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Research Article / Araştırma Makalesi

An Overview of the Flora of Siirt Province

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Abstract: In this study, the plant taxa that spread within the provincial borders of Siirt were determined by scanning the existing literature and as a results of the 2-year (2018-2019) field study. In the literature review, the existence of 786 taxa within the borders of Siirt province was determined. As a result of the field studies, 447 of these taxa were collected and recorded. In addition, with this study, it was determined that 89 new taxa are distributed in Siirt province. According to the results, it was determined that a total of 875 taxa are distributed within the provincial borders of Siirt and 71 of these 875 taxa are endemic (endemism rate 8.11%). Out of 95 taxa that are not endemic or endemic and are considered endangered are evaluated in categories as follows: 6 taxa "CR" Critical, 10 taxa are "EN" Endangered, 33 taxa are "VU" Vulnerable, 18 taxa are in "NT" Near Threatened, 23 taxa are in "LC" Least Concern, and 5 taxa are in "DD" Data Deficient. Of the detected taxa, 329 are Iran-Turan elements, 90 are Mediterranean elements, 35 are European-Siberian elements, and 4 are Euxine elements while 417 belong to a phytogeographical region or multi-regions. As a result of the study, the first five families with the most taxa are, respectively, Poaceae (115 taxa), Fabaceae (109 taxa), Asteraceae (95 taxa), Apiaceae (56 taxa), and Lamiaceae (53 taxa).

Keywords: Biodiversity, endemic, plant, taxon.

Siirt İli Florasına Genel Bir Bakış

Öz: Bu çalışmada mevcut literatürlerin taranması ve 2 yıllık (2018-2019) arazi çalışmaları sonucunda Siirt il sınırları içerisinde yayılış gösteren bitki taksonları araştırılmıştır. Literatür araştırmalarında Siirt ili sınırları içerisinde 786 taksonun varlığı belirlenmiştir. Arazi çalışmaları sonucunda bu taksonlardan 447 tanesi toplanmış ve kayıt altına alınmıştır. Ayrıca bu çalışma ile Siirt ilinde 89 taksonun yeni yayılışı tespit edilmiştir. Elde edilen sonuçlara göre Siirt il sınırları içerisinde toplam 875 taksonun yayılışı belirlenmiştir ve bu 875 taksondan 71 tanesi (%8.11) endemiktir. Endemik veya endemik olmayıp tehlike altında olduğu değerlendirilen toplam 95 taksonun 6'sı "CR" Kritik; 10'u "EN" Tehlikede; 33'ü "VU" Hassas; 18'si "NT" Tehdide açık, 23'si "LC" Düşük riskli ve 5'i "DD" yetersiz veri kategorilerinde değerlendirilmektedir. Tespit edilen taksonlardan 329'u İran-Turan elementi, 90'ı Akdeniz elementi, 35'i Avrupa-Sibirya elementi, 4'ü Karadeniz elementi iken 417'si ise fitocoğrafik bölgesi bilinmeyen veya çok bölgeli türler kategorisinde yer almaktadır. Çalışma sonucunda en fazla takson içeren ilk beş familya sırasıyla Poaceae (115 takson), Fabaceae (109 takson), Asteraceae (95 takson), Apiaceae (56 takson) ve Lamiaceae (53 takson)'dir.

Anahtar kelimeler: Biyoçeşitlilik, endemik, bitki, takson.

1. Giriş

Dünyada tanımlamış ve kabul edilen bitki türlerinin sayısı yaklaşık 308.312'si vasküler bitki olmak üzere yaklaşık 374.000 olarak bilinmektedir. 295.383 çiçekli bitki taksonundan kapalı tohumlu 284.281 takson ve bu taksonlardan 74.273'ü monokotiledon 210.008'i ise dikotiledondur. Algler yaklaşık 44.000, ciğerotları yaklaşık 9.000, boynuz otları yaklaşık 225, yosunlar 12.700, likopodlar 1.290, eğrelti otları 10.560 ve açık tohumlular 1.079 takson olarak tespit edilmiştir (Christenhusz & Byng, 2016). Bitkilerin isimlendirilmesi ve takson sayılarının belirlenmesi sürekli devam eden çalışmalardır. Her yıl yaklaşık 2.000 yeni bitki türü tespit edilip literatüre kazandırılmaktadır (Christenhusz & Byng, 2016).

Türkiye'nin, Asya ile Avrupa kara parçalarının birleşim noktasında olması ve Akdeniz, Avrupa-Sibirya ve İran-Turan fitocoğrafik bölgelerinin kesiştiği bölgede yer alması farklı vejetasyon tiplerine ve zengin bir floraya sahip olmasına vesile olmuştur. Sistematik botanik alanında Türkiye'de ilk olarak Tournefort tarafından yapılan çalışma 1702 yılında yayımlanmıştır. Türkiye'de yapılmış en kapsamlı flora çalışması Türkiye ve Doğu Ege Adaları Florası isimli eserdir. Bu eser 9 cilt ve 2 ek ciltten oluşmaktadır (Davis, 1965-1985; Davis et al., 1988; Güner et al., 2000). Güner ve arkadaşlarının 2012 yılında yaptıkları "Türkiye Bitkileri Listesi, Damarlı Bitkiler" isimli çalışmada 2012 yılına kadar yapılan tüm floristik çalışmaların derlenmesi sonucunda Türkiye'de toplam 11.707 takson olduğunu bildirmiştir. Bu taksonların endemizm oranı ise %31.82 olarak belirtilmiştir (Güner et al., 2012).

Siirt ili kuzeyden Batman ve Bitlis, batıdan Batman, güneyden Mardin ve Şırnak, doğudan Şırnak ve Van illeri ile çevrili bir konumdadır. Siirt ili 41° 57' doğu boylamı ve 37° 55' kuzey enlemi üzerinde yer almaktadır (Özyazıcı et al., 2014). Doğuya doğru ilerledikçe yükselen dağlardan Herekol (Yazlıca) Dağı 2943 m, Körkandil Dağı 2821 m,

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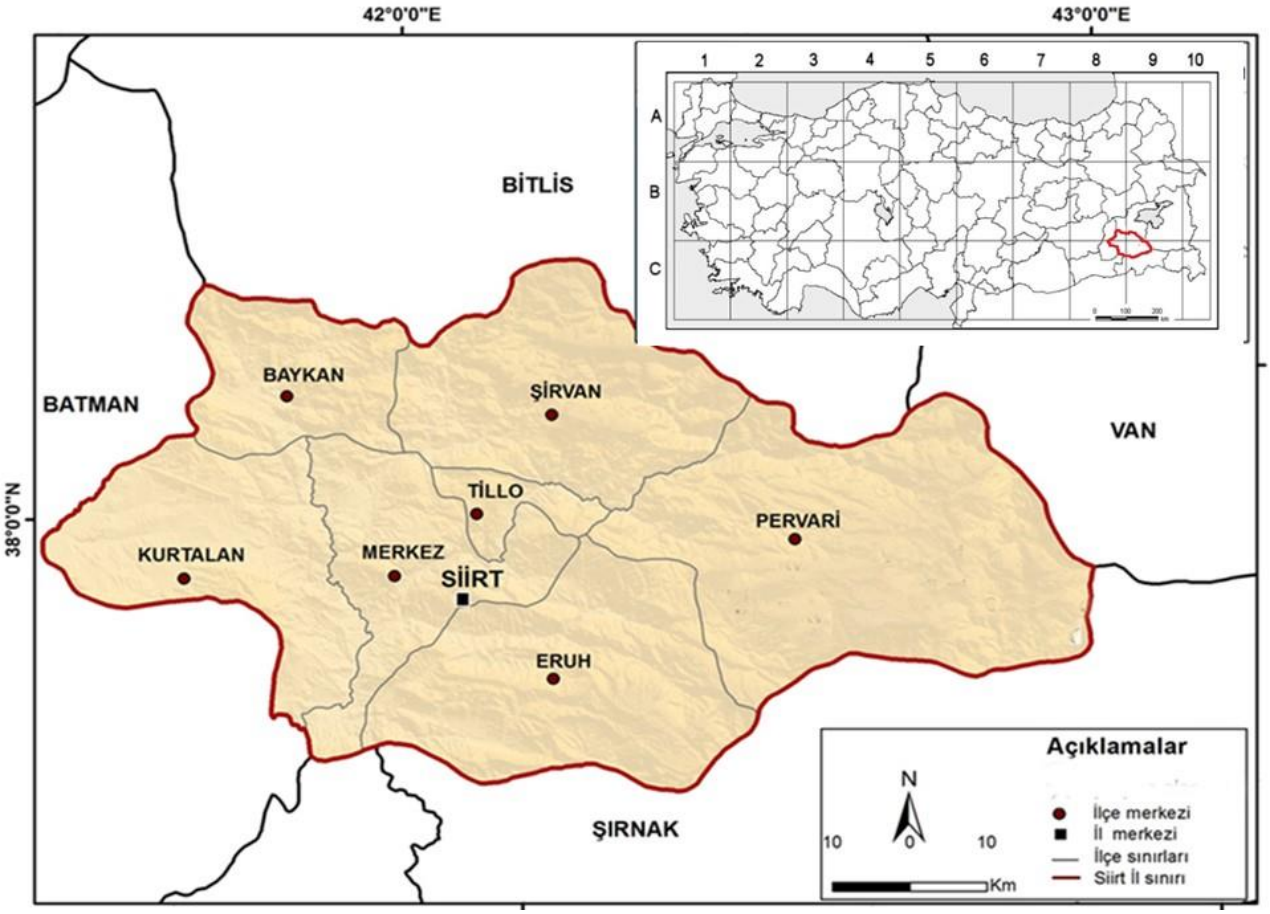
Şirvan Bekravi Dağı 2650 m, Hastelli Dağı 2700 m ve Çıraf Dağı 2268 m yüksekliklere sahip önemli yükseltilerdir. (Anonim, 1997). Farklı topoğrafik yapısı ile birçok farklı bitki türünün doğal yaşam alanı olan Siirt ilinde, yapılan az sayıdaki floristik çalışma (Yangın, 2001; Tugay & Öztürk, 2003; Yapıcı, 2006; Dönmez, 2006; Özgökçe & Ünal, 2007; Doğan & Akaydın, 2007; Yıldırım & Ertekin, 2008; Kaya & Ertekin, 2009; Kahraman et al., 2009; Dönmez, 2009; Yapıcı et al., 2009; Aslan et al., 2010; Kahraman et al., 2011; Özhatay et al., 2011; Özçelik & Korkmaztürk, 2012; Behçet et al., 2012; Karabacak et al., 2012; Karabacak et al., 2014; Fırat, 2015; Karabacak et al., 2015; Celep et al.,2016; Şahin et al., 2016; Ünalan, 2017; Fırat et al., 2018; Fırat, 2019; Pınar et al., 2019; Fidan et al., 2019; Sırrı et al., 2020) nedeniyle olması gerekenden çok daha az sayıda bitki taksonu kayıt altına alınmıştır. Bunlarda en önemlisi olan Türk sistematikçileri tarafından tasarımlanan Türkiye Bitkileri Veri Servisi (TUBIVES) isimli veri tabanında Türkiye Florasında Siirt iline kayıtlı 43 tanesi endemik 320 taksonun ismi listelenmiştir. Her bir taksonun ayrı ayrı taksonomik hiyerarşisi, genel taksonomik özellikleri ve ülkemizdeki dağılış haritası sunulmuştur (Bakış et al., 2011).

Bu çalışma sonucunda Grid kareleme sistemine göre B8, B9, C8, C9 karelerine giren (Şekil 1) Siirt ili sınırları içerisinde yayılış gösteren bitkilerin listesi, endemizim durumları, fitocoğrafik bölgeleri ve tehlike kategorileri verilmiştir.

2. Materyal ve Metot

Çalışmanın materyalini Siirt ili sınırları içerisinde (Şekil 1) yapılmış floristik çalışmalara ait literatürler araştırmaları ile yazarlar tarafından 2018-2019 yıları arasında yapılmış arazi çalışmaları sonucu elde edilen bitki listeleri ve bitki örnekleri oluşturmaktadır. Arazi çalışmaları sonucunda toplanan bitki örnekleri, herbaryum tekniklerine uygun olarak preslenip kurutulduktan sonra Siirt Üniversitesi Flora ve Fauna merkezi herbaryumunda (SUFAF) koruma altına alınmışlardır.

Bitkilerin Türkçe bilimsel isimlendirmelerinde Türkiye Bitkileri Listesi (Damarlı Bitkiler) temel kaynak alınmış (Güner et al., 2012), bu kaynaklardan sonra Siirt ili sınırları içerisinden bilim dünyasına tanıtılan yeni türlerin Türkçe isimleri ise ilgili yayınlardan alınmıştır (Fırat, 2015; Şahin et al., 2016; Fırat et al., 2018; Fırat, 2019). Literatürde Türkçe bilimsel ismi kayıt altında olmayan türlerin halk tarafından kullanılan yerel isimleri veya bu çalışmada verilen yeni Türkçe isimleri kullanılmış ve Tablo 1'de Türkçe adı sütununda ismin yanına (*) işareti konularak belirtilmiştir.



Şekil 1. Siirt il haritası

Figure 1. Map of Siirt province

3. Bulgular ve Tartışma

Literatür araştırmalarında Siirt ili sınırları içerisinde 786 taksonun varlığı tespit edilmiştir. Arazi çalışmaları sonucunda bu taksonlardan 447 tanesi toplanmış ve kayıt altına alınmıştır. Ayrıca bu çalışma ile Siirt ilinde 89 taksonun yeni yayılışı keşfedilmiştir. Yapılan arazi çalışmaları ve literatürlerin değerlendirmeleri sonucunda Siirt ili için 88 familya 397 cinse ait toplam 875 taksonun yayılışının olduğu sonucuna varılmıştır (Tablo 1).

Tablo 1. Siirt ilinde yayılış gösteren bitki taksonları

Table 1. Plant taxa distributed in Siirt province

S. No	Familya ve Takson Adı	Türkçe Adı	Tespit Şekli*	Fitocoğrafik Bölge**	IUCN ***	Endemizm ****
	Equisetaceae					
1	*Equisetum giganteum* L.	Kırkkilitotu	A+L	-	NE	-
	Cystopteridaceae					
2	*Cystopteris fragilis* (L.) Bernh.	Gevrekeğrelti	A+L	-	NE	-
	Pteridaceae					
3	*Adiantum capillus-veneris* L.	Baldırıkara	A+L	-	NE	-
	Cupressaceae					
4	*Juniperus oxycedrus* L. subsp. *oxycedrus*	Katranardıcı	A+L	-	NE	-
	Pinaceae					
5	*Pinus sylvestris* L. var. *hamata* Steven	Sarıçam	A	-	NE	-
	Acanthaceae					
6	*Acanthus dioscoridis* L. var. *dioscoridis*	Lokmanayıpençesi	A+L	-	NE	-
	Alismataceae					
7	*Damasonium alisma* Mill.	Sukaranfili	A+L	-	NE	-
	Amaranthaceae					
8	*Amaranthus albus L.*	Kömüşmancarı	A+L	-	NE	-
9	*Amaranthus graecizans* L. subsp. *silvestris* (Vill.) Brenan	Ormanohraşanı	A+L	-	NE	-
10	*Chenopodium album* L. subsp. *album* var. *album*	Aksirken	A+L	-	NE	-
	Amaryllidaceae					
11	*Allium akaka* S.G.Gmel. ex Schult. & Schult.f.	Yersoğanı	A+L	İr.-Tur.	NE	-
12	*Allium ampeloprasum* L.	Pırasa	A+L	Akd.	NE	-
13	*Allium armerioides* Boiss.	Mardinsoğanı	A	İr.-Tur.	DD	+
14	*Allium chrysantherum* Boiss. & Reut.	Sarıkafa	A+L	İr.-Tur.	NE	-
15	*Allium dictyoprasum* C.A.Mey. ex Kunth	Topsoğan	A+L	İr.-Tur.	NE	-
16	*Allium flavum* L. subsp. *tauricum* (Besser ex Rchb.) Stearn var. t*auricum*	Torossarısı	A+L	Akd.	NE	-
17	*Allium guttatum* Stev. subsp. *sardoum* (Moris) Stearn	Solgunsoğan	A+L	Akd.	NE	-
18	*Allium paniculatum* L. subsp. *paniculatum*	Sürüsalkım	A+L	Akd.	NE	-
19	*Allium pervariensis* Fırat & Koyuncu	Sirik*	A+L	İr.-Tur.	EN	++
20	*Allium scorodoprasum* L. subsp. *rotundum* (L.) Stearn	Delipırasa	A+L	-	NE	-
21	*Allium stamineum* Boiss.	Yabansarmısağı	A+L	D.Akd	NE	-
22	*Narcissus tazetta* L. subsp. *tazetta*	Nergis	A+L	-	NE	-
23	*Sternbergia clusiana* (Ker Gawl.) Ker Gawl. ex Spreng.	Vargetgülü	A+L	İr.-Tur.	NE	-
24	*Sternbergia colchiciflora* Waldst. & Kit.	Tavukçiçeği	A	-	NE	-
25	*Sternbergia vernalis* (Mill.) Gorer & J.H.Harvey	Kışnergisi	A	-	NE	-
	Anacardiaceae					
26	*Pistacia khinjuk* Stocks	Bıttım	A+L	İr.-Tur.	NE	-
27	*Pistacia vera* L.	Antepfıstığı	A+L	İr.-Tur.	NE	-
28	*Rhus coriaria* L.	Sumak	A+L	-	NE	-
	Apiaceae					
29	*Actinolema eryngioides* Fenzl	Aklema	A+L	İr.-Tur.	NE	-
30	*Ammi visnaga* (L.) Lam.	Hıltan	A+L	Akd.	NE	-
31	*Anthriscus caucalis* M.Bieb.	Deligımı	A+L	-	NE	-
32	*Apium nodiflorum* (L.) Lag.	Bendik	A+L	-	NE	-
33	*Artedia squamata* L.	Karabenek	A+L	-	NE	-
34	*Bunium elegans* (Fenzl) Freyn var. *brevipes* Freyn & Sint.	Hoşaksar	L	-	LC	+
35	*Bunium elegans* (Fenzl) Freyn var. *elegans*	Hoşaksar	L	-	NE	-

S. No	Familya ve Takson Adı	Türkçe Adı	Tespit Şekli*	Fitocoğrafik Bölge**	IUCN ***	Endemizm ****
36	*Bunium elegans* (Fenzl) Freyn var. *involucratum* Ö.Saya	Hoşaksar	L	-	VU	+
37	*Bunium paucifolium* DC.	Koçkuzu	A+L	İr.-Tur.	NE	-
38	*Bupleurum cappadocicum* Boiss.	Perişeytanı	A+L	İr.-Tur.	NE	-
39	*Bupleurum croceum* Fenzl	Çiğdemşeytanı	A+L	İr.-Tur.	NE	-
40	*Bupleurum kurdicum* Boiss.	Tavşandili	A+L	İr.-Tur.	NE	-
41	*Caucalis platycarpos* L.	Kavkal	A+L	-	NE	-
42	*Chaerophyllum crinitum* Boiss.	Saçılakotu	A+L	İr.-Tur.	NE	-
43	*Conium maculatum* L.	Baldıran	A+L	-	NE	-
44	*Coriandrum sativum* L.	Kişniş	A+L	-	NE	-
45	*Daucus broteri* Ten.	Çocukboğanotu	A+L	Akd.	NE	-
46	*Daucus carota* L.	Yabanihavuç	A+L	-	NE	-
47	*Daucus guttatus* Sibth. & Sm.	Beneklihavuç	A+L	-	NE	-
48	*Eryngium campestre* L. var. *virens* Link	Kırsenet	A+L	-	NE	-
49	*Eryngium creticum* Lam.	Gözdikeni	A+L	-	NE	-
50	*Eryngium pyramidale* Boiss. & Hausskn.	Sivriboğadikeni	A+L	İr.-Tur.	NE	-
51	*Ferulago angulata* (Schlecht.) Boiss. subsp. *angulata*	Olukluçakışır	A+L	İr.-Tur.	NE	-
52	*Ferulago angulata* (Schlecht.) Boiss. subsp. *carduchorum* (Boiss. & Hausskn.) D.F.Chamb.	Olukluçakışır	A+L	İr.-Tur.	VU	-
53	*Grammosciadium macrodon* Boiss.	Kocakami	A+L	İr.-Tur.	NE	-
54	*Heptaptera anisoptera* (DC.) Tutin	Kanatlıçakşır	A+L	-	NE	-
55	*Lagoecia cuminoides* L.	Pülüskün	A+L	Akd.	NE	-
56	*Lisaea heterocarpa* (DC.) Boiss.	Akgelinpıtrağı	A	İr.-Tur.	NE	-
57	*Lisaea strigosa* (Banks & Sol.) Eig	Dikgelinpıtrağı	A+L	İr.-Tur.	NE	-
58	*Malabaila lasiocarpa* Boiss.	Şabulgan	A+L	İr.-Tur.	LC	+
59	*Malabaila secacul* (Mill.) Boiss. subsp. *secacul*	Davarotu	A+L	-	NE	-
60	*Oenanthe silaifolia* M.Bieb.	Attohumu	A+L	-	NE	-
61	*Ormosciadium aucheri* Boiss.	Ayıeli	A+L	-	NE	-
62	*Pastinaca armena* Fisch. & C.A.Mey.	Kelemenkeşir	L	-	VU	+
63	*Pimpinella corymbosa* Boiss.	Salkımanason	A+L	İr.-Tur.	NE	-
64	*Pimpinella eriocarpos* Banks & Sol.	Meyane	A+L	İr.-Tur.	NE	-
65	*Pimpinella flabellifolia* (Boiss.)Benth. & Hook. ex Drude	Yelanasonu	L	İr.-Tur.	VU	+
66	*Pimpinella kotschyana* Boiss.	Kıranasonu	A+L	İr.-Tur.	NE	-
67	*Pimpinella peregrina* L.	Elanasonu	A+L	-	NE	-
68	*Pimpinella puberula* (DC.) Boiss.	Tüylüanason	A+L	İr.-Tur.	NE	-
69	*Pimpinella nudicaulis Trautv.*	Köseanason	A+L	İr.-Tur.	NE	-
70	*Pimpinella sintenisii* H.Wolff	Kayaanasonu	A+L	-	VU	-
71	*Prangos ferulacea* (L.) Lindl.	Eşek Çakşırı	A+L	-	NE	-
72	*Prangos peucedanifolia* Fenzl	Kayaçakşırı	A+L	İr.-Tur.	NE	-
73	*Scaligeria meifolia* (Fenzl) Boiss.	Uzunanason	L	-	NE	-
74	*Scandix iberica* M.Bieb.	Atkişnekotu	A+L	-	NE	-
75	*Scandix pecten-veneris* L.	Zühretarağı	A+L	-	NE	-
76	*Scandix stellata* Banks & Sol.	Dağ Kişkişi	A+L	-	NE	-
77	*Smyrnium cordifolium* Boiss.	Kokarbaldıran	A+L	İr.-Tur.	NE	-
78	*Tordylium hasselquistiae* DC.	Ekindavulotu	A+L	İr.-Tur.	NE	-
79	*Tordylium trachycarpum* (Boiss.) Al-Eisawi	Bozkafkalida	A+L	D.Akd	NE	-
80	*Torilis arvensis* (Huds.) Link subsp. *neglecta* (Spreng.) Thell.	Şeytanhavucu	A+L	-	NE	-
81	*Torilis leptocarpa* (Hochst.) C.C.Towns.	Narindercikotu	L	İr.-Tur.	NE	-
82	*Torilis leptophylla* (L.) Rchb.f.	İncedercikotu	L	-	NE	-
83	*Torilis tenella* (Delile) Rchb.f.	Zarifdercikotu	A+L	-	NE	-
84	*Turgenia latifolia* (L.) Hoffm.	Karaheci	A+L	-	NE	-

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	Apocynaceae					
85	*Apocynum venetum*L. subsp. *armenum* (Pobed.) ined.	Pembekız	A+L	-	NE	-
86	*Cionura erecta* (L.) Griseb.	Babrik	L	D.Akd	NE	-
87	*Cynanchum acutum* subsp. acutum L.	Bacırgan	L	-	NE	-
88	*Nerium oleander* L.	Zakkum	A	-	NE	-
89	*Periploca graeca* L. var. *graeca* L.	Gariplerurganı	L	D.Akd	NE	-
90	*Vincetoxicum tmoleum* Boiss.	Hıyaluk	A	İr.-Tur.	NE	-
	Aracaceae					
91	*Arum rupicola* Boiss. var. *virescens (Stapf)* P.C.Boyce	Dağsorsalı	L	İr.-Tur.	NE	-
92	*Biarum carduchorum* (Schott) Engl.	Kardi	A+L	-	NE	-
	Aristolochiaceae					
93	*Aristolochia bottae* Jaub. & Spach	Köpektaşağı	L	İr.-Tur.	NE	-
	Asparagaceae					
94	*Asparagus verticillatus* L.	Gılemşe	L	-	NE	-
95	*Bellevalia fominii* Woronow	Benlisümbül	A+L	İr.-Tur.	VU	-
96	*Bellevalia koyuncui* Karabacak & Yıldırım	Şirvansümbülü*	A+L	İr.-Tur.	VU	++
97	*Bellevalia longistyla* (Miscz.) Grossh.	Kellelisümbül	A+L	İr.-Tur.	VU	-
98	*Bellevalia pseudolongipes* Karabacak & Yıldırım	Yalancısaçaksümbül*	A+L	İr.-Tur.	LC	+
99	*Bellevalia paradoxa* (Fisch. & C.A.Mey.) Boiss.	Aşpenceri	A+L	İr.-Tur.	NE	-
100	*Bellevalia sasonii* Fidan	Sasonkırsümbülü	A	İr.-Tur.	VU	+
101	*Bellevalia siirtensis* Fırat	Siirtsümbülü*	A+L	İr.-Tur.	VU	+
102	*Bellevalia vuralii* B.Şahin & Aslan	Diclekırsümbülü	A+L	İr.-Tur.	CR	++
103	*Hyacinthella siirtensis* B.Mathew	Siirtkopçası	A+L	İr.-Tur.	NT	+
104	*Muscari armeniacum* Leichtlin ex Baker	Gavurbaşı	A	-	NE	-
105	*Muscari comosum* (L.) Mill.	Morbaş	A+L	Akd.	NE	-
106	*Muscari neglectum* Guss. ex Ten.	Arapüzümü	A+L	-	NE	-
107	*Ornithogalum narbonense* L.	Akbaldır	A+L	Akd.	NE	-
108	*Ornithogalum oligophyllum* E.D.Clarke	Kurtsoğanı	A+L	-	NE	-
109	*Puschkinia scilloides* Adams	Serhişing	A+L	İr.-Tur.	NE	-
110	*Scilla hyacinthoides* L.	Dağsoğanı	L	Akd.	NE	-
111	*Scilla leepii* Speta	İncesümbül	A+L	İr.-Tur.	NT	+
112	*Scilla persica* Hausskn.	Çatalsümbül	A+L	İr.-Tur.	NE	-
113	*Scilla siberica* Haw. subsp. *armena* (Grossh.) Mordak	Camışkıran	A+L	İr.-Tur.	NE	-
114	*Asplenium ceterach* L.	Dalakotu	L	-	NE	-
115	*Asplenium haussknechtii* Godet & Reut.	Karabacak	L	-	NE	-
116	*Asplenium onopteris* L.	Kalkaneğreltisi	L	-	NE	-
117	*Asplenium ruta-muraria* L.	Duvarsaçağı	L	-	NE	-
	Asteraceae					
118	*Achillea millefolium* L.subsp. *millefolium* var. *millefolium*	Civanperçemi	L	Avr.-Sib.	NE	-
119	*Achillea vermicularis* Trin.	Püşan	A+L	İr.-Tur.	NE	-
120	*Arctium minus* (Hill) Bernh.	Löşlek	A	Avr.-Sib.	NE	-
121	*Bellis perennis* L.	Koyungözü	A+L	Avr.-Sib.	NE	-
122	*Calendula arvensis* (Vaill.) L.	Portakalnergisi	L	-	NE	-
123	*Carlina lanata* L.	Keygana	A	Akd.	NE	-
124	*Carlina oligocephala* Boiss. & Kotschy subsp. *oligocephala*	Domuzdikeni	A+L	-	NE	-
125	*Carthamus glaucus* M.Bieb. subsp. *glaucus*	Karakızdikeni	A	-	NE	-
126	*Carthamus lanatus* L.	Sarıdiken	A	-	NE	-
127	*Carthamus tinctorius* L.	Aspir	A+L	-	NE	-
128	*Centaurea aggregata* Fisch. & C.A.Mey. ex DC subsp. *aggregata*	Kümedüğme	L	-	NE	-

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129	*Centaurea balsamita* Lam.	Süslüsarıbaş	A+L	İr.-Tur.	NE	-
130	*Centaurea behen* L.	Zerdalidikeni	A+L	İr.-Tur.	NE	-
131	*Centaurea bruguierana* (DC.) Hand.-Mazz. subsp. *bruguierana*	Akçakavgalaz	L	İr.-Tur.	NE	-
132	*Centaurea fenzlii* Reichardt	Battalbaş	A	İr.-Tur.	LC	+
133	*Centaurea iberica* Trev. ex Spreng.	Deligözdikeni	A+L	-	NE	-
134	*Centaurea kurdica* Reichardt	Pamukdikeni	A	İr.-Tur.	NT	+
135	*Centaurea persica* Boiss.	Acemkavgalazı	A	İr.-Tur.	NE	-
136	*Centaurea polypodiifolia* Boiss. var. *polypodiifolia*	Akbehmen	A	-	NE	-
137	*Centaurea regia* Boiss. subsp. *cynarocephala* (Wagenitz) Wagenitz	Topaçkavgalazı	A	İr.-Tur.	NE	-
138	*Centaurea regia* Boiss. subsp. *regia*	Şahkavgalaz	A	İr.-Tur.	NE	-
139	*Centaurea rigida* Banks & Sol.	Gürbüzdiken	A	İr.-Tur.	NE	-
140	*Centaurea solstitialis* L. subsp. *solstitialis*	Çakırdikeni	A+L	-	NE	-
141	*Centaurea spectabilis* (DC.) Sch.Bip. var. *araneosa* (Boiss.) Wagenitz	Turanbaşı	A	-	NE	-
142	*Centaurea urvillei* DC. subsp. *urvillei*	Alakötürüm	A+L	D.Akd	NE	-
143	*Centaurea virgata* Lam.	Acısüpürge	A+L	İr.-Tur.	NE	-
144	*Chardinia orientalis* (L.) Kuntze	Çağlaotu	L	İr.-Tur.	NE	-
145	*Chondrilla juncea* L.	Karakavuk	A	-	NE	-
146	*Chrysophthalmum montanum* (DC.) Boiss.	Tutça	A+L	İr.-Tur.	NE	-
147	*Cichorium pumilum* Jacq.	Dünek	A+L	D.Akd	NE	-
148	*Cnicus benedictus* L.	Topdiken	A+L	-	NE	-
149	*Conyza canadensis* (L.) Cronquist	Selviotu	L	-	NE	-
150	*Cota tinctoria* var. *tinctoria* (L.) J.Gay	Boyacıpapatyası	A+L	-	NE	-
151	*Cousinia eriocephala* Boiss. & Hausskn. ex Boiss.	Yünlükızan	A	İr.-Tur.	LC	+
152	*Crepis alpina* L.	Yürekotu	A+L	-	NE	-
153	*Crepis commutata* (Spreng.) Greuter	Delikıskıs	L	-	NE	-
154	*Crepis pulchra* L. subsp. *pulchra*	Zarifkıskıs	L	-	NE	-
155	*Crepis sahendi* Boiss. & Buhse	Azerikıskısı	L	İr.-Tur.	NE	-
156	*Crepis sancta* (L.) Bornm. subsp. *obovata* (Boiss. & Noë) Babc.	Yumurtakıskısı	A+L	-	NE	-
157	*Crepis willdenowii* Czerep.	Bozkıskıs	L	İr.-Tur.	NE	-
158	*Crupina crupinastrum* (Moris) Vis.	Gelindöndüren	L	-	NE	-
159	*Cyanus triumfettii* (All.) Dostál ex Á.Löve & D.Löve subsp. *triumfettii*	Delikapele	A+L	-	NE	-
160	*Echinops orientalis* Trautv.	Dağşekeri	A+L	İr.-Tur.	NE	-
161	*Echinops phaeocephalus* Hand.-Mazz.	Botantopuzu	A+L	İr.-Tur.	DD	-
162	*Eupatorium cannabinum* L.	Koyunpıtrağı	A	Avr.-Sib.	NE	-
163	*Filago pyramidata* L.	Ateşpamuğu	A+L	#YOK	NE	-
164	*Geropogon hybridus* (L.) Sch.Bip.	Melezyemlik	L	Akd.	NE	-
165	*Gundelia siirtica* Fırat	Kereng*	A+L	İr.-Tur.	CR	+
166	*Gundelia tournefortii* L. var. *armata* Freyn & Sint.	Haskenger	A+L	İr.-Tur.	NE	-
167	*Gundelia tournefortii* L. var. *tournefortii* L.	Kenger	A+L	İr.-Tur.	NE	-
168	*Hedypnois rhagadioloides* (L.) F.W.Schmidt subsp. *cretica* (L.) Hayek	Sünnetlice	L	Akd.	NE	-
169	*Helichrysum plicatum* DC. subsp. *polyphyllum* (Ledeb.) P.H.Davis & Kupicha	Kalisarçiçeği	A+L	-	NE	-
170	*Inula acaulis* Schott & Kotschy ex Boiss var. *acaulis*.	Bodurandızotu	L	-	NE	-
171	*Inula helenium* L.subsp. *vanensis* Grierson	Vanandızotu	A	İr.-Tur.	NT	+
172	*Jurinea cataonica* Boiss. & Hausskn. subsp. *cataonica*	Alageyikgöbeği	L	İr.-Tur.	VU	+
173	*Klasea cerinthifolia* (Sm.) Greuter & Wagenitz	Topbaş	A	-	NE	-
174	*Klasea coriacea* (Fisch. & C.A.Mey. ex DC.) Holub	Çıtotu	A	İr.-Tur.	NE	-
175	*Klasea serratuloides* (DC.) Greuter & Wagenitz	Etlitopbaş	A+L	İr.-Tur.	NE	-
176	*Lactuca scarioloides* Boiss.	Meleto Marulu	L	İr.-Tur.	NE	-

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177	*Lactuca hispida* DC.	Kıllımarul	L	-	NE	-
178	*Lactuca tuberosa* Jacq.	Toparmarul	L	-	NE	-
179	*Lapsana communis* L. subsp. *intermedia* (M.Bieb.) Hayek var. *intermedia*	Şebrek	A+L	-	NE	-
180	*Leontodon asperrimus* (Willd.) Endl.	Aşyemliği	L	İr.-Tur.	NE	-
181	*Matricaria chamomilla* L.var. *recutita* (L.) Fiori	Almanpapatyası	L	-	NE	-
182	*Notobasis syriaca* (L.) Cass.	Yavankenger	A+L	Akd.	NE	-
183	*Onopordum carduchorum* Bornm. & Beauverd	Kavdikeni	A+L	İr.-Tur.	NE	-
184	*Phagnalon kotschyi* Sch.Bip. ex. Boiss.	Telbozçalı	L	İr.-Tur.	NE	-
185	*Picris kotschyi* Boiss.	Arapşirosu	L	-	NE	-
186	*Psephellus karduchorum* (Boiss.) Wagenitz	Müküstülübaşı	L	İr.-Tur.	VU	+
187	*Reichardia dichotoma* (Vahl) Freyn	Karasakız	A	İr.-Tur.	VU	-
188	*Rhagadiolus stellatus* (L.) Gaertn.	Çatlakçanak	A+L	Akd.	NE	-
189	*Rhaponticum insigne* (Boiss.) Wagenitz	Sarıkekre	L	İr.-Tur.	NE	-
190	*Siebera pungens* (Lam.) J.Gay	Fezaçiçeği	A+L	İr.-Tur.	NE	-
191	*Silybum marianum* (L.) Gaertn. subsp. *anatolicum* Meriçli	Anadevedikeni	A	D.Akd	NE	+
192	*Silybum marianum* (L.) Gaertn. subsp. *marianum*	Devedikeni	A	Akd.	NE	-
193	*Scorzonera papposa* DC.	Tekecan	A+L	İr.-Tur.	NE	-
194	*Scorzonera veratrifolia* Fenzl	Nerebent	A	İr.-Tur.	NE	-
195	*Senecio doriiformis* DC. subsp. *orientalis* (Fenzl) V.A.Matthews	Atkanaryaotu	L	İr.-Tur.	NE	-
196	*Senecio racemosus* (M.Bieb.) DC.	Şiro	L	İr.-Tur.	NE	-
197	*Senecio vernalis* Waldst. & Kit.	Kanaryaotu	A	-	NE	-
198	*Sonchus asper* (L.) Hill subsp. *glaucescens* (Jord.) Ball	Gevirtlek	L	-	NE	-
199	*Tanacetum kotschyi* (Boiss.) Grierson	Ateşpireotu	L	İr.-Tur.	NE	-
200	*Taraxacum androssovii* Schischk.	Zeze	A+L	-	NE	-
201	*Taraxacum assemanii* Blanche ex Boiss.	Anamasçıtlığı	L	İr.-Tur.	NE	-
202	*Taraxacum stevenii* DC.	Gelingöbeği	L	İr.-Tur.	NE	-
203	*Taraxacum montanum* (C.A.Mey.) DC.	Dağhindibası	A+L	İr.-Tur.	NE	-
204	*Taraxacum scaturiginosum* G.E.Haglund	Kıvırkıvır	A+L	-	NE	-
205	*Tragopogon buphthalmoides* (DC.) Boiss. var. *latifolius* Boiss.	Tarlayemliği	A+L	-	NE	-
206	*Tragopogon porrifolius* (Sch.Bip.) Greuter subsp. *longirostris*	Helevan	A+L	-	NE	-
207	*Turanecio eriospermus* (DC.) Hamzaoğlu	Bozturanotu	A+L	-	NE	-
208	*Xanthium spinosum* L.	Pıtrak	A	-	NE	-
209	*Xanthium strumarium* L.subsp. *strumarium*	Kocapıtrak	A	-	NE	-
210	*Xeranthemum annuum* L.	Kâğıtçiçeği	A+L	-	NE	-
211	*Xeranthemum longipapposum* Fisch. & C.A.Mey.	Uslukağıtçiçeği	L	İr.-Tur.	NE	-
212	*Zoegea leptaurea* L.	Sarıdüğme	A	İr.-Tur.	NE	-
	Berberidaceae					
213	*Bongardia chrysogonum* (L.) Spach	Çatlakotu	A+L	İr.-Tur.	NE	-
	Boraginaceae					
214	*Alkanna froedinii* Rech.f.	Gedikhavacivaotu	A+L	-	LC	+
215	*Alkanna kotschyana* A.DC.	Meşehavacivası	A+L	D.Akd	LC	+
216	*Alkanna orientalis* (L.) Boiss. var. *orientalis* (L.) Boiss.	Sarısormuk	A+L	İr.-Tur.	NE	-
217	*Alkanna trichophila* Hub.-Mor. var. *mardinensis* Hub.-Mor.	Gorız	L	İr.-Tur.	LC	+
218	*Alkanna trichophila* Hub.-Mor. var. *trichophila*	Gorız	L	İr.-Tur.	NE	-
219	*Anchusa azurea* Mill. var. *kurdica* (Guşul.) D.F.Chamb.	Sığırdili	A+L	-	NE	-
220	*Anchusa azurea* Mill. var. *azurea* Mill.	Sığırdili	A+L	-	NE	-
221	*Asperugo procumbens* L.	Nevazilotu	L	-	NE	-
222	*Buglossoides incrassata* (Guss.) I.M.Johnst. subsp. *incrassata*	Toktaşkesen	A	Akd.	NE	-
223	*Cerinthe minor* L. subsp. *auriculata* (Ten.) Domac	Livarotu	A+L	-	NE	-

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224	*Echium italicum* L.	Kurtkuyruğu	L	Akd.	NE	-
225	*Heliotropium ferrugineogriseum* Nábělek	Paslıbambulotu	L	İr.-Tur.	EN	+
226	*Heliotropium europaeum* L.	Akrepotu	A+L	İr.-Tur.	NE	-
227	*Heliotropium lasiocarpum* Fisch. & C.A.Mey.	Bozkırbambulotu	A+L	İr.-Tur.	NE	-
228	*Myosotis stricta* Roem. & Schult.	Yitikunutmabeni	A+L	Avr.-Sib.	NE	-
229	*Onosma alborosea* Fisch. & C.A.Mey. subsp. *alborosea* var. *alborosea*	Kayaemceği	A+L	İr.-Tur.	NE	-
230	*Onosma davisii* Riedl	Mıjmıjok	L	İr.-Tur.	EN	+
231	*Onosma lanceolata* Boiss. & Hausskn.	Taşmıjmıjok	L	İr.-Tur.	NE	-
232	*Onosma neglecta* Riedl	Bahaemziği	L	İr.-Tur.	NT	+
233	*Onosma nemoricola* Hausskn. & Bornm.	Koruşincarı	L	İr.-Tur.	NE	-
234	*Onosma proballanthera* Rech.f.	Yaylaemziği	L	İr.-Tur.	NT	+
235	*Onosma rascheyana* Boiss.	Vanemceği	L	İr.-Tur.	NE	-
236	*Onosma rechingeri* Riedl	Geçmıjmıjok	A+L	İr.-Tur.	LC	+
237	*Onosma rostellata* Lehm.	Kirlimıjmıjok	L	İr.-Tur.	NE	-
238	*Onosma sericea* Willd.	Kâğıtemcek	L	İr.-Tur.	NE	-
239	*Onosma trachytricha* Boiss.	Kabaşircan	L	İr.-Tur.	NE	-
240	*Paracaryum strictum* (K.Koch) Boiss.	Meşeçarşakotu	L	İr.-Tur.	NE	-
241	*Phyllocara aucheri* (A.DC.) Guşul.	Karadindik	A+L	-	NE	-
242	*Symphytum kurdicum* Boiss. & Hausskn.	Kürtkafesotu	A	İr.-Tur.	NE	-
243	*Trichodesma incanum* (Bunge) A.DC.	Gökkaldirik	A	-	NE	-
	Brassicaceae					
244	*Aethionema arabicum* (L.) Andrz. ex DC.	Araptaşçantası	A+L	-	NE	-
245	*Aethionema armenum* Boiss.	Taşçantası	A	İr.-Tur.	NE	-
246	*Aethionema froedinii* Rech.f.	Diclekayagülü	A+L	İr.-Tur.	NE	-
247	*Aethionema grandiflorum* Boiss. & Hohen var. *grandiflorum*	Kocakayagülü	A	-	NE	-
248	*Alliaria petiolata* (M.Bieb.) Cavara & Grande	Sarmısakhardalı	A+L	-	NE	-
249	*Alyssum alyssoides* (L.) L.	Deliotu	A+L	-	NE	-
250	*Alyssum desertorum* Stapf.	Dumanotu	A+L	-	NE	-
251	*Alyssum linifolium* Stephan ex. Willd. var. *linifolium*	Çıplak Kuduzotu	L	-	NE	-
252	*Alyssum sibiricum* Willd.	Kedidili	L	-	NE	-
253	*Alyssum simplex* Rudolph	Sadekuduzotu	L	-	NE	-
254	*Alyssum stapfii* Vierh.	Acemkuduzotu	A+L	-	NE	-
255	*Alyssum strictum* Willd.	Dikkuduzotu	A+L	İr.-Tur.	NE	-
256	*Alyssum strigosum* Banks & Sol. subsp. *cedrorum* (Schott & Kotschy) T.R.Dudley	Kayakuduzotu	A+L	-	NE	-
257	*Arabis nova* Vill.	Tıfılkazteresi	A	-	NE	-
258	*Arabis verna* (L.) R.Br.	Morkazteresi	A+L	Akd.	NE	-
259	*Aubrieta parviflora* Boiss.	Acemobrizyası	A+L	İr.-Tur.	NE	-
260	*Biscutella didyma* L.	Çıtçıtotu	A+L	-	NE	-
261	*Barbarea brachycarpa* Boiss. subsp. *minor* (K.Koch) Parolly & Erenvar. *minor*	Nicarcık	A	-	NE	-
262	*Brossardia papyracea* Boiss.	Zıylan	A	İr.-Tur.	NE	-
263	*Calepina irregularis* (Asso) Thell.	Tophardal	L	-	NE	-
264	*Capsella bursa-pastoris* (L.) Medik.	Çobançantası	A+L	-	NE	-
265	*Clypeola jonthlaspi* L.	Akçeotu	A+L	-	NE	-
266	*Clypeola lappacea* Boiss.	Yolukakçeotu	A+L	İr.-Tur.	NE	-
267	*Conringia clavata* Boiss.	Topuztelkari	A+L	-	NE	-
268	*Descurainia sophia* (L.) Webb ex Prantl subsp. *sophia*	Sadırotu	A+L	-	NE	-
269	*Draba verna* L.	Çırçırotu	A+L	-	NE	-
270	*Erysimum repandum* L.	Çatalzarife	A+L	-	NE	-
271	*Fibigia clypeata* (L.) Medik. subsp. *clypeata* var. *clypeata*	Sikkeotu	A+L	-	NE	-

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272	*Hirschfeldia incana* (L.) Lagr.-Foss.	Nadasturpu	L	-	NE	-
273	*Isatis aucheri* Boiss.	Posçivitotu	L	İr.-Tur.	LC	+
274	*Isatis cochlearis* Boiss.	Adıyamançivitotu	L	İr.-Tur.	NE	-
275	*Lepidium cartialgineum*(J.Mayer) Thell. subsp. *crassifolium* (Waldst. & Kit.) Thell.	Meşinteresi	L	-	NE	-
276	*Lepidium chalepense* L.	Kormik	A+L	-	NE	-
277	*Lepidium draba* L.	Diğnik	A+L	-	NE	-
278	*Lepidium ruderale* L.	Tuzık	L	-	NE	-
279	*Microthlaspi perfoliatum* (L.) F.K.Mey.	Giyle	A+L	-	NE	-
280	*Matthiola longipetala* (Vent.) DC. subsp. *bicornis* (Sibth. & Sm.) P.W.Ball	Boynuzluşebboy	A+L	-	NE	-
281	*Neslia paniculata* (L.) Desv. subsp. *thracica* (Velen.) Bornm.	Göçmenhardalı	A+L	-	NE	-
282	*Noccaea valerianoides* (Rech.f.) F.K.Mey.	Müküsdağarcığı	L	İr.-Tur.	CR	+
283	*Parlatoria cakiloidea* Boiss.	Bitteresi	L	-	NE	-
284	*Sinapis arvensis* L.	Hardal	A+L	-	NE	-
285	*Sisymbrium septulatum* DC.	Harranbülbülotu	L	-	NE	-
286	*Thlaspi bornmuelleri* (Rech.f.) Hedge	Firenkdağarcığı	L	İr.-Tur.	VU	+
287	*Zuvanda exacoides* (DC.) Askerova	Çölemerikteresi	L	-	VU	-
	Campanulaceae					
288	*Asyneuma amplexicaule* (Willd.) Hand.-Mazz. subsp. *amplexicaule* var. *angustifolium* (Boiss.) Bornm.	Hoşdeğnek	L	İr.-Tur.	NE	-
289	*Asyneuma filipes* (Nábělek) Damboldt	Yayldeğneği	L	İr.-Tur.	NE	-
290	*Campanula conferta* A.DC.	Saklıçançiçeği	A	-	NE	-
291	*Campanula erinus* L.	Çatalçançiçeği	L	-	NE	-
292	*Campanula flaccidula* Vatke	Sarkıkçıngırak	A+L	-	NE	-
293	*Campanula glomerata* L. subsp. *hispida* (Witasek) Hayek	Yumakçanı	L	Avr.-Sib.	NE	-
294	*Campanula mardinensis* Bornm. & Sint.	Mardinçıngırağı	A	-	NE	-
295	*Campanula propinqua* Fisch. & C.A.Mey.	Kumçanı	L	-	NE	-
296	*Campanula retrorsa* Labill.	Yozçıngırak	L	-	NE	-
297	*Campanula reuteriana* Boiss. & Balansa	Selçançiçeği	L	-	NE	-
298	*Campanula saxonorum* Gand.	İnceçıngırak	A	-	LC	+
299	*Campanula tridentata* Schreb.	Gökçeçançiçeği	L	-	NE	-
300	*Legousia falcata* (Ten.) Fritsch ex Janch.	Eğrikadınaynası	A	Akd.	NE	-
301	*Legousia pentagonia* (L.) Thell.	Kadınaynası	A+L	D.Akd	NE	-
302	*Michauxia nuda* A.DC.	Cıbılkeşir	A+L	-	CR	-
	Cannabaceae					
303	*Celtis tournefortii* Lam.	Dardağan	A+L	-	NE	-
	Capparaceae					
304	*Capparis spinosa* L.	Kebere	A+L	-	NE	-
	Caprifoliaceae					
305	*Cephalaria procera* Fisch. & Avé-Lall.	Ganteper	A+L	-	NE	-
306	*Cephalaria syriaca* (L.) Schrad.	Pelemir	A+L	-	NE	-
307	*Pterocephalus kurdicus* Vatke var. *kurdicus*	Pembecücükotu	A+L	-	VU	-
308	*Pterocephalus plumosus* (L.) Coulter	Gökcücükotu	A+L	-	NE	-
309	*Pterocephalus pyrethrifolius* Boiss. & Hohen.	Yaylacücükotu	A+L	İr.-Tur.	NE	-
310	*Pterocephalus strictus* Boiss. & Hohen.	Akcücükotu	A+L	İr.-Tur.	VU	-
311	*Scabiosa argentea* L.	Yazısüpürgesi	A+L	-	NE	-
312	*Scabiosa calocephala* Boiss.	Çayıruyuzotu	L	İr.-Tur.	NE	-
313	*Scabiosa rufescens* Freyn & Sint.	Kızılpuk	L	İr.-Tur.	NT	+
314	*Scabiosa sicula* L.	Adauyuzotu	L	Akd.	NE	-
315	*Scabiosa persica* Boiss.	Acemzivanı	L	İr.-Tur.	NE	-
316	*Valeriana dioscoridis* Sm.	Çobanzurnası	A+L	D.Akd	NE	-

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317	*Valeriana officinalis* L.	Kediotu	A+L	-	NE	-
318	*Valeriana speluncaria* Boiss. var. *speluncaria*	İnkediotu	A+L	-	NT	+
319	*Valerianella coronata* (L.) DC.	Taçlıkuzugevreği	A+L	-	NE	-
320	*Valerianella dactylophylla* Boiss. & Hohen.	Elkuzugevreği	A+L	İr.-Tur.	NE	-
321	*Valerianella vesicaria* (L.) Moench	Kuzugevreği	A+L	-	NE	-
	Caryophyllaceae					
322	*Agrostemma githago* L.	Buğdaykaramuğu	A+L	-	NE	-
323	*Arenaria serpyllifolia* L. subsp. *leptoclados* (Rchb.) Nyman	Kurukumotu	A+L	-	NE	-
324	*Bufonia calyculata* Boiss. & Balansa	Özgehatunotu	A+L	-	LC	+
325	*Cerastium glomeratum* Thuill.	Boynuzotu	A+L	-	NE	-
326	*Dianthus floribundus* Boiss.	Kırkkaranfil	L	İr.-Tur.	NE	-
327	*Dianthus strictus* Banks & Sol. var. *subenervis* (Boiss.) Reeve	Dimisok	A+L	-	NE	-
328	*Holosteum umbellatum* L. var. *umbellatum*	Şeytanküpesi	A+L	-	NE	-
329	*Minuartia hamata* (Hausskn.) Mattf.	Koruotu	L	-	NE	-
330	*Paronychia kurdica* Boiss. subsp. *kurdica* var. *kurdica*	Bozkepekotu	A+L	-	NE	-
331	*Saponaria suffruticosa* Nábělek	Çalıköpürgen	L	İr.-Tur.	NE	-
332	*Saponaria tridentata* Boiss.	Üşmen	L	İr.-Tur.	NE	-
333	*Saponaria viscosa* C.A.Mey.	Şenak	L	İr.-Tur.	NE	-
334	*Silene aegyptiaca* (L.) L.f. subsp. *ruderalis* Coode & Cullen	Kababallıca	A	-	NE	-
335	*Silene ampullata* Boiss.	Pörtlekkıyışak	A	İr.-Tur.	NE	-
336	*Silene capitellata* Boiss.	Kavuklunakıl	A+L	İr.-Tur.	LC	+
337	*Silene conoidea* L.	Şıvananotu	A+L	-	NE	-
338	*Silene dichotoma* Ehrh. subsp. *dichotoma*	Çatalnakıl	L	-	NE	-
339	*Silene longipetala* Vent.	Ballısüpürge	L	-	NE	-
340	*Silene monerantha* F.N.Williams	Botannakılı	L	İr.-Tur.	NE	-
341	*Vaccaria hispanica* (Mill.) Rauschert	Ekinebesi	A+L	-	NE	-
342	*Velezia rigida* L.	Tığotu	L	-	NE	-
	Cistaceae					
343	*Helianthemum ledifolium* (L.) Mill.	Kurugüngülü	L	-	NE	-
	Cleomaceae					
344	*Cleome ornithopodioides* L.	Taşsaçakgülü	A+L	D.Akd	NE	-
	Colchicaceae					
345	*Colchicum crocifolium* Boiss.	Urfamahruru	A	İr.-Tur.	NE	-
346	*Colchicum kotschyi* Boiss.	Acıçiğdem	A+L	İr.-Tur.	NE	-
347	*Colchicum szovitsii* Fisch. & C.A.Mey. subsp. *szovitsii*	Katırçiğdemi	A	-	NE	-
	Convolvulaceae					
348	*Convolvulus arvensis* L.	Tarlasarmaşığı	A+L	-	NE	-
349	*Convolvulus galaticus* Rost. ex Choisy	Bozsarmaşık	A	İr.-Tur.	NE	-
350	*Convolvulus reticulatus* Choisy subsp. *reticulatus*	Dolaşgan	L	İr.-Tur.	NE	-
351	*Ipomoea purpurea* (L.) Roth	Kahkahaçiçeği	A	-	NE	-
	Cornaceae					
352	*Cornus sanguinea* L. subsp. *australis* (C.A.Mey.) Jáv.	Kansiğdiren	A	Avr.-Sib.	NE	-
	Crassulaceae					
353	*Rosularia sempervivum* (M.Bieb.) A.Berger subsp. *sempervivum*	Somkayakoruğu	A+L	İr.-Tur.	NE	-
354	*Sedum pallidum* M.Bieb.	Koyunörmece	A+L	Kar.	NE	-
355	*Umbilicus intermedius* Boiss.	Kandilyaprağı	A+L	-	NE	-
356	*Umbilicus tropaeolifolius* Boiss.	Kaplıkotu	L	-	NE	-
	Cucurbitaceae					
357	*Bryonia multiflora* Boiss. & Heldr.	Ülüngür	A	İr.-Tur.	NE	-

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	Cuscutaceae					
358	*Cuscuta approximata* Bab.	Bağboğanotu	A	-	NE	-
359	*Cuscuta babylonica* Aucher ex Choisy var. *babylonica*	Gelinsaçı	A+L	-	NE	-
360	*Cuscuta campestris* Yunck.	Kâfirsaçı	A	-	NE	-
361	*Cuscuta hyalina* Roth	Zarbostanbozan	A+L	-	NE	-
	Cyperaceae					
362	*Carex distans* L. subsp. *distans*	Sinaayakotu	L	Avr.-Sib.	NE	-
363	*Carex kurdica* Kük. ex Hand.-Mazz.	Yaylasazı	L	İr.-Tur.	NE	-
364	*Cyperus fuscus* L.	Maydanozbağı	L	Avr.-Sib.	NE	-
365	*Cyperus difformis* L.	Göcelebüken	L	-	NE	-
	Datiscaceae					
366	*Datisca cannabina* L.	Renkotu	A	-	NE	-
	Dioscoraceae					
367	*Dioscorea communis* (L.) Caddick & Wilkin	Dolanbaç	L	-	NE	-
	Euphorbiaceae					
368	*Euphorbia denticulata* Lam.	Karasütlük	A+L	İr.-Tur.	NE	-
369	*Euphorbia helioscopia* L. subsp. *helioscopia*	Feribanotu	L	-	NE	-
370	*Euphorbia macrocarpa* Boiss. & Buhse	Meşesütleğeni	L	-	NE	-
371	*Euphorbia macroclada* Boiss.	Neblul	A	İr.-Tur.	NE	-
372	*Euphorbia orientalis* L.	Gezersütleğen	A+L	İr.-Tur.	NE	-
373	*Euphorbia oxyodonta* Boiss.	Hiloşirik	L	D.Akd	NE	-
374	*Euphorbia sanasunitensis* Hand.-Mazz.	Aksisütleğen	A	İr.-Tur.	NT	+
375	*Euphorbia szovitsii* Fisch. & C.A.Mey. var. *szovitsii*	Urussütleğeni	A+L	İr.-Tur.	NE	-
	Fabaceae					
376	*Anagyris foetida* L.	Zivircik	A+L	Akd.	NE	-
377	*Astragalus amblolepis* Fisch.	Kütgeven	L	İr.-Tur.	NE	-
378	*Astragalus angustiflorus* K.Koch subsp. *angustiflorus*	İncegeven	A+L	İr.-Tur.	NE	-
379	*Astragalus basianicus* Boiss. & Hausskn. ex Boiss. var. *glabrescens* Širj.	Arapgeveni	L	İr.-Tur.	NE	-
380	*Astragalus capito* Boiss. & Hohen.	Dağgeveni	L	İr.-Tur.	NE	-
381	*Astragalus decurrens* Boiss.	Kulaklıgeven	L	İr.-Tur.	NT	-
382	*Astragalus delanensis* Širj. & Rech.f.	Delangeveni	L	İr.-Tur.	DD	+
383	*Astragalus densifolius* Lam. subsp. *densifolius*	Gümüşgeven	L	İr.-Tur.	NE	-
384	*Astragalus echinops* Aucher ex Boiss.	Topuzgeveni	A+L	İr.-Tur.	NE	-
385	*Astragalus elongatus* Willd. subsp. *elongatus*	Yazıyoncası	L	İr.-Tur.	NE	-
386	*Astragalus emarginatus* Labill.	Oyukgeven	A+L	İr.-Tur.	NE	-
387	*Astragalus ermineus* V.A.Matthews	Sansargeveni	A+L	İr.-Tur.	NT	+
388	*Astragalus hamosus* L.	Koçboynuzu	L	-	NE	-
389	*Astragalus hirticalyx* Boiss. & Kotschy ex Boiss.	Tüylüçanak	L	İr.-Tur.	NE	-
390	*Astragalus macrocephalus* Willd. subsp. *finitimus* (Bunge) D.F.Chamb.	Topaçgeven	L	İr.-Tur.	NE	-
391	*Astragalus macrostachys* DC.	Kocakafa	L	İr.-Tur.	NE	-
392	*Astragalus mardinensis* Nábelek	Mardingeveni	A	İr.-Tur.	VU	+
393	*Astragalus mesites* Buhse	Esergeven	L	İr.-Tur.	NE	-
394	*Astragalus microcephalus* Willd. subsp. *microcephalus*	Anadolukitresi	L	İr.-Tur.	NE	-
395	*Astragalus neurocarpus* Boiss.	Çizikgeven	L	İr.-Tur.	NE	-
396	*Astragalus oocephalus* Boiss. subsp. *stachyophorus* Hub.-Mor. & D.F.Chamb	Oğulgeveni	L	İr.-Tur.	NT	+
397	*Astragalus oxyglottis* Steven ex M.Bieb.	Irmakgeveni	L	-	NE	-
398	*Astragalus russelii* Banks & Sol.	Ballan	L	İr.-Tur.	NE	-
399	*Astragalus suberosus* Banks & Sol.	Yemengeveni	A+L	-	NE	-
400	*Astragalus xanthogossypinus* Hand.-Mazz.	Lifgeveni	L	-	VU	-

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401	*Cicer pinnatifidum* Jaub. & Spach	Çakılnohutu	L	-	NE	-
402	*Colutea cilicica* Boiss. & Balansa	Patlangaç	A+L	-	NE	-
403	*Coronilla scorpioides* (L.) W.D.J.Koch	Akrepburçağı	A+L	-	NE	-
404	*Dorycnium pentaphyllum* Scop. subsp. *haussknechtii* (Boiss.) Gams	Gervenük	A+L	-	LC	+
405	*Glycyrrhiza glabra* L.var. *glabra*	Meyan	A+L	-	NE	-
406	*Hedysarum aucheri* Boiss.	Altınbatalak	L	İr.-Tur.	VU	+
407	*Hedysarum erythroleucum* Boiss.	Kırbaçbatalağı	L	İr.-Tur.	LC	+
408	*Hedysarum kotschyi* Boiss.	Sarıbatalak	L	İr.-Tur.	NE	-
409	*Hedysarum spinosissimum* L.	Dişlekbatalağı	L	Akd.	NE	-
410	*Hedysarum varium* Willd. subsp. s*yriacum* (Boiss.) Townsend	Şam Batalağı	L	İr.-Tur.	NE	-
411	*Hippocrepis unisiliquosa*L. subsp. *unisiliquosa*	Atnalı	L	-	NE	-
412	*Hymenocarpos circinnatus* (L.) Savi	Pulluot	L	-	NE	-
413	*Lathyrus aphaca* L. var. *biflorus* Post	Sarıburçak	A+L	Akd.	NE	-
414	*Lathyrus boissieri* Širj.	Ermürdümük	A+L	İr.-Tur.	NE	-
415	*Lathyrus cicera* L.	Colban	A+L	Akd.	NE	-
416	*Lathyrus cassius* Boiss.	Kelimirdik	L	Akd.	NE	-
417	*Lathyrus gorgoni* Parl. var. *gorgoni*	İmirdik	A+L	Akd.	NE	-
418	*Lathyrus inconspicuus* L.var. *inconspicuus*	Yılanmürdümüğü	L	-	NE	-
419	*Lens culinaris* Medik. subsp. *orientalis* (Boiss.) Ponert	Yasmık	L	-	NE	-
420	*Lotus aegaeus* (Griseb.) Boiss.	Nohudak	L	-	NE	-
421	*Lotus gebelia*Vent. var. *hirsutissimus* (Ledeb.) Dinsm.	Gülgazalboynuzu	A+L	İr.-Tur.	NE	-
422	*Medicago minima* (L.) Bartal. var. *minima*	Gurnik	L	-	NE	-
423	*Medicago monantha* (C.A.Mey.) Trautv.	Dağgurniği	L	İr.-Tur.	NE	-
424	*Medicago noeana* Boiss.	Çevrince	L	İr.-Tur.	NE	-
425	*Medicago orbicularis* (L.) Bartal.	Paralık	L	-	NE	-
426	*Medicago polymorpha* L.var. *polymorpha*	Kırkyonca	L	-	NE	-
427	*Medicago polymorpha* L.var. *vulgaris* (Benth.) Shinners	Kırkyonca	L	-	NE	-
428	*Medicago radiata* L.	Hilalyonca	A+L	İr.-Tur.	NE	-
429	*Medicago rigidula* (L.) All. var. c*inerascens* (Jord.) Rouy	Kabayonca	L	-	NE	-
430	*Medicago rigidula* (L.) All. var. *rigidula*	Kabayonca	A+L	-	NE	-
431	*Medicago rigidula* (L.) All. var. *submitis* (Boiss.) Heyn	Kabayonca	L	-	NE	-
432	*Medicago rhytidiocarpa* (Boiss. & Balansa) E.Small	Buruşukyonca	L	İr.-Tur.	NT	+
433	*Medicago rotata* Boiss. var. *eliezeri* Eig	Topaçyonca	A+L	D.Akd	NE	-
434	*Medicago sativa* L.subsp. *sativa* L.	Karayonca	A+L	-	NE	-
435	*Melilotus indicus* (L.) All.	Otuzluyonca	L	-	NE	-
436	*Onobrychis aequidentata* (Sibth. & Sm.) d Urv.	Dişlekkorunga	A+L	Akd.	NE	-
437	*Onobrychis caput-galli* (L.) Lam.	Pıtrakkorunga	A+L	Akd.	NE	-
438	*Onobrychis galegifolia* Boiss.	Darpkorungası	A+L	İr.-Tur.	NE	-
439	*Onobrychis megataphros* Boiss.	Bağkorungası	A+L	İr.-Tur.	NE	-
440	*Onobrychis radiata* (Desf.) M.Bieb.	Tekerkorunga	A+L	#YOK	NE	-
441	*Onobrychis transcaucasica* Grossh.	Kafkorungası	A+L	İr.-Tur.	NE	-
442	*Ononis adenotricha* Boiss.	Karayandırak	L	D.Akd	NE	-
443	*Ononis pusilla* L.	Yaltakdikeni	L	Akd.	NE	-
444	*Ononis spinosa* L.subsp. *leiosperma* (Boiss.) Sirj.	Demirdelen	A	-	NE	-
445	*Ononis viscosa* L. subsp. *breviflora* (DC.) Nyman	Siyekdikeni	L	Akd.	NE	-
446	*Oxytropis persica* Boiss.	Acemgagageveni	L	Avr.-Sib.(Dağ)	NE	-
447	*Pisum sativum* L. subsp. *elatius* (M.Bieb.) Aschers. & Graebn. var. *pumilio* Meikle	Bezelye	A+L	-	NE	-
448	*Pisum fulvum* Sibth. & Sm.	Esmerbezelye	A+L	D.Akd	NE	-
449	*Prosopis farcta* (Banks & Sol.) J.F.Macbr.	Çediotu	A+L	-	NE	-

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450	*Scorpiurus subvillosus* L.var. *subvillosus*	Koyundücüğü	L	-	NE	-
451	*Trifolium argutum* Sol.	Dirfil	A+L	-	NE	-
452	*Trifolium batmanicum* Katzn.	Batmanüçgülü	L	-	EN	-
453	*Trifolium campestre* Schreb. subsp. *campestre* var. *campestre*	Üçgül	A+L	-	NE	-
454	*Trifolium fragiferum* L. var. *fragiferum*	Çileküçgülü	L	-	NE	-
455	*Trifolium haussknechtii* Boiss. var. *haussknechtii*	Antepüçgülü	L	İr.-Tur.	NE	-
456	*Trifolium hirtum* All.	Deliyonca	A+L	Akd.	NE	-
457	*Trifolium lappaceum* L.	Yivliyonca	L	Akd.	NE	-
458	*Trifolium leucanthum* M.Bieb.	Tapışıküçgül	L	-	NE	-
459	*Trifolium nigrescens*Viv. subsp. *petrisavii* (Clementi) Holmboe	Yelüçgülü	A+L	-	NE	-
460	*Trifolium physodes* Steven & M.Bieb var. *psilocalyx* Boiss.	Meşeüçgülü	L	D.Akd	NE	-
461	*Trifolium purpureum* Lois. var. *purpureum*	Morüçgül	A+L	D.Akd	NE	-
462	*Trifolium repens* L.var. *repens*	Aküçgül	A+L	-	NE	-
463	*Trifolium resupinatum* L. var. *resupinatum*	Anadoluüçgülü	A+L	-	NE	-
464	*Trifolium scabrum* L.	Hıyardücük	L	Akd.	NE	-
465	*Trifolium grandiflorum* Schreb.	Hanımüçgülü	L	D.Akd	NE	-
466	*Trifolium spumosum* L.	Keseyonca	L	Akd.	NE	-
467	*Trifolium stellatum* L.var. *stellatum*	Yıldızyonca	L	-	NE	-
468	*Trifolium tomentosum* L.var. *tomentosum*	Yünlüyonca	L	-	NE	-
469	*Trigonella coelesyriaca* Boiss.	Handekok	L	-	LC	-
470	*Trigonella mesopotamica* Hub.-Mor.	Dicleboyotu	L	-	NE	-
471	*Trigonella monspeliaca* L.	Somçemenotu	A+L	-	NE	-
472	*Trigonella spicata* Sibth. & Sm.	Başakboyotu	L	-	NE	-
473	*Trigonella strangulata* Boiss.	Düğmeliboyotu	L	-	NE	-
474	*Vicia alpestris* Steven subsp. *alpestris*	Dağbaklası	A+L	-	NE	-
475	*Vicia cracca*L. subsp. *stenophylla* Vel.	Meşefiği	L	-	NE	-
476	*Vicia ervilia* (L.) Willd.	Küşne	A+L	-	NE	-
477	*Vicia galeata* Boiss.	Sombakla	L	-	NE	-
478	*Vicia hyrcanica* Fisch. & C.A.Mey.	Acembaklası	L	İr.-Tur.	NE	-
479	*Vicia narbonensis* L.var. *narbonensis*	Kocafiğ	L	-	NE	-
480	*Vicia noeana* Boiss.& Reut. ex Boiss. var. *megalodonta* Rech.f.	Salkımbakla	L	İr.-Tur.	NE	-
481	*Vicia sativa* L.subsp. *sativa*	Fiğ	A+L	-	NE	-
482	*Vicia sativa* L. *subsp. nigra* (L.) Ehrh. var. *nigra*	Ekinfiği	A+L	-	NE	-
483	*Vicia sativa* L. *subsp. nigra* (L.) Ehrh. var. *segetalis* (Thuill.) Ser. ex DC.	Eşekgürülü	A+L	-	NE	-
484	*Vicia villosa* Roth subsp. *villosa*	Tüylüfiğ	L	-	NE	-
	Fagaceae					
485	*Quercus brantii* Lindl.	Karameşe	A+L	-	NE	-
486	*Quercus infectoria*	Zindiyen	A+L	İr.-Tur.	NE	-
	Gentianaceae					
487	*Centaurium tenuiflorum* (Hoffmanns. & Link) Fritsch subsp. *acutiflorum* (Schott) Zeltner	Sivrigelindüğmesi	A	-	NE	-
488	*Gentiana olivieri* Griseb.	Afat	A+L	İr.-Tur.	NE	-
	Geraniaceae					
489	*Erodium acaule* (L.) Becherer & Thell.	Leylekgagası	A+L	Akd.	NE	-
490	*Erodium cicutarium* (L.) L Hér. subsp. *cicutarium*	İğnelik	A+L	-	NE	-
491	*Erodium laciniatum* (Cav.) Willd. subsp. *laciniatum*	Kıyığneliği	L	-	NE	-
492	*Geranium dissectum* L.	Dilimlııtır	L	-	NE	-
493	*Geranium libanoticum* Schenk	Pelgizer	L	-	NE	-
494	*Geranium rotundifolium* L.	Helilok	L	-	NE	-
495	*Geranium tuberosum* L.	Çakmuz	L	İr.-Tur.	NE	-

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	Hypericaceae					
496	*Hypericum amblysepalum* Hochst.	Kantülçiçeği	L	İr.-Tur.	NE	-
497	*Hypericum capitatum* Choisy var. *luteum* N.Robson	Bantof	L	İr.-Tur.	NE	+
498	*Hypericum lysimachioides* Boiss.& Noë var. *lysimachioides*	Eğinkantaronu	L	İr.-Tur.	NE	-
499	*Hypericum pseudolaeve* N.Robson	Keşişkantaronu	L	İr.-Tur.	LC	-
500	*Hypericum retusum* Aucher	Aran	L	İr.-Tur.	NE	-
501	*Hypericum scabrum* L.	Karahasançayı	L	İr.-Tur.	NE	-
502	*Hypericum spectabile* Jaub. & Spach	Tarlakantaronu	L	İr.-Tur.	NT	+
503	*Hypericum triquetrifolium* Turra	Pırpırotu	L	-	NE	-
	Iridaceae					
504	*Crocus biflorus* Mill. subsp. *pseudonubigena* B.Mathew	Siirtçiğdemi	A+L	İr.-Tur.	LC	+
505	*Crocus biflorus* Mill. subsp. *tauri* (Maw) B.Mathew	Berfan	A+L	İr.-Tur.	NE	-
506	*Crocus cancellatus*Herb. subsp. *damascenus* (Herb.) B.Mathe	Pivok	A+L	İr.-Tur.	NE	-
507	*Crocus karduchorum* Kotschy ex Maw	Koyakçiğdemi	A+L	İr.-Tur.	EN	+
508	*Gladiolus antakiensis* A.P.Ham.	Alkılıçotu	L	D.Akd	VU	-
509	*Gladiolus atroviolaceus* Boiss.	Kıraçsüseni	A+L	İr.-Tur.	NE	-
510	*Gladiolus humilis* Stapf	Bodurkılıçotu	A	İr.-Tur.	EN	+
511	*Gladiolus italicus* Mill.	Kılıçotu	L	-	NE	-
512	*Gladiolus kotschyanus* Boiss.	Çayırkılıçotu	A+L	-	NE	-
513	*Gynandriris sisyrinchium* (L.) Parl.	Keklikçiğdemi	L	İr.-Tur.	NE	-
514	*Iris aucheri* (Baker) Sealy	Kayanavruzu	A+L	İr.-Tur.	VU	-
515	*Iris pseudocaucasica* Grossh.	Vannavruzu	A+L	İr.-Tur.	NE	-
516	*Iris x germanica* L.	Göksüsen	A+L	İr.-Tur.	NE	-
517	*Iris persica* L.	Buzala	A+L	-	NE	-
518	*Iris reticulata* M.Bieb. var. *reticulata*	Karakörpeze	A+L	İr.-Tur.	NE	-
	Ixioliriaceae					
519	*Ixiolirion tataricum* (Pall.) Schult. & Schult.f. var. *tataricum*	Köpekotu	A+L	İr.-Tur.	NE	-
	Juglandaceae					
520	*Juglans regia* L.	Ceviz	A+L	İr.-Tur.	NE	-
521	*Pterocarya pterocarpa* (Michx.) Kunth ex I.Iljinsk.	Yalankoz	A+L	Hir.-Kar.	NE	-
	Juncaceae					
522	*Juncus inflexus* L.subsp. *inflexus*	Sazak	L	-	NE	-
	Lamiaceae					
523	*Ajuga chamaepitys* (L.) Schreb. subsp. *chia* (Schreb.) Arcang.	Acıgıcı	A+L	-	NE	-
524	*Ajuga chamaepitys* (L.) Schreb. subsp. *laevigata* (Banks & Sol.) P.H.Davis	Kelmayasıl	L	İr.-Tur.	NE	-
525	*Ajuga chamaepitys* (L.) Schreb. subsp. *rechingeri* (Bilik) P.H.Davis	Yünmayasılı	L	İr.-Tur.	NE	-
526	*Clinopodium nepeta*(L.) Kuntze subsp. *nepeta*	Kedifesleğeni	A+L	İr.-Tur.	NE	-
527	*Cyclotrichium longiflorum* Leblebici	Tüylüçekme	A+L	Avr.-Sib.	NE	-
528	*Lallemantia iberica* (M.Bieb.) Fisch. & C.A.Mey.	Ajdarbaşı	L	İr.-Tur.	NE	-
529	*Lamium album* L. subsp. *crinitum* (Montbret & Aucher ex Benth.) Mennema	Kovanlık	A+L	Kar.	NE	-
530	*Lamium amplexicaule* L.var. *amplexicaule*	Baltutan	A+L	-	NE	-
531	*Lamium garganicum* L.subsp. *striatum* (Sm.) Hayek	Telbalıcak	A	-	NE	-
532	*Marrubium anisodon* K.Koch	Yayaotu	A+L	-	NE	-
533	*Marrubium cuneatum* Banks & Sol.	Elkurtaran	L	-	NE	-
534	*Mentha longifolia* (L.) L.subsp. *typhoides* (Briq.) Harley	Derenanesi	A+L	İr.-Tur.	NE	-
535	*Nepeta betonicifolia* C.A.Mey. subsp. *betonicifolia*	Sizvripisikotu	L	İr.-Tur.	NE	-
536	*Nepeta cataria* L.	Kedinanesi	L	Avr.-Sib.	NE	-
537	*Nepeta italica* L.	Eşekçayı	A	Akd.	NE	-
538	*Nepeta obtusicrena* Boiss. & Kotschy ex Hedge	Kumpisiği	L	İr.-Tur.	NT	+

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539	*Nepeta trachonitica* Post	Kızılpiskotu	L	İr.-Tur.	NE	-
540	*Phlomis bruguieri* Desf.	Kabaçalba	A+L	İr.-Tur.	NE	-
541	*Phlomis kurdica* Rech.f.	Gubel	A+L	İr.-Tur.	NE	-
542	*Phlomis pungens* Willd. var. *pungens*	Silvanok	A+L	-	NE	-
543	*Phlomis rigida* Labill.	Diriçalba	A+L	İr.-Tur.	NE	-
544	*Salvia candidissima* Vahl	Galabor	A	-	NE	-
545	*Salvia ceratophylla* L.	Tarakşalba	L	İr.-Tur.	NE	-
546	*Salvia ertekinii* Yıld.	Erşalba	A+L	İr.-Tur.	EN	++
547	*Salvia indica* L.	Sultanşalba	A+L	İr.-Tur.	NE	-
548	*Salvia limbata* C.A.Mey.	Maldili	A	İr.-Tur.	NE	-
549	*Salvia macrochlamys* Boiss. & Kotschy	Gevrekşalba	A+L	İr.-Tur.	NE	-
550	*Salvia multicaulis* Vahl	Kürtreyhanı	A+L	İr.-Tur.	NE	-
551	*Salvia palaestina* Benth.	Sürmelişalba	A+L	İr.-Tur.	NE	-
552	*Salvia sclarea* L.	Paskulak	L	-	NE	-
553	*Salvia siirtica* Kahraman, Celep & Doğan	Siirtadaçayı, Şefera*	A+L	İr.-Tur.	CR	+
554	*Salvia syriaca* L.	Çevlikotu	L	İr.-Tur.	NE	-
555	*Salvia trichoclada* Benth.	Meşeşalbası	A+L	İr.-Tur.	NE	-
556	*Salvia viridis* L.	Zarifşalba	A+L	Akd.	NE	-
557	*Satureja avromanica*Maroofi	Kayakekiği	A+L	Anix*	NE	-
558	*Scutellaria orientalis*L. subsp. *porphyrostegia* J.R.Edm.	Kınalıkaside	A+L	İr.-Tur.	VU	+
559	*Sideritis montana* L.subsp. *montana*	Karaçay	A+L	D.Akd	NE	-
560	*Sideritis vulcanica* Hub.-Mor.	Madençayı	A+L	İr.-Tur.	VU	+
561	*Stachys annua* (L.) L.subsp. *annua* var. *annua*	Hacıosmanotu	A+L	-	NE	-
562	*Stachys brantii* Benth.	Yitikçayçe	A+L	İr.-Tur.	DD	+
563	*Stachys lavandulifolia* Vahl	Tüylüçay	A	-	NE	-
564	*Stachys mardinensis* (Post) R.R.Mill	Kayapungu	A+L	İr.-Tur.	NE	-
565	*Stachys saturejoides* Montbret & Aucher ex Benth.	Çarşakdelisi	A+L	İr.-Tur.	NE	-
566	*Teucrium chamaedrys* L. subsp. *sinuatum* (Celak.) Rech.f.	Sancıotu	A+L	İr.-Tur.	NE	-
567	*Teucrium chasmophyticum* Rech.f.	Gürpüntüotu	L	-	CR	-
568	*Teucrium orientale* L. var. *puberulens* Ekim	Kirveotu	A+L	İr.-Tur.	NE	-
569	*Teucrium parviflorum* Schreber	Koyunotu	A+L	İr.-Tur.	NE	-
570	*Teucrium polium* L.	Acıyavşan	A+L	-	NE	-
571	*Thymbra spicata* L.subsp. *spicata*	Zahter	A	Akd.	NE	-
572	*Thymus fallax* Fisch. & C.A.Mey.	Catri	A+L	İr.-Tur.	NE	-
573	*Vitex agnus-castus* L.	Hayıt	A+L	Akd.	NE	-
574	*Ziziphora capitata* L.	Anuk	A+L	-	NE	-
575	*Ziziphora persica* Bunge	Karareyhan	A+L	İr.-Tur.	NE	-
	Liliaceae					
576	*Fritillaria assyriaca* Baker subsp. *assyriaca*	Donuklale	L	İr.-Tur.	NE	-
577	*Fritillaria carica* Rix	Bodursarı	A	D.Akd	NE	-
578	*Fritillaria imperialis* L.	Ağlayangelin	A+L	İr.-Tur.	VU	-
579	*Fritillaria minuta* Boiss. & Noë	Kınalılâlesi	A+L	İr.-Tur.	NE	-
580	*Fritillaria pinardii* Boiss.	Mahçuplâle	A+L	İr.-Tur.	NE	-
581	*Fritillaria uva-vulpis* Rix	Ayılalesi	L	İr.-Tur.	VU	-
582	*Gagea luteoides* Stapf	Altınyıldız	L	-	NE	-
583	*Gagea reticulata* (Pall.) Schult. & Schult.f.	Ağyıldızı	L	İr.-Tur.	NE	-
584	*Gagea tenera* Pascher	Titrekyıldız	L	İr.-Tur.	NE	-
585	*Gagea uliginosa* Siehe & Pascher	Batakyıldızı	L	İr.-Tur.	NE	-
586	*Gagea villosa* (M.Bieb.) Sweet var. *villosa*	Tüylüyıldız	A+L	Akd.	NE	-

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587	*Tulipa armena* Boiss. var. *armena*	Dağlâlesi	A+L	İr.-Tur.	NE	-
588	*Tulipa sintenisii* Baker	Muşlalesi	A+L	İr.-Tur.	NE	+
589	*Linum mucronatum* Bertol. subsp. *armenum* (Bordz.) P.H.Davis	Sarıkamışketeni	L	İr.-Tur.	NE	-
	Linaceae					
590	*Linum nervosum* Waldst. & Kit.	Bayırketen	L	-	NE	-
591	*Linum pubescens*Banks & Sol. subsp. *anisocalyx* (P.H.Davis) Yılmaz & Kaynak	Bezir	A+L	D.Akd	NE	-
592	*Linum strictum* L. var. *spicatum* Pers.	Tokketen	L	-	NE	-
593	*Linum trigynum* L.	Otlakketeni	L	Akd.	NE	-
	Loranthaceae					
594	*Loranthus europaeus* Jacq.	Ardıçburcu	L	-	NE	-
	Lythraceae					
595	*Lythrum salicaria* L.	Hevhulma	A+L	Avr.-Sib.	NE	-
	Malvaceae					
596	*Alcea fasciculiflora* Zohary	Kümehatmi	L	İr.-Tur.	DD	-
597	*Alcea striata* (DC.) Alef. subsp. *striata*	Yivlihatmi	L	İr.-Tur.	NE	-
598	*Malva neglecta* Wallr.	Çobançöreği	L	-	NE	-
599	*Malva sylvestris* L.	Ebegümeci	L	-	NE	-
	Meliaceae					
600	*Melia azedarach* L.	Tesbihağacı	A	-	NE	-
	Moraceae					
601	*Ficus carica* L. subsp. *rupestris* (Hausskn.) Browicz	İtinciri	L	İr.-Tur.	NE	-
	Oleaceae					
602	*Jasminum fruticans* L.	Boruk	A	Akd.	NE	-
	Onagraceae					
603	*Epilobium hirsutum* L.	Hasanhüseyinçiçeği	A+L	-	NE	-
	Orchidaceae					
604	*Anacamptis pyramidalis* (L.) Rich.	Sivrisalep	A+L	-	NE	-
605	*Cephalanthera longifolia* (L.) Fritsch	Kuğusalebi	A+L	Avr.-Sib.	NE	-
606	*Cephalanthera kurdica* Bornm. ex Kraenzl.	Kurtkuşçuğu	A+L	İr.-Tur.	NE	-
607	*Dactylorhiza romana* (Seb.) Soó subsp. *georgica* (Klinge) Soó ex Renz & Taubenheim	Çamkökü	A+L	-	NE	-
608	*Dactylorhiza umbrosa* (Karelin & Kirilow) Nevskivar. *umbrosa*	Gövdelisalep	A+L	İr.-Tur.	NE	-
609	*Epipactis helleborine* (L.) Crantz subsp. *helleborine*	Bindallıçiçeği	A+L	-	NE	-
610	*Himantoglossum affine* (Boiss.) Schltr.	Keşkeşçiçeği	A+L	Akd.	NE	-
611	*Himantoglossum comperianum* (Steven) P.Delforge	Meşekeşkeşi	A+L	İr.-Tur.	NE	-
612	*Limodorum abortivum* (L.) Sw. var. *abortivum*	Saçuzatan	A+L	-	NE	-
613	*Ophrys bornmuelleri* M.Schulze subsp. *bornmuelleri*	Ebemsalebi	A+L	Akd.	NE	-
614	*Ophrys bornmuelleri* M.Schulze subsp. *carduchorum* Renz & Taubenheim	Öküzsalebi	A+L	İr.-Tur.	NT	+
615	*Ophrys levantina*Gölz & H.R.Reinhard subsp. *grandiflora* (H.Fleischm. & Soó) Kreutz	Kocakazankara	A+L	Akd.	NE	-
616	*Ophrys cilicica* Schltr.	Tülekdokuyan	A+L	Akd.	LC	+
617	*Ophrys holoserica* (Burm.f.) Greuter subsp. *heterochila* Renz & Taubenheim	Deşdiyesalebi	A+L	Akd.	NE	-
618	*Ophrys oestrifera* M.Bieb. subsp. *oestrifera*	Sineksalebi	A+L	-	NE	-
619	*Ophrys phrygia* H.Fleischm. & Bornm.	Yunussalebi	A+L	İr.-Tur.	LC	-
620	*Ophrys reinholdii* Spruner ex Fleischm. subsp. *straussii* (H.Fleischm.) E.Nelson	Sidiklisalep	A+L	İr.-Tur.	NE	-
621	*Ophrys schulzei* Bornm. & Fleischm.	Dağablamutu	A+L	-	NE	-
622	*Ophrys transhyrcana* Czerniak. subsp. *mouterdeana* (B.Baumann & H.Baumann) Kreutz	Hazarsalebi	A+L	-	NE	-
623	*Ophrys umbilicata* Desf. subsp. *khuzestanica* Renz & Taubenheim	Acemsalebi	L	İr.-Tur.	NE	-
624	*Orchis anatolica* Boiss.	Dildamak	A+L	D.Akd	NE	-
625	*Orchis collina* Banks & Sol. ex Russell	Tepesalebi	A	Akd.	NE	-

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626	*Orchis coriophora* L.subsp. *coriophora*	Pirinççiçeği	A+L	-	NE	-
627	*Orchis laxiflora* Lam. subsp. *laxiflora*	Salepsümbülü	A+L	Akd.	NE	-
628	*Orchis mascula* (L.) L. subsp. *pinetorum* (Boiss. & Kotschy) G.Camus	Çamsalebi	A+L	D.Akd	NE	-
629	*Orchis simia* Lam.	Saleppüskülü	A+L	Akd.	NE	-
630	*Orchis spitzelii* Sauter ex W.D.J.Koch.	Dağsalebi	L	Akd.	NE	-
631	*Orchis tridentata* Scop.	Katranalacası	A+L	Akd.	NE	-
632	*Platanthera chlorantha* (Cruster) Rchb.	Çarpıksalep	L	-	NE	-
	Orobanchaceae					
633	*Bartsia trixago* L.	Karaballıbaba	L	-	NE	-
634	*Bornmuellerantha aucheri* (Boiss.) Rothm.	Sadırlıdavunotu	A	İr.-Tur.	NE	-
635	*Orobanche aegyptiaca* Pers.	Dinlendiren	A	-	NE	-
636	*Orobanche crenata* Forssk.	Zıpırotu	L	-	NE	-
637	*Orobanche ramosa* L.	Narincanavarotu	A	-	NE	-
638	*Parentucellia latifolia* (L.) Caruel subsp. *flaviflora* (Boiss.) Hand.-Mazz.	Sarıüçdilotu	A+L	-	NE	-
639	*Rhynchocorys kurdica* Nábelek	Şarkfilburnu	L	İr.-Tur.	NT	+
	Papaveraceae					
640	*Corydalis integra* Barbey & Fors.-Major	Yamaçtarlakuşu	A+L	-	NE	-
641	*Corydalis oppositifolia* DC. subsp. *oppositifolia*	İparkazgagası	A	-	VU	+
642	*Fumaria asepala* Boiss	Akşahtere	L	İr.-Tur.	NE	-
643	*Fumaria densiflora* DC.	Ergendöşeği	L	-	NE	-
644	*Fumaria officinalis* L. subsp. *cilicica* (Hausskn.) Lidén	Şahtere	L	İr.-Tur.	NE	-
645	*Fumaria parviflora* Lam.	Tarlaşahteresi	L	-	NE	-
646	*Fumaria vaillantii* Loisel.	Güvercingöğsü	L	-	NE	-
647	*Hypecoum procumbens* Å.E.Dahl subsp. *procumbens*	Yavruağzı	L	Akd.	NE	-
648	*Papaver arenarium* M.Bieb.	Karagöz	A+L	-	NE	-
649	*Papaver yildirimlii* Ertekin	Hüddüdü	A+L	-	EN	++
650	*Papaver rhoeas* L.	Gelincik	A+L	-	NE	-
651	*Roemeria hybrida* (L.) DC. subsp. *hybrida*	Morgelincik	A+L	-	NE	-
	Phyllanthaceae					
652	*Andrachne telephioides* L.	Duvarnohutu	A+L	-	NE	-
	Plantaginaceae					
653	*Globularia sintenisii* Hausskn. & Wettst.	Dicleküreçiçeği	L	İr.-Tur.	NE	-
654	*Globularia trichosantha* Fisch. & C.A.Mey. subsp. *trichosantha*	Köseyayılımı	L	İr.-Tur.	NE	-
655	*Lagotis stolonifera* Maxim.	Sururotu	L	İr.-Tur.	NE	-
656	*Plantago lanceolata* L.	Damarlıca	A+L	-	NE	-
657	*Plantago major* L. subsp. *intermedia* (Gilib.) Lange	Yedidamarotu	A+L	-	NE	-
658	*Veronica acinifolia* L.	Benlik	L	-	NE	-
659	*Veronica anagallis-aquatica* L.	Sugedemesi	A+L	-	NE	-
660	*Veronica gentianoides* Vahl subsp. *glacialis* (Nábelek) A.Öztürk & M.A.Fisch.	Yaylakandili	L	İr.-Tur.	NE	-
661	*Veronica orientalis* Mill. subsp. *orientalis*	Gözmumcuğu	L	-	NE	-
662	*Veronica polita* Fr.	Mavişot	L	-	NE	-
663	*Veronica syriaca* Roem. & Schult.	Arapmavişi	L	Akd.	NE	-
	Platanaceae					
664	*Platanus orientalis* L.	Çınar	A+L	-	NE	-
	Plumbaginaceae					
665	*Acantholimon caryophyllaceum* Boiss.	Kirpidikeni	A	İr.-Tur.	NE	-
666	*Acantholimon latifolium* Boiss.	Zapkirpiotu	L	İr.-Tur.	NE	-
667	*Plumbago europaea* L.	Karakına	A	Avr.-Sib.	NE	-
668	*Psylliostachys spicata* (Willd.) Nevski	Tuzgülü*	L	-	LC	-

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	Poaceae					
669	*Aegilops biuncialis* Vis.	İkikılçık	L	-	NE	-
670	*Aegilops columnaris* Zhukovsky	Kılbuğday	A+L	İr.-Tur.	NE	-
671	*Aegilops cylindrica* Host	Kirpikliot	A+L	İr.-Tur.	NE	-
672	*Aegilops triuncialis* L.subsp. *triuncialis*	Üçkılçık	L	-	NE	-
673	*Agropyron cristatum* (L.) Gaertn. subsp. *incanum* (Nab.) Melderis	Kopayrığı	L	İr.-Tur.	NE	-
674	*Agrostis gigantea* Roth	Kocatavusotu	L	Avr.-Sib.	NE	-
675	*Agrostis olympica* (Boiss.) Bor	Ulutavusotu	A+L	Avr.-Sib.(Dağ)	NE	-
676	*Agrostis stolonifera* L.	Tavusotu	A+L	Avr.-Sib.	NE	-
677	*Alopecurus arundinaceus* Poir.	Kamış Tilkikuyruğu	L	Avr.-Sib.	NE	-
678	*Alopecurus aucheri* Boiss.	Kabatilkikuyruğu	L	İr.-Tur.	NE	-
679	*Alopecurus laguroides* Balansa	Kartilkikuyruğu	L	Avr.-Sib.(Dağ)	NE	-
680	*Alopecurus textilis* Boiss. subsp. *textilis*	Saçaklıtilkikuyruğu	L	İr.-Tur.	NE	-
681	*Alopecurus vaginatus* (Willd.) Kunth	Beneklitilkikuyruğu	L	-	NE	-
682	*Apera intermedia* Hack.	Pusluipekçimi	L	İr.-Tur.	NE	-
683	*Avena fatua* L.var. *fatua*	Deliyulaf	L	-	NE	-
684	*Avena sterilis* L.subsp. *sterilis*	Şifan	A+L	-	NE	-
685	*Avena wiestii* Steudel	Farazotu	L	-	NE	-
686	*Brachypodium distachyon* (L.) P.Beauv.	Tekkılcan	L	Akd.	NE	-
687	*Briza humilis* M.Bieb.	Kadındili	A+L	-	NE	-
688	*Bromus armenus* Boiss.	Acemkılcanı	L	İr.-Tur.	NE	-
689	*Bromus arvensis* L.	Tarlabromu	L	-	NE	-
690	*Bromus danthoniae* Trin. subsp. *danthoniae*	İbubukotu	A+L	-	NE	-
691	*Bromus erectus* Huds.	Dikbrom	A+L	-	NE	-
692	*Bromus japonicus* Thunb. subsp. *anatolicus* (Boiss. & Heldr.) Penzes	Aniyeotu	A+L	-	NE	-
693	*Bromus macrocladus* Boiss.	Delikılcan	A+L	D.Akd	EN	+
694	*Bromus scoparius* L.	İbubuk Ekini	A+L	-	NE	-
695	*Bromus sterilis* L.	Sağırilcan	A+L	-	NE	-
696	*Bromus tectorum* L.	Kırbromu	A+L	-	NE	-
697	*Bromus tomentellus* Boiss. subsp. *tomentellus*	Bozkırbromu	A+L	İr.-Tur.	NE	-
698	*Bromus variegatus* M.Bieb. subsp. *variegatus*	Sarıbrom	A+L	İr.-Tur.	NE	-
699	*Calamagrostis pseudophragmites* (Haller) Koeler	Sazçimi	A+L	Avr.-Sib.	NE	-
700	*Catabrosa aquatica* (L.) P. Beauv.	Çipil	A+L	-	NE	-
701	*Phalaris paradoxa* L.	Topuzlukanyaş	A+L	Akd.	NE	-
702	*Catabrosella araratica* (Lipsky) Tzvelev	Ağrıçipilciği	A+L	İr.-Tur.(Dağ)	NE	-
703	*Catabrosella parviflora* (Boiss. & Buhse) E.B.Alexeev ex R.R.Mill subsp. *parviflora*	Çipilcik	A+L	İr.-Tur.	NE	-
704	*Catabrosella parviflora* (Boiss. & Buhse) E.B.Alexeev ex R.R.Mill subsp. *calvertii* (Boiss.) E.B.Alexeev ex R.R.Mill	Çayırçipilciği	A+L	İr.-Tur.	NE	-
705	*Chrysopogon gryllus* (L.) Trin. subsp. *gryllus*	Buzağıotu	A+L	-	NE	-
706	*Crypsis alopecuroides* (Piller & Mitterp.) Schrad.	Derebakakotu	A+L	-	NE	-
707	*Crypsis schoenoides* (L.) Lam.	Bakakotu	A+L	-	NE	-
708	*Cynodon dactylon* (L.) Pers. var. *dactylon*	Köpekdişi	A+L	-	NE	-
709	*Cynodon dactylon* (L.) Pers. var. *villosus* Regel	Köpekdişi	A+L	-	NE	-
710	*Dactylis glomerata* L. subsp. *glomerata*	Domuzayrığı	A+L	Avr.-Sib.	NE	-
711	*Dactylis glomerata* L. subsp. *hispanica* (Roth) Nyman	Kıllıdomuzayrığı	A+L	-	NE	-
712	*Deschampsia caespitosa* (L.) P.Beauv.	Çayırsaçı	A+L	-	NE	-
713	*Echinaria capitata* (L.) Desf.	Dikenbaşotu	A+L	-	NE	-
714	*Echinochloa crus-galli* (L.) P. Beauv.	Darıcan	A+L	-	NE	-
715	*Echinochloa oryzoides* (Ard.) Fritsch	Karacinek	A+L	-	NE	-

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716	*Elymus repens* (L.) Gould	Sabankıran	A+L	-	NE	-
717	*Elymus hispidus* (Opiz) Melderis subsp. *podperae* (Nábelek) Melderis	Gavurelimotu	A+L	-	NE	-
718	*Elymus hispidus* (Opiz) Melderis subsp. *hispidus*	Eimotu	A+L	-	NE	-
719	*Elymus hispidus* (Opiz) Melderis subsp. *barbulatus* (Schur) Melderis	Ilamuk	A+L	-	NE	-
720	*Elymus kosaninii* (Nábelek) Melderis	Kireçbuğdayı	A+L	İr.-Tur.	NE	-
721	*Eremopoa altaica* (Trin.) Roshev.	Dağsalkımı	A+L	İr.-Tur.	NE	-
722	*Eremopoa multiradiata* (Trautv.) Roshev.	Deresalkımı	A+L	İr.-Tur.	NE	-
723	*Eremopoa persica* (Trin.) Roshev.	Acemsalkımı	A+L	İr.-Tur.	NE	-
724	*Festuca brunnescens* (Tzvelev) Galushko	Esmeryumak	A+L	İr.-Tur.	NE	-
725	*Festuca oreophila* Markgr.-Dann.	Parlakyumağı	A+L	İr.-Tur.	NE	-
726	*Gaudiniopsis macra* (M.Bieb.) Eig subsp. *macra*	Somyulaf	A+L	İr.-Tur.	NE	-
727	*Glyceria notata* Chevall.	Kıvrıktatlıçim	A+L	-	NE	-
728	*Hordeum brevisubulatum* (Trin.) Link subsp. *violaceum* (Boiss. & Huet) Tzvelev	Çayırarpası	A+L	İr.-Tur.	NE	-
729	*Hordeum bulbosum* L.	Boncukarpa	A+L	-	NE	-
730	*Hordeum geniculatum* All.	Yatıkarpa	A+L	Avr.-Sib.	NE	-
731	*Hordeum murinum* L. subsp. *glaucum* (Steud.) Tzvelev	Duvararpası	A+L	-	NE	-
732	*Hordeum murinum* L. subsp. *murinum* L.	Pisipisiotu	A+L	-	NE	-
733	*Hordeum spontaneum* K.Koch	Yabaniarpa	A+L	İr.-Tur.	NE	-
734	*Koeleria eriostachya* Pančić	Yaylakırnalı	A+L	-	NE	-
735	*Lolium perenne* L.	Çim	A+L	Avr.-Sib.	NE	-
736	*Lolium rigidum* Gaudin var. *rigidum*	Sertçim	A+L	-	NE	-
737	*Melica ciliata* L. subsp. *ciliata*	Kirpikliinci	A+L	-	NE	-
738	*Melica persica* Kunth subsp. *jacquemontii* (Decne. ex Jacquem.) P.H. Davis	Çayırinciotu	A+L	İr.-Tur.	NE	-
739	*Melica persica*Kunth subsp. *inaequiglumis* (Boiss.) Bor	Kireçinciotu	A+L	-	NE	-
740	*Nardus stricta* L.	Kılotu	A+L	Avr.-Sib.	NE	-
741	*Oryza sativa* L.	Çeltik	A+L	-	NE	-
742	*Oryzopsis holciformis* (M.Bieb.) Hack.	Kadifepirinçotu	A+L	-	NE	-
743	*Oryzopsis lateralis* (Regel) Stapf ex Hook.f.	Yanpirinçotu	L	İr.-Tur.	NE	-
744	*Phalaris aquatica* L.	Sukanyaşı	A+L	-	NE	-
745	*Phleum alpinum* L.	Alpitkuyruğu	A+L	Avr.-Sib.	NE	-
746	*Phleum montanum* K.Koch subsp. *montanum*	Dağitkuyruğu	A+L	-	NE	-
747	*Phleum montanum* K.Koch subsp. *serrulatum* (Boiss.) Doğan	Dişlekitkuyruğu	A+L	D.Akd	NE	-
748	*Phleum pratense* L.	Çayıritkuyruğu	A+L	Avr.-Sib.	NE	-
749	*Phleum subulatum* (Savi) Asch. & Graebn. subsp. *subulatum*	Telitkuyruğu	A+L	-	NE	-
750	*Phragmites australis* (Cav.) Trin. ex Steud.	Kamış	A+L	Avr.-Sib.	NE	-
751	*Poa alpina* subsp. *fallax* F. Herm.	Yaylasalkımotu	A+L	-	NE	-
752	*Poa angustifolia* L.	Darsalkımotu	A+L	-	NE	-
753	*Poa araratica* Trautv.	Ağrısalkımı	A+L	İr.-Tur.	NE	-
754	*Poa bulbosa* L.	Yumrulusalkım	A+L	-	NE	-
755	*Poa pratensis* L.	Çayırsalkımotu	A+L	-	NE	-
756	*Poa sterilis* M.Bieb.	Kösesalkımotu	L	-	NE	-
757	*Poa supina* Schrad.	Sulusalkımotu	L	Avr.-Sib.	NE	-
758	*Poa timoleontis* Heldr. ex Boiss.	Gürsalkımotu	L	D.Akd	NE	-
759	*Poa trivialis* L.	Kabasalkımotu	L	-	NE	-
760	*Psathyrostachys fragilis* (Boiss.) Nevskisubsp. *secaliformis* Tzvelev	Çavdararpası	L	İr.-Tur.	NE	-
761	*Psilurus incurvus* (Gouan) Schinz & Thell.	Eğrikuyrukotu	L	-	NE	-
762	*Puccinellia distans* (Jacq.) Parl. subsp. *sevangensis* (Grossh.) Tzvelev	Kaftuzçimi	L	-	NE	-
763	*Rostraria cristata* (L.) Tzvelev var. *cristata*	Gagaotu	L	-	NE	-

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764	*Saccharum ravennae* (L.) L.	Usluşekerkamışı	L	-	NE	-
765	*Secale anatolicum* Boiss.	Anadoluçavdarı	L	-	NE	-
766	*Secale cereale* var. *cereale* L.	Çavdar	L	-	NE	-
767	*Secale vavilovii* Grossh.	Gevrekçavdar	L	-	NE	-
768	*Secale montanum* Guss.	Dağçavdarı	L	-	NE	-
769	*Setaria viridis* (L.) P.Beauv.	Yeşilsıçansaçı	A+L	-	NE	-
770	*Sorghum halepense* (L.) Pers. var. *halepense*	Ekinsüpürgesi	L	-	NE	-
771	*Spodiopogon pogonanthus* (Balansa) Boiss.	Bozsakal	A+L	D.Akd	NE	-
772	*Stipa arabica* Trin. & Rupr.	Buzağılık	L	İr.-Tur.	NE	-
773	*Stipa ehrenbergiana* Trin. & Rupr.	Sorguçotu	A+L	İr.-Tur.	NE	-
774	*Stipa holosericea* Trin.	Dirgenkılaç	L	İr.-Tur.	NE	-
775	*Stipa pontica* P.A.Smirn.	Körpekılaç	L	-	NE	-
776	*Taeniatherum caput-medusae* (L.) Nevski subsp. *crinitum* (Schreb.) Melderis	Kılçıkarpası	A+L	İr.-Tur.	NE	-
777	*Trisetum flavescens* (L.) P.Beauv.	Palah	A+L	Avr.-Sib.	NE	-
778	*Trisetum thospiticum* Chrtek	Özpalah	A+L	İr.-Tur.	VU	+
779	*Triticum baeoticum* Boiss.	Yabanisiyez	A+L	-	NE	-
780	*Triticum turgidum* L.	Şişikbuğday	L	-	NE	-
781	*Ventenata dubia* (Leers) Coss. & Durieu	Ventenotu	L	-	NE	-
782	*Zingeria biebersteiniana* (Claus) P.A.Smirn. subsp. *biebersteiniana*	Oyalısalkım	L	İr.-Tur.	NE	-
783	*Zingeria pisidica* (Boiss.)Tutin	Burduroyalısalkımı	L	İr.-Tur.	NE	-
	Polygonaceae					
784	*Polygonum setosum* Jacq. subsp. *luzuloides* (Jaub. & Spach) Leblebici	Uzunebemekmeği	L	İr.-Tur.	NE	-
785	*Rumex crispus* L.	Labada	L	-	NE	-
	Primulaceae					
786	*Anagallis arvensis* L.var. *caerulea* (L.) Gouan	Farekulağı	A+L	-	NE	-
	Punicaceae					
787	*Punica granatum* L.	Nar	L	-	NE	-
	Ranunculaceae					
788	*Aconitum cochleare* Vorosch.	Gökboğanotu	L	İr.-Tur.	NE	-
789	*Adonis aestivalis* L. subsp. *aestivalis*	Kandamlası	A+L	İr.-Tur.	NE	-
790	*Adonis aleppica* Boiss.	Taşçiçeği	A+L	İr.-Tur.	NE	-
791	*Adonis flammea* Jacq.	Cinlâlesi	L	-	NE	-
792	*Anemone coronaria* L.	Manisalalesi	A+L	Akd.	NE	-
793	*Ceratocephala falcata* (L.) Pers.	Yelotu	A+L	-	NE	-
794	*Clematis orientalis* L.	Köpektutağı	L	-	NE	-
795	*Consolida oliveriana* (DC.) Schrödinger	Kıllımahmuz	L	-	NE	-
796	*Consolida orientalis* (J.Gay) Schrödinger	Morçiçek	L	-	NE	-
797	*Delphinium albiflorum* DC.	Akhezaren	L	-	NE	-
798	*Delphinium kurdicum* Boiss. & Hohen.	Şahhezaren	A+L	İr.-Tur.	NE	-
799	*Nigella arvensis* L. var. *caudata* Boiss.	Tarlaçörekotu	A+L	-	NE	-
800	*Nigella orientalis* L.	Şarkçörekotu	A+L	-	NE	-
801	*Nigella sativa* L.	Çörekotu	A+L	-	NE	-
802	*Ranunculus argyreus* Boiss.	Çitemik	L	-	NE	-
803	*Ranunculus arvensis* L.	Mustafaçiçeği	L	-	NE	-
804	*Ranunculus asiaticus* L.	Şakayıklalesi	A	-	NE	-
805	*Ranunculus chius* DC.	Meremcik	L	-	NE	-
806	*Ranunculus cornutus* DC.	Evlimemedotu	L	-	NE	-
807	*Ranunculus cuneatus* Boiss.	Körükotu	L	-	NE	-
808	*Ranunculus diversifolius* Boiss. & Kotschy	Cilodüğünçiçeği	L	-	NE	-

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809	*Ranunculus kochii* Ledeb.	Karçiçeği	A+L	İr.-Tur.	NE	-
810	*Ranunculus macrorrhynchus* Boiss. subsp. *macrorrhynchus*	Yamaçyağotu	L	İr.-Tur.	NE	-
811	*Ranunculus macrorrhynchus* Boiss. subsp. *trigonocarpus* (Boiss.) P.H.Davis	Üçyağotu	L	İr.-Tur.	NE	-
812	*Ranunculus millefolius* Sol. subsp. *millefolius*	Bindüğünçiçeği	L	-	NE	-
813	*Ranunculus munzurensis* S.Erik & Yıld.	Munzurdüğünçiçeği	A	İr.-Tur.	NT	+
814	*Ranunculus muricatus* L.	Kutsaldefne	L	-	NE	-
815	*Ranunculus obesus* Trautv.	Ardanuçyağotu	L	-	NE	-
816	*Ranunculus sphaerospermus* Boiss. & Blanche	Suçiçeği	A	-	NE	-
	Resedaceae					
817	*Reseda lutea* L. var. *lutea*	Muhabbetçiçeği	A+L	-	NE	-
	Rhamnaceae					
818	*Paliurus spina-christi* P. Mill.	Karaçalı	A+L	-	NE	-
	Rosaceae					
819	*Agrimonia eupatoria* L. subsp. *eupatoria*	Fıtıkotu	L	-	NE	-
820	*Alchemilla buseriana* Rothm.	Beritpençesi	L	-	LC	+
821	*Amygdalus arabica* Oliv.	Arapbademi	L	İr.-Tur.	NE	-
822	*Amygdalus communis* L.	Badem	A+L	-	NE	-
823	*Amygdalus kotschyi* Boiss. & Hohen.	Çalıbademi	L	İr.-Tur.	VU	-
824	*Amygdalus orientalis* Mill.	Payam	L	İr.-Tur.	NE	-
825	*Cerasus brachypetala* Boiss. var. *bornmuelleri* (Schneider) Browicz	Yırtıkkiraz	L	İr.-Tur.	NE	-
826	*Cerasus microcarpa* (C.A.Mey.) Boiss. subsp. *tortuosa* (Boiss. & Hausskn.) Browicz	Sarıdağkirazı	L	İr.-Tur.	NE	-
827	*Crataegus azarolus* L. *var.* azarolus	Müzmüldek	A+L	-	NE	-
828	*Crataegus orientalis* Pall. ex M.Bieb. subsp. *orientalis*	Alıç	L	-	NE	-
829	*Potentilla aucheriana* Th.Wolf ex Bornm.	Bayırparmakotu	L	İr.-Tur.	NE	-
830	*Potentilla lignosa* Willd. ex Schltdl.	Odunparmakotu	L	İr.-Tur.	NE	-
831	*Potentilla pannosa* Boiss. & Hausskn. ex Boiss.	Yünparmakotu	L	İr.-Tur.	VU	-
832	*Potentilla recta* L.	Suparmakotu	L	-	NE	-
833	*Potentilla reptans* L.	Reşatınotu	L	-	NE	-
834	*Pyrus syriaca* Boiss. var. *syriaca*	Çakalarmudu	L	-	NE	-
835	*Rosa canina* L.	Kuşburnu	L	-	NE	-
836	*Rosa foetida* J.Herrm.	Acemsarısı	A+L	İr.-Tur.	NE	-
837	*Rosa phoenicia* Boiss.	Fenikegülü	L	D.Akd	NE	-
838	*Rubus sanctus* Schreb.	Böğürtlen	A+L	-	NE	-
839	*Sanguisorba minor* L. subsp. *lasiocarpa* (Boiss. & Hausskn.) Nordborg	Karagöndürme	L	-	NE	-
	Rubiaceae					
840	*Asperula orientalis* Boiss. & Hohen.	Gökçebelumotu	L	İr.-Tur.	NE	-
841	*Asperula xylorrhiza* Nábelek	Siirtbelumotu	L	İr.-Tur.	NE	-
842	*Callipeltis cucullaris* (L.) Steven	Nermik	L	İr.-Tur.	NE	-
843	*Cruciata taurica* (Pall. ex Willd.) Ehrend.	Kırımgüzeli	A+L	İr.-Tur.	NE	-
844	*Galium aparine* L.	Çobansüzgeci	L	-	NE	-
845	*Galium haussknechtii* Ehrend.	Çitiplikçiği	L	İr.-Tur.	NE	-
846	*Galium tricornutum* Dandy	Havotu	A+L	İr.-Tur.	NE	-
847	*Galium verum* L. subsp. *verum*	Boyalık	L	Avr.-Sib.	NE	-
848	*Rubia tenuifolia* d'Urv. subsp. *doniettii* (Griseb.) Ehrend. & Schönb.-Tem.	Çöpboyası	A	D.Akd	NE	-
849	*Rubia tinctorum* L.	Kökboyası	L	İr.-Tur.	NE	-
850	*Sherardia arvensis* L.	Gökörenotu	L	Akd.	NE	-
	Rutaceae					
851	*Haplophyllum ptilostylum* Spach	Tüylüsedo	L	İr.-Tur.	NE	-

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	Salicaceae					
852	*Populus alba* L. var. *alba*	Akkavak	A	Avr.-Sib.	NE	-
853	*Populus euphratica* Olivier	Fıratkavağı	A+L	-	NE	-
854	*Salix acmophylla* Boiss.	Acemsöğüdü	L	İr.-Tur.	NE	-
	Sapindaceae					
855	*Acer campestre*L. subsp. *campestre*	Ovaakçağacı	L	Avr.-Sib.	NE	-
	Saxifragaceae					
856	*Saxifraga hederacea* L. *var. libanotica*(Bornm.) Matthews	Cılıztaşkıran	L	-	NE	-
	Scrophulariaceae					
857	*Scrophularia mesopotamica* Boiss.	Sahrasıracası	A+L	İr.-Tur.	LC	+
858	*Scrophularia striata* Boiss.	Eşekpancarı	L	İr.-Tur.	NE	-
859	*Scrophularia xanthoglossa* Boiss. var. *decipiens* (Boiss. & Kotschy) Boiss.	Serkele	L	İr.-Tur.	NE	-
860	*Verbascum agrimoniifolium* (K.Koch) Hub.-Mor. subsp. *agrimoniifolium*	Majak	A	-	NE	-
861	*Verbascum froedinii* Murb.	Siirtsığırkuyruğu	A+L	İr.-Tur.	VU	-
862	*Verbascum globiferum* Hub.-Mor.	Topsığırkuyruğu	A+L	İr.-Tur.	EN	+
863	*Verbascum laetum* Boiss. & Hausskn. ex Boiss.	Simsığırkuyruğu	A+L	İr.-Tur.	VU	-
864	*Verbascum sinuatum* L. subsp. *sinuatum* var. *adenosepalum* Murb.	Bodanotu	L	Akd.	NE	-
	Solanaceae					
865	*Datura stramonium* L.	Boruçiçeği	L	-	NE	-
866	*Physalis philadelphica* Lam.	Pırıp	A	-	NE	-
867	*Solanum dulcamara* L.	Sofur	L	Avr.-Sib.	NE	-
	Tamaricaceae					
868	*Tamarix smyrnensis* Bunge	Ilgın	A+L	-	NE	-
	Thymelaeaceae					
869	*Daphne mucronata* Royle subsp. *mucronata*	Tevri	A	İr.-Tur.	NE	-
870	*Thymelaea gussonei* Boreau	Yamaççekemi	L	Akd.	NE	-
	Typhaceae					
871	*Typha domingensis* Pers.	Şeytanmumu	L	-	NE	-
	Ulmaceae					
872	*Ulmus minor* Mill.	Ovakarağacı	L	-	NE	-
873	*Zelkova carpinifolia* (Pall.) K.Koch	Zelkova	L	Hir.-Kar.	NE	-
	Urticaceae					
874	*Urtica dioica* L. subsp. *dioica*	Isırgan	A	Avr.-Sib.	NE	-
	Violaceae					
875	*Viola modesta* Fenzl	Sahramenekşesi	A+L	-	NE	-

*A+L (Arazi+Literatür – Field+ Literature), A (Arazi- Field), L (Literatür - Literature)

**-(Fitocoğrafik bölgesi bilinmeyen veya çok bölgeli - Unknown phytogeographic zone or multizone), Akd. (Akdeniz elementi - Mediterranean element), D.Akd (Doğu Akdeniz elementi - Eastern Mediterranean element), Avr.-Sib. (Avrupa-Sibirya elementi - Euro-Siberian element), Avr.-Sib.(Dağ) (Avrupa-Sibirya dağ elementi - Euro-Siberian mountain element), Hir.-Kar. (Hirkanya-Karadeniz elementi - Hirkania-Black Sea element), İr.-Tur. (İran-Turan elementi - Iran-Turan element), İr.-Tur.(Dağ) (İran-Turan dağ elementi - Iran-Turan mountain element), Kar. (Karadeniz elementi - Black Sea element)

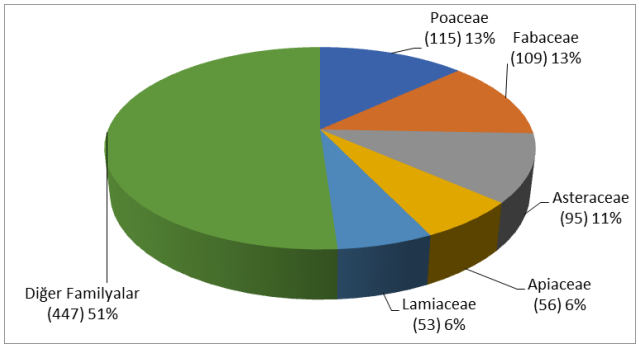
***CR (Critical-Kritik), EN (Endangered-Tehlikede), VU (Vulnerable-Hassas), NT (Near Threatened- Tehdide açık), LC (Least Concern-Düşük riskli), DD (Data Deficient- Yetersiz veri), NE (Not Evaluated-değerlendirilmedi)

****-(Endemik değil - Not Endemic), + (Endemik - Endemic), ++ (Lokal Endemik - Local Endemic)

En fazla takson içeren ilk beş familya sırasıyla Poaceae (115 takson), Fabaceae (109 takson), Asteraceae (95 takson), Apiaceae (56 takson) ve Lamiaceae (53 takson)'dir (Şekil 2). Türkiye Florası'nda takson sayısı yönünden en zengin familyalara bakıldığında ilk sıraları Asteraceae, Fabaceae, Lamiaceae, Brassicaceae ve Poaceae almaktadır. En zengin ilk 10 familya, toplam floranın yaklaşık %65'ini oluşturmaktadır. Bu oran Siirt ilinde%47.49'dur.

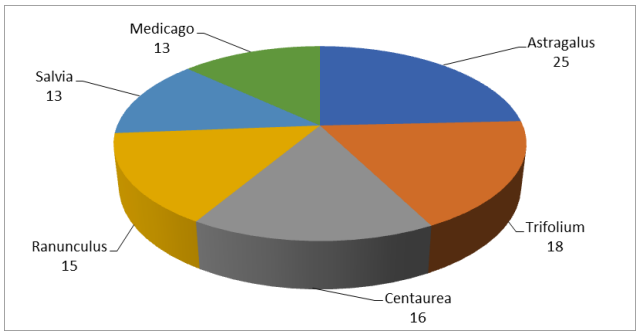
Elde edilen verilere göre Siirt ilinde en çok takson içeren ilk beş cins sırasıyla *Astragalus* (24 takson), *Trifolium* (18 takson), *Centaurea* (16 takson), *Ranunculus* (15 takson), *Medicago* ve *Salvia* (13'er takson)'dır (Şekil 3). İç, Doğu ve

Güneydoğu Anadolu Bölgesi'ni de kapsayan İran-Turan fitocoğrafik bölgesi, *Astragalus* cinsinin gen merkezidir. Türkiye Florası'na göre; en fazla takson içeren *Astragalus* cinsi, çalışma alanımızda da en fazla takson içeren cinstir. Türkiye Florası'na göre içerdiği takson sayısı bakımından 9. sırada bulunan *Trifolium* cinsi bu çalışmada ikinci sırada yer almaktadır. Çalışma alanının orman formasyonu, step ve step meşelik alanların varlığı bu cinsin üyelerinin fazla olmasında etkili olmuştur.



Şekil 2. En fazla takson içeren ilk beş familya

Figure 2. The first five families with the most taxa



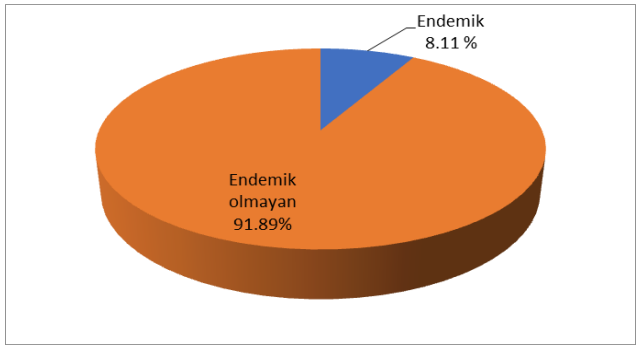
Şekil 3. En fazla takson içeren ilk beş cins

Figure 3. The first five genus with the most taxa

Siirt ilinde yayılış gösteren taksonlardan 22 familyaya ait 71 takson (%8.11) endemiktir (Şekil 4). Endemik veya endemik olmayıp tehlike altında olduğu değerlendirilen toplam 95 bitkinin 6'sı "CR" Kritik; 10'u "EN" Tehlikede; 33'ü "VU" Hassas; 18'si "NT" Tehdide açık, 23'ü"LC" Düşük riskli ve 5'i "DD" veri yetersiz tehlike kategorilerinde değerlendirilmektedir (Tablo 2). Listelerde verilen takson isimleri ve tehlike kategorileri Güner et al. (2012) tarafından yazılan Türkiye Bitkileri Listesi baz alınarak oluşturulmuş olan ve güncellenerek elektronik ortamda sunulan https://bizimbitkiler.org.tr/yeni/demos/technical/ adresinden alınmıştır (Tablo 2).

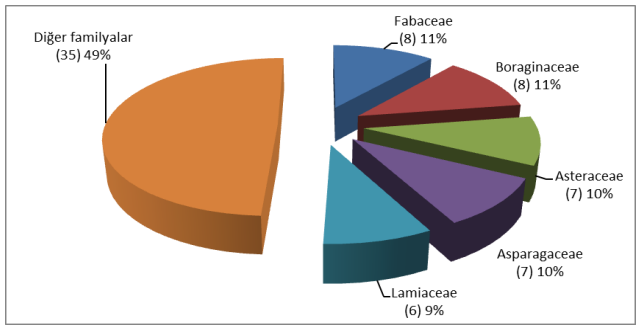
Endemik taksonların yer aldığı en fazla takson içeren ilk beş familya sırasıyla Fabaceae (8 takson), Boraginaceae (8 takson), Asteraceae (7 takson), Asparagaceae (7 takson), Lamiaceae (6 takson) olarak tespit edilmiştir (Şekil 5).

Çalışma alanında tespit edilen bitkilerden 329 takson İran-Turan, 90 takson Akdeniz, 35 takson Avrupa-Sibirya ve 4 takson Karadeniz elementi elemanıdır. 417takson ise fitocoğrafik bölgesi bilinmeyen veya çok bölgelidir (Şekil 6).



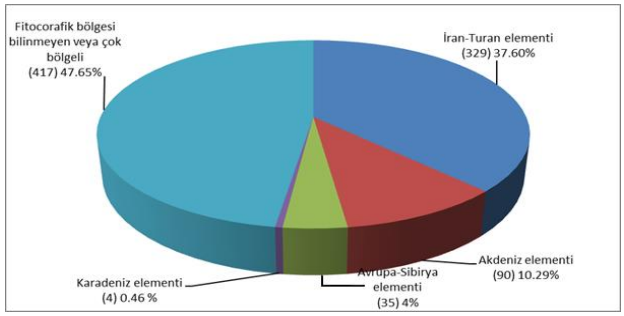
Şekil 4. Siirt ili endemik bitkilerinin % grafiği

Figure 4. Percentage graph of endemic plants in Siirt province



Şekil 5. En fazla endemik takson içeren ilk beş familyaya ait grafik

Figure 5. Graph of the first five families containing the most endemic taxa



Şekil 6. Taksonların fitocoğrafik bölgelere göre dağılım grafiği

Figure 6. Distribution graph of taxa according to phytogeographic regions

Tablo 2. Endemik veya endemik olmayan nadir taksonların tehdit kategorilerinin listesi

Table 2. The list of threat categories of endemic and non endemic rare taxa

Familya	Takson	IUCN	Endemik
Amaryllidaceae	*Allium armerioides* Boiss.	DD	Endemik
Amaryllidaceae	*Allium pervariensis* Fırat & Koyuncu	EN	Endemik
Apiaceae	*Bunium elegans* (Fenzl) Freyn var. *brevipes* Freyn & Sint.	LC	Endemik
Apiaceae	*Bunium elegans* (Fenzl) Freyn var. *involucratum* Ö.Saya	VU	Endemik
Apiaceae	*Ferulago angulata* (Schlecht.) Boiss. subsp. *angulata*	VU	Endemik değil

Familya	Takson	IUCN	Endemik
Apiaceae	*Malabaila lasiocarpa* Boiss.	LC	Endemik
Apiaceae	*Pastinaca armena* Fisch. & C.A.Mey.	VU	Endemik
Apiaceae	*Pimpinella flabellifolia* (Boiss.) Benth. & Hook. ex Drude	VU	Endemik
Apiaceae	*Pimpinella sintenisii* H.Wolff	VU	Endemik değil
Asparagaceae	*Bellevalia fominii* Woronow	VU	Endemik değil
Asparagaceae	*Bellevalia koyuncui* Karabacak & Yıldırım	VU	Endemik
Asparagaceae	*Bellevalia longistyla* (Miscz.) Grossh.	VU	Endemik değil
Asparagaceae	*Bellevalia pseudolongipes* Karabacak & Yıldırım	LC	Endemik
Asparagaceae	*Bellevalia sasonii* Fidan	VU	Endemik
Asparagaceae	*Bellevalia siirtensis* Fırat	VU	Endemik
Asparagaceae	*Bellevalia vuralii* B.Şahin & Aslan	CR	Endemik
Asparagaceae	*Hyacinthella siirtensis* B.Mathew	NT	Endemik
Asparagaceae	*Scilla leepii* Speta	NT	Endemik
Asteraceae	*Centaurea fenzlii* Reichardt	LC	Endemik
Asteraceae	*Centaurea kurdica* Reichardt	NT	Endemik
Asteraceae	*Cousinia eriocephala* Boiss. & Hausskn. ex Boiss.	LC	Endemik
Asteraceae	*Echinops phaeocephalus* Hand.-Mazz.	DD	Endemik değil
Asteraceae	*Gundelia siirtica* Fırat	CR	Endemik
Asteraceae	*Inula helenium* L. subsp. *vanensis* Grierson	NT	Endemik
Asteraceae	*Jurinea cataonica* Boiss. & Hausskn. subsp. *cataonica*	VU	Endemik
Asteraceae	*Psephellus karduchorum* (Boiss.) Wagenitz	VU	Endemik
Asteraceae	*Reichardia dichotoma* (Vahl) Freyn	VU	Endemik değil
Boraginaceae	*Alkanna froedinii* Rech.f.	LC	Endemik
Boraginaceae	*Alkanna kotschyana* A.DC.	LC	Endemik
Boraginaceae	*Alkanna trichophila* Hub.-Mor. var. *mardinensis* Hub.-Mor.	LC	Endemik
Boraginaceae	*Alkanna trichophila* Hub.-Mor. var. *mardinensis* Hub.-Mor.	VU	Endemik değil
Boraginaceae	*Heliotropium ferrugineogriseum* Nábělek	EN	Endemik
Boraginaceae	*Onosma davisii* Riedl	EN	Endemik
Boraginaceae	*Onosma neglecta* Riedl	NT	Endemik
Boraginaceae	*Onosma proballanthera* Rech.f.	NT	Endemik
Boraginaceae	*Onosma rechingeri* Riedl	LC	Endemik
Brassicaceae	*Isatis aucheri* Boiss.	LC	Endemik
Brassicaceae	*Noccaea valerianoides* (Rech.f.) F.K.Mey.	CR	Endemik
Brassicaceae	*Thlaspi bornmuelleri* (Rech.f.) Hedge	VU	Endemik
Brassicaceae	*Zuvanda exacoides* (DC.) Askerova	VU	Endemik değil
Campanulaceae	*Campanula saxonorum* Gand.	LC	Endemik
Campanulaceae	*Michauxia nuda* A.DC.	CR	Endemik değil
Caprifoliaceae	*Pterocephalus kurdicus* Vatke var. *kurdicus*	VU	Endemik değil
Caprifoliaceae	*Pterocephalus strictus* Boiss. & Hohen.	VU	Endemik değil
Caprifoliaceae	*Scabiosa rufescens* Freyn & Sint.	NT	Endemik
Caprifoliaceae	*Valeriana speluncaria* Boiss. var. *speluncaria*	NT	Endemik
Caryophyllaceae	*Bufonia calyculata* Boiss. & Balansa	LC	Endemik
Caryophyllaceae	*Silene capitellata* Boiss.	LC	Endemik
Euphorbiaceae	*Euphorbia sanasunitensis* Hand.-Mazz.	NT	Endemik
Fabaceae	*Astragalus decurrens Boiss.*	NT	Endemik değil
Fabaceae	*Astragalus delanensis* Širj. & Rech.f.	DD	Endemik
Fabaceae	*Astragalus ermineus* V.A.Matthews	NT	Endemik
Fabaceae	*Astragalus mardinensis* Nábelek	VU	Endemik
Fabaceae	*Astragalus oocephalus* Boiss. subsp. *stachyophorus* Hub.-Mor. & D.F.Chamb	NT	Endemik
Fabaceae	*Astragalus xanthogossypinus* Hand.-Mazz.	VU	Endemik değil

Familya	Takson	IUCN	Endemik
Fabaceae	*Dorycnium pentaphyllum* Scop. subsp. *haussknechtii* (Boiss.) Gams	LC	Endemik
Fabaceae	*Hedysarum aucheri* Boiss.	VU	Endemik
Fabaceae	*Hedysarum erythroleucum* Boiss.	LC	Endemik
Fabaceae	*Medicago rhytidiocarpa* (Boiss. & Balansa) E.Small	NT	Endemik
Fabaceae	*Trifolium batmanicum* Katzn.	EN	Endemik değil
Fabaceae	*Trigonella coelesyriaca* Boiss.	LC	Endemik değil
Hypericaceae	*Hypericum pseudolaeve* N.Robson	LC	Endemik değil
Hypericaceae	*Hypericum spectabile* Jaub. & Spach	NT	Endemik
Iridaceae	*Crocus biflorus* Mill. subsp. *pseudonubigena* B.Mathew	LC	Endemik
Iridaceae	*Crocus karduchorum* Kotschy ex Maw	EN	Endemik
Iridaceae	*Gladiolus antakiensis* A.P.Ham.	VU	Endemik değil
Iridaceae	*Gladiolus humilis* Stapf	EN	Endemik
Iridaceae	*Iris aucheri* (Baker) Sealy	VU	Endemik değil
Lamiaceae	*Nepeta obtusicrena* Boiss. & Kotschy ex Hedge	NT	Endemik
Lamiaceae	*Salvia ertekinii* Yıld.	EN	Endemik
Lamiaceae	*Salvia siirtica* Kahraman, Celep & Doğan	CR	Endemik
Lamiaceae	*Scutellaria orientalis*L. subsp. *porphyrostegia* J.R.Edm.	VU	Endemik
Lamiaceae	*Sideritis vulcanica* Hub.-Mor.	VU	Endemik
Lamiaceae	*Stachys brantii* Benth.	DD	Endemik
Lamiaceae	*Teucrium chasmophyticum* Rech.f.	CR	Endemik değil
Liliaceae	*Fritillaria imperialis* L.	VU	Endemik değil
Liliaceae	*Fritillaria uva-vulpis* Rix	VU	Endemik değil
Malvaceae	*Alcea fasciculiflora* Zohary	DD	Endemik değil
Orchidaceae	*Ophrys bornmuelleri* M.Schulze subsp. *carduchorum* Renz & Taubenheim	NT	Endemik
Orchidaceae	*Ophrys cilicica* Schltr.	LC	Endemik
Orchidaceae	*Ophrys phrygia* H. Fleischm. & Bornm.	LC	Endemik değil
Orobanchaceae	*Rhynchocorys kurdica* Nábelek	NT	Endemik
Papaveraceae	*Corydalis oppositifolia* DC. subsp. *oppositifolia*	VU	Endemik
Papaveraceae	*Papaver yildirimlii* Ertekin	EN	Endemik
Plumbaginaceae	*Psylliostachys spicata* (Willd.) Nevski	LC	Endemik değil
Poaceae	*Bromus macrocladus* Boiss.	EN	Endemik
Poaceae	*Trisetum thospiticum* Chrtek	VU	Endemik
Ranunculaceae	*Ranunculus munzurensis* S.Erik & Yıld.	NT	Endemik
Rosaceae	*Alchemilla buseriana* Rothm.	LC	Endemik
Rosaceae	*Amygdalus kotschyi* Boiss. & Hohen.	VU	Endemik değil
Rosaceae	*Potentilla pannosa* Boiss. & Hausskn. ex Boiss.	VU	Endemik değil
Scrophulariaceae	*Scrophularia mesopotamica* Boiss.	LC	Endemik
Scrophulariaceae	*Verbascum froedinii* Murb	VU	Endemik değil
Scrophulariaceae	*Verbascum globiferum* Hub.-Mor.	EN	Endemik
Scrophulariaceae	*Verbascum laetum* Boiss. & Hausskn. ex Boiss.	VU	Endemik değil

Siirt ili yaklaşık olarak 500 m'den başlayıp 3000 m'ye kadar yükselen topoğrafik yapısıyla birçok farklı bitki taksonuna ev sahipliği yapmaktadır. Teknolojinin gelişmesi ve insanların doyumsuzluğu bir araya gelince canlıların birçok doğal yaşam alanı yok olmaktadır. Tarım alanlarının genişletilmesi, yol yapım ve genişletme çalışmaları, baraj yapımları, enerji santralleri (termik santraller), maden ocakları, potansiyelin çok üstünde ve erken otlatma, halkın yakacak temini ve en önemlisi de her sene meydana gelen yangınlar, acil tedbirlerin alınmaması durumunda alanda yayılışı olan pek çok endemik ve nadir taksonun kaybolmasına sebep olacaktır. Halk tarafından gıda ve tıbbî amaçlarla kullanılan bazı bitkilerin (sirik, lale, salep gibi) aşırı ve bilinçsiz şekilde toplanması ve satılması nesillerinin yok olmasına neden olmaktadır. Bilinçsiz toplamalar ülkemizde bitki çeşitliliği için büyük tehdit oluşturmaktadır. Stepler, hayvanlar tarafından ilkbaharda filiz halindeyken ve sonbaharda ıslanıp yumuşadıkları zamanlarda otlanmakta ve otsu stepler ile biçeneklerden biçilen otlar kışın hayvanlara verilmek için yığınlar halinde etrafları çevrilmektedir. Yoğun ve aşırı otlatma, bu stepleri tehdit etmektedir. İlkbaharın ilk günlerinde bitkiler tohum bağlayamadan, daha kar örtüsü alandan kalkmadan yapılan otlatmalarla step zarar görmekte ve

hayvanların sevmediği veya dikenli bitkilerin alandaki yayılışı artmaktadır. Özellikle yüksek dağ yamaçlarındaki yerleşim alanlarında halkın yakacak olarak kullandığı ve araziden söktüğü çeşitli türler ile sahada aşırı ve erken yapılan otlatma erozyonu kolaylaştıran, dolayısıyla bitki örtüsünü tehdit eden en önemli sebeplerdir. Dağların zirvesinde yerleştirilen askerî amaçlı radar, karakol ve gözetleme istasyonları, bu istasyonlara yol yapımları ve bu istasyonların güvenliği için yer alacak güvenlikle ilgili yapılaşmalar sonucu, önemli ölçüde alanın tabii özelliğine zarar verilmiştir. Bitkilere yönelik tehditler şu şekilde özetlenebilir.

İnsan Faaliyetleri: İnsanoğlunun hayatı kendisi için daha kolay hale getirmek için yaptığı faaliyetlerin tamamına yakını doğaya ve diğer canlılara direkt veya dolaylı olarak olumsuz etkiler yapmaktadır.

Tarımsal Uygulamalar: Tarım alanları açmak için birçok bitkinin doğal yaşam alanı tahrip edilmektedir.

Yol Yapma ve Genişletme Çalışmaları: Yol yapma ve yol genişletme çalışmaları ile çoğu bitkinin yaşama alanı daraltılmakta ya da tamamen yok edilmektedir.

Doğadan Toplama: İnsanların gıda, ilaç, kozmetik, süs eşyası gibi amaçlarla doğadan topladıkları bitkilerin doğal populasyonları zamanla azalmakta ve hatta bazen tamamen yok olmaktadır.

Hayvancılık Faaliyetleri (Aşırı Otlatma ve Biçme): Bilinçsiz erken otlatma ve ot biçme ile birçok bitki tahrip edilmekte ve populasyonlardaki birey sayısı azalmaktadır.

Orman Yangınları: Bölgede güvenlik gerekçesiyle meydana gelen orman yangınları bitkileri tehdit eden etmenlerin başında gelmektedir.

Enerji ve Madencilik Faaliyetleri: Yapılan enerji ve madencilik faaliyetleri de diğer etkenler gibi birçok bitki taksonunun doğal yaşam alanlarının bozulmasına neden olmaktadır.

Araştırma alanında yapılan arazi çalışmaları sırasında Orman vejetasyonu, Step vejetasyonu, Kayalık alan vejetasyonu, Çayır vejetasyonu, Sulak alan vejetasyonu, Alpin vejetasyonu ve Kumul alan vejetasyonu olmak üzere 7 farklı vejetasyon tipi tespit edilmiştir. Çalışmalarımız sırasında tespit edilen vejetasyon tipleri aşağıda verilmiştir.

Orman Vejetasyonunda *Quercus infectoria* subsp. *veneris* (A.Kern.) Meikle (Ziyden-Mazı meşesi) ve *Quercus brantii* Lindl. (Karameşe) taksonlarının hakim olduğu ormanlık alanlar 500-1100 m'den başlar 2000-2100 m'ye kadar devam eder. Baykan-Ziyaret arasında *Pinus sylvestris* L. (Sarıçam) ağaçlarından oluşan ormanlık alanlar da bulunmaktadır. *Astragalus* sp. (Geven) yastık formasyonu, *Euphorbia* sp. (Sütleyen) otsu formasyonu ve *Quercus* sp (Meşe) çalı formasyonlarının hakim olduğu Siirt ilinde step vejetasyonu bir çok yerde görülmektedir. *Aubrieta parviflora* Boiss. (Acemobrizyası), *Paronychia kurdica* subsp. *kurdica* Boiss. (Bozkepekotu), *Onosma alborosea* subsp. *alborosea* Fisch. & C.A.Mey. (Kayaemceği) taksonlarının hakim olduğu Siirt Aydınlar ilçesi sınırlarından Şırnak il sınırına kadar olan Botan Vadisi boyunca uzanan alanlar, Kovanağzı kayalıkları, Beykent radar bölgesi, Şirvan Şeytan Kapısı kayalıkları, Beğendik Kanyonu, Köprüçay Kanyonu, Bilgili Köyü kayalıkları, Kuşdalı kayalıkları ve Gündoğdu Vadisi başlıca kayalık alanlardır. Pervari-Çemikari, Kovanağzı, Sarıyaprak, Kurtalan-Beykent, Eruh-Birini Geçidi bazı bölgeleri, Çırav Dağı bazı kesimleri, Şirvan-Nallıkaya çevresi bazı bölgeleri başlıca çayır vejetasyonunun görüldüğü alanlardır. Sulak alan vejetasyonu Gölköy Gölü, Botan, Kezer ve Başur Çayları'nın kıyılarında görülen vejetasyon tipi olup yoğunluk olarak *Typha domingensis* Pers., *Phragmites australis* (Cav.) Trin. ex Steud., *Lythrum salicaria* L. ve *Ranunculus sphaerospermus* Boiss. & Blanche taksonlarının bulunduğu alanlardır. Özellikle 2000 m'nin üstündeki yüksekliklerde orman bitki örtüsünün bittiği yerlerde *Colchicum szovitsii* Fisch. & C.A.Mey., *Bellevalia paradoxa* (Fisch. & C.A.Mey.) Boiss., *Gladiolus atroviolaceus* Boiss. taksonlarının yoğunlukta olduğu Pervari'nin yüksek dağlık alanları (Herekol dağı, Körkandil Dağı), Şirvan Bekravi Dağı ve Şeytan Kapısı zirveleri, Eruh Çıraf Dağı ve Brini Geçidi üst kısımlarıAlpin vejetasyonun görüldüğü alanlardır. *Tamarix smyrnensis* Bunge, *Juncus inflexus* L. subsp. *inflexus* ve *Cyperus fuscus* L. taksonlarının yoğunlukta yayılış gösterdiği Botan, Kezer ve Başur Çayları'nın kıyılarında kumul alan vejetasyonları gözlenmiştir.

Sonuç olarak, yeteri kadar araştırılmamış bu bakir alanlarda yapılacak yeni floristik çalışmalar ile Siirt ilinin bitki çeşitliliğinin zenginleştirilmesinin yanı sıra Türkiye ve Dünya literatürüne de yeni bitki türlerinin kazandırılabileceği düşünülmektedir. Bu nedenle daha fazla floristik çalışmanın yapılması önem arz etmektedir. Floristik çalışmaların yanında bitkilerin zarar görmesini ve vejetasyon yapısının bozulmasını önlemek için de ilgili paydaşlar ile beraber şehir merkezleri ve kırsal alanlarda biliçlendirme çalışmaları yapılmalıdır.

Teşekkür: Siirt İli'nin karasal ve iç su ekosistemleri biyolojik çeşitlilik envanter ve izlemesi projesi kapsamında arazi çalışmaları için destek sağlayan Tarım ve Orman Bakanlığı'na, arazi çalışmalarında yardımcı olan Siirt İli Doğa Koruma ve Milli Parklar Müdürlüğü ve çalışanlarına teşekkür ederiz.

Etik kurul onayı: Bu çalışma için etik kurul onayı alınmasına gerek yoktur.

Çıkar çatışması: Yazarlar, çıkar çatışması olmadığını beyan etmiştir.

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Research Article / Araştırma Makalesi

Lipid Production by *Yarrowia lipolytica* B9 Using Crude Glycerol as Carbon Source

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Abstract: The present study was carried out to optimize some culture conditions to increase lipid production from *Yarrowia lipolytica* B9 in crude glycerol-based medium. The experiments displayed that too high concentrations of ammonium sulfate, KH_2PO_4, and $MgSO_4$ increased cell growth but inhibited lipid synthesis. In contrast, more synthesis of lipids was determined to be achived at high concentrations of NaCl. The optimum concentrations of glycerol, ammonium sulfate, KH_2PO_4, $MgSO_4$, and NaCl for lipid synthesis were determined as 50, 3, 1.5, 1 and 5 g/L, respectively. The optimal incubation time for lipid synthesis was found to be 6 days. Lipid concentration of 2.69 g/L and lipid content of 60.1%, were reached under optimal culture conditions.

Keywords: Oleaginous, yeast, mineral salt, optimization.

Kaynaklarından Ham Gliserolü Kullanarak *Yarrowia lipolytica* B9 ile Lipit Üretimi

Öz: Mevcut çalışma, ham gliserol bazlı besiyerinde *Yarrowia lipolytica* B9'dan lipit üretimini artırmak için bazı kültür koşullarını optimize etmek amacıyla gerçekleştirilmiştir. İnceleme sonucunda amonyum sülfat, KH_2PO_4 ve $MgSO_4$'ün çok yüksek konsantrasyonlarının hücre büyümesini artırdığı fakat lipit sentezini inhibe ettiği gözlemlendi. Buna ek olarak, daha fazla lipit sentezinin yüksek NaCl konsantrasyonlarında başarıldığı ortaya çıktı. Lipit sentezi için gliserol, amonyum sülfat, KH_2PO_4, $MgSO_4$ ve NaCl'nin optimum konsantrasyonları sırasıyla 50; 3; 1,5; 1 ve 5 gr/lt olarak belirlendi. Lipid sentezi için optimal inkübasyon süresinin 6 gün olduğu bulundu. Optimal kültür koşullarında 2.69 gr/lt lipit konsantrasyonuna ve %60.1 lipit içeriğine ulaşıldı.

Anahtar kelimeler: Yağlı, maya, mineral tuz, optimizasyon.

1. Introduction

Today, microorganisms are used in the production of various substances such as single cell protein, enzyme, recombinant protein, antibiotics, ethanol, glutathione, pigments, organic acids, and polysaccharides. In addition to these valuable products, microorganisms have recently been used in the production of biolipids which are also called single cell oils (Fakas et al., 2009).

The term "oleaginous" is extensively used for microorganisms accumulating lipids over 20% on dry weight basis. Some genera of microalgae, yeasts, molds, and bacteria show oleaginous property; however, most of oleaginous microorganisms belong to the yeast genera such as *Candida, Cryptococcus, Rhodotorula, Lipomyces,* and *Yarrowia* (Taskin et al., 2015; Ortucu et al., 2017). For instance, many investigators have documented that strains of *Yarrowia lipolytica* are good lipid producers (Gao et al., 2017; Dobrowolski et al., 2019).

It has been well documented that some nutritional and environmental culture conditions significantly trigger lipid synthesis in oleaginous yeasts, thereby causing the lipid accumulation over 70% on dry weight basis (Beopoulos et al., 2009; Taskin et al., 2016). For example, low amounts of nitrogen and phosphorus sources but high amounts of carbon sources increase lipogenesis in oleaginous yeasts (Amaretti et al., 2010; Taskin et al., 2016; Wang et al., 2018).

The industrial production of biodiesel is mainly performed from vegetable oils. Recently, lipids of oleaginous microorganisms have also been reported to be used as alternative biodiesel feedstock. However, high cost of carbon sources which are employed for cultivation of oleaginous microorganisms significantly limits economical production of microbial lipids (Meng et al., 2009; Santek et al., 2018). To solve this problem, cheap agricultural byproducts and organic wastes such as molasses, whey, glycerol, and fruit peels have been suggested to be utilized as alternative carbon sources in the production of microbial lipids (Papanikolaou et al., 2007; Fakas et al., 2009; Meng et at., 2009).

Glycerol is a by-product that is released from vegetable and animal oils during the production of biodiesel. It is stated that an average 1 kg of glycerol is produced during the production of per 10 kg biodiesel. Therefore, it is produced in high amounts every year by the biodiesel industry (Papanikolaou & Aggelis, 2010; Anand & Saxena, 2011). In addition to 60-90% glycerol, crude glycerol contains water, methanol, mineral elements, and fatty acids. Although crude glycerol is produced in large quantities, it is not of much medical and industrial importance (Swiatkiewicz & Koreleski, 2009;

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Chen & Walker, 2011; Yang et al., 2012). As an alternative approach, crude glycerol is employed as a carbon source for production of various microbial substances today. For example, many studies have demonstrated that this substance is effectively employed as a carbon source in synthesis of lipids from various yeasts, including *Y. lipolytica* strains (André et al., 2009; Gao et al., 2016; Kumar et al., 2020). Accordingly, this work was conducted to produce lipids from *Y. lipolytica* B9 in crude glycerol-based medium and to optimize some culture conditions for enhancement of lipid production.

2. Material and Methods

2.1. Chemicals and microorganism

Chloroform, methanol, HCl, and mineral salts were purchased from Sigma (USA) but potato dextrose agar (PDA) and potato dextrose broth (PDB) from Merck (Germany). The strain *Yarrowia lipolytica* B9 (Taskin et al., 2015) was selected as the test microorganism for lipid production in glycerol-based medium.

2.2. Preparation of yeast preculture

The yeast was firstly activated on PDA medium at 15°C for 48 h. Then, one loopful of the yeast biomass on PDA was inoculated into 250 mL flask containing 100 mL of PDB medium. After the flask was placed into shaking incubator, it was incubated at 15°C and 200 rpm for 48 h. The prepared preculture was then used for the inoculation of glycerol-based production medium described below.

2.3. Lipid production in glycerol-based medium

Experiments for production of lipids from *Y. lipolytica* B9 were performed in 250 mL flasks containing 100 mL of the production medium that was composed crude glycerol, ammonium sulfate, 0.5 g/L KH_2PO_4, and 0.5 g/L $MgSO_4$ (pH 6.0). The initial experiments were focused on determining the most favorable concentrations of glycerol (20-60 g/L) and ammonium sulfate (2-5 g/L) for lipid synthesis. After determining the most favorable concentrations of glycerol and ammonium sulfate, the effects of different concentrations of KH_2PO_4 (0.5-2.5 g/L), $MgSO_4$ (0.5-2.5 g/L), and NaCl (0-6 g/L) on yeast growth and lipid synthesis were examined. Final experiments were done to elucidate the influence of incubation time (up to 7 days) on lipid synthesis and cell growth. During the optimization experiments, the flasks were inoculated with 2 mL of preculture (OD_{600}=2.0) and they were incubated at 15°C and 200 rpm.

2.4. Analysis of lipid production and cell growth

At the end of the specified incubation time, the yeast cultures were centrifuged at 5000 rpm for 10 min. The supernatant was removed and the precipitated wet cells were dried to constant weight at 70°C. Final weight of dried cells was termed as cell concentration (g/L). Yeast lipids were extracted from dry cells with chloroform-methanol treatment. For this purpose, dried cells (0.1 g) were firstly hydrolyzed with 10 mL of 4 N HCl at 60°C for 2 h in a water bath. The prepared suspension was centrifuged at 5000 rpm for 10 min and the supernatant was discharged (Tasselli et al., 2018). Then, the precipitated cells in the tube were extracted with 5 mL of chloroform-methanol mixture (2V\V). After the suspension was vortexed for 5 min, it was centrifuged at 5000 rpm for 5 min. Afterwards, the supernatant was discharged and 5 mL of chloroform-methanol mixture was re-added into the tube. After chloroform-methanol extraction and centrifugation were applied four cycles, the precipitated wet cells in tubes were dried to constant weight at 70°C. After the final weight of dried cells was subtracted from their initial weight, and the decrease in dry weight was expressed as lipid concentration (g/L). Lipid content was determined according to the following formula. Lipid content (%) = [lipid concentration (g/L) / cell biomass (g/L)] x 100.

2.5. Statistical analysis

Each analysis was performed in three biological and two technical replicates. All the measurements were mean ± standard deviation (±SD) of six determinations (n = 6). Statistical difference was analyzed in the SPSS 15.0 package program using $P < 0.05$ significance level one-way ANOVA.

3. Results

3.1. Influence of ammonium sulfate and crude glycerol concentrations on lipid production

Different concentrations of ammonium sulfate and crude glycerol were tested for enhancement of lipid synthesis in the yeast.

As seen from Table 1, increasing concentrations of ammonium sulfate resulted in a continuous increase in cell biomass. For example, even at the lowest and highest concentrations of glycerol, the maximum cell biomass was measured at a concentration of 5 g/L of ammonium sulfate. On the contrary, excessive concentrations of ammonium sulfate decreased lipid production. For example, when glycerol concentration was kept constant at 20 g/L, maximum lipid synthesis was detected in the presence of 2 g/L ammonium sulfate and higher concentrations of ammonium sulfate decreased lipid synthesis. It was found that crude glycerol concentrations up to 50 g/L increased both cell growth and lipid synthesis. When the production medium contained 50 g/L glycerol, the maximum lipid concentration (1.55 g/L) and lipid content (36.4%) were accomplished at an ammonium sulfate concentration of 3 g/L. On the other hand, it was observed that when crude glycerol concentration was increased to 60 g/L, both lipid synthesis and cell growth slightly reduced. Therefore, subsequent experiments were performed in the culture medium containing 50 g/L glycerol and 3 g/L ammonium sulfate.

Table 1. Effect of glycerol and ammonium sulfate concentrations on cell growth and lipid synthesis in *Yarrowia lipolytica* B9

Crude glycerol (g/L)	Ammonium sulfate (g/L)	Cell mass (g/L)	Lipid concentration (g/L)	Lipid content (%)
20	2	3.22±0.07e	0.86±0.03fg	26.7
	3	3.31±0.09de	0.82±0.04h1	24.7
	4	3.41±0.07d	0.78±0.04h1	22.8
	5	3.56±0.08cd	0.74±0.021	20.7
30	2	3.43±0.1d	0.99±0.05e	28.9
	3	3.57±0.1cd	0.96±0.03e	26.9
	4	3.71±0.12c	0.92±0.03fg	24.8
	5	3.93±0.11bc	0.82±0.05h1	20.9

Crude glycerol (g/L)	Ammonium sulfate (g/L)	Cell mass (g/L)	Lipid concentration (g/L)	Lipid content (%)
40	2	3.72±0.15c	1.15±0.03d	30.9
	3	3.89±0.14bc	1.25±0.04c	32.1
	4	4.11±0.14bc	1.11±0.04d	27.0
	5	4.23±0.13b	0.98±0.05e	23.2
50	2	3.98±0.1bc	1.35±0.06bc	33.9
	3	4.25±0.1b	1.55±0.08a	36.4
	4	4.56±0.12a	1.45±0.05b	31.8
	5	4.72±0.17a	1.33±0.08bc	28.2
60	2	3.81±0.09c	1.25±0.06c	32.8
	3	4.05±0.1bc	1.37±0.06bc	33.8
	4	4.22±0.11b	1.42±0.06b	33.6
	5	4.03±0.13bc	1.19±0.03cd	29.5

3.2. Influence of $MgSO_4$, KH_2PO_4 and NaCl concentrations on cell growth and lipid synthesis in *Yarrowia lipolytica* B9

When experiments were carried out at optimal concentrations of glycerol and ammonium sulfate, maximum values for lipid concentration (1.79 g/L) and lipit content (40.9%) were -reached in the medium containing 1 g/L magnesium sulfate, but higher concentrations of magnesium sulfate gradually reduced lipid synthesis. For example, the highest concentration (2.5 g/L) of magnesium sulfate gave rise to the lowest lipid concentration (1.36 g/L). In contrast to lipid synthesis, increasing concentrations of magnesium sulfate caused continuous increases in cell concentration. For instance, the maximum value (4.72 g/L) for cell concentration was measured at the highest concentration (2.5 g/L) of magnesium sulfate (Fig. 1).

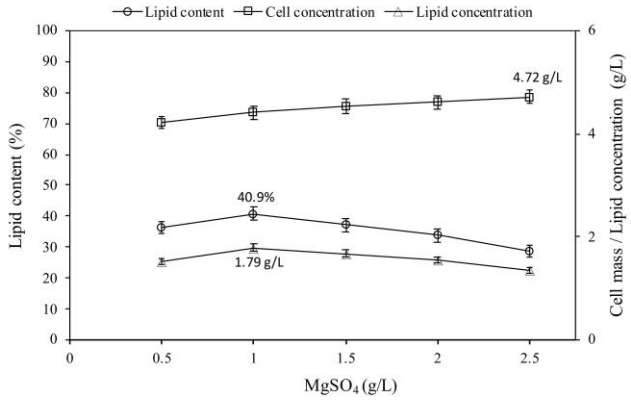

Figure 1. Effect of $MgSO_4$ concentration on cell growth and lipid synthesis in *Yarrowia lipolytica* B9. Culture conditions: Glycerol 50 g/L, ammonium sulfate 3 g/L, KH_2PO_4 0.5 g/L, temperature 15°C, shaking speed 200 rpm, initial pH 6.0 and incubation time 7 days. All the measurements were mean ± standard deviation (±SD) of six determinations (n = 6).

Figure 1 shows that the maximum values for both lipid concentration (2.12 g/L) and lipid content (45.9%) were recorded in the presence of 1.5 g/L KH_2PO_4, while supplementation of KH_2PO_4 over 1.5 g/L significantly decreased lipid synthesis. For example, the lowest values for lipid concentration and lipid contents were measured at the highest concentration (2.5 g/L) of KH_2PO_4. On the contrary, a continuous enhancement in cell concentration occured as KH_2PO_4 concentration was increased. For example, the maximum value (4.81 g/L) for cell concentration was reached at the highest concentration (2.5 g/L) of KH_2PO_4 (Fig. 2).

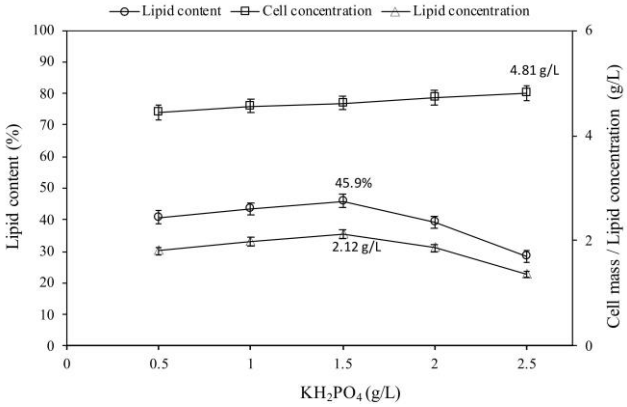

Figure 2. Effect of KH_2PO_4 concentration on cell growth and lipid synthesis in *Yarrowia lipolytica* B9. Culture conditions: Glycerol 50 g/L, ammonium sulfate 3 g/L, $MgSO_4$ 1 g/L, temperature 15°C, shaking speed 200 rpm, initial pH 6.0 and incubation time 7 days. All the measurements were mean ± standard deviation (±SD) of six determinations (n = 6).

Experiments revealed that in comparison with the control medium (without addition of NaCl), the supplementation of NACl upto 3 g/L increased cell concentration. However, NaCl concentrations over 3 g/L caused gradual decreases in cell concentrations. Lipid synthesis increased as NaCl concentration increased and the maximum values for lipid concentration (2.68 g/L) and lipid content (59.7%) were obtained at 5 g/L NaCl concentration (Fig. 3). However, NaCl concentrations above 5 g/L were found to reduce lipid synthesis.

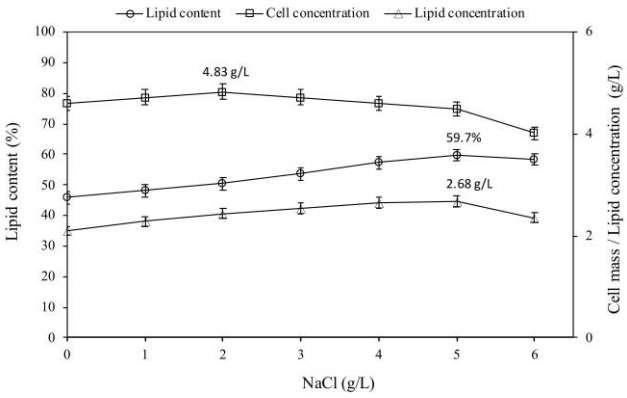

Figure 3. Effect of NaCl concentration on cell growth and lipid synthesis in *Yarrowia lipolytica* B9. Culture conditions: Glycerol 50 g/L, ammonium sulfate 3 g/L, KH_2PO_4 1.5 g/L, $MgSO_4$ 1 g/L, temperature 15°C, shaking speed 200 rpm, initial pH 6.0 and incubation time 7 days. All the measurements were mean ± standard deviation (±SD) of six determinations (n = 6).

As seen from Figure 4, the yeast cells showed the best growth performance in the first three days (especially in the first day) of incubation. The cell concentration reached to maximum value (4.48 g/L) on day 4 and no increase was detected in the following days. Unlike cell growth, no significant lipid accumulation was detected within the first three days and an important increment in lipid synthesis was observed after day 3. Both lipid concentration and lipid content reached to maximum values (respectively 2.69 g/L and 60.1%) at the end of day 6. On the other hand,

both lipid concentration and lipid content showed a small reduction when incubation time was increased from 6 to 7 days (Fig. 4).

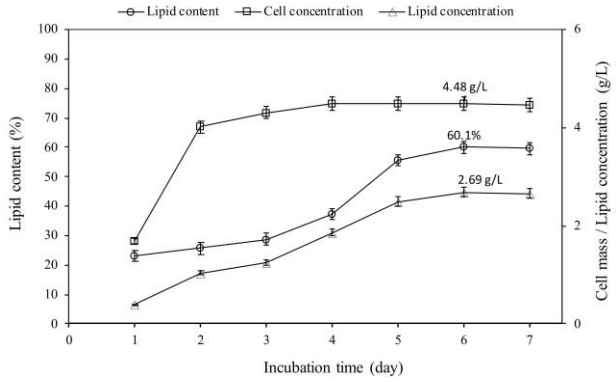

Figure 4. Effect of incubation time on cell growth and lipid synthesis in *Yarrowia lipolytica* B9. Culture conditions: Glycerol 50 g/L, ammonium sulfate 3 g/L, KH_2PO_4 1.5 g/L, $MgSO_4$ 1 g/L, temperature 15°C, shaking speed 200 rpm and initial pH 6.0. All the measurements were mean ± standard deviation (±SD) of six determinations (n = 6).

4. Discussion

It is well known that *Yarrowia lipolytica* strains utilize glycerol as a cheap carbon source for lipid synthesis. Therefore, in this work, crude glycerol was selected as a carbon source for lipid production. In order to increase lipid synthesis, different concentrations of carbon (crude glycerol) and nitrogen (ammonium sulfate) sources were tested primarily. It was determined that low concentrations of ammonium sulfate and high concentrations of glycerol, in other words, nitrogen-limited but carbon excess conditions increased the lipid synthesis in the yeast. This result was similar to those of previous studies (Taskin et al., 2015; Ortucu et al., 2017; Zhang et al., 2019).

The present study revealed that excessive concentrations of $MgSO_4$ and KH_2PO_4 increased cell growth but decreased lipid synthesis. This was mainly attributed to the presence of P and S in these mineral salts. Because, in the literature, it is stated that carbon source is directed to cell growth in the presence of excessive P and S but to lipid synthesis under P and S limited conditions (Bandhu et al., 2014; González-García et al., 2017; Ortucu et al., 2017; Elfeky et al., 2019). The experiments revealed that NaCl concentrations up to 5 g/L significantly stimulated lipid synthesis. This finding is consistent with the fact that NaCl increases lipid accumulation by causing stress in yeasts (Tchakouteu et al., 2017; Guo et al., 2019).

The experiments showed that although cell growth stopped at the end of day 3, lipid synthesis continued until the end of day 6. The result is in parallel with the knowledge that extended incubation times are more favorable for lipid synthesis in oleaginous microorganisms (Ortucu et al., 2017; Abghari & Chen, 2017; Radha et al., 2020; Altun et al., 2020). A small decrease in lipid concentration was observed after day 6. This decrease can be explained by depletion of carbon source (glycerol) in the culture medium. Namely, storage lipids might have been used as carbon source by the yeast since glycerol was exhausted in the medium. The lipid content of the yeast was determined to be 60.1% under optimized culture conditions. This value was higher than the lipid contents of other *Y. lipolytica* strains which were cultivated in glycerol-based medium in the previous studies (Sriwongchai et al., 2013; Rakicka et al., 2015; Sara et al., 2016; Gajdoš et al., 2017; Niehus et al., 2018). Furthermore, it was higher than lipid contents of other oleaginous yeasts in the previous studies (Karatay & Donmez, 2010; Amaretti et al., 2010; Gao et al., 2017; Uprety et al., 2017). Taskin et al. (2015) informed that lipids of *Y. lipolytica* B9 included oleic acid, cis-10-heptadecenoic acid, palmitoleic acid, and palmitic acid but not polyunsaturated fatty acids and therefore could be used as a biodiesel feedstock. It was shown in the current work that lipids of this yeast could be produced using crude glycerol as a cheap carbon source. Since crude glycerol is a waste material that does not find much use today, it is thought that performing lipid production using crude glycerol can contribute to reducing the environmental pollution problem as well as cost of biodisel feedstock.

Ethics committee approval: Ethics committee approval is not required for this study.

Conflict of interest: The authors declare that there is no conflict of interest.

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Research Article / Araştırma Makalesi

Length-Weight Relationship and Condition Factor of *Alburnus sellal* Heckel, 1843 Population in the Tigris River (Şırnak-Turkey)

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Abstract: This study was aimed to investigate the length-weight relationship and condition factor of *Alburnus sellal* population from Güçlükonak to Cizre (Şırnak) location of Tigris River between January and December 2015. A total of 107 specimen of *A. sellal* length-weight relationships were determined as $W=0.0129*L^{2.925}$ ($R^2=0.8803\pm0.115$) in females, $W=0.0076*L^{3.0908}$ ($R^2=0.7033\pm0.151$) in males, and $W=0.0092*L^{3.0363}$ ($R^2=0.8332\pm0.133$) all individuals. The type of growth for all individuals, females, and males were isometric growth ($p>0.05$). The average condition factor was found to be 1.032±0.012 (0.480-1.259) for all individuals, 1.055±0.014 (0.707-1.259) for females, and 0.99±0.020 (0.480-1.217) for males. According to the length-weight relationship and the condition factor of the *A. sellal* population from Güçlükonak to Cizre (Şırnak) location of Tigris River, it can be said that the environment is ecologically sufficient for nutritional development.

Keywords: Growth, sellal bleak, nutrition, performance.

Dicle Nehri'ndeki (Şırnak-Türkiye) *Alburnus sellal* Heckel, 1843 Populasyonunun Boy-Ağırlık İlişkisi ve Kondisyon Faktörü

Öz: Bu çalışmada Dicle Nehri'nin Güçlükonak ile Cizre arasındaki Mevkiinde (Şırnak) Ocak 2015 ile Aralık 2015 tarihleri arasında yakalanan *Alburnus sellal* bireylerinin boy-ağırlık ilişkisi ile kondisyon faktörü araştırılmıştır. İncelenen 107 adet *A. sellal* bireyinin boy-ağırlık ilişkisi dişilerde $W=0.0129*L^{2.925}$ ($R^2=0.8803\pm0.115$), erkeklerde $W=0.0076*L^{3.0908}$ ($R^2=0.7033\pm0.151$) ve tüm bireyler için $W=0.0092*L^{3.0363}$ ($R^2=0.8332\pm0.133$) olarak hesaplanmıştır. Dişi, erkek ve tüm bireylerde izometrik büyüme gösterdiği belirlenmiştir (t-testi, $p>0.05$). Ortalama kondisyon faktörü tüm bireylerde 1.032±0.012 (0.480-1.259), dişilerde 1.055±0.014 (0.707-1.259) ve erkeklerde 0.99±0.020 (0.480-1.217) olarak tespit edilmiştir. Dicle Nehri'nin Güçlükonak'tan Cizre (Şırnak) mevkiine kadar olan bölgeden yakalanan *A. sellal*'in boy-ağırlık ilişkisi ve kondisyon faktörüne göre ortamın ekolojik açıdan besleme gelişimi için yeterli olduğu söylenebilir.

Anahtar kelimeler: Büyüme, inci balığı, beslenme, verim.

1. Giriş

Balıkçılık ve balık biyolojisinde önemli bir veri olan boy-ağırlık ilişkileri, farklı lokalitedeki veya aynı popülasyondaki balık boyundan ağırlığının tahmin edilmesini ve balık büyümesinin karşılaştırılmasını sağlar (Koutrakis & Tsikliras, 2003). Boy-ağırlık ilişkileri tahminleri stok değerlendirmesi ve stok yönetimleri için bilinmesi gereklidir (Pauly, 1983; Petrakis & Stergiou, 1995; Gonçalves et al., 1997; Koutrakis & Tsikliras, 2003; Başusta et al., 2013).

Hızla artan dünya nüfusunun zengin proteine ihtiyacı bulunmaktadır. Proteince de zengin olan balıklar insan gıdası için önemli türlerdir. Asi, Fırat ve Dicle Nehir sisteminde dağılım gösteren gümüş balığı *Alburnus sellal* Heckel, 1843, bununla birlikte Kor, Mahurlu Gölü, Persis ve Hormuz havzalarında da dağılım göstermektedir (Çiçek et al., 2015; Jouladeh-Roudbar et al., 2020). Bu tür ticari bir tür olmamakla birlikte Irak'ta tüketilmektedir (Coad, 2020).

Önceki çalışmalarda *Alburnus mossulensis* ve *A. sellal* olarak tanımlanan ve son revizyon çalışmalarında (Mohammadian-Kalat et al., 2017; Mangıt & Yerli, 2018; Jouladeh-Roudbar et al., 2020) *A. sellal* olduğu belirtilen türle ilgili Türkiye içsularında popülasyon ve ekolojisi üzerine bir çok çalışma (Türkmen & Akyurt, 2000; Başusta, 2000; Yıldırım et al., 2003; Çiçek, 2013; Alkan Uçkun & Gökçe, 2015; Keskin, 2016; Serdar et al., 2017; Parmaksız et al., 2018; Özcan, 2019; Yakut, 2019) (Tablo 1) yapılmasına rağmen Dicle Nehri'nin bu kesiminde boy-ağırlık ilişkileri ve kondisyon faktörünü değerlendiren bir çalışmaya rastlanmamıştır. Bu çalışma ile Türkiye'nin Dicle Nehri (Şırnak) bölgesindeki *A. sellal* türünün boy-ağırlık ilişkisi ve kondisyon faktörü hakkında önemli bilgiler sağlanması amaçlanmaktadır.

2. Materyal ve Metot

Bu araştırma, Dicle Nehri'nin Güçlükonak ile Cizre arasında kalan bölgede (Şırnak/Türkiye) (Şekil 1) Ocak 2015 ile Aralık 2015 tarihleri arasında mevcut populasyonu temsil edecek şekilde aylık olarak gerçekleştirilmiştir. Araştırmada kullanılan *Alburnus sellal* bireylerinin örnekleme işlemi; yöresel balıkçıların bölgede avlamış olduğu balıklar içerisinden rastgele yapılmıştır.

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Tablo 1. *Alburnus sellal* ile ilgili farklı bölgelerde yapılmış çalışmalar

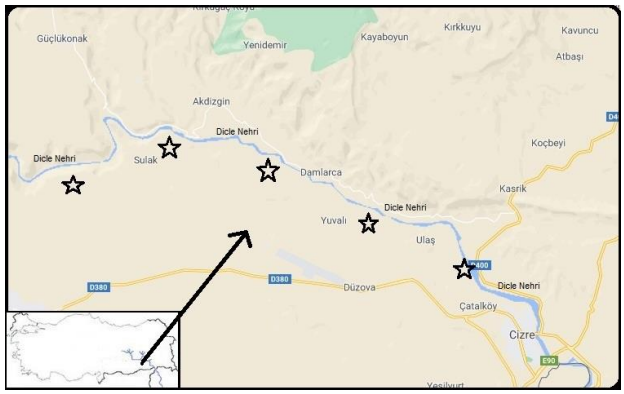
Table 1. Studies on *Alburnus sellal* in different regions

Araştırma Alanı	Araştırıcı	Eşey	Boy	Boy-ağırlık ilişkisi		L_∞	ϕ	Kondisyon
				a	b			
Keban Baraj Gölü	Başusta (2000)	Dişi	12.5-17.0*	0.000004	3.126			0.49-0.89
		Erkek	11.2-16.5	0.000003	3.144			0.51-0.85
Karasu Irmağı	Türkmen & Akyurt, 2000	Dişi	8.5-18.5	0.008	3.082	21.59	1.95	0.56-1.48
		Erkek	8.9-18.1	0.01	2.828	20.41	2.00	0.58-1.55
Karasu Irmağı	Yıldırım et al., 2003	Dişi	19.5	0.0073	3.136	19.88	1.91	0.186
		Erkek	18.9	0.0129	2.913	21.87	1.87	0.16
Dicle Nehri	Çiçek, 2013	Dişi	8.8-20.2	0.000003	3.272	20.97	1.87	0.81-1.54
		Erkek	10.3-16.9	0.00001	2.965	17.55	2.04	0.79-1.47
Karakaya Baraj Gölü	Alkan Uçkun & Gökçe, 2015	Dişi	10.8-18.6	0.206	2.065	15.21	1.73	0.54-1.96
		Erkek	12.5-19.0	0.119	2.138	14.78	2.75	0.51-1.31
Gamsiab Baraj Gölü, İran	Hedayati et al., 2016	Tüm	3.1-11.59*	0.007	3.135			
Aşağı Fırat	Keskin, 2016	Tüm	3.3-16.5*	0.0078	3.032	19.27		0.53-1.10
Karasu Nehri	Serdar et al., 2017	Dişi	7.5-19.9*	0.0131	2.80			
		Erkek	8.0-21.4	0.0125	2.82			
Hamzeh-Ali Bölgesi	Radkhah, 2016	Tüm	1.92-7.41*	0.00006	3.09			
Bibi-Sayyedan Nehri	Keivany et al., 2017	Dişi	4.5-16.80*	0.0061	3.175			0.61-0.84
		Erkek	4.6-14.10*	0.0084	3.0355			0.49-0.98
Atatürk Baraj Gölü	Parmaksız et al., 2018	Dişi	14.0-20.5	-5.55	3.30			0.45-1.48
		Erkek	4.0-20.5	-5.37	3.21			0.19-1.49
Komasi Nehri, İran	Fazlı et al., 2019	Tüm	7.0-11.6					0.99-1.61
Azad Baraj Gölü, İran		Tüm	7.5-17.5	0.00005	2.743	17.03	2.12	0.93-1.82
Murat Nehri	Özcan, 2019	Dişi	8.7-19.9	0.0097	2.812			0.43-0.91
		Erkek	7.2-19.6	0.0168	2.599			0.46-0.98
Keban Baraj Gölü	Yakut, 2019	Dişi	10.4-17.8	0.0018	3.555	22.97	4.15	0.56-1.15
		Erkek	9.5-18.2	0.0042	3.213	22.05	4.35	0.41-1.05
Dicle Nehri	Bu çalışma	Dişi	13.8-19.0	0.0129	2.925	25.30	1.97	0.71-1.26
		Erkek	13.3-19.5	0.0076	3.091	29.99	2.04	0.48-1.22

*Total boy değeri

Alburnus sellal popülasyonun yaşları pullardan yararlanılarak belirlenmiştir. Pullar, yumuşak bir fırça yardımıyla temizlendikten sonra alkol serisinden geçirilerek preparatları hazırlanmıştır. Hazırlanan preparatlar mikroskopta incelenerek yaş tayinleri gerçekleştirilmiştir (Türkmen et al., 2005). Balıkların cinsiyetleri, iç muayene otopsi tekniğine göre makroskobik inceleme ile gonadlarına bakılarak tespit edilmiştir (Timur, 2008).

Elde edilen örneklerin çatal boy değerleri ±1 mm hassasiyetli ölçüm tahtası kullanılarak ağırlıkları ise ±0.01 gram hassasiyetli dijital teraziyle yapılmıştır. Boy-ağırlık ilişkisi parametrelerini hesaplanmada $W=a*L^b$ (Le Cren, 1951) denklemi, kondisyon faktörü hesaplanmasında $K=W/L^3*100$ (Le Cren, 1951) denklemi kullanılmıştır. Burada W: balığın total ağırlığını (g), L: balığın çatal boyunu (cm) ifade eder, a ve b: regresyon parametreleridir. Büyüme tipini belirlemede *b* değerinin 3'ten farklılığı t-testi ile hesaplanmıştır. Boy ve ağırlık bakımından von Bertalanffy büyüme formülü $L_t= L_\infty[1-e^{-k(t-t0)}]$ ile $W_t= W_\infty[1-e^{-k(t-t0)}]^b$ (Von Bertalanffy, 1938) kullanılmıştır. Farklı bölgelerdeki büyüme oranlarını karşılaştırmada ise büyüme performans indeksi (ϕ) $=\log_{10}(k)+2\log_{10}(L_\infty)$ formülünden (Pauly & Munro, 1984) yararlanılmıştır.



Şekil 1. Örnekleme alanı haritası

Figure 1. Sampling area map

3. Bulgular ve Tartışma

İncelenen *Alburnus sellal* bireyleri II-VI yaş grupları arasında dağılım göstermiştir, 0 ve I yaş grubunda herhangi bir bireye rastlanmamıştır.

Alburnus sellal bireylerinin çatal boyları 13.3 ile 19.5 cm (ortalama 16.34±0.121 cm) arasında değişirken, ağırlıkları ise 19.66 ile 83.3 g (46.03±1.22 g) arasında değişim göstermiştir (Tablo 2). İncelenen *A. sellal* bireylerinin %62.62'si dişi ve %37.38'si erkek olmak üzere, dişi:erkek oranı 1:0.60 olarak hesaplanmıştır.

Boy-ağırlık ilişkisi dişi bireyler için $W=0.0129*L^{2.925}$ ($R^2=0.8803\pm0.115$), erkek bireyler için $W=0.0076*L^{3.0908}$ ($R^2=0.7033\pm0.151$) ve tüm bireyler için $W=0.0092*L^{3.0363}$ ($R^2=0.8332\pm0.133$) olarak hesaplanmıştır. Boy-ağırlık ilişkisinin b değerinin dişi (2.925±0.134), erkek (3.091±0.326) ve tüm bireylerde (3.036±0.133) 3'ten farkının önemsiz olduğu gözlemlenmiştir (t-testi, $p>0.05$). Dişi, erkek ve tüm bireylerde izometrik büyüme gösterdiği belirlenmiştir (t-testi, $p>0.05$) (Tablo 2).

Alburnus sellal'in yaş-boy ve yaş-ağırlık değerleri kullanılarak hesaplanan von Bertalanffy büyüme denklemleri dişilerde $L_t= 25.30[1-e^{-0.145(t+5.10)}]$ ile $W_t= 163.95[1-e^{-0.145(t+5.10)}]^{2.925}$, erkeklerde $L_t= 29.99[1-e^{-0.123(t+4.29)}]$ ile $W_t= 279.35[1-e^{-0.123(t+4.29)}]^{3.091}$ ve tüm bireyler için $L_t= 29.02[1-e^{-0.119(t+4.74)}]$ ile $W_t= 253.83[1-e^{-0.119(t+4.74)}]^{3.036}$ olarak bulunmuştur. Büyüme performans indeksi (ϕ) 1.97 (dişi); 2.04 (erkek); 2.00 (tüm bireylerde) olarak hesaplanmıştır (Tablo 2).

Kondisyon faktörü tüm bireylerde 1.032±0.012 (0.480-1.259), dişilerde 1.055±0.014 (0.707-1.259) ve erkeklerde 0.99±0.020 (0.480-1.217) olarak tespit edilmiştir. Yaşlara göre ortalama kondisyon faktörü en düşük II. yaşta (dişi:1.039; erkek:0.969) hesaplanırken, yaşın artması ile ortalama kondisyon faktörü değerleri artarak IV. yaşta en yüksek değere (dişi:1.063; erkek:1.006) ulaşmıştır (Tablo 3).

Tablo 2. *Alburnus sellal*'in boy ve ağırlık parametreleri

Table 2. Height and weight parameters of *Alburnus sellal*

Eşey	n	Çatal Boy (cm)	Ağırlık (g)	Boy-ağırlık ilişkisi parametreleri						
		min-max	Min-max	a	b	b'nin güven sınırı	R^2	$L\infty$	$W\infty$	ϕ
Dişi	67	13.8-19.0	27.2-71.62	0.0129	2.925	2.657-3.193	0.880	25.30	163.95	1.97
Erkek	40	13.3-19.5	19.66-83.3	0.0076	3.091	2.765-3.416	0.703	29.99	279.35	2.04
Tüm	107	13.3-19.5	19.66-83.3	0.0092	3.036	2.773-3.99	0.833	29.02	253.83	2.00

Tablo 3. *Alburnus sellal*'in yaşlara göre kondisyon faktörü

Table 3. Condition factor according to age of *Alburnus sellal*

Yaş Grubu	Dişi		Erkek	
	min-max	Ortalama±SH	min-max	Ortalama±SH
II	0.899-1.183	1.039±0.058	0.827-1.033	0.969±0.024
III	0.707-1.252	1.055±0.018	0.479-1.217	0.989±0.026
IV	0.842-1.259	1.063±0.025	0.929-1.083	1.006±0.077
V	1.131	-	1.123	-
VI	0.911	-		
Toplam	0.707-1.259	1.055±0.014	0.480-1.217	0.99±0.020

4. Tartışma ve Sonuç

Araştırma bölgesinde yapılan örneklemeler sonucunda incelenen *Alburnus sellal* bireyleri II-VI yaş grupları arasında dağılım göstermiştir, 0 ve I yaş grubunda herhangi bir bireye rastlanmamıştır. Ayrıca bu durumun kullanılan ağ materyalinin ağ gözü açıklığından kaynaklandığı tahmin edilmektedir. Farklı çalışmalarda *A. sellal* bireylerinde yaşın; 0-IV (Alkan Uçkun & Gökçe, 2015; Keskin, 2016; Keivany et al., 2017), I-IV (Fazlı et al., 2019), I-VI (Türkmen & Akyurt, 2000), I-VII (Yıldırım et al., 2003; Parmaksız et al., 2018), II-VI (Başusta, 2000) ve III-IX (Çiçek, 2013) arasında dağılım gösterdiği bildirilmiştir. Populasyonların yaş dağlımı aralıkları ile yaşadığı çevrenin beslenme kapasitesi yakından ilişkilidir (Fazlı et al., 2019).

İncelenen *Alburnus sellal* bireylerinin dişi:erkek oranı 1:0.60 olarak hesaplanmıştır. Dişi:Erkek oranları Keban Baraj Gölü'nde 1:0.87 (Başusta, 2000), Dicle Nehri Bismil lokalitesinde 1:0.18 (Çiçek, 2013), Karasu Nehri'nde 1:1.21 (Serdar et al., 2017), Murat Nehri'nde 1:0.79 (Özcan, 2019) ve Keban Baraj Gölü'nde 1:0.82 (Yakut, 2019) olarak bildirilmiştir. Çalışmamızda dişilerin erkeklerden daha fazla sayıda olduğu, bu durumun da Başusta (2000), Çiçek (2013), Özcan (2019) ve Yakut (2019) verileri ile benzer olduğu fakat Serdar et al. (2017) verilerinden ise farklı olduğu görülmüştür.

Ölçümleri yapılan *Alburnus sellal* bireylerinde maksimum çatal boy 19.5 cm olarak bulunmuştur. Başusta (2000), Türkmen & Akyurt (2000), Hedayati et al. (2016), Keskin (2016), Radkhah (2016), Keivany et al. (2017), Fazlı et al. (2019) ve Yakut (2019)'un yaptıkları çalışmalarında elde ettikleri maksimum boy verileri bizim verilerden küçük çıkmıştır. Maksimum boy Serdar et al. (2017) tarafından 21.4 cm ve Parmaksız et al. (2018) tarafından ise 20.5 cm olarak tespit edilmiş olup, bizim verilerden büyük çıkmıştır. Diğer taraftan ise Yıldırım et al. (2003), Çiçek (2013), Alkan Uçkun & Gökçe (2015) ve Özcan (2019)

tarafından bildirilen maksimum boy değerleri bizim verilere benzerdir (Tablo 1).

Alburnus sellal bireylerinin boy-ağırlık ilişkisindeki *b* değeri bakımından izometrik büyüme görüldüğü tespit edilmiştir. Farklı bölgelerde gerçekleştirilen çalışmalardaki (Türkmen & Akyurt, 2000; Keskin, 2016; Radkhah, 2016) *A. sellal* bireylerinin büyüme tipleri izometrik büyüme olup bu çalışmadaki verileri desteklemektedir. Alkan Uçkun & Gökçe (2015), Serdar et al. (2017) ve Fazlı et al. (2019) büyüme tipini negatif allometrik olarak tespit ederken Başusta (2000), Yıldırım et al. (2003), Hedayati et al. (2016), Keivany et al. (2017), Parmaksız et al. (2018) ve Yakut (2019) çalışmalarında pozitif allometrik büyüme görüldüğünü tespit etmişlerdir (Tablo 1). Büyüme tipinde görülen bu farklılık balığın yaşı, cinsi, olgunluk durumu, eşeye, mevsime ve beslenmeye göre değişmektedir (Erkoyuncu, 1995).

Dicle Nehri'nin Güçlükonak ile Cizre arasındaki bölgedeki *Alburnus sellal* bireylerinin sonsuzdaki boyu (L_∞) dişilerde 25.30 cm ve erkeklerde 29.99 cm olup erkek bireylerin L_∞ değeri dişilerden daha yüksek bulunmuştur. Bu durum Yıldırım et al. (2003) tarafından elde edilen verilere benzer olup, Türkmen & Akyurt (2000), Çiçek (2013), Alkan Uçkun & Gökçe (2015) ve Yakut (2019) verilerinden ise farklıdır. Büyüme performans indeksi (ϕ) dişilerde 1.97 ve erkeklerde ise 2.04 olarak tespit edilmiştir. Bizim verilerimiz Türkmen & Akyurt (2000), Yıldırım et al. (2003) ve Çiçek (2013) verilerine benzer olup Alkan Uçkun & Gökçe (2015), Fazlı et al. (2019) ve Yakut (2019) verilerinden ise farklıdır (Tablo 1). Bu durum habitat farklılığı, populasyon yapısı, yaş grupları ve yakalanan örneklerin temsil ettikleri grup sayılarından kaynaklanabilir.

Bu çalışmada kondisyon faktörü dişilerde 0.707 ile 1.259 arasında, erkeklerde ise 0.480 ile 1.217 arasında değiştiği tespit edilmiştir. Kondisyon faktörü bakımından Keskin (2016), Özcan (2019) ve Yakut (2019) çalışmalarındaki verileri bizim verilere benzer olup Başusta (2000) ve Keivany et al. (2017) verileri bizim verilerden düşük olduğu, buna karşın Türkmen & Akyurt (2000), Çiçek (2013), Alkan Uçkun & Gökçe (2015), Parmaksız et al. (2018) ve Fazlı et al. (2019) çalışmalarındaki verilerin bizim değerlerden yüksek olduğu gözlemlenmiştir (Tablo 1). Ayrıca, Dicle Nehri Bismil lokalitesinde Çiçek (2013) tarafından elde edilen kondisyon faktörü (Dişi: 1.1045 ve Erkek: 1.1016) bizim verilerden (Dişi:1.055 ve Erkek:0.99) yüksek değere sahip olması, Bismil lokalitesinin besin çeşitliliği ve yoğunluğu bakımından Güçlükonak lokalitesinden daha iyi durumda olduğu ve bunun sonucunda da daha iyi kondisyon değerlerine sahip olabileceği düşünülmüştür.

Sonuç olarak Dicle Nehri'nin Güçlükonak ile Cizre arasında kalan bölgede (Şırnak) yakalanan *Alburnus sellal* popülasyonu ile büyüme özellikleri ve kondisyon değerleri belirlenmiştir. Mevcut değerler ışığında Dicle Nehri'nin Güçlükonak ile Cizre arasında kalan bölgede popülasyon uzerinde aşırı avcılık baskısının bulunmadığı söylenebilir.

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Research Article / Araştırma Makalesi

Distinguishing the Protected Designation of Origin Apricot (*Prunus armeniaca* L. cv. Şalak) from Closely Related Cultivars by High Resolution Melting

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Abstract: The apricot cultivar *Prunus armeniaca* cv. Şalak (registered as "Iğdır Kayısısı") is a Protected Designation of Origin (PDO) apricot and produced in Aras Basin (Iğdır, Turkey) region. Authenticating the special products, which has adulteration potential, by DNA based methods is reliable and cost-effective. The aim of the current study is to distinguish the PDO apricot from closely related cultivars by High Resolution Melting. We tested 12 SSR markers previously validated for *Prunus* spp. by means of distinguishing the ability of five closely related apricot cultivars that are Şalak (AS), Hasanbey (HB), Hacıhaliloğlu (HH), Kabaaşı (KB), and Şekerpare (SK) produced in Turkey. Capillary electrophoresis validation showed 11 of 12 markers amplified unique fragments for the cultivars. HRM analysis combined with the Principal Component Analysis (PCA) successfully distinguished the PDO AS from closely related cultivars. HRM analysis combined with PCA can be a cost-effective and reliable authenticating method for PDO food products.

Keywords: Genotyping, microsatellites, authenticity, food adulteration, SSR-HRM.

Coğrafi İşarete Sahip Şalak Kayısı Çeşidinin (*Prunus armeniaca* L. cv. Şalak) Yakın İlişkili Çeşitlerde Yüksek Çözünürlüklü Erime Yöntemi ile Ayırt Edilmesi

Öz: Şalak kayısı çeşidi (*Prunus armeniaca* cv. Şalak) Aras Havzası'nda üretimi yapılan ve coğrafi işarete sahip bir kayısı çeşididir. Tescilli ismi Iğdır Kayısısı olarak belirlenmiştir. Özellikle gıda aldatmacasına maruz kalma potansiyeli olan özel gıda ürünlerinin DNA temelli yöntemler ile tanımlanması güvenilir ve görece ucuz maliyetlidir. Bu çalışmanın amacı Şalak kayısı çeşidinin, yakın ilişkili kayısı çeşitlerden Yüksek Çözünürlüklü Erime (HRM) yöntemi kullanarak ayırt edilmesi için bir protokol geliştirmektir. Çalışmada, daha önceden *Prunus* türleri için doğrulanmış 12 adet SSR belirtecinin, Türkiye'de üretimi yapılan Şalak (AS), Hasanbey (HB), Hacıhaliloğlu (HH), Kabaaşı (KB) ve Şekerpare (SK) kayısı çeşitlerini ayırt etme başarısı test edilmiştir. Çalışmada ayrıca HRM verilerinden Temel Bileşenler Analizi yapılabilmesi için R yazılımında kullanılabilecek bir komut dosyası oluşturulmuştur. Kılcal elektroforez ile doğrulanmış 12 SSR belirtecinden 11 tanesinin, her kayısı çeşidi için farklı fragmentler çoğalttığı tespit edilmiştir. Temel Bileşenler Analizi ile desteklenmiş HRM sonuçlarının Şalak kayısı çeşidini diğer çeşitlerden başarılı bir şekilde ayırt ettiği belirlenmiştir.

Anahtar kelimeler: Genotipleme, mikrosatelitler, genetik doğrulama, gıda aldatmacası, SSR-HRM.

1. Introduction

Apricot is an important drupe fruit and it has been cultivated in Asia since 2000 BC. The plant is cultivated in warm to subtropical regions throughout the world. *Prunus armeniaca* L. is the commonly cultivated apricot species and thousands of genotypes are cultivated. Turkey is the leading country in apricot production with 677,000 tons of average production (FAOSTAT, 2020). With this production capability, Turkey supplies approximately 20% of the world's total apricot production. In Turkey, apricot production is specialized in Malatya, Erzincan, and Iğdır regions (Ercişli, 2004). According to the Turkish Statistical Institute 2019 data, Malatya province is leading the apricot production with 391,000 tons in Turkey (Fig. 1) (TÜİK, 2020). Turkey has 28 registered apricot cultivars (Turkish Apricot Research Institute, 2019) and numerous genotypes. Iğdır is also an important province for apricot production. *P. armeniaca* cv. Şalak, which is cultivated in Iğdır province is awarded the mark of Protected Designation of Origin (PDO) and named as "Iğdır Kayısısı" (Iğdır Apricot) by the Turkish Patent and Trademark Office (Registration number 385, dated 17 September 2018).

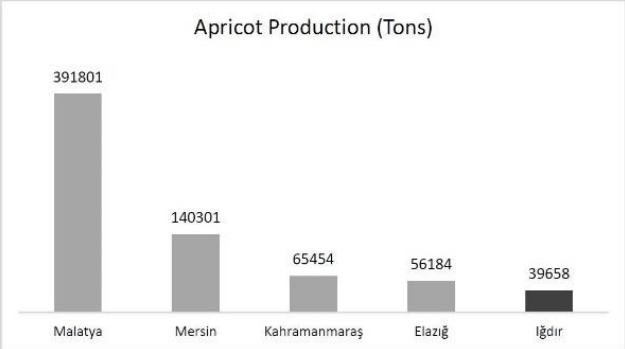

Figure 1. Top five apricot producing regions in Turkey for the 2019 year (TÜİK, 2020).

The PDO products have higher economic value than the non-PDO ones due to their relatively smaller

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production scale and harder production processes, globally. Therefore, it is necessary to ensure that consumers trust PDO authenticity. This causes the emergence of the "traceability of PDO" term. The quantitative traceability of PDO originated food products are important for consumer's protection against food adulteration.

DNA-level tracing of authenticity is quite reliable since it is a stable macromolecule and exists in each tissue and organs and not affected by environmental factors (Li et al., 2018). There are various molecular methods that have the capability of traceability of PDO products such as DNA barcoding, SSR genotyping, and etc. Nevertheless, due to both having specialized downstream processes needed (i.e. bioinformatics skills and computing skills) and being relatively expensive, cheaper and faster methods are needed. High Resolution Melting (HRM) is a technique based on monitoring the DNA denaturation and quantification by a Real-Time PCR instrument. Its advantages can be listed as being cost-effective, having high throughput, requiring less laboratory duty, and no complicated software knowledge needed compared to other marker methods. Another advantage of HRM is detecting the sequence variation without sequencing or hybridization processes (Tindall et al., 2009). Either microsatellite markers or barcoding regions have been employed for HRM analysis successfully (Druml & Cichna-Markl, 2014). HRM was successfully applied to the detection of herbal medicine products (Li et al., 2018), genotyping of peach genotypes (Chou et al., 2020), authenticity testing of sweet cherry products (Ganopoulos et al., 2011), and species identification of ginseng (Osathanunkul & Madesis, 2019). In a previous study, researchers developed novel HRM markers to detect plum pox virus (PPV) resistance by targeting PPV resistance locus in *Prunus armeniaca*. According to the results, PPV resistance locus could be detected by using a reliable and user-friendly method HRM (Passaro et al., 2017).

This study aims to develop an HRM based protocol, which distinguishes the PDO Şalak cultivar from closely related cultivars, and validate the Şalak at the cultivar level.

2. Material and Methods

2.1. Plant materials and DNA extraction

In this study, we chose closely related Turkish apricot cultivars Şalak (AS), Hasanbey (HB), Hacıhaliloğlu (HH), Kabaaşı (KB), and Şekerpare (SK). According to the information we obtained from the local community and Apricot Research Institute authority, those cultivars are rather close in terms of morphology and taste. Therefore, we would like to test the HRM performance on those cultivars. We obtained AS, HB, HH, KB, and SK cultivars from the Republic of Turkey Ministry of Agriculture and Forestry Apricot Research Institute (Malatya, Turkey).

We extracted total DNA from 100 mg leaf tissue of the samples using the modified CTAB protocol as described in the literature (Aydın et al., 2018). We confirmed the DNA concentration and integrity by NanoDrop (Maestrogen) and agarose gel electrophoresis and stored the DNA samples at -20°C.

2.2. Primers mining

Twelve sets of SSR markers that were previously validated for apricot were chosen (Table 1), and checked the amplification success and optimized the PCR conditions for all the primers on each apricot cultivar. The optimum PCR reaction was carried out in a total volume of 20 µl containing 2X Reaction Buffer (Thermo Scientific, USA), 0.1 mM dNTPs, 0.2 µM both primers, 1 U Taq DNA polymerase (Thermo Scientific, USA), 1 mM Mg^{+2}, 10 ng total DNA and nuclease-free water. Thermal cycling (Sensoquest Labcycler Gradient, Germany) condition was 95°C 3 min first denaturation, 35 cycles of 95°C 30 s denaturation, 55°C 30 s annealing, 72°C 1 min extension, and thermal cycling was finalized by 72°C 10 min extension step. PCR products were validated by capillary electrophoresis (CE) (Qiaxcel Advanced, Germany) with QIAxcel DNA High Resolution Kit (Qiagen, Germany). The software settings were used as follows; Process profile: Default High Res v2.0; Method: 0H1200; Size marker: GeneRuler 100bp Plus, Thermo, USA (run by side the samples); Alignment marker: QX 15bp-3kb. The results were visualized and analyzed by the ScreenGel 1.2 software.

Table 1. SSR primers used in the study and specifications. Lower / Upper limits are starting and ending points of melting which is filtered for PCA analysis in the R script.

Primer	Sequence (Forward and Reverse, 5′→3′)	Melting Temperature	Temperature Optimization for Principal Component Analysis		Expected Size (bp)	Reference
			Lower Limit	Upper Limit		
pchgms1	GGGTAAATATGCCCATTGTGCAATC GGATCATTGAACTACGTCAATCCTC	55°C	71.22	78.39	~194	Sosinski et al., 2000
pchgms2	GTCAATGAGTTCAGTGTCTACACTC AATCATAACATCATTCAGCCACTGC	55°C	72.93	79.42	~163	
pchgms4	ATCTTCACAACCCTAATGTC GTGGAGGCAAAAGACTTCAAT	55°C	73.16	80.71	~174	
UDP96-001	AGTTTGATTTTCTGATGCATCC TGCCATAAGGACCGGTATGT	57°C	76.92	82.7	~120	Cipriani et al., 1999
UDP96-003	TTGCTCAAAAGTGTCGTTGC ACACGTAGTGCAACACTGGC	57°C	76.92	82.7	~143	
UDP96-005	GTAACGCTCGCTACCACAAA CCTGCATATCACCACCCAG	57°C	76.92	82.7	~155	
UDP96-010	CCCATGTGTGTCCACATCTC TTGATGATTCCATGCGTCTC	57°C	78.53	82.13	~131	
UDP97-402	TCCCATAACCAAAAAAAACACC TGGAGAAGGGTGGGTACTTG	57°C	71.17	75.49	~136	
UDP98-406	TCGGAAACTGGTAGTATGAACAGA ATGGGTCGTATGCACAGTCA	57°C	72.41	79.69	~101	
UDP98-409	GCTGATGGGTTTTATGGTTTTC CGGACTCTTATCCTCTATCAACA	57°C	74.51	79.51	~129	

Primer	Sequence (Forward and Reverse, 5′→3′)	Melting Temperature	Temperature Optimization for Principal Component Analysis Lower Limit	Upper Limit	Expected Size (bp)	Reference
UDP98-021	AAGCAGCAATTGGCAGAATC GAATATGAGACGGTCCAGAAGC	57°C	72.0	82.04	~145	Testolin et al., 2000
PS12A02	GCCACCAATGGTTCTTCC AGCACCAGATGCACCTGA	60°C	76.86	84.56	~200	Downey & Iezzoni, 2000

2.3. HRM-PCR amplification and Data Analysis

Firstly, normalization the concentration of all the DNA samples was adjusted to 10ng/µl before HRM analysis. HRM amplifications were performed on Rotor-Gene-Q 5plex thermal cycler (Qiagen, Germany) with a 72-well carousel. The HRM mix was prepared as 10 µl total volume consisting of 5µl Luminaris Colour HRM Master Mix (Thermo Scientific, USA), 0.5 µl of 10 mM each primer (Sentebiolab, Turkey), 10 ng DNA, and nuclease-free water to 10 µl. We used a three-step cycling protocol as 95°C 10 min initial denaturation followed by 45 cycles of 95°C 10 s denaturation, 60°C 30 s annealing, and 72°C 30 s extension. Data acquiesced following each extension step. We added 95°C 30 s and 50°C 30 s steps for heteroduplex formation to the end of the cycle. We performed HRM immediately after the amplification in increments of 0.1°C s^{-1} hold time from 65°C to 95°C and data acquiesced continuously. All the reactions were performed as three replicates and no template control (NTC) was included in the reactions.

We analyzed the HRM data using both Rotor-Gene-Q Software (2.3.5). We first normalized the HRM curves by removing the background fluorescence; then, drew difference plots of AS against the other cultivars for each SSR primer. Next, the software calculated Genotype Confidence Percentages (GCPs) for each cultivar against AS by setting each cultivar as "genotype". We set the confidence threshold to 90% for more reliable results.

2.4. Developing R Script for Principal Component Analysis of HRM data

Although there is a well-developed R Script available for HRM-PCA analysis (Chou et al., 2020), we needed to improve it with different libraries to improve visualization performance. The improved script uses the new R Script to visualize the data by Principal Component Analysis (PCA) to be used to help distinguish the cultivars, which HRM failed. Following the HRM reaction, raw HRM temperature and normalized fluorescence data were exported with the Rotor-Gene-Q (2.3.5) software as comma-separated values (csv) file. Clustering and PCA were performed in RStudio (RStudio Team, 2020). The samples were clustered with 'mclust' (Scrucca et al., 2016) and visualized with 'ggplot2' (Wickham, 2016) packages. The script was improved by means of compatibility and better visualization by commonly used and recent packages. The PCA results can be exported as high-quality image formats as well as PDF for better publishing. We also added self-explanatory comments to the script in both English and Turkish to increase usability. The RStudio script is publicly available on GitHub (https://github.com/biologisthurkan/hrm_pca) and in Supplementary Material 1. The script can be used with all the HRM supported devices. A sample data file is also available on both GitHub and in Supplementary Material 2.

3. Results

3.1. DNA extraction and PCR validation of SSR markers by Capillary Electrophoresis (CE)

DNA extraction from each cultivar was successfully performed with the used protocol. The DNA concentration that was obtained varied between 39.32 to 95.06 ng μl^{-1} and A260/230 ratio ranged from 1.234 to 1.766, which is sufficient for HRM analysis. Each SSR marker successfully amplified for five studied apricot cultivars. The CE validation of the markers showed that each marker had different patterns among Turkish apricot cultivars. The PCR fragments sizes and counts generally varied among the cultivars (Table 2). The SSR markers pchgms4 and UDP96-001 amplified only one fragment for all the cultivars while the remaining markers amplified more than one fragment. The longest fragment (347 bp) was amplified by the UDP96-005 marker on KB and the shortest one (96 bp) was amplified by UDP96-010 on HB. According to the fragment sizes, the UDP96-001 marker could distinguish AS, HB, and KB but not HH and SK. We present an example CE comparison electropherogram for the pchgms4 marker which compares the fragment peaks (Fig. 2). According to the peaks in Figure 2 and fragment data in Table 2, cultivars HB and HH have identical fragments (169 bp) and AS and SK also have identical fragments (194 bp). Therefore, based on CE results, the pchgms4 marker could not distinguish the cultivars HB and HH, and AS and SK.

Table 2. SSR fragment analysis by capillary electrophoresis. Amplicon size of each primer per cultivars showed in the table. Cultivar abbreviations AS: Aprikoz Şalak, HB: Hasanbey, HH: Hacıhaliloğlu, KB: Kabaaşı, SK: Şekerpare.

	Amplicon sizes for each cultivar (bp)				
Primer	AS	HB	HH	KB	SK
pchgms1	185	173, 180, 184, 190	180, 189, 193	173, 179, 184, 190	180
pchgms2	159, 170, 172	149, 163, 202	191, 196	165, 170, 181	158, 169, 172, 176
pchgms4	194	196	169	169	194
UDP96-001	137	133	135	198	135
UDP96-003	136	106	102, 106, 108, 116, 126	103, 136, 147	133
UDP96-005	139, 144, 150, 156, 158, 162, 167	125, 138, 144, 176	125, 138, 143, 149, 178	257, 276, 318, 347	126, 159, 164, 177, 179, 182

	Amplicon sizes for each cultivar (bp)				
Primer	AS	HB	HH	KB	SK
UDP96-010	105, 113, 117, 121	96, 105	98, 105	107, 114	98, 105
UDP97-402	146, 152	168, 173	164	165, 170	165
UDP98-406	107	100, 105, 117	105, 108, 119	104, 108	107, 119, 123
UDP98-409	153, 158, 173, 185, 188	168, 173, 182, 187, 190	176, 179, 185, 198, 206	223, 227	150, 155, 159
UDP98-021	141, 143	139, 141	139, 141	117, 138, 143, 147	118
PS12A02	173, 184, 187, 193	169, 172, 176, 178	168, 179, 182, 188	169, 179, 181, 186	176, 181, 187, 193, 196, 199, 201

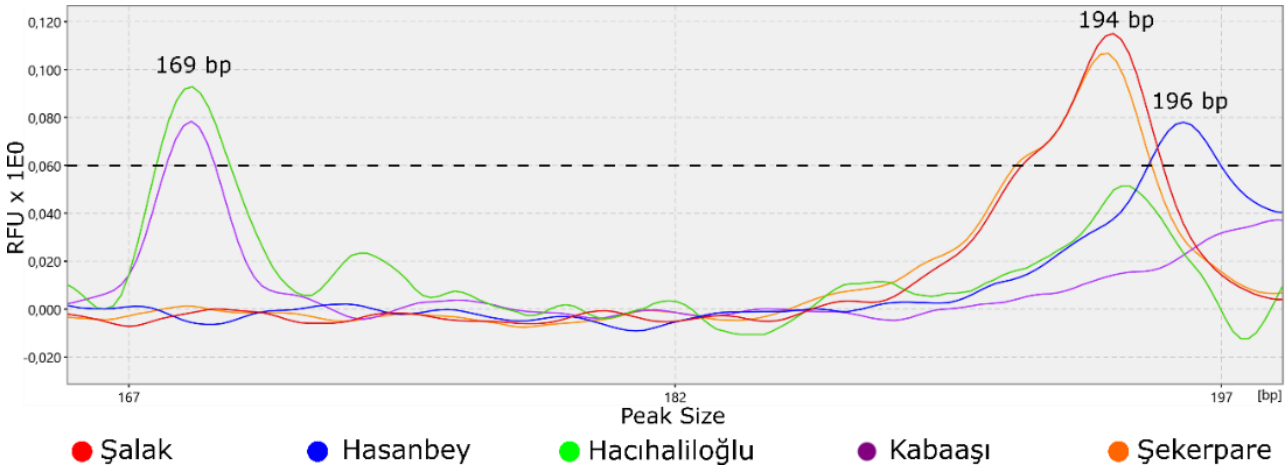

Figure 2. Capillary Electrophoresis comparison electropherogram view of pchgms4 marker for five studied cultivars. Each cultivar is color-coded and peak sizes marked on each peak. Black dashed line represents the threshold for RFU signal.

3.2. HRM Results

The HRM analysis results of five closely related apricot cultivars scanned by 12 SSR markers are shown as difference plot curves (Fig. 3) and as GCP table (Table 3). The plot curves showed that 11 of the 12 SSR markers generated unique HRM curves for AS and distinguished it from the other cultivars. In a detailed look on the pchgms4 marker, which could not distinguish AS and SK cultivars according to the CE results, HRM curves of AS and SK differs from each other. The marker UDP96-001 generated almost identical curves for AS and KB and could not distinguish the cultivars. Auto calling function of the Rotor-Gene Q software calculates an R-value to provide a percentage of confidence. This percentage is used to call other genotypes as the positive control (AS in this study). The GCP lower than 90% were accepted as different genotypes while higher ones were accepted as the same genotype. The GCPs support the distinction among the five cultivars for 11 markers, except UDP96-001. The pchgms4 marker could clearly distinguish AS and SK from each other with 24.34 GCP. For the UDP96-001 marker, the GCP for KB was calculated as 99.73%, which is quite higher than the 90% threshold and supports the plot curve result. Therefore, the UDP96-001 is indeed the only marker that could not distinguish AS from the others. The most distinguishing marker for AS was UDP98-409 based on GCPs.

Generally, HRM performed greater in the study. All the markers we tested except UDP96-001 generated unique melting shapes and this resulted in differentiation of the PDO cultivar. The marker pchgms4, which could not distinguish AS and SK by CE, worked much better on HRM analysis. HRM distinguished AS from SK with 24.34% GCP (Table 3) and drew different melting shape (Fig. 3) even they generated identically sized fragments in CE results.

Table 3. Genotype distinguishing performance of each marker for five studied apricot cultivars. The confidence threshold is 90%. Cultivar abbreviations AS: Aprikoz Şalak, HB: Hasanbey, HH: Hacıhaliloğlu, KB: Kabaaşı, SK: Şekerpare.

pchgms1			pchgms2			pchgms4			UDP96-001		
Cultivar	Genotype	Confidence %	Cultivar	Genotype	Confidence %	Cultivar	Genotype	Confidence %	Cultivar	Genotype	Confidence %
AS	AS	100.00	AS	AS	100.00	AS	AS	100.00	AS	AS	100.00
HB	Variation	32.72	HB	Variation	0.00	HB	Variation	15.25	HB	Variation	10.08
HH	Variation	10.58	HH	Variation	0.06	HH	Variation	62.59	HH	Variation	76.60
KB	Variation	72.04	KB	Variation	0.01	KB	Variation	0.05	KB	AS	99.73
SK	Variation	3.38	SK	Variation	74.04	SK	Variation	24.34	SK	Variation	35.95

UDP96-003			UDP96-005			UDP96-010			UDP97-402		
Cultivar	Genotype	Confidence %	Cultivar	Genotype	Confidence %	Cultivar	Genotype	Confidence %	Cultivar	Genotype	Confidence %
AS	AS	100.00	AS	AS	100.00	AS	AS	100.00	AS	AS	100.00
HB	Variation	51.62	HB	Variation	72.75	HB	Variation	31.64	HB	Variation	50.69
HH	Variation	27.10	HH	Variation	22.19	HH	Variation	15.44	HH	Variation	19.31
KB	Variation	8.06	KB	Variation	46.00	KB	Variation	50.32	KB	Variation	18.03
SK	Variation	6.43	SK	Variation	75.47	SK	Variation	38.35	SK	Variation	9.62
UDP98-406			UDP98-409			UDP98-021			PS12A02		
Cultivar	Genotype	Confidence %	Cultivar	Genotype	Confidence %	Cultivar	Genotype	Confidence %	Cultivar	Genotype	Confidence %
AS	AS	100.00	AS	AS	100.00	AS	AS	100.00	AS	AS	100.00
HB	Variation	11.38	HB	Variation	4.80	HB	Variation	71.30	HB	Variation	72.15
HH	Variation	64.33	HH	Variation	15.19	HH	Variation	22.45	HH	Variation	75.72
KB	Variation	73.80	KB	Variation	4.75	KB	Variation	21.84	KB	Variation	66.76
SK	Variation	68.16	SK	Variation	30.52	SK	Variation	0.63	SK	Variation	30.34

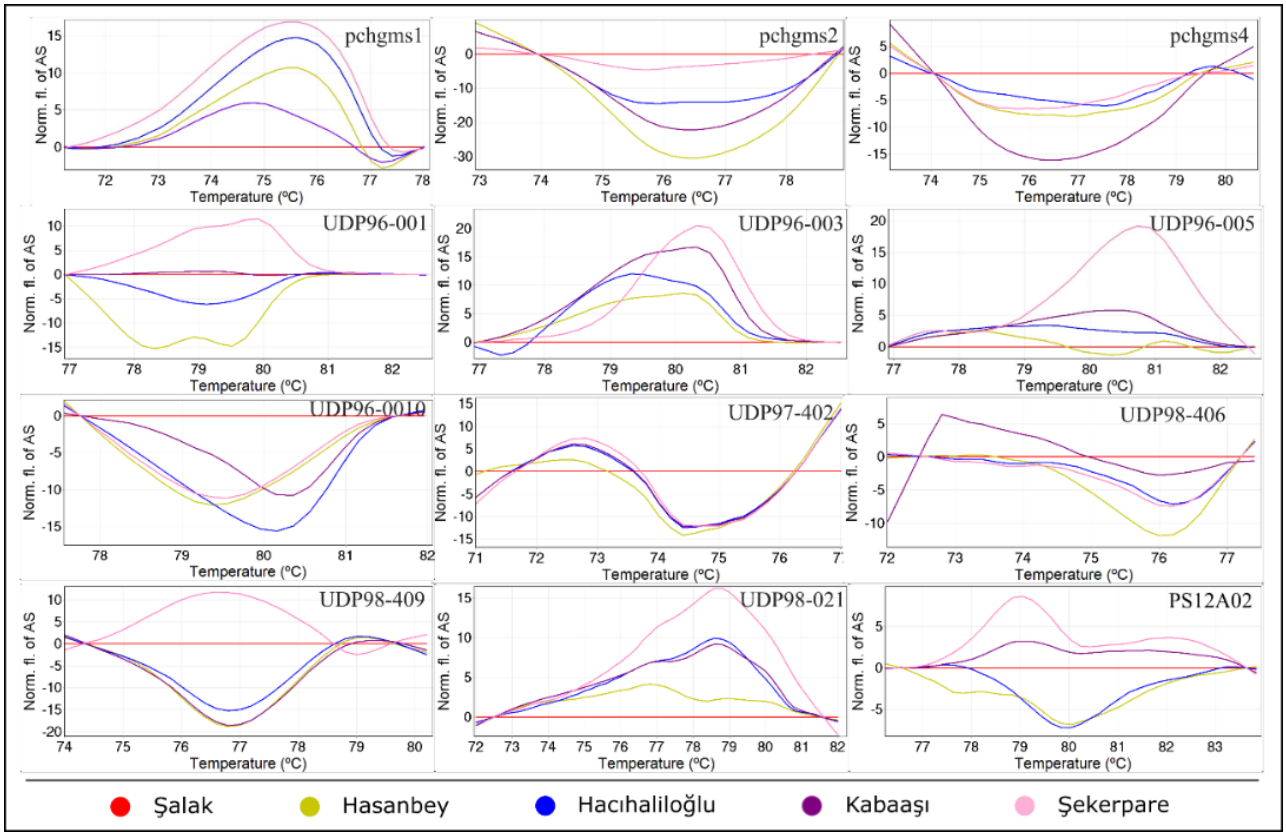

Figure 3. HRM analysis of five closely related apricot cultivars with 12 SSR marker. Difference plot curves were drawn based on AS cultivar.

To deeper analyze the HRM results, we used a third tool PCA clustering the data for failed marker UDP96-001. PCA could successfully cluster AS and KB and distinguished them (Fig. 4). According to the results, we were successfully able to distinguish the PDO AS apricot from closely related cultivars by HRM using 12 markers. Supporting HRM data with PCA resulted better resolution and distinguishing power.

4. Discussion

Distinguishing the special food products from closely related varieties is quite important due to high adulteration potential. "Iğdır Kayısısı" (*Prunus armeniaca* cv. Şalak) is a PDO product special to Iğdır region, which is the fifth ranking apricot producing region in Turkey (TÜİK, 2020). DNA based authentication methods are reliable and provide traceability. DNA barcoding, which uses the nucleotide sequences of short DNA fragments, is a gold standard to distinguish closely related species and reconstructing the phylogeny for many organism groups (Lahaye et al., 2008; Hollingsworth et al., 2011) particularly for animals and most plants (Pentinsaari et al., 2016).

Nevertheless, it is not a convenient method for distinguishing at cultivar-level due to the slow mutation rate. Moreover, DNA barcoding requires experience in bioinformatics and specialization in computer software. SSR markers are more useful for screening genetic differences at the cultivar level since they are more stable and co-dominant transmission and conserved microsatellites are still widely used in taxonomy studies (Tuler et al., 2015). Due to those advantages, we combined the stability of SSR markers and distinguishing power of HRM on five closely related apricot cultivars including the PDO "Iğdır Kayısısı" with 12 potential SSR markers that were validated before for *Prunus* species and cultivars (Cipriani et al., 1999; Downey & Iezzoni 2000; Testolin et al., 2000; Sosinski et al., 2000). This study is the first to use SSR-HRM to detect a PDO apricot and distinguish closely related apricot cultivars.

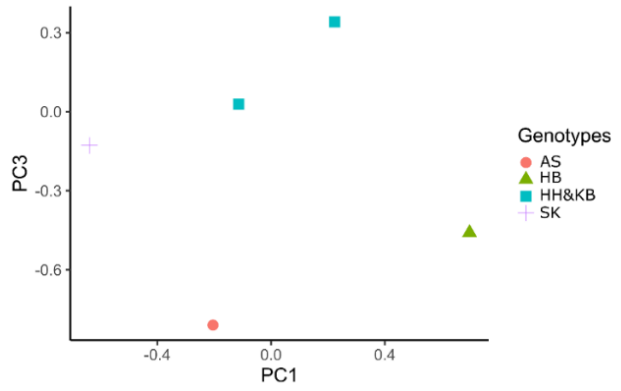

Figure 4. Principal Component Analysis, calculated by the R Script, for UDP96-001 marker distinguishes AS and KB cultivars.

We first validated the SSR markers whether they are successfully amplified on *Prunus armeniaca* cultivars by CE. The obtained fragment sizes are quite similar to the literature (Table 1 and Table 2) except for two markers. The primer pairs of the marker UDP97-402 amplified larger fragments than defined in the literature (expected: ~136 bp,) for HB (168 and 173 bp), HH (164 bp), KB (165 and 170 bp), and SK (165 bp) cultivars. The amplified fragments of the UDP98-409 marker ranging between 150 – 227 bp among cultivars were also larger than reported in the literature (expected fragment size ~129 bp). Due to the nature of microsatellites, it varies by means of length due to repeating regions (Li et al., 2018) thus, microsatellite markers could distinguish the species or cultivars. Although most of the markers generated different fragments for each cultivar, the pchgms4 marker amplified identical fragments for AS and SK (194 bp), and HH and KB (169 bp). Therefore, even scanning by high resolution CE, it is not possible to distinguish those cultivars. HRM generates melting curve shapes by continuously screening the level of fluorescence dye that intercalates with dsDNA during melting, the software can measure the distance between curves. The curve shapes depend on GC content, amplicon size, and the nucleotide sequence (Wittwer 2009). Thus, although the fragment sizes of AS and SK amplified by the primers of pchgms4 marker are identical, different GC content or/and different nucleotide sequence separated AS and SK easily by HRM. HRM behaves quite different for the UDP96-001 marker. According to the CE results, the sizes of the fragments for AS (137 bp) and KB (198 bp) were different but HRM failed to distinguish those cultivars. We investigated the melting curve shape and determined that the shape is almost identical although the signal level is different (Fig. 5). Therefore, the failure of HRM might be because of the identical melting shape.

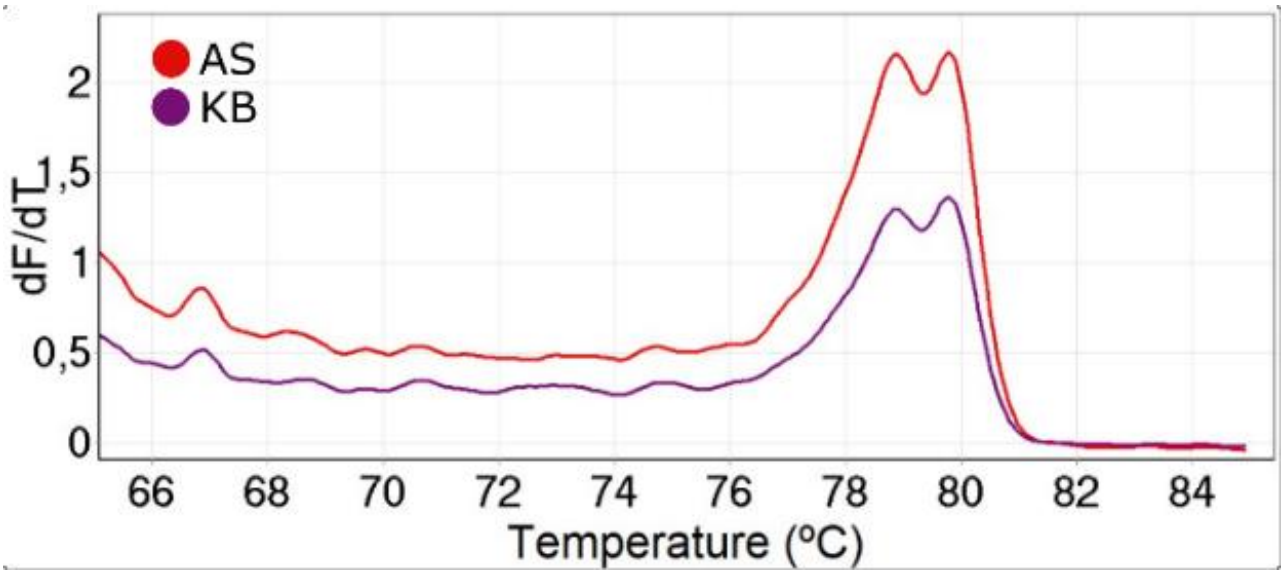

Figure 5. Melting Curve shapes of AS and KB cultivars.

New special food products, such as PDO products, bring new potential adulterants. Therefore, taking advantage of new molecular biology methods would help to manage food safety. In this study, we showed that HRM-SSR is a powerful approach for distinguishing closely related apricot cultivars. Moreover, when HRM failed, the raw HRM fluorescence data can be used on PCA for advanced analysis to distinguish the cultivars.

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Research Article / Araştırma Makalesi

Chemical Compositions, Antioxidant Activities, and Mineral Matter Contents of *Achillea collina* Becker ex Rchb from the Flora of Bulgaria

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Abstract: Medicinal plants are mostly used in pharmaceutical, nutrition, and nutraceutical fields. *Achillea* spp. is widely used as nutraceuticals and food. In this study, chemical composition of essential oils, mineral composition, and antioxidant activities of different parts of *A. collina* were investigated. Flavonoids and polyphenols were detected using total flavonoid and polyphenolic contents assays. The Antioxidant activities were identified using 1,1-Diphenyl-2-picrylhydrazyl (DPPH), Ferric Reducing Antioxidant Power (FRAP), and Trolox Equivalent Antioxidant Capacity (TEAC) assays. The essential oil chemical compositions were determined by Gas Chromatography–Mass Spectrometry (GC-MS) analysis. According to the results, the most active plant part was *A. collina* leaves with a value of IC_{50}=62.32±1.53 µg/mL. The highest total phenolic and flavonoid contents (171.66±0.47 mg GAE /g and 8.50±0.43mg QE/g) were found in leaf parts. The essential oil was only obtained from the flower part of the plant. Besides, the major components found in *A. collina* were 1,8-cineole, camphor, *β*-linalool, *γ*-terpinene, *γ*-terpineol, *α*-pinene, epi-cyclocolorenone, epi-cyclocolorenone, and *p*-cymene. The results revealed that leaf samples of *A. collina* obtained from Bulgarian flora had higher antioxidant effect. The highest macro and microelement contents were Potassium (K)>Calcium (Ca)>Manganese (Mn)>Phosphor (P)>Magnesium (Mg)>Sodium (Na)> Copper (Cu)> Iron (Fe)> Boron (B), respectively.

Keywords: Asteraceae, essential oils, antioxidant activity, mineral matter.

Bulgaristan Florasında Yetişen *Achillea collina* Becker ex Rchb'ın Kimyasal Bileşeni, Antioksidan Özellikleri ve Mineral Madde İçeriği

Öz: Tıbbî bitkiler daha çok ilaç, gıda ve nutrasötik alanlarda kullanılmaktadır. *Achillea* spp. nutrasötikler veya gıdalar halinde yaygın olarak kullanılan bir bitkidir. Bu çalışmada *A. collina*'nın farklı kısımları kullanılarak uçucu yağ bileşenleri, mineral maddeleri, toplam flavonid, toplam fenolik içeriği ve antioksidan aktivitesi belirlenmiştir. Antioksidan aktivitesinin belirlenmesinde; 1,1-Difenil-2-pikrilhidrazil (DPPH), Ferrik İndirgeyici Antioksidan Gücü (FRAP) ve Trolox Eşdeğer Antioksidan Kapasitesi (TEAC) analizleri yapılmıştır. Uçucu yağın kimyasal kompozisyonu Gaz Kromatografisi-Kütle Spektrometrisi (GC-MS) analizi ile belirlenmiştir. Sonuçlara göre *A. collina*' nın bitki kısımlarından en yüksek antioksidan aktivitesi IC_{50}=62.32±1.53 µg/mL değeri ile yapraklarından elde edilmiştir. En yüksek toplam fenolik ve flavonoid içerikleri (171.66±0.47 mg GAE/g ve 8.50±0.43 mg QE/g) yaprak kısımlarında tespit edilmiştir. Uçucu yağ sadece bitkinin çiçek kısmından elde edilmiştir. *A. collina*'da bulunan ana bileşenler 1,8-sineole, kafur, *β*-linalool, *γ*-terpinen, *γ*-terpineol, *α*-pinen, epi-siklokolorenon, epi-siklokolorenon ve *p*-simen olarak tespit edilmiştir. Sonuçlar, Bulgaristan florasından elde edilen *A. collina*' nın yaprak örneklerinin daha yüksek antioksidan etkiye sahip olduğunu ortaya koymuştur. En yüksek makro ve mikro element içerikleri sırasıyla Potasyum (K)>Kalsiyum (Ca)>Mangan (Mn)>Fosfor (P)>Magnezyum (Mg)>Sodyum (Na)>Bakır (Cu)>Demir (Fe)>Bor (B) olarak belirlenmiştir.

Anahtar kelimeler: Asteraceae, uçucu yağlar, antioksidan aktivite, mineral madde.

1. Introduction

The development of modern perceptions about the role of herbal medicine and a healthy lifestyle leads to the rational use of medicinal plants both in Bulgaria and in all developed countries. New health tendencies require the development of national strategies for preserving their diversity and sustainable use. In Bulgaria, the management plan emphasizes its goals on the image and quality management of the Bulgarian flora. The competition on the herbal market in the international plan is high, which requires the rational use of the wide varieties and introduced samples, to create sustainable

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programs for various maintenance and to build zoning points in places suitable for growing medicinal plants (Dzhabarova & Peneva, 2017).

Achillea spp., known as yarrow, is widely used as a medicinal and aromatic plants in the world. There are about 110-140 species in the genus *Achillea* (*Asteraceae*). Most of them are located in Europe, Asia, and North Africa (Konemann, 1999; Oberprieler et al., 2007). Medicinal plants of *A. millefolium* L. group in Bulgarian wild flora are widely used in both traditional medicine and pharmaceutical, cosmetics, and nutritive industries (Edreva et al., 2017).

Achillea species in European folk medicine are widely used in the treatment of inflammatory diseases. *Achillea* ssp. contains flavonoids that increase saliva and stomach acid helping to improve digestion. The flowers are often used to treat hay fever and various allergic mucus problems. The dark blue essential oil is obtained from its flowers and often used as an anti-inflammatory or chest rub for colds and influenza. The leaves encourage clotting so it can be used fresh for nosebleeds (Choudhary et al., 2007; Lakshmi et al., 2011; Ali et al., 2017; Chávez-Silva et al., 2018).

Concerning the bioactivity of *Achillea millefolium*, recent studies reported antioxidant and antimicrobial activities, antiphlogistic, hepatoprotective, gastrointestinal, antispasmodic, diuretic, urinary antiseptic, and calcium antagonist activities of its polar extracts (Stojanović et al., 2005; Georgiev & Stoyanova, 2006; Yaeesh et al., 2006; Ali et al., 2017).

Most of the components obtained from *Achillea* species are highly bioactive (Mockute & Judzentiene, 2010). *A. millefolium* is considered as one of the most important species that is represented with the species of *A. rosea* alba Ehrend., *A. asplenifolia* Vent., *A. setacea* Walds. & Kit., *A. collina* Becker ex Rchb, *A. pratensis* Saukel & Länger, *A. millefolium* L., and *Achillea pannonica* Scheele (Bocevska & Sovova, 2007; Rehus & Neugebauerova, 2011). *A. collina* is a common species for the Central Europe and rich in monoterpenes and sesquiterpenes and more than 120 chemical compounds have been identified (Nemeth, 2005). Various assays have been used in order to evaluate the antioxidant capacity of herbal products and foods (Somogyi et al., 2007; Dzhabarova & Peneva, 2017).

The presence of a wide variety of medicinal plant species and their preservation as an element of traditional homeopathic practices for the prevention and treatment of the local population are the subject of various analyses. Social change, globalization, and the industrialization of agriculture, including the production of aqueous extracts of herbs, increase the need for in-depth knowledge of the composition and possible methods of technological preparation. The increased demand for some valuable medicinal plant species with not widespread distribution leads to targeted cultivation. The incentives offered by several Euro-projects for small and medium-sized businesses to reduce unemployment have also significantly increased the interest of young Bulgarian farmers in herbal production and, through it, the development of alternative forms of tourism as part of industries of national importance (Vitkova et al., 2005; Trendafilova et al., 2006). On the other hand, natural resources particularly the availability of medicinal plant species popularize the regions by creating adequate marketing tools for synergy between natural resources and alternative forms of tourism.

The aim of the present study was to investigate the chemical properties, mineral compositions, and antioxidant capacities of essential oil components obtained by using different parts (leaf and flower) of *Achillea collina* from Bulgaria.

2. Material and Methods

2.1. Material

The plants were collected in October 2016 from the region of Sliven town (42.865844°N 26.133691°E, Bozhevtsi). The plant species was identified by Paisii Hilendarski University of Plovdiv, Faculty of Biology, the Department of Botany and Methods of Biology Teaching. The identification of the species is based on morphological features. The raw material has been submitted to the herbarium of the Bulgarian Academy of Sciences; however, we still have not received the required number. The plants are dried at room temperature (25°C), in the shade away from direct sunlight, until equilibrium humidity is reached (8-10%). The dried raw material is stored in closed paper two-layer bags, in cabinets in the dark and away from heat. Before processing, the raw material is cut to size 1 cm.

2.2. Extraction

The plant (leaf and flower) sample (4 g) were mixed by methanol (40 mL) (1/10 w/v). The prepared samples were incubated for 24 hours at 40°C in an oven (Electo-mag M 5040 P). Then, it was filtered into balloon flasks (Whatman No 1 filter paper). The methanol in the samples was removed with the help of a rotary evaporator (Heating Bath B-491, BUCHI). The balloon bottles, which were blown up, were kept in the oven for 24 hours and completely dried. The extracts were taken into falcon tubes and closed with parafilm and stored at +4°C to be used in the analysis.

2.3. Chemical composition

The plants moisture was determined by drying up to the constant weight at 105°C and the results from the chemical analyses were given on a dry weight (DW) basis. The ash content was determined according to AOAC (2005), by mineralization of the samples at 550°C for 5 hours.

2.3.1. Isolation of essential oil

The essential oil was isolated by hydrodistillation (50 g of flowers) (the ratio of flowers: water = 1:10) for 3 hours using a Clevenger apparatus (Balinova & Diakov, 1974). The essential oil was stored in dark vials at 4°C until analysis. Essential oil could not be obtained from leaf samples.

2.3.2. Chromatography–Mass Spectrometry (GC-MS) analysis

The compounds of the essential oil were detected with gas chromatography (GC) (Agilent 7890A), temperature; 35°C/3 min, 5°C/min to 250°C (3 min), total 49 minutes, HP-5 column MS (30 m x 250 µm x 0.25 µm), 1 mL/min constant speed, 30:1 split ratio. Helium was used as carrier

gas. The GC-MS analysis (Agilent 5975C mass spectrometer) was used the same column and temperature as in the GC analysis. The essential oil components were identified by comparing their relative retention time and library data (NIST 08 database) (Adams, 2007). And then, compounds retention indices (Kovat's) were listed.

2.3.3. Protein content

The total protein content (the samples 1 g each) was determined by the method of AOAC (2016) with a UDK 152 Kjeldahl System.

2.3.4. Cellulose content

The cellulose content (crude fiber) in the leaf and flower samples was determined by the method of Brendel et al. (2000).

2.3.5. Chlorophylls and carotenoid contents

For evaluation of chlorophyll a, chlorophyll b, and the total carotenoids content, 0.5 g of plant samples were homogenized with 10 mL extract (80% alkaline acetone) and stored in the dark at 25°C for 24 hours. Then centrifuged at 1500 g for 10 minutes. Absorbance was measured at 470 nm, 645 nm and 663 nm. Then the results were calculated according to the method proposed by Corte Real et al. (2017):

$$\text{Chlorophyll a (mg/L)} = 9.784*A663-0.990*A645 \quad (1)$$

$$\text{Chlorophyll b (mg/L)} = 21.426*A645-4.650*A663 \quad (2)$$

$$\text{Total carotenoids content (mg/L)} = 4.695*A470-0.268*(\text{chl a} + \text{chl b}) \quad (3)$$

2.3.6. Nutrient contents

Flower plant parts were dried and 0.5 g was weighed. Then, each sample was put into a porcelain crucible. All samples were burned until gray ash (550°C). After burning the 0.5 g weighed samples, the ashes were dissolved in 4 mL 0.1 N HCl and filtered (Whatman No. 1), and completed with distilled water (10 mL) (Kaçar & İnal, 2010). Mineral and heavy metal contents were determined by Yozgat Bozok University, Science and Technology Application and Research Center using iCAP-Qc ICP-MS spectrometer (Thermo Scientific). The analysis was not performed for leaf samples due to the insufficient amount of leaf sample.

2.3.7. Total phenol contents assay

Folin-Ciocalteu Reagent (FCR) method was used to determine the total phenolic content of the 40 mL of methanol extracts (Singleton et al., 1999). For the study, 100 mL of sodium carbonate solution was prepared. To prepare the saturated sodium carbonate solution, 20 g of sodium carbonate was first weighed and 80 mL of hot distilled water was added to it. The lid of this solution was covered by boiling and dissolved thoroughly. After dissolution, the temperature of the solution was cooled down to room temperature. Approximately 7 g of sodium carbonate was added on top and the solution was saturated. The resulting solution was left in the dark for 24 h. Samples were prepared for later analysis. First, 2.4 mL of pure water was placed in glass tubes and 40 µL of extract was added. 40 µL methanol was added to the prepared control groups instead of extracts. Then, 200 µL of folin and 600 µL of saturated sodium carbonate were added to the samples. In the next step, 760 µL of distilled water was added and vortexed for complete mixing of the added chemicals. The prepared samples were incubated at room temperature for 2 h and absorbance measurement was performed at 765 nm. Gallic acid was used for standard phenolic substance control. The values obtained are expressed as gallic acid conjugate. Spectrophotometric measurements to determine the total phenolic content PerkinElmer Lambda 25 UV/VIS made in spectrophotometer device.

2.3.8. Total flavonoids content assay

The total flavonoid compound amounts of the 40 mL methanol extracts were determined by optimizing the aluminum chloride colorimetric method of Biju et al. (2014). 1 mg/mL extract was prepared. Plant extract 50 µL was mixed with methanol 950 µL. Then, 4 ml of distilled water was added and mixed. 0.3 mL sodium nitrate NaNO2 (5%) was added and incubated for 5 min. Then, 0.3 mL of aluminum chloride (10%) was added and incubated for 6 min. After incubation, 2 mL of 1 mol/L sodium hydroxide was added. To the resulting solution, 2.4 mL of distilled water was added and completed to 10 mL, then the solution was incubated for 15 min. Later, absorbance at 510 nm was evaluated. As result of quercetin equivalents (QE)/g of extract was calculated.

2.3.9. DPPH (1,1-diphenyl-2- picrilhydrazyl) free radical assay

The free radical activities of the extracts were determined using DPPH (1,1-diphenyl-2- picrilhydrazyl) free radical, a known, and commonly used radical (Gezer et al., 2006). Firstly, the amount of extract that defines a certain amount of DPPH radical has been determined and a comparison has been made between these samples. 16 mg DPPH radical solution was prepared in 100 mL methanol. The DPPH solution was used in the analysis and was prepared as 0.1 µM. By setting 517 nm in the spectrophotometer, DPPH reading was done and dilution was made with methanol until the absorbance value was 1.000. 1 mg/mL extract solution was prepared as main stock and 6 different concentrations were obtained by dilution. 3 mL samples were taken from each concentration (50, 75, 100, 150, 200, 300) and 1 mL 0.1 µM DPPH was added on top. The reaction mixture was incubated for 30 min in the dark. BHT (butyl hydroxytoluene) and BHA (butyl hydroxyanisol) were used as reference. The DPPH was determined as the inhibition percentage and the following formula is used:

$$\text{Radical scavenging activity DPPH \%} = [\text{A blank} - \text{A sample})/\text{A blank}] \times 100.$$

Spectrophotometric measurements for DPPH radical scavenging activity determination were performed with the aid of PerkinElmer Lambda 25 UV/VIS spectrophotometer device.

2.3.10. Ferric reducing antioxidant power (FRAP) assay

Ferric reducing antioxidant power assay (FRAP) was determined according to the method of Benzie & Strain (1999). Leaf and flower samples (4 g) were extracted with water distilled (40 mL) (temp.: from 80 to 105°C) during 20 min (Fraction I). The crop residues were extracted with distilled water (60 ml) (temperature from 100 to 130°C) during 30 min (Fraction II). Both fractions were filtered when cooled to 25°C (Benzie and Strain, 1999). This analysis evaluated the change in absorbance at 620 nm for

the production of FeII-tripyridyltriazine from oxidised FeIII. The reagent was prepared via mixing 300 mmol/L acetate buffer with 10 mmol/L 2,4,6-tripyridyl-s-triazine with 40 mmol/L HCl and 20 mmol/L ferric chloride at low pH. Trolox was used as standard. Samples were quantified by a spectrophotometer (PerkinElmer Lambda 25 UV/VIS).

2.3.11. Trolox equivalent antioxidant capacity (TEAC) assay

Trolox Equivalent Antioxidant Capacity (TEAC) assay is consisted in the reducing of the absorbance of the $ABTS^{+\bullet}$ at 734 nm (Re et al., 1999). $ABTS^{\bullet+}$ was prepared by reacting ABTS solution with potassium persulfate (2.45 mM). The $ABTS^{\bullet+}$ solution at 734 nm was diluted with phosphate buffer. After addition to the diluted $ABTS^{\bullet+}$ Trolox standard, the mixture was incubated for 15 minutes. Next, inhibition in absorbance at 734 nm was evaluated. samples were examined with a PerkinElmer Lambda 25 UV/VIS spectrophotometer.

2.3.12. Hydroxyl radical scavenging capacity assay

This method was analyzed according to the method of Halliwell and Gutteridge (2007). Hydroxyl radical scavenging capacity was assessed by detecting the ability of sample (flower) extracts to reduce the generation of 2-hydroxy terephthalate which is a strong fluorescent in a reaction between hydroxyl radical and terephthalic acid ($C_8H_6O_4$).

2.3.13. Superoxide scavenging capacity assay

This method was specified as the superoxide radical inhibition caused to be decrescent of nitro blue tetrazolium to formazan (McCord & Fridovich, 1969).

2.4. Statistical analysis

All measurements were carried out in triplicates. The results were expressed as mean ± SD and analyzed using MS-Excel software.

3. Results

Results of the proximate composition of the analyzed *Achillea collina* flower and leaf samples are presented in Table 1. According to the results, the moisture and cellulose content of *A. collina* leaf samples was higher than that obtained for the flower samples. However, the chlorophyll a, b and total carotenoids flower samples were higher than leaf samples. To the best of our knowledge, there were no data in the literature regarding the proximate composition of *A. collina* plant parts from different geographical regions.

Nemeth et al. (2008) reported that the essential oil yield ratio of *A. collina* varied between 0.07-1.77%. According to other studies the yield varied between 0.73%, 0.28-0.63%, and 0.09-0.80% (Spinarová & Petrikova, 2003; Konakchiev et al., 2006). The variations in the obtained results related to the essential oil yield could be due to the ecological and geographical factors in which the samples were obtained (Nemeth, 2005; Bozin et al., 2008).

Kindlovits et al. (2016) reported that the phenol content of *A. collina* ranged from 139 to 220 mg GAE/100 g. The results of the antioxidant activity of *A. collina* are presented in Table 2. IC_{50} value, µg/mL BHA (19.662±0.34) and BHT (13.818±0.50) were used as standards. The antioxidant capacity of leaf samples was higher than the flower samples (Table 2). According to other studies from the literature, the essential oils expressed stronger scavenging effects and IC_{50} value was found to be 0.62 mg/mL for *A. collina* (Bozin et al., 2008). Georgieva et al. (2015) studied the antioxidant activity (DPPH, ABTS, FRAP and CUPRAC assays) of *A. millefolium* (leaves and stems) and reported that the highest free radical scavenging activity was observed against CUPRAC (55.08±0.85 to 148.99±1.94 µM TE/g dw), followed by FRAP (38.16±0.47 to 132.71±1.86 µM TE/g dw), DPPH (24.15±0.15 to 116.74±0.21 µM TE/g dw) and ABTS (18.59±0.22 to 125.75±2.24 µM TE/g dw). Their results revealed that *A. millefolium* may be used as an easily accessible source of natural antioxidants and also as a possible food supplement or in pharmaceutical industry (Ali et al., 2017)

Table 1. The proximate composition of *Achillea collina samples*

Parameters	leaves	flowers
Moisture, %	8.84±0.08	8.32±0.07
Yield of essential oil, % (v/w)	*	0.344±0.0
Protein, %	*	10.57±0.09
Cellulose, %	20.04±1.00	19.45±0.90
Ash, %	*	8.37±0.08
Chlorophyll (a), µg/ g dw	32.25±1.06	42.22±1.05
Chlorophyll (b), µg/ g dw	56.19±5.71	221.35±2.80
Total carotenoids, µg/ g dw	2.80±1.96	16.39±0.80
Total phenol, mg GAE /g	171.66±0.47	137.33±8.73
Total flavonoid, mg QE/g	8.50±0.43	7.39±0.32

* Insufficient sample quantity

Table 2. Antioxidant activity of *Achillea collina*

Methods	leaves	flowers
FRAP assay, µmol/L	710.17±35.19	511.96±39.71
TEAC assay, µmol/L	232.26±31.08	85.43±19.30
DPPH assay (IC_{50} value), µg/mL	62.32±1.53	102.28±1.27
Hydroxyl radical scavenging capacity, mM EtOH/mL	*	22.9±6.1
Superoxide scavenging capacity, unit SOD/mL	*	31.7±7.2

* Insufficient sample quantity

The chemical composition of the *Achillea collina* essential oil is shown in Table 3. According to the results, 66 constituents representing 98.35% of the total oil content were identified in *A. collina* essential oil. As 24 of the essential oil components were with concentrations above 1%. The main compounds (over 3%) were: 1,8-cineole (21.60%), camphor (6.58%), *β*-linalool (5.25%), *γ*-terpinene (4.65%), *γ*-terpineol (4.36%), *a*-pinene (3.96%), epi-cyclocolorenone (3.30%), epi-cyclocolorenone (3.25%), and *p*-cymene (3.03%).

Distribution of major groups of essential oil components are shown in Table 3. Oxygenated monoterpenes (52.80%) are the dominant group in the oil, followed by (oxygenated sesquiterpenes (21.36%), monoterpene hydrocarbons (15.86%), sesquiterpene hydrocarbons (4.73%), phenyl propanoids (3.92%), and oxygenated aliphatics (1.33%).

Table 3. The chemical composition of the essential oil from the flowers of *Achillea collina*

№	RT, min	RI [a]	Compounds	Content (% of TIC[b])
1	9.02	908	Santolina triene	0.39±0.0
2	9.85	923	*a*-Thujene	0.90±0.0
3	10.05	930	*a*-Pinene	3.96±0.03
4	10.57	944	Camphene	0.27±0.0
5	10.70	952	Thuja-2,4(10)-diene	0.36±0.0
6	11.34	970	Sabinene	1.40±0.01
7	11.50	976	*β*-Pinene	2.68±0.02
8	11.70	982	(3*E*)-Octen-2-ol	0.12±0.0
9	11.90	988	*β*-Myrcene	0.30±0.0
10	12.13	997	Yomogi alcohol	0.45±0.0
11	12.79	1013	*a*-Terpinene	0.58±0.0
12	13.07	1018	*p*-Cymene	3.03±0.03
13	13.22	1024	D-Limonene	0.11±0.0
14	13.40	1027	1,8-cineole	21.60±0.20
15	14.13	1052	*γ*-Terpinene	4.65±0.04
16	14.51	1066	(*Z*)-Sabinene hydrate	1.22±0.01
17	14.99	1077	Tolualdehyde	0.39±0.0
18	15.45	1095	*β*-Linalool	5.25±0.05
19	16.28	1110	6-Campholenol	0.26±0.0
20	16.77	1136	(*Z*)-Verbenol	0.75±0.0
21	16.91	1141	Camphor	6.58±0.06
22	17.27	1156	(*Z*)-Chrysanthenol	1.23±0.01
23	17.36	1162	(*E*)-Chrysanthenol	2.74±0.02
24	17.65	1166	Borneol	1.29±0.01
25	17.93	1176	1-Terpinen-4-ol	2.55±0.02
26	18.38	1197	*γ*-Terpineol	4.36±0.04
27	18.66	1203	Verbenone	0.11±0.0
28	19.36	1230	Pulegone	0.33±0.0
29	19.44	1235	Chrysanthenyl acetate	0.21±0.0
30	20.63	1283	(*E*)-*a*-Necrodol acetate	1.15±0.01
31	20.84	1287	Lavandulyl acetate	1.66±0.01
32	20.96	1290	*p*-Cymen-7-ol	0.44±0.0
33	22.72	1324	Myrtenyl acetate	0.19±0.0
34	23.63	1385	*β*-Cubebene	0.26±0.0
35	23.77	1390	*β*-Elemene	0.89±0.0
36	25.06	1441	(*Z*)-β-Farnesene	0.13±0.0
37	25.49	1454	*a*-Caryophyllene	0.24±0.0
38	25.60	1458	allo-Aromadendrene	0.32±0.0
39	25.94	1477	*β*-Chamigrene	0.27±0.0
40	25.97	1479	*a*-Curcumene	0.41±0.0
41	26.17	1482	Germacrene D	0.38±0.0
42	26.35	1488	*β*-Selinene	0.13±0.0
43	26.64	1509	*γ*-Cadinene	0.52±0.0
44	27.02	1523	*δ*-Cadinene	0.63±0.0
45	27.34	1536	*a*-Cadinene	0.29±0.0
46	27.57	1545	*a*-Calacorene	0.18±0.0
47	27.88	1562	(*E*)-Nerolidol	0.56±0.0
48	28.25	1573	Germacrene D-4-ol	0.15±0.0
49	28.37	1577	Spathulenol	0.37±0.0
50	28.46	1579	(*E*)-Sesquisabinene hydrate	2.26±0.02
51	28.58	1581	Caryophyllene oxide	3.25±0.03
52	29.00	1594	Viridiflorol	0.64±0.0
53	29.12	1602	Ledol	0.58±0.0
54	29.29	1630	*γ*-Eudesmol	0.66±0.0
55	29.38	1638	epi-α-Muurolol	0.48±0.0
56	29.53	1652	*a*-Cadinol	1.51±0.01
57	29.76	1665	14-hydroxy-(*Z*)-Caryophyllene	0.60±0.0
58	29.84	1669	14-hydroxy-(*E*)-Caryophyllene	0.85±0.0
59	29.93	1674	(*Z*)-Nerolidyl acetate	2.75±0.02
60	30.25	1683	(2*Z*,6*Z*)-Farnesal	1.28±0.01
61	30.29	1685	Germacra-4(15),5,10(14)-trien-1-α-ol	1.15±0.01
63	31.12	1757	Cyclocolorenone	0.43±0.0
64	32.62	1778	epi-Cyclocolorenone	3.30±0.03
65	34.17	1861	(*Z*,*Z*)-Farnesyl acetone	0.19±0.0
66	36.69	1957	*n*-Hexadecanoic acid	1.18±0.01
Oxygenated aliphatics,%				1.33
Monoterpene hydrocarbons,%				15.86
Oxygenated monoterpenes,%				52.80
Sesquiterpene hydrocarbons,%				4.73
Oxygenated sesquiterpenes,%				21.36
Phenyl propanoids,%				3.92

RI[a] – retention index(Kovat's); TIC[b] – total ion current

Bozin et al. (2008) reported that monoterpene (27.19%), sesquiterpene hydrocarbons (28.02%), oxygenated monoterpenes (20.83%), and proazulenes (19.42%) were determined in *Achillea collina* essential oil. The main components in the essential oil were *β*-pinene (22.52%), chamazulene (19.42%) and *E*-caryophyllene (14.92%). The flowering tops containing essential oil are the most active part of the plant, used mainly for the treatment of influenza, hemorrhage, dysmenorrhea, diarrhea and as a homeostatic agent (Benedek et al., 2008).

Macro and micro element contents have been reported to play important roles in plant growth and development, including cell wall formation, photosynthesis, and respiration. At the same time, these nutrients provide the cofactors needed by numerous enzymes of primary and secondary metabolism. Moreover, the limitation or absence of an element can cause changes in the plant biosynthetic capacity (Figueiredo et al., 2008). Total dry matter, the order of limiting nutrients was K>Ca>P>Mg>Na for macro-elements and Mn>Cu>Fe>B for micro-elements (Figs. 1-2). In addition, the order of heavy metals was Sr>Rb>Ni>Ba>Co>Zn (Fig. 3).

There were data for the mineral content of *Achillea millefolium* in the literature and it contained the highest concentrations of Cu (O'Dell & Claassen, 2015). The level of Ca was reported as low that affected the growth of *A. millefolium* more than other elements. Alvarengaa et al. (2015) reported the macro and micromineral composition content in the following order Zn>Fe>B>Cu>Mn>Mo (for micro-elements) and Ca=K=N>P>S>Mg (for macro-

elements), respectively.

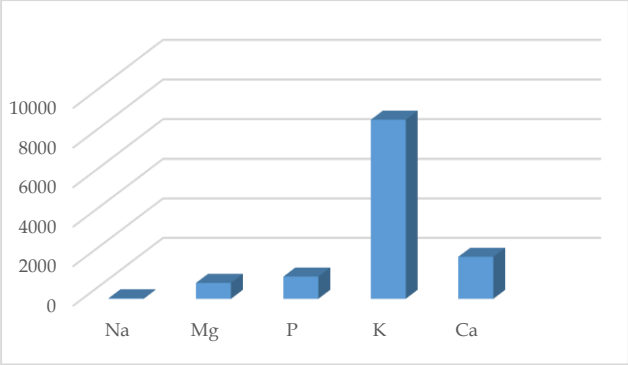

Figure 1. Macro-element content of the flowers of *Achillea collina* (ppm)

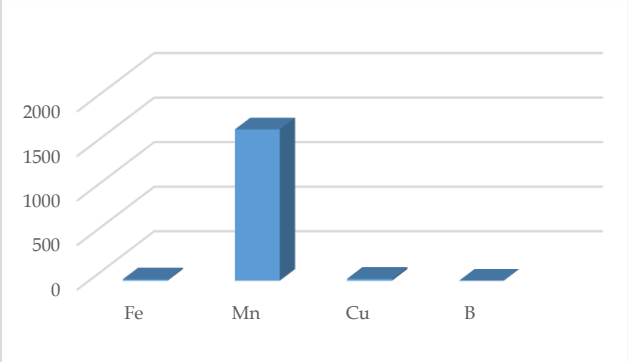

Figure 2. Micro-element content of the flowers of *Achillea collina* (ppm)

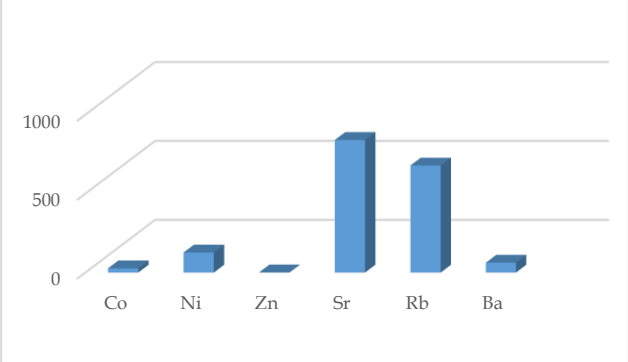

Figure 3. Heavy metals in the flowers of *Achillea collina* (ppm)

4. Conclusion

This study has shown that *Achillea collina* leaf samples obtained from Bulgarian flora has an higher antioxidant effect. Our study provided extensive evidence regarding the antioxidant activity and mineral composition of *A. collina*. The limiting mineral order for total dry matter was as follows K>Ca>P>Mg>Na (for macro elements) and Mn>Cu>Fe>B (for micro elements). *A. collina* EO content was 0.3% and the most abundant chemical compound was 1,8-cineole (21.60%), followed by camphor (6.58%), *β*-linalool (5.25%) and *γ*-terpinene (4.65 %). The most active plant parts were *Achillea collina* leaves with a value of IC_{50}=62.32±1.53 µg/mL. The highest total phenolic and flavonoid contents (171.66±0.47 mg GAE /g and 8.50±0.43mg QE/g) were found in leaf parts.

In recent years, especially in Europe, *Achillea collina* subspecies was the most effective plant group in terms of utilization in pharmacy and food industry. The knowledge of herbal medicine together with the traditions and ecological resources of the regions as well as the effects of the biochemical and photochemical profile of plants could be a subject for further research.

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Research Article / Araştırma Makalesi

Alterations on the Activities of Ion ATPases in the Gill and Muscle of Freshwater Mussel (*Unio tigridis*) Exposed to Copper

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Abstract: Ion ATPases in the tissues of aquatic animals are sensitive to metal exposures. Mussels are filter-feeding animals and have a sedentary lifestyle that makes them good bio-indicator animals. Thus, the present study was carried out to investigate the effects of copper (0, 30, 90 µg/L) on the activities of Na-ATPase, Mg-ATPase, and Ca-ATPase in the gill and muscle of freshwater mussels (*Unio tigridis*) in different exposure durations (0, 7, 14, 21 days). Feeding of mussels during the experiments were done with the cultured unicellular algae (*Cholorella vulgaris*), serving them approximately 300,000 algae/ml. At the end of 21 days of exposure period, no mussel mortality was recorded. Total protein concentrations in the gill and muscle did not change significantly ($P>0.05$) in any exposure groups. Likewise, control ATPase activities did not alter significantly during different exposure periods. However, ATPase activities in the gill and muscle of mussels altered significantly ($P<0.05$) following exposure to copper, especially at the higher concentration. Although there were significant increases and decreases in the activity of Mg-ATPase in both tissues, Na-ATPase and Ca-ATPase activities did not fluctuate as there were only significant decreases at the higher exposure concentration. The present data demonstrated that copper at environmentally realistic concentrations was able to alter the activities of ion ATPases in the gill and muscle of mussels and emphasized osmoregulatory stress which mussels might face in waters contaminated with copper.

Keywords: Metal, mollusk, enzyme, osmoregulation, toxicity.

Bakır Etkisinde Tatlı Su Midyelerinin (*Unio tigridis*) Solungaç ve Kaslarındaki İyon ATPaz Aktivitelerinin Değişimi

Öz: Suda yaşayan hayvanların dokularındaki iyon ATPazlar metal etkilerine karşı hassastır. Midyeler suyu filtre ederek yaşadıklarından ve yerleşik yaşam tarzları nedeniyle iyi biyoindikatör hayvanlar olarak bilinmektedir. Bu nedenle bu çalışma, tatlı su midyelerinin (*Unio tigridis*) solungaç ve kaslarındaki Na-ATPaz, Mg-ATPaz ve Ca-ATPaz aktiviteleri üzerine bakırın farklı derişimlerde (0, 30, 90 µg/L) ve farklı sürelerdeki (0, 7, 14, 21 gün) etkilerini araştırmak amacıyla yapılmıştır. Midyeler deney süresince kültür alglerle (*Cholorella vulgaris*) beslenmişlerdir (300.000 alg/ml). Yirmi bir günlük deney süresi sonunda herhangi bir midye ölümü kaydedilmemiştir. Solungaç ve kastaki toplam protein düzeyleri herhangi bir deney koşulunda istatistiki olarak anlamlı ($P>0.05$) bir değişim göstermemiştir. Benzer şekilde, kontrol ATPaz aktiviteleri de farklı deney süreçlerinde anlamlı bir değişim göstermemiştir. Ancak, midyelerin solungaç ve kaslarındaki ATPaz aktiviteleri, özellikle yüksek bakır etkisinde istatistiki olarak anlamlı ($P<0.05$) azalmalar göstermiştir. Mg-ATPaz aktivitesinde her iki dokuda da anlamlı artışlar ve azalışlar görülmesine rağmen, Na-ATPaz ve Ca-ATPaz aktivitelerinde dalgalanmalar görülmemiştir. Çünkü bu enzimlerin aktivitelerinde sadece anlamlı azalmalar olmuştur. Bu deneyin verileri, çevresel olarak düşük derişimlerdeki bakırın bile midyelerin solungaç ve kaslarındaki iyon ATPazların aktivitelerini değiştirebildiğini ve midyelerin bakırla kontamine olmuş sularda karşılaşabilecekleri osmoregülasyon sistem stresini vurgulamıştır.

Anahtar kelimeler: Metal, mollusca, enzim, osmoregulasyon, toksisite.

1. Introduction

The final stop of all kinds of pollutants released into the environment are usually the aquatic environments. For this reason, monitoring the aquatic environments and the health of organisms living in these environments are important issues that environmental scientists care about. Although metals have been used by humans for many years, their release to the environment in large quantities started with the industrial revolution. This means that metal pollution mostly started with the beginning of the 20th century. Clark (1989) stated that there are some differences between the terms "pollution" and "contamination" of waters. According to this, water pollution is explained as various kinds of damage to aquatic organisms as a result of direct or indirect release of metals to the environment by human activities while contamination is explained as higher metal levels in the water than normal values. In other words, contamination may give a warning signal but for pollution to occur it must have been caused by human activities and adverse effects on organisms must be observed. Since fresh waters have very small volumes compared to the seas, they can be exposed to the contaminants more than the seas, emphasizing the freshwaters are more sensitive ecological environments than seas or oceans.

It is often difficult to directly observe the effect of metal contamination on aquatic environments. For this reason, various enzymatic or non-enzymatic molecules called biomarkers are frequently used to determine the effects of pollutants. Thus, concrete data can be obtained

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about the stress experienced by aquatic organisms and their physiological state (Wood et al., 2012a; 2012b). Mussels are very important indicator organisms and are frequently used to determine environmental pollution or contamination (Nugroho & Frank, 2012; Goswami et al., 2014; Zhou et al., 2021). The most important reasons for this are feeding strategy and sedentary lifestyle of mussels. Since they filter the water, metals in the water can be intensively taken up by mussels and transported through the food-chain, making them very important organisms for biomonitoring of waters. In order to determine the potential effects of pollution, important data can be obtained both in terms of mussel biology and as a food source for humans by performing biomarker analyses in the organs of mussels (Doyotte et al., 1997; Sukhovskaya et al., 2021). For this reason, it has been suggested that the determination of enzyme activities specific to different systems in various organs can be used as an "early warning signal" in terms of very serious damage that may occur later (Canli & Stagg, 1996).

Studies on metal accumulation and toxicity in mussels in natural aquatic environments have demonstrated that metal accumulations in mussels are not at toxic levels, except specimens from industrial areas. Similarly, metal concentrations in tissues of control *Unio tigridis* were found to be very low (Canli et al., 2021), suggesting there was no considerable metal contamination in the natural environment of mussels used in the present study. Laboratory experiments have shown that exposures to metals, including copper, affect the osmoregulation system of mussels, with significant fluctuations in ion ATPase activities (Viarengo et al., 1996; Vijayavel et al., 2007; Chandurvelan et al., 2013; Jorge et al., 2013; Le et al., 2021). These studies emphasized that while metals affected the osmoregulation system in mussels, several other factors such as metal concentration, duration, chemical properties of water, and biology of the species were found to be important. Thus, this study was carried out to investigate the effects of copper on the ion ATPases in the gill and muscle of freshwater mussels *U. tigridis*. For this aim, the osmoregulation enzyme activities such as Na-ATPase, Mg-ATPase, and Ca-ATPase in the gill and muscle were measured following exposure to different copper concentrations (0, 30, 90 µg/L) at different durations (0, 7, 14 and 21 days).

2. Material and Methods

2.1. Experimental animals and exposure conditions

In this study, mussels (*Unio tigridis*) belonging to the family Unionidae were used. Mussels were collected from Gölbaşı lake of Hatay region (36° 30'17.6 "N 36° 29'10.8" E) at depths of 1-6 meters by professional divers. The collected mussels were quickly brought to the laboratory where the experiments were carried out and left for two weeks for adaptation to the new conditions (pH; 8.3±0.08, total hardness; 304.2±21.2 mg $CaCO_3$/L, alkalinity; 195±11.4 mg Ca_2CO_3/L, conductivity; 580±10.8 µS/cm, temperature; 22±1.0°C, oxygen; 6.0±1.0 mg/L, light regime; 12 h light/12 h dark). Physical and chemical quality controls of the exposure waters were done regularly with the Thermo Scientific Orion 5-Star device. All the chemical used in the experiments were purchased from Sigma (Germany) and of analytical purity.

2.2. Algae Culturing

Freshwater algae (*Cholorella vulgaris*) were cultured in the laboratory to feed the mussels both during adaptation and experiments. Algae culture medium (8 L of 3NBBM+V media) were mixed with air continuously and the ambient temperature was kept at 22±2°C, enlighting them with fluorescent lamps (Philips TLM 40W/54RS) using a photoperiod of 16:8 (L:D). The irradiance level was determined using a radiation sensor LI-COR (LI-250). Algae numbers were determined daily by optical density measurements at 680 nm using a UV-vis spectrophotometer (Schimadzu UV-1800) and algae culturing continued for one week until reaching 1513±30 absorbance at 680 nm. These algae were called stock algae and were used in mussel feeding.

2.3. Experiment Protocol

At the end of the adaptation, the mussels were distributed in glass aquariums with 33x33x40 cm dimensions containing 20 L of tap water. Since these distributions were made randomly, there was no significant difference between the groups in terms of weight (30.21±1.33 g) and length (58.65±0.97 mm) of mussels ($P>0.05$). Then, the mussels (12 for each group) were exposed to different concentrations of copper (as $CuSO_4$) (0, 30, 90 µg/L) for different periods (0, 7, 14, 21 days). Preliminary experiments showed that studied copper levels were sublethal for the mussels. During the experiments, copper concentrations in the aquariums were renewed every second day for reasons such as adhesion to the glass surface, evaporation of the water, and precipitation of copper. Mussels were fed with unicellular freshwater algae (*Cholorella vulgaris*) before each water change (approximately 300,000 algae/ml), serving the algae at total darkness for 5 h. At each exposure duration, mussels were taken from the aquariums and checked whether they were alive or not. Mussels were considered alive if their shells were tightly closed. In addition, the viability of the animals was confirmed during the dissection. Then, the mussels were opened by cutting the anterior and posterior adductor muscles which connect the shells and the tissues were dissected out and stored at –85°C (Esco UUS-480A) until they were used.

2.4. Measurements of Ion ATPase Activities

Measurements of ATPase activities in *Unio tigridis* were detailed in our previous paper (Canli & Canli, 2021). According to data, Na-ATPase activity was measured at Na^+ (100 mM), Mg^{2+} (4 mM), ATP (6 mM), pH (7), temperature (37°C), incubation time (30 min), supernatant volume (50 µl). For Ca-ATPase, all the parameters used in Na-ATPase were the same but instead of Na^+, 4 mM Ca^{2+} was added to the medium. Residual activities were accepted as Mg-ATPase activity. Samples were placed in a water bath (37°C) with a shaker (Wise Bath WSB-30) to start the reaction. The reaction was stopped after 30 min with 0.5 ml ice-cold distilled water. Determination of ATPase activity was done by the method of Atkinson et al. (1973). A series of phosphate concentrations (50-600 µM) were prepared to calculate the phosphate liberated from ATP and enzyme activity and the blanks were subtracted from total absorbance obtained at 390 nm. Protein levels were measured by the method of Lowry et al. (1951) using bovine serum as standard. Finally, ATPase activity was

calculated as µmol Pi/mg protein/h.

2.5. Statistical Analysis

Firstly, the distributions of data were determined and appropriate statistical tests were used using a package program (SPSS 20). Nonparametric data were analyzed with Kruskal Wallis and/or Mann-Witney U test and data showing normal distribution were analyzed with One Way Anova and/or t-test. The figures demonstrating the mean and associated standard error of data were drawn with Microsoft Excel Program and statistical significance levels were indicated on the figures.

3. Results

During the experiments, no mortality was observed in the control or copper exposed groups. Experimental results showed that copper exposures caused significant ($P<0.05$) alterations in the osmoregulation system in the gill and muscle of mussels as there were significant decreases or increases in ATPase activities. However, there was no significant change ($P>0.05$) in ATPase activities of controls between 0-21 days. Similarly, no significant difference was observed in the feeding of mussels during the experiments. Activities of Na-ATPase and Mg-ATPase (µmol Pi/mg prot./h) in the gill of controls at day 0 were 5.01±0.49 and 9.98±0.52 respectively while these were 4.75±0.61 and 11.5±0.71 at day 21, respectively. However, ATPase activities in the gill of mussels altered significantly ($P<0.05$) following exposure to copper, especially at the higher concentration. Although there were significant increases and decreases in the activity of Mg-ATPase in in the gill, Na-ATPase activities did not fluctuate as there were only significant decreases at the higher exposure concentration. Total ATPase activity in the gill significantly increased at the lower exposure concentration of copper; although, it decreased at the higher exposure concentration (Figs. 1-3). Similarly, activities of Ca-ATPase and Mg-ATPase (µmol Pi/mg prot./h) in the muscle of controls at day 0 were 2.50±0.53 and 6.51±0.39 respectively while these were 3.01±0.42 and 6.49±0.68 respectively at day 21. Ca-ATPase activity decreased only at the higher copper exposure especially after 14. day while Mg-ATPase activity significantly increased at the lower copper exposure concentration and decreased at the higher exposure concentration. Total ATPase activity in the muscle significantly decreased at the higher exposure concentration (Figs. 4-6). Total protein concentrations in the gill and muscle did not change significantly ($P>0.05$) in any exposure groups (Figs. 7 and 8). Mean algae counts among control and Cu exposed mussels were 292,000±34,000 algae/ml and there was no significant difference ($P>0.05$) among the groups.

4. Discussion

No mussel mortality was observed after exposure to copper for 21 days, indicating that the copper concentrations used were sublethal for the animals. It was shown that similar copper concentrations were also reported to be sublethal for some other mussel species (Rajalakshmi & Mohandas, 2005; Company et al., 2007; Zhou et al., 2021). However, taking the mortality as the end point of a study does not mean that animals are not affected by copper exposures. The present data demonstrated that despite alterations in ATPases activities following copper exposures, there was no significant

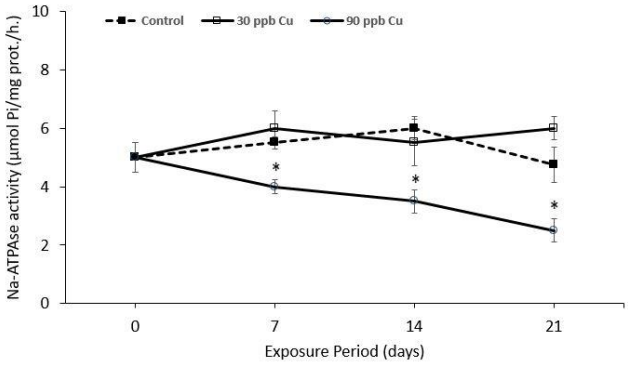

Figure 1. Activities of Na-ATPase in the gill of mussels (*Unio tigridis*) exposed to copper in differing concentrations and durations. Data demonstrate the mean and associated standard errors of 6 measurements. * indicates significant differences ($P<0.05$) among individual control group and its copper exposure groups.

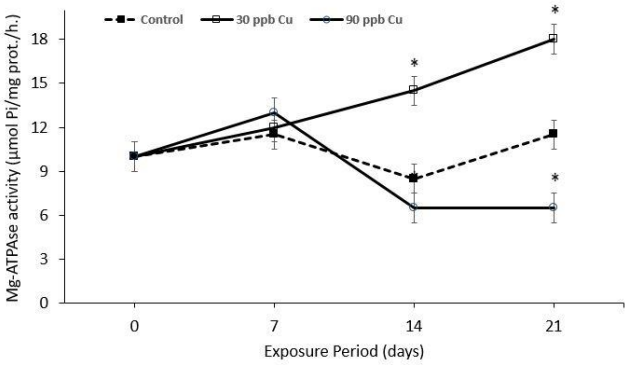

Figure 2. Activities of Mg-ATPase in the gill of mussels (*Unio tigridis*) exposed to copper in differing concentrations and durations. Data demonstrate the mean and associated standard errors of 6 measurements. * indicates significant differences ($P<0.05$) among individual control group and its copper exposure groups.

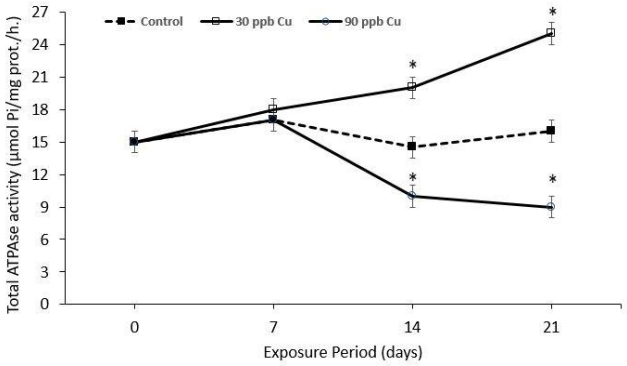

Figure 3. Activities of total ATPase in the gill of mussels (*Unio tigridis*) exposed to copper in differing concentrations and durations. Data demonstrate the mean and associated standard errors of 6 measurements. * indicates significant differences ($P<0.05$) among individual control group and its copper exposure groups.

changes in the appetite of mussels as algae consumption did not differ among exposure groups and controls. This suggests that low copper exposures affect ATPases but not filtration capacity of mussels. However, Jorge et al. (2013) found that filtration rate of juvenile freshwater mussels (*Lampsilis siliquoidea*) altered significantly following chronic Cu exposures. Similarly, Vijayavel et al. (2007) found significant alterations in the filtration rates of in the

green mussel (*Perna viridis*) after exposure to Ag and Cr. There were significant decreases in all ATPase activities in both gill and muscle tissues indicating the metabolism of mussels responded to copper exposures and tried to cope with physiological stress. Nevertheless, alterations in ATPase activities were inevitable after copper exposures. Since freshwater mussels are a good bioindicator animal in the determination of water pollution, they have been frequently used in natural and laboratory studies using stress biomarkers (Doyotte et al., 1997; Falfushynska et al., 2018; Al-Fanharawi et al., 2019). There are some difficulties in doing chronic experiments with mussels. For example, supplying of a suitable food that mussels can filter easily is not easy and should be reproduced in laboratory conditions to prevent feeding experimental mussels with contaminated feed collected from the field.

Literature data demonstrated that ATPase activities in tissues of mussels altered by metal exposures, most important factors being metal types, concentrations, durations and biology of animals. Pagliarani et al. (1996) showed that both the Na,K-ATPase and Na-ATPases in tissues of mussel *(Mytilus galloprovincidis*) showed dose-dependent response to mercury (0.1 and 0.25 mg/L), zinc (0.5 and 1.0 mg/L), and ATPases being more susceptible to metals in the gills than in the mantle. Similarly, Viarengo et al. (1996) carried out an experiment on the same species, assessing in vivo effects of Cu on the Ca homeostasis mechanisms of gill cell plasma membranes after exposure to Cu for 1, 4, and 7 days. The activity of Ca-ATPase and Na,K-ATPase on a plasma membrane-enriched subcellular fraction showed an initial decrease reaching a minimum after four days of metal exposure, followed by a recovery. The authors indicated that the inhibition of the plasma membrane Ca-ATPase activity was a direct evidence of metal effects on Ca homeostasis processes while the inhibition of Na,K-ATPase could also involve an impairment of the Na/Ca antiporter. Toxicity of metals seems more severe in young animals comparing to the older ones. Jorge et al. (2013) studied the effect of chronic Cu exposures on the juvenile freshwater mussels (*Lampsilis siliquoidea*) and found that there were several physiological disturbances during chronic copper exposure, predominant toxicity occurring as a decrease in whole body sodium content paralleled by an inhibition of Na,K-ATPase activity. Similarly, Giacomin et al. (2013) demonstrated that juvenile (6–12 months old) freshwater mussels (*L. siliquoidea*) were very sensitive to Cu exposures as whole-body ion contents (Na, K, Ca and Mg) and enzyme (Na,K-ATPase, H-ATPase, and carbonic anhydrase) activities altered significantly. The effects of Cd exposures in the green-lipped mussel (*Perna canaliculus*) were demonstrated by Chandurvelan et al. (2013) after exposing the animals to acute and chronic exposures. They pointed out that following exposures, the levels of glycogen in the digestive gland and Na,K-ATPase activity in the gill were significantly altered by Cd exposure relative to levels in mussels exposed to Cd-free seawater. The effects of Ag and Cr were remarkable as the activities of Na,K-ATPase, Ca-ATPase, and Mg-ATPase in the mussels (*Perna viridis*) as inhibitions of enzymes were evident (Vijayavel et al., 2007). It is commonly agreed that chronic toxicity of Cu at sublethal levels was associated with ionoregulatory disturbance (Le et al., 2021). The authors indicated that Cu might inhibit Na,K-ATPase activity by reducing the number of functional pump sites and the limited Cu-bound Na,K-ATPase turnover rate.

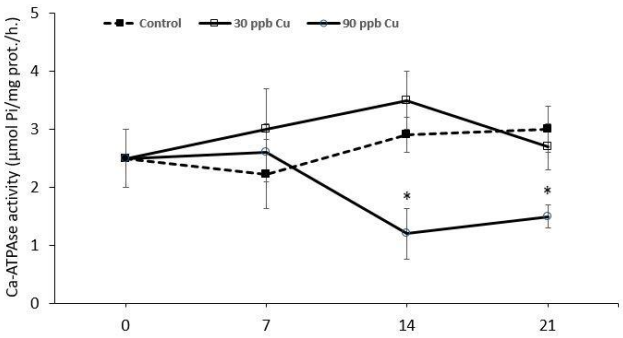

Figure 4. Activities of Ca-ATPase in the muscle of mussels (*Unio tigridis*) exposed to copper in differing concentrations and durations. Data demonstrate the mean and associated standard errors of 6 measurements. * indicates significant differences (P<0.05) among individual control group and its copper exposure groups.

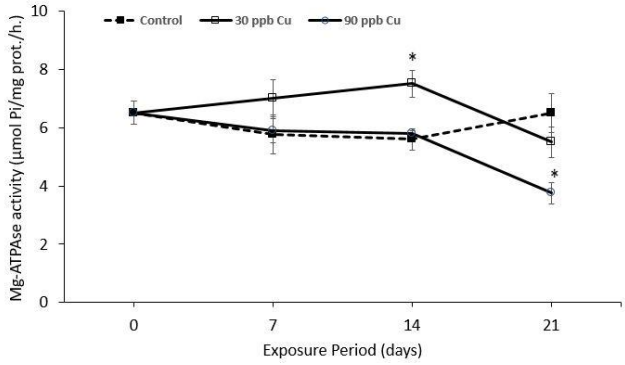

Figure 5. Activities of Mg-ATPase in the muscle of mussels (*Unio tigridis*) exposed to copper in differing concentrations and durations. Data demonstrate the mean and associated standard errors of 6 measurements. * indicates significant differences (P<0.05) among individual control group and its copper exposure groups.

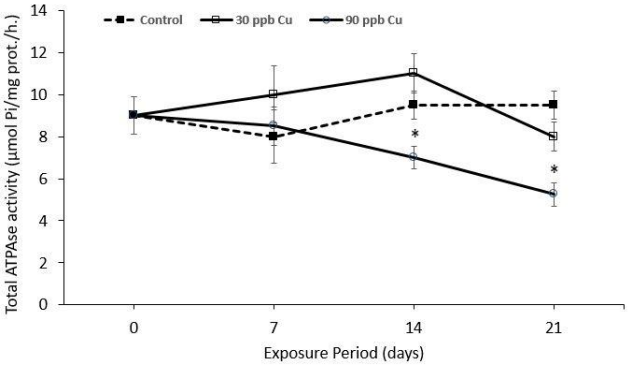

Figure 6. Activities of total ATPase in the muscle of mussels (*Unio tigridis*) exposed to copper in differing concentrations and durations. Data demonstrate the mean and associated standard errors of 6 measurements. * indicates significant differences (P<0.05) among individual control group and its copper exposure groups.

5. Conclusion

The present data demonstrated that ion ATPases of freshwater mussel (*Unio tigridis*) were very sensitive to copper exposures as their activities altered significantly following exposure to sublethal concentrations of copper. Nevertheless, algae filtration capacity of mussels did not

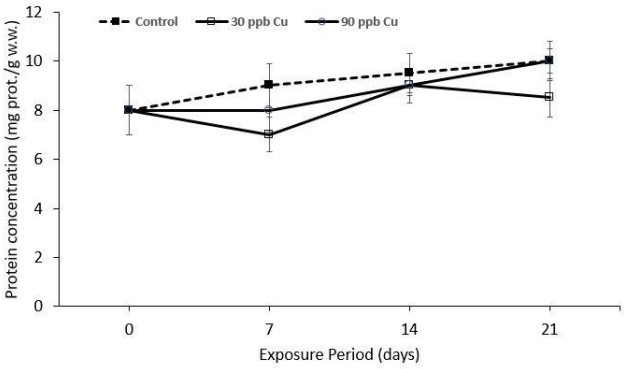

Figure 7. Levels of total protein in the gill of mussels (*Unio tigridis*) exposed to copper in differing concentrations and durations. Data demonstrate the mean and associated standard errors of 6 measurements. No significant difference (P>0.05).

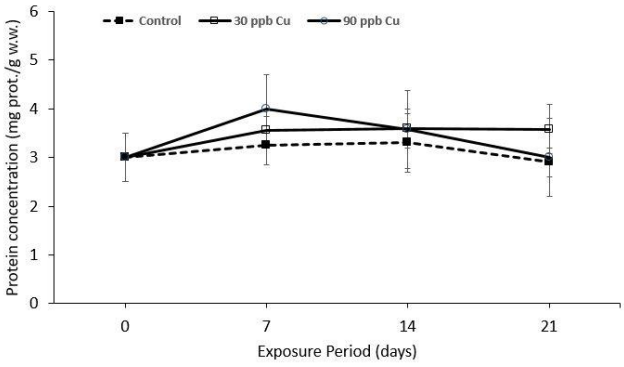

Figure 8. Levels of total protein in the muscle of mussels (*Unio tigridis*) exposed to copper in differing concentrations and durations. Data demonstrate the mean and associated standard errors of 6 measurements. No significant difference (P>0.05).

change significantly after 21 days of exposure durations. This was found to be significant in terms of emphasizing the chronic damage that may occur in mussels exposed to metals in natural aquatic environments. Although metal concentrations are generally at very low levels in many natural aquatic environments, the present study emphasized the environmental awareness once again due to the toxic effects of Cu at very low levels. As a continuation of this study, copper toxicity in mussels can be examined from different aspects such as the transport of metals in the food-chain.

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Ethics committee approval: Ethics committee approval is not required for this study.

Conflict of interest: The author declares that there is no conflict of interest.

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Research Article / Araştırma Makalesi

Determination of toxic effects of deltamethrin on the primary gill cell culture of Lake Van fish [*Alburnus tarichi* (Güldenstadt 1814)]

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Abstract: Lake Van fish is one of few vertebrate species living in the lake, therefore it plays a very useful role as an indicator in determining lake pollution. Deltamethrin (DLT) is one of the pesticides used extensively in the Lake Van basin. In this study, it was aimed to determine the effects of DLT on the primary gill cell culture. Different concentrations of DLT (0.01, 0.1, 1, and 10 µM) were added to primary gill cell culture and the total antioxidant and oxidant levels, malondialdehyde (MDA), and 8-hydroxy-2'-deoxyguanosine (8-OHdG) levels were determined at the end of 24 and 48 h of administration. While the highest concentration of applied DLT (10 µM) caused a significant increase in the total oxidant level at 48 h, a decrease in the total antioxidant level was detected at 24 and 48 h ($P < 0.05$). The increase in MDA level, which is the product of lipid peroxidation of unsaturated fatty acids, with DLT concentrations of 1–10 µM at the end of 24 and 48 h was found to be significant ($P < 0.05$). As a result, it can be said that DLT caused oxidative stress in the primary gill cell culture of Lake Van fish exposed to concentrations of 1 and 10 µM, but no genotoxic effect was observed.

Keywords: Pesticide, Oxidative stress, Lipid peroxidation, DNA damage, Lake Van.

Van Gölü Balıklarının [*Alburnus tarichi* (Güldenstadt 1814)] Birincil Solungaç Hücre Kültüründe Deltametrinin Toksik Etkilerinin Belirlenmesi

Öz: Van Gölü balığı, Van Gölü'nde yaşayan az sayıdaki omurgalı türlerinden biridir bu yüzden gölün kirliliğinin belirlenmesinde çok önemli bir gösterge olarak rol almaktadır. Deltamethrin (DLT), Van Gölü havzasında yaygın olarak kullanılan pestisitlerden biridir. Bu çalışmada, DLT'nin primer solungaç hücre kültürü üzerine etkilerinin belirlenmesi amaçlanmıştır. Primer solungaç hücre kültürüne farklı konsantrasyonlarda (0.01, 0.1, 1 ve 10 µM) eklenen DLT'nin 24 ve 48 saat sonunda toplam antioksidan, oksidan, malondialdehit (MDA) ve 8-hidroksi-2'-deoksiguanozin (8-OHdG) seviyeleri belirlenmiştir. Uygulanan en yüksek DLT konsantrasyonu (10 µM) 48 saatte toplam oksidan seviyesinde önemli bir artışa neden olurken, 24 ve 48 saatte toplam antioksidan seviyesinde bir azalma tespit edildi ($P < 0.05$). Doymamış yağ asitlerinin lipid peroksidasyonunun ürünü olan MDA seviyesindeki artış, 24 ve 48 saat sonunda 1-10 µM DLT konsantrasyonları ile anlamlı bulunmuştur ($P < 0.05$). DLT uygulama konsantrasyonlarının hiçbirinde 8-OHdG seviyesinde herhangi bir değişiklik gözlenmedi ($P > 0.05$). Sonuç olarak, 1 ve 10 µM konsantrasyonlara maruz bırakılan Van Gölü balıklarının primer solungaç hücre kültüründe DLT'nin oksidatif strese neden olduğu ancak herhangi bir genotoksik etkinin gözlenmediği söylenebilir.

Anahtar kelimeler: Pestisit, Oksidatif stres, Lipid peroxidasyon, DNA hasarı, Van Gölü.

1. Introduction

Although pesticides are produced for agricultural pests, they are chemicals that have destructive effects on non-target organisms as a result of water, food, and different contaminations. Deltamethrin (DLT) [(S)-α-cyano-3-phenoxybenzyl-(S)-2-(4-chlorophenyl)-3-methyl butyrate], which has been produced synthetically since 1974 to control pests in agricultural production, is widely used worldwide as a pyrethroid insecticide (Lu et al., 2019). Like other pyrethroids, it has highly toxic effects on fish and other aquatic organisms. In particular, these pyrethroids have a median lethal dose (LD_{50}) of 100 to 1000 times lower in fish than in mammals and birds (Bradbury & Coats, 1989; Paul & Simonin, 2006). These pyrethroids have been reported to have adverse effects on fish reproduction, development, behavioral physiology, and different organs (Köprücü & Aydın, 2004; Cengiz, 2006; Benli et al., 2009). It has been reported that enzymes that are effective in pesticide detoxification, especially in fish liver, are quite low when compared to those in mice (Glickman & Lech, 1982; Wheelock et al., 2005). DLT can accumulate in sediment, benthic organisms, and food particles due to its lipophilicity. There have been studies showing that it causes toxicity in many fish species (Yildirim et al., 2006; Amin & Hashem, 2012; Hedayati & Tarkhani, 2014; Petrovici et al., 2020). Reactive oxygen species (ROS) can react with biological macromolecules, such as DNA, lipids, and proteins, in living things. As a result of this reaction, it can cause cell death. If ROS cannot be removed from the cells by the antioxidant defense system, it causes oxidative stress. It is known that pesticides cause a great deal of damage in living creatures by increasing ROS (Bagchi et al., 1995; Monteiro et al., 2006). Animal cell culture is the process of cells continuing to live outside of the body under certain conditions. Studies with cell cultures constitute an important part of popular research topics today. Cell cultures are conducted to determine the effects of a particular substance or the functions of a certain biological molecule or molecules produced in a cell or tissue. Various studies can be

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performed on cells propagated from a specific cell line in cell culture or in primary cultures made from a specific organ and trials that cannot be done *in vivo* can be made and results can be obtained. It has many advantages over *in vivo* studies. One of the most important of these advantages is that it minimizes the number of living specimens used in *in vivo* studies (Freshney, 2015). Lake Van is located in the eastern Anatolian region of Turkey and is also among the largest soda lakes in the world. Only one vertebrate species, the Lake Van fish, has adapted to the extreme characteristics of the lake. Although attempts have been made to adapt other fish species to the extreme conditions of the lake, they were not successful (Danulat & Selçuk, 1992). There has been a decrease in the population level of Lake Van fish in recent years (Freyhof, 2014). Although overfishing and habitat destruction have been shown as the reason for the decrease in the population level, the effect of the increasing pollution in the lake cannot be ignored. Lake Van fish is the only vertebrate species living in the lake; thus, it plays a very useful role as an indicator in determining lake pollution. *In vitro* studies are more advantageous than *in vivo* studies as they use fewer living things and create a more controllable environment. In this study, changes in the total antioxidant status (TAS), total oxidant status (TOS), malondialdehyde (MDA), and 8-hydroxy-2'-deoxyguanosine (8-OHdG) levels in Lake Van fish primary gill cell culture of DLT, which is one of the pesticides used extensively in agricultural production in the Lake Van basin, were investigated.

2. Material and Methods

2.1. Fish

The living fish (3+ years old) used in this study were caught using sprinkle nets from the Karasu Stream that pours into Lake Van during the breeding season (Figs. 1A & 1B). The fish were adapted for seven days by attaching an air stone to fiberglass tanks with 300 L of rested chlorine-free tap water (temperature: 13.1±2°C, pH of 8.57±0.4, dissolved oxygen: 6.41±0.14 mg/L, oxygen saturation: 61.1% L, conductivity: 731 mS/cm, salinity: 0.47%). During the experiment, they were fed commercial trout feed once a day. Animal experimental procedures were conducted in accordance with the animal study protocols (2018/5) approved by the Van Yüzüncü Yıl University Animal Experiments Local Ethics Committee.

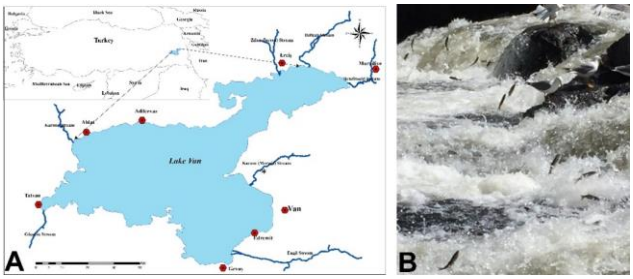

Figure 1. Karasu stream flowing into the lake where the Lake Van fish were caught using a sprinkle net (A) and breeding migration of the fish (B).

2.2. Primary gill cell isolation and cell culture

In order to prevent the study from being affected by anesthetic agents, the fish were euthanized and decapitation was performed. The gill springs were removed and placed into phosphate-buffered saline (PBS) (136.9 mM NaCl, 8.06 mM Na_2PO_4, 2.68 mM KCl, 1.47 mM KH_2PO_4). The gill filaments were cut into small pieces using a scalpel. They were then treated with PBS containing antibiotics and antimycotic (18°C, 10 min). The filaments were placed in a solution containing trypsin enzyme and kept in a shaker for 20 min (this process was repeated twice). The cell suspension was then filtered through an 80-µm filter into PBS containing fetal bovine serum (FBS). The suspension was centrifuged at 250 *Xg*. Cells were washed with PBS containing 2% FBS and centrifuged at 250 *Xg* for 8 min. Next, the cells were suspended in 5% FBS and medium containing antibiotic-antimycotic and; then, they were placed into appropriate cell culture dishes (Part et al., 1993). Dead-alive cell count was performed under a microscope by staining the isolated cell suspension with trypan blue. After it was determined that more than 90% of the cells were viable, they were placed in poly-L-lysine-coated 48-well cell dishes. The pH change and microbial contamination in the cell culture dishes were observed via microscopic observations during the experimental period. DLT (0.01, 0.1, 1, and 10 µM) was added onto the cells. The proportion of solvent used in the culture medium was set not to exceed 1%.

2.3. Determination of TAS and TOS levels

The TAS and TOS levels, developed by Erel (2004), were determined by mass using the Rel Diagnostics Assay kit (Mega Medicine, Gaziantep, Turkey). According to the method used for the TAS measurement, the antioxidant effect of the sample against strong free radical reactions of the generated hydroxyl radical was measured. Results were expressed in µmol Trolox Eq/L (Erel, 2004). According to the method used for the TOS measurement, the total amount of oxidant molecules in the sample was related to the color density that could be measured spectrophotometrically. Calibration of the test was done using hydrogen peroxide (H_2O_2). Results were expressed as micromolar hydrogen peroxide equivalent per liter (µmol H_2O_2 Eq/L) (Erel, 2005).

2.4. Determination of MDA concentrations

MDA is a product of lipid peroxidation (LPO) which was measured in terms of the MDA concentration using the thiobarbituric acid reaction (John & Steven, 1978)

2.5. 8-OHdG assay

DNA damage in gill cells was determined using ready-made commercial ELISA kits for 8-OHdG content. Samples taken at the end of 24 and 48 h were extracted and evaluated according to the commercial kit procedures. Finally, the absorbance values of the samples were measured at 450 nm in a microplate reader. Values were calculated using the graphics obtained from the standards in the kit (Bioassay Technology Laboratory, Shanghai, China). The 8-OHdG content was expressed as ng/mL.

2.6. Statistical analysis

The data were expressed as the mean±standard error of the mean (SEM). Raw data were analyzed by using statistical package software (SPSS 11.5, IBM Corp., Armonk, NY, USA). Between group comparisons were performed with a one-way ANOVA and Duncan's multiple comparison

tests. Significance was accepted at $P < 0.05$.

3. Results

As a result of the primary gill cell isolation of Lake Van fish, it was determined that the gill cell morphology was round in appearance under the microscope. Erythrocytes were observed in the culture because the tissue contained much blood. However, the total erythrocytes did not exceed 10% of the gill cells. No contamination was observed in the cell culture during the experiment. There was no change in the TOS level at 24 h of exposure to DLT; however, but it was determined that it caused an increase with 1 and 10 µM of DLT at 48 h ($P < 0.05$) (Fig. 2). When the groups exposed to DLT and the control were compared in terms of the TAS level, the decrease with all of the DLT concentrations at 48 h and with 10 µM at 24 h was found to be significant ($P<0.05$) (Fig. 3). For the MDA levels, which is a product of LPO, an increase was observed with 10 µM of DLT at 24 h and with 1–10 µM of DLT at 48 h ($P<0.05$) (Fig. 4). No significant change was observed in the 8-OHdG level, which is a marker of DNA damage, with any of the DLT concentrations of at 24 or 48 h ($P>0.05$) (Fig. 5).

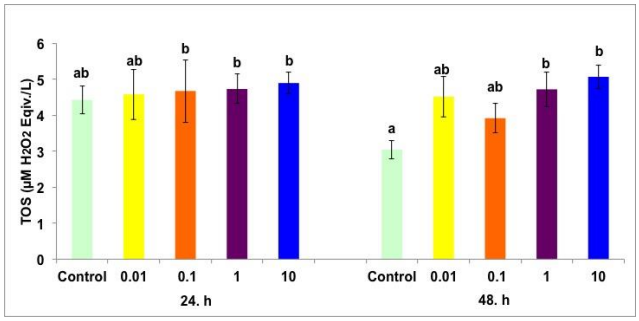

Figure 2. Changes in total oxidant status activity in Lake Van fish gill primary cell culture exposed to different concentrations of deltamethrin. Different letters represent statistical significance at $P<0.05$. Values are expressed as the mean ± SEM (n: 6).

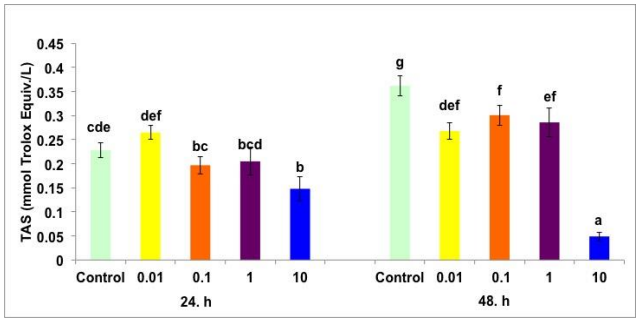

Figure 3. Changes in total antioxidant status activity in Lake Van fish gill primary cell culture exposed to different concentrations of deltamethrin. Different letters represent statistical significance ($P<0.05$). Values are expressed as the mean±SEM (n: 6).

4. Discussion

Pesticides play an important role in the formation of ROS. The immune systems of fish are significantly affected by toxic substances. Reaction to toxic substances can be either activating or suppressive. However, this activation does not mean that the immune system becomes stronger or resistant (Ghelichpour et al., 2019; Mirghaed et al., 2020). Gills are multifunctional organs in fish (Wang et al., 2016). Oxidative damage caused by chemicals in aquatic ecosystems can be evaluated by measuring the enzymatic

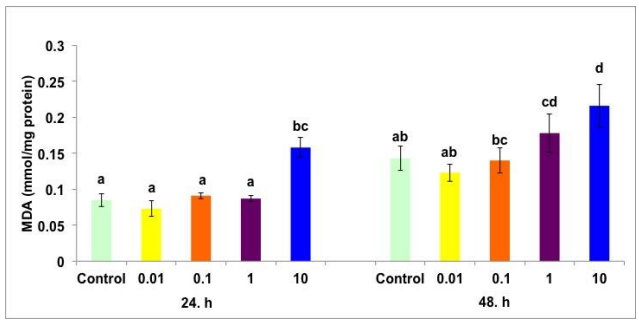

Figure 4. Changes in malondialdehyde level in Lake Van fish gill primary cell culture exposed to different concentrations of deltamethrin. Different letters represent statistical significance at $P<0.05$. Values are expressed as the mean ± SEM (n: 6).

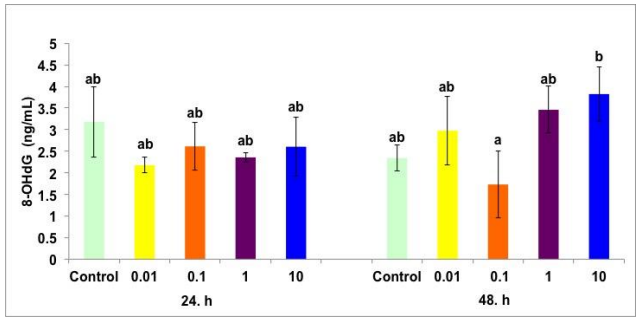

Figure 5. Changes in 8-hydroxy-2'-deoxyguanosine level in Lake Van fish gill primary cell culture exposed to different concentrations of deltamethrin. Different letters represent statistical significance at $P<0.05$). Values are expressed as the mean±SEM (n: 6).

and nonenzymatic antioxidant profiles in fish (Slaninova et al., 2009). There have been many studies with DLT on fish and it has been reported that this pyrethroid is a source of oxidative stress. In this context, in a study conducted with DLT, it was suggested that it reduced hepatic and renal antioxidant levels [catalase (CAT), superoxide dismutase (SOD), glutathione (GSH)] in the liver and kidneys, and increased MDA levels (Abdel-Daim et al., 2013). In a study on primary hepatocyte culture in Lake Van fish, fish primary cell culture exposed to 10 µM of DLT for 24 h was found to have a significant increase ($P<0.05$) while the increase seen in fish exposed to 10 µM of DLT for 48 h was nonsignificant (Kiraççakali & Oğuz, 2020). DLT (65.6 µg/L) has been reported to reduce the activity of antioxidant enzymes, such as SOD, CAT, and glutathione reductase, in common carp (*Cyprinus carpio* L) exposed for 96 h and cause oxidative damage in the gills, liver, and kidneys (Stara et al., 2015). In freshwater fish (*Channa punctatus* Bloch) exposed to DLT (0.75 µg/L) for 46 h, an increase was observed in the levels of glutathione peroxidase and glutathione-S-transferase against ROS damage in the liver and kidneys while a significant decrease in CAT activity was reported. Significant increases were observed in LPO (MDA) levels in all tissues (Sayeed et al., 2003). Increased LPO is an indicator of oxidative damage (Pinedo-Gil et al., 2018). ROS increase the oxidation of polyunsaturated fatty acids in the cell membrane structure (Blahova et al., 2013). It has been observed that LPO increases due to various pesticides in fish (Rao, 2006; Üner et al., 2006). LPO is one of the most important biomarkers of oxidative stress in fish and it causes the impairment of cellular function through its destructive effects on biomolecules under oxidant conditions (Nunes et al., 2018). The current study showed

that the increase in the TOS, decrease in the TAS, and increase in MDA levels in the Lake Van fish primary cell culture were associated with the increased concentration of DLT and prolonged exposure (Figs. 2–4). These results were similar to those of studies related to DLT exposure. The increased 8-OHdG activity of the pyrethroids in the gill tissues of fish may be a response to oxidative stress. 8-OHdG is an indicator used in the determination of the toxicity of many chemicals. In this study, it was observed that DLT did not cause DNA damage at the end of 24 and 48 h in the Lake Van fish primary gill culture. The reason why this pyrethroid did not show a significant increase in the 8-OHdG level in the gill cell culture may have been due to the short exposure time.

Gonad anomalies in Lake Van fish sampled from Lake Van were shown in previous studies (Ünal et al., 2007; Oğuz & Yeltekin, 2014). It was reported that DLT causes toxic effects on the gonads in addition to oxidative stress in fish (Petrovici et al., 2000). Gonad anomalies observed in Lake Van fish may have been caused by DLT or similar pyrethroids. For this reason, studies and measures such as the quantification of pesticides in Lake Van and restrictions on the use of these pyrethroids should be taken.

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Ethics committee approval: Animal experimental procedures were conducted in accordance with animal study protocols (2018/5) approved by the Van Yüzüncü Yıl University Animal Experiments Local Ethics Committee.

Conflict of interest: The author declares that there is no conflict of interest.

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Research Article / Araştırma Makalesi

DNA Barcoding and Phylogeny of *Ambystoma mexicanum* Cultivated as a Model Organism in Regenerative Medicine Research at Mersin University Aquaculture Units of the Faculty of Fisheries

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Abstract: *Ambystoma mexicanum* belongs to the family Ambystomatidae. It is one of the 30 species of the genus *Ambystoma*, which lives in a wide geography from southern Mexico to southern Alaska. It is accepted as a model organism in evolutionary biology, developmental biology, and regenerative medicine research. It can regenerate the brain, heart, and kidney organs as well as limb regeneration. Accurate identification of the model organism is important for the reproducibility and comparability of experiments. We aimed to confirm the species identification of axolotls using integrated taxonomic methods that were grown at Mersin University Aquaculture Units of the Faculty of Fisheries. Cytochrome oxidase subunit 1 (*COI*) and cytochrome b (*Cytb*) gene fragments of mtDNA sequences were used as molecular markers for phylogenetic analyses and species delimitation methods and compared with the sequences that were submitted to NCBI GenBank as species of *Ambystoma*. In the analyses that were conducted with different data sets, the individuals in question were grouped as a candidate species with the *A. mexicanum* species whose sequences were given in previous studies. All sequences obtained in this study and *A. mexicanum* sequences obtained from NCBI GenBank were grouped as haplotypes and their genetic distances were found to be 0 and it was determined that the individuals which were the subject of this study definitely belong to the *A. mexicanum* species. The results revealed that some species within the genus *Ambystoma*, especially *A. barbouri* and *A. texanum*, may be species complexes. On the other hand, *A. mexicanum* was grouped together with *A. andersoni* as candidate species in all analyses performed with the combined datasets of *COI*, *Cytb*, and *COI+Cytb*. These results revealed that the taxa in question are paraphyletic and should be assigned to the *A. mexicanum* species.

Keywords: Regenerative medicine, axolotl, phylogeny, species delimitation, cytochrome b, cytochrome oxidase subunit I.

Mersin Üniversitesi Su Ürünleri Fakültesi Uygulama Birimleri'nde Rejeneratif Tıp Araştırmalarında Model Organizma Olarak Yetiştirilen *Ambystoma mexicanum*'un DNA Barkodlaması ve Filogenisi

Öz: *Ambystoma mexicanum* Ambystomatidae familyasında yer alır. Güney Meksika'dan Güney Alaska'ya kadar geniş bir coğrafyada yaşayan *Ambystoma* cinsinin 30 türünden biridir. Limb rejenerasyonunun yanında beyin, kalp, böbrek organlarını rejenere edebilmeleri nedeniyle, evrimsel biyoloji, gelişim biyolojisi ve rejeneratif tıp araştırmalarında model organizma olarak kabul edilir. Model organizmaların kullanıldığı araştırmalarda canlının tür teşhisinin doğru yapılması deneylerin tekrarlanabilirliği ve karşılaştırılabilirliği açısından önemlidir. Bu çalışmada; Mersin Üniversitesi Su Ürünleri Fakültesi Uygulama Birimleri'nde yetiştirilen aksolotlların tür teşhisini bütünleşik taksonomik yöntemler kullanarak kesinleştirmek amacıyla mtDNA sitokrom oksidaz alt ünite 1 (*COI*) ve sitokrom b (*Cytb*) gen fragmentleri moleküler belirteç olarak kullanılmış ve NCBI GenBank'ta daha önce dizisi verilmiş olan *Ambsytoma* türleri ile filogenetik analizler ve tür sınırlarını belirleme yöntemleri ile karşılaştırılmıştır. Farklı veri setlerinin kullanıldığı analizlerin tamamında söz konusu bireyler daha önceki çalışmalarda elde edilen *A. mexicanum* türü ile aday tür olarak gruplanmıştır. Bu çalışmada elde edilen tüm diziler ile NCBI GenBank'tan elde edilen *A. mexicanum* dizileri haplotip olarak gruplanmış olup genetik uzaklıkları 0 bulunmuş ve bu çalışmanın konusu olan bireylerin kesin olarak *A. mexicanum* türüne ait olduğu belirlenmiştir. Sonuçlar *Ambystoma* cinsi içerisinde bazı türlerin özellikle *A. barbouri* ve *A. texanum* 'un tür kompleksi olabileceğini ortaya koymuştur. Diğer yandan *A. mexicanum*, *COI*, *Cytb* ve *COI+Cytb* birleştirilmiş veri setleri ile yapılan tüm analizlerde *A. andersoni* ile aday tür olarak gruplanmıştır. Bu sonuçlar söz konusu taksonların parafiletik olduğunu ve *A. mexicanum* türüne atanması gerektiğini ortaya koymuştur.

Anahtar kelimeler: Rejeneratif tıp, aksolotl, filogeni, tür sınırlarını belirleme, sitokrom b, sitokrom oksidaz alt ünite I.

1. Giriş

Ambystoma mexicanum (Aksolotl) rejeneratif tıp, evrimsel biyoloji ve onkoloji çalışmalarında model organizma olarak sıklıkla kullanılan bir semender türüdür (Gresens, 2004; Lust & Tanaka, 2019; Roy & Levesque, 2006). *A. mexicanum*, Amphibia sınıfının, Urodela takımına ait, 10 semender ailesinin içerisinde yer alır. Güney Meksika'dan, Güney Alaska'ya kadar geniş bir alanda yayılım gösteren Ambystomatidae familyasının, *Ambystoma* cinsine ait 30

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türünden biridir ve yaygın adıyla köstebek semenderleri olarak da bilinirler (Farkas & Monaghan, 2015). Meksika Vadisi'nde, Xochimilco gölü civarında endemik olarak bulunurlar (Gresens, 2004).

Aksolotllar neotenik amfibiler olup, larval dönemlerinde cinsel olgunluğa ulaşırlar (Farkas & Monaghan, 2015). Kolonyalizm öncesi, yerli uygarlıkların halkları tarafından besin olarak tüketildikleri, astım ve bronşit gibi hastalıkları tedavi etmede terapötik amaçla kullanıldıklarına dair raporlar bulunmaktadır (Soriano-Lopez et al., 2006). Aksolotların günümüzde de hala deney hayvanı olarak bu kadar popüler ve vazgeçilmez olmasının temel nedeni rejenerasyon kapasiteleridir (Roy & Levesque, 2016; Stamm et al., 2018; Reiß et al., 2015) Birçok semender türünün aksine metamorfoz geçirmedikleri için, embriyonik hücre benzeri yapılara sahiptirler ve bu sayede yaşamları boyunca rejenerasyon kapasitelerini koruyabilirler.

Memeliler ile karşılaştırıldıklarında, kompleks organ ve dokuları rejenere edebilmekte ve derin yaraları, memelilerde görünenin aksine, yara izi olmaksızın iyileştirebilmektedirler (Levesque et al., 2010). Yara iyileşmesi kapasitelerini aydınlatmak üzere yapılan çalışmalar *Ambystoma mexicanum* epidermal lipooksijenaz (AmbLOXe) proteinine odaklanmıştır (Mashkouli et al., 2020; Menger et al., 2011). Bu protein farklı insan hücre hatları ile oluşturulan in-vitro yara iyileşmesi modellerinde denenmiş, hücre proliferasyonu ve migrasyonunu artırarak yara iyileşmesini anlamlı derecede artırdığı gösterilmiştir (Sibai et al., 2019; Mashkouli et al., 2020). Beyin ve omurilik gibi insanda rejenerasyon kapasitesi çok düşük olan dokuları kolayca yenileyebilmeleri nedeniyle de sinir bilim çalışmalarında popüler organizma haline gelmişlerdir (Lust & Tanaka, 2019; Demircan et al., 2020). Sinir doku rejenerasyon kapasitelerinin araştırıldığı çalışmalar, rejenerasyonda aktif rol alan hücresel yolaklar, hedef genler, kodlanmayan RNA'lar gibi moleküler belirteçlere odaklanmıştır (Sibai et al., 2019; Sabin et al., 2019; Diaz & Echeverri, 2013; Echeverri, 2020).

Bu canlının rejeneratif kapasitesinin sırlarını aralamak ve bu bilgilerden yola çıkarak insanda rejeneratif kapasiteyi artırabilmek amacıyla yapılacak bilimsel çalışmalarda model organizma olarak kullanımı popüler hale gelmektedir. Bu sebeple özellikle genetik temelli araştırmalar için model organizma olarak kullanılacak canlının tür sınırlarının bilinmesi ayrıca önem taşımaktadır.

Tür sınırlarını belirleme (Species delimitation) metotları, bütünleşik taksonomi ve sistematik çalışmalarında son yıllarda sıklıkla kullanılan güncel yaklaşımlardır (Sites & Marshall, 2003; Flot, 2015). Özellikle analiz edilen bireylerin aynı türü temsil ettiklerinden emin olunması için neredeyse bir ön koşul niteliğindedir (Bortolus, 2008). Büyük ölçüde morfolojik karakterlere dayanan geleneksel tür tanımlama yöntemleri halen yaygın bir biçimde kullanılsa da her geçen gün türlerin tanımlanmasında ve tür sınırlarının belirlenmesinde yeni nesil yaklaşım ve yöntemler geliştirilmektedir. Moleküler verilerin büyük hacimlerde ve kısa sürede üretilebilmesi ve biyoinformatik alanındaki gelişmelere paralel olarak tür sınırlarının belirlenmesinde uygulamalı yaklaşımlar geliştirilmekte ve kullanılmaktadır (Sites & Marshall, 2003).

Bu çalışmanın amacı Mersin Üniversitesi Su Ürünleri Fakültesi Uygulama Birimleri'nde Rejeneratif Tıp Araştırmalarında Model Organizma Olarak Yetiştirilen *Ambystoma mexicanum*'un tür teşhisini bütünleşik taksonomik yöntemler kullanarak kesinleştirmektir. Bu amaçla mitokondri genomunda bulunan ve hayvanlar için DNA barkodlama bölgesi olarak bilinen sitokrom oksidaz alt ünite 1 (*COI*) ve sitokrom b (*Cytb*) gen fragmentleri moleküler belirteç olarak kullanılmış ve NCBI GenBank'ta daha önce dizisi verilmiş olan *Ambsytoma* türleri ile filogenetik analizler ve tür sınırlarını belirleme testleri yardımıyla karşılaştırılmıştır.

2. Materyal ve Metot

2.1. DNA İzolasyonu

DNA izolasyonu Mersin Üniversitesi Su Ürünleri Fakültesi Uygulama Birimleri'nde yetiştiricilik faaliyetleri sırasında ölen 3 adet 15-20 g ağırlığında juvenil aksolotl kuyruk biyopsisinden Clinic SV, GeneAll, 108-101 kiti kullanılarak gerçekleştirilmiştir. DNA izole edilen bireyler Mersin Üniversitesi Su Ürünleri Fakültesi Koleksiyon'unda MEUFR-21-11-001, MEUFR-21-11-002 ve MEUFR-21-11-003 numaraları ile saklanmaktadır. 200 mg kuyruk dokusu otomatik cam boncuklu doku homojenizatörü ile parçalandıktan sonra kit protokolü uygulanarak DNA izolasyonu gerçekleştirilmiştir. Elde edilen DNA örneği -20°C'de saklanmıştır.

2.2. PCR reaksiyonu

PCR reaksiyonu PCR 2X MasterMix (WizPure™ (Katalog No: W1401) kullanılarak Applied Biosystems™ ProFlex termalcycler cihazında gerçekleştirilmiştir. Total reaksiyon hacmi 20 µl olacak şekilde, 10 µl PCR 2X MasterMix, 2 µl template DNA, 6 µl DNase/RNase içermeyen moleküler su, 1 µl ileri yönlü primer ve 1 µl geri yönlü primer kullanılmıştır. Çalışmada evrensel barkodlama bölgelerinden *COI* ve *Cytb* seçilmiştir. Kullanılan primerlere ait diziler ve referansları Tablo 1'de verilmiştir, *COI* gen fragmentleri amplifikasyonu için AmphF2_t1/AmphR3_t1 primer çifti kullanılmıştır. PCR reaksiyonu koşulları Tablo 2'de verilmiştir. *Cytb* gen fragmentleri amplifikasyonu için ise MVZ15/MVZ16 primer çifti kullanılmıştır ve PCR reaksiyonu koşulları Tablo 3'te verilmiştir. PCR sonrasında AmphF2_t1/AmphR3 ve MVZ15/MVZ16 bölgelerinin PCR ürünleri %2'lik agaroz jel elektroforezi ile görüntülenmiştir (110V, 150mA, 45 dk.).

2.3. PCR Ürünlerinin Saflaştırılması ve Sanger Dizileme

PCR reaksiyonundan sonra saflaştırma aşamasında ExoSAP-IT Express PCR Cleanup Reagents, Thermo (Katalog No: 75001.200.UL) kiti kullanılmıştır. 10 µl PCR ürünü, 4 µl kit bileşeni ile sırasıyla 37°C'de 4 dk, 80°C'de 1 dk, 4°C'de 4 dk inkübasyona bırakılmıştır. Sanger dizileme reaksiyonları PCR aşamasında kullanılan primer çiftleri ile gerçekleştirilmiş olup bu aşamada Atlas Biyoteknoloji'den hizmet alımı yapılmıştır.

2.4. *COI* ve *Cytb* Gen Fragmanlarının Kontrolü ve Hizalanması

Dizi analizi sonucu elde edilen ileri ve geri yönlü gen dizileri BioEdit v7.0 (Hall, 2004) programında göz ile

kontrol edilmiş, okuma kalitesi düşük 5′ ve 3′ uçları silinmiştir. Daha sonra ileri ve geri yönlü okumalar yine BioEdit v7.0 programında hizalanmış, göz ile kontrol edilip belirsiz bölgeler kromatogramlardan kontrol edilerek düzenlenmiştir. Oluşturulan konsensus diziler NCBI Blast (Altschul et al., 1990) algoritması kullanılarak *Ambystoma* cinsine ve dizilenmesi hedeflenen gen bölgesine ait olup olmadıkları kontrol edilmiştir.

Tablo 1. Polimeraz Zincir Reaksiyonları için kullanılan primerler ve dizileri.

Table 1. Primers and their squences that were used for Polymerase Chain Reaction

Gen	Primer	Dizi (5′ – 3′)	Referans
COI	AmphF2_t1	TGTAAAACGACGGCCAGTTTCAACWAAYCAYAAAGAYATYGG	Chambers & Hebert, 2016
	AmphR3_t1	CAGGAAACAGCTATGACTADACTTCWGGRTGDCCRAARAATCA	
Cytb	MVZ15	GAACTAATGGCCCACACWWTACGNAA	Moritz et al., 1992
	MVZ16	AAATAGGAAATATCATTCTGGTTTAAT	

Tablo 2. AmphF2_t1/AmphR3_t1 Primer çifti için PCR reaksiyonunun koşulları.

Table 2. PCR conditions for AmphF2_t1/AmphR3_t1 primer couple

	Sıcaklık (°C)	Zaman	Döngü Sayısı
İlk Denatürasyon	94	5 dk.	1
Denatürasyon	94	40 sn.	
Primer Bağlanma	45	40 sn.	5
Primer Uzama	72	60 sn.	
Denatürasyon	94	40 sn.	
Primer Bağlanma	51	40 sn.	35
Primer Uzama	72	60 sn.	
Son Uzama	72	5 min.	1

Tablo 3. MVZ15/MVZ16 Primer çifti için PCR reaksiyonunun koşulları.

Table 3. PCR conditions for MVZ15/MVZ16 primer couple

	Sıcaklık (°C)	Zaman	Döngü Sayısı
İlk Denatürasyon	94	5 dk.	1
Denatürasyon	94	40 sn.	
Primer Bağlanma	48	40 sn.	40
Primer Uzama	72	60 sn.	
Son Uzama	72	5 dk.	1

2.5. Analizler İçin Veri Setlerinin Oluşturulması

Analizlerde 4 farklı veri seti kullanılmıştır. Tüm veri setlerine *Dicamptodon aterrimus* taksonu dış grup olarak dahil edilmiştir. Veri setine eklenen dizilere ait NCBI Genbank erişim numaraları ve bu dizilere atanmış türler Tablo 4 ve 5'te verilmiştir. Analizler için hazırlanan verisetleri:

1- *COI* veri seti: DNA izole edilen 3 bireyin 2'sinden *COI* dizisi elde edilebilmiştir (MEUFR-21-11-001 ve MEUFR-21-11-003). Bunun yanında Tablo 4'te bilgileri verilen diziler analizlere dahil edilmiştir.

2- *Cytb* veri seti: DNA izole edilen 3 bireyden başarılı bir şekilde elde edilen diziler ve Tablo 5'te erişim numaraları verilen diziler analizlere dahil edilmiştir.

3- *COI* ve *Cytb* birleştirilmiş veri seti: Bu veri setinde DNA izole edilen 3 bireyden hem *COI*, hem de Cytb'nin dizilerine sahip olan 2 birey kullanılmıştır (MEUFR-21-11-001 ve MEUFR-21-11-003). Bunun yanında Tablo 4 ve 5'te erişim numaraları "*" ile işaretlenen 11 *Ambystoma* tür/alttürüne ve 1 dış gruba ait total mitogenom dizisinden ilgili gen bölgeleri ayrı ayrı hizalanarak alınan toplam 14 dizi dahil edilmiştir.

4- Total mitogenom veri seti: Yukarıdaki veri setleri ile gerçekleştirilen tür sınırlarını belirleme testlerinde *A. mexicanum* ve bu çalışmada dizisi verilen bireyler ile aday tür olarak gruplanan *Ambystoma* türlerin (bkz: Sonuçlar), birlikte gruplanma sebeplerinin, veri setinin tür sınırlarını belirlemede yetersiz olmasından kaynaklanıp kaynaklanmadığının karşılaştırmasını yapabilmek için oluşturulmuştur. Bu amaçla yukarıda birleştirilmiş veri setinde kullanılan total mitogenom dizisi bir bütün olarak analiz edilmiştir.

Tablo 4. *COI* veri seti haplotip grupları, ait oldukları türler ve diziler.

Table 4. *COI* data set haplotype groups, attended species and sequences.

Haplotip	Tür	GenBank Erişim Numarası Referansı	
1	*Ambystoma mexicanum*	AY659991*	1
		AJ584639*	2
	MEUFR-21-11-001,	OK605096*	Bu çalışma
	MEUFR-21-11-003	OK605097*	

Haplotip	Tür	GenBank Erişim Numarası Referansı	
2	*A. andersoni*	AY659993*	1
3	*A. mavortium stebbinsi*	KP013120*	Yayınlanmamış
4	*A. tigrinum*	KU986212, KU986050, KU985809, KU986072	3
5	*A. dumerilii*	AY659994*	1
6	*A. tigrinum tigrinum*	AY659992*	1
7	*A. californiense*	AY659995*	1
8	*A. californiense*	KU986196, KU986165, KU986077, KU986040	3
9	*A. californiense*	KU985632	3
10	*A. laterale*	AY728218*	4
11	*A. laterale jeffersonianum* tür kompleksi	EF525710	5
12	*A. laterale jeffersonianum* tür kompleksi	EF525709	5
13	*Ambystoma* sp.	GU078472	6
14	*A. barbouri*	GU078469*	6
15	*A. barbouri*	GU078470*	6
16	*A. texanum*	GU078471*	6
17	*A. texanum*	MN135418, MN135417, MN135416	Yayınlanmamış
18	*A. annulatum*	KU986254, KU985678	3
19	*A. annulatum*	MT878234, MT878235	7
20	*A. macrodactylum*	MG421393	Yayınlanmamış
21	*A. bishopi*	KP289200*	Yayınlanmamış
22	*A. cingulatum*	KU986180, KU986169	3
23	*A. opacum*	MT878233, MT878232	7
24	*A. opacum*	MN135585	Yayınlanmamış
		KU986081, KU985732, KU985606	3
25	*A. talpoideum*	MG822788*	8
26	*Dicamptodon aterrimus*	GQ368657*	9

1: Samuels et al., 2005; 2: Arnason et al., 2004; 3: Chambers & Hebert, 2016; 4: Mueller et al., 2004; 5: Smith et al., 2008; 6: Bi, K., & Bogart 2010; 7: Siler et al., 2021; 8: Ren et al., 2019; 9: Zhang, & Wake, 2009. **COI+Cytb* ve total mitogenom veri setinde kullanılan diziler.

1: Samuels et al., 2005; 2: Arnason et al., 2004; 3: Chambers & Hebert, 2016; 4: Mueller et al., 2004; 5: Smith et al., 2008; 6: Bi, K., & Bogart 2010; 7: Siler et al., 2021; 8: Ren et al., 2019; 9: Zhang, & Wake, 2009. *Sequences also included in *COI+Cytb* and complete mitogenome data sets.

Tablo 5. *Cytb* veri seti haplotip grupları, ait oldukları türler ve diziler.

Table 5. *Cytb* data set haplotype groups, attended species and sequences.

Haplotip	Tür	GenBank Erişim Numarası ve Referansı	
1	*Ambystoma mexicanum*	AY659991*	1
	MEUFR-21-11-001, MEUFR-21-11-002, MEUFR-21-11-003	OK631828*, OK631829, OK631830*	Bu çalışma
2	*A. mexicanum*	AJ584639*	2
3	*A. andersoni*	AY659993*	1
4	*A. mavortium stebbinsi*	KP013120*	Yayınlanmamış
5	*A. tigrinum*	EF036666	3
6	*A. dumerilii*	AY659994*	1
7	*A. tigrinum tigrinum*	AY659992*	1
8	*A. tigrinum*	EF036665, EF036667	3
9	*A. californiense*	AY659995*	1
10	*A. laterale*	AY728218*	4
		EF036629	3
11		EF036628	3
12		EF036624	3
13		EF036625	3
14		EF036631	3

Haplotip	Tür	GenBank Erişim Numarası ve Referansı	
15		EF036630	3
16		EF036626	3
17	*A. jeffersonianum*	EF036685	3
18		EF036686	3
19		EF036678, EF036677, EF036681	3
20		EF036687	3
21		EF036676	3
22		KT780869	Yayınlanmamış
23	*A. macrodactylum*	EF036633, EF036634	3
24	*A. barbouri*	GU078484	5
		EF036599	3
25		GU078474	5
		EF036596	3
26		GU078505	5
		EF036619	3
27		GU078513, GU078512	5
		EF036613	3
28		GU078470*, GU078511	5
29		GU078500	5
30		GU078499, GU078482, GU078483	5
		EF036605	3
31		GU078486, GU078487, GU078503, GU078485	5
		EF036607	3
32		GU078501	5
33	*Ambystoma sp.*	GU078472, GU078476, GU078475, GU078509, GU078507	5
	A. barbouri	EF036609	3
34	*Ambystoma sp.*	GU078473	5
		EF036668, EF036669	3
35	*A. barbouri*	EF036612	3
36	*Ambystoma sp.*	EF036674	3
37	*Ambystoma sp.*	EF036673	3
38	*Ambystoma sp.*	EF036675	3
39	*A. barbouri*	EF036617	3
40		GU078490	5
41		GU078492	5
42		GU078496, GU078497, GU078480, GU078502, GU078488	5
		EF036600	3
43		GU078479	5
		EF036603	3
44		GU078478	5
		EF036601	3
45		GU078481	5
46		GU078469*	5
47		EF036610	3
48		GU078471*	5
49		GU078506	5
		EF036642	3
50		EF036655	3
51		EF036663	3
52		EF036656, EF036648, EF036652, EF036653	3

Haplotip	Tür	GenBank Erişim Numarası ve Referansı	
53		EF036654	3
54		EF036643	3
55		EF036646	3
56		EF036644	3
57		GU078495	5
		EF036616	3
58	*A. texanum*	EF036664	3
59		EF036662	3
60	*A. mabeei*	EF036632	3
61	*A. bishopi*	KP289200*	Yayınlanmamış
62	*A. cingulatum*	EF036621	3
63	*A. maculatum*	EF036637	3
64		EF036636	3
65		EF036635	3
66	*A. gracile*	AY691729	6
67	*A. gracile*	EF036622	3
68	*A. gracile*	EF036623	3
69	*A. opacum*	KT780868	Yayınlanmamış
70	*A. talpoideum*	MG822788	7
		EF036639	3
71	*Dicamptodon aterrimus*	GQ368657*	8

1: Samuels et al., 2005; 2: Arnason et al., 2004; 3: Robertson et al., 2006; 4: Mueller et al., 2004; 5: Bi & Bogart 2010; 6: Chippindale et al., 2004; 7: Ren et al., 2019; 8: Zhang, & Wake, 2009. **COI+Cytb* ve total mitogenom veri setinde kullanılan diziler.

1: Samuels et al., 2005; 2: Arnason et al., 2004; 3: Robertson et al., 2006; 4: Mueller et al., 2004; 5: Bi & Bogart 2010; 6: Chippindale et al., 2004; 7: Ren et al., 2019; 8: Zhang, & Wake, 2009. *Sequences also included in *COI+Cytb* and complete mitogenome data sets.

2.6. Veri Setlerinin Analizlere Hazırlanması

Tüm veri setlerinin çoklu hizalamaları MAFFT (Katoh & Standley, 2013) algoritması kullanarak gerçekleştirilmiştir. Veri setlerinin çoklu hizalamaları Mesquite V3.6 (Maddison, 2021) programında kontrol edilerek düzenlenmiş ve ileriki analizlerde kullanılacak dosya formatlarına uygun şekilde dışarı aktarılmıştır. Bu amaçla *COI*, *Cytb* ve *COI+Cytb* veri setleri bu çalışmada elde edilen dizilerin uzunluğu dikkate alınarak kırpılmıştır. *COI* ve *Cytb* veri setlerinde aynı populasyona ait olduğu bilinen (erişim numaralarından kontrol edilmiştir), veri setine göre çok kısa olan ve çok miktarda belirsiz bölge içeren diziler veri setinden çıkarılmıştır. Daha sonra *COI* ve *Cytb* veri setleri DnaSP 6 (Rozas et al., 2017) programında daha sonraki analizler için haplotiplere gruplanmış ve analizler bu haplotipler kullanılarak gerçekleştirilmiştir.

Haplotip olarak gruplanan *COI* ve *CytB* veri setleri tekrar Mesquite V3.6 programında işlenerek kodon pozisyonları belirlenmiştir. *COI+Cytb* birleştirilmiş veri setinde ise haplotip gruplandırması yapılmamıştır. Bu veri setinde ilgili gen bölgeleri tekrar çoklu hizalandıktan sonra birleştirilmiş ve her bir gen bölgesi için kodon pozisyonları belirlenmiştir.

Total mitogenom dizilerini içeren 4. Veri setinde ise hizalama sonrası DnaSP 6 programında boşluk içeren ve belirsiz bölgeler çıkarılmış ve sonuç olarak 16101 bç uzunluğunda bir veri seti elde edilmiştir. Bu veri seti gen bölgeleri ve kodonlara göre bölümlenmeden analiz edilmiştir.

2.7. Genetik Uzaklıkların Belirlenmesi

COI ve *Cytb* veri setleri için ikili genetik uzaklıklar MEGA-X (Kumar et al., 2018) programında Kimura-2 parametresi (K2P) (Kimura, 1980) kullanılarak belirlenmiştir. Belirlenen bu uzaklıklar tür sınırlarını belirleme testlerinden ABGD (Automatic Barcode Gap Discovery) (Puillandre et al., 2012) analizinde kullanılmak üzere MEGA uzaklık dosyası olarak dışarı aktarılmıştır.

2.8. Nükleotid Substitüsyon Modellerinin Belirlenmesi

Maksimum Olasılık ve Bayesian çıkarsama analizleri için kullanılacak olan nükleotid substitüsyon modelleri PartitionFinder V2.1 (Lanfear et al., 2017) programında BIC (Bayesian information criterion) a göre belirlenmiştir. Bölümleme şeması *COI*, *Cytb* ve *COI+Cytb* veri setleri için kodon pozisyonlarına göre belirlenmiş, total mitogenom veri seti için ise bütün olarak analiz edilmiştir. Bayesian çıkarsama analizlerinin gerçekleştirildiği MrBayes programı PartitionFinder V2.1 tarafından denenen tüm modelleri desteklemediği için Bayesian çıkarsama analizlerinde kullanılacak olan modeller belirlenirken "models=mrbayes" parametresi kullanılmıştır. Veri setlerinde analizlere göre kullanılan nükleotid substitüsyon modelleri Tablo 6'da özetlenmiştir. Bunların yanında GMYC (Generalized Mixed Yule Coalescent) (Pons et al., 2006) analizlerinde kullanılmak üzere gerekli olan ultrametrik ağaçlar için gerekli substitüsyon modeli Beast v2.6.6 (Bouckaert et al., 2014) programında yer alan bModelTest v1.2.1 paketi (Bouckaert & Drummond, 2017)

kullanılarak otomatik olarak belirlenmiştir.

2.9. Filogenetik Ağaçların İnşası

Maksimum Olasılık ağaçları IQTree 2 (Minh et al., 2020) programı kullanılarak inşa edilmiştir. Dal destek değerleri aynı programda yer alan Ultrafast Bootstrap Approximation 2 (UFBoot2) (Hoang et al., 2018) algoritması kullanılarak 1000 tekrarlı ve "-bnni" parametresi ile hesaplanmıştır. Bayesian ağaçları ise MrBayes 3.2.7 (Ronquist et al., 2012) programı kullanarak inşa edilmiştir. *COI*, *Cytb* ve total mitogenom veri setleri için 10^6 jenerasyon, *COI+Cytb* birleştirilmiş veri için 500000 jenerasyon her biri 3 sıcak ve 1 soğuk 4 zincirli birbirinden bağımsız 2 süreç ile analiz gerçekleştirilmiştir. Tüm veri setleri için ağaçlar her 100 döngüde bir örneklenmiştir. Jenerasyon sayısı 2 bağımsız süreçteki ayrılma frekanslarının standart sapmasının 0.02'nin altında olup olmadığı değerlendirilerek aşamalı olarak arttırılıp belirlenmiştir. Ayrıca model parametrelerinin 2 bağımsız süreç için Efektif Örneklem Büyüklüğü (ESS) Tracer V1.17 (Rambaut et al., 2018) programı yardımıyla kontrol edilmiştir (önerildiği üzere tüm analizlerde her bir süreç tüm parametrelerde ESS'nin 200'ün üzerinde olduğu görüldükten sonra analiz sonlandırılmıştır). Örneklenen ağaçların ilk %25'i analizden uzaklaştırılmış (burn-in), kalan ağaçlardan %50 çoğunluk ağacı (50% majority rule tree) inşa edilmiştir. Ultrametrik ağaçlar Beast v2.6.6 (Bouckaert et al., 2014) programı kullanılarak inşa edilmiştir. Tüm veri setlerinde "Relaxed Clock Log Normal" saat modeli ön tanımlı öncüller ile kullanılmıştır. İnşa edilen ultrametrik ağaçlarda türlerin ayrılma zamanlarının belirlenmesinden ziyade dalların birbirine görece ayrılma zamanlarının belirlenmesi amaçlandığından tüm veri setleri için substitüsyon oranı "1" olarak kullanılmıştır. Ağaç öncülü için "Yule Model" ön tanımlı parametreler ile seçilmiştir. Analiz için jenerasyon sayısı yukarıda anlatıldığı şekilde Tracer V1.7.1 programı ile belirlenmiştir (*COI* veri seti için $15X10^6$, *Cytb* veri seti için $25X10^6$, *COI+Cytb* ve total mitogenom veri setleri için $2X10^7$ jenerasyon). Analiz sonucu inşa edilen ağaçlardan %10 'u yakılarak mcc ağacı (Maximum Clade Credibility Tree) TreeAnnotator v2.6.2 programı kullanılarak hesaplanmıştır. Elde edilen nexus formatındaki ağaç dosyaları GMYC analizinde kullanılmak üzere newick formatına FigTree v1.4.4 (Rambaut, 2018) programı yardımıyla çevrilmiştir.

Tablo 6. Filogenetik analizler için kullanılan modeller

Table 6. Models used in phylogenetic analyses

Veri Seti	Bölümleme Tablosu		Model	
			IQTree	MrBayes
COI	1-616\3		TRNEF+I	SYM+I
	2-617\3		F81+I	
	3-615\3		TRN+G	HKY+G
Cytb	1-697\3		GTR+G	
	2-698\3		SYM+G	
	3-696\3		HKY+I	
COI+Cytb	*COI*	*Cytb*	IQTree	MrBayes
	1-709\3	711-1461\3	HKY+I	
	2-707\3	712-1462\3	GTR+G	
	3-708\3	710-1460\3	TRNEF+G	SYM+G
Total mitogenom	-		-	GTR+I+G

2.10. Tür Sınırlarını Belirleme Testleri

Bu çalışmada dizileri elde edilen bireyler ile karşılaştırmak üzere dizileri NCBI GenBank veri tabanından indirilen türlerin (Tablo 4 ve 5) ayrı birer türü temsil edip etmediğinin belirlenmesi için 4 farklı yaklaşım kullanılmıştır. *COI* ve *Cytb* veri setleri için uzaklık tabanlı ABGD (Automatic Barcode Gap Discovery = Otomatik Barkod Boşluğu Keşfi) (Puillandre et al., 2012) yukarıda anlatıldığı şekilde MEGA X programında hesaplanan ikili uzaklıklar üzerinden gerçekleştirilmiştir. Göreceli barkod boşluğu ön tanımlı olan X=1.5 değerinde bölümleme oluşmadığı için programda önerildiği şekilde 0.1'lik basamaklar ile 1'e düşürülmüştür. Ayrıca istatistiksel parsimoni ile şebeke analizleri TCS (Crandall et al., 2000) programı ile %90 ve %99 bağlantı limiti ile gerçekleştirilmiştir. Bunlar dışında tüm veri setleri için ağaç tabanlı bir yaklaşım olan bPTP (Poisson Tree Processes) analizleri pPTP web sunucusunda (Zhang et al., 2013), Bayesian ağaçları girdi olarak kullanılarak ön tanımlı parametreler ile (jenerasyon sayısı farklı olarak 500000 seçilmiştir) gerçekleştirilmiştir. Tüm testlerde bütün veri setleri için dış grup analize dahil edilmemiştir. GMYC (Pons et al., 2006) analizleri ise tek ve çoklu eşik değerleri ile ayrı ayrı GMYC web sunucusunda (Zhang et al., 2013) gerçekleştirilmiştir.

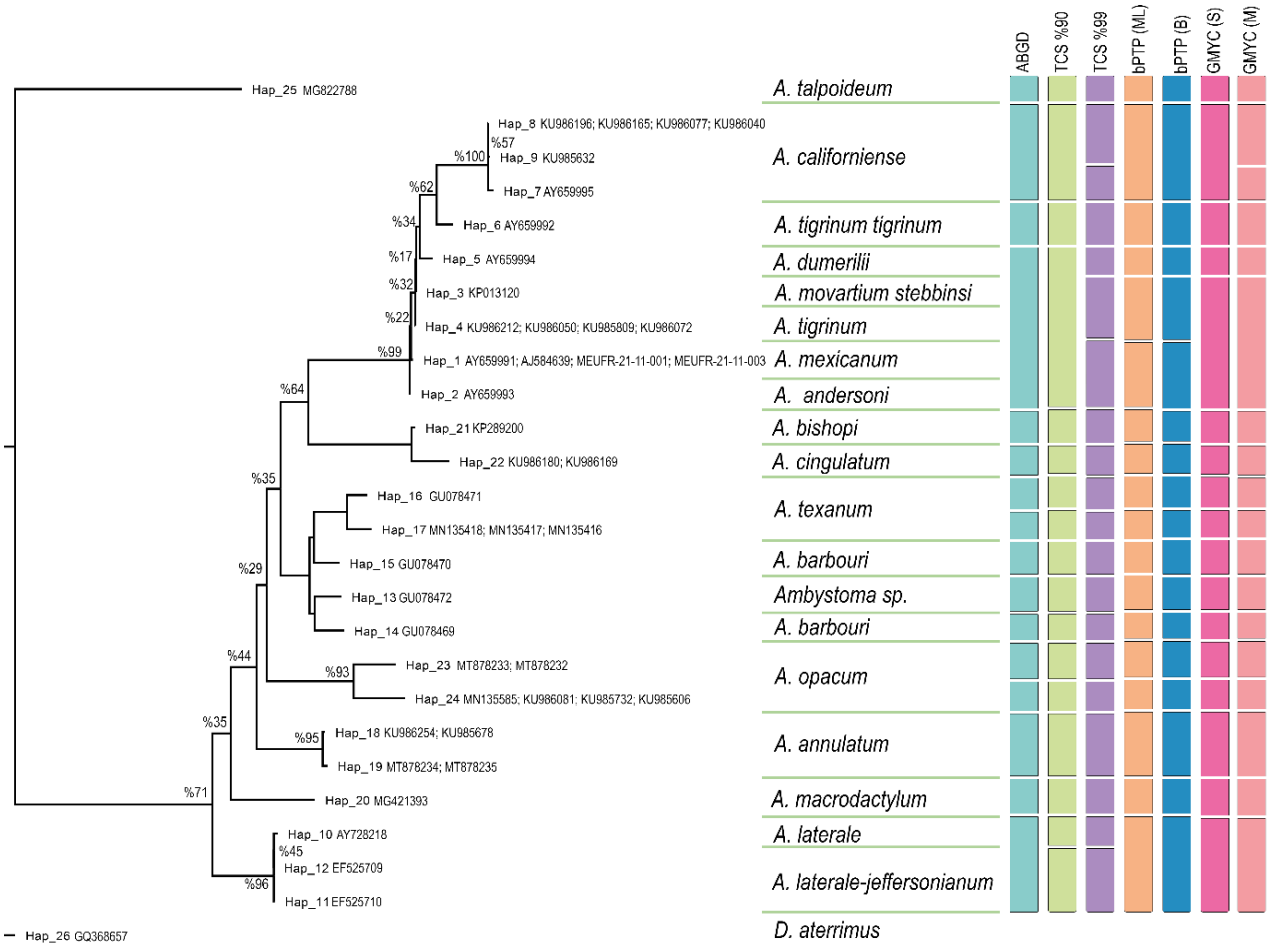
3. Bulgular

3.1. *COI* veri seti

Veri setinin oluşturulduğu toplam 43 *COI* dizisi 26 haplotip olarak gruplanmıştır (Tablo 4). Bu çalışmada dizisi elde edilen bireyler daha önceki çalışmalardan dizileri verilen *A. mexicanum* (AY659991 ve AJ584639) ile Haplotip 1 olarak gruplanmış olup genetik uzaklıkları 0'dır. Haplotip 1'e en yakın grup *A. andersoni* türünün tek yer aldığı Haplotip 2 olup genetik uzaklığı 0,00651 olarak hesaplanmıştır. Haplotip 1'e *Ambystoma* türlerinin yer aldığı haplotiplerden en uzak grup ise *A. talpoideum*'un tek yer aldığı Haplotip 25 olup genetik uzaklığı 0,19649 olarak

hesaplanmıştır. Haplotip 1'in dış gruba (*Dicamptodon aterrimus*, Haplotip 26) olan genetik uzaklığı ise 0,25830 olarak hesaplanmıştır.

Elde edilen Maksimum Olasılık ağacında Haplotip 1 ve 2 kardeş takson olarak gruplanmış olup dal destek değeri %99'dur (Şekil 1). Bayesian analizleri sonucunda elde edilen ağacın topolojisi benzer olup (Şekil 2) Haplotip 1 ve 2 0,52 soncul olasılık değeri ile kardeş takson olarak dallanmıştır.



Şekil 1. *COI* veri seti ile inşa edilen maksimum olasılık ağacı (solda), % rakamlar UFBoot2 ile hesaplanan dal destek değerlerini göstermektedir. Sağdaki renkli sütünlar tür sınırlarını belirleme testlerine göre aday tür gruplarını göstermektedir.

Figure 1. Maximum likelihood tree constructed with *COI* data set (left), numerals show UfBoot2 clad supports. Coloured columns on the right show candidate species groupings according to species delimitation methods

Otomatik barkod boşluğu keşfi (ABGD) analizi sonucunda 0.039 uzaklığında bir barkod boşluğu (Barcode gap distance) hesaplanmış ve bu boşluğa göre 16 aday tür gruplanmıştır. Bu gruplamada Haplotip 1-5 (MEUFR-21-11-001, MEUFR-21-11-003, *A. mexicanum, A. andersoni, A. mavortium stebbinsi, A. tigrinum, A. dumerilii*) birlikte yer almaktadır (Şekil 1).

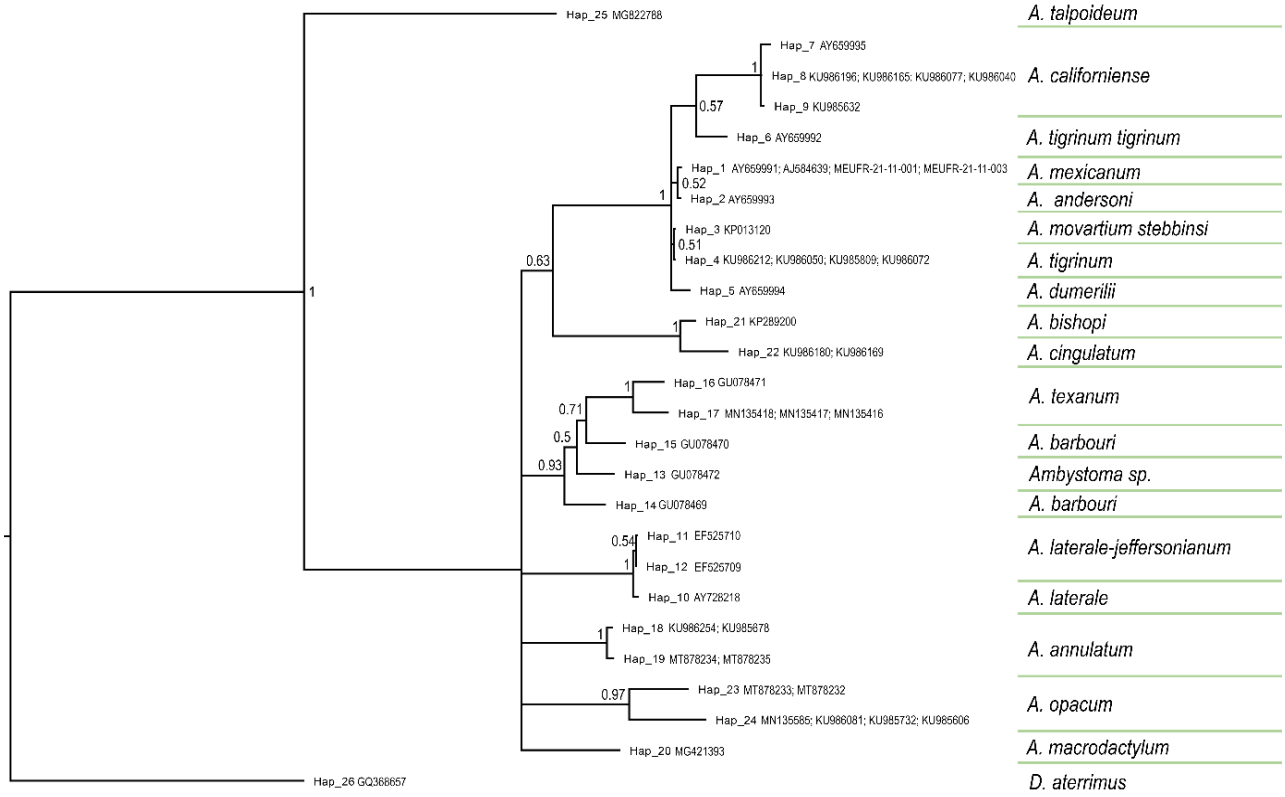
TCS programı ile gerçekleştirilen istatiksel parsimoni analizlerinde ise %90 bağlantı limitindeki gruplama ABGD ile aynıdır. %99 bağlantı limiti ile yapılan analizde ise beklenen şekilde daha fazla aday tür hesaplanmış (21 aday tür) olup haplotip 1 ve 2 birlikte gruplanmıştır (MEUFR-21-11-001, MEUFR-21-11-003, *A. mexicanum, A. andersoni*) (Şekil 1)

PTP (Poisson Tree Processes) analizleri için Bayesian çıkarsama sonucunda elde edilen ağaç kullanılmıştır. Maksimum olasılık ve Bayesian çözümü aynı sonuçları vermiş olup 18 aday tür belirlenmiştir. Haplotip 1 ve 2 (MEUFR-21-11-001, MEUFR-21-11-003, *A. mexicanum, A. andersoni*) birlikte gruplanmıştır (Şekil 1).

GMYC analizinde tekli eşik değerinde (Single threshold) 17, çoklu eşik değerinde (Multi threshold) ise 18 aday tür tespit edilmiştir. Her iki eşik değerinde de Haplotip 1, 2, 3 ve 4 (*A. mexicanum, A. andersoni, A. tigrinum*, ve *A. movartium stebbinsi*) birlikte aday tür olarak gruplanmıştır (Şekil 1).

3.2. *Cytb* veri seti

Veri setinin oluşturulduğu toplam 115 *Cytb* dizisi 71 haplotip olarak gruplanmıştır (Tablo 5). Bu çalışmada dizisi elde edilen 3 birey daha önceki çalışmalardan dizisi verilen *A. mexicanum* (AY659991) ile Haplotip 1 olarak gruplanmış olup genetik uzaklıkları 0'dır. Haplotip 1'e en yakın grup diğer bir *A. mexicanum* (AJ584639) dizisini içeren Haplotip 2 olup genetik uzaklığı 0,0014347212 olarak hesaplanmıştır. Haplotip 1'e *Ambystoma* türlerinin yer aldığı haplotiplerden en uzak grup ise *Ambystoma maculatum* (EF036636)'un tek yer aldığı Haplotip 64 olup genetik uzaklığı 0,1906534778 olarak hesaplanmıştır. Haplotip 1'in dış gruba (*Dicamptodon aterrimus*, Haplotip 71) olan genetik uzaklığı ise 0,2671068700 olarak hesaplanmıştır.



Şekil 2. *COI* veri seti ile inşa edilen Bayesian çıkarsama ağacı. Düğümler üzerindeki numaralar kladların soncul olasılık değerlerini göstermektedir.

Figure 2. Bayesian tree constructed with *COI* data set. Numerals above the nodes shows the posterior probability of the clades

Elde edilen Maksimum Olasılık ağacında bu çalışmada dizilenen 3 örnek ile AY659991 erişim numaralı *A. mexicanum* dizisi (Haplotip 1) ve AJ584639 erişim numaralı *A. mexicanum* (Haplotip 2) kardeş takson olarak gruplanmış olup dal destek değeri %76'dır Haplotip 3 (*A. andersoni*) ise Haplotip 1 ve 2'nin oluşturduğu klada kardeş takson olarak dallanmış olup dal destek değeri %99 'dur (Şekil 3). Bayesian analizleri sonucunda elde edilen ağacın topolojisi benzer olup (Şekil 4) Haplotip 1 ve 2 0,87 soncul olasılık değeri ile kardeş takson olarak dallanmıştır. Haplotip 3 (*A. andersoni*) ise %91 dal destek değeri ile bu klada kardeş takson olarak yer almaktadır.

Otomatik barkod boşluğu keşfi (ABGD) analizi sonucunda 0.032 uzaklığında bir barkod boşluğu (Barcode gap distance) hesaplanmış ve bu boşluğa göre 21 aday tür gruplanmıştır. Bu gruplamada Haplotip 1-5 (MEUFR-21-11-001, MEUFR-21-11-002, MEUFR-21-11-003, *A. mexicanum, A. andersoni, A. mavortium stebbinsi, A. tigrinum*) birlikte yer almaktadır (Şekil 3).

TCS programı ile gerçekleştirilen istatiksel parsimoni analizlerinde ise %90 bağlantı limitindeki aday tür sayısı ve gruplama ABGD ile aynıdır. %99 bağlantı limiti ile daha fazla aday tür tespit edilmiş olup (29 aday tür) olup haplotip, 1, 2 ve 3 birlikte gruplanmıştır (MEUFR-21-11-001, MEUFR-21-11-003, *A. mexicanum, A. andersoni*) (Şekil 3)

PTP (Poisson Tree Processes) analizleri için Bayesian çıkarsama sonucunda elde edilen ağaç kullanılmıştır. Maksimum olasılık çözümü sonucunda 24, Bayesian çözümü sonucunda ise 25 aday tür hesaplanmıştır. Her iki çözümde de Haplotip 1-5 (MEUFR-21-11-001, MEUFR-21-11-002, MEUFR-21-11-003, *A. mexicanum, A. andersoni, A. mavortium stebbinsi, A. tigrinum*) birlikte aday tür olarak gruplanmıştır (Şekil 3).

GMYC analizinde tekli eşik değerinde (Single threshold) 24, çoklu eşik değerinde (Multi threshold) ise 23 aday tür tespit edilmiştir. Her iki eşik değerinde de *COI* veri setindekine benzer şekilde çalışmanın konusu olan *A. mexicanum* ile *A. andersoni*, *A. tigrinum*, ve *A. movartium stebbinsi* birlikte aday tür olarak gruplanmıştır (Şekil 3).

3.3. *COI+Cytb* birleştirilmiş veri seti

Bu veri setinde aynı bireylerden izole edilen DNA'lardan dizilenmiş *COI* ve *Cytb* dizilerinin birleştirilmesi hedeflendiğinden, NCBI Gen Bank'ta hali hazırda bulunan total mitokondri genomları ile bu çalışmada her iki gen bölgesinin başarılı bir şekilde dizilenebildiği MEUFR-21-11-001 ve MEUFR-21-11-003 katolog numaralı bireylere ait dizileri kullanılmıştır (Analizde kullanılan diziler Tablo 4 ve 5'te * ile işaretlenmiştir). Maksimum olasılık analizleri sonucunda elde edilen ağaçta elimizdeki örnekler ile *A. mexicanum*'a ait diziler %99 dal destek değeri ile aynı kladda yer almaktadır. Bu klada kardeş takson olarak yer olan *A. andersoni*'nin de yer aldığı kladın ise dal destek değeri %95'tir (Şekil 5). Bayesian çıkarsama ile elde edilen ağaçta da topoloji benzer olup yukarıda bahsedilen kladların soncul olasılıkları sırası ile 0,98 ve 1 'dir (Şekil 6).

Elde edilen Bayesian çıkarsama ağacının girdi olarak kullanıldığı bPTP analizinde maksimum olasılık ve Bayesian çözümleri aynı sonucu vermiş olup 10 aday türe işaret etmiştir. Elimizdeki örnekler ile *A. mexicanum*, *A. andersoni*, ve *A. mavortium stebbinsi* birlikte aday tür olarak gruplanmıştır (Şekil 5).

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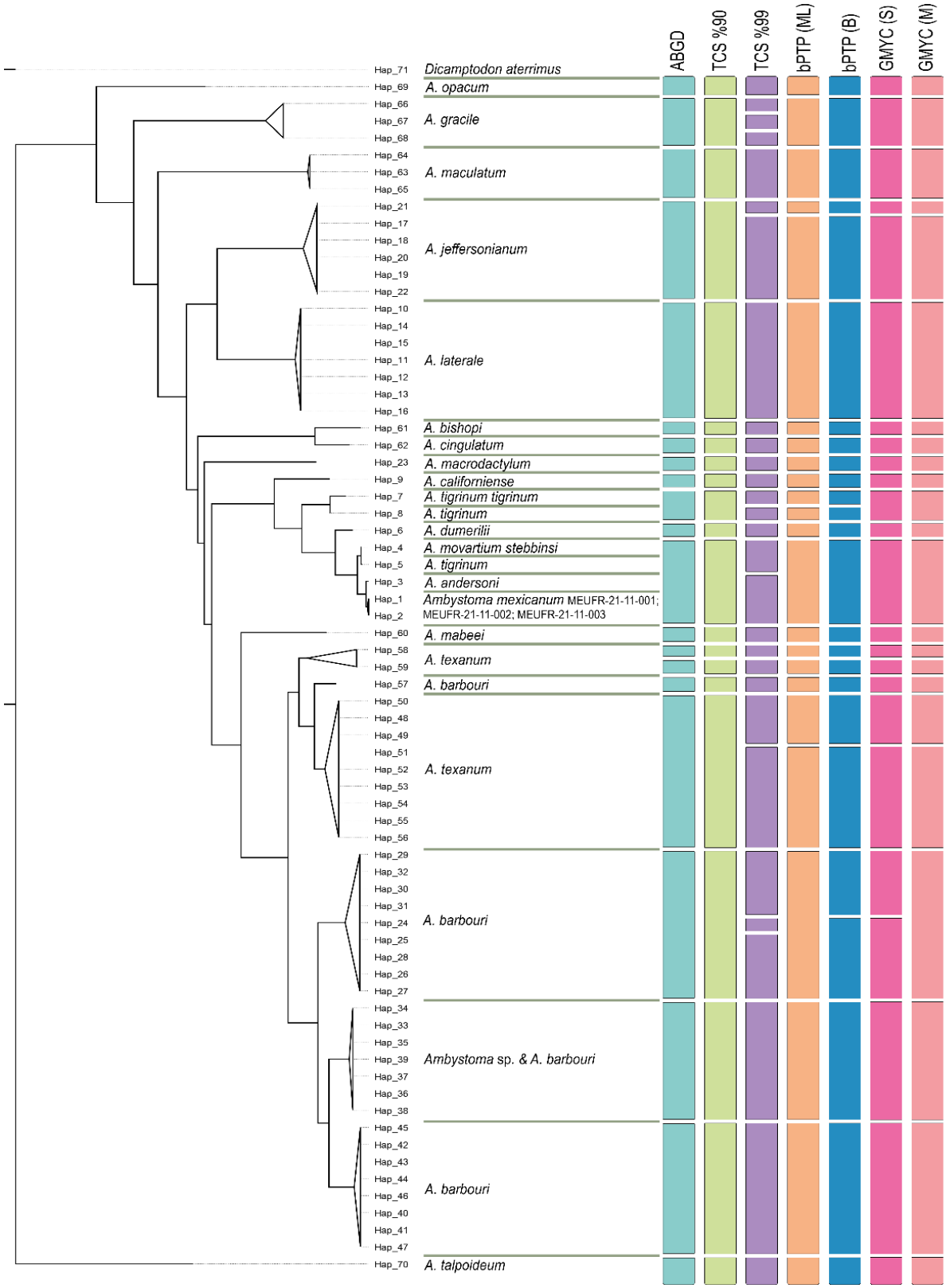

Şekil 3. *Cytb* veri seti ile inşa edilen maksimum olasılık ağacı (solda), % rakamlar UFBoot2 ile hesaplanan dal destek değerlerini göstermektedir. Sağdaki renkli sütünlar tür sınırlarını belirleme testlerine göre aday tür gruplarını göstermektedir.

Figure 3. Maximum likelihood tree constructed with *Cytb* data set (left), numerals show UfBoot2 clad supports. Coloured columns on the right show candidate species groupings according to species delimitation methods.

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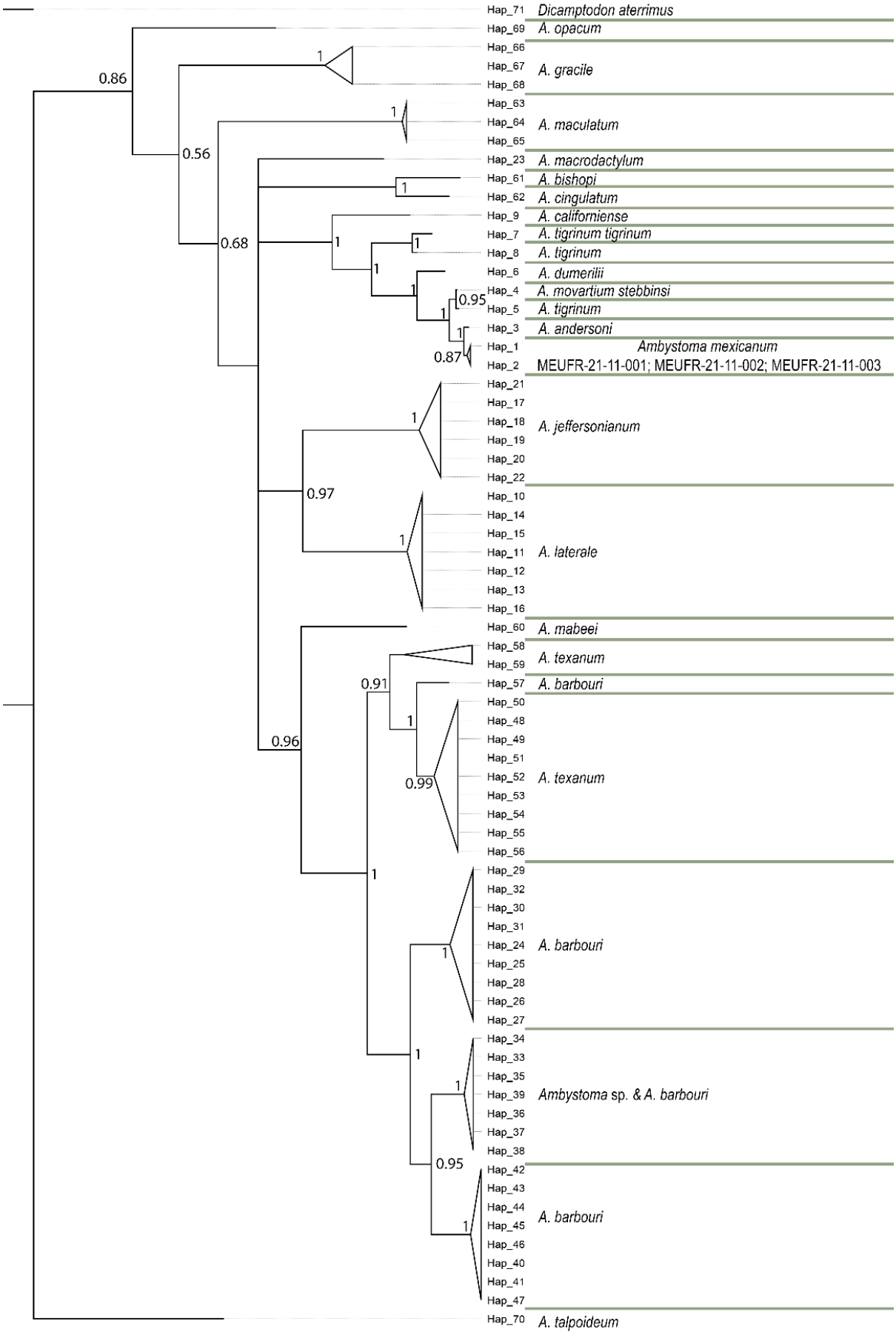
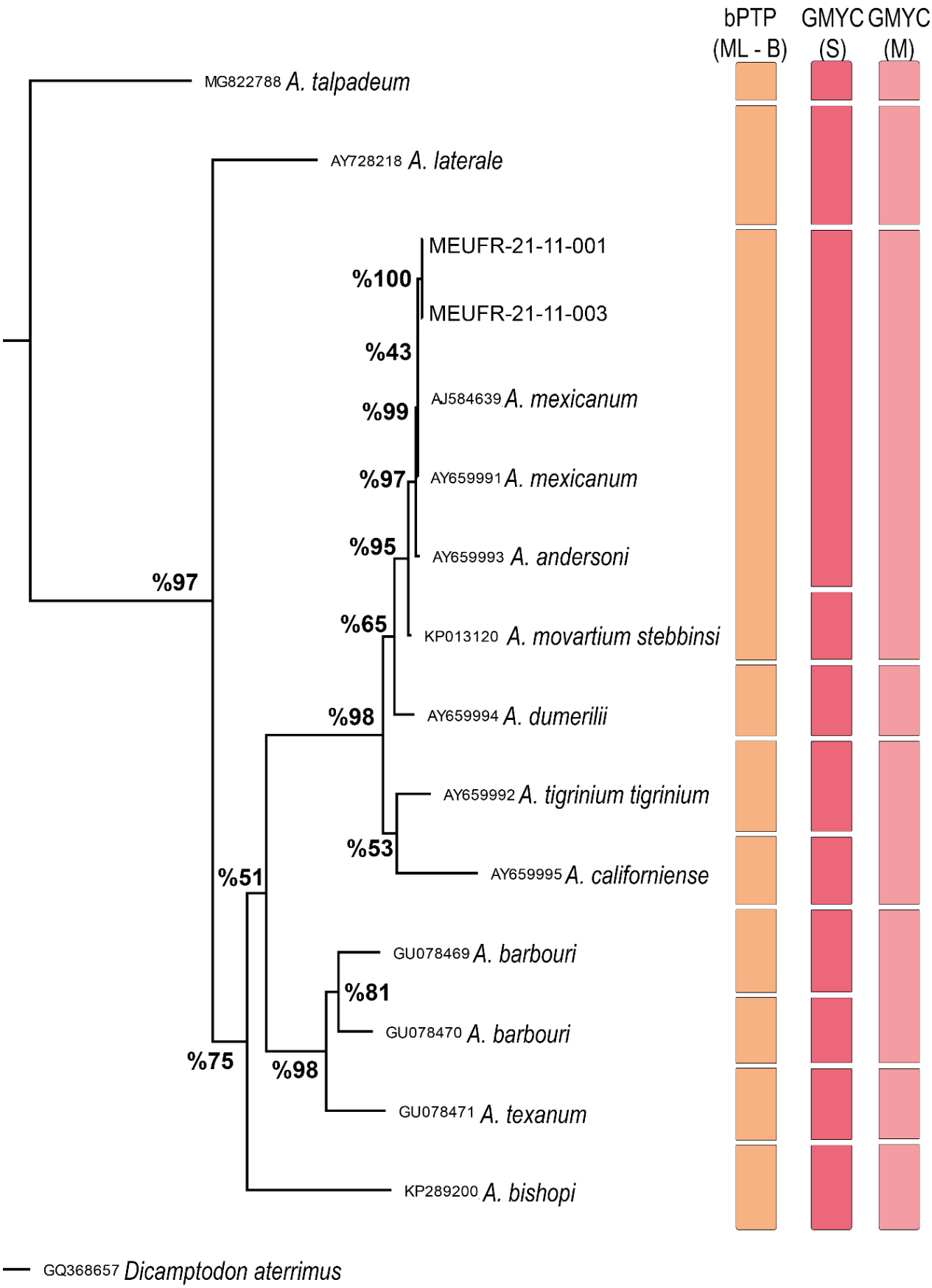


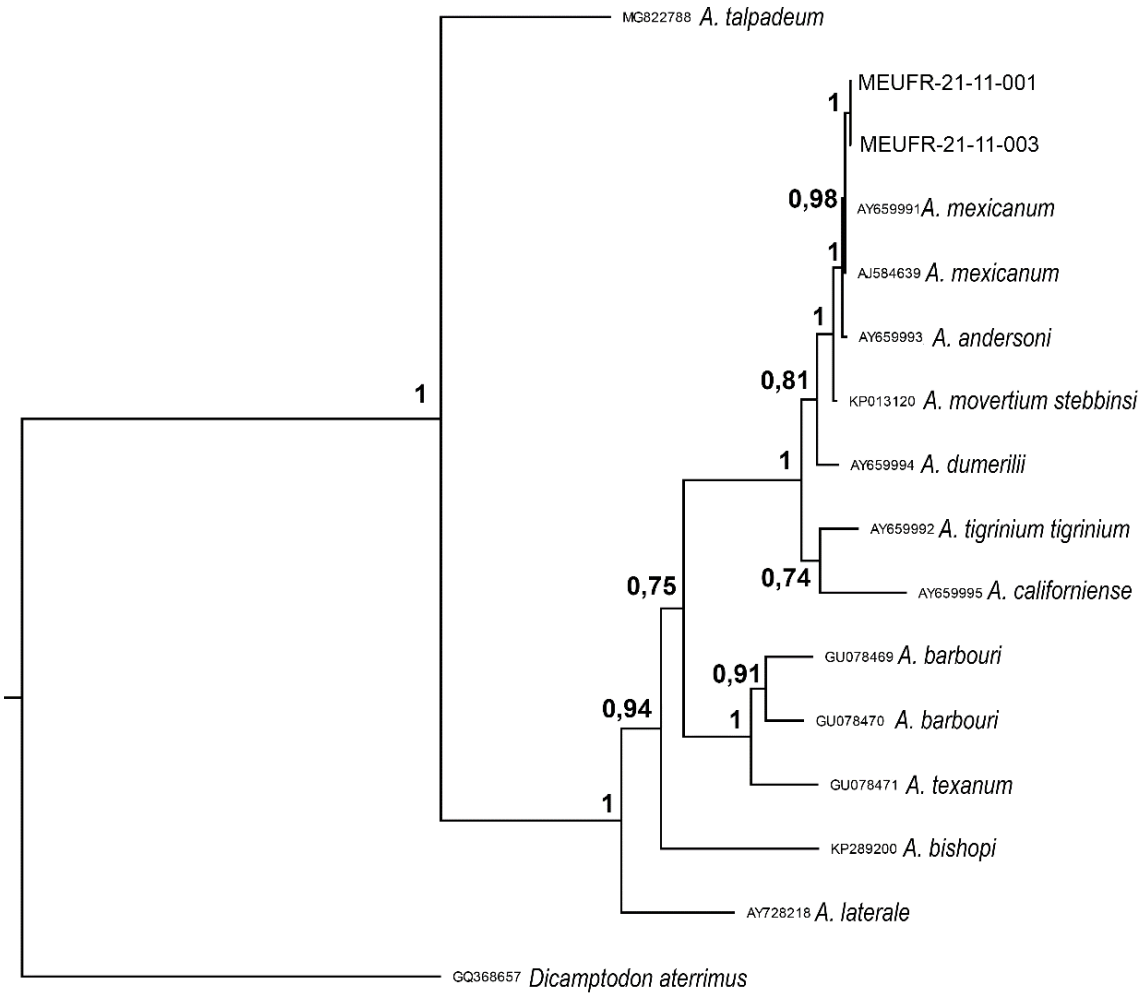
Şekil 4. *Cytb* veri seti ile inşa edilen Bayesian çıkarsama ağacı. Düğümler üzerindeki numaralar kladların soncul olasılık değerlerini göstermektedir.

Figure 4. Bayesian tree constructed with *Cytb* data set. Numerals above the nodes shows the posterior probability of the clades.



Şekil 5. *COI+Cytb* birleştirilmiş veri seti ile inşa edilen maksimum olasılık ağacı (solda), %rakamlar UFBoot2 ile hesaplanan dal destek değerlerini göstermektedir. Sağdaki renkli sütun bPTP testine göre aday tür gruplarını göstermektedir.

Figure 5. Maximum likelihood tree constructed with *COI+Cytb* concatenated data set (left), numerals show UfBoot2 clad supports. Coloured column on the right show candidate species groupings according to bPTP test.



Şekil 6. *COI+Cytb* birleştirilmiş veri seti ile inşa edilen Bayesian çıkarsama ağacı. Düğümler üzerindeki numaralar kladların soncul olasılık değerlerini göstermektedir.

Figure 6. Bayesian tree constructed with *COI+Cytb* concatenated data set. Numerals above the nodes shows the posterior probability of the clades.

GMYC analizinde tekli eşik değerinde (Single threshold) 11, çoklu eşik değerinde (Multi threshold) ise 8 aday tür tespit edilmiştir. Bu çalışmada elde edilen diziler için çoklu eşik değerindeki gruplandırma bPTP analizindeki gruplandırma ile (*A. mexicanum*, *A. andersoni*, ve *A. mavortium stebbinsi*) ile aynıdır. Tekli eşik değerinde ise bu gruplandırmadan *A. mavortium stebbinsi* aday tür olarak ayrılmıştır (Şekil 3).

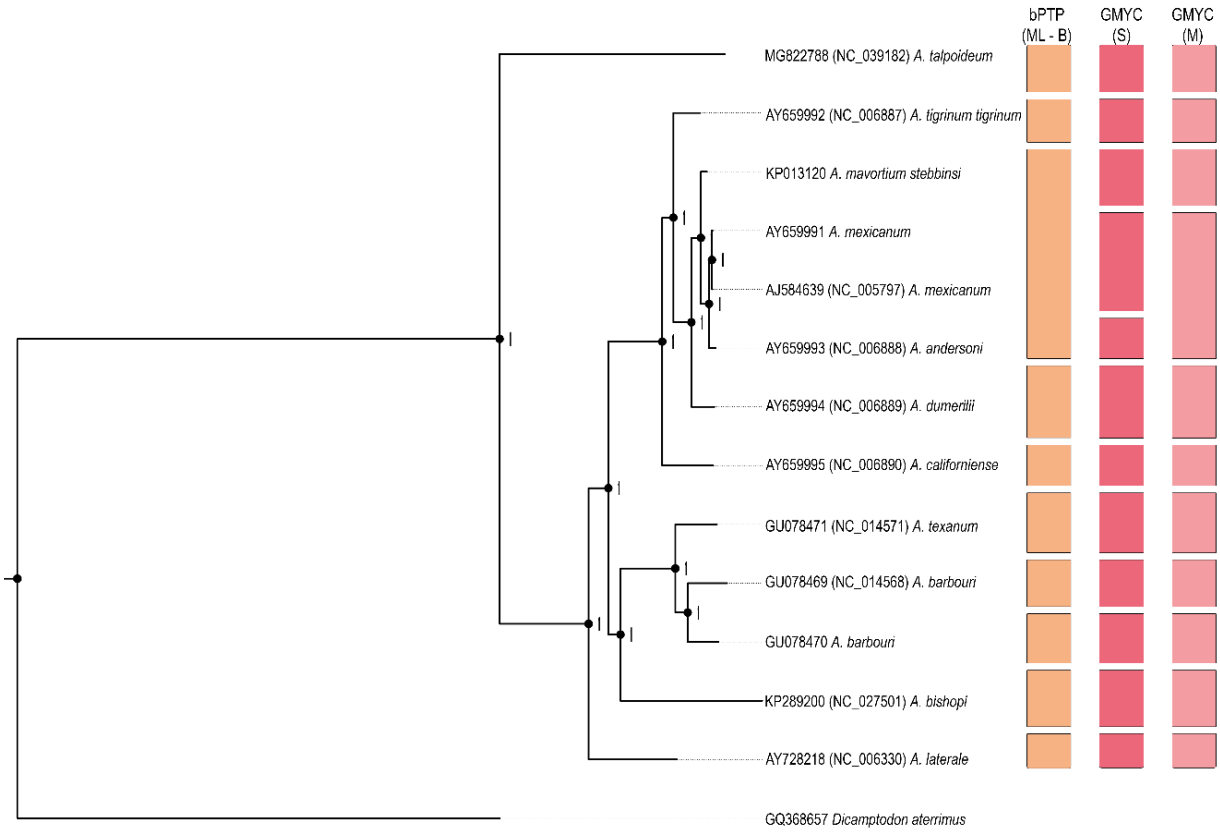
3.4. Total mitogenom veri seti

Yukarıda detayları verilen ve 3 farklı veri seti ile gerçekleştirilen analizlerde bu çalışmada dizilenen örnekler ile daha önceki çalışmalardan elde edilen *A. mexicanum*'a ait diziler her ne kadar birlikte gruplansalar da beklenilen şekilde yalın ve diğer morfolojik türlerden farklı bir grup oluşturmadıkları görülmüştür. Bu durumun elimizdeki sınırlı uzunlukta ve 2 gen bölgesi ile sınırlı dizilerden kaynaklanıp kaynaklanmadığının test edilmesi amacı ile NCBI GenBank veri tabanında bulunan ve içerisinde referans genomların da yer aldığı total mitogenomlar ile ek analizler gerçekleştirilmiştir (referans genom olan dizilerin numaraları ağaç görsellerinde erişim numaraları yanında parantez içerisinde verilmiştir). Bir dış grup dahil toplam 14 taksona ait 16101 bç uzunluğunda dizi ile gerçekleştirilen Bayesian çıkarsama analizinde tüm kladlar 1 soncul olasılık değeri ile ayrılmıştır. Yukarıda farklı veri setleri ile gerçekleştirilen analizlere benzer şekilde *A. mexicanum, A. andersoni, A. mavortium stebbinsi* ve *A. dumerilii* taksonlarının bulunduğu klad benzer topolojiye sahiptir. Bu ağacın girdi olarak kullanıldığı bPTP analizinin her iki çözümünde de 11 *Ambystoma* taksonu 10 aday türe ayrılmıştır. Bu 11 taksondan *A. mexicanum, A. andersoni, A. mavortium stebbinsi* yukarıdaki analizlere benzer şekilde aday tür olarak gruplanmıştır. GMYC analizinde tekli eşik değerinde 12, çoklu eşik değerinde ise 11 aday tür tespit edilmiştir. *A. mexicanum, A. andersoni, A. mavortium stebbinsi* taksonlarının dışında diğer taksonlar bPTP analizindeki şekli ile gruplanmıştır. Tekli eşik değerindeki gruplama morfolojik türler ile aynıdır. Çoklu eşik değerinde ise diğer tüm analizlerde olduğu gibi *A. mexicanum* ve *A. andersoni* taksonları birlikte aday tür olarak gruplanmıştır (Şekil 7).

4. Tartışma

Ambystoma mexicanum türü yüksek rejeneratif kapasitesi nedeniyle bilim insanlarının dikkatini çekmiş ve rejenerasyon kapasiteleri birçok araştırmaya konu

olmuştur. Diğer semender türlerinin aksine bu tür uygun yaşam koşullarında metamorfoz geçirmeden kalabilir ve bu sayede embriyonik hücre benzeri rejenerasyon potansiyelini koruyabilir. Kompleks organ ve dokuları rejenere edebilir ve bunu yara izi olmadan gerçekleştirebilirler (Gresens, 2004; Lust & Tanaka, 2019). Bu özellikleri sebebi ile sıklıkla model organizma olarak yetiştirilmektedirler.



Şekil 7. Total mitogenom veri seti ile inşa edilen Bayesian çıkarsama ağacı (solda). Düğümler üzerindeki numaralar kladların soncul olasılık değerlerini göstermektedir. Sağdaki renkli sütun bPTP testine göre aday tür gruplarını göstermektedir.

Figure 7. Bayesian tree constructed with complete mitogenome data set. Numerals above the nodes shows the posterior probability of the clades. Coloured column on the right show candidate species groupings according to bPTP test.

Genel olarak canlıların konu olduğu tüm araştırmalarda olduğu gibi özellikle rejeneratif tıp alanı gibi model organizmaların kullanıldığı alanlarda hem çalışma sonuçlarının tekrar edilebilirliği, hem de farklı araştırmacılar tarafından yapılan çalışmaların sağlıklı karşılaştırılabilirliği açısından, üzerinde çalışılan canlının tür teşhisinin doğru yapılması önemlidir (Bortolus, 2008). Bu sebeple bu çalışmada Mersin Üniversitesi Su Ürünleri Fakültesi Uygulama Birimleri'nde rejeneratif tıp araştırmalarında kullanılmak üzere model organizma olarak yetiştirilen ve morfolojik olarak *Ambystoma mexicanum* olarak teşhis edilmiş olan bireylerin tür teşhisleri yukarıda detayları verilen moleküler filogenetik analizler ile test edilmiştir. Farklı veri setlerinin kullanıldığı bu analizlerin tamamında söz konusu bireyler daha önceki çalışmalarda elde edilen *A. mexicanum* türü ile aday tür olarak gruplanmıştır.

Bunun yanında *COI* veri seti için bu çalışmalardan elde edilen diziler ile NCBI GenBank'tan elde edilen *A. mexicanum* dizileri haplotip olarak gruplanmış olup genetik uzaklıkları 0'dır. Benzer şekilde *Cytb* veri setinde de bu çalışmada dizi bilgisi elde edilmiş olan örnekler *A. mexicanum*'a ait bir dizi ile (AY659991) ile haplotip olarak gruplanmış (genetik uzaklık 0) olup diğer bir *A. mexicanum* (AJ584639) örneği ile ise genetik uzaklığı 0,0014'tür. Bu uzaklık ise toplam 698 bç uzunluğundaki dizide 1 nükleotid substitüsyonundan kaynaklanmaktadır. Bu substitüsyon 3. kodon pozisyonunda yer almakta olup kodlanan aminoasiti (lösin) değiştirmemektedir (TTA → TTG, transisyonel sessiz mutasyon). Bu veriler göz önünde bulundurulduğunda bu çalışmanın konusu olan bireyler *A. mexicanum* türüne aittir.

Bununla birlikte *COI* ve *Cytb* veri setlerini birlikte kullanarak yapılan analizlerde *Ambystoma* cinsi içerisinde *A. barbouri* ve *A. texanum* 'un tür kompleksi olabileceğine işaret etmektedir. Bu çalışmanın amacı *Ambystoma* cinsinin filogenisi olmadığından bu duruma ayrıntılı değinilmemiştir, fakat bu çalışmanın konusunu oluşturan *A. mexicanum*'un durumu aşağıda ayrıntılı tartışılmıştır.

COI, *Cytb* ve *COI+Cytb* birleştirilmiş veri setleri ile yapılan tüm analizlerde *A. mexicanum* (ve bu çalışmanın konusu bireyler, bundan sonra *A. mexicanum* olarak geçecektir) en geniş anlamda *A. andersoni, A. mavortium stebbinsi, A. tigrinum* ve *A. dumerilii* ile birlikte, en dar anlamda ise *A. andersoni* ile birlikte bir tür olarak gruplanmıştır. Bu durumun iki gen bölgesi ile sınırlı veri setinden mi kaynaklandığının anlaşılabilmesi için söz konusu taksonların total mitogenomları ile oluşturulan dördüncü bir veri setinden bPTP ve GMYC testleri gerçekleştirilmiştir. Bu testin sonucundaki gruplama *COI+Cytb* birleştirilmiş veri seti ile yapılan gruplama ile aynı olup gerçekleştirilen analizlerde (tek eşik değerlikli

GMYC analizi hariç) *A. mexicanum,* tekrar *A. andersoni* ile aday tür olarak gruplanmıştır. Bu durumun üç sebebi olabilir; i) NCBI veri bankasında mitogenomu verilen dizi (*A. andersoni*; AY659993) yanlış taksona atanmıştır, ii) bu takson parafiletik olup *A. mexicanum* türüne atanmalıdır, veya çok az olası bir ihtimal olarak iii) total mitogenom dahil veri setleri *Ambystoma* cinsi için tür sınırlarını belirlemede yetersizdir. Elde edilen tüm ağaçlardaki dal uzunlukları da göz önünde bulundurulduğunda birinci ve ikinci senaryonun olabilirliği ağır basmaktadır. Söz konusu iki taksona ait dizilerin sadece birer total mitogenom ile sınırlı olması kesin bir yargıya varmayı güçleştirmektedir.

Ambystoma andersoni' nin bu makalede kullanılan dizisi diğer dört *Ambystoma* total mitogenom dizisi ile birlikte (*A. californiense*, *A. tigrinum*, *A. dumerilii*, *A. mexicanum*, *A. andersoni*) Ambystomidae familyasının filogenetik pozisyonunu çözmek amacıyla Samuels et al. (2005) tarafından dizilenmiş ve aynı çalışmada analiz edilmiştir. Çalışma sonucu bu 5 taksondan oluşan ağaçta bu çalışmada elde edilen ağaçlara benzer şekilde *A. mexicanum* ve *A. andersoni* kardeş takson olarak yer almış (soncul olasılığı 1), *A. dumerilii* ise bu klada kardeş takson olarak yer almıştır (soncul olasılık 1). Beş taksonun tümü birlikte değerlendirildiğinde analiz sonucunda elde ettikleri akrabalık ilişkileri bu çalışmada elde edilen ile benzer sonuçları vermektedir. Fakat Samuels et al. (2005) tarafından gerçekleştirilen bu çalışmanın hedefi *Ambystoma* içerisindeki tür sınırlarının belirlenmesinden ziyade Ambystomidae familyasının filogenisini belirlemek olduğundan bu iki türün (*A. mexicanum* ve *A. andersoni*) yakın ilişkisini ayrıntılı tartışmamışlardır.

Farklı veri setleri ile elde edilen ağaçlarda birlikte gruplanan *A. californiense*, *A. tigrinum*, *A. dumerilii*, *A. moveratum stebbinsi* ve *A. mexicanum* uzun süredir *A. tigrinum* tür kompleksinin birer üyesi olarak bilinmektedir (Gehlbach, 1967). *Ambystoma* içerisinde durumun çözümü ile ilgili sınırlı çalışmalar bulunmakta olup (Shaffer & McKnight, 1996; O'Neill et al., 2013) bu çalışmada da elde edilen sonuçlar tür kompleksinin henüz çözülemediğini göstermiştir.

Son olarak 3 sınırlı veri seti ile yapılan analizler total mitogenom veri seti ile yapılan analizler ile karşılaştırıldığında, farklı veri setleri arasındaki toplam aday tür sayısındaki değişimin analizlere dahil edilebilen takson sayısı (NCBI GenBank'ta söz konusu genin dizisine sahip takson sayısı) ile orantılı olarak arttığı görülmüştür. Bu bağlamda dış grup hariç, *COI* veri setine dahil edilen 19 takson 16-20 aday türe, *Cytb* veri setine dahil edilen 24 takson 21-29 aday türe, *Cytb+COI* ve total mitogenom veri setine dahil edilen 11 takson ise sırasıyla 8-11 ve 10-12 aday türe gruplanmıştır. Fakat tüm veri setlerinde ortak olan 11 takson düşünüldüğünde aday tür gruplamalarının benzer olduğu görülmüştür. Bu sebeple en azından çalışma konusu olan *Ambystoma* cinsi için tek bir gen bölgesinden oluşan sınırlı veri setinin de aday türlerin belirlenmesinde yeterli olduğu düşünülmektedir.

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Etik kurul onayı: Bu çalışma için etik kurul onayı alınmasına gerek yoktur.

Çıkar çatışması: Yazarlar, çıkar çatışması olmadığını beyan etmiştir.

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Research Article / Araştırma Makalesi

The Effect of Methylparaben on Development and Fecundity of *Drosophila melanogaster*

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Abstract: Parabens are a group of chemicals used as preservatives in many industrial products such as foods, cosmetics, and pharmaceuticals. Recent studies have revealed that these chemicals have endocrine disrupting properties. *Drosophila melanogaster* is one of the suitable model organisms used to test the toxic effect of various chemicals in vivo. In this study, the effects of methylparaben (MP) (50 mM, 100 mM, and 200 mM), one of the most commonly used parabens, on the developmental parameters and fecundity of *D. melanogaster* were examined. No significant difference was observed in the viability rates from larva to pupa and from pupa to adult. However, experimented MP concentrations caused a delay in larva to pupa and pupa to adult developmental times ($p<0.05$). While MP did not cause a significant change in mean offspring number, it significantly decreased fecundity at all doses ($p<0.05$).

Keywords: Paraben, endocrine disrupting compounds, developmental delay, offspring number, egg number.

Metilparabenin *Drosophila melanogaster*'in Gelişimi ve Fekunditesi Üzerine Etkileri

Öz: Parabenler; gıdalar, kozmetik ürünler ve ilaçlar gibi birçok endüstriyel üründe koruyucu olarak kullanılan bir kimyasal madde grubudur. Son yıllarda yapılan çalışmalar, bu kimyasalların endokrin bozucu özelliği olduğunu ortaya koymuştur. *Drosophila melanogaster* çeşitli kimyasalların toksik etkisini in vivo olarak test etmek için kullanılan uygun model organizmalardan biridir. Bu çalışmada, en sık kullanılan parabenlerden biri olan metilparabenin (MP) (50 mM, 100 mM ve 200 mM) *D. melanogaster*'in gelişimi ve fekunditesi üzerine etkileri incelenmiştir. Pupalaşma ve erginleşme yüzdelerinde anlamlı bir fark gözlenmemiştir. Fakat MP uygulaması pupalaşma ve erginleşme sürelerinde gecikmeye yol açmıştır ($p<0.05$). MP ortalama yavru döl sayısında anlamlı bir değişime yol açmazken, tüm dozlarda fekunditeyi önemli ölçüde azaltmıştır ($p<0.05$).

Anahtar kelimeler: Paraben, endokrin bozucular, gelişim zamanı gecikmesi, yavru döl sayısı, yumurta sayısı.

1. Introduction

In today's world, the increase in human population, the tendency to raise people's living standards, and rapid urbanization have increased the demand for ready-made foods and various cosmetic products. At this point, the use of protective additives has increased at the same rate. Parabens, which are para-hydroxybenzoic acid esters, are among the most widely used additives in the food, cosmetic, and pharmaceutical industries. The fact that it has a wide range of activity, low cost, and safe use for a long time has made these compounds very popular as antimicrobial protective additives (Soni et al., 2005). Parabens are also found in low concentrations in environmental samples such as rivers, air, and dust. Humans can be exposed to parabens through inhalation, oral ingestion, and dermal absorption (Chen et al., 2016). Methylparaben (MP), ethylparaben (EP), propylparaben (PP), and butylparaben (BP) are the most commonly used parabens (Soni et al., 2005).

Although they are thought to be safe, various toxicological and carcinogenic biological effects of parabens have been determined. Recent studies have shown that parabens can have endocrine disrupting effects and may be harmful to human health by showing weak estrogenic effects (Soni et al., 2005; Boberg et al., 2010). It has been determined that they can activate estrogen receptors (Erα and Erβ) in human reporter cell lines (Gomez et al., 2005) and have an antagonistic relationship with human estrogen related receptor gamma (EERγ) (Zhang et al., 2013). Moreover, the estrogenic effects of parabens have been detected in vivo by using a receptor binding assay and in vitro by using yeast-based estrogenic assay (Routledge et al., 1998). A decrease in sperm motility and fertility was also observed after paraben administrations (Tavares et al., 2009; Riad et al., 2018). Because of these properties, parabens have been classified by the Endocrine Society as potential endocrine disrupting chemicals (EDCs) (Chen et al., 2016).

Drosophila melanogaster is one of the most widely used organisms in biological studies due to its easy cultivation, the large number of offspring, small size, and well-known genetic characteristics (Li et al., 2015). In addition, it has been determined that this organism has similar toxin metabolic pathways with humans and vertebrates, and; therefore, it has been frequently used in toxicological studies (Gao et al., 2020). In the study investigating the effects of MP on growth, development, and egg production in *D. melanogaster*, it was determined that 2% MP concentration had a toxic effect, significantly reducing the number of eggs, larvae, pupae and eclosion and delaying the development time. In the same study, at a low concentration of 0.02%, contrary to these results, it was

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observed that MP had potential estrogenic activity, increased the number of eggs, larvae, pupae and eclosion, and shortened the development time. (Gu et al., 2009). In a similar study, it was found that EP increased fertility at low concentrations (0.02%) while it shortened lifespan and decreased fecundity at high concentrations (0.10%, 0.20%). It was also determined that EP treatments changed the expression levels of estrogen-related receptor gene (ERR), ecdysone receptor gene (EcR), and yolk protein receptor gene (YPR) (Liu et al., 2014). In a study by Gao et al. (2020), it was determined that EP delayed the development of *D. melanogaster* and caused changes in hormone levels. In another study, Chen et al. (2016) investigated MP and EP mixture on lifespan and preadult development period in *D. melanogaster* and found that it reduced lifespan and affected preadult development.

In the present study, it was examined that the effects of MP on developmental parameters and fertility of *D. melanogaster.* Larva to pupa and pupa to adult viability rates, developmental times, mean offspring numbers, and mean egg numbers were determined. Thus, it is aimed to contribute to the literature on the effects of parabens, which we can take into our bodies with the foods we eat and the drugs and the cosmetic products we use. More research is needed to examine the potential additive toxicity of parabens.

2. Material and Methods

2.1. Fly strains and media

In the current study, the Canton S (CS) wild-type strain of *Drosophila melanogaster* was used as the model organism. The flies were reared on a standard cornmeal *Drosophila* medium. The stock cultures and experimental groups were kept in an incubator at 25±1°C temperature, with 50-60% humidity, and 12-h light and 12-h dark cycle.

2.2. Chemicals and exposure procedure

Methylparaben (MP) (99.0% purity, CAS No. 99-76-3) was purchased from Sigma-Aldrich (Steinheim, Germany). All doses of MP were dissolved in 1 mL ethanol and completed to 1 L with 5% (w/v) sucrose solution to prepare the stock solutions. The same volume of ethanol was added to the stock solution without MP as the control group.

The CS strain virgin females and males of the same age were mated in culture bottles. Parent flies were removed after 8 h and the third instar larvae were obtained 72±4 h later. These larvae were treated with 50 mM, 100 mM, and 200 mM MP for 6 h in tubes containing drying papers that had absorbed 5 mL stock solutions.

Dose selection was made based on the results from previous studies. In studies performed by Ayar and Uysal (2013), the LD50 dose for MP was determined as 300 mM in *D. melanogaster*. The doses used in this study (50 mM, 100 mM, and 200 mM) were chosen lower than the determined LD50 dose. As these doses did not cause a lethal effect on the *D. melanogaster* larvae, the developmental and reproductive effects of this chemical could be examined.

2.3. Pre-adult viability and developmental time assay

The larvae of control and MP groups were placed in tubes that contained a standard cornmeal medium. There were ten tubes in each group and every tube contained ten larvae. Two repetitions were done for each group. The development of control and experimental groups was observed at 6-h intervals. The number of individuals to reach pupal and adult stages and their developmental times were recorded separately. From the adults that emerged, virgin females were collected to use in "offspring number assay" experiment.

2.4. Offspring number assay

The virgin females hatched from treated larvae were used to examine the effects of MP on the average daily number of offspring. A female and 3 males collected from an untreated stock of the same age were mated. After the first pupa was seen, parents were removed. When the first adults hatched the number of emerged offsprings was counted at 24-h intervals for 10 days.

2.5. Fecundity assay

The CS strain third instar larvae of *D. melanogaster* were treated with 50 mM, 100 mM, and 200 mM MP for 6 h. Virgin females developed from the treated larvae were used to determine the effects of MP on fecundity. A female (treated) and three males (non-treated) of the same age (3 days old) were crossed in empty glass culture bottles. Then, plastic spoons containing standard cornmeal medium were placed in these culture bottles. These spoons were changed every 24-h and the eggs laid in the spoons were counted with a stereomicroscope for 10 days.

2.6. Statistical analysis

The statistical analyses were carried out by using the Statistical Package for the Social Sciences (SPSS) 15.0 program. The larva to pupa and pupa to adult viability rates were compared by using a one-way ANOVA followed by the Games-Howell multiple comparison test. Statistical comparisons of developmental times were performed with a two-variable t-test. The ANOVA followed by LSD test analysis test was used for calculating the daily mean offspring number. The differences of daily mean egg production in each group were checked with ANOVA test by the Games-Howell multiple comparison test. The significance level for all statistical analyses was set at $p<0.05$.

3. Results

3.1. Effects of the MP on the pupation period

The pupae that developed from larvae with and without MP were counted and viability rates of larva to pupa were determined. As a result of statistical analysis (ANOVA), it was seen that MP treatments were not effective on larva to pupa viability rates (Table 1).

Pupae were counted at 6-h intervals to determine the effect of MP on the developmental time of larva to pupa. It was determined that the mean larva to pupa developmental times were significantly prolonged in all MP treatment groups compared to control ($p<0.05$) (Table 2).

3.2. Effects of the MP on the maturation period

The pupa to adult viability rates of the control and MP treatment groups were determined and then compared

using one-way ANOVA. Statistical analyses showed that MP treatments did not affect the pupa to adult viability rates (Table 3).

The developmental times of pupa to adult were also determined for all groups and were compared with a two-variable t-test. In the 50 mM MP group, the mean pupa to adult developmental time was significantly prolonged compared to the control ($p<0.05$). The mean pupa to adult developmental times of 100 and 200 mM groups were longer than that of the control group. However, the differences were not significant (Table 4).

3.3. Effects of the MP on daily mean offspring numbers

To determine the effect of MP on the daily mean offspring number, offspring counting was performed for 10 days. As a result of the statistical analysis, it was determined that MP treatments did not cause significant changes in the mean number of offspring (Table 5).

3.4. Effects of the MP on daily mean egg numbers

The effects of MP on the fecundity of *D. melanogaster* were examined daily over a 10-day period. As seen in Table 6, all MP concentrations (50 mM, 100 mM, and 200 mM) reduced the daily mean egg productions significantly ($p<0.05$).

Table 1. Larva to pupa viability rates and significance levels of control and treatment groups

Groups	Rate ± S.E.	S.D.	p
Control	100±0.00	0.000	0.288
50 mM	98±0.01	0.042	
100 mM	97±0.02	0.048	
200 mM	99±0.01	0.032	

S.E.: Standard error, S.D.: Standard deviation

Table 2. The effect of MP on larva to pupa developmental time

Group No.	Groups	Larva to Pupa Developmental Time (h)	Significant Differences of the Means (p value)
1	Control	69.2	
2	50 mM	71.7	1-2* (0.003)
3	100 mM	70.8	1-3* 0.028)
4	200 mM	70.4	1-4* (0.026)

*: Indicates significant delay in larva to pupa developmental time compared to control

Table 3. Pupa to adult viability rates and significance levels of control and treatment groups

Groups	Rate ± S.E.	S.D.	p
Control	99±0.01	0.032	0.163
50 mM	99±0.02	0.048	
100 mM	96±0.03	0.083	
200 mM	95±0.03	0.090	

S.E.: Standard error, S.D.: Standard deviation

Table 4. The effect of MP on mean pupa to adult developmental time

Group No.	Groups	Mean Pupa to Adult Developmental Time (h)	Significant Differences of the Means (p value)
1	Control	70.5	
2	50 mM	73.2	1-2* (0.003)
3	100 mM	71.2	
4	200 mM	72.3	

*: Indicates significant delay in mean pupa to adult developmental time compared to control

Table 5. Effect of MP on daily mean offspring number of *D. melanogaster*

Group No.	Groups	No. of Female	No. of Offspring	Daily Mean Offspring Number ± S.E.	S.D.	Significant Differences of the Means
1	Control	22	2896	13.16±0.73	10.828	
2	50 mM	24	3098	12.91±0.74	11.428	
3	100 mM	25	3314	13.26±0.74	11.642	
4	200 mM	25	3455	13.30±0.69	10.967	

S.E.: Standard error, S.D.: Standard deviation

Table 6. Effect of MP on daily mean egg number of *D. melanogaster*

Group No.	Groups	No. of Female	No. of Egg	Daily Mean Egg Number ± S.E.	S.D.	Significant Differences of the Means (p value)
1	Control	22	1560	7.09±0.56	8.308	
2	50 mM	23	1035	4.50±0.38	5.735	1-2* (0.001)
3	100 mM	23	911	3.96±0.38	5.693	1-3* (0.000)
4	200 mM	22	972	4.42±0.40	5.979	1-4* (0.001)

S.E.: Standard error, S.D.: Standard deviation

4. Discussion

Whether the additives used in the food, cosmetics, and pharmaceutical industries have a toxic effect is one of the most researched subjects. Considering the amount of substances added to foods and cosmetics and the number of people exposed to it, the importance of this issue is evident. For this reason, scientists are trying to determine the possible carcinogenic, mutagenic, and toxic effects of additives with in vivo and in vitro test methods. In this context, parabens are one of the most used and most studied chemical groups.

In this study, the effects of methylparaben (MP), one

of the protective additives, on the developmental parameters and fecundity of *Drosophila melanogaster* were investigated. Although the effects of MP on larva to pupa and pupa to adult viability rates were not observed, MP treatments caused significant ($p<0.05$) delays in larva to pupa and pupa to adult developmental times. No significant change was observed in the daily mean number of offspring. However, daily average egg numbers were significantly decreased in the treatment groups compared to the control ($p<0.05$).

Many synthetic chemicals are known to mimic the effects of natural estrogens. These chemicals, defined as endocrine disruptors (EDCs), exert their effects by binding to estrogen receptors (Routledge et al., 1998). Parabens are also compounds that have the ability to bind to vertebrate estrogen receptors. Thus, they can act as EDCs (Vo et al., 2010).

Ecdysteroids are steroid hormones that play an important role in insects. 20-hydroxyecdysone (20E), an ecdysteroid hormone, exerts its effect by binding to ecdysone receptors. This hormone regulates developmental changes (such as metamorphosis) in fruit flies and other insects (Kozlova & Thummel, 2000; Gáliková et al., 2011). The ecdysone receptor of *D. melanogaster* was determined to be homologous to the vertebrate estrogen receptor (Zou & Fingerman, 1997). It is also noted that EDCs that can bind to steroid hormone receptors can also bind to ecdysteroid receptors of invertebrates (Watts et al., 2001). Liu et al. (2014) determined that EP treatments caused changes in ecdysone receptor gene (EcR) and estrogen-related receptor gene (ERR) expression levels of *D. melanogaster*. Another study by Gao et al. (2020) found that EP increased EcR expression and decreased the amount of 20E. They stated the reason for this is that EP mimics the 20E and induces EcR expression. Based on these published studies and the results of this study, it can be said that the MP may have bound to EcR by mimicking the 20E; thus, preventing 20E from binding to its receptor, causing delays in developmental time.

Another striking effect of MP on *D. melanogaster* in the present study was on the mean egg number. It was significantly decreased in all treatment groups compared to the control. Fecundity of *D. melanogaster* can be affected by many internal (age, genetic factors, and etc.) and external (population density, nutrition, humidity, temperature, and etc.) factors (Ashburner et al., 2011). Stress is one of the external factors affecting fecundity. Studies have shown that EDCs induce oxidative stress by causing the formation of free radicals in cells (Roy et al., 1997; Obata & Kubota, 2000; Herrero et al., 2015). It is determined that parabens cause the formation of reactive oxygen species (ROS) and; thus, induce oxidative stress (Pop et al., 2011; Samarasinghe et al., 2018). It is also determined that ecdysteroid hormones are also effective on fecundity. It was reported that any effect that will increase the amount of ecdysteroids will decrease egg production (Rauschenbach et al., 2000). In addition, the balance between 20E and juvenile hormone (JH) is of great importance for the normal oogenesis process in *Drosophila* (Soller et al., 1999). A study by Gu et al. (2009) showed that 2% MP reduced the number of eggs, but 0.02% MP increased them. In a similar study, it was determined that EP increased fertility at low concentrations (0.02%), while it decreased fecundity at high concentrations (0.10%, 0.20%) (Liu et al., 2014). Based on the results of the current study and previous studies, we can say that MP may have affected fecundity by imitating the ecdysone hormone and disrupting the balance between JH and ecdysone. The MP treatments in the present study also may have increased the amount of ecdysteroid hormones by causing oxidative stress in the cells and this may have decreased egg production.

The results of this study showed that MP did not affect the survival of *D. melanogaster* but prolonged development time and decreased egg production. It can be said by looking at the results of both this study and previous studies that parabens, which are widely used in the food, pharmaceutical, and cosmetic industries, have toxic effects. Especially considering their endocrine disrupting effects, the use of these chemicals should be kept under control.

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Research Article / Araştırma Makalesi

Effects of *Microcystis viridis* and *Aphanizomenon gracile* Mixed Culture on the Growth of Vetch, Chickpea, and Barley

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Abstract: In this study, the effects of different doses of mixed cultures of *Microcystis viridis* and *Aphanizomenon gracile*, which are densely found in some Şanlıurfa Dam Lakes, were investigated to determine their effects on the growth of vetch (*Vicia sativa* L.), chickpea (*Cicer arietinum* L.), and barley (*Hordeum vulgare* L.). The doses prepared from the cyanobacteria mixture were applied to the soil by spraying. In terms of root length, 1.5% application dose in vetch and 2% dose in chickpea and barley were found to be effective. The 2% application dose of the cyanobacteria mixture increased the plant height of all three plants compared to the control. While 2% application dose was found to be effective on root dry and wet weight in chickpea and barley, 1.5% application dose was found to be effective on shoot weight in vetch and chickpea.

Keywords: *Cyanobacteria,* biofertilizer, plant growth, dose.

Microcystis viridis ve *Aphanizomenon gracile* Karışık Kültürün Fiğ, Nohut ve Arpa Gelişimine Etkileri

Öz: Bu çalışmada fiğ (*Vicia sativa* L.), nohut (*Cicer arietinum* L.) ve arpa (*Hordeum vulgare* L.) gelişimi üzerine etkilerini belirlemek amacıyla, bazı Şanlıurfa Baraj Göllerinde yoğun olarak bulunan *Microcystis viridis* ve *Aphanizomenon gracile*'in karışık kültürünün farklı dozlarının etkisi araştırılmıştır. Siyanobakteri karışımından hazırlanan dozlar, topraklara püskürtülerek uygulanmıştır. Kök uzunluğu bakımından fiğ de %1.5 uygulama dozu, nohut ve arpada ise %2'lik doz etkili bulunmuştur. Siyanobakteri karışımının %2'lik uygulama dozu her üç bitkinin bitki boyunu kontrole göre arttırmıştır. Kök kuru ve yaş ağırlığına %2'lik uygulama dozu nohut ve arpada etkili bulunurken, yeşil aksam ağırlığına ise fiğ ve nohutta %1.5 uygulama dozu etkili bulunmuştur.

Anahtar kelimeler: *Cyanobacteria,* biyogübre, bitki gelişimi, doz.

1. Giriş

Tarımsal üretim üzerindeki küresel baskı, insan gıdası, hayvan yemi ve enerji üretimine sürekli artan talebi karşılamaya yönelik yeni ve sürdürülebilir yaklaşımlar gerektirmektedir. Geleneksel tarım uygulamaları, büyük ölçüde yenilenemeyen gübre ve pestisit girdilerine dayanmaktadır (Renuka et al., 2018). Tarımsal kimyasallar, insanoğlu için önemli ilerlemeler sağlasa da (Cooper & Dobson, 2007), bu kimyasallar insan sağlığı ve çevre için ciddi, çözülmemiş bir tehdit oluşturmaktadır (Fenner et al., 2013). Ayrıca, bu girdilerin temini ve uygulanması, kaynakların tükenmesi ve mineral gübrelere yönelik artan talep nedeniyle giderek daha maliyetli hale gelmektedir. Bu nedenle, tarımda kimyasal girdilerin kullanımı ve artan endişe nedeniyle alternatif arayışlara yönelinmiştir. Bitki biyouyarıcıları, gübrelere, özellikle de çiftlik dışı kimyasal girdilere olan bağımlılığı azaltabildikleri için tarımsal sürdürülebilirliğinin ele alınmasında çok önemli bir rol oynamaktadır (Fenner et al., 2013).

Mikroalg türevli ürünler tarımda çok işlevli özelliklere sahiptir, besin alımını kolaylaştırarak, ürün performansını, bitkinin fizyolojik durumunu ve abiyotik strese toleransı iyileştirmiştir (Renuka et al., 2018). Son yıllarda, tarla ve sera koşullarında mikroalg ekstraktlarının etkisini test eden çalışmalar artmıştır. Yapılan çalışmalarda siyanobakteriyal uygulamaların; marul, amaranth, domates ve biberin çimlenme, fide büyümesini, sürgün ve kök biyokütlesini uyardıklarını göstermiştir. (Faheed & Abd-El Fattah, 2008; Garcia-Gonzalez & Sommerfeld, 2016; Barone et al., 2018; 2019, El Arroussi et al., 2018). *Chlorella vulgaris* içeren bir ortamda (2 ve 3 g/kg toprakta kuru mikroalg ekstresi) marulun çimlenmesini hızlandırdığı, marulun gelişimini (hem taze hem de kuru ağırlık bazında) teşvik edildiği rapor edilmiştir (Faheed & Abd-El Fattah, 2008). Aynı çalışmada, bitki büyümesinin (yani sürgün, kök kuru ağırlığı ve uzunluğu) iyileştirilmesi, fotosentetik aktiviteyi etkileyen karotenoid ve klorofil pigment biyosentezinin uyarılmasıyla ilişkilendirilmiştir. Benzer şekilde, *Spirulina platensis*'in uygulanması, roka bitki büyümesini arttırmıştır (Wuang et al., 2016). Garcia-Gonzalez and Sommerfeld (2016) ve El Arroussi et al. (2018), domates ve biberin de mikroalgal ekstraktlarının uygulanmasından olumlu etkilendiğini belirtmiştir. Örneğin, *Acutodesmus dimorphus* kuru biyokütlesi veya sulu hücre ekstraktları farklı konsantrasyonlarda (0, 0.75, 1.875, 3.75, 5.625 ve 7.5 g/ml) tohum ve yapraktan uygulandığında; tohum çimlenmesini, bitki büyümesini ve bitkinin çiçek açmasını teşvik etmiştir (Garcia-Gonzalez & Sommerfeld, 2016).

Ülkemizde önemli arpa üretim merkezlerinden biri

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Şanlıurfa'dır. Şanlıurfa'da üretilen arpa ülke ekonomisi için oldukça önemli olup, insan ve hayvan beslenmesinde de önem taşımaktadır (Kızılgeçi et al., 2016). Yemeklik tane baklagiller içerisinde en fazla ekim ve üretim alanına sahip olan nohut ise kuru koşullarda yetiştirilmektedir (Demirci & Bildirici, 2020). Ülkemizde en fazla nohut üretimi; İç Anadolu, Ege ve Güneydoğu Anadolu bölgelerinde yapılmaktadır (Demirci & Bildirici, 2020). Kuraklığa oldukça dayanıklı olan fiğ ise tahıl üretiminin yapıldığı yerlerde de kolaylıkla yetiştirilebilmektedir (Sağlamtimur et al., 1991). Doğal mera alanlarında da kendiliğinden yetişebilen fiğin hayvan beslenmesinde kullanıldığı gibi, yeşil gübre bitkisi olarak da kullanılarak toprağın organik madde içeriğini arttırdığı bilinmektedir (Sağlamtimur et al., 1991).

Bitkisel üretimde kimyasal ilaçların yerine mikroorganizmalardan hazırlanan gübrelerin kullanımı ile ilgili yapılan pek çok çalışmalar bulunmaktadır (Barone et al., 2018; 2019; El Arroussi et al., 2018). Mikroorganizmaların kullanımı gübre kullanımını azaltmak ve verimi arttırmada alternatif olarak karşımıza çıkmaktadır. Özellikle mikroalglerin biyogübre olarak kullanımı son yıllarda dikkat çekmektedir. Çevre dostu olan mikroorganizmaların endüstriyel ölçekli üretiminin yapılabilmesi hem organik hem de sürdürülebilir tarım için önem arz etmektedir. Ülkemizde mikroalg bazlı biyogübrelerin üretimi olmakla birlikte, etkileri bitki, iklim ve toprak koşullarına göre de değişiklik göstermektedir. Bu nedenle çalışmamızda; bölgemiz koşullarına uyumlu ve bazı Şanlıurfa Baraj Göllerinde yoğun olarak bulunan olan mikroalglerden *Microcystis viridis* ve *Aphanizomenon gracile*'ın karışık kültürünün endüstriyel ölçütte üretilmesinden önce; sera koşullarında, kimyasal gübre yerine biyostimülant olarak farklı dozlarının nohut, fiğ ve arpa gelişimi üzerine etkilerinin belirlenmesi amaçlanmıştır.

2. Materyal ve Metot

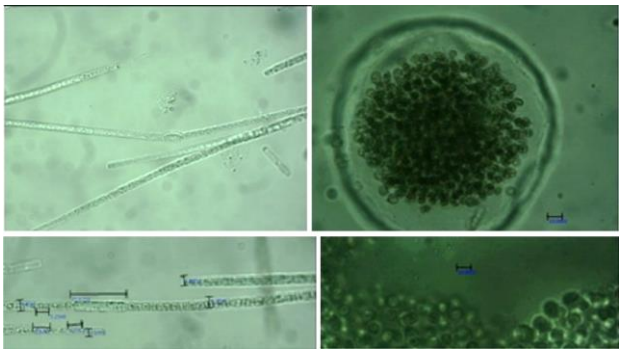
2.1. Denemede Kullanılan Materyal

Denemede nohut, arpa ve fiğ tohumları kullanılmıştır. Tohumlar Ege Üniversitesi Tarla Bitkileri bölümünden sağlanmıştır.

2.2. Kullanılan Siyanobakteri

Şanlıurfa baraj gölünden alınan kavanozlara alınan su örnekleri hemen laboratuvara getirilmiş, daha önce hazırlanmış BG11, BGA besiyerleri içeren petri kutularına 1'er ml olacak şekilde püskürtme şeklinde ekilmiştir (Pereira et al., 2009). Petri kutuları sürekli ışık altında oda sıcaklığında 15 gün süre ile inkübe edilmiştir. İnkübasyon sonrası gelişen siyanobakteri kültürlerinden örnek alınmış, BG11 besiyerine ekilerek saflaştırılmıştır. Gelişen kültürler mikroskopta Tablo 1'de (Komárek & Komárková 2006; Komárek & Anagnostidis 2008; Komárek, 2013; Kociński et al., 2013; Cires & Ballot, 2016) göre tanımlanmıştır. Şanlıurfa baraj gölünde yaz aylarında aşırı çoğalma yapan, su örneklerinden izole ettiğimiz türler *Microcystis viridis* ve *Aphanizomenon gracile* olarak tanımlanmıştır. Bu mikroalgleri içeren sular ile bazı Şanlıurfa baraj göllerinin etrafındaki tarlaların sulandığı ve çiftçilerin tarlalarından bu sular ile sulanan ürünlerden yüksek verim alındığı için, çalışmamızda her iki alg türünün eşit oranda karışımı hazırlanarak kullanılmıştır.

Saf kültürlerin her biri BG11 besiyerine ekilmiş, oda sıcaklığında sürekli ışık altında 15 gün boyunca inkübe edilmiştir. İnkübasyon sonunda örnekler, 5000 x g'de 15 dk santrifüjlenmiştir. Hücreler steril su ile beş kez yıkanmıştır. 500 ml steril distile suya 5 g taze alg materyalini içeren ekstrakt %1 uygulama dozu olarak kullanılmıştır. Benzer olarak ekstraktların %1.5 ve %2'lik uygulama dozları hazırlanmıştır. Uygulama dozlarının her biri %50 *M. viridis* ve %50 *A. gracile* içerecek şekilde ayarlanmıştır. *M. viridis* ve *A. gracile* 'in mikroskobik görünümleri Şekil 1'de ve teşhislerinde kullanılan ölçümleri Tablo 1'de (Komárek & Komárková, 2006; Komárek & Anagnostidis, 2008; Komárek, 2013; Kociński et al., 2013; Cires & Ballot, 2016) verilmiştir.



Şekil 1. *Aphanizomenon gracile* ve *Microcystis viridis*'in mikroskobik görünümleri

Figure 1. Microscopic views of *Aphanizomenon gracile* and *Microcystis viridis*

Tablo 1. *Aphanizomenon gracile ve Microcystis viridis*'in tanımı, morfolojisi ve ölçümleri

Table 1. Description, morphology and measurements of *Aphanizomenon gracile* and *Microcystis viridis*

Tür	Tanımı, morfolojisi ve ölçümleri
Aphanizomenon gracile Lemmermann 1907	Metamerik bir yapıya sahiptir. trikomlar düz, soliterdir, çapraz duvarda daralır ve uçlara doğru hafifçe daralır. Hücreler çok sayıda küçük granül ve gaz vezikülü ile kısa ve silindiriktir (5-8 mikron uzunluğunda ve 2-4 mikron genişliğinde). Apikal hücreler hafif yuvarlak veya koniktir (3-5 µm uzunluğunda ve 1.5-2,5 µm genişliğinde). Heterositler soliter, interkalar, genellikle kısa ve silindiriktir, bazı zamanlar neredeyse küreseldir (3.5–6 µm). Akinetler heterositlerden uzak, interkalar, silindirik ve kutuplarda yuvarlaktır. Akinetler yalnızdırlar ve her zaman genişlikten (4.5–7.5 µm) daha uzundurlar (10-35 µm).
Microcystis viridis (A. Braun in Rabenhorst) Lemmermann 1903	Koloniler serbest yüzen, mikroskobik, paket benzeri ve üç boyutlu koloniler, daha sonra bazen düzensiz kümelerle makroskopik, ancak deliksiz, düzensiz küresel veya uzun yoğun kümelenmiş hücreler. Müsilaj renksiz, hücre kümelerinden biraz uzakta ve hücre anahatlarını aşağı yukarı kopyalayarak dalgalı kenar boşluğu, genellikle 5-10 µm genişliğinde sınırlı ve hafif kırılma sınırı. Hücreler küresel, aerotoplu, (3) 3.5-7.0 (7.9-8.4) µm çapındadır.

2.3. Saksı Denemeleri

Denemede kullanılan topraklar, Harran Üniversitesi kampüs alanında daha önce herhangi bir uygulama ve ekimin yapılmadığı alandan alınmıştır. Toprakların bazı fizikokimyasal özellikleri GAP Araştırma Enstitüsü'nde yaptırılmıştır. Saksı denemesinde kullanılan toprakların

pH'ı 7.71, toprak tekstürü %51.62 kil, %23 kum ve %25.38 silt içeriğine sahip olup killi bünyeye sahiptir. Kullanılan toprakların organik madde içeriği %1.62, kireç içeriği %22.08, EC 0.86 ds/m ve azot içeriği %0.07'dir.

Topraklar 3 ardışık gün 121°C'de 15 dk otoklavda steril edildikten sonra 2 kg'lık saksılara doldurulmuştur. Tohumların yüzey sterilizasyonu %1'lik sodyum hipoklorit (NaOCl)'te 1 dk bekletildikten sonra alkolde 5 sn bekletilmiş ve steril distile sudan 5 kez geçirilerek yapılmıştır. Tohumların her birinden saksılara 5'er tane ekilmiş, çimlenme sonunda 2'ye seyreltilmiştir. Hazırlanan ekstraktlar toprağa püskürtme şeklinde su yerine verilmiştir. Kontrol olarak mikroalg ekstraktları yerine çeşme suyu verilmiştir. Saksı denemeleri tesadüf parselleri deneme desenine göre 3 tekerrürlü olarak kurulmuştur. Bitkiler ekimden 90 gün sonra hasat edilmiştir. Saksılar gerektiğinde hazırlanan ekstraktlar ile sulanmıştır.

Uygulamalar arasındaki farklılık ve gruplamalar her bir bitki için ayrı ayrı JUMP11 istatistik programı kullanılarak belirlenmiştir.

3. Bulgular ve Tartışma

Mikroalgler; su biyoremediasyon etmenleri (Oswald, 1992), su ürünleri yetiştiriciliğinde (De Pauw et al., 1992), insanlar ve hayvanlar için gıda (Becker, 1992), pigment üretiminde (Johnson & An, 1991), ağır metallerin biyolojik olarak uzaklaştırılmasında (Wilde & Benemann, 1993) ve tarım (Renuka et al., 2018) gibi birçok alanda kullanılmaktadır. Mikroalgler tarımsal açıdan önemli bitkilerin verimini artırma potansiyeli açısından son yıllarda dikkat çekmektedir (Rachidi et al., 2020). Prokaryotik siyanobakteriler, bitki büyümesini ve ürün verimini artırmak için biyogübre olarak uygulanmıştır (Innok et al., 2009; Renuka et al., 2018). *Anabaena variabilis* ve *Nostoc* sp. ile muamele edilmiş çeltik bitkilerinin inorganik gübreye göre bitki boyu, yaprak uzunluğu ve tane verimini daha fazla arttırdığı rapor edilmiştir (Singh & Datta, 2007; Innok et al., 2009). *A. laxa* ve *Calothrix elenkinii* ile aşılama, kişniş tohumlarının çimlenmesini arttırmış; kişniş, kimyon ve rezenede kök ve sürgün gelişimini desteklemiştir (Kumar et al., 2013). Ayrıca, siyanobakteriler *Calothrix* sp., *Hapalosiphon* sp., *Nostoc* sp. ve *Westiellopsis* sp.'nin süpernatantlarının kontrol olarak su uygulandığında buğdayda kök uzunluğunu ve tohum çimlenmesini kontrole göre sırası ile 2.7, 2.1 ve 1.1 kat arttırdığı bildirilmiştir (Karthikeyan et al., 2009).

Çalışmamızda *Microcystis viridis* ve *Aphanizomenon gracile* karışık kültürünün farklı dozlarının fiğ, nohut ve arpa bitkilerinin gelişimleri üzerine etkileri Tablo 2'de verilmiştir. Denememizde test edilen bitkilerin kök uzunluğu üzerine siyanobakteri karışımının farklı dozlarının etkileri farklılık göstermiştir. Kök uzunluğunu fiğde %1.5 oranındaki uygulama dozu etkili bulunurken, arpa ve nohutta %2'lik uygulama dozu etkili olmuştur. Mikroalg karışımının farklı dozları kontrolle karşılaştırıldığında fiğ, arpa ve nohutun bitki boyunu arttırmıştır. Nohut ve fiğ bitki boyunu; %2'lik doz, kontrolle karşılaştırıldığında önemli ölçüde arttırırken, arpada en yüksek bitki boyu %1.5 uygulama dozunda elde edilmiştir (Tablo 2). Bu sonuçlar, Zhang et al. (2017) tarafından bildirildiği gibi *Chlorella vulgaris* ve *Scenedesmus quadricauda* ile aşılı domates bitkilerinin kontrole göre bitki boyu ve bitki ağırlığının artması ile benzerlik göstermektedir. Siyanobakteriler ile aşılı fiğ, nohut ve arpanın bitki boyu ve kök uzunluğunun kontrole göre artmasının alglerin fitouyarıcı olmasından kaynaklanabileceği düşünülmektedir.

Tablo 2. Siyanobakteriyal karışımın farklı dozlarının fiğ, nohut ve arpa gelişimi üzerine etkileri

Table 2. Effects of different doses of cyanobacterial mixture on the growth of vetch, chickpea and barley

Özellik	Siyanobakteri doz (%)	Fiğ	Nohut	Arpa
Bitki boyu (cm)	0	97.3 C	51 B	19.7 C
	1	103.3 BC	56.3 AB	23.7 B
	1.5	109.7 AB	56.7 AB	27.7 A
	2	113.7 A	60.7 A	29.0 A
Kök uzunluğu (cm)	0	14 C	8.8 C	8.6 B
	1	17.3 BC	13.7 B	8.6 B
	1.5	23 A	15.7 AB	12 AB
	2	20 AB	20.3 A	14.3 A
Kök kuru ağırlığı (g)	0	0.020 C	0.04 D	0.03 B
	1	0.077 B	0.06 C	0.09 B
	1.5	0.167 A	0.17 B	0.32 A
	2	0.029 C	0.21 A	0.36 A
Kök yaş ağırlığı (g)	0	0.43 B	0.18 D	0.33 C
	1	0.77 A	0.36 C	0.48 C
	1.5	0.82 A	0.84 B	1.23 B
	2	1.4 A	0.99 A	1.60 A
Gövde yaş ağırlığı (g)	0	0.74 B	3.5 B	0.95 C
	1	0.95 AB	4.1 B	1.84 B
	1.5	1.41 A	6 A	3.26 C
	2	1.27 AB	5.9 A	3.15 A
Gövde kuru ağırlığı (g)	0	0.17 B	0.7 B	0.19 C
	1	0.22 AB	0.8 B	0.40 B
	1.5	0.28 A	1.2 A	0.63 A
	2	0.23 AB	1.3 A	0.61 A

Zhang et al. (2017), mikroalg uygulanan domates bitkilerinde ölçülen fizyolojik parametreler üzerindeki olumlu etkinin mikroalgler tarafından yavaşça salınan biyouyarıcı maddelerin veya allelokimyasalların varlığından da kaynaklanabileceğini bildirmişlerdir. Allelopati, hedef organizmalar üzerindeki faydalı veya zararlı etkiye bağlı olarak olumlu veya olumsuz etki gösterebilmektedir (Zhang et al., 2017).

Mikroalglerden elde edilen polisakkaritler, tarımsal ürünlerin geliştirilmesi ve korunması için potansiyel olarak bir biyolojik kaynak olarak tanımlanmıştır (Rossi & De Philippis, 2016). Mikroalg polisakkaritlerini bitki biyouyarıcı olarak kullanma olasılığının araştırıldığı çalışmada; üç mikroalg türünden elde edilen ham polisakkarit özü, domates bitkilerine sulama yoluyla uygulanmış, sürgün ve kök uzunluğu, boğum sayısı, sürgün ve kök kuru ağırlığı üzerindeki etkilerine göre karşılaştırılmıştır (Rachidi et al., 2020). *Arthrospira platensis*, *Dunaliella salina* ve *Porphorydium* sp.'nin elde edilen polisakkarit (1 mg/ml) uygulamasının domates bitkilerinde, kontrole oranla yeşil aksam kuru ağırlığı ve

yeşil aksam uzunluğunu sırasıyla %46.6 ve %25.26 oranında önemli ölçüde iyileştirdiği belirlenmiştir (Rachidi et al., 2020).

Siyanobakteri karışımının farklı dozlarının fiğ, arpa ve nohut yaş ve kuru ağırlıkları üzerine etkileri kontrolle karşılaştırıldıklarında etkili bulunmuştur. En yüksek yeşil aksam yaş ağırlığı her üç bitkide de %1.5 uygulama dozunda belirlenmiştir (Tablo 2).

Yapılan benzer çalışmada mikroalg *Chlorella vulgaris* ve *Scenedesmus quadricauda*'dan elde edilen ekstraktların, şeker pancarı bitki ağırlığı üzerinde biyouyarıcı bir etki gösterdiği gösterilmiştir (Barone et al., 2018). Ayrıca Barone et al. (2018), şeker pancarı bitkisinin, gelişme ortamına *S. quadricauda* ekstraktının eklenmesi ile kök ağırlığı ve kök uzunluğunu kontrole oranla önemli ölçüde arttırdığını rapor etmişlerdir. Puglisi et al. (2020) *S. quadricauda* 'nın marul gelişimine olan etkisini araştırdıkları çalışmalarında da marul fidelerinin gelişimini olumlu etkilediklerini, kuru madde, karotenoid, protein ve klorofil içeriğini arttırdığı incelenmiştir. Bizim çalışmamızda da siyanobakteri karışımının uygulanan üç farklı dozlarının kontrole göre bitki boyu, kök uzunluğu ile yaş ve kuru ağırlıklarını arttırmaları; araştırıcıların bulguları ile desteklenmektedir.

Bakteriler ve funguslar bitki gelişimini iyileştirmek için kullanılmakta olsa da, yeni veriler alglerin de bitki büyümesini desteklediğini, patojen büyümesini doğrudan inhibe ederek, bitki bağışıklığını aktive ederek patojenlere karşı biyolojik kontrol ajanları olarak hareket ettiğini göstermektedir (Kumar et al., 2013; Rossi & De Philippis, 2016; Renuka et al., 2018). Bu nedenle, algler, biyogübreler ve bitki koruyucular olarak kullanılabilen yeni bir biyoaktif materyal olarak karşımıza çıkmaktadır. Çalışmamızda siyanobakterilerin farklı dozları test edilen bitkilerin gelişmelerini kontrole göre arttırmıştır. Mikroalglerin bitki gelişimini destekleyici olarak kullanımı ile ilgili araştırmaların yapılması kimyasal gübrelerin kullanımını da azaltacaktır. Çalışmamızın yapılacak olan çalışmalara yol göstereceği düşünülmektedir.

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Research Article/Araştırma Makalesi

Examination of Behavioral Traits of Monocultures and Polycultures of Two Different Trout Species (*Oncorhynchus mykiss*, *Salmo* sp.) at Different Ratios Depending on Various Factors

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Abstract: The present study aimed to determine both the effects of monocultures of rainbow trout (R) and brown trout (B) and polycultures (R 75%-B 25%; R 66%-B 34% and R 50%-B 50%) on behavior. In the study, the area used vertically by the fish in the tank, the mobility rate of fish in the tank, the rate of tendency of fish in eating as soon as they were fed, whether the fish test the feed, the interspecies feed competition, the time they start to take the first feed, the duration of the feed consumption of the fish and the feed area of the fish have been considered as behavioral evaluation criteria. The trout were monitored with a camera to determine their behavior. Considering all behavioral criteria, the best polyculture rate was determined as R 66%-B 34%. Brown trout were found to be more mobile and exhibit more relaxed behavior compared to other groups in polyculture. In addition, interspecies feed competition was mostly encountered in this group. As a result, in this study, in which two different trout species were monocultured and treated at different polyculture ratios, the main factor causing behavioral change in fish was found to be the different stocking rates of fish to each other in the same tank. Different rates applied in polyculture caused unpredictable changes in behavior in both species. The ratio of fish used in polyculture was found to be a considerable factor affecting the final product and their behavior for aquaculture.

Keywords: Behavioral change, rainbow trout, brown trout, polyculture.

İki Farklı Alabalık Türünün (*Oncorhynchus mykiss*, *Salmo* sp.) Monokültürleri ile Farklı Oranlarda Polikültürlerinin Çeşitli Faktörlere Bağlı Davranış Özelliklerinin İncelenmesi

Öz: Bu çalışmada gökkuşağı alabalığı (G) ve kahverengi alabalığın (K) monokültürleri ile farklı oranlarda (G%75-K%25; G%66-K%34 ve G%50-K%50) polikültürlerinin davranış üzerine etkilerinin belirlenmesi amaçlanmıştır. Çalışmada balıkların; tankta dikey olarak kullandığı bölge, tanktaki hareketlilik oranları, yemin ilk verildiği anda yeme karşı ilgi yüzdesi, yemi test edip etmediği, türler arası yem rekabeti, ilk yem almaya başlama süresi, yemi tüketme süresi ve yem aldığı bölge davranış değerlendirme ölçütleri olarak dikkate alınmıştır. Alabalıkların davranışlarını belirlemek için kamera kayıtları kullanılmıştır. Tüm davranış ölçütleri dikkate alındığında en iyi polikültür oranı G%66-K%34 olarak belirlenmiştir. Bu gruptaki kahverengi alabalıkların polikültürdeki diğer gruplara kıyasla daha hareketli olduğu ve daha rahat davranış sergiledikleri görülmüştür. Ayrıca türler arası yem rekabeti de en fazla bu grupta görülmüştür. Sonuç olarak iki farklı alabalık türünün monokültürünün ve farklı oranlarda polikültürünün yapıldığı bu çalışmada balıklardaki davranış değişikliğine sebep olan temel etken, balıkların aynı tanktaki birbirlerine olan farklı stoklama oranlarıdır. Polikültürde uygulanan farklı oranların iki türün davranışlarında önceden tahmin edilemeyecek şekilde değişikliklere sebep olmuştur. Polikültürde kullanılan balıkların birbirlerine olan oranları, yetiştiricilikte davranışı nihai ürünü etkileyen ve dikkate alınması gereken önemli bir faktördür.

Anahtar kelimeler: Davranış değişikliği, gökkuşağı alabalığı, kahverengi alabalık, polikültür.

1. Introduction

Changes in environmental conditions in aquaculture may cause several differences in fish behavior. Observation and evaluation of these differences are crucial for aquaculture producers and experts. Many systems, therefore, have been developed by researchers to evaluate fish behavior (Hong, & Zha, 2019; Han et al., 2020). Due to cost effectiveness and their reliability, video technologies that form the basis of most of these systems are widely applied in both research and commercial aquaculture facilities to monitor and analyze fish behavior (Kane et al., 2004; Stien et al., 2007; Wagget & Buskey, 2007; Salierno et al., 2008; Papadakis et al., 2012; Papadakis et al., 2014; Hong & Zha, 2019). Moreover, monitoring and analyzing the fish behavior provide economic benefits for fish farmers (Stien et al., 2007; Papadakis et al., 2014). As in all farming fields, an important purpose in aquaculture is to obtain maximum product from the unit area with the least cost. Although it may be underestimated, the factors that cause sudden changes in fish behavior may affect the profitability of the business (Karataş, 2015). Understanding fish behavior, therefore, is crucial for the effective management of aquaculture facilities (Pinkiewicz et al., 2011).

Researchers have conducted many comprehensive studies on feeding, swimming, and group behavior of fish. In addition to investigating fish interactions with other species, they examined fish behavior under environmental stress and in polyculture aquacultures (Xu et al., 2006; Delcourt et al., 2009; Sadoul et al., 2014; Karataş, 2015; Føre et al., 2018; Li et al., 2020). These studies on fish behavior have contributed to the development of healthy

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aquaculture practices in terms of improving breeding conditions, identifying individual or environmental negativities. Studies on the behavior of cultured fish species in polyculture breeding studies, however, is extremely scarce. Polyculture breeding studies mostly focused on the growth and survival rates of the cultured fish rather than their behavior (Barki & Karplus, 2016). However, the behavior of the fish in the polyculture studies may significantly affect the breeding results (e.g., growth, feed consumption, and etc.) (Rahman & Verdegem, 2010; Karataş, 2015).

In this study, the effects of monocultures of rainbow trout (R) and brown trout (B), which are two carnivorous species cultivated in Turkey, and polycultures (R 75%-B 25%; R 66%-B 34% and R 50%-B 50%) on behavior are aimed to be investigated in a comparative manner.

2. Material and Methods

This study was carried out at Fisheries Research and Application Facility, Yüzüncü Yıl University Research and Application Farm Directorate. The study was approved by the Animal Experiments Local Ethics Committee (No: 27552122-80) of Van Yüzüncü Yıl University. In the study, rainbow trout (*Oncorhynchus mykiss*) and stream ecotype of brown trout (*Salmo sp.*) were used. The fish used in the research were obtained from the Avcil Trout Farm in Bitlis Province. The study was carried out between July and October 2014. In the current examination, ten PVC aquaculture tanks with a volume of 500 liters were used. During the study, the amount of dissolved oxygen in water and the temperature and pH of the water were measured on daily basis just after 30 minutes of feeding. During the study, fish were fed with 2 mm and 3 mm trout grower feed produced by a private company (Skretting) twice every day, once in the morning and once in the evening. To determine the exact environmental and food consumption effects of polyculture, "over nutrition" method was used as nutritional regime. To prevent the feed to affect the behavior of the fish and to be ever present in the environment whenever they needed, the feed was delivered more than the amount required by the fish. During this experiment, the amount of dissolved oxygen ranged from 5.8 mg/L to 7.4 mg/L whereas the pH of water varied from 7.9 to 8.1. While the temperature was about 18°C at the beginning of the experiment, it was measured as about 15°C at the end. The water flow rate of tanks was adjusted to an average of 2 L/ min in the present study.

A total of 140 rainbow trout (24.7±0.2 g) and 100 brown trout (41.8±0.8 g) were tested in the study. The weight difference between the rainbow trout and brown trout utilized in the current experiment was found higher than the intended level. This is because the trial layout was corrupted seven times due to the difficulties in material supply, the deterioration of water quality, and the recurrent diseases. Five different stockings were applied at different rates and each stocking rate was assessed as a group. These groups were planned as two replications. Rainbow trout (R) and Brown trout (B) ratios in polyculture were established in line with the recommendations of the commercial fish farms. A total of 240 fish were used and 24 fish were placed in each group. Table 1 shows the groups applied in the current study and the stocking rates.

Table 1. The groups applied in the current study and stocking rates.

Groups	Stocking Rates (Species and number of fish in groups)
R	100% Rainbow Trout (24)
B	100% Brown Trout (24)
R50B50	50% Rainbow Trout (12) + 50% Brown Trout (12)
R66B34	66.6%Rainbow Trout (16) + 33.3%Brown Trout (8)
R75B25	75%Rainbow Trout (18) + 25% Brown Trout (6)

The study was launched after 10 days of adaptation of the fish. Then, the study was proceeded for another 80 days. In order to examine the effects of two different trout breeding in the same environment at different rates on the behavior, the fish behaviors in the experimental tanks were monitored every three weeks using a GoPro Hero II underwater camera during and after feeding. Video cameras were placed in the tanks just 5 minutes before the feeding procedure and recorded until the camera batteries died (approximately 60 minutes). The criteria for the behavior of the trout during and after feeding procedures were specified according to the results of the comparative monitoring of the groups.

In the study, to determine the behavior of the fish, the edges of the experimental tanks were marked creating white rings at 15 cm intervals. The bottom of the tank was calibrated as 0 cm whereas the surface of the tank was adjusted as 50 cm. The water level in each tank was set to 40 cm (Fig. 1).

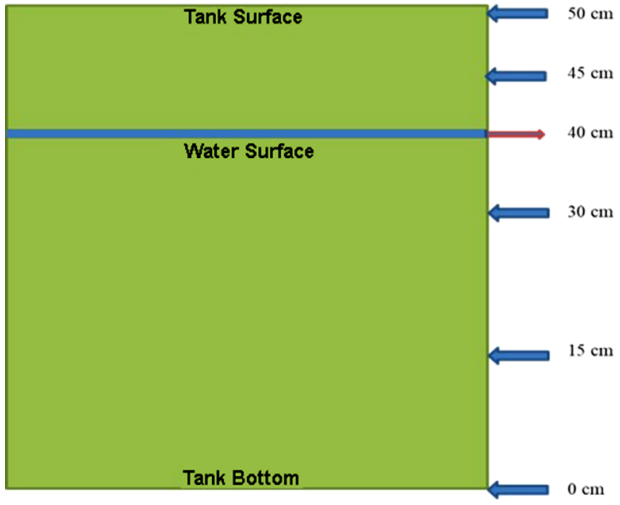

Figure 1. Markings made at 15 cm intervals from the bottom of the tank towards the surface of the tank to determine the vertical use of the fish in the tank.

In the present study, data on behavior were obtained watching the camera records. Then, the records were assessed to respond to the quantitative behavioral criteria stated below. These assessments were carried out by the same observer to be consistent through the current experiments. Since it was difficult to identify fish in camera records at first glance, the videos were watched repeatedly at first and; then, the assessments were carried out after becoming familiar with these records.

In the study, 8 behavioral evaluation criteria, which were quantitatively classified as discussed below, were carried out.

(i) The area used vertically by the fish in the tank;

In monoculture farming, brown trout generally use the bottom of the tank whereas rainbow trout mostly use the middle part of the tank. In some polyculture groups, however, the distribution of fish in the water column varied for both trout species. This situation, therefore, was defined as the area used vertically by the fish in the tank and this has been digitized in centimeters from the bottom of the tank upwards.

(ii) The mobility rate of the fish in the tank;

In monoculture aquaculture, rainbow trout are usually very active in the tank and they tend to swim quickly and actively around the tank. Brown trout, on the other hand, are not very mobile in the tank compared to rainbow trout and even some of them tend to stay still on the bottom of the tank. This behavior of the fishes, however, shows alterations in polyculture groups as they compete with each other for food and area. These alterations in the movement of fish were observed particularly in brown trout in polyculture groups. This situation has defined as the mobility rate of fish in the tank. Based on the number of fish in the polyculture groups, the number of fish swimming quickly and actively during and after feeding were calculated. Then, these alterations were assessed as minimum +, maximum +++++ based on numerical values.

(iii) The rate of tendency of the fish in eating as soon as they were fed;

In monoculture aquaculture, while the majority of rainbow trout tend to eat feed quickly and actively (like gobbling and rushing for feed), most of the brown trout remain stable and do not actively eat feed. This situation differs in polyculture groups. Based on the number of fish in the polyculture groups, the number of fish tending to feed (regardless of whether the fish take the feed or not) was determined and given as percentage.

(iv) Whether the fish test the feed;

In monoculture, the brown trout which did not show interest in the feed as soon as they were fed tested the feed. This test involved playing with the feed, gathering the feed using their tails, and hanging around the feed. In this way, whether the fish test the feed was evaluated as "yes" or "no".

(v) The time the fish start to take the first feed;

The time after the start of feeding the fish was expressed as the time the fish start to take the first feed and this was given in minutes (in other words, it was determined in what minute the fish started to take the first feed). This situation showed alterations in different video records taken at different times for the same group. Therefore, the time the fishes start to take the first feed was given as the minimum start minute and the maximum start minute.

(vi) The interspecies feed competition;

In polyculture groups, on the other hand, the interspecies competition during the feed intake was assessed as present or not present.

(vii) The duration of the feed consumption of the fish;

The period between the first and last feed intake of the fish was determined as the duration of the feed consumption. This period was evaluated in minutes. This also varied in different video records taken at different times for the same group. Therefore, the duration of the feed consumption of fishes was given as the minimum feed consumption time and the maximum feed consumption time.

(viii) The feed area of the fish;

In monoculture farming, brown trout commonly grab the feed from the bottom of the tank (ground) whereas rainbow trout take the feed from both the tank bottom (ground) and the water column. In some polyculture groups, however, the feed area differed for both trout species. This was defined as the feed area of the fishes and considered starting from the bottom of the tank or water column.

3. Results

The findings of the present study on the effects of two different trout species farming in the same environment at different rates on behavior were evaluated separately for each group and given in figures and tables. The areas used vertically by the fish in the test tanks were given in Figure 2 and Table 2.

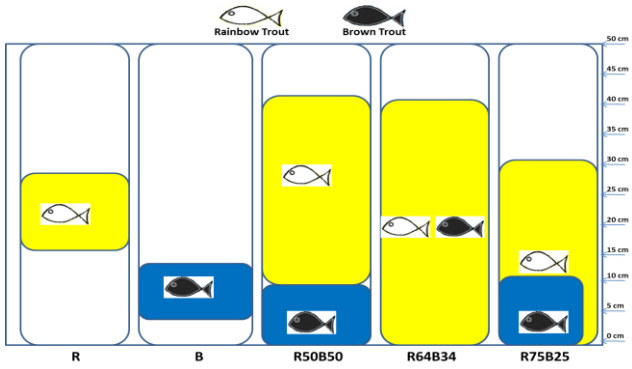

Figure 2. The areas used vertically by the fish in the test tanks.

The findings on the areas used vertically by the fish and the mobility rate of fish in the tanks were given in Table 2.

The findings on the rate of tendency of the fish in eating as soon as they were fed, whether the fish test the feed, the time the fish start to take the first feed were given in Table 3.

The findings on the interspecies feed competition, the duration of the feed consumption of the fish, and the feed area of the fish were given in Table 4.

4. Discussion and Conclusions

In the present study, two different trout species in experimental tanks were monitored using a GoPro underwater camera during and after feeding to examine the effects of breeding (monoculture and polyculture) of these fish in the same environment at different rates on behavior. In line with the behavioral results obtained, when considering the areas used vertically by the fish in the tank it was observed that the monocultured rainbow trout actively used every part of the tank and mainly preferred the areas between 15-30 cm from the bottom of the tank. The monocultured brown trout, on the other hand, mostly used the area of 5-15 cm and rarely preferred the area of 20-25 cm from the bottom of the tank. This

situation, however, varied in other groups when polyculture was performed. Unpredictable behavior patterns were even encountered in each group. In R50B50 group where polyculture was performed, it was observed that rainbow trout used the area of 10-40 cm whereas brown trout utilized the area of 0-10 cm from the bottom of the tanks. This shows that two different trout species recognized each other, allocated the tank between themselves, and did not poach on each other's territory. In the polyculture of rainbow trout and brown trout in the R66B34 group, both species in the tanks with a water height of 50 cm mainly used the areas of 0-40 cm from the bottom of the tanks. This demonstrates that these different species were completely accustomed to each other and shared every part of the tank. In the R75B25 group, it was observed that the rainbow trout mainly used the area of 0-30 cm whereas brown trout preferred the area of 0-10 cm from the bottom of the tank. This situation can be evaluated as those two different trout species allocated the tank between themselves; however, rainbow trout also actively disturbed the area of brown trout; that is, they pressured on the brown trout.

Table 2. The areas used vertically by the fish and the mobility rate of fish in the tanks.

Groups	Fish species	The areas used vertically by the fishes (maximum depth: 50 cm)	The mobility rate of fishes in the tanks (maximum value +++++)
R	Rainbow trout	All areas of the water column in the tank (Mostly between 15-30 cm)	+++++
B	Brown trout	The distance between 0-25 cm (Mostly between 5-15 cm)	+ + - - -
R50B50	Rainbow trout	Between 10-40 cm	++++-
	Brown trout	Between 0-10 cm	+++ --
R66B34	Rainbow trout/Brown trout	Between 0-40 cm/Between 0-40 cm	+++++/+++++
R75B25	Rainbow trout/Brown trout	Between 0-30 cm/Between 0-10 cm	+++++/+ + + - -

Table 3. The rate of tendency of the fish in eating as soon as they were fed whether the fish test the feed and the time the fish start to take the first feed.

Groups	Fish species	The rate of tendency of fishes in eating as soon as they were fed	Whether the fishes test the feed (Yes/No)	The time fishes start to take the first feed (Minimum-Maximum)
R	Rainbow trout	90% interested when fed	No	0 min (Started straight away)
B	Brown trout	20% interested when fed	Yes (Playing with the feed, gathering the feed using their tails)	After 20-30 min
R50B50	Rainbow trout	90% interested when fed	No	0 min (Started straight away)
	Brown trout	Not interested when fed. 50% interested after 5- 10 min	No	Between 5-10 min
R66B34	Rainbow trout	90% interested when fed	No	0 min (Started straight away)
	Brown trout	90% interested when fed	No	0 min (Started straight away)
R75B25	Rainbow trout	90% interested when fed	No	0 min (Started straight away)
	Brown trout	Not interested when fed. 60% interested after 25- 30 min	No	Between 20-25 min

Table 4. The interspecies feed competition, the duration of the feed consumption of the fish, and the feed area of the fish.

Groups	Fish species	The interspecies feed competition (present/not present)	The duration of the feed consumption of fishes (minimum-maximum)	The feed area of the fishes
R	Rainbow trout	-	Between 20- 25 min	Water column and tank bottom
B	Brown trout	-	Between 30-40(+)* min	Tank bottom
R50B50	Rainbow trout	Present	Between 15 -20 min	Water column and tank bottom
	Brown trout	Present	Between 35-55(+)* min	Tank bottom
R66B34	Rainbow trout	Present	Between 25-30 min	Water column and tank bottom
	Brown trout	Present	Between 25-30 min	Water column and tank bottom
R75B25	Rainbow trout	Present	Between 20-25 min	Water column and tank bottom
	Brown trout	Not present	Between 35-40(+)* min	Tank bottom

*Recording could not be continued due to the camera running out of charge. However, the feed consumption of the fish continued to be monitored by the researcher and it was observed that the feed consumption also continued after the video recording.

In the present study, the application of polyculture caused changes in the areas used vertically by the fish in the tanks. Similarly, Rahman & Verdegem (2010) investigated the polyculture of two different carp species (*Labeo calbasu* and *Cirrhinus cirrhosus*) in their study. They stated that both species were fed from the bottom of the tank, however, this situation changed in polyculture due to the competition between species. In addition, they reported that one of the species, *L. calbasu, started f*eeding from both the tank bottom and the water column. In another study conducted by Barki & Karplus (2016), the behavior of tilapia and crayfish in polyculture was examined and it was reported that while tilapia species used every part of the tank in monoculture, they preferred to use mostly the water

column due to the presence of crayfish in polyculture.

Assessing the study groups in terms of the mobility rate of the fish in the tank, it was observed that the monocultured rainbow trout were very active in the tank. Moreover, it was noticed that the fishs were swiftly and actively swimming around the tank. On other hand, it was observed that monocultured brown trout were not very active in the tank and some of them were dormant on the bottom of the tank. Rainbow trout and brown trout in the R66B34 group, however, were very active in the tank. In particular, the brown trout, which were calmer and shier, were found to be very comfortable in this group (R66B34) like rainbow trout. In the other two groups (R50B50, R75B25), the brown trout exhibited recessive behavior similar to the group B.

The polyculture application conducted in this study caused changes in the mobility rate of the fish in the tanks. In a previous study showing similarities with the results of this study, Rahman & Verdegem, (2010) investigated the polyculture of two different carp species (*Labeo calbasu* and *Cirrhinus cirrhosus)* and they reported that one of the species (*C. cirrhosus*) exhibited recessive behavior in polyculture compared to monoculture while the other species (*L. calbasu*) was more active. As a result, they asserted that this situation has a positive effect on the growth of *L. calbasu* species. In another study, Barki & Karplus (2016) investigated polycultures of tilapia and crayfish and reported that tilapia species were more active in polyculture compared to monoculture and this increased the growth of tilapias.

When the study groups were evaluated in terms of the rate of tendency of the fish in eating as soon as they were fed, it was observed in monocultured rainbow trout that most of the fish (about 90%) showed interest in eating quickly and actively (by attacking the bait) as soon as the feeding process started. In monocultured brown trout, it was observed that most of the fish (about 80%) remained stable after the start of feeding and they did not show interest in feeding quickly and actively. This shows that the brown trout were very sensitive and tested the feed (picking the feed up by wiggling it using their tails). After examining the video records, it was determined that these fish started to eat the feed in small amounts during the first 20-30 minutes after feeding and they aggregated the feed in an area on the bottom of the tank using their tails and then tested. After this process, testing and recognizing the feed, it was observed that the feed intake of the fish increased. It was monitored that the rainbow trout in all groups did not test the feed and reckon the objects thrown into the tanks as feed. The brown trout in the R66B34 group were found to be very comfortable towards the feed like rainbow trout. This may indicate that the brown trout in this group learned this behavior from rainbow trout. In the other two groups (R50b50, R75B25), it was observed that the brown trout waited for a while after feeding and then took the feed (Table 3). The fish in these two groups (R50B50, R75B25), however, did not test the feed. The rainbow trout's ingestion of the feed may have caused the brown trout to take the bait without testing it.

In this study, the application of polyculture caused changes in the percentage of interest of the fish towards the feed at the first feeding. Similarly, Sırtkaya (2013) compared the growth performance, daily feed consumption, and feed conversion ratios of monocultured and polycultured rainbow trout (*Oncorhynchus mykiss*) and turbot (*Psetta maxima*). She observed that monocultured turbot fish were relatively less active than turbot in the polyculture group, while turbot in the polyculture group were more active in feed intake. Furthermore, Sırtkaya (2013) reported that the fish in the polyculture group grouped among themselves, stood in different directions, and were positioned opposite to each other on both sides of the tank. In another polyculture study, it was reported that due to presence of the crayfish at the bottom of the tank, tilapias moved faster in the water column in polyculture compared to monoculture (Barki & Karplus, 2016). It was also stated that the tilapias attacked to the feed as a group and consumed more feed before the feed sinks into the tank bottom (Barki & Karplus, 2016).

When the study groups were evaluated in terms of the interspecies feed competition, feed competition between fish in mixed farms was observed. In the R50B50 group, the rainbow trout, which did not leave their area during non-feeding periods, put pressure on brown trout by entering their area to take the feed on the ground during feeding. Under these circumstances, the brown trout remained stable and formed a protection area by covering the feed. In the R66B34 group, on the other hand, there was a competition between brown trout and rainbow trout during feeding due to the feed intake from both the ground and the water column. It was observed that the fish chased each other during feed intake and prevented each other taking the feed. In the R75B25 group, some of the brown trout (approximately 20%) approached the feed 5-10 minutes after feeding; however, the rainbow trout did not allow them and prevented the brown trout from approaching the feed. This situation in the R75B25 group can be interpreted that a sense of competition was formed in rainbow trout however this feeling did not form in the brown trout because of not attempting to take the feed as they submitted themselves to this situation.

Some studies reported that even albinism is a factor affecting the aquaculture process in rainbow trout farming. For example, when normal pigmented rainbow trout and albino rainbow trout were reared in separate environments, the growth and feed utilization of albino rainbow trout were not decent. When they were reared in the same pond; however, albinos competed with normal rainbow trout for feed intake and subsequently the growth of albinos increased (Okumuş et al., 2001).

Considering the study groups with regards to the duration of the feed consumption of the fish, this duration showed differences between groups. While rainbow trout did not consume the feed after the first 25 minutes in all groups, this period was 30-40(+) minutes in monocultured brown trout. This period, however, shortened in some groups in polyculture. The shortening of feed consumption time of brown trout in polyculture groups could be due to learning from rainbow trout.

Considering the behaviors observed in all groups during the current study, it was observed that the R66B34 group exhibited the most comfortable behavior among the brown trout. Nevertheless, this kind of comfortable behavior was not observed in groups with other brown trout. However, some differences were observed in the motility rates, the areas used vertically in the tank, the rate

of tendency of fish in eating as soon as they were fed, the areas where they were fed and the duration of the feed consumption of brown trout in polyculture. On the other hand, when the behavioral differences in rainbow trout were evaluated in the present study, it is determined that behavioral differences occurred in the mobility rates of the fish in the tank, the area used vertically by the fishes in the tank, and the duration of the feed consumption of fishes. Indeed, in our publication containing other results of the same experiment, where the data on growth and feed evaluation conducted in detail, Karataş et al. (2017) reported that the feed consumption amounts and growth rates of brown trout in all polyculture groups increased comparing to monoculture of brown trout due to feed competition between fish. Karataş et al. (2017) recommended especially the R66B34 group along with other polyculture groups rather than the monoculture of brown trout to fish farmers for rapid growth of brown trout. This is because, when this ratio of polyculture was applied, brown trout's comfortable behavior like rainbow trout reduced the stress of brown trout and increased feed competition and feed consumption. In addition, in the study of Karataş et al. (2017), where monoculture and polyculture of rainbow trout and brown trout were experimented, it was reported that the highest amount of final product was observed in monoculture of rainbow trout. Therefore, they recommended monoculture of rainbow trout rather than polyculture of rainbow trout with brown trout to the fish farmers who desires the largest amount of final product.

In conclusion, in the present study, in which two different trout species were monocultured and polycultured at different rates, the main factor causing behavioral changes in trout was the ratio of fish to each other. Different ratios applied in polyculture caused unpredictable changes in behavior in both fish species in the current examination. Therefore, the ratio of the fishes used in polyculture is a significant factor affecting the final product.

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Conflict of interest: The authors declare that there is no conflict of interest.

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Research Article/Araştırma Makalesi

Bioactive Properties of Different Parts of *Vitis labrusca* L. Fruit

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Abstract: *Vitus labrusca* L. is a fragrant grape variety that is widely grown in our country and the world. In addition to being consumed as a table grape, it is also consumed by processing into different products such as wine, vinegar, and molasses. This fruit and its residues, revealed after processing into the product, are rich in phenolic compounds. The residues of the grape can be evaluated due to their bioactive potential. In this study, the bioactive characteristics of *V. labrusca* L. grown in Samsun were investigated. Total phenolic, antioxidative activity properties and oleanolic acid levels were determined in order to reveal their bioactive properties. For this purpose, the whole fruits, skin, and seed parts of the fruits were analyzed separately. It is concluded that the highest values as the total phenolics (1519.68±280.05 mg kg^{-1}), oleanolic acid (351.32±91.42 mg kg^{-1}), and antioxidant values (FRAP: 22.13±6.77 µmol $Fe^{2+}g^{-1}$) were determined in the skin of fruit. Furthermore, when the relationship between the natural antioxidant contents and the antioxidant activity examined, there was a negative correlation between the total phenolics and DPPH reducing value (as EC_{50}). In conclusion, it is considered that the residues of the grape should be evaluated for their bioactive potential due to *V. labrusca* L. Fruits being rich in bioactive compounds, and these compounds are mainly concentrated in the skin part.

Keywords: Antioxidant activity, grape, oleanolic acid, triterpenoids.

Vitis labrusca L. Meyvesinin Farklı Kısımlarının Biyoaktif Özellikleri

Öz: *Vitus labrusca* L. ülkemizde ve dünyada yaygın olarak yetiştirilen kokulu bir üzüm çeşididir. Sofralık üzüm olarak tüketilmesinin yanı sıra şarap, sirke ve pekmez gibi farklı ürünlere işlenerek de tüketilmektedir. Bu meyve ve ürüne işlendikten sonraki kalıntıları fenolik bileşikler açısından zengindir. Üzüm kalıntıları biyoaktif potansiyelleri nedeniyle değerlendirilebilir. Bu çalışmada, Samsun'da yetiştirilen *V. labrusca* L.'nın biyoaktif özellikleri araştırılmıştır. Biyoaktif özelliklerini ortaya koymak için toplam fenolik madde, antioksidan aktivite özellikleri ve oleanolik asit miktarları belirlenmiştir. Bu amaçla meyveler bütün olarak, kabuk ve çekirdek kısımları ayrı ayrı analiz edilmiştir. Toplam fenolik madde (1519.68±280.05 mg kg^{-1}), oleanolik asit (351.32±91.42 mg kg^{-1}) ve antioksidan aktivite değerleri (FRAP: 22.13±6.77 µmol $Fe^{2+}g^{-1}$) en fazla meyvelerin kabuk kısmında belirlenmiştir. Ayrıca, doğal antioksidan içerikleri ile antioksidan aktivite arasındaki ilişki incelendiğinde, toplam fenolik madde ile DPPH indirgeme değeri (EC_{50} olarak) arasında negatif bir ilişki olduğu görülmüştür. Sonuç olarak, *V. labrusca* L. meyvelerinin biyoaktif bileşiklerce zengin olması ve bu bileşiklerin ağırlıklı olarak kabuk kısmında yoğunlaşması nedeniyle üzüm kalıntılarının biyoaktif potansiyelleri açısından değerlendirilmesi gerektiği düşünülmektedir.

Anahtar kelimeler: Antioksidan aktivite, üzüm, oleanolik asit, triterpenoidler.

1. Introduction

Studies have shown an inverse relationship between age-related diseases and fruit and vegetable consumption due to antioxidants and especially phenolics in fruits and vegetables (Boas et al., 2014).

Grape is widely consumed and used to produce wine, vinegar, and molasses in the world. It has also been used to cure various diseases in traditional medicine (Liu et al., 2012). Mainly, its therapeutic potential has resulted from numerous phenolic compounds like flavonoids (anthocyanins, flavanols, and flavonols), phenolic acid, and resveratrol found in grapes. In addition, the grape phenolics have antiatherosclerotic, antithrombotic, and anti-inflammatory effects due to antioxidant activities (Carrieri et al., 2013).

The grape skin is present at about 5-10% of the total dry weight of grape berry. It is a hydrophobic barrier against UV-C light, dehydration, physiological and climatic injuries, and fungal infection. Generally, there are five layers on the grape skin from outside to inside: 1. epicuticular wax layer; 2. cuticle proper; 3. cutinized layer; 4. pectic layer, and 5. non cutinized cellulose layer or cellulose layer. Next to the epidermis, hypodermis including many phenolic compounds, has a few cell layers (Fava et al., 2011).

The main functions of the fruit cuticle, the hydrophobic coating on the epidermis are the prevention of dehydration by diminishing water loss and the inhibition of leaching of organic and inorganic compounds from inner tissues (Pensec et al., 2014). The first preventive barrier against abiotic and biotic environmental stresses is the cuticle. The cuticle is the exterior mechanical support, hence maintaining fruit wholeness. This support is crucial for fruits that have soft tissue (Pensec et al., 2014; Lara et al., 2019).

Cuticles are formed from two classes of lipids: very

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long-chain fatty acids and lipids derived from isoprene. In cuticular waxes, the lipids, derived from isoprene, are pentacyclic triterpenes and steroids. These triterpenoids have many biological functions and a lot of pharmacological effects against biotic stress (Pensec et al., 2014).

Oleanolic acid, a natural triterpenoid, is widely distributed in food, therapeutic herbs, and other plants. The oleanolic acid is in grape berries, especially in their skin (Chronopoulou et al., 2013). In recent years, there have been studies on its biological and pharmacological significance. The oleanolic acid and some of its derivatives show exciting properties such as hepatoprotective, anti-inflammatory, anti-HIV, and antitumor activities (Chronopoulou et al., 2013; Ayeleso et al., 2017). Kang et al. (2021) determined that oleanolic acid prevents allergic inflammatory reaction by blocking the NF-κB and STAT1 signaling pathways and oleanolic acid diminishes the levels of histamine and immunoglobulin-E.

The parts rich in terpenoids and phenolic compounds are separated when processing into grape juice and wine (Dwyer et al., 2014). It has been reported that the phenolics in grapes and red wines, which are known to have positive effects on health, inhibit the oxidation of low-density lipoproteins (LDL) *in vitro* (Jayaprakasha et al., 2003). For example, during wine production, approximately 25% of the grape weight is separated as waste. Currently, this residue part of the grape is used as compost in agricultural areas. Therefore, it is important to know the potential of such a waste that is rich in bioactive compounds to be converted into products with high added value in food industry as functional foods, in cosmetic industry as cosmetic agents, and as food supplements due to their pharmaceutical properties (Jayaprakasha et al., 2003; Dwyer et al., 2014).

This study aimed to assess the natural antioxidant compounds, total phenolics, the relationship between the oleanolic acid presence, and the *in vitro* antioxidant activities of whole fruit, seed, and skin extracts of *Vitis labrusca* L. grape (common name "kokulu üzüm") variety from Samsun, Turkey.

2. Material and Methods

This study was carried out in the food engineering department laboratories of Ondokuz Mayis University. Grape *(Vitis labrusca* L.*)* samples, cultivated in Samsun, were purchased from seven different local markets at the end of the summer season of 2016. After the samples were washed and the unnecessary parts were removed, each fruit sample was separated into two groups of 200 g. The first group was analyzed as whole. These fruits were homogenized by waring blender. In the second group, however, the seeds and skins of the fruits were manually separated. Then, the seeds and the skins were weighed and ground in a conventional electric grinder just before the analysis while the second one was analyzed separately as skins and seeds. The fruits in first group were homogenized by waring blender.

2.1. Extraction of Antioxidants

Ultrasound-assisted extraction was done by the use of ultrasonic bath (Sonorex, Bandelin). The samples (10 g) were placed into a conical flask including 25 mL of methanol as solvent, and sonicated for 15 min at 40°C temperature. The extraction process was carried out three times with fresh portions of solvent in the conditions mentioned above.

2.2. Physical-chemical analysis

The whole fruits were analyzed for soluble solid content (Brix), pH, and dry matter according to AOAC (2000). Dry matter analysis were done in the skin and seed parts of grape. For dry matter analysis, the sample was dried in the oven with a vacuum (100 mm-Hg) at 70°C until obtaining the constant weight.

2.3. Color Analysis

The surface color of grape berry was measured with a tristimulus reflectance colorimeter Model CR-400 (Minolta Co., Japan). Values were obtained for C illuminant and 2° observer. Before the test, the instrument was calibrated with a standard white provided by the manufacturer. The *L*, a*, b** components are CIELAB space. *L** indicates lightness or luminance. a* represents green (−) to red (+) axis, and *b** represents blue (−) to yellow (+) axis.

2.4. Determination of natural antioxidants

2.4.1. Total phenolic compounds

The total phenolic content of each extract was determined according to the Folin–Ciocalteau colorimetric method (Singleton & Rossi, 1965). Absorbance was read at 760 nm and results were expressed as gallic acid equivalents (mgGAE kg^{-1}).

2.4.2. Total anthocyanins content

Total anthocyanins content was determined by the pH-differential method (Wrolstad, 1976). For this aim, the extracts were set to pH 1.0 and 4.5 with buffers. The absorbance of each solution was measured at a wavelength of maximum absorption and 700 nm. The difference in the absorbance values at pH 1.0 and 4.5 was directly proportional to the total anthocyanin concentration expressed in malvidin 3.5-diglucoside.

2.4.3. Oleanolic acid determination

The oleanolic acid was determined using HPLC-DAD according to the procedure described by Zhang et al. (2013). HPLC-DAD analyses were performed on a Shimadzu HPLC system equipped with Class Vp software (ver. 6.14 SP2), a binary gradient (LC20 AT) pump a DAD detector (SPD-M20A), and a system controller (CBM-20A). Also, there was an autosampler (SIL-20A) with a 20 µL sample loop. The column used was PAH Ultimate XB-PAH column (4.6×250 mm, 5 µm, Welch Materials, USA). The wavelength was set at 210 nm. The mobile phase containing 85% acetonitrile and 15% ultra-distilled water was used at the flow rate of 1.0 mL min^{-1}. A stock solution containing 200 µg mL^{-1} of oleanolic acid standard was prepared in methanol and diluted to a series of convenient concentrations to set the calibration curve.

2.4.4. 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity analysis

The DPPH method was performed as described by Tural and Koca (2008) with some modifications. The reduction in the absorbance of 100 µM DPPH radicals, (1 mL) dissolved in 80% methanol, by the addition of each extract

was measured at 515 nm after 180 minutes.

Trolox (a water soluble vitamin E analog) was used as positive control. Radical scavenging activity was expressed as the inhibition percentage and was calculated using the following formula:

$$Inhibition\ \% = [(A_{blank} - A_{sample})/A_{blank}] \times 100$$

Where A_{blank} is the absorbance of the blank (containing methanol instead of sample) and A_{sample} is the absorbance of the extracts or control. The half maximal inhibitory concentration (EC_{50}) value for each extract was calculated from the plotted graph of scavenging activity against the concentrations of the sample.

2.4.5. The ferric reducing/antioxidant power (FRAP) analysis

The direct measurement of antioxidant (reducing) ability thanks to the reduction of the complex Fe^{3+}/tripyridyltriazine (TPTZ) to Fe^{2+} at acid pH was carried out by using the FRAP method, which is defined by Benzie and Szeto (1999). FRAP was calculated from a calibration curve using $FeSO_4$ as the standard and expressed as µmol $FeSO_4$ equivalents per g (µmol $Fe^{2+}g^{-1}$).

2.5. Statistical analysis

The data were statistically evaluated by ANOVA and Duncan tests (ver. 21.0, SPSS) at a 0.05 level of significance. All the data was also subjected for correlation analyses using Pearson's correlation coefficient.

Table 1. Some physical properties of whole fruits analyzed

Sample number	Skin%	Seed%	*L**	+*a**	+*b**	Soluble solids % (Brix)	pH
1	24.43	9.20	20.68	4.91	2.44	20.00	3.99
2	32.14	14.70	16.56	5.35	1.73	20.00	3.67
3	31.26	8.50	19.57	5.99	0.97	18.00	3.73
4	26.56	6.71	19.16	5.44	1.74	19.00	3.60
5	25.76	6.95	20.92	7.11	2.32	19.00	3.74
6	30.58	10.93	22.63	6.30	3.77	16.00	3.68
7	26.63	5.85	21.04	8.25	1.79	18.50	4.06

Where L* is luminescens (*L**=0, black; *L**=100, white); *a** is redness and greenness (+60, red; -60, green) and the *b** value represents yellowness and blueness (+60, yellow; -60, blue).

Table 2. Antioxidant properties of the whole fruits and their skin and seeds

	Sample number	Dry matter %	Total phenolics mg kg^{-1}	Total antocyanins mg 100 g^{-1}	Oleanolic acid mg kg^{-1}	FRAP µmol Fe^{2+} g^{-1}	EC_{50} mg mL^{-1}
Whole fruits	1	25.60	409.44	32.73	128.54	7.63	9.24
	2	25.80	418.33	79.81	122.07	9.30	10.25
	3	25.20	515.56	56.83	161.24	11.27	8.75
	4	23.40	431.67	113.97	187.04	10.64	11.25
	5	24.00	476.67	69.41	139.16	10.92	9.49
	6	18.25	355.56	14.91	272.22	10.27	14.79
	7	22.55	504.44	18.60	238.99	11.96	9.14
Mean		23.54±2.62c	444.52±57.25b	55.18±35.90ab	178.47±57.79b	10.28±1.43b	10.42±2.10b
Skin	1	32.47	1868.89	29.81	337.33	26.03	2.21
	2	50.30	1626.67	44.87	314.75	26.48	2.23
	3	34.14	1333.33	110.70	245.74	19.16	3.01
	4	30.53	1686.11	247.65	522.08	32.65	1.75
	5	30.28	1497.78	397.49	417.90	21.32	2.53
	6	31.39	1005.56	27.48	293.72	12.54	4.68
	7	33.03	1619.44	104.49	327.71	16.75	3.01
Mean		34.59±7.06b	1519.68±280.05a	137.49±137.73a	351.32±91.42a	22.13±6.77a	2.77±0.95c
Seed	1	40.95	407.78	15.37	40.95	5.03	39.68
	2	40.56	322.22	20.80	40.55	6.67	13.37
	3	53.91	304.44	32.29	53.90	5.98	21.53
	4	42.83	372.78	13.04	42.82	7.49	16.39
	5	40.24	457.22	2.17	40.23	8.01	10.81
	6	44.53	161.11	4.34	44.53	3.42	18.53
	7	39.80	149.44	10.71	39.80	3.84	18.52
Mean		43.26±4.98a	310.713±117.83b	14.10±10.23b	43.25±4.98c	5.78±1.76b	19.83±9.44a

*There is no statistical difference between those shown with the same letter in the same column (p>0.05).

**As positive control, Trolox were 50.70 µmol g^{-1} and 66.54 µg mL^{-1} for FRAP and EC_{50}, respectively.

3. Results and Discussion

The physical properties of the fruits were given in Table 1, while the antioxidant analysis results for whole fruits, the seeds, and skins of the samples were shown in Table 2. As seen in Table 1, the skin weight of fruits and seed weight were ranged between 24.43 and 32.14%, 5.85-14.70%, respectively. The total soluble solids of the samples ranged between 16.0 and 20.0% and their pH values ranged between 3.60 and 4.06.

As it is known, the color of food affects the taste of the consumer (Benmeziane et al., 2016). Anthocyanins, the phenolic compounds, are responsible for the color of grapes (Yamamoto et al., 2015). Anthocyanins are affected by many factors including temperature, light, pH, metal ions, and oxygen, etc. (Benmeziane et al., 2016). Color values in fruits are expressed with L^* (brightness: 100, white; 0, black), a^* (+, red; -, green), and b^* (+, yellow; -, blue). A lower L^* value of grapes means a darker skin color (Yamamoto et al., 2015). In this study, the color values were examined and it was seen that the fruit color was dark purple or close to black. Additionally, it has been thought that the waxy layer on the surface of a grape creates a difference between the color values. It was found that there was no statistically significant correlation between the total anthocyanin content and the color values in the studied samples. However, it was observed that the total amount of anthocyanin was highest in the skin.

As seen in Table 2, the total phenolics, the total anthocyanins, oleanolic acids, and FRAP values were the highest while the EC_{50} value was the lowest in the skin part. These values were statistically significant ($p<0.05$).

Carrieri et al. (2013) studied 12 table grape varieties (white, red, and black) and found that the total polyphenols in the grape extracts ranged from 81 mg kg^{-1} to 355 mg kg^{-1} in fresh weight. The highest polyphenolic content (297 mg kg^{-1}) was found in the black grapes while the lowest (138 mg kg^{-1}) phenolic value was for red grapes. The white grapes had no anthocyanins, but there was an intermediate content (196 mg kg^{-1}). In present study, total phenolics and anthocyanins were higher than their findings.

In a study involving *Vitis labrusca* L., antioxidant activity, proximate composition, phenolic compounds, trans-resveratrol, and fatty acid contents of peel, pulp, and seeds of four grape varieties were determined by Santos et al. (2011). They found that for 50% inhibition of DPPH, the lowest concentrations necessary were (9.26 to 126.91 µg mL^{-1}) in seeds. Additionally, these researchers concluded that the total phenolic content varied between 0.04 and 122.35 mg g^{-1}, the highest phenolic concentrations (89.83 to 122.35 mg g^{-1}) were in seeds, and the pulp had no antioxidant activity (Santos et al., 2011). Baydar et al. (2004) found that the quantities of total phenolics extracted with various solvent mixtures varied from 627.98 to 667.87 mgGAE g^{-1} in grape seed extracts and 29.55 to 45.44 mgGAE g^{-1} in grape bagasse extracts. The highest phenolic contents were in the grape seed extracts while grape bagasse extracts gave the lowest phenolic contents. They concluded that the phenolic compound contents of the seed extracts were higher than the phenolic contents of the grape skin extracts. According to their findings, the phenolic values for seed were higher than the ones in this study. On the contrary, the total phenolic content was the highest in the skin part of the samples in the present study. There is increasing evidence about grape skins stating that they are rich in phenolics such as anthocyanins and flavonols (Georgiev et al., 2014; Shahidi & Ambigaipalan, 2015; Sabra et al., 2021). Lago-Vanzela et al. (2011) determined that 93.7% of the total phenolic content was distributed in the skin of the Bordô grape (*V. labrusca*) cultivated in South Brazil. As a result of this study, it was revealed that the total phenolic matter contents of the whole fruit, skin, and seed parts were lower than the findings of Baydar et al. (2004), Rockenbach et al. (2011a), and Santos et al. (2011) but higher than the findings of Carrieri et al. (2013).

In another study, Rockenbach et al. (2011b) found that the total phenolic, anthocyanin, DPPH, and FRAP values of the *Vitis labrusca* L. pomace in dry weight were 32.62 mg g^{-1}, 1.84 mg g^{-1}, 188.02 µmol g^{-1} and 4117.79 µmol g^{-1}, respectively. The anthocyanin values of the present study were higher than the findings of Rockenbach et al. (2011b).

There are two mechanisms based on single-electron transfer and hydrogen atom transfer that can be used to determine the antioxidant activity. *In vitro* antioxidant activity of foods should be performed based on both mechanisms (Samavardhana et al., 2015). Ferric ion is frequently used as a mark of electron-donating activity in the FRAP method. The antioxidants in the samples act as reductants in a redox-linked colorimetric reaction. The value reflects the reducing power of the antioxidants (Shao et al., 2016). The extract has the ability to scavenging of free radicals thanks to hydrogen donation. DPPH is a stable free radical that is reduced to diphenyl picryl hydrazine through a reaction with antioxidant compounds. The initial reaction mixture has a deep violet color that changed to light yellow after reducing DPPH (Kobra et al., 2019). In the present study, DPPH and FRAP methods were used and the results were given in Table 2. DPPH radical scavenging activities of the extracts were accounted as EC_{50} values. EC_{50} is an efficient concentration of the extract required for 50% scavenging of DPPH. Trolox was 66.54 µg mL^{-1} for 50% scavenging of DPPH (EC_{50}) as a positive control. However, the EC_{50} mean values were 10.42±2.10 mg mL^{-1} in whole fruits, 2.77±0.95 mg mL^{-1} in the skin, and 19.83±9.44 mg mL^{-1} in the seed samples. The skin parts had the highest radical scavenging power. The values of the skin parts were lower than the Trolox (66.54 µg mL^{-1}) about 40 times. As comparing the FRAP values with the Trolox (positive control 50.70 µmol g^{-1}), the FRAP mean value (22.13±6.77 µmol Fe^{2+} g^{-1}) was about half of Trolox in the skin parts. The FRAP values of the grape skin in our study were higher than the findings of Rockenbach et al (2011a). They determined the total phenolic and FRAP values of *Vitis labrusca* L. as 1839 mg $100g^{-1}$ dry weight (skin), 2128 mg $100g^{-1}$ dry weight (seed), and 4362 µmol $100g^{-1}$ (skin), 2942 µmol 100 g^{-1} (seed), respectively.

Oleanolic acid, which has antioxidant properties especially in the skin part, was determined using HPLC-DAD. In the present study, according to the obtained chromatograms, the oleanolic acid retention times in the samples ranged from 14.0 minutes to 14.5 minutes (Fig. 1).

Oleanolic acid treatment is effective on oxidative stress which is a significant factor in developing different diseases such as liver disorders, inflammation, cancer, and diabetes (Ayeleso et al., 2017). However, there are relatively few studies regarding grape triterpenic acid contents, including oleanolic acid. The quantitative oleanolic acid data obtained were lower than the findings reported by Pensec et al. (2014) while they were higher than results reported by Orbán et al. (2009).

The triterpenoid contents and compositions of the waxy cuticular layer of eight grape cultivars were investigated by Pensec et al. (2014) at some phenological stages: young grapes, grapes at veraison, and mature grapes. According to their findings, the total triterpenoid levels were high in young grapes and fruit development the neutral triterpenoid levels showed a slight increase; however, the total triterpenoid levels progressively decreased. They stated that the oleanolic aldehyde was the most dominant triterpenoid among neutral triterpenoids. They concluded that the oleanolic acid levels, which were the highest in the wax extract of young grapes, were 406 µg mg^{-1} in Muscat d'Alsace and 782 µg mg^{-1} in Sylvaner, and these levels decreased during fruit maturation (Pensec et al., 2014).

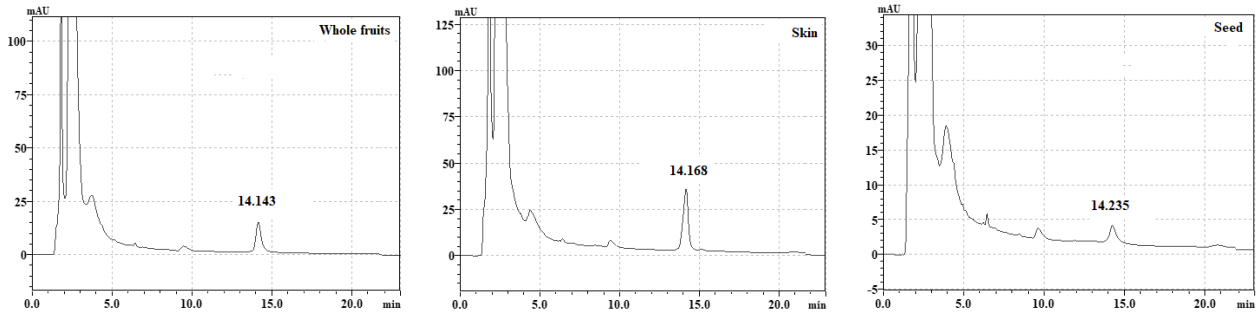

Figure 1. Oleanolic acid Chromatograms of the whole fruits and their skin and seeds

In another investigation, Orbán et al. (2009) revealed that the content of the dominant triterpenes in the skin and cuticular wax of grape berries highly varied related to grape varieties by liquid chromatography-mass spectrometry. The oleanolic acid levels ranged from 157.43 mg kg^{-1} to 239.19 mg kg^{-1} in red grape skin and 31.53 mg kg^{-1} to 162.01 mg kg^{-1} in cuticular wax. In the present study, the lowest oleanolic acid values (Table 2) were in the seed parts in our samples but the oleanolic acid values in the skin were higher than their findings.

De Nisco et al. (2013) reported that the total antioxidant activity was higher for fruit skin extracts than the whole fruits (about 2- to 4-fold). Burin et al. (2010) have reported that the phenolic composition of grape fruits influenced their antioxidant activities.

In the present study, to find out the contribution of natural antioxidants to the antioxidant activity of the fruits, a correlation coefficient was obtained between the total phenolics, total anthocyanins, oleanolic acid values, and antioxidant activity values in different parts of the grape fruits. When the results for these parts of the fruit were analyzed among themselves, there was a very significant correlation ($p<0.01$) between total phenolics and EC_{50} values (r=-0.886**) and between FRAP and EC_{50} values (r=-0.912**) in the skin (according to Pearson correlation). Besides, there was a significant ($p<0.05$) relation between total phenolics and FRAP values (r=0.831*) as well as between total phenolics and EC_{50} (r=-0.802*) in the seed part and the whole fruit. In a study investigating total phenolic content, color, and antioxidant activity in commercial, organic, and homemade grape juices, Burin et al. (2010) found a vigorously positive correlation between the antioxidant activity and total phenolic content for the commercial juice. Our results were consistent with the literature.

Vitis labrusca L. grape variety is consumed by people as table grape. In addition, this grape is used to produce fruit juice, molasses, vinegar, and wine. The residue of grapes, which is released when processed into products such as fruit juice, molasses, vinegar, and wine, are rich in bioactive compounds. Therefore, the whole fruit, the skin, and the grape seed were separately analyzed in this study. It was determined that bioactive compounds such as oleanolic acid and total phenolic compounds were concentrated in the skin. In the study, skin and seed separation was carried out under controlled conditions in the laboratory. Therefore, it may not fully reflect the losses of bioactive components during processing in the actual production process. From this point of view, further studies are needed to determine the losses in the production process and the bioactive components in the residue. The characteristics of the soil in which the plant is grown, agricultural production conditions, and the region where it is grown affect the composition of plant products. The study only reflects the results of samples from different local markets in Samsun.

4. Conclusion

As a result of this study, it was found that natural antioxidant substances of *Vitis labrusca* L. Fruits such as total phenolics, total anthocyanins, and oleanolic acids concentrated in the skin part, and the antioxidant activity was very high compared with fruit seeds. These findings are parallel with general literature information. When compared with whole fruit, it was seen that the fruit skin had about three times more total phenolic and two times more oleanolic acid. According to the findings, the grape skin is a potential source of oleanolic acid, an important bioactive compound. In conclusion, *V. labrusca* L. fruits are rich in bioactive compounds and these compounds are mainly concentrated in the skin part. Therefore, the residues of the grape should be evaluated due to their bioactive potential.

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Research Article / Araştırma Makalesi

Investigation of *Coxiella burnetii* and *Ehrlichia canis* by Molecular Methods in Ticks Removed from Patients Admitted to Hospital with Tick Bite Complaints

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Abstract: Ticks have to suck blood from their hosts during their developmental stages, except for the egg period. They also transmit the pathogens to their host while feeding. A total of 100 ticks obtained from 42 female and 58 male patients admitted to a hospital were included in the study. After the morphological examination of the ticks, DNA extraction was performed. Conventional PCR for *Coxiella burnetii* and Nested-PCR for *Ehrlichia canis* were performed using species-specific primers. In the morphological examination, it was determined that all of the ticks belonged to the Ixodidae family and 95 (95%) were *Rhipicephalus sanguineus* and 5 (5%) were *Ixodes ricinus* species. *C. burnetii* was detected in 36 ticks and *E. canis* was found in 26 ticks out of 100 ticks included in the study. It was determined that 33 (91.67%) of the *C. burnetii* infected ticks were *R. sanguineus* and 3 (8.33%) were *I. ricinus*. It was also determined that 25 (96.15%) of the *E. canis* infected ticks were *R. sanguineus* and one (3.85%) was the *I. ricinus*. When the incidence of *C. burnetii* and *E. canis* was examined according to the place of residence of the patients, it was found that *C. burnetii* was seen at a higher rate in people living in rural areas and as a result of the statistical analysis, it was found that the difference was statistically significant. In conclusion, ticks pose a significant risk as vectors of *C. burnetii* and *E. canis*.

Keywords: Tick contact, PCR, Ixodidae, zoonosis.

Kene Isırığı Şikâyetiyle Hastaneye Başvuran Hastalardan Çıkarılan Kenelerde *Coxiella burnetii* ve *Ehrlichia canis*'in Moleküler Yöntemlerle Araştırılması

Öz: Keneler, yumurta dönemleri hariç diğer gelişme safhalarında konaklarından kan emmek zorundadırlar. Beslendikleri esnada vektörlük yaptıkları patojenleri de konağa bulaştırırlar. Bu çalışmada kene ısırığı şikâyetiyle hastaneye başvuran hastalardan elde edilen kenelerde *Ehrlichia canis* ve *Coxiella burnetii* yaygınlığının moleküler yöntemlerle araştırılması amaçlandı. Çalışmaya hastaneye başvuran 42 kadın ve 58 erkek hastadan elde edilen toplam 100 kene dâhil edildi. Keneler morfolojik olarak incelendikten sonra DNA ekstraksiyonları yapıldı. Türlere özgü primerler kullanılarak *C. burnetii* için Konvansiyonel PCR ve *E. canis* için Nested-PCR yöntemi uygulandı. Yapılan morfolojik incelemede kenelerin tamamının Ixodidae ailesinden olduğu ve 95'nin (%95) *Rhipicephalus sanguineus*, 5'inin (%5) ise *Ixodes ricinus* türü olduğu saptandı. Çalışmaya dâhil edilen 100 kenenin 36'sında *C. burnetii*, 26'sında *E. canis* saptandı. *C. burnetii* ile enfekte kenelerin 33'ünün (%91,67) *R. sanguineus*, 3'ünün (%8,33) *I. ricinus* türü olduğu belirlendi. *E. canis* ile enfekte kenelerin 25' inin (%96,15) *R. sanguineus*, birinin (3,85) *I. ricinus* türü kene olduğu belirlendi. Hastaların ikamet ettiği yere göre *C. burnetii ve E. canis*'in görülme sıklığı incelendiğinde kırsal kesimde yaşayan insanlarda *C. burnetii*'nin daha yüksek oranda görüldüğü ve yapılan istatistiksel değerlendirmede anlamlı bir fark olduğu saptandı. Sonuç olarak keneler *C. burnetii* ve *E. canis* vektörlükleri için önemli risk oluşturmaktadırlar.

Anahtar kelimeler: Kene teması, PCR, Ixodidae, zoonoz.

1. Giriş

Keneler, sivrisineklerden sonra, dünya çapında birçok insan ve hayvan patojeninin en önemli vektörleri olarak kabul edilmektedir (Abdelkadir et al., 2019). Amerika Birleşik Devletleri'nde her yıl bildirilen vektör kaynaklı hastalıkların yaklaşık %95'inden keneler sorumludur (Eisen et al., 2017). Dünyada 900 kene türü bulunmakta olup bunların yaklaşık 25 türü tıbbî ve veterinerlik açısından büyük önem taşımaktadır (Nava et al., 2009).

Keneler, yumurta dönemleri hariç diğer gelişme safhalarında konaklarından kan emmek zorundadırlar (Dumanli et al., 2016). Beslendikleri esnada vektörlük yaptıkları patojenleri de konağa bulaştırırlar. Keneler kanla beslenen diğer artropodlardan daha fazla patojen etkenleri konaklarına aktarırlar. Örneğin Kırım-kongo kanamalı ateşi, Lyme hastalığı, Rocky Mountain benekli ateşi, insan granülositik anaplazmozu, insan monositik anaplazmozu, kene kaynaklı ensefalit, babesiosis, theileriosis, ehrlichiosis, Q ateşi etkenlerini insanlara

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bulaştırırlar. Ayrıca, kene ısırıkları önemli miktarda kan kaybına, şiddetli toksik reaksiyonlara ve hatta kene felci nedeniyle konağının ölümüne neden olabilmektedir (Sonenshine & Roe, 2014; Gürbüz et al., 2021).

Keneler, hastalık etkenini transovarial, transstadial, venereal ve nonviremik nakil şekilleriyle yeni nesillerine veya bir sonraki gelişim evrelerine aktarabilir. Ayrıca keneler çiftleşme esnasında birbirlerini ve aynı konak üzerinde beslenen fakat enfekte olmayan keneleri de enfekte edebilirler. Böylece, kene ile bulaşan hastalıklar nesilden nesile, hatta birçok coğrafi bölgelere de aktarılabilmektedir. Bundan dolayı, kenelerin hayat döngülerini tamamlayana kadar kan emmek için yerleştikleri tüm canlılar risk altındadır (Aydın & Coşkun, 2019). Bu özellikleri ile keneler, özellikle Türkiye'yi de kapsayan tropik ve subtropik iklim kuşağında bulunan bölgelerde hem insan hem de hayvan sağlığını tehdit etmektedirler (Dumanli et al., 2016).

Köpeklerde monositik ehrlichiosis *Ehrlichia canis*'in ve Q ateşi *Coxiella burnetii*'nin neden olduğu kene kaynaklı hastalıklardır. Zorunlu hücre içi gram negatif bakteri olan *E. canis*, evcil-vahşi köpeklerin ve nadiren de insanların monositlerini enfekte eder. Kahverengi köpek kenesi *Rhipicephalus sanguineus*, *E. canis*'in birincil vektörüdür (Ayan et al., 2020; Santoro et al., 2016). Keneler arasında *Ehrlichia* bulaşı, transstadiyal geçiş ile mümkünken, transovaryal geçiş ile mümkün değildir. *E. canis*'in daha önce sadece köpekleri enfekte ettiği düşünülüyordu ancak, Venezuela'da bir grup asemptomatik insandan da izole edilip moleküler olarak tanımlanmıştır. Böylece *E. canis*, insanlar için de klinik hastalıkların potansiyel bir ajanı olarak düşünülmüştür (Laušević et al., 2019). *E. canis*, Akdeniz'e kıyısı olan tüm Avrupa ülkelerinde endemiktir (Santoro et al., 2016).

Coxiella burnetii küçük, pleomorfik, hareketsiz, kapsüllenmemiş, gram negatif zorunlu hücre içi bir bakteridir. *C. burnetii*, insanlarda zoonotik bir hastalık olan Q ateşinden sorumlu patojendir. İnsanlarda *C. burnetii* enfeksiyonu asemptomatik veya semptomatik olabilirken kendini sınırlayan ateşli hastalık, akut Q ateşi (atipik pnömoni, hepatit) ve kronik Q ateşi (endokardit) şeklinde hastalığın üç farklı klinik formu mevcuttur. Hastalık insanlara daha çok *C. burnetii* ile kontamine olmuş havanın solunması ile bulaşmaktadır. Bunun dışında, enfekte koyun, keçi veya sığırlarla doğrudan temas yoluyla veya bu enfekte hayvanların idrarları, dışkıları ve doğum sonrası atıklarına dolaylı olarak teması ile de bulaşabilmektedir. Ayrıca, *C. burnetii* enfeksiyonlarının pastörize edilmemiş süt ürünlerinden, kan transfüzyonundan ve korunmasız cinsel ilişkiden de bulaşabileceği bildirilmiştir (Cikman et al., 2017).

2007-2010 yılları arasında Hollanda'da 3500'den fazla kişiyi etkileyerek rapor edilen en büyük Q ateşi salgını, dikkatleri keneler üzerine çekerek sağlık kurumları arasında yeniden önem kazanmıştır. Dünya Sağlık Örgütü (WHO), Q ateşini "önemi artan bir enfeksiyon" olarak tanımlamıştır (Cikman et al., 2017).

Bu çalışmanın amacı kene ısırığı şikâyetiyle hastaneye başvuran insanlarda bulunan kenelerde *Ehrlichia canis* ve *Coxiella burnetii* yaygınlığının moleküler yöntemlerle araştırılmasıdır.

2. Materyal ve Metot

2.1. Kene toplama ve morfolojik tanımlama

Çalışma için Muş Alparslan Üniversitesi Bilimsel Araştırma ve Yayın Etiği Kurulunda izin alındı (29.12.2020/27). Çalışmaya kene ısırığı şikâyetiyle Mayıs 2020- Eylül 2021 tarihleri arasında Kelkit Devlet Hastanesine başvuran 42 kadın ve 58 erkek hastadan elde edilen 100 kene dâhil edildi. Keneler Estrada-Pena ve ark. (2004) tarafından tanımlanan teşhis anahtarlarına göre identifiye edildi.

2.2. DNA ekstraksiyonu

Keneler DNA ekstraksiyonuna başlanmadan önce ependorf tüplere alınarak dondur çözdür işlemine tabi tutuldu. Ependorf içerisindeki keneler steril bir çubuk ile ezildi. Daha sonra DNA izolasyon kiti kullanılarak (İnvitrogen, ABD) üretici firmanın protokolüne uygun bir şekilde DNA ekstraksiyonu yapıldı. DNA örnekleri PCR yapılana kadar -20°C'de saklandı.

2.3. *Coxiella burnetii* için konvesiyonel PCR amplifikasyonu

Coxiella burnetii'yi saptamak için IS1111 geninin 687 bç uzunluğundaki bölgesini çoğaltan Trans1 (5'-TATGTATCCACCGTAGCCAGTC-3') ve Trans2 (5'-CCCAACAACACCTCCTTATTC-3') primerleri kullanıldı (Mares-Guia et al., 2014). Amplifikasyon, her biri 95°C'de 30 sn, 60°C'de 30 sn, 72°C'de 1 dk olmak üzere, toplam 35 döngü olarak programlandı. İlave olarak birinci döngü öncesi 95°C'de 15 dk denatürasyon aşaması, son döngüyü takiben de 72°C'de 7 dk ekstansiyon aşaması uygulandı.

2.4. *Ehrlichia canis* için Nested-PCR amplifikasyonu

Ehrlichia canis'i saptamak için Nested-PCR yapıldı. Nested-PCR'ın birinci aşamasında; 477 bç'lik DNA bölgesini çoğaltan, *Ehrlischia* spp'nin 16S rRNA gen bölgesine spesifik ECC (5´-AGAACGAACGCTGGCGGCAAGC-3´) ve ECB (5´-CGTATTACCGCGGCTGCTGGCA-3´) primerleri kullanıldı (Rojas-Triviño et al., 2013). Nested PCR'ın ikinci aşamasında 365 bç'lik bölgeyi çoğaltan, *E. canis*'e spesifik ECAN5 (5-CAATTATTTATAGCCTCTGGCTATAGGA-3´) ve HE3 (5´-TATAGGTACCGTCATTATCTTCCCTAT-3´) primerleri kullanıldı. Nested-PCR'ın birinci aşamasında amplifikasyon, her biri 95°C'de 90 sn, 55°C'de 90 sn, 72°C'de 90 sn olmak üzere, toplam 37 döngü olarak programlandı. Nested-PCR'ın ikinci aşamasında ise amplifikasyon, her biri 95°C'de 1 dk, 55°C'de 1 dk, 72°C'de 1 dk olmak üzere, toplam 40 döngü olarak programlandı. Her iki PCR aşamasında da ilave olarak birinci döngü öncesi 95°C'de 15 dk denatürasyon aşaması, son döngüyü takiben de 72°C'de 10 dk ekstansiyon aşaması uygulandı.

Daha sonra %1.5'luk agaroz jel hazırlanıp Safe-T-Stain (BioShop, Canada) ile boyandı. Ardından PCR ürünleri agaroz jelde yürütülüp elde edilen görüntülere göre pozitiflikler belirlendi.

2.5. İstatistiksel analiz

Tanımlayıcı istatistikler, sürekli değişkenler için ortalama, standart sapma, minimum ve maksimum olarak, kategorik değişkenler için sayı ve yüzde olarak ifade edildi. Sürekli değişkenler bakımından, bağımsız iki grup karşılaştırmalarında, normal dağılım koşulu sağlanan

durumlarda T-Test, normal dağılım koşulu sağlanmayan durumlarda Mann Whitney U test istatistiği kullanıldı. Kategorik değişkenler Ki-kare testi ile karşılaştırıldı. İstatistiksel analizler SPSS 22.0 versiyonu ile yapıldı ve $p<0.05$ istatistiksel olarak anlamlı kabul edildi.

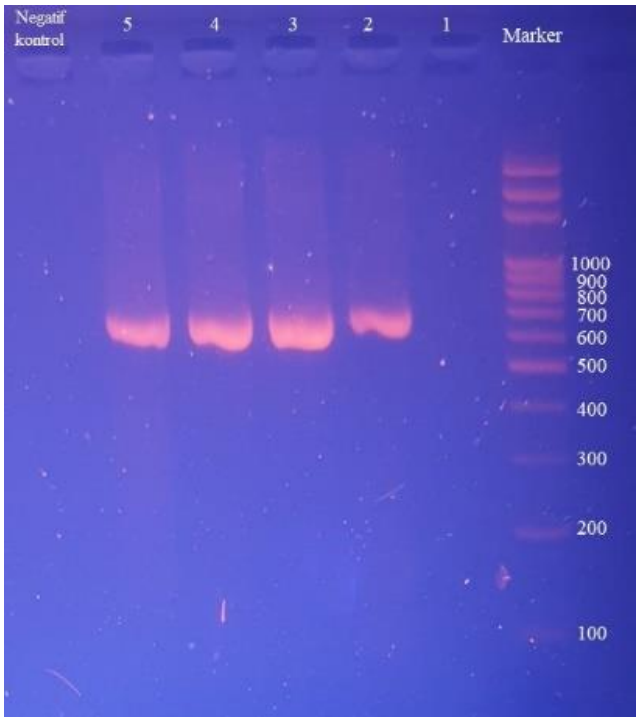
3. Bulgular

Hastalardan toplanan 100 kenenin tamamının Ixodidae ailesinden olduğu ve 95'nin (%95) *Rhipicephalus sanguineus*, 5'inin (%5) ise *Ixodes ricinus* türü olduğu belirlendi. PCR sonucunda 100 kenenin 36'sında *Coxiella burnetii* (Şekil 1), 26'sında *Ehrlichia canis* (Şekil 2) saptandı. *C. burnetii* ile enfekte kenelerin 33'ünün (%91.67) *R. sanguineus*, 3'ünün (%8.33) *I. ricinus* türü olduğu belirlendi. *E. canis* ile enfekte kenelerin 25' inin (%96.15) *R. sanguineus*, 1'inin (3.85) *I. ricinus* türü kene olduğu belirlendi (Tablo 1).

Tablo 1. Kene türlerinde *Coxiella burnetii* ve *Ehrlichia canis* görülme sıklığı

Table 1. Incidence of *Coxiella burnetii* and *Ehrlichia canis* in tick species

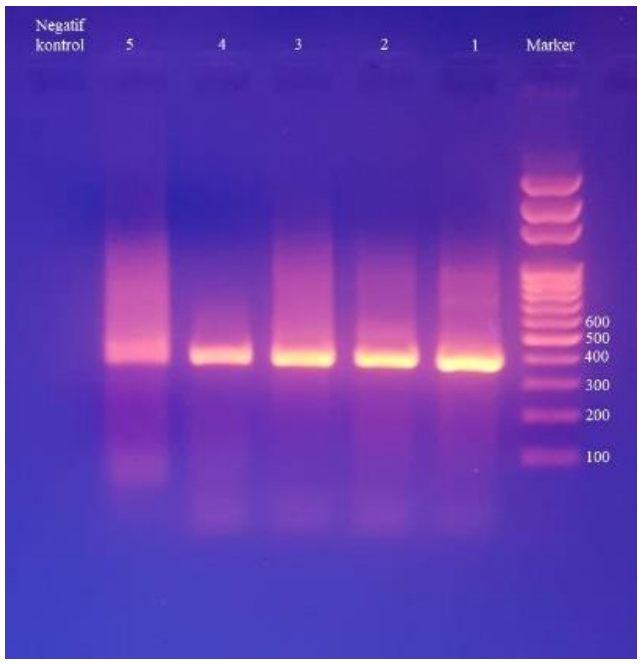
Kene Türü (n)	*C. burnetii* n (%)	*E. canis* n (%)
R. sanguineus (95)	33 (34,7)	25 (26,3)
I. ricinus (5)	3 (60)	1 (20)
Toplam (100)	36 (36)	26 (26)



Şekil 1. *Coxiella burnetii* pozitif örneklere ait agaroz jel görüntüsü

Figure 1. Agarose gel image of positive samples of *Coxiella burnetii*

Bu çalışmada hastaların ikamet ettiği yere göre *Coxiella burnetii* ve *Ehrlichia canis*'in görülme sıklığı incelendiğinde kırsal kesimde yaşayan insanlarda *C. burnetii*'nin daha yüksek oranda görüldüğü ve yapılan istatistiksel değerlendirmede anlamlı bir fark olduğu saptandı (Tablo 2). *E. canis*'in ise hastaların ikamet ettiği yere göre görülme sıklığında anlamlı bir farkın olmadığı belirlendi.



Şekil 2. *Ehrlichia canis* pozitif örneklere ait agaroz jel görüntüsü

Figure 2. Agarose gel image of positive samples of *Ehrlichia canis*

Tablo 2. *Coxiella burnetii* sıklığının yerleşim yerine göre değerlendirilmesi

Table 2. Evaluation of the frequency of *Coxiella burnetii* by place

	C. burnetii		
İkamet yeri	Pozitif n (%)	Negatif n (%)	P değeri
Kırsal (n: 51)	21 (41.2)	30 (58.8)	<0.05
Kentsel (n: 49)	15 (30.6)	34 (69.4)	
Toplam	36 (36)	64 (64)	

4. Tartışma

Keneler dünyanın hemen her yerinde görülebilmektedir. Kene teması özellikle iklim şartlarının uygun olduğu ve hayvancılığın yaygın olarak yapıldığı bölgelerde artmaktadır (Çıtıl et al., 2020). Ülkemiz, kenelerin rahatlıkla yerleşerek üreyebilecekleri ve yayılım sağlayabilecekleri iklim ve coğrafi koşullara sahip olmasına rağmen, Türkiye'deki kene türleri hakkında çok az taksonomik bilgi bulunmaktadır (Yücesan et al., 2019). Türkiye'nin kene faunası, Ixodidae ailesinde altı soyda 39 tür ve Argasidae ailesinde üç soyda sekiz tür olmak üzere toplam 47 türden oluşmaktadır (İnci et al., 2016). Bu kene türleri ülkenin yedi bölgesinde dağılım göstermekle birlikte *Ixodes* türleri daha çok ülkenin kuzeyinde görülmektedir (Aydın & Bakırcı, 2007). Bu çalışmada Ixodidae ailesinden *Ixodes* ve *Rhipicephalus* soylarından kene türleri belirlenmiştir. Belirlenen bu soylar insan tutulumu açısından sık karşılaşılan soylar olduğu diğer çalışmalarda da bildirilmiştir (İnci et al., 2016; Yücesan et al., 2019).

Ixodidae ailesine bağlı keneler insan ve hayvan sağlığını etkileyen çok sayıda hastalık etkenine vektörlük yapma potansiyeline sahiptirler (Yücesan et al., 2019). Bu etkenler arasında zoonotik önemi olan *Coxiella burnetii* (Cikman et al., 2017) ve son zamanlarda zoonoz olduğu düşünülen *Ehrlichia canis* (Laušević et al., 2019) bakterileri de yer almaktadır.

Ülkemizde kene temas öyküsü olan veya veteriner hekim ve kasap gibi risk grubunda bulunan insanlarda *Coxiella burnetii* seropozitifliğinin araştırıldığı çalışmalarda %10-%42.2 oranlarında faz II IgG seropozitifliği saptanmıştır (Eyigör et al., 2006; Arabacı et al., 2017; Ertürk et al., 2017; Kireçci & Uğuz, 2019). Bu oranlar keneler ile insanlara bulaşan *C. burnetii*'nin kenelerin bulaştaki önemini bir kez daha ortaya koymaktadır. Ancak bu alanda ülkemizde sınırlı sayıda çalışma yapılmıştır. Samsun, Sinop, Ordu, Giresun, Trabzon, Amasya, Tokat ve Sivas illerindeki sığır ve koyunlarda toplanan kenelerin %2.71'inde PCR yöntemi ile *C. burnetii* varlığı saptanmıştır (Kılıçoğlu et al., 2020). Türkiye'nin 38 ilinden toplanan kenelerdeki *C. burnetii* varlığının araştırıldığı çalışmada kenelerin 1-7 adedi bir araya getirilerek gruplandırılmış ve Denizli'ye ait 56 kene grubunun altısında ve Ankara'ya ait 53 kene grubunun birinde PCR yöntemi ile *C. burnetii* varlığı saptanmıştır (Altay et al., 2013). Ülkemizde daha önce insanlar üzerinde toplanan kenelerde *C. burnetii* varlığını araştıran herhangi bir çalışma yapılmamış olup, bu çalışmada ise insanlar üzerinden toplanan kenelerin %36'sında *C. burnetii* saptanmıştır. Belirlenen oran ve kene temas öyküsü olan veya veteriner hekim ve kasap gibi risk grubunda olan insanlarda *C. burnetii* seropozitifliğinin araştırıldığı çalışmalar bulaş riskinin yüksek olduğunu göstermektedir.

Köpek, kedi ve kemirgenler *Ehrlichia* türlerinin doğal rezervuarlarıdırlar. *Rhipicephalus sanguineus* kene türünün enfekte hayvanlardan kan emmesi ve daha sonra insanlarda konaklaması sonucu insanlar enfekte olabilir. Vektörle bulaşan hastalıklar şüphesi olan 250 hasta, kan serumunun analiz edildiği bir çalışmada 64 hastanın serumunda *E. canis* saptanmıştır. Çalışmada ehrlichiosis enfeksiyonundan sonra bağışıklık sistemi ile ilgili sorunların geliştiği de bildirilmiştir (Andrić, 2014). Çalışma dikkate alındığında zoonoz önemi olan bu bakterinin kenelerdeki yaygınlığı önem kazanmaktadır. Edirne, Kırklareli, Tekirdağ ve İstanbul köpeklerinde toplanan kenelerin %21.25'inde (27/127) (Çetinkaya et al., 2016), Van köpeklerinde toplanan kenelerin %22.72'sinde (50/220) (Ayan et al., 2020) *E. canis* saptanmıştır. Ülkemizde insanlar üzerinde toplanan kenelerde *E. canis* varlığını araştıran herhangi bir çalışma yapılmamış bu çalışmada ise insanlar üzerinden toplanan kenelerin %26'sında *E. canis* saptanmıştır. Gerek köpeklerden toplanan kenelerde belirlenen oran (Çetinkaya et al., 2016; Ayan et al., 2019) gerekse bu çalışmada belirlenen oran *E. canis*'in ülkemizdeki kenelerde yaygın olduğunu düşündürtmektedir.

Sonuç olarak keneler, *Coxiella burnetii* ve *Ehrlichia canis* vektörlükleri için önemli risk oluşturmaktadırlar. Bundan dolayı özellikle hastalığın artış gösterdiği yaz aylarında şüpheli klinik bulgularla gelen hastalarda hayvanlarla temas ve kene tutunması öyküsü mutlaka sorgulanmalı ve muayenede kene aranmalıdır. Ayrıca kene vektörlüğü riskinden dolayı kenelerin tutundukları yerden çıkarılması işleminin mutlaka uzman sağlık personeli tarafından yapılması konusunda halk bilinçlendirilmelidir. Bu hastalıkların endemik olarak görüldüğü kırsal kesimlerde yaşayan insanlara ve özellikle çiftçilik-hayvancılık ile uğraşan kişilere bilgilendirme toplantıları, görsel ve medya aracılığı ile duyurular yapılarak kene vektörlükleri ve kene teması hakkında farkındalık arttırılmalıdır.

Etik kurul onayı: Bu çalışma için, Muş Alparslan Üniversitesi Bilimsel Araştırma ve Yayın Etiği Kurulunda izin alınmıştır (29.12.2020/27).

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Research Article/Araştırma Makalesi

Three New and Two Confirmed Records for the Iraqi Spider Fauna (Arachnida: Araneae)

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Abstract: The Iraqi spider fauna includes several dozens of species described and reported mostly by local researchers but still there are considerable sampling gaps. For the first time in Iraq, *Agelena orientalis* C. L. Koch 1837 (Agelenidae), *Oxyopes globifer* Simon, 1876 (Oxyopidae), and *Thanatus formicinus* (Clerck, 1757) (Philodromidae) are recorded. In addition, newly collected material of *Bassaniodes tristrami* (O. Pickard-Cambridge, 1872) (Thomisidae) and *Eusparassus mesopotamicus* Moradmand & Jäger, 2012 (Sparassidae) are also presented. The characteristic features, habitus and copulatory organs of all species are presented here along with their geographic distribution.

Keywords: Grass spiders, lynx spider, crab spiders, huntsman spider, first records.

Irak Örümcek Faunası için Üç Yeni Kayıt ve İki Kayıt Doğrulaması

Öz: Irak örümcek faunası, çoğunlukla yerel araştırmacılar tarafından tanımlanan ve rapor edilen birkaç düzine türü içerir fakat yine de birçok örneklenmemiş alan bulunmaktadır. Irakta ilk kez *Agelena orientalis* C. L. Koch 1837 (Agelenidae), *Oxyopes globifer* Simon, 1876 (Oxyopidae), ve *Thanatus formicinus* (Clerck, 1757) (Philodromidae) kayıt edilmiştir. Buna ilaveten *Bassaniodes tristrami* (O. Pickard-Cambridge, 1872) (Thomisidae) ve *Eusparassus mesopotamicus* Moradmand & Jäger, 2012 (Sparassidae) türlerinin yeni toplanan örnekleri sunulmuştur. Tüm türlerin karakteristik özellikleri, habitatları ve üreme organları, coğrafi dağılımları ile birlikte burada sunulmuştur.

Anahtar kelimeler: Huni ağ örümcekleri, vaşak örümceği, yengeç örümcekleri, avcı örümceği, ilk kayıtlar.

1. Introduction

Araneae Clerck, 1757 is the largest order of arachnids with 4219 genera and 49583 described species around the world (World Spider Catalog, 2021). Still, the knowledge on diversity and distribution of spider fauna of Iraq is sparse, especially in comparison to the other countries of the Middle East. The most recently list of Iraqi spiders is that of Fomichev et al. (2018) who listed only 53 species belonging to 42 genera and 22 families. During the three-year period following that publication, several papers (e.g. Al-Khazali, 2018, 2020, Al-Khazali & Najim, 2018; Al-Khazali & Jäger, 2019; Najim et al., 2019; Al-Khazali & Fomichev, 2021) have reported data and new records of spiders as a result of which the number of Iraqi spider species has risen to 61.

The aim of the present study is to present records of three species new to the list of Iraqi spiders and additional distribution records of two species based on the new material collected in northern and southern Iraq.

2. Material and Methods

All specimens were collected from three different provinces: Dohuk, Erbil (both in northern Iraq) and Dhi Qar (southern Iraq) (Fig. 1). The specimens were deposited at the College of Basic Education, University of Sumer, Dhi Qar, Iraq. Spiders were preserved in 75% ethanol and examined using a Nikon camera connected to a dissecting microscope. The epigynes were cleared in a KOH/water solution until the soft tissues were dissolved. Photographs of the copulatory organs were taken in a dish with white cotton at the bottom, filled with alcohol. Identification of specimens depended on comparison with figures of Levy (1996, 1999, 1976), Logunov (1996), Szita and Samu (2000), and Moradmand and Jäger (2012). The measurements of legs are provided as total length (femur, patella, tibia, metatarsus, and tarsus). All measurements are in millimeters. The Iraq map that was used in this study obtained from the website Country Wise Codes.

3. Results

Family: Agelenidae C. L. Koch, 1837

Genus: *Agelena* Walckenaer, 1805

Agelena orientalis C. L. Koch, 1837 (Fig. 2a–c)

Agelena orientalis: Levy, 1996: 86, f. 4–8.

Material examined: 2 ♀♀, Iraq, Dohuk Province, Zakho

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District, agricultural region, 37.139336° N 42.707545° E, 2. Apr.2020, leg. H. S. Kachel.

Determination: Levy, 1996.

Diagnosis: The female of this species is very similar to *A. labyrinthica*; however, it can be distinguished by the shape of the epigynal septum, the posterior edge of the epigyne, and the shape and position of the spermathecae (see Levy, 1996; figs. 7-8).

Figure 1. Map of Iraq showing specimens sampling localities: Dohuk, Erbil, and Dhi Qar provinces (dark circles).

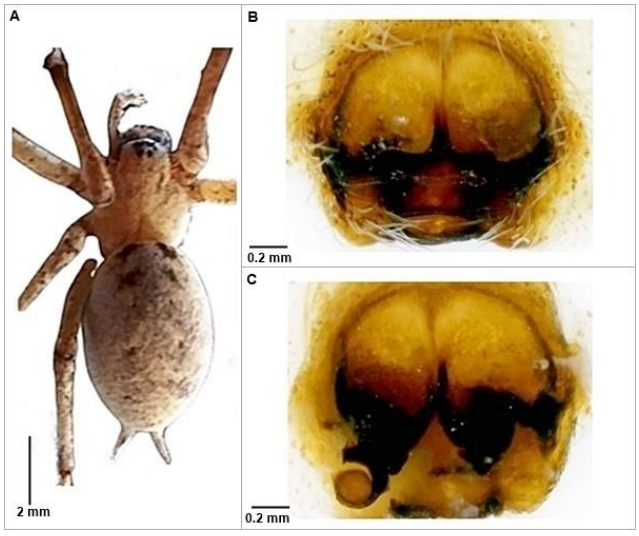

Figure 2. *Agelena orientalis* C. L. Koch, 1837, female; A. Habitus (live specimen), dorsal view; B-C. Epigyne, ventral and dorsal views respectively.

Description of female: General appearance as in Fig. 2a. Measurements: Body length 8.87; carapace 6.65 long, 5.02 wide; opisthosoma 8.08 long, 4.40 wide. Leg measurements: I: 20.56 (6.01, 1.50, 5.02, 5.65, 2.38), II: 20.35 (6.10, 1.78, 4.72, 5.34, 2.41), III: 19.52 (6.07, 1.40, 5.14, 4.68, 2.23), IV: 22.03 (7.72, 1.51, 5.47, 4.95, 2.38). General coloration brown-yellow, carapace yellow, brownish frontally, covered with fine setae. Sternum and coxae pale yellow, maxillae and chelicerae brown. Opisthosoma elongated and expanded in the middle part, dorsally yellow with thin brown stripes at the top, covered with fine setae, ventrally pale yellow. Spinnerets brownish. Legs uniform yellow.

General distribution: Widely distributed, from Italy to Central Asia, Iran (World Spider Catalog, 2021), and Iraq (current paper).

Family: Oxyopidae Thorell, 1869

Oxyopes globifer Simon, 1876 (Fig. 3a-d).

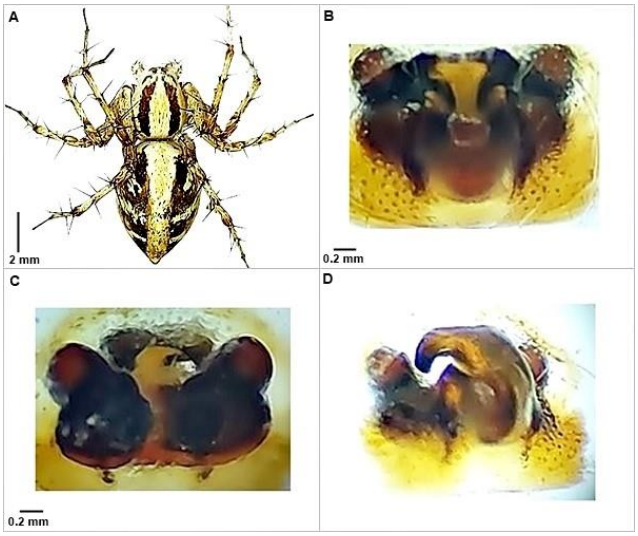

Figure 3. *Oxyopes globifer* Simon, 1876, female; A. Habitus (live specimen), dorsal view; B-D. Epigyne, ventral, dorsal and retrolateral views respectively.

Material examined: 2 ♀♀, Iraq, Dhi Qar Province, AL-Nassr District, from the outside wall of my house, 31.534582° N 46.120739°E, 12 m a.s.l. (Fig. 1), 2. July. 2021, leg. A. M. Al-Khazali.

Determination: Levy (1999).

Diagnosis: The female of *O. globifer* can be recognized from congeners by having a strong laterally extending median protrusion of the epigyne (Fig. 3b-d).

Description of female: General appearance as in Fig. 2a. Measurements: Body length 8.87; carapace 3.21 long, 2.34 wide; opisthosoma 5.66 long, 3.15 wide. Leg measurements: I: 9.95 (2.79, 0.81, 2.33, 2.64, 1.38), II: 3.56 (2.59, 1.02, 2.15, 2.60, 1.06), III: 6.43 (2.12, 0.77, 1.45, 2.03, 0.76), IV: 10.78 (3.32, 1.22, 2.23, 3.07, 0.94). Carapace elongated, light-yellow in the midline, with a wide reddish-brown strip on each lateral side, the outer edges light-yellow, covered with fine setae. Sternum, labium, maxillae and chelicerae light yellow. Opisthosoma oval, with a semi-pointed end, dorsal coloration as in carapace, laterally with a pattern of three pairs of light-grey stripes, ventrally yellow with small pale spots on sides, with a broad, pale brown medial stripe. Spinnerets yellow. Legs uniform reddish yellow.

General distribution: This species distributed in Southern Spain, Greece, Kazakhstan, Turkmenia, Uzbekistan, Tadzhikistan, Turkey, Israel, Egypt, Algeria, Tunisia, Libya (Levy, 1999), Iran (Zamani et al, 2015), and Iraq (current paper).

Family: Philodromidae Thorell, 1870

Genus: *Thanatus* C. L. Koch, 1837

Thanatus formicinus (Clerck, 1757) (Fig. 4a-c)

Determination: Logunov (1996), Szita & Samu (2000).

Specimens examined: 2 ♀♀, Iraq, Erbil Province, Zanko Village, agricultural land, 36.190073° N, 43.993030° E, 12 m a.s.l. (Fig. 1), 15. Feb. 2020, leg. F. S. Hussein.

Diagnosis: See Logunov (1996).

Figure 4. *Thanatus formicinus* (Clerck, 1757), female; A. Habitus, dorsal view; B, C. Epigyne, ventral view (A, after maceration in KOH), D. dorsal view.

Description of female: General appearance as in Fig. 4a. Measurements: Body length 8.6; prosoma 3.83 long, 2.87 wide; opisthosoma 4.77 long, 3.62 wide. Leg measurements: I: 4.28 (1.30, 0.47, 1.12, 1.01, 0.38), II: 4.96 (1.44, 0.43, 1.33, 1.05, 0.71), III: 4.09 (1.52, 0.36, 1.42, 0.98, 0.62), IV: 5.83 (1.72, 0.56, 1.22, 1.45, 0.88). The general coloration brownish yellow to light reddish brown, carapace brownish, with a pair of wide longitudinal brown bands, covered with fine setae. Ocular area mostly light reddish. Legs, coxae and sternum reddish brown, maxillae and chelicerae light brown. Opisthosoma oval, brownish yellow, dorsum with clear reddish brown cardiac strip extending from the anterior to the middle, covered with fine brownish setae, ventrally pale brownish. Spinnerets brownish yellow.

General distribution: North America, Europe, North Africa, Turkey, Caucasus, Russia (Europe to Far East), Iran, Kazakhstan, Central Asia, China, Japan (World Spider Catalog, 2021), and Iraq (current paper).

Family: Thomisidae Sundevall, 1833

Genus: *Bassaniodes* Pocock, 1903

Bassaniodes tristrami (O. Pickard-Cambridge, 1872) (Fig. 5. a-c)

Xysticus tristrami Kiany et al., 2017: 7, f. 14a-c (♂).

Bassaniodes tristrami Breitling, 2019: 203.

B. tristrami Naumova, 2020: 5, f. 9-11 (♂).

Determination: Levy (1976), Dippenaar-Schoeman (1989), Kiany et al. (2017)

Specimens examined: 1 ♂ and 1 ♂ subadult, Northern Iraq, Dohuk Province, Zakho district, agricultural land, under dry mud piles, 37.139336° N, 42.707545° E, 12 m a.s.l. (Fig. 1), 2. Mar. 2020, leg. H. S. Kachel.

Diagnosis: See Levy (1976) and Kiany et al. (2017).

General distribution: Greece, Turkey, Caucasus, Russia (Europe) to Central Asia, Middle East (World Spider Catalog, 2021), Kyrgyzstan, Tajikistan (Mikhailov, 2013), and Iraq (current paper).

Distribution in Iraq: Dohuk province.

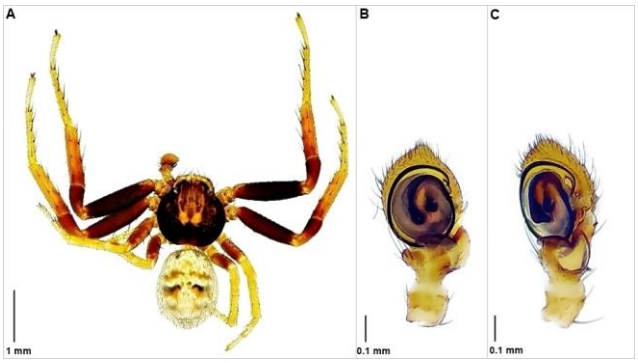

Figure 5. *Bassaniodes tristrami* (O. Pickard-Cambridge, 1872), male; A. Habitus, dorsal view; B, C. Palp, ventral and nearly retrolateral views.

Comments: This species was recorded in Iraq for the first time by Fomichev et al. (2018) under the genus *Xysticus* based on material collected from Dohuk province, northern Iraq.

Family: Sparassidae Bertkau, 1872

Genus: *Eusparassus* Simon, 1903

Eusparassus mesopotamicus Moradmand & Jäger, 2012 (Fig. 6a-d)

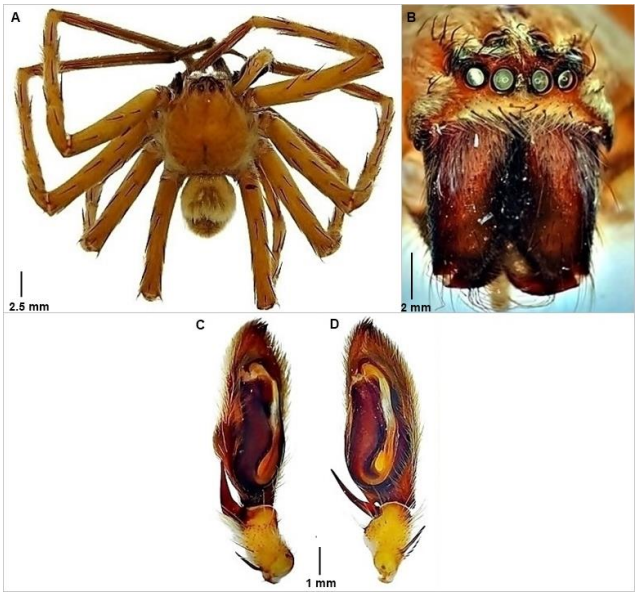

Figure 6. *Eusparassus mesopotamicus* Moradmand & Jäger, 2012, male; A. Habitus, dorsal view; B. Prosoma, nearly anterior; C, D. Palp, ventral and retro-ventral views.

Determination: Moradmand & Jäger, (2012), Moradmand, (2013).

Specimens examined: 2 ♂♂, southern Iraq, Dhi Qar Province, AL-Nassr district, From inside a rural house, 31.534582° N

46.120739°E, 12 m a.s.l. (Fig. 1), 10. July. 2020, leg. A. M. Al-Khazali.

Diagnosis: See Moradmand & Jäger (2012).

General distribution: Known from Iran, Iraq, and Turkey (World Spider Catalog, 2021).

Distribution in Iraq: AL-Najaf and Dhi Qar provinces.

Comments: According Moradmand & Jäger (2012), only one female was collected from AL-Najaf province in Iraq, in the current study two males were collected from Dhi Qar province, southern Iraq.

4. Discussion

According to the recent data, there are approximately 61 species of Iraqi spiders known so far (AL-Khazali, 2020). The present findings represent the first records of three species of spiders: *Agelena orientalis* C. L. Koch 1837, *Thanatus formicinus* (Clerck, 1757), and *Oxyopes globifer* Simon, 1876 in Iraq and the last two also represent the first records of these genera. Thus, the number of species within spider fauna of Iraq has risen to 64 known species. However, the spider fauna of Iraq is still poor when it is compared with some neighboring countries such as Iran that contains 780 known species (Zamani et al., 2020) and Turkey where there are 1129 known species (Demir & Seyyar, 2017; Danışman et al., 2021). This is due to the scarcity of studies and researchers interested in this group of arachnids. It is expected to find many other species of spiders in Iraq due to the fact that many regions are still not studied at all and; therefore, any material collected from those regions are expected to include new records of spider species and genera. *Bassaniodes tristrami* (O. Pickard-Cambridge, 1872) was recorded for the first time in Iraq under genus *Xysticus* by Fomichev et al. 2018 where three males were collected from Dohuk Province, north foothill of Chiaje-Spizakho-Dag Mountain Range. Both males in this study were found in another place in the same Dohuk Province but it is a geographically different region (agricultural land) to the previous study by Fomichev et al. (2018); no female was found so far. In this study, two male *E. mesopotamicus* specimens were collected from Dhi Qar province, which is a new locality for this species in southern Iraq, despite Moradmand and Jäger reporting a single female of this species from AL-Najaf province in central Iraq (2012).

Therefore, according to the current study as well as the previous studies interested in Iraqi spiders, it can be concluded that this group of arachnids is still very poor, especially when compared with some neighboring countries such as Iran and Turkey. Fomichev et al. (2018) indicated that the number of species may not exceed 10% of the actual number of spider species in Iraq. In this regard and to increase knowledge about the spider fauna of Iraq, more arachnological studies of this country must be conducted.

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Conflict of interest: The authors declare that there is no conflict of interest.

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Research Article/Araştırma Makalesi

Antioxidant Properties and Monoterpene Composition of 13 Different Pine Resin Samples from Turkey

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Abstract: Pine resin has been used as a traditional health-promoting medicinal food in Turkey and some countries for centuries. In the present study, the antioxidant potential and the monoterpene profile of 13 pine resin samples purchased from herbalists in different provinces of Turkey were investigated. According to the analysis results, there were differences between the pine resin samples, especially in terms of color and antioxidant properties. The experimental results demonstrated that the pine resin had strong *in-vitro* antioxidant effects. Total phenolic, FRAP, and DPPH (EC_{50}) values ranged between 23.19 and 379.44 mgGAE g^{-1}, 68.85 and 758.80 µmol Fe^{2+} g^{-1}, 54.36 and 1006.97 µg g^{-1}, respectively. Total phenolic content values correlated well with the FRAP values. In contrast, it was found that there was a negative correlation between FRAP values and EC_{50} values (r=-0.719**) and between total phenolic substance and EC_{50} values (r=-0.688**). Also, the monoterpene compounds of the resin were determined by applying solid-phase microextraction (SPME) and gas chromatography-mass spectroscopy. Eight monoterpene compounds were identified in different pine resin samples, including *a*-pinene, camphene, *β*-pinene, 3-carene, *β*-myrcene, cymene, D-limonene, and *p*-cymene. Generally, the main monoterpenes were *a*-pinene and *β*-pinene.

Keywords: Terpenes, total phenolic matter, volatile compounds, bioactive compounds, pinen.

Türkiye'den 13 Farklı Çam Reçine Örneğinin Antioksidan Özellikleri ve Monoterpen Bileşimi

Öz: Çam reçinesi, yüzyıllardır Türkiye'de ve bazı ülkelerde geleneksel, sağlığı geliştirici tıbbi gıda olarak kullanılmaktadır. Bu çalışmada, Türkiye'nin farklı illerindeki aktarlardan satın alınan 13 adet çam reçinesi örneğinin antioksidan özellikleri ve monoterpen profili araştırılmıştır. Analiz sonuçlarına göre çam reçinesi örnekleri arasında özellikle renk ve antioksidan özellikler açısından farklılıklar tespit edilmiştir. Deneysel sonuçlar, çam reçinesinin güçlü *in vitro* antioksidan etkilere sahip olduğunu göstermiştir. Toplam fenolik, FRAP ve DPPH (EC_{50}) değerleri sırasıyla 23.19 ve 379.44 mgGAE g^{-1}, 68.85 ve 758.80 µmol Fe^{2+} g^{-1}, 54.36 ve 1006.97 µg g^{-1} arasında değişmiştir. Toplam fenolik içerik değerleri, FRAP değerleri ile iyi bir korelasyon göstermiştir. Buna karşılık, FRAP değerleri ile EC_{50} değerleri (r=-0.719**) arasında ve toplam fenolik madde ile EC_{50} değerleri (r=-0.688**) arasında negatif korelasyon olduğu tespit edilmiştir. Ayrıca reçinenin monoterpen bileşikleri, katı faz mikroekstraksiyon (SPME) ve gaz kromatografisi-kütle spektroskopisi uygulanarak belirlenmiştir. Farklı çam reçinesi numunelerinde *a*-pinen, camphene, *β*-pinene, 3-carene, *β*-myrcene, cymene, D-limonen ve p-cymene olmak üzere sekiz monoterpen bileşiği tanımlanmıştır. Genel olarak, örneklerdeki başlıca monoterpenler *a*-pinen ve *β*-pinendir.

Anahtar kelimeler: Terpenler, toplam fenolik madde, uçucu bileşikler, biyoaktif bileşikler, pinen.

1. Introduction

Plants and herbal products are used in many areas of human life, including as a source of food and as medicine, fuel, and clothing (Tetik et al., 2013). It is known that many plants have been used for medicinal purposes since ancient times. The oldest information about the medicinal use of plants is found in Chinese, Egyptian, and Greek historical sources. Some drugs were produced and exported in Anatolia during the Hittites civilization (Sarı et al., 2008). Herbal medicines, widely used in ancient civilizations, are still used today. Exact figures on how much of the world's population uses local and traditional medicines are unknown. However, according to WHO, herbal products' global market value size is 62 billion USD that is expected to reach 5 trillion USD by 2050 (Hayta et al., 2014). Nowadays, it is reported that the number of plants used in the world is around 20000. 4000 of them are widely used for medicinal purposes and about 400 of them are traded (Sarı et al., 2008).

Turkey has a rich flora due to its variable climate and many ecological regions. This diversity in vegetation provides a rich source of medicinal plants used by the Anatolian civilization (Hayta et al., 2014). *Pinus* spp., which naturally grows in our country, belongs to Pinaceae, the most prominent family of conifers. The genus *Pinus* includes about 100 species, used from its leaves to its bark and seeds in many countries in the Northern hemisphere. (Mármol et al., 2019; Dziedziński et al., 2021). The resin produced from pine (*Pinus* spp.) has been widely used in adhesives, soaps, insecticide, construction materials, artworks, and even in embalming fluid throughout history (Alkan et al., 2016; Neis et al., 2019). It has been observed that natural resins obtained from trees and herbaceous plants used in archaeological materials cannot be

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destroyed by fungi, insects, and microorganisms (Alkan et al., 2016). There are various pine-based food additives and food products marketed in Korea. Some of them are beverages, wine, tea, and candy (Yu et al., 2004). Pine resin is used to treat skin diseases, burns and scald wounds, tracheitis, pulmonary tuberculosis and is an excellent antiseptic. In Turkey, the resin is boiled and used externally in abscesses or chewing gum to clean teeth and to prevent bad breath. The resin is dissolved in hot water and applied externally to wounds and cuts (Ulukanlı et al., 2014).

Resin is secreted to survive against abiotic effects in plants. It contains many bioactive chemicals, including main terpenoids. Thanks to these bioactive compounds, the resin has protective effects against external factors such as pathogenic microorganisms and insects (Neis et al., 2019). The resin biosynthesized by epithelial cells is found in channels called schizogenous or in special vesicles in the cells of trees (Alkan et al., 2016).

Monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpenes, oxygenated sesquiterpenes, and carbonyl compounds can be mentioned as volatile compounds of *Pinus* (Yu et al., 2004). It is known that the composition and amounts of many compounds in plants vary according to the parts of the plant (leaf, stem, branch, root, and similar parts), where it grows, according to the season and location. Tiberi et al. (1999) listed the monoterpenes in the extracts of *P. pinea* needles as *a*-pinene, *β*-pinene, *δ3*-carene, myrcene, limonene, and phellandrene.

To the best of our knowledge, in Turkey, few studies have been carried out on the resin, popularly called pine resin, rich in secondary metabolites and used in many fields from medicine to food. This study investigated the antioxidant properties and monoterpene compounds of pine resin. For this purpose, pine resin samples were obtained from herbalists in different provinces of Turkey. Two antioxidant activity methods, FRAP (Ferric reducing antioxidant power) and DPPH (diphenyl-1-picrylhydrazyl) radical scavenging activity, were used. Folin's method was used to determine the total phenolic contents in the samples. It was carried out by spectrophotometric measurement of the blue color formed by phenolic compounds in alkaline medium with Folin reagent. Furthermore, the monoterpene compositions of the samples were determined by applying solid-phase microextraction (SPME) and gas chromatography-mass spectroscopy.

2. Material and Methods

In the present study, the thirteen pine resin samples investigated were purchased from herbalists in different provinces of Turkey (Table 1). The samples were analyzed in the Food Engineering Department Laboratory of Ondokuz Mayis University. The color measurement was performed before crushing them into powder with a glass mortar.

Table 1. Color, dry matter, and antioxidant properties of pine resin samples

Samples	City	*L**	*a**	*b**	Moisture %	Total phenolics mgGAE g^{-1}	FRAP µmol g^{-1}	EC_{50}, µg g^{-1}
1	Mersin	75.60	-0.52	19.47	3.53	29.82	78.76	618.00
2	Bolu	35.87	4.42	9.16	4.80	42.01	183.07	154.55
3	Bolu	73.15	-0.37	13.01	3.79	36.07	75.05	401.15
4	Bolu	60.94	1.42	15.36	2.61	378.77	712.87	54.36
5	Bolu	47.17	7.15	14.06	3.50	73.16	442.36	152.45
6	Bolu	70.95	2.27	22.72	3.39	23.19	68.85	451.03
7	Giresun	69.40	2.70	14.78	3.90	341.94	758.80	96.31
8	Artvin	53.47	5.20	7.43	3.42	379.44	752.60	92.06
9	Samsun	61.44	1.42	16.31	3.78	25.82	147.22	681.99
10	Aydın	75.74	-0.02	18.48	4.23	24.94	146.47	1006.97
11	İzmir	68.43	1.06	19.81	3.32	27.69	73.81	496.07
12	İstanbul	73.88	-0.31	17.29	3.51	32.26	141.85	572.40
13	Edirne	70.62	1.30	19.97	3.47	26.44	83.96	459.24
	Mean	64.36	1.98	15.99	3.65	110.89	281.97	402.81
	Std dev	12.23	2.35	4.38	0.51	146.67	279.62	284.09

Where *L** is luminescens (*L**=0, black; *L**=100, white); *a** is redness and greenness (+60, red; -60, green) and the *b** value represents yellowness and blueness (+60, yellow; -60, blue).

**EC_{50} value of positive control (trolox) was 66.54 µg mL^{-1}

2.1. Color analysis

The color of the samples was measured by Minolta CR 400 (Japan) color measuring device. The color was expressed as *L** (100, lightness; 0, darkness), *a** (+, redness; -, greeness), *b** (+, yellowness; -, blue). White ceramic (No:19633162) was used in the standardization of the device.

2.2. Moisture content

The moisture of the samples was determined by drying in an oven with vacuum at 70°C to constant weight (AOAC, 2000).

2.3. Extraction procedure

One gram of the powder sample was mixed with 25 mL of 80% methanol and the mixture was left to dissolve for 12 h

at room temperature and then filtered by 0.45 micron disposable filter.

2.4. Total phenolic analysis

According to Singleton and Rossi (1965), the total phenolics of the extracts were determined. 50 µL of the filtrate was mixed with 50 µL of Folin-Ciocalteau reagent and 250 µL of 10% sodium carbonate and placed in the dark for two h; its absorbance was read at 760 nm in a spectrophotometer. The calibration curve was drawn using gallic acid (Sigma) as a standard and the total phenolic content was expressed as mgGAE g^{-1}.

2.5. Antioxidant activity

Ferric reducing antioxidant power (FRAP)

According to Gao et al. (2000), the FRAP assay was determined with some modifications. FRAP reagent consists of 10 mM TPTZ (tripyridyltriazine) in 40 mM HCl, 20 mM ferric chloride and 300 mM acetate buffer (pH 3.6) in the ratio of 1:1:10 (v/v/v). FRAP solution (50 µL) was mixed with methanolic extract (950 µL) for this analysis. After 5 min, the absorbance of the colored mixture was measured at 593 nm. FRAP was calculated from a calibration curve using $FeSO_4$ as the standard and expressed as µmol $FeSO_4$ equivalents per g (µmol Fe^{2+} g^{-1}).

Radical scavenging effect test

DPPH (1,1-diphenyl-2-picrylhydrazyl) is a free radical with unpaired electrons. The radical scavenging effect of the resin was estimated by published methods (Nakajima et al., 2004; Thaipong et al., 2006). 50 µL of different concentrations of resin extracts were mixed with 1000 µL of 100 µM DPPH and incubated at room temperature for 120 min and absorbance was read spectrophotometrically at 515 nm. Trolox (a water-soluble vitamin E analog) was used as a positive control. The inhibition of free radicals from DPPH as a percentage was calculated with the following equation:

$$\text{DPPH radical scavenging activity (\%)} = [(A_0-A_1)/A_0] \times 100$$

Where; A_0 is the absorbance of the control reaction, A_1 is the absorbance of the extracts. The EC_{50} value was defined as the concentration of 50% of DPPH radical scavenging activity.

2.6. Monoterpene profiles determination

The sample (1 g) and 22 mL of water were placed in the headspace vial (Perkin Elmer, USA). The headspace vial was tightly sealed with a silicone/polytetrafluoroethylene (PTFE) septum (Perkin Elmer, USA) and aluminum cover (Perkin Elmer, USA). The sample was heated at 80°C for one hour and then put into headspace autosampler. The transfer line was a fused silica 1 m x 320 µm. The vial pressure was fixed at 10 psi and column pressure was kept at 25 psi. The needle temperature was set at 90°C and transfer line temperatures at 100°C. The trap hold time was 6 min and the outlet split was on. Desorbed compounds were automatically injected into a GC column (Optima-Wax, 60 m length, 0.25 mm inner diameter, 0.25 µm film thickness). The oven temperature was 70°C. The flow rate of the helium carrier gas was 1 mL min^{-1}. The injection was performed in the splitless mode (200°C injection port temperature). The GC column temperature was programmed as follows: 1. holding for 5 min at 35°C, 2. increasing from 35°C to 160°C at a rate of 3°C min^{-1}, and 3. holding at 160°C for 15 min. The MS conditions were: 200°C for ion source temperature; 70 eV for ionization energy; 33-300 amu for mass scan range; 350 V electron multiplier voltage; 0.25 s for scan time, 0.05 for standby time; and electron ionization (EI) as ion mode. The analyses were performed in triplicate.

The monoterpene composition was determined by comparing their retention index and mass spectra with a commercial spectra database (Wiley 6, NBS 75k) and the instrument's internal library. 10 µL methyl alcohol: water (1:1) mixture was used as internal standard. The standard peaks were compared to those of samples based on their retention time and mass spectra. The unknown chromatograms were identified using Mass Spectral Libraries according to the retention index calculated thanks to the n-alkane series (C6-C20) (Alasalvar et al., 2012).

2.7. Statistical analysis

The data were evaluated as mean value±standard deviation. The data was also subjected to a correlation analysis using Pearson's correlation coefficient.

3. Results and Discussion

The results showing the color characteristics, moisture contents, and antioxidant properties of the examined 13 different pine resin samples were given in Table 1.

*L** values of the samples varied between 35.87 and 75.74, *a** values between -0.52 and +7.15, *b** values between +7.43 and +22.72. There are huge differences between the color values of the samples. It was determined that the color of 3 samples (2, 5, and 8 numbers) was dark, except for 4 samples (1, 3, 10, and 12 numbers), the redness values of the other samples were higher. Generally, the yellowness values were high in all samples. This difference in colors is probably due to different places where the trees grow as well as different drying conditions and harvesting times. Moisture contents of pine resin varied between 2.61 and 4.80%. It was observed that some samples (2 and 10 numbers) had higher moisture content than the other samples. These values were closely related to the conditions of drying the pine resin.

In the human body, reactive oxygen species such as free radicals, superoxides, hydroxyl radicals, and hydrogen peroxide are produced during the endogenic metabolic processes. The reactive matters readily react with DNA, lipids, and proteins resulting in cell damage. However, the antioxidants give hydrogen atoms to free radicals. Thus, the antioxidants prevent the cell from harmful effects of the radicals. Today, interest in natural antioxidants has increased due to their positive effects on health. This interest has led to a focus on research on the antioxidants in various disciplines. Pine products are rich in antioxidants. To date, different parts of the trees (bark, needles, shoots, seeds), various extraction methods, and solvents have been used in the studies investigating the antioxidant properties of *Pinus* trees (Dziedziński et al., 2021).

Phenolic compounds, including flavonoids, have biological effects. The source of antioxidant activity is polyphenolic compounds. The radical scavenging

activities and inhibition of lipid peroxidation are measured to determine the antioxidant activity resulting from the polyphenolic contents of the plant material. The total phenolic content can be determined by measuring the color intensity of the phenolic compound extract with the Folin-Ciocalteau reagent. As a result of many studies, it has been observed that the best solvent for the extraction of phenolics is the methanol: water mixture (Kwak et al., 2006). In this study, total phenolic substances were investigated with the Folin technique and it was found between 23.19 mgGAE g^{-1} and 379.44 mgGAE g^{-1}. As can be seen in Table 1, there were significant variations among the samples compared with each other. Such a considerable variation is presumably due to the location and climatic conditions where it grows. Compared with other researchers, Kwak et al. (2006) found total phenolic contents as 57.71 mg g^{-1} dry weight basis in pine needles of *Pinus densiflora* extracted by 75% ethanol. This value was within the limits of our findings.

FRAP values of pine resin ranged from 68.85 to 758.80 µmol Fe^{2+} g^{-1}. The FRAP values of 3 samples (4, 7, and 8 numbers) are much higher than the others as seen in Table 1. Antioxidant activities of the samples were analyzed by a DPPH free radical assay using spectrophotometric. EC_{50} value indicates the concentration of sample that could inhibit 50 percent reduction of DPPH radical (Table 1). The EC_{50} value of the samples ranged from 54.36 to 1006.97 µg g^{-1}. Generally, the antioxidant power of the samples in our study was lower than the antioxidant power of Trolox (EC_{50} value 66.54 µg mL^{-1}) used as a positive control. The lower the EC_{50} value, the higher the antioxidant activity. The samples used had less potential antioxidant activity than positive control "Trolox".

Park et al. (2011) extracted *Pinus densiflora* needles in hot water, ethanol, hexane, hot water-hexane, and hot water-ethanol. They analyzed the proanthocyanidin contents and antioxidant activities of the sample extracts. At the end of this research, they found that the hot water extract (EC_{50} value=0.27 mg mL^{-1}) had superior antioxidant activity than the other extracts.

Tillah et al. (2017) determined the antibacterial and antioxidant activities of *Pinus merkusii*, *P. oocarpa*, *P. insularis*, *Agathis loranthifolia* resins. They found that *P. oocarpa* resin extracted in n-hexane had the most antibacterial potential compared with the other samples. For the antioxidant activity, they expressed that *P. merkusii* resin extracted in *n*-hexane had the lowest EC_{50} value (60.203 mg mL^{-1}) compared with the other extracts and the EC_{50} value changed in relation to the solvent.

Kwak et al. (2006) determined that the EC_{50} value was 95.12 mg mL^{-1} in the extracts with ethyl alcohol 75%. They concluded that pine needles would have potent antioxidative activity due to the high phenolic compounds.

Dziedziński et al. (2021) reported that the total phenolics of alcoholic extract for *Pinus brutia* tree barks were 412.42 mgGAE g^{-1} and the aqueous extracts obtained from the shoots of *P. sylvestris* was 0.86 mgGAE g^{-1} dry weight basis. It was expressed that the alcoholic extracts have higher total phenolic values than the aqueous extracts. They declared that the value of EC_{50}, which expresses free radical tests, for *P. koraiensis* seeds extracts in 40% aqueous ethanol and *P. brutai* bark extracts in 80% aqueous methanol were 0.023 mg mL^{-1} and 9.17 µg mL^{-1}, respectively. Many previous studies clearly stated that the antioxidant activity of the plant products was affected by whether extraction solvents contain water. The use of aqueous mixtures of water and organic solvents, such as ethanol, methanol, acetone, isopropanol, or acetonitrile, remarkably increases the antioxidant efficacy of the extracts (Dziedziński et al., 2021).

Vankatesan et al. (2019) investigated the antioxidant activity of *Pinus densiflora* needle extracts in water:ethanol mixture ratios of 0, 20, 40, 60, 80, and 100 percent. They determined that the highest radical scavenging capacity for the extract contained 40% of ethanol. It was followed by the values of the extracts containing 60%, 20%, 80%, 0%, and 100% ethanol, respectively.

According to our findings, FRAP values the extracts correlated well with the total phenolic content (r=0.958**), while a high negative correlation was found between FRAP values and EC_{50} values (r=-0.719**). However, the correlation between total phenolic substance and EC_{50} values was not as high as in FRAP values (r=-0.688**). Xie et al. (2015) reported that these differences could be attributed to the different stoichiometry of the DPPH and FRAP assay reactions. In addition, the phenolic composition differences in extracts, which solvent used, and their different solubility in the test systems may also affect their antioxidant activity.

The monoterpene compositions of the samples were determined by applying solid-phase microextraction (SPME) and gas chromatography-mass spectroscopy and the results were shown in Table 2. The monoterpene composition (%) was determined by comparing of their retention index and retention time (Fig. 1).

Eight monoterpene compounds were identified in the different samples of the resin, including *a*-pinene, camphene, *β*-pinene, 3-carene, *β*-myrcene, cymene, D-limonene, and *p*-cymene. Most of these volatile compounds have been reported previously in various species of *Pinus* (Ustun et al., 2012; Kadri et al., 2015; Xie et al., 2015; Kurti et al., 2019; Sharma et al., 2020).

In contrast, cymene was identified in *Pinus* samples for the first time in the present study. The compounds *a*-pinene, *β*-pinene, and 3-carene were the most dominant monoterpene compounds determined in the studied samples. *a*-pinene was found as the most abundant monoterpene compound in *Pinus* samples accounting for 21.17-51.37%. *a*-pinene was followed by *β*-pinene of 0.96-40.36%. These results were found to be in agreement with many previous studies, which reported *a*-pinene and *β*-pinene as the significant volatile substances in *Pinus* species with concentrations ranging from 8.16 to 50.40% and from 0.35 to 47.50%, respectively (Ustun et al., 2012; Xie et al., 2015; Kurti et al., 2019; Sharma et al., 2020). *a*-pinene and *β*-pinene exhibit diverse biological activities such as antifungal, antiviral, and antimicrobial and are generally recognized as safe (GRAS) (Dziedziński et al., 2021).

As shown in Table 2, the percent monoterpene composition of the pine resin samples varied significantly

according to the herbalist from which it was purchased. The highest amount of *a*-pinene was found in the samples numbered as 4 (51.37%), 10 (48.54%), 3 (47.05%), 5 (46.27%), 2 (44.08%), 11 (41.37%), 13 (41.28%), 6 (41.07%), 1 (38.01%), 8 (37.33%), and 12 (32.55%). The highest amount of *β*-pinene was determined in samples 8 (40.36%) and 4 (30.09%), while the most significant amount of 3-carene was found in sample 7 (39.27%). Tiberi et al. (1999) listed the monoterpenes in the extracts of *P.pinea* needles as *a*-pinene, *β*-pinene, *δ*3-carene, myrcene, limonene, and phellandrene. Also, the correlation between monoterpene composition and antioxidant activity was examined, and generally, no statistically significant relationship was found in the present study.

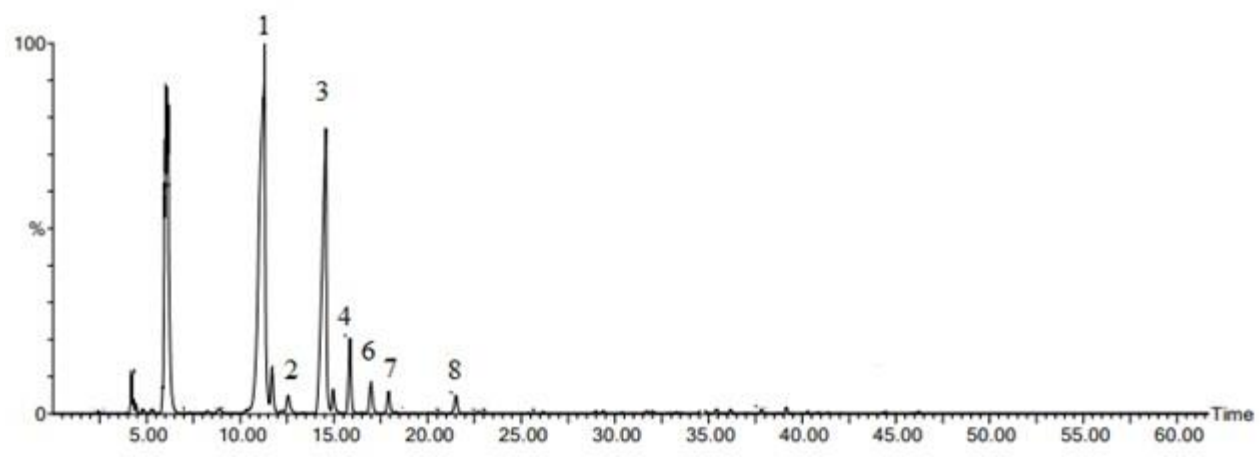

Figure 1. Typical GC-MS chromatogram of pine resin (1: *a*-pinene, 2: camphene, 3: *β*-pinene, 4: 3-carene, 6:cymene, 7: D-limonene, 8: p-cymene) (5: *β*-myrcene is not clear for only this chromatogram but RT for *β*-myrcene is 16.44 min.)

Table 2. Monoterpene profiles of the resin samples (%)

		a-pinene	camphene	*β*-pinene	3-carene	β-myrcene	cymene	D-limonene	p-cymene
	RT, min	11.13	12.15	14.56	15.85	16.44	16.99	17.92	21.52
	RI	1100	1124	1182	1214	1240	1252	1269	1355
Samples	1	38.01	0.1	21.53	3.66	0.05	1.8	1.2	1.08
	2	44.08	0	6.82	0.64	0.77	0.27	0.69	1.15
	3	47.05	0.08	1.58	0.45	0.54	0.52	0.12	0.56
	4	51.37	0.02	30.09	0.92	0.11	0.03	0.85	0.44
	5	46.27	0.07	0.96	0.44	0.96	0.01	1.12	0.2
	6	41.07	0.54	13.2	4.95	0.03	1.29	1.53	1.47
	7	21.17	0.34	8.67	39.27	0.84	1.72	1.79	0.97
	8	37.33	0.96	40.36	1.3	1.42	0.12	2.49	0.11
	9	26.65	0.22	5.07	3.54	0.08	1.49	2.01	2.99
	10	48.54	0.69	26.19	0.77	0.11	0.77	1.34	0.26
	11	41.37	2.25	12.05	4.13	0.04	1.52	1.49	1.56
	12	32.55	0.76	18.17	5.51	0.09	3.29	1.04	1.75
	13	41.28	1.14	14.69	6.94	0.1	1.68	0.77	0.1
	mean	39.75	0.55	15.34	5.58	0.40	1.12	1.27	0.97
	std	8.71	0.64	11.70	10.36	0.46	0.95	0.62	0.84

*RT; retention time, RI; retention index

The investigated pine resin samples were obtained in line with the statements of herbalists in different cities. Therefore, the information about the process of resin before it reaches the herbalist is not clear. We can clearly state that the difference between the analysis results of the present study is that the samples were taken from different cities. However, this difference may be caused by many factors such as pine species, altitude level, seasonal conditions where the resin is collected, drying conditions of the resin, characteristics of the soil where the pine trees grow, and duration of sunshine. The current study was carried out on a limited number of materials. For this reason, the bioactive potential of pine resin could be revealed more clearly by conducting studies under controlled conditions according to these factors that can directly affect the bioactive substance and its properties.

4. Conclusion

In the present study, the antioxidant potential and the monoterpene profile of 13 pine resin samples purchased from different provinces of Turkey were investigated. FRAP, which shows antioxidant potential, positively

correlated with total phenolic content while DPPH (EC_{50}) negatively correlated with total phenolic content. Both conditions (negative or positive correlation) revealed that the antioxidant activity of the samples was well correlated with the total phenolic content; thus, all the resin samples exhibited good antioxidant potential. Pine resin samples exhibited significant color values and antioxidant properties in the present study. It is known that the composition and amounts of many compounds in plants vary according to the parts of the plant (leaf, stem, branch, root, and similar parts), where it grows, and according to the season and location. The use of volatile compounds obtained from pine needles in the food industry is mentioned in the literature. However, information is needed about their usage in this industry. The antioxidant potential of pine resin, which is mainly mentioned for its use in folk medicine, and the antimicrobial properties of terpenoids allow it to be used as a food additive in today's food industry. Its use as antioxidant, antimicrobial, and flavoring in food should be investigated.

Ethics committee approval: Ethics committee approval is not required for this study.

Conflict of interest: The authors declare that there is no conflict of interest.

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Review / Derleme

The Biosynthesis of Silver Nanoparticles and their Use as a Biosensor Material

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Abstract: Various synthesis methods are being developed in order to increase the number of scientific fields where nanoparticles can be used. Recently, the biosynthesis methods have eliminated the limitations of the traditional synthesis methods such as physical and chemical ones. They have been also developed as an alternative synthesis method. With green synthesis called herbal nanofactories, primary and secondary metabolites in plants enable the reduction and capping of nanoparticles. The functional groups of alkaloids, phenolics, terpenoids, ketones, polysaccharides, proteins, vitamins, and amino acids in plants react with silver metals in ionic form and reduce "+" valued metals to "0" valued nanostructures. At the same time, functional groups of secondary metabolites form bonds with "0" valued silver nanometals and cover the surface of silver nanometals; thus, stabilization is achieved. Synthesis by biological methods provides high efficiency and rapid synthesis and the production cost of silver nanoparticle decreases. Moreover, biosynthesis is an environment-friendly technique as it takes place inside a living being. With the latest technology, silver nanoparticles stand out in the fields of biosensor and photoimaging. In this review, in which areas silver nanoparticles are used and their biosynthesis, stabilization, characterization, antibacterial mechanism, and use as a biosensor will be discussed.

Keywords: Green synthesis, bionanotechnology, characterization, secondary metabolite, antibacterial activity.

Gümüş Nanopartiküllerinin Biyosentezi ve Biyosensör Materyali Olarak Kullanımı

Öz: Nanopartiküllerin kullanılabileceği bilim alanlarını arttırmak amacıyla son zamanlarda çeşitli sentezleme metotları geliştirilmeye çalışılmaktadır. Bu metotlardan biri nanopartiküllerin bitkiler aracılığıyla sentezlenmesidir. Günümüzde biyosentez yönteminin kullanılması, fiziksel ve kimyasal yöntemler gibi geleneksel sentez yöntemlerinin sınırlamalarını ortadan kaldırmış, alternatif bir sentez yolu olarak geliştirilmiştir. Bitkisel nanofabrikalar olarak adlandırılan yeşil sentez ile bitkilerde bulunan primer ve sekonder metabolitler nanopartiküllerin indirgenmesi ve kapatıcılığını mümkün kılmaktadır. Bitkilerde bulunan alkaloidler, fenolikler, terpenoidler, ketonlar, polisakkaritler, proteinler, vitaminler, amino asitlerin fonksiyonel grupları iyon halindeki gümüş metalleri ile tepkimeye girerek "+" değerlikli metalleri "0" değerlikli nanometal yapılara indirgemektedir. Aynı zamanda sekonder metabolitlerin fonksiyonel grupları "0" değerlikli gümüş nanopartiküller ile bağlar oluşturarak gümüş nanopartiküllerin yüzeyini kaplar, böylece gümüş nanopartiküllerinin stabilizasyonu sağlanmış olur. Biyolojik yöntemler ile sentez hızlıdır, yüksek verim sağlar ve gümüş nanopartikülü üretimi maliyeti düşer. Aynı zamanda, biyosentez yoluyla nanopartikül üretimi canlı içinde gerçekleştiğinden çevre dostu bir tekniktir. Son teknoloji ile gümüş nanopartiküller, biyosensör ve fotogörüntüleme alanlarında öne çıkmıştır. Gümüş nanopartiküller ile bazı belirteçlerin spesifik olarak tespiti çeşitli çalışmalarla kanıtlanmıştır. Bu derlemede gümüş nanopartiküllerinin kullanım alanları, biyosentezi, stabilizasyonu, karakterizasyonu, antibakteriyel mekanizması ve biyosensör olarak kullanımına değinilecektir.

Anahtar kelimeler: Yeşil sentez, biyonanoteknoloji, karakterizasyon, sekonder metabolit, antibakteriyel aktivite.

1. Giriş

Gümüş (Ag^+) iyonunun; nötron sayısı 61, atom numarası (proton sayısı) 47, elektron sayısı 47, atom ağırlığı 107.87'dir. Argentum olarak da bilinen Ag^+ yüksek elektriksel ve termal iletkenliğe sahip, yumuşak, beyaz, parlak ve saf formda bulunan bir geçiş metalidir (Howe & Dobson, 2002). Eski çağlardan beri madenî paralar, mutfak eşyaları, solüsyonlar, köpükler, merhemler ve losyonların içeriğinde kullanılmaktadır; bununla birlikte, tıp, elektronik ve ev uygulamaları gibi çeşitli alanlarda da kullanımı yaygındır. Ag^+'nin kullanım alanı geniş olduğundan oldukça büyük öneme sahiptir. Gümüş sülfadiazin yara bölgesinde biyofilm oluşumunu önlemek için yanık yaralarının tedavisinde sıklıkla kullanılmaktadır.

Gümüş serbest oksijen moleküllerine maruz kaldığında kendiliğinden oksitlenir (Sallah et al., 2020). Bu durum nanobilim ve nanoteknoloji uygulamalarında, nanomalzemelerin üretiminde önemli ve dikkat çeken bir ilerlemeye yol açmıştır. Gümüşün farklı kimyasal reaksiyonlarının anlaşılması gümüş nanopartiküllerin (AgNP'ler) sentezi çalışmalarını hızlandırmıştır.

AgNP'ler atık sularda veya bitki materyallerinde doğal olarak birikmektedir (Howe & Dobson, 2002), bununla birlikte deneysel çalışmalar için fiziksel, kimyasal ve biyolojik olmak üzere çeşitli yöntemler kullanılarak AgNP'lerin sentezi gerçekleştirilmektedir. Fiziksel yöntemlerde buhar yoğunlaştırma, lazer ablasyon, gama ışıması ve elektron ışınlaması AgNP'lerin sentezinde en sık kullanılan yaklaşımlardır (Iravani et al., 2014; Sallah et al., 2020). Kimyasal yöntemlerde metal tuzlarını indirgemek için su veya organik çözücülere ihtiyaç vardır.

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Bu yöntem üç önemli kimyasal madde üzerine yoğunlaşır: indirgeyici maddeler, metal öncüler ve stabilize edici veya kapatıcı ajanlar. Biyolojik yöntemlerde ise bitki ekstraktları, funguslar ve bakteriler kullanılarak AgNP'lerin biyosentezi gerçekleştirilmektedir (Gudikandula & Maringanti, 2016). AgNP'lerin sentezi için; fiziksel ve kimyasal yöntemlerde daha fazla enerji, yüksek basınç, sıcaklık ve kimyasallara ihtiyaç duyulurken biyolojik yöntemlerde AgNP'lerin sentezi *in vivo* gerçekleşir. Fiziksel ve kimyasal yöntemlerin dezavantajları göz önünü alındığında AgNP'lerin sentezi için biyolojik yöntemler tercih edilmektedir (Gudikandula & Maringanti, 2016; Sharifi-Rad et al., 2020). Biyolojik yöntemler; çevre dostu, ucuz, tek basamaklıdır, bununla birlikte tehlikeli veya toksik kimyasallar kullanılmadığı için güvenlidir (Nabikhan et al., 2010).

AgNP'lerin biyosentezinde kullanılan bitki ekstraktları; fungus ve bakteriler ile karşılaştırıldığında daha fazla AgNP üretim potansiyeline sahiptir (Gudikandula & Maringanti, 2016). Bunun nedeni bitki ekstraktlarının içerdiği biyoaktif bileşiklerdir [primer (proteinler, amino asitler) ya da sekonder metabolitlerdir (alkaloidler, fenolikler, terpenoidler, ketonlar, vitaminler)]. Bu bileşikler AgNP'lerin sentezinde indirgeyici ve kapatıcı ajan olarak rol oynar, aynı zamanda AgNP'lerin sentezlenmesi süresini kısaltır (Mukherjee et al., 2014; Sharifi-Rad et al., 2020).

AgNP'lerin farklı bitki türlerinden alınan eksplant kaynaklarının yanında bitki doku kültürü metodlarını kullanarak da üretildiği rapor edilmiştir (Mude et al., 2009; Nabikhan et al., 2010; Xia et al,. 2016; Aref & Salem, 2020; Rashmi et al., 2021) (Tablo 1). Bitki doku kültürü, aseptik koşullar altında katı veya sıvı besin ortamlarında bitkilerin, dokuların, organların büyümesi ve çoğalması olarak tanımlanır. Bitki doku kültürü; kallus üretimi, sekonder metabolit üretimi, bitki gelişimi gibi çalışmalarda önemli bir rol oynamaktadır. Ek olarak, hızlı çoğalma, gen kaynağı korunması, somaklonal varyasyon, dihaploid bitki üretimi, somatik hibridizasyon, genetik mühendisliği için doku kültürü uygulamaları kullanılabilir.

Tablo 1. Bitki ekstraktları ile gümüş nanopartiküllerinin biyosentezi ve biyolojik aktivitesi.

Table 1. The biosynthesis and the biological activity of silver nanoparticles by plant extracts.

NP	Boyut (nm)	Morfoloji	pH	Isı (°C)	Bitki	Aktivite	Karakterizasyon	Kaynak
Ag	5-104	Heksagonal, Nanotriangles	2,11	30	*Medicago sativa*'nın Tohum Ekstraktı	Antibakteriyal Aktivite	TEM, XRD, SEM, XPS, UV-Vis	Lukman et al., 2011
Ag	55-80 5-40	Küresel	-	35	*Cinnamonum camphora*'nın Kallus Kültürü Ekstraktı	Antibakteriyal Aktivite	FT-IR, UV-Vis, XRD, TEM, EDX, SEM, DLS	Aref & Salem, 2020
Ag	60-80	Küresel	-	35	*Carica papaya*'nın Kallus Ektraktı	-	FTIR, SEM, UV-Vis	Mude et al., 2009
Ag	32.9	Küresel	6	32	*Centella asiatica L.*'nin Kallus Kültürü Ekstraktı	Antioksidan Aktivite	UV-Vis, XRD, TEM, EDAX, FTIR	Rashmi et al., 2021
Ag	5-20	Küresel	-	25	*Sesuvium portulacastrum* L.'nin Kallus ve Yaprak Ekstraktı	Antimikrobiyal Aktivite	TEM, XRD, FTIR	Nabikhan et al., 2010
Ag	44	Küresel	-	Oda Sıcaklığı	*Coleus aromaticus*'un Yaprak Ekstraktı	Bacterisidal Aktivite	XRD, UV-Vis, EDAX, SEM, FTIR	Vanaja & Annadurai, 2013
Ag	6-27	Küresel	7,8,9,10,11	Oda Sıcaklığı	*Taxus yunnanensis*'ın Kallus Ekstraktı	Antibakteriyal ve Sitotoksik Aktivite	XRD, TEM, FTIR	Xia et al., 2016

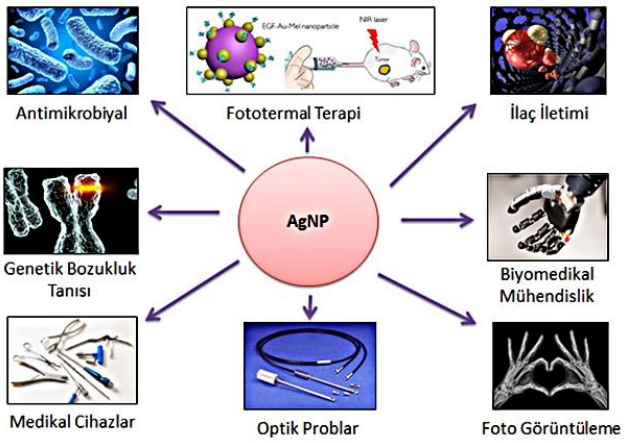
AgNP'ler birçok moleküle kıyasla daha az kırılma indisine sahiptir. Biyomoleküller AgNP'lere bağladığında yerel kırılma indisinde bir artış gösterir ve Ag'nin yok olma (soğurma veya saçılma) spekturumu gözle görülür bir şekilde artar. AgNP'lerdeki bu değişim ile çeşitli sensörlerin hedef moleküle etkili bir şekilde bağlandığı kanıtlanmıştır. Aynı zamanda AgNP'ler üzerindeki çeşitli kaplama (silika gibi) ajanları biyomoleküler saptamada etkili görev görür (Sotiriou & Pratsinis, 2011).

AgNP'lerin biyosensör materyali olarak kullanımı AgNP'lerin kimyasal stabilitesi, elektriksel iletkenliği ve katalitik aktivitesinin yüksek olması nedeniyle hedef maddenin tespit edilmesini güçlendirir. Algılama yüzeylerine elektro-statik çekim ile bağlanması AgNP'lerin stabilitesi ile yakından ilgilidir. Yüzeyde güçlü bir tutunma oluşturmak için genellikle yüzey kimyasal modifikasyonunun yapılması gerekir. Bu açıdan bakıldığında AgNP'ler yüzey kimyasal modifikasyonları için uygundur. Örneğin; AgNP'lerin yüzeyine bağlanması için trietoksisilan (3-Aminopropil), dallanmış polietilenimin (BTI), sitrat, lipoik asit, polietilen glikol (PEG) ve polivinilpirolidon en yaygın kullanılan kimyasaldır (Toh et al., 2015; Tan et al., 2020).

Bu derlemede AgNP'lerin; kullanım alanları, bitki ekstraktları ve bitki doku kültürü yöntemleri ile biyosentezi, biyosentezi için kritik parametreler, karakterizasyonu, antimikrobiyal aktivite üzerindeki etki mekanizmaları ve AgNP'lerin biyosensör alanında kullanımını değerlendirmek amaçlanmıştır.

2. Gümüş Nanopartiküllerin Kullanım Alanları

AgNP'ler; fototermal terapi, genetik hastalık teşhisi, biyomedikal mühendislik, tıbbi cihazlar, optik problar, foto görüntüleme gibi mekanik, elektrik, manyetik, katalitik, fotokimyasal alanlarda (Calderón-Jiménez et al., 2017), bunlara ek olarak, ilaç taşıyıcı ve biyosensör olarak, yiyecek ve ürün paketlemede, su arıtma ya da su sterilizasyonunda, UV ışığın zararlı etkisini önlemede, deterjanlardaki antibakteriyel spreylerde, plastiklerde, kozmetik ve tekstilde kullanılmaktadır (Şekil 1) (Calderón-Jiménez et al., 2017; Gonzalez et al., 2017). AgNP'lerin kullanım alanının geniş olması verimli üretim tekniklerinin anlaşılmasını ve farklı yöntem yaklaşımlarını önemli kılmıştır.



Şekil 1. AgNP'lerin uygulama alanları.

Figure 1. Applications of AgNPs.

3. Gümüş Nanopartiküllerin Biyosentezi

Bitki materyali ya da bitki doku kültürü ile AgNP'lerin biyosentezi basit, hızlı, verimli ve çevre dostu olduğundan önemli bir tekniktir. Bitki doku kültürü teknikleriyle kallus üretimi; bitkinin yaprak, sürgün, kök veya hipokotil, epikotil, kotiledon, kök, apikal meristem ve ilk yapraklardan gerçekleştirilebilir. Bitki materyali ya da bitkiden elde edilen kallus ekstraktları kapatıcı ve indirgeyici ajan olarak çok sayıda polar grup içerdiğinden AgNP'lerin stabilizasyonu için önemlidir.

Arabidopsis thaliana, *Centella asiatica*, *Hordeum vulgare*, *Linum usitatissimum*, *Sesuvium portulacastrum*, *Taxus yunnanensis*, *Cinnamonum camphora* gibi birçok bitkisel materyal ve bitki doku kültürü ile üretilen kallus ekstraktları AgNP'lerin biyosentezi için kullanılmıştır (Nabikhan et al., 2010; Mandeh et al., 2012; Netala et al., 2015; Anjum & Abbasi, 2016; Xia et al., 2016; Abbasi et al., 2017; Aref & Salem, 2020) (Tablo 1).

4. Gümüş Nanopartiküllerin Stabilitesi

Biyomoleküllerin AgNP'ler yüzeyine tutunması elektrostatik ve sterik etkileşimlerle olmaktadır. Bu reaksiyonlar pozitif Ag iyonuna bağlanan uygun stabilizör ile gerçekleşir. DNA, RNA, antikor, aptamer ve peptid gibi biyomoleküller bağlanarak AgNP'nin yüzeyine AgNP'ler tutuklanabilmektedir (immobilizasyon) (Tan et al., 2020). Aynı zamanda tiol grupları AgNP'lerin yüzeyinde immobilizasyon için kullanılmaktadır (Tan et al., 2020). Fakat AgNP'lerin yüzeyine biyomoleküllerin immobilizasyonunu sağlamak için AgNP'lerin agregatlaşmasının en aza indirilmesi önemlidir. Çözüm olarak AgNP'lerin optimum koşullarda üretilmesi gerekmektedir. Proteinler de hidrofobik olarak elektrostatik etkileşim ve değişken bağlar ile AgNP'lere bağlanabilmektedir (Szymanski & Porter, 2013). Sitrat kaplı AgNP'ler; tiol, antikor, amin, protein ve polimer olmak üzere çeşitli moleküllerle kolayca tutunmaktadır. Bununla birlikte PVP, AgNP'lerin yüzeyine çok güçlü bir şekilde bağlanır bu nedenle tanik asit veya sitrat kaplı AgNP'ye kıyasla daha yüksek stabilite gösterir. Biyomoleküllerin AgNP'lerin yüzeyine bağlanması genel olarak karboksil (COOH) veya amin grupları ile gerçekleşmektedir.

4.1. Bitki Ekstraktlarının İndirgeyici Ajan Olarak Kullanılması

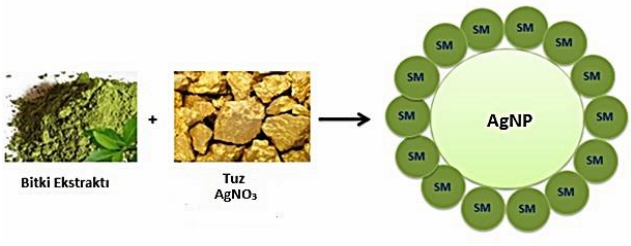
Bitki ekstraktları içerdiği çeşitli biyoaktif bileşikler sayesinde AgNP'lerin biyosentezinde indirgeyici ajan olarak rol oynar, dolayısıyla AgNP'lerin biyolojik olarak sentezlenmesi AgNP'lerin stabilitesini arttırır. Aynı zamanda biyolojik olarak sentezlenen AgNP'ler immünojenik olmayan özellikler gösterir. Bu durum tıbbî uygulamalarda kullanılmak üzere geliştirilen biyosensörler için bir avantajdır.

İndirgeme, bir maddenin bileşimindeki hidrojen miktarının artması ya da oksijen miktarının azalmasıdır. Başka bir deyişle, iyon ya da atomun bir elektron ilâvesi ile daha düşük değerliğe inmesidir. AgNP'lerin indirgenebilmesi için indirgeme ajanı seçilmelidir. İndirgeme ajanı kimyasal indirgeme hızını etkilerken, stabilize edici ajan, sentezlenen AgNP'lerin yüzeyine seçici olarak bağlanacak ajanları öncelikli olarak düzenlemektedir (Oliveira et al., 2020). Hem indirgeme hem stabilize edici ajanın dikkatli bir şekilde seçilmesi AgNP'lerin morfolojileri üzerindeki kontrolü sağlayacaktır (Toh et al., 2015). Bu nedenle AgNP'lerin biyosentezi için kullanılacak bitki içerdiği metabolitler göz önüne alınarak değerlendirilmelidir. Bitki ekstraktlarının içerdiği biyoaktif bileşiklerden (fenolik asitler, oksalik, malik, askorbik asit, çözünür karbonhidratlar, tanen, omega-3, flavonoidler, alkaloidler, lignan) hangisinin AgNP'leri indirgediği kesin olarak bilinmediğinden bazı araştırıcılar bitkinin farklı kısımları (gövde, yapraklar, kök ve doku kültürü ürünleri) ile AgNP biyosentezleyerek bitkinin organları arasında farklılık olup olamadığını gözlemlemiştir (Gholamreza et al., 2014; Xia et al., 2016).

Rasheed et al. (2017) AgNP'lerin biyosentezi için indirgeme ajanı olarak *Artemisia vulgaris* yaprak ekstraktını kullanmıştır. Bununla birlikte, biyosentez ürünü olan AgNP'lerin biyolojik aktivite çalışmaları yapılmıştır (Rasheed et al., 2017). Biyosentez ürünü AgNP'ler güçlü antibakteriyel aktivite göstermiştir. Aynı zamanda AgNP'lerin antioksidan ve sitotoksisite etkisi, MCF-7 ve HeLa hücre hatlarına karşı umut verici olduğu bildirilmiştir.

4.2. Bitki Ekstraktlarının Kapatıcı Ajan Olarak Kullanılması

İlaç iletimi, biyo-algılama ve biyo-görüntüleme gibi tıbbi uygulamalarda AgNP'leri kullanmak için AgNP'lerin stabilitesinin kusursuz olması zorunludur. Fakat AgNP'ler ile ilgili en önemli dezavantaj uzun süre kararlılıklarını koruyamamalarıdır. Ayrıca AgNP'ler çok reaktif oldukları için hava ile uzun süre temas sonucu kolayca oksitlenebilir. Monodispers ve polidispers AgNP'ler elde etmek için AgNP'lerin yüzey modifikasyonu gerekli olduğundan AgNP'leri yan ürünlerden saflaştırabilmek endüstriyel ölçekte AgNP'lerin kullanılmasının önünde bir engeldir. Bu nedenle çoğu uygulamada AgNP'lerin saf olarak sentezlendikten sonra aşınmaya ve oksitlenmeye karşı kararlı yapılarını korumak için yüzey aktif maddeler, polimerler, bitki ekstraktlarında bulunan metabolitler gibi organik bileşikler ya da silika ve karbon vb. inorganik tabakayla kaplanması gerekmektedir (Javed et al., 2020) (Şekil 2).



Şekil 2. Sekonder metabolitler, AgNP'lerin kapatıcı ve indirgeyici ajanlarıdır. SM: Sekonder Metabolitler, NP: Nanopartiküller.

Figure 2. Secondary metabolites are the capping and reducing agents of AgNPs. SM: Secondary Metabolites, NP: Nanoparticles.

AgNP'lerin biyosentezi için kullanılan *Solanum trilobatum* kabuğu kapama ajanı olarak kabul edilmiştir (Ramanathan et al., 2018). Rashid et al. (2019) AgNP'ler için kapama ajanı olarak dört farklı bitkiyi (*Rumex dantatus, R. Hastatus, Bergenia stracheyi* ve *B. ciliata*) kullanmıştır. Aynı zamanda farklı formüle edilmiş AgNP'lerin altı farklı bakteri suşuna (*Staphylococcus haemolyticus, S. aureus, E. coli, Bacillus cereus, Salmonella typhi* ve *Pseudomonas aeruginosa*) karşı güçlü antibakteriyel aktivite gösterdiği bildirilmiştir. AgNP'lerin aktivitesi, derişimlerinin artmasıyla artmıştır.

5. Gümüş Nanopartiküllerin Biyosentezi İçin Kritik Parametreler

Bilim insanları çeşitli alanlarda kullanmak üzere bütün AgNP bileşimleri; biyoyararlanım ve hareketliliği ciddi şekilde azaltan agregatlaşma potansiyeline sahiptir. AgNP'lerin agregat davranışı ve stabilizasyonu, pH, sıcaklık, inkübasyon periyodu, tuz konsantrasyonu, redoks koşulu, karışım oranı, iyonik güç, doğal organik madde konsantrasyonu ve AgNP'lerin yüzey kimyası gibi birçok değişkenin etkileşimine büyük ölçüde bağlıdır, bu değişkenler AgNP'ler arasındaki elektrostatik ve sterik etkileşimi etkilemektedir (Singh et al., 2016; Siddiqi & Husen, 2017).

5.1. pH'ın Gümüş Nanopartiküller Üzerindeki Etkisi

AgNP'lerin ortalama boyutu ve agregatlaşma eğilimi reaksiyon sisteminin pH'ına yüksek oranda bağlıdır, pH'ın etkisi çözünmüş oksijen konsantrasyonunun etkisinden daha güçlüdür (Alqadi et al., 2014; Fernando & Zhou, 2019). Lukman et al. (2011) 0.1 mM ve 0.01 mM $AgNO_3$ çözeltileri ile AgNP'lerin biyosentezi için *Medicago sativa* tohum ekstraktını kullanmıştır. AgNP'ler 2.0 ve 11.0 pH aralığında incelenmiştir. Numuneler 30°C'de inkübe edilmiştir. Çalışma, pH 11.0'daki reaksiyonda yüksek bir monodispersitenin elde edildiğini göstermiştir, pH 11.0'da ortalama boyut 11.5 nm'dir, ancak pH 2.0'da reaksiyon meydana gelmemiştir (Lukman et al., 2011).

Hegazy et al. (2014) ve Hegazy et al. (2015) 1 mM $AgNO_3$ çözeltisi ile AgNP'lerin biyosentezi için *Medicago sativa* L.'nin kallus ekstraktını kullanmıştır. Numuneler oda sıcaklığında yaklaşık 24 saat inkübe edilmiştir. Farklı pH aralıkları ve 2.0, 5.0, 7.0, 9.0, 10.0 ve 11.0 çalışılmıştır. pH 2.0'de 24 saatlik inkübasyondan sonra ekstraktın rengi değişmemiştir, bu yüzden araştırmacılar reaksiyonun devam etmediğini yorumlamıştır. Araştırmacılar, pH'ı ayarlanmamış örneklerde 2 ila 50 nm, pH 5.0'de 5 ila 60 nm, pH 10.0'da 35 ila 40 nm arasındaki boyut aralıkları gözlemlemiştir ve AgNP'lerin morfolojisi her iki durumda da (pH değeri ayarlanmamış ve pH değerleri ayarlanmış) küresel, disk ve düzensizdir (Hegazy et al., 2014; Hegazy et al., 2015).

Taxus yunnanensis'in kallus ekstraktları kullanılarak AgNP'ler sentezlenmiştir. Farklı pH aralıkları çalışılmış ve 7.0, 8.0, 9.0, 10.0 ve 11.0 pH'lı AgNP'ler, 439, 425, 411, 409 ve 450 nm'de maksimum absorbans göstermiştir. pH 10.0'da daha küçük boyutlu AgNP'lerin oluştuğu gözlenmiştir. Bu nedenle AgNP'lerin biyosentezi için en uygun koşul olarak pH 10.0'u tercih etmişlerdir (Xia et al., 2016). Xia et al. (2016), 7.0-10.0 pH'da AgNP'lerin ortalama boyutunun küçülmesini absorpsiyon pikinin kısa dalga boyuna kaymasıyla ilgili olabileceğini bildirmiştir.

5.2. Sıcaklığın Gümüş Nanopartiküller Üzerindeki Etkisi

1 mM gümüş nitrat ($AgNO_3$) çözeltisi ile AgNP'leri üretmek için *Carica papaya*'nın kallus ekstraktı kullanılmıştır. Numuneler, yaklaşık 24 saat boyunca 35°C'de inkübe edilmiştir (Mude et al., 2009). 1 mM $AgNO_3$ çözeltisi ile AgNP'leri üretmek için *Citrullus colocynthis* (L.) Schrader'ın kökten elde edilen kallus ekstraktı kullanılmıştır. İndirgeme için numuneler yaklaşık 24 saat boyunca 35°C'de inkübe edilmiştir (Satyavani et al., 2011). Selenyum ve $AgNO_3$ kullanarak *Spermacoce hispida*'nın yaprak sulu ekstraktlarından selenyum NP'ler ve AgNP'ler sentezlenmiştir. Ekstraktlar farklı sıcaklık (4, 20, 40 ve 60°C'de 15 dakika), farklı pH aralıkları (6.0, 7.0, 8.0, 9.0 veya 10.0), farklı $AgNO_3$ ve selenyum konsantrasyonları (0.5: 49.5, 2:48, 4:46 ve 6:44) ve inkübasyon zamanı ile optimize edilmiştir. Sonuçlar, Sh-SeNP'lerin sentezi için optimum koşulun, pH 9.0'da, 4:46 oranında ve Sh-ALE için 30 mM selenious asit çözeltisi, 10 dakika boyunca 40°C'de inkübe edildiğinde bulunmuşken, AgNP'ler için optimum şartın pH 8.0'de, 4:46 oranında 1 mM $AgN0_3$'ta 10 dakika boyunca 40°C'de inkübe edildiğinde bulunmuştur (Vennila et al., 2018).

5.3. İnkübasyon Süresinin Gümüş Nanopartiküller Üzerindeki Etkisi

$AgNO_3$ kullanarak *Sesuvium portulacastrum* L.'nin kallus ve yaprak ekstraktlarından AgNP'ler sentezlenmiştir. Ekstraktlar farklı inkübasyon süresi ile inkübe edilmiştir (0-10-20-30-40-50dk, 1-2-3-4-6-24-48 s). Sonuçlar, sarıdan kahverengiye kademeli olarak renk değişimi göstermiştir ve inkübasyon sırasında AgNP'lerin yoğunluğu artmıştır. 420 nm'de renk yoğunluğunun inkübasyon süresi ile arttığını bildirmiştir. 24 saatlik inkübasyondan sonra renk değişmemiştir. Araştırmacılar, renk yoğunluğunun, yaprak ekstraktları ile karşılaştırıldığında kallus ekstraktlarında daha yüksek olduğunu belirlemişlerdir (Nabikhan et al., 2010).

5.4. Tuz Konsantrasyonunun Gümüş Nanopartiküller Üzerindeki Etkisi

Linum usitatsimum'nun sulu ekstraktları ve TDZ-içerikli kallus kullanılarak AgNP'lerin biyosentezinin optimum aralığı bulmak için farklı konsantrasyonlarda 1 mM $AgNO_3$ (1:1, 1:2, 1:5, 1:10 v/v) denenmiştir. AgNP'li kallus ekstraktının biyoindirgenmesi AgNP'li bitki ekstraktının biyoindirgenmesinden daha kısa sürede tamamlanmıştır. Ayrıca, AgNP'lerin biyoindirgenmesi için farklı zamanlarda (0-10-20-30-40-50dk, 1-2-3-4-6-24s) çalışılmıştır. Sulu ekstrelerin reaksiyon karışımı 6 saat içinde tamamlanmıştır. TDZ içerikli kallus

reaksiyonundaki Ag iyonlarının indirgenmesi 2 saat içerisinde tamamlanmıştır (Anjum & Abbasi, 2016).

6. Gümüş Nanopartiküllerin Karakterizasyonu

AgNP'ler morfolojilerine (boyut, şekil, yüzey alanı) ve dağılma özelliklerine göre karakterize edilir. Genel karakterizasyon teknikleri: ultraviyole görünür spektroskopi (UV-Vis), atomik kuvvet mikroskopisi (AFM), taramalı elektron mikroskobu (SEM), geçirimli elektron mikroskobu (TEM), X-ışını toz difraksiyonu (XRD), fourier dönüşümü kızılötesi spektroskopisi (FTIR), indüktif eşleşmiş plazma kütle spektroskopisi (ICP-MS), dinamik ışık saçılımı (DLS), enerji dağılımlı spektroskopi (EDS), brunauer-emmett-teller tekniği (BET)'dir (Nabikhan et al., 2010; Khatami et al., 2015; Netala et al., 2015; Bao et al., 2016; Moldovan et al., 2016; Patra et al., 2016; Vennila et al., 2018) (Tablo 2).

AgNP'lerin spektrumları UV-Vis ile 250 ila 800 nm dalga boyu arasında taranarak belirlenmektedir. AgNP'lerin morfolojisini SEM ile belirlemek için AgNP'ler kurutularak stamplara yapıştırılmaktadır. AgNP'li ekstraktların TEM ile analizinde ise ekstraktlar kaplı gridler üzerine damlatılarak kurutulmaktadır. AgNP'lerin kristal yapısını belirlemek için XRD tarama aralığı 20° ile 80° arasında olmalıdır. AgNP'leri FTIR ile analiz edebilmek için kurutulmuş numune kullanılmalı, bununla birlikte FTIR 4cm^{-1} çözünürlükte ve spekturumu 450-4000cm^{-1} aralığında ayarlanmalıdır (Mude et al., 2009).

Tablo 2. Gümüş nanopartiküllerin karakterizasyon teknikleri ve temel işlevleri.

Table 2. The characterization techniques and main functions of silver nanoparticles.

Karakterizasyon Tekniği	Amaç	Referans
UV-Spektrofotometre	AgNP'lerin karakterizasyonunu ve stabilitesini değerlendirir.	Zook et al., 2011; Gorham et al., 2012
SEM	AgNP'lerin morfolojisini belirler. Görüntülerden histogram elde edilir. NP'ler manuel olarak ölçülür ve sayılır.	Chen et al., 2017
TEM	AgNP'lerin boyutu, morfolojisi ve boyut dağılımını ölçer. SEM ile karşılaştırıldığında daha iyi çözünürlük sağlar.	Parvathiraja et al., 2021
XRD	Atomik ölçekte kristallik derecesini ölçer. AgNP'lerin yapısını, partikül boyutlarını analiz eder, bileşikleri tanımlar.	Pal et al., 2017
FTIR	AgNP'lerin oluşturduğu çeşitli kimyasal bağları karakterize eder.	Baudot et al., 2010
ICP-MS	Ag+ iyonlarının konsantrasyonunu belirler.	Bao et al., 2016
DLS	AgNP'lerin boyutunu ölçer. Farklı pH ve sıcaklık koşullarında zaman içindeki stabilitelerini değerlendirir.	Zhang & Zhang, 2014

AgNP'lerin karakterizasyon yöntemleri ile ilgili birçok araştırmacı çalışmıştır. Nabikhan et al. (2010) AgNP'leri XRD kullanarak yoğun olarak belirlemiştir. AgNP'lerin şekli ve boyutu TEM kullanılarak karakterize edilmiştir. Sentezlenmiş AgNP'lerin 5 ila 20 nm arasında değişen ve farklı büyüklükte küre şeklinde olduğu görülmüştür. Proteinlere işaret eden amid I, II ve III'e karşılık gelen ekstraktlarda yüksek pikler elde edilmiştir ve bunlar FTIR analizi ile belirlenmiştir. Araştırmacılar, flavonların ve terpenoidlerin olduğuna işaret eden aromatik halkalara, geminal metillere ve eter bağlarına karşılık gelen pikleri de kaydetmişlerdir (Nabikhan et al., 2010). Satyavani et al. (2011) AgNP'lerin morfolojisini 20-80 nm^{-1} yay sabiti ve rezonans frekansı 209-286 kHz olan AFM ile karakterize etmiştir. Numunenin spektrumu FTIR kullanılarak 4 cm^{-1} çözünürlükte 400-4000 cm^{-1} aralığında kaydedilmiştir. AgNP'lere bağlanan aromatik halka ve bağlı amid bölgesi içeren polifenoller FTIR ile belirlenmiştir (Satyavani et al., 2011). Xia et al. (2016) XRD kullanılarak AgNP'leri nano-kristaller şeklinde belirlemiştir. Sentezlenen küresel AgNP'ler, TEM kullanılarak 6.4 ila 27.2 nm arasındaki boyut aralığı ile karakterize edilmiştir. Kallus ekstraktlarında fitokimyasallar FTIR kullanılarak incelenmiştir (Xia et al., 2016). Vennila et al. (2018) AgNP'leri UV-Vis kullanılarak karakterize etmiştir. AgNP'lerin morfolojisi ve boyutu SEM ile araştırılmıştır. Element analizi için EDX analizi yapılmıştır. AgNP'lerin toplam yüzey alanı, ASAP 2010 yüzey alanı analizörü metrometrileri kullanılarak BET yöntemi ile belirlenmiştir. AgNP'lerin kristal yapısı, tarama aralığı 20° ile 80° arasında olan XRD kullanılarak belirlenmiştir. AgNP'lerin yüzey fonksiyonel grupları FTIR ile 4000-500 cm^{-1} dalga boyu taraması yapılarak incelenmiştir (Vennila et al., 2018). Birçok araştırmacı benzer yöntemler ile AgNP'lerin karakterizasyonunu yapmıştır.

7. Gümüş Nanopartiküllerin Antibakteriyal Aktivite Mekanizması

Bakteriler AgNP'lere maruz kaldığında, AgNP'lerin oksidasyonundan üretilen gümüş iyonlarının pozitif yükü ile bakterilerin negatif yüklü hücre zarı arasındaki elektrostatik çekim nedeniyle bakterilerin hücre duvarına veya zarına yapışma eğilimindedir (Choi et al., 2010). Aynı zamanda, AgNP'ler bakteri hücre duvarındaki kükürt içeren proteinlere karşı güçlü bir afiniteye sahiptir. Böylece, AgNP'ler hücre içine kolayca girebilmektedir (Şekil 3). Bununla birlikte, antibakteriyal çalışmalarda AgNP'lerin yüzeyine bağlanan kapatıcı ajanların kullanımı da önemlidir. Araştırmacılar, Tween-80, sodyum dodesil sülfat (SDS), polivinilpirolidon ile AgNP'ler kapatıldığında AgNP'lerin antibakteriyal aktivitesinin arttığını bildirmişlerdir (Kvitek et al., 2008; Toh et al., 2015).

AgNP'lerin bakteri zarına bağlanması hücre zarının yapısında geri dönüşü olmayan morfolojik değişikliklere neden olmaktadır (Abdalla et al., 2020). Dolayısıyla bakteri lipit çift tabakasının bütünlüğünde ve hücre zarının geçirgenliğinde bir kayba neden olabilir. Hücre yapısındaki değişiklik, hücre zarının geçirgenliğinin artmasına neden olabilir ve bu da hücrenin çeşitli aktivitelerini düzenli olarak yapabilmesini olumsuz etkiler. Örneğin, AgNP'lerden gümüş iyonlarının salınması; potasyum iyonlarının taşınmasını ve salınmasını değiştirir böylece, hücrelerin taşıma aktivitesini etkiler. Hücre zarı geçirgenliğinin artması sitoplazma, proteinler, iyonlar ve hücresel enerji rezervuarı ATP gibi hücresel içeriklerin kaybına da neden

olabilir (Wakshlak et al., 2015). TEM görüntüleri AgNP'lerin, membran depolarizasyonu ve destabilizasyonu yoluyla gram-negatif bakterilerin (*E. coli* ve *S. typhimurium*) bütünlüğünü etkilediğini göstermiştir. *Lactobacilli*'de AgNP'lerin bakterisit etkisi araştırılmış, asidik büyüme koşullarının AgNP'lerin çözünürlüğünü ve hidroksil radikallerinin (-OH) üretimini uyardığı bildirilmiştir (Tian et al., 2018). –OH radikalleri, hücresel reaktif oksijen türlerinde (ROS) bir artışa neden olarak DNA ve mitokondri hasarıyla hücrenin ölümüne yol açmaktadır (Ahmed et al., 2016) (Şekil 4).

Lawsonia inermis (Henna)'nın sulu ekstraktı ile AgNP'ler biyosentezlenmiş ve AgNP'lerin insan patojenlerine karşı antimikrobiyal aktivitesi olduğu bildirilmiştir (Kumar & Kathireswari, 2016). *Taxus yunnanensis* kallus ekstraktı kullanılarak AgNP'ler sentezlenmiş ve insan kanser hücrelerinde sitotoksisitesi ve antibakteriyel aktivitesi incelenmiştir (Xia et al., 2016).

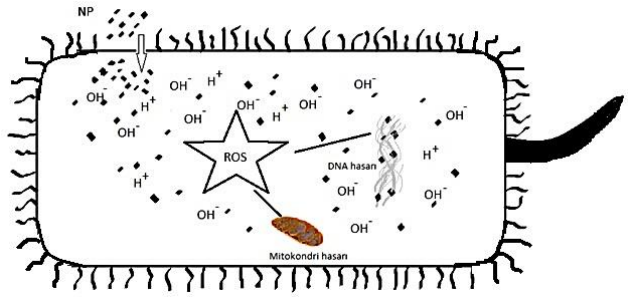
8. Gümüş Nanopartiküllerin Biyosensör Alanında Kullanımı ve Bakterilerin Tespiti

Viral, bakteriyel enfeksiyon ve kanser gibi çeşitli hastalıkların etkili bir şekilde tedavi edilebilmesi için erken teşhis önemlidir. Bu hastalıklar; problar, DNA, protein, antikor ya da enzim salgılanmasıyla tespit edilmektedir. Diğer NP'ler gibi, AgNP'ler de biyosensör alanında aktif olarak kullanılmaktadır (Loiseau et al., 2019). AgNP-konjuge biyomoleküller, hedef moleküllere karşı yüksek derecede stabilite ve hassasiyet gösterir. Bu nedenle AgNP-konjuge probların (Tablo 3), hedeflenmiş biyomolekülleri daha hızlı tespit ettiği kanıtlanmıştır.

Tablo 3. Biyomolekülleri tespit eden AgNP-konjuge probları.

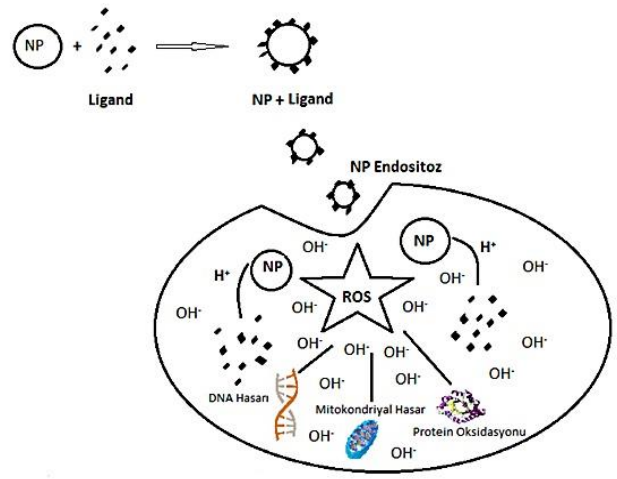
Table 3. AgNP-conjugated probes detect biomolecules.

Hedef	Prob	Sensör	Sınır limiti	Kaynak
Melamin	Sülfanilik asit	Kolorimetrik	10.6 nM	Song et al., 2015
C-reaktif protein	Antikor	İmmunofloresans	30 pg/mL	Zhao et al., 2017
DNA	DNA	Elektrokimyasal	50 pM	Cheng et al., 2009
Alfa fetal	Antikor	Eliza	0.23 ng/mL	Xuan et al., 2016
Biyotin	Streptavidin	LSPR	-	Kim & Lee, 2012
Glukoz	Glukoz oksidaz	Elektrokimyasal	-	Chen et al., 2012



Şekil 3. AgNP'lerin antibakteriyal aktivitesi.

Figure 3. The antibacterial activity of AgNPs.



Şekil 4. AgNP'lerin sitotoksik aktivitesi.

Figure 4. The cytotoxic activity of AgNPs.

Bakteri tespiti için bakteri hücresi üzerindeki dış yüzey proteininin AgNP'ler tarafından tespit edilmesi hedeflenmektedir. Bakteriyi tespit etmek için prob olarak antikor, DNA veya aptamer kullanılmaktadır. Etkili bir tespit için problar AgNP'lerin yüzeyine konjuge edilmektedir. Tüm bakteriyi tespit edebilmek için genellikle Surface Enhanced Raman Scattering (SERS) ya da lokalize yüzey plazmon rezonansı teknikleri kullanılmaktadır (Naja et al., 2007). Bakteri ile birlikte aynı zamanda influenza, insan immün yetmezlik virüsü, herpes simpleks virüsü ve Ebola virüsü gibi viral hastalıkların erken teşhisi de bu teknikler sayesinde kolaylaştırılmıştır, böylece kişilerin erken evrede tedavi edilmesi sağlanmaktadır. AgNP'ler kullanılarak bakteri tespiti, algılama sınırını geliştirmek için ihtiyaç duyulan bir tekniktir. Araştırmacılar AgNP-konjuge prob molekülü ile bakterilerin tespitini kanıtlamıştır (Naja et al., 2007; Liu et al., 2010). *Rhodococcus rhodochrous* ve *E. coli* bakteri türleri AgNP'ler ile SERS tekniği kullanılarak tespit edilmiştir (Naja et al., 2007). Bununla birlikte, diğer tekniklere kıyasla *E. coli*'nin AgNP'ler ile 10 kat daha iyi tespit edildiği kanıtlanmıştır. AgNP'ler altın nanopartikül ile birleştirilerek *E. coli* üzerinde denenmiştir. AgNP'ler ve altın nanopartikülleri kompleksi yüzey plazmon rezonansı ile *E. coli*'yi daha etkili bir şekilde tespit etmiştir.

9. Sonuçlar

Son on beş yıldır bitki ekstraktları ve bitki doku kültürü yöntemleri ile AgNP'lerin üretimi, stabilizasyonu ve karakterizasyonu çalışmaları aktif bir şekilde yapılmaktadır. Bitkisel tabanlı nanoteknoloji ile AgNP'lerin üretimi, toksik kimyasallar içermediğinden ve çevre dostu, ucuz bir yöntem olduğundan önemli ve ilgi çekici bir konudur. Özellikle kallus ve süspansiyon kültüründe daha fazla miktarda AgNP'nin üretilebileceği konusunda pek çok çalışma bulunmaktadır. AgNP'lerin üretimi ile ilgili yeni metodlar keşfetmek AgNP'lerin toksisitesinin en aza indirilmesi açısından önemlidir. Bununla birlikte, yeni sentez teknikleri sayesinde en basitten en olağandışı şekillere kadar farklı şekilli partiküller üretilebilmektedir. Bu, AgNP'lerin daha birçok özelliğini keşfetmek için bir avantajdır.

AgNP'ler AuNP'lere kıyasla kimyasal olarak daha az kararlı ve daha az biyo-uyumlu olmalarına rağmen, LSPR özelliklerinden dolayı biyosensör olarak daha hassastır, bu nedenle biyosensör tasarımında AgNP'lerin kullanımı yaygınlaşmıştır. Bu araştırmaların çoğu yeni-güncel konu olduğundan farklı stratejiler denenmelidir: örneğin; genel strateji LSPR bandının kayması esasına dayanırken daha fazla hassas olan RIS'ler sayesinde nanopartiküllerin biyosensör alanında uygulanmasına olan ilginin artması beklenmektedir.

Nanoteknoloji gelecekte tahmin dahi edilemeyen birçok yeni gelişmelere imkân sağlayacaktır. Biyoteknoloji ve nanomateryallerin kullanılmasıyla şaşırtıcı önemli gelişmeler yaşanacaktır. Yeni teşhis ve tedavi yöntemleri geliştirilebilecek, akıllı ilaçlar tasarlanabilecek, kanser tedavilerinde kullanım potansiyeli olan farklı metotlar belirlenebilecek ve insan ömrünün uzaması, yaşam kalitesinin artması nanoteknolojik gelişmeler ile gerçekleştirilebilecektir.

Etik kurul onayı: Bu çalışma için etik kurul onayı alınmasına gerek yoktur.

Çıkar çatışması: Yazarlar, çıkar çatışması olmadığını beyan etmiştir.

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Erratum / Düzeltme

Erratum to: Molecular Characterization and Determination of Some Biochemical Properties of Endemic *Serratula olygocephala* Growing in Adıyaman

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Son okuma sırasında Tablo 1'de ve eklenmesi gereken referanslar gözden kaçmış olup aşağıda düzeltilmiştir. Aynı zamanda Tablo 2'de PZR reaksiyon koşullarında tabloda sütunlar arasındaki kayma gözden kaçmış olup aşağıdaki şekilde düzeltilmiştir.

2.2.4. PZR sonuçlarının dizilenmesi

Örnekler, BM Laboratuarı (Ankara) gibi dizi analizi yapan ticari kuruluşlara hizmet alımı şeklinde gönderildi. Elde edilen diziler; Windows 95/98/NT/2000/XP için yazılmış olan BioEdit (Hall, 1999) biyolojik dizi sıralama editörü ile kontrol edildi. Sol (5', forward) ve sağ (3' revers) primerler ile okunan diziler eşleştirildi. National Center for Biotechnology Information (NCBI, http://www.ncbi.nlm.nih.gov) veri bankasında BLAST analizi ile hangi bitki oldukları teyit edildi ve veri bankasındaki yakın benzerlik gösterdiği diğer bitkiler kullanılarak Paup 4.0 (Swofford, 2000) ve BioEdit (Hall, 1999) programları yardımıyla filogenetik analiz yapıldı.

Tablo 1. PZR'de Kullanılan Primerler ve Özellikleri

Primer	Nükleotid Dizisi(5'-3')	Tm Değeri	Referans
ITS-4	TCCTCCGCTTATTGATATGC	52.1ºC	(White et al., 1990)
ITS-5	GGAAGGAGAAGTCGTAACAAG	55.0ºC	(Sang et al., 1995)
TrnL-F	ATTTGAACTGGTGACACGAG	55.0ºC	(Taberlet et al., 1991)
TrnL-R	GGTTCAAGTCCCTCTATCCC	55.0ºC	(Taberlet et al., 1991)

Tablo 2. PZR Reaksiyonları

Basamak	Sıcaklık	Zaman	Devir Sayısı
Ön ısıtma	94ºC	5 dak.	1 devir
1. basamak	94ºC	45 sn.	
2. basamak	50ºC	45 sn.	35 devir
3. basamak	72ºC	2 dak.	
4. basamak	72ºC	10 dak.	1 devir
5. basamak	4ºC	25 saat	

Kaynaklar

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