Volume / Cilt / 16 Issue / Sayı / 3 December /Aralık / 2023

BIOLOGICAL DIVERSITY AND CONSERVATION

It is a peer-reviewed international journal that publishes on biological diversity and conservation
Biyolojik çeşitlilik ve koruma üzerine yayın yapan hakemli uluslararası bir dergidir

Print: ISSN 1308-5301
Online: ISSN 1308-8084

http://www.biodicon.com

BIOLOGICAL DIVERSITY AND CONSERVATION
BİYOLOJİK ÇEŞİTLİLİK VE KORUMA

Biyolojik çeşitlilik ve koruma üzerine yayın yapan hakemli uluslararası bir dergidir
It is a peer-reviewed international journal that publishes on biological diversity and conservation
Volume / Cilt / 16 Issue / Sayı / 3 December/Aralık / 2023

Editor-in-Chief/ Editör*:* Prof.Dr. Ersin YÜCEL
ISSN 1308-5301 Print; ISSN 1308-8084 Online

Description

The journal "BIOLOGICAL DIVERSITY AND CONSERVATION" is an international journal that publishes in accordance with the principles of independent, impartial and double-blind peer review. The journal publishes experimental studies on all aspects of biodiversity, original articles, case reports, editorial comments and letters to the editor prepared in accordance with ethical rules. This journal accepts research in science and basic sciences, biodiversity conservation, biology, ecology, medicine, biochemistry, pharmacy and biotechnology. The editorial and publishing processes of the "BIOLOGICAL DIVERSITY AND CONSERVATION" journal are in line with the Council of Science Editors (CSE), Committee on Publication Ethics (COPE), European Association of Science Editors (EASE), International Committee of Medical Journal Editors (ICMJE) guidelines, World Association of Medical Journal Editors (WAME) and National Information Standards Organization (NISO). Complies with Journal Openness Principles and Best Practice in Scientific Publishing. Three issues are published annually; place of publication is Türkiye.

Açıklama

"BİYOLOJİK ÇEŞİTLİLİK VE KORUMA" dergisi, bağımsız, tarafsız ve çift-kör akran değerlendirme ilkelerine uygun olarak yayın yapan uluslararası bir dergidir. Dergide biyolojik çeşitliliğin tüm yönleriyle ilgili deneysel çalışmalar, orijinal makaleler, vaka raporları, editör yorumları ve etik kurallara uygun olarak hazırlanan editöre mektuplar yayınlamaktadır. Bu dergi fen ve temel bilimler, biyolojik çeşitliliğinin korunması, biyoloji, ekoloji, tıp, biyokimya, eczacılık ve biyoteknoloji üzerine yapılmış araştırmaları kabul eder. "BİYOLOJİK ÇEŞİTLİLİK VE KORUMA" dergisinin editörlük ve yayın süreçleri Council of Science Editors (CSE) doğrultusunda, Committee on Publication Ethics (COPE), European Association of Science Editors (EASE), International Committee of Medical Journal Editors (ICMJE) yönergeleri, World Association of Medical Editors (WAME) ve National Information Standards Organization (NISO) göre şekillendirilmektedir. Dergi Açıklık İlkeleri ve Bilimsel Yayıncılıkta En İyi Uygulama ile uyumludur. Yılda üç sayı yayımlanır; yayımlanma yeri Türkiye'dir.

***Abstracted-Indexed in*/Dergiyi tarayan veri tabanları**

Web of Science {Zoological Records Indexed] Clavariate Analytic, CiteFactor, JournalSeek Database, arastirmax, acarindex, Index Copernicus, IPindexing, Google Scholar, Medical Reads (RRS), CrossRef;10.46309/biodicon.

***Libraries*/Kütüphaneler**

Aberystwyth University; All libraries; Bath University; Birmingham University; Cardiff University; City University London; CONSER (Not UK Holdings); Edinburgh University; Essex University; Eskişehir Teknik Üniversitesi Library, Exeter University; EZB Electronic Journals Library; Feng Chia University Library; GAZİ Gazi University Library; Glasgow University; HEC-National Digital Library; Hull University; Imperial College London; Kaohsinug Medical University Library; ANKOS; Anadolu University Library; Lancaster University; Libros PDF; Liverpool University; London Metropolitan University; London School of Economics and Political Science; Manchester University; National Cheng Kung University Library; National ILAN University Library; Nottingham University; Open University; Oxford University; Queen Mary,University of London;Robert Gordon University; Royal Botanic Gardens, Kew; Sheffield Hallam University; Sheffield University; Shih Hsin University Library; Smithsonian Institution Libraries; Southampton University; Stirling University; Strathclyde University; Sussex University; The National Agricultural Library (NAL); The Ohio Library and Information NetWork; Trinity College Dublin; University of Washington Libraries; Vaughan Memorial Library; York University.

Web of Science-Clarivate Analytics, Zoological Record

This journal is a CrossRef;10.46309/biodicon. Member/ Bu dergi CrossRef;10.46309/biodicon. üyesidir.

*Please visit " http:// www.biodicon.com***"** *for instructions about articles and all of the details about journal/* Makale yazım kuralları ve dergi ile ilgili diğer ayrıntılar için " http:// www.biodicon.com**"** adresini ziyaret ediniz.

Publisher: ERSİN YÜCEL
***Address*:** Ersin YÜCEL, Sazova Mahallesi, Ziraat Caddesi, No.277 F Blok, 26005, Tepebaşı-Eskişehir/Türkiye
E-mail* / E-posta / :** biodicon@gmail.com ; ***Web Address: http://www.biodicon.com
https://dergipark.org.tr/en/pub/biodicon

Biological Diversity and Conservation/ **Biyolojik Çeşitlilik ve Koruma** ***Volume / Cilt / 16 Issue / Sayı / 3 December/Aralık / 2023***
ISSN 1308-5301 Print; ISSN 1308-8084 Online ---------- *Derginin Yayına Başlama Tarihi / Start Date Published 2008*

Publisher: ERSİN YÜCEL
This journal is published three numbers in a year. Printed in Eskişehir/*Türkiye /*Bu dergi yılda üç sayı yayınlanır. Yayınlanma yeri Eskişehir/Türkiye'dir.
All sorts of responsibilities of the articles published in this journal are belonging to the authors/ Dergide yayınlanan makalelerin her türlü sorumluluğu yazarlarına aittir

Biological Diversity and Conservation/* Biyolojik Çeşitlilik ve Koruma** ***Volume / Cilt / 16 Issue / Sayı / 3 December/Aralık / 2023
ISSN 1308-5301 Print; ISSN 1308-8084 Online ---------- Derginin Yayına Başlama Tarihi / Start Date Published 2008

Publisher: ERSİN YÜCEL
Bu dergi yılda üç sayı yayınlanır. Yayınlanma yeri Eskişehir/Türkiye'dir. / *This journal is published three numbers in a year. Printed in* Eskişehir/*Türkiye.*
Dergide yayınlanan makalelerin her türlü sorumluluğu yazarlarına aittir / *All sorts of responsibilities of the articles published in this journal are belonging to the authors*

Reviewer

***Executive Editor/* Genel Yayın Yönetmeni:** Ersin YÜCEL, Mine YÜCEL
***Production Editor*/Yayın Editörü**: İlkin YÜCEL ŞENGÜN
***Layount Editor/* Mizampaj Editörü:** Dilge YÜCEL
***Spelling and Language Editor*/Yazım ve Dil Editörü**: Mine YÜCEL
***Web Consultant/* Veb düzeni**; Alper YARGIÇ, Didem AYDAN, Sibel Tabanlıoğlu, Elif Çağla DELİ, Şerife AKINCI
***Technical Editor/* Teknik Editör:** Recep Sulhi ÖZKÜTÜK
***Editorial Office/* Editörlük Ofisi**; Yasemin YÜCEL, Umut KILINÇ
***Subscription Manager*/Abonelik Yöneticisi**: Yasemin YÜCEL,
***Cover Layout*/Kapak Düzeni**; Mine YÜCEL
***Secretary/* Genel Sekreter:** Faik CEYLAN
***Printing Press*/Baskı**; Cetemenler, Eskişehir/Türkiye

BIOLOGICAL DIVERSITY AND CONSERVATION (ISSN: 1308-5301; e-ISSN: 1308-8084)
Journal General Information (Year 2008-2023)

Journal name	BIOLOGICAL DIVERSITY AND CONSERVATION / BİYOLOJİK ÇEŞİTLİLİK VE KORUMA
CODEN	BIODICON
Frequency	Tri-annual
Publisher	ERSİN YÜCEL
ISSN	ISSN: 1308-5301
e-ISSN	e-ISSN: 1308-8084
Journal Barcode No.	977130853001
Starting Year	2008
Editors-in-Chief	Prof.Dr. Ersin YÜCEL
Subject	Biological Diversity, Conservation, Ecology, Environment and Human Health
Language	English and/or Turkish
Type of Access	Print and Online
Phone No.	+905364189988
e-mail	biodicon@gmail.com ; info@ersinyucel.com.tr
Journal Internet Access	http://www.biodicon.com ; https://dergipark.org.tr/en/pub/biodicon
Publisher Address	ERSİN YÜCEL, Sazova Mahallesi, Ziraat Caddesi, No.277 F Blok, Eskisehir, Türkiye, 26005
Ethical Principles	The Copyright Agreement Form Conflict of Interest Form (ICMJE Form) Reviewer Evaluation Form
Authors Declaration and sign these forms	The Copyright Agreement Form Author Contribution Form Conflict of Interest Form (ICMJE Form)
Copyright © 2008-2023	© 2023 ERSİN YÜCEL All rights reserved
Owner	ERSİN YÜCEL
Publisher	ERSİN YÜCEL
Memberships	CrossRef;10.46309/biodicon. Medical Reads (RRS) Medical Journal Editors (ICMJE) PubMed
Publishing Processes	Council of Science Editors (CSE) Committee on Publication Ethics (COPE) European Association of Science Editors (EASE) World Association of Medical Journal Editors (WAME) National Information Standards Organization (NISO)
Editorial Board, Reviewers	Postal address, ORCID ID
Ethical issues	Permissions and copyright transfer policy specified
Corresponding Author Contact Information	Postal address, Phone, Fax, E-mail, ORCID ID
Authors Contact Information	Postal address, ORCID ID
Publication Process	3-15 week
Process of Review	Double-blind review
Editorial policy of the journal	This journal is an international, scientific, online and print published in accordance with independent, unbiased, and double-blinded peer-review principles.
Indexes	Web of Science {Zoological Records Indexed] Clavariate Analytic,
Price Police	The article processing is free

www.biodicon.com BIOLOGICAL DIVERSITY AND CONSERVATION

ISSN 1308-8084 Online ISSN 1308-5301 Print

Research article/Araştırma makalesi
DOI: 10.46309/biodicon.2023.1258866
16/3 (2023) 172-179

A preliminary assessment of faunal mortality on a road through Mannur Reserve Forest, Kanchipuram, Tamil Nadu, India

Chezhian YOSEPH [*1], Prabakaran SARANGAN [2], Muzafar RIYAZ [3], Chidambaram TAMILSELVAN [4]
ORCID: 0000-0002-6202-9971**;** 0000-0002-8366-5168**;** 0000-0001-9372-681X; 0000-0002-4193-418X

[1] Jeevan Research Foundation, Chennai- 600056, Tamil Nadu, India
[2] Zoological Survey of India, MBRC, Chennai-600028, Tamil Nadu, India
[3] Xavier Research Foundation, St. Xavier's College, Palayamkottai-627002, Tamil Nadu, India
[4] Bioscience Research Foundation, Chennai-600116, Tamil Nadu, India

Abstract

Many studies have shown that anthropological acts driven by human demands have led to the extinction of numerous plant and animal species, or have put them in danger of becoming extinct. Deforestation is a serious occurrence that damages the ecosystem permanently, reduces biodiversity, and interferes with an entire ecological niche's capacity to function. Evidence on the causes and impacts of vehicle mobility in a forest reserve area is provided by the present research. Animal mortality was shown to be higher during the lockdown with traffic relaxation and somewhat lower during the lockdown without relaxation. The present study shows how unregulated automobile access into the Mannur Protected Forest results in significant faunal fatalities. The state has to be persuaded to exclusively adopt Good Environmental Practices (GEP) while constructing roads through the forest, and the appropriate authorities must keep an eye on the movement of automobiles through the remote forest zone.

Keywords: road mortality, fauna, reserve forest, kanchipuram district, tamil nadu

---------- * ----------

Mannur Rezerv Ormanı, Kanchipuram, Tamil Nadu, Hindistan'dan geçen bir yolda fauna ölümlerinin ön değerlendirmesi

Özet

Birçok araştırma, insan taleplerine dayalı antropolojik eylemlerin, çok sayıda bitki ve hayvan türünün yok olmasına veya yok olma tehlikesiyle karşı karşıya kalmasına yol açtığını göstermiştir. Ormansızlaşma, ekosisteme kalıcı olarak zarar veren, biyolojik çeşitliliği azaltan ve tüm ekolojik nişin işlev görme kapasitesine müdahale eden ciddi bir olaydır. Bir orman koruma alanındaki araç hareketliliğinin nedenleri ve etkilerine ilişkin kanıtlar bu araştırma tarafından sağlanmaktadır. Hayvan ölümlerinin, tecrit sırasında gevşeme ile daha yüksek ve gevşeme olmadan tecrit sırasında biraz daha düşük olduğu gösterildi. Bu çalışma, Mannur Koruma Altındaki Ormana düzensiz otomobil erişiminin nasıl önemli faunal ölümlere yol açtığını göstermektedir. Devlet, ormanın içinden yollar inşa ederken yalnızca İyi Çevresel Uygulamaları (GEP) benimsemeye ikna edilmeli ve ilgili makamlar, uzak orman bölgesinde otomobillerin hareketini kontrol etmelidir.

Anahtar kelimeler: karayolu ölümleri, fauna, rezerv ormanı, kanchipuram bölgesi, tamil nadu

[*] *Corresponding author* / Haberleşmeden sorumlu yazar: Tel.: +918681879975; Fax.: +918681879975; E-mail: chezhianzsi@gmail.com
 Received: 14.03.2023; Published: 15.12.2023; BioDiCon. 1086-140323

1. Introduction

One of the most complex obstacles that our planet is coping with at the present moment is the depletion of its biological diversity. Loss of biodiversity refers to the decline in the number and variety of living organisms on Earth, including plants, animals, and microorganisms. This loss is caused by a variety of factors, including habitat destruction, pollution, climate change, and over-exploitation of natural resources [1, 2]. Habitat destruction is one of the main causes of biodiversity loss, as it destroys the natural homes of many species, making it difficult for them to survive. Climate change is also a significant contributor to biodiversity loss, as it causes changes in temperature, precipitation, and sea level, which can affect the distribution of species. Additionally, pollution and over-exploitation of natural resources can damage ecosystems, making it difficult for many species to survive [3].

The loss of biodiversity has a number of negative consequences, including the loss of ecosystem services, the disruption of the delicate balance of ecosystems, and the extinction of many species. Additionally, the loss of biodiversity can also have negative effects on human well-being, as it can lead to the loss of important ecosystem services, such as pollination and pest control, and can also disrupt the delicate balance of ecosystems. Therefore, it is important to take action to protect biodiversity and to preserve the natural world for future generations [4].

It is well known that the global assessment of natural resources is not predictable. Fauna directly and indirectly plays a vital role in the ecosystem by being part of food webs and thereby influences the economic status of the country. Some examples include soil arthropods which enrich the texture and nutrition of soil in the forest ecosystem, agricultural fields and litter regions [5]. Insects serve as the backbone of any ecosystem as the entire vegetation depends on natural pollinators. Reserve forests are pockets of resources for faunal and floral diversity that help conserve and protect threatened species. Most nocturnal organisms in these forests are active during the night in search of potential mates, food, shelter and seasonal migration. All of these activities are disrupted by the noise caused by traffic, light and vibration caused by the motor vehicles that traverse the forests.

Faunal mortality on roads, also known as wildlife-vehicle collisions, is a significant problem that affects both wildlife and human populations [6, 7, 8, 9]. These collisions can result in the death or injury of both animals and people, as well as damage to vehicles and infrastructure. One of the main causes of faunal mortality on roads is habitat fragmentation. As human populations continue to expand and urban areas continue to grow, natural habitats are being destroyed and replaced with roads and other infrastructure. This fragmentation of habitats can lead to animals being forced to cross roads in order to find food, mates, or other resources. Additionally, many animals are attracted to the roadside vegetation, which can be more abundant and diverse than the surrounding habitat, further increasing the risk of collisions. Climate change also plays a role in faunal mortality on roads as it causes changes in temperature, precipitation, and sea level, which can affect the distribution of species. These changes can lead to animals moving to new areas in search of food or suitable habitats, which can increase the risk of collisions with vehicles.

The vehicular movement is a major cause of concern as it is the only reason for the increasing number of road kill in these forests. The present study aims to quantify the loss of biodiversity due to vehicular movement and road construction in the middle of Mannur Reserve Forest, Tamil Nadu. The study is significant as it highlights the negative impact of roads on wildlife and underscores the importance of conservation measures in mitigating such impacts. The study also provides valuable data that can be used to inform policy decisions and conservation strategies in the region.

2. Materials and methods

Mannur Reserve Forest (MRF) is located 39 km eastwards from the district headquarters in Kanchipuram and 13 km from Sriperumbudur and 37 km from the state Capital Chennai. Mannur is surrounded by Kadambathur block on the West, Poonamallee block towards the East, Tiruvallur block towards the North. Mannur junction is in the border of the Kanchipuram and Thiruvallur districts. Mannur is near from national highway (SH 116 - Kanchipuram - Vandavasi highway) of 8.5 km. Mannur Reserve forest is located between Thandalam road junctions (Thandalam Koot Road) to Mannur road junction (Kattu Koot Road). Mannur Reserve Forest (Figure 1) under Forest (Conservation) Act 1980, and norms is the forest area used by protected, nesting, foraging, over wintering, resting, migration, important or sensitive species of flora and fauna for breeding. MRF is a dense scrub forest with grass, herbs, scrub, bush and shrubs. Total forest area 7.38 Km2 and the road was constructed between forest of 1.6 km and width of the road is 24 ft. which is a two-way road without divider with rich vegetation along both sides of the road.

Motor cycles, Cars, Bikes, Scooters, Vans, Lorries, Buses, Heavy Duty Lorries, Trucks, Tractors and almost all type of petrol, diesel motor vehicles use this road. Vehicles move with a minimum of 40 kmph to a maximum speed of 100 kmph across this 1.68 km stretch running through MRF. Road kill data was obtained for 30 days, from mid-June to mid-July, 2020; the data includes periods before and after COVID-19 lockdown. The data was collected from 15th to 25th June, due to containment zones that were in district territories in Chennai, Kanchipuram, Chengalpattu and Thiruvallur districts by Tamil Nadu Government.

After 26th June 2020, the lockdown was further extended with some relaxations till 31st August, 2020. The roads were systematically surveyed in two sessions i.e. morning (6:00 am to 7:00 am) and evening (5:00 pm to 6:00 pm) throughout the study period. Line Transect sampling method (Karanth & Nichols, 2017) was followed in the present study

and data was collected on both sides of the road and dead samples/species were observed, number of mortality was recorded and photograph were taken for identification. Pictorial handbooks and literatures were consulted to identify the species [10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21]. Overall percentage of mortality rate was calculated and reported using statistical tool/software.

Figure 1. A map of the study area and road through the Mannur Reserve Forest (Google Maps)

3. Results

Mannur Reserve Forest, characterized by its dense vegetation, hosts a diverse array of plant life, encompassing an abundance of herbs and shrubs. Within this lush environment, the "Gloriosa superba" plant, commonly referred to as the "flame lily" or the "glory lily," thrives in abundance. Notably, this plant holds significance as the state flower of Tamil Nadu and the national flower of Zimbabwe. The impact of vehicular encounters on the resident wildlife within the reserve forest is evident, as illustrated in Figure 2. Animals across various groups experienced direct collisions with vehicles while traversing roads within the forest, resulting in the loss of vital organs and, consequently, a substantial number of fatalities.

Table 1 provides a comprehensive overview of the common names, phyla, classes, and orders of species documented throughout the study. Among the diverse animal groups affected by mortality are Clitellata, Mollusca, Orthoptera, Blattodea, Lepidoptera, Odonata, Hymenoptera, Diptera, Coleoptera, Arachnida, Chilopoda, Diplopoda, Amphibia, Reptilia, Carnivora, Aves, and Mammalia. A total of 55 species, spanning various taxa, were identified and are enumerated below. The documentation of species mortality during the lockdown, both with and without relaxation, is detailed in Table 1. The distribution of mortality percentages during the lockdown without relaxation is visually represented in the pie diagrams (Figure 3, 4).

Table 1. List of species observed in the road mortality with thier common names and taxonomic position)

S. No.	Species Name	Common Name	
1.	*Eisenia fetida* (Savigny, 1826)	Earthworm	Mollusca
2.	*Achatina fulica* Bowdich, 1822	Giant African land snail	
3.	*Laevicaulis alte* (Férussac, 1822)	Leather-leaf slug	
4.	*Poekilocerus pictus* (Fabricius,1775)	Grasshopper	Arthropoda
5.	*Oxya hyla hyla* Serville, 1831		
6.	*Xylocopa violacea* (Linnaeus, 1758)	Violet Carpenter Bee	

Table 1. Continued

7.	*Chrysocoris stollii* (Wolff, 1801)	Beetles	
8.	*Sternocera chrysis* (Fabricius, 1775)		
9.	*Copris (Paracopris) cribratus* Gillet, 1927		
10.	*Onthophagus amphinasus* Arrow, 1931		
11.	*Hasora Chromus* (Cramer, 1782)	Butterflies	
12.	*Eurema brigitta* (Cramer, 1780)		
13.	*Hebomoia glaucippe* (Linnaeus, 1758)		
14.	*Danaus chrysippus* (Linnaeus, 1758)		
15.	*Junonia almanac* (Linnaeus, 1758)		
16.	*Junonia lemonias* (Linnaeus, 1758)		
17.	*Tirumala limniace* (Cramer, 1775)		
18.	*Euploea core* (Cramer, 1780)		
19.	*Pachiliopta aristolochiae* (Linnaeus, 1775)		
20.	*Pachliopta hector* (Linnaeus, 1758)		
21.	*Mycalesis subdita* (Moore, 1892)		
22.	*Papilio demoleus* Linnaeus, 1758		
23.	*Diplacodes trivialis* (Rambur, 1842)	Dragonfly	
24.	*Orthetrum sabina* (Drury, 1770)		
25.	*Trithemis pallidinervis* (Kirby, 1889)		
26.	*Apis mellifera* Linnaeus, 1758	Honey bees	
27.	*Apis cerana indica* (Fabricius, 1798)		
28.	*Musca domestica* Linnaeus, 1758	Housefly	
29.	*Periplaneta americana* (Linnaeus, 1758)	Cockroaches	
30.	*Periplaneta australasiae* (Fabricius, 1775)		
31.	*Blattella germanica* Linnaeus, 1767		
32.	*Therea petiveriana* (Linnaeus, 1758)		
33.	*Blatta orientalis* Linnaeus, 1758		
34.	*Rhysida nuda nuda* (Newport, 1845)	Centipedes	
35.	*Cormocephalus pygmaeus* Pocock, 1892		
36.	*Xenobolus carnifex* (Fabricius, 1775)	Millipede	
37.	*Anoplodesmus saussurii* (Humbert, 1865)		
38.	*Trigoniulus corallinus* (Gervais, 1847)		
39.	*Heterometrus flavimanus* (Pocock, 1900)	Scorpion	
40.	*Heterometrus madraspatensis* (Pocock 1900)		
41.	*Daboia russelii* (Shaw & Nodder, 1797)	Snakes	Reptilia
42.	*Bungarus caeruleus* (Schneider, 1801)		
43.	*Coluber naja* Linnaeus, 1758		
44.	*Enhydris enhydris* (Schneider, 1799)		

Table 1. Continued

45.	*Ptyas mucosa* (Linnaeus, 1758)		
46.	*Calotes versicolor* (Daudin, 1802)	Garden lizard	
47.	*Melanochelys trijuga* (Schweigger, 1812)	Indian black turtle	
48.	*Duttaphrynus melanostictus* (Schneider, 1799)	Frog	Amphibia
49.	*Duttaphrynus microtympanum* (Boulenger, 1882)		
50.	*Corvus splendens* Vieillot, 1817	House crow	Aves
51.	*Athene cunicularia* (Molina, 1782)	Burrowing owl	
52.	*Herpestes javanicus* (É. Geoffroy Saint-Hilaire, 1818)	Mongoose (small)	Mammalia
53.	*Funambulus palmarum* (Linnaeus, 1766)	Indian squirrel	
54.	*Bandicota bengalensis* Gray, 1835	Rat - Mammal	
55.	*Golunda ellioti* Gray, 1837		

Figure 2. Mortality of Higher taxa on Mannur RF road.
4A. *Funambulus palmarum* (Linnaeus, 1766), 4B. *Daboia russelii* (Shaw & Nodder, 1797), 4C. *Melanochelys trijuga* (Schweigger, 1812), 4D. *Athene cunicularia* (Molina, 1782), 4E. *Calotes versicolor* (Daudin, 1802), 4F. *Ptyas mucosa* (Linnaeus, 1758)

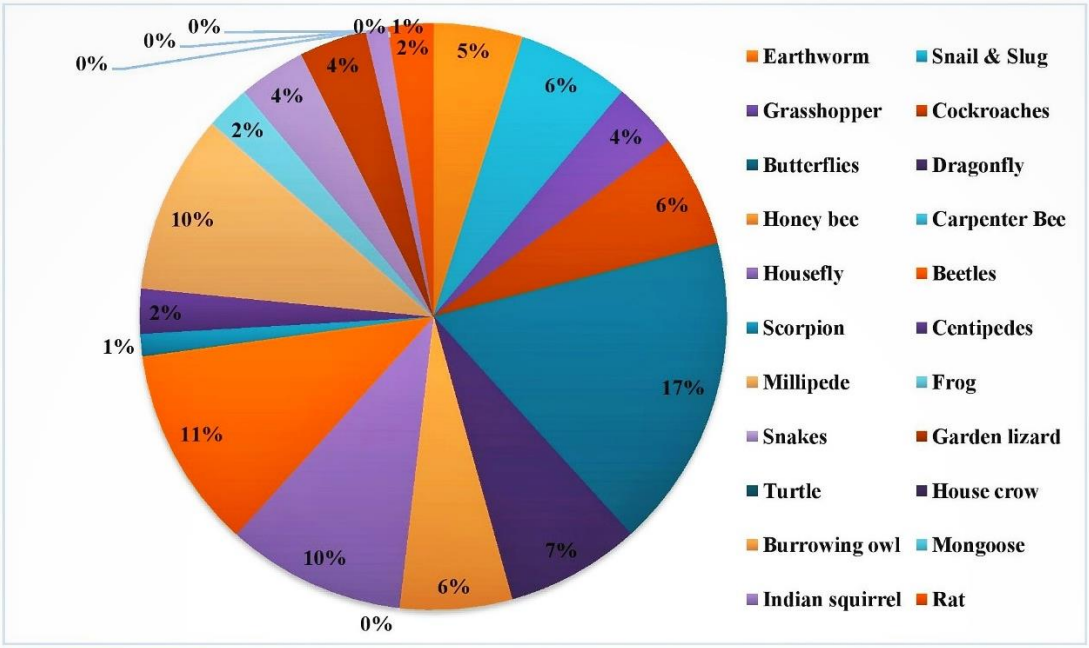


Figure 3. Pie diagram showing the percentage of casualties during Lockdown without traffic relaxation

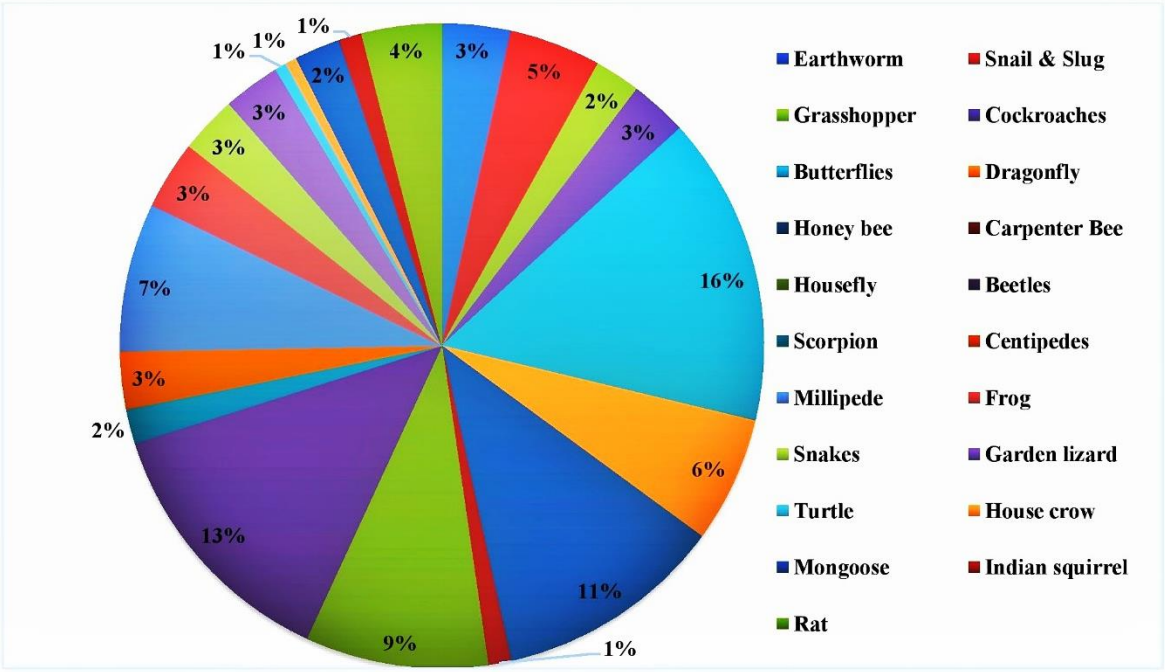


Figure 4. Pie diagram showing the percentage of casualties during Lockdown with traffic relaxation

4. Conclusions and discussion

Various factors have led to the loss of biodiversity in the reserved forest, the most significant being the laying of new roads, widening and repairing of the existing roads. This leads to unnecessary traffic wherein animals met with accidents and thus resulting in a severe diversity imbalance due to adverse decrease in population. Due to the unmonitored automobile activities which are steadily on the rise, there is loss of endangered/threatened species, endemic species, protected species, and a drastic drop in the reproductive capacity because of loss of mates and an increase in pollution of air, water and noise.

The study was undertaken to assess the extent of faunal mortality on a 7-kilometer stretch of a road that cuts through the forest. We surveyed the road twice a month for one month and recorded the number and species of animals

that were killed by vehicles. We also collected data on the traffic density and speed of vehicles on the road. The results of the study showed that a total of 55 animal species, including mammals, birds, and reptiles, were killed during the survey period. The study also found that the highest number of fatalities occurred during the traffic relaxation, which corresponded to peak traffic hours.

In order to prevent such adverse effects from happening, it is mandatory to implement the Good Environmental Practices (EPI) as it will help in minimizing the direct impacts of faunal diversity as a result of road construction project. Incorporation of biodiversity conservation into the design and implementation of road projects is also a necessary strategy for environmentally sustainable development.

Results show that mortality is higher (182 casualties) during the lockdown period with traffic relaxation and comparatively less (82 casualties) during lockdown period without relaxation. Thus, the passage of vehicles within the secured forest area needs to be controlled by the traffic rules and regulations and Good Environmental Practices (EPI) has to be followed exclusively to develop roads for modern civilization.

In order to address faunal mortality on roads, a number of different strategies can be employed. These include wildlife crossings, such as underpasses and overpasses, which can provide safe passage for animals across busy roads. Additionally, wildlife warning signs and speed limits can be used to alert drivers to areas where collisions are more likely to occur. Habitat restoration and conservation efforts can also help to reduce the risk of collisions by providing animals with more suitable habitats and resources.

In conclusion, faunal mortality on roads is a significant problem that affects both wildlife and human populations. It is caused by a variety of factors, including habitat fragmentation, climate change, and human activity. To address this problem, a number of strategies can be employed, including wildlife crossings, warning signs, speed limits, and habitat restoration and conservation efforts. By taking action to protect wildlife and reduce the risk of collisions, we can help to preserve the natural world for future generations. The study underscores the need for conservation measures to mitigate the impact of roads on wildlife and provides valuable data that can be used to inform policy decisions and conservation strategies.

Acknowledgements

The author wishes to thank Jeevan Research Foundation, Chennai- 600056, Tamil Nadu, India for exteneded support.

Funding Acknowledgement

The present study received no specific grant from any funding agency.

References

[1] Wilcove, D. S., Giam, X., Edwards, D. P., Fisher, B., & Koh, L. P. (2013). Navjot's nightmare revisited: Logging, agriculture, and biodiversity in Southeast Asia. *Trends in Ecology and Evolution*, *28*(9), 531–540. https://doi.org/10.1016/j.tree.2013.04.005

[2] Riyaz, M., Shah, R. A., & Packiam, S. M. (2022). Insect conservation and management: A need of the hour. In H. A. F. El-Shafie (Ed.), *Global decline of insects*. IntechOpen. https://doi.org/10.5772/intechopen.100023

[3] Cardinale, B. J., Duffy, J. E., Gonzalez, A., Hooper, D. U., Perrings, C., Venail, P., Narwani, A., Mace, G. M., Tilman, D., Wardle, D. A., Kinzig, A. P., Daily, G. C., Loreau, M., Grace, J. B., Larigauderie, A., Srivastava, D. S., & Naeem, S. (2012). Biodiversity loss and its impact on humanity. *Nature*, *486*(7401), 59–67. https://doi.org/10.1038/nature11148

[4] Laurance, W. F., Sayer, J., & Cassman, K. G. (2014). Agricultural expansion and its impacts on tropical nature. *Trends in Ecology and Evolution*, *29*(2), 107–116. https://doi.org/10.1016/j.tree.2013.12.001

[5] Culliney, T. (2013). Role of arthropods in maintaining soil fertility. *Agriculture*, *3*(4), 629–659. https://doi.org/10.3390/agriculture3040629

[6] Forman, R. T. T., & Alexander, L. E. (1998). Roads and their major ecological effects. *Annual Review of Ecology and Systematics*, *29*(1), 207–231. https://doi.org/10.1146/annurev.ecolsys.29.1.207

[7] Fahrig, L., Rytwinski, T., & Desrochers, A. (2011). Effects of roads on animal abundance: An empirical review and synthesis. *Ecology and Society*, *16*(1), 21.

[8] Erritzoe, J., Mazgajski, T. D., & Rejt, Ł. (2003). Bird casualties on European roads—A review. *Acta Ornithologica*, *38*(2), 77–93. https://doi.org/10.3161/068.038.0204

[9] Santos, S. M., Lourenço, A., Mira, A., & Beja, P. (2011). Patterns of road mortality and mitigation measures in a highly fragmented landscape in Mediterranean Europe. *Environmental Management*, *48*(4), 834–847.

[10] Aswathi, K., & Sureshan, P. M. (2017). Additions to the scorpion fauna (Arachnida: Scorpiones) of Kerala, India, with an illustrated key to the genera. *Journal of Threatened Taxa*, *9*(2), 9844–9850. https://doi.org/10.11609/jott.2039.9.2.9844-9850

[11] Bhowmik, H. K., Saha, B. C., & Bhargava, R. (1990). Contribution to the Acridid fauna (Orthoptera) of north-eastern states of India. *Records of the Zoological Survey of India*, *86*(2), 217–227. https://doi.org/10.26515/rzsi/v86/i2/1990/161655

[12] Chezhian, Y., & Prabakaran, S. (2016). Diversity of millipedes (Myriapoda: Diplopoda) in Yelagiri hills, Eastern Ghats, Vellore district, Tamil Nadu. *International Journal of Fauna and Biological Studies*, *3*(2), 91–97.

[13] Daniel, J. A. (2017). Commonly found avifauna of Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India. *Journal of Entomology and Zoology Studies*, *5*(4), 46–52.

[14] Das, I., & Bhupathy, S. (2009). Melanochelys trijuga (Schweigger 1812). Indian black turtle. In Chelonian Research Monographs A. G. J. *Conservation biology of Freshwater turtles and tortoises: A Compilation Project of the IUCN/SSC Tortoise and Freshwater Turtle Specialist Group* Rhodin et al. (Eds.), *5* (pp. 038.1–038.9).

[15] Fabricius. (1775). Systema entomologiae, sistens insectorum classes, ordines, genera, species, adiectis synonymis, locis, descriptionibus, observationibus, *289*.

[16] Prabakaran, S., Chezhian, Y., Ezhumalai, P., & Ramesh, G. (2015). Diversity of cockroaches in Yelagiri hills part of Eastern Ghats of Tamil Nadu, India. *International Journal of Fauna and Biological Studies*, *2*(3), 30–35.

[17] Subramanian, K. A., Dinesh, K. P., & Radhakrishnan, C. (2013). Atlas of endemic amphibians of Western Ghats: 1–246. Published by the Director. *Zoological Survey of India*. Kolkata, India.

[18] Pratihar, S., Patra, B., Samarasinghe, S. A., & Dinal, J. S. (2014). Duttaphrynus in India, Bangladesh and Sri Lanka. *Indian Forester*, *140*, 1035–1037.

[19] Surender, G., Kumar, R. S., Swamy, K., Naresh, B., & Vasudeva, R. V. (2015). Partial albinism in house crow (Corvus splendens) at Baswapur village of Nizamabad District, Telangana. *International Journal of Advances in Case Reports*, *2*(1), 28–31.

[20] Mukherjee, S., Banerjee, S., Saha, G. K., Basu, P., & Aditya, G. (2015). Butterfly diversity in Kolkata, India: An appraisal for conservation management. *Journal of Asia-Pacific Biodiversity*, *8*(3), 210–221. https://doi.org/10.1016/j.japb.2015.08.001

[21] Ammanna, V. H. F. (2013). *Calotes versicolor* (Asian garden lizard). *Herpetological Bulletin*, *126.*

www.biodicon.com BIOLOGICAL DIVERSITY AND CONSERVATION

ISSN 1308-8084 Online ISSN 1308-5301 Print

Research article/Araştırma makalesi
DOI: 10.46309/biodicon.2023.1212529
16/3 (2023) 180-191

Assessment of some shrubs plant seeds in terms of mineral contents for feed purposes

Celalettin AYGÜN [*1], Murat OLGUN[2], Metin TURAN[3]
ORCID: 0000-0002-1308-7796**;** 0000-0001-6981-4545**;** 0000-0002-4849-7680

[1] Geçit Kuşağı Tarımsal Araştırma Enstitüsü Müdürlüğü- 26100 Eskişehir, Türkiye
[2] Eskişehir Osmangazi Üniversitesi, Ziraat Fakültesi- 26040 Eskişehir, Türkiye
[3] Yeditepe Üniversitesi, Uygulamalı Bilimler Yüksek Okulu, İstanbul, Türkiye

Abstract

Seeds of 51 bush species, which are plants that can be an alternative feed source, were evaluated in terms of macro and micro elements. In terms of macro and micro elements, the relations, similarities and differences of the species with each other have been revealed. As a result of the research, N, K, P, Ca, Mg and Fe were determined as remarkable minerals in bush seeds. From shrub plants; Deeds of *Clematis orientalis* L., *Cotoneaster integerrimus* Medik., *Crataegus monogyna* Jacq., *Crataegus rotundifolia* Moench., *Daphne oleoides* Schreb., *Ephedra major* Host., *Lonicera caucasica* Pall., *Sambucus ebulus* L., *Smilax excelsa* L., *Sorbus torminalis* (L.) Crantz., L., *Ziziphus vulg*aris Lam., were determined as possible seeds to be used as animal feed.

Key words: shrub, seed, mineral, content

---------- * ----------

Bazı çalımsı bitki tohumlarının yem amaçlı mineral içerikleri açısından değerlendirilmesi

Özet

Alternatif yem kaynağı olabilecek bitkilerden çalıların yaprak, sap-sürgün gibi kısımlarının yanında meyve ve tohumlarının kullanılması amacıyla 51 adet bitki tohumları değerlendirilmiştir. Makro ve mikro elementler açısından tohumların içerikleri, birbirleri ile ilişkileri ve benzerlikleri ve farklılıkları ortaya konulmuştur. Neticede; N, K, P, Ca, Mg ve Fe çalı tohumlarında dikkate değer mineraller olarak belirlenmiştir. Çalı bitkilerinden; *Clematis orientalis* L., *Cotoneaster integerrimus* Medik., *Crataegus monogyna* Jacq., *Crataegus rotundifolia* Moench., *Daphne oleoides* Schreb., *Ephedra major* Host., *Lonicera caucasica* Pall., *Sambucus ebulus* L., *Smilax excelsa* L., *Sambucus nigra* L., *Sorbus torminalis* (L.) Crantz., *Viscum album* L., *Ziziphus vulgaris* Lam., ın tohumlarının hayvan yemi olarak kullanılması muhtemel tohumlar olarak belirlenmiştir.

Anahtar kelimeler: çalı, tohum, mineral, içerik

1. Giriş

Ülkemizin fiziki olarak engebeli bir yapıya sahip olmasından dolayı farklı iklim ve bitki örtüsü çeşitliliğine sahiptir. Bu olumsuz yapının hayvancılık açısından olumlu etkilerinden birisi de uygun mera ve yaylak-kışlakların yer almasıdır. Dünyanın kurak ve yarı kurak bir çok mera alanları verimsiz hale gelmiş meralarda bulunan kaliteli türlerin yerini lezzetsiz istilacı türler almıştır. Yapılan bir çok çalışmada meraların verimlilik ve mera sağlığı bakımından riskli ve sorunlu olduğu ortaya konulmuştur [20]. Dünya'da kurak ve yarı kurak bölgelerde bulunan mera alanlarında genelde

* *Corresponding author* / Haberleşmeden sorumlu yazar: Tel.: +902223240300; Fax.: +90223240301; E-mail: caygune25@hotmail.com
 Geliş tarihi: 30.12.2022; Yayın tarihi: 15.12.2023 BioDiCon. 1076-301222

bitkiyle kaplılık oranının yaklaşık olarak % 50-80 arasında olup, kaliteli azalıcı bitkiler oranının yaklaşık % 5-20 arasında değişmesine rağmen, istilacı bitkilerin % 70-90 arasında değiştiği ve mera kalitesinin istilacı türlerden dolayı bozulduğu ortaya konmuştur. Dünyada değişik stres faktörlerinden dolayı zayıflayan meraların iyileştirilmesi ve besleme kalitesinin arttırılması çalışmaları yoğun olarak devam etmesi sorunu çözmekten uzaktır. Bu konuda bir çok çalışma yapılmasına rağmen mera kalitesi ve verimliliği dünyada gittikçe düşmektedir [12, 23].

Meraların verimliliğinin arttırılması ve kalitesinin iyileştirilmesinde çalımsı bitkiler önemli bir potansiyel teşkil etmekte olup, bu alanlar için çalımsı bitkiler kullanılarak gerek ıslah, gerek kurak zamanlarda sağlanan yem katkıları ile önemli bir ıslah materyali oldukları aşikârdır. Çalımsı bitkilerin yaprak, sap-sürgün gibi kısımları özellikle küçükbaş hayvanlar için yem kaynağı oluştururken, bu bitkilerin tohumları da meşe palamudunda olduğu gibi yem değeri açısından samandan daha iyi durumda olan palamudun doğal üretimin bol olduğu yerlerde yem olarak kullanılabildiği bildirilmiştir [30]. Çalımsı bitkiler gerek yaprakları, gerek sap-sürgünleri ve gerekse tohumları ile tüketilebilen ek yem kaynakları olup, mera alanlarına kullanımlarının artmasıyla meraların verim ve kalitesinin arttırılmasında önemli bir potansiyel bitki guruplarıdır. Özelllikle çalımsı bitkilerin tohumları içeridikleri lezzetli biyokimyasal ve mineral maddeler vasıtasıyla hayvan beslenmesinde önemli role sahip olup, içerdikleri mineraller hayvansal verim ve kaliteye önemli katkı sağlamaktadırlar [8, 32]. Çalımsı bitkilerle ilgili dünyada bir çok çalışma yapılmasına rağmen bu çalışmalar sadece bitki tanımlaması ve besin içeriklerinin tek bitkide belirlenmesi bağlamında yürütülmüştür [29, 16, 2]. Dünyada bu bitkilerle ilgili ıslah çalışmaları oldukça yeni ve yetersiz olup çalımsı bitkilerin meralarda kullanılabilirliklerinin belirlenmesi, yüksek verimli ve kaliteli çeşit geliştirme çalışmalarına ihtiyaç vardır. Islah çalışmalarında, merada kullanılabilecek çeşit geliştirme amacıyla yapılan çalışmaların yanı sıra bu çalıların biyokimyasal ve mineral madde içeriklerinin belirlenmesi ıslah çalışmalarının başarısını arttıracaktır [11, 15, 13, 24, 3, 26]. Mineraller (N, P, K, Ca, Mg, S, Na, Fe, Cu, Mn, Zn, B) bitki gelişmesinde önemli bir yer tutmakta olup, bitkilerin yüksek verim ve kalite gibi önemli unsurlarını ortaya çıkaran, protein karbonhidrat ve yağ gibi organik bileşiklerin oluşumu, bitkinin genetik kapasite ve çevreyle olan interaksiyonunda hayati önem arz eden organik bileşikler, amino asitler gibi bileşiklerin biyokimsayal aktivitesinin ortaya konulmasında önemli yer tutarlar [24, 3]. Minerallerin, çalımsı bitkilerin değişik kısımlarındaki içeriklerinin belirlenmesi çalımsı birtkilerle yürütülen ıslah çalışmasında kullanılan bitkilerin kalitesinin ortaya konmasında önemli rol oynayacaktır. Özellikle çalımsı bitkilerin tohumlarının içerdikleri mineral maddelerin belirlenmesi tohumların kullanım ve değerlendirilme potansiyelinin arttıracaktır [30, 13].

Çalılarla ilgili yürütülen çalışmalarda başarı şansını arttırmak amacıyla yapılan değerlendirmelerde değişik istatistik metotları kullanıabileceği gibi bu metotların birlikte değerlendirilmesi başarı derecesini ve seleksiyon başarısını arttıracaktır. Bu amaçla korelasyon, faktör, biplot, hiyerarşik clustering ve Conditional formatting analiz yöntemleri, yürütülen ıslah çalışmalarında başarıyla kullanılan yöntemlerdir [28, 14]. Bu çalışmada değişik analiz metotları (korelasyon, faktör, biplot, hiyerarşik clustering ve Conditional formatting) kullanılarak bazı çalımsı bitki tohumları içerikleri mineral maddeler yönünden benzerlikleri/farklılıkları ve mineral madde yönünden performansları ortaya konmuştur.

2. Materyal ve yöntem

Deneme alanına ait toprak özelliklerini gösterir analiz sonucundanda anlaşıldığı gibi toprakta organik madde miktarı 0-20-20 40 cm derinlikte % 1.53-1.17 arası değişmiştir. Aynı şekilde yarayışlı fosfor ve potasyum miktarları 0-20 cm de 10.04 ve 7.17 kg/da, 82.95 ve 60.30 kg/da arası değişmiştir. Dikkat edilir ise organik madde, fosfor ve potasyum mktarları derinlik arttıkça azalmış ve dolayısıyla bitkiye en yarayışlı toprak derinliği 0-20 cm olarak ortaya konmuştur. Diğer taraftan araştırmada çalışılan tohumların elde edildiği bitkilerin listesi Tablo 1' de verilmiştir. Bitkiler Geçit Kuşağı Tarımsal Araştırma Enstitüsü Müdürlüğü Çalımsı Bitkiler Plantasyonu ve Suriye'den (ICARDA) temin edilmiştir. Bitkilerin olgunlaşma dönemleri birbirlerinden farklılık gösterdiği için tohumlar sonbaharda elde edilmiştir.

Tablo 1. Çalışılan bitkilerin listesi

Sıra No	Cins /Tür	Sıra No	Cins /Tür
1	*Acantholimon acerosum* (Willd.) Boiss.	**27**	*Elaeagnus angustifolia* L.
2	*Acer campestre* L.	**28**	*Ephedra major* Host.
3	*Alhagi pseudalhagi* (M. Bieb.) Fisch.	**29**	*Euonymus europaeus* L.
4	*Atriplex canescens* (Pursh) Nutt.	**30**	*Globularia trichosantha* Fisch. & C.A.Mey.
5	*Atriplex halimus* L.	**31**	*Gonocytisus angulatus* (L.) Spach.
6	*Atriplex hortensis* L.	**32**	*Jasminum fruticans* L.
7	*Atriplex lentiformis* (Torr.) S.Watson.	**33**	*Lonicera caucasica* Pall.
8	*Atriplex leucoclada* Boiss.	**34**	*Mahonia aquifolium* (Pursh) Nutt.
9	*Atriplex nummularia* Lindl.	**35**	*Malus floribunda* Siebold ex Van Houtte.
10	*Atriplex polycarpa* (Torr.) S.Watson.	**36**	*Paliurus spina-christi* Mill.
11	*Atriplex undulata* (Moq.) D.Dietr.	**37**	*Phillyrea latifolia* L.

Tablo 1. Devam ediyor

12	*Berberis vulgaris* L.	**38**	*Pyracantha coccinea* M. Roem.
13	*Buxus sempervirens* L.	**39**	*Rosa pulverulenta* M. Bieb.
14	*Cephalaria media* Litv.	**40**	*Salsola Vermiculata* L.
15	*Cistus creticus* L.	**41**	*Sambucus ebulus* L.
16	*Cistus laurifolius var. atlanticus* Pit.	**42**	*Smilax excelsa* L.
17	*Clematis orientalis* L.	**43**	*Sambucus nigra* L.
18	*Clematis vitalba* L.	**44**	*Sorbus torminalis* (L.) Crantz.
19	*Colutea cilicica* Boiss. & Balansa.	**45**	*Rhamnus frangula* L.
20	*Cotoneaster integerrimus* Medik.	**46**	*Rhus coriaria* L.
21	*Cotoneaster horizontalis* Decne.	**47**	*Rosa canina* L.
22	*Cotoneaster lacteus* W.W. Sm.	**48**	*Rubus caesius* L.
23	*Crataegus marginatus* Jacq.	**49**	*Viscum album* L.
24	*Crataegus monogyna* Jacq.	**50**	*Vitex agnus-castus* L.
25	*Crataegus rotundifolia* Moench.	**51**	*Ziziphus vulgaris* Lam.
26	*Daphne oleoides* Schreb.		

Tohumlarda mineral madde analizleri (N, P, K, Ca, Mg, S, Na, Fe, Cu, Mn, Zn, B) Endüktif olarak eşleştirilmiş plazma spektrometresi (Perkin-Elmer, Optima 2100 DV, ICP/OES, Shelton, CT) kullanarak 06484-4794, ABD [22]'e göre yapılmıştır.

Denemede kullanılan korelasyon [21, 28], faktör [28, 19] biplot analizi [35] ise GenStat 14th paket programı, AGGL. HİE JULSPEr analizi, conditional Formating analizleri Excel, Minitab, Statistica paket programları kullanılarak yapılmıştır [28].

3. Bulgular

Deneme de kullanılan çalı tohumlarının minerallerinin birbirleri ile olan ilişkilerini gösteren koralasyon anlizi Tablo 2'de verilmiştir.

Tablo 2. Çalı tohumlarının minerallerinin birbirleri ile olan ilişkilerini gösteren koralasyon tablosu

	N	**P**	**K**	**Ca**	**Mg**	**S**	**Na**	**Fe**	**Cu**
	2	**5**	**6**	**5**	**5**	**2**	**3**	**4**	**2**
P	0,112**öd**								
K	-0,003**öd**	0,182**öd**							
Ca	-0,098**öd**	-0,535****	-0,005**öd**						
Mg	-0,178**öd**	0,258*	0,591****	0,018**öd**					
S	0,759****	0,112**öd**	-0,003**öd**	-0,098**öd**	-0,178**öd**				
Na	0,090**öd**	0,602****	0,540****	-0,417****	0,245**öd**	0,090**öd**			
Fe	-0,188**öd**	0,531****	0,526*	-0,307*	0,551****	-0,188**öd**	0,553****		
Cu	-0,010**öd**	-0,036**öd**	0,547****	0,189**öd**	0,204**öd**	-0,010**öd**	0,120**öd**	-0,097**öd**	
Mn	-0,186**öd**	-0,278*	0,022**öd**	0,447****	0,462****	-0,186**öd**	-0,328*	0,135**öd**	-0,166**öd**
Zn	-0,298*	-0,156**öd**	0,496****	0,337*	0,443****	-0,298*	-0,048**öd**	0,104**öd**	0,332*
B	-0,010**öd**	-0,036**öd**	0,547****	0,189**öd**	0,204**öd**	-0,010**öd**	0,120**öd**	-0,097**öd**	0,523****
	Mn	**Zn**							
Zn	0,326*								
B	-0,166**öd**	0,332*							

öd: önemli değil, *: P ≤ 0.05, **: P ≤ 0.01.

Tablo 2'de verilen korelasyon ilişkileri incelendiğinde; N ile S arasında, P ile Na, K ila Na, K ile Cu, K ile Zn ve B arasında, Ca ile Mn arasında, Mg ile Fe, Mg ile Mn ve Zn arasında, Na ile Fe arasında, Cu ile B arasında olumlu ve çok önemli (P< 0.01) ilişki belirlenirken, N ile Zn arasında, K ile Fe arasında, Ca ile Zn arasında, Mg ile Zn arasında, Cu ile Zn arasında, N ile Zn arasında, P ile b arasında olumlu ve % 5 düzeyinde önemli ilişki belirlenmiştir. Ayrıca, mineraller arasında P ile Ca arasında, Ca ile Na arasında olumsuz ve (P< 0.01) düzeyinde önemli ilişki, P ile Mn arasında, N ile Zn arasında olumsuz ve önemli (P< 0.05) düzeyinde önemli ilişki belirlenmiştir.

Mineralller her ne kadar analizlerde olumlu ve olumsuz, önemli ve önemsiz ilişki gösterlerse de minerallerin bitkisel ve hayvansal metabolizmada bütünleşik etkiye sahiptirler, yani herhangi bir mineral eksikliğinde metabolik, büyüme ve gelişme faaliyetleri sekteye uğrar, durma noktasına gelir dolyısıyla bu ekiden dolayı bitki ve hayvan metabolizmasında belli düzeyde bulunması lazımdır. Vitamin sentezi, hormon üretimi, enzim aktivitesi, kolajen oluşumu, doku sentezi, oksijen taşınması, enerji üretimi ve büyüme, üreme ve sağlıkla ilgili diğer fizyolojik süreçler için iz mineralleri gereklidir. Bir veya birkaç elementin eksik ya da fazla alınması normal işlevleri bozduğu gibi elementler arasındaki oranların bozulması da organizmada fizyolojik değişikliklere neden olabildiği vurgulanmıştır [25, 1].

Minerallerin proteinlerin, karbonhidratların, yağların temel bileşenleri olduğunu unutmamak gerekir. Çalışmamızda da sinerjistik/antagonistik etkiye sahip minerallerin birbiri ile negative ve pozitif önemlilikleri bu durumu

destekler niteliktedir. Çalılar mera verimliliğinin ve kalitesinin arttırılmasında, erozyonun önlenmesinde ve toprağın fiziko kimyasal yapılarının iyileştirilmesinde önemli rol oynayan ve gelecektede oynayacak olan önemli bitkilerdir. Çalılar sap yaprak gövde ve kök sürgünleri ve tohumları ile hayvancılık için çok önemli bir yem kaynağı olup önem arz etmektedir. Özellikle hayvanlar tatafından severek yenilen lezzetli çalılar mera ortamının kalitesinin artmasında oldukça etkilidir [15, 3, 26]. Çalıların hayvanlar tatarfından tercih edilebilirliğine kalitesinin artmasına çalının gelişim ve büyümesinde, biyotik ve abiyotik streslere dayanımında mineraller önemli rol oynamaktdır. Mineraller ayrıca hayvanların beslenmesinde sağlıklı olmasında ve yeterli verim vermesinde yüksek kaliteli ve verimli bitki meydana getirilmesine önemli rol oynamaktdır. Mineraller bitki bünyesinde belirli oranda bulunmakla hayvanların ihtiyacının karşılanmasında temel teşkil ederler.

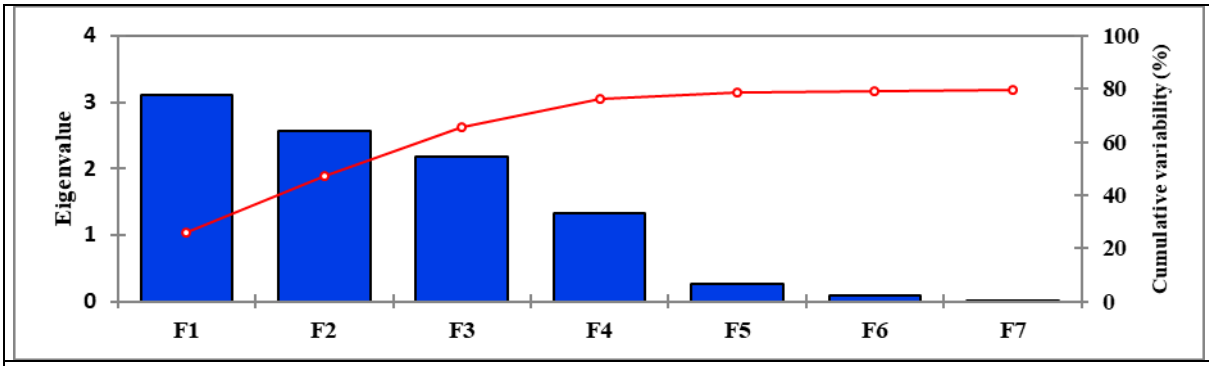
Makro ve mikro elementlerin bitkilerin büyümesinde önemli rolleri olduğu gibi, bitkilerde yeterli oranlarda bulunarak hayvanlar tarafından alınmasının da o kadar önemli olduğunu, çalımsı bitkilerin kurak sezonda özellikle küçükbaş hayvanların beslenmesine katkılarının izahı ve kullanımının yaygınlaştırılması amacıyla bazı çalı bitkilerinin sezonluk (ilkbahar, yaz, sonbahar) yaprak örneklerindeki makro ve mikro besin elementi içerikleri hususunda yaptıkları çalışma neticesinde incelenen çalı türlerinin besin içeriklerinin sezonlara göre değişmekle birlikte bazı türlerde, bitkilerde bulunması gereken sınır değerlerin üstünde, bazı türlerde ise nispeten düşük olduğu belirlenmiştir. Ancak; mevsimsel olarak düşünüldüğünde hayvanların besin ihtiyaçlarının kurak sezonda karşılanması hususunda ilave yemler olarak kullanılabileceklerini belirtmişlerdir [5].

Patlangaç çalısının büyüme mevsimi boyunca yeşil kalabildiğini bitkide yaprak ve sürgünler yaz sonundan itibaren küçükbaş hayvanlar tarafından otlandığını, ilkbahardan sonbahara doğru yaprak ve sürgün üretimi azalan çalıda mevsime göre yaprak ve sürgünde ham protein oranı %10-20 arasında değiştiğini, yaprak ve sürgünlerde tanen içeriğinin gelişme mevsiminin ilerlemesine bağlı olarak düşüş gösterdiği, Sonuç olarak bozkır meralarında yaz aylarında yemin kıt olduğu dönemde patlangaç çalısı kaliteli kaba yem temini açısından kayda değer bir alternatif olabileceğini bildirmişlerdir [6].

Çalıların diğer ot türlerinin kuru olduğu zamanlarda yeterli miktarda yeşil yem sağladıkları, bu yeşil materyalin sadece kuraklığın olduğu periyotta tek besin kaynağı olduğu, bu yüzden odunumsu türlerin dominant olarak bulunduğu meralarda birçok çalımsının otlayan hayvanlar için önemli bir yem kaynağı olarak mera alanlarında yem sağlayıcı olarak düşünülebileceği, tesis edilmesinden sonra mikroklima ve toprak yapısının da değişeceği, daha fazla çevresel avantajları olan alanların oluşacağı vurgulanmıştır [33, 34].

24 adet çalı bitkisi ile yapılan bir çalışmada, bitkilerinin sezonluk (ilkbahar, yaz, sonbahar) yaprak verimleri, besin madde içerikleri ve hazmolunabilirlikleri belirlemek amacıyla ilkbahar, yaz ve sonbaharda ayrı ayrı incelenmiş ve elde edilen değerlere göre çalı bitkilerinin özellikle kurak sezonlarda otlatılabileceği ve ilave yemler olarak katkı sağlayabileceği belirlenmiştir [7]. Çalı tohumlarının mineral yönünden karşılaştırmalı faktör analizi Tablo 3' te verilmiştir.

Tablo 3. İncelenen çalı tohumlarının mineral yönünden karşılaştırmalı faktör analizi



	F_1	F_2	F_3	F_4	Communality
N (%)	-0,312	**0,611**	0,552	0,474	0,676
P (%)	0,256	**0,647**	-0,295	-0,098	0,581
K (%)	**0,851**	0,185	0,105	0,175	0,800
Ca (%)	0,021	**-0,614**	0,276	0,275	0,529
Mg (%)	**0,680**	-0,027	-0,259	0,410	0,698
S (%)	-0,312	**0,611**	0,552	0,474	0,476
Na (%)	0,453	**0,639**	-0,181	-0,114	0,660
Fe (ppm)	0,537	0,361	**-0,567**	0,165	0,767
Cu (ppm)	0,664	-0,098	**0,674**	-0,240	0,962
Mn (ppm)	0,110	-0,519	-0,241	**0,630**	0,738
Zn (ppm)	**0,525**	-0,392	0,039	0,190	0,467

Tablo 2. Devam ediyor

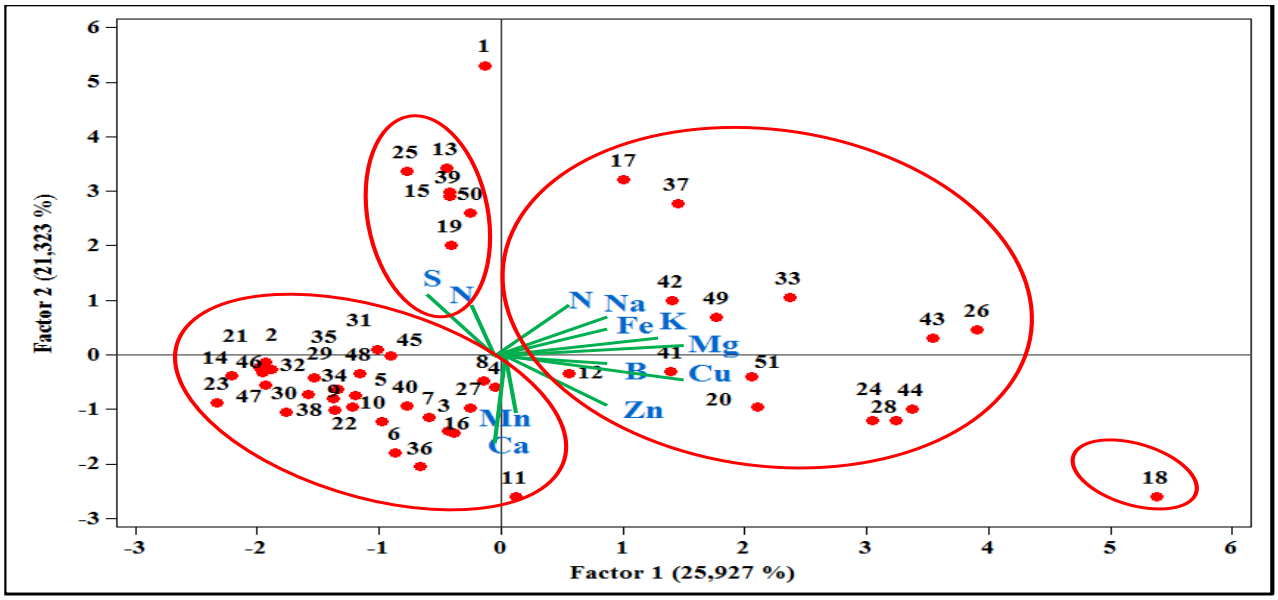
B (ppm)	0,664	-0,098	**0,674**	-0,240	0,962
Latent Root	3,111	2,559	2,173	1,322	9,165
Factor Variance (%)	25,927	21,323	18,110	11,014	76,374

Variables	**Loading**	**% Total Communality**	**Suggested Factor Name**
Factor₁			***K***
K	**0,851**	0,800	***Mg***
Mg	**0,680**	0,698	
Zn	**0,525**	0,467	
Factor₂			**P**
N	**0,611**	0,676	**Na**
P	**0,647**	0,581	***Ca***
Ca	**-0,614**	0,529	
S	**0,611**	0,476	
Na	**0,639**	0,660	
Factor₃			***Cu***
Fe	**-0,567**	0,767	***B***
Cu	**0,674**	0,962	
B	**0,674**	0,962	
Facto₂₄			***Mn***
Mn	**0,630**	0,738	

Tablo 3'den de anlaşıldığı gibi kümülatif varyabiliteyi en iyi anlatan dört faktör seviyesi ele alınmış olup, bu faktör seviyeleri çalıların mineral yönünden % 76.374 oranında açıklayıclık sağlamaktadır. Faktör 1, aşamasında çalıları en çok açıklayan mineraller K ve Mg olurken faktör 2 düzeyinde P, Na ve Ca olmuştur. Faktör 3 aşamasında Cu ve B, faktör 4 seviyesinde ise Mg en çok açıklayıcılık sağlayan ve çalıları en iyi temsil eden mineraller olarak belirlenmiştir. Dolayısıyla bu analiz sonucunda çalı tohumları yönünden göz önünde tutulan ve ön plana çıkan mineraller K, Mg, P, Na, Ca, Cu, B ve Mn olarak belirlenmiştir.

Makro ve mikro elementler olarak sınıflandırılan çok sayıda inorganik elementin hayvan beslenmesi ve büyümesindeki önemi günümüzde kabul görmektedir [4]. İz elementler, yaşamın devam ettirilmesinin yanında büyüme, gelişme ve üretim faaliyetleri ile canlının diğer hayati fonksiyonlarını yerine getirmede önemlidir. Vücut için gerekli olan iz elementlerin kandaki miktarları kritik düzeyin altına düştüğünde hastalıklara özgü klinik semptomlar ortaya çıkmaya başlamaktadır [27, 17].

Makro ve mikro elementlerin hayvan vücudunda ki görevleri incelendiğinde; üreme dâhil olmak üzere hayvanlardaki tüm fizyolojik süreçler için önemli olup [10], yeterli miktarda mineralin sağlanması gerektiği, ancak eksikliğinin zararlı olduğu gibi fazlalığının da zararlı olduğu bildirilmektedir [31].



Şekil 1. Çalışmada incelenen minerallerin ve çalı genotiplerinin benzerliklerini, farklılıklarını performanslarını ve stabilitelerini gösteren biplot analiz

Makro ve mikro elementler sağlık, büyüme, üretim ve yeniden üretim için gereklidir ve bağışıklık sisteminin birçok bileşeninin işleyişi için gereklidirler [5]. Böylece, uygun sağlık ve bağışıklığın korunmasına katkıda bulunurlar

Tarımsal çalışmalarda önemini gittikçe arttırarak kullanıma giren biplot analizi incelenen değişkenlerin birbiri ile olan ilişkisini, performansını ve stabilite durumlarını gösteren çok yararlı bir metot olarak ortaya çıkmaktadır. Biplot analizinde çeşitlerin incelenen genotiplerin unsurlar yönünden performanslarının birbirleri ile olan sinerjistik/antagonistic etkileşimleri; genotipleri ile incelenen unsurların birliktelikleri açıklanabilmektedir. Ayrıca hem genotiplerin hemde incelenen unsurların stabil olup olmadıkları belirlenebilmektedir [35]. Bu bağlamda çalışmamızda gerek minerallerin ve çalı genotiplerinin benzerliklerini farklılıklarını performanslarını ve stabilitelerini gösteren biplot analizi şekil 1 de verilmiştir.

Şekil 1 den de görüleceği gibi mineraller üç farklı grup altında toplanmıştır. S ve N aynı gurupta yer alırken, Mn ve Ca farklı gurubu oluşturmuştur. Bunu yanısıra N, Na, Fe, K, Mg, B, Zn ve Cu aynı gurupta yer almış olup, bu mineraller en stabil ve genotiplerde en çok bulunan mineraller olarak belirlenmişlerdir. Genotiplere bakılacak olursa *Acantholimon acerosum* (Willd.) Boiss. ve *Clematis vitalba* L. türleri mineraller yönünden diğer türlerden ayrı tek başına gurubu oluşturmuşlardır. *Buxus sempervirens* L., *Cistus creticus* L., *Colutea cilicica* Boiss. & Balansa., *Crataegus rotundifolia* Moench., *Rosa pulverulenta* M. Bieb. ve *Vitex agnus-castus* L. türleri mineraller yönünden benzerlik göstermiş olup aynı grubu oluşturmuşlardır. Diğer taraftan, *Berberis vulgaris* L., *Clematis orientalis* L., *Cotoneaster integerrimus* Medik., *Crataegus monogyna* Jacq., *Daphne oleoides* Schreb., *Ephedra major* Host., *Lonicera caucasica* Pall., *Phillyrea latifolia* L., *Sambucus ebulus* L., *Sambucus nigra* L., *Sorbus torminalis* (L.) Crantz., *Viscum album* L ve *Ziziphus vulgaris* Lam. genotipleri mineraller yönünden benzer özelik göstererek aynı grubu oluşturmuşlardır. Bu grubu oluşturan genotipler aynı zamanda mineral yönünden yüksek performanslı ve stabil genotipler olarak belirlenmiştir. Yine, *Acer campestre* L., *Alhagi pseudalhagi* (M. Bieb.) Fisch., *Atriplex canescens* (Pursh) Nutt., *Atriplex halimus* L., *Atriplex hortensis* L., *Atriplex lentiformis* (Torr.) S.Watson., *Atriplex leucoclada* Boiss., *Atriplex nummularia* Lindl., *Atriplex polycarpa* (Torr.) S. Watson., *Atriplex undulata* (Moq.) D.Dietr., *Berberis vulgaris* L., *Cephalaria media* Litv., *Cistus laurifolius var. atlanticus* Pit., *Cotoneaster horizontalis* Decne., *Cotoneaster lacteus* W.W. Sm., *Crataegus marginatus* Jacq., *Elaeagnus angustifolia* L., *Euonymus europaeus* L., *Globularia trichosantha* Fisch. & C. A. Mey., *Gonocytisus angulatus* (L.) Spach., *Jasminum fruticans* L., *Mahonia aquifolium* (Pursh) Nutt., *Malus floribunda* Siebold ex Van Houtte., *Paliurus spina-christi* Mill., *Phillyrea latifolia* L., *Pyracantha coccinea* M. Roem., *Salsola Vermiculata* L., *Smilax excelsa* L., *Rhamnus frangula* L., *Rhus coriaria* L., *Rosa canina* L., *Rubus caesius* L. ve *Ziziphus vulgaris* Lam. türleri aynı grubu oluşturan ve düşük performanslı türler olarak belirlenmiştir. Yine *Berberis vulgaris* L., *Clematis orientalis* L., *Cotoneaster integerrimus* Medik., *Crataegus monogyna* Jacq., *Daphne oleoides* Schreb., *Ephedra major* Host., *Lonicera caucasica* Pall., *Phillyrea latifolia* L., *Sambucus ebulus* L., *Sambucus nigra* L., *Sorbus torminalis* (L.) Crantz., *Viscum album* L. ve *Ziziphus vulgaris* Lam. türleri yüksek performanslı türler olarak belirlenmiştir.

Denemede incelenen çalı bitkilerine ait tohumların içerdiği mineral maddeler Agglomerative Hierarchical Clustering metoduna göre analiz edilmiş, sonuçlara göre benzer etkiye sahip olan mineraller ve bu mineraller yönünden benzer özellik gösteren çalımsı bitkiler ortaya konarak Tablo 4'de verilmiştir.

Tablo 4'den de görüleceği gibi çalımsı bitkilerin tohumları mineral özellikleri yönünden benzer veya farklı özellik gösterebilirler. Dolayısıyla yüksek performansa sahip çalımsı bitkilerin mineral özelliği yönünden benzerlik gösteren çalımsı bitklerin belirlenmesiyle zengin içeriğe sahip yüksek verimli çalıların belirlenmesi mümkün olacaktır. Bu bağlamda N ve Ca bir gurubu oluştururken ikinci gurubu Mg. S, Na, Fe, Cu, Mn, Zn oluşturmuştur. P ve K ise kendi başlarına ayrı birer grup oluşturmuşlardır. Çalımsı türlerden de 1 gurup; *Acantholimon acerosum* (Willd.) Boiss., *Alhagi pseudalhagi* (M. Bieb.) Fisch., *Atriplex canescens* (Pursh) Nutt., *Atriplex halimus* L., *Atriplex hortensis* L., *Atriplex nummularia* Lindl., *Atriplex polycarpa* (Torr.) S. Watson., *Berberis vulgaris* L., *Buxus sempervirens* L., *Cistus creticus* L., *Cistus laurifolius var. atlanticus* Pit., *Colutea cilicica* Boiss. & Balansa., *Crataegus rotundifolia* Moench., *Phillyrea latifolia* L., *Pyracantha coccinea* M. Roem., *Rosa pulverulenta* M. Bieb., *Salsola Vermiculata* L., *Sambucus ebulus* L., *Rhamnus frangula* L., *Vitex agnus-castus* L. 2. gurup; *Acer campestre* L., *Atriplex lentiformis* (Torr.) S. Watson., *Atriplex leucoclada* Boiss., *Atriplex undulata* (Moq.) D.Dietr., *Cephalaria media* Litv., *Cotoneaster horizontalis* Decne., *Cotoneaster lacteus* W.W. Sm., *Crataegus marginatus* Jacq., *Elaeagnus angustifolia* L., *Euonymus europaeus* L., *Globularia trichosantha* Fisch. & C.A.Mey., *Gonocytisus angulatus* (L.) Spach., *Jasminum fruticans* L., *Mahonia aquifolium* (Pursh) Nutt., *Malus floribunda* Siebold ex Van Houtte., *Paliurus spina-christi* Mill., *Rhus coriaria* L., *Rosa canina* L., *Rubus caesius* L.ve *Clematis orientalis* L., *Clematis vitalba* L., *Cotoneaster integerrimus* Medik., *Crataegus monogyna* Jacq., *Daphne oleoides* Schreb., *Ephedra major* Host., *Lonicera caucasica* Pall., *Smilax excelsa* L., *Sambucus nigra* L., *Sorbus torminalis* (L.) Crantz., *Viscum album* L. ve *Ziziphus vulgaris* Lam. 3. gurubu oluşturmuşlardır.

Bu analiz neticesinde; yüksek mineral içeriğine sahip türlerin belirlenmesi mümkün olurken; türlerdeki minerallerin dağılımı benzerlik ve farklılığı ortaya konmuştur. Nitekim Aggromeratif Hierarchical Cluster analizi ile gerek incelenen unsurların ve gerekse ele alınan materyallerin benzerlikleri ve farklılıkları ortaya konarak yüksek değere sahip olan materyaller ve etkin unsurları ortaya konması mümkün olmaktadır. Bu özelliği ile bu analiz metodu tarımsal çalışmalarda etkin şekilde kullanılabilir [18].

Tablo 4. Hiyerachical Clustural analizine göre benzerlik gösteren mineraller ve çalımsı bitkiler

Class	**Genotypes**			**Minerals**			
	1	2	3	1	2	3	4
Within-class variance	996062,443	387509,944	1434928,857	10508688,802	0,000	0,000	2594428,612
Min. dis. to centroid	553,447	265,147	236,449	2292,236	0,000	0,000	478,219
Av. Dis. to centroid	920,847	570,796	1009,599	2292,236	0,000	0,000	1333,168
Max, dis. to centroid	1783,983	893,480	2179,134	2292,236	0,000	0,000	2404,763
	Gen 1	**Gen 2**	**Gen 17**	**N**	**P**	**K**	**Mg**
	Gen 3	**Gen 7**	**Gen 18**	**Ca**			**S**
	Gen 4	**Gen 8**	**Gen 20**				**Na**
	Gen 5	**Gen 11**	**Gen 24**				**Fe**
	Gen 6	**Gen 14**	**Gen 26**				**Cu**
	Gen 9	**Gen 21**	**Gen 28**				**Mn**
	Gen 10	**Gen 22**	**Gen 33**				**Zn**
	Gen 12	**Gen 23**	**Gen 42**				
	Gen 13	**Gen 27**	**Gen 43**				
	Gen 15	**Gen 29**	**Gen 44**				
	Gen 16	**Gen 30**	**Gen 49**				
	Gen 19	**Gen 31**	**Gen 51**				
	Gen 25	**Gen 32**					
	Gen 37	**Gen 34**					
	Gen 38	**Gen 35**					
	Gen 39	**Gen 36**					
	Gen 40	**Gen 46**					
	Gen 41	**Gen 47**					
	Gen 45	**Gen 48**					
	Gen 50						

İstatistikel olarak incelenen unsurların benzerliklerinin farklılıklarının ortaya koymada conditional formating metot başarı ile kullanılacak bir metottur. Bu metodun kullanılması ile incelenen unsurlar gerek sütun ve gerekse satır yönünden iki yönlü olarak analiz edilerek benzerlik ve farklılıklar ortaya konabilir [9]. Tablo 5'dan da görüldüğü gibi yapılan anlaiz sonucunda K, P, Mg, Na, Cu, Zn ve B bir gurubu oluştururken, N, S, Ca, Fe ve Mn'da diğer bir gurubu oluşturmuştur. Diğer taraftan, çalımsı bitkiler de çeşitli gurupları oluşturmuştur. Bunlardan birinci gurupta *Euonymus europaeus* L., *Paliurus spina-christi* Mill, ikinci gurupta *Acer campestre* L., *Atriplex lentiformis* (Torr.) S. Watson., *Atriplex undulata* (Moq.) D. Dietr., *Buxus sempervirens* L., *Cephalaria media* Litv., *Cotoneaster horizontalis* Decne., *Cotoneaster lacteus* W.W. Sm., *Crataegus marginatus* Jacq., *Globularia trichosantha* Fisch. & C. A. Mey., *Gonocytisus angulatus* (L.) Spach., *Jasminum fruticans* L., *Mahonia aquifolium* (Pursh) Nutt., *Rhamnus frangula* L., *Rhus coriaria* L., *Rosa canina* L., *Rubus caesius* L., üçüncü gurupta ise *Alhagi pseudalhagi* (M.Bieb.) Fisch., *Alhagi pseudalhagi* (M.Bieb.) Fisch., *Atriplex canescens* (Pursh) Nutt., *Atriplex halimus* L., *Atriplex hortensis* L., *Atriplex leucoclada* Boiss., *Atriplex nummularia* Lindl., *Atriplex polycarpa* (Torr.) S. Watson., *Berberis vulgaris* L., *Cistus creticus* L., *Cistus laurifolius var. atlanticus* Pit., *Colutea cilicica* Boiss. & Balansa., *Crataegus rotundifolia* Moench., *Elaeagnus angustifolia* L., *Malus floribunda* Siebold ex Van Houtte., *Phillyrea latifolia* L., *Pyracantha coccinea* M. Roem., *Rosa pulverulenta* M. Bieb., *Salsola Vermiculata* L., *Vitex agnus-castus* L., dördüncü gurupta *Clematis orientalis* L., *Clematis vitalba* L., *Cotoneaster integerrimus* Medik., *Ephedra major* Host., *Sambucus ebulus* L., *Smilax excelsa* L., *Sambucus nigra* L., *Viscum album* L., *Ziziphus vulgaris* Lam., beşinci gurupta ise *Crataegus monogyna* Jacq., *Daphne oleoides* Schreb., *Lonicera caucasica* Pall., *Sorbus torminalis* (L.) Crantz. den oluşmaktadır. Yukarıda yapılan analizler neticesinde minerallerin birleşik sınıflaması Tablo 6'da, minerallerin benzerlik/farklılıkları Tablo 7'de verilmiştir.

Tablo 5. Yapılan analizler neticesinde minerallerin birleşik sınıflaması

Genotypes	N	P	K	Ca	Mg	S	Na	Fe	Cu	Mn	Zn	B	Mean
Acantholimon acerosum (Willd.) Boiss.	2,50	3342	12326	2981	1965	585	711	132,08	32,65	18,99	34,35	9,77	3
Acer campestre L.	2,27	2501	10845	4512	1748	531	431	75,69	46,52	22,37	26,58	13,93	2
Alhagi pseudalhagi (M. Bieb.) Fisch.	1,94	2510	12200	4526	2126	455	421	122,96	30,43	34,48	36,61	9,11	3
Atriplex canescens (Pursh) Nutt.	2,12	2551	12905	4247	2115	495	462	108,50	35,12	29,06	42,76	10,52	3
Atriplex halimus L.	2,20	2078	12322	4515	1928	515	475	112,47	35,96	34,45	32,18	10,77	3
Atriplex hortensis L.	2,02	2323	12060	4567	2081	473	451	97,29	30,44	35,11	40,72	9,11	3
Atriplex lentiformis (Torr.) S.Watson	1,94	2569	11351	4067	2098	455	475	111,15	29,95	40,86	36,02	8,97	2
Atriplex leucoclada Boiss.	2,04	2697	11003	4572	2087	477	527	121,46	34,25	32,78	37,63	10,26	3
Atriplex nummularia Lindl.	2,16	2379	12450	4859	1880	506	415	110,28	32,02	28,70	37,50	9,59	3
Atriplex polycarpa (Torr.) S.Watson	2,07	2293	12490	4371	2059	484	425	115,94	30,37	36,38	34,58	9,09	3
Atriplex undulata (Moq.) D.Dietr.	1,76	2469	11459	4526	2086	412	391	113,96	36,44	30,73	41,54	10,91	2
Berberis vulgaris L.	1,95	2712	13092	4456	1813	456	501	118,00	45,63	20,45	34,69	13,66	3
Buxus sempervirens L.	2,35	3131	12342	3023	2013	550	453	136,00	41,89	21,39	22,10	12,54	2
Cephalaria media Litv.	2,26	2330	11598	4475	1612	529	410	79,48	42,31	20,10	32,00	12,67	2
Cistus creticus L.	2,14	2982	12344	3420	1813	501	645	123,00	36,45	20,13	24,36	10,91	3
Cistus laurifolius var. atlanticus Pit.	2,05	2103	12362	4103	2235	480	426	136,29	30,12	40,28	35,24	9,02	3
Clematis orientalis L.	2,18	3112	14865	3713	2181	510	691	126,35	32,65	18,04	32,14	9,77	4
Clematis vitalba L.	1,67	2783	16340	4012	2382	391	532	130,74	62,31	34,35	52,31	18,66	4
Colutea cilicica Boiss. & Balansa.	1,78	2708	12322	3221	1743	417	613	145,00	28,78	14,32	25,40	8,62	3
Cotoneaster integerrimus Medik.	1,98	2543	15645	4532	2213	463	540	113,00	48,76	27,13	36,57	14,60	4
Cotoneaster horizontalis Decne.	2,24	2341	11542	4255	1756	524	425	82,36	43,15	25,62	25,87	12,92	2
Cotoneaster lacteus W.W. Sm.	2,03	2458	11365	4168	1823	475	416	88,62	40,21	24,15	31,24	12,04	2
Crataegus marginatus Jacq.	2,12	2419	10524	4951	1792	496	451	76,57	38,59	25,61	24,15	11,55	2
Crataegus monogyna Jacq.	2,13	2545	17216	4532	2003	498	516	107,73	56,78	20,43	55,48	17,00	5
Crataegus rotundifolia Moench.	2,06	2999	13243	2881	1752	483	611	128,31	28,43	18,96	25,47	8,51	3
Daphne oleoides Schreb.	2,06	2621	17592	4430	2543	481	642	154,00	51,44	29,66	30,13	15,40	5
Elaeagnus angustifolia L.	2,06	2707	11023	4858	2280	482	452	116,76	33,74	33,05	36,07	10,10	3
Ephedra major Host.	1,95	2362	16396	3988	2385	456	523	119,88	55,47	25,95	40,03	16,61	4
Euonymus europaeus L.	2,03	2516	10574	3746	1799	475	469	80,11	39,58	28,62	30,74	11,85	1
Globularia trichosantha Fisch. & C.A.Mey.	2,11	2275	11234	4206	1784	494	446	70,12	40,22	24,59	34,75	12,04	2
Gonocytisus angulatus (L.) Spach.	2,13	2641	10250	4211	1789	498	521	87,54	45,26	22,96	32,11	13,55	2
Jasminum fruticans L.	2,3	2035	11478	4158	1653	538	469	81,06	47,51	24,13	27,46	14,22	2
Lonicera caucasica Pall.	2,30	2771	18548	4373	2014	538	566	142,00	45,93	29,80	38,91	13,75	5
Mahonia aquifolium (Pursh) Nutt.	2,02	2415	11263	4102	1875	473	412	92,06	40,12	20,16	30,85	12,01	2
Malus floribunda Siebold ex Van Houtte	2,17	2554	11642	4899	2039	509	436	105,02	35,21	31,94	31,15	10,54	3
Paliurus spina-christi Mill.	1,84	2418	10674	4168	1788	431	386	82,13	48,15	21,52	35,45	14,42	1
Phillyrea latifolia L.	1,97	3420	11926	2981	2430	461	540	147,00	40,14	25,26	22,52	12,02	3
Pyracantha coccinea M. Roem.	2,1	2211	12110	4023	1912	491	402	80,72	42,12	22,31	26,59	12,61	3
Rosa pulverulenta M. Bieb.	2,21	2540	13233	3298	1751	517	614	129,41	42,13	12,54	22,87	12,61	3
Salsola vermiculata L.	2,02	2397	12152	4181	2149	473	429	124,11	26,80	34,45	37,57	8,02	3
Sambucus ebulus L.	1,92	2648	14255	4384	1813	450	633	113,00	45,52	24,45	38,87	13,63	4
Smilax excelsa L.	2,43	2811	15433	4433	2375	569	521	107,71	48,34	28,71	34,67	14,47	4
Sambucus nigra L.	2,01	2613	16577	4283	2231	470	671	148,00	50,72	25,92	39,35	15,19	4
Sorbus torminalis (L.) Crantz	2,06	2387	17321	4564	2377	481	602	103,59	54,18	20,14	46,59	16,22	5
Rhamnus frangula L.	2,21	2498	12021	3911	1813	517	457	78,20	46,41	20,35	36,49	13,90	2
Rhus coriaria L.	2,16	2245	11635	4026	1765	505	420	83,56	40,13	25,48	29,51	12,01	2
Rosa canina L.	2,11	2347	11320	3851	1842	494	424	89,45	35,41	24,13	32,06	10,60	2
Rubus caesius L.	2,15	2651	10268	4474	1955	503	447	84,15	44,58	23,15	30,78	13,35	2
Viscum album L.	2,24	2711	15433	4069	2243	524	634	103,00	50,11	36,54	29,65	15,00	4
Vitex agnus-castus L.	2,10	2710	13242	2813	2183	491	642	102,00	28,79	18,76	32,44	8,62	3
Ziziphus vulgaris Lam.	2,19	2540	15400	3356	2433	512	413	115,00	46,71	29,61	48,71	13,98	4
Mean	4	3	3	4	3	4	3	4	3	4	3	3	

N (%), diğer elementler için (ppm)

Tablo 6. İncelenen minerallerin değişik istatistiki analizlerle hesaplanan benzerlik ve farklılıkları

Minerals	Correlation	Factor	Bi-plot	Aggl. Hie. Clus.	Conditi. Format.	Total
N			*	*	*	**3**
P	*	*		*		**3**
K	*	*	*	*		**4**
Ca	*	*			*	**3**
Mg	*	*	*	*		**4**
S				*	*	**2**
Na		*	*			**2**
Fe	*		*		*	**3**
Cu		*	*			**2**
Mn		*			*	**2**
Zn			*			**1**
B		*	*			**1**

Tablo 6'dan da görüldüğü gibi yapılan analizlerin birleşik sonucu olarak Zn ve B, bir gurubu oluştururken, Mg, S, Na, Cu ve Mn bir gurubu oluşturmuştur. Diğer taraftan N, P, Ca ve Fe aynı gurubu oluştururken K ve Mg bir gurubu oluşturmuştur. Çalımsı bitkilerin mineral yönünden benerlik ve farklılıklarının ortaya konulması bitki seçiminde bitkilerin mineral içeriği yönünden değerlendirilmesinde büyük kolaylık sağlayacaktır. Aynı zamanda çalımsı bitkiler içerisinde mineral dağılımı ve miktarının belirlenerek benzerlik ve farklılıklarının ortaya konulması, zengin mineral içerikli çalımsı bitkilerin belirlenmesinde benzer özellik gösteren bitkilerin belirlenmesinde büyük kolaylık sağlayacaktır. Benzer şekilde çalımsı bitkilerin en çok Mg, N, P, K, Ca ve Fe yönünden benzerlik gösterdikleri ortaya konmuştur.

Tablo 7. İncelenen çalımsı bitkilerin değişik istatistiki analizlerle hesaplanan benzerlik ve farklılıkları

Genotypes	Bi-plot	Aggl. Hier. Clus.	Conditi. Format.
Acantholimon acerosum (Willd.) Boiss.		*	
Acer campestre L.			
Alhagi pseudalhagi (M. Bieb.) Fisch.		*	
Atriplex canescens (Pursh) Nutt.		*	
Atriplex halimus L.		*	
Atriplex hortensis L.		*	
Atriplex lentiformis (Torr.) S.Watson			
Atriplex leucoclada Boiss.			
Atriplex nummularia Lindl.		*	
Atriplex polycarpa (Torr.) S.Watson		*	
Atriplex undulata (Moq.) D.Dietr.			
Berberis vulgaris L.		*	*
Buxus sempervirens L.		*	
Cephalaria media Litv.			
Cistus creticus L.		*	
Cistus laurifolius var. atlanticus Pit.		*	
Clematis orientalis L.	*	*	*
Clematis vitalba L.	*	*	
Colutea cilicica Boiss. & Balansa.		*	
Cotoneaster integerrimus Medik.	*	*	*
Cotoneaster horizontalis Decne.			
Cotoneaster lacteus W.W. Sm.			
Crataegus marginatus Jacq.			
Crataegus monogyna Jacq.	*	*	*
Crataegus rotundifolia Moench.		*	
Daphne oleoides Schreb.	*	*	*
Elaeagnus angustifolia L.			
Ephedra major Host.	*	*	*
Euonymus europaeus L.			
Globularia trichosantha Fisch. & C.A.Mey.			
Gonocytisus angulatus (L.) Spach			

Tablo 7. Devam ediyor

Jasminum fruticans L.			
Lonicera caucasica Pall.	*	*	*
Mahonia aquifolium (Pursh) Nutt.			
Malus floribunda Siebold ex Van Houtte			
Paliurus spina-christi Mill.			
Phillyrea latifolia L.		*	*
Pyracantha coccinea M. Roem.		*	
Rosa pulverulenta M. Bieb.		*	
Salsola Vermiculata L.		*	
Sambucus ebulus L.	*	*	*
Smilax excelsa L.	*	*	*
Sambucus nigra L.	*	*	*
Sorbus torminalis (L.) Crantz	*	*	*
Rhamnus frangula L.		*	
Rhus coriaria L.			
Rosa canina L.			
Rubus caesius L.			
Viscum album L.	*	*	*
Vitex agnus-castus L.		*	
Ziziphus vulgaris Lam.	*	*	*

Tablo 7 dikkate alındığında mineral madde içeriği yönünden *Clematis orientalis* L., *Cotoneaster integerrimus* Medik., *Crataegus monogyna* Jacq., *Crataegus rotundifolia* Moench., *Daphne oleoides* Schreb., *Ephedra major* Host., *Lonicera caucasica* Pall., *Sambucus ebulus* L., *Smilax excelsa* L., *Sambucus nigra* L., *Sorbus torminalis* (L.) Crantz., *Viscum album* L., *Ziziphus vulgaris* Lam., çalımsı bitkilerin mineral içeriklerinden benzerlik gösterdiği, bu bitkilerin zengin mineral içeriğine sahip bitkiler olarak ele alınabilecekleri sonucuna varılmıştır.

Ülkemiz'de 260.000 ha. alana yayılışı bulunan [36] meşeliklerden yılda 60-70 bin ton meşe pamlamudu üretiminin olduğu bildirilmiştir. Tarafımızdan Kütahya ve çevresinde yaptığımız arazi gözlemlerinde çobanların uzun sopalarla palamutların dökülmesine yardımcı olduğu, özellikle keçiler tarafından tercih edildikleri gözlemlenmiştir.

Avakado (*Persea Americana* Mill.) tohumunun besin kompozisyonunun fitokimyasal ve antioksidan özellikleride nem içeriğinin % 8.6, yağ % 14.1, lif % 7.1, kül % 2.4, protein % 23.0 ve karbonhidrat içeriğinin % 44.70 mg / 100g. magnezyum 0.10, kalsiyum 0.82, çinko 0.18, potasyum 4.16, sodyum 1.41 ve fosfor 0.09 olarak bildirilmiştir [16].

Üzüm çeşitlerinin çekirdeklerinin biyoaktif özllikleri, asit yağ kompozisyonları ve mineral içerikleri ile ilgili olarak yapılan çalışmada tohumların Fe içeriklerinin farklı çeşitlerde 29.96 mg/kg - 73.82 mg/kg arasında olarak belirlemişlerdir [2].

Tropik bitki tohumlarının besin değerleri ile ilgili olarak yapılan bir çalışmada 5 çalı, 10 ağaç ve bir çok otsu tür incelemiş olup, *Afzelia bella* Harms, *Daniellia ogea* (Harms) Rolfe ex Holland, *Gliricidia sepium* (Jacq.) Walp, *Millettia Thonningii* (Schum. Et Thonn.) Bak, *Pericopsis elata* (Harms) Van Meeuwen.'da Na içeriği sırasıyla 175.3, 133.9, 201, 103ve 250 mg/100 g, K içeriği sırasıyla 733.94, 565.5, 987, 406 ve 1250 mg/100 g, Ca içeriği sırasıyla 307.06, 342.52, 421, 862 ve 250 mg/100 g, Mg içeriği sırasıyla 223.78, 150.05, 145, 202 ve 160 mg/100 g. P içeriği sırasıyla 368, 297.5, 584.71, 244.6 ve 370 mg/100 g. F içeriği sırasıyla; 9.4, 12.69, 14.28, 24.58 ve 30 mg/100 g. Cu içeriği sırasıyla; 0.92, 0.49, 0.95, 1.12 ve nd mg/100 g. Zn içeriği sırasıyla; 2.56, 1.77, 1.77, 1.9 ve 2 mg/100 g. Mn içeriği sırasıyla 1.1, 1.17, 2.5, 2.17 mg/100 g. olarak bildirmiştir [11].

Moringa oleifera Lam. ın besin değeri ve tıbbi özelikleri ile ilgili çalışmalarında; bitki tohumlarında sırasıyla Ca, Mg, P, Zn, ve S içerikleri sırasıyla 45, 8.66, 75, 0.15, 0.05 mg/100g olarak tespit ettiklerini bildirmişlerdir [13].

Kuzeydoğu Brezilya'da üretilen noni (*Morinda citrifolia* L) 'in kimyasal karakterizasyonu, beslenme özellikleri ve antioksidan kapasitesi çalışmalarında bitki tohumlarına ait içerikleri ile ilgili olarak Na : 1.13 Mg/15 g, Mg: 19.76 Mg/15 g lık örnekte, Ca: 43.76 Mg/15 g lık örnekte, Mn: 24.24 Mg/15 g lık örnekte ve Fe: 0.67 Mg/15 g lık örnekte olarak bildirmişlerdir. Buna göre bitki tohumlarının zengin mineral ve lif kaynağı olduğunu vurgulamışlardır [24].

Mesquite pod' un besin değeri ve ruminal fermantasyonunun değerlendirilmesi çalışmasında tohum kapsülü olgunlaştıktan sonra rengi sarı olan ve kayda değer protein ve karbonhidrat içeren tohumlar içermekte olup, bitkinin farklı bölümlerinin kimyasal bileşimini, rumen fermentasyonunu ve sindirilebilirliğini değerlendirmesinde; tohumlarının en yüksek ham protein, eter ekstresi ve lignin değerlerine sahip oduğunu, toplam bakla, kabuk ve tohumdaki ham protein içeriğinin sırasıyla % 12.15, % 10.65 ve % 36.53 olduğunu tespit etmişlerdir [3].

Shorea robusta Gaertn. çekirdeğinin kimyasal bileşimini ve besleyici değeri hususunda iyi bir ham protein kaynağı olduğunu, bitki tohum kekinin yüksek oranda parçalanabilir protein içeren yemleri iyileştirmek için

kullanılabileceğini bildirmişlerdir [15]. *Enterolobium Cyclocarpum* Jacq Griseb. tohumlarının işlenerek ruminant üretimi için yem olarak kullanılması çalışmasında; tohumların yem kalitei değerlendirilmiş olup, tohumlar besin bakımından zengin olduğu bildirilmiştir [26].

4. Sonuçlar ve tartışma

Sonuç olarak Mineral oranı yüksek bitkilerin belirlenmesinde N, K, P, Ca, Mg ve Fe göz önünde tutulması gereken mineraller olarak belirlenmiştir. Yine *Clematis orientalis* L., *Cotoneaster integerrimus* Medik., *Crataegus monogyna* Jacq., *Crataegus rotundifolia* Moench., *Daphne oleoides* Schreb., *Ephedra major* Host., *Lonicera caucasica* Pall., *Sambucus ebulus* L., *Smilax excelsa* L., *Sambucus nigra* L., *Sorbus torminalis* (L.) Crantz., *Viscum album* L., *Ziziphus vulgaris* Lam., gibi çalımsı bitkilerin kullanılması yüksek mineralli çalıların belirlenerek mera ıslah çalışmalarında kullanılması hayvan beslemede kaliteyi arttıracaktır. Çalımsı bitki tohumlarının mineral analizlerinin yapılarak ortaya konması, çalımsı bitkierin mineral içerikleri yönünden benzerlik/farklılıklarının belirlenmesi yüksek mineral kalitesine sahip türlerin belirlenmesine katkıda bulunacaktır. Bu suretle yapılacak seleksiyon ve ıslah çalışmalarında kaliteli türlerin belirlenmesi daha kolay olacaktır. Yüksek mineral içeriğine sahip türlerin hayvan beslemede de kullanılmasıyla hayvansal ürün kalitesinin arttırılması mümkün olacaktır.

Teşekkür

Bu çalışma Tarımsal Araştırmalar ve Politikalar Genel Müdürlüğü tarafından TA / 11 / 02 /003 proje numarası ile desteklenmiştir.

Kaynaklar

[1] Adkins, Y., Zinker, SC., Lepine, A. & Lonnerdal, B. (1997). Changes in nutrient and protein composition of cat milk during lactation. *American Journal of Veterinary Research*, 58(4), 370-375.

[2] Al Juhaimi, F., Geçgel, Ü., Gülcü, M., Hamurcu, M. & Özcan, M.M. (2017). Bioactive properties, fatty acid composition and mineral contents of grape seed and oils. *South African Journal Enology and Viticulture*, 38 (1).

[3] Aliarabi, H., Alipour, F., Zamani, P., Alipour, D., Maleki, M. & Zaboli, K. (2018). Evaluation of nutritional value and ruminal fermentation of mesquitepod. *İranian Journal of Animal Science Research*, 10(1), 35-46.

[4] Altıntaş, A., Uysal, H., Yıldız, S. & Goncagül, T. (1990). Akkaraman ve melezlerinde serum ve yapağı örneklerinde karşılaştırmalı mineral durumu. *Lalahan Hayvancılık Araştırma Enstitüsü Dergisi*, 30(1), 40-56.

[5] Aygün, C., Kara, İ., Oral, H.H., Erdoğdu, İ., Atalay, A.K. & Sever, A.L. (2018a). Bazı çalı bitkilerinin sezonluk (ilkbahar, yaz, sonbahar) yaprak örneklerindeki makro ve mikro besin elementi içerikleri. *Journal of Bahri Dagdas Crop Research* 7(1), 51-65.

[6] Aygün, C., Koç, A. & Turan M. (2018b). Patlangaç (Colutea Cilicica Boiss. & Bal.) çalısının yem değeri ve bozkır meraları açısından önemi. *Biyoloji Bilimleri Araştırma Dergisi*, 11(1), 38-41.

[7] Aygün, C., Oral, HH., Kara, İ., Erdoğdu, İ., Atalay, A.K. & Sever, A.L. (2018c). Bazı çalı bitkilerinin sezonluk (ilkbahar, yaz, sonbahar) yaprak verimleri, besin madde içerikleri ve hazmolunabilirlikleri. *Journal of Bahri Dagdas Animal Research*, 7 (1):7-17.

[8] Bouzid, S.M. & Papanastasis, V.P. (1996). Effects of seeding rateand fertilizer on establishment and growth of Atriplex halimusand Medicago arborea. *Journal of Arid Environments*, 33, 109–115.

[9] Excel, (2007). https://www.actx.edu/web/ files/filecabinet/folder7/Excel 2007. Erişim Tarihi: 24.02.2022.

[10] Elrod, C.C. & Butler, W.R. (1993). Reduction of fertility and alteration of uterine pH in heifers fed excess ruminally degradable protein, *Journal of Animal Science*, 1993, 71(3), 694-701.

[11] Ezeagu, I.E. (2009). Nutritional value of tropical plant seeds. *Agronomy Monograph* 51. American Society of Agronomy, Crop Science Society of America, Soil Science Society of America, 677 S. Segoe Rd., Madison, WI 53711, USA.

[12] FAO, (2018). Grassland perspectives, http://www.fao.org/docrep/008/y8344e/y8344e0j.htm. Erişim Tarihi: 31.12.2018.

[13] Gopalakrishnan, L., Doriya, K. & Kumar, D.S. (2016). *Moringa oleifera*: A review on nutritive importance and its medicinal application. *Food Science and Human Wellness*, 5(2), 49-56.

[14] Gürsakal, N. (2002). Bilgisayar uygulamalı istatistik II. (1. Baskı). Alfa Yayınları 1097. Dizi No: 147. İstanbul.

[15] Hossain, M.D. Emran., Kabir, M.D., Sarma, S.M. & Sikder, H. (2015). Nutrıtıve value of sal seed (*Shorea robusta*). *Journal of Animal and Feed Research*, 5(1), 28-32.

[16] Ifesan, B.O.T., Olorunsola, B.O. & Ifesan, B.T. (2015). Nutritional composition and acceptability of candy from avocado seed (Persea americana). *International Journal of Agriculture Innovations and Research*, 3(6), 2319-1473.

[17] İmren, H.Y. & Şahal, M. (1991). Veteriner iç hastalıkları. Ankara, Medisan.

[18] Jat, M.L., Jat, R.K., Singh, P., Jat, S.L., Sidhu, H.S., Jat, H.S., ... & Gupta, R. (2017). Predicting yield and stability analysis of wheat under different crop management systems across agro-ecosystems in India. *American Journal of Plant Sciences*, 8(8), 1977-2012.

[19] Kalaycı, Ş. (2006). SPSS Uygulamalı çok değişkenli istatistik teknikleri, 2. Baskı, Asil Yayın Dağıtım Ltd. Şti.

[20] Koç, A., Gökkuş, A. & Altın, M. (2003). Mera durumu tespitinde dünyada yaygın olarak kullanılan yöntemlerin mukayesi ve türkiye için bir öneri. Türkiye 5. Tarla Bit. Kon., 13-17 Ekim, Diyarbakır.

[21] Massart, D.L., Vandeginste, B.G.M., Buydens, L.M.C., de Jong, S., Lewi, P.J. & Smeyers-Verbeke, J. (1997). Straight line regression and calibration. In Handbook of chemometrics and qualimetrics, Part A, pp 171–231, Amsterdam, The Netherlands: Elsevier.

[22] Mertens, D. (2005). AOAC official method 975.03. in: W Horwitz, GW Latimer (eds) Metal in plants and pet foods. Official methods of analysis, 18th edn. Maryland, USA.

[23] Mnene, W.N., Wandera, F.P. & Lebbie, S.H**. (**2000). Arresting environmental degradation through accelerated on-site soil sedimentation and revegetation using micro-catchment and re-seeding. Paper presented at the 3rd All-Africa Conference on Animal Agriculture, Alexandria, Egypt, 6-9 November 2000.

[24] Nascimento, L.C.S., Rodrigues, D.R.N., Alves, M.P.C., Sabaa Srur, A.U.O., Barbosa, J., J.L. & Barbosa, M.I.M.J. (2018). Chemical characterization, nutritional aspects and antioxidant capacity of noni (*Morinda citrifolia* L) produced in northeastern Brazil. *International Food Research Journal*, 25(2), 870-875.

[25] Nockels, C.F., DeBonis, J. & Torrent, J. (1993). Stress induction affects copper and zinc balance in calves fed organic and inorganic copper and zinc sources. *Journal of Animal Science*, 71, 2539-2545.

[26] Ojo, V.O.A., Akinade, G.A., Fasae, O.A. & Akinlolu, A.O. (2018). Effect of treatment methods on the nutritive quality of elephant-ear seeds (*Enterolobium Cyclocarpum* Jacq Griseb) as feed for ruminant production. *Pertanika Journal of Tropical Agricultural Science*, 41(1), 453-462.

[27] Okatan, A.G., Çam, Y. & Leblebici, Z. (2008). Kayseri yöresinde dil oynatma hastalığı olan sığırlarda bazı iz elementlerin serum düzeylerinin değerlendirilmesi. *Sağlık Bilimleri Dergisi*,17(1), 16-22.

[28] Özdamar, K. (1999). Paket programlar ile istatistiksel veri analizi, Vol: I-II, 2. Baskı, Eskisehir, Kaan Basımevi.

[29] Rodrigues, M.A.M., Lourenço, A.L., Cone, J.W., Nunes, F.M., Santos, A.S., Cordeiro, J.M.M., Guedes, C.M.V. & Ferreira, L.M.M. (2014). Evaluation of the nutritive value of muiumba (*Baikiaea plurijuga*) seeds: chemical composition, *in vitro* organic matter digestibility and *in vitro* gas production. *Springerplus*, 3, 311.

[30] Sarıçiçek, Z. & Kılıç, Ü. (2002). Meşe palamudunun yem değerinin belirlenmesi üzerine bir çalışma. *Hayvansal Üretim*, 43(1), 32-44.

[31] Schweigert, F.J. & Zucker, H. (1988). Concentratio of vitamin A, beta-carotene and vitamin E in individual bovine follicles of different quality. *Journal of Reproduction and Fertility*, 82(2), 575-579.

[32] Snyman, H.A. (2003). Fire and the dynamics of semi-arid grassland: influence on plant survival, productivity and water-use efficiency. *African Journal of Range & Forage Science*, 20(1), 29–39.

[33] Spatz, G., Papachristou, T.G., Papanastasis V.P., Frame, J. & Nastis, A.S. (1999). Grassland and woody plants in Europe. Proceedings of the International Occasional Symposiumof the European Grassland Federation, , 27-29 May.1999. 27-39; 24 ref. Thessaloniki, Greece.

[34] Tahtacıoğlu, L., Aygün, C. & Karaman, Y. (2009). Kuraklık riskine karşı çalımsı yem bitkileri, I. Ulusal Kuraklık ve Çölleşme Sempozyumu, 16-19 Haziran 2009- Konya.

[35] Yan, W. & Rajcan, I. (2002). Biplots analysis of the test sites and trait relations of soybean in Ontario. Crop Science 42, 11-20.

[36] Bozkurt, Y., Göker, Y. (1986). Orman Ürünlerinden Faydalanma Ders Kitabı. İstanbul Üniversitesi Orman Fakültesi Yayın No: 3402-379, İstanbul.

www.biodicon.com BIOLOGICAL DIVERSITY AND CONSERVATION

ISSN 1308-8084 Online ISSN 1308-5301 Print

Research article/Araştırma makalesi
DOI: 10.46309/biodicon.2023.1223530
16/3 (2023) 192-202

Studies on genetic diversity of caprifig: caprifig genetic resources (*Ficus carica* var. *caprificus*) conservation and characterization project

Arzu AYAR [*1], Aytekin BELGE [1], Mesut ÖZEN [1], Berrin ŞAHİN [1], Çağlar KARACAOĞLAN [1]
ORCID: 0000-0002-2080-209X**;** 0000-0002-4470-833X; 0000-0002-2699-3827;
0000-0002-0258-7066; 0000-0003-1746-1455

[1] İncir Araştırma Enstitüsü Müdürlüğü, 09600 Aydın, Türkiye

Abstract

In this research, male fig (caprifig) genotypes collected within the scope of the project from Marmara, Mediterranean Regions, and mainly from the Aegean Region of Türkiye were evaluated according to some identification criteria for fig. Male fig trees produce in three different periods as Profichi (spring), Mammoni (summer), and Mamme (winter) fruits in a year. The most important of these are the Profichi fruits, which are used to ensure caprification in female figs. For the caprification process, Profichi fruits are hung on the female fig trees. Caprification occurs via fig wasp (*Blastophaga psenes* L.), which emerges from Profichi fruits. Quality characteristics and phenological observations of Profichi fruit was evaluated in this research. The average fruit weight of Profichi fruits is 7.7- 49.9 g; fruit width is 23.4-54.9 mm; fruit length is 27.7-59.7 mm. The number of fruits on the shoot was determined to be in the range of 2-10. The number of male flowers in Profichi fruits is 76-269, and the number of female (gall) flowers varied between 194-1193. In conclusion, this study has shown that there is a significant genetic diversity among domestic male figs in the Türkiye Fig Field Gene Bank.

Keywords: male fig, genetic resources, fig field gene bank, conservation

---------- * ----------

Erkek incir genetik çeşitliliği üzerine çalışmalar: erkek incir genetik kaynakları (*Ficus carica* var. *caprificus*) muhafaza ve karakterizasyonu projesi

Özet

Bu araştırmada, proje kapsamında Türkiye'nin ağırlıklı olarak Ege Bölgesi olmak üzere, Marmara ve Akdeniz Bölgelerinden toplanan erkek incir (caprifig) genotipleri, çeşit tanımlama kriterlerine göre değerlendirilmiştir. Erkek incir ağaçları, bir yıl boyunca ilek (bahar), ebe (yaz) ve boğa (kış) olmak üzere üç farklı periyotta meyve verir. Bunlardan en önemlisi ilek meyveleridir ve bu meyveler dişi incirlerde döllenmeyi sağlamak amacıyla kullanılır. Dişi incirlerde döllenme; ağaca file torbalar içinde asılan ilek meyvelerinden çıkan ilek arıcığı (*Blastophaga psenes* L.) vasıtasıyla gerçekleşir. Çalışmada genel olarak ilek meyve kalite özellikleri ve fenolojik gözlemleri değerlendirilmiştir. İlek meyvelerinin ortalama meyve ağırlığı 7.7-49.9 gr; meyve genişliği 23,4-54,9 mm; meyve büyüklüğü 27.7-59.7 mm ve sürgündeki meyve sayısının 2-10 aralığında olduğu belirlenmiştir. İlek meyvelerinde erkek çiçek sayısı 76-269; dişi (gal) çiçek sayısı ise 194-1193 arasında değişmektedir. Sonuç olarak; bu çalışma, Türkiye İncir Arazi Gen Bankası'nda bulunan yerel erkek incirler arasında büyük bir genetik çeşitlilik olduğunu ortaya koymuştur.

Anahtar kelimeler: erkek incir, genetik kaynaklar, incir arazi gen bankası, muhafaza

[*] *Corresponding author* / Haberleşmeden sorumlu yazar:Tel.: +902565811123-114; Fax.: +902565811124; E-mail:arzu.gocmez@tarimorman.gov.tr
 Geliş tarihi: 04.01.2023; Yayın tarihi: BioDiCon. xxx-xxxxxx

1. Introduction

Türkiye contains rich wild and cultivated fig forms. Male fig (caprifig) trees constitute one of the vaulable genetic resources in Türkiye. Caprifigs, used as pollinators, are essential to protect genetic resources and transfer the future. According to reference [3, 5], In the 1960s and 70s, by Ölçer (1968), Eroğlu (1982) and Aksoy et al. (1994), selection researches was initiated in Türkiye to develop new female fig varieties including pollinator figs. In 1960, 18 caprifig cultivars were evaluated according to phenological and pomological characteristics [17]. Eroğlu (1982) determined 58 caprifig genotypes in his selection study in the Aegean Region.

Fig Research Institute Directorate (Aydın/ Türkiye) is the institution primarily responsible for the conserving of caprifig genetic resources. It has aimed to conserve the caprifig cultivars and genotypes collected by survey and selection studies, etc., carried out in the fig growing regions of Türkiye, enrich the collection with new cultivars and genotypes, characterize them and provide genetic diversity for further breeding studies. The creation of the caprifig genetic resources plot began in the 1960s. As of 2022, the number of varieties and genotypes in caprifig genetic resources is 70 [2].

Characterization of male fig cultivars and genotypes, conserved ex-situ and in-situ due to selection and breeding studies, is accepted as a primary and essential step in carrying out any future breeding program [20].

Morphological and pollinator characteristics of 6 standard cultivars and 90 caprifig genotypes grown in the Eastern Mediterranean region of Türkiye were determined. In the principle component analysis (PCA), the number of female (gall) flowers per fruit; *Blastophaga*'s-exit time, pollen viability, and germination percentages; the amount of pollen per flower and fruit were taken as the prominent parameters. Significant morphological and pollinator variability was seen among caprifig genotypes. Mersin06 and Osmaniye02 genotypes were found to be parthenocarpic among 90 caprifig genotypes. In addition to their use as pollinators, it has been concluded that they are valuable genetic resources to include in breeding programs [5].

In the Fig Research Institute Directorate, Umurlu caprifig field gene bank, 12 superior caprifig were determined suitable pollinators in Sarılop and Bursa Siyahı fig cultivars. Fruit and pollen size, shape class, and surface structure were determined in 12 caprifig genotypes. It was determined that the Kızılay-1 cultivar had larger fruit (33.5g) and larger ostiole opening (6.0mm) compared to other genotypes. SEM images of pollen grains of all samples except Mıstık and Mor Demirtaş were characterized as suboblate. On the other hand, the Mor Demirtaş cultivar was the longest in terms of pollen length (11.25 µm), and the Yanako-2 cultivar with the largest pollen width (13.34 µm) and pori diameter (2.26 µm). All genotypes were determined 2-porate. PC1 and PC2 (PCA) defined 73% of the genotypes. According to cluster analysis, genotypes were divided into groups [4].

Pollen was collected from a total of 24 individuals, including 20 caprifig genotypes sampled from Adana, Hatay, Kahramanmaraş, Mersin, and Osmaniye provinces in the Eastern Mediterranean Region and 4 standard caprifig varieties from Aydın in the Aegean Region of Türkiye to determine the pollen characteristics. Polar length, equatorial diameter, colpus width, pollen shape, porate number, porate width, exine thickness, and abnormal pollen ratio were determined as the most important distinguishing criteria [6].

310 fig genotypes in the Fig Research Institute Directorate fig field gene bank collected from 6 geographic regions (including caprificus) were analyzed with 14 SSR loci. 7 similarities, 54 clone-level similarities, 36 synonyms, and 22 homonymous differences were detected [8].

As in Türkiye, studies on the collection, characterization, and conservation of genetic resources have been made and continue to be conducted in many countries with caprifig genetic diversity.

The National Clonal Germplasm Repository (NCGR) fig collection in Davis, California, currently consists of 190 species and 78 fruit varieties. It contains a selection from 44 different regions, 40 advanced selections from plant breeders, 28 caprifigs, and a few species and hybrids [21].

The lack of sufficient caprifig trees to pollinate female figs in Tunisia is considered an essential constraint for developing the fig industry. The study focused on characterizing 8 local caprifig cultivars grown in coastal and continental conditions [12]. In Tunisia, fruit set, yield, characteristics, and shoot length data of female figs were recorded and evaluated in 2008 and 2009 to determine the efficiency of the caprification on Zidi, Bidhi, and Bither Abiadh female fig cultivars [11]. The identical genotypes which were grown in both regions (terrestrial and coastal regions) were compared. It was determined that tree growth, yield, fruit characteristics, TSS, and earliness situations were different [11, 13, 22].

By crossing male and female figs that produce parthenocarpic fruit, hybrid individuals that produce parthenocarpic fruit at a rate of 50% can be obtained. For this purpose, caprifigs UCR 228-20, 271-I and 347, which produce parthenocarpic fruit, were used as parents in California and Israel [5, 21].

The productivity of three pollen sources in Kazerun, Iran, was evaluated to determine the most effective pollinator in female figs ('Payves' and 'Sabz' (Smyrna type- female figs)). As a result, fruit length, skin color, total soluble dry matter content (TSS), total phenolics, flavonoids, and anthocyanins were significantly affected by pollen sources. There was no significant effect of pollen source on fruit size, weight, ostiole opening, fruit ripening time, and antioxidant capacity [19].

In Spain, 15 reference samples were fingerprinted using 21 SSR markers, along with 42 lines corresponding to 2 caprifig and 12 local varieties. A total of 77 alleles were uncovered, detecting a beneficial level of genetic variability in local germplasm pools [18].

In Iran, 53 caprifig accessions were analyzed for phenotypic diversity using 32 morphological criteria. Significant phenotypic diversity was detected among caprifig accessions according to morphological characteristics. As a result of PCA it was determined that the top ten components that contributed the most to petiole length and thickness, leaf length and width, fruit width, fruit shape, central lobe length, number of lobes and leaf shape explained 76.09% of the total morphological variation [14].

In Tunisia, 20 caprifigs were analyzed using improved SSR markers. The 13 pairs of primers were found to amplify 37 alleles in the studied accessions. The results obtained showed a low genetic diversity in the studied figs. Samples from different geographical regions were analyzed. No clear grouping based on geographic origin has been observed, which suggests an extensive exchange of caprifig plant material via vegetative propagation [9].

In Tunisia, 53 morphological features and 4 pollen descriptors of caprifig were studied to select the most distinctive features. Except for the number of anthers/flower and pollen viability, there were significant differences between genotypes in almost quantitative traits. It was stated that 40 characters among 53 morphological features and 4 pollen descriptors showed good discrimination power and could be used to distinguish caprifig trees [10].

Identification was performed using molecular markers and morphological characters to analyze genetic diversity and related relationships between female and caprifig genotypes grown under the same environmental conditions in the Tunisia ex situ fig germplasm bank. This study showed that there is significant genetic diversity among local figs [1]. For the purpose of collection, conservation, caracterization, documentation and evaluation of wild caprifig genetic resources in Tirana, different populations were evaluated in the northwest, central, and southwest (3 regions) of the country. Genotypes with different fruit colors were found. The number of gall flowers was found to be about 800-1200, and the other flowers were found in the range of 300-400 [15].

The phenotypic variability of wild female fig and caprifig genotypes in Risso was evaluated. Significant variations were observed among figs. Due to the increasingly pronounced effects of caprifigs on fruit quality and phytochemical properties of female figs, it has been found important to conserve caprifig genetic materials not only for pollination but also for fig breeding studies [16].

Measurements, observations, and evaluations were made using the identification list developed by UPOV and IPGRI in all domestic and foreign caprifig and female fig varieties and genotypes to be conserved with the collection program in 9 locations and 26 sub-locations in the Kerkennah Archipelago of Tunisia. In particular, the research showed that it is imperative to pay attention to threatened and rare varieties. The conservation program in farmer's orchards was found to be important for the conservation of traditional knowledge, the rehabilitation of varieties, and sustainable agriculture. The region was stated to be a suitable alternative [7, 11, 13].

In this project, to enrichment of genotypes with new survey, handing them down to the next generations, and to conserving and keeping the documentation for use in future breeding programs, and to carrying out annual maintenance operations in the collection was sustainable important target.

This research aimed at metric and non-metric characterization of caprifig genotypes/varieties which conserved in Umurlu caprifig field gene bank.

2. Material and methods

Caprifig genotypes found in Fig Research Institute Directorate fig field gene bank constituted the study material (Figure 1).

70% of the male figs used as material were collected from Aydın and İzmir Provinces of the Aegean Region, 11% from Balıkesir Province of the Marmara Region, and 17% from Hatay, Osmaniye, Mersin and Adana Provinces of the Mediterranean Region in Türkiye.

Abalı İlek, Hamza İlek, Ak İlek, Elma İlek, Büyük Konkur, Ak Erkek 2, Taşlık, Bardakçı and Bardacık caprifigs were registered variety in the caprifig field gene bank [2] .

Average fruit weight (g), fruit size (mm) (width, length and ostiole opening), fruit number per shoot (pieces/shoot), amount of *Blastophaga psenes* L., amount of male and female (gall) flowers, harvest time (day), foliation date, the birth date and ripening time criterias of Profichi fruit were evaluated (Table 1).

Figure 1. Male fig (caprifig) field gene bank (planted date:2009)

Table 1. Some descriptions used in caprifig genotypes

Fruit number per shoot /tree	The fruits amount were counted and determined on shoots of the trees.
Harvest date / period	The date on which the Fig wasp, which comes out of the of the Profichi fruit, was seen, was taken as the harvest start date. The last date of Fig wasp emergence was determined as the last harvest date.
Blastophaga psenes L. (pieces)	5 Profichi fruits, were placed in glass jars covered with tulle. *Blastophaga psenes* L. amount which comes out of 5 fruits, was counted every day.
Amount of male and gall flowers	Male and gall flowers in 5 Profichi fruits were counted one by one.
Profichi fruit weight (g)	Fruits were weighed with a precision scale sensitive to 0.01 g and their fruit weights were determined in g. It was determined by dividing the total weight of fruits from each variety by the number of fruits.
Profichi fruit width-length (mm) and ostiole width (opening) (mm)	Determined in mm with digital caliper. Fruit width was determined by measuring from the equatorial region, which is the middle part of the fruit. Fruit length was found by measuring the distance between the ostiole and the point where the neck of the fruit meets the petiole.
Fruit cavity	If the inside is completely full; there is no gap; The gaps up to the lentil volume; very small, The gaps between the lentil-chickpea volume; small; The voids up to the chickpea volume; medium, The voids larger than the chickpea volume; large.
Fruit shape index	It is expressed as the ratio of fruit width to fruit length. this value; If <0.9 oblonge, = 0.9-1.1 globose, >1.1 fruit shape is oblate.

3. Results

The leafing date generally was observed when two-leaf emergence appears in 50% of the tree between March 09 and April 14.

The birth of the profichi fruits generally was observed between March 05 and April 08, when budding and leafing occured (Figure 2).

The ripening of profichi fruits generally coincides with the period when female figs became receptive. Generally, skin colour of Profichi fruit turns to bright and light green as a sign of maturity. The scales around the ostiole opening begin to open. This date is also on which the female fig wasp, which comes out of the Profichi fruit, was seen. Generally, this period was observed between June 10-27 (2015), May 29- June 19 (2016) and May 29- June 25 (2022). In general, Şeytan 1, Mıstık İlek, Bozdoğan Kaba İlek, Sarı İlek, Frenk, Kıbrıslı, Damarlı and Gabalı genotypes had matured profichi fruits the earliest among the caprifigs.

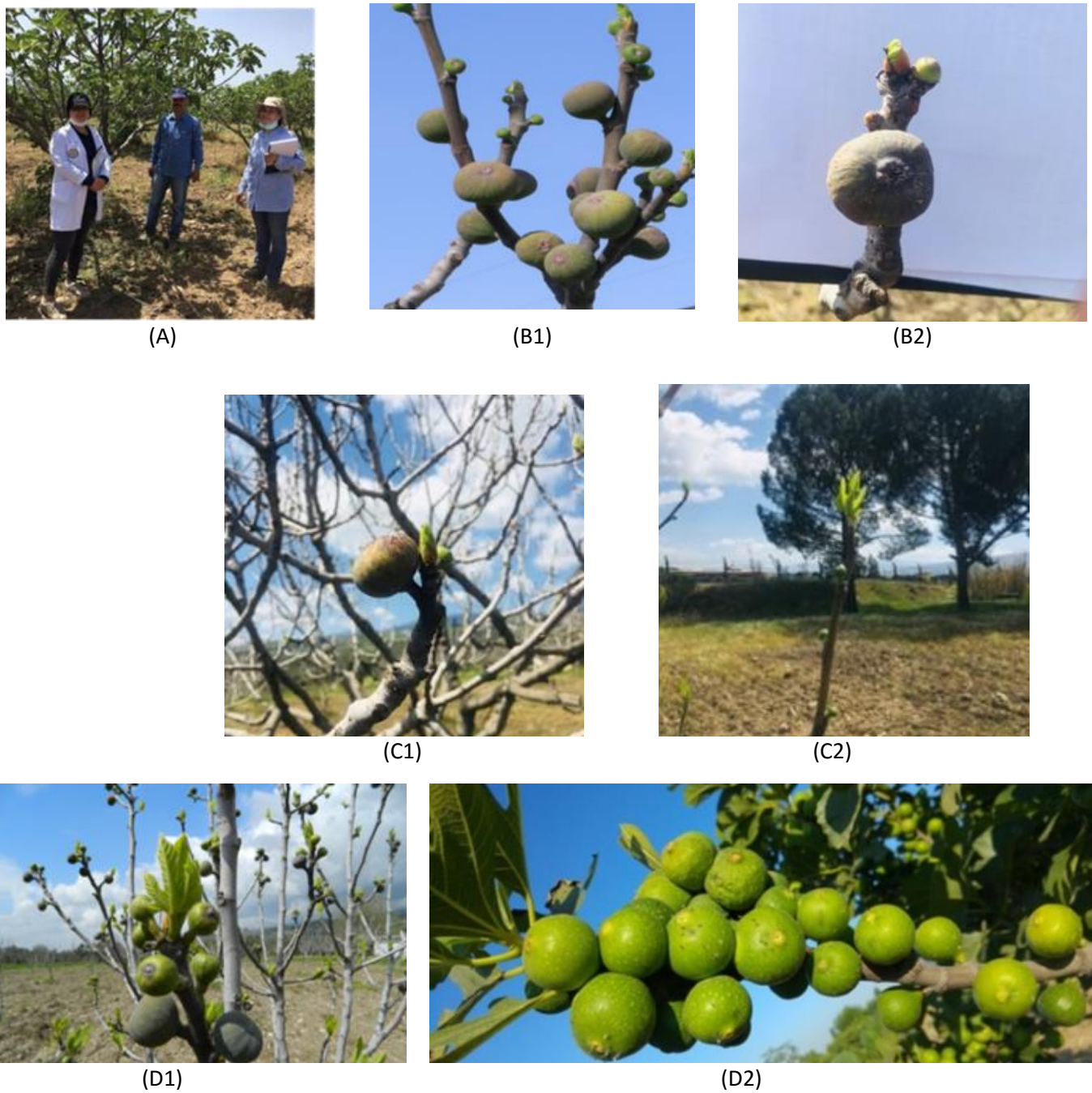


Figure 2. Phenological observation studies (A), mamme fruits on the branch (B1, B2), onset of leafing (C1, C2) and newly formed profichi fruits (D1, D2)

Generally, the average amount of profichi fruits on the shoot was detected between 2-10. It was counted as 3-8 (2015) and 2- 13 (2016) pieces/shoot.

The number of Mamme fruits on the caprifig tree varied according to years, genotype, tree age, and vigor. According to the phenological observations of 2021; approximately 100 or more in Bardakçı İlek, Hamza, Ak Erkek 1, Taşlık, Frenk, Mehmet Tosun genotypes, approximately 60-80 in Kızılay 1, Çaçaron, Şeytan 2, Çakın 1, Sarı İlek genotypes; It was determined in the range of 30-50 in Ömerbeyli Kaba İlek, Kuyucak İlek, Gabalı, Damarlı, Ak İlek, Aydın 2, Karabulut, Conkurt, Armut İlek, Kızılburun, Yanako 1, Adalı, Eşref 1, Eşref 2 genotypes. It was determined as 20 in other genotypes. The amount of mamme fruit is almost non-existent in Mor Demirtaş, Kara İlek, Hatay 20, Adana 10, Hatay 35, Mersin 05, Osmaniye 02, Hatay 13 and Adana 03 genotypes.

The fruit cavity of the genotypes was generally in the middle group. Harvest times generally were determined between 8 days (Aydın2, Ak Erkek 1, Körpe İlek) and 12 days (Ömerbeyli Kaba Ilek, Yanako 1).

The average fruit size values of some genotypes are given in Table 2 (2015-2022). Average fruit weights of Profichi fruits were determined in the range of 7.7 (oblonge) (Barbaros)- 49.4 g (globoose) (Hacı Yusuf). Profichi fruit width 23.4 (Osmaniye 02)- 54.9 (Ayar Dolduran); fruit lengths were determined in the range of 27.7 (Osmaniye 02) - 59.7 mm (Bozdoğan Kaba İlek). Fruit shape indexes were determined as 0.7 (Damarlı)- 1.1 (Hatay 35). Regarding the ostiole opening, Mersin 05 had the smallest (0.6 mm), and Hatay 06 had the largest (6.4 mm) ostiole opening.

Table 2. Fruit quality metric datas in some caprifig genotypes

Genotypes	Average fruit weight (g)	Average fruit width (mm)	Average fruit lengths (mm)	Average ostiole opening (mm)	Fruit shape indexes
Bostancı	31.5	46.2	50.7	3.1	0.91
Hacı Yusuf	49.4	53.7	57.9	5.9	0,93
Çakın-2	34.0	49.2	49.4	3.8	1.00
Damarlı	18.1	38.0	53.4	2.7	0.71
Aydın-2	18.3	38.6	43.9	3.6	0.88
Ak Erkek 1	24.5	40.7	48.9	2.9	0.83
Ayar Dolduran	43.7	54.9	55.3	3.3	0.99
Bozdoğan Kaba İlek	42.1	49.1	59.7	2.4	0.82
Yanako 1	21.3	41.7	46.9	3.7	0.89
Körpe İlek	25.6	41.1	52.1	3.3	0.79
Barbaros	7.7	27.8	36.7	2.2	0.76
Mehmet Tosun	14.9	33.9	37.7	3.5	0.90
Hatay 06	29.0	41.9	46.9	6.4	0.89
Hatay 35	23.7	43.5	41.0	0.7	1.06
Mersin 05	15.9	35.7	42.6	0.6	0.84
Osmaniye 02	8.1	23.4	27.7	2.9	0.85

The number of male flowers in profichi fruits was determined in the range of 76 (Barbaros)- 269 (Hacı Yusuf). Gall flower number was found in the range of 194 (Hatay 20) - 1193 (Bozdoğan Kaba Ilek). The gall flowers are generally located near the stalk of fruit. Male flowers are usually located near the ostiole opening of fruit (Figure 3).

During the Profichi fruit ripening period with the swelling of the scales around the ostiole opening, female *Blastophaga psenes* L., which has wings and can fly, to continue its generation comes out of the Profichi fruits. As it emerges from the ostiole opening, it rubs against the pollens of the male flowers. Pollens is moved to female fig fruits via fig wasp.

The amount of *Blastophaga psenes* L was determined in the range of 21 (M.Tosun)- 501 (Çakın 2). Fig wasp exit times was generally determined as 3- 4 days (Figure 4).

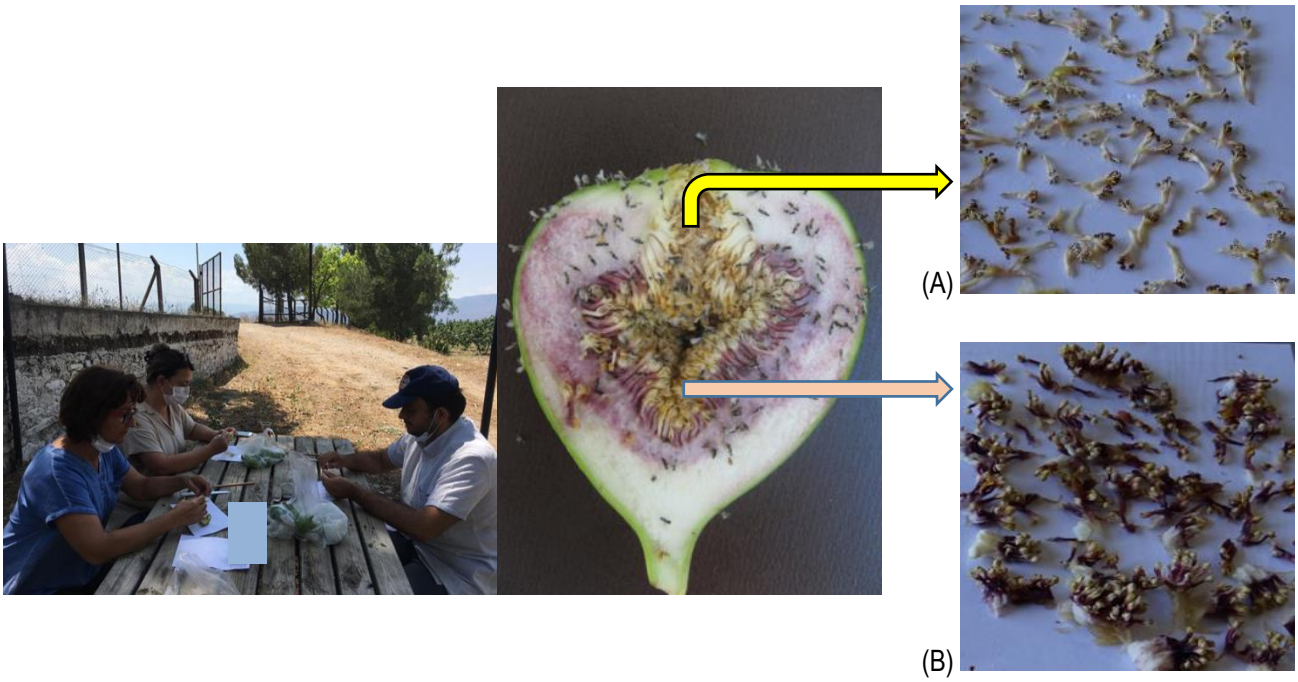


Figure 3. Detection of male flowers (A) and gall flowers (B) in fruit

Figure 4. Healthy profichi fruits, *Blastophaga pseneses* L. counting and emergence times observations

Every year, instead of the genotypes that dry out for any reason in the collection, their cuttings are taken, and their saplings are produced and planted. In this way, renewing the collection and continuing the conservation program in the project (Figure 5).

Figure 5. Production and planting of saplings of genotypes that are missing in the collection for any reason

In addition to the characterization and documentation information of the caprifig field gene bank to be passed on to future generations, the status of fruit sections, trees, and fruit branches of genotypes were recorded with photographs (Figure 6). As an example; the cross-section of the fruit, its condition on the branch and tree growth habit photos of some genotypes were given in Figure 7.

Figure 6**.** Photography and archive studies on male fig fruits and trees

Figure 7. Sections of fruit and fruit on the branch and tree growth habit photos of some caprifigs

4. Conclusion and discussion

In a different research, the first leafing dates were between March 10 and April 15 in 2015 under the conditions of the Eastern Mediterranean Region, and the fruiting of the Profichi fruit was between March 8 and April 16 [5].

It has been reported that some caprifig genotypes do not produce Mammoni (summer crop) and Mamme (winter crop) fruits on the tree or their numbers are low [5]. Similarly, in this research, it was observed that some genotypes did not produce/low produced Mamme fruit.

Some data ranges obtained from Profichi fruits by different researchers are given below.

Profichi fruit weight; 11.52-38.12 g [14]; 17.21-36.89 g [23]; 32 (Jrani)-29.5 (Dijebba2) g [12] and 8.96 (Kmaraş05)- 55.14 (Hatay02) g [5].

Profichi fruit width; 18.60-34.70 mm [14]; 37.54-50.25 mm [23] and 28.55 (Mersin15)- 60.66 (Hatay02) mm [5].

Profichi fruit length; 25.30-55.60 mm [14]; 40.54-64.04 mm [23] and 25.64 (Mersin15)- 67.68 (Hatay33) mm [5].

Ostiole opening; 5.80 (Caprifig1)-15 (Caprifig53) mm [14]; 6.6 (Assafri)- 8.1(Jrani) mm [12] ; 2.26- 5.80 mm [5] and 0.65-5.11 mm [23].

The number of Profichi fruits on the shoot; 5.71 (Jrani) - 6.32 (Assafri) pieces/20 cm [12] and 1.33 (Kmaraş06) - 9.70 (Hatay06) pieces/shoot [5].

Number of *Blastophaga*; 168-1,700 pieces/fruit (Eroğlu, 1982), 1350 pieces/fruit (Condit, 1947) [5]; 267 (Caprifig35)- 4 (Caprifig3) pieces/fruit [14]; 76 (Dijebba3)-450 (Assafri) (fertile galls per syconium counted) [12]; It was determined as 119-480 pieces/fruit in another study conducted in Hatay Province of Turkey [23].

Number of male flowers; 300-400 pieces/fruit [15]; 350 pieces/fruit (Condit, 1947), 6.0 (Kmaraş03)- 258.2 (Adana03) pieces/fruit and,

Number of female (gall) flowers; 75-450 pieces/fruit in a study conducted in Tunisia [12], 167.70 (Mersin16)-1121.2 (Hatay13) pieces/fruit, and it was stated that there is no female (gall) flower in Osmaniye02 genotype [5]; 244-771 [23]; 800-1200 pieces/fruit [15]. Flower pollen viability rates were found in the range of 64.99-91.53% [23]; 40.81%

(Mersin16)-100% (Kmaraş01) in different studies. The vitality rate of plants to be used as pollinator varieties is required to be >50% [5].

The caprifig genotypes in this research were generally examined in terms of phenological, pomological, etc. It can be said that their properties are generally similar when compared to the varieties in other studies.

Literature studies on male figs generally include studies on determining suitable pollinators in order to increase the yield and quality of female figs. However, as there is a need to develop new fig varieties in line with market needs changes day by day, the importance given to caprification studies is increasing. The fact that Turkish female fig genotypes need caprification reveals once again the importance of male figs. Provided that excessive caprification is avoided, caprification has an effect on improving fruit quality.

This project was referred to as an evaluation and research project in previous years. Therefore, male fig genetic resources also include studies conducted by different researchers. Therefore, some of the important studies carried out are given below as archive information.

310 fig genotypes in the Fig Research Institute Directorate fig field gene bank collected from 6 geographic regions (including caprificus) were analyzed with 14 SSR loci. 7 similarities, 54 clone-level similarities, 36 synonyms, and 22 homonymous differences were detected [8].

In conclusion some differences (1 identical [(Yanako 1 (57, Aydın) - Yanako 2 (59, Aydın)], 1 synonim [(Kara Ilek (28) - Kavun İlek (29)] and, 6 homonyms [(Kızılay 1 - Kızılay 2); (Şeytan 1 - Şeytan 2); (Çakın 1 - Çakın 2), (Ak Erkek 1 - Ak Erkek 2), (Kaba İlek (Bozdoğan) (53) - Kaba İlek (Ömerbeyli) (1)); (Büyük Konkur (34) - Küçük Konkur (38)] were found among caprifigs.

In terms of pollen amount, genotypes generally produced a moderate amount of pollen. The pollen germination power of the genotypes was found to be >78.90%. High pollen viability was found in Bostancı Ilek (96%) [4].

The vitality of caprificus genotypes in this project are found to be >50%.

The threat of genetic erosion poses a risk for caprifig genetic diversity. In reducing this risk, the field gene bank-collection orchards is significant in ensuring genotypes' sustainability and has become a universal corservation approach. In Türkiye, most female fig genotypes require caprification. The preservation and enrichment of the existing genetic richness and similar approaches are essential in developing high-quality fruit varieties in new breeding studies.

This research was carried out to collect, describe, protect, document and evaluate caprifig genetic resources. Also, it is an archive of fig gene sources. Rich genetic variation was found between genotypes in the metric and non-metric definitions of genotypes. The 'Fig (*Ficus carica* var *caprificus*) ‘The Genetic Resources Conservation and Characterization Project' is an ongoing project.

The collection of multiple data for the characterization of caprificus genetic resources in field conditions and the laboratory environment continues with similar to approaches and studies of other countries/researchers [4, 5, 6, 7, 11, 12, 13, 14, 15, 16, 19].

Acknowledgements

In this research, a part of 'Sarılop ve Bursa Siyahı İncir Çeşitlerine Uygun Tozlayıcıların Tespit Edilmesi Project final report' (2015-2018) (TAGEM/BBAD/14/A08/P07) data and ‘Fig (*Ficus carica* L.) Genetic Resources Conservation and Characterization Project’ (2015-.2022) (TAGEM/TBAD/Ü/20/A7/P9/1815) caprificus multiple data were used. About 70 caprifig genotypes were evaluated collectively in terms of metric and non-metric criteria.

References

[1] Aljane F., & Essid, (2018). Characterization and assessment of fig (*Ficus carica* L.) genetic resources in Tunisia: an overview. In Kallel A., Ksibi M., Dhia, H.B., Khelifi, N (Eds). A. Recent Advances in Environmental Science from the Euro-Mediterranean and Surrounding Regions, EMCEI 2017. Advances in Science, Technology & Innovation. (pp. 1355-1356) Springer, Cham, Switzerland.

[2] Ayar, A. (2022). İncir (*Ficus carica* L.) arazi gen bankaları ve çalışmaları. Biyolojik Çeşitlilik ve Koruma, 15 (3) , 308-319. DOI: 10.46309/biodicon.2022.1144871.

[3] Ayar, A., Seferoğlu, H. & Belge, A. (2022). Sarılop İncir Klonlarının Fenolojik Gözlem Verileri ve Ağaç Gelişim Özellikleri Yönünden Değerlendirilmesi. Uluslararası Anadolu Ziraat Mühendisliği Bilimleri Dergisi , 4 (2) , 42-53 . Retrieved from https://dergipark.org.tr/tr/pub/uazimder/issue/70074/1030007.

[4] Bilgin, AA., Mısırlı, A., Belge, A., & Özen, M. (2020). The pollen and fruit properties of *Ficus carica* caprificus. International Journal of Fruit Science 20(3), 1696-1705. DOI: 10.1080/15538362.2020.1828226

[5] Caliskan, O., Bayazid, S., Ilgin, M., & Karatas, N. (2017). Doğu Akdeniz Bölgesi doğal popülasyonundaki erkek incir (*Ficus carica* var. *caprificus*) genotiplerinin tanımlanması, moleküler karakterizasyonu ve muhafazası. TÜBİTAK TOVAG Proje No:114O034, Hatay, Türkiye.

[6] Caliskan, O., Bayazit, S., Kılıc, D., Ilgin, M., & Karatas, N. (2021). Pollen morphology and variability of caprifig (*Ficus carica* var. *caprificus*) genetic resources in Turkey using multivariate analysis. Scientia Horticulturae, 287, 110283. DOI: 10.1016/j.scienta.2021.110283

[7] Debbabi, O. S., Khanfir, E., Mohamed, A.D., & Massaoud, M.M. (2021). Ethnobotanical and on farm genetic surveys of Fig (*Ficus carica* L.) genetic resources in Kerkennah Islands. International Journal of Hortic. Sci. and Techology 8(2),153-163. DOI: 10.22059/ijhst.2020.310191.396

[8] Ergül, A., Büyük, BP., Hazrati, N. Yılmaz F., Kazan, K., Arslan, N., … & Çobanoğlu F. (2021). Genetic characterisation and population structure analysis of Anatolian figs (*Ficus carica* L.) by SSR markers. Folia Horticulturae. 33(1), 49–78. DOI: 10.2478/fhort-2021-0005

[9] Essid, A., F. Aljane, A. Ferchichi, & J.I. Hormaza. (2015). Analysis of genetic diversity of Tunisian caprifig (*Ficus carica* L.) accessions using simple sequence repeat (SSR) markers. Hereditas 2015, 152:1. DOI: 10.1186/s41065-015-0002-9

[10] Essid A., Aljane, F., & Ferchichi, A. (2017). Morphological characterization and pollen evaluation of some Tunisian ex situ planted caprifig (*Ficus carica* L.) ecotypes. South African Journal of Botany, 111, 134-143. DOI: 10.1016/j.sajb.2017.03.001.

[11] Gaaliche, B., Hfaiedh, L., Trad, M., & Mars, M. (2011). Caprification efficiency of three Tunisian fig (*Ficus carica* L.) cultivars. J. Nat. Prod. Plant Resour., 1(3), 20-25.

[12] Gaaliche, B., Zarrouk, I., & Mars, M. (2017). Agro-phenological behaviour of several caprifigs grown in two different ecological areas in Tunisia. Acta Hortic. 1173(1173), 149-156.DOI: 10.17660/ActaHortic.2017.1173.25

[13] Gaaliche, B., Saddoud, O., & Mars, M. (2012). Hindawi, International Scholarly Research Notices In: Xu, X., Koba, T., Aitken, K. (Academic Eds.) Morphological and pomological diversity of fig (*Ficus carica* L.) cultivars in Northwest of Tunisia. DOI: 10.5402/2012/326461

[14] Khadivi- Khub, A., & Anjam, K. (2014). Characterization and evaluation of male fig (caprifig) accessions in Iran. Plant Systematics and Evolution, 300, 2177–2189. DOI: 10.1007/s00606-014-1038-4

[15] Kokaj, T. (2018). Genotype identıfıcatıon of Roshnık Varıety. Agriculture University of Tirana/ Institute of Plant Genetic Resources. International Journal of Agriculture, Environment and Bioresearch, 3(06).136-145.

[16] Mirheidari, F., Khadivi, A., Moradi, Y., & Paryan, S. (2020). Phenotypic variability of naturally grown edible fig (*Ficus carica* L.) and caprifig (*Ficus carica* var. *caprifi*cus Risso) accessions. Scientia Horticulturae, 267(1).109320. DOI: 10.1016/j.scienta.2020.109320

[17] Öncel, H. (1969). İncir yetiştiriciliğinde ilek ve ilekleme. Ziraat İşleri Genel Müdürlüğü Yayınları, İstanbul, Türkiye.

[18] Perez-Jiménez M., López B., Dorado G., Pujadas-Salvá A., Guzmán G., & Hernandez P. (2012). Analysis of genetic diversity of southern Spain fig tree (*Ficus carica* L.) and reference materials as a tool for breeding and conservation. Hereditas, 149 (3), 108-113. DOI: 10.1111/j.1601-5223.2012.02154.x

[19] Pourghayoumi, M., Bakhshi, D., Rahemi, M., & Jafari, M. (2012). Effect of pollen source on quantitative and qualitative characteristics of dried figs (*Ficus carica* L.) cvs 'Payves' and 'Sabz' in Kazerun – Iran. Scientia Horticulturae, 147, 98-104. DOI: 10.1016/j.scienta.2012.08.026

[20] Rezq Salimia, B., Awad, M, Hamdan, Y., & Shtaya, M. (2013). Genetic variability of some Palestinian fig (*Ficus carica* L.) genotypes based on pomological and morphological descriptors. An-Najah Univ. J. Res. (N.Sc.). 27, 84-110

[21] Stover E., & Aradhya M. (2005-2008, May,16). Fig genetic resources and research at the US National Clonal Germplasm repository in Davis, California. III. International Symposium on Fig. Vilamouran, Algarve (Portugal). DOI: 10.17660/ActaHortic.2008.798.6.

[22] Trad, M., Gaaliche, B., Renard, C.M.G.C, & Mars, M. (2013). Plant natural resources and fruit characteristics of fig (*Ficus carica* L.) change from coastal to continental areas of Tunisia. Journal of Agricultural Research and Development.3(2), 022-025, Retrieved from http://www.e3journals.org.

[23] Yaman, S. & Çalışkan, O. (2016). Hatay'dan seçilmiş bazı erkek incir genotiplerinin (*Ficus carica* var.*caprificus*) tozlayıcı özellikleri. Anadolu. J. Agr. Bilim. 31:315–320. DOI: 10.7161/omuanajas.269983.

www.biodicon.com BIOLOGICAL DIVERSITY AND CONSERVATION

ISSN 1308-8084 Online ISSN 1308-5301 Print

Research article/Araştırma makalesi
DOI: 10.46309/biodicon.2023.1255384
16/3 (2023) 203-209

Determination of genetic diversity of *Astragalus* L. species distributed in Erzincan and its surroundings by ISSR method

Engin DAĞ[1], Nalan YILDIRIM DOĞAN [*1], Mustafa KORKMAZ[1]
ORCID: 0000-0002-6302-7222; 0000-0002-5344-5367; 0000-0001-6732-7874

[1]Erzincan Binali YILDIRIM University, Faculty of Arts and Science, Department of Biology, Erzincan, Türkiye

Abstract

In this study, plant samples of *Astragalus* L. genus were collected from 42 different locations of Erzincan in the vegetation period of 2021. As a result of the plant identification studies, 12 taxa (*A. pennatulus, A. compactus, A. aduncus, A. xylobasis, A. angustifolius* subsp. *angustifolius, A. karamasicus,A. kurdicus, A. cancellatus, A. eriocephalus* subsp. *Eriocephalus, A. lycius, A. microcephalus, A. lagurus)* were determined. 3 of them (*Astragalus pennatulus, Astragalus karamasicus, Astragalus lycius*) are endemic. The genetic relationship between the identified species was investigated with ISSR (inter-simple sequence repeat) molecular markers. 16 primers (UBC868, UBC817, UBC807, UBC812, UBC816, UBC825, UBC824, UBC830, UBC836, UBC840, UBC848, UBC855, UBS856, UBC841, UBC842, UBC61) were used in the ISSR method. According to the PCR results, the primers studied gave bands of 100-2000 bp in length. Polymorphism was calculated as 90.8% according to ISSR primers. Dendrograms were created using the ISSR data and the UPGMA method. While the highest genetic similarity was observed between *A. kurdicus* and *A. angustifolius supsp angustifolius* taxa, the lowest genetic similarity was observed between *A. angustifolius* subsp *angustifolius* and *A. pennatulus* taxa. These studied markers turned out to be suitable for characterizing genetic diversity between *Astragalus* species.

Keywords: *Astragalus*, Erzincan, genetic diversity, ISSR, taxonomy

---------- * ----------

Erzincan ve çevresinde yayılış gösteren Astragalus L. türlerinin genetik çeşitliliğinin ISSR yöntemi ile belirlenmesi

Özet

Bu çalışmada, 2021 yılının vejetasyon döneminde Erzincan'daki 42 farklı lokaliteden *Astragalus* L. cinsine ait bitki örnekleri toplanmıştır. Yapılan bitki tayini çalışmaları sonucunda 12 takson (*A. pennatulus, A. compactus, A. aduncus, A. xylobasis, A. angustifolius* subsp. *angustifolius, A. karamasicus,A. kurdicus, A. cancellatus, A. eriocephalus* subsp. *eriocephalus, A. lycius, A. microcephalus, A. lagurus)* belirlenmiştir. Bunlardan 3'ü (*Astragalus pennatulus, Astragalus karamasicus, Astragalus lycius*) endemiktir. Belirlenen türler arasındaki genetik ilişkiler ISSR moleküler markırları ile araştırılmıştır. ISSR (kısa dizi tekrarları arası) yönteminde 16 primer (UBC868, UBC817, UBC807, UBC812, UBC816, UBC825, UBC824, UBC830, UBC836, UBC840, UBC848, UBC855, UBS856, UBC841, UBC842, UBC61) kullanılmıştır. PCR sonuçlarına gore, çalışılan primerler 100-2000 bç uzunluğunda bantlar vermiştir. ISSR primerlerine göre polimorfizm % 90.8 hesaplanmıştır. ISSR verileri ile UPGMA yöntemi kullanılarak dendrogramlar oluşturulmuştur. En yüksek genetik benzerlik *A. kurdicus* ile *A. angustifolius supsp angustifolius* taksonları arasında gözlenirken, en düşük genetik benzerlik *A. angustifolius* subsp *angustifolius* ile *A. pennatulus* taksonları arasında gözlenmiştir. Çalışılan bu markerlar *Astragalus* türleri arasındaki genetik benzerliği karakterize etmede uygun markırlar olduğunu ortaya çıkarmıştır.

Anahtar kelimeler: *Astragalus*, Erzincan, genetik çeşitlilik, ISSR, taksonomi

[*] *Corresponding author* / Haberleşmeden sorumlu yazar: Tel.: +904462243032; Fax.: +904462243016; E-mail: : nyildirim@erzincan.edu.tr
 Geliş tarihi: : 23.02.2023; Yayın tarihi: 15.12.2023 BioDiCon. 1087-230223

1. Giriş

Bitki zenginliği ve endemizm bakımından Doğu Anadolu bölgesi Türkiye'nin önemli bölgeleri arasındadır. Doğu Karadeniz, Doğu Anadolu ve Orta Anadolu coğrafi bölgeleri arasında köprü konumunda olan Erzincan ili, zengin florası ile Türkiye'nin önemli alanları arasında gelmektedir [1]. Erzincan, Türkiye'nin 13 endemik bitki merkezinden 2'sine ve 6 önemli bitki alanına sahiptir [2]. Erzincan'da çok fazla mikroiklim görülmesi, dağların uzanış yönleri ve oluşma şekillerinden dolayı farklı habitatlara sahip olması nedeniyle bitki çeşitliliği açısından oldukça zengin bir bölgededir. Bölge Avrupa-Sibirya ve İran-Turan fitocoğrafi bölgelerinin kesişim noktasında ve Anadolu Çaprazı'nın (Anadolu Diyagonali) geçiş noktaları üzerinde bulunmaktadır. Bu durum Erzincan'ın birçok endemik türe ev sahipliği yapmasına imkan sağlamıştır [3].

Astragalus L. Dünya üzerinde yaklaşık 3000 taksonu ile Fabaceae (Baklagiller) familyasının en büyük cinsidir[4, 5]. Türkiye'de de *Astragalus* en büyük cins olup cinse ait türler genel olarak İran –Turan bölgesinde yayılış göstermektedirler. Bu bölge *Astragalus* için çeşitlilik merkezi olarak bilinmektedir. Ülkemizde Geven olarak bilinen *Astragalus* cinsinin 485 taksonu bulunmaktadır. Bu taksonların içerisinde 224 tanesi endemik olup, endemizim oranı % 47'lerdedir [6]. *Astragalus* türleri günümüzde çok çeşitli amaçlar ile kullanılmakta olup çeşitli faydaları bulunmaktadır. Ülkemizin İç Anadolu ve Doğu Anadolu bölgelerinde *Astragalus* L. köklerinden hazırlanan sıvı karışım kanser ve yara iyileştirmede, geleneksel olarak tedavilerde kullanılır. *Astragalus*'un birkaç türü şifalı bitkiler olarak bilinir. Bu türler; analijezik, ağrı dindirici, mide ve bağırsak iltihabı ve tansiyon gibi hastalıklarda kulanılmaktadır. Örneğin *Astragalus gummifer* L. mukoza üzerine koruyucu etkisinden dolayı boğaz enfeksiyonlarında etkilidir [7]. Aynı zamanda bazı *Astragalus* türleri yakıt, sıvı yağlar, kereste, lif, gübre, bazı kimyasal maddelerin üretiminde ve bahçecilik alanında kullanılmaktadır [8]. Ayrıca insanlar ve hayvanlar tarafından gıda maddesi olarak kulllanılan türleride vardır. Bazı türleri ilaç ve süs bitkisi olarak kullanılmaktadır [9]. Erzincan çevresinde yapılan bazı etnobotanik çalışmalarda da bazı *Astragalus* türlerinin yöresel kullanımlarına yer verilmiştir. Yörede *Astragalus microcephalus* Willd. gövde ve yapraklarından çay yapılarak iltihap sökücü ve tansiyon düşürücü olarak kullanıldığı, bitkinin yağı çıkarılarak mide ve solunum yolları rahatsızlıklarının, ağız yaralarının tedavisinde, kansere karşı ve yakacak olarak kullanıldığı bildirilmektedir. *Astragalus angustifolius* subsp. *pungens* ve *Astragalus plumosus* Lam. subsp. *kurugeanus* (Freyn & Bornm.) Ponert bitkileri yörede kozmetik ve tutkal yapımında kullanıldığı belirtilmekte, *Astragalus plumosus* Lam. subsp. *kurugeanus* (Freyn & Bornm.) ve *A. lagurus* Willd. taksonlarının arılar tarafından bal yapımında kullanıldığı ve bazı *Astragalus* taksonlarının hayvan yemi olarak kullanıldığı belirtilmektedir [10].

Astragalus cinsi, yıllık ve çok yıllık forma sahiptir. Morfolojik olarak otsu ve odunsu dikenli yastıklar şeklinde çeşitli gruplar içermektedirler. Bu kısa ömürlü bitkilerin yaşam alanı açık ve yarı kurak çöller, dağlık bozkırlar, Akdeniz bölgesi, Güneybatı Asya, Orta Asya ve Kuzey Amerika'nın dağlık alanlarıdır [11]. İklimin daha nemli ve daha soğuk olduğu Doğu Asya ve Kuzey Avrupa'da, *Astragalus* türleri nadir bulunmaktadır [4].

Astragalus türlerinin, morfolojik sinapomorfiler tarafından desteklenmeyen, ancak cinste uzun yıllardır uygulanan alt jenerik konseptle yakından eşleşen on bir ana dal oluşturduğu gösterilmiştir. Şu anda cinste bilinen bu ana dallar gayri resmi olarak *Glottis, Phaca, Hamosa, Trimeniaeus, Conturtoplicata, Ophiocarpus, Cercidothrix, Astracantha, Diholcos, Hypoglottis* ve *Neo-Astragalus* olarak adlandırılır [12].

Türlerin genetik çeşitliliğini belirlemek amacı ile birçok yöntem kullanılmaktadır. Fakat son yıllarda en çok tercih edilen ve güvenilir sonuçlar veren tekniklerin başında moleküler markır yöntemleri gelmektedir. Büyük bir organizasyona sahip olan bitki genomlarının genetik çeşitliliği moleküler markır yöntemleri kullanılarak araştırılmaktadır. Moleküler markırlar bitki popülasyonları veya populasyon içindeki birey genotipleri arasındaki çeşitliliğin belirlenmesinde %100'e yakın güvenirlilikle sonuçlar vermektedir [13]. ISSR (kısa dizi tekrarları arası) yönteminde 2-5 arasında tekrarlanan nükleotitleri içeren primerler kullanılmaktadır. Tekniğin temel prensibi bu primerler kulanılarak iki mikrosatellit noktası arasındaki bölgenin çoğaltılmasıdır. ISSR yöntemi gen haritalama çalışmaları, genetik çeşitlilik ve taksonomik analizlerde kullanılabilmektedir [14].

Erzincan ve çevresinde daha önce yapılan floristik çalışmalarda, bölgenin *Astragalus* (Geven) taksonlarının yayılışı bakımından oldukça zengin olduğu belirlenmiştir [15]. Çalışmamızda Erzincan ve çevresinde yayılış gösteren A*stragalus* L. türlerinin genetik çeşitliliği ISSR yöntemi ile belirlenmesi hedeflenmiştir.

2. Materyal ve yöntem

2.1. Çalışmada kullanılan bitkisel materyal

Erzincan ve çevresinde 2021 yılı vejetasyon döneminde yürütülen arazi çalışmalarında *Astragalus* L. cinsine ait taksonların toplanması için birçok lokaliteye gidilerek bitki örnekleri toplanmıştır. Her bir tür için iki ayrı numune toplanmış, toplanan örnekler numaralandırılmış ve lokaliteleri ile beraber kayda alınmıştır. Çalışmamızın materyalini oluşturan bu bitki örneklerinin bir numunesi laboratuvar çalışmaları için -80º C derecede muhafaza edilmiş, diğer numunesi de tür teşhisleri için muhafaza edilmiştir. Örnekler bilinen herbaryum metodları uygulanarak preslenmiş, kurutulmuş ve teşhise hazır hale getirilmişlerdir. Daha sonra Doç. Dr. Mustafa KORKMAZ tarafında tür teşhisleri

yapılan bitki örnekleri Erzincan Binali Yıldırım Üniversitesi Fen Edebiyat Fakültesi Biyoloji Bölümü Herbaryumunda muhafaza altına alınmışlardır.

2.2. DNA izolasyonu ve ISSR protokolü

DNA izolasyonu Lin et al. [16]'e göre yapılmıştır. DNA örneklerinin miktar ve kalite tayini Nanodrop spektrofotometre cihazı kullanılarak gerçekleştirilmiştir. ISSR analizi için 16 primer (UBC868, UBC817, UBC807, UBC812, UBC816, UBC825, UBC824, UBC830, UBC836, UBC840, UBC848, UBC855, UBS856, UBC841, UBC842, UBC61) kullanılmıştır. ISSR-PCR analizi için master mix karışımı; 2 µl PCR buffer (10 x), 1.15 µl MgCl2 (50 mM), 0.3 µl mix dNTP (2 mM), 1 µl primer (60 ng/µl), 5 units Taq DNA polimeraz (0.20 µl), 14.05 µl steril saf su ile 20µl'ye tamamlanmıştır. Hazırlanan karışımlar kullanılan primer bağlanma sıcaklığına göre Bio-Rad C1000 Touch PCR cihazında Başlangıç denatürasyonu 1 döngü 95 ºC'de 2 dakika; 35 döngü 95 ºC'de 30 saniye denatürasyon, primer bağlanma sıcaklığında 60 saniye ve 72 ºC'de 120 saniye uzama; 1 döngü 72 ºC'de 5 dakika olarak yapılmıştır. PCR işlemi sonrası elde edilen ürünler agaroz jel elektroforezinde yürütülmüştür. ISSR yöntemi için agaroz jel yoğunlu % 1.5 olarak ayarlanmıştır. PCR örneklerinin yüklemesinin ardından elektroforez işlemi için 90 dakika boyunca 90 volta 60 amps de yürütülmüştür. Yürütme işleminin ardından görüntüleme cihazında görüntülenmiştir.

2.3. Veri analizi

ISSR primerlerinden elde edilen amplifikasyon ürünleri var (1) ya da yok (0) şeklinde değerlendirilmiş, veriler NTSYSpc 2.11V [17] (bilgisayar paket programında analiz edilmiştir. Çalışmada kullanılan genotipler için soyağaçları Jaccard [18] genetik benzerlik indeksine göre UPGMA yöntemi kullanılarak elde edilmiştir.

3. Bulgular

3.1. *Erzincan ve çevresinden örneklenen Astragalus Türleri*

Çalışmamızda Erzincan ve çevresinden toplanan *Astragalus* L. cinsine ait taksonlar herbaryum tekniklerine uygun olarak preslenerek kurutulmuştur. Daha sonra teşhis çalışmaları yapılmıştır. Çalışmalar sonucu belirlenen türler ve taksonomik özellikleri Tablo 1' de verilmiştir.

Tür İsmi	Ömür	Rakım	Herb.Kodu	Lokalite	Habitat	Elementi	Endemizm
Astragalus microcephalus	Çok Yıllık	2050 m	EBYU-E.D. 10	Ergan Dağı, kayak tesisi, 2. istayon. civarı	Kayalık alanlar	İran-Turan	
Astragalus kurdicus	Çok Yıllık	2293 m	EBYU-E.D. 7	Erzincan, Çayırlı, dağ yolu 32. km	Yamaçlar	İran-Turan	
Astragalus lycius	Çok Yıllık	2293 m	EBYU-E.D. 5	Erzincan, Çayırlı, dağ yolu 32. km	Kireçli step alanlar	Bilinmiyor	Endemik [21]
Astragalus cancellatus	Çok Yıllık	2280 m	EBYU-E.D. 4	Erzincan, Çayırlı, Yaylalar Köyü üstü, Aksu Deresi civarı	Dere yatağı kenarlıklar	İran-Turan	
Astragalus compactus	Çok Yıllık	1450 m	EBYU-E.D. 2	Ergan Dağı etekleri	Meyili step alanlar	İran-Turan	Endemik [22]
Astragalus pennatulus	Çok Yıllık	1550 m	EBYU-E.D. 11	Ergan Dağı etekleri, 5. Km	Meyili step alanlar	İran-Turan	Endemik [21]
Astragalus angustifolius subsp. *angustifolius*	Çok Yıllık	2100 m	EBYU-E.D. 9	Erzincan, Spikor Dağı	Zayıf vejetasyon gösteren step alanlar	Bilinmiyor	
Astragalus aduncus	Çok Yıllık	1450 m	EBYU-E.D. 12	Dedek Köyü yolu, Step	Akıntılı yamaçlar	İran-Turan	
Astragalus eriocephalus subsp. *eriocephalus*	Çok Yıllık	2250 m	EBYU-E.D. 1	Ergan Dağı, kayak tesisi, 2. istasyon civarı	Çayırlık step alanlar	İran-Turan	Endemik [22]
Astragalus karamasicus	Çok Yıllık	1850 m	EBYU-E.D. 3	Ergan Dağı, kayak tesisi, 1. istasyon civarı	Yol kenarı ve yamaçlar	İran-Turan	Endemik [21]
Astragalus lagurus	Çok Yıllık	1650 m	EBYU-E.D. 8	Binkoç Köyü üstleri	Yol kenarı step	İran-Turan	
Astragalus	Çok	2000 m	EBYU-	Erzincan, Spikor	Korunmuş	İran-Turan	Endemik [22]

xylobasis	Yıllık		E.D. 6	Dağı	alanlar		

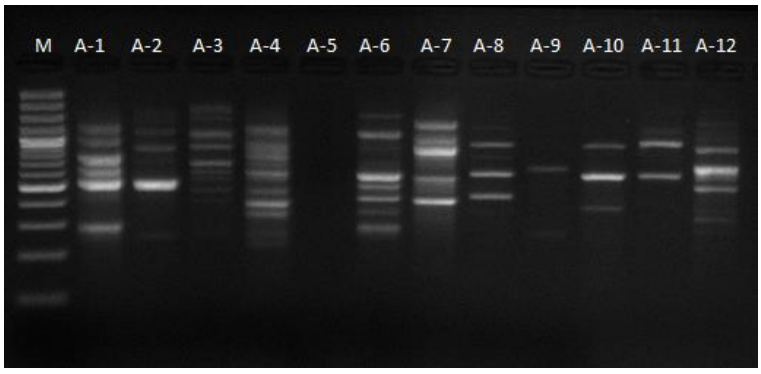
Tablo 1. Erzincan ve çevresinden toplanan *Astragalus* cinsine ait taksonlar

3.2. ISSR PCR analizi

ISSR-PCR tekniğinde 12 *Astragalus* türü üzerinden 16 ISSR primeri (UBC868, UBC817, UBC807, UBC812, UBC816, UBC825, UBC824, UBC830, UBC836, UBC840, UBC848, UBC855, UBS856, UBC841, UBC842, UBC861) çalışılmıştır. Bant büyüklükleri 100-2000 bç arasında gözlenmiştir. Toplamda 141 bant elde edilirken, polimorfik bant sayısı 128 ve yüzde polimorfizm % 90.8 olarak hesaplanmıştır. En fazla bant veren primerler UBC848, UBC836, UBC817 (12 bant) olurken, en düşük bant veren primer UBC824 nolu primer (3 bant) olmuştur. ISSR primerlerinin meydana getirdği bant büyüklüğü, bant sayısı ve polimorfizm oranları Tablo 2.' de verilmiştir. UBC848 primerine ait jel görüntüsü Şekil 1. de verilmiştir.

Tablo 2. ISSR primerlerinin meydana getirdiği bant büyüklüğü, bant sayısı ve polimorfizm

Primer adı	Molekül büyüklüğü (bç)	Toplam bant sayısı	Polimorfik bant sayısı	% Polimorfizm
UBC807	600-2000	9	8	88.8
UBC812	400-1500	8	8	100
UBC816	500-1500	8	7	87.5
UBC817	300-2000	12	10	83.3
UBC824	900-1500	3	3	100
UBC868	100-400	5	4	80
UBC825	400-1500	11	10	90.9
UBC830	250-1000	9	8	88.8
UBC836	150-1200	12	10	83.3
UBC840	200-1500	11	10	90.9
UBC848	250-2000	12	12	100
UBC855	300-1200	10	9	90
UBC856	200-800	6	5	83.3
UBC841	200-2000	11	11	100
UBC842	100-1000	9	8	88.8
UBC861	250-500	5	5	100
Toplam	100-2000	141	128	90.8



Şekil 1**.** UBC848 primerine ait jel görüntüsü

-1 *A. microcephalus*

-2 *A. lagurus*

-3 *A. aduncus*

-4 *A. xylobasis*

-5 *A. angustifolius* subsp. *angustifolius*

-6 *A. compactus*

A-7 *A. pennatulus*

A-8 *A. karamasicus*

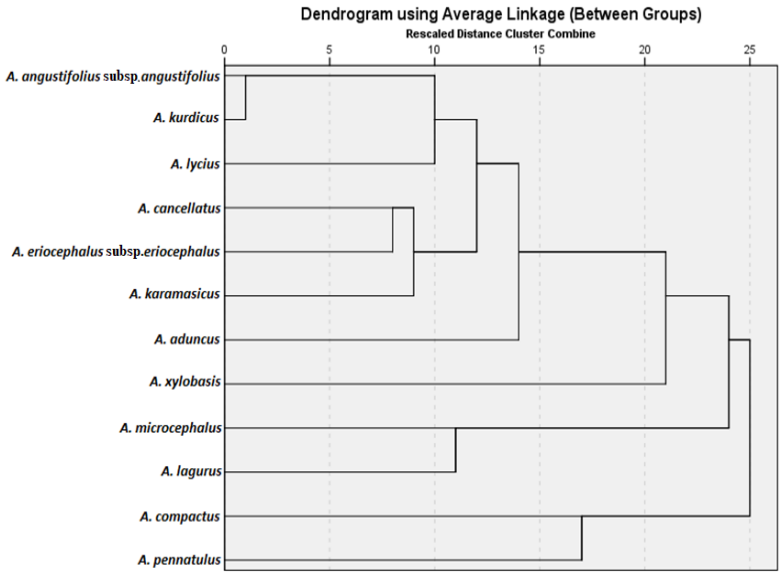
A-9 *A. kurdicus*

A-10 *A. cancellatus*

A-11 *A. eriocephalus* subsp. *eriocephalus*

A-12 *A. lycius*

Jaccard [18] genetik benzerlik indeksine göre UPGMA yöntemi kullanılarak elde edilen dendrogram Şekil 2'de verilmiştir. Dendrogram verilerine göre çalışılan türler 2 grupta toplanmıştır. 1. Grupta *A. pennatulus* ve *A. compactus* yer alırken, 2. Grup 2 alt gruba ayrılmıştır. 1. Alt grupta *A. aduncus, A. xylobasis, A. angustifolius* subsp. *angustifolius, A. karamasicus,A. kurdicus, A. cancellatus, A. eriocephalus* subsp. *eriocephalus ve A. lycius* yer alırken, 2. Alt grupta *A. microcephalus* ve *A. lagurus* yer almıştır. En yüksek genetik benzerlik *A. kurdicus* ile *A. angustifolius supsp angustifolius* türleri arasında olurken, en düşük genetik benzerlik *A. angustifolius* subsp *angustifolius ile A. pennatulus* arasında olmuştur.



Şekil 2. ISSR yöntemi ile elde edilen Erzincan ve çevresinde yayılış gösteren 12 *Astragalus* türü arasındaki genotipik bağlantı

Çalışılan türlerde yapılan genetik benzerlik indeksine göre *A. angustifolius* subsp *angustifolius* ile *A. kurdicus* arasındaki genetik benzerlik 0.09 olurken, *A. angustifolius* subsp *angustifolius* ile *A. pennatulus* arasındaki genetik benzerlik 0.967 olarak hesaplanmıştır.

4. Sonuçlar ve Tartışma

Dünya'da ve Türkiye'de *Astragalus* taksonlarının farklı özelliklerini ortaya koymak amacıyla çok sayıda çalışma yapılmıştır. Bunlardan bazıları taksonomik içerikli [19, 20], fenolojik bileşik ve biyoaktivite içerikli [21], kullanım alanları ve önemi [7], karyolojik [22] ve anatomik, palinolojik [23] çalışmalardır. Floristik bakımdan oldukça zengin olan ülkemiz, jeolojik yapısı, coğrafik konumu ve topoğrafik yapısı ile her çeşit toprak türünü bulundurmaktadır. Çeşitli toprak yapısına sahip olması, farklı iklim türlerinin oluşması ve aynı zamanda İran-Turan kökenli bitki türlerinin gen merkezi olması ile çok değişik vejetasyon tiplerini barındırmaktadır [24, 25].

Yapılan bu çalışmada Erzincan ve çevresinde toplanan *Astragalus* L. türleri arasındaki genetik çeşitlilik ISSR moleküler markır yöntemi kullanılarak belirlenmiştir. Erzincan ve çevresinde *Astragalus* cinsine ait 42 farklı lokaliteden örnekler toplanmıştır. Bu örneklerden 3'ü endemik (*Astragalus pennatulus, Astragalus karamasicus, Astragalus lycius*) olmak üzere toplam 12 farklı takson belirlenmiştir. Bu taksonlar; *Astragalus microcephalus* (Erzincan-Ergan Dağı), *Astragalus kurdicus* (Erzincan-Çayırlı), *Astragalus lycius* (Erzincan-Çayırlı), *Astragalus cancellatus* (Erzincan-Çayırlı Yaylalar köyü), *Astragalus compactus* (Ezincan-Ergan dağı), *Astragalus pennatulus* (Erzincan-Ergan Dağı), *Astragalus angustifolius* subsp. *angustifolius* (Erzincan-Spikor Dağı), *Astragalus aduncus* (Erzincan- Dedek köyü), *Astragalus eriocephalus* subsp. *eriocephalus* (Erzincan-Ergan Dağı), *Astragalus karamasicus* (Erzincan-Eran Dağı), *Astragalus lagurus* (Erzincan-Binkoç köyü), *Astragalus xylobasis* (Erzincan- Spikor Dağı) olarak teşhis edilmiştir. Çalışılan 12 taksondan ikisi (*Astragalus microcephalus* ve *Astragalus compactus*) sinonim olmuşlardır. Bu taksonların geçerli isimleri *Astragalus microcephalus* Willd. subsp. *microcephalus* ve *Astragalus compactus* Lam. subsp. *compactus* olmuştur. Çalışılan taksonların endemizm durumları araştırıldığında TÜBİVES (Türkiye Bitkileri Veri Servisi) [26] kaynağına göre altı tür (*Astragalus pennatulus, Astragalus karamasicus, Astragalus lycius, Astragalus eriocephalus* subsp. *eriocephalus, Astragalus compactus* ve *Astragalus xlobasis*) endemik olarak verilmesine karşın, Bizim Bitkiler [27] adlı sitede üç tür (*Astragalus pennatulus, Astragalus karamasicus, Astragalus lycius*) endemik olarak verilmektedir.

Jie vd. [28]. tarafından 2019 yılında yapılan çalışmada *Astragalus membranaceus*'un 8 populasyonu arasındaki genetik ilişkiyi analiz etmek için ISSR yöntemini kullanmışlardır. UPGMA kümelemesinin sonuçlarına göre 2 grup oluşturmuşlardır. Bu çalışmada *A. membranaceus'* un genetik çeşitliliğini belirlemede ISSR yönteminin uygun olduğunu göstermişlerdir. Baykal Gölü bölgesine özgü nadir bir tür olan *Astragalus sericeocanus*'un altı popülasyonunda genetik çeşitlilik ve popülasyonun genetik yapısını değerlendirilmek amacı ile ISSR belirteçleri kullanıldığı çalışmada Mantel (Nathan Mantel)'in testi ile *A. sericeocanus* popülasyonları arasında genetik uzaklık ile coğrafi uzaklık arasında anlamlı bir ilişki görülmüştür [29]. Kayseri'nin Erciyes Dağı'nda yetişen, nesli kritik tehlike altında olan endemik tür *A. argaeus*'un 4 populasyonundaki genetik çeşitliliği tespit etmek için ISSR belirteçleri kullanılmıştır [30]. Aynı şekilde 2011 yılında İspanya'nın güneydoğusunda endemik ve nesli tükenmekte bir tür olan *Astragalus nitidiflorus*' un 5 populasyonundaki genetik çeşitliliği ve populasyon yapısını değerlendirmek için yapılan çalışmada ISSR belirteçleri kullanılmıştır. İstatistiksel sonuçlar, populasyon ve tür düzeyinde düşük bir genetik çeşitlilik olduğunu gösterirken, populasyonlar arasında ise düşük bir genetik farklılaşma seviyesi tespit etmişlerdir [31]. Çin'in farklı eyaletlerinden toplanan *Astragalus sinicus* L.'nin 22 türü arasındaki genetik çeşitliliği belirlemek amacı ile ISSR belirteçlerinin kullanıldığı çalışmada UPGMA analiz sonuçlarına göre *Astragalus sinicus* L.'nin 22 türünün 4 farklı gruba ayrıldığı saptanmıştır [32].Sonuç olarak yapılan literatür araştırmasında, Erzincan ve çevresinde *Astragalus* türleri ile ilgili genetik çeşitlilik üzerinde bir çalışma bulunmamıştır. Bu çalışma ile bölgedeki *Astragalus* türleri arasındaki genetik çeşitlilik ISSR yöntemi kullanılarak detaylı bir şekilde araştırılmış ve bu yöntemin *Astragalus* türleri üzerinde genetik çeşitliliği belirlemede ideal bir yöntem olduğu tespit edilmiştir.

Kaynaklar

[1] Yücel, C. (2002). Turizmde yükselen değer: Ekoturizm. *TÜRSAB Dergisi*, 219, 1-7.

[2] Korkmaz, M., Özçelik, H., Kandemir, A., & İlhan, V. (2013). Erzincan ve çevresinde yayılış gösteren doğal Gül (Rosa L.) taksonları. *Süleyman Demirel Üniversitesi Fen Bilimleri Enstitüsü Dergisi*, 17(1), 49-59.

[3] Kandemir, A., Sevindi, C., Korkmaz, M., & Çelikoğlu, Ş. (2015). Erzincan Türkiye'a özgü endemik bitki taksonlarının IUCN tehdit kategorileri. *Bağbahçe Bilim Dergisi*, 2(1), 43-65.

[4] Podlech, D. (2013). Zarre Sh. A taxonomic revision of the genus Astragalus L.(Leguminosae) in the Old World. Vienna, 1.

[5] Fırat M., 2005. "Contributions To The Check-List Of Astragalus Species in Van, Turkey ", *International Symposium 7 th Plant Life of South West Asia,* Eskişehir, Türkiye, 25 Haziran 2005 - 29 Haziran 2007, pp.65-65.

[6] Chamberlain, D. F., & Matthews, V. (1970). Astragalus L. Flora of Turkey and the East Aegean islands, 3, 49-254.

[7] Kadıoğlu, B., Kadıoğlu, S., & Turan, Y. (2012). Gevenlerin (Astragalus sp.) farklı kullanım alanları ve önemi. *Alinteri Journal of Agriculture Science*, 14(1), 17-26.

[8] Lewis, G., Schrire, B., Mackinder, B., & Lock, M. (2005). Legumes of the world. Royal botanic gardens. Kew, Surrey, UK.

[9] Seçmen, Ö., Gemici, Y., Gök, G., Bekât, L., & Leblebici, E. (2008). Tohumlu Bitkiler Sistematiği, *Ege Üniversitesi Fen Fakültesi Kitaplar Serisi*, No: 116, 286.

[10] Korkmaz, M., Karakuş, S., Selvi, S., Çakılcıoğlu, U. (2016). Traditional knowledge on wild plants in Üzümlü (Erzincan-Turkey). *Indian Journal of Traditional Knowledge*,15(4), 538-545.

[11] Podlech, D. (1986). Taxonomic and phytogeographical problems in Astragalus of the Old World and South-West Asia. *Proceedings of the Royal Society of Edinburgh, Section B: Biological Sciences*, 89, 37-43.

[12] Azani, N., Babineau, M., Bailey, C. D., Banks, H., Barbosa, A. R., Pinto, R. B., ... & Zimmerman, E. (2017). A new subfamily classification of the Leguminosae based on a taxonomically comprehensive phylogeny: The Legume Phylogeny Working Group (LPWG). Taxon, 66(1), 44-77.

[13] Gülşen, O., & Mutlu, N. (2005). Bitki biliminde kullanılan genetik markırlar ve kullanım alanları. *Alatarım*, 4(2), 27-37.

[14] Başçiftçi, Z., Olgun, M., Sunar, S., Yıldırım, N., Ayter, N., & Ağar, G. (2021). Determination of genetic divergence in some bread wheat varieties by IRAP and ISSR analyses. *Biyolojik Çeşitlilik ve Koruma*, 14(1), 35-39.

[15] Korkmaz, M., & Karacan, S. (2015). Erzincan'ndaki bazı erozyonlu alanlar ve bu alanların dominant bitki türleri. *Biyoloji Bilimleri Araştırma Dergisi*, 8(2), 12-23.

[16] Lin, Z. X., He, D., Zhang, X. L., Nie, Y., Guo, X., Feng, C., & Stewart, J. M. (2005). Linkage map construction and mapping QTL for cotton fibre quality using SRAP, SSR and RAPD. *Plant Breeding*, 124(2), 180-187.

[17] Rohlf, F. J. (2004). NTSYSpc version 2.11 w. Applied Biostatistics. Inc. Port Jefferson, NY.

[18] Jaccard, P. (1908). Nouvelles recherches sur la distribution florale. Bull. Soc. Vaud. Sci. Nat., 44, 223-270.

[19] Ekici, M., Akan, H., & Aytac, Z. (2015). Taxonomic revision of Astragalus L. section Onobrychoidei DC. (Fabaceae) in Turkey. *Turkish Journal of Botany*, 39(4), 708-745.

[20] Castillón, E. E., González-Elizondo, M. S., & Quintanilla, J. Á. V. (2016). A new species of Astragalus (Fabaceae, Faboideae) from Durango, Mexico. *Phytotaxa*, 288(1), 91-95.

[21] Işik, N. (2012) Bazı Astragalus (Geven) L. türlerinin tohum yağ asidi kompozisyonlarının araştırılması (Master's thesis, Fen Bilimleri Enstitüsü).
[22] Martin, E., Karaman Erkul, S., & Aytaç, Z. (2015). Karyological studies on Oxytropis (Fabaceae) from Turkey. *Caryologia*, 68(4), 357-362.
[23] Özbek, F. (2012). Türkiyedeki Astragalus L.(Fabaceae) cinsine ait Uliginosi gray ve Ornithopodium bunge seksiyonları üzerinde anatomik ve palinolojik araştırmalar. *Fen Bilimleri Enstitüsü*, Yüksek Lisans Tezi, Ankara, 172s.
[24] Davis, P. H. (1970). Flora of Turkey and the East Aegean Islands. Vol. 3. Flora of Turkey and the East Aegean Islands. Vol. 3. Edinburgh University Press.
[25] Ekici, M., Aytac, Z., Akan, H., & Pinar, M. (2008). A new species of Astragalus L.(section Onobrychoidei DC.: Fabaceae) from Turkey. *Botanical Journal of the Linnean Society*, 157(4), 741-747.
[26] Tübives, 194.27.225.161, 06.08.2022.
[27] Bizim bitkiler. 'Damarlı Bitkiler', *Bizimbitkiler.org.tr*, 06.08.2022.
[28] Cui, J., Wang, D., Wang, Q., Chen, T., Zhang, F., & Qin, X. (2019). ISSR system optimization and genetic diversity analysis of Astragalus membranaceus produced in Shanxi and Inner Mongolia. *Natural Product Research and Development*, 31(11), 1941-1948.
[29] Selyutina, I. Y. E., Konichenko, E. S., Dorogina, O. V., & Sandanov, D. V. (2016). Genetic diversity of the endangered endemic milkvetch Astragalus sericeocanus Gontsch., Fabaceae from the Lake Baikal region. *Biochemical Systematics and Ecology*, 68, 163-169.
[30] Atasagun, B., Aksoy, A., & Gürcan, K. (2018). Genetic diversity in Astragalus argaeus, a critically endangered species from Turkey. *Biologia*, 73, 927-936.
[31] Vicente, M. J., Segura, F., Aguado, M., Migliaro, D., Franco, J. A., & Martínez-Sánchez, J. J. (2011). Genetic diversity of Astragalus nitidiflorus, a critically endangered endemic of SE Spain, and implications for its conservation. *Biochemical Systematics and Ecology*, 39(3), 175-182.
[32] Zhang, H., Jichen, C. H. E. N., & Xinjian, L. I. N. (2014). Genetic Variability of Astragalus sinicus L. Based on ISSR Markers. *Agricultural Science & Technology*, 15(8).

www.biodicon.com BIOLOGICAL DIVERSITY AND CONSERVATION

ISSN 1308-8084 Online ISSN 1308-5301 Print

Research article/Araştırma makalesi
DOI: 10.46309/biodicon.2023.1263014
165/2 (2023) 210-217

Investigation of climate and vegetation change in Göbekli Tepe (Türkiye) region using pollen data

Abdulkadir GÜZEL[1], Arif PARMAKSIZ [*2], Mehmet ÖZCANLI[1]
ORCID: 0000-0002-4168-4803; 0000-0003-0321-8198; 0000-0003-2228-8298

[1] Harran University, Faculty of Science-Literature, Department of Geography, 63290 Şanlıurfa, Türkiye
[2] Harran University, Faculty of Science-Literature, Department of Biology, 63290 Şanlıurfa, Türkiye

Abstract

Today, it is known that there is a change in the world's climate system and the living things that cause the deterioration of the natural balance are human beings. Human beings have always made changes in the environment they live in to meet their nutritional and shelter needs. These changes have affected the habitat in the region over time, causing both flora and fauna to change and have affected the level of biodiversity. In order to understand this change, pollen records from past periods are valuable archives of vegetation dynamics and provide important information about vegetation. In this study, pollen belonging to the ancient period was investigated from the wells drilled in near Göbekli Tepe, which is described as the "zero point of history". Wells were dug from 3 different localities and samples were taken from different depths. Pollen was found in the 1st and 3st wells, but no pollen was found in the 2st well. The pollen analyzes obtained from the samples in the 1st well between 6 meters and 7-7.5 meters were approximately B.C. It covers the years 11126 and 13354. The pollen analyzes made at 6, 10, 12 meters in the samples in the 3rd well were B.C. It reflects the changes in vegetation and climate between 10634-12418. Although individuals belonging to *Quercus, Salix, Juglans, Abies, Pinus* and *Juniperus* species of forest vegetation were encountered in Ancient Göbekli Tepe, no individuals belonging to these tree species were encountered today. In this study, data were obtained supporting that the region was forest-steppe in the past, and the presence of forest-forming taxa *Juglans, Abies, Pinus*, and *Juniperus* was determined for the first time and the vegetation of the past period was made more evident. The information obtained in this study shows that there is a significant change in the region and will be a source for detailed studies to be done in the future.

Key words: Göbekli Tepe, human impact, oak steppe-forest, pollen

---------- * ----------

Göbekli Tepe (Türkiye) bölgesinin iklim ve bitki örtüsü değişiminin polen verileri kullanılarak araştırılması

Özet

Günümüzde dünya iklim sisteminde bir değişim olduğu ve doğal dengenin bozulmasına neden olan canlıların insanoğlu olduğu bilinmektedir. İnsanlar, beslenme ve barınma ihtiyaçlarını karşılamak için yaşadıkları çevrede her zaman değişikler yapmışlardır. Bu değişiklikler zaman içerisinde bölgedeki habitatı etkileyerek hem floranın hem de faunanın değişmesine neden olmuş ve biyoçeşitlilik düzeyini etkilemiştir. Bu değişimin anlaşılması için geçmiş dönemlere ait polen kayıtları, bitki örtüsü dinamiklerinin değerli arşivleridir ve bitki örtüsünün hakkında önemli bilgiler sağlamaktadır. Bu çalışmada "tarihin sıfır noktası" olarak nitelendirilen Göbekli Tepe bölgesinde açılan kuyulardan eski döneme ait polenler araştırılmıştır. 3 farklı lokaliteden kuyular kazılmış ve farklı derinliklerden örnekler alınmıştır. 1. ve 3. kuyularda polenlere rastlanmış olup, 2. kuyuda polen bulunmamıştır. 1. kuyudaki örneklerinden 6 m ve 7-7.5 m'ler arasında elde edilen polen analizleri yaklaşık G.Ö. 11126 ve 13354 yıllarını kapsamaktadır. 3. kuyudaki örneklerinde 6, 10, 12 m'lerde yapılan polen analizleri G.Ö. 10634-12418 yılları arasındaki vejetasyon ve iklimdeki değişimleri yansıtmaktadır. Orman bitki örtüsüne ait ağaçlardan *Quercus, Salix, Juglans, Abies, Pinus* ve *Juniperus* türlerine ait bireylere Eski Dönem Göbekli Tepe'de rastlanmış olmasına rağmen günümüzde bu ağaç cinslerine ait hiç bir bireye rastlanmamıştır. Bu çalışmada bölgenin geçmiş dönemde orman-

[*] *Corresponding author* / Haberleşmeden sorumlu yazar: Tel.: +904143183562; Fax.: +904143183541; E-mail: aprmksz@gmail.com
 Received: 10.13.2023; Published: 15.12.2023 BioDiCon. 1088-100323

bozkır olduğunu destekler şekilde veriler elde edilmiş ve orman oluşturan *Juglans*, *Abies*, *Pinus*, ve *Juniperus* taksonlarının varlığı ilk defa belirlenerek geçmiş döneme ait bitki örtüsü daha da belirgin bir hale getirilmiştir. Bu çalışmada elde edilen bilgiler bölgede önemli bir değişimin olduğunu göstermekte olup, ileride yapılacak detaylı çalışmalar için bir kaynak teşkil edecektir.

Anahtar kelimeler: Göbekli Tepe, insan etkisi, meşe bozkır-ormanı, polen,

1. Introduction

The formation of today's climatic conditions started from the last glacial period, that is, 20-22 thousand years ago. During this period, the temperatures in the world decreased, the pattern and amount of precipitation changed, changes-growth-shrinkage-displacements were experienced in the pressure and wind systems. During this period, all vegetation migrated and cold-resistant species became dominant. Not only the climatic variables have changed, but also the soil formation processes and plant species have changed with the climate [1].

Plant species migrated from the poles to the equator and from high to low, and took shelter in places where they could find an environment to survive. Today's biodiversity features have emerged as a result of these warming-cooling periods [2]. Climate changes have a huge impact on all ecosystems, especially temperature increases, leading to habitat changes, reduction or extinction of local species, and biodiversity losses. In addition to climate change, anthropogenic pressures significantly affect the composition of vegetation, and a better understanding of past vegetation dynamics is required to resolve the impact of these forcing factors and to assess future vegetation change [3].

In order to understand how both the climate and the vegetation of people change over time, it is important to investigate the regions where people settled down. In this context, it is known that Göbekli Tepe, near Örencik village, 18 km northeast of Şanlıurfa, in the Southeastern Anatolia Region of Turkey, is a Neolithic archaeological site and is the oldest known historical structure in the world so far and located within the borders of the Fertile Crescent, is also at the intersection of domestication areas of sheep, cattle, goats and pigs, and this confirms that Göbekli Tepe is the region where animal domestication first started [4]. Thus, human beings have started to make choices by changing the environment in which they live by domesticating both plants and animals for their needs such as nutrition and shelter. Domestication is to enable animals or plants to be used for the benefit of humans and to adapt them to live in a close relationship with humans [5]. Similarly, in the domestication of animals, it is likely that some changes were made in this region both for the feeding of these animals and for the needs of people. Therefore, climate was not the only factor affecting ecosystem dynamics in Göbekli Tepe; It is estimated that vegetation cover is also greatly affected by anthropogenic pressures. There is evidence that humans have altered the landscape and vegetation for thousands of years [6,7,8]. Anthropogenic pressures not only change tree position, but also change the composition of vegetation, increasing fire frequency and grazing activities, leading to a decrease in stress-sensitive species [9]. Pollen analysis makes an important contribution to our understanding of how plant populations around the world change over time, the distribution and abundance of pollen taxa reflect spatial changes in the composition of plant communities and biomes at a regional scale [10].

In this study, the change of climate and vegetation from that period to the present has been investigated by digging wells in areas close to Göbekli Tepe and taking plant pollen from the old period. The pollen-based scientific approach was made for the first time for the Göbekli Tepe Region and a basis was established for the evaluation of the results.

2.Materials and methods

2.1.Study site

The research area is Göbekli Tepe Basin, in which Örencik Village is located in Haliliye district of Şanlıurfa (Figure 1,2,3). The establishment of this settlement here thousands of years ago was not accidental. Due to the natural conditions of the environment, security, water-food supply and the vital and social functions that follow, this place has been chosen as a settlement. The samples taken from the three wells by drilling from the sandy sedimentation area detected during the fieldwork were sent to the laboratory in order to provide information about the presence of pollen depending on the examination of the flora fossils in it.

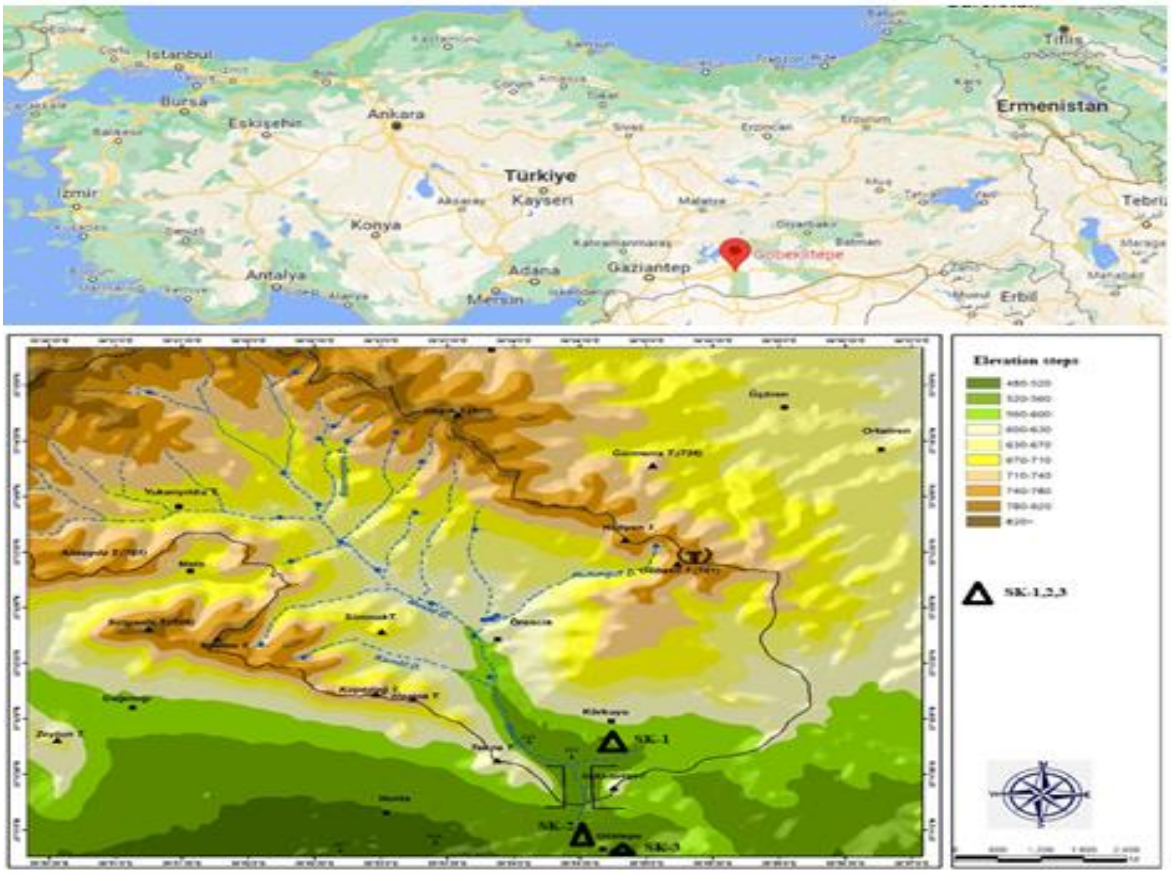


Figure 1. Map showing the location of Göbekli Tepe in Türkiye and the distribution of drilling points in the basin

Figure 2. Satellite photo of Göbekli Tepe basin

Figure 3. General view of the Göbekli Tepe region

Depending on the field investigations, the areas where the alluviums flowing over the Göbekli Tepe basin are filled were especially preferred. Because these alluviums, together with plant seeds and living remains, are dragged by external forces and stacked in a layered manner on pits or flat areas where the slope decreases. These areas are also very important in terms of paleoenvironmental research from past to present, from a hoarding organization. The patterns taken from the mentioned field were numbered according to their depth and placed in protective sampling bags. These patterns were sent to TUBITAK Marmara Research Center. The submitted samples were first subjected to age determination. Based on the period we examined from these samples, then the samples were subjected to pollen analysis at the Institute of Geosciences of Istanbul Technical University and information about the vegetation of the period was obtained.

2.2. Pollen Analysis

Pollen analyzes were carried out on 8 samples. Since there is not enough pollen in SK-2 in the samples, it is not shown in the diagram. Fossil pollen and spore analysis consists of two stages: "Sampling and preparation of pollen sections" and "Identification and counting of pollen and spore grains".

2.3. Sampling and preparation of pollen sections

Approximately 20 g samples were taken from the samples. The samples taken were prepared with the standard pollen preparation method [11]. Firstly, 35% HCl acid to eliminate carbonate content and 47% HF acid to eliminate silica content were added to the samples, respectively. Then, ZnCl2 (density>2.0) solution was added to the samples to separate the palynomorphs. Microscope slides were prepared by passing the remaining sediment sample through 200 µm and 10 µm nylon sieves and placing some glycerin on glass coverslips. Pollen identifications were analyzed using a Leica transmitted light microscope at Istanbul Technical University Eurasia Institute of Earth Sciences, using immersion oil in different lenses (x40 and x100).

2.4. Identification and counting of Pollen and spore grains

Within the scope of the research, classification of fossil spores and pollen was made depending on the degree of differentiation of morphological characteristics between genera. Pollen atlases [12] and pollen photographs were used for counting and pollen identification. The results obtained are shown in detailed pollen diagrams and pollen diagrams were created using the TiliaIT program [13]. The photographs of the obtained some pollen are shown in Figure 4.

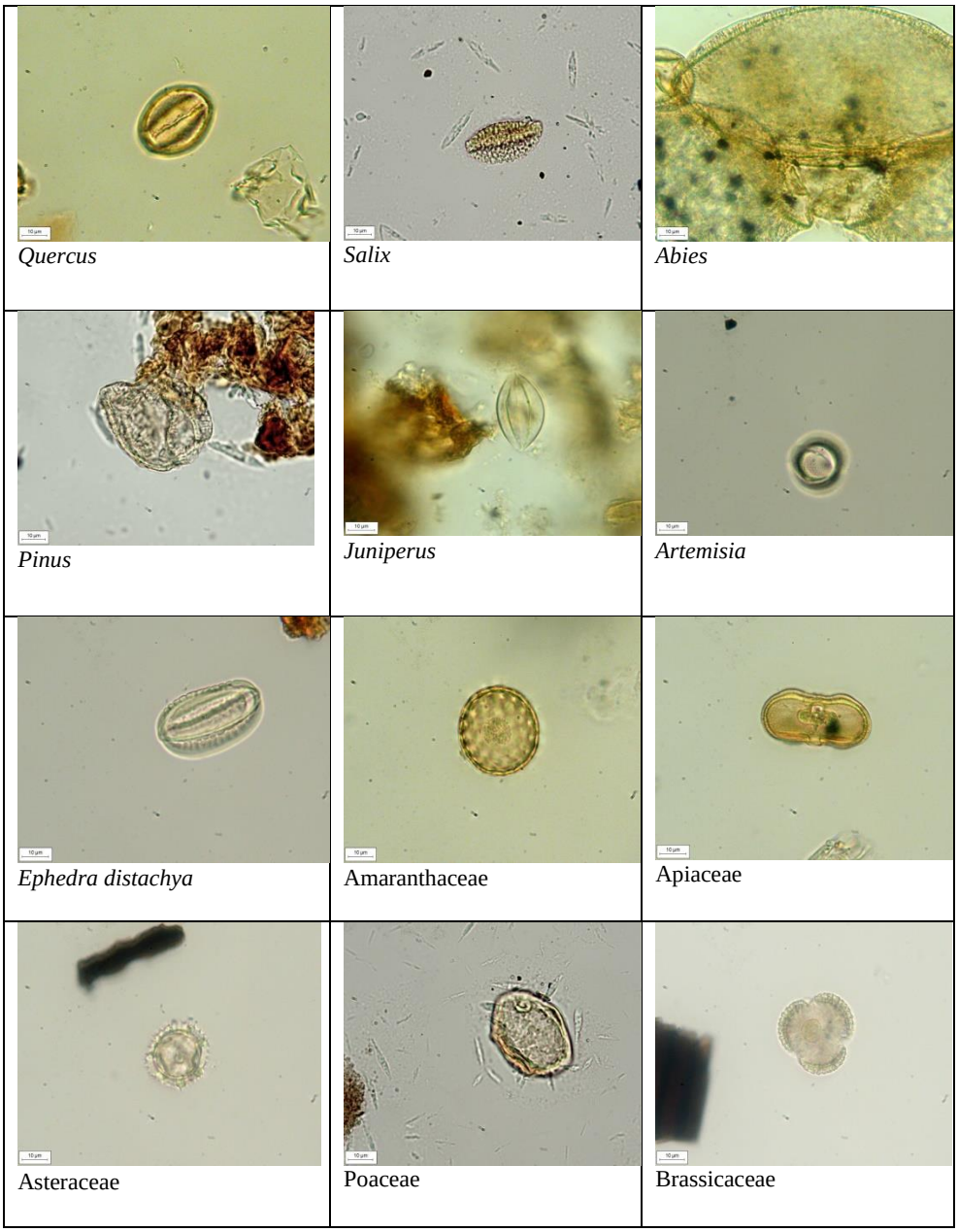


Figure 4. Some pollen photographs used for the identification of taxa

3. Results

The pollen analyzes obtained from the SK-1 samples between 6 m and 7-7.5 m. It covers the years B.C. 11126 and 13354 and gives information about vegetation-climatic changes (Figure 5). The SK-1, 6 meter specimen covers the Younger Dryas period. According to pollen analysis, plant communities seen in this period are mainly *Artemisia* steppes and herbaceous plants of Asteraceae-Cichorioideae, Asteraceae-Asteriodeae and Poaceae are numerous. While *Artemisia* steppes were around 14%, Asteraceae Cichorioideae from herbaceous plants was determined as 45.5%. While the pollen belonging to the Poaceae family reached 13%, the pollen belonging to the Asteraceae-Asteriodeae family was observed as 5.9%. The increase in herbaceous and steppe plants in this period indicates that the study area was cold and dry in 11126 before today (B.C.). In the forest community, there are *Quercus*, *Salix*, *Juglans*, *Pinus*, *Abies* and *Juniperus* species, albeit few. Similarly, the density of herbaceous and steppe plants in the SK-1 7-7.5 meters sample shows that the region was under the influence of a cold and arid climate in 13354 before today (B.C.). The non-pollinated palynomorph (NNP) assemblage includes Pseudoschizaea Phytolith, Glomus, Anabea, Trilet spore.

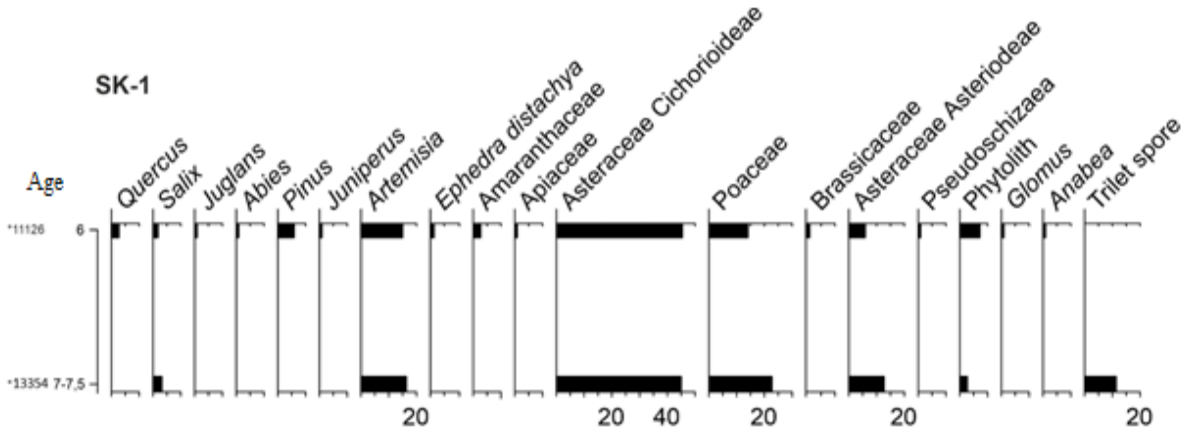


Figure 5. Detailed pollen diagram of SK-1 samples

Pollen analyzes of SK-3 samples at 6, 10, 12 meters reflect the changes in vegetation and climate between the years 10634-12418 before the present day (Figure 6). Among the plant communities, *Artemisia* steppes, Asteraceae-Cichorioideae, Asteraceae-Asteroideae and Poaceae herbaceous plants are numerous. While Artemisia steppes reached 44% at 12 m in samples, Asteraceae-Cichorioideae from herbaceous plants were recorded between 38-41%. While the pollens belonging to the Poaceae family reached 16.1%, the pollens belonging to the Asteraceae-Asteriodeae family were found to be between 7-12.9%. The increase in herbaceous and steppe plants in this period indicates that the study area was cold and arid between the years 10634-12418 before the present day. In the forest community, there are Quercus and Salix species, albeit few. Phytolith, Glomus and Botryococcus algae were detected in the non-pollen palynomorph (NNP) assemblage.

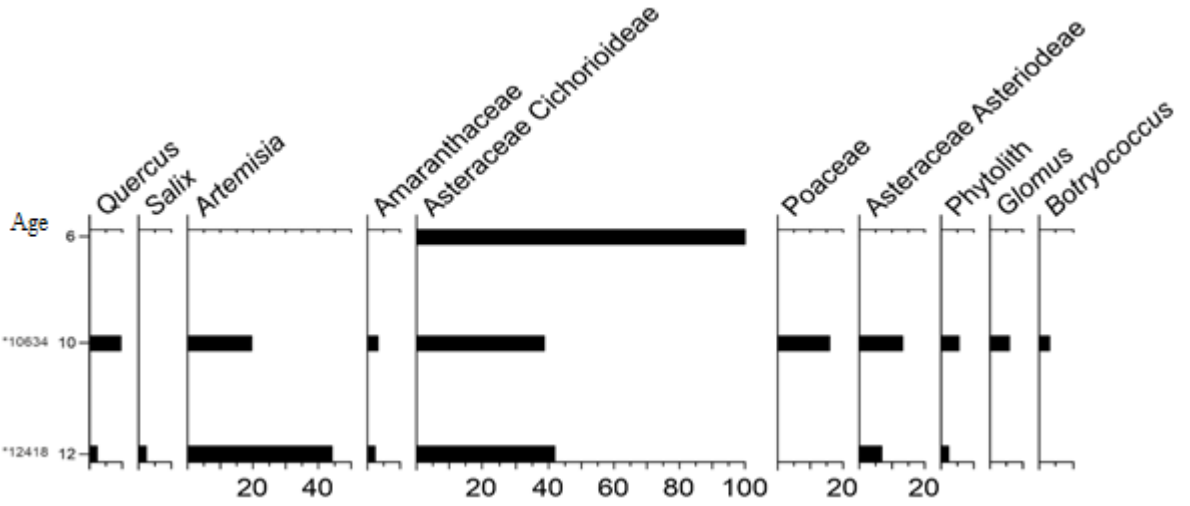


Figure 6. Detailed pollen diagram of SK-3 samples.

Fossil pollen records are well-established indicators of past vegetation changes, the prevalence of pollen in environmental environments has made palynology one of the most common and valuable tools for studying past environmental and climatic changes globally for decades [14]. In this study, the characteristics of the obtained fossil pollen were investigated using current techniques and revealed the data on the vegetation that lived in the Göbekli Tepe region in the past. In addition, the plants living in this region today were determined both by literature review and as a result of individual observations and compared in Table 1. In addition, the plant species that are naturally distributed in this region today were determined by both literature review and individual observations, and the taxa determined as a result of pollen analyzes were compared in Table 1.

Table 1. The status of the taxa of Göbekli Tepe region in the past-present and present-day Şanlıurfa

Taxon names	Ancient Göbekli Tepe	Nowadays Göbekli Tepe	Today's Şanlıurfa Spread
Quercus	+	-	+
Salix	+	-	+
Juglans	+	-	-
Abies	+	-	-
Pinus	+	-	-
Juniperus	+	-	-
Artemisia	+	-	+
Ephedra distachya	+	-	-
Amaranthaceae	+	+	+
Apiaceae	+	+	+
Asteraceae	+	+	+
Poaceae	+	+	+
Brassicaceae	+	+	+

In Table 1, although individuals belonging to *Quercus*, *Salix*, *Juglans*, *Abies*, *Pinus*, and *Juniperus* species belonging to forest vegetation were encountered in Ancient Period Göbekli Tepe, no individuals belonging to these trees have been observed today. However, it was determined that only *Quercus* [15] and *Salix* [16] individuals were distributed in Şanlıurfa. Thus, trees belonging to the forest were found in a few communities in the old period, and these have completely disappeared from the region due to the changes that have occurred until today. Both climatic and anthropogenic influences have caused them. Because anthropogenic pressures cause a decrease in pressure sensitive species such as *Abies* [9].

The genus *Artemisia* is a genus of the Asteraceae family and is a hardy herbaceous or shrubby plant group that grows in dry or semi-arid habitats. Plants belonging to this group were found in the Göbekli Tepe region in the past but not seen today, and their distribution continues in Şanlıurfa [16]. *Ephedra distachya* is a shrub that protects the soil against erosion with its strong roots, and although it was found in the past, it is not even found in Şanlıurfa today.

Species belonging to Amaranthaceae, Apiaceae, Asteraceae, Poaceae and Brassicaceae families are encountered both in the past and today. In addition, the detection of Phytolith, *Glomus* and *Botryococcus* algae in the non-pollen palynomorph (NNP) assemblage in the samples obtained is an indication that there are also water resources in the Göbekli Tepe region. As a result of these determinations, it also shows that the people around Göbekli Tepe benefit from these resources and may engage in some economic activities depending on these resources. Recent research has highlighted that the region between the upper reaches of the Euphrates and Tigris was a region where the transition to food-producing subsistence took place in the early Epipalaeolithic and Pottery Neolithic Period [17].

In the study of Neef (2003), it was stated that since taxa such as *Pistacia*, *Prunus*, *Quercus* and Poaceae were identified in Göbekli Tepe, it indicates the existence of a forest-steppe dominated by pistachio-almond oaks around Göbekli Tepe in the Early Neolithic Age [18]. This forest-steppe is typical of arid areas bordering a steppe with a slightly continental character. It is relatively open with widely spaced trees, also supported by results from zoological remains that indicate relatively open landscapes near the site [19].

4. Conclusions and discussion

In this study, we have obtained data supporting that it is a forest-steppe and the existence of forest-forming *Juglans*, *Abies*, *Pinus* and *Juniperus* has been determined for the first time and the vegetation of the past period has been made more evident. Comparable steppe forest types have disappeared in the Göbekli Tepe region today, because human intervention has often led to irreversible degradation of vegetation, especially in these areas where tree growth is limited by water stress [18]. Human beings are trying to survive and benefit from the ecological texture they live in, and accordingly exhibit various practices. This situation continues from the past to the present. It is certain that there is deterioration in the world climate system and if the human being, who is the main source of this deterioration, continues his various activities such as production and consumption habits without taking the necessary precautions, this deterioration in the climate will continue to increase [20]. Destruction or change of habitats can cause populations to shrink and disappear over time [21].

In this study, important information was obtained by performing fossil pollen analyzes of the past vegetation of Göbekli Tepe region. The change of climate and vegetation was examined and the data obtained, especially some taxa belonging to forest vegetation, were determined for the first time based on pollen analysis. This study will help to better determine the vegetation of that period by scanning more regions and investigating pollen from more wells in future studies. In addition, it has been revealed that there are deep differences between the natural vegetation of the past and the natural vegetation of today.

References

[1] Atalay, I. (1996). Palaeosols as indicators of the climatic changes during Quaternary period in S. Anatolia. *Journal of Arid Environments*, 32(1), 23–35. https://doi.org/10.1006/jare.1996.0003

[2] Brauer, A., Haug, G. H., Dulski, P., Sigman, D. M., & Negendank, J. F. W. (2008). An abrupt wind shift in western Europe at the onset of the Younger Dryas cold period. *Nature Geoscience*, 1(8), 520–523. https://doi.org/10.1038/ngeo263

[3] Van Vugt, L., Garcés-Pastor, S., Gobet, E., Brechbühl, S., Knetge, A., Lammers, Y., & Schwörer, C. (2022). Pollen, macrofossils and sedaDNA reveal climate and land use impacts on Holocene mountain vegetation of the Lepontine Alps, Italy. *Quaternary Science Reviews*, 296, 107749.

[4] Demirci, S., Koban Baştanlar, E., Dağtaş, N.D., Pişkin, E., Engin, A., Özer, F., & Togan, I. (2013). Mitochondrial DNA diversity of modern, ancient and wild sheep (*Ovis gmelinii anatolica*) from Turkey: new insights on the evolutionary history of sheep. *PloS one*, 8(12), e81952. https://doi.org/10.1371/journal.pone.0081952

[5] Parmaksiz, A., Oymak, S., Yuncu, E., Demirci, S., Bastanlar, E., Unal, E., & Ozer, F. (2018). Y-Chromosome Polymorphisms in 12 Native, Karagul, Karacabey Merino Breeds from Turkey and Anatolian Mouflon (*Ovis gmelinii anatolica*). *Kafkas Universitesi Veteriner Fakultesi Dergisi*, 24 (6): 821-828.

[6] Rey, F., Schworer, C., Gobet, E., Colombaroli, D., van Leeuwen, J.F.N., Schleiss, S., & Tinner, W. (2013). Climatic and human impacts on mountain vegetation at Lauenensee (Bernese Alps, Switzerland) during the last 14,000 years. *Holocene*, 23(10), 1415e1427. https://doi.org/10.1177/0959683613489585

[7] Schworer, C., Colombaroli, D., Kaltenrieder, P., Rey, F., & Tinner, W. (2015). Early human impact (5000-3000 BC) affects mountain forest dynamics in the Alps. *Journal of Ecology*, 103(2), 281e295. https://doi.org/10.1111/1365-2745.12354

[8] Vescovi, E., Tinner, W., Beer, R., van Leeuwen, J.F.N., Steinhauser, U., Ziegler, S., Gilli, A., Wirth, S.B., Samartin, S., Jacquat, C., & Carraro, G. (2018). Vegetation history of the upper Leventina valley from neolithic to Roman times, recorded by pollen, spores, plant macrofossils, and charcoal. In: Della Casa, P. (Ed.), The Leventina Prehistoric Landscape (Alpine Ticino Valley, Switzerland). *Chronos Zürich*, 207e225.

[9] Conedera, M., Tinner, W., Neff, C., Meurer, M., Dickens, A.F., & Krebs, P. (2009). Reconstructing past fire regimes: methods, applications, and relevance to fire management and conservation. *Quaternary Science Reviews*, 28 (5e6), 555e576. https://doi.org/10.1016/j.quascirev.2008.11.005

[10] Connor, S. E., & Kvavadze, E. V. (2008). Modelling late Quaternary changes in plant distribution, vegetation and climate using pollen data from Georgia, Caucasus. *Journal of Biogeography*, 36(3), 529-545.

[11] Cour, P. (1974). Nouvelles techniques de détection des flux et des retombées polliniques: étude de la sédimentation des pollens et des spores à la surface du sol. *Pollen et Spores*, 16, 103-141.

[12] Reille, M. (1992). Pollen et spores d'Europe et d'Afrique du Nord. Laboratoire de botanique historique et de palynologie, Marseille, France.

[13] Grimm, E.C. 1991-2015. TiliaIT. https://www.tiliait.com.

[14] Chevalier, M., Davis, B. A., Heiri, O., Seppä, H., Chase, B. M., Gajewski, K., & Kupriyanov, D. (2020). Pollen-based climate reconstruction techniques for late Quaternary studies. *Earth-Science Reviews*, 210, 103384.

[15] Akan, H., Kaya, Ö.F., Eker, İ. & Cevheri, C. (2005). The flora of Kaşmer Dağı (Şanlıurfa). *Turkish Journal of Botany*, 29 (4), 291-310.

[16] Atamov, V., Aslan, M., Cevheri, C., & Çetin, E. (2007). Contribution to the flora of Fatik mountain (Sanliurfa-Turkey). *Asian Journal of Plant Science*, 6(1), 1-11.

[17] Dietrich, L., Meister, J., Dietrich, O., Notroff, J., Kiep, J., Heeb, J., & Schütt, B. (2019). Cereal processing at Early Neolithic Göbekli Tepe, southeastern Turkey. *PLoS One*, 14(5), e0215214.

[18] Neef, R. (2003). Overlooking the Steppe-Forest: A Preliminary Report on the Botanical Remains from Early Neolithic Göbekli Tepe (Southeastern Turkey) The Newsletter of Southwest Asian Neolithic Research. *NeoLithics* 2/03, 13-16.

[19] Driesch, A., & Peters, J. (1999). Vorläufiger Bericht über die archäozoologischen Untersuchungen am Göbekli Tepe und am Gürcütepe bei Urfa, Türkei. *Istanbuler Mitteilungen*, 49, 23-39.

[20] Demir, A. (2009). The effects of global climate change on biodiversity and ecosystems resources. *Environmental Sciences of Ankara University*, 1, 037-054.

[21] Parmaksız, A., Yiğin, A., & Bozkaya, F. (2023). Determination of genetic structure of Bald ibis population living in Birecik (Türkiye) using mtDNA COI sequences. *Biological Diversity and Conservation*, 16(1), 31-37.

www.biodicon.com BIOLOGICAL DIVERSITY AND CONSERVATION

ISSN 1308-8084 Online ISSN 1308-5301 Print

Research article/Araştırma makalesi
DOI: 10.46309/biodicon.2023.1266407
16/3 (2023) 218-223

Aphrodes diminuta Ribaut, 1952 (Hemiptera: Auchenorrhyncha: Cicadellidae): a new record for Turkish leafhoppers

Rukiye TANYERİ [*1], Ünal ZEYBEKOĞLU[2], Emine DEMİR ÖZDEN [3]
ORCID: 0000-0001-9994-8763**;** 0000-0001-7595-9572**;** 0000-0003-4486-003X

[1] Sinop University, Arts and Science Faculty, Department of Biology, Sinop, Türkiye
[2] Ondokuz Mayıs University, Arts Faculty, Department of Biology, Samsun, Türkiye
[3] Duzce University, Agriculture Faculty, Department of Plant Protection, Düzce, Türkiye

Abstract

In this study, specimens which belong to genus *Aphrodes* Curtis (Hemiptera: Cicadellidae: Aphrodinae), collected from Kastamonu provinces between 2016-2018 have been examined. Materials were collected by a sweeping net and a hand aspirator on the plants during daytime. Two species were found distributed in this area; *Aphrodes bicinctus* (Schrank, 1776), *Aphrodes diminuta* Ribaut, 1952. *Aphrodes diminuta* is a new record for the Cicadellidae fauna of Türkiye. The distribution of the species both in Türkiye and the world, the photograps of aedeagus and the coordinates of the specimens were given. In order to show the differences in aedeagus, photographs and measurements of the *A. makarovi* Zachvatkin,1948 have also been added.

Key words: Cicadellidae, Türkiye, biodiversity, *Aphrodes diminuta*

---------- * ----------

Aphrodes diminuta Ribaut, 1952 (Hemiptera: Auchenorrhyncha: Cicadellidae): Türkiye Cicadellidae faunası için yeni bir kayıt

Özet

Bu çalışmada 2016-2018 yılları arasında Kastamonu'dan toplanan *Aphrodes* (Hemiptera: Cicadellidae: Aphrodinae) cinsine ait örnekler incelenmiştir. Materyal gün içinde bitkilerin üzerinden atrap ve aspiratör yardımıyla toplanmıştır. Çalışma alanında bu cinse ait 2 tür tespit edilmiştir: *Aphrodes bicinctus* (Schrank, 1776), *Aphrodes diminuta* Ribaut, 1952. *A. diminuta*, Türkiye Cicadellidae faunası için yeni bir kayıttır. Türlerin Türkiye ve Dünya'daki dağılımları, aedeagus fotoğrafları ve örneklerin toplandıkları lokalitelerin koordinatları verilmiştir. Aedegustaki farklılıkları gösterebilmek için *A. makarovi* Zachvatkin, 1948'nin fotoğrafları ve ölçümleri de eklenmiştir.

Anahtar kelimeler: Cicadellidae, Türkiye, biyolojik çeşitlilik. *Aphrodes diminuta*

[*] *Corresponding author* / Haberleşmeden sorumlu yazar: Tel.: +905333956239; Fax.: +905333956239; E-mail: rtanyeri@sinop.edu.tr
 Geliş tarihi: 16.03.2023; Yayın tarihi: 15.12.2023 BioDiCon. 1091-160323

1. Introduction

Cicadellidae (Hemiptera), being one of the largest insect family, comprises more than 22000 described species is grouped into about thirty-six subfamilies [1]. It has 2300 species described in 338 genera in the Palearctic region [2]. According to Demir [3], Türkiye Cicadellidae fauna is considered to have 473 species. This number reached to 482 with the new records and new species in the following years [4, 5, 6, 7, 8, 9, 10, 11, 12].

Leafhoppers of the genus *Aphrodes* Curtis (Hemiptera: Cicadellidae: Aphrodinae) are common and widely distributed in the Palearctic [13]. They are not only vectors of some plant diseases [14] but are also evaluated as an appropriate indicator group in grassland communities [15]. Due to the similarity and variation in the morphological characters of coloration, size and aedeagus, some species were grouped under the name of *A. bicinta* species group. Nickel [16] suggested that this species group consists of 4 species; *A. bicincta* (Schrank, 1776); *A. makarovi* Zachvatkin, 1948; *A. diminuta* Ribaut, 1952 and *A. aestuarina* (Edwards, 1908). The genus *Aphrodes* comprise at least four species which are very similar morphologically and often live syntopically [17]. The genus *Aphrodes* is a taxonomically challenging taxon, different species are considered as the ecotype or subspecies of those species. For example, *A. aestuarina* has been accepted as a different ecotype of *A. makarovi* [18]. To solve problems in the species status Tishechkin [19]. used the the male vibrational signal in The Central European Russia *bicincta* species group by combining it with morphological characters and founded that *A. makarovi*, *A. bicincta* ve *A. centrorossica* (= *A. diminuta*) were separated from each other. Bluemel *et al*. [20]. used a combination of different criteria (vibration signals, mitochondrial DNA, aedeagus morphology) samples collected from Slovenia and U.K, and stated that *A. aestuarina*, *A. bicincta*, *A. diminuta* and *A. makarovi* are genetically and morphologically distinct. In the same study, morphological key to male *Aphrodes* was constituted according to the morphometric measurements of some body parts and morphological characters of the aedeagus (body length/ aedeagus length ratio and distance between aedegal spines). In this study, the measurements mentioned above were carried out and it has been demonstrated that morphometric measurements of aedeagus are very useful parameters in the differentiation of the species.

According to the literature, *Aphrodes* fauna of Türkiye consists of 11 species. Oshanin [21] listed *A. bicinctus*, Dlabola [22, 23] listed *A. bifasciatus* (Linnaeus, 1758) and *A. histrionicus* (Fabricius, 1794) and Nast [24] *A. nigritus* (Kirschbaum, 1868) from Türkiye. Lodos and Kalkandelen [25] and Özgen and Karsavuran [26] added 6 new records for this genus; *A. albiger* (Germar, 1821), *A. albifrons* (Linnaeus, 1758), *A. angusticeps* Emelyanov, 1964, *A. elongatus (Lethierry, 1876), A. trifasciatus* (Fourcrcoy, 1785) and *A. flavostriatus* (Donavan, 1799). Başpınar & Uygun [27] reported *A. makarovi* from Adana for the first time.

2. Material and method

The specimens were collected from Kastamonu provinces between May 2016 and October 2018. The sampling of the adults was made by a sweeping net and a hand aspirator over the plants during the daytime. All the material was collected by the first author. The samples were taken in insect killing jars, labeled and brought to the laboratory and placed in insect storage packages. The specimens were prepared by standard insect preparation and identified according to Bluemel *et al*. [20]. The photos of the general view of dry samples were taken by Canon Eos 70D model camera connected to Zeiss Stem 2000-C stereomicroscope. The body length of the males, the length of the aedeagus, the distance between the upper and lower spines were measured for identification. Specimens of *A.makarovi* that has been in the personal collection of the first author have also been added for comparison. Specimens (9♂♂, 5♀♀) of *A. makarovi* were collected from Sinop before [28]. General views of the species from the dorsal are given in Figure 1, the pictures of the aedeagus taken from the ventral are given in Figure 2.

Specimens are stored at Sinop University, Faculty of Arts and Sciences, Department of Biology, Invertebrata Laboratory.

3. Results

A total of 30 specimens belonging to *Aphrodes* genus collected from Kastamonu provinces. The following information is given for the material listed: Administrative district (town, village or specific locality), date, altitude, (coordinate), number of specimens. Additionally, distribution in Turkey andthe world and locality remarks are added.

Aphrodes diminuta recorded in Türkiye for the first time. Morphometric measurements used for identification of species are shown in Table 1.

***Aphrodes bicinctus* (Schrank, 1776)**

Material examined: Kastamonu: Araç, 20.vii.2017, 41° 18' 01.2" N, 33° 31' 54.4" E, 1101 m, (2♂♂); İhsangazi, 20.vii.2017, 41° 13' 21.9" N, 33° 25' 38.0" E, 780 m, (3♂♂).

Distribution of Türkiye: Adana, Afyon, Ankara, Antalya, Bilecik, Bursa, Çanakkale, Diyarbakır, Edirne, Elazığ, Erzincan, Erzurum, Gaziantep, Giresun, Gümüşhane, Hakkari, İstanbul, İzmir, Kars, Kırklareli, Konya, Kütahya, Manisa, Muğla, Ordu, Rize, Samsun, Sinop, Tekirdağ, Tokat, Trabzon, Uşak, Van, Zonguldak [29].

Distribution of the world: Afghanistan, Albania, Algeria, Austria, Bulgaria, Cyprus, Czechoslovakia, Denmark, England, Finland, France, Germany, Greece, Hungary, Iran, Ireland, Italy, Lebanon, Madeira Islands, Mongolia, Morocco, Netherlands, North America, Norway, Spain, Sweden, Switzerland, Poland, Portugal, Romania, Syria, Tunisia, Türkiye, Yugoslavia [29].

***Aphrodes diminuta* Ribaut, 1952**

Material examined: Kastamonu: Centre, 06.viii.2017, 41° 32' 24.5" N, 33° 46' 33.3" E, 1041 m, (4♂♂, 3♀♀); 06.viii.2017, 41° 43' 39.2" N, 34° 01' 55.9" E, 1331 m, (9♂♂, 5♀♀); Yaralıcilvegöz, 06.viii.2017, 41° 47' 35.8" N, 34° 04' 53.2" E, 1400 m, (4♂♂).

Distribution of the world: Austria, Central Asia, Kazakhstan, Russian Far East, Siberia, Slovenia, Switzerland [13, 30,31,32].

Table 1. The range of obtained values for male body and aedeagus characters

Character (mm)	***A. bicinctus***			***A. makarovi***			***A. diminuta***		
	Min.	Max.	**Ave.**	Min.	Max.	**Ave.**	Min.	Max.	**Ave.**
Body length	5,7	6,6	**6,1**	6,9	7,6	**7,2**	4,6	5,2	**4,9**
Aedeagus length	0,66	0,72	**0,7**	0,88	1	**0,95**	0,78	0,92	**0,87**
Distance between spines	0,08	0.15	**0,09**	0,04	0,09	**0,06**	0,18	0,25	**0,23**

It is evident that the length of the aedeagus and the distance between the spines of aedeagus are more than the other two species according to the body length of *A. diminuta*. *A. makarovi* is distinctly larger than the other two species. The spines of the aedeagus are very close to each other. In *A. bicincta*, the spines are similar to each other but the aedeagus is smaller (Table 1).

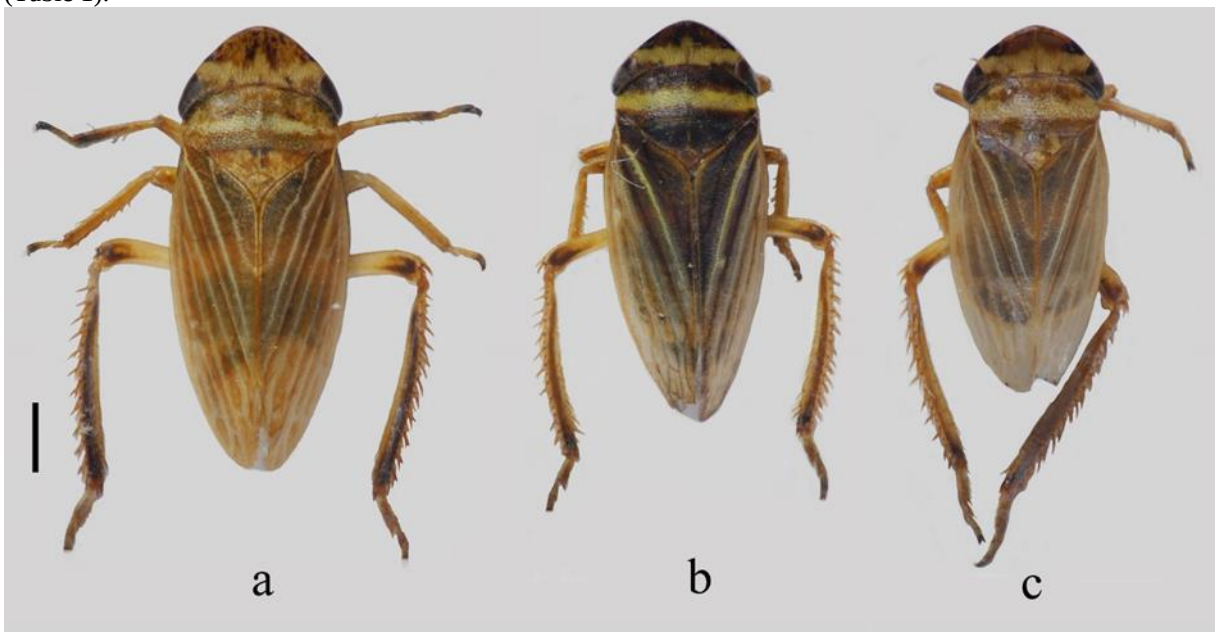


Figure 1. Habitus of three *Aphrodes* spp. (♂) a) *Aphrodes makarovi*, b) *Aphrodes bicinctus*, c) *Aphrodes diminuta* (Scale bar:1 mm)

Figure 2. Frontal wiev of aedeagus of three *Aphrodes* spp. a) *Aphrodes makarovi*, b) *Aphrodes bicinctus*, c) *Aphrodes diminuta* (Scale bar: 0,1 mm)

4. Conclusions and discussion

A. bicincta species group is characterized by consisting of wide transverse bands on the head and pronotum. This pattern shows variations according to light and darkness. The aedeagus is elongated with two pairs of spines in its central section [18, 33].The position of the spines in the aedeagus and their distance from each other are used in the differentiation of the species [34]. To eliminate taxonomic gap due to variation within and between species, different parameters were used. Within these parameters, one of the important characters for distinction in *Aphrodes* species is known as the body length /aedeagus length ratio and the location of the spines in the aedeagus. *Aphrodes diminuta* is distinguished by having the largest aedeagus according to body size. *A. macarovi*, *A. bicincta* specimen are similar in distance from the spines in the aedeagus, but *A. makarovi* is significantly different from *A. bicincta* in both body size and aedeagus size [20]. The diagnosis of the species evaluated in this study was made according to this study. Morphometric measurements were parallel with the study [20] mentioned above.

The samples belonging to *A. makarovi* species has the largest size of 3 species with its 7.2 mm size. In the U.K and Slovenia samples, this ratio is 5.93 mm. The males of *A. diminuta* differ from other *Aphrodes* species by being small in size [19]. Similar results were obtained from the samples in this study. *A. diminuta* has the largest aedeagus in terms of body size when body lengths are compared to aedeagus length. In *A. diminuta*, the distance between the upper spines and the lower spines in the aedeagus is greater than the average species. (mean: 2.33 mm).

Bluemel *et al*. [20] stated that this rate is also an important character in the identification of the species. The results obtained in this study are similar. In *A. makarovi* and *A. diminuta* samples, the transverse bands in the head and pronotum are light colored and the variation is small. In *A. bicinta*, it is bright yellow in general but the variation is quite high. Although the samples were collected from a limited area, the results may vary according to coloration. Since *A. bicinta* species group belongs to taxonomically problematic groups it is very possible that they have already been recorded under different names in other parts of the country. The fact that this species has not been registered in our country so far may be related to this situation. When all the results are evaluated, the ratio of body length to aedeagus length is a very useful character in distinguishing 3 species.

No data were found on the ecology of *A. diminuta*. There is an evidence which shows that it was collected only from the higher areas. It is known that *A. diminuta* is generally located at 1200 m and at least 1500 m in Bovyera Alps [16]. According to Seljak [32] in Slovenia *A. diminuta* seems to have a more mountainous distribution, being

collected mainly at altitudes between 600 - 1400 m. Similarly, the samples collected in this study are at an altitude of 1000-1400 m.

Türkiye *Aphrodes* fauna has reached to 12 species after the record of *A. diminuta* from Kastamonu. Leafhoppers of the genus *Aphrodes*, are abundant, widely distributed over the Palearctic and also in North America. *Aphrodes diminuta* is placed into the European chorotype [35]. and distributed at Austria, Kazakhstan, Central Asia, Siberia, Russian Far East, Slovenia and Switzerland. When its distribution in the world is examined, although this record seems to be new for the Türkiye Cicadellidae fauna, it is thought that it may have been evaluated as another species due to variation within the genus before.

References

[1] Dietrich, C. H. (2004). Phylogeny of the leafhopper subfamily Evacanthinae with a review of Neotropical species and notes on related groups (Hemiptera: Membracoidea: Cicadellidae). *Systematic Entomology*, 29(4), 455-487.

[2] Başpınar, H. Uygun, N. &Hermoso De Mendoza, A. (2012). Cicadellidae, 119-125. In Vacande, V. (eds), Integrated control of Citrus pests in the Mediterranean Region, Bentham Science Publishers.

[3] Demir, E. (2006a). Contributions to the knowledge of Turkish Auchenorrhyncha with twelve new records (Homoptera, Cicadellidae). *Munis Entomolgy & Zoo*logy,1(2), 215-236.

[4] Demir, E. (2006b). A new species of *Thamnotettix* Zetterstedt, 1840 (Homoptera: Auchenorrhyncha: Cicadellidae) from Turkey. *Journal of the Kansas Entomological Society*, 79(3), 283-287.

[5] Demir, E. (2006c). *Bobacella* (Homoptera: Auchenorrhyncha: Cicadellidae): a new genus record for Turkey. *Entomological News*, 117(4), 455-456.

[6] Demir, E., & Demirsoy, A. (2008). Some interesting and new records of Cicadellidae (Insecta: Hemiptera) from eastern Turkey. *Zoology in the Middle East*, 45(1), 116-117.

[7] Güçlü, Ş. (2010). *Verdanus artvinensis* (Hemiptera: Cicadellidae), a new leafhopper species from northeastern Turkey. *Turkish Journal of Zoology*, 34(1), 45-48.

[8] Zeybekoğlu, Ü. (2010). A new species of the bug genus *Cicadula* Zetterstedt, 1838 from Turkey: (Hemiptera: Cicadomorpha: Cicadellidae). *Zoology in the Middle East*, 50(1), 107-110.

[9] Karavin, M., Zeybekoğlu, Ü., & Kartal, V. (2011). First record of *Bilusius valiko* Logvinenko, 1974 (Hemiptera, Cicadomorpha, Cicadellidae) from Turkey, with redescription of the species. *Turkish Journal of Zoology*, 35(6), 893-895.

[10] Demir, E. (2016). Deltocephalinae (Hemiptera, Cicadellidae) species in southwestern Turkey with new records. *Entomologica Romanica*, 20, 49-55.

[11] Gnezdilov, V. M., & Özgen, I. (2021). A new species of *Balcanocerus* Maldonado-Capriles (Hemiptera: Auchenorrhyncha: Cicadellidae: Eurymelinae) from Eastern Anatolia, with a key to Palaearctic species and new records. *Proceedings of the Entomological Society of Washington*, 123(3), 529-537.

[12] Gnezdilov, V. M., Özgen, I., Emeljanov, A. F., & Neimorovets, V. V. (2021). First record of the leafhopper tribe Adelungiini Baker (Hemiptera, Auchenorrhyncha, Cicadellidae: Megophthalminae) from Turkey, with notes on emAchrus/em emalbicosta/em (Kusnezov, 1929). *Zootaxa*, 4950(3), 580-588.

[13] Tishechkin, D.Y. (2017). On the taxonomy and distribution of *Aphrodes bicincta* (Schrank, 1776) species group (Homoptera: Auchenorrhyncha: Cicadellidae: Aphrodinae) in Eastern Palaearctic. *Zootaxa* 4318(1), 167-176.

[14] Weintraub, P.G. & Beanland, L.A. (2006). Insect vectors of phytoplasmas. *Annual Review of Entomology*, 51, 91–111.

[15] Nickel, H. & Achtziger, R. (2005). Do they ever come back? Response of leafhopper communities to extensification of land use. *Journal of Insect Conservation*, 9, 319–333.

[16] Nickel, H. (2003). The leafhoppers and planthoppers of Germany (Hemiptera Auchenorrhyncha): patterns and strategies in a highly diverse group of phytophageous insects. *Deutshe Entomologische Zeitschrift Banner*, 50, 259-260.

[17] Derlink, M., Pipan, B., Pavlovčič, P., Jones, L. E., Meglič, V., Symondson, W. O., & Virant-Doberlet, M. (2014). Characterization of eleven polymorphic microsatellite markers for leafhoppers of the genus *Aphrodes* (Hemiptera: Cicadellidae). *Conservation Genetics Resources*, 6(4), 933-935.

[18] Guglielmino, A. & Bückle, C. (2015). Revision of Errhomeninae and Aphrodinae (Hemiptera, Cicadomorpha) in Italy with remarks on their variability and distribution in adjacent regions and description of three new taxa. *Zootaxa*, 3906, 1-66.

[19] Tishechkin, D.Y. (1998). Acoustic signals and morphological characters of leafhoppers belonging to Aphrodes bicinctus group (Homoptera, Cicadellidae) from European Russia. *Entomological Review*, 78, 370–377.

[20] Bluemel, J. K., Derlink, M., Pavlovčič, P., Russo, I. R. M., Andrew King, R.,Corbett, E., ... & Symondson, W. O. (2014). Integrating vibrational signals, mitochondrial DNA and morphology for species determination in the genus *Aphrodes* (Hemiptera: C icadellidae). *Systematic Entomology*, 39(2), 304-324.

[21] Oshanin, B. (1908). Verzechnis der Palaearktischen Hemipteren. Mit Besonderer Berücksichtigung Ihrer Verteilung im Russischen Reiche. II. Band Homoptera. Beilage zum -Annuaire de Musee Zoologue de l'Academie Imperiale des Scierıces, St. Petersburg, 493pp.

[22] Dlabola, J. (1957). Results of the Zoological Expedition of the National Museum in Prague to Turkey 20. Homoptera, Auchenorrhyncha. *Acta Entomologica Musei Nationale Pragae*, 31(469), 19-68.

[23] Dlabola, J. (1981). Ergebnisse der Tschechoslowakisch-Iranischen Entomologischen Expeditionen nach dem Iran (1970 und 1973). *Acta Entomologica Musei Nationale Pragae*, 40, 127-311.

[24] Nast, J. (1972). Palearctic Auchenorrhycha (Homoptera.l. An Annotated Check List. Polish Academy of Sciences Institute of Zoology. Polish Scientific Publishers, Warszava, 550pp.

[25] Lodos, N. & Kalkandelen, A. (1982). Preliminary list of Auchenorrhyncha with notes on distribution and importance of species in Turkey IX. Family Cicadellidae: Iassinae, Penthiminae, Dorycephalinae, Hecalinae and Aphrodinae. *Türkiye Bitki Koruma Dergisi*, 6, 147-159.

[26] Özgen, İ. & Karsavuran, Y. (2009). The Cicadellidae (Homoptera) species which found at the vineyards in Diyarbakır, Elazığ and Mardin provinces (Turkey). *Türkiye Entomolojisi Dergisi*, 33(3), 217-240.

[27] Başpınar, H. & Uygun, N. (1991). Doğu Akdeniz Bölgesi turunçgil bahçelerindeki Cicadellidae türleri üzerinde faunistik ve sistematik çalışmalar II. *Türkiye Entomoloji Dergisi,* 15(3), 157-172.

[28] Tanyeri, R. & Zeybekoğlu, Ü. (2022). Contributions of the Cicadellidae (Hemiptera: Cicadomorpha) Fauna of Sinop and Black Sea Region of Turkey. *Kahramanmaraş Sütçü İmam Üniversitesi Tarım ve Doğa Dergisi*, 25(2), 292-296.

[29] Demir, E. (2008). The Fulgoromorpha and Cicadomorpha of Turkey. Part I: Mediterranean region (Hemiptera). *Munis Entomology & Zoology*, 3(1), 447-522.

[30] Mühlethaler, R., Günthart, H., Burckhardt, D., Holzinger, W., Kunz, G., Lauterer, P., ... & Seljak, G. (2007). Zur fauna der Zikaden, blattflöhe und deren parasitoide der Alp Flix (Graubünden, Schweiz). *Beiträge zur Zikadenkunde*, 9, 45-53.

[31] Holzinger, W. E. & Kunz, G. (2006). New records of leafhoppers and planthoppers from Austria (Hemiptera: Auchenorrhyncha). *Acta Entomologica Slovenica*, 14(2), 163-174.

[32] Seljak, G. (2016). New and little known plant-and leafhoppers of the fauna of Slovenia (Hemiptera: Fulgoromorpha and Cicadomorpha). *Acta Entomologica Slovenica*, 24(2), 151-200.

[33] Quartau, J. A. & Borges, P.A. (2003). A new species of the genus *Aphrodes* Curtis from the Azores (Hemiptera,Cicadellidae). *Bocagiana*, 213, 1-11.

[34] Hamilton, K.G.A. (1983). Introduced and native leafhoppers common to the old and new worlds (Rhynchota: Homoptera: Cicadellidae). *Canadian Entomologist*, 115(5), 473–511.

[35] Nickel, H. & Remane, R. (2002). Checklist of the planthoppers and leafhoppers of Germany, with notes on food plants, diet width, life cycles, geographic range and conservation status (Hemiptera, Fulgoromorpha and Cicadomorpha). *Beiträge zur Zikadenkunde*, 5, 27-64.

www.biodicon.com BIOLOGICAL DIVERSITY AND CONSERVATION

ISSN 1308-8084 Online ISSN 1308-5301 Print

Research article/Araştırma makalesi
DOI: 10.46309/biodicon.2023.1270516
16/3 (2023) 224-228

Antioxidant and anticandidal effects of *Pedicularis comosa* L. var. *acmodonta* Boiss.

Nagehan SALTAN[1], Zeynep GÜLCAN[*1], Gökalp İŞCAN[2], Yavuz Bülent KÖSE[1]
ORCID: 0000-0002-1207-909X; 0000-0002-4379-3253; 0000-0003-1210-0490; 0000-0002-3060-7271

[1] Anadolu University, Faculty of Pharmacy, Department of Pharmaceutical Botany, Eskişehir, Türkiye
[2] Anadolu University, Faculty of Pharmacy, Department of Pharmacognosy, Eskişehir, Türkiye

Abstract

The aim of this study is to measure the antioxidant and anticandidal efficiency of *Pedicularis comosa* var. *acmodonta* (Orobanchaceae). Using a spectrophotometric test on extracts from *P.comosa* var. *acmodonta* grown in Türkiye, the antioxidant efficiency of the plant extracts was estimated by measuring the violet coloration in a methanol solution containing 1,1-diphenylpyryl hydrazil (DPPH·). In addition, the results of the anticandidal effect tests using the CLSI M27-A2 method are also included in this study. The 70% ethanol extract of *P. comosa* var. *acmodonta* showed the highest antioxidant activity (0.143 mg/mL). According to the anticandidal activity test findings on five *Candida* species, it was determined that 70% ethanol extract of *P. comosa* var. *acmodonta* inhibited *C. utilis* at a dose of 31.25 µg/mL (MIC).

Keywords: anticandidal, antioxidant, *Pedicularis,* Orobanchaceae.

---------- * ----------

Pedicularis comosa L. var. *acmodonta* Boiss. türünün antioksidan ve antikandidal etkilerinin belirlenmesi

Özet

Bu çalışmanın amacı, *Pedicularis comosa* var. *acmodonta* (Orobanchaceae) türünün antioksidan ve antikandidal aktivitesini değerlendirmektir. Türkiye'de yetişen *P. comosa* var. *acmodonta* ekstreleri üzerinde bir spektrofotometrik test kullanılarak, bitki ekstrelerinin antioksidan aktivitesi, 1,1-difenilpiril hidrazil (DPPH·**)** içeren bir metanol solüsyonundaki mor rengin açılması ölçülerek değerlendirilmiştir. Ayrıca CLSI M27-A2 yöntemi kullanılarak yapılan antikandidal etki testlerinin sonuçları da bu çalışmada yer almaktadır. *P. comosa* var. *acmodonta* 'nın %70 etanol ekstresi en yüksek antioksidan aktiviteyi (0.143 mg/mL) göstermiştir. Beş *Candida* türü üzerinde yapılan antikandidal aktivite testi bulgularına göre *P. comosa* var. *acmodonta* 'nın %70 etanol ekstresinin *C. utilis*'i 31.25 µg/mL (MIC) dozunda inhibe ettiği belirlenmiştir.

Anahtar kelimeler: antikandidal, antioksidan, *Pedicularis*, Orobanchaceae.

1. Introduction

The *Orobanchaceae* family consists mostly of parasitic plants. Currently, it is represented worldwide by 98 genera and 2233 species [1]. It is observed that ethnobotanical studies on parasitic plants are limited worldwide. O'Neill and Rana conducted ethnobotanical research on parasitic plants found in Nepal (Himalayas). As a result of the research, it was determined that more than 150 taxa are distributed in Nepal. Through interviews with 141 informants, it was observed that 43 parasitic plants were ethnobotanically used by the local population for medicinal, animal feed, food, religious ceremonies, and various materials [2, 3].

Pedicularis L. is a genus of typically grows to a height of up to 50 cm and is perennial herbaceous plant. *Pedicularis* species have historically been used for a very long time as folk medicines to cure tiredness and digestive problems, as well as to clear heat and remove toxins while preserving vitality and removing edema [4]. Different

[*] *Corresponding author* / Haberleşmeden sorumlu yazar: Tel.: +902223350580-3704 Fax.: ++902223350580-3704; E-mail:zgulcan@anadolu.edu.tr
 Received: 24.03.2023; Published: 15.12.2023 BioDiCon. 1093-240323

therapeutic uses for *Pedicularis* species exist in Traditional Chinese Medicine [5]. For the treatment of collapse, weariness, and senility, several of them have roots and stems that are utilized in Chinese folk medicine and as heart tonics. Iridoids, phenylethanoids, phenylpropanoids, and flavonoids [6-9] all of which are produced by the genus *Pedicularis*, are known to have antioxidant effects.

Pedicularis, known as “lousewort” or “wood concrete” in the literature, is popularly known as 'bitotu sormuk, sorma, sormuk otu' in Türkiye [10, 11]. The plant is used as a registered food in Eastern Anatolia due to the nectar in its flowers. It is one of the most commonly used classes of medicinal herbs and has many therapeutic benefits on fatigue, spontaneous perspiration, digestion issues, and heart issues. Additionally, some species of this genus have been found to exhibit hepatoprotective, anticancer, anti-oxidative, anti-haemolysis, and antibacterial properties [5, 6, 10]. In Flora of Turkey *Pedicularis* is represented by 11 species, 2 of which are endemic.

Due to the theuropatic benefits on digestion issues in the traditional use of the *Pedicularis* species, the antioxidant and anticandidal effects of *Pedicularis comosa* var. *acmodonta* extracts are emphasized in this study. To the best of our knowledge, methanolic extract of antibacterial efficacy was proven in earlier studies however there has been no research done on *Pedicularis* anticandidal effects [12].

2. Materials and methods

2.1. P. comosa L. var. acmodonta Boiss.

The plant was picked up from Han/Eskişehir in Türkiye on 26.05.2021 (Figure 1). Sample has been kept at the Herbarium in the Anadolu University, Eskisehir, Türkiye (ESSE 15818).

Figure 1. *Pedicularis comosa* var. *acmodonta*

2.2. Plant extract

The aerial parts were used. For using both polar and nonpolar solvents, 70% ethanol and *n*-hexane were used. Samples were held to macerate for 48 hours at 24°C temperature in an orbital shaker at 150 rpm. The solvents from the macerated were eliminated with a rotary evaporator under lower pressure after they had been filtered through filter paper. By lyophilizing, a 70% ethanolic extract was produced. The extracts were kept at +4 ºC after being released from their solvents until usage.

2.3. Antioxidant activity

The DPPH radical assay was put to use to evaluate the antioxidant properties of plant extracts made from the aerial parts using the techniques developed by Kumarasamy et al. with some modifications [13]. For standard matter, Gallic acid made used. All determinations were carried out in triplicate.

2.4. Antimicrobial activity

P. comosa var. *acmodonta* extracts were tested for their capability to inhibit the upgrowth of five different strains of *Candida*, including *C. utilis* (NRRL Y- 900), *C. albicans* (ATCC 90028), *C. tropicalis* (ATCC 750), *C. parapsilosis* (ATCC 22019) and *C. krusei* (ATCC 6258). Standard method (CLSI M27-A2) was used to identify the minimum inhibitory (MIC) concentrations of the extracts. Dilutions of the extracts were prepared between the concentration of 8000 to 15.6 µg/mL while the standard antifungals were 16 to 0.003 µg/mL. Minimal fungicidal concentration (MFC) was determined for the most active extract and the candida. A loop of medium from all clear wells including MIC was transferred into fresh PDA plates. After 24h incubation MFC was determined.

3. Results

Free radical scavenging activity of the 70% ethanol and *n*-hexane extracts were assessed spectrophotometrically by use of DPPH•. The *in vitro* antioxidant and anticandidal activities of the extracts obtained from the plant are presented in Tables 1 and 2. The results in Table 1 reveal that 70% ethanol extract (IC_{50} 0.143 mg/mL) was more capable of scavenging DPPH radical.

Table 1**.** DPPH• radical sweeping activity of the extracts (mg/mL)

Extracts	**DPPH test IC_{50} (mg/mL)**
70% ethanol	0.143 ±0.046
n-hexane	0.835 ±0.088
Gallic acid	0.002 ±0.001

The literature records indicate that the chemical compositions and biological activities of many *Pedicularis* species have been studied [14, 15, 16, 17, 18, 19]. Antioxidant activity of *P. sibthorpii* and *P. wilhelmsiana* were determined between the range of 0.01-0.7 mg/mL and 0.01-1.02 mg/mL by Khodaie et al. (2012). In a different investigation, the DPPH· radical scavenging and ABTS assays revealed that *P. mexicana* was active (0.883 0.032 mg/mL; 1.242 0.020 mg/mL) [20]. The DPPH radical photometric test was used in a study to evaluate antioxidant activity, and it was revealed that *P. comosa* L. var. *sibthorpii* (Boiss.) Boiss extracts had a very high ability to scavenge free radicals (IC_{50} <12.5 µg/mL) [21]. In a study evaluating the antidiabetic and antioxidant effect of the ethanol extract of *P. longiflora* Rudolph, it was reported that the increase in antioxidants and decrease in oxidants in the test groups may be due to the antioxidant features of the plant [22]. It was shown that the total polyphenol content of plants in water and methanol extracts ranged between 4.6 and 183.8 and 8.2 and 270.1 mg/g, respectively, and that *P. resupinata* had a high level of radical scavenging action against DPPH (IC_{50} <25 µg/mL) radical in Korea [23]. According to DPPH radical scavenging activity, extracts and various fractions from *P. longiflora* var. *tubiformis* (Klotzsch) P. C. Tsoong were concentrated and screened by Lan et al. It was shown that the 40% aqueous ethanol fraction demonstrated a remarkable antioxidant efficiency. The application of six major constituents (boschnaloside, alyssonoside, leucosceptoside A, isoverbascoside, leucosceptoside B and verbascoside) with high content revealed a clear antioxidant ability towards the DPPH radical [24].

Five *Candida* species were used for anticandidal assay according to the CLSI M27-A2 method. As regards the study results given in Table 2, it was determined that *C. utilis* (MIC 31.25 µg/mL) was quite sensitive to the ethanol extract prepared from the aerial parts of the plant.

Table 2. Anticandidal effect of *P. comosa* var. *acmodonta* extracts MIC (µg/mL)

Candida	**70% ethanol**	***n*-hexane**	**Amp-B**	**Keto**
C. utilis NRRL Y-900	31.25*	1000	1	0.5
C. albicans ATCC 90028	2000	4000	1	0.5
C. tropicalis ATCC 750	500	500	2	0.25
C. parapsilosis ATCC 22019	1000	1000	2	0.125
C. krusei ATCC 6258	500	500	1	1

*MFC: Minimum fungicidal concentration =1000 µg/mL
AMP-B: Amphotericin B, Keto: Ketoconazole

Studies on the antimicrobial effects of *Pedicularis* species primarily focus on bacteria. To the best of our knowledge, there is no study on the anticandidal effect of the species that is the subject of the study.

Opportunistic infections of *Candida* species are an important threat to public health such as systemic infections, vaginitis, oral and cutaneous candidiasis, and candidemia. To overcome the problem, it is a big need to find new natural substances with antifungal potential, more potent and less harmful.

In a previous study disc diffusion method was carried out to evaluate the antibacterial effect of the water and methanol extracts of *P. comosa* var. *sibthorpii* against ten pathogenic bacteria by Turker et al. (2021). It has been reported that plant extracts generally show antibacterial activity against *S. aureus*, *S. epidermidis* and *S. pyogenes* [21]. In another study, methanol extract of *P. sibthorpii* Boiss. was found to be effective against *P. aeruginosa*, *S. aureus* and *S. epidermidis*, however not sensitive to *C. albicans*. Moreover, methanolic extract from *P. wilhelmsiana* showed inhibitory activity against *P. aeroginosa, S. epidermidis*, *S. aureus* and *M. luteus*, whereas it did not show any inhibitory effect against *E. coli, S. paratyphi* and *B. cereus* [12]. Dulger and Ugurlu reported that the antifungal effect of *P. olympica* Boiss. extracts is less than that of standard antifungal antibiotics [25].

4. Conclusions and discussion

The biological activity data of the *P. comosa* var. *acmodonta*, revealed for the first time, is important to highlight our assumption that the plant can be exploited as a potential antioxidant, as well as contain positive components to ameliorate the negative effects of *Candida* spp. In this study, *P. comosa* var. *acmodonta* was evaluated in terms of anticandidal and antioxidant potential. As far as we know, the absence of anticandidal and antioxidant activity studies on *P. comosa* var. *acmodonta* increases the originality of this study. According to the activity results, the substances responsible for the effect are thought to be phenolic compounds [12]. It is foreseen that this study will constitute a source for comprehensive studies to be done in the future.

References

[1] https://www.worldfloraonline.org/ date of access: 09.10.2023.

[2] Satıl, F. , Tümen, G. & Selvi, S. (2017). Balıkesir'de yayılış gösteren parazit bitkilerin etnobotanik özellikleri. *Biological Diversity and Conservation*, 10 (3), 7-11. Retrieved from https://dergipark.org.tr/en/pub/biodicon/issue/55729/762124

[3] O'Neill, A.R., Rana, S.K. (2016). An ethnobotanical analysis of parasitic plants (Parijibi) in the Nepal Himalaya. *Journal of Ethnobiology and Ethnomedicine*,12(14), 1-15.

[4] Shao, M.H., Dai, W., Yuan, S.W., Lu, Y., Chen, D.F., Wang, Q. (2018). Iridoids from *Pedicularis* verticillata and their anti-complementary activity. *Chemistry and Biodiversity*, 15, e1800033. https://doi.org/10.1002/cbdv.201800033.

[5] Jiang, T.F., Ou, Q.Y., Shi, Y.P. (2003). Separation and determination of phenylpropanoid glycosides from *Pedicularis* species by capillary electrophoresis. *Journal of Chromatography A*, 986(1), 163-167. https://doi.org/10.1016/S0021-9673(02)01918-0.

[6] Zhang, Z.X., xie, W.D., Jia, Z.J. (2008). Glycosids from two *Pedicularis* species. *Biochemical Systematics and Ecology*, 36(5-6), 467-472.

[7] Akdemir, Z., Çalis, I., Junior, P. (1991). Iridoids and phenylpropanoid glycosides from *Pedicularis nordmanniana*. *Planta Medica*, 57(6), 584-585. DOI: 10.1055/s-2006-960215.

[8] Fujii, M., Miyaichi, Y., Tomimori, T. (1995). Flavonoid, phenylethanoid and iridoid constituents of the whole plant of *Pedicularis longiflora* var. *tubiformis*. *Planta Medica*, 61(6), 584. DOI: 10.1055/s-2006-959387.

[9] Su, B.N., Ma, L.P., Jia, Z.J. (1998). Iridoid and phenylpropanoid glycosides from *Pedicularis artselaeri*. *Planta Medica*, 64(8), 720-723. DOI: 10.1055/s-2006-957565.

[10] Li, M., He, X., Tao, R., Cao, X. (2014). Phytochemistry and pharmacologyof the genus *Pedicularis* used in traditional Chinese medicine. *The American Journal of Chinise Medicine*, 42, 1071-95. https://doi.org/10.1142/S0192415X14500670.

[11] Şen, B., Gürdal, B., Taşkın, T., Hacıoğlu, M., Bozkurt Güzel, Ç., Tufan, S., Mat, A. (2017). *Pedicularis condensata* Türüne ait Anatomi ve Aktivite Çalışmaları. *Marmara Pharmaceutical Journal*, 21(3), 485-492.

[12] Khodaiea, L., Delazara, A., Lotfipour, F., Nazemiyehc, Nazemiyeh, H. (2012). Antioxidant and antimicrobial activity of *Pedicularis sibthorpii* Boiss. and *Pedicularis wilhelmsiana* Fisch ex. *Advanced Pharmaceutical Bulletin*, 2(1), 89-92. https://doi.org/10.5681/apb.2012.012.

[13] Kumarasamy, Y., Byres, M., Cox, P.J., Jaspars, M., Nahar, L., Sarker, S.D. (2007). Screening seeds of some Scottish plants for free radical scavenging activity. *Phytotherapy Resarch*, 21 (7), 615-621. https://doi.org/10.1002/ptr.2129.

[14] Zheng, R.L., Wang, P.F., Li, J., Liu, Z.M., Jia, Z.J. (1993). Inhibition of the autoxidation of linoleic acid by phenylpropanoid glycosides from *Pedicularis* in micelles. *Chemistry and Physics of Lipids*, 65(2), 151-154. https://doi.org/10.1016/0009-3084(93)90048-8.

[15] Scalbert, A., Johnson, I.T., Saltmarsh, M. (2005). Polyphenols: antioxidants and beyond. *American Journal of Clinical Nutrition*, 81(suppl), 215S-217S. https://doi.org/10.1093/ajcn/81.1.215S.

[16] Hosoya, T., Yun, Y.S., Kunugi, A. (2008). Antioxidant phenylpropanoid glycosides from the leaves of Wasabia japonica. *Phytochemistry*, 69, 827-832. https://doi.org/10.1016/j.phytochem.2007.08.021.

[17] Ahmad, I., Ahmad, N., Wang, F. (2009). Antioxidant phenylpropanoid glycosides from Buddleja davidii. Journal of Enzyme Inhibition and Medicinal Chemistry, 24(4), 993-997. https://doi.org/10.1080/14756360802565072.

[18] Biao, C.H., Hua, T.N., Sheng, P.C. (2009). Progress in research on *Pedicularis* plants. *China Journal of Chinese Materia Medica*, 34(19), 2536-2546.

[19] Zhu, M., Tan, N., Zhu, H., Zeng, G., He, W., Yu, B., Chen, X. (2010). Anti-sports anaemia effects of verbascoside and martynoside in mice. *International Journal of Sports Medicine*, 31(8), 537- 541. DOI: 10.1055/s-0030-1254115.

[20] Moreno-Escobar, J.A., Bazaldúa, S., Villarreal, M.L., Bonilla-Barbosa, J.R., Mendoza, S., Rodríguez-López, V. (2011). Cytotoxic and antioxidant activities of selected Lamiales species from Mexico. *Pharmaceutical Biology*, 49(12), 1243–1248. https://doi.org/10.3109/13880209.2011.589454.

[21] Turker, A.U., Yildirim, A.B., Taş, I., Ozkan, E., Turker, H. (2021). Evaluation of some traditional medicinal plants: phytochemical profile, antibacterial and antioxidant potential. *Romanian Biotechnological Letters*, 26(2), 2499-2510. DOI: 10.25083/rbl/26.2/2499.2510 https://doi.org/10.25083/rbl/26.2/2499.2510.

[22] Yatoo, M.I., Dimri, U., Gopalakrishan, A., Saminathan, M., Dhama, K., Mathesh, K., Saxena, A., Gopinath, D., Husain, S. Antidiabetic and oxidative stress ameliorative potential of ethanolic extract of *Pedicularis longiflora* Rudolph. *International Journal of Pharmacology*, 12(3), 177-187, 2016. https://doi.org/10.3923/ijp.2016.177.187.

[23] Lee, Y.M., Bae, J.H., Jung, H.Y., Kim, J.H., Sik, D. (2011). Antioxidant activity in water and methanol extracts from Korean edible wild plant. *Journal of the Korean Society of Food Science and Nutrition*, 40(1), 29-36.

[24] Lan, Y., Chi, X., Zhou, G., Zhao, X. (2018). Antioxidants from *Pedicularis longiflora* var. *tubiformis* (Klotzsch) P. C. Tsoong. *Record of Natural Products*, 12(4), 332-339. http://doi.org/10.25135/rnp.35.17.08.142.

[25] Dulger, B., Ugurlu, E. (2005). Evaluation of antimicrobial activity of some endemic scrophulariaceae members from Turkey. *Pharmaceutical Biology*, 43(3), 275–279. https://doi.org/10.1080/13880200590928870.

www.biodicon.com BIOLOGICAL DIVERSITY AND CONSERVATION

ISSN 1308-8084 Online ISSN 1308-5301 Print

Research article/Araştırma makalesi
DOI: 10.46309/biodicon.2023.1344361
16/3 (2023) 229-236

Neuroprotective effect of Cistus laurifolius on hydrogen peroxide-induced neurodegeneration in differentiated SH-SY5Y cells

Hamiyet ECIROGLU[*1], Fatma YILDIZ[1], Ersin YUCEL[2]
ORCID: 0000-0002-3555-3946; 0000-0002-9270-9062; 0000-0001-8274-7578

[1]Department of Medical Laboratory Techniques, Vocational School of Health Services, Alanya Alaaddin Keykubat University, Antalya, Turkey
[2]Department of Biology, Faculty of Science, Eskisehir Technical University, Eskişehir, Turkey

Abstract

Neurodegeneration is an important finding of various neurological diseases such as Alzheimer's Disease and Parkinson's Disease. MAP2 and Rbfox3/NeuN genes are also two important neuronal markers. In this study, our aim is to investigate the neuroprotective effect of *Cistus laurifolius L.* extract in neurodegeneration caused by hydrogen peroxide (H_2O_2) treatment in differentiated SH-SY5Y (f-SH-SY5Y) cells.

In the study, the effects of H_2O_2 (62.5-1000µM) and *Cistus laurifolius* (15.62-1000 mg/ml) doses on cell viability were determined by the 3-(4,5-dimethylthiazol-2-yl)2,5-diphenyl tetrasodium bromide (MTT) method. The effect of *Cistus laurifolius* in d-SH-SY5Y cells followed by H_2O_2 treatment on cell viability was determined by the MTT test. The effect of the extract on MAP2 and Rbfox3/NeuN gene expressions in 24-hour periods was evaluated by the Real-Time Quantitative Reverse Transcription Polymerase Chain Reaction (RT-qPCR) method.

According to our findings, *Cistus laurifolius* extract significantly reduced cell viability in SH-SY5Y cells at 24 h and 48 h ($p<0.05$). H_2O_2 caused a decrease in d-SH-SY5Y cell viability at all doses ($p<0.05$). We showed that *Cistus laurifolius* extract applied for 24 hours and 48 hours before toxicity significantly increased cell viability at doses of 31.25 and 62.5 mg/ml in 24 hours compared to the H_2O_2 group. In d-SH-SY5Y cells, MAP2 gene expression in the H_2O_2 group was significantly decreased compared to the control group, however, it was upregulated in *Cistus laurifolius* groups at 31.25 and 62.5 mg/ml administered before toxicity compared to the H_2O_2 group ($p<0.05$). However, there was no significant difference between the groups in terms of NeuN gene expression ($p>0.05$).

The neuroprotective effect of *Cistus laurifolius* in the in-vitro neurodegeneration model has been extensively investigated for the first time. According to the data we obtained, *Cistus laurifolius* increased the cell viability and MAP2 gene expression and showed a neuroprotective effect. However, further studies are still needed to confirm the therapeutic value of the extract.

Keywords: SH-SY5Y, *Cistus laurifolius*, neuroprotective effect, MAP2, NeuN, cytotoxicity[*]

---------- * ----------

Cistus laurifolius'un farklılaşmış SH-SY5Y hücrelerinde hidrojen peroksit kaynaklı nörodejenerasyon üzerindeki nöroprotektif etkisi

Özet

Nörodegenerasyon, Alzheimer ve Parkinson gibi çeşitli nörolojik hastalıkların önemli bir bulgusudur. MAP2 ve Rbfox3/NeuN genleri de çeşitli nörodejeneratif hastalıkların patogenezinde rol alan iki önemli nöronal belirteçtir. Bu çalışmada amacımız olgun nöron benzeri hücreye farklılaşmış SH-SY5Y (f-SH-SY5Y) hücrelerinde hidrojen peroksit

[*] *Corresponding author* / Haberleşmeden sorumlu yazar: Tel.: +902425106060-7025; Fax.: +902425106009; E-mail: hamiyet.eciroglu@alanya.edu.tr
 Received: 16.08.2023; Published: 15.12.2023 BioDiCon. 1116-091623

(H_2O_2) muamelesi kaynaklı gelişen nörodejenerasyonda *Cistus laurifolius* L. ekstresinin nöroprotektif etkisinin araştırılmasıdır.

Çalışmada H_2O_2 (62.5-1000uM) ve *Cistus laurifolius* (15.62-1000mg/ml) dozlarının hücre canlılığı üzerine etkileri MTT yöntemi ile belirlendi. f-SH-SY5Y hücrelerinde *Cistus laurifolius'*un, ardından H_2O_2 uygulamasının hücre canlılığı üzerindeki etkisi 3-[4,5-dimetiltiyazol-2-il]-2,5-difenil-tetrazolyum bromür (MTT) testi ile belirlendi. Ekstrenin 24 saatlik sürede MAP2 ve Rbfox3/NeuN gen ifadeleri üzerindeki etkisi Gerçek Zamanlı Kantitatif Revers Transkriptaz Polimeraz Zincir Reaksiyonu (RT-qPCR) yöntemi ile değerlendirildi.

Bulgularımıza göre *Cistus laurifolius* ekstresi uygulaması ile SH-SY5Y hücrelerinde 24 ve 48 saatte hücre canlılığı anlamlı düzeyde azalmıştır ($p<0.05$). H_2O_2 tüm dozlarda d-SH-SY5Y hücre canlılığında azalmaya neden olmuştur ($p<0.05$). Toksisite öncesi 24 saat ve 48 saatlik sürelerde uygulanan *Cistus laurifolius* ekstresinin 24 saatte 31.25 ve 62.5 mg/ml'lik dozlarda hücre canlılığını H_2O_2 grubuna göre anlamlı derecede artırdığını gösterdik. d-SH-SY5Y hücrelerinde, H_2O_2 grubunda MAP2 gen ekspresyonu kontrol grubuna göre anlamlı derecede azaldı, buna karşılık toksisite öncesinde uygulanan 31.25 ve 62.5 mg/ml'lik *Cistus laurifolius* gruplarında H_2O_2 grubuna göre MAP2 gen ekspresyonu upregüle edildi ($p<0.05$). Ancak gruplar arasında NeuN gen ekspresyonu açısından anlamlı bir fark görülmedi ($p>0.05$).

Cistus laurifolius'un *in vitro* nörodejenerasyon modelindeki nöroprotektif etkisi ilk kez kapsamlı bir şekilde araştırılmıştır. Elde ettiğimiz verilere göre *Cistus laurifolius* hücre canlılığı ve MAP2 gen ekspresyonunda artışa neden olmuş ve nöroprotektif etki göstermiştir. Bununla birlikte, *Cistus laurifolius* ekstresinin terapötik değerini doğrulamak için daha fazla çalışmaya ihtiyaç vardır.

Anahtar kelimeler: SH-SY5Y, *Cistus laurifolius*, nöroprotektif etki, MAP2, NeuN, sitotoksisite

1. Introduction

The most prominent feature of neurological diseases such as Alzheimer's disease (AD), and Parkinson's disease (PD) is the development of neurodegeneration in the brain tissue due to various reasons. As a result of neurodegeneration, pathophysiological changes occur in neurons such as morphological disorders, loss of function, microtubule dysfunction, and neuronal atrophy [1,2].

Microtubules (MT) are one of the main elements of the cytoskeleton, which has many structural and functional roles in cells [3]. In addition, Microtubule-associated proteins (MAPs) have been identified, which have various roles such as neuronal development and signaling, binding to MTs, and stabilizing them [1,3]. One of these proteins, MAP2, provides stabilization by binding to microtubules, is a regulator in forming dendritic structures, mediates MT and actin cross-linking, and plays a role in cell signal transmission. It has been shown that irregularities in MAP2 expression and immunoreactivity are associated with neurodegenerative diseases such as AD, Prion disease, and neurodegenerative diseases [1,4,5]. Neuronal nuclei (NeuN) is a specific neuronal marker found in nuclei of mature neurons [6]. Although the physiological roles of NeuN have not been fully elucidated, it has been used in many studies to directly assess neuronal death or loss [6-8]. A study by Kim et al. explained that Neun is the gene product of Rbfox-3 [9]. Based on the available information, it is understood that MAP2 and NeuN genes are good therapeutic targets in neurodegeneration models.

In subjects such as investigating the pathogenesis of neurodegenerative diseases or developing treatment strategies, SH-SY5Y neuroblastoma cells, from which neuron-like cell models are obtained by applying differentiation protocol, are often used. SH-SY5Y cells can be differentiated into morphologically and structurally neurons with inducers such as retinoic acid (RA) and brain-derived neurotrophic factor (BDNF) [10-12]. In addition, since hydrogen peroxide (H_2O_2) causes neuron damage and increases Reactive oxygen species (ROS) in cells, it is often preferred to create an *in-vitro* neurotoxicity model [13]. Our study used the neurotoxicity model induced by H_2O_2 in differentiated SH-SY5Y (d-SH-SY5Y) cells.

Cistaceae is a plant family containing approximately 180 different species with antimicrobial, antiulcerogenic, antidiarrheal, antirheumatic, and vasodilator effects [14,15]. *Cistus laurifolius* ., one of the members of this family, is known to be used in traditional treatment methods for the treatment of rheumatic pain, high fever and urinary tract inflammation [16]. Studies have reported that *Cistus laurifolius* extracts are rich in flavonoids and have anti-inflammatory, antimicrobial, antioxidant, and anticarcinogenic effects [16-19]. In addition, the anti-cholinesterase and antioxidant effects of various Cistus species (*C. libanotis, C. creticus, C. Salvifolius*) were demonstrated in the AD model [20]. In another study, phytochemical compounds and antioxidant capacity of 5 different Cistus species were shown, according to the results obtained from the SH-SY5Y cells [21]. According to our research, limited reports in the literature describe the functional role of Cistus species in the prevention of neurodegeneration.

In this study, we aimed to examine the neuroprotective effect of the extract obtained from the leaves of *Cistus laurifolius* in the model of H_2O_2-induced neurodegeneration. In the study, the cytotoxic effects of *Cistus laurifolius* extract on d-SH-SY5Y cells were evaluated and the IC_{50} dose was calculated. Then, its protective effect on cell viability and its effects on MAP2 and NeuN gene expression were investigated in the neurodegeneration model.

2. Materials and methods

2.1. Plant material and extract preparation

Fresh leaves of *Cistus laurifolius* were collected in July 2013 by Prof. Dr. It was collected by Ersin YÜCEL. This sample was coded with ANES No. 15518 from Anadolu University Herbarium. The leaves of *Cistus laurifolius* were first dried in the sun for 3 days, then in an oven at 40-45 ºC for 48 hours, and then they were obtained in powder form. 40 g of powdered leaves were boiled in 800 ml of water for 60 minutes and the extract was evaporated in a vacuum evaporator and lyophilized. The final lyophilized form was stored at + 4°C until used in the tests [16].

2.2. Cell culture and neuron-like differentiation

A human SH-SY5Y neuroblastoma cell line was obtained from the Culture Collection Animal Cells, FMD Institute, Ankara, Turkey. The cells were grown in DMEM/F12 Hams (1:1) medium (Capricorn, China) supplemented with 10% v/v fetal bovine serum (FBS) (Cegrogen, Germany) and 1 units/ml penicillin-streptomycin (Cegrogen, Germany) at 37°C in an incubator containing 5% CO_2. The medium content was replaced every two days and cells were sub-cultured once they reached 70-80% confluence.

For cell differentiation, culture dishes were first coated with 50 mg/ml type I collagen (Gibco, USA). Next, cells were cultured as described above. After 24 hours of plating, the percentage of FBS in the culture medium was reduced to 1% and supplemented with 10 µM Retinoic acid (RA) (Bldpharm, China). The medium content was replaced per 2 days and differentiation was completed in 5 days [10]. Morphological changes and cell density were examined under inverted light microscopy (Zeiss Axio, Germany).

2.3. Cell Viability Assay

For cell viability tests, $1x10^3$ cells were added to each well in a 96-well plate and a differentiation protocol was applied. First, *Cistus laurifolius* extract (up to 15.62-1000 mg/ml) was applied to d-SH-SY5Y cells for 24 and 48 hours, and the cytotoxic effect of the extract on d-SH-SY5Y cells was evaluated. Then, H_2O_2 (up to 50-100µM) was treated to d-SH-SY5Y cells to determine the toxicity model, and the appropriate dose was determined. Finally the cells were treated with increasing concentration (up to 15.6-1000 mg/ml) of the extract for 24 and 48 hours. After incubation, cells were treated with a medium containing 250 µM H_2O_2 for 24 hours. After cell viability tests, protective doses of *Cistus laurifolius* extract on viability were determined. All experiments were repeated three times.

In all experiments, MTT (3-(4,5-dimethylthiazol-2-yl)2,5-diphenyl tetrasodium bromide) test was used to determine cell viability at the end of the incubation period. 100 µl of MTT solution was added to each well with a final 5 mg/ml concentration and incubated for 3 hours at 37 ^{0}C. After the incubation period, 100 µl of DMSO was added to each well and the tetrazolium salts formed were dissolved. Then, it was read spectrophotometrically in an ELISA Reader device (SynergyTM H1, Biotek, USA) at 630 nm and 570 nm absorbance. Cell viability was evaluated on a percentage basis compared to the control group [17].

2.4. Gene expression analysis

For further experiments, extract doses (31.25 mg/ml and 62.5 mg/ml) and time (24 hours) at which the highest viability was detected in the neurotoxicity model were determined. A total of four groups were formed for gene expression: control group, H_2O_2 group, extract doses of 31.25 mg/ml and 62.5 mg/ml. Cells were cultured at the specified doses and times and harvested at the end of the incubation periods. Gene expression analyses were performed by the Real-Time Quantitative Reverse Transcription Polymerase Chain Reaction (RT-qPCR) method.

2.4.1. RNA extraction and cDNA synthesis

For the evaluation of gene expression, firstly, total RNA isolation was performed from cell groups using the Total RNA Purification Isolation Kit (Jena Bioscience, Germany). The purity of isolated RNAs was measured with ELISA Plate Reader (260/280 nm= 1.8-2.1). RNAs were stored at -80 ^{0}C until further use.

In the second step, cDNA synthesis was performed from Totatl RNA using the cDNA synthesis kit (ABT, Turkey) in accordance with the manufacturer's instructions. cDNAs were stored at -20 ^{0}C until RT-qPCR studies.

2.4.2. Real-Time Quantitative Reverse Transcription Polymerase Chain Reaction (RT-qPCR) Analysis

MAP2 and NeuN gene expression in d-SHSY5Y cells treated with *Cistus laurifolius* extract was evaluated by RT-qPCR (LightCycler®96 Instrument - Roche Diagnostics). This test was performed using the SYBR-Green Master Mix (A.B. T™ 2X, Turkey) kit in accordance with the manufacturer's instructions. Glyceraldehyde 3-Phosphate Dehydrogenase (GAPDH) gene was used as housekeeping gene. The relative expression values were calculated using

the Cycle threshold (Ct) method according to the $2^{-\Delta\Delta Ct}$ formula and the fold change in mRNA expressions of target genes was determined [22].

2.5. Statistics

We used SPSS 21.0 for Windows software to analyze study data. The normality of data distributions was confirmed using the Shapiro–Wilk test. The groups showed normal distribution and the continuous variables were presented as means and standard error. One-way ANOVA and Tukey Test were used to test the differences between the groups. Other statistical analyses were calculated using GraphPad Prism (Version 7.04 for Windows, GraphPad Software, USA) software. A result of $p<0.05$ and $p<0.001$ was considered statistically significant.

3. Results

3.1. Cell Viability Analysis

To investigate the protective effects of *Cistus laurifolius* extract against H_2O_2-induced cytotoxicity in d-SH-SY5Y cells, cell viability was evaluated by MTT assay. First, to understand the optimum concentration range of *Cistus laurifolius* extract, SH-SY5Y cells were treated with the extract at logarithmically decreasing concentrations (15.62-1000 mg/ml) for 24 and 48 hours. According to our findings, there was a regular dose-dependent decrease in cell viability at 24 h exposure, and a statistically significant decrease in cell viability was observed at doses of 125 mg/ml and above ($p<0.05$). In 48-hour extract exposure, a significant decrease was observed at all doses compared to the control group ($p<0.05$). The 24 h and 48 h IC_{50} dose of *Cistus laurifolius* extract in SH-SY5Y cells was determined as 154.4 mg/ml and 122.4 mg/ml, respectively.

In order to determine the toxic dose of H_2O_2 in d-SH-SY5Y cells, doses of 62.5 µM, 125 µM, 250 µM, 500 µM and 1000 µM were tested in 24 hours (Figure 1. B). A significant decrease was observed in all doses compared to the control group ($p<0.05$) and the IC_{50} dose of H_2O_2 was determined as 212.7 µM. It was decided to use 250 µM as the neurotoxicity dose to be used in later experiments.

Finally, to evaluate the protective effect of *Cistus laurifolius* extract, d-SH-SY5Y cells were treated with increasing doses (15.62-1000 mg/ml) of the extract for 24 and 48 hours. The cells were then exposed to 250 µM H_2O_2 for 24 hours. After 24 hours of *Cistus laurifolius* treatment, a significant increase in cell viability was observed at concentrations of 31.25 and 62.5 mg/ml compared to the H_2O_2 toxicity group ($p<0.05$) (Figure 1.C.). At increasing doses, cell viability decreased with the effect of H_2O_2 and extract, and the toxicity rate increased significantly at concentrations of 500 and 1000 mg/ml ($p<0.05$). After 48 hours of *Cistus laurifolius* treatment, there was no significant increase in cell viability compared to the H_2O_2 toxicity group ($p>0.05$), on the contrary, the toxicity rate increased significantly at 500 and 1000 mg/ml concentration ($p<0.05$) (Figure 1.D.).

3.2. Analysis of gene expression in H_2O_2-induced d-SH-SY5Y cells pretreated with Cistus laurifolius

To evaluate the neuroprotective effect of *Cistus laurifolius* extract in H_2O_2-induced d-SH-SY5Y cells, mRNA expression levels of MAP2 and NeuN genes were evaluated by RT-qPCR method. According to our results, MAP2 gene expression level in the H_2O_2 toxicity group decreased 5.9-fold compared to the control group without any drug treatment ($p<0.001$). The MAP2 gene expression level in the 31.25 mg/ml and 62.5 mg/ml *Cistus laurifolius* extract group administered 24 hours before H_2O_2 treatment increased 2.2 and 2.4-fold, respectively, compared to the H_2O_2 toxicity group ($p<0.05$). MAP2 level in the d-SHSY5Y control group was still significantly higher than all groups ($p<0.05$; $p<0.001$) (Figure 2.A.).

On the other hand, NeuN gene expression level in the H_2O_2 toxicity group decreased 1.54-fold compared to the control group, but this was not statistically significant ($p>0.05$). In the treatment groups, the NeuN gene expression level in 31.25 mg/ml and 62.5 mg/ml *Cistus laurifolius* extract applied for 24 hours increased 1.2 and 1.1 fold, respectively, but these results were not statistically significant ($p>0.05$).

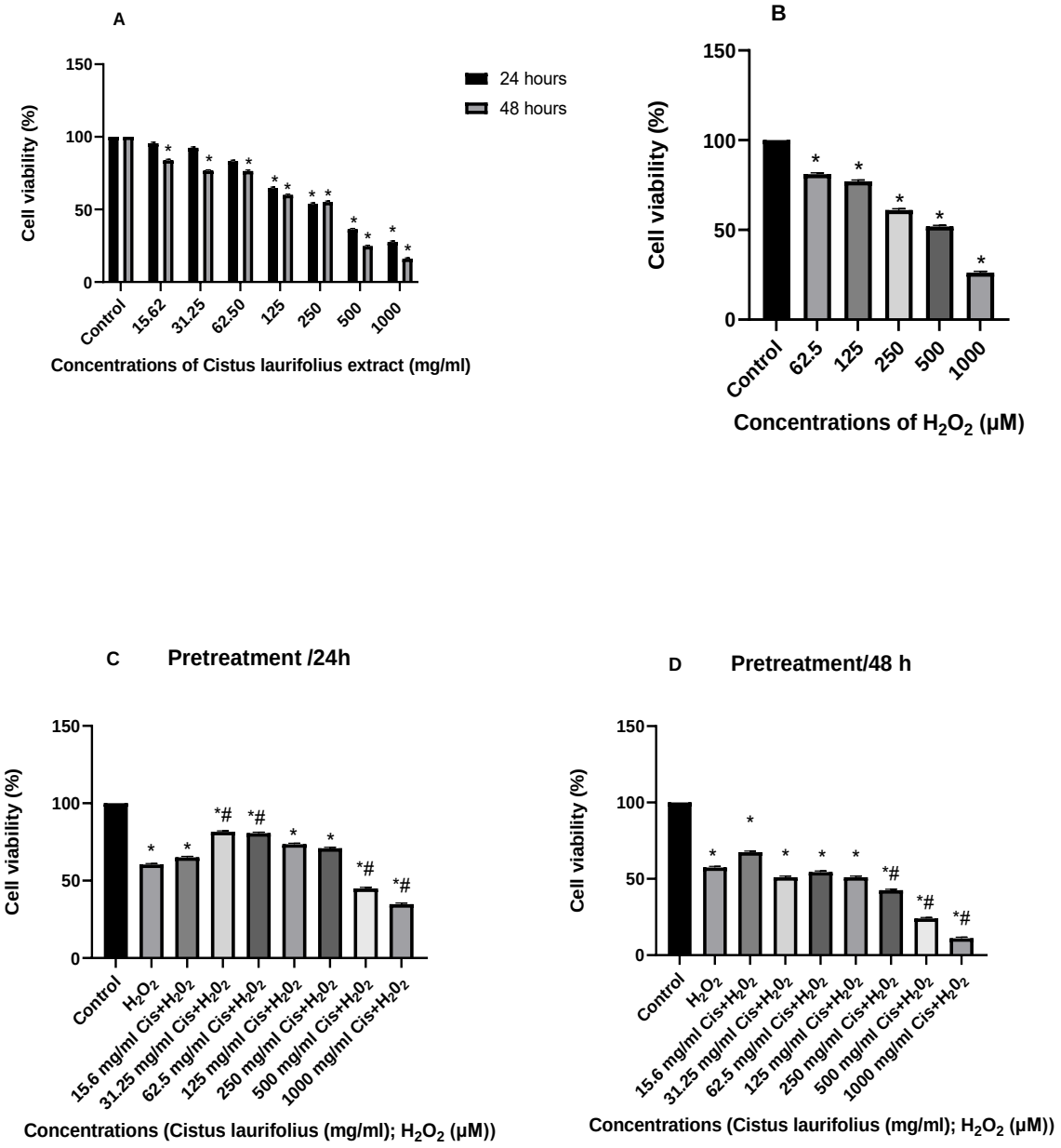


Figure 1. Evaluation of the effect of *Cistus laurifolius* extract on H_2O_2-induced cell death in d-SH-SY5Y cells by MTT test. A) Dose (up to 15.62-1000 mg/ml) and time (24h and 48 h)-dependent cytotoxic effect of *Cistus laurifolius* extract on SH-SY5Y cell viability. B) Dose-dependent cytotoxic effect of H_2O_2 for 24 h in d-SH-SY5Y cell. C) Effect of *Cistus laurifolius* extracts on d-SH-SY5Y cell viability against H_2O_2 (250 µM) neurotoxicity in 24-hour treatment. D) Effect of *Cistus laurifolius* extracts on d-SH-SY5Y cell viability against H_2O_2 (250 µM) neurotoxicity in 48-hour treatment. d-SH-SY5Y cells were pretreated with *Cistus laurifolius* before the toxicity of H_2O_2.) (Cis: *Cistus laurifolius*) Values are presented as mean±SEM. ∗p<0.05 compared to control group; #p<0.05 compared to H_2O_2 group. (Sample size of 4 per group; n=4).

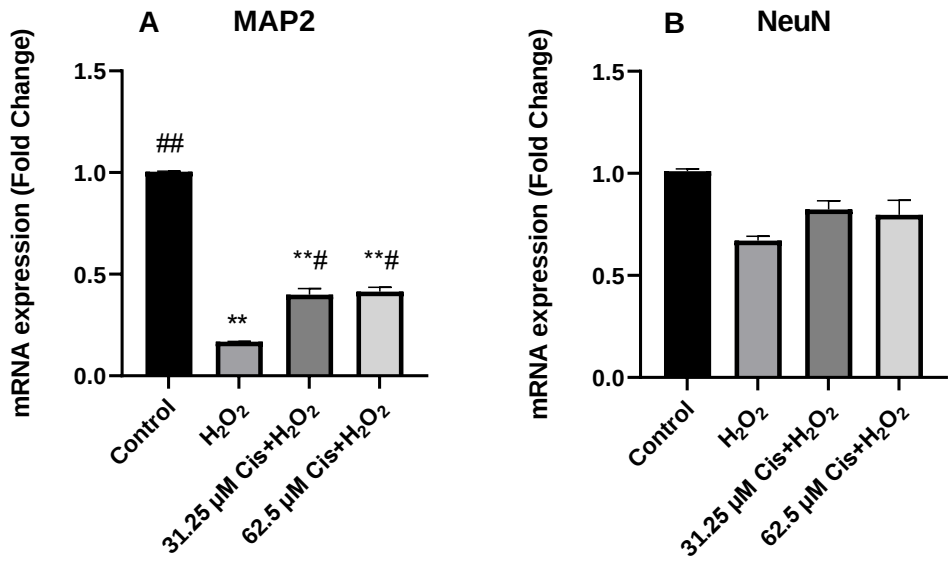


Figure 2. Effect of 24 h *Cistus laurifolius* treatment against H_2O_2 (24h) toxicity on the expression of MAP2 and NeuN genes in d-SH-SY5Y neuroblastoma cells. A) MAP2 gene expression B) NeuN gene expression analysis by RT-QPCR. (Cis: *Cistus laurifolius*) Values are presented as mean±SEM. **p<0.001 compared to control group; #p<0.05, ##p<0.05 compared to H_2O_2 group. (n=4).

4. Conclusions and discussion

In the pathogenesis of neurodegenerative diseases, cell damage, loss of function and even neuronal cell death are observed due to various causes such as oxidative stress, inflammation or accumulation of toxins [1,2]. Therefore, the discovery of potential neuroprotective agents that can reduce neurotoxicity is very important in reducing or treating the symptoms of neurodegenerative diseases [23]. In this study, we investigated the neuroprotective effect of *Cistus laurifolius* extract against H_2O_2-induced neurotoxicity in d-SH-SY5Y cells. In the study, we first evaluated the cytotoxic effect of *Cistus laurifolius* extract in SH-SY5Y cells by the MTT method. Then, we examined the cytotoxic effect of H_2O_2 at a certain dose range to determine the dose to be used to create a neurotoxicity model. Then, we evaluated the neuroprotective effect of pretreatment administration of *Cistus laurifolius* extract by the MTT method. We also evaluated the effect of *Cistus luurifolius* extract on the expression levels of MAP2 and NeuN genes in d-shSY5Y cells by RT-qPCR method.

H_2O_2 is a frequently used agent to create a neurodegeneration model *in vitro* studies. This agent causes oxidative stress and neuronal damage in cells [13,23]. In studies, an H_2O_2 dose of around 250 to 300 uM is preferred to induce neurotoxicity in SH-SY5Y cells [23, 24]. In this study, we examined the 24-hour cytotoxic effect of H_2O_2 at increasing doses up to 62.5-1000uM. A significant cytotoxic effect was observed at all doses compared to the control group. The IC_{50} dose of H_2O_2 was determined as 212.7 µM and 250 uM H_2O_2 dose was selected as the neurotoxicity dose to be used in further experiments. It is seen that the results we obtained are also compatible with the literature.

The cytotoxic effects of various Cistus species (such as *Cistus ladanifer, C. creticus, C. parviflorus*) have been studied in cancer types such as breast cancer, prostate cancer, cervical cancer, and neuroblastoma under *in vitro* conditions and reported to show antiproliferative effects [17, 21, 25]. SH-SY5Y cells, the IC_{50} dose of essential oils of *Cistus ladanifer* was 92.8 ppm [25], and the IC50 dose of C. parviflorus extract was 7.89 µg / ml [21]. The cytotoxic effects of *Cistus laurifolius* L. extract were examined in mouse fibrosarcoma cells (Wehi 164), human cervical adenocarcinoma cells (Hep2C), human muscle rhabdomyosarcoma cells (RD) cells, and it was reported that it showed significant antiproliferative effect at doses of 250 mg/ml and above in all these cell lines [17]. We found only one study evaluating the cytotoxic effect of *Cistus laurifolius* in SH-SY5Y cells. In this study conducted by Onal et al., the 24-hour IC_{50} dose of *Cistus laurifolius* in SH-SY5Y cells was determined as 45,73 mg/ml [21].

In our study, we firstly observed that *Cistus laurifolius* had a significant toxic effect on SH-SY5Y cell viability at doses of 125 mg and above in 24 and 48 hours applications, and we determined the IC_{50} doses as 154.4 mg/ml and 122.4 mg/ml for 24h and 48 hours, respectively. Our study results showed antiproliferative effect in cancer cells as in other species available in the literature. However, our main aim in this study was to investigate its protective effect against the neurotoxicity model in neuron-like d-SH-SY5Y cells. Therefore, we examined the protective effect of the treatment of the extract before toxicity on cells. The doses that increased cell viability the most in the pretreatment *Cistus laurifolius* treatment were 31.25 and 62.5 mg/ml for 24 hours. In these groups, cell viability increased significantly compared to the H_2O_2 group. However, at higher doses of *Cistus laurifolius*, cell viability decreased with

the combined effect of H_2O_2. It probably provides a protective effect with the effect of antioxidant activity at lower doses.

Anti-inflammatory and antioxidant properties of *Cistus laurifolius* have been shown in various studies [18, 21]. However, we found a limited number of studies in the literature on its neuroprotective effect. Akkol et al. showed that *Cistus* L. has acetylcholinesterase (AChE) butyrylcholinesterase (BChE) inhibitory effect and neuroprotective effect in the AD model [20]. In this study, we also examined the changes in MAP2 and NeuN gene expressions to see the effects of *Cistus laurifolius* extract on neuro markers. MAP2 and NeuN genes are among the important neuro markers in neurodegeneration models [1,6]. It has been shown that irregularities in MAP2 expression and its immunoreactivity are also associated with neurodegenerative diseases such as AD, Prion disease, or neuropsychiatric disorders such as schizophrenia and autism [1, 4, 5]. Although the physiological roles of NeuN have not been fully elucidated, it has been used in many studies to directly assess neuronal death or loss [6, 7, 8]. In this study, MAP2 gene expression of Cistus L. was upregulated against 250 μM H_2O_2 neurotoxicity at doses of 31.25 and 62.25 mg/ml, but no significant change was observed in NeuN gene expression. In fact, NeuN gene expression in the H_2O_2 group did not show a significant change compared to the control group. According to these results, we can say that Cistus has a positive effect on MAP2 gene against neuronal toxicity, while it is not effective for NeuN

In conclusion, the neuroprotective effect of *Cistus laurifolius* in the in-vitro neurodegeneration model has been extensively investigated for the first time. According to the data we obtained, *Cistus laurifolius* caused an increase in cell viability and MAP2 gene expression and showed a neuroprotective effect. In this context, our results are quite promising. However, further studies are still needed to confirm the therapeutic value of Cistus extracts.

References

[1] DeGiosio, R. A., Grubisha, M. J., MacDonald, M. L., McKinney, B. C., Camacho, C. J., & Sweet, R. A. (2022). More than a marker: Potential pathogenic functions of MAP2. *Frontiers in Molecular Neuroscience*, *15*, 974890. https://doi.org/10.3389/fnmol.2022.974890

[2] Dubey, J., Ratnakaran, N., & Koushika, S. P. (2015). Neurodegeneration and microtubule dynamics: death by a thousand cuts. *Frontiers in Cellular Neuroscience*, *9*, 343. https://doi.org/10.3389/fncel.2015.00343

[3] Bodakuntla, S., Jijumon, A. S., Villablanca, C., Gonzalez-Billault, C., & Janke, C. (2019). Microtubule-associated proteins: structuring the cytoskeleton. *Trends in Cell Biology*, *29*(10), 804-819. https://doi.org/10.1016/j.tcb.2019.07.004

[4] Shelton, M. A., Newman, J. T., Gu, H., Sampson, A. R., Fish, K. N., MacDonald, M. L., … & Sweet, R. A. (2015). Loss of microtubule-associated protein 2 immunoreactivity linked to dendritic spine loss in schizophrenia. *Biological Psychiatry*, *78*(6), 374-385. https://doi.org/10.1016/j.biopsych.2014.12.029

[5] Sontag, J. M., Nunbhakdi-Craig, V., White, C. L., Halpain, S., & Sontag, E. (2012). The protein phosphatase PP2A/Bα binds to the microtubule-associated proteins tau and MAP2 at a motif also recognized by the kinase Fyn. *Journal of Biological Chemistry*, *287*(18), 14984-14993. https://doi.org/10.1074/jbc.M111.338681

[6] Duan, W., Zhang, Y. P., Hou, Z., Huang, C., Zhu, H., Zhang, C. Q., & Yin, Q. (2016). Novel insights into NeuN: from neuronal marker to splicing regulator. *Molecular Neurobiology*, 53, 1637-1647. https://doi.org/10.1007/s12035-015-9122-5

[7] Long, X., Olszewski, M., Huang, W., & Kletzel, M. (2005). Neural cell differentiation in vitro from adult human bone marrow mesenchymal stem cells. *Stem Cells And Development*, 14(1), 65-69. https://doi.org/10.1089/scd.2005.14.65

[8] Safford, K. M., Safford, S. D., Gimble, J. M., Shetty, A. K., & Rice, H. E. (2004). Characterization of neuronal/glial differentiation of murine adipose-derived adult stromal cells. *Experimental Neurology*, 187(2), 319-328. https://doi.org/10.1016/j.expneurol.2004.01.027

[9] Kim, K. K., Adelstein, R. S., & Kawamoto, S. (2009). Identification of neuronal nuclei (NeuN) as Fox-3, a new member of the Fox-1 gene family of splicing factors. *Journal of Biological Chemistry*, *284*(45), 31052-31061. https://doi.org/10.1074/jbc.M109.052969

[10] Schneider, L., Giordano, S., Zelickson, B. R., Johnson, M. S., Benavides, G. A., Ouyang, X., ... & Zhang, J. (2011). Differentiation of SH-SY5Y cells to a neuronal phenotype changes cellular bioenergetics and the response to oxidative stress. *Free Radical Biology and Medicine*, 51(11), 2007-2017. https://doi.org/10.1016/j.freeradbiomed.2011.08.030

[11] Lopez-Suarez, L., Al Awabdh, S., Coumoul, X., & Chauvet, C. (2022). The SH-SY5Y human neuroblastoma cell line, a relevant in vitro cell model for investigating neurotoxicology in human: Focus on organic pollutants. *Neurotoxicology*, 92, 131-155. https://doi.org/10.1016/j.neuro.2022.07.008

[12] Uberti, D., Piccioni, L., Colzi, A., Bravi, D., Canonico, P. L., & Memo, M. (2002). Pergolide protects SH-SY5Y cells against neurodegeneration induced by H2O2. *European Journal of Pharmacology*, *434*(1-2), 17-20. https://doi.org/10.1016/S0014-2999(01)01537-0

[13] Lingappa, S., Shivakumar, M. S., Manivasagam, T., Somasundaram, S. T., & Seedevi, P. (2021). Neuroprotective effect of epalrestat on hydrogen peroxide-induced neurodegeneration in SH-SY5Y cellular model. *Journal of Microbiology and Biotechnology,* 31(6), 867. doi: 10.4014/jmb.2101.01002.

[14] Emerce, E., Gürbüz, P., DOĞAN, Ş., Kadioglu, E., & Suntar, I. (2019). Cytotoxic Activity-Guided Isolation Studies on Fumana procumbens (Dunal) Gren. & Godr. *Records of Natural Products,* 13(3). doi: 10.25135/rnp.98.18.07.331

[15] Yücel, E., (2014). Recognition Guide of Medicinal Plants Growing in Turkey, Türmatsan, Eskisehir, 1. Baskı, ISBN:978-975-93746-8-6

[16] Sadhu, S. K., Okuyama, E., Fujimoto, H., Ishibashi, M., & Yesilada, E. (2006). Prostaglandin inhibitory and antioxidant components of Cistus laurifolius, a Turkish medicinal plant. *Journal of Ethnopharmacology*, *108*(3), 371-378. https://doi.org/10.1016/j.jep.2006.05.024

[17] Aydın, S. S., & Yücel, E. (2021). Anti-proliferative effect of Cistus laurifolius on human cervical adenocarcinoma (Hep2C), human muscle rhabdomyosarcoma (RD), mouse fibrosarcoma (Wehi 164) cell line. *Biyolojik Çeşitlilik ve Koruma,* 14(2), 236-241. https://doi.org/10.46309/biodicon.2021.908458

[18] Küpeli, E., & Yesilada, E. (2007). Flavonoids with anti-inflammatory and antinociceptive activity from Cistus laurifolius L. leaves through bioassay-guided procedures. *Journal of Ethnopharmacology*, *112*(3), 524-530. https://doi.org/10.1016/j.jep.2007.04.011

[19] Hacioglu, C., Kar, F., Kara, Y., Yucel, E., Donmez, D. B., Sentürk, H., & Kanbak, G. (2021). Comparative effects of metformin and Cistus laurifolius L. extract in streptozotocin-induced diabetic rat model: oxidative, inflammatory, apoptotic, and histopathological analyses. *Environmental Science and Pollution Research*, *28*(41), 57888-57901. https://doi.org/10.1007/s11356-021-14780-y

[20] Akkol, E. K., Orhan, I. E., & Yeşilada, E. (2012). Anticholinesterase and antioxidant effects of the ethanol extract, ethanol fractions and isolated flavonoids from Cistus laurifolius L. leaves. *Food Chemistry*, *131*(2), 626-631. https://doi.org/10.1016/j.foodchem.2011.09.041

[21] Onal, F., Ozturk, I., Aydin Kose, F., Der, G., Kilinc, E., & Baykan, S. (2023). Comparative evaluation of polyphenol contents and biological activities of five Cistus L. species native to Turkey. *Chemistry & Biodiversity*, *20*(1), e202200915. https://doi.org/10.1002/cbdv.202200915

[22] Livak, K. J., & Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2− ΔΔCT method. 25(4), 402-408. https://doi.org/10.1006/meth.2001.1262

[23] Tian, W., Heo, S., Kim, D. W., Kim, I. S., Ahn, D., Tae, H. J., ... & Park, B. Y. (2021). Ethanol extract of Maclura tricuspidata fruit protects SH-SY5Y neuroblastoma cells against H2O2-induced oxidative damage via inhibiting MAPK and NF-κB signaling. *International Journal of Molecular Sciences*, *22*(13), 6946. https://doi.org/10.3390/ijms22136946

[24] Ismail, N., Ismail, M., Fathy, S. F., Musa, S. N. A., Imam, M. U., Foo, J. B., & Iqbal, S. (2012). Neuroprotective effects of germinated brown rice against hydrogen peroxide induced cell death in human SH-SY5Y cells. *International journal of molecular sciences*, *13*(8), 9692-9708. https://doi.org/10.3390/ijms13089692

[25] Najar, B., Shortrede, J. E., Pistelli, L., & Buhagiar, J. (2020). Chemical composition and in vitro cytotoxic screening of sixteen commercial essential oils on five cancer cell lines. *Chemistry & Biodiversity*, *17*(1), e1900478. https://doi.org/10.1002/cbdv.201900478

www.biodicon.com BIOLOGICAL DIVERSITY AND CONSERVATION

ISSN 1308-8084 Online ISSN 1308-5301 Print

Research article/Araştırma makalesi
DOI: 10.46309/biodicon.2023.1373439
16/3 (2023) 237-251

Exhibition collection species list of Eskişehir Osmangazi University Zoology Museum

Hakan ÇALIŞKAN[*1]
ORCID: https://orcid.org/0000-0001-7879-6449

[1] Eskişehir Osmangazi University, Faculty of Science, Department of Biology, 26040 Eskişehir, Türkiye

Abstract

Biodiversity museums are biological libraries that introduce life thanks to the specimens they have. ESOGÜ Zoology Museum is one of the few biodiversity museums in Türkiye. It has been serving as a university museum since it was established in 2007. This study aims to share the existing records in the museum by preparing the first list of the species exhibited in the ESOGÜ Zoology Museum. In the study, 205 samples were collected from 46 provinces in Türkiye , and 5 different foreign countries between 1987 and 2012. Inventory registration numbers are provided for the specimens in a code pattern consisting of six letters and six numbers. In the list, the phylum, class, order and families of the species, the date of collection or acquisition, the locality, and the person who collected it are provided. There are 24,241 specimens, 21,411 of which are Insecta, registered in the inventory of the museum. As of June 2023, 698 examples are presented to the visitors in the exhibition hall of the collection. 205 of 317 specimens from Cnidaria, Mollusca, Arthropoda, Echinodermata, and Chordata phylum, constituting the main collection, were evaluated. As a result of this study 169 species, 14 of which were at the genus level, belonging to the 18 classes, 69 orders, and 136 families were listed.

Key Words: Biodiversity, Cnidaria, Mollusca, Arthropoda, Echinodermata, Chordata

---------- * ----------

Eskişehir Osmangazi Üniversitesi Zooloji Müzesi sergi koleksiyonu tür listesi

Özet

Biyoçeşitlilik müzeleri, sahip oldukları numuneler sayesinde yaşamı tanıtan birer biyolojik kütüphanelerdir. ESOGÜ Zooloji Müzesi Türkiye'de bulunan az sayıdaki biyoçeşitlilik müzelerinden birisidir. Kurulduğu 2007 yılından günümüze bir üniversite müzesi olarak hizmet vermektedir. Bu çalışma, ESOGÜ Zooloji Müzesi bünyesinde sergilenen türlerin ilk listesini hazırlayarak müzede var olan kayıtların paylaşıma açılması amaçlamaktadır. Çalışmada 205 numune, 1987-2012 yılları arasında Türkiye'de 46 il ve 5 farklı yurt dışı ülkeden bir araya getirilmişlerdir. Numuneler için altı harf ve altı rakamdan oluşan bir kod düzeniyle envanter kayıt numaraları verilmiştir. Listede, türlerin şube, sınıf, takım ve familyalarına, toplandığı ya da elde edildiği tarih, lokalite ve toplayan kişi bilgisine yer verilmiştir. Müzenin envanterine kayıtlı 21 411'i Insecta olmak üzere 24 241 numune bulunmaktadır. Kolleksiyona ait sergi salonunda Haziran 2023 itibariyle 698 örnek ziyaretçilere tanıtılmaktadır. Bunların içinde yer alan ve sergilenen ana koleksiyonu oluşturan Cnidaria, Mollusca, Arthropoda, Echinodermata, Chordata şubelerine ait 317 numunenin 205'i değerlendirilmiştir. Bu çalışma sonucunda 18 sınıf, 69 takım ve 136 familyaya ait 14'ü cins düzeyinde olmak üzere 169 tür listelenmiştir.

[*] *Corresponding author* / Haberleşmeden sorumlu yazar: Tel.: +905055617945; Fax.: +902222393578; E-mail: hakan@ogu.edu.tr
 Geliş tarihi: 09.10.2023; Yayın tarihi: 15.12.2023 BioDiCon. 1124-091023

Anahtar Kelime: Biyoçeşitlilik, Cnidaria, Mollusca, Arthropoda, Echinodermata, Chordata

1. Giriş

Biyoçeşitlilik müzeleri, diğer müzeler gibi insan ve doğanın birikimlerini gelecek nesillere aktaran mucizevi mekânlardır. Dünyamızın ve üzerindeki yaşam formlarının yorumlanmasında ziyaretçilerine harikulade deneyimler yaşatan biyolojik kütüphanelerdir. Yaşamın dinamiklerini tanıtırlar. Türkiye çok zengin biyoçeşitliliğe sahip olmasına rağmen sahip olduğu biyolojik çeşitliliğini tanıtacak müzeleri bir kaç ilde bulunmakta olup, kapasiteleri son derece sınırlıdır [1]. Ülkemizde biyoçeşitlilik koleksiyonlarının sergilenmesi ve açık erişeme sunulması ile ilgili olarak Bicer ve diğerleri [2], Alhasan ve Akan [3], Kesici ve Dalyan [4], Kaya ve diğerleri [5], Dikmen ve Özuluğ [6], Özuluğ ve Saç [7], Gönülal ve Güreşen [8], Dalyan [9], Baycan ve Tosunoğlu [10] ile Kaya ve Özuluğ [11]'un müze koleksiyonlarına ait liste çalışmaları yer almaktadır.

Ülkemizin üniversite müzeleri arasında ilk Zooloji Müzesi, İstanbul Üniversitesi Zooloji Müzesidir. Koleksiyonu 19. yy.'a kadar gitmektedir. 1933 yılında ülkemiz zooloji bilimine ve Anadolu faunasının tespitine büyük katkı sağlayan Ord. Prof. Dr. Curt Kosswig tarafından alınan ZMUI (Zoologischen Museums der Universitat Istanbul) uluslararası kodla müze ismini bilim dünyasına duyurmuştur [12]. ZMUI'dan 74 yıl sonra üniversite bünyesinde faaliyet gösteren az sayıda biyoçeşitlilik müzelerinden birisi olan ESOGÜ Zooloji Müzesi, 30 Nisan 2007 yılında kurulmuş ve hizmet vermektedir. Müzede 24241 numune yer almaktadır. Numuneler, Eskişehir başta olmak üzere Türkiye'nin birçok yerinden, 1987 yılından günümüze kadar toplanarak bir araya getirilmiştir. Koleksiyonda numuneler taksidermi, iskelet, sıvı çözelti içinde ya da kurutulmuş olarak tutulmaktadırlar. Envanterde, 9 Porifera, 6 Anthozoa, 2611 Mollusca, 21411 Arthropoda, 32 Echinodermata, 31 Pisces, 8 Amphibia, 14 Reptilia numuneleri kuru ya da sıvı solüsyonlarda saklanırken, 48 Aves, 8 Mammalia taksidermi olarak, 25 Mammalia, 2 Aves numunesi iskelet olarak bulunmaktadır. Ayrıca 36 adet fosil numune koleksiyonda yer almaktadır. Sergi salonunda Haziran 2023 itibariyle 698 numune tanıtım amacıyla yerleştirilmiştir.

Müzede yer alan Cnidaria, Mollusca, Arthropoda, Echinodermata, Chordata şubelerine ait 317 numune müzenin sergilenen ana koleksiyonunu oluşturmaktadır. Bu çalışmada söz konusu ana koleksiyon içinde yer alan ve yazar tarafından 1988-2012 yılları arasında toplanan 195, temin edilen 10 numune olmak üzere toplam 205 numune değerlendirilmiş ve 18 sınıf, 69 takım, 136 familyaya ait 14'ü cins düzeyinde, 169 tür ve alt türlerden oluşan liste hazırlanmıştır. Tür listesi ESOGÜ Zooloji Müzesinin paylaşılan ilk verileridir. Müzede bulunan koleksiyonun çok büyük bir kısmı halen teşhis edilmeyi beklemektedir.

2. Materyal ve yöntem

Çalışmada listelenen türler 1988-2012 yılları arasında bir araya getirilmiştir. 195 numune, ülkemizde Afyon, Antalya, Balıkesir, Bilecik, Bolu, Bursa, Çanakkale, Düzce, Eskişehir, Gaziantep, İstanbul, İzmir, Mardin, Muğla, Urfa, Sakarya illerinden toplam 46 lokalitede yazarın araştırma amaçlı yaptığı biyolojik araştırma çalışmaları sırasında toplanmıştır. Temin edilen 10 numunenin 6'sı Çin, Batı Pasifik, Tayland, Nijerya ve Peru menşelidir. Çalışma toplam 205 numunenin değerlendirilmesiyle elde edilmiştir.

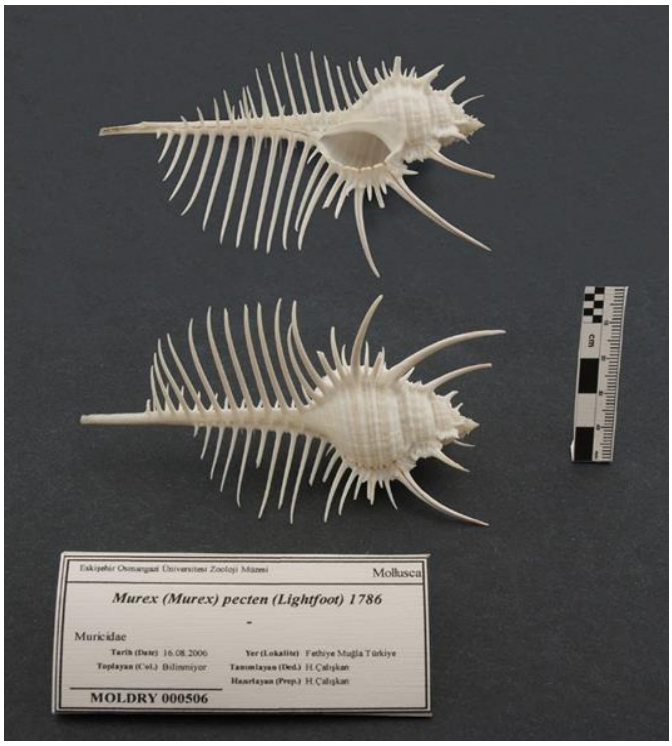
Temin edilen numunelerden, MOLDRY000468 *Harpago sp.*, *MOLDRY000333 Laevistrombus canarium* (Linnaeus, 1758) satın alınmış; REPLIC000346 *Iguana iguana* (Linnaeus, 1758), REPTAX *000469 Caretta caretta* (Linnaeus, 1758), AVSTAX000472 *Neophron percnopterus* (Linnaeus, 1758), AVSTAX000623 *Anas crecca* Linnaeus, 1758, AVSTAX000479 *Calidris pugnax* (Linnaeus, 1758), MAMTAX000619 *Lama glama* (Linnaeus, 1758) numuneleri yazara hediye edilmiş ancak ne yazık ki müzede yaşanan su baskınları sırasında etiket ve kayıt defterlerinin zarara uğraması nedeniyle listede kişi bilgileri eksik verilmiştir. Bu örnekler listede " toplayan" sütununda (?) ile belirtilmişlerdir. CRSDRY000627 *Tachypleus tridentatus* (Leach, 1819), G. Ortuğ, AVSTAX000048 *Columba livia var. domestica* J.F.Gmelin, 1789, Ü. Remzi Dinçer tarafından yazara hediye edilmiştir.

Taksidermi ve iskelet numuneleri, doğada ölü ya da yaralanmış olarak bulunup kurtarılamayan numunelerdir. Taksidermi ve sıvı örnekleri Gülen [13]'e göre ve iskelet numuneleri Çalışkan ve diğerleri [14]'e göre yazar tarafından hazırlanmışlardır. Sıvı numunelerin saklanmasında %70 Alkol ve %4 formaldehit çözeltisi kullanılmıştır.

Listelenen türlerin nomeclaturu, Küresel Biyoçeşitlilik Bilişim Tesisi GIBIF (the Global Biodiversity Informatics Facility) [15], Dünya Deniz Türleri Kaydı WoRMS (World Register of Marine Species) [16] ve Ramazzotti ve diğerleri [17]'ne göre verilmiştir.

Türlerin teşhisleri, Türkiye Kelebek Gözlemcileri & Fotoğrafçıları Topluluğu [18], Bakır & Çevingen [19], Çıplak ve diğerleri [20], Çıplak & Demirsoy [21], Coppard, [22], Demirsoy [23, 24, 25, 26, 27], Devetak [28], Önder ve diğerleri [29], Dyachko & Nedoev [30], Öztürk & Bağdatlı [31], Topçu & Öztürk [32], Flutsch & Tauzin [33], Furtun ve diğerleri [34], Özalp & Alparslan [35], Hristo & Chobanov [36], Holthuis [37], Karataş ve diğerleri [38], Kirvan ve diğerleri. [39], Korotyaev ve diğerleri [40], Özgür [41], Neimorovets [42], Néraudeau ve diğerleri [43], Tüzün & Kekilioğlu [44], Özbek & Ustaoğlu [45], Öztürk ve diğerleri [46], Rasmont ve diğerleri [47], Turin ve diğerleri [48] ve

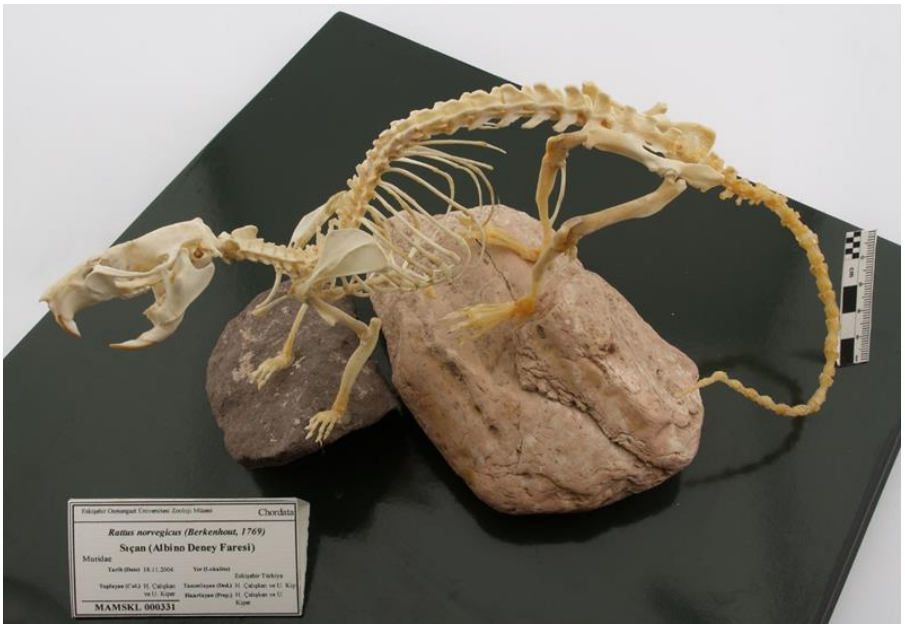
Zariquiey Alvarez [49]'e göre yapılmıştır. Teşhislerinde karşılaştırma materyaline ihtiyaç duyulan 14 örnek cins düzeyinde bırakılmıştır.



Şekil 1. MOLDRY 000506 numaralı *Murex* (Murex) *pecten* (Lightfoot) 1786. Numunelerin envantere kayıtlarında kullanılan kodun yer aldığı etiket ve dijital görsel için yerleşim planı (ölçek 5 cm)

Numunelerin müze envanter kayıtları için kullanılan kodlama sistemi 6 harf ve 6 rakamdan oluşan iki bölüme ayrılmıştır (Şekil 1). Harflerin ilk üçü şube, alt şube ya da sınıfı tanımlar, [Porifera (POR), Cnidaria (CND), Anthozoa (ANT), Mollusca (MOL), Crustacea (CRS) Chelicerata (CHL), Myriapoda (MYR), Insecta (INS), Echinodermata (ECH)] son üçü örneğin kuru (DRY), sıvı (LIC), taksiderim (TAX), iskelet (SKL) olduğunu belirtir. Numaralandırma 0-999 arasında her yeni numuneye sırayla verilen kayıt numarasını ifade eder [50]. Müze örneklerinin dijitalleştirilmesi çalışması kapsamında görsel kayıtları da alınmaktadır (Şekil 1-3).

Şekil 2. INSDRY 000220 kodlu *Saturnia* (Saturnia) *pyri* (Denis & Schiffermüller) 1775 ve numune için hazırlanan genel kullanım veri etiketi (ölçek 5 cm)



Şekil 3. MAMSKL000331 nolu *Rattus norvegicus* (Berkenhout, 1769) ve özel hazırlık gerektiren numuneler için tasarlanan numune veri etiketi (ölçek 5 cm)

Numuneler için Çalışkan ve Yalçın [50]'a göre farklı tipte etiketler tasarlanmıştır. Numunenin önüne bilgilendirme amaçlı hareketli iki farklı tip [Şekil 1, 2], numunenin içine ya da tabla altına yerleştirilen sabit (Şekil 4) bir adet etiket tasarımı kullanılmaktadır. Taksidermi gibi büyük örneklerin platformlarının altına yerleştirilen ve Arthropoda özellikle Insecta gibi daha ufak numuneler ile birlikte iğnelenen, küçük az yer kaplayan etiketler kullanılmıştır

Şekil 4: Küçük numuneler ve numune tablalarının altına yerleştirmek için kullanılan veri etiketi

3. Bulgular

Bu çalışmada yer alan 14'ü cins düzeyinde, 169 tür, teşhisleri yapılarak Ek'de, Tablo 1 olarak listelenmiştir. Bazı türlerin hem taksidermisi, hem iskeleti sergilendiği için listede ayrı ayrı yer almaktadırlar. Tekrarlanan türler sayıya dahil edilmemiştir. Teşhisleri cins düzeyinde bırakılmış olan numuneler farklı vücut özellikleri nedeniyle sergi numunesi olarak seçilen örneklerdir.

4. Sonuç

ESOGÜ Zooloji Müzesi sergi salonunda sergilenen Cnidaria, Mollusca, Arthropoda, Echinodermata, Chordata şubelerine ait 205 biyolojik çeşitlilik numunesi değerlendirilmiştir. Bu çalışmada, 18 sınıf, 69 takım, 136 familyaya ait 14'ü cins düzeyinde, 169 tür ve alt türlerden oluşan liste hazırlanmıştır.

Uzun yıllar saklama dolaplarında beklemiş biyolojik türlere ait birey numunelerinin teşhisleri için hassas taksonomik vücut kısımlarına ihtiyaç duyulmaktadır. Moleküler yöntemler gibi teknolojik yaklaşımlar bu tür örnekler için kullanılamayabilir. Bu nedenle koleksiyon olarak saklanacak tüm numunelerin toplanması ve saklanmasında morfolojik vücut kısımlarının korunması, zarar görmemesi büyük önem taşır. Bu hassasiyet müze koleksiyonlarının sürdürülebilirliğinin en büyük zorlukları arasındadır.

Hızlanan iklim değişikliği ile birlikte müzelerde ya da şahsi koleksiyonlarda var olan biyoçeşitlilik kayıtlarının listelerinin açık erişime sunulmalı ve dijital veriye dönüştürülmelidir. Bu çalışmada hazırlanan liste, ESOGÜ Zooloji Müzesinin paylaşılan ilk verileridir. Müzede bulunan koleksiyonun çok büyük bir kısmı halen teşhis edilmeyi beklemektedir.

Teşekkür

Çalışmada yer alan koleksiyonun korunması ve bakımı konusundaki destekleri için Eskişehir Osmangazi Üniversitesi Biyoloji Bölümü öğretim üyeleri Doç. Dr. D.Ümit ŞİRİN, Doç. Dr. Figen ÇALIŞKAN, Dr. Arş. Gör. Ebru Ceren Fidan'a, çalışmadaki türlerin teşhislerinin kontrolünde Niğde Halisdemir Üniversitesi Fen Ebebiyat Fakültesi Biyoloji Bölümü öğretim üyesi Prof. Dr. Ahmet KARATAŞ'a, Akdeniz Üniversitesi Fen Fakültesi Biyoloji Bölümü öğretim üyesi Prof. Dr. Battal ÇIPLAK'a ve ESOGÜ Zooloji Müzesi Koleksiyonu için yeni yer sağlayan Eskişehir Osmangazi Üniversitesi Rektörlüğüne şükranlarımı sunarım.

Kaynaklar

[1] Çalışkan, H. (2020) Biodiversity Museums: Place and Importance of Biodiversity Museums in Turkey: Recommentations and Practices for Zoology Museums of Eskişehir Osmangazi University. In F. Erbay, M. Erbay & N. Ö. Erbay (Eds.), *Post-Modern University Museums*. (pp. 172-173). İstanbul: Kadir Has University,

[2] Bicer, S., Odabasi, D. A., & Ozturk, B. (2022). The Catalog of Aquatic Mollusca (Bivalvia and Gastropoda) in the Limnology Museum of Çanakkale Onsekiz Mart University (COMULM). *Turkish Journal of Bioscience and Collections*, 6(1), 7–13. https://doi.org/10.26650/tjbc.20221017242

[3] Alhasan, N. & Akan, H. (2021). Harran Üniversitesi Herbaryumu (HARRAN)'ndaki Geofit Koleksiyonu. *Turkish Journal of Bioscience and Collections*, 5(1), 22-82. https://doi.org/10.26650/tjbc.2021717711

[4] Kesici, N. B., & Dalyan, C. (2018). An updated list of cryptobenthic fishes (Syngnathidae, Gobiidae, Gobiesocidae, Tripterygiidae, Blenniidae, Scorpaenidae) in the IUSHM fish collection. *Turkish Journal of Bioscience and Collections*, 2(2), 1-10.

[5] Kaya, N., Ergün Bacak, E., Özuluğ, O., & Arslangündoğdu, Z. (2018). The Bird Collection of İstanbul University Biology Department (Turkey). *Turkish Journal of Bioscience and Collections*, 2(2), 20-32.

[6] Dikmen, F., & Özuluğ, O. (2018). Insect (Coleoptera and Orthoptera) species of İstanbul in the Zoology Collection of Istanbul University. *Turkish Journal of Bioscience and Collections*, 2(1), 27-43.

[7] Özuluğ, M., & Saç, G. (2018). İstanbul Üniversitesi Fen Fakültesi Hidrobiyoloji Müzesi Balık koleksiyonu Tip Örnekleri Kataloğu. *Turkish Journal of Bioscience and Collections*, 2(1), 44-50.

[8] Gönülal, O., & Güreşen, S. O. (2017). A catalogue of the marine species: Gökçeada Marine Museum. *Turkish Journal of Bioscience and Collections* , 1(1), 1-15.

[9] Dalyan, C.(2017). The collection of Lessepsian Fishes in the Istanbul University, Science Faculty, Hydrobiology Museum. *Turkish Journal of Bioscience and Collections*, 1(1),16-20.

[10] Baycan, B., & Tosunoğlu, M. (2017). The Catalog of Amphibia and Reptilia Specimens in the Çanakkale Onsekiz Mart University Zoology Museum (COMU-ZM). *Turkish Journal of Bioscience and Collections*, 1(1),38-55.

[11] Kaya, N., & Özuluğ, O. (2017). The Herpetological collection of Zoology Museum, İstanbul University. *Anadolu University Journal of Science and Technology C- Life Science and Biotechnology*, 6 (2), 55-63. https://doi.org/ 10.18036/aubtdc.286684

[12] Özuluğ, O., Dökümcü, N., & Kaya, N., (2013). İstanbul Üniversitesi Zooloji Müzesi'nin ülkemiz zooloji tarihindeki önemi. *Ordu Üniversitesi Bilim ve Teknoloji Dergisi*, 3(1), 69-78.

[13] Gülen, Ö. K. (1985). Biyolojik Koleksiyonlar ve Laboratuvar Tekniği. Ankara: Taş Kitapçılık.

[14] Çalışkan, H., Şirin, D. Ü., Fidan, E., & Ulutaş, V. (2017, 07-08 Nisan). Biyoçeşitlilik Müzelerinde örneklerin sergi amaçlı hazırlanmaları. Büyük Değişimler Çağında Üniversite Müzeleri. 4. Uluslararası Üniversite Müzeleri Birliği Platformu Konferansı, Anadolu Üniversitesi, Eskişehir.

[15] The Global Biodiversity Informatics Facility (GIBIF).(2023, Temmuz 25). https://www.gbif.org/

[16] World Register of Marine Species (WoRMS). (2023, Temmuz 5). https://www.molluscabase.org/

[17] Fischer, W., Schneider, M., & Bauchot., M.L. (1987). *Méditerranée et Mer Noire (Zone de Pêche 37).* Rev.1. (1 volumes). Rome: Organisation des Nations Unies pour l'Alimentation et l'Agriculture.

[18] Türkiye Kelebek Gözlemcileri & Fotoğrafçıları Topluluğu (2023, 10 Ekim). http://www.adamerkelebek.org/.

[19] Bakır, K., & Çevirgen, F. (2010). İzmir Körfezi'nde Bulunan Krustase Türleri. *Ege University Journal of Fisheries & Aquatic Sciences*, 27(2), 79-90.

[20] Çıplak, B., Demirsoy, A., & Bozcuk, A. N. (1996). Caelifera Fauna of (Orthoptera, Insecta) Malatya District. *Turkish Journal of Zoology*, 20(1), 17-31.

[21] Çıplak, B., & Demirsoy, A. (1997). Mantodea (Insecta) fauna of Malatya vicinity (Turkey) and some remarks on the mantises of Anatolia. – *Journal of Orthoptera Research*, 6, 105-111.

[22] Coppard, S.E. (2008). A comparative analysis of the spatangoid echinoid genera Brissopsis and Metalia: A new genus and species of spatangoid (Echinodermata: Echinoidea: Brissopsidae) from the Philippines and the reassignment of Brissopsis persica to Metalia. *Zootaxa* 1760, 1–23. https://doi.org/10.11646/zootaxa.1760.1.1

[23] Demirsoy, A. (1975). Erzurum Bölgesi Orthoptera (Insecta) faunasının tesbiti and taksonomik incelenmesi. Yay. No: 347, Erzurum: Atatürk Üniversitesi yayınları.
[24] Demirsoy, A. (1996). Türkiye Omurgalıları: Amfibiler. Ankara: Meteksan.
[25] Demirsoy, A. (1997). Türkiye Omurgalıları: Sürüngenler. Ankara: Meteksan.
[26] Demirsoy, A. (2001). Yaşamın Temel Kuralları Omurgalılar / Anamniyota. Cilt III, kısım I. Ankara: Meteksan,.
[27] Demirsoy, A. (2003). Türkiye Omurgalıları: Memeliler. Ankara: Meteksan.
[28] Devetak, D. (1997). Genus Macronemurus Costa, 1855 in the northwestern part of the Balkan Peninsula (Neuroptera: Myrmeleontidae). Annales, Ann. *Istrian Mediterr. Studies* 11, 203–208.
[29] Önder, F., Karsavuran, Y., & Tezcan, S. (2014). *Türkiye Heteroptera (Insecta) Kataloğu*. İzmir: Meta Basım
[30] Dyachkov, Y. V., & Nedoev, K. (2021). A contribution to the centipede (Chilopoda: Geophilomorpha, Scolopendromorpha) fauna of Uzbekistan and Turkmenistan. – *Ecologica Montenegrina* 41, 41–50 http://dx.doi.org/10.37828/em.2021.41.6
[31] Öztük, Ö., & Bağdatlı, E. (2018). Investigations on morphology of the European Hornet (Vespa crabro) nest and biological development situations of the larvae. *Acta Biologica Turcica*, 31(2), 36-41.
[32] Topçu, N.E., & Öztürk, B. (2015). Composition and abundance of octocorals in the Sea of Marmara, where the Mediterranean meets the Black Sea. Scientia Marina, 79(1), 12-135. http://dx.doi.org/10.3989/scimar.04120.09A
[33] Flutsch, P. G., & Tauzin. P.H. (2009). Une nouvelle sous-espèce de Cetonia aurata Linnaeus, 1761 de Chine (Coleoptera, Cetoniinae, Cetoniini). *Cetonimania*, 6(2), 101-108.
[34] Furtun, Ö. L., Erciyas-Yavuz, K., & Karataş, A. (2021).Trakuş-Türkiye'nin Kuşları. İstanbul: Türkiye İş Bankası Kültür Yayınları.
[35] Özalp, H., & Alparslan, M. (2011). The first record of Cladocora caespitosa (Linnaeus, 1767) (Anthozoa, Scleractinia) from the Marmara Sea. *Turkish Journal of Zoology*, 35(5), 701 - 705.
[36] Hristov, G.H., & Chobanov, D.P. (2016). An annotated checklist and key to the Bulgarian cockroaches (Dictyoptera: Blattodea). *Zootaxa*, 4154 (4): 351–388. https://doi.org/10.11646/zootaxa.4154.4.1
[37] Holthuis, L.B. (1961). Report on a collection of Crustacea Decapoda and Stomatopoda from Turkey and the Balkans. *Zool. Verh.*, Leiden, 47: 1-67.
[38] Karataş, A., Çalışkan, F., Çalışkan, H., Şirin, D.Ü., & Karataş, A. (2005 Mart 17-19). The Scorpion Fauna of Turkey. 7. International Meeting of Experts on Envenomation from Poisonous Animals.
[39] Kirwan, G. M., Boyla, K. A., Castell, P., Demirci, B., Özen, M., Welch, H., & Marlow, T. (2010). *The distribution, taxonomy and breeding of Turkish birds*. London: Christopher Helm, an imprint of A&C Black Publishers Ltd.
[40] Korotyaev, B. A., Gültekin, N., & Gültekin, L. (2020). A new species Asproparthenis omeri sp. nov. (Coleoptera: Curculionidae: Lixinae) from the Aras valley in northeastern Turkey. *Journal of Insect Biodiversity*, 17(1): 12-27.
[41] Özgür, E. (2016). Echinoderm Fauna of the Sea of Marmara. In E. Ozsoy, M. N. Çağatay, B. Neslihan, N. Balkıs, & B. Öztürk (Eds.), *The Sea of Marmara; Marine Biodiversity, Fisheries, Conservation and Governance* (42th ed., pp. 517-532). İstanbul: Turkish Marine Research Foundation (TUDAV)
[42] Neimorovets, V. (2020). Review of the genus Eurygaster (Hemiptera: Heteroptera: Scutelleridae) of Russia, *Zootaxa*, 4722(6), 501-539. https://doi.org/10.11646/zootaxa.4722.6.1
[43] Néraudeau, D., Dudicourt, J.C., Frédéric Boutin, F., Ceulemans. L., & Nicolleau, P. (2010). The Spatangus of Miocene and Pliocene in the West of France. *Annales de Paléontologie*, 96(4), 159-170. https://doi.org/10.1016/j.annpal.2011.05.001
[44] Tüzün, A., & Kekilioğlu, A. (2003). Ankara Vespidae (Insecta, Hymenoptera) türleri üzerine faunistik çalışmalar ve ekolojik gözlemler. *Selçuk Üniversitesi Fen Edebiyat Fakültesi Fen Dergisi,* 22, 97-105.
[45] Özbek, M., & Ustaoğlu, M.R. (2001). Freshwater Malacostraca (Crustacea) fauna of İzmir province and adjacent areas (Except Amphipoda). *Anadolu University Journal of Science and Technology*, 2(1), 19-25.
[46] Öztürk, B., Doğan, A., & Önen, M. (2008). Türkiye denizel mollusca türleri tayin atlası (Cephalopoda hariç). *TÜBİTAK TIBAG 103T154*, p 1-466. Erişim Tarihi: 04.07.2023 https://search.trdizin.gov.tr/tr/yayin/detay/607437/
[47] Rasmont, P., Coppée, A., Michez, D., & De Meulemeester, T. (2008). An overview of the Bombus terrestris (L. 1758) subspecies (Hymenoptera: Apidae). *Annales de la Société entomologique de France* , 44(2), 243-250. https://doi.org/10.1080/00379271.2008.10697559
[48] Turin, H., Penev, L., & Casale, A. (2003). The Genus Carabus in Europa. Fauna Europaea Evertebrata, No 2. Sofia: Pensoft Publishers.
[49] Zariquiey-Álvarez,R. (1968). Crustaceos Decapodos Ibéricos. Barcelona: Investigación Pesquera.
[50] Çalışkan, H., & Yalçın, H.K. (2023). Biyoçeşitlilik Koleksiyonlarının Dijitalleştirilmesi ve Aydınlatma Kabini Tasarımı. *Turkish Journal of Bioscience and Collections*, 7(2), 59–68. https://doi.org/10.26650/tjbc.1318177

Ek

Tablo 1. ESOGÜ Zooloji Müzesinde sergilenen Cnidaria, Mollusca, Arthropoda, Echinodermata ve Chordata şubelerine ait tür listesi

		Familya	Kayıt Numarası		Tür Adı	Tarih	Lokalite	Toplayan
Phylum	**: Cnidaria**							
Classis	**: Anthozoa**							
Ordo	: Alcyonacea	Gorgoniidae	CNDDRY	000503	*Eunicella sp.*	16.08.2006	Fethiye, Muğla, Türkiye	H. Çalışkan
Ordo	: Scleractinia	Cladoceridae	CNDDRY	000085	*Cladocora caespitosa* (Linnaeus, 1767)	16.08.1997	Bodrum, Muğla, Türkiye	H. Çalışkan
			CNDDRY	000350	*Cladocora caespitosa* (Linnaeus, 1767)	20.08.2005	Bodrum, Muğla, Türkiye	H. Çalışkan
Phylum	**: Mollusca**							
Classis	**: Bivalvia**							
Ordo	: Adapedonta	Solenidae	MOLDRY	000209	*Solen marginatus* Pulteney, 1799	14.07.2002	Geyikli, Çanakkale, Türkiye	H. Çalışkan
		Solenidae	MOLDRY	000504	*Solen marginatus* Pulteney, 1799	16.08.2006	Fethiye, Muğla, Türkiye	H. Çalışkan
Ordo	: Arcida	Glycymeridіdae	MOLDRY	000450	*Glycymeris glycymeris* (Linnaeus, 1758)	24.08.2005	Marmaris, Muğla, Türkiye	H. Çalışkan
Ordo	:Cardiida	Donacidae	MOLDRY	000360	*Donax vittatus* (da Costa, 1778)	20.08.2005	Türkbükü, Bodrum, Muğla, Türkiye	H. Çalışkan
Ordo	:Mytilida	Mytilidae	MOLDRY	000496	*Mytilus galloprovincialis* Lamarck, 1819	01.07.2006	Bodrum, Muğla, Türkiye	H. Çalışkan
		Mytilidae	MOLDRY	000657	*Mytilus galloprovincialis* Lamarck, 1819	01.09.2010	İzmir, Türkiye	H. Çalışkan
Ordo	:Ostreida	Margaritidae	MOLDRY	000354	*Pinctada imbricata* Röding, 1798	20.08.2005	Türkbükü, Bodrum, Muğla, Türkiye	H. Çalışkan
		Ostreidae	MOLDRY	000461	*Ostrea sp.*	25.08.2005	Fethiye, Muğla, Türkiye	H. Çalışkan
		Pinnidae	MOLDRY	000660	*Pinna carnea* Gmelin, 1791	01.09.2011	İzmir, Türkiye	H. Çalışkan
		Pinnidae	MOLDRY	000515	*Pinna nobilis* Linnaeus, 1758	22.08.2006	Türkbükü, Bodrum, Muğla, Türkiye	H. Çalışkan
Ordo	:Pectinida	Pectinidae	MOLDRY	000460	*Pecten jacobaeus* (Linnaeus, 1758)	25.08.2005	Fethiye, Muğla, Türkiye	H. Çalışkan
Ordo	:Venerida	Glossidae	MOLDRY	000547	*Glossus humanus* (Linnaeus, 1758)	30.06.2007	Datça, Muğla, Türkiye	H. Çalışkan
		Veneridae	MOLDRY	000462	*Venus verrucosa* Linnaeus, 1758	25.08.2005	Fethiye, Muğla, Türkiye	H. Çalışkan
Classis	**:Cephalopoda**							
Ordo	:Octopoda	Argonautidae	MOLDRY	000665	*Argonauta argo* Linnaeus, 1758	20.08.2012	Bodrum, Muğla, Türkiye	H. Çalışkan
		Octopodidae	MOLLIC	000499	*Octopus vulgaris* Cuvier, 1797	01.07.2006	Bodrum, Muğla, Türkiye	H. Çalışkan
Classis	**:Gastropoda**							
Ordo	:Arcida	Arcidae	MOLDRY	000597	*Arca zebra* Swainson, 1833	30.08.2008	Fethiye, Muğla, Türkiye	H. Çalışkan
			MOLDRY	000598	*Arca zebra* Swainson, 1833	31.08.2008	Fethiye, Muğla, Türkiye	H. Çalışkan
Ordo	:Lepetellida	Haliotidae	MOLDRY	000211	*Haliotis tuberculata* Linnaeus, 1758	22.08.2002	Fethiye, Muğla, Türkiye	H. Çalışkan
			MOLDRY	000344	*Haliotis tuberculata* Linnaeus, 1758	18.06.2005	Geyikli, Çanakkale, Türkiye	H. Çalışkan
Ordo	:Littorinimorpha	Aporrhaidae	MOLDRY	000662	*Aporrhais pespelecani* (Linnaeus, 1758)	01.09.2009	İzmir, Türkiye	H. Çalışkan

Tablo 1. Devam ediyor

		Familya	Kayıt Numarası		Tür Adı	Tarih	Lokalite	Toplayan
		Cassidae	MOLDRY	000663	*Galeodea echinophora* (Linnaeus, 1758)	01.09.2009	İzmir, Türkiye	H.Çalışkan
		Charoniidae	MOLDRY	000595	*Charonia tritonis* (Linnaeus, 1758)	30.08.2008	Fethiye, Muğla, Türkiye	H. Çalışkan
		Cypraeidae	MOLDRY	000596	*Naria erosa* (Linnaeus, 1758)	30.08.2008	Fethiye, Muğla, Türkiye	H. Çalışkan
		Naticidae	MOLDRY	000457	*Neverita josephinia* Risso, 1826	25.08.2005	Fethiye, Muğla, Türkiye	H. Çalışkan
			MOLDRY	000464	*Neverita josephinia* Risso, 1827	21.09.2005	Manavgat, Antalya, Türkiye	H. Çalışkan
		Strombidae	MOLDRY	000355	*Conomurex persicus* (Swainson, 1821)	20.08.2005	Türkbükü, Bodrum, Muğla, Türkiye	H. Çalışkan
			MOLDRY	000455	*Conomurex persicus* (Swainson, 1821)	25.08.2005	Fethiye, Muğla, Türkiye	H. Çalışkan
			MOLDRY	000468	*Harpago sp.*	07.10.2005	Çin	??
			MOLDRY	000333	*Laevistrombus canarium* (Linnaeus, 1758)	01.01.2005	Çin	??
		Tonnidae	MOLDRY	000267	*Tonna galea* (Linnaeus, 1758)	02.08.2004	Datça, Muğla, Türkiye	H. Çalışkan
Ordo	:Neogastropoda	Columbellidae	MOLDRY	000343	*Columbella rustica* (Linnaeus, 1758)	18.06.2005	Geyikli, Çanakkale, Türkiye	H. Çalışkan
		Muricidae	MOLDRY	000210	*Bolinus brandaris* (Linnaeus, 1758)	14.07.2002	Geyikli, Çanakkale, Türkiye	H. Çalışkan
			MOLDRY	000352	*Bolinus brandaris* (Linnaeus, 1758)	20.08.2005	Türkbükü, Bodrum, Muğla, Türkiye	H. Çalışkan
			MOLDRY	000449	*Bolinus brandaris* (Linnaeus, 1758)	23.08.2005	Datça, Muğla, Türkiye	H. Çalışkan
			MOLDRY	000073	*Hexaplex trunculus* (Linnaeus, 1758)	16.07.1996	Fethiye, Muğla, Türkiye	H. Çalışkan
			MOLDRY	000516	*Hexaplex trunculus* (Linnaeus, 1758)	22.08.2006	Türkbükü, Bodrum, Muğla, Türkiye	H. Çalışkan
			MOLDRY	000506	*Murex pecten (*Lightfoot) 1786	16.08.2006	Fethiye, Muğla, Türkiye	H. Çalışkan
			MOLDRY	000184	*Rapana venosa* (Valenciennes, 1846)	02.06.2001	Gelibolu, Çanakkale, Türkiye	H. Çalışkan
			MOLDRY	000661	*Rapana venosa* (Valenciennes, 1846)	03.09.2011	Geyikli, Çanakkale, Türkiye	H. Çalışkan
Ordo	:Trochida	Trochidae	MOLDRY	000088	*Phorcus turbinatus* (Born, 1778)	18.08.1997	Fethiye, Muğla, Türkiye	H. Çalışkan
Phylum	**:Arthropoda**							
Classis	**:Malacostraca**							
Ordo	:Decapoda	Astacidae	CRSDRY	000068	*Pontastacus leptodactylus (*Eschscholtz, 1823)	18.07.1995	Bursa, Türkiye	H. Çalışkan
			CRSDRY	000083	*Pontastacus leptodactylus* (Eschscholtz, 1823)	14.08.1997	İzmir, Türkiye	H. Çalışkan
		Calappidae	CRSDRY	000087	*Calappa granulata* (Linnaeus, 1758)	16.08.1997	Bodrum, Muğla, Türkiye	H. Çalışkan
		Carcinidae	CRSDRY	000264	*Carcinus maenas* (Linnaeus, 1758)	27.07.2004	İzmir, Türkiye	H. Çalışkan
		Eriphiidae	CRSDRY	000079	*Eriphia verrucosa* (Forskål, 1775)	11.08.1997	Çanakkale, Türkiye	H. Çalışkan
		Grapsidae	CRSDRY	000263	*Pachygrapsus sp*	27.07.2004	İzmir, Türkiye	H. Çalışkan
		Majidae	CRSDRY	000153	*Maja squinado* (Herbst, 1788)	07.09.2000	Çanakkale, Türkiye	H. Çalışkan

Tablo 1. Devam ediyor

		Familya	Kayıt Numarası		Tür Adı	Tarih	Lokalite	Toplayan
			CRSDRY	000208	*Maja squinado* (Herbst, 1788)	14.07.2002	Geyikli, Çanakkale, Türkiye	H. Çalışkan
		Penaeidae	CRSDRY	000150	*Penaeus sp*	07.09.2000	Çanakkale, Türkiye	H. Çalışkan
		Potamidae	CRSDRY	000078	*Potamon potamios* (Olivier, 1804)	09.08.1997	Bursa, Türkiye	H. Çalışkan
Ordo	:Sessilia	Balanidae	CRSDRY	000111	*Balanus sp.*	17.08.1998	Ayvalık, Balıkesir, Türkiye	H. Çalışkan
			CRSDRY	000151	*Balanus sp.*	07.09.2000	Çanakkale, Türkiye	H. Çalışkan
Ordo	:Stomatopoda	Squillidae	CRSDRY	000080	*Squilla mantis* (Linnaeus, 1758)	11.08.1997	Çanakkale, Türkiye	
Classis	**:Merostomata**							
Ordo	:Xiphosurida	Limulidae	CRSDRY	000627	*Tachypleus tridentatus* (Leach, 1819)	02.11.2009	Batı Pasifik	G. Ortuğ
Classis	**:Arachnida**							
Ordo	:Ixodida	Ixodidae	CHLDRY	000639	*Hyalomma marginatum* C.L.Koch, 1844	14.11.2009	Yenisofça, Eskişehir, Türkiye	H. Çalışkan
Ordo	:Scorpiones	Buthidae	CHLDRY	000198	*Aegaeobuthus gibbosus* (Brullé, 1832)	07.06.2001	Ekincik, Köyceğiz, Muğla, Türkiye	H. Çalışkan
			CHLDRY	000658	*Aegaeobuthus gibbosus* (Brullé, 1832)	24.07.2011	Sarıcakaya, Eskişehir, Türkiye	H. Çalışkan
			CHLDRY	000655	*Androctonus crassicauda* (Olivier, 1807)	23.08.2010	Harran, Urfa, Türkiye	H. Çalışkan
			CHLDRY	000654	*Buthacus macrocentrus* (Ehrenberg, 1828)	23.08.2010	Harran, Urfa, Türkiye	H. Çalışkan
			CHLDRY	000656	*Hottentotta saulcyi* (Simon, 1880)	27.07.2008	Mardin, Türkiye	H. Çalışkan
			CHLDRY	000653	*Leiurus abdullahbayrami* Yagmur, Koc & Kunt, 2009	21.08.2010	Nizip, Gaziantep, Türkiye	H. Çalışkan
		Iuridae	CHLDRY	000444	*Iurus kinzelbachi* Kovarik, Fet, Soleglad & Yagmur, 2010	23.08.2005	Kuşadası (Dilekyarımadası), Aydın, Türkiye	H. Çalışkan
Ordo	:Solifugae	Galeodidae	CHLDRY	000657	*Galeode sp.*	23.07.2011	Meşelik Yerl., Eskişehir, Türkiye	H. Çalışkan
Classis	**:Diplopoda**							
Ordo	:Julida	Julidae	MYRDRY	000021	*Julus sp* .	20.04.1989	Köyceğiz, Muğla, Türkiye	H. Çalışkan
Classis	:Chilopoda							
Ordo	:Scolopendromorpha	Scolopendridae	MYRDRY	000020	*Scolopendra cingulata* Latreille, 1829	20.04.1989	Köyceğiz, Muğla, Türkiye	H. Çalışkan
Classis	**:Insecta**							
Ordo	:Blattodea	Blattidae	INSDRY	000335	*Periplaneta americana* (Linnaeus, 1758)	24.04.2005	Alanya, Antalya, Türkiye	H. Çalışkan
			INSDRY	000336	*Blatta orientalis* Linnaeus, 1758	08.05.2005	Eskişehir, Türkiye	H. Çalışkan
Ordo	:Orthoptera	Acrididae	INSDRY	000194	*Oedipoda miniata* (Pallas, 1771)	07.06.2001	Köyceğiz, Muğla, Türkiye	H. Çalışkan

Tablo 1. Devam ediyor

		Familya	Kayıt Numarası	Tür Adı	Tarih	Lokalite	Toplayan
		Gryllidae	INSDRY 000247	*Gryllus bimaculatus* De Geer, 1773	30.05.2004	Sultandere, Eskişehir, Türkiye	H. Çalışkan
		Gryllotalpidae	INSDRY 000433	*Gryllotalpa gryllotalpa* (Linnaeus, 1758)	23.08.2005	Bayramiç, Çanakkale, Türkiye	H. Çalışkan
		Tettigoniidae	INSDRY 000097	*Saga sp.* ♂	05.05.1998	Fethiye, Türkiye	H. Çalışkan
			INSDRY 000098	*Saga sp.* ♀	05.05.1998	Fethiye, Türkiye	H. Çalışkan
Ordo	: Mantodea	Mantidae	INSDRY 000664	*Mantis religiosa* (Linne, 1758)	15.08.2022	Meşelik Yerl., Eskişehir, Türkiye	H. Çalışkan
Ordo	:Hemiptera	Scutelleridae	INSDRY 000250	*Eurygaster integriceps* Puton, 1881	10.06.2004	Meşelik Yerl., Eskişehir, Türkiye	H. Çalışkan
		Pentatomidae	INSDRY 000425	*Aelia rostrata* Boheman, 1852	23.08.2005	Eskişehir, Türkiye	H. Çalışkan
			INSDRY 000422	*Graphosoma lineatum* (Linnaeus, 1758)	23.08.2005	Eskişehir, Türkiye	H. Çalışkan
		Pyrrhocoridae	INSDRY 000424	*Pyrrhocoris apterus* (Linnaeus, 1758)	09.06.2004	Meşelik Yerl., Eskişehir, Türkiye	H. Çalışkan
Ordo	:Hymenoptera						
		Apidae	INSDRY 000262	*Bombus terrestris* (Linnaeus, 1758)	05.07.2004	Meşelik Yerl., Eskişehir, Türkiye	H. Çalışkan
		Scoliidae	INSDRY 000541	*Megascolia maculata* (Drury, 1773)	29.05.2007	Meşelik Yerl., Eskişehir, Türkiye	H. Çalışkan
		Vespidae	INSDRY 000538	*Vespa crabro* (Linnaeus, 1758)	20.05.2007	Hasan Çavuş K., Akçakoca, Düzce, Türkiye	H. Çalışkan
			INSDRY 000528	*Vespula germanica* (Fabricius, 1793)	11.05.2007	Meşelik Yerl., Eskişehir, Türkiye	H. Çalışkan
Ordo	:Coleoptera	Buprestidae	INSDRY 000586	*Capnodis miliaris* (Klug, 1829)	20.08.2007	Sarıcakaya, Eskişehir, Türkiye	H. Çalışkan
		Carabidae	INSDRY 000260	*Carabus scabrosus* Olivier, 1790	04.07.2004	Meşelik Yerl., Eskişehir, Türkiye	H. Çalışkan
			INSDRY 000280	*Calosoma sycophanta* (Linnaeus, 1758)	18.09.2004	Bayramiç, Çanakkale, Türkiye	H. Çalışkan
			INSDRY 000281	*Cicindela campestris* Linnaeus, 1758	28.10.2009	Bayramiç, Çanakkale, Türkiye	H. Çalışkan
			INSDRY 000252	*Zabrus sp.*	11.06.2004	Çukurhisar, Eskişehir, Türkiye	H. Çalışkan
		Cerambycidae	INSDRY 000411	*Morimus funereus* Mulsant, 1863	23.08.2005	Bayramiç, Çanakkale, Türkiye	H. Çalışkan
			INSDRY 000131	*Purpuricenus budensis* (Götz, 1783)	07.07.2000	Çanakkale, Türkiye	H. Çalışkan

Tablo 1. Devam ediyor

		Familya	Kayıt Numarası	Tür Adı	Tarih	Lokalite	Toplayan
		Chrysomelidae	INSDRY 000638	*Leptinotarsa decemlineata* (Say, 1824)	13.11.2009	Şuhut, Afyon, Türkiye	H. Çalışkan
		Coccinellidae	INSDRY 000226	*Coccinella septempunctata* Linnaeus, 1758	14.08.2003	Meşelik Yerl., Eskişehir, Türkiye	H. Çalışkan
		Curculionidae	INSDRY 000248	*Asproparthenis punctiventris* (E.F.Germar, 1823)	02.06.2004	Meşelik Yerl., Eskişehir, Türkiye	H. Çalışkan
		Meloidae	INSDRY 000261	*Muzimes collaris* (Fabricius, 1787)	04.07.2004	Eskişehir, Türkiye	H. Çalışkan
			INSDRY 000271	*Mylabris calida* (Pallas, 1782)	09.08.2004	Meşelik Yerl.,Eskişehir, Türkiye	H. Çalışkan
		Scarabaeidae	INSDRY 000584	*Cetonia aurata (Linnaeus, 1758)*	20.08.2007	Mayıslar, Eskişehir, Türkiye	H. Çalışkan
			INSDRY 000253	*Oryctes nasicornis (Linnaeus, 1758)*	18.06.2004	Mayıslar, Eskişehir, Türkiye	H. Çalışkan
			INSDRY 000272	*Polyphylla fullo (Linnaeus, 1758)*	09.08.2004	Meşelik Yerl., Eskişehir, Türkiye	H. Çalışkan
			INSDRY 000108	*Polyphylla olivieri (Castelnau, 1840)*	10.07.1998	Fethiye, Muğla, Türkiye	H. Çalışkan
			INSDRY 000230	*Tropinota hirta (Poda, 1761)*	06.04.2004	Meşelik Yerl., Eskişehir, Türkiye	H. Çalışkan
Ordo	:Lepidoptera	Erebidae	INSDRY 000236	*Arctia festiva (Hufnagel, 1766)*	09.05.2004	Meşelik Yerl., Eskişehir, Türkiye	H. Çalışkan
		Nymphalidae	INSDRY 000368	*Limenitis reducta Staudinger, 1901*	23.08.2005	Meşelik Yerl., Eskişehir, Türkiye	H. Çalışkan
		Pieridae	INSDRY 000039	*Gonepteryx cleopatra (Linnaeus, 1767)*	08.07.1990	Gökçeada, Çanakkale, Türkiye	H. Çalışkan
		Pieridae	INSDRY 000391	*Pieris brassicae (Linnaeus, 1758)*	23.08.2005	Bayramiç, Çanakkale, Türkiye	H. Çalışkan
		Papilionidae	INSDRY 000380	*Iphiclides podalirius (Linnaeus, 1758)*	23.08.2005	Bayramiç, Çanakkale, Türkiye	H. Çalışkan
			INSDRY 000219	*Papilio machaon Linnaeus, 1758*	02.06.2003	Meşelik Yerl., Eskişehir, Türkiye	H. Çalışkan
			INSDRY 000076	*Zerynthia cerisy (Godart, 1824)*	09.05.1997	Bolu, Türkiye	H. Çalışkan
		Saturnidae	INSDRY 000266	*Saturnia pavonia Linnaeus, 1758*	02.08.2004	Eskişehir, Türkiye	H. Çalışkan
		Saturnidae	INSDRY 000363	*Saturnia pyri (Denis & Schiffermüller) 1775*	23.08.2005	Bayramiç, Çanakkale, Türkiye	H. Çalışkan
Ordo	:Neuroptera	Myrmeleontidae	INSDRY 000409	*Palpares libelluloides (Linnaeus, 1764)*	23.08.2005	Bayramiç, Çanakkale, Türkiye	H. Çalışkan
		Nemopteridae	INSDRY 000223	*Nemoptera sinuata Olivier, 1811*	28.07.2003	Mudanya, Bursa, Türkiye	H. Çalışkan

Tablo 1. Devam ediyor

		Familya	Kayıt Numarası		Tür Adı	Tarih	Lokalite	Toplayan
Phylum	**:Echinodermata**							
Classis	**:Asteroidea**							
Ordo	:Paxillosida	Astropectinidae	ECHDRY	000104	*Astropecten aranciacus (Linnaeus, 1758)*	05.05.1998	Fethiye, Muğla, Türkiye	H. Çalışkan
		Familya	Kayıt Numarası	Tür Adı	*Tarih*	Lokalite	Toplayan	
			ECHDRY	000511	*Astropecten spinulosus (Philippi, 1837)*	22.08.2006	Türkbükü, Bodrum, Muğla, Türkiye	H. Çalışkan
Ordo	:Valvatida	Chaetasteridae	ECHDRY	000512	*Chaetaster longipes (Bruzelius, 1805)*	22.08.2006	Türkbükü, Bodrum, Muğla, Türkiye	H. Çalışkan
		Goniasteridae	ECHDRY	000507	*Peltaster placenta (Müller & Troschel, 1842)*	22.08.2006	Bodrum, Muğla, Türkiye	H. Çalışkan
Classis	**:Echinoidea**							
Ordo	:Spatangoida	Brissidae	ECHDRY	000138	*Metalia sternalis (Lamarck, 1816)?*	09.08.2000	Ortakent, Bodrum, Muğla, Türkiye	H. Çalışkan
Ordo	:Spatangoida	Spatangidae	ECHDRY	000143	*Spatangus purpureus O.F.Müller, 1776*	09.08.2000	Ortakent, Bodrum, Muğla, Türkiye	H. Çalışkan
Ordo	:Camarodonta	Parechinidae	ECHDRY	000099	*Paracentrotus lividus (Lamarck, 1816)*	05.05.1998	Fethiye, Muğla, Türkiye	H. Çalışkan
			ECHDRY	000140	*Paracentrotus lividus (Lamarck, 1816)*	09.08.2000	Ortakent, Bodrum, Muğla, Türkiye	H. Çalışkan
Phylum	**:Chordata**							
Classis	**:Elasmobranchii**							
Ordo	:Carcharhiniformes	Scyliorhinidae	PSCLIC	000614	*Scyliorhinus canicula (Linnaeus, 1758)*	11.11.2008	Edremit, Balıkesir, Türkiye	H. Çalışkan
Ordo	:Hexanchiformes	Hexanchidae	PSCSKL	000588	*Hexanchus griseus (Bonnaterre, 1788)*	18.10.2007	Kuzey Ege Denizi, Türkiye	H. Çalışkan
Ordo	:Rhinopristiformes	Rhinobatidae	PISDRY	000186	*Rhinobatos rhinobatos (Linnaeus, 1758)*	19.05.2001	Sarıyer, İstanbul, Türkiye	H. Çalışkan
Ordo	:Squaliformes	Squalidae	PISDRY	000185	*Squalus acanthias Linnaeus, 1758*	19.05.2001	Sarıyer, İstanbul, Türkiye	H. Çalışkan
Classis	**:Teleostei**							
Ordo	:Anguilliformes	Muraenidae	PSCLIC	000617	*Muraena helena Linnaeus, 1758*	20.06.2009	Geyikli, Çanakkale, Türkiye	H. Çalışkan
Ordo	:Beloniformes	Belonidae	PISDRY	000109	*Belone belone (Linnaeus, 1760)*	15.07.1998	İstanbul, Türkiye	H. Çalışkan
Ordo	:Perciformes	Triglidae	PSCLIC	000615	*Trigla lyra Linnaeus, 1758*	11.11.2008	Edremit, Balıkesir, Türkiye	H. Çalışkan
Ordo	:Pleuronectiformes	Soleidae	PSCLIC	000608	*Solea solea (Linnaeus, 1758)*	03.09.2008	İstanbul, Türkiye	H. Çalışkan
Ordo	:Scombriformes	Scombridae	PSCSKL	000066	*Thunnus thynnus (Linnaeus, 1758)*	10.11.1994	Kuzey Ege Denizi, Türkiye	H. Çalışkan
Ordo	:Synbranchiformes	Mastacembelidae	PSCLIC	000613	*Mastacembelus mastacembelus (Banks & Solander, 1794)*	30.10.2009	Urfa, Türkiye	H. Çalışkan

Tablo 1. Devam ediyor

		Familya	Kayıt Numarası		Tür Adı	Tarih	Lokalite	Toplayan
Ordo	:Syngnathiformes	Syngnathidae	PISDRY	000187	*Hippocampus sp.*	04.06.2001	Ayvalık, Balıkesir, Türkiye	H. Çalışkan
Classis	**:Amphibia**							
Ordo	:Anura	Bombinatoridae	AMBLIC	000590	*Bombina bombina (Linnaeus, 1761)*	25.06.2008	Eskişehir, Türkiye	H. Çalışkan
		Bufonidae	AMBLIC	000589	*Bufo bufo (Linnaeus, 1758)*	25.06.2008	Eskişehir, Türkiye	H. Çalışkan
			AMBLIC	000592	*Bufotes viridis (Laurenti, 1768)*	25.06.2008	Eskişehir, Türkiye	H. Çalışkan
		Hylidae	AMBLIC	000606	*Hyla orientalis Bedriaga, 1890*	02.09.2008	Sapanca, Sakarya, Türkiye	H. Çalışkan
		Ranidae	AMBLIC	000591	*Pelophylax ridibundus (Pallas, 1771)*	25.06.2008	Eskişehir, Türkiye	H. Çalışkan
Ordo	:Caudata	Salamandridae	AMBLIC	000594	*Lyciasalamandra luschani (Steindachner, 1891)*	30.08.2008	Fethiye, Muğla, Türkiye	H. Çalışkan
			AMBLIC	000641	*Ommatotriton ophryticus (Berthold, 1846)*	16.11.2009	Osmaneli, Bilecik, Türkiye	H. Çalışkan
			AMBLIC	000607	*Triturus ivanbureschi Arntzen & Wielstra, 2013*	03.09.2008	Büyükçekmece, İstanbul, Türkiye	H. Çalışkan
Classis	**:Squamata**	Agamidae	REPLIC	000148	*Laudakia stellio* (Linnaeus, 1758)	09.08.2000	Ortakent, Bodrum, Muğla, Türkiye	H. Çalışkan
			REPSKL	000274	*Laudakia stellio* (Linnaeus, 1758)	14.08.2004	Köyceğiz, Muğla, Türkiye	H. Çalışkan
		Anguidae	REPLIC	000214	*Pseudopus apodus* (Pallas, 1775)	17.05.2003	Bayramiç, Çanakkale, Türkiye	H. Çalışkan
		Chamaeleonidae	REPLIC	000604	*Chamaeleo chamaeleon* (Linnaeus, 1758)	31.08.2008	Fethiye, Muğla, Türkiye	H.Çalışkan
		Colubridae	REPLIC	000215	*Dolichophis caspius* (Gmelin, 1789)	18.05.2003	Edremit, Balıkesir, Türkiye	H. Çalışkan
			REPDRY	000517	*Dolichophis caspius* (Gmelin, 1789)	22.08.2006	Bodrum, Muğla, Türkiye	H. Çalışkan
			REPLIC	000519	*Natrix natrix* (Linnaeus, 1758)	28.08.2006	Eskişehir, Türkiye	H. Çalışkan
			REPLIC	000500	*Natrix tessellata* (Laurenti, 1768)	05.07.2006	Eskişehir, Türkiye	H. Çalışkan
		Iguanidae	REPLIC	000346	*Iguana iguana* (Linnaeus, 1758)	02.08.2005	Tayland-Türkiye	??
		Lacertidae	REPLIC	000117	*Lacerta trilineata* Bedriaga, 1886	10.06.1999	Eskişehir, Türkiye	H. Çalışkan
		Typhlopidae	REPLIC	000602	*Xerotyphlops vermicularis* (Merrem, 1820)	20.06.2009	Bayramiç, Çanakkale, Türkiye	H. Çalışkan
		Viperidae	REPLIC	000204	*Montivipera xanthina* (Gray, 1849)	27.08.2001	Eskişehir, Türkiye	H. Çalışkan
Classis	**:Testudines**	Cheloniidae	REPTAX	000469	*Caretta caretta* (Linnaeus, 1758)	08.10.2005	Fethiye, Muğla, Türkiye	??
		Geoemydidae	REPLIC	000524	*Mauremys rivulata* (Valenciennes, 1833)	18.11.2006	Adrasan Antalya Türkiye	H. Çalışkan
		Testudinidae	REPDRY	000609	*Testudo graeca* Linnaeus, 1758	01.01.2009	Eskişehir, Türkiye	H. Çalışkan
Classis	**:Aves**							
Ordo	:Accipitriformes	Accipitridae	AVSTAX	000473	*Aquila heliaca* Savigny, 1809	01.01.1997	Eskişehir, Türkiye	H. Çalışkan

Tablo 1. Devam ediyor

		Familya	Kayıt Numarası		Tür Adı	Tarih	Lokalite	Toplayan
			AVSTAX	000474	*Buteo rufinus* (Cretzschmar, 1829)	01.10.2006	Eskişehir, Türkiye	H. Çalışkan
			AVSTAX	000472	*Neophron percnopterus* (Linnaeus, 1758)	01.01.2006	Eskişehir, Türkiye	??
Ordo	:Anseriformes	Anatidae	AVSTAX	000050	*Anas acuta* Linnaeus, 1758	20.03.1992	Eskişehir, Türkiye	H. Çalışkan
			AVSSKL	000490	*Anser anser subsp. domesticus* Linnaeus, 1758	04.05.2006	Eskişehir, Türkiye	H. Çalışkan, S. Oluçay, M. Topkaya, C. Destire
			AVSTAX	000623	*Anas crecca* Linnaeus, 1758	29.10.2009	Eskişehir, Türkiye	??
			AVSTAX	000630	*Anas platyrhynchos* Linnaeus, 1758	05.11.2009	Eskişehir, Türkiye	H. Çalışkan
			AVSTAX	000481	*Spatula clypeata* (Linnaeus, 1758)	01.01.2006	Eskişehir, Türkiye	H. Çalışkan
			AVSTAX	000125	*Tadorna ferruginea* (Pallas, 1764)	01.05.2000	İzmir, Türkiye	H. Çalışkan
Ordo	:Charadriiformes	Scolopacidae	AVSTAX	000479	*Calidris pugnax* (Linnaeus, 1758)	01.01.2006	Eskişehir, Türkiye	??
Ordo	:Columbiformes	Columbidae	AVSTAX	000051	*Columba livia* J.F.Gmelin, 1789	19.04.1992	Eskişehir, Türkiye	H. Çalışkan
			AVSTAX	000048	*Columba livia var. domestica* J.F.Gmelin, 1789	05.05.1991	Eskişehir, Türkiye	Ü.R.Dinçer
			AVSTAX	000494	*Streptopelia decaocto* (Frivaldszky, 1838)	04.06.2006	Meşelik Yerl., Eskişehir, Türkiye	H. Çalışkan
Ordo	:Cuculiformes	*Cuculidae*	AVSTAX	000482	*Clamator glandarius* (Linnaeus, 1758)	01.01.2006	Eskişehir, Türkiye	H. Çalışkan
Ordo	:Falconiformes	Falconidae	AVSTAX	000027	*Falco tinnunculus* Linnaeus, 1758	21.06.1989	Eskişehir, Türkiye	H. Çalışkan
Ordo	:Galliformes	Phasianidae	AVSTAX	000052	*Alectoris chukar* (J.E.Gray, 1830)	28.04.1992	Eskişehir, Türkiye	H. Çalışkan
			AVSTAX	000127	*Lophura nycthemera* (Linnaeus, 1758)	01.05.2000	İzmir, Türkiye	H. Çalışkan
			AVSTAX	000485	*Perdix perdix* (Linnaeus, 1758)	01.01.2006	Eskişehir, Türkiye	H. Çalışkan
Ordo	:Passeriformes	Corvidae	AVSTAX	000047	*Garrulus glandarius* (Linnaeus, 1758)	05.05.1991	Eskişehir, Türkiye	H. Çalışkan
		Sturnidae	AVSTAX	000060	*Sturnus vulgaris* Linnaeus, 1758	17.10.1993	Eskişehir, Türkiye	H. Çalışkan
Ordo	:Pelecaniformes	Ardeidea	AVSTAX	000126	*Ardea purpurea* Linnaeus, 1766	01.05.2000	İzmir, Türkiye	H. Çalışkan
			AVSTAX	000119	*Ardeola ralloides* (Scopoli, 1769)	10.08.1999	İzmir, Türkiye	H. Çalışkan
		Pelecanidae	AVSTAX	000123	*Pelecanus onocrotalus* Linnaeus, 1758	01.05.2000	İzmir, Türkiye	H. Çalışkan
		Threskiornithidae	AVSTAX	000124	*Plegadis falcinellus* (Linnaeus, 1766)	01.05.2000	İzmir, Türkiye	H. Çalışkan
Ordo	:Phoenicopteriformes	Phoenicopteridae	AVSTAX	000129	*Phoenicopterus ruber* Linnaeus, 1758	01.05.2000	İzmir, Türkiye	H. Çalışkan
Ordo	:Psittaciformes	Psittacidae	AVSTAX	000069	*Psittacus erithacus* Linnaeus, 1758	01.01.1996	Nijerya	H. Çalışkan
Ordo	:Strigiformes	Strigidae	AVSTAX	000053	*Asio otus* (Linnaeus, 1758)	03.06.1992	Eskişehir, Türkiye	H. Çalışkan

Tablo 1. Devam ediyor

		Familya	Kayıt Numarası		Tür Adı	Tarih	Lokalite	Toplayan
			AVSTAX	000064	*Bubo bubo* (Linnaeus, 1758)	18.06.1994	Eskişehir, Türkiye	H. Çalışkan
Classis	**:Mammalia**							
Ordo	:Artiodactyla	Bovidae	MAMSKL	000121	*Bos taurus* Linnaeus, 1758	28.09.1999	Eskişehir, Türkiye	H. Çalışkan
			MAMSKL	000254	*Capra hircus subsp. aegagrus* Erxleben, 1777	25.06.2004	Eskişehir, Türkiye	H. Çalışkan
			MAMSKL	000610	*Gazella subgutturosa* (Güldenstaedt, 1780)	01.01.2009	Hayvanat Bahçesi, Eskişehir, Türkiye	H. Çalışkan
			MAMTAX	000067	Ovis aries Linnaeus, 1758	27.05.1995	Eskişehir, Türkiye	H. Çalışkan
			MAMSKL	000491	*Ovis aries* Linnaeus, 1758	19.05.2006	Eskişehir, Türkiye	H. Çalışkan
		Camelidae	MAMTAX	000619	*Lama glama* (Linnaeus, 1758)	25.10.2009	Peru	??
		Suidae	MAMTAX	000040	*Sus scrofa* Linnaeus 1758	09.10.1990	İnönü, Eskişehir, Türkiye	H. Çalışkan
			MAMSKL	000120	*Sus scrofa* Linnaeus, 1758	03.09.1999	Eskişehir, Türkiye	H. Çalışkan
Ordo	:Diprotodontia	Macropodidae	MAMSKL	000618	*Macropus rufogriseus* (Desmarest, 1817)	01.01.2009	Hayvanat Bahçesi, Eskişehir, Türkiye	H. Çalışkan
Ordo	:Carnivora	Canidae	MAMSKL	000002	*Canis lupus subsp. familiaris* Linnaeus, 1758	28.05.1988	Eskişehir, Türkiye	H. Çalışkan
			MAMSKL	000095	*Vulpes vulpes* (Linnaeus, 1758)	02.05.1998	Eskişehir, Türkiye	H. Çalışkan
		Felidae	MAMSKL	000001	*Felis silvestris* Schreber, 1777	10.08.1987	İstanbul, Türkiye	H. Çalışkan
			MAMTAX	000118	*Felis catus* Linnaeus, 1758	10.08.1999	İzmir, Türkiye	H. Çalışkan
Ordo	:Charadriiformes	Scolopacidae	AVSTAX	000479	*Calidris pugnax* (Linnaeus, 1758)	01.01.2006	Eskişehir, Türkiye	??
			MAMSKL	000463	*Martes foina* (Erxleben, 1777)	01.09.2005	Seyitgazi, Eskişehir, Türkiye	H. Çalışkan
Ordo	:Cetacea	Phocoenidae	MAMSKL	000059	*Phocoena phocoena* (Linnaeus, 1758)	03.09.1993	İstanbul, Türkiye	H. Çalışkan
Ordo	:Erinaceomorpha	Erinaceidae	MAMTAX	000122	Erinaceus sp.	01.05.2000	İzmir, Türkiye	H. Çalışkan
Ordo	:Perissodactyla	Equidae	MAMSKL	000637	*Equus caballus* Linnaeus, 1758	12.11.2009	Eskişehir, Türkiye	H. Çalışkan
			MAMSKL	000642	*Equus caballus* Linnaeus, 1758 (TAY)	17.11.2009	Eskişehir, Türkiye	H. Çalışkan
			MAMSKL	000077	*Equus mulus (*Erkek *Equus asinus L*innaeus, 1758 *x* Dişi *Equus caballus* Linnaeus, 1758)	03.08.1997	Eskişehir, Türkiye	H. Çalışkan
Ordo	:Rodentia	Muridae	MAMTAX	000229	*Rattus norvegicus* (Berkenhout, 1769)	19.11.2003	Eskişehir, Türkiye	H. Çalışkan
			MAMSKL	000331	*Rattus norvegicus* (Berkenhout, 1769)	18.11.2004	Eskişehir, Türkiye	H. Çalışkan ve U. Kiper
		Sciuridae	MAMTAX	000207	*Sciurus vulgaris* Linnaeus, 1758	14.07.2002	Eskişehir, Türkiye	H.Çalışkan

www.biodicon.com BIOLOGICAL DIVERSITY AND CONSERVATION

ISSN 1308-8084 Online ISSN 1308-5301 Print

Research article/Araştırma makalesi
DOI: 10.46309/biodicon.2023.1273996
165/2 (2023) 252-257

Isolation and molecular characterization of non-*Azotobacter* bacteria using *Azotobacter* isolation protocols from pastures in the south of Türkiye

Ebru ÇELEN*[1]
ORCID: 0000-0002-8452-5933

[1]Department of Biology, Faculty of Arts and Science, Bolu Abant İzzet Baysal University, Gölköy, 14280 Bolu, Türkiye

Abstract

Soil is a biodiversity-rich ecosystem. Nitrogen-fixing *Azotobacter* bacteria, an important component of this ecosystem, are frequently isolated using various methods. The aim of the present study was to perform partial molecular characterization of non-*Azotobacter* isolates derived during two *Azotobacter* isolation protocols, as well as to determine which bacteria can be obtained using this method. A total of 800 isolates were acquired from soil samples collected from various pastures in Antalya province of Turkey, with 92 of them being molecularly characterized. These isolates were clustered through RFLP analysis of 16S rRNA gene and the DNA sequences of the isolates representing different groups were performed. According to these results, the bacteria belonging to various genera (*Agrobacterium*, *Phyllobacterium*, *Variovorax*, *Acinetobacter*, *Pseudomonas*, *Agromyces*, and *Arthrobacter*) were identified. The results show that similar bacteria could be obtained through two isolation protocols used in this study. However, more diverse bacteria were encountered on the Brown-medium than on soil-past.

Keywords: *Azotobacter*, isolate, soil, 16S rDNA

---------- * ----------

Türkiye'nin güneyindeki meralardan *Azotobacter* izolasyon protokolleri kullanılarak *Azotobacter* olmayan bakterilerin izolasyonu ve moleküler karakterizasyonu

Özet

Toprak, biyolojik çeşitlilik açısından zengin bir ekosistemdir. Bu ekosistemin önemli bir bileşeni olan azot bağlayan *Azotobacter* bakterileri çeşitli yöntemlerle sıklıkla izole edilir. Bu çalışmanın amacı, iki *Azotobacter* izolasyon protokolü sırasında elde edilen *Azotobacter* olmayan izolatların kısmi moleküler karakterizasyonunu gerçekleştirmek ve bu yöntemle hangi bakterilerin elde edilebileceğini belirlemektir. Türkiye'nin Antalya ilindeki çeşitli meralardan toplanan toprak örneklerinden toplam 800 izolat elde edilmiş ve bunlardan 92'si moleküler olarak karakterize edilmiştir. İzolatlar, 16S rRNA genlerinin RFLP analizi ile gruplandırılmış ve farklı grupları temsil eden izolatların DNA dizileri gerçekleştirilmiştir. Bu sonuçlara göre çeşitli cinslere (*Agrobacterium*, *Phyllobacterium*, *Variovorax*, *Acinetobacter*, *Pseudomonas*, *Agromyces* ve *Arthrobacter*) ait bakteriler tespit edilmiştir. Sonuçlar, bu çalışmada kullanılan iki izolasyon protokolü ile benzer bakterilerin elde edilebileceğini göstermektedir. Bununla birlikte, Brown Besi ortamında Soil-Past'a göre daha çeşitli bakteriler tespit edilmiştir.

Anahtar kelimeler: *Azotobacter*, izolat, toprak, 16S rDNA

1. Introduction

Soil is an ecosystem having great biodiversity, including many diverse organisms belonging to numerous groups on the soil's surface and underground. A gram of soil has been estimated to contain several thousand species of bacteria [1]. Bacteria, a significant part of this enormous diversity, play a variety of roles in elemental cycles and

* *Corresponding author* / Haberleşmeden sorumlu yazar: Tel.: +905533529792; Fax.: +903742534642; E-mail: celen_e@ibu.edu.tr
 Received: 21.03.2023; Published: 15.12.2023 BioDiCon. 1112-310323

biochemical reactions [2], and also they establish unique interactions with the other organisms in the soil. Nitrogen, one of the basic elements of living organisms, is essential for the survival of all organisms. Earth's atmosphere contains almost 78 % nitrogen [3]. But many organisms are not able to use atmospheric nitrogen. The fixation of nitrogen, one of the most significant processes on Earth, from the atmosphere to soil or other ecosystems, is performed by only prokaryotes [4]. Bacteria (such as *Rhizobium*, *Azotobacter*, and *Cyanobacteria*) having the ability of nitrogen fixation can take place in different phylogenetic groups [5]. These bacteria can convert atmospheric nitrogen (N_2) to ammonia, which is essential for plant growth and supports the nutrition of plants. Therefore, agricultural activities utilize nitrogen-fixing bacteria for enhancing plant crops. Isolation of nitrogen-fixing bacteria such as *Azotobacter* is very significant for their diverse contributions to plant growth and ecosystems [6, 7]. Since it has been shown that bacteria, can grow in microbial cultures, corresponding to less than 1% of the entire microbial diversity [8, 9] we know that cultivation methods are relatively limited. On the other hand, it may be an obligation to isolate some bacteria for the purpose of utilizing them or investigating their properties. The *Azotobacter* genus is a Gram-negative free-living nitrogen-fixing bacteria that has been commonly studied for a long time. There are various isolation media such as Winogradsky, Brown, and Burk [10] to isolate them. But diverse bacteria may be encountered in these enhancement and semi-selective media. This study aims to identify and characterize bacteria growing in the media used for the isolation of *Azotobacter*.

2. Materyal ve yöntem

2.1.Sampling Site and Sample Collection

Rhizosphere and bulk soil samples were collected from four pastures (Elmalı-Eymir, Manavgat-Demirciler, İbradı-Eynif and Akseki-Çimi) Antalya in Turkey. The rhizosphere soil was picked up using a shovel that took a profile of the soil together with the plant roots. Bulk soil was taken from 10 cm depth of top soil. All samples were transported to the laboratory in a cool ice chest on the same day and stored at +4 °C for a maximum of one night until the isolation step. The rhizosphere soil taken with the plant root was removed from the part of the plant root under septic conditions in the laboratory. All soil samples were sieved with a mesh size of 4 mm.

2.2. Isolation of Bacteria

In the study, two distinct culturing methods were utilized for the isolation of bacteria. Firstly, a 10 g soil sample from each sample was placed in a 250 mL erlenmayer flask containing 90 mL of sterilized 0.85% (w-v) NaCl solution and glass beads and shaken at 100 rpm for 15 minutes at room temperature [11]. For soil suspensions, 10-2-10-5 dilutions were spread on the selective-Brown medium (pH 6.8-7) of *Azotobacter* and incubated at 29–30 °C for 5–9 days [12, 10]. The mucoid colonies on plates at 10-2-10-5 dilutions were transmitted to TSA (Triptic Soy Agar) medium. Second, in a sterilized plate, 30-50 g of soil samples were completely mixed with mannitol solution (1 %) and the surface was smoothed [13]. Plates were incubated for 5-9 days at 29-30 °C. Isolates from these plates were transferred to nitrogen-free Brown medium. All isolates, from both isolation methods, were tested by the KOH method for Gram properties [14], and then they were grown on the selective LG medium [10] of *Azotobacter*. The bacteria with yellow pigment growing on LG medium were chosen and characterized by molecular methods.

2.3. DNA Extraction and PCR analysis

Two or three bacterial colonies from TSA medium were suspended in 500 µL double sterilized water, then precipitated by centrifuge. The precipitate was suspended in 100 µL water and mixed with 100 µL of 10 mM Tris-HCl (pH, 8.2). The suspension was treated with 1 mg-ml proteinaz K and incubated at 55 °C for 2 hours [13]. The supernatant obtained was stored at -20 °C. The 16S rRNA gene was amplified using universal bacterial primers 27f and 1495r by PCR [15]. PCR was performed in a 25 µL mix with 0.5 U GoTaq of DNA polymerase enzyme and 2 µL of DNA sample.

2.4. RFLP (Restriction Fragment Length Polimorfizm) and Electrophoresis

Three different restriction enzymes (Alu I, Rsa I and Hha I) were used according to manufacturer's protocol (Promega) for RFLP analysis of 16S rRNA gene. The 16S rRNA gene PCR products were mixed with 10 U of enzyme, BSA, and NaCl and incubated at 37 °C for 12 hours. The PCR products and the fragments of 16S rDNA from RFLP were run on 0.5 % and 2.5 % agarose gels, respectively, using TBE buffer. Gels were stained with Ethidium Bromide and viewed using a UV transilluminator.

2.5. Analysis of RFLP and DNA Sequences

A matrix was constituted using the profile of the DNA bands from RFLP of 16S rDNA according to the absence or presence of DNA bands. For clustering of all isolates, the matrix was performed with UPGMA (Unweighted Pairgroup Method with Aritmetic Average) analysis by Bioedite 5 and PHYLIP 3.66 programs [16, 17]. The distance between groups was accounted for according to Nei ve Li'e [18]. The dendrogram of clustering was obtained using the Treeview 1.66 program. DNA sequences were conducted for isolates representing different groups using Macrogen sequencing service (Macrogen Ltd., Seoul, South Korea). DNA sequences were compared with nBlast analysis [19]. All the 16S rRNA gene sequences reported in this paper have been deposited in the NCBI GenBank database.

3. Results

A total of 800 isolates were derived using two distinct nitrogen-free media (Brown and soil-past) from various pasture soils in Turkey. The 92 isolates were chosen depending on the characteristics of pigment and growth on the media. For molecular characterization of these isolates, the RFLP method was performed using the 16S rRNA gene. All isolates were clustered using UPGMA analysis according to RFLP results (Figure 1). According to the cluster diagram, isolates were separated into at least seven major groups and the substitution of branches was clustered into subgroups. The 16S rDNA of 11 isolates representing separate groups was sequenced and identified partially (Table 1). The results of 16S rDNA sequences show that some isolates of these groups correspond to Variovorax, Phyllobacterium, Acinetobacter, Agromyces, Agrobacterium, Arthrobacter and Pseudomonas. The majority of isolates (88%) were recovered by Brown-medium isolation, while only a few isolates were acquired through soil-past isolation. (Figure 1).

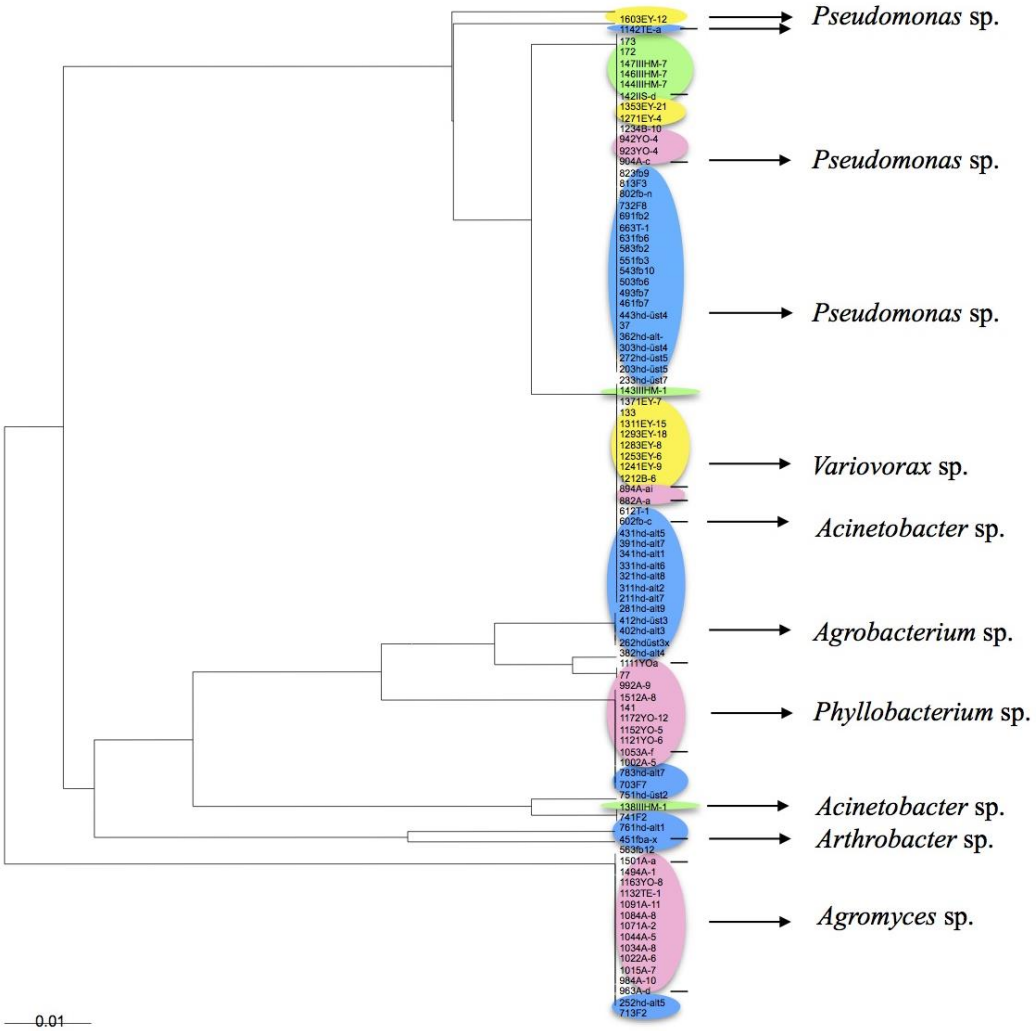


Figure 1. Dendrogram generated using UPGMA cluster analysis. - : isolates from soil past isolation methods, Others were isolated from Brown medium. Colors point distinct pastures, Blue: Çimi; Yellow: Eynif; Pink: Eymir; Green: Demirciler. Accession numbers for 16S rDNA sequences; OP686576 (1hdalt-5), OP686577 (2TE-a), OP686578 (3EY-12), OP686579 (3hdust-4), OP686580 (4A-c), OP686581 (IIIHM-1), OP686582 (1EY9), OP686583 (1fbax), OP686585 (2hdalt3), OP686586 (2YO12) and OP696653 (4A-c)

Table 1. Blast results for 16S rDNA sequences from isolates

NCBI accession numbers	identification	isolate	similarity (%)
JN989304.1	*Agromyces* sp. G4342	1A2	99
CP002417.1	*Variovorax paradoxus* EPS	1EY-9	99
NR_026233.1	*Arthrobacter aurescens* strain DSM	1fbax	99
GQ169811	*Agrobacterium tumefaciens*	2hdalt3	100
NR_043192.1	*Phyllobacterium ifriqiyense*	2YO12	99
AJ633632.1	*Acinetobacter calcoaceticus*	1hdalt-5	99
NR_025228.1	*Pseudomonas koreensis*	2TE-a	99
NR_025228.1	*Pseudomonas koreensis*	3EY-12	99
NR_025228.1	*Pseudomonas* sp.	3hdust-4	99
MK883099.1	*Pseudomonas* sp.	4A-c	93
X81661.1	*Acinetobacter calcoaceticus*	IIIHM-1	99

4. Conclusions and discussion

The present study shows that many diverse bacteria can be isolated from the selective media practically. No doubt, if more restriction enzymes in RFLP analysis had been used, the groups of isolates in cluster analysis would have been branched more. Thus, that could have provided further enhanced the diversification of bacteria. Despite the fact that two isolation procedures were used in the study, most isolates were derived from Brown-medium. As a result, it appears that isolating a specific bacteria using Brown-medium may be more difficult.

It seems that numerous *Pseudomonas* were recovered from Brown-medium. The cluster diagram shows that *Pseudomonas* was also isolated from all pastures. Since *Azotobacter* and *Pseudomonas* are closely related [20, 21], it is not surprising that many and diverse *Pseudomonas* are obtained using these isolation steps. *Acinetobacter* and *Pseudomonas* bacteria could be commonly isolated together from different environments [22, 23]. Regarding the isolates from the present study, we do not completely know whether they are nitrogen-fixing bacteria. But, somehow, these isolates were able to grow on nitrogen-free media. Some strains of *Phyllobacterium* and *Acinetobacter* have been reported to fix atmospheric nitrogen [24, 25]. Because of the abundance of organic molecules, growth factors for *Variovorax* are not necessary [26]. As a result, *Variovorax* isolates may have had a chance to thrive on the Brown-medium that was utilized. On the other hand, it is remarkably significant to identify these bacteria in the Ibrad-Eynif pasture sampled here because Finkel et al. [27] reported that a single genus, *Variovorax* is efficient for maintaining plant root growth in a complex microbiome, and cor bacteria of *Variovorax* in plant-microbe interactions. *Arthrobacter* and *Agromyces* are Gram-positive bacteria, and although Gram-negative isolates were chosen using the KOH test in the present study, the reason we encountered them might have been a false negative. The KOH test is a simple, rapid, and reliable method [28], but some bacteria may give a false negative KOH reaction [29].

Culture-independent methods are becoming more common and help us comprehend the microbial diversity in various ecosystems [30, 31]. However, the ability to isolate and grow the bacteria is a significant step toward utilizing them and learning their features. It seems that the bacteria isolated in this study are able to grow in a medium containing specific nutrition sources. Consequently, this research also aids in our comprehension of the efficiency of the nutrient media employed in the isolation of bacteria. Microbial populations in soil play a central role in the productivity and health of terrestrial ecosystems [32], and affect their ecosystems due to their various metabolic activities. Assuming that each of these bacteria may have a specific function in the soil ecosystem, determining the presence of these bacteria may contribute to our understanding of pasture soils in the future.

It is exceedingly difficult to isolate a specific bacteria from soil that has an enormous diversity of microorganisms using cultural methods. In general, many studies focus on the relevant microorganism when they use cultural methods for the isolation of a particular microorganism. But, the priority of the present study is to determine what kinds of microorganisms can grow on the nitrogen-free media used for the isolation of *Azotobacter*. Moreover, this study shows, albeit partially, the diversity of soil and various bacterial groups occurring in the different pasture soils.

Acknowledgment

Isolates in the current study were isolated at the Department of Biology, Akdeniz University, Turkey, and molecular characterization of isolates was performed at the Department of Biology, York University, UK. I am grateful to Prof. Dr. J. Peter W. Young and Dr. Cyril Bontemps for their help with the molecular characterization of isolates.

Conflict of Interest

The author declares no conflict of interest for current research article.

References

[1] Torsvik, V., Goksøyr, J., Daae, F. L., Sørheim, R., Michalsen, J. & Salte, K. (1994). Use of DNA analysis to determine the diversity of microbial communities. In: K. Ritz, J. Dighton & K.E. Giller (Eds.), Beyond the Biomass. Compositional and Functional Analysis of Soil Microbial Communities (pp. 39-48). Chichester: John Wiley.

[2] Falkowski, P.G., Fenchel, T. & Delong, E.F. (2008). The microbial engines that drive Earth's biogeochemical cycles. *Science*, 320:1034-1039. https://doi.org/10.1126/science.1153213.

[3] Schlesinger, W.H. (1991). Biogeochemistry: An Analysis of Global Change, Academic, San Diego, Calif: Academic.

[4] Young, J.P.W. (1992). Phylogenetic classification of nitrogen-fixing organisms. In: G. Stacey, R.H. Burris & H.J. Evans. (Eds.), Biological nitrogen fixation (pp. 43-86). New York: Chapman and Hall Inc.

[5] Raymond, J., Siefert, J.L., Staples, C.R. & Blankenship, R.E. (2004). The natural history of nitrogen fixation. Molecular Biology and Evolution, 21:541-554. https://doi.org/10.1093/molbev/msh047.

[6] Postgate, J.R. (1998). Nitrogen Fixation (3nd ed.), Cambridge: Cambridge University Press.

[7] Revillas, J.J., Rodelas, B., Pozo, C., Martínez-Toledo, M.V., & González-López, J. (2000). Production of B-group vitamins by two *Azotobacter* strains with phenolic compounds as sole carbon source under diazotrophic and adiazotrophic conditions. *Journal of Applied Microbiology*, 89:486-493. https://doi.org/10.1046/j.1365-2672.2000.01139.x.

[8] Ward, D.M., Bateson, M.M., Weller, R. & Ruff-Roberts, A.L. (1992). Ribosomal RNA analysis of microorganisms as they occur in nature. *Advanced Microbial Ecology*, 12:220–286. https://doi.org/10.1007/978-1-4684-7609-5_5.

[9] Pham, V.H. & Kim, J. (2012). Cultivation of unculturable soil bacteria. Trends in Biotechnology, 30:475-484.

[10] Kennedy, C., Rudnick, P. Macdonald, M.L. & Melton, T. (2005). Genus III. *Azotobacter* Beijerinck 1901. In: N.R. Krieg, J.T. Staley & G.M. Garrity (Eds.), Bergey's Manual of Systematic Bacteriology – The Proteobacteria (2nd ed, pp 384–402). New York: Springer.

[11] Wollum, A.G. (1982). Cultural methods for soil microorganisms. In: A.L. Page, R.H. Miller & D.R. Keeney (Eds.), Methods of Soil Analysis: Part 2 Chemical and Microbiological Properties (pp. 781-802). Madison: American Society of Agronomy.

[12] Knowles, R. (1982). Free-living dinitrogen-fixing bacteria. In: A.L. Page, R.H. Miller & D.R. Keeney (Eds.), Methods of Soil Analysis: Part 2 Chemical and Microbiological Properties (pp. 1071-1092). Madison: American Society of Agronomy.

[13] Aquilanti, L., Favilli F. & Clementi F. (2004). Comparison of different strategies for isolation & preliminary identification of *Azotobacter* from soil samples. *Soil Biology and Biochemistry*, 36: 1475-1483. https://doi.org/10.1016/j.soilbio.2004.04.024.

[14] Ryu, E. (1938). On the Gram-differentiation of bacteria by the simplest method. Journal of the Japanese Society of Veterinary Science, 17: 205-207.

[15] Weisburg, W.G., Barns, S.M., Pelletier, D.A. & Lane D.J. (1991). 16S ribosomal DNA amplification for phylogenetic study. Journal of Bacteriology, 173:697-703. https://doi.org/10.1128/jb.173.2.697-703.1991.

[16] Sneath, P.H. & Sokal, R.R. (1973). Numerical taxonomy: The principles and practice of numerical classification. (1nd ed), San Francisco: W. H. Freeman.

[17] Felsenstein, J. (1990). PHYLIP manual version 3.3. Berkeley, CA: University of California Herbarium.

[18] Nei, M. & Li, W.H. (1979). Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proceedings of the National Academy of Sciences*, 76:5269-5273. https://doi.org/10.1073/pnas.76.10.5269.

[19] Altschul, S.F., Gish, W., Miller, W., Myers, E.W. & Lipman, D.J. (1990). Basic local alignment search tool. *Journal of Molecular Biology*, 215: 403-410. https://doi.org/10.1016/S0022-2836(05)80360-2.

[20] Young, J.M. & Park, D.C. (2007). Probable synonymy of the nitrogen-fixing genus *Azotobacter* and the genus *Pseudomonas*. *International Journal of Systematic and Evolutionary Microbiology*, 57:2894-2901. https://doi.org/10.1099/ijs.0.64969-0.

[21] Hesse, C., Schulz, F., Bull, C., Shaffer, B., Yan, Q., Shapiro, N., et al. (2018). Genome-based evolutionary history of *Pseudomonas* spp. *Environmental Microbiology*, 20: 2142– 2159. https://doi.org/10.1111/1462-2920.14130.

[22] Heo, S.M., Haase, E.M., Lesse, A.J., Gill, S.R. & Scannapieco, F.A. (2008). Genetic relationships between respiratory pathogens isolated from dental plaque and bronchoalveolar lavage fluid from patients in the intensive care unit undergoing mechanical ventilation. *Clinical Infectious Diseases*, 47:1562-70. https://doi.org/10.1086/593193.

[23] Chiellini, C., Chioccioli, S., Vassallo, A., Mocali, S., Miceli, E., Fagorzi, C., et al. (2019). Exploring the bacterial communities of Infernaccio waterfalls: a phenotypic and molecular characterization of *Acinetobacter* and *Pseudomonas* strains living in a red epilithic biofilm. *Diversity*, 11:175. https://doi.org/10.3390/d11100175.

[24] Rojas, A., Holguin, G., Glick, B. R. & Bashan, Y. (2001). Synergism between *Phyllobacterium* sp. (N_2-fixer) and *Bacillus licheniformis* (P-solubilizer), both from a semiarid mangrove rhizosphere. *FEMS Microbiology Ecology*, 35:181-187. https://doi.org/10.1111/j.1574-6941.2001.tb00802.x.

[25] Chaudhary, H.J., Peng, G., Hu, M., He, Y., Yang, L., Luo, Y. & Tan, Z. (2012). Genetic diversity of endophytic diazotrophs of the wild rice, Oryza alta and identification of the new diazotroph, *Acinetobacter oryzae* sp. nov. *Microbial Ecology*, 63: 813-821. https://doi.org/10.1007/s00248-011-9978-5.

[26] Willems, A. Mergaert, J. & Swings, J. (2015). Variovorax. In: W.B. Whitman, P. DeVos, S. Dedysh, B. Hedlund, P. Kämpfer, F. Rainey, M.E. Trujillo, J.P. Bowman, D.R. Brown & F.O. Glöckner et al., (Eds.), Bergey's Manual of Systematics of Archaea and Bacteria (pp. 1–9). Hoboken, NJ, USA: American Cancer Society.

[27] Finkel, O.M., Salas-González, I., Castrillo, G., Conway, J.M., Law, T.F., Teixeira, P., et al. (2020). A single bacterial genus maintains root growth in a complex microbiome. *Nature*, 587: 103-108. https://doi.org/10.1038/s41586-020-2778-7.

[28] Powers, E.M. (1995). Efficacy of the Ryu nonstaining KOH technique for rapidly determining gram reactions of food-borne and waterborne bacteria and yeasts. *Applied and Environmental Microbiology*, 61: 3756-3758. https://doi.org/10.1128/aem.61.10.3756-3758.1995.

[29] Halebian, S., Harris, B., Finegold, S.M. & Rolfe, R.D. (1981). Rapid method that aids in distinguishing Gram-positive from Gram-negative anaerobic bacteria. *Journal of Clinical Microbiology*, 13:444-448. https://doi.org/10.1128/jcm.13.3.444-448.1981.

[30] Çelik, P. A., Mutlu, M. B., Korkmaz, F., Yaman, B. N., Gedikli, S., & Çabuk, A. (2021). Boron mine ponds: metagenomic insight to bacterial diversity. *Biyolojik Çeşitlilik ve Koruma*, 14(2): 229-235. https://doi.org/10.46309/biodicon.2021.902221.

[31] Su, C., Lei, L., Duan, Y., Zhang, K. Q., & Yang, J. (2012). Culture-independent methods for studying environmental microorganisms: methods, application, and perspective. Applied microbiology and biotechnology, 93: 993-1003.

[32] Copley, J. (2000). Ecology goes underground. Nature, 406(6795): 452-455.

BIOLOGICAL DIVERSITY AND CONSERVATION
Volume / Cilt / 16 Issue / Sayı / 3 December/Aralık / 2023

Contents **/ İçindekiler**

***Abstracted-Indexed in* / Dergiyi tarayan veri tabanları:** Web of Science {Zoological Records Indexed] Clavariate Analytic, CiteFactor, Turkey Citation Index, JournalSeek Database, arastirmax, Medical Reads (RRS), CrossRef;10.46309/biodicon.

***Libraries* / Kütüphaneler:** *Aberystwyth University; All libraries; Bath University; Birmingham University; Cardiff University ;City University London; CONSER (Not UK Holdings); Edinburgh University; Essex University; Exeter University; Eskişehir Teknik Üniversitesi Library, EZB Electronic Journals Library; Feng Chia University Library; GAZİ Gazi University Library; Glasgow University; HEC-National Digital Library; Hull University; Imperial College London; Kaohsinug Medical University Library; KYBELE Anadolu University Library; Lancaster University; Libros PDF; Liverpool University; London Metropolitan University; London School of Economics and Political Science; Manchester University; National Cheng Kung University Library; National ILAN University Library; Nottingham University; Open University; Oxford University; Queen Mary,University of London;Robert Gordon University; Royal Botanic Gardens, Kew; Sheffield Hallam University; Sheffield University; Shih Hsin University Library; Smithsonian Institution Libraries; Southampton University; Stirling University; Strathclyde University; Sussex University; The National Agricultural Library (NAL); The Ohio Library and Information NetWork; Trinity College Dublin; University of Washington Libraries; Vaughan Memorial Library; York University.*

Web of Science-Clarivate Analytics, Zoological Records Indexed

This journal is a CrossRef member (DOI;10.46309/biodicon (*Bu dergi CrossRef* (DOI;10.46309/biodicon) *üyesidir)*

"BIOLOGICAL DIVERSITY AND CONSERVATION" has been added to the journal list of the Medical Reads (RRS) program together with the "Biology and Medical Journals**".**
Dergide yayınlanan makalelere" http:// www.biodicon.com" adresinden ulaşabilir.
This journal is available online at http:// www.biodicon.com

ISSN 1308-5301 Print / ISSN 1308-8084 Online

ISSN 1308-5301
9 771308 530001

INSTRUCTIONS FOR AUTHORS

"BIOLOGICAL DIVERSITY AND CONSERVATION (BioDiCon)" is an international, peer-reviewed scientific journal published in online and printed form, in accordance with the principles of independency and impartiality. This journal is a peer reviewed journal on the topics of biological diversity and conservation that uses independent, unbiased double-blinded peer review principles.

"BIOLOGICAL DIVERSITY AND CONSERVATION" publishes experimental studies, compilations on current issues, case reports, editorial comments and editorial letters prepared in accordance with the ethical guidelines, about on all areas of biological diversities. Descriptive or experimental studies presenting clear research questions are accepted. 'BIOLOGICAL DIVERSITY AND CONSERVATION' has been added to the journal list of the Medical Reads (RRS) program together with the "Biology and Medical Journals". The journal's publication language are English and Turkish.

The manuscripts submitted to Biological Diversity and Conservation will go through a double-blind peer-review process. Each submission will be reviewed by at least two external, independent peer reviewers who are experts in their fields in order to ensure an unbiased evaluation process. The editorial board will invite an external and independent editor to manage the evaluation processes of manuscripts submitted by editors or by the editorial board members of the journal.

An approval of research protocols by the Ethics Committee in accordance with international agreements is required for experimental, clinical, and drug studies and for some case reports. If required, ethics committee reports or an equivalent official document will be requested from the authors.

A declaration text including the written consent should be added in the manuscripts concerning experimental research on humans related to that the patients and the volunteers were informed about the procedures of the performed and to be performed studies on them. In addition, information about patients' approval, the name of the ethics committee, and the ethics committee approval number should be addressed in the Materials and Methods section of the manuscript. In the studies carried out on animals, the precautions taken to prevent pain and suffering of the animals should be clearly indicated.

Authorship Contribution Form

The journal of Biological Diversity and Conservation, in order to protect the rights of the authors and to prevent such situations as ghost / honorary authorship, requires the author to submit a signed and a scanned copy of the **Authorship Contribution Form** (which can be downloaded from http://www.biodicon.com).

Policy of Screening for Plagiarism

All submissions are checked by a similarity detection software iThenticate program for determination of plagiarism and non-ethical situations. In cases where the application is alleged or suspected as plagiarism, citation manipulation and data forgery/production, the Editorial Board will follow and act in accordance with the COPE rules.

If the editorial board gets suspicious about a case of 'gift authorship', the application will be rejected without being reviewed. As a part of article submission the responsible author should send a short statement text affirming that s/he takes all the responsibilty of the article during the submission and review stages of the manuscript.

ICMJE Conflict of Interest form

Biological Diversity and Conservation journal asks and encourages its authors and submitted articles to disclose any existing or potential conflicts of interests, including financial, consultant and institutional ones, that may lead to potential bias or conflicts among individuals involved in the evaluation process. Any material grants or other support received for a study sent from individuals or institutions should be reported to the Editorial Board. In order to disclose a potential conflict of interest, **The Potential Conflict of Interest Disclosure Form (ICMJE)** must be signed by all contributing authors and forwarded to the journal editorial board.

Appeals and Complaint

All appeals and complaint cases are dealt with in the framework of the COPE trial rules by The Editorial Board of the journal. In such cases, the authors should directly make a contact with the editorial office in relation to their appeal and complaints.

Copyright

When submitting an article to the journal of Biological Diversity and Conservation, the authors agree to give the copyright of their articles to the journal. If the publication is rejected, the copyright of the article will be assigned back to the authors. Biological Diversity and Conservation requires each article application to be carried out together with the **Copyright Transfer Form** (which can be downloaded from

When using previously published content, permission from the copyright owner must be obtained by the authors.

The statements or opinions contained in the articles published in the Journal of Biological Diversity and Conservation reflect the views of the author(s); the editors, the editorial board, and the publisher don't accept any responsibility or liability for such materials. All the responsiblity belongs to the authors about the published content.

PREPARATION OF MANUSCRIPT

Authors are required to prepare manuscripts in accordance with the STROBE guidelines for observational original research studies, STARD guidelines for studies on diagnostic accuracy, PRISMA guidelines for systematic reviews and meta-analysis, ARRIVE guidelines for experimental animal studies, TREND guidelines and CONSORT guidelines for randomized research studies for non-randomized public behaviour. The manuscripts should be prepared in accordance with ICMJE-Recommendations for the Conducting (http://www.icmje.org/icmje-recommendations).

Manuscripts can only be submitted through the journal's online manuscript submission and evaluation system, available at Submissions that do not comply with the rules of the journal will be returned to the submitting author with the requests of correction requets.

Authors are required to submit the following

a) Copyright Transfer Form,
b) Author Contributions Form,
c) ICMJE Potential Conflict of Interest Disclosure Form (should be filled in by all contributing authors) during the initial submission. These forms are available for download at
d) Similarity Form (iThenticate: According to the Plagiarism Detection Software programme the similarity index of the article should not exceed 20% after all references and less than 1% matches have been removed.)

Preparation of the Manuscript (General rules for all manuscript types)

Title page: The first page of the article should include the title of manuscript, authors' names and institutions, an abstract, and keywords. Title, abstract, and keywords must be provided both in English and Turkish. The corresponding author should be indicated by a footnote and besides his/her full open mail address, and an e-mail address should also be provided.
A separate title page should be submitted for all submissions and this page should include:

- Full title of manuscript as well as a short title of up to 50 characters (title)
- Name(s) , address and highest academic degree of the author (s),
- Providing information about supporting institutions (if any),
- Name, address, telephone, fax numbers and e-mail address of the corresponding author,
- Acknowledgment of the individuals who contributed to the preparation of the manuscript.

Abstract: The abstract of Original Articles should be structured with subheadings (Objective, Methods, Results, and Conclusion). Abstract should be written both in English and in Turkish up to 300 words. First the English name of the article, then the English abstract should be written. After that the Turkish name of the article and a summary in Turkish should be written If the article is written in English and sent from outside Turkey, Turkish abstract it is not necessary. Please check Table 1 below for word count specifications.

Keywords: Key words should not exceed 3-5 words. Keywords should start with lowercase letters except for italic words and special names.

MANUSCRIPT TYPES

Original Research

The first page of the article should include the title of manuscript, authors' names and institutions, an abstract, and keywords. Title, abstract, and keywords must be provided both in English and Turkish. . If the article is written in English and sent from outside Turkey, Turkish Title, abstract and Keywords it is not necessary. The corresponding author should be indicated by a footnote and besides his/her full open mail address, and an e-mail address should also be provided.

Title: Must be provided both in English and Turkish.
Abstract: It should not include subheadings and should be limited to 350 words
Keywords: This section should contain at least three and at most six items and should be provided just below the abstract.
Main Text: The text should start with the Introduction, and be divided into appropriate sections. Sections must be numbered consecutively. Section headings must be written minisculely flush left and bold with their numbers (as, 1. Introduction, 2. Material and method, 3. Results, 4. Conclusions and discussion). Subsections must be numbered as "1.1", "1.2", etc., using the section number and must be written minisclulely flush left. All acronyms and abbreviations used in the manuscript should be defined at first use, both in the abstract and in the main text. In the final section there must be Acknowledgements (if any) , References and Appendices (if any).Please check Table 1 for the limitations. It should include Introduction, other subheadings and Conclusion sections and should be limited to 5000 words except for the references.
References: The reference list should be placed just below the main text and the number of references should not exceed 50.
Tables, Figures and Images: The appearance of Tables, Figures and Images should be provided in the text. The visuals should be original or the written permission obtained from the copyright holder should be sent to the Editor-in-Chief's Office.

Review

Reviews prepared by the authors who have a broad knowledge in a specific area and represented in the international literatüre by a high number of publications and citations, are evaluated.
Title: Must be provided both in English and Turkish.
Abstract: Abstract shouldn't include subheadings and should be limited to 350 words.
Key words: This section should include at least three and at most six items and should be provided just below the abstract.
Main Text: It should include Introduction, other subheadings and Conclusion sections and should be limited to 5000 words excluding the references.
References: The reference list should be placed below the main text and the number of references should not exceed 55.
Tables, Figures and Images: Tables, Figures and Images: The appearance of Tables, Figures and Images sholud be provided in the text. The visuals should be original or the written permission obtained from the copyright holder should be sent to the Editor-in-Chief's Office. Please check Table 1 for the limitations.

Research note, Case Reports, Case Series and Literature Review

The text should include Introduction, Case reports, Discussion and Conclusion subheadings. Please check Table 1 for the limitations for Research Note.

Letters to the Editor

Discusses important parts, unobserved aspects or missing parts of a previously published article. Letters to the Editor should not include Abstract, Keywords and Tables, Figures, Images, and other media. The text should be unstructured. The commented article must be properly identified in the letter to the editor (Table 1).

Table 1. Limitations for each manuscript type

Type of manuscript	Word limit	Abstract word limit	Reference limit	Table limit	Figure limit
Original Research	5000	350	25	7	maximum 20 images
Review Article	5000	350	35	7	maximum 20 images
Research note, Case Report	1000	250	15	No tables	maximum 15 images
Case Series	1500	250	20	maximum 15 images	maximum 15 images
Literature of Review	3500	250	35	maximum 15 images	maximum 15 images
Letter to the Editor	500	No abstract	7	No tables	No figure

Tables

Tables should be included in the main document, and they should be numbered consecutively in the order they are referred to within the main text. Their appearance in the text should be provided. A descriptive title must be placed above the tables. Abbreviations used in the tables should be defined below the tables by footnotes. Tables should be created using the "insert table" command of the word processing software.

Figures, Graphics and Photographs

Figures, graphics, and photographs should be submitted as separate files (JPEG format) through the submission system. The files should not be embedded in a Word document or in the main document. Any information within the images that may indicate an individual or institution should be blacked out. The minimum resolution of each submitted figure should be 300 DPI. All submitted figures should be clear in resolution and large in size. Figures , tables and illustrations should be inserted to the appropriate positions where they are mentioned in the text. All drawings, graphics, photographs, etc. should be regarded as figures. Figures should be numbered consecutively (as Figure 1.).

References

While citing publications, the latest and the most current publications should be preferred. At least half (50%) of the references should be published within the last 10 years. Unpublished results and personal communications are not recommended in the reference list, but may be mentioned in the text. Journal titles should be abbreviated in accordance with the journal abbreviations in Index Medicus/ MEDLINE/PubMed. When there are 6 or fewer authors, all authors should be listed. If there are 7 or more authors, the first 6 authors should be written, the space should be **"..."** left behind and the last author should be written. In the main text of the manuscript, references should be cited by using numbers in parentheses [as 3]. Authors are responsible for the accuracy of references. The reference styles for different types of publications are presented in the following examples. Reference must be made according to APA 6th. Please refer to the details;

Reference form

Reference form

No author

[1] *Regional perspectives on aid for trade*. (2014). Paris, FR: Organisation for Economic Cooperation and Development.

Journal article with DOI

[1] Sengün, Y. (2012). The wild plants consumed as a food in Afyonkarahisar. *Biological Diversity and Conservation*, 5(*2*), 95-105. https://doi:10.1242/jeb.103937
[2] Yücel, E., & Ezer, T. (2018). The bryophyte flora of Eskisehir Province (Turkey). *Arctoa* 27(*2*), 164–171. https://doi: 10.15298/arctoa.27.16
[3] Author, A. (year). Title of article: *Title of Journal*, volume(issue), page range. Doi:xx xxxxxxxxxx.

Journal article with DOI

[1] Eroğlu, G., Kaşik, G., & Öztürk, C. (2015). Three new myxomycete records from Turkey. *Biological Diversity and Conservation*, 8(*1*), 16-18.

[2] Brown, L. F., Yeo, K., Berse, B., Yeo, T. K., Senger, D. R., Dvorak, H. F., & Van De Water, L. (1992). Expression of vascular permeability factor. *The Journal of Experimental Medicine*, 176(*5*), 1375-1379.

Journal article published in a supplement

[1] Lock, M. (2015). Eclipse of the gene and the return of divination. *Current Anthropology*, *46*(Suppl. 5), S47-S70. Retrieved from https://www.jstor.org/stable/10.1086/432452

Books

[1] Fleer, M. (2015). *Science for children*. Cambridge, UK: Cambridge University Press.
[2] Berkes, F. & Kışlalıoğlu, M. (1990). *Ekoloji ve çevre bilimleri*. İstanbul: Remzi Kitabevi.
[3] Langlais, B., Reckhow, D. A., & Brink, D. R. (1991). *Ozone in water treatment*. London: UK: CRC Press.
[4] Brown, M., Shields, J., Kaine, S., Dolle-Samuel, C., North-Samardzic, A., McLean, P., … O'Leary, P. (2016). *Managing employee performance and reward: Concepts, practices, strategies (2nd ed.).* Port Melbourne, VIC: Cambridge University Press.

eBook

[1] Bull, M. (2015). *Punishment and sentencing: Risk, rehabilitation and restitution*. Retrieved from http://CURTIN.eblib.com.au/patron/FullRecord.aspx?p=1985996

Chapter in an edited print book

[1] Petermann, E. (2015). Monster mash-ups: Features of the horror musical. In L. Piatti-Farnell & D. L. Brien (Eds.), *New directions in 21st century gothic: The gothic compass* (4th ed., pp. 71-83). New York, NY: Taylor and Francis.

Conference paper

[1] Novak, D., & Verber, D. (2015, July 21). *Assessment of the influence caused by random events within real- time strategy game engine on a game bot gameplay*. Paper presented at the 8th Annual International Conference on Computer Games, Multimedia and Allied Technology, Singapore. https://doi.org/10.5176/2251-1679_CGAT15.27

www.biodicon.com
ISSN: 1308-8084 Online

Biological Diversity and Conservation
Biyolojik Çeşitlilik ve Koruma
ISSN: 1308-5301 Print

Hakem Değerlendirme Formu / Reviewer Evaluation Form

Makale adı / Article Title:
Makale No / Article No:

Lütfen sizin için uygun olan seçeneği "**X**" koyarak işaretleyiniz / Please indicate your answer with an "**X**".

1. Makale orijinal mi? / Is the article original?
   - □ Evet / Yes
   - □ Hayır / No
2. Problem uygun şekilde belirlenmiş mi? / Is the problem properly stated?
   - □ Evet / Yes
   - □ Hayır / No
3. Problem uygun şekilde ele alınmış mı veya çözülmüş mü? / Is the problem adequately treated or solved?
   - □ Evet / Yes
   - □ Değişiklikler veya ekler gerekli /Changes or elaborations required
   - □ Hayır / No
4. Belgeleme / Documentation:

   Tablolar / Tables:
   - □ İyi / Good
   - □ Zor anlaşılır (Tablo no:) / Unclear (Table no:)
   - □ Gereksiz (Tablo no:) / Unnecessary (Table no:)
   - □ Doğru değil (Tablo no:) / Incorrect (Table no:)

   Grafikler / Graphs:
   - □ İyi / Good
   - □ Zor anlaşılır (Şekil no:) / Unclear (Figure no:)
   - □ Gereksiz (Şekil no:) / Unnecessary (Figure no:)
   - □ Doğru değil (Şekil no:) / Incorrect (Figure no:)

   Diğer çizimler / Other illustrations:
   - □ İyi / Good
   - □ Değişiklik gerekli (Şekil no:) / Change needed (Figure no:)
   - □ Gereksiz (Şekil no:) / Unnecessary (Figure no:)
   - □ Düşük kalitede (Şekil no:) / Poor quality (Figure no:)

   İstatistikler / Statistics:
   - □ Uygun / Suitable
   - □ Uygun değil / Unsuitable
5. Sonuçların yorumu / Interpretation of results:
   - □ Doğru veya uygun / Correct or appropriate
   - □ Düzeltilmeli / Should be amended
   - □ Bulunamadı / Not found
6. Literatür alıntısı / Literature cited:
   - □ Uygun / Appropriate
   - □ Çok geniş / Too broad
   - □ Tam değil / Incomplete
7. Dil ve üslup / Language and style:
   - □ İyi yazılmış / Well written
   - □ Daha kısa ve öz olmalı / Should be made more concise
   - □ Tekrar yazılmalı veya düzenlenmeli / Should be rewritten or edited
8. Makale başlığı / Article title:
   - □ Uygun / Appropriate
   - □ Çok uzun / Too long
   - □ Çok genel / Too general

9. Özet / Abstract:
   - □ Uygun / Appropriate
   - □ Çok uzun / Too long
   - □ Çok genel / Too general
   - □ Makalenin içeriğini yansıtmıyor / Does not reflect the paper's content
10. Anahtar kelimeler / Key words:
   - □ Uygun / Appropriate
   - □ Uygun değil / Inappropriate
11. Makale hakkında genel fikir / General opinion about the paper:
   - □ Yeni bulgular sağlıyor / Provides new findings
   - □ Yeni bulgular sağlıyor ama az etkili / Provides new findings but is of marginal interest
   - □ Önceki bulguların gerekli bilgisini sağlıyor / Provides needed confirmation of previous findings
   - □ Önceden bilinen çalışmaların tekrarı / Repeats already known work
12. Öneriler / Recommendations:
   - □ Değiştirilmeden basılmalı / Should be published without changes
   - □ Değişikliklerden sonra basılmalı / Should be published after changes
   - □ Yeniden yazım veya düzeltme sonrasında bir karar için yeniden sunulmalı / Should be resubmitted for a decision after rewriting or editing
   - □ Basılmamalı / Should not be published
13. Detaylı işaretlemeler (Lütfen eğer gerekliyse sayfaları ekleyin) / Detailed remarks (Please attach pages if necessary):

Belirtmek istediğiniz diğer ayrıntılar (varsa) / Other details wanted to be indicated (if exist)

Hakemin adı / Name of reviewer:
Adres / Address:
Tel / Fax no:
e-mail:

(Hakem adı gizli tutulur / Reviewer name is kept secret)

Telif Hakkı Devir Formu/ *The Copyright Agreement Form*

Biyolojik Çeşitlilik ve Koruma/*Biological Diversity and Conservation*
ISSN 1308-5301 Print; ISSN 1308-8084 Online
Prof. Dr. Ersin YÜCEL, P.K. 86, İki Eylül PTT Merkez, 26000 Eskişehir / Türkiye

Derginin Adı / *Journal Title*: Biyolojik Çeşitlilik ve Koruma/*Biological Diversity and Conservation*
Makalenin Adı / *Manuscript title*: ……
……
……
……
Yazarların Adı / *Full Names of All Authers*: ……
……
……
Yazışmaların Yapılacağı Yazarın Adı ve Adresi / *Name, Adres Of Corresponding Author*: ……..
……
……
TC Kimlik No. / *ID Number*: …… e-posta: ……

Yazar(lar) / The Author(s) warrant(s) that:
Sunulan makalenin orijinal olduğunu; makalenin başka bir yerde basılmadığını veya basılmak için sunulmadığını; diğer şahıslara ait telif haklarını ihlal etmediğini taahüt eder. /
The manuscript submitted is his own orijinale work; the manuscript has not been published and is not being submitted or considered for publication elsewhere; the manuscript do not infringre upon any existing copyright.

"Telif Hakkı Devir Formu" tüm yazarlarca imzalanmalıdır. / *This copyright form must be signed by all authors.*

TC Kimlik No. / *ID Number*:……
Adı Soyadı / *Full name*:……
Tarih / *Date*: ……İmza / *Signature*……

TC Kimlik No. / ID *Number*:……
Adı Soyadı / *Full name*:……
Tarih / Date: ……İmza / *Signature*……

TC Kimlik No. / *ID Number*:……
Adı Soyadı / *Full name*:……
Tarih / *Date*: ……İmza / *Signature*……

TC Kimlik No. / *ID Number*:……
Adı Soyadı / *Full name*:……
Tarih / *Date*: ……İmza / *Signature*……

TC Kimlik No. / *ID Number*:……
Adı Soyadı / *Full name*:……
Tarih / *Date*: ……İmza / *Signature*……

TC Kimlik No. / *ID Number*:……
Adı Soyadı / *Full name*:……
Tarih / *Date*: ……İmza / *Signature*……

TC Kimlik No. / *ID Number*:……
Adı Soyadı / *Full name*:……
Tarih / *Date*: ……İmza / *Signature*……

Address:
Prof. Dr. Ersin YÜCEL
Sazova Mahallesi, Ziraat Caddesi, No.277 F Blok, Eskisehir, Türkiye, 26005
Phone: +90364189988; E-mail: biodicon@gmail.com

www.biodicon.com
ISSN: 1308-8084 Online

Biological Diversity and Conservation
Biyolojik Çeşitlilik ve Koruma
ISSN: 1308-5301 Print

ABONE FORMU / SUBSCRIPTION FORM

Adı /	Name	:
Soyadı /	Surname	:
Adres /	Address	:
Semt – İlçe /	City- State	:
Posta kodu /	Postal Code	:
İl /	Country	:
Telefon /	Telephone	:
Faks /	Fax	:
e-posta /	e-mail	:

* Yurtiçi Abone Ücreti, **Yıllık 3 Sayı 150TL** / Annual Subscirption Rate for Outside Turkey is **70 USD or 60 EUR for 3 issues.**

*Abone olmayanlar için her bir sayı 100 TL 'dir (Türkiye içi) / Each volume is **50 USD or 40 EUR** for non-subscribers (Outside of Turkey).

Sadece belirttiğim sayıyı olmak istiyorum / I would like to have an issue;

Vol. 1/1…….( ), Vol. 1/2 …….. ( )
Vol. 2/1 ……..( ) , Vol. 2/2 ……. ( ) , Vol. 2/3…..…. ( )
Vol 3/1. ……( ) , Vol. 3/2……. ( ) , Vol .3/3…….( )
Vol 4/1. ……( ) , Vol. 4/2……. ( ) , Vol .4/3……..( )
Vol 5/1. ……( ) , Vol. 5/2……. ( ) , Vol .5/3…….. ( )
Vol 6/1. ……( ) , Vol. 6/2……. ( ) , Vol .6/3…….. ( )
Vol 8/1. ……( ) , Vol. 8/2……. ( ) , Vol .8/3…….. ( )
Vol 9/1. ……( ) , Vol. 9/2……. ( ) , Vol .9/3…….... ( )
Vol 10/1. ..…( ) , Vol. 10/2….. ( ) , Vol .10/3……. ( )
Vol 11/1. ….( ) , Vol. 11/2….. ( ) , Vol .11/3……..….( )
Vol 12/1. ….( ) , Vol. 12/2….. ( ) , Vol .12/3……..….( )
Vol 13/1. ….( ) , Vol. 13/2….. ( ) , Vol .13/3……..….( )
Vol 14/1. ….( ) , Vol. 14/2….. ( ) , Vol .14/3……..….( )
Vol 15/1. ….( ) , Vol. 15/2….. ( ) , Vol .15/3……..….( )
Vol 16/1. ….( ) , Vol. 16/2….. ( ) , Vol .16/3……..….( )

Abone olmak istiyorum / I would like to have an annual subscription;
……...…… ( ) 2023…….( ) 20

Dergi aboneliğiniz ile ilgili ücreti **"VAKIFBANK, Ersin YÜCEL, IBAN: TR17 0001 5001 5800 7308 0978 79"** numaralı hesaba yatırdıktan sonra bu formu "**biodicon@gmail.com**" adresine ulaştırınız. Dergi adresinize posta ile adresinize gönderilecektir.

The payment of the article offering, please transfer total amount to the following bank account: "**VAKIFBANK, Ersin YÜCEL, IBAN: TR17 0001 5001 5800 7308 0978 79".** After that, this form is going to be sent the following address "**biodicon@gmail.com**". The journal with cash on delivery will be sent your address.

Tarih / Date: ……/……/20 İmza / Signature:
(Abone formunu Word belgesi olarak gönderiniz/Please send subscription form as a Word document)

www.biodicon.com
ISSN: 1308-8084 Online

Biological Diversity and Conservation
Biyolojik Çeşitlilik ve Koruma
ISSN: 1308-5301 Print

Adı /	Name	:
Soyadı /	Surname	:
Adres /	Address	:
Semt – İlçe /	City- State	:
Posta kodu /	Postal Code	:
İl /	Country	:
Telefon /	Telephone	:
Faks /	Fax	:
e-posta /	e-mail	:

* Yurtiçi Abone Ücreti, **Yıllık 3 Sayı 150TL** / Annual Subscirption Rate for Outside Turkey is

70 USD or 60 EUR for 3 issues.

*Abone olmayanlar için her bir sayı 100 TL 'dir (Türkiye içi) / Each volume is **50 USD or 40 EUR** for non-subscribers (Outside of Turkey).

Sadece belirttiğim sayıyı olmak istiyorum / I would like to have an issue;

Vol. 1/1…….( ), Vol. 1/2 …..… ( )
Vol. 2/1 ….....( ) , Vol. 2/2 ……. ( ) , Vol. 2/3…..…. ( )
Vol 3/1. ……( ) , Vol. 3/2……. ( ) , Vol .3/3……..…( )
Vol 4/1. ……( ) , Vol. 4/2……. ( ) , Vol .4/3……..…..( )
Vol 5/1. ……( ) , Vol. 5/2……. ( ) , Vol .5/3……..… ( )
Vol 6/1. ……( ) , Vol. 6/2……. ( ) , Vol .6/3……..… ( )
Vol 8/1. ……( ) , Vol. 8/2……. ( ) , Vol .8/3……..… ( )
Vol 9/1. ……( ) , Vol. 9/2……. ( ) , Vol .9/3……... ( )
Vol 10/1. ..…( ) , Vol. 10/2….... ( ) , Vol .10/3……. ( )
Vol 11/1. ….( ) , Vol. 11/2….... ( ) , Vol .11/3……..…( )
Vol 12/1. ….( ) , Vol. 12/2….... ( ) , Vol .12/3……..…( )
Vol 13/1. ….( ) , Vol. 13/2….... ( ) , Vol .13/3……..…( )
Vol 14/1. ….( ) , Vol. 14/2….... ( ) , Vol .14/3……..…( )
Vol 15/1. ….( ) , Vol. 15/2….... ( ) , Vol .15/3……..…( )
Vol 16/1. ….( ) , Vol. 16/2….... ( ) , Vol .16/3……..…( )

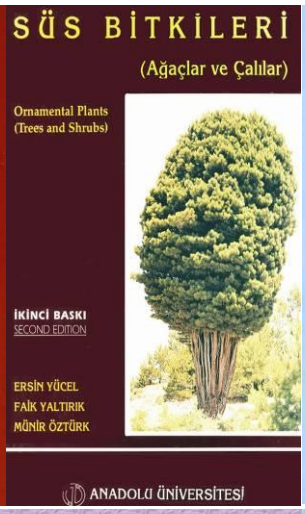
Abone olmak istiyorum / I would like to have an annual subscription;
……...…… ( ) 2023…….( ) 20

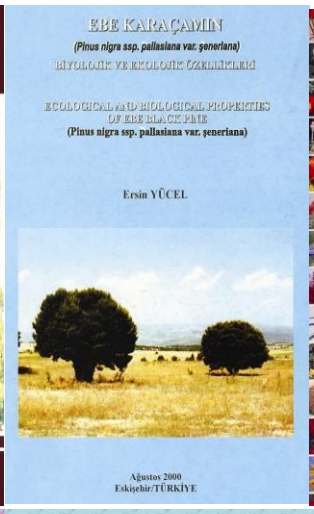
Dergi aboneliğiniz ile ilgili ücreti **"VAKIFBANK, Ersin YÜCEL, IBAN: TR17 0001 5001 5800 7308 0978 79"** numaralı hesaba yatırdıktan sonra bu formu "**biodicon@gmail.com**" adresine ulaştırınız. Dergi adresinize posta ile adresinize gönderilecektir.

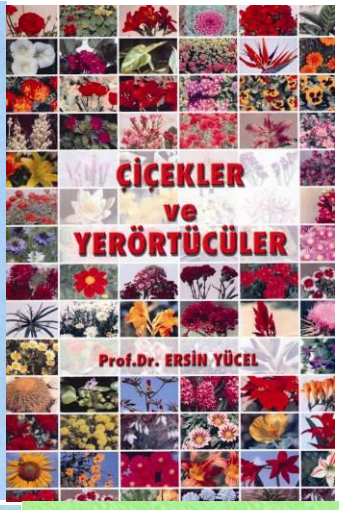
The payment of the article offering, please transfer total amount to the following bank account: "**VAKIFBANK, Ersin YÜCEL, IBAN: TR17 0001 5001 5800 7308 0978 79".** After that, this form is going to be sent the following address "**biodicon@gmail.com**". The journal with cash on delivery will be sent your address.

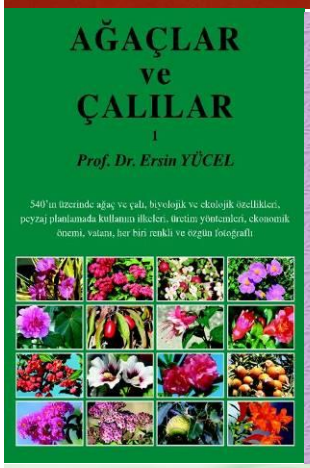
Tarih / Date: ……/……/20 İmza / Signature:

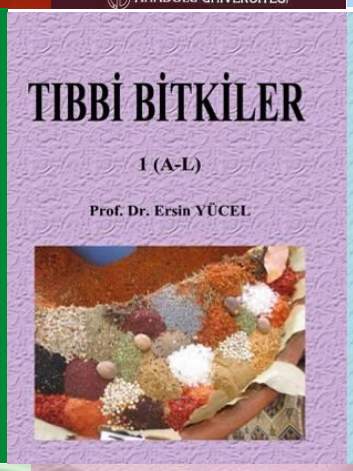
(Abone formunu Word belgesi olarak gönderiniz/Please send subscription form as a Word document)

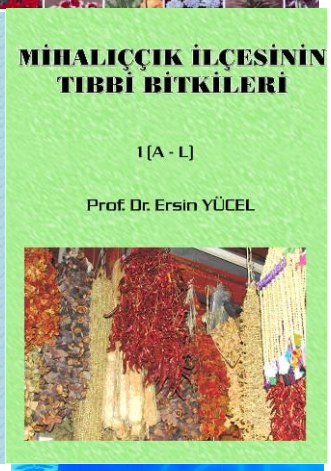


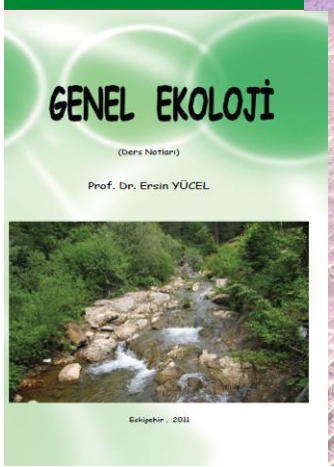


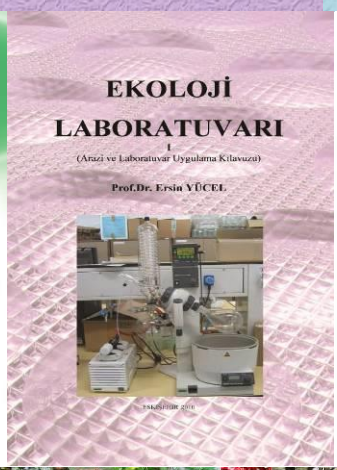


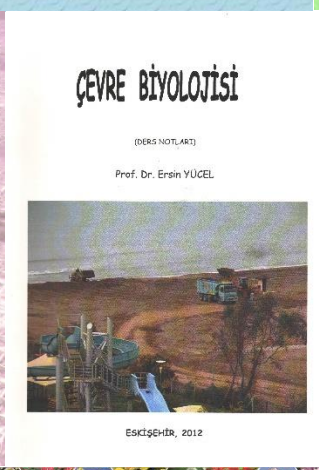


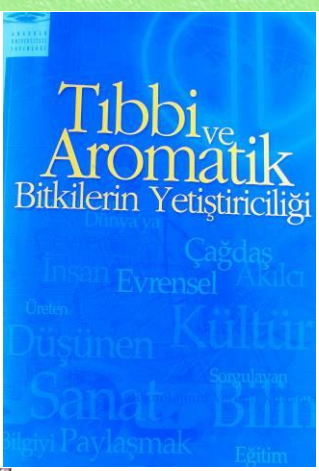


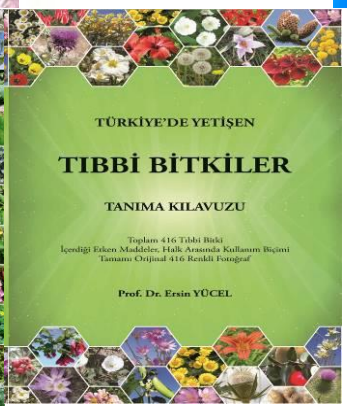












İletişim Adresi: ebitki@gmail.com
http://www.bitkilerim.com