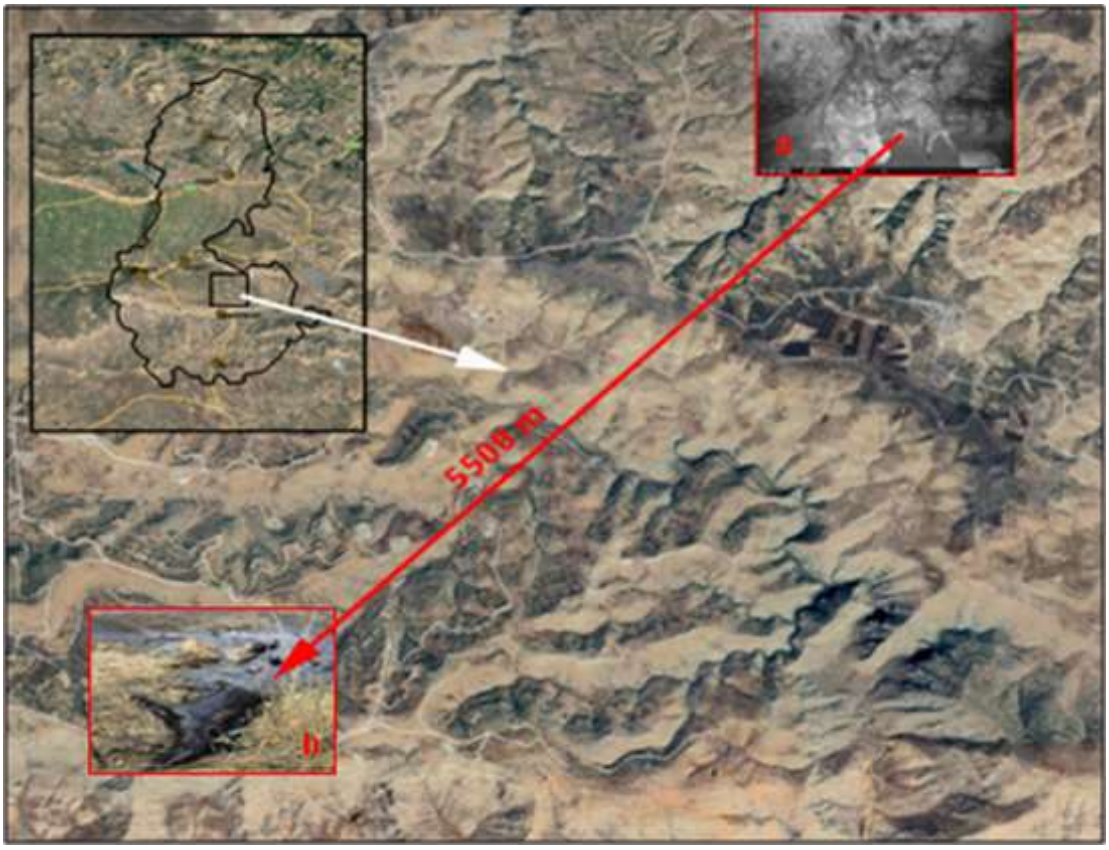

Figure 4. A dead striped hyena in an oil waste 5500 meters away from its nest (a. nest, b. oil waste).
Şekil 4. Yuvasından 5500 metre uzaklıktaki bir petrol atığında ölmüş çizgili sırtlan (a. yuva, b. petrol atığı).

DISCUSSION

The family Hyaenidae is represented by 4 species (*H. hyaena*, *H. brunnea*, *C. crocuta*, *P. cristata*) throughout the world. Only the striped hyena is distributed in Türkiye (Leakey et al., 1999; Yıldırım, 2010; Atay et al., 2017; Kılıç, 2018).

Striped hyenas prefer semi-deserts, steppes, rocky areas, and sparsely forested valleys (Mills & Hoffer, 1998). They have similar natural habitats in Batman Province

Compared to the other three hyena species, fewer studies have been conducted on striped hyenas (Abi-Said & Abi-Said, 2007; Alam et al., 2015; Wagner, 2006). The population of the striped hyena is declining rapidly in the world (Çoğal et al., 2021; Dadashi-Jourdehi et al., 2020; Alam et al., 2015; Kasparek et al., 2004), in addition, it was reported that the population is extinct in many countries (Mills & Hofer, 1998). Among Türkiye's neighbours, the most data on the status of *H. hyaena* comes from Iran. However, recent studies (Özkurt et al., 1998; Kasparek et al., 2004; Akay et al., 2010; Yıldırım, 2010; Kılıç, 2018; Çoğal et al., 2021) have revealed the existence of striped hyena populations in Türkiye. This study showed that striped hyenas are distributed in the southern part of the Batman province (approx. 1000 km^2), which is inside the Dicle Valley Important Natural Area (approx. 1355 km^2).

Wagner (2006) reported that about half of hyena deaths in Kenya were caused by humans, most deaths in Niger were due to poisoning, and the main cause of population decline in Tanzania was road accidents. Similar to this case, three of the four striped hyenas detected as deceased in this study were road kill.

CONCLUSION

Anatolia's location at the intersection of three continents and its complex topography and geomorphology offer high habitat and species diversity. The Batman province has rich biodiversity because of its geographical structure, vegetation, and different ecosystems. One of the most important species of this biodiversity is the striped hyena. In this study, as a result of studies carried out in 104 locations in Batman province, striped hyenas were detected in 20 localities by direct and indirect observation methods. This study is the first and most detailed research to determine the local distribution areas of striped hyenas in Türkiye. However, this knowledge of their ecology and behaviour has been limited by the challenges of studying them in their habitats.

The striped hyena, which inhabits caves and burrows in the valleys along the Tigris River, is one of the mammal

species that should be prioritized for protection in the Tigris Valley Important Natural Area, encompassing the districts of Hasankeyf and Gercüş.

The most important factor threatening striped hyenas is roadkill. The cause of death of three of the four striped hyenas found dead was roadkill. The locality of occurred the three roadkill occurred is the Hasankeyf-Gercüş region. In addition, farmers are converting natural habitats into agricultural land, which reduces hyenas' access to food and disrupts the predator-prey balance. Determining the distribution areas of striped hyenas in Türkiye may help manage conservation activities and determine population densities.

According to the IUCN (International Union for Conservation of Nature), the striped hyena is listed as "Near Threatened" (AbiSaid and Dloniak, 2015) globally, while in Türkiye it is listed as "Vulnerable" (Jdeidi et al., 2010). It is also under protection in Türkiye by the Land Hunting Law No. 4915.

The measures to be taken should not be limited to raising awareness, but also the species should be protected by the authorized units and the application of dissuasive punishments.

To give examples of these actions;

a. To prevent road kills, ecological bridges or underpasses can be built in these areas by determining the passing route of the striped hyena. These passages are important not only for the striped hyena but also for other wildlife. In addition, potential harm to humans will be prevented.

b. The education of people in rural areas engaged in agriculture, livestock farming, and hunting will be an important factor in the conservation of the striped hyena.

c. A species action plan should be developed for the striped hyena in Batman province.

d. The disposal of waste to nature that would be harmful to wildlife should be prevented, and the sanctions in this regard should be increased.

e. More comprehensive studies should be carried out to determine the population density of the striped hyena distributed in the Batman province and other regions of Türkiye.

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Contribution Rate Statement Summary of Researchers

The authors have contributed equally to the article.

Conflict of Interest

The authors have declared no conflict of interest.

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Investigation of Possible Genotoxicity of Surface and Tap Waters Using the Comet Assay

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ABSTRACT

We tested the hypothesis that chronic exposure to total heavy metal loads in Cevizdere surface water and Ünye District tap water of Ordu Province (Türkiye) may cause a genetic toxic effect in erythrocyte cells of carp (*Cyprinus carpio*) individuals. For this purpose, total heavy metal (loid)s contents in the surface and tap waters were determined seasonally. It was determined that both Cevizdere surface water and Ünye District tap water caused DNA damage in erythrocyte cells of carp fish, and this damage was statistically significant when compared to control groups (P<0.05). As a result of the comet analysis, the DNA damage detected in the erythrocyte cells of *C. carpio* showed the presence of genotoxic potential of Cevizdere Stream surface water and Ünye District tap water. The genotoxicity of the surface waters of the Cevizdere Stream, which is the main source of drinking water for the Ünye District in Ordu City, as well as the Ünye District tap waters, was evaluated *in vivo* for the first time using the Comet assay. This study revealed genotoxic damage in *C. carpio* due to total heavy metal(loid) pollution in the surface water of Cevizdere Stream and tap water of Ünye District, contributing to a better understanding of the relationship between genotoxicity and heavy metal(loid) pollution.

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Comet Analizi Kullanılarak Yüzey ve Musluk Sularının Olası Genotoksisitesinin İncelenmesi

ÖZET

Ordu İli (Türkiye) Cevizdere yüzey suyu ve Ünye İlçesi musluk suyundaki toplam ağır metal yüklerine kronik maruz kalmanın sazan (*Cyprinus carpio*) bireylerinin eritrosit hücrelerinde genetik toksik etkiye neden olabilme hipotezini test ettik. Bu amaç doğrultusunda yüzey ve musluk sularındaki toplam ağır metal(loid) içerikleri mevsimsel olarak belirlenmiştir. Hem Cevizdere yüzey suyunun hem de Ünye İlçesi musluk suyunun sazan balıklarının eritrosit hücrelerinde DNA hasarına neden olduğu ve bu hasarın kontrol gruplarıyla karşılaştırıldığında istatistiksel olarak anlamlı olduğu belirlenmiştir (P<0,05). Comet analizi sonucunda *C. carpio*'nun eritrosit hücrelerinde tespit edilen DNA hasarı, Cevizdere yüzey suyu ve Ünye İlçesi musluk suyunun genotoksik potansiyelinin varlığını göstermiştir. Ordu İli, Ünye İlçesi'nin içme suyunun ana kaynağı olan Cevizdere'nin yüzey sularının ve Ünye İlçesi musluk sularının genotoksisitesi Comet yöntemi kullanılarak ilk kez *in vivo* olarak değerlendirilmiştir. Bu çalışma, Cevizdere yüzey suyu ve Ünye İlçesi musluk suyunda toplam ağır metal(loid) kirliliğine bağlı olarak *C. carpio*'da genotoksik hasar olduğunu ortaya koyarak, genotoksisite ile ağır metal(loid)ler arasındaki ilişkinin daha iyi anlaşılmasına katkı sağlamıştır.

Genetik

Araştırma Makalesi

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Anahtar Kelimeler
DNA hasarı
Comet Analizi
Yüzey ve musluk suları
Genotoksisite
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INTRODUCTION

Water pollution, caused by various types of pollutants, is mostly the result of human activities like industry, agriculture, and urbanization. This is one of the most critical problems for all organisms, including humans, in terms of protecting and sustaining water resources. In addition, another important concern about water pollution is the potential negative effects of genotoxic pollutants on humans and native biota. Different chemicals, such as heavy metals and metalloids, directly contribute to water pollution. These substances can cause DNA damage and mutations in living organisms. Even chronic exposure to low doses of these chemicals can negatively affect biodiversity and increase the risk of developing cancer in humans (Mitra et al., 2022). Heavy metals, which are among the leading pollutants in aquatic systems, can be found everywhere because they are formed because of atmospheric and geological processes as well as human activities. Water bodies around the world have long been affected by heavy metal pollution, making it a global issue. Heavy metals can also be used as environmental monitoring factors and are often preferred in monitoring the state of the environment and organisms. Heavy metals and metalloids (heavy metal(loid)s) receive significant attention due to their toxicity, accumulation, and their harmful effects on aquatic ecosystems, fish, and human health (Fikirdesici Ergen et al., 2023; Khan et al, 2023).

Heavy metal(loid)s can cause DNA damage in organisms in different ways, such as breaking DNA strands and DNA-protein cross-links (Gebel et al., 1998; Kousar & Javed, 2015; Karaismailoğlu, 2022). Therefore, it is vital to determine the genotoxic effects of these pollutants for environmental impact assessment and organism monitoring studies. Comet assay has found wide application in the literature as a sensitive method used to evaluate DNA damage in organisms in terrestrial and aquatic systems exposed to various pollutants (Mokhamer et al., 2019; Kontaş, 2022). Since genotoxic effects can be detected in fish species, as in many organisms, even in cases of exposure to low concentrations of pollutants, with the help of this method, it is among the effective indicators in monitoring environmental health (Bolognesi & Cirillo, 2014; Kontaş, 2022).

Ordu is an important province in the Black Sea Region of Türkiye, which attracts tourists due to its natural, historical places, sea, rivers, lakes, ponds, waterfalls, and the opportunity for many cultural and touristic activities. Cevizdere valley is one of the most important valleys of Ordu city, and Ünye district is a distinguished district located in this valley. Cevizdere Stream, located within the borders of Ünye district, is the most important stream source providing drinking water to this district. Ünye Drinking Water Treatment Plant, one of the main drinking water sources of the city, is located in the Cevizdere Stream basin. Agricultural land is cultivated in the basin, and animal husbandry and beekeeping activities are carried out. Additionally, the Ünye wastewater treatment plant is located on the east side of the downstream part of the study area (Oy, 2018; Beden & Ulke Keskin, 2021; Yedier et al., 2022). Cevizdere Stream and the basin in which it is located are also important for the economy of Ordu city. Ünye Cement Factory, located on the southern slopes of the valley where the Cevizdere Stream is located, is an important industrial facility for both the region and Türkiye (Ünye Municipality (MU), 2018). Ünye district's water resources are exposed to different pollutants such as agriculture, cement, textile, mining, and domestic waste (Kurucu & Bostancı, 2022; Bostancı et al., 2024). There are a limited number of studies in the literature investigating the genotoxicity of surface and tap water (Vazquez Boucard et al., 2017; Feretti et al., 2020). In addition, no detailed study was reported investigating the genotoxicity of surface water used as a drinking water source, as well as tap water in cities in the Black Sea region. Therefore, within the scope of this study, we tested the hypothesis that chronic exposure to total heavy metal loads in Cevizdere surface water and Ünye District tap water in Ordu Province (Türkiye) may cause genetic toxic effects on erythrocyte cells of carp (*Cyprinus carpio*) individuals.

MATERIAL and METHOD

Sampling Areas and Collection of Water Samples

The sampling areas in the study were selected as the local consumers in the Ünye district for tap water samples and the water intake area of the drinking water treatment plant on the Cevizdere Stream, which provides drinking water to the Ünye district for surface water samples (Figure 1). Within the scope of the study, water samples were collected seasonally from several random points at the stations, representing these sampling points in 2020, and quickly transported to the laboratory in sterile polyethylene jerricans, where they were mixed in the laboratory to form a representative sample and transferred to the control and experimental tanks.

Water Chemical Analysis

In order to prepare the water samples for heavy metal analysis, firstly, the water sample representing each group was filtered with the help of a 0.45 µm nitrocellulose membrane filter, and nitric acid was added to keep the pH of the samples below 2. Then, the water samples were taken into Falcon tubes, labeled, and stored at +4 C° until analysis. The heavy metal(loid) concentrations (µg/L) in these water samples were measured on Inductively

Coupled Plasma Mass Spectrometry (ICP-MS) at Sinop University Scientific and Technological Research Application and Research Center (SUBITAM). Since the toxic effects will be evaluated based on the total heavy metal load within the scope of the study, the heavy metals in the relevant water samples were evaluated not individually but by taking into account the total heavy metal (loid) content.

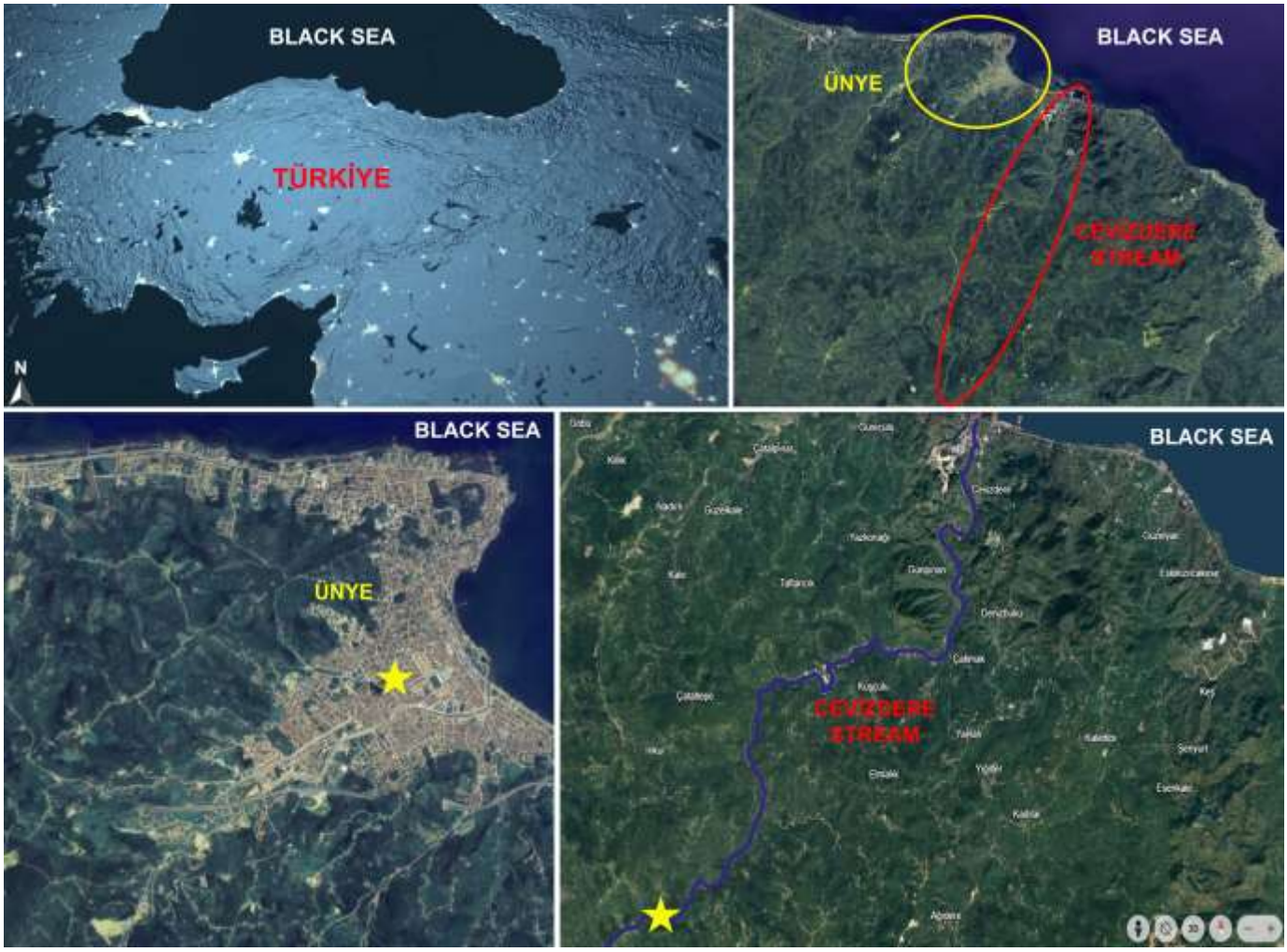

Figure 1. Sampling areas.
Şekil 1. Örnekleme alanları.

Test Organisms

Fish samples, *Cyprinus carpio*, were obtained from Suluova Yedikır Aquaculture Production and Research Station, Amasya, Türkiye. The experimental procedures of the study were approved by the Animal Experiments Local Ethics Committee, Ordu University (approval number: 82678388/5). The length and weight of *C. carpio* individuals were selected to be between 4.5 - 5.5 cm and 1.00 - 1.70 g, respectively, and the fish were transferred to 80 L aquariums in the laboratory where they were acclimated to the environmental conditions for a month. Aquariums were aerated with air stone diffusers and sponge air pumps. Oxygen concentration, pH, and temperature in aquarium water are between 80-90%, 7-8, and 24-26°C during the day and night periods, respectively. Fish samples were fed with commercial feed without additives twice a day, at 8 am and 5 pm, throughout the experiment. In this study, the experimental setup was designed into three main groups: Group I: Cevizdere Stream (Surface water), Group II: Ünye (Tap water), and Group III: Control groups (dechlorinated clean water). Fish samples were placed in the relevant tanks of these three groups, 20 fish in each tank. All experiments were carried out in three replicates. Water samples in the tanks were renewed every ten days. Three fish samples were randomly taken from each tank for comet analysis on the 10^{th}, 20^{th}, and 30^{th} days after the start of the experiment.

In vivo Comet Assay

At the end of the 10^{th}, 20^{th}, and 30^{th} days, three fish samples from each tank were anesthetized with clove oil, and blood samples were immediately collected from the hearts of these samples with heparinized syringes, and the

procedure of Comet assay was immediately performed (Sing et al., 1988; Kontaş, 2022). After the erythrocyte cells were stained, comet images were captured using a fluorescence microscope (Leica DM2500, Leica Microsystems, Germany) at 20X objective. Quantification of the DNA single-strand breaks in the stored images was analyzed using Comet Score 2.0 software (Tritek Corp, Sumerduck, VA, USA). A hundred erythrocyte cells were randomly counted for each fish sample. The % DNA in the tail (tDNA%) is a reliable parameter of DNA damage because both the extent and amount of DNA in the tail are considered (Hartmann et al., 2003; Kumaravel & Jha, 2006; Tung et al., 2024). Therefore, % tail DNA, which is a valid and reliable reflection of genetic damage, is used to assess DNA damage in erythrocyte cells of fish samples.

Statistical Analysis

The DNA damage, as recorded by the alkaline comet assay, was analyzed considering the mean (± standard error) of the % tail DNA measured. The data were normally distributed (Kolmogorov–Smirnov normality test) and therefore Student's t test was performed for comparison of means between groups (control and treated groups). Then, comparisons according to the groups (Group I, Group II, Group III), the exposure times (10^{th}, 20^{th}, and 30^{th} days), and the seasons (autumn, winter, spring, and summer) were tested using the Minitab 18.0 Statistical Software. P-values lower than 0.05 were considered statistically significant.

RESULTS and DISCUSSION

Total Heavy Metal(loid)s Contents of Water Samples

When the heavy metal data in tap water and surface water samples were evaluated according to the relevant water quality criteria based on each heavy metal data (except for copper in winter and spring seasons), it was determined that they were generally within the range suitable for human use (Official Gazette of the Republic of Türkiye, 2005; 2019; 2022). However, since heavy metals together will create more toxic effects in the water environment, it was deemed more appropriate to compare the total heavy metal loads for water samples in each season for the purpose of the study. Total heavy metal(loid)s contents in the Cevizdere Stream surface water and the Ünye District tap water of Ordu province were determined as 26.703 ± 4.785 µg / L for Cevizdere Stream and 134.135 ± 7.128 µg / L for Ünye District tap water in spring, 47.955 ± 3.942 µg/L for Cevizdere Stream and 57.521 ± 5.412 µg / L for Ünye District tap water in summer, 27.684 ± 4.842 µg / L for Cevizdere Stream and 31.212 ± 4.587 µg / L for Ünye District tap water in Autumn, 40.052 ± 4.235 µg / L for Cevizdere Stream and 96.101 ± 6.053 µg / L for Ünye District tap water in winter.

When the total heavy metal(loid)s contents of the surface waters of the Cevizdere Stream and the tap waters of Ünye District were compared, it was determined that the total load contents of the tap waters were higher than the surface waters. A similar situation in terms of total heavy metal load was reported in a study conducted in the Black Sea region, which covers the sample area of the current study and is consistent with the data in the current study (Bostancı et al., 2024). It was stated that parameters such as temperature, pH, DO, and water salinity can affect the concentration of heavy metals in water (Wisha et al., 2018). Increasing temperature values in water may have different effects on heavy metals. Depending on the chemical properties of the heavy metal, it may show a parallel to the temperature increase or the opposite may be the case (Gati et al., 2016; Lazăr et al., 2024). This situation may also lead to their seasonal toxicological effects being different. When the total heavy metal(loid)s contents were evaluated seasonally, the total heavy metal(loid)s contents in the surface waters of the Cevizdere stream were determined to be lower than the value in the tap water in their respective groups in all seasons. The differences between the total heavy metal(loid)s amounts in the Cevizdere Stream surface water and Ünye District tap water were statistically significant in spring, summer, and winter seasons ($P < 0.05$), and it was determined that there was no statistical difference in the autumn season ($P > 0.05$).

Heavy metalloids are significant pollutants due to their high potential to spread from various sources. In addition, heavy metalloids pose a particular concern in aquatic systems and humans because they can show high toxicity to organisms even at low concentrations (Marcovecchio et al., 2007). Therefore, heavy metalloid pollution in water is not only a worldwide environmental problem but also a critical issue for human health (Sekabira et al., 2010). For this reason, it is crucial to continuously assess the quality of water sources. Therefore, analyzing and quantifying these toxic agents in water resources is of great importance. In order to protect human health globally, various international organizations such as USEPA, WHO, EPA, and the European Union Commission have established guidelines for the presence of heavy metals in drinking and surface waters (Mohod & Dhote, 2013; Eissa et al., 2023). The coastal districts of Ordu city, like Ünye, are heavily polluted with metalloid-based pollutants. These pollutants, which can have bioaccumulative and toxic effects on organisms, come from natural sources like rock erosion and human activities such as domestic and industrial wastewater discharge, as well as the use of herbicides and pesticides (Kurucu & Bostancı, 2022; Bostancı et al., 2024). The variation in the total load of heavy metals (loids) in surface water samples in the Ünye district between seasons could be due to changes in climate as well as

an increase in the district's population because of tourism activities. Additionally, surface waters are more affected by factors such as agricultural drainage, domestic sewage, and municipal wastewater. In addition to population growth, the concentration of heavy metal(loid)s in surface waters has increased due to industrialization in the coastal regions of Ünye District. Similar to this ur study, many studies have stated that domestic and industrial wastewaters are the primary contributors to heavy metal pollution in coastal cities and districts, posing a significant genotoxic risk to aquatic systems (Bashar & Fung, 2020; Liu et al., 2022). Sunjog et al. (2012) reported that industrial and domestic wastewaters in Serbia have genotoxicity potential because it is not treated before being released into waterways, and that they can be effectively monitored with comet analysis in aquatic ecosystems in Serbia. Silva et al. (2020) found that elevated levels of certain metals in urban water can lead to genetic damage in *Astyanax lacustris*, consistent with this study's findings.

It was reported that contaminants in tap and drinking waters used for consumption are related to the presence of disinfection by-products and corrosion in pipes used in distribution systems, and genotoxic damage may occur in organisms exposed to these pollutants (Richardson et al., 2003; Zegura et al., 2009; Cortés & Marcos, 2018). A similar situation may also be the case in terms of heavy metal pollution in the tap water of Ünye District. Ünye District's main water is provided by long-distance water transportation through pipes. The district's water distribution system is old and worn, making it more susceptible to corrosion, and as a result, contaminants from pipes in the distribution system can contaminate tap water. In this study, it is thought that this may be one of the main reasons for heavy metal(loid) pollution in tap water in the Ünye district of Ordu province. In addition, after many connection points in plumbing fixtures and pipes in water distribution systems are joined by welding processes, heavy metals in these parts can migrate into water due to aging in these parts over time. Moreover, contamination in the water may result from potential issues such as breakage and cracking at these connection points. Many studies have found that lead is commonly used for soldering in water distribution systems and plumbing pipes, potentially leading to increased levels of heavy metals in tap water (Jaishankar et al., 2014; Sorlini et al., 2014; Ghoochani et al., 2023). In addition, it was reported that in residences using plastic pipes, possible breakage and leakage of plastic pipes in close contact with domestic garbage dumps may cause heavy metal-containing leachate to mix with tap water and may be responsible for the increase in heavy metal concentration in tap water (Duplay et al., 2013; Chowdhury et al., 2021). Moreover, soft acidic water may cause some complications in pipelines due to its contact with plumbing, taps, and water fittings in residences. These situations may cause corrosion in water transmission and installation pipes and increase the concentration of some heavy metals such as Fe, Cu, Pb, and Ni in tap water (WHO, 2005; USEPA, 2011; WHO, 2022). In addition, high levels of heavy metals such as Cu in tap water may have a corrosive effect on the increased dissolved oxygen level in tap water and heavy metal(loid)s leakage from pipes in buildings. The pollution of heavy metals (loid)s in the surface water of Cevizdere Stream and tap water in the Ünye district can be attributed to various reasons.

Genotoxicity of Cevizdere Stream surface water and Ünye tap water

In the present study, the comet test was successfully performed in *Cyprinus carpio* erythrocyte cells. The values of DNA in the tail (%) used to determine DNA damage in fish exposed to Cevizdere Stream and Ünye tap water, are summarized in detail according to the experimental groups (tap water, surface water, and control), and seasons (spring, summer, autumn, and winter), and exposure times (10^{th}, 20^{th}, and 30^{th} days) (Figure 2). In the present study, it was found that the surface waters of Cevizdere Stream and tap waters of Ünye District exhibited significant genotoxic effects in *C. carpio* samples compared to the negative control, as determined by comet analysis. It was determined that genotoxic damage increased in direct proportion to the total heavy metal content in water samples and exposure times, and showed a positive correlation. Comet analysis data of the fish individuals exposed to Cevizdere Stream surface waters in the spring revealed that tail DNA% values were 13.31% on the 10^{th} day, 13.96% on the 20^{th} day, and 15.70% on the 30^{th} day (Figure 2A). These results also show that the genotoxic effect of Cevizdere Stream surface water in the spring season on the fish increases depending on the exposure time. There are statistical differences in tail DNA% between exposure times in the Cevizdere Stream surface water group in the spring season ($P<0.05$). According to the comet analysis results of *C. carpio* individuals exposed to Ünye tap waters in spring, tail DNA% values were 13.43% on the 10^{th} day, 14.55% on the 20^{th} day, and 15.94% on the 30^{th} day (Figure 2A). These results show that the genotoxic effect of Ünye tap waters in the spring season on the fish increases depending on the exposure time. There are statistical differences in tail DNA% between exposure times in the Ünye tap water group in the spring season ($P<0.05$). It was also determined that Ünye tap water causes more DNA damage than Cevizdere surface water in the spring season. In addition, it was determined that there were statistical differences in tDNA% when both Cevizdere Stream surface water and Ünye tap water were compared with the negative control group at all exposure periods (10^{th}, 20^{th}, and 30^{th} days) in the spring season (Figure 2A).

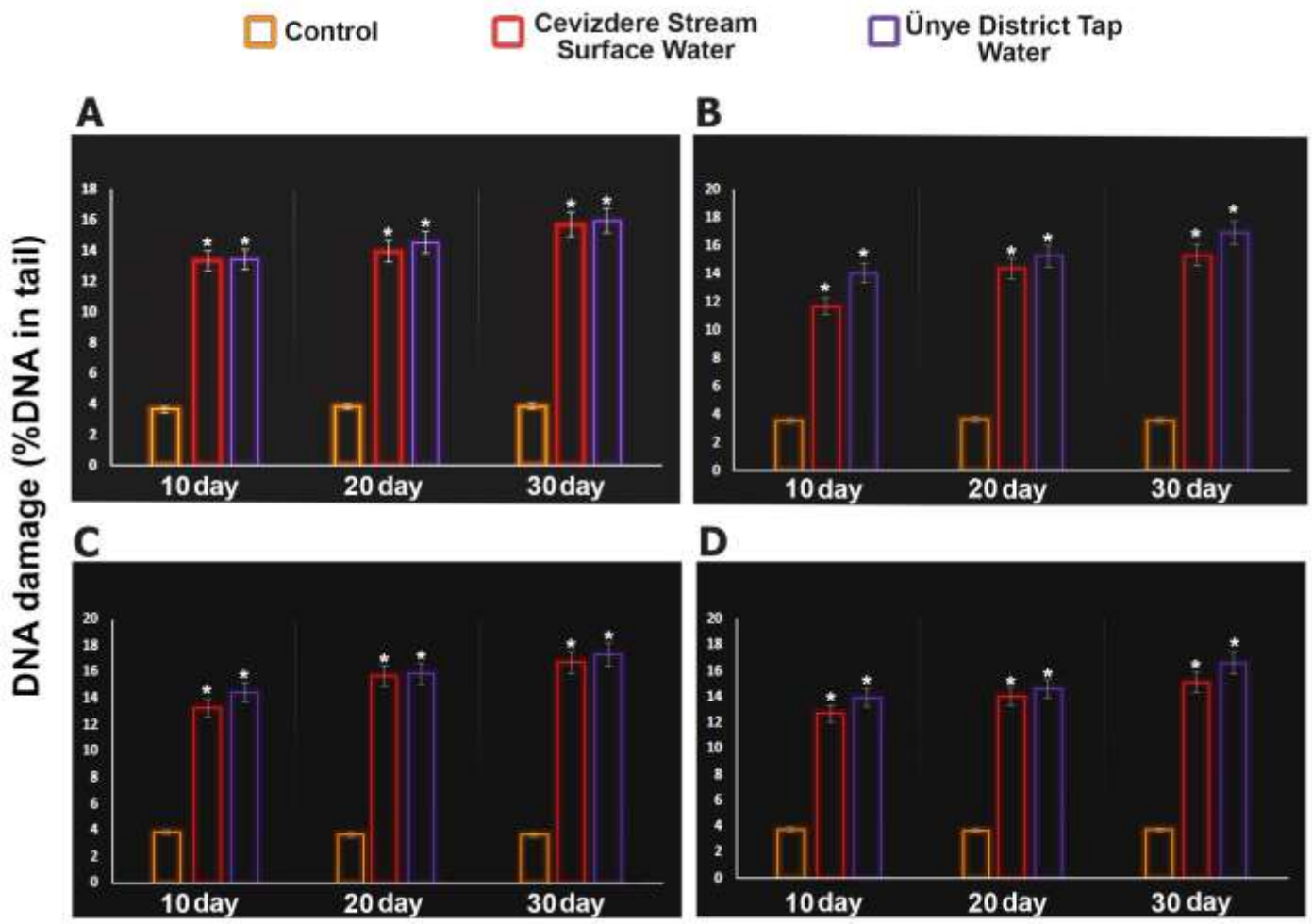

Figure 2. DNA percentages in the tail in erythrocyte cells of Cyprinus carpio subjected to increasing exposure time to water extracts in Cevizdere Stream surface water, Ünye District tap water, and control groups in spring (A), summer (B), autumn (C), and winter (D) seasons.

Şekil 2. Cevizdere Deresi yüzey suyu, Ünye İlçesi musluk suyu ve kontrol gruplarında ilkbahar (A), yaz (B), sonbahar (C) ve kış (D) mevsimlerinde su ekstraktlarına artan sürelerle maruz bırakılan Cyprinus carpio'nun eritrosit hücrelerindeki kuyruktaki DNA yüzdeleri.

According to the comet analysis results of *C. carpio* individuals exposed to Cevizdere Stream surface water samples in summer, tail DNA% values were 11.68% on the 10th day, 14.41% on the 20th day, and 15.31% on the 30th day (Figure 2B). These results also show that the genotoxic effect of Cevizdere Stream surface water in the summer season on the fish increases depending on the exposure time. It was determined that Cevizdere Stream surface water samples in the summer season caused statistical differences in tDNA% examined in the study, according to the exposure time in *C. carpio* ($P<0.05$). Comet analysis data of fish individuals exposed to Ünye tap water in the summer revealed that tail DNA% values were 14.06% on the 10th day, 15.27% on the 20th day, and 16.93% on the 30th day (Figure 2B). These results show that the genotoxic effect of Ünye tap waters in the summer season on the fish increases depending on the exposure time. There are statistical differences in tail DNA% between exposure times in the Ünye tap water group in the summer season ($P<0.05$). Ünye tap water causes more DNA damage than Cevizdere Stream surface water in the summer season, similar to the spring season. Moreover, statistical differences in tDNA% values were found when comparing both Ünye tap water and Cevizdere surface water with the negative control group at all exposure times in the summer (Figure 2B).

As a result of Comet analysis of *C. carpio* individuals exposed to Cevizdere Stream surface water samples in autumn, tail DNA% values were determined as 13.25% on the 10th day, 15.70% on the 20th day, and 16.71% on the 30th day (Figure 2C). These results show that the genotoxic effect of Cevizdere Stream surface water in the autumn season on the fish increases depending on the exposure time. Cevizdere Stream surface water samples in the autumn season caused statistical differences in tDNA% examined in the study, according to the exposure time in

C. carpio (P<0.05). Tail DNA% values in erythrocyte cells of *C. carpio* individuals exposed to Ünye tap water samples in the autumn were 14.45% on the 10th day, 15.84% on the 20th day, and 17.35% on the 30th day (Figure 2C). These results show that the genotoxic effect of Ünye tap waters in the autumn season on the fish increases depending on the exposure time. There are statistical differences in tail DNA% between exposure times in the Ünye tap water group in the autumn season (P<0.05). It was found that in autumn, Ünye tap water causes more DNA damage than Cevizdere Stream surface water, similar to the other seasons. Furthermore, it was determined that there were statistical differences in tDNA% values when both Ünye tap water and Cevizdere Stream surface water were compared with the negative control group at all exposure times in the autumn (Figure 2C).

Tail DNA% values in erythrocyte cells of *C. carpio* individuals exposed to Cevizdere Stream surface water samples in the winter were 12.73% on the 10th day, 14.02% on the 20th day, and 15.10% on the 30th day (Figure 2D). These results show that the genotoxic effect of Cevizdere Stream surface water in the winter season on the fish increases depending on the exposure time. There are statistical differences in tail DNA% between exposure times in the Cevizdere Stream surface water group in the winter season (P<0.05). According to the comet analysis results of erythrocyte cells of fish individuals exposed to Ünye tap water in winter, tail DNA% values were determined as 13.92% on the 10th day, 14.62% on the 20th day, and 16.63% on the 30th day (Figure 2D). These results show that the genotoxic effect of Ünye tap waters in the winter season on the fish increases depending on the exposure time. There are statistical differences in tail DNA% between exposure times in the Ünye tap water group in the winter season (P<0.05). It was also determined that Ünye tap water causes more DNA damage than Cevizdere Stream surface water in winter, similar to other seasons. Moreover, it was determined that there were statistical differences in tDNA% values when both Ünye tap water and Cevizdere surface water were compared with the negative control group at all exposure times in the winter (Figure 2D). In this study, the genotoxic effects of tap and surface waters were successfully carried out using the Comet assay. Like this study, the comet assay was used in many studies on the genotoxicity of water and has proven to be a sensitive and rapid method to detect DNA damage in organisms (Yuan et al., 2005; Zani et al., 2005; Buschini et al., 2008).

DNA damage in the erythrocyte cells of carp fish exposed to the surface waters of the Cevizdere Stream, which is the main drinking water source of Ünye District of Ordu province, and the tap water of Ünye district, may be caused by organic and inorganic pollutants. In addition, in monitoring studies in surface water and tap water, it is difficult to identify the compounds that may be responsible for possible negative effects associated with exposure to environmental pollutants in aquatic environments in mixed form and to directly attribute the genotoxic effect to a single type of pollutant. Heavy metals are among the most important pollutants affecting organisms and ecosystem health in aquatic systems. Moreover, the interactions of the relevant heavy metals (loid) with each other are quite complex, and these substances can show different effects when they are mixed in the same environment, as well as their effects when they are found alone in the environment (Kocadal et al., 2020; Kontaş, 2022; Mitra et al., 2022; Kontaş Yalçınkaya et al., 2025). In the current study, chemical analysis results of water samples showed the presence of metals in Cevizdere surface waters and Ünye district tap water. Even if they are not at very high levels, these pollutants have the potential to cause DNA damage to erythrocyte cells when evaluated as total heavy metal(loid)s. It is widely reported in the literature that exposure to these heavy metals may cause toxicogenic damage and carcinogenesis in organisms. When the DNA in the tail is evaluated, it is clear that these water samples cause DNA damage in the erythrocyte cells of carp fish. Similar to the current study, DNA damage caused by heavy metals (such as Cu, Fe, Mn, and Cd) was reported in *Clarias gariepinus* sampled from the Orontes River (Turan et al., 2020). There are many studies in the literature that mainly use the average value of tail length and tDNA% to evaluate genotoxicity (Alink et al., 2007; Pereira et al., 2012; Osman et al., 2012; Nwani et al., 2013). The mean tail length and tDNA% values were reported as 35.17 ± 1.370 % and 45.14 ± 5.610% in *Alburnus chalcoides* from Melet River (Kontaş & Bostancı, 2020). Moreover, the mean tDNA% value in *Clarias gariepinus* exposed to the surface waters of the Orontes River was calculated as 17.746 ± 1.072 % (Turan et al., 2020). In this study, this value was calculated as 14.323 ± 1.236 % for spring, 13.800 ± 1.890 % for summer, 15.220 ± 1.779 % for autumn, and 13.950 ± 1.187 % for winter in the samples exposed to Cevizdere Stream surface water seasonally. In the *Channa punctatus*, it was determined that the percentage of tail DNA% in individuals in the control group was 1.6 ± 0.32, while it was 16.5 ± 0.61 in the treatment group, and this increase was reported to indicate significant DNA damage in this species (Mehra & Chadha, 2021).

Increasing exposure time in carp fish in all seasons caused an increase in DNA damage, and in this study, a positive relationship was observed between exposure time and DNA damage in all groups except the control group. A similar positive relationship between DNA damage and exposure time has been reported previously (Ahmed et al., 2005). Moreover, we observed an increase in DNA damage in erythrocyte cells of the *C. carpio*, depending on the total metalloid amount. It revealed a similar trend in *Labeo rohita* after exposure to organophosphate pesticides in blood samples (Mohanty et al., 2011) and in *Channa punctatus* after exposure to tetrabromobisphenol A (Sharma et al., 2019) and profenophos (Pandey et al., 2011). The increases in DNA damage observed in fish, depending on

the exposure time as a result of metalloid accumulation in their environment, are quite understandable. In high-temperature seasons (spring and summer), the concentration of pollutants and especially heavy metal(loid)s in water increases due to the decrease in the amount of surface water. This was also confirmed by the total heavy metal(loid)s load determined in the surface and tap water samples in this study. Therefore, this situation can be associated with the DNA damage detected in *C. carpio* in warm seasons. In low-temperature seasons (winter and autumn), especially as a result of rainfall, pollutants from agricultural areas and environmental wastes enter surface water resources in the form of oil. In this study, DNA damage caused by surface water in the erythrocyte cells of *C. carpio* during low-temperature seasons may be associated with excessive rainfall in these seasons. This study is also consistent with previous data reporting an increase in DNA damage in fish from waters contaminated with different pollutants, such as industrial, agricultural, and domestic wastes. In addition, it also supports the results emphasized in many genotoxic studies that the amount of DNA damage may vary depending on exposure time and seasonal differences (Dhawan et al., 2009; Andem et al., 2013; Kontaş, 2022).

CONCLUSION

This study revealed that the surface and tap waters of Ünye district, one of the important towns of Ordu province in the Black Sea Region, were polluted with heavy metal(loid)s and that the total heavy metal load in these waters caused genotoxic effects on carp fish. This study is the first to use comet analysis to evaluate the possible genotoxicity of the surface water of Cevizdere Stream, used as a drinking water source in Ünye district of Ordu province, and the tap water in these regions on carp fish. Concentrations of such contaminants in drinking water can often vary from nanograms per liter to micrograms per liter, often resulting in such contaminants falling below detection limits in analysis and their genetic toxic effects remaining undetected. In addition, although heavy metals do not cause genotoxic effects individually and in low amounts, these heavy metals have the potential to come together and create genotoxic effects as a total heavy metal load. Therefore, instead of evaluating the pollutants individually in such aquatic environments, it is necessary to evaluate them based on total accumulation, which can provide more detailed information about the relevant environment. In addition, it is important to analyze the pollutants in water with alternative analyses, such as comet analysis, to obtain detailed information about the current conditions of these environments. For this reason, local consumers should also be sure that the distributed water is of good quality until it reaches users, even if the surface water is thoroughly purified in treatment plants and offered for use as tap water. It is recommended that users use this water by taking the necessary precautions instead of using it directly in order to prevent negative health effects.

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Contribution Rate Statement Summary of Researchers

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

Conflict of Interest

The authors declare no conflicts of interest.

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Determination of Macro and Microelement Content of Some Virginia Market Type Peanut Varieties

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ABSTRACT
This study was designed to investigate the macro and micronutrient contents of 10 different Virginia market peanut cultivars under Kahramanmaras conditions for 2 years. Arıoğlu 2003, Halisbey, Osmaniye 2005, and Sultan varieties registered by Çukurova University, Batem 5025, Batem Cihangir, Brantley, NC-7, and Wilson varieties registered by Western Mediterranean Agricultural Research Institute, Brantley, NC-7, and Wilson varieties originating from the USA, and lastly Flower-22 variety originating from China were used as material. The research was conducted for two years (2018-2019) under main crop conditions in the experimental fields of Kahramanmaraş Sütçü İmam University, Faculty of Agriculture, Department of Field Crops, Application and Research Centre. It was observed that the cultivars were considerably different in terms of macro and micronutrient contents, and the variety-year interactions were significant. The two-year average results showed that the highest N, P, Fe, Ni, and Cu contents were obtained from the Flower-22 variety, the highest K and Ca contents were obtained from the Batem Cihangir variety, and the highest Zn contents were obtained from Sultan and Osmaniye-2005 varieties. Principal component biplot analyses (PCA) accounted for 52.7% of the relationships between the studied traits. As a consequence of the study, it was observed that P value had positive and important relationships with Fe, Zn, Mo and Cu contents, Ca content had positive and important relationships with Fe, Mn, Ni, and Cu, and K values had negative and important relationships with Fe, Mn and Ni.

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Bazı Virginia Pazar Tipi Yer Fıstığı Çeşitlerinin Makro ve Mikro Element İçeriklerinin Belirlenmesi

ÖZET
Bu çalışmada, Kahramanmaraş koşullarında 10 farklı Virginia Pazar tipi yerfıstığı çeşitlerinin makro ve mikro besin element içerikleri 2 yıl süreyle araştırılmıştır. Çukurova Üniversitesi tarafından tescil edilen Arıoğlu 2003, Halisbey, Osmaniye 2005 ve Sultan çeşitleri, Batı Akdeniz Tarımsal Araştırma Enstitüsü tarafından tescil edilen Batem 5025, Batem Cihangir, Brantley, NC-7 ve Wilson çeşitleri, ABD orijinli Brantley, NC-7 ve Wilson çeşitleri ve son olarak Çin orijinli Flower-22 çeşidi materyal olarak kullanılmıştır. Araştırma, Kahramanmaraş Sütçü İmam Üniversitesi Ziraat Fakültesi Tarla Bitkileri Bölümü Uygulama ve Araştırma Merkezinde ana ürün koşullarında iki yıl (2018-2019) süreyle yürütülmüştür. Çeşitlerin makro ve mikro besin element içerikleri bakımından önemli derecede farklı olduğu ve çeşit-yıl interaksiyonlarının önemli olduğu görülmüştür. İki yıllık ortalama sonuçlar, en yüksek N, P, Fe, Ni ve Cu içeriklerinin Flower-22 çeşidinden, en yüksek K ve Ca içeriklerinin Batem Cihangir çeşidinden, en yüksek Zn içeriklerinin ise Sultan ve Osmaniye-2005 çeşitlerinden elde edildiğini göstermiştir. Çalışılan özellikler arasındaki ilişkilerin %52,7'si temel bileşen biplot analizi

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(PCA) ile açıklanmıştır. Çalışma sonucunda, P değerinin Fe, Zn, Mo ve Cu içerikleriyle, Ca içeriğinin Fe, Mn, Ni ve Cu içerikleriyle, K değerlerinin ise Fe, Mn ve Ni ile negatif ve önemli ilişkilere sahip olduğu görülmüştür.

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INTRODUCTION

Peanut (*Arachis hypogaea* L.) is a one-year oilseed plant from the legume family, originating from South America, grown as a main and second crop. Peanut, which is widely used in human and animal nutrition, is an important oil plant that enriches the soil where it is grown with nitrogen (Arıoğlu, 2014). It is a legume plant grown in tropical and temperate climates (Ayoola & Adeyeye, 2012), emphasized due to its high protein content (27%-29%) and consumed worldwide due to its nutritive properties (Guo et al., 2020; Mattil et al., 1964; Zhang et al., 2020).

Peanut oil is superior to many vegetable oils in terms of flavor and durability. For this reason, it is consumed very much. Peanut varieties, which are grouped due to differences such as flavour, oil content, size, shape, and tolerance to diseases, are preferred for specific and special uses. Different varieties can be used for many uses, but the most preferred varieties are Spanish, Runner, Virginia, and Valencia (Woodroof, 1983). In spite of the fact that peanut is a legume plant, it is usually classified among oilseeds because of its very high oil content, rich in protein, fat, as well as fibre (Suchoszek-Lukaniuk et al., 2011). Nutritionally, peanut seeds are rich in oil, protein, niacin, fibre, magnesium, vitamins, manganese, and phosphorus (Davis & Dean, 2016; Fletcher & Shi, 2016). Peanut seeds are a rich source of minerals (phosphorus, calcium, magnesium, and potassium) and vitamins (E, K, and group B) (Arya et al., 2016; Grosso & Guzman, 1995; Kholief, 1987; Singh & Singh, 1991). Peanut is an important source of mineral elements for the nutritional requirements of humans and animals (Asibuo et al., 2008). In addition, studies conducted in Turkey have shown that different peanut genotypes exhibit significant differences in terms of yield and quality characteristics under various environmental conditions (Uçak et al., 2017).

Peanuts are an affordable and rich source of essential nutrients, including proteins, carbohydrates, fats, vitamins, minerals, and fiber. Often called the "poor man's protein," peanuts provide vital nutrients that support growth, energy, and disease prevention when consumed in sufficient amounts. They contain essential vitamins, metal ions for enzyme activity, and fatty acids that boost heart-healthy HDL cholesterol. Additionally, peanuts supply essential amino acids and carbohydrates, crucial for protein synthesis and energy production. Including peanuts in the diet can help prevent illness and promote overall health (Settaluri et al., 2012).

The chemical composition of peanut seeds, especially the macro (K, Ca, Mg, P) and micro (Fe, Zn, Cu, Mn) elements it contains, directly affects both the nutritional value when consumed as food and the germination ability, seedling development and seed quality when used as seed material (Fageria, 2009; Welch & Graham, 2004). In a study by Bakal & Arıoğlu (2021), agronomic and quality characteristics of different groundnut varieties were examined, and the effects of mineral composition on seed quality were emphasized. While nutrient deficiencies may lead to micronutrient deficiencies in terms of human health, the toxic accumulation of some elements may pose a health risk (White & Broadley, 2009). At the same time, adequate levels of minerals in the seed increase the vigor of the seed and make the young plant more resistant to stress conditions (Farooq et al., 2012). In this context, the determination of macro and micro elements in peanut seeds is of great importance for both nutritional security and quality seed production. The main objective of this research was to evaluate the macro and micro element values of different Virginia market peanut varieties for two years under Kahramanmaras conditions. For this purpose, ten different peanut cultivars were grown in the main crop production conditions for two years, and the mineral performances of the cultivars used in the study were determined by PCA and correlation analyses.

MATERIAL and METHOD

The experiment was established in Kahramanmaraş conditions within the main crop peanut growing season for two years (2018 and 2019). Kahramanmaraş is located in the Mediterranean Region on the coordinates of north latitude 37°35'40.77‘ and east longitude 36°48'51.43’.

Soil samples taken from the experimental area at 0-30 cm depth before planting were analyzed in the laboratories of Kahramanmaraş Sütçü Imam University, University-Industry-Public Cooperation Development Application and Research Center, and the results of these analyses are given in Table 1.

The soils of the test area have a clay loamy texture and are flat and nearly flat sloping. There is no salinity problem in the test area with high lime content. The phosphorus and potassium content is low, and the organic matter

content is at a medium level. In addition, the pH value of the soil is 7.50, and the soil is basic (Table 1).

Table 1. Soil analysis (0-30 cm depth) results of the research area (*)
Tablo 1. Araştırma alanının toprak analiz (0-30 cm derinlik) sonuçları (*)

Location	Texture (% Sat.)	Salinity (%)	Organic Matter %	Lime CaCO3 (kg/da)	Phosphorus mg/kg	Potassium (mg/kg)	pH	Total Nitrogen (%)
Kahramanmaras	59.40	0.13	2.65	2.19	5.78	112.10	7.50	0.08

* Soil analyses were carried out in the laboratory of Kahramanmaraş Sütçü İmam University, University-Industry-Public Cooperation Development Application and Research Centre.

The trials were conducted in 2018 and 2019 at Kahramanmaraş Faculty of Agriculture, Department of Field Crops Research and Application Centre. Kahramanmaraş, with its geographical location and other factors, shows a climate characteristic closer to the Mediterranean climate among the three different climate types.

Table 2. Mean temperature, precipitation, and relative humidity data for the experimental years and long years (April-October average during the main crop growing season).
Tablo 2. Deneme yılları ve uzun yıllar için ortalama sıcaklık, yağış ve bağıl nem verileri (ana ürün yetiştirme sezonu boyunca Nisan-Ekim ortalaması).

Climate values	Years	April	May	June	July	August	September	October	Total	Average
Average temperature (°C)	2018	20.10	24.40	26.40	29.10	29.60	27.90	22.90	180.40	25.77
	2019	17.00	24.10	27.10	28.40	29.60	27.30	24.20	177.70	25.39
	Long Years	18.50	21.75	26.28	29.23	29.50	26.73	22.43	174.42	24.92
Average precipitation (mm)	2018	33.00	29.20	23.40	0.00	0.00	1.20	64.00	150.80	21.54
	2019	59.40	2.60	13.80	28.00	0.00	0.00	22.80	126.60	18,09
	Long Years	50.27	52.38	25.09	5.71	6.26	25.73	29.52	194.96	27.85
Average relative humidity (%)	2018	61.20	62.80	70.20	69.80	68.80	63.60	58.60	455.00	65.00
	2019	67.00	57.60	68.70	68.80	68.00	62.10	61.60	453.80	64.83
	Long Years	63.46	67.94	67.60	68.85	69.58	64.58	58.17	460.18	65.74

Monthly precipitation, temperature, and relative humidity data for the 2018-2019 production year and for many years are given in Table 2. In the 2018-2019 growing season, the average temperature values reached 25.77 °C in 2018, which was higher than the long-term average. When the average precipitation is analyzed, it is seen that there is lower precipitation compared to long years. In addition, it was determined that there were no significant differences between the relative humidity values during the growing period and long-year averages (Table 2).

A total of ten genotypes registered by Çukurova University and Western Mediterranean Agricultural Research Institute were used. These genotypes are given in Table 3.

Table 3. Variety names, market type, origin, and growth forms of the varieties
Tablo 3. Çeşit isimleri, pazar tipi, orijin ve çeşitlerin büyüme formları

Variety names	Market type	Origin	Growth forms
Arıoğlu- 2003	Virginia	Türkiye	Semi-spreading
Batem 5025	Virginia	Türkiye	Semi-spreading
Batem Cihangir	Virginia	Türkiye	Semi Upright
Brantley	Virginia	ABD	Semi-spreading
Flower-22	Virginia	Çin	Semi-spreading
Halisbey	Virginia	Türkiye	Semi-spreading
NC-7	Virginia	ABD	Semi-spreading
Osmaniye-2005	Virginia	Türkiye	Semi-spreading
Sultan	Virginia	Türkiye	Semi-spreading
Wilson	Virginia	ABD	Semi-spreading

The research was designed as a randomized block design with three replications. Sowing was arranged in 4 rows with 70 cm between rows and 15 cm above rows, and 9500 seeds per decare.

The plot size was applied as 5 m long-2.8 m wide (5 m x 2.8 m) and 14 m^2. Before sowing, 30 kg/da 18-46-0 (N-P-K) DAP (Diammonium phosphate) fertilizer was applied by hand sprinkling, and 15 kg/da urea (46% N) fertilizer was applied approximately 60 days after emergence. Other maintenance operations (irrigation, weed and pest control) were carried out considering plant and soil moisture.

Data collection

The peanut plants in the experimental area were mechanically harvested after reaching physiological ripeness (in October), the peanut plants were inverted and left to dry, and finally, peanut fruits were harvested by hand after three days of drying. Seed samples were separated and dried in an air dryer at 65 °C until reaching constant weight. Seeds were digested at 180° with 4 ml HNO_3 and 3 ml H_2O_2 (Berghof MWS 2 DAP 60 K microwave oven). Macro and micronutrients were analyzed from the extracts using ICP OES device (Kacar & Inal, 2014).

Data Evaluation

The data were analyzed by analysis of variance for two years using LSD test to compare the means. Correlation coefficients and principal component analyses were calculated and evaluated on average data (JMP 17 SAS Institute Inc, 2020).

RESULTS and DISCUSSION

Average values and analysis of variance findings of N, P, K and Ca among macro elements and Fe, Mn, Zn, Ni, Mo and Cu among micro elements of the peanut varieties used in the study for two years are reported in Table 4.

Macro elements

Nitrogen is a necessary element for vegetative growth, nutrient absorption, photosynthesis, and capsule improving (Sing, 1999). N, P, K, Ca, Fe, Mn, Zn, Ni, Mo, and Cu are obligatory for plants (Gascho & Davis, 1994), and these nutrients should be present in sufficient amounts in the soil to obtain high yields (Aşık & Aşık, 2023). Peanuts are a good food source of macro minerals, which are needed in quantities of more than 100 mg/day (Derise et al., 1974; Settaluri et al., 2012). In this study, the varieties produced different results in terms of N content, and all varieties had higher N content in the second year (Figure 1). When the two-year cultivar averages (Table 4 and Figure 1) were analyzed, it was observed that N content varied between 3.51-3.98%. Flower-22 variety had the highest N content, followed by Sultan (3.84%), NC-7 (3.80%), and Brantley (3.76%) varieties. Wilson (3.51%) and Arıoğlu-2003 (3.53%) varieties had the lowest nitrogen content. In their study, Aşık & Aşık (2023), determined the nitrogen content between 3.67 and 4.38%. Nitrogen content in the seed is affected by plant applications, variety characteristics, and environmental conditions (Steer & Hocking, 1984).

The phosphorus value of plants varies from 0.1-0.8 percent of the dry matter. Phosphorus is present in low quantities in peanut plants; however, plants can absorb phosphorus even in soils very poor in phosphorus (Aşik, 2023a). Phosphorus is also involved in energy utilization, storage as well and transport in plants (Tasso et al., 2004). Feitosa et al. (1993) concluded that over 70% of the phosphorus uptake by peanut plants from pellets accumulates in the fruit and that the element has a significant effect on fruit formation. In this study, the varieties produced different results in terms of phosphorus (P) contents (Table 1). All varieties except Batem-5025 and Sultan varieties had lower P content in the second year (Figure 1). When the two-year variety averages (Table 4 and Figure 1) are analyzed, it is seen that P content varied between 0.397-0.548%. Brantley (0.548%) and Flower-22 (0.547%) varieties had the highest P content, followed by Halisbey (0.540%) and Osmaniye-2005 (0.531%) varieties. The lowest phosphorus content was found in Batem-5025 (0.397%). In the study conducted by Abd EL-Kader (2013), it was determined that the phosphorus content in peanut seeds was between 0.40-0.50%.

Potassium is another element that is important for plants and is absorbed in significant amounts. It plays a significant role in capsule formation and grain weight increase (Taiz & Zeiger, 2013). Potassium values of the varieties ranged between 0.052 and 0.074 % (Table 4). All varieties except Sultan had similar or higher K content in the second year (Figure 1). According to the two-year variety averages (Table 1 and Figure 1), the highest potassium value was obtained from Batem Cihangir variety (0.522%), and the lowest potassium value was obtained from Wilson variety (0.320%). Oerise et al. (1974) reported an average of 0.63% potassium element in seeds of 3 Virginia-type peanut varieties; Aşik (2023b) reported that seed potassium content varied between 0.37% and 0.43%.

Table 4. Mean squares and values of macro and microelement contents for peanut varieties from two years (2018 and 2019) average.
Tablo 4. Yer fıstığı çeşitlerinin makro ve mikro element içeriklerinin iki yıllık (2018 ve 2019) ortalama değerler ve kareler ortalaması

Varieties	N (%)	P (%)	K (%)	Ca (%)	Fe(mg kg^{-1})	Mn(mg kg^{-1})	Zn(mg kg^{-1})	Ni(mg kg^{-1})	Mo(mg kg^{-1})	Cu(mg kg^{-1})
Arıoğlu-2003	3.53±0.22	0,450±0.02	0.517±0.01	0.054±0.00	17.86±0.81	13.94±0.42	25.14±0.54	2.37±0.13	1.13±0.02	4.42±0.04
Batem 5025	3.69±0.37	0.397±0.06	0.464±0.02	0.059±0.00	13.23±0.36	16.82±0.41	21.72±0.32	5.33±0.09	1.08±0.00	3.55±0.06
Batem Cihangir	3.59±0.21	0.523±0.04	0.522±0.02	0.072±0.00	23.34±1.10	17.64±0.59	23.61±0.33	8.05±0.15	0.65±0.02	4.49±0.05
Brantley	3.76±0.14	0.548±0.03	0.463±0.01	0.056±0.00	22.58±0.68	16.76±0.22	25.98±0.77	5.30±0.04	2.22±0.10	3.50±0.16
Flower-22	3.98±0.19	0.547±0.02	0.363±0.01	0.074±0.00	24.68±0.55	19.76±0.32	25.14±0.87	8.35±0.10	0.63±0.01	7.08±0.02
Halisbey	3.75±0.25	0.540±0.01	0.415±0.01	0.052±0.00	17.52±0.65	18.04±0.34	20.90±0.28	5.19±0.01	2.14±0.01	3.52±0.08
NC-7	3.80±0.19	0.459±0.01	0.349±0.01	0.065±0.00	23.15±0.52	21.13±0.21	24.46±1.02	8.28±0.04	0.31±0.00	3.17±0.05
Osmaniye 2005	3.61±0.25	0.531±0.02	0.453±0.02	0.067±0.00	21.89±0.33	17.12±0.34	28.48±0.11	5.20±0.03	1.09±0.02	3.65±0.11
Sultan	3.84±0.34	0.526±0.01	0.405±0.01	0.060±0.00	24.38±0.31	17.14±0.26	28.90±0.78	7.25±0.08	2.19±0.06	3.11±0.00
Wilson	3.51±0.25	0.450±0.00	0.320±0.01	0.064±0.00	21.37±0.46	18.18±0.44	20.35±0.61	6.29±0.06	0.35±0.02	3.39±0.14
LSD (P=0.05)	2.47	6.07	8.25	7.81	6.77	5.59	6.58	3.08	7.35	4.84
Analysis of variance for macro and microelement contents combined over years *Makro ve mikro element içerikleri için yıllara göre birleştirilmiş varyans analizi*										
Cultivars (C)	1.18**	0.15**	0.25**	0.002**	716.06**	196.56**	461.89**	190.51**	30.55**	75.48**
Years (Y)	17.03**	0.002	0.06**	1.66	9.87	2.35	10.38*	0.01	0.02	0.10**
C x Y	1.40**	0.009	0.10**	0.0004*	26.38	1.91	12.07	0.86*	0.11	0.81*

*, **: Significant at the 0.05 and 0.01 level of probability

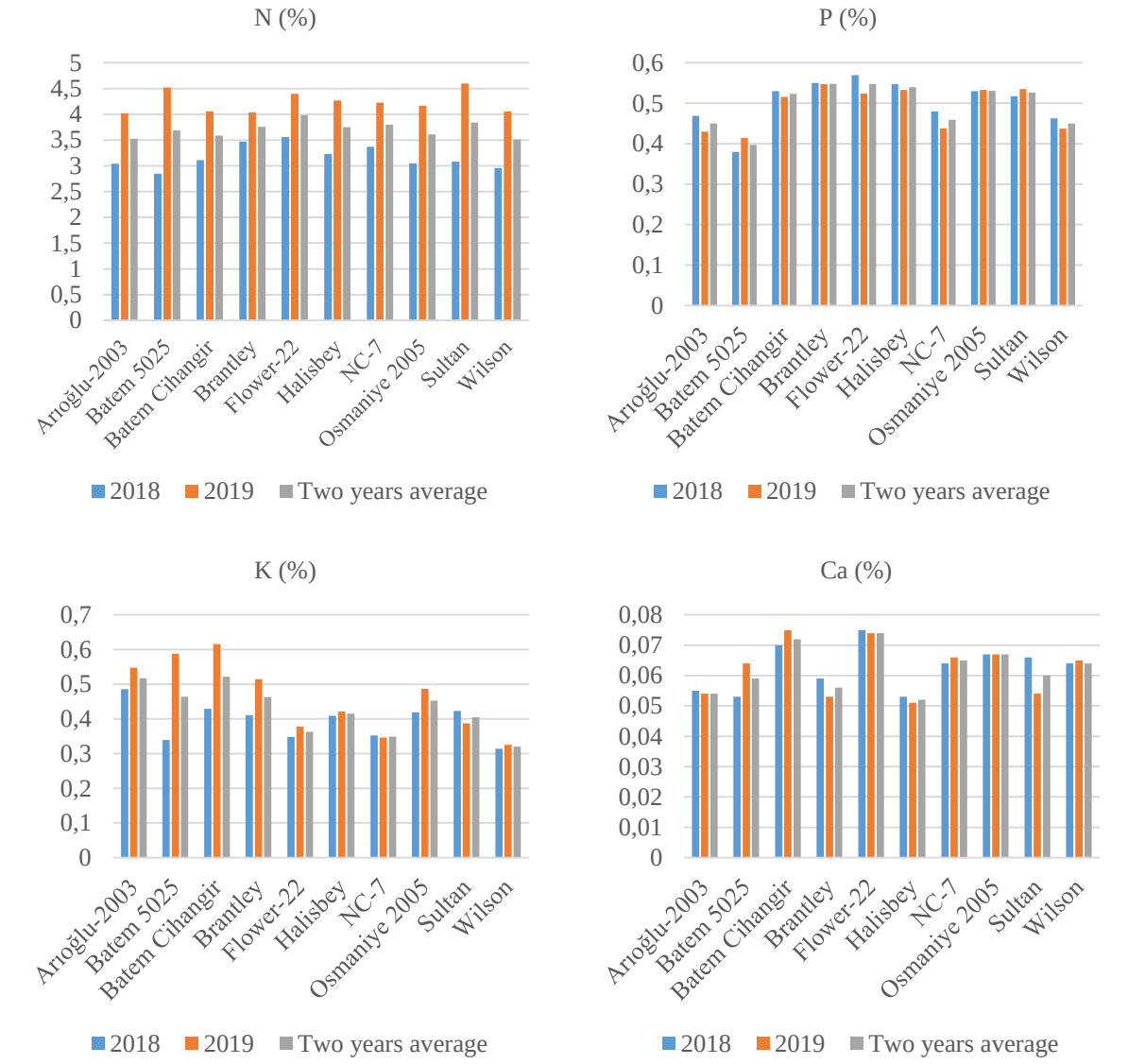

Figure 1. Macroelement contents of peanut varieties
Şekil 1. Yer fıstığı çeşitlerinin makroelement içerikleri

Calcium is one of the crucial elements in peanut generation, and its deficiency causes yield and quality losses (Cox et al., 1982). It is a necessary and important element for the formation of gynophores and filling of capsules in the plant (Aşık, 2023a). Calcium values of the cultivars ranged between 0.052 and 0.074% (Table 1). According to the two-year cultivar averages (Table 4 and Figure 1), the highest calcium value was obtained from Flower-22 (0.074%) and Batem Cihangir (0.072%), and the lowest was obtained from Halisbey (0.052%) and Arıoğlu-2003 (0.054%). Although peanuts do not remove much calcium from the soil, it has a special role in regulating many aspects of plant growth and development (Arıoğlu, 2014). Branch and Gaines (1983) reported that seed calcium contents varied between 0.04% and 0.08% in a study with 26 different peanut germplasms.

Micro elements

Variances of cultivar, year, and year-cultivar interactions were found to be significant in terms of micronutrients (Table 1). Peanut cultivars showed significant differences in the micronutrients (Fe, Mn, Zn, Ni, Mo, and Cu) analyzed (Table 4 and Figure 2). When the two-year averages were analyzed, it was determined that the Flower-22 variety had high values in terms of iron (24.68 mg kg^{-1}), nickel (8.35 mg kg^{-1}), and copper (7.08 mg kg^{-1}) contents. In addition, NC-7 variety had high values in terms of Mn (21.13 mg kg^{-1}) and Ni (8.28 mg kg^{-1}) value, Sultan variety had high values in terms of Zn (28.90 mg kg^{-1}) and Mo (2.19 mg kg^{-1}) value, Osmaniye-2005 variety had high values in terms of Zn (28.48 mg kg^{-1}) content, Brantley (2.22 mg kg^{-1}) and Halisbey (2.14 mg kg^{-1}) varieties had high values in terms of Mo content.

Figure 2. Microelement contents of peanut varieties
Şekil 2. Yer fıstığı çeşitlerinin mikroelement içerikleri

Batem-5025 (13.23 mg kg^{-1}) had the lowest Fe content, Arıoğlu-2003 had the lowest Mn (13.94 mg kg^{-1}) and Ni (2.37 mg kg^{-1}) contents, Wilson (20.35 mg kg^{-1}) and Halisbey (20.90 mg kg^{-1}) varieties had the lowest Zn content.

In addition, NC-7 (0.37 mg kg^{-1}) and Wilson (0.35 mg kg^{-1}) varieties had the lowest Mo content, and Sultan (3.11 mg kg^{-1}) and NC-7 (3.17 mg kg^{-1}) varieties had the lowest Cu content (Table 4 and Figure 2). Iron, an important micro mineral in human and animal nutrition, is implicated in many biological functions like the transport, storage, and utilization of oxygen by red blood cells and redox potentials (İnce & Çağındı, 2020). Fe composition is important in peanuts, and its absence leads to chlorosis of the leaves (Aşık, 2023a). In this study, Flower-22 variety attracted attention with its high Fe content. Zinc, which has important physiological effects in living organisms and performs a role in many biological functions, is an important micronutrient in human nutrition and is associated with many enzyme systems in the human body (Kınık et al., 2001). Zinc is the second-highest trace element in the human body after iron and is essential for the function of over 300 enzymes in the body (Akdeniz et al., 2016). Zinc deficiency is an important problem worldwide (Hambidge, 2000). In this study, Sultan and Osmaniye-2005 varieties stood out with high Zn content. This findings regarding Fe and Zn values are consistent with the results of Chen et al. (2022). The values of Mn, Ni, Mo and Cu obtained in this study are in agreement with the findings of many researchers (Hallock et al., 1971; Gaines & Hammons, 1981; Chen et al., 2022).

Principal Component Analysis

PCA (Principal component analysis), a dimensionality minimization method, was applied utilizing the dataset of the examined agricultural traits. Total variation was obtained from 10 principal component axes, and the Eigenvalues, Variability (%), and Cumulative values (%) are shown in Table 5. The first principal component (PC1) accounts for 33.101% of the overall variation. The secondary principal component (PC2) accounts for 19.599% of the overall variation. The third principal component explains 13.532 of the overall variation (PC3). The cumulative proportion of the three main components in the overall variation is 66.7233%. Remaining principal components (PC4=11.266%, PC5=7.672%, PC6=5.503%, PC7=3.091%, PC8=2.634%, PC9=2.478% and PC10=1.124%) have 33.22% of the overall variation. As a consequence of PCA analysis, 10 principal component axes were identified, and these axes showed the total variation. All 10 principal components revealed 100% of the overall variation.

Table 5. Eigenvalues, Variability and Cumulative Values
Tablo 5. Ekovalans, varyabilite ve kümülatif değerler

	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10
Eigenvalue	3,31009	1,959925	1,353241	1,1266	0,76719	0,550319	0,30908	0,263369	0,247809	0,112378
Variability (%)	33,101	19,599	13,532	11,266	7,672	5,503	3,091	2,634	2,478	1,124
Cumulative (%)	33,101	52,7	66,233	77,499	85,17	90,674	93,764	96,398	98,876	100

The Scree Plot (graphical representation of eigenvalues) is presented in Figure 3. Eigenvalues obtained for PC1 are 3.310. Other eigenvalues were 1.959 (PC2), 1.353 (PC3), 1.126 (PC4), 0.767 (PC5), 0.550 (PC6), 0.309 (PC7), 0.263 (PC8), 0.247 (PC9), and 0.112 (PC10), respectively. Eigenvalues above 1 indicate that the considered principal component weight values are found to be reliable (Mohammadi & Prasanna, 2003). Moreover, Iezzoni and Pritts (1991) reported that PCs with eigenvalues greater than 1 (PCs with eigenvalues >1.0) are more informative than the original variable.

Principal component analysis is used to see the variation among the varieties used in the research and the relationships between the examined traits of these varieties visually more clearly and enough to be understood (Chakravorty et al., 2013; Tekdal et al., 2018). According to PCA (Principal Component Analysis) analysis, it is possible to see the relationships between the varieties and the traits examined visually together. According to the PCA biplot analysis performed at the 2-year average results of the features examined in terms of nutrients in this research, principal component 1 (PC1) was found to be 33.1%, principal component 2 (PC2) 19.6%, and 52.7% in total. It was observed that there was a positive relationship between Molybdenum and Potassium contents, Zn, N, P, Fe, and Cu contents, while there was a negative relationship with other properties (Ca, Mn, and Ni) (Figure 4).

Correlation Coefficient Analysis

Correlation coefficient values between variables are given in Table 6. It is seen that nitrogen content is positively correlated with potassium, and potassium is negatively and significantly correlated with Fe, Mn, and Ni. Phosphorus content was positively correlated with Fe, Zn, Mo, and Cu element contents. It was found that among the macronutrients analyzed, Ca content had a positive and prominent correlation with Fe, Mn, Ni, and Cu content and a negative and significant correlation with Mo content. Among micronutrients, Fe has a positive and important correlation with Mn, Zn, and Ni. A positive and important correlation was found between Mn content and Ni content, and a negative and important correlation was found among Mo content. In comparison, a negative and significantly correlation was found among Mo content and Cu and Ni content (Table 6).

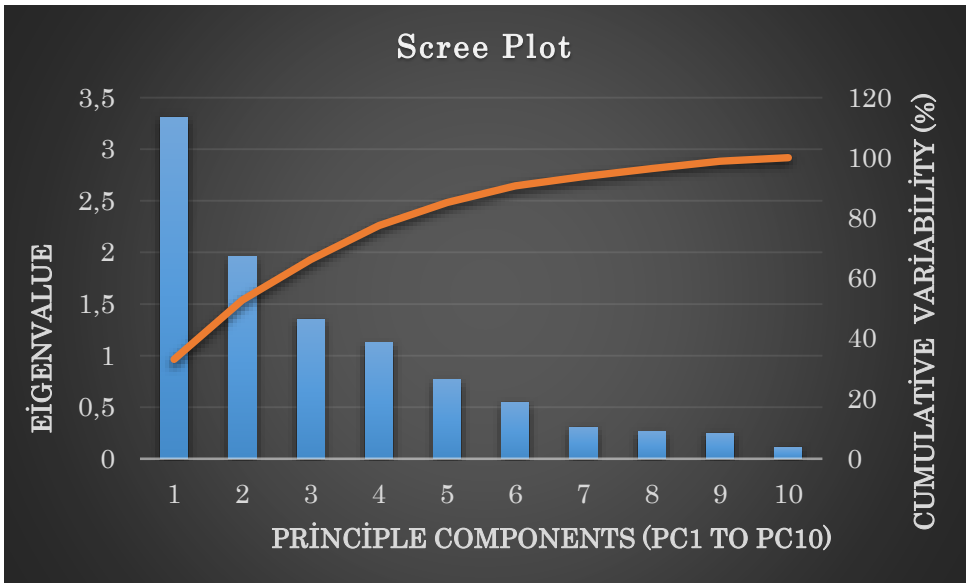

Figure 3. Graphical representation of Eigenvalues
Şekil 3. Ekovalans değerlerinin grafiksel gösterimi

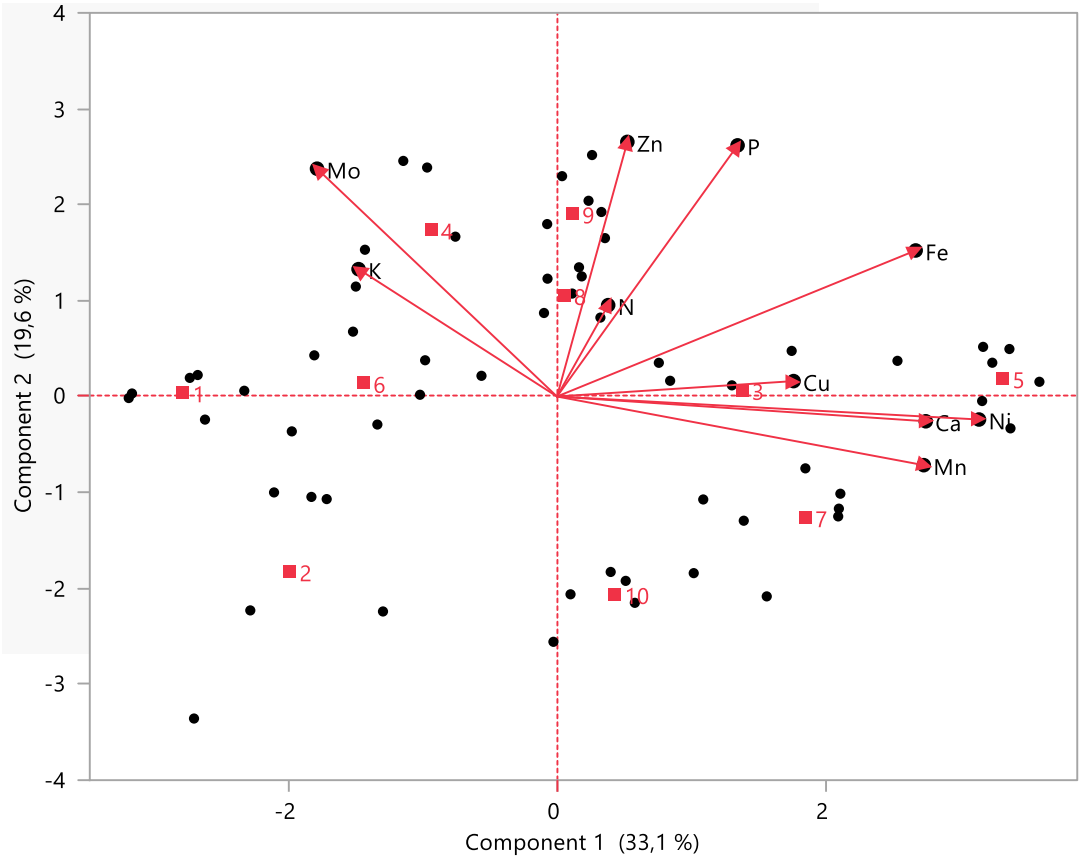

Figure 4. Relationships between genotypes and traits analyzed according to principal component analysis
Şekil 4. Temel bileşenler analizine göre genotipler ve incelenen özellikler arasındaki ilişkiler

CONCLUSION

In this study, macro and micronutrient contents of 10 peanut cultivars were determined for 2 years, and PCA analysis and correlation coefficients were estimated for the traits. It was determined that the varieties were significantly different for each macro- and micronutrient element investigated. Flower-22 cultivar had high values in terms of N, P, Fe, Ni, and Cu contents, and Sultan and Osmaniye-2005 cultivars had high values in terms of Zn contents. When the two-year averages were analyzed in terms of macro and micronutrient contents, it was determined that the Flower-22 variety showed a superior performance compared to other varieties.

Table 6. Relationships and correlation coefficients between the analysed traits of peanut varieties.
Tablo 6. Yerfıstığı çeşitlerinin incelenen özellikleri arasındaki ilişkiler ve korelasyon katsayıları.

	N (%)	K (%)	P (%)	Ca (%)	Fe (mg kg^{-1})	Mn (mg kg^{-1})	Zn (mg kg^{-1})	Ni (mg kg^{-1})	Mo (mg kg^{-1})	Cu (mg kg^{-1})
N	1									
K	0.3156*	1								
P	0.0382	0.0048	1							
Ca	0.0285	-0.0523	0.2001	1						
Fe	0.02	-0.2759*	0.5448**	0.4459**	1					
Mn	0.2111	-0.4388**	0.1556	0.3823**	0.3981**	1				
Zn	0.1734	0.1743	0.3382**	0.0884	0.4287**	-0.0626	1			
Ni	0.1429	-0.3637**	0.2304	0.5996**	0.5942**	0.7539**	0.0373	1		
Mo	0.0367	0.2134	0.3872**	-0.5484**	-0.1062	-0.3771**	0.2504	-0.3511**	1	
Cu	0.0639	-0.0161	0.261*	0.4771**	0.2433	0.1404	0.0343	0.2373	-0.3155*	1

According to biplot analysis, principal component 1 (PC1) was found to be 33.1%, principal component 2 (PC2) 19.6%, and 52.7% in total. As a consequence of the correlation analysis, it was determined that N value had a positive and meaningful relationship with K content, P value had a positive and meaningful relationship with Fe, Zn, Mo and Cu content, Ca value had a positive and meaningful relationship with Fe, Mn, Ni and Cu, and K content had a negative and meaningful relationship with Fe, Mn and Ni.

There are numerous factors known to influence the nutrient composition of plants in foods, including hereditary factors, climate, topography, soil chemistry, agricultural practices such as fertilizer application, the stage of ripeness, and the growing season. A significant variety of peanut cultivars are used in the study, and this diversity provides the opportunity to select genetic types with desirable traits for use in fertilizer programs. Further research should be carried out in more than one location and on these cultivars to show the effects of agricultural practices on the nutrient composition of seeds.

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Contribution Rate Statement Summary of Researchers

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

Conflict of Interest

The authors declare that he/she has no conflict of interest.

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Post-Feeding Behaviors of Newborn and Peak Lactation Dairy Cows

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ABSTRACT
This study compared the behavioral characteristics of newborn and peak lactation dairy cows in the barn after feeding. The study used 10 newborn and 10 peak lactation Holstein cows. Behavior was directly observed for 10 consecutive days between 11:00-13:00 at five-minute intervals using the time sampling method. After the morning feeding, eating, lying down, locomotion, lying, and standing rumination, friendly interaction, and abnormal stereotypic behavior of newborn and peak dairy cattle were significantly different (P≤0.05). Cows in the peak period (33.7%) showed more feeding behavior than cows in the neonatal period (12.4%) (P=0.0001). Lying behavior was 21.9% in the neonatal group and 15.7% in the peak group (P=0.0561). Locomotion behavior was 12.3% in the neonatal group and 17.1% in the peak group (P=0.0028). Lying rumination was 19.8% in the newborn group and 8.7% in the peak group (P=0.0022). Standing rumination was significantly different in the neonate (8.2%) and peak (1.2%) groups (P=0.0008). In conclusion, it was observed that the transition and peak periods in dairy cattle caused differences in the behavioral characteristics of the animals. It can be said that the difference in feeding visits and activities due to the continuity of feeding behavior and the difference in the ratio of roughage in the daily rations is the most important source of behavioral differences between the groups, in which different physiological and metabolic reactions occur.

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Yeni Doğuran ve Pik Dönemindeki Süt Sığırlarının Beslenme Sonrası Davranışları

ÖZET
Bu çalışmada, yeni doğuran ve laktasyonun pik dönemindeki süt sığırlarının yemleme sonrasında barınak içindeki davranış özellikleri karşılaştırmalı olarak irdelenmiştir. Çalışmada, 10 yeni doğuran ve 10 pik dönemindeki Siyah Alaca (Holstein) sığırlarından yararlanılmıştır. Davranışlar ardışık 10 gün boyunca 11:00-13:00 arasında beşer dakika aralıklılarla zaman örneklemesi yöntemiyle doğrudan gözlenmiştir. Sabah yemleme sonrasında yeni doğuran ve pik dönemlerinde bulunan süt sığırlarının beslenme, yatma, lokomosyon, yatarak ve ayakta geviş getirme, arkadaşça etkileşim ve anormal stereotipik davranışları önemli ölçüde farklılaşmıştır (P≤0.05). Pik (%33.7) dönemindeki sığırlar yeni doğuran (%12.4) dönemindeki sığırlardan daha fazla beslenme davranışı göstermiştir (P=0.0001). Yeni doğuran grubunda yatma davranışı ortalama %21.9, pik grubunda ortalama %15.7 olmuştur (P=0.0561). Dikilme davranışı benzer gerçekleşen gruplarda, lokomosyon davranışı yeni doğuran grubunda %12.3, pik grubunda ise %17.1 olarak saptanmıştır (P=0.0028). Yatarak geviş getirme davranışı yeni doğuran grubunda ortalama %19.8, pik grubunda ortalama %8.7 olmuştur (P=0.0022). Ayakta geviş getirme davranışı yeni doğuran (%8.2) ve pik (%1.2) grubuna göre önemli ölçüde farklılık göstermiştir (P=0.0008). Sonuçta, süt sığırları için geçiş dönemi ve pik dönemlerinin hayvanların davranış özelliklerinde farklılığa neden olduğu

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görülmüştür. Farklı fizyolojik ve metabolik reaksiyonların gerçekleştiği iki grup davranış farklılıklarının en önemli kaynağının beslenme davranışının devam etmesine bağlı yemlik ziyareti ve aktiviteleri ile günlük rasyonlarındaki kaba/kesif yem oranı farklılığından olduğu söylenebilir.

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INTRODUCTION

It is possible to state that the 'annual production cycle' for dairy cattle consists of the sum of 'lactation' and 'dry period' that it goes through consecutively following the first calving, at which point it joins the milking herd. In the postpartum period, milk yield increases rapidly in proportion to the growth needs of the newborn, after which it begins to decrease gradually. The period when milk yield peaks in the first 60-100 days after calving is defined as "early lactation" (Hutjens, 2016). The 3-week period before and 3 weeks after birth is considered the "transition period" (Grummer, 1995; Neave et al., 2017). It has been reported that dairy cows during the transition period face very important rapid changes in nutritional requirements, internal physiological conditions, and the social environment to which they are exposed, with the risk of triggering health problems of infectious and metabolic origin (Goff & Horst, 1997; LeBlanc et al., 2006; Cattaneo et al., 2023).

The productivity of the lactation period can be greatly influenced by the success of transition period management. During this process, the constraints of feeding herd management and systematic environmental factors (lactation order, calving season, calving year, service period etc.) can have a significant impact on the level of peak performance of dairy cattle (Tüzemen & Tankal, 2024). The most fundamental change occurring during this period is the sudden increase in nutrients needed to produce milk. Dry matter consumption, and therefore nutrient consumption, has lagged in this increase over the same period. It is stated that the net energy of lactation and metabolic protein consumption lagged the net energy of lactation and metabolic protein consumption by 26% and 25%, respectively (Bell, 1995). Total dry matter intake in dairy cows is reported to increase rapidly from early lactation, +0.8 kg dry matter/cow per week by week 6, and +0.3 kg dry matter/cow per week from week 7 to week 12 (Walsh et al., 2024).

Huzzey et al. (2005) emphasized that the changes in feed intake during the transition period are regulated together with the changes in feeding behavior, but explained that the scientific knowledge on this subject is insufficient. Keyserlingk & Weary (2011), who state that changes in animal behavior during the transition period can also be used to predict the risk of health and foot problems, point to the existence of previous studies (Sowell et al., 1999) describing the relationships between morbidity and feeding behavior in beef cattle. The authors also emphasize the nature of the changes in dietary and related behaviors.

It can be said that the behavior of cattle under grazing conditions is usually coordinated. In addition, feed intake, rumination, and subsequent resting behavior may be associated with a significant proportion of the group (Arave & Albright, 1981; Miller & Wood-Gush, 1991). It has been reported that dairy cattle exhibit diurnal feeding activity, and that feeding activity increases at sunrise and sunset (Albright, 1993). It has also been reported that under indoor conditions, an individual within a group can significantly stimulate the entire group to go to the feeder (DeVries & Keyserlingk, 2008). Primiparous, multiparous, or parity status has been found to influence postpartum behavior in cattle (Neave et al., 2017; Peiter et al., 2021; Cattaneo et al., 2023).

One of the most important aspects that has a determining influence on the general characteristics of daily feeding behavior is the level of yield (Peiter et al., 2021). Therefore, all sorts of directive aspects related to the individual and the animal that have a directive effect on this trait can create identifiable differences in behavioral characteristics as a means of adaptation (Dado & Allen, 1994; Peiter et al., 2021). While studies in dairy cattle have focused on the transition period, including the prenatal and postnatal periods (Neave et al., 2017; Peiter et al., 2021), it has been noted that there is limited research on behavior during the peak period (DeVries et al., 2003). In addition, dairy cattle during the neonatal period and the peak period can suffer from a high incidence of diseases of metabolic origin. A comparative study of the behavior of cattle during this period will help in the early diagnosis of these diseases. The objective of the current study was to compare the post-feeding behavior of newborn and peak lactation dairy cows in shelters.

MATERIAL and METHOD

Animals and study design

This study was carried out in a dairy cattle farm in the Yenişehir district of the province of Bursa with a total of 20 head of dairy cattle. There were no deviations from routine farming practices in the study. Cows that gave birth at the beginning of lactation were grouped as "newborn" and those in the highest milk yield period of lactation were grouped as "peak". The study used 10 newborn and 10 1st and 2nd lactation peak Holstein cows. Cows were numbered and marked with spray paint for each group, selected from a group of approximately 200 cows. The newborn group was selected from cows that gave birth within 1 week, while the peak group was selected from cows in the 85th-90th day of lactation. At the beginning of the study, the average milk yield of the cows in the newborn group was 22.7±2.17 liters, and the average milk yield of the animals in the peak period was 28.5±1.83 liters. Newborn and peak period cows were fed according to the values reported by NRC (1989).

Feeding and Management

On the farm, cows are divided into the dry period, newborn, and peak groups. The barn has no resting stalls and consists of an enclosed feeding system followed by a manure cleaning alley and an open resting area. The resting area consists of a compact area of soil and a certain amount of accumulated manure. It is equipped with fans and scratching brushes. The area immediately behind the locked feeding system is concrete and is cleaned with the help of a tractor in the morning and evening when the cows are milked. The water needs of the cows are met with automatic drinkers.

The shelter is semi-open, and the animals are free to roam around the inside of the shelter. The cows are fed twice in the lock system, once in the morning and once in the evening, and the cows that come out of milking stay in the lock system for 1 hour. Animals can pass from the resting place to the milking unit with special partitions. Milking is carried out in 2 × 24 parallel milking units.

Behavior Observation

The cows were observed by two observers using the direct observation method for a period of 10 consecutive days in July. After milking in the morning, each group was taken to feed. Observations began after the locks were opened. Behaviors were sampled for five minutes between 11:00 and 13:00 by the time sampling method. In the study, feeding (feed consumption, tendency to feed, and attend to with feed), lying down, standing up, locomotion, lying rumination, standing rumination, aggressive interactions (butting, head shaking, mouthing, threatening, etc.), friendly interactions (licking hair, rubbing, etc.), abnormal stereotypies (biting and licking equipment, manipulation to litter, etc.), tendency to water (consumption, tendency, etc.), and other behaviors (urination, defecation, scratching, etc.) were observed.

Statistical Analysis

The behavioral traits of the cows were recorded and analyzed as "1" for present and "0" for absent. A discrete model (GEE) based on the repeated binomial distribution method was used to analyze the behavioral traits. The model included group (newborn, peak), day of observation (1,...,10), interaction, and individual repeated effect. Pairwise contrast based on the Wald Chi-square test was used for significant factors (SAS, 1999).

RESULTS and DISCUSSION

Table 1 presents the mean behavioral traits of newborn and peak cattle, while Table 2 presents the results of the statistical analysis of behavioral traits. The factors observation day and group x observation day did not have a statistically significant effect on any behavior ($P>0.05$; data not shown). It was found that the cows in the peak group (33.7%) showed more feeding behavior than the cows in the newborn group (12.4%) (Table 1). Nielsen (1999) stated that the increase in energy requirements during the lactation process, beginning with calving, may be the main reason for the increase in dry matter intake and that differentiation in the number of meals, meal duration, and intake rate parameters can be used strategically to increase the level of intake. It is known that dairy cattle during the transition period experience very important and rapid changes in terms of nutrient requirements, physiology, and social environment (Goff & Horst, 1997; Neave et al., 2017; Cattaneo et al., 2023). The dry matter intake of cattle is estimated to be 10-12 kg/day, increasing to 21 kg/day during lactation, according to NRC (1989). In the study, it was observed that feeding activity (consumption, tendency, feeder visits) was significantly higher in cows in the peak group than in cows in the newborn group. Huzzey et al. (2005) stated that cattle spend less time consuming at the feeder during the post-calving transition period compared to the prenatal period. The authors state that the reason for this may be an increase in the feed consumption rate. This was the case for animals in the newborn group of the present study, but not for animals in the peak period. It is likely that the

increase in the rate of consumption during the peak period of daily milk production may not have been sufficient for nutrition. A higher level of nutritional activity has been found in cows with a higher milk yield (Johnston & DeVeries, 2018). It has been observed that cows spend a greater proportion of their mealtimes at the feeder during the peak period and reduce the number of meals spent away from the feeder (DeVeries et al., 2003).

The average lying behavior was 21.9% in the newborn group and 15.7% in the peak group (Table 1). This difference between the groups was found to be statistically significant (Table 2). The difference between the groups is further increased by the addition of the behavior of the lying ruminants. This may be since cows in the peak period exhibit more feeding behavior (consumption and tendency to eat). Lying time and feeding behavior have been found to be inversely related to dairy cows, with lying behavior being at its lowest during peak feeding (Fregonesi et al., 2007). On the other hand, it has been observed that there is an increasing relationship between lying down and ruminating behavior in cattle during the newborn period (Schirmann et al., 2012).

It was found that the standing behavior of cows, which showed an average of 16% standing behavior, was close between the groups (Table 1; P=0.1030). Huzzey et al. (2005), who studied behavioral changes in dairy cows during the transition period, found that standing behavior increased after calving and that the increase in standing time was expected. It was found that cows in the peak group (17.1%) exhibited locomotor behavior at a higher rate than cows in the newborn group (12.6%) (Table 1). The fact that the cows in the peak group showed more locomotor behavior than the cows in the newborn group may be due to the mobility of the cows during their visits to the feeder, when they show more feeding behavior. Cows with higher milk yield have been found to have increased feeding behavior, as well as increased feeder head activity and number of meals (Johnston & DeVeries, 2018).

Table 1. Mean observation rates and standard errors (SE) of behaviors by lactation period group, %.
Çizelge 1. Laktasyon dönemi gruplarına göre davranışlara ait ortalama gözleme oranları ve standart hata (SH) değerleri, %

Behavior (*Davranış*)	Newborn (*Yenidoğan*)		Peak (*Pik*)		Overall (Genel)
	Mean (*Ortalama*)	SE (*SH*)	Mean (*Ortalama*)	SE (*SH*)	
Feeding (*Beslenme*)	12.4	0.66	33.7	0.96	23.1
Lying (*Yatma*)	21.9	0.84	15.7	0.74	18.8
Standing (*Dikilme*)	16.8	0.76	14.9	0.72	15.9
Locomotion (*Lokomosyon*)	12.6	0.67	17.1	0.76	14.9
Lying rumination (*Yatarak geviş*)	19.8	0.81	8.7	0.57	14.3
Standing rumination (*Ayakta geviş*)	8.2	0.56	1.2	0.22	4.7
Tendency to water (*Suya yönelim*)	3.7	0.38	4.0	0.40	3.9
Abnormal Stereotype (*Anormal stereotipi*)	0.8	0.18	0.2	0.08	0.5
Friendly interaction (*Arkadaşça etkileşim*)	0.5	0.15	1.6	0.25	1.1
Aggressive interaction (*Agresif etkileşim*)	0.5	0.15	0.3	0.11	0.4
Other (*Diğer*)	2.8	0.33	2.6	0.32	2.7

It is known that cattle ruminate by lying down (Cooper et al., 2007). The standing ruminations were 14.3%, while lying ruminations were 4.7% (Table 1). It was found that the cows in the newborn group had significantly more ruminating behavior in both the lying and standing positions than the cows in the peak group (Table 2; P≤0.05). Cows in the peak period had higher total dry matter intake than cows in the newborn period, while time spent feeding was higher in behavioral observations. Although total dry matter intake determines rumination behavior (Schirmann et al., 2012), it can be said that decreasing the ratio of roughage to concentrate feed during the peak period decreases rumination behavior in cows. Dado & Allen (1994) found that each 1 kg of NDF consumed resulted in approximately 66 minutes of rumination, while Maekawa et al. (2002) reported that changes in the ratio of roughage to compound feed had a linear effect on rumination. In addition, the fact that the cows in the newborn group stood up more than the peak group may have caused higher levels of standing rumination behavior.

In addition to several factors such as dry matter intake and ambient temperature, characteristics related to the physiological phase the animal is in are important factors in determining the water requirements of dairy cattle. In this sense, lactation has been identified as the physiology that most stimulates water demand (NRC, 2001). Huzzey et al. (2005) reported that the time allocated to water consumption increased by 20% during the transition period compared to the pre-calving period, and this was accompanied by an increase in the number of water-

consuming meals. While water tendencies were similar between the groups, slightly higher water tendency behavior was observed in the peak group with higher milk yield (Table 1).

Table 2. Estimates (b), standard errors (SE), odds ratio (Ψ), and p values of behaviors according to lactation period groups*.

Çizelge 2. Laktasyon dönemi gruplarına göre davranışlara ait tahmin (b), standart hata (SH), odds (Ψ) ve P değerleri.*

Behavior (*Davranış*)	Newborn (*Yenidoğan*)			
	b	SE (*SH*)	Ψ	P
Feeding (*Beslenme*)	-1.31	0.07	0.27	<0.0001
Lying (*Yatma*)	0.37	0.17	1.45	0.0281
Standing (*Dikilme*)	0.14	0.07	1.15	0.1030
Locomotion (*Lokomosyon*)	-0.38	0.08	0.68	0.0028
Lying rumination (*Yatarak geviş*)	0.93	0.08	2.53	0.0022
Standing rumination (*Ayakta geviş*)	1.93	0.19	6.89	0.0008
Tendency to water (*Suya yönelim*)	0.17	0.15	0.84	0.4731
Abnormal Stereotype (*Anormal stereotipi*)	1.56	0.55	4.76	0.0050
Friendly interaction (*Arkadaşça etkileşim*)	-1.16	0.31	0.31	0.0103
Aggressive interaction (*Agresif etkileşim*)	0.69	0.58	1.99	0.2568
Other (*Diğer*)	0.01	0.08	1.01	0.4330

*:b value for peak group is 0.00 Ψ value and 1.00. *: Pik grubuna ait b değeri 0.00 Ψ değeri ve 1.00'dır.

Although at a lower level, cows in the newborn group exhibited more abnormal stereotypic behavior than did cows in the peak group (Table 1, 2; P = 0.0050). The transition period between very different physiological processes, such as pregnancy and lactation, witnesses very important changes in terms of metabolic processes in different tissues of the organism. In this regard, it would not be wrong to say that the nutritional conditions within this process are very critical for dairy cattle (NRC, 2001; Overton & Waldron, 2004). From this point of view, it can be said that the cows in the newborn group are under pressure during the "transition period". However, in the present study, although no clear conclusion can be drawn due to the time sampling of behaviors and the low rate of abnormal stereotypic, the fact that the newborn group is clearly separated from the peak group suggests the need for careful herd management of dairy cows in this group. Redbo et al. (1992) found an increase in oral stereotypic behavior as milk yield increased in dairy cows.

The cows in the peak group (1.6 %) showed more friendly interaction behavior than the cows in the newborn group (0.5 %) (Table 1). The fact that the cows in the peak group were in estrus may have increased the level of friendly interaction. Although cows in the peak group showed significantly more feeding and locomotor behavior than cows in the newborn group, they showed lower levels of aggressive interaction (Table 2; P=0.2568). Although the level of aggressive interactions between animals varies according to the presence of competitive resources, food is one of the most important of these resources (Tölü & Savaş, 2007).

CONCLUSIONS

The feeding, lying down, locomotion, lying and standing, rumination, friendly interactions, and abnormal stereotypic behaviors of dairy cows whose post-feeding behaviors were compared between early lactation (newborn) and peak period were significantly different. It can be said that the physiological activities of newborn dairy cows in the transition period, the nutrient requirements of dairy cows in the peak period, and changes in the ratio of roughage/concentrate in the daily ration cause differences. It appears that the higher feeding behavior of the cows during the peak period and the low roughage content in their rations resulted in lower levels of lying and ruminating behavior and higher levels of locomotor behavior. It is also likely that the level of friendly interaction increased during the peak period because the cows were in estrus. The fact that cows in the newborn group exhibit significantly more abnormal stereotypic behavior than the peak group, even at low levels, may put practices that can reduce abnormal stereotypic behavior during this period on the herd management agenda.

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Contribution Rate Statement Summary of Researchers

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

Conflict of Interest

The authors declare no conflicts of interest.

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