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ARAŞTIRMA MAKALESİ **RESEARCH PAPER**

Contributions to the Genus *Clubiona* (Araneae: Clubionidae) in Türkiye, with a New Record and Additional Notes

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Abstract: The family Clubionidae, referred to as sac spiders, comprises numerous genera and species. These spiders are widely distributed worldwide. *Clubiona* Latreille, 1804 is the largest genus in the family Clubionidae, with 528 species. The genus has 47 species distributed in Europe. The genus, which has many species worldwide, has only 14 species in Türkiye. The detailed study of these species contributes significantly to our understanding of the biodiversity in the region. To make new contributions to the genus *Clubiona*, specimens were collected and systematically studied in Bayburt province. Samples were collected from bushes, between the plant roots and under the stone. The species were examined and measured using an Olympus SZX-16 stereomicroscope. This study reports some members of the genus *Clubiona* from Bayburt province. *C. diversa* O. Pickard-Cambridge, 1862 is recorded for the first time from Türkiye. Together with the new record, *C. brevipes* Blackwall, 1841, *C. neglecta* O. Pickard-Cambridge, 1862 and *C. pseudoneglecta* Wunderlich, 1994 are given as new locality records from Bayburt province. Morphological characters and photos of the species are presented with a detailed description of the new record. In addition, the world distribution of the new record is shown with a map.

Keywords: Bayburt, biodiversity, *Clubiona diversa*, sac spiders.

Yeni Bir Kayıt ve İlave Notlar ile Türkiye'deki *Clubiona* (Araneae: Clubionidae) Cinsine Katkılar

***Sorumlu yazar:**
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Öz: Kese örümcekleri olarak adlandırılan Clubionidae ailesi, çok sayıda cins ve tür içerir. Bu örümcekler dünya çapında geniş bir dağılıma sahiptir. *Clubiona* Latreille, 1804, 528 tür ile Clubionidae familyasının en fazla türe sahip cinsidir. Bu cinsin Avrupa'da dağılım gösteren 47 türü vardır. Dünyada çok sayıda türü bulunan cinsin, Türkiye'de yalnızca 14 türü bulunmaktadır. Bu türlerin detaylı bir şekilde incelenmesi bölgedeki biyoçeşitliliği anlamamıza önemli katkılar sağlayacaktır. *Clubiona* cinsine yeni katkılar sağlamak amacıyla Bayburt ilinden örnekler toplanmış ve sistematik açıdan incelenmiştir. Örnekler çalılıklardan, bitki köklerinin arasından ve taş altından toplanmıştır. Türler Olympus SZX-16 stereomikroskop kullanılarak incelenmiş ve ölçümleri yapılmıştır. Bu çalışmada, Bayburt ilinden *Clubiona* cinsinin bazı üyeleri rapor edilmiştir. *C. diversa* O. Pickard-Cambridge, 1862 Türkiye'den ilk kez kaydedilmiştir. Yeni kayıtla birlikte *C. brevipes* Blackwall, 1841, *C. neglecta* O. Pickard-Cambridge, 1862 ve *C. pseudoneglecta* Wunderlich, 1994 Bayburt ilinden yeni lokalite kaydı olarak verilmiştir. Türlerin morfolojik karakterleri ve fotoğrafları, yeni kaydın detaylı tanımıyla birlikte sunulmuştur. Ayrıca yeni kaydın dünya dağılımı harita ile gösterilmiştir.

Anahtar kelimeler: Bayburt, biyoçeşitlilik, *Clubiona diversa*, kese örümcekleri.

INTRODUCTION

The family Clubionidae, known as sac spiders, comprises 665 species of 18 genera (World Spider Catalog, 2025). In Türkiye, 15 species of this family are recognised, 14 belonging to the genus *Clubiona* (Danışman et al., 2024; Nentwig et al., 2025). These species are: *C. brevipes* Blackwall, 1841, *C. caucasica* Mikhailov & Otto, 2017, *C. comta* C. L. Koch, 1839, *C. corticalis* (Walckenaer, 1802), *C. frutetorum* L. Koch, 1867, *C. golovatchi* Mikhailov, 1990, *C. lutescens* Westring, 1851, *C. marmorata* L. Koch, 1866, *C. neglecta* O. Pickard-Cambridge, 1862, *C. pseudoneglecta* Wunderlich, 1994, *C. pseudosimilis* Mikhailov, 1990, *C. reclusa* O. Pickard-Cambridge, 1863, *C. similis* L. Koch, 1867 and *C. terrestris* Westring, 1851 (Avezbayeva & Türkeş, 2022; Coşar et al., 2022; Danışman et al., 2012; Danışman et al., 2018; Danışman et al., 2024; Helsdingen, 2013; Marusik & Kunt, 2010;

Russell-Smith, 2009; Türkeş & Atlı, 2022). In this study, four species of the genus *Clubiona* were identified as the first locality records for Bayburt province, with one species being a new record for the araneofauna of Türkiye.

MATERIAL AND METHOD

Samples were collected from the bushes, through the plant roots and under the stone using a hand aspirator and stored in 70% ethanol. The samples were examined and measured using an Olympus SZX-16 stereomicroscope. The female genitalia were removed and cleared with 10% KOH before illustration. The examined specimens were deposited in the Arachnology Museum of the Bayburt University (BUAM). All measurements are given in millimetres (mm). The world distribution of the species is based on the World Spider Catalog (2025). Habitus and copulatory organs were taken by means of an Olympus SP-810UZ digital camera and compared mainly with Almquist (2007), Azheganova (1968), Bosmans et al. (2017), Brændegaard (1966), Gajdoš et al. (2020), Gaymard and Lecigne (2018), Locket and Millidge (1951), Merrett (2001), Pozzi and Hänggi (1998), Reimoser (1937), Roberts (1998), Sterghiu (1985) and Wiehle (1965). Leg measurements of *Clubiona diversa* O. Pickard-Cambridge, 1862 are given in Table 1.

The following abbreviations are used in the text: Fe, femur; Pt, patella; Ti, tibia; Mt, metatarsus; Ta, tarsus; E, embolus; Ta, tibial apophysis; Co, copulatory opening; Re, receptacles.

RESULTS

Family: Clubionidae Simon, 1878

Genus: *Clubiona* Latreille, 1804

Clubiona brevipes Blackwall, 1841

Material Examined: 1♀, (BUAM CLU-01/01), Bayburt Province, Akşar Town, around Korgan Bridge, 40°20'8.6"N 40°00'5.0"E, 1636 m., 02.11.2024, among the bushes, leg. N. Demircan Aksan.

Description: Female (Figure 1A-C) Total length 5.0. Carapace length 2.2, width 1.3. Abdomen length 2.8, width 2.0. Carapace and chelicerae dark brown. Legs yellowish. Abdomen brownish with dark median pattern. Epigyne and vulva are shown in Figure 1B, C.

Distribution: Europe, Caucasus, Japan (World Spider Catalog, 2025).

The first record of the species in Türkiye was reported from the Marmara region (Helsdingen, 2013). In this study, *C. brevipes* Blackwall, 1841 is identified as the first locality record for Bayburt province (Eastern Black Sea).

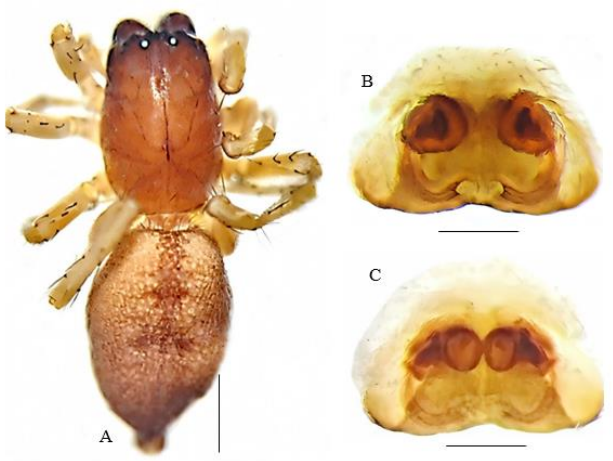

Figure 1. *Clubiona brevipes* (♀), A. Habitus, dorsal view B. Epigyne, ventral view C. Vulva, dorsal view (Scale: A. 1.0, B-C 0.2).

Clubiona diversa O. Pickard-Cambridge, 1862

Material Examined: 2♀♀, 2♂♂ (BUAM CLU-01/02), Bayburt Province, Akşar Town, around Korgan Bridge, 40°20'8.6"N 40°00'5.0"E, 1636 m., 23.11.2024, between the plant roots around the rocks, leg. N. Demircan Aksan.

Description: Female (♀) (Figure 3A-D). Total length 3.7. Carapace length 1.4, width 0.8. Abdomen length 2.3, width 1.0. Carapace brown, darker anteriorly with dense fine hairs. Chelicerae dark brown. Sternum light brown. Legs brown. Abdomen yellowish-white with dense fine hairs. Epigyne with single copulatory opening (Co). Each pair of receptacles (Re) close to each other.

Male (♂) (Figure 3E-I). Total length 3.1. Carapace length 1.3, width 1.0, brown. Abdomen length 1.8, width 0.8. Carapace brown, darker anteriorly with dense fine hairs. Chelicerae dark brown. Sternum light brown. Legs brown. Abdomen yellowish-white with dense fine hairs. Palp pale brown with dark cymbium. Tibial apophysis (Ta) pointed towards the tip. Embolus (E) long with curved distally.

Distribution: Europe, the Caucasus, Russia (from Europe to the Far East), Kazakhstan, Pakistan, Korea and Japan (World Spider Catalog, 2025).

In this study, *C. diversa* is recorded for the first time from Türkiye.

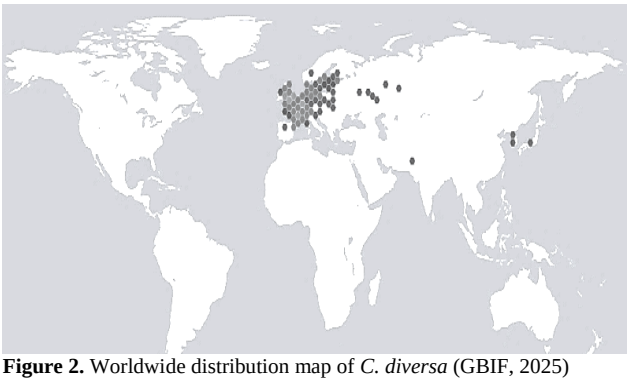

Figure 2. Worldwide distribution map of *C. diversa* (GBIF, 2025)

Table 1. *Clubiona diversa*, total body length and leg measurements (♂/♀)

Male / Female	Total body length	Leg	Fe	Pt	Ti	Mt	Ta	Total
♂	3.1	I	0.9	0.2	0.9	0.5	0.4	2.9
		II	0.9	0.3	0.9	0.5	0.4	3.0
		III	0.9	0.2	0.5	0.7	0.2	2.5
		IV	1.1	0.2	0.9	1.0	0.2	3.4
♀	3.7	I	0.9	0.3	0.8	0.7	0.3	3.0
		II	0.9	0.3	0.9	0.7	0.3	3.1
		III	0.8	0.2	1.0	0.6	0.2	2.8
		IV	1.2	0.3	1.0	1.2	0.3	4.0

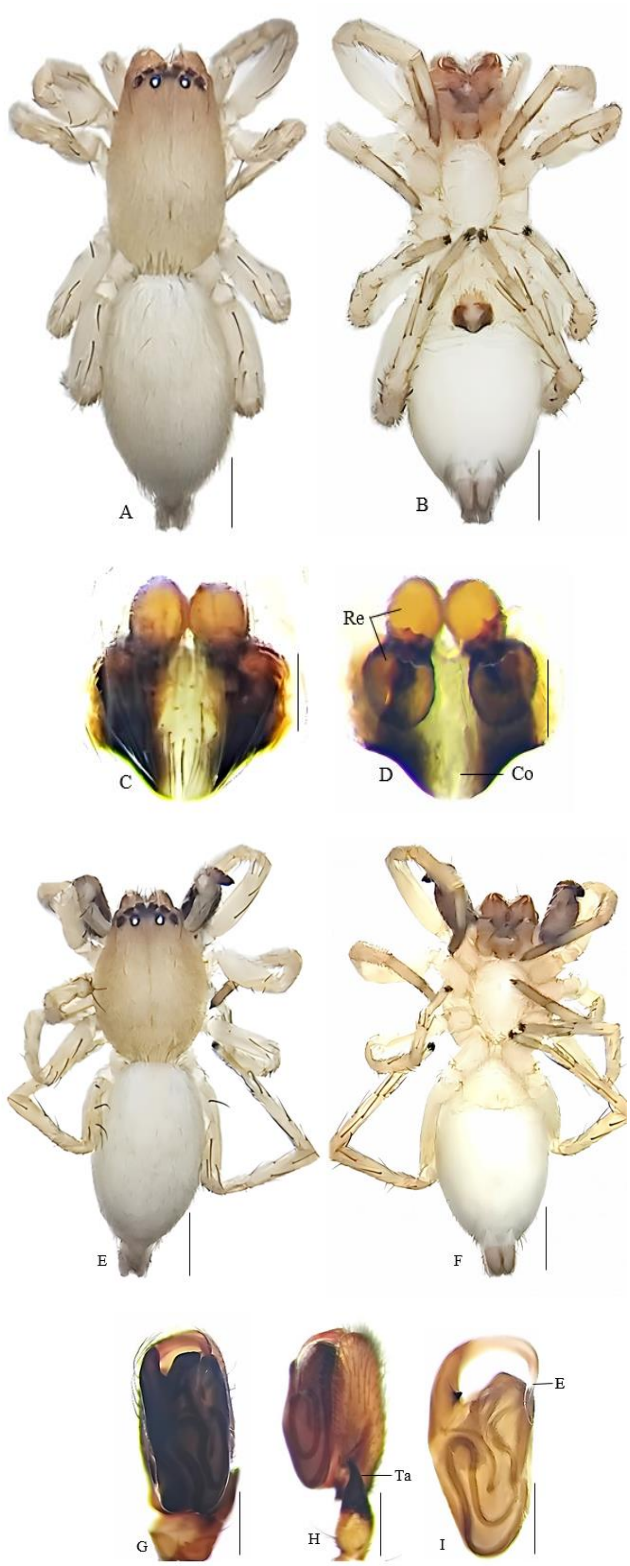

Figure 3. *Clubiona diversa* (♀, ♂), Female, A. Habitus, dorsal view B. Habitus, ventral view C. Epigyne, ventral view D. Vulva, dorsal view (Scale: A, B. 0.5, C, D. 0.1). Male, E. Habitus, dorsal view F. Habitus, ventral view G. Palp, ventral view H. Palp, retrolateral view; I. Bulbus, ventral view (Scale: E, F. 0.5, G-I. 0.2).

Clubiona neglecta O. Pickard-Cambridge, 1862

Material Examined: 1♀, (BUAM CLU-01/03), Bayburt Province, Demirözü District, around Demirözü Dam, 40°07'46"N 39°53'54"E, 1720 m., 09.11.2024, under the stone, leg. N. Demircan Aksan.

Description: Female (Figure 4A-C), Total length 5.5. Carapace length 2.5, width 1.7. Abdomen length 3.0, width 2.0. Carapace and legs brown. Chelicerae dark brown. Abdomen reddish brown with median dark pattern and light dots. Epigyne and vulva are shown in Figure 4B, C.

Distribution: Europe, Türkiye, the Caucasus, Russia (from Europe to South Siberia), Iran, Central Asia, China and Korea (World Spider Catalog, 2025).

The first record of the species in Türkiye was reported from the Mediterranean coast (Russell-Smith, 2009). In this study, *C. neglecta* is presented as the first locality record for Bayburt province (Eastern Black Sea).

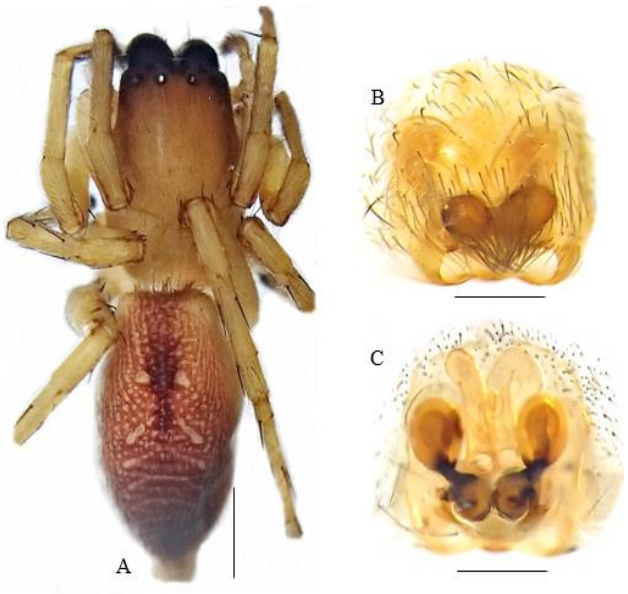

Figure 4. Clubiona neglecta (♀), A. Habitus, dorsal view B. Epigyne, ventral view C. Vulva, dorsal view (Scale: A. 1.0, B-C 0.2).

Clubiona pseudoneglecta Wunderlich, 1994

Material Examined 1♀, (BUAM CLU-01/04), Bayburt Province, Akşar Town, 40°20'8.6"N 40°00'5.0"E, 1645 m., 23.11.2024, among the bushes, leg. N. Demircan Aksan.

Description. Female (Figure 5A-C). Total length 5.0. Carapace length 2.0, width 1.2. Abdomen length 3.0, width 1.8. Prosoma brown. Legs yellowish brown. Abdomen brownish with median dark pattern. Epigyne and vulva are shown in Figure 5B, C.

Distribution: Morocco, Algeria, Europe, Caucasus, Iran (World Spider Catalog, 2025).

The first record of C. pseudoneglecta in Türkiye was reported from the Marmara region (Helsdingen, 2013). In this study, C. pseudoneglecta is given as the first locality record for Bayburt province (Eastern Black Sea).

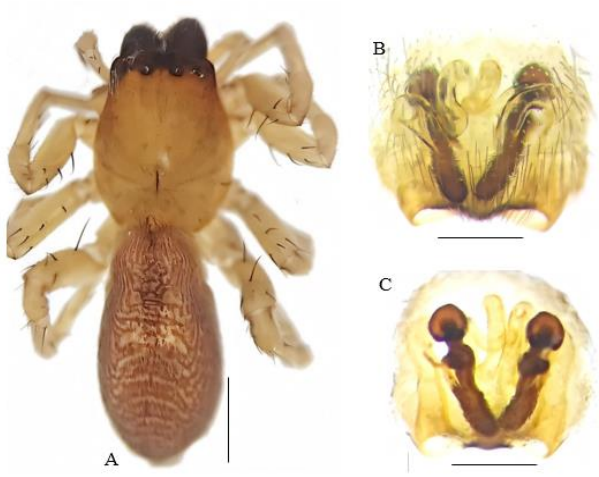

Figure 5. *Clubiona pseudoneglecta* (♀), A. Habitus, dorsal view B. Epigyne, ventral view C. Vulva, dorsal view (Scale: A. 1.0, B-C. 0.2).

DISCUSSION

In this study, a total of 7 specimens belonging to the genus *Clubiona* were examined, and 4 species were identified. Among these species, *C. diversa* is reported from Türkiye for the first time, increasing the number of known *Clubiona* species in the country to 15. Additionally, *C. brevipes*, *C. neglecta*, and *C. pseudoneglecta*, which are widespread in Türkiye, are recorded for the first time from Bayburt province in this study. Thus, the study provides both a regional contribution with species whose distribution is already known in Türkiye and a new record for the country.

C. diversa has a Palearctic distribution, ranging from Europe to the Far East and typically inhabits heathland of dunes, as well as litter and grass on limestone (Almquist, 2007; World Spider Catalog, 2025). The species was found only in one locality, among plant roots, during the survey. It has been observed that *C. diversa* is largely consistent with previously described specimens in terms of its morphological characteristics.

These new records contribute significantly to the expansion of the geographical range of *Clubiona* species in Türkiye. The results highlight the importance of continued field surveys to document the biodiversity of spiders, especially in less-explored areas such as Bayburt province.

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ARAŞTIRMA MAKALESİ **RESEARCH PAPER**

Kızıl Tilkilerde (*Vulpes vulpes*) Ossa Coxae'nın Morfometrik ve Radyografik İncelenmesi

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Öz: Bu çalışma, yetişkin kızıl tilkilerde ossa coxae'nın morfolojisini tanımlamak ve radyografi kullanarak pelvisin morfometrik değerlendirmelerini yapmak amacıyla yapıldı. Bu amaçla 4 erişkin erkek tilki kullanıldı. Diseksiyonları yapıldıktan sonra makro-anatomik olarak da incelendi. Bu incelemelere göre geniş bir incisura acetabuli mevcuttu. Acetabulum'un cranialinde, m. rectus femoris'in orijini için pürüzlü bir kabartı bulunmaktaydı. İlium içbükey bir kanada sahipti. Tuber ischiadicum tek bir çıkıntıdan oluşmaktaydı ve os pubis craniocaudal olarak dar ve dorsoventral olarak basıktı. Daha sonra ossa coxae'nın dorsal, lateral ve ventral yönlü olarak radyografileri çekildi ve görüntü üzerinde 11 ölçüm noktası belirlendi. Bu ölçümlere göre tilkinin pelvis uzunluğunun en büyük uzunluk (GL (Greatest length)) ortalama 90,44±5,9 mm, en küçük genişlik ise (SB (Smallest breadth)) 5,48±0,97 mm idi. Tür ayrımı noktasında önemlilik arz edeceği ispatlanmıştır. Elde edilen bulgular, mevcut bilgi tabanına katkıda bulunmakta ve farklı araştırma ve mesleki uygulama alanları için pratik çıkarımlar sunmaktadır.

Anahtar kelimeler: Kızıl tilki, morfometri, ossa coxae.

Morphometric and Radiographic Investıgation of Ossa Coxae in Red Foxes (*Vulpes vulpes*)

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Abstract: This study was conducted to describe the morphology of the ossa coxae in adult red foxes and to make morphometric evaluations of the pelvis using radiography. For this purpose, 4 adult male foxes were used. After dissection, they were examined macro-anatomically. According to these examinations, a large incisura acetabuli was present. Cranial to the acetabulum, there was a jagged ridge for the origin of the m. rectus femoris. The ilium had a concave wing. The tuber ischiadicum consisted of a single ridge and the os pubis was narrow craniocaudally and flattened dorsoventrally. Radiographs of the ossa coxae were then taken in dorsal, lateral and ventral directions and 11 measurement points were determined on the image. According to these measurements, the greatest length (GL (Greatest length)) of the pelvic length of the fox was 90.44±5.9 mm and the smallest width (SB (Smallest breadth)) was 5.48±0.97 mm. It proved to be important for species distinction. The findings contribute to the existing knowledge base and have practical implications for different areas of research and professional practice.

Keywords: Morphometry, ossa coxae, red fox.

GİRİŞ

Kızıl tilki (*Vulpes vulpes*) kürk taşıyan bir hayvan olarak büyük bir ekonomik değere sahiptir. Osteoarkeologlar ve yaban hayatı ile ilgilenler, popülasyon yönetimi çalışmaları için veya arkeolojik alanlardaki kemik kalıntılarının tanımlanması amacıyla osteolojik parametrelere ihtiyaç duymaktadır (Bisaillon & Deroth, 1979).

Kalça kemiği veya os coxae; os pubis, os ilium ve os ischii kemiklerinden oluşmaktadır. Bu üç kemiğin corpusu acetabulum adı verilen bir oyuk oluşturur ve femurun caput ossis femoris ile eklem yapmasını sağlamaktadır. Sağ ve sol tarafta ossa coxae ve symphysis pelvina tarafından oluşturulan pelvis, bu kemiklerin

ventralde birleşmesiyle ve ayrıca sacrum ve dorsalden ilk birkaç caudal vertebra (coccygeal) ile meydana gelmektedir. Pelvis kemikleri tarafından çevrelenen boşluk cavum pelvis olarak tanımlanmaktadır (Binici, 2005; König & Liebich; 2015;Bahadır & Yıldız, 2016; Dursun, 2008; Liebich vd.,2007).

Farklı yaban hayvanı iskeletleri üzerine çok sayıda çalışma yapılmıştır (Yılmaz vd., 1999; Yılmaz vd., 2000; Özdemir & Karan, 2001; Atalar & Özdemir, 2002; Atalar & Yılmaz, 2004; Özüdoğru vd., 2012; Karan vd., 2016; Pagh vd., 2018; Özüdoğru vd., 2018; Rizwan vd., 2021). Ancak, gelişen görüntüleme teknikleri ile alınan morfometrik veriler; taksonomik sınıflandırılma, türler arasındaki farklılıkların ayırt edilmesi, zooarkeoloji alanındaki çalışmalar için değerli bilgiler sağlamaktadır (Sporri vd., 1994; Dyce vd., 2009).

Pelvis kemiklerinin değerlendirilmesinde manuel olarak kullanılan pelvimetreden başlayan süreç, görüntüleme tekniklerinin ilerlemesi sonucunda yerini daha net görüntüler verebilen X-ray, bilgisayarlı tomografi (BT), manyetik rezonans görüntüleme (MRG) ve ultrasonografi (USG) gibi görüntüleme sistemlerine bırakmıştır (Sporri vd., 1994; Dyce vd., 2009). Pelvis morfometrisi arkeoloji ve adli bilimlerin yanı sıra obstetrik ve jinekoloji alanında da oldukça önemlidir (Monteiro vd., 2013;Federle vd.,1982; Ohlerth &Scharf, 2007; Decker vd., 2011; Duetsch & Peterson, 2012).

Bu çalışmanın amacı, yetişkin erkek kızıl tilkinin ossa coxae'sının morfometrik özelliklerini, radyografi kullanılarak değerlendirmelerini ortaya koymaktır.

MATERYAL VE METOT

Bu çalışma anatomik bir çalışma olup gözlemsel bir tasarıma sahiptir. Çalışma, hem Tarım ve Orman Bakanlığı Doğa Koruma ve Milli Parklar Genel Müdürlüğü 24.06.2024-494637, hem de Fırat Üniversitesi Hayvan Deneyleri Yerel Etik Kurulu 17.10.2024-28157 tarih ve sayılı kararlarına uygun olarak yürütülmüştür. Bu çalışmada toplam 4 adet erişkin erkek (ortalama 6.3 kg ağırlığında) tilkinin pelvis radyografik görüntüleri kullanılmıştır.

Doğada ölmüş halde öğrenciler tarafından bulunan Kızıl Tilkiler toplanarak Anatomi Anabilim dalına getirilmiştir. Diseksiyon işlemlerini takiben makro-anatomik yapıları incelenen kemikler birleştirilerek iskelet oluşturulmuştur.

Tilki iskeletlerinin radyoloji görüntüleri Fırat Üniversitesi Hayvan Hastanesinde çekilmiştir. Röntgen masasına yerleştirilen kemikler cranio-dorsal, dorso-medial ve ventro-lateral pozisyonda konumlandırılarak görüntüleri alınmıştır.

Terminolojide Nomina Anatomica Veterinaria (World Association of Veterinary Anatomists, 1973) kullanılmıştır.

Görüntülerin ölçümleri, anatomik referans noktaları kısaltma ve tanımlarıyla Tablo 1 de gösterilmiştir. Pelvis metrik ölçüm noktalarına (Von Den Driesch, 1976) dayalı olarak 11 ölçüm noktasında (Tablo 1) gerçekleştirilmiştir.

Tablo 1. Tilki coxa'sının dorsal, ventral ve lateral yüzeylerinden elde edilen ölçüm parametreleri
Table 1. Measurement parameters obtained from the dorsal, ventral and lateral surfaces of the fox coxa.

Kısaltma	Tanımı
GL	Greatest length (En büyük uzunluk)
LA	Length of the acetabulum (Acetabulum uzunluğu)
LAR	Length of the acetabulum on the rim (Acetabulumun kenar üzerindeki uzunluğu)
LS	Length of the symphysis (Symphysis uzunluğu)
SH	Smallest height (En küçük yükseklik)
SB	Smallest breadth (En küçük genişlik)
LFo	Length of foramen obturatum (Foramen obturatum uzunluğu)
GBTc	Greatest breadth across the tubera coxarum (Tubera coxarum boyunca en büyük genişlik)
GBA	Greatest breadth across the acetabula (Acetabula boyunca en büyük genişlik
GBTİ	Greatest breadth across the Tubera ischiadica (Tubera ischiadica boyunca en büyük genişlik)
SBI	Smallest breadth across the bodies of the ischia (İschia gövdeleri boyunca en küçük genişlik)

BULGULAR

Makro-anatomik incelemelere göre; uzun bir kemik olan os coxae'da kemikleşmiş bir sympyhysis pelvina bulunmaktaydı. Acetabulum oldukça derin ve pürüzsüz olan facies lunata'nın medialinde geniş bir incisura acetabuli mevcuttu. Oval şekle sahip foramen obturatum'un craniomedial kenarı tırtıklı, diğer kenarları düzenli ve pürüzsüz olduğu görüldü.

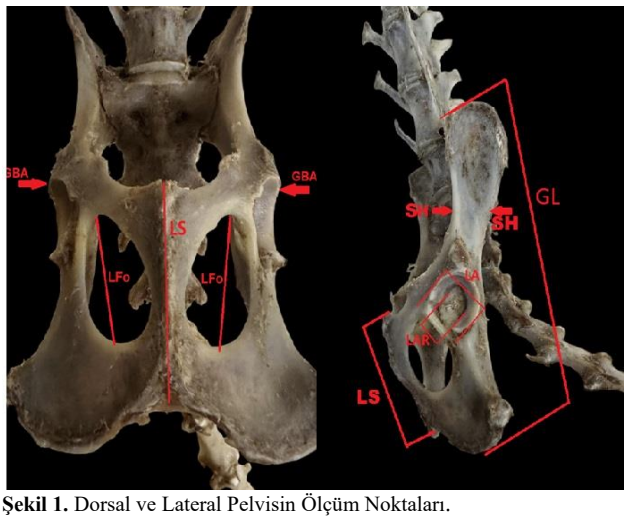
İlium'un içbükey bir kanada sahipti. Kemiğin gövdesi kalındı ve acetabulumun cranialinde, m. rectus femoris'in orijini için pürüzlü bir kabartı bulunmaktaydı. Area lateralis m. recti femoris ve area medialis m. recti femoris belirgin değildi.

İlium'un craniodorsal kenarı dışbükeydi. İlium'un dorsal kenarın cranial yarısı içbükeydi ve incisura ischiadica major mevcuttu. Spina ischiadicayı oluşturduktan sonra daha yüzeysel bir incisura ischiadica minor görülmekteydi. Sacropelvinal yüzün caudalinde facies auricularis, craniodorsalinde de küçük bir tuberositas ilaca mevcuttu.

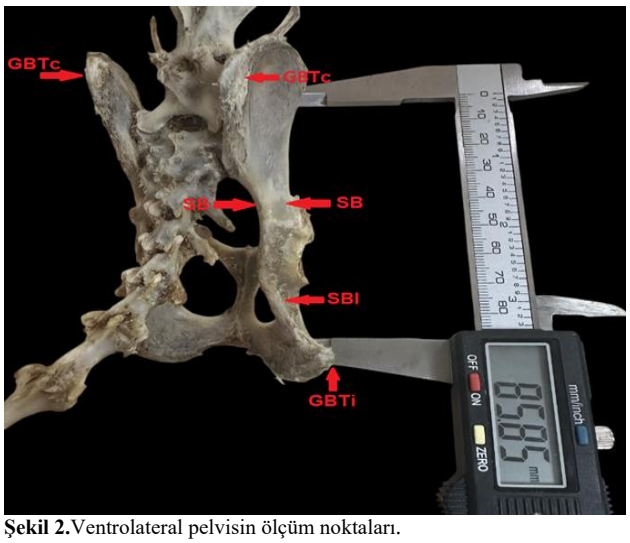
Os ischii kalın bir gövde ve ince bir ramus şeklindeydi. Kemiğin caudolateral kısmında belirgin ve tek bir çıkıntıdan oluşan tuber ischiadicum mevcuttu.

Os pubis craniocaudal olarak dar ve dorsoventral olarak basıktı. Belirgin bir eminentia iliopubica bulunmaktaydı.

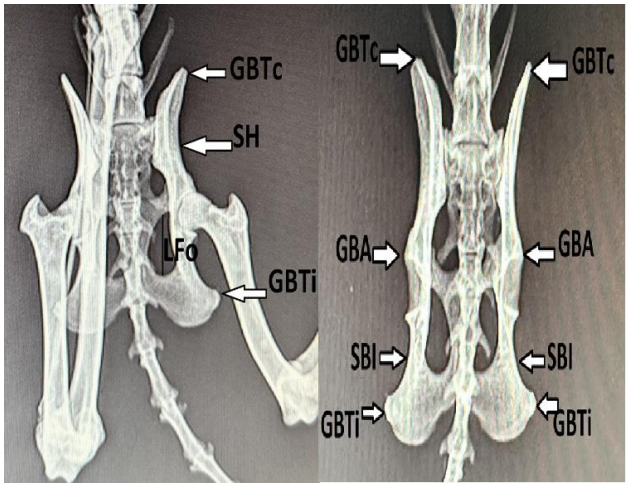
Pelvislerin ölçüm noktaları hem pelvis üzerinde (Şekil 1 ve Şekil 2), hem de radyografi üzerinde (Şekil 3 ve Şekil 4) işaretlenmiştir.



Şekil 1. Dorsal ve Lateral Pelvisin Ölçüm Noktaları.
Figure 1. Measurement Points of the Dorsal and Lateral Pelvis.

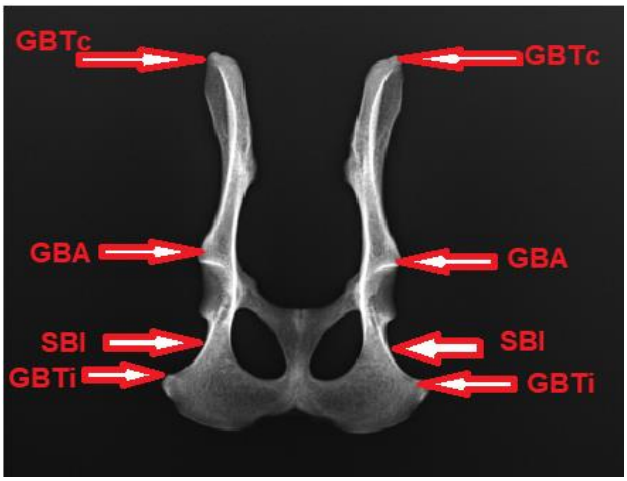


Şekil 2. Ventrolateral pelvisin ölçüm noktaları.
Figure 2 . Measurement points of the ventrolateral pelvis.



Şekil 3. Pelvisin dorsal ve lateral ölçüm noktalarının radyografik görüntülenmesi.
Figure 3. Radiographic visualization of the dorsal and lateral measurement points of the pelvis.

Tilkilerin pelvislerinin uzunluk ve genişlik ölçümlerine dayalı olarak 11 parametre ölçüm ortalamaları hesaplanmıştır ve Tablo 2 de sunulmuştur.



Şekil 4. Pelvisin dorsoventral yönde ölçüm noktalarının radyografik görüntülenmesi
Figure 4. Radiographic visualization of the dorsoventral measurement points of the pelvis

Tablo 2. Tilkinin os coxa'sının ölçüm noktalarının ortalama değerleri (mm)
Table 2. Mean values of the measurement points of the os coxa of the fox (mm)

Ölçüm noktaları	Ortalama	Standart Sapma (±)
GL	90,44	5,9
LA	13,87	0,55
LAR	14,47	0,70
LS	28,62	5,83
SH	11,68	1,79
SB	5,48	0,97
LFo	21,79	2,35
GBTc	48,60	4,29
GBA	47,59	4,54
GBTi	69,49	1,42
SBI	47,69	6,62

Tablo 2'ye göre tilkinin pelvis uzunluğunun en büyük uzunluk (GL (Greatest length)) ortalama 90,44±5,9 mm, en küçük genişlik ise (SB (Smallest breadth)) 5,48±0,97 mm olarak ölçülmüştür. Tubera ischiadica boyunca en büyük genişlik de (GBTi (Greatest Breadth across the Tubera ischiadica)) ikinci en yüksek değer olarak belirlenmiştir.

Tubera coxarum boyunca en büyük genişlik (GBTc), Acetabula boyunca en büyük genişlik (GBA) ve ischia gövdeleri boyunca en küçük genişlik (SBI) ölçümleri de birbirlerine çok yakın değerler olduğu görüldü (Tablo 2).

Acetabulum uzunluğu (LA), Acetabulumun kenar üzerindeki uzunluğu (LAR) ve en küçük yükseklik (SH) değerlerinin de birbirlerine yakın olduğu tespit edildi.

TARTIŞMA VE SONUÇ

Hayvan pelvislerinden elde edilen osteometrik veriler, taksonomik sınıflandırma, tür içi morfolojik varyasyonların incelenmesi ve cinsel dimorfizm özelliklerinin belirlenmesi için yaygın olarak kullanılmaktadır. Ayrıca, elde edilen bu osteometrik veriler, evrimsel çalışmalar, gelişimsel araştırmalar ve adli

bilimler gibi önemli bilimsel alanlara önemli bilgiler sunmaktadır (Pitakamnop vd.,2017; Yılmaz & Demircioğlu, 2021). Etçillerde pelvisin morfometrik ve morfolojik özelliklerinin incelenmesi, bu türlerin filogenik ve allometrik özelliklerinin belirlenmesi, lokomotor davranışlarının ortaya konması, ekolojik çeşitliliklerinin belirlenmesi ve pelvis bölgesi ile ilgili çeşitli doğumsal veya edinsel patolojik değişikliklerin değerlendirilmesi açısından oldukça önemlidir (Jurgelenas, 2015; Fealey vd., 2017; Nganvongpanit vd., 2017).

Atalar ve Özdemir (2002) sansarda tuber ischiadicum'un tek çıkıntı; Özdemir ve Karan (2001) ise porsukta iki çıkıntıya sahip olduğunu bildirmişlerdir. Vaşaklarda (Karan vd.,2016) ise çalışmamızdaki gibi tuber ischiadicum bir çıkıntıya sahiptir.

Spina ischiadica küttür; caudal ucu ischiadica minor'a doğru kalınlaşmaktadır. Eminentia iliopubica, symphysis pelvina'nın ön ucu ile corpus ossis ilii arasındaki mesafenin merkezinde yer almaktaydı. Fossa acetabuli derin, incisura acetabuli ise geniş bir çentik şeklindeydi. Bu veriler literatürlerle (Miller vd., 1964; Özdemir & Karan, 2001;Atalar & Özdemir, 2002; Parés-Casanova, 2014) uyumluluk göstermekteydi.

Kızıl tilkinin apendiküler iskeletinin morfolojisi kutup tilkisi (Miller vd., 1964) ve evcil köpeğinkine benzerdir (Hildebrand, 1952). Kızıl tilkinin uzuv kemikleri belirli bir hareket tarzı için kendine özgü biçim özellikleri göstermez, çünkü olağandışı morfolojik özellikler gözlemlenmemiştir.

Nganvongpanit ve arkadaşları (2017) tarafından Retriever cinsi köpeklerin pelvik osteometrik ölçümleri üzerine yapılan bir çalışmada GL, GBTc, GBTi, LS, LFo ölçüm değerleri erkeklerde sırasıyla 161,50 mm, 88,44 mm, 105,22 mm, 47,15 mm, 29,13 mm iken dişilerde 145,93 mm, 80,05 mm, 98,93 mm, 42,33 mm, 27,11 mm olarak bulunmuştur. Pitakarnnop ve arkadaşları (2017) tarafından evcil kedilerin pelvik morfometrik ölçümleri üzerine yapılan bir başka çalışmada, bu ölçüm değerlerinin erkeklerde sırasıyla 79,46 mm, 42,97 mm, 37,23 mm, 16,70 mm, 18,01 mm; dişilerde ise 74,61 mm, 39,73 mm, 39,58 mm, 18,21 mm, 18,41 mm olduğu bildirilmiştir.

Van kedilerinde (Yılmaz & Demircioğlu, 2021) bu değerler sırasıyla 80,40 mm, 43,54 mm, 46,06 mm, 31,73 mm, 20,01 mm; erkeklerde 76,64 mm, 41,36 mm, 43,47 mm, 29,38 mm, 19,76 mm olarak tespit edilmiştir. Genel olarak bu değerler arasında küçük farklılıklar olmasının tür, yaş, boy, ırk ve vücut ağırlığı özelliklerinden kaynaklandığı düşünülmektedir. Erkek kızıl tilkilerin pelvik osteometrik ölçümlerinin Retriever cinsi köpeklerinden düşük, Van kedisi ve evcil kedilerden ise yüksek değerde olduğu görüldü.

Rakun köpeklerinde yapılan bir çalışmada (Jurgelenas, 2015) Rakun köpeklerinde GL dişilerde 92,31mm iken erkeklerde 94.91mm, LAR dişilerde 15.29 mm iken erkeklerde 15.55 mm, SB dişilerde 6,01 mm iken erkeklerde 5,95 mm, LFo dişilerde 16,79mm iken erkeklerde 17,69mm, GBTc dişilerde 56,89mm iken erkeklerde 57,28mm, GBA dişilerde 53,10mm iken erkeklerde 53,33 mm, GBTi dişilerde 58,47 mm iken erkeklerde 59,29mm, SBI dişilerde 44,60 mm iken erkeklerde 45,22 mm 'dir. Erkek kızıl tilkilerde ise LFo, GBTi ve SBI haricindeki tüm değerlerin Rakun köpeklerinden daha yüksek olduğu görüldü.

Bu veriler, erkek rakun köpeklerinde ilium şaftı arasındaki genişliğin kızıl tilkilerden daha fazla olduğunu göstermektedir. Schutz ve diğer yazarlar (2009), tazı gibi hızlı koşan köpeklerin, daha küçük, daha kısa ve daha geniş pelvik kemiklere sahip olan Amerikan Pit Bull Terrier gibi daha kısa bacaklı köpeklerden daha büyük, daha uzun ve daha dar pelvik kemiklere sahip olduğunu bildirmiştir (Schutz vd., 2009;Macdonald, 2004). Rakun köpekleri daha kısa bacaklara sahip yavaş hayvanlar iken, kızıl tilkiler saatte 48 km hızla koşabilen hızlı hayvanlardır (Schutz vd., 2009;Macdonald, 2004). Ayrıca, kızıl tilkinin uzun kemiklerinin rakun köpeğinden daha uzun olduğu tespit edilmiştir ki bu da kısa bacaklı köpeklerde pelvik kemiklerin daha geniş olduğu yönündeki yukarıdaki öneriyi doğrulamaktadır.

Yine aynı çalışmada (Jurgelanas, 2015) kızıl tilki ölçümleri ise GL dişilerde 92,24 mm iken erkeklerde 99,57 mm, LAR dişilerde 14,05 mm iken erkeklerde 15.06 mm, SB dişilerde 5,77 mm iken erkeklerde 5,96 mm, LFo dişilerde 20,17 mm iken erkeklerde 22.00 mm, GBTc dişilerde 49,65 mm iken erkeklerde 52,45 mm, GBA dişilerde 49,35 mm iken erkelerde 52,43 mm, GBTi dişilerde 66,79 mm iken erkeklerde 70,58 mm, SBI dişilerde 45,96 mm iken erkeklerde 48,12 mm 'dir. Yaptığımız çalışmada ise erkek kızıl tilkideki bazı değerlerin Jurgelenaz'ın ölçümlerindeki dişilerle daha uyumlu olduğu görüldü.

Özkadif ve Halıgür (2022) yaptığı çalışmada kızıl tilkilerde GL değeri dişlerde 80,30 mm iken erkeklerde 120,09 mm, LFo değeri dişilerde 18,29 mm iken erkeklerde 26,65 mm, LS değeri dişilerde 28,09 iken erkeklerin 59,12 mm olarak bulunmuştur.

Bu çalışmaya istinaden ölçümlerimizin dişilerle erkeklerin ölçümlerinin arasında bir değer olduğu belirlendi.

Cinsiyetlere ve türlere ilişkin verilerin karşılaştırılmasında oldukça önemli foramen obturatorum uzunluğu farklılıkları tespit edilmiştir. Sajjarengpong ve arkadaşları (2003) evcil köpeklerin foramen obturatorumlarının erkeklerde dişilere göre çok az daha büyük olduğunu ve aradaki farkın istatistiksel olarak önemsiz olduğunu belirlemişlerdir. Evans (2012) evcil köpeklerde ovalden üçgene kadar çeşitli foramen obturator

formları belirlemiştir. Çalışmamızda foramen obtoratum evcil köpeklerdeki gibi oval şeklindeydi. Foramenin obturator uzunluk ölçümlerinin cinsiyetler ve türler arasında ayrım yapmak için yetersiz olduğu belirtilmiştir.

Jurgelėnas (2015); kızıl tilki, rakun köpeği ve evcil köpek aynı familyaya (Canidae) ait olduğunu, ancak cinsiyetler arasındaki farklar türlere göre değişkenlik gösterdiğini bildirmiştir. Ayrıca Kızıl tilkilerin pelvik kemik ölçümlerindeki farklılıklar çok belirgin, ancak rakun köpekleri ve evcil köpeklerde bu farklılıklar çok az olduğunu belirtmiştir. Pelvik kemikle ilgili cinsel dimorfizm birçok memeli türünde bildirilmiş olmasına rağmen, bazı sonuçlar cinsiyetler arasında ilişkilendirilmemiştir.

Parés-Casanova (2014) atlarda pelvik kemiklerin cinsiyetler arasında önemli ölçüde farklı olmadığını bildirmiştir. Bu sebeple yapılan çalışmalara bakıldığında yapılan ölçümlerin dişi ve erkek ayrımı ile ilişkilendirilmesinde kullanılamayacağı yönündedir. Ancak buna istinaden tür ayrımı noktasında önemlilik arz edeceği yine çalışmalarla ispatlanmıştır.

Sonuç olarak, bu çalışmada kızıl tilkilerde ossa coxae'sının osteometrik ölçümlerini belirlemek ve uzunluk/genişlik oranlarını hesaplamak için X-ray görüntüleri kullanılmıştır. Bu çalışmanın bulguları, kızıl tilkilerin taksonomik sınıflandırılması, türler arasındaki farklılıkların ayırt edilmesi, zooarkeoloji alanındaki çalışmalar ve klinik uygulamalarda veteriner hekimlere yardımcı olmak gibi çeşitli uygulamalar için değerli olabilecek temel morfometrik veriler sağlamaktadır. Bu bulgular, mevcut bilgi tabanına katkıda bulunmakta ve farklı araştırma ve mesleki uygulama alanları için pratik çıkarımlar sunmaktadır.

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ARAŞTIRMA MAKALESİ **RESEARCH PAPER**

Developing An Effective Training Protocol For Biodetector Rats: A Preliminary Study

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Abstract: Rats have increasingly been employed as biodetector animals due to their keen olfactory capabilities, similar to dogs, for tasks including disease diagnosis, search-and-rescue operations, and detecting hazardous substances such as explosives and prohibited materials. This preliminary study aimed to develop a reliable and efficient training protocol for biodetector rats by integrating classical and operant conditioning methods. Ten female Sprague Dawley rats were trained using classical and operant conditioning paradigms within a specially modified Skinner box. The animal was expected to ring the bell in the odor chamber, then was rewarded. Following the socialization phase, a training procedure was prepared including a clicker as a conditioned stimulus at each stage and reward food which functioned as an unconditioned stimulus and later served as a positive reinforcer. Training sessions were conducted for 15 minutes daily, five days per week, with rats receiving a 60% reduction in their food intake 24 hours prior to training to increase motivation. The frequency of target behaviors and non-target behaviors was recorded, showing a significant increase in target behavior realization by 628 % and an increase in non-target behaviors by 98 %. These findings demonstrate that integrating classical and operant conditioning is an effective and practical approach for training biodetector rats. Future studies are planned to incorporate advanced technology such as machine learning and artificial intelligence to further refine training methodologies and enhance outcomes.

Keywords: Biodetector, rats, biodetector training, skinner box.

Biyodedektör Sıçanlar İçin Etkili Bir Eğitim Protokolünün Geliştirilmesi: Bir Ön Çalışma

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Öz: Sıçanlar, köpeklerde olduğu gibi üstün koku alma yetenekleri nedeniyle hastalık teşhisi, arama-kurtarma operasyonları ve patlayıcı veya yasak maddelerin tespiti gibi çeşitli görevlerde biyodedektör hayvan olarak giderek daha fazla kullanılmaktadır. Bu ön çalışmada, klasik ve operant koşullanma yöntemlerini entegre ederek biyodedektör sıçanlar için güvenilir ve verimli bir eğitim protokolü geliştirmek amaçlanmıştır. On adet dişi Sprague Dawley sıçanı, özel olarak modifiye edilmiş bir Skinner kutusu içinde klasik ve operant koşullanma paradigmaları kullanılarak eğitilmiştir. Hayvanın koku odasındaki zili çalması beklenmiş ve ardından ödüllendirilmiştir. Her eğitim aşamasında koşullu uyarıcı olarak tıklayıcı kullanılırken, yiyecek ödülleri başlangıçta koşulsuz uyarıcı işlevi görmüş ve ardından istenilen davranışın pozitif pekiştiricisi olarak görev yapmıştır. Eğitim seansları haftada beş gün, her sıçan için günde 15 dakika olarak uygulanmış ve sıçanların motivasyonunu artırmak amacıyla seanslardan 24 saat önce gıda alımları %60 oranında azaltılmıştır. Eğitim prosedürü, zili her iki pençeyle çalma davranışını hedefledi. Hedef davranışların ve hedef dışı davranışların gerçekleşme sıklıkları kaydedilmiş; hedef davranışların gerçekleşmesinde %628, hedef dışı davranışlarda ise %98 oranında artış görülmüştür. Elde edilen bulgular, klasik ve operant koşullanma yöntemlerinin biyodedektör sıçanların eğitimi için etkili ve pratik bir yaklaşım olduğunu göstermektedir. Gelecekte yapılacak çalışmalarda, eğitim yöntemlerini daha da geliştirmek ve sonuçları iyileştirmek amacıyla makine öğrenmesi ve yapay zekâ gibi ileri teknolojilerin kullanılması planlanmaktadır.

Anahtar kelimeler: Biyodedektör, sıçanlar, biyodedektör eğitimi, skinner kutusu

INTRODUCTION

Throughout rat training processes, animals acquire novel skills and behaviors required to perform detection tasks in response to specific targets, as documented in previous studies. To effectively address research questions, understanding learning and memory mechanisms during the training process is critical. Learning and memory are the major issues in neuroscience (Mayes & Roberts, 2001). Learning is defined as the acquisition of knowledge and skills, and it is also considered a change in behavior resulting from experiences. Memory can be classified according to motivational context as reward-based or punishment-based memory. Furthermore, memory is divided into relational and non-relational categories based on its structural characteristics. Relational memory involves the experiencer organizing and controlling relationships and responses between stimuli, while non-relational memory pertains to creating conditions conducive to specific responses. Classical and operant conditioning paradigms, commonly observed in behavioral research, fall within the non-relational memory category (Quillfeldt, 2006).

Commonly employed training methodologies include the open field habituation test, passive avoidance test, contextual fear conditioning, two-way active avoidance test, maze tests, and novel object recognition tests (More et al., 2016; Quillfeldt, 2006; Tanila, 2018). Pavlov's classical conditioning studies primarily focused on involuntary responses elicited by known stimuli. In contrast, Skinner's operant conditioning research concentrated on voluntary behaviors and their associations with consequences, where stimuli may be less explicit (Skinner, 1938). Operant conditioning involves establishing associations between behaviors and subsequent outcomes. The Skinner box, developed by Skinner, is a pivotal experimental apparatus for observing operant conditioning processes (Skinner, 1938). Operant conditioning comprises repeated trials where behavior frequency is either increased or decreased based on the outcomes. Positive reinforcement, involving pleasant stimuli, increases behavior probability, while negative or aversive stimuli can temporarily suppress behaviors. While pleasant stimuli or effects are positive reinforcements for the behavior, unpleasant stimuli have a meaning as punishment and can stop the behavior for a certain period of time. According to studies focused on memory and operant conditioning, training experimental animals significantly enhances research validity (Dalkiran et al., 2022).

Skinner advocated for the incorporation of auditory stimuli in behavioral training, citing their perceptibility across various distances and contexts, a distinct advantage over visual stimuli (Skinner, 1961). Presently, training methods that employ clickers or auditory cues are acknowledged as efficacious techniques in animal training within the realm of behavioral psychology. These methods are particularly noted for their effectiveness in marking and reinforcing desired behaviors (Feng et al., 2016). A standard clicker is usually a small plastic apparatus containing a metal piece that emits a brief, sharp, dual-tone click when pressed. This auditory signal typically precedes the immediate provision of food reinforcement. Research examining the correlation between clicker cues and food intake remains limited; while some studies find no substantial differences when compared to exclusive food reinforcement, others have noted improved response rates (Pfaller-Sadovsk et al., 2020; Martin & Friedman, 2020).

In recent years, there has been a growing interest in biomaterials and engineering technologies aimed at enhancing system quality while simultaneously reducing operational costs. Among these, biosensors represent a particularly promising avenue for the analysis of both biological and non-biological systems. Biosensors are analytical devices that translate biological signals into quantifiable outputs, such as volatile organic compounds (VOCs). These VOCs, also referred to as odorant molecules, are distinct chemical entities produced by biological systems. Various types of chemical and biological analyzers have been developed to detect and interpret these molecules. Several studies have highlighted the potential applications of biosensors in applied sciences and medical diagnostics (Oh et al., 2015). Nevertheless, analytical instruments currently in use are reported to have significant limitations, including high costs, slow response times, operational complexity, substantial power requirements, and the need for extensive optimization (Oh et al., 2015; Tomsic & Musevic 2013; D'Amico et al., 2010). Given these constraints, there is a need for alternative and innovative sensory tools—particularly those involving biodetector animals. Animals such as dogs, rats, insects, and honeybees, which possess exceptionally sensitive olfactory systems, have been employed for biodetection purposes in recent years. Notably, the olfactory sensitivity of dogs and rats has been shown to exceed that of humans by up to several hundred thousand times (Berg et al., 2024).

Rats have long been utilized as model organisms in scientific research; however, their olfactory capabilities have remained relatively underexplored. Recent studies have begun to investigate their potential as biodetector animals in a wide array of applications, including the diagnosis of diseases and pathological conditions, search and rescue operations, and the detection of flammable, explosive, or prohibited substances—similar to the roles

traditionally assigned to dogs (Poling et al., 2011; Schoenberg et al., 2019; Leidinger et al., 2017). To effectively employ an animal as a biodetector, the implementation of an appropriate behavioral training protocol is essential. In this context, behavioral modeling conducted in conjunction with structured training programs plays a critical role. Biodetector rats, in particular, must be capable of executing a series of task-specific behaviors acquired through such training. The present study aimed to develop a refined training model using a modified Skinner box, integrating classical and operant conditioning paradigms to assess and address gaps in behavioral training. Furthermore, the study sought to construct optimized behavioral models for biodetector training by systematically identifying and correcting deficiencies encountered during the training process. Ultimately, this research also serves as a preliminary investigation into the feasibility of utilizing rats as biodetector animals.

MATERIAL AND METHOD

Animals and housing: In the study, 10 female Sprague Dawley rats, 1-2 months old, 150-250 g live weight, were used as experimental animals. The rats were housed in Bursa Uludag University Experimental Animal Application and Research Center. All necessary approvals were obtained from the University Experimental Animals Local Ethics Committee (Ethics Committee Decision No: 2024-06/03).

The animals were kept for 1 week to get used to the environment before the experiment. Each rat was housed individually in boxes measuring 20×30×55 cm. The cages were outfitted with enrichment materials, including tunnels, ladders, running wheels, and platforms constructed from wood and metal, to promote physical and mental stimulation for the animals. Additionally, compact cotton material was provided to facilitate nesting behaviors (Figure 1). The rats were kept under standard lighting (12 hours light / 12 hours dark) and room temperature (22-23 ^{0}C) conditions during the study. Sufficient standard pellet food and water were available *ad libitum*.

Skinner training box: A Skinner box was modified and designed for the experiment. The box was rectangular prisms measuring 30×30×40 cm, crafted from transparent materials to allow for unobstructed observation of the interior by the operator. There was a bell, a bait and an odor chamber inside the box. The chain with the bell attached hangs from the top of the odor chamber. The bell was used as a discriminative stimulus that the subjects interacted with during the operant conditioning phase. The bait and the odor chamber were in a location that is accessible from the outside (Figure 2).

Figure 1. Rats house with housing materials.

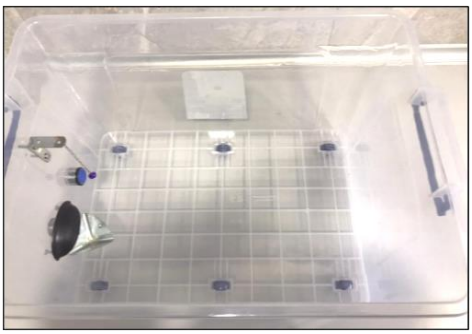

Figure 2. Modified skinner box

Reward food: Solid biscuit pieces were given as a reward food. This reward food served as a reinforcer for the rats when they successfully performed the target behavior in the study.

Clicker: A standard commercial model clicker was used. It was held in a plastic or metal housing, had a piece that was depressed at one end, returned to alignment when released, and maked a sharp "click" sounded each time it was depressed.

Test procedure: The overall procedure for training rats consisted of two phase: (i) a socialization phase, followed by (ii) a continuous training phase, in which reward was delivered via classical and operant conditioning paradigms.

Socialization phase: The initial phase encompassed the adaptation and socialization process. This phase was designed to help the rats acclimate to the experimental environment, enhance their confidence in interacting with humans, and promote their effective participation in the experimental procedures. To achieve this, a 7-day socialization protocol was implemented for the rats. Throughout this process, no food restrictions were imposed on the animals. Following the adaptation and socialization phase, the rats' food intake was reduced by 60%, while water intake remained unrestricted for 24 hours before the training sessions.

Training phase: The training schedule involved one session per animal per day, conducted five days a week, with each session limited to 15 minutes. The training protocol was meticulously developed by integrating and adapting the core principles of classical and operant conditioning paradigms.

Target and non-target behaviors were recorded across all training stages. Additionally, the percentage changes in the frequency of these behaviors were calculated and analyzed. The observed behaviors during the training sessions included: circling the cage, waiting in the reward chamber, inspecting a corner of the cage unconsciously touching the bell, unintentionally touching the bell, and ringing the bell with both forepaws that modified by literatures (Rautio et al., 2024; Figure 3-7). In the study, "ringing the bell with both forepaws" behavior was targeted for the training procedure. Non-target behaviors were defined as any actions that deviated from the specified target behavior. Furthermore, to monitor the welfare of the rats throughout the training process, indicators of anxiety-specifically spontaneous urination, defecation, and vocalization-were systematically recorded.

a. First stage: In the initial phase of the training procedure, the odor chamber was physically separated from the section designated for reward delivery using a partition. During this stage, the rats were subjected to classical conditioning, wherein a commercial clicker was employed as a conditioned stimulus. The food reward was administered immediately following the click sound to establish the association between the auditory cue and reinforcement.

b. Second stage: In the second and subsequent stages, the partition separating the odor chamber from the reward area was removed. When the animal made contact with the bell—using a paw, its nose, or another part of the body—the clicker was activated, and the food reward was immediately delivered. Through repeated trials, the rats began to form an association between bell activation, the clicker sound, and the subsequent reward. At this stage, however, there was no odor awareness.

c. Third stage: During this stage, the rats were reinforced for touching the bell with either one or both forepaws. The aim was for rat to learn to lift its body. The partition remained removed during this stage to maintain environmental continuity and facilitate learning.

d. Fourth stage: In this final stage, the rats were required to assume an upright posture with their noses oriented toward the odor chamber and ring the bell using both forepaws. Upon successful execution of this specific behavior, the clicker was sounded and the food reward was delivered. This stage represented the complete acquisition of the target behavior in the context of the training apparatus.

Figure 3. Circling the cage behavior.

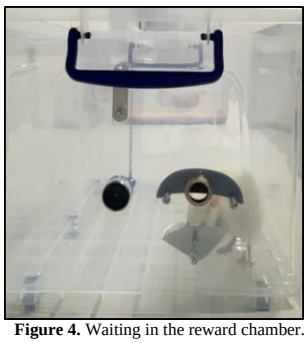

Figure 4. Waiting in the reward chamber.

Figure 5. Inspecting a corner of the cage unconsciously touching the bell.

Figure 6. Unintentionally touching the bell.

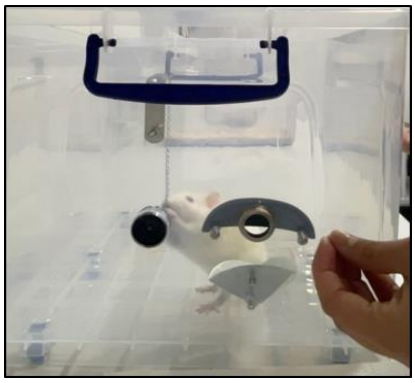

Figure 7. Ringing the bell with both forepaws.

Statistical analysis: Statistical analyses were conducted using GraphPad Prism version 5. Analysis of variance (ANOVA) will be used to determine the effects of operant conditioning protocols on the number of shaping trials, and the frequency of target and non-target behaviors. Response rates will be calculated with the application of the conditioned stimulus. Parameters were grouped and means and standard errors were calculated.

Prior to hypothesis testing, the Kolmogorov–Smirnov and Shapiro–Wilk tests were applied to evaluate the normality of data distribution. For data exhibiting normal distribution, Levene's test was used to assess homogeneity of variances. Depending on the outcomes of these preliminary tests, either parametric methods—including ANOVA and the paired samples t-test—or non-parametric methods—such as the Kruskal–Wallis test and Mann–Whitney U test—were used for group comparisons. A p-value of less than 0.05 was considered statistically significant.

RESULTS

Target behaviors were successfully performed, and non-target behaviors were recorded throughout all stages of the training phases (Figure 8). Percentage changes in behavior frequencies were calculated by comparing data from the beginning and end of the study (Figure 9). No statistically significant difference was observed between the overall frequencies of target and non-target behaviors across all rats (p:0.209; 41.29±6.71 ve 24.57±9.61). However, the target behavior realization rate increased markedly by 628%, while the frequency of non-target behaviors showed a more moderate increase of 98%.

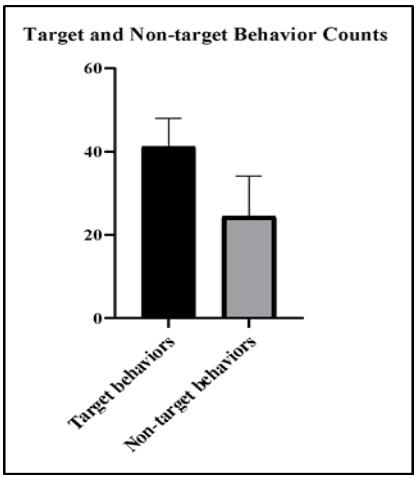

Figure 8. The counts of target and non-target behaviors during traning (p>0.005; Mean ± S.E; n=10).

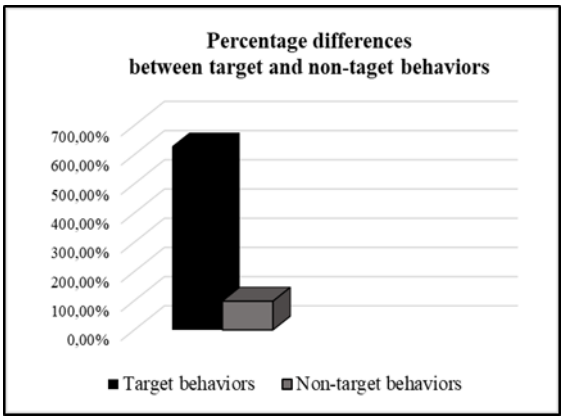

Figure 9. The percentage differences between target and non-target behaviors during traning (p>0.005; Mean ± S.E; n=10).

Following the socialization phase, initial training (Stage 1) began, during which the association between the clicker sound and food reward was established. Some rats successfully learned this association by Day 3, while others required up to Day 6. Although each stage was structured to occur over one training day, rest intervals were incorporated between stages. As a result, Stage 1 training was conducted between Days 6 and 12.

Stage 2 commenced on Day 12, at which point rats were introduced to the bell. On Days 12 and 13, food rewards were given when the rats rang the bell unintentionally. By Day 14, however, the animals had begun to associate bell-ringing—whether performed with a paw, the nose, or another part of the body—with the clicker sound and the subsequent reward.

On Day 15 (Stage 3), the rats began receiving rewards only when they touched the bell with either one or both forepaws. By Day 18 (Stage 4), all animals were able to perform the full target behavior—ringing the bell with both forepaws in response to the odor stimulus—followed by the clicker sound and reward delivery.

Throughout the training process, no signs of distress, such as urination, defecation, or vocalization, were observed in any of the rats, with the exception of one instance of urination on Day 1 by a single animal.

DISCUSSION AND CONCLUSION

In this study, we demonstrated a fundamental training approach in which rats successfully learned to perform a sequence of novel behaviors involving bell ringing, auditory cues (clicker), and food reinforcement. The animals observed high performance and learning success during the study. Previous studies have reported that the effects of clicker training with several species, including dogs and horses. Early research on clicker-based methodologies was firmly grounded in behavioral analysis, particularly in the context of reinforcement learning. However, it has been noted that key terms commonly used in clicker training—such as cueing, bridging, and conditioned reinforcement—are often interpreted inconsistently across different studies, which complicates the analysis of behavioral outcomes. The earliest device resembling a modern clicker—a buzzer—was developed by McCall and Burgin (2002), who found that bell training paired with food rewards was more effective in horses than providing food alone. Similarly, a study by Thorn et al. (2006) investigated whether a clicker paired with food was more effective than verbal praise in dog training. Their findings suggested that verbal praise elicited higher levels of sustained response than the clicker. In contrast, Chiandetti et al. (2016) found no significant difference between clicker-plus-food, verbal praise-plus-food, and food-only conditions, highlighting ongoing inconsistencies in the literature. Given the limited but growing body of research exploring the association between clicker cues and food reinforcement (Pfaller-Sadovsky et al., 2020; Martin & Friedman, 2020), one of the most notable findings in the present study is that training success was achieved across all individual rats, reinforcing the utility and reproducibility of this conditioning model in a rodent population.

In the present study, the initial and most critical step involved training the rats to associate the clicker sound with the subsequent food reward—a key phase

representing the completion of classical conditioning. The success of this association was found to be influenced by the individual rats' engagement and responsiveness to training. Despite the incorporation of rest periods between training stages, most rats were able to establish the clicker–reward connection between the 3rd and 6th training days.

In subsequent stages, particularly Stage 2—when the partition was removed—rats began to unintentionally contact the bell using various parts of their bodies. Upon doing so, they received both the clicker sound and the food reward. This phase represented the second major milestone of the study, as it marked the beginning of the operant conditioning process. Repeated reinforcement led the rats to gradually associate the act of bell-ringing with the reward mechanism.

By Day 12, although inconsistently, rats began to actively ring the bell. By Day 14, most animals had learned to associate the clicker sound and food reward with physical contact with the bell, whether through one or both forepaws, their nose, or other body parts. From Day 15 onward, food rewards were only administered when the bell was touched using one or both forepaws. Notably, by Day 18, the animals consistently performed the complete target behavior—ringing the bell using both forepaws—successfully linking it to the clicker cue and the ensuing reward.

The behavior of "ringing the bell with both forepaws" represented the most critical component of the training process, as it served as the primary action through which a clear association between the behavior and the reinforcement could be established. It was observed that rats acquired this target behavior relatively quickly, regardless of the trainer's level of experience. Notably, efficient training outcomes were achieved even under the guidance of less experienced trainers.

Previous studies have reported common errors in clicker training, such as reinforcing inappropriate behaviors or failing to present the food reward promptly following the clicker cue (Leidinger et al., 2017). In the present study, similar mistakes were identified during the early training stages—particularly the initial failure of some rats to respond to the clicker before the association was learned. However, these issues were progressively resolved as the training advanced.

The individual temperament of rats constitutes an uncontrollable variable in the training process. Although all animals in the present study were born and raised under identical environmental conditions, they nonetheless exhibited a wide range of behavioral characteristics. Previous research has highlighted such variability, noting differences in taste preferences, appetite, and exploratory behavior among individual rodents (Crawley, 2007; Loos et al., 2015; Leidinger et al., 2017).

Based on the current findings, the type of food reward emerged as another critical factor influencing training outcomes. Although solid food rewards were employed in this study, training effectiveness appeared to be affected by individual differences in taste preference and feeding behavior. Moreover, the use of solid rewards was found to reduce motivation in some animals, likely due to the longer time required for consumption. Therefore, it is proposed that utilizing liquid food rewards may enhance the acquisition of target behaviors and improve overall training efficiency.

A key limitation of this study is that it was conducted using only one rat strain. Considering that behavioral traits such as learning ability, stress response, and reward sensitivity can differ significantly between strains, future studies should include additional strains to enhance the generalizability of the findings (Loos et al., 2015; Leidinger et al., 2017). Moreover, further research is warranted to evaluate the broader behavioral repertoire and physiological stress responses associated with the training process, in order to better understand both performance and animal welfare outcomes.

In conclusion, this study demonstrated that the application of a clicker-based operant conditioning protocol in female Sprague Dawley rats is both practical and effective. Despite certain limitations, including a modest sample size and individual learning variability, the findings offer a clear proof of principle for training paradigms based on observational and operant learning in rodents. The high success rate observed across the majority of subjects highlights the reliability of the model. Looking ahead, future studies will aim to refine the behavioral framework further and explore the integration of automated systems, including machine learning and artificial intelligence, to enhance precision, efficiency, and scalability in biodetector training protocols.

Conflict of interest statement: None of the authors have any conflicts of interest to disclose.

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ARAŞTIRMA MAKALESİ **RESEARCH PAPER**

Comprehensive Analysis of Publications on the Use of Artificial Intelligence in Aquaculture Published in Web of Science

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Abstract: Aquaculture is gaining importance due to the increasing population and food demand. However, one of the biggest challenges in the sector is the need for innovative technologies. Artificial intelligence (AI) offers important solutions in environmental process management, early disease detection, water quality monitoring and optimizing feeding strategies. This study examines the evolution of AI in aquaculture by analyzing 202 publications in the Web of Science database between 1998 and 2024. Academic productivity has increased rapidly in recent years, reaching 64 articles in 2024. The most common document types are "*Articles*" (124) and "*Reviews*" (41), with research focused on environmental disciplines such as *Fisheries* (41) and *Marine Freshwater Biology* (29), as well as technical fields such as *Engineering Electrical Electronics* (26) and *Computer Science* (25). The leading journals are *Aquaculture* and *Computers and Electronics in Agriculture*. China (52) and the US (28) are the top contributors, with Li Daoliang (7 publications) being the most prolific author. Keyword analysis reveals central themes such as "*Aquaculture*" (66), "*Artificial Intelligence*" (61), and "*Machine Learning*" (36), while concepts such as "*Smart Fish Farming*" and "*Sustainability*" indicate a shift toward technology-driven green solutions. Citation networks reveal strong connections but some fragmentation. The findings suggest that AI is increasing its role in the industry, encouraging sustainability and collaboration.

Key words: Aquatic animal, bibliometric analysis, citations, smart fish farming, VoSviewer.

Web of Science'da Yayımlanan Su Ürünleri Yetiştiriciliğinde Yapay Zekanın Kullanımı Konusundaki Yayınların Kapsamlı Analizi

***Sorumlu yazar:**
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Öz: Su ürünleri yetiştiriciliği, artan nüfus ve gıda talebi nedeniyle önem kazanmaktadır. Ancak sektördeki en büyük zorluklardan biri, yenilikçi teknolojilere duyulan ihtiyaçtır. Yapay zeka (YZ), çevresel süreçlerin yönetimi, hastalıkların erken tespiti, su kalitesinin izlenmesi ve beslenme stratejilerinin optimize edilmesi gibi konularda önemli çözümler sunmaktadır. Bu çalışma, 1998-2024 yılları arasında Web of Science veri tabanındaki 202 yayını analiz ederek yapay zekanın su ürünleri yetiştiriciliğindeki evrimini incelemektedir. Son yıllarda akademik üretkenlik hızla artmış, 2024'te 64 makaleye ulaşmıştır. En yaygın belge türleri "*Makaleler*" (124) ve "*İncelemeler*" (41) olup, araştırmalar *Balıkçılık* (41) ve *Deniz Tatlı Su Biyolojisi* (29) gibi çevre disiplinlerinin yanı sıra *Mühendislik Elektrik Elektroniği* (26) ve *Bilgisayar Bilimi* (25) gibi teknik alanlarda yoğunlaşmıştır. *Aquaculture* ve *Computers and Electronics in Agriculture* en önde gelen dergilerdir. Çin (52) ve ABD (28) en fazla katkı sağlayan ülkeler olup, Li Daoliang (7 yayın) en üretken yazarlardandır. Anahtar kelime analizi, "*Su Ürünleri Yetiştiriciliği*" (66), "*Yapay Zeka*" (61) ve "*Makine Öğrenimi*" (36) gibi merkezi temaları ortaya koyarken, "*Akıllı Balık Çiftliği*" ve "*Sürdürülebilirlik*" gibi kavramlar teknoloji odaklı çevreci çözümlere yönelimi göstermektedir. Atıf ağları, güçlü bağlantılar olsa da bazı parçalanmaların sürdüğünü ortaya koymaktadır. Bulgular, yapay zekanın sektördeki rolünü artırarak sürdürülebilirlik ve iş birliğini teşvik ettiğini göstermektedir.

Anahtar kelimeler: Akıllı balık çiftçiliği, atıflar, bibliyometrik analiz, su hayvanları, VoSviewer.

INTRODUCTION

Aquaculture production has gained significant momentum on a global scale due to factors such as the rapidly increasing world population, the need for sustainable food sources, the spread of healthy eating habits, technological innovations and the decrease in natural aquaculture stocks. This increase is of vital importance for humanity due to the multidimensional benefits of aquaculture, such as contributing to food security with its high nutritional value, supporting economic activities, encouraging environmental sustainability and having a cultural value for many societies.

Aquaculture, the cultivation of aquatic organisms, is vital for global food security and an important source of protein (Boyd et al., 2022). As the global population increases, there is an urgent need for sustainable expansion in aquaculture. In 2022, global aquatic animal production reached 185 million tons, of which 94 million tons were derived from aquaculture activities. It is estimated that this increase in aquaculture production will continue in the coming years and will increase by 10% by 2032 and reach 205 million tons (FAO 2024).

In recent years, the increasing world population and the need for sustainable food sources have brought about significant growth in aquaculture production. This growth has necessitated not only the efficient use of natural resources but also the integration of innovative technologies that will optimize production processes. Artificial intelligence (AI), in particular, draws attention with its versatile applications in the aquaculture sector, such as increasing production efficiency, reducing environmental impacts, improving stock management and improving quality control processes.

The term "Artificial Intelligence" was first introduced at the 1955 Dartmouth Conference, where John McCarthy proposed a study based on the hypothesis that " every aspect of learning or any other feature of intelligence can in principle be so precisely described that a machine can be made to simulate it" (Zha, 2020). Artificial intelligence, one of the main areas of computer science today, has penetrated into such diverse areas as education, healthcare, finance and manufacturing due to its ability to very quickly address problems that humans cannot solve. Today, AI technologies are widely used in areas such as increasing efficiency, improving decision support systems, and encouraging innovation, leading to a continuous expansion of AI's sectoral applications.

The first study on the use of AI and aquaculture, "*A fuzzy logic application to aquaculture environment control*" was written by Lea et al. (1998). Lee, (2000) in his article titled "*Process Control and Artificial Intelligence Software for Aquaculture*," reported that aquaculture farmers recognized that by controlling environmental conditions and system inputs (such as water, oxygen, temperature, feed rate, and stocking density), they could regulate the physiological rates of reared species and the final process outputs (such as ammonia, pH, and growth). Chen et al. (2003) reported how they developed a neural network model-based approach to more accurately predict nitrogen content in treated wastewater used in wastewater recycling and how the model was useful in evaluating the groundwater recharge process by increasing cost-effectiveness. In the following years, Carbajal and Sánchez (2008), Hernández et al. (2010) and Hernández et al. (2011) published articles on the use of artificial intelligence in shrimp farming.

Wangle et al. (2020) conducted a comprehensive review on the integration of machine learning and remote sensing for water quality monitoring, emphasizing the effectiveness of RS-based geospatial data and machine learning ML algorithms, particularly support vector machine regression and artificial neural networks, in accurately estimating water quality parameters and improving real-time decision-making in aquaculture.

Karimanzira and Rauschenbach (2021), investigated innovative solutions such as digitalization, advanced technologies and double water recirculation system (DRAPS) to overcome the stability, standardization and economic profitability problems of large-scale aquaponic systems and evaluated the potential to increase the efficiency and sustainability of these systems.

Zhao et al. (2021) reviewed the machine learning algorithms applied in smart fish aquaculture in the past five years, detailing their applications in areas such as fish biomass assessment, fish identification and classification, behaviour analysis, and water quality parameters prediction. Wang et al. (2021a) conducted a review examining the integration of AI in aquaculture, its applications to key challenges for the industry, existing commercial AI products, technical and financial barriers to AI use, and suggesting future research directions to increase efficiency and sustainability in aquaculture systems.

Vo et al. (2021) carried out an extensive review of smart aquaculture, evaluating 100 research articles from the last ten years to explore the methodologies, findings, and progress in machine learning applications within the field.

In order to reduce feed waste and water pollution in fish farming, Chiu et al. (2022a) developed an artificial intelligence objects (AIoT)-based precision feeding management system that automatically adjusts the feed amount by detecting fluctuations on the water surface and revealed that this system reduces aquaculture costs by optimizing the fish feeding process.

Lim et al. (2022) examined the microalgae biomass production process and evaluated the contributions of IoT and artificial intelligence integration to microalgae agriculture, and discussed the transition to smart microalgae

agriculture, especially by addressing the stages of IoT hardware installation and process optimization with machine learning. Nguyen et al. (2022) compared the accuracies of multi-trait genomic prediction models (ssGBLUP), Bayesian (BayesCpi), random forest (RF) and multilayer perceptron (MLP) in a commercially important population of banana shrimp (*Fenneropenaeus merguiensis*), showing that machine and deep learning-based models provide higher prediction performance compared to the traditional ssGBLUP method and that the BayesCpi model in particular is more effective in large-scale genetic improvement programs.

Lu et al. (2022), emphasizing the importance of water quality in aquaculture, designed a low-cost and artificial intelligence-supported buoy system that measures parameters such as dissolved oxygen, salinity, water temperature and flow rate in real time and evaluated the effectiveness of this system in terms of wireless data transmission, short-term water quality predictions and cost-effectiveness.

Emphasizing the importance of water quality in fish farming, Guo et al. (2023) generated a dataset of 5203 images based on water colour and developed a deep transfer learning-based classification model, proposing an automatic water quality monitoring system that provides higher accuracy (99%) compared to traditional observational methods.

Bi et al. (2023) developed a new artificial intelligence-based method due to the labor-intensive process of manual control of buoys in mussel farms in New Zealand and provided automatic detection of buoys using image processing and a convolutional neural network (CNN) and reported that this proposed method performed better compared to other baseline methods.

Abdullah et al. (2024) reviewed various IoT applications in aquaculture, covering water quality monitoring, feeding strategies, and smart health monitoring, and highlighted the potential of IoT in aquaculture by focusing on sensor advancements, artificial intelligence (AI) integration, and increased productivity.

In this study, 202 publications in the Web of Science database between 1998 and 2024 were analysed to evaluate the evolution, current trends and impacts of artificial intelligence in aquaculture and to suggest future research directions.

MATERIAL AND METHOD

The data were collected from WoS databases viz., Science Citation Index Expanded (SCI Expanded), Conference Proceedings Citation Index (CPCI-S), Emerging Sources Citation Index (ESCI), Book Citation Index-Science (BKCI-S) and Social Sciences Citation Index (SSCI) for the period between 1989-2024. Advance search was adopted and use the following keywords for searching and retrieving data “aquaculture*”* and *“*artificial intelligence*”.*

The query path is; TS=("aquaculture" and "artificial intelligence"). The query link is: https://www.webofscience.com/wos/woscc/summary/4a2b833d-aefe-4b8c-a5f6-e0adb3e158ab-013bc4a758/times-cited-descending/1. Data on publications related to “aquaculture” and “artificial intelligence” include publication trends, publication types, contribution of countries, most productive authors, most cited publications, most publication titles, research fields, Web of Science Index and publication languages. 202 bibliographic records were retrieved and bibliographic records were analyzed and mapped using the VoSviewer version 1.6.20 and Microsoft Excel 2016.

RESULTS

Evolution in Publication Trends: This subsection focuses on the number of publications on aquaculture and AI, document types, and overall participation of authors, institutions, journals, and countries. The analysis shows that a total of 202 publications were published in the Web of Science from 1998 to December 31, 2024 (Figure 1).

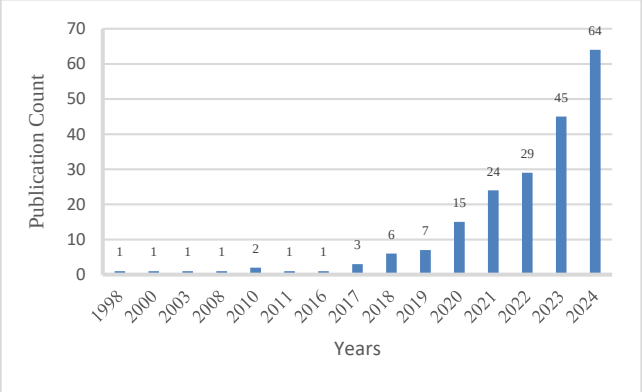

Figure 1. Number of publications on aquaculture and AI use recorded in WoS between 1998 and 2024.

The first publication on the use of Aİ and aquaculture, " *A fuzzy logic application to aquaculture environment control*” was written by Lea et al. (1998). The data in the Table 1 show that there has been a significant increase in the number of publications over the years, especially since 2020. While a limited number of publications were published between 1998 and 2019, this number increased rapidly after 2020 and reached 64 in 2024. This indicates a significant acceleration in academic productivity in recent years.

Publication types: When the distribution of publications on the use of aquaculture and AI between 1998 and 2024 is examined according to their types, among the document types, the most published ones are "*Article*" with 124 and "*Review*" with 41. These are followed by "*Proceedings Paper*" with 37, "*Early Access*" with 3, "*Book*

Chapter" with 2 and "*Editorial Material*" with one "*Data Paper*" each. This distribution shows that the research outputs largely consist of articles and compilation studies (Figure 2).

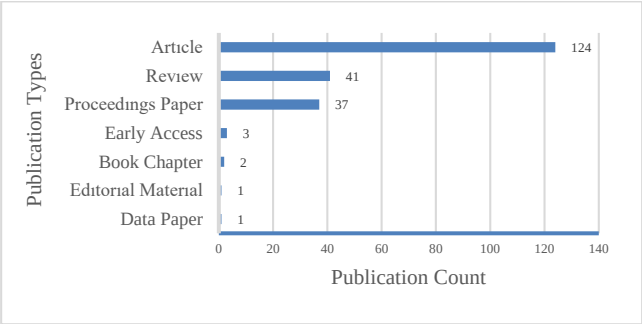

Figure 2. Distribution of publication types and their counts about on aquaculture and AI.

Research areas: The research findings show that technical disciplines such as Computer Science (57) and Engineering (55) have the highest density, while environmental fields such as Fisheries (41), Agriculture (29) and Marine Biology (29) hold an important place, with other fields exhibiting a lower but balanced distribution (Figure 3).

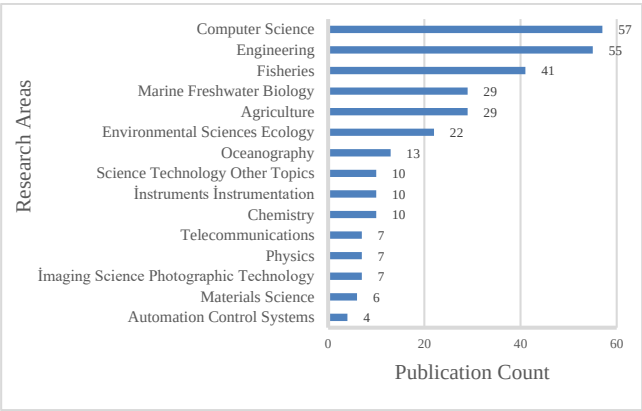

Figure 3. The 15 most published research areas and number of publications on aquaculture and AI.

***Publication titles*:** Figure 4. lists the top 15 publication titles with the most publications on aquaculture and AI. The journals with the most publications are *Aquaculture* and *Computers and Electronics in Agriculture*, each with 9 publications. Other important journals include *Sensors* (6), *Aquacultural Engineering, Fishes, Frontiers in Marine Science, Journal of Marine Science and Engineering,* and *Reviews in Aquaculture* (5 publications). Additionally, *Aquaculture İnternational, İEEE Access (Inst. Electrıcal Electronıcs Engıneers Inc.*), and *Scientific Reports* have 4 publications, while *Aquaculture Research, Applied Sciences Basel* and *Agriculture Basel* have 3 publications. These data provide an overview of the journals in which academic studies in the relevant field are concentrated.

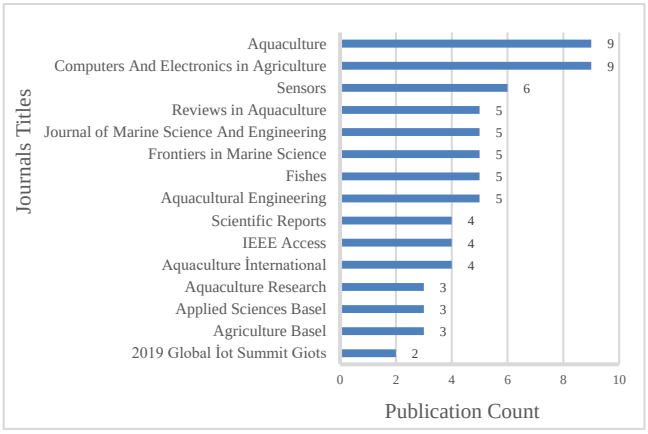

Figure 4. Top 15 journals titles and publication count in which studies on aquaculture and AI.

Countries' contribution

Analysis of the Web of Science database showed that the country with the most publications in the field of aquaculture and AI is the People's Republic of China (52 publications), followed by the USA (28 publications) and Taiwan (16 publications). Among other prominent countries on the list, Brazil comes in fourth place with 11 publications, followed by Australia 10 publications and İndia with 10 publications, respectively. Table 1 shows that Peoples R. China has taken an important leadership role in scientific publications covering aquaculture and artificial intelligence (Table 1).

Table 1. Ranking of countries with a minimum of two publications or more in the fields of aquaculture and artificial intelligence.

No	Countries	Publication Count	No	Countries	Publication Count
1	Peoples R China	52	23	Scotland	4
2	USA	28	24	South Africa	4
3	Taiwan	16	25	Tunisia	4
4	Brazil	11	26	Denmark	3
5	Australia	10	27	Egypt	3
6	İndia	10	28	Finland	3
7	England	9	29	İndonesia	3
8	Japan	9	30	İreland	3
9	Mexico	9	31	Poland	3
10	Spain	9	32	Thailand	3
11	South Korea	8	33	Türkiye	3
12	İtaly	7	34	Vietnam	3
13	Norway	7	35	Chile	2
14	France	6	36	İran	2
15	Malaysia	6	37	İraq	2
16	Philippines	5	38	Nepal	2
17	Qatar	5	39	Nigeria	2
18	Bangladesh	4	40	Palestine	2
19	Canada	4	41	Peru	2
20	Germany	4	42	Russia	2
21	Greece	4	43	Singapore	2
22	Saudi Arabia	4	44	Slovenia	2

Most productive authors: The top 15 most productive authors publishing on aquaculture and AI are shown in Figure 5. According to the data in the table, the researcher with the most publications in the field of aquaculture and artificial intelligence is Li Daoliang with 7 publications. He is followed by C. Cheng S., Chang Chin-

Chun and Ubina Naomi A.with 5 publications each. Ranjan Rakesh., Good Christopher., Hsun-Yu Lan., Tsukuda Scott and Sharrer Kata have 4 publications each. Among the other researchers, Sanchez-Fernandez Luis Pastor., Hamzaoui Mahdi., Xu Xianbao., Dupont Charlotte., and Li Juan and Wu Junfeng have contributed with 3 publications each. These data show the contributions of leading researchers who are actively working in the relevant field.

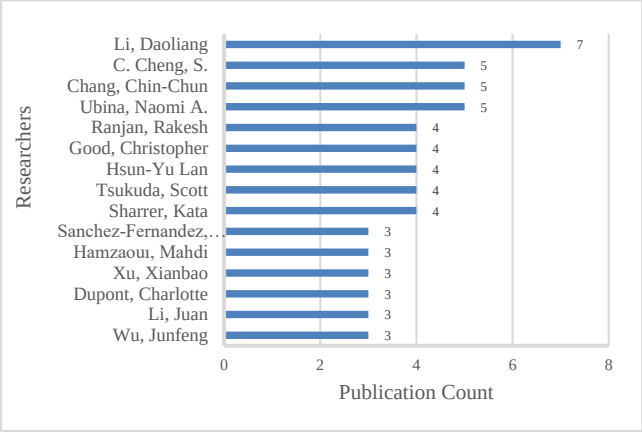

Figure 5. Top 15 most productive authors involved in aquaculture and AI.

Most cited articles: According to WoS data; of the 202 articles published on aquaculture and AI, 42 articles were not cited at all, while 155 articles were cited between 1 and 158. The most cited publication was Yang et al. (2021a) with 158 citations, Zenger et al. (2019) came second with 145 citations, and Yang et al. (2021) came third with 125 citations. The data in the table show the differences in the ranking, number of citations and number of links of the documents. "Yang et al. (2021a)", which has the highest number of citations, has 158 citations and also attracts attention with 28 links. In second place is "Zenger et al. (2019)", which has 145 citations but zero links. In third place is "Yang et al. (2021b)" with 125 citations and 16 links (Table 2).

Table 2. Highly cited articles on aquaculture and AI in WoS.

Rank	Documents	Citations ▼	Links
1	Yang et al. (2021a)	158	28
2	Zenger et al. (2019)	145	0
3	Yang et al. (2021b)	125	16
4	Lu et al. (2022)	114	0
5	Zhao et al. (2021)	111	3
6	Fernandes et al. (2020)	89	0
7	Wang et al. (2021a)	76	13
8	Chen et al. (2003)	60	1
9	Mustapha et al. (2021)	58	10
10	Gladju et al. (2022)	53	1
11	Wang et al. (2021b)	53	2
12	Lim et al. (2022)	49	0
13	Jin et al. (2021)	45	0
14	Lee (2000)	43	3
15	Chiu et al. (2022b)	42	1

Citation network of publications: The bibliographic citation network of publications in aquaculture and AI researches is shown in Figure 6. This figure created using WoSviewer, based on data from the Web of Science Database, assessed on January 5, 2025, presents a network analysis of bibliographic citation across 202 publications. Publications that received at least 1 citation were included in the visualization. Of the 202 documents, 156 meet the threshold. The largest set of connected documents consist of items. The network illustrates the relationships between documents, with the distance between nodes indicating their bibliographic relatedness and the node sizes representing the frequency of citation occurrences within each document.

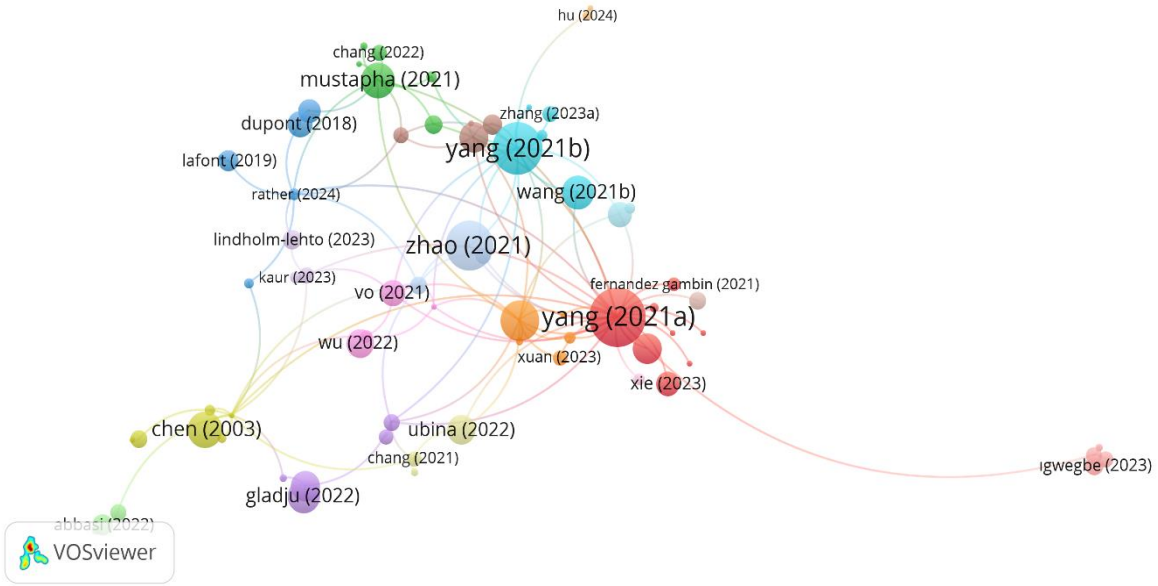

Figure 6. Citation network of publications featuring aquaculture and AI with at least 1 or more citations

Citation network of authors' total link strength: Table 3 and Figure 7, created using WoSviewer, presents a network analysis of the total link strength among 937 authors. Author with at least 1 publication were included in the visualization. The minimum number of citations for an author is calculated as 5. For each author, the total strength of citation connections to other authors was calculated and the authors with the largest total link strength were selected. It was calculated that some of the 432 authors in the network were not connected to each other and the largest connected item cluster consisted of 61.

The data in Table 3 and Figure 7 presents the metrics such as the number of publications, number of citations, nd total link strength of the authors. Song Zhang stands out with 270 citations and 135 link strength despite having only two publications. Daoliang Li is the author with the most documents with 7 publications and has a significant impact with 385 citations and 132 link strength. Authors such as Shuanglin Dong, Qinfeng Gao, Jintao Liu, Xinting Yang, and Chao Zhou stand out with high citations (158) and link strength (123) with a single publication. Xianbao Xu has high link strength (63) with 3 publications and 130 citations. Other authors with lower link strength metrics include Jincun Liu, Dean R. Jerry, David B. Jones, and Mehar S. Khatkar.

Table 3. Scientific performance and total link strength of the citations 15 most influential researchers publishing on aquaculture and AI.

	Author	Documents	Citations	Total link strength▼
1	Zhang, Song	2	270	135
2	Li, Daoliang	7	385	132
3	Dong, Shuanglin	1	158	123
4	Gao, Qinfeng	1	158	123
5	Liu, Jintao	1	158	123
6	Yang, Xinting	1	158	123
7	Zhou, Chao	1	158	123
8	Xu, Xianbao	3	130	63
9	Liu, Jincun	2	149	17
10	Jerry, Dean R.	1	145	0
11	Jones, David B.	1	145	0
12	Khalilisamani, Nima	1	145	0
13	Khatkar, Mehar S.	1	145	0
14	Raadsma, Herman W.	1	145	0
15	Zenger, Kyall R.	1	145	0

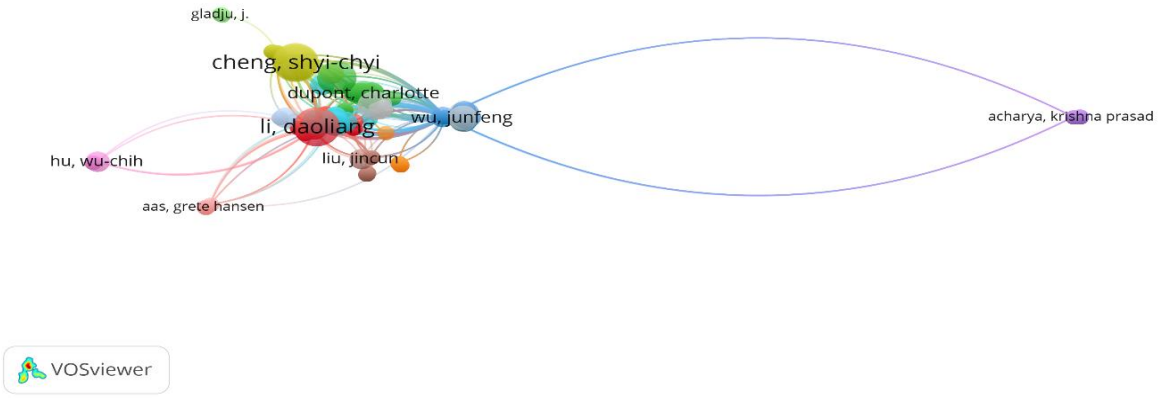

Figure 7. Citation network of authors' total link strength of publications in aquaculture and AI.

Analysis of the most used keywords: Table 4 and Figure 8 show the analysis results of 91 keywords used in articles written in the field of aquaculture and AI. When the 15 most frequently used keywords in Table 4 are examined, it is revealed that artificial intelligence and related technologies are increasingly used in the field of aquaculture. The data shows that the most frequently used keyword is "Aquaculture" (66 times, 156 connection strength). This is followed by advanced technologies such as "Artificial Intelligence" (61 times, 144 connection strength), "Machine Learning" (36 times, 96 connection strength) and "Deep Learning" (26 times, 68 connection strength). Applications such as "Computer Vision" (15 times, 42 connection strength) and "Internet of Things" (14 times, 40 connection strength) stand out especially in smart fish farming and precision aquaculture applications. In addition, environmental factors such as "Water Quality" (11 times, 34 connection strength) and "Sustainability" (7 times, 15 connection strength) play an important role in the development of aquaculture technologies. These findings show that a technology-oriented and environmentally aware transformation process is taking place in the aquaculture sector.

Table 4. The 15 most used keywords in articles published on aquaculture and AI.

	Keyword	Occurrences	Total Link Strength ▼
1	Aquaculture	66	156
2	Artificial İntelligence	61	144
3	Machine Learning	36	96
4	Deep Learning	26	68
5	Computer Vision	15	42
6	Internet Of Things	14	40
7	Water Quality	11	34
8	Artificial Intelligence	11	13
9	Precision Aquaculture	8	24
10	Fish	8	23
11	Sustainability	7	15
12	Convolutional Neural Networks	6	20
13	Iot	6	20
14	Smart Fish Farming	6	17
15	Object Detection	6	15

In Figure 8, keywords of scientific research conducted in the field of aquaculture and AI were analysed

via VOSviewer. The findings show that the terms *Aquaculture* and *Machine Learning* are at the center of the research and have strong relationships with many other keywords. In addition to advanced artificial intelligence techniques such as *Deep Learning*, *Computer Vision* and *Object Detection*, technological approaches such as the *Internet of Things and Automation* are also frequently used in aquaculture applications. In the analysis, where environmental sustainability themes also draw attention, it is seen that keywords such as *Water Quality*, *Dissolved Oxygen* and *Sustainability* have an important place. In addition, when the time scale is examined, it is determined that the interest in *Smart Fish Farming* and *Environmental Monitoring* has increased in recent years.

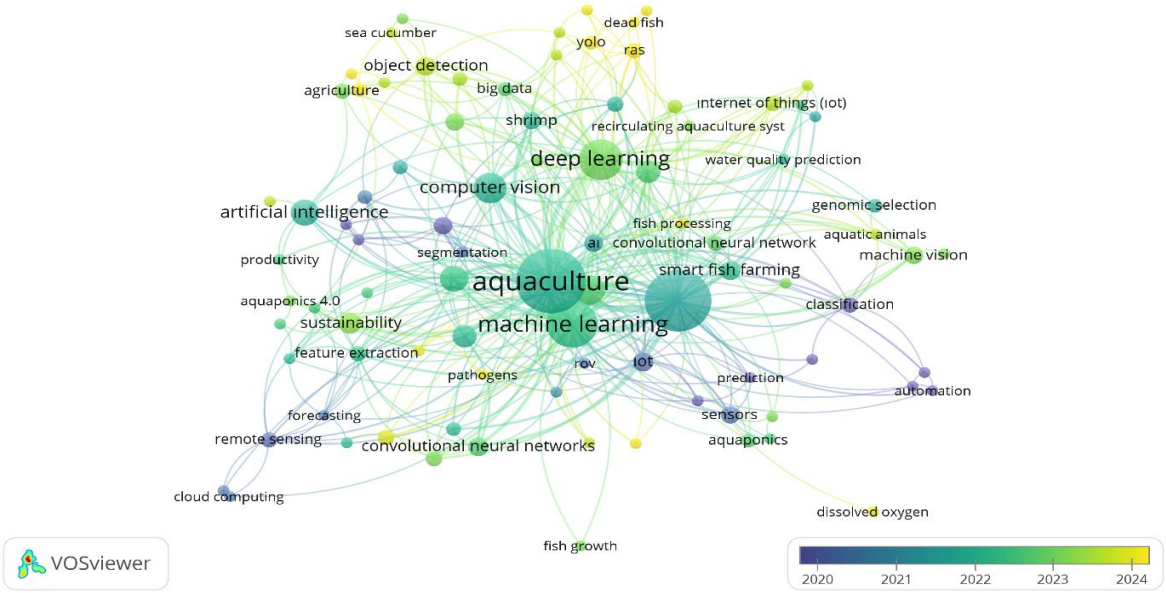

Figure 8. Analysis of keywords in articles published on aquaculture and AI.

DISCUSSION

The analysis of publication trends in aquaculture and artificial intelligence (AI) from 1998 to 2024 reveals a dynamic evolution in this interdisciplinary field. Between 1988 and 2019, research in the field of artificial intelligence and aquaculture initially focused on fuzzy logic and process control systems, but over time, it has become integrated with advanced technologies such as big data, signal processing, machine learning, and the internet of things (IoT) (Lea et al. 1998; Lee, 2000). In the early 2000s, the use of artificial intelligence software in aquaculture management increased, and after 2010, water quality monitoring systems and artificial intelligence-supported analyses came to the fore. During this period, information fusion, image processing, and IoT-based systems were used in areas such as disease detection, smart feeding, and real-time water monitoring, and processes for smart aquaculture were developed (Dupont et al. 2018).

The modest beginnings, marked by the pioneering work of Lea et al. (1998) on fuzzy logic for aquaculture environmental control, have translated into a significant increase in academic output after 2020, reaching 60 publications in 2024. This acceleration is in line with global advances in AI technologies such as machine learning (ML), deep learning (DL), and the Internet of Things (IoT), and reflects their increasing integration into agricultural and environmental sciences. The dominance of "Articles" (120) and "Reviews" (41) among publication types suggests a solid original research base supported by synthetic overviews, facilitating knowledge dissemination and consolidation in the field.

In the 2010s, advanced technologies such as information fusion, image processing, and IoT-based systems transformed the industry by offering innovative solutions such as disease detection, real-time monitoring, and smart feeding systems. In the 2020s, advanced AI techniques such as deep learning (DL) and computer vision (CV) have become widely used in areas such as fish behaviour analysis, biomass estimation, and remote monitoring of aquatic ecosystems (Hu et al., 2020; Chen et al., 2020). In particular, the integration of autonomous systems and underwater unmanned vehicles has made aquaculture processes more efficient and sustainable. These developments indicate a transition from approaches focused on the analysis of specific parameters in previous years to ecosystem-based, multi-data source solutions (Uz et al., 2020).

Studies published in 2021 and 2022 have shown that AI, ML, IoT, and CV technologies are playing an

increasing role in aquaculture (Zhao et al., 2021; Wu et al., 2022). Applications such as smart fish farms, water quality monitoring, and automatic feeding systems have provided higher accuracy and efficiency compared to traditional methods, while AIoT-based solutions have created new opportunities in monitoring environmental impacts (Chiu et al., 2022b). In 2023, more complex and integrated systems such as digital twin technologies, AI-assisted fish health monitoring, and water pollution analysis have come to the fore (Ubina et al., 2023; Igwegbe et al., 2023). This year stands out as a transition period in which technologies offer interactive and data-driven solutions compared to previous periods.

Studies from 2024 show that AI and ML techniques are becoming more widespread in aquaculture and their sectoral applicability is expanding (Cai et al., 2024; Alprol et al., 2024). While deep learning algorithms increase operational efficiency in areas such as fish counting, behavioural analysis, and disease diagnosis, AIoT solutions have provided optimized processes in precision feeding and water quality management. These technologies have significant potential for the future of aquaculture by reducing labour costs and supporting environmental sustainability.

The distribution of research areas highlights a synergy between technical disciplines (e.g., Computer Science, 57; Engineering, 53) and applied fields like Fisheries (41) and Marine Biology (29). This interdisciplinary nexus underscores AI's role as a transformative tool in addressing practical aquaculture challenges, such as water quality management, species monitoring, and precision farming. The concentration of publications in leading journals like *Aquaculture* and *Computers and Electronics in Agriculture* (9 each) reflects the field's maturation, with these outlets serving as key platforms for advancing both theoretical and applied insights. However, the diversity of journals, including *Sensors* and *Frontiers in Marine Science*, indicates a broadening scope, encompassing sensor-based technologies and marine ecosystem sustainability.

Geographically, the dominance of the People's Republic of China (52 publications) and the USA (28) points to significant investments in AI-driven aquaculture research, likely driven by economic stakes in seafood production and technological innovation. Emerging contributions from countries like Taiwan (16), Brazil (11), and India (10) suggest a globalizing trend, potentially fuelled by regional needs for sustainable aquaculture amid growing food security demands. This aligns with the prolific output of researchers like Li Daoliang (7 publications), whose work exemplifies leadership in integrating AI into aquaculture systems, and the high citation impact of authors like Zhang Song (270 citations), indicating influential contributions despite fewer publications.

Citation analyses further illuminate the field's intellectual structure. The most cited articles, such as Yang et al. (2021a) with 158 citations and Zenger et al. (2019) with 145, highlight seminal works that have shaped discourse, likely due to their innovative applications of AI in precision aquaculture and genetic prediction. The citation network (156 connected publications) and authors' link strength (e.g., Li Daoliang, 132) reveal a tightly knit research community with robust knowledge exchange, albeit with some fragmentation, as 432 authors formed a largest cluster of only 61. This suggests opportunities for greater collaboration to enhance connectivity and impact.

Keyword analysis reinforces the technological and ecological priorities driving this field. "Aquaculture" (66 occurrences) and "Artificial Intelligence" (61) anchor the research, while "Machine Learning" (36), "Deep Learning" (26), and "Computer Vision" (15) reflect the adoption of cutting-edge tools for applications like fish detection and behaviour analysis. The prominence of "Water Quality" (11) and "Sustainability" (7) underscores an environmental consciousness, aligning with global sustainability goals. The temporal shift toward "Smart Fish Farming" and "Environmental Monitoring" in recent years indicates a maturing focus on automation and real-time ecosystem management, consistent with broader trends in smart agriculture.

These findings demonstrate that AI in aquaculture has evolved from rudimentary applications in the late 1990s to a sophisticated, technology-driven field by 2024. The rapid post-2020 growth reflects not only technological advancements but also increasing recognition of AI's potential to enhance efficiency, reduce labour costs, and promote sustainable practices in aquaculture. However, challenges remain, including the need for broader adoption in less-represented regions and the integration of fragmented research efforts into cohesive frameworks. The reliance on technical disciplines also suggests a need for greater involvement of social and economic perspectives to address scalability and accessibility issues.

The integration of AI into aquaculture has reached a pivotal stage, characterized by significant academic productivity, interdisciplinary collaboration, and a focus on sustainability. The leadership of countries like China, the influence of key researchers, and the dominance of technology-oriented journals and keywords collectively signal a transformative trajectory. Future research should prioritize bridging regional disparities, enhancing cross-disciplinary collaboration, and addressing practical barriers to large-scale implementation. By doing so, AI can fully realize its potential to revolutionize aquaculture,

contributing to global food security and environmental resilience in an increasingly digitalized world.

As a result, AI applications in aquaculture have evolved from simple systems to complex and integrated technologies in an evolutionary process that extends from the 1980s to the present. Today, the combination of deep learning, IoT, and autonomous systems is making the sector more efficient, sustainable, and digital. However, more research is needed on issues such as standardization, cost-effectiveness, and infrastructure requirements for the wide-scale applicability of these technologies. In the future, AI-supported aquaculture systems have a high potential to contribute to global food security and environmental sustainability goals. In this context, increasing academic and industrial collaboration stands out as a critical element that will accelerate the sectoral adaptation of the technology.

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ARAŞTIRMA MAKALESİ RESEARCH PAPER

Evidence-Based Confirmation of Alien Species Caramote Prawn *Penaeus kerathurus* on the Turkish Coast of the Black Sea

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Abstract: This study provides the evidence-based confirmation of historical records of the prawn species *Penaeus kerathurus* on the Black Sea coast of Türkiye, highlighting a notable geographical extension of its known distribution range. A female (12.81 cm in TL) and a male (13.56 cm in TL) were captured 22 days apart using a gill net at a depth of 8 meters off the coast of Fatsa district (Ordu, Black Sea). Although the species is known for its adaptability and ecological significance, the findings suggest that its presence in the Black Sea is more likely due to anthropogenic factors, particularly introduction via ballast water from ships, rather than active swimming through the Turkish Straits System. Its rare occurrence in the Marmara Sea further supports this hypothesis. The specie's high fecundity and adaptability may facilitate its colonization in the Black Sea, while the low salinity and cooler sea water temperatures may challenge its long-term establishment.

Keywords: Range expansion, alien species, Penaeidae, ballast water, Türkiye.

Karadeniz'in Türkiye Kıyılarında Yabancı Karides Türü *Penaeus kerathurus*'un Kanıta Dayalı Doğrulanması

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Öz: Bu çalışma, *Penaeus kerathurus* karidesinin Türkiye'nin Karadeniz kıyılarındaki tarihi kayıtlarını kanıta dayalı olarak doğrulamakta ve türün bilinen dağılım alanında dikkate değer bir coğrafi genişlemeyi ortaya koymaktadır. Toplam uzunluğu 12,81 cm olan bir dişi ve 13,56 cm olan bir erkek birey, Fatsa ilçesi (Ordu, Karadeniz) açıklarında, 8 metre derinlikte fanyalı ağ kullanılarak 22 gün arayla yakalanmıştır. Yüksek adaptasyon yeteneği ve ekolojik önemi ile bilinen bu türün Karadeniz'deki varlığının, Türk Boğazlar Sistemi üzerinden aktif göçten ziyade, büyük olasılıkla gemilerin balast suyu yoluyla insan kaynaklı taşınmadan kaynaklandığı düşünülmektedir. Marmara Denizi'ndeki sınırlı dağılımı da bu hipotezi desteklemektedir. Bu türün yüksek üreme kapasitesi ve adaptasyon yeteneği, Karadeniz'de kolonileşmesini kolaylaştırırken, düşük tuzluluk ve daha soğuk deniz suyu sıcaklıkları uzun vadeli yerleşimini zorlaştırabilir.

Anahtar Kelimeler: Coğrafi genişleme, yabancı tür, Penaeidae, balast suyu, Türkiye.

INTRODUCTION

The caramote prawn, *Penaeus kerathurus* (Forsskål, 1775), is a prominent member of the family Penaeidae. This species predominantly inhabits shallow estuarine and marine environments, preferring sandy, muddy, and shell gravel substrates at common depths ranging from shoreline to 40 meters and occasionally up to 90 meters (d'Udekem d'Acoz, 1999). The caramote prawn is considered a valuable species in small-scale fisheries (Sartol et al., 2018; Şen, 2025).

The geographical distribution of the caramote prawn extends across the eastern Atlantic, from southern England to Angola, including Mauritania, and Morocco and throughout the Mediterranean Sea (González & Santana, 2014). In Turkish seas, it was also distributed in the Aegean

Sea and the Sea of Marmara (Ihsanoglu, 2020). This study presents a validated record of caramote prawn in the Turkish Black Sea, occurring eight years after the first confirmed report by Gönülal and Türetken (2019). This confirmation contributes significantly to our understanding of the species' potential establishment and persistence in the Black Sea.

MATERIAL AND METHOD

Two specimens of the caramote prawn were caught as bycatch during commercial fishing operations off the coast of Fatsa district, Ordu, in the Black Sea on October 3, 2024 (Specimen 1; 41°02'16"N – 37°30'01"E), and October 25, 2024 (Specimen 2; 41°02'21"N – 37°29'37"E), respectively. The sampling point was very close to the Fatsa Pier, 200 m away from where ships in international circulation load and unload. Both specimens were caught at a depth of 8 meters using a 40 mm mesh size (stretched measure) monofilament gill net deployed during routine commercial fishing activities. Identification of the specimens was performed using taxonomic keys specific to *Penaeus* prawns (Fischer et al., 1987; Galil et al., 2002). The scientific name was further cross-referenced with SeaLifeBase (Palomares & Pauly, 2024). Morphometric measurements included total body length (TL in cm) and carapace length (CL in cm). TL was measured in a stretched condition from the tip of the rostrum to the end of the telson while CL was measured from the tip of the rostrum to the mid-posterior dorsal margin of the carapace using digital Vernier caliper (Conides et al., 2008). Total body weight (TW in g) was recorded using a digital scale with an accuracy of 0.01 g. Sex was determined based on the presence of a petasma in males and a thelycum in females (Conides et al., 2008; Ihsanoglu et al., 2021).

RESULTS AND DISCUSSION

The captured specimens of the caramote prawn included a female (Specimen 1) and a male (Specimen 2). The female measured 12.81 cm in TL, 5.03 cm in CL, and weighed 14.27 g in TW. The male measured 13.56 cm in TL, 5.21 cm in CL, and weighed 15.35 g in TW (Figure 1).

Froglia and Scanu (2023), through an extensive literature review, revealed that the first two records of caramote prawn reported in the Black Sea (one in 2005 and the other in 2014) were initially misidentified as *P. semisulcatus*. These records were later retrospectively confirmed as *P. kerathurus*, with the individuals likely introduced into the Black Sea as larvae or post-larvae via ballast water discharge from ships. Subsequently, in 2017, Gönülal and Türetken (2019) reported the first confirmed record of *P. kerathurus* along the Turkish coast of the Black Sea. However, no additional data have been presented regarding the species' presence, population continuity, or ecological impact. This study provides a verified record of caramote prawn in the Turkish Black Sea, confirming its presence eight years after the initial documented report by Gönülal and Türetken (2019).

While some Penaeidae members have a notable swimming capacity, estimated at approximately 100 km per month (Klima, 1963; Altuve et al., 2008), the rare presence in the Marmara Sea, region it would have needed to traverse to reach the Black Sea, makes the hypothesis of natural range expansion highly unlikely. The Ordu coast of the Black Sea, located over 2500 km away from the species' established populations in the Levantine and Aegean Seas, presents a significant geographical barrier to direct swimming. This is further supported by the lack of rare historical or contemporary records of the caramote prawn in intermediate regions, despite extensive fisheries activities that would likely have detected the species. Both specimens in this study were captured near the Fatsa pier, an active hub for maritime cargo operations, further reinforcing the likelihood of introduction via anthropogenic vectors. Ships operating in international waters often serve as vectors for invasive marine species, particularly through ballast water discharge. During transport, larval stages, such as nauplius, zoea, mysis, or early post larvae, can survive and later establish populations upon release into favorable habitats. The proximity of the collection site to an active pier increases the probability that these specimens were transported as larvae or juveniles in ballast tanks or potentially attached to the hulls of ships. Froglia and Scanu (2023) also highlighted that the prior observations of the caramote prawn in the Black Sea involved a juvenile specimen likely introduced to the Black Sea via ballast water from ships.

The discovery of mature female, with body size (5.3 cm in this study) exceeding CL_{50} thresholds for reproductive maturity (3.9 cm for females) (Ihsanoglu et al., 2021), suggests that the specimens had time to settle and grow following their introduction to the Black Sea. This observation implies that the introduction occurred some time ago, allowing the shrimp to adapt and mature in their new habitat. Furthermore, environmental changes driven by climate change may have facilitated the establishment of this non-native species. The process of Mediterraneanization, characterized by warming sea temperatures and other climatic shifts, has been increasingly documented in the Black Sea (Oğuz & Öztürk, 2011). These changes have supported the introduction and proliferation of various alien migrants and other non-indigenous species in recent years (Öztürk, 2021; Eyüboğlu, 2022; Aydın et al., 2024). In this context, the ongoing warming of the Black Sea likely created more favorable conditions for the survival and establishment of the caramote prawn, which thrives in a broad range of environmental conditions.

Figure 1. The male *Penaeus kerathurus* specimen caught on 25 October 2024 (a) with distinctive rostrum (b)

The potential for the caramote prawn to colonize the Black Sea remains uncertain, as it depends on several environmental and biological factors. Body size of specimens confirm the presence of sexually mature individuals, highlighting the potential for reproduction and further establishment of the species in the Black Sea, provided environmental conditions are favorable. The presence of opposite sexes in the population increases the possibility of reproduction, as the caramote prawn exhibits high fecundity (approximately 156,000 oocytes/g) (Conides et al., 2008). This reproductive capacity may provide the foundation for the species to establish a population in the Black Sea. However, the ability of the species to complete its life cycle under the Black Sea's environmental conditions is less certain. The Black Sea's low salinity and relatively low winter temperatures could pose significant challenges, particularly during the larval stages. Therefore, while the high reproductive potential and adult adaptability of the caramote prawn suggest it could colonize the Black Sea under favorable conditions, its long-term establishment and population sustainability remain uncertain.

The introduction and presence of the caramote prawn in the Black Sea could lead to significant ecological and economic impacts. These effects can be both direct and indirect, influencing native ecosystems and fisheries in various ways. The caramote prawn dominates the ecosystem among the prawn species due to its carnivorous behavior. Its introduction may increase competition for resources with native prawn species and other benthic fauna, potentially leading to declines in native populations. If the species becomes established and thrives, it could emerge as a target species for fisheries due to its high economic value. Given its high market value and desirability, the establishment of the caramote prawn in the Black Sea could provide an alternative income source for fisheries.

CONCLUSION

This study documents the first record of the caramote prawn on the Turkish Black Sea coast, marking a significant milestone in its geographic range expansion. The findings underscore the species' ecological adaptability yet strongly support the hypothesis that its introduction occurred via ballast water discharge rather than active migration through the Turkish Straits System. Despite its high fecundity and resilience, the Black Sea's unique environmental conditions, such as lower salinity and cooler temperatures, may limit the species' potential for long-term establishment. To mitigate risks and capitalize on potential benefits, continued monitoring, coupled with genetic studies, is essential to track its population dynamics and inform management strategies for both ecological conservation and fisheries sustainability.

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COMPLIANCE WITH ETHICAL STANDARDS

Authors' Contributions: All authors contributed equally to the final version of the manuscript.

Conflict of Interests: The authors declare that they have no actual, potential, or perceived conflict of interests for this article.

Statement on the Welfare of Animals: This study does not imply any responsibility for animal welfare.

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ARAŞTIRMA MAKALESİ RESEARCH PAPER

The Role and Importance of Additional Vitamin A Supplementation in the Treatment Protocol for Crop Candidiasis in Budgerigars

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Abstract: This study evaluated the effectiveness of adding oral Vitamin A to the standard treatment for crop candidiasis in budgerigars. A total of 60 budgerigars aged 1-4 years with confirmed crop candidiasis were divided into two groups. Group 1 received Nystatin, Metoclopramide, and Enrofloxacin, while Group 2 received the same treatment along with oral Vitamin A for 14 days. Clinical scoring was used to assess symptoms pre- and post-treatment on a scale of 1 to 5. Statistically significant improvements were observed in both groups ($p<0.05$). However, post-treatment clinical scores showed a significantly greater improvement in Group 2 ($p<0.05$). The recurrence rate of the disease was 33.3% in Group 1, compared to only 13.3% in Group 2 within 365 days. These results suggest that the addition of Vitamin A to the standard treatment protocol for crop candidiasis leads to faster recovery and a lower recurrence rate. The aim of the study was the investigation of the potential benefits of incorporating Vitamin A in managing crop candidiasis in budgerigars.

Keywords: Budgerigars, candida, treatment, vitamin A.

Muhabbet Kuşlarında Kursak Kandidiazisi Tedavi Protokolüne Ek Olarak Verilen A Vitamininin Rolü ve Önemi

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Öz: Bu çalışmada muhabbet kuşlarında kursak kandidiyazı için standart tedaviye oral A Vitamini eklenmesinin etkinliği değerlendirildi. 1-4 yaş arası, kursak kandidiazis tanısı konan 60 muhabbet kuşu iki gruba ayrıldı. Grup 1, Nystatin, Metoclopramid ve Enrofloksasin alırken, Grup 2 aynı tedaviyi 14 gün boyunca oral A Vitamini ile birlikte aldı. Semptomları tedavi öncesi ve sonrası 1'den 5'e kadar bir ölçekte değerlendirmek için klinik skorlama kullanıldı. Her iki grupta da istatistiksel olarak anlamlı farklar gözlendi ($p<0,05$). Tedavi sonrası klinik skorlama Grup 2'de anlamlı derecede daha fazla iyileşme gösterdi ($p<0,05$). Hastalığın tekrarlama oranı Grup 1'de %33,3 iken, Grup 2'de 365 gün içinde sadece %13,3 idi. Bu sonuçlar, kursak kandidiazisi için standart tedavi protokolüne A Vitamini eklenmesinin daha hızlı iyileşmeye ve daha düşük bir tekrarlama oranına yol açtığını göstermektedir. Çalışmanın amacı, muhabbet kuşlarında kursak kandidiazisinin tedavisinde A Vitamini kullanımının potansiyel faydalarının incelenmesidir.

Anahtar Kelimeler: Muhabbet kuşu, kandidiazis, tedavi, A vitamin.

INTRODUCTION

There are various gastrointestinal system diseases in budgerigars (Hollwarth and Prieto, 2024). These include stasis or dilatation of the esophagus and crop, neoplasms, gastrointestinal system foreign bodies, bacterial, viral, parasitic diseases, diarrhea, constipation and cloacal prolapse.

Crop candidiasis in budgerigars is one of the common diseases of the gastrointestinal system (Terry and Campbell, 2017). Candidiasis is a mycotic disease seen in the upper digestive system (mouth, esophagus and crop) of budgies. The disease is also called moniliasis or crop mycosis. The most commonly isolated species in patients is Candida albicans (Talazadeh et al., 2022). Poor hygiene

in the bird's environment and food preparation, prolonged use of antibiotics, concurrent immunosuppressive conditions (e.g., malnutrition, debilitation), failure to clean excess formula from the skin or mouth of hand-reared chicks, high concentrations of sugar in fruit and hand-rearing formula, which provide an ideal medium for yeast growth, and alkaline crop contents, which are observed when crop stasis occurs for any reason, are reported to be predisposing factors for overgrowth of the agents (Doneley, 2018). Pigeons, turkeys, chickens, geese, pheasants, quail, parrots, budgerigars, guinea fowls, and other birds have all been found to have candidiasis (Samanta, and Bandyopadhyay, 2017). Another contributing factor is nutritional inadequacies, particularly a lack of vitamin A, which can affect the body's defense processes as a mediator of resistance against major diseases and cause candidiasis (Velasco, 2000).

The disease can occur as primary or secondary in birds, with secondary bacterial infections also accompanying the infection. In patients with candidiasis, yellow-white necrotic plaques in the mouth are seen in the form of lines (Ladds, 2009). In the chronic phase, thickening and irregularity of the crop mucosa are characteristic. Crop mucosa is covered with exudate ranging from catarrhal to mucoid. The clinical signs of the disease varies depending on the factors that cause predisposition and the pathogenicity of the agent. Crop candidiasis is usually characterized by weight loss, slow growth, crop thickening, enlargement, delayed crop emptying, regurgitation, hoarseness or abnormal vocalization (Talazadeh et al., 2022; Garces, 2023).

By detecting Candida species on a Gram, Romanowsky-type, or methylene blue stain of the excrement, crop contents, or regurgitated material, candidiasis can be diagnosed. In extreme situations where tissue invasion has taken place, the budding yeast will create hyphae that are visible in feces or scrapings taken from the throat or crop (Ibrahim, 2020).

Treatment of candidiasis includes the administration of antifungal agents. Additionally, the use of metoclopramide (0.5mg/kg for 5 days) may help promote crop motility and prevent regurgitation (Apsemidou et al., 2020; McCready, 2024). Nystatin (10 mg/kg every 12 hours for 14 days), an antifungal drug, is the most commonly used drug in treatment due to its low cost and low toxicity (Kafrashi and Babazadeh, 2022; Garces, 2023). In addition, antibiotic use is recommended against secondary bacterial infections. For this purpose, enrofloxacin can be used at a dose of 10 mg/kg body weight for 5 days. Patients may also be given vitamin A for its positive contribution to the treatment process. (Kurtdede et al., 2008).

Vitamin A is a micronutrient that is essential for supporting growth and development and maintaining the integrity of mucosal epithelial cells. Furthermore, because of its vital role in boosting immune function by controlling humoral and cellular immunological processes, vitamin A is referred to as an anti-inflammatory vitamin (Huang et al., 2018). Vitamin A is one of the antioxidant vitamins that neutralizes free radicals by reducing them in the body (Blaner et al., 2021). Oxidative stress occurs as a result of various diseases (Chatterjee, 2016). Oxidative stress reduces antioxidant levels due to increased production of reactive oxygen species (Esin et al., 2024). Therefore, in situations that create oxidative stress, the antioxidant properties of vitamin A can be used. Secretory immunoglobulin A (IgA) is an antibody that plays an important role in the immune function of mucosal membranes. It is found in all mammals and birds. It is one of the main lines of defense of our body. It is found in all mucosal membranes in our body. In vitamin A deficiency, IgA production is negatively affected. Therefore, in vitamin A deficiency, IgA production, especially in mucosal membranes, is disrupted and the immune system is negatively affected (Li and Chen, 2020). In addition, from this point of view, vitamin A will have positive effects on the healing process in diseases, especially mucosal diseases (Kurtdede et al., 2008). Vitamin A deficiency also hinders the maturation and proliferation of T and B lymphocytes, lowers the generation of natural killer cells, monocytes, and macrophages, and influences the release of cytokines and antibodies (Munteanu and Schwartz, 2022).

The aim of this study was to investigate the contribution of vitamin A added to the routine treatment protocol used in budgerigars with crop candidiasis on the healing process.

MATERIAL AND METHOD

The study material consisted of 60 budgerigars aged between 1 and 4 years old, brought to Ondokuz Mayıs University Animal Hospital with complaints of anorexia, weight loss, crop dilatation, hoarseness/abnormal vocalization, regurgitation and vomiting, and diagnosed clinically with crop candidiasis. As a result of physical examination, crops of birds suspected of crop candidiasis were gently massaged and some crop content was taken. The diagnosis was confirmed by the observation of blastospores and pseudohyphae in the native examination of smear preparations prepared from crop content, and by the observation of the agent itself or pseudohyphae in Giemsa staining of smears (Figure 1). A clinical scoring chart was made for the budgerigars with crop candidiasis included in the study. Clinical scores ranged from 1 to 5 based on symptom severity. The clinical score criteria were

based on the five most commonly observed clinical signs in crop candidiasis: anorexia, weight loss, regurgitation/vomiting, crop distension, and abnormal vocalization. (Table 1). In this study, a clinical scoring system was developed to assess clinical signs associated with crop candidiasis in budgerigars, inspired by previously established scoring systems for feather condition and sedation levels in psittacine species (Abreu et al., 2024; van Zeeland et al., 2013). Clinical scoring was performed on both groups before and after treatment.

Table 1. Clinical scoring table for budgerigars included in the study.

Symptoms	No	Yes
Anorexia	0	1
Weight loss	0	1
Regurgitation/vomiting	0	1
Crop distension	0	1
Abnormal vocalization	0	1

The study consisted of two groups consequently, treatment 1 group and treatment 2 group. Treatment 1 group received nystatin (10 mg/kg for 14 days), metoclopramide (0.5mg/kg for 5 days) and enrofloxacin (10 mg/kg for 5 days). Treatment 2 group received oral vitamin A 8.000IU/kg (Zhang et al., 2023) for 14 days in addition to these drugs. The same diet was applied to both groups during the treatment period. The treatment duration was 14 days in both groups.

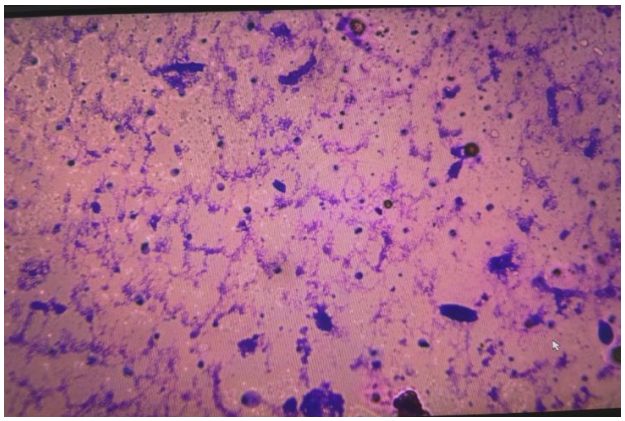

Figure 1. Microscopic appearance of Candida albicans in a smear from a budgerigar under 400× magnification (40× objective). The yeast cells appear as loosely aggregated clusters, indicative of fungal overgrowth (Black arrows).

Statistical Analysis: Clinical scores for both groups were recorded as numerical data before and after treatment. The data were analyzed using the IBM SPSS Statistics for Windows (IBM Corp., Armonk, N.Y., USA). Quantitative data were assessed for normality using the Shapiro-Wilk test. Since the data has a normal distribution, before using the independent t-test, the two groups were assessed for equality of variances. For this purpose, Levene's test was used to determine whether the standard deviation of the treatment 1 group was expected to be the same as the standard deviation of the treatment 2 group. Independent t-test was used because the data values had a normal distribution and showed variance equality. Paired sample t test the was used for comparative statistics of pre-treatment and post-treatment clinical scores within the group. For continuous variables, means and standard deviations are given. P values less than 0.05 were considered significant.

RESULTS

The statistical evaluation results of pre and post treatment scores in the groups were shown in table 2. In within-group measurements, the clinical score of budgerigars with crop candidiasis in treatment 1 group was 4 ± 0.83 before treatment, but with the decrease in symptoms after treatment, the clinical score decreased to 1 ± 0.83, and this difference was found to be statistically significant (a, b) ($p<0.05$). In within-group measurements, the clinical score of budgerigars with crop candidiasis in treatment 2 group was 3.9 ± 0.75 before treatment, while the clinical score after treatment was 0.4 ± 0.24, consistent with improvement, and this difference was found to be statistically significant (c, d) ($p<0.05$). When the measurements between the groups were examined, no statistically significant difference was found between the pre-treatment clinical scores of the treatment 1 group and treatment 2 group ($p>0.05$). However, the difference between the post-treatment clinical scores of the treatment 1 group and treatment 2 group (1 ±0.53 and 0.4 ±0.24, respectively) was found to be statistically significant ($p<0.05$).

The recurrence rate of the disease within 365 days after treatment was evaluated in both groups. In the treatment 1 group, recurrence was observed in 10 out of 30 budgerigars (33.3%), whereas in the treatment 2 group, recurrence occurred in 4 out of 30 budgerigars (13.3%). (Table 3).

Table 2. The statistical evaluation results of pre and post treatment scores in the groups.

Groups	Pre-Treatment Score	Post-Treatment Score
Treatment 1	4 ± 0.83^{a}	1 ± 0.53^{b}
Treatment 2	3.9 ± 0.75^{c}	0.4 ± 0.24^{d}
P Value	>0.05	**<0.05**

The P value in the table shows the statistical difference in measurements between groups. Different lower case letters (a, b and c, d) show the statistical difference in measurements within groups.

Table 3. Disease recurrence rates among groups within 365 days after treatment.

	Treatment 1 (n:30)	Treatment 2 (n:30)	P Value
Recurrence	10	4	**<0.05**
Percentile (%)	33.3	13.3	

DISCUSSION

Candida spp., a type of yeast, is a disease that affects almost all bird species. Symptoms of candidiasis in avian species are similar, including anorexia, weight loss, crop enlargement, hoarseness/abnormal vocalizations, regurgitation, and vomiting (Gibbons, 2020; Schmidt et al.,

2024). In our study, budgerigars with crop candidiasis showed symptoms consistent with similar publications.

In diagnosing crop candidiasis, oral, vomitus, crop, or fecal content can be used to diagnose Candida agents microscopically (Kurtdede et al., 2008; Ibrahim 2020). In addition, the diagnosis can be supported by the 'honeycomb' appearance of the crop with contrast radiography (Kurtdede et al., 2008; Krautwald et al., 2017). In addition, yellow-white necrotic plaques can be seen in the oral mucosa of birds with crop candidiasis (Hauck, 2024). The disease can be diagnosed with these diagnostic tools. In this study, candidiasis diagnose was comfirmed by observing blastospores and pseudohyphae in the natural examination and Giemsa staining of smears prepared crop content similar to previous studies in patients.

The most commonly preferred antifungal drug for avian crop candidiasis is nystatin (McCready, 2024). Nystatin binds to a compound called ergosterol in the cell membrane of Candida species. Ergosterol is a structural component of the cell membrane of fungi/yeasts and is necessary for the integrity of the fungal membranes (Rai et al., 2022). Nystatin also binds with ergosterol to create holes (pores) in the cell membrane. These holes in the cell membrane disrupt the normal ionic balance between the cell's internal and external environment. These holes cause substances contained in the cell (such as potassium, water, and other ions) to leak out. This leads to cell death or loss of growth ability (Prasad et al., 2016). As a result, nystatin prevents Candida species from multiplying in the crop, which makes it effective in the treatment of crop candidiasis. In crop candidiasis, the use of metoclopramide can help increase sluggish crop motility and prevent regurgitation. Therefore, it finds a place in the treatment protocol (Apsemidou et al., 2020; McCready, 2024).

Talazadeh et. Al. (2022), reported that, using vitamin A in crop candidiasis in birds has positive effects particularly in the immune system and potential benefits in treating fungal infections (Talazadeh et al., 2022). Vitamin A has important role associated with functions such as protecting mucosal tissues, strengthening the immune response, and healing epithelial cells (Özkanlar et al., 2016; Stephensen and Lietz, 2021). Therefore, it is thought that vitamin A supplementation may be a potential treatment tool against infections of mucosal surfaces such as the crop. Considering these effects, vitamin A was added to the treatment protocol in this study.

Vitamin A supports the regeneration of crop epithelial cells and their healthy structure. The crop mucosa is an important part of the digestive system and the epithelial cells found here play a critical role in digestion and protection from infections (Kierończyk et al., 2016). Vitamin A increases the durability of the mucosal barrier by helping cells differentiate and proliferate properly. In addition, strengthens the mucosal immune response by helping to increase the production of IgA antibodies. Vitamin A can increase the resistance of the crop mucosa to fungi (Bos et al., 2022). Diseases such as crop candidiasis, especially caused by yeasts such as Candida spp., are common in birds with weak immune systems. Adequate vitamin A intake can increase protection against such infections and can help heal mucosal damage by reducing inflammation during fungal infections (Campione et al., 2020). Probably, faster and better regression was observed in the symptoms in the vitamin A treatment group compared with the other treatment group for these reasons and the lower recurrence rate of the disease in the group using vitamin A can be explained, as well.

CONCLUSION

In this study detected that, vitamin A was added to the routine treatment protocol in the treatment of crop candidiasis, it provided faster and more effective recovery. In addition, when vitamin A was used, a significant decrease was observed in the recurrence rate of the disease. Therefore, the use of vitamin A in addition to the treatment of crop candidiasis in birds is recommended as a result of this study.

Contributions: ÇE contributed to the study's conception and design. Material preparation and data collection were performed by all authors. ÇE contributed data analysis, writing, and editing the last draft of the manuscript. The manuscript has been read and approved by all named authors.

Conflict of interest: The authors declare no competing interests.

Ethics Committee Permission: This study was approved by the Ondokuz Mayıs University Animal Experiments Local Ethics Committee (2024-49).

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ARAŞTIRMA MAKALESİ **RESEARCH PAPER**

Determination of Fatty Acids in Seeds of *Morus alba* L. (White Mulberry), *Morus nigra* L. (Black Mulberry) and *Morus nigra-pandula* L. (Flanking Mulberry) Grown in Şanlıurfa province

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Atıf yapmak için: **Aslan, M. (2025).** Şanlıurfa ilinde yetiştirilen *Morus alba* L. (Beyaz dut), *Morus nigra* L. (Siyah dut) ve *Morus nigra-pandula* L. (Sarkık dut) türlerinin tohumlarında yağ asitlerinin belirlenmesi. *Anadolu Çev. Hay. Bil. Derg.*, ***10***(3), 256-260. https://doi.org/10.35229/jaes.1608561

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Abstract: In this study, the fatty acid profiles of three mulberry species white (Morus alba L.), black (Morus nigra L.), and floppy mulberry (Morus nigra pandula L.) cultivated in Şanlıurfa in 2021 were analyzed. Oils were extracted from the seeds using the Soxhlet method, and the fatty acid compositions were identified through gas chromatography. A total of ten different fatty acids were detected in the mulberry seeds. Among them, linoleic acid was the most abundant across all three species, followed by significant amounts of palmitic acid, oleic acid, and stearic acid. The study also calculated the atherogenic and thrombogenic indices, finding that the values were well below the recommended limits. Overall, the results highlight that all three mulberry species are rich sources of linoleic acid, an essential fatty acid, and have potential applications in the food, pharmaceutical, and cosmetic industries.

Keywords: Black mulberry, fatty acid, flankıng mulberry, Şanlıurfa, white mulberry.

Şanlıurfa İlinde Yetiştirilen *Morus alba* L. (Beyaz dut), *Morus nigra* L. (Siyah dut) ve *Morus nigra-pandula* L. (Sarkık dut) Türlerinin Tohumlarında Yağ Asitlerinin Belirlenmesi

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Öz: Bu çalışmada 2021 yıllında Şanlıurfa'da yetiştirilen beyaz *(Morus alba* L.), siyah (*Morus nigra* L.) ve sarkık dut (*Morus nigra pandula* L.) türlerinin yağ asitleri kompozisyonu belirlenmiştir. Dut çekirdeklerinden sokshelet ekstraksiyon yöntemiyle yağlar ekstrakte edilmiş ve gaz kromatografisi yöntemiyle yağ asidi bileşimleri tespit edilmiştir. Dut meyvelerinde toplam on farklı yağ asidi tanımlanmıştır. Her üç türde de linoleik asit en yüksek oranda tespit edilen yağ asidi olmuştur. Palmitik asit, oleik asit ve stearik asit tespit edilen diğer önemli yağ asitleridir. Çalışmada ayrıca aterojenik ve
trombojenik indeksler de belirlenmiştir. Elde edilen değerlerin önerilen limitin oldukça altında olduğu saptanmıştır. Sonuçlar her üç dut çeşidinin de esansiyel bir yağ asidi olan linoleik asit bakımından önemli bir kaynak olduğunu ve gıda, ilaç ve kozmetik gibi alanlarda kullanılabileceğini göstermiştir.

Anahtar kelimeler: Beyaz dut, siyah dut, sarkık dut, Şanlıurfa, yağ asidi.

INTRODUCTION

Fatty acid composition in plants can vary even among individuals of the same species. An increase in temperature during seed development tends to reduce linoleic acid levels while raising the concentrations of oleic, palmitic, and stearic acids (Samanci & Ozkaynak, 2003). Research has shown that seeds produced in colder climates generally contain higher levels of linoleic acid (Nagaraj & Reddy, 1997), whereas seeds from warmer climates have elevated oleic acid levels (Pritchard et al., 2006). High temperatures negatively impact the synthesis of linoleic and linolenic acids but enhance the production of oleic acid (Weiss, 1983; Stryer, 1986; Röbbelen et al., 1989). In sesame populations, stearic and oleic acid contents increase from northern to southern latitudes, while palmitic and linoleic acid contents decrease. These shifts are largely attributed to environmental differences such as climate and soil conditions across different ecological regions (Uppstrom, 1995; Cuniberti et al., 2004; Fatwa & Namzer,

2023). The fatty acid profiles of seeds are influenced by a variety of factors, including ecological, morphological, physiological, and agricultural conditions, as well as genetic variation (Anastasi et al., 2000). Furthermore, the ongoing changes in fatty acid distribution from seed formation through maturation are referred to as ontogenetic variability (Baydar, 2000; Baydar et al., 2006). The Moraceae family includes 24 species of mulberry. Plants of the *Morus* genus are either monoecious or dioecious, milky-sapped, and can be either deciduous trees or shrubs. Their leaves are typically alternate, though they can occasionally be opposite, simple, or palmately lobed. The flowers are arranged in cymes, and they produce aggregate fruits of various shapes (Davis, 1982). Mulberries thrive in temperate, tropical, and subtropical climates, adapting to a wide range of environmental and soil conditions. They have become naturalized across Asia, Europe, and the Americas, with major cultivation regions including the Middle East, East and Southeast Asia, Africa, Europe, and the Americas (Ercisli & Orhan, 2007; Hussain et al., 2017; Zhang et al., 2018; Ustun-Argon et al., 2019). In Turkey, mulberry cultivation has a history spanning over 400 years, with *Morus alba* (white mulberry), *Morus nigra* (black mulberry), and *Morus rubra* (red mulberry) being the most commonly grown species. Mulberries are enjoyed fresh and are also processed into products such as molasses, pestil (fruit leather), köme (a walnut-filled fruit roll), marmalade, juice, and liqueur (Aydin et al., 2015; Gecgel et al., 2011; Hussain et al., 2017; Ustun-Argon et al., 2019). Between 2017 and 2021, Turkey's annual mulberry production across 15 provinces averaged 73,383 tons, with specific yearly outputs of 66,647 tons in 2018, 69,317 tons in 2019, 70,620 tons in 2020, and 69,475 tons in 2021.

Mulberry fruits have long been used in traditional medicine across Turkey and many other countries for various purposes, including as an antihelminthic, treatment for toothaches, expectorant, laxative, emetic, and remedies for sore throat, fever, thirst, dysentery, and oral lesions (Turan et al., 2017; Ustun-Argon et al., 2019). They are also recognized for their potential pharmacological benefits in managing cholesterol, diabetes, oxidative stress, and obesity, owing to their rich content of polyphenolic compounds (Chan et al., 2016; Turan et al., 2017; Zhang et al., 2018). Mulberry seed oil is a source of linoleic and linolenic acids essential fatty acids that humans cannot synthesize and must obtain through diet. These fatty acids are vital for building cell membranes, supporting brain and nervous system development and function, and regulating hormone production (Sonta et al., 2021; Ercisli & Orhan, 2007; Hussain et al., 2017). The purpose of this study was to analyze the fatty acid profiles, as well as the atherogenicity (AI) and thrombogenicity (TI) indices, of oils extracted from three mulberry varieties White mulberry (BD) (*Morus alba* L.), Black mulberry (KD) (*Morus nigra* L.), and Flanking mulberry (SD) (*Morus nigra-pendula* L.) and to assess their potential health impacts

MATERIAL AND METHOD

On May 20, 2021, samples of white mulberry, black mulberry, and drooping mulberry were collected from trees located at the Osmanbey Campus of Harran University. The collected plants were assigned collector numbers: white mulberry as Aslan 5044, black mulberry as Aslan 5045, and drooping mulberry as Aslan 5046. Photographs of the mulberry samples are shown in Figure 1. The sampling area, Osmanbey settlement, lies north of the Harran Plain, where the plain meets the Germüş Plateau (Benek et al., 2016), as shown in Figure 2. Seeds were separated from the mulberry fruits by washing with water and subsequently dried. A sample of 5–10 grams of dried and ground seeds was placed into a filter paper cartridge and loaded into an extractor connected to a balloon. The cartridge was set up to enable the circulation of petroleum ether. After six hours of extraction, the petroleum ether was recovered through distillation. The resulting oil, contained in a glass flask, was placed in an oven at 103°C to evaporate any remaining ether, then cooled in a desiccator and weighed. The oil content was calculated relative to the dry weight of the seeds (Uylaşer and Başoğlu, 2000). The oils from the ground seeds of the mulberry varieties were extracted using the Soxhlet extraction method.

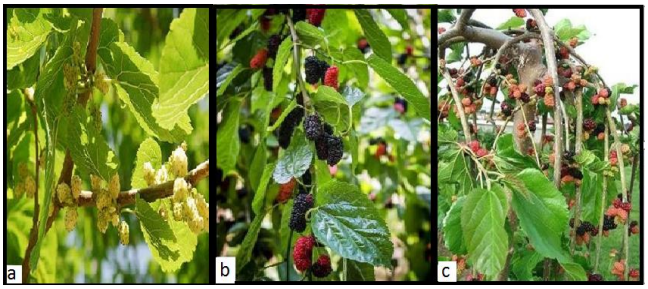

Figure 1. *Morus alba* (White mulberry), b; *Morus nigra* (Black mulberry) c; *Morus nigra-pandula* (Flankıng mulberry)

All laboratory analyses in this study were conducted at the Central Laboratory of Harran University (HUBTAM). A 100 µL oil sample was placed into a 20 mL test tube, and 2 mL of isooctane was added. The tube was sealed and shaken thoroughly. Next, 200 µL of 2 N KOH solution in methanol was added to the tube and vortexed for 1 minute. After approximately 30 minutes, the upper clear layer was transferred into a vial. A 1 µL sample was extracted using an injector and injected into the GC-FID device (TS-EN ISO 12966-2, 2017). The analysis was performed using a Thermo Trace GC Ultra model gas chromatograph with FID.

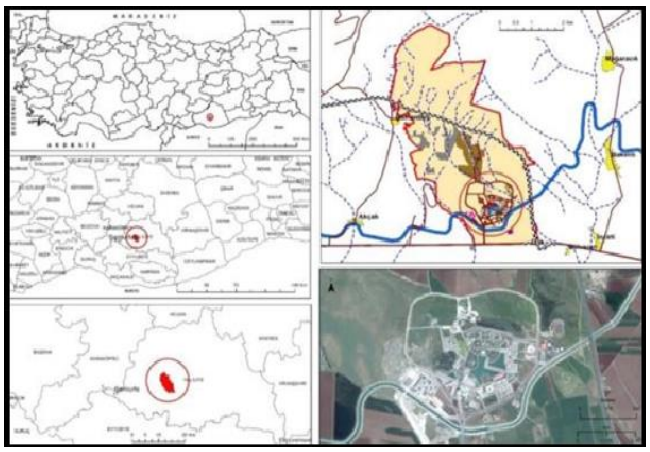

Figure 2. Osmanbey campus area where mulberry samples were taken (Map by Benek et al., 2016)

A Flame Ionization Detector (FID) was used for the analysis. A 60 m HP-88 column was employed to separate the fatty acids. The temperatures for the detector and injection block were set at 280°C and 250°C, respectively. A temperature program was applied to the column, starting at an initial temperature of 100°C. This temperature was raised by 10°C/min to 180°C, then by 5°C/min to 220°C, where it was held for 5 minutes. The split ratio was set at 1/100, and the injection volume was 1 µL.

Atherogenicity (AI) and thrombogenicity (TI) indices are used to evaluate the potential effects of fatty acids on cardiovascular health. These indices are widely recognized and reliable for assessing fats in both solid and liquid forms. Analyzing these indices provides valuable insight into the different impacts of individual fatty acids on human health, particularly concerning atherosclerosis, blood clot formation, increased risk of atheroma, and thrombus development (Tilami, 2022). The indices were calculated using the formulas proposed by Ulbricht (1991).

$$AI=(4\times C14:0+C16:0)/(\sum MUFA+\sum PUFA);$$

$$TI=[(C14:0+C16:0+C18:0)/(0.5\times\sum MUFA+0.5\times\sum n6\ PUFA+3\times\sum n3\ PUFA+(n3/n6))].$$

A computer program was employed to statistically analyze the results. The outcomes from three replicates are presented as the mean value ($\bar{x}$) and standard deviation (SD). Fatty acids were evaluated using analysis of variance (ANOVA), and mean comparisons were conducted using the Tukey test. Differences between means were considered significant at $p<0.05$.

RESULTS AND DISCUSSION

The fatty acid components of the 3 mulberry varieties used in our study are given in Table 1.

Table 1. Fatty acid compositions and AI and TI indices of mulberries of BD, KD and SD cultivars.

Fatty acids (%)	White mulberry (BD) *($\bar{x}$±ss)*	Black mulberry (KD) *($\bar{x}$±ss)*	Drooping mulberry (SD) *($\bar{x}$±ss)*
Myristic Acid (C14:0)	0.057±0.012	0.047±0.006	0.050±0.010
Palmitic Acid (C16:0)	10.843±0.230^{c}	10.287±0.225^{b}	8.777±0.129^{a}
Palmitoleic Acid (C16:1)	0.090±0.010^{a}	0.073±0.015^{ab}	0.057±0.006^{b}
Heptadecanoic Acid (C17:0)	0.063±0.006	0.073±0.012	0.073±0.006
Heptadecanoic Acid (C17:0)	0.063±0.006	0.073±0.012	0.073±0.00
Heptadecanoic Acid (C17:0)	0.063±0.006	0.073±0.012	0.073±0.006
Heptadecenoic Acid (C17:1)	0.037±0.006^{b}	0.027±0.006^{a}	0.030±0.000^{ab}
Stearic Acid (C18:0)	3.520±0.070^{ab}	3.393±0.086^{a}	3.560±0.070^{b}
Oleic Acid (C18:1n9c)	8.003±0.675^{a}	9.280±0.735^{b}	9.463±0.225^{b}
Linoleic Acid (C18:2n6c	76.733±0.469	7.,307±0.856	76.987±0.180
α-Linolenic Acid (C18:3n3)	0.443±0.021^{a}	0.413±0.015^{a}	0.813±0.050^{b}
Arachidic Acid (C20:0)	0.147±0.006^{b}	0.090±0.020^{a}	0.137±0.006^{b}
SFA	14.630±0.140^{c}	13.890±0.123^{b}	12.597±0.047^{a}
MUFA	8.130±0.671^{a}	9.380±0.755^{b}	9.550±0.229^{b}
PUFA	77.177±0.461	76.720±0.862	77.800±0.226
ω3	0.443±0.021^{a}	0.413±0.015^{a}	0.813±0.050^{b}
ω6	76.733±0.469	76.307±0.856	76.987±0.180
ω3/ ω6	0.006±0.000^{a}	0.005±0.000^{a}	0.011±0.001^{b}
AI	0.130±0.003^{c}	0.122±0.003^{b}	0.103±0.002^{a}
TI	0.329±0.004^{c}	0.311±0.004^{b}	0.271±0.002^{a}

Sybols: a-b: Different letters indicate significant differences in column SFA: saturated fatty acids.
MUFA: monounsaturated fatty acids PUFA: Polyunsaturated fatty acids among species.

A total of 10 distinct fatty acids were found in mulberry seed oils. The most prevalent fatty acids were linoleic acid, palmitic acid, oleic acid, and stearic acid. Linoleic acid had the highest concentration among the species, ranging from 76.307% to 76.987%. The linoleic acid content in BD (76.733%) aligns with the values reported by Ustun-Argon, İlhan, Gökyer, Ozturk, and Koparal, Rahman (2019) and Sánchez-Salcedo et al. (2016), but is higher than those found by Ercisli & Orhan (2007). The linoleic acid levels in KD are similar to those reported by Gecgel et al. (2011) and Sánchez-Salcedo et al. (2016), though they exceed the values from Ercisli & Orhan (2007).

The palmitic acid content was highest in SD (10.843%) and lowest in BD (8.777%). The BD values in this study align with those reported by Rahman and Sánchez-Salcedo et al. (2016). However, Ustun-Argon et al. (2019) found lower values, while Ercisli & Orhan (2007) observed higher values. The KD values in this study were consistent with those of Gecgel et al. (2011), but lower than those reported by Ercisli & Orhan (2007) and Sánchez-Salcedo et al. (2016). The lowest oleic acid content was found in BD (8.003%), and the highest in SD (9.463%). In comparison, Zhang et al. (2010), Ustun-Argon et al. (2019), and Sánchez-Salcedo et al. (2016) reported lower values for BD, while Ercisli & Orhan

(2007) reported higher values. For the KD species, the oleic acid levels reported by Gecgel et al. (2011) and Sánchez-Salcedo et al. (2016) were lower, while those from Ercisli & Orhan (2007) were higher. Stearic acid values were similar across species, averaging around 3.5%. In BD species, the values from Ustun-Argon et al. (2019) and Sánchez-Salcedo et al. (2016) matched those of this study, while they were lower than those reported by Rahman and Ercisli & Orhan (2007). For KD species, the values were similar to those of Sánchez-Salcedo et al. (2016) but lower than those from Gecgel et al. (2011) and Ercisli & Orhan (2007). No significant differences in polyunsaturated fatty acids (PUFA) were found between species, although saturated fatty acids (SFA) were higher in BD, and monounsaturated fatty acids (MUFA) were higher in SD.

The AI and TI indices are values that reflect the interactions between various fatty acids and their impact on human coronary health. The AI value represents the potential to prevent plaque aggregation, reduce cholesterol and phospholipid levels, thereby protecting against coronary diseases. On the other hand, the TI index indicates the blood's tendency to clot (Ulbricht, 1991; Busova & Dorko, 2021). Consuming foods with low AI and TI values is advantageous for cardiovascular health, with recommendations suggesting these values should be below 1 (Ouraji et al, 2009; Karslı, 2021). The AI and TI indices for the current study are provided in Table 1, which shows that both indices are below the recommended value of 1. The AI index was lowest in SD (0.103) and highest in BD (0.130). Similarly, the TI index was lowest in SD (0.271) and highest in BD (0.329). Our literature review did not find any studies on this topic related to mulberry seed oils. However, Tilami (2022) found that the AI and TI values for most seed oils were under 1 in his research.

CONCLUSION

This study analyzed the fatty acid profiles of white, black, and drooping mulberry varieties, as well as their atherogenicity (AI) and thrombogenicity (TI) indices. The main fatty acids identified were linoleic, palmitic, oleic, and stearic acids, with linoleic acid—an essential polyunsaturated fatty acid—being the most prevalent, making up over 76% of all varieties. The AI and TI values were notably lower than the recommended threshold of 1, suggesting positive health implications. These results imply that mulberry varieties could be a valuable natural source of essential fatty acids, particularly linoleic acid. In comparison, previous studies on fig and mulberry seed oils reported different fatty acid compositions, including palmitic acid (6–7%), oleic acid (15-16%), linoleic acid (29-31%), and linolenic acid (41-42%) (Duman & Yazıcı, 2018). In contrast, this study found higher levels of linoleic acid (76-77%) and linolenic acid (44-52%) in mulberry seeds, while palmitic and oleic acid levels were slightly higher and lower, respectively. These findings suggest a distinct fatty acid composition in mulberry kernel oil compared to fig kernel oil, with mulberry oil being especially rich in linolenic acid. Overall, the fatty acid profile and the balance between saturated and unsaturated fats are consistent with existing research. Linoleic acid, known for its cardiovascular benefits, is particularly noteworthy (Boelhouwer, 1983; Slama et al., 2020; Akgün & Başhan, 2024). Similar variability was observed in the seed oil of Opuntia ficus indica, which contains 9.32% palmitic, 3.11% stearic, 16.8% oleic, and 70.3% linoleic acid (Ennouri et al., 2005). This highlights that fatty acid profiles can vary among plant species and may change depending on various factors. Understanding these differences is crucial for evaluating oil quality and determining the best uses. Thus, knowledge of fatty acid composition can aid in cultivating specific mulberry varieties suited for particular applications and environmental conditions.

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ARAŞTIRMA MAKALESİ **RESEARCH PAPER**

A Comparative Study for Some Chemical Properties of Packaged Natural Mineral Water Sold in Sivas (Türkiye)

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Abstract: In the study, some chemical properties of packaged natural mineral water sold in Sivas were researched and compared with sticker values. The paired t-test statistic was used for comparisons between the sticker and laboratory findings. There were statistically significant differences for fluoride (p=0.042*), magnesium (p=0.009**) and sulfate (p=0.000**) between sticker and laboratory findings. The maximum values were observed to be as pH (8.30), calcium (85.60 ppm), chloride (6.60 ppm), fluoride (0.12 ppm), iron (0.03 ppm), magnesium (21.10 ppm), potassium (1.50 ppm), sulphate (8.00 ppm) in sticker findings and pH (8.14), calcium (39.98 ppm), chloride (4.00 ppm), fluoride (0.01 ppm), iron (0.30 ppm), magnesium (23.97 ppm), potassium (0.00 ppm), sulphate (0.00 ppm) in laboratory findings for water samples. These results obtained from all packaged natural mineral water samples were below the proposed standards of national and international for drinking water. At the same time, the fluoride content of packaged natural mineral waters in Sivas was determined to be fairly small which may be detrimental in the health of local inhabitants for dental carries. Hereby, the people of Sivas should be selective when deciding which packaged natural mineral waters to drink.

Keywords: Health, mineral, natural, packaged, water, quality.

Sivas (Türkiye)'da Satılan Ambalajlanmış Doğal Mineralli Suların Bazı Kimyasal Özellikleri için Karşılaştırmalı Bir Çalışma

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Öz: Bu çalışmada, Sivas'da satılan ambalajlanmış doğal mineralli suların bazı kimyasal özellikleri araştırılmış ve etiket değerleri ile karşılaştırılmıştır. Etiket ve laboratuvar bulguları arasındaki karşılaştırmalar için eşleştirilmiş t-testi kullanılmıştır. Etiket ve laboratuvar bulguları arasında florür (p=0.042*), magnezyum (p=0.009**) ve sülfat (p=0.000**) için istatistiksel olarak önemli farklar bulunmuştur. Maksimum değerler, etiket bulguları için pH (8.30), kalsiyum (85.60 ppm), klorür (6.60 ppm), florür (0.12 ppm), demir (0.03 ppm), magnezyum (21.10 ppm), potasyum (1.50 ppm), sülfat (8.00 ppm); laboratuvar bulguları için pH (8.14), kalsiyum (39.98 ppm), klorür (4.00 ppm), florür (0.01 ppm), demir (0.30 ppm), magnezyum (23.97 ppm), potasyum (0.00 ppm), sülfat (0.00 ppm) olarak belirlenmiştir. Tüm ambalajlanmış doğal mineralli su örneklerinden elde edilen bu sonuçlar, içme suyu için önerilen ulusal ve uluslararası standartların altındadır. Aynı zamanda, Sivas'da ambalajlanmış doğal mineralli suların florür içeriğinin oldukça düşük olduğu belirlenmiştir ve bu da yerel halk sağlığına diş çürümeleri şeklinde olumsuz etkiler yapabilir. Bu vesile ile Sivas halkı, hangi ambalajlı doğal mineralli suları içeceğine karar verirken seçici davranmalıdır.

Anahtar kelimeler: Ambalajlanmış, doğal, kalite, mineral, sağlık, su.

INTRODUCTION

One of the most important reasons that distinguishes the Earth from other planets and why it is a living environment for living things is the presence of water. Water is one of the indispensable needs of human life on earth. There are no calories, sugar, oil, cholesterol and caffeine in water. Water is of increasing strategic importance. Water, which is of vital importance for people and is also of great importance for health, must first of all be reliable. In this regard, individuals generally turn to consuming packaged water (Karakuş et al. 2016). Water consumption per capita in the world is around 800 m^3 per year on average. Approximately 1.4 billion people, corresponding to approximately 20 percent of the world's population, lack adequate drinking water, and 2.3 billion

people long for healthy water. Some estimates show that more than 3 billion people will face water scarcity by 2025. According to The Food and Agriculture Organization, in 2025 the ratio of the population experiencing water scarcity and water stress to the world population is estimated to increase by 34 percent and 15 percent, respectively (Tümer et al. 2016). Packaged waters are a source of drinking water that is increasingly used in both developing and developed countries. Due to the excessive variability in the mineral content of packaged waters, the public should have access to information about the minerals, since the total dissolved solids range from almost zero to several thousand milligrams per liter (WHO, 2009). Despite its economic costs and environmental damage, the use of packaged water is increasing worldwide. This is linked to the accessibility of packaged water, its convenience, taste and the belief that it is safer than tap water. Packaged water production in Türkiye in 2020 is 4.6 billion liters and this production amount is expected to increase year by year (Ulvi et al. 2022). Unfortunately, Türkiye is a country on the verge of water scarcity. Türkiye's rainfall rate is below the world average, and rapid increase in migration and climate changes cause the picture to gradually deteriorate. For this reason, water management must be in a way that eliminates the problem within the country (Ögenler and Okuyaz, 2017).

The quality of consumed water directly affects health (Şavik et al. 2012). Packaged drinking waters in Türkiye are defined in four different classes. These are natural spring water, drinking water, processed drinking water and natural mineral water. The natural mineral water is usually seen as a product that has medical value because of the high amounts of dissolved mineral (Güler, 2007). Natural mineral water is the groundwater, which is naturally occurring under suitable geological conditions at various depths of the earth's crust, is extracted by one or more sources from the earth spontaneously or by technical methods, is defined by mineral content, residue elements and other components and protected against all kinds of pollution risks. The most important feature that separates natural mineral waters from other drinking waters is that they contain a specific amount of mineral at the time they are obtained from the source. Natural mineral waters have different chemical compositions and their mineralization can range from a few milligrams to several grams per one liter of water. Due to some substances and minerals they carry, mineral waters have additional positive effects on health as compared to spring waters (Karagülle, 2004). To date, no studies have been conducted regarding the quality of packaged natural mineral waters sold in the province of Sivas. In the study, some chemical properties of six different brands of packaged natural mineral water widely consumed by the people of Sivas were researched and compared with sticker values.

MATERIAL AND METHOD

***Description of Study Area*:** The study area is Sivas province located in the central Anatolian part of Türkiye. According to the 2018-year address based population registration system, the population of the province center is 470589. The province covers an area of 27202 square kilometers. The altitude of the province is about 1285 meter. It is bordered by Erzincan to the east, Yozgat to the west, Ordu and Tokat to the north, Kahramanmaraş and Malatya to the south, Giresun to the northeast and Kayseri to the southwest. Türkiye's road and railway network linking the four side passes through Sivas. In Sivas, agriculture, husbandry, weaving, leatherwork, mining and small handcrafts are the most important sources of income (Doğan, 2007). Sivas is one of the coldest provinces in Türkiye. The winter months are freezing cold and the average winter temperature is around 0 °C in Sivas. It is observed that the temperature decreased to -36.4 °C. In summer the temperature is usually above 19 °C. However, it is seen that the temperature in summer is over 38 °C. As can be understood from this, the annual temperature difference shows a big difference of 74 °C. The average number of days the temperature drops below 0 °C is 132 days in Sivas (Pürlü, 2013).

Sampling Design and Procedure: Brands of packaged natural mineral waters were bought through randomly sampling from local markets and shops in Sivas province of Türkiye. All water samples were collected during May 2018 - April 2019. Container sizes of the collected samples are 0.5 L. The packaged mineral water samples of six different brands were collected in Sivas. Water samples are given a code number between 1 and 6 to keep their brand names secret. Permite numbers of packaged natural mineral water samples were recorded as MS.66.01 for brand number of 1, MS.54.08 for brand number of 2, MS.01.01 for brand number of 3, MS.80.01 for brand number of 4, MS.54.01 for brand number of 5 and MS.54.05 for brand number of 6. Similarly, permission dates of packaged natural mineral water samples were determined as 08 September 2009 for brand number of 1, 03 February 2012 for brand number of 2, 09 January 2010 for brand number of 3, 26 October 2016 for brand number of 4, 06 October 2009 for brand number of 5 and 20 July 2010 for brand number of 6. The analysis results on the stickers of the packaged mineral water samples were used as comparison data. Packaged water sticker values are the license values obtained from Provincial Health Directorates of the Türkiye Ministry of Health. Provincial Public Health Directorates make taken water samples analyzes. The consequences of the analysis are based on the mineral water samples taken from the final filling point, which is the last point to be used for water consumption in the labels of the natural mineral waters.

Concentrations of parameters are in ppm, except pH. In this study the amount of pH, calcium, chloride, fluoride, iron, magnesium, potassium, sulphate of six different brands were analyzed and compared with labeled values. The pH of the packaged mineral water samples was measured by a digital HI 2211 Hanna pH meter. Fluoride and iron were measured by colorimetric method using Chembio test kits CB5100 and CB5090 respectively. Potassium and sulphate of the packaged water samples were analyzed by titrimetric method using Chembio test kits CB5390 and CB5450, respectively. Chloride and total hardness were measured by titrimetric method using Aquamerck test kits 1.11106.0001 and 1.08039.0001 respectively. Calcium and magnesium of the packaged water samples values were reckoned according to the formula of total hardness change (Höll, 1979; Kasımlıoğlu & Yılmaz, 2014). Also, laboratory and sticker values of six packaged natural mineral water samples widely consumed in Sivas were compared and discussed with the international drinking water regulations (ECDWD, 1998; TWRHC, 2005; EPA, 2014; CDWQ, 2017; WHO, 2017).

Statistical Analysis: The dependent sample paired t-test was performed to assessment the differences of packaged natural mineral waters in Sivas between the sticker and laboratory findings. The Kolmogorov Smirnov test was used to evaluate the normal distribution of observations. Statistical analyzes were performed with IBM SPSS 22.0 package program. There was a statistically significant difference between the laboratory and sticker values in cases $p<0.05$ (at 95 percent confidence level) and $p<0.01$ (at 99 percent confidence level).

RESULTS AND DISCUSSION

Nowadays, people drink bottled waters by buying. Water is very important for human health. But it is even more important to know what is the property of drinking water is. However, it is very difficult to know the nature and property of the water flowing through the creek, fountain or any resource. The characteristics of packaged water are written on the stickers. Drinkers of packaged water should read the stickers well and know what they drink. The natural mineral water is rich in mineral content. Minerals in these waters help to meet the body's mineral needs. This type of water in the stickers "Natural Mineral Water" is written in capital letters. In addition, what are the minerals in these waters and their quantities are written on the stickers. When the natural mineral water is consumed, the body's water needs are mixed. In addition, the essential minerals in the body help us to meet our daily mineral needs by getting into the blood through rapid absorption in the intestines. It is not possible to supply water with other liquids and foods. Nothing replaces the water. The most important supporting evidence for this is that all minerals in natural mineral waters are present in certain proportions. The packaged natural mineral water is widely used for drinking due to its potential benefits. All of the samples researched packaged natural mineral water is produced in different cities of Türkiye. There is no packaged natural mineral water company in Sivas. Sold in Sivas, the mentioned packaged water resources are mostly located in Sakarya province of Türkiye followed by Adana, Osmaniye and Yozgat provinces. Samples of six brands of packaged natural mineral water of 0.5 litres capacity were collected randomly from Sivas. Descriptive statistics and comparisons of sticker and laboratory findings of some chemical properties in packaged natural mineral water samples are summarized in Table 1. The highest pH (8.30), calcium (85.60 ppm), chloride (6.60 ppm), fluoride (0.12 ppm), potassium (1.50 ppm) and sulphate (8.00 ppm) levels were determined in sticker findings of packaged natural mineral water, whereas the highest iron (0.30 ppm) and magnesium (23.97 ppm) levels were measured in the laboratory findings of packaged natural mineral water in Sivas (Table 1). It was found that calcium was the highest parameter in the sticker and laboratory findings. Also, sticker mean findings of pH, calcium, chloride, fluoride, potassium and sulphate were found to be higher than laboratory mean findings. However, sticker mean values of iron and magnesium were determined to be lower than in laboratory mean findings.

Table 1. Descriptive statistics and chemical of sticker and laboratory findings of packaged natural mineral waters.

Parameters	Sticker Findings				Laboratory Findings				Paired t-test	
	Min	Max	Mean	±SD	Min	Max.	Mean	±SD	t-value	p-value
pH	7.55	8.30	8.05	0.27	7.53	8.14	7.89	0.21	1.217	0.278
Calcium	19.37	85.60	39.36	23.66	18.56	39.98	29.75	8.11	0.955	0.384
Chloride	1.07	6.60	2.69	2.23	1.00	4.00	2.00	1.26	1.680	0.154
Fluoride	0.00	0.12	0.06	0.04	0.01	0.01	0.01	0.00	2.712	0.042*
Iron	0.00	0.03	0.01	0.01	0.00	0.30	0.10	0.12	-2.022	0.099
Magnesium	1.70	21.10	6.48	7.26	11.13	23.97	17.83	4.86	-4.098	0.009**
Potassium	0.00	1.50	0.36	0.56	0.00	0.00	0.00	0.00	1.581	0.175
Sulphate	2.74	8.00	5.83	1.77	0.00	0.00	0.00	0.00	8.052	0.000**

Min: Minimum, Max: Maximum, SD: Standard Deviation, Asterisks Notation (*: $p<0.05$; **: $p<0.01$) indicate the significance level of the comparisons in the Table 1.

According to the ECDWD (1998), TWRHC (2005) and EPA (2014) standards the tolerable range of pH in drinking water should be 6.50-9.50 unit. The pH ranged from 7.53 to 8.30 in sticker and laboratory findings in Sivas (Table 1) it also observed within the ECDWD (1998), TWRHC (2005) and EPA (2014) standards for drinking water. According to the results of the dependent sample t-test conducted to test whether the difference between the

mean values of the sticker and laboratory values of the chemical parameters were statistically significant (Table 1), there was no significant difference in pH between the sticker and the laboratory mean values (p=0.278). The mean pH (8.05±0.27) was determined in the stickers, whereas the mean pH (7.89±0.21) was measured in the laboratory (Table 1). Therefore, packaged natural mineral water samples are alkaline in Sivas. One of the most important factors determining the valuable of water for the body is pH. In recent years, various drops and carbonates have been recommended to make drinking water alkaline. On this subject, a comment can be made by reading the pH value of water in stickers. Alkaline water to pH 9.5 means that it is healthier for the body. In other words, the pH value of the packaged waters should be from 7.1 to 9.5 for public health. There is no need to use such drops and carbonates to make the water alkaline. When buying packaged natural mineral water, the pH value of 7.1 and above should be preferred for drinking. Therefore, it is crucial to check the pH value of the packaged natural mineral water in stickers.

In this study, calcium cation ranged from 18.56 to 85.60 ppm were determined in sticker and laboratory findings, whereas magnessium cation ranged from 1.70 to 23.97 ppm were determined in sticker and laboratory findings for packaged natural mineral water samples (Table 1). Calcium and magnesium are very important for human health. Calcium and magnesium help the vessels and muscles work regularly. Insufficient intake of the calcium and magnesium may adversely affect health. Food is the main source of calcium and magnesium. The available proof shows that, due to food habits, many people in most countries are unable to obtain the recommended intake of one or both of these nutrients from their diet. Calcium and magnesium concentrations in drinking water vary significantly from one source to another. In some populations, rich mineral drinking water can contribute significantly to the total intake of these nutrients (WHO, 2009). For calcium and magnesium have not suggested any guideline value by the WHO (2017). According to the results of the dependent sample t-test conducted to test whether the difference between the mean values of the sticker and laboratory values of the chemical parameters were statistically significant (Table 1), the mean difference between the sticker and the laboratory values of the magnesium value was found to be statistically important at the 99 percent confidence level (t=-4.098; p<0.01). For magnesium in Sivas, the sticker mean value ($\bar{x}$=6,48) is lower than the mean of the laboratory value ($\bar{x}$=17,83). However, there was no significant difference in calcium between the sticker and the laboratory mean values (p=0.384).

According to the by ECDWD (1998), TWRHC (2005), EPA (2014), CDWQ (2017) and WHO (2017) standards of chloride anion should not exceed 250 ppm for drinking water. High levels of chloride cause a salty taste in water and beverages. The taste levels for the chloride are due to the associated cation. Chloride values exceeding 250 ppm increase the likelihood of taste detection. A guideline value is not suggested for chloride (WHO, 2017). In this study, chloride anion varied from 1.00 to 6.60 ppm in sticker and laboratory findings for packaged natural mineral water samples (Table 1). The results show that the chloride anion in Sivas is below the standard limit. According to the results of the dependent sample t-test conducted to test whether the difference between the mean values of the sticker and laboratory values of the chemical parameters were statistically significant (Table 1), there was no significant difference in chloride between the sticker and the laboratory mean values (p=0.154).

According to the ECDWD (1998), TWRHC (2005), CDWQ (2017) and WHO (2017) standards the permissible guideline value of fluoride anion should be 1.50 ppm for drinking water. Fluoride is an element that has harmful effects on the body in its deficiency and excess. Low amounts of fluoride have negative effects on dental health, while high amounts of fluoride accumulate in teeth and bones and cause a disease known as fluorosis. The most important feature of fluoride is the protective effect against dental caries. Addition of fluoride to water sources at levels above 0.6 ppm indicates a decrease in tooth decay in children. Optimum beneficial effects of fluoride are realized at about 1.0 ppm. The parametric level for the fluoridated supplies is 1.0 ppm. However, the parametric level for the supplies with naturally occurring fluoride is 1.5 ppm (EPA, 2014). Fluoride is widely used in areas of high sugar intake to reduce tooth decay. These are tablets, mouthwashes, toothpaste, varnishes or gels. In addition, fluoride can be added to table salt or drinking water to provide protection against tooth decay. The amounts added to the drinking water are generally between 0.5 and 1 ppm. Above 1.5 ppm increase the risk of dental fluorosis. Higher levels cause skeletal fluorosis (WHO, 2017). According to Dissanayake (1991), if fluoride falls below 0.5 ppm in drinking water, it causes dental caries; If fluoride is between 0.5 and 1.5 ppm, it is optimal for dental health; if fluoride is between 1.5 and 4 ppm, it causes fluorosis in the teeth; If fluoride is between 4 and 10 ppm, it causes dental and skeletal fluorosis and if fluoride is above 10 ppm, it causes crippling fluorosis. In this study, fluoride anion varied from 0.00 to 0.12 ppm in sticker and laboratory findings for packaged natural mineral water samples (Table 1). The results show that the fluoride anion in Sivas is below the standard permissible limit by ECDWD (1998), TWRHC (2005) and WHO (2017). According to the results of the dependent sample t-test conducted to test whether the difference between the mean

values of the sticker and laboratory values of the chemical parameters were statistically significant (Table 1), the difference of fluoride value between the sticker value and laboratory value was found to be statistically significant at 95 percent confidence level (t=2.712; p<0.05). For fluoride in Sivas, the sticker mean value ($\bar{x}$=0,06) is higher than the mean of the laboratory value ($\bar{x}$=0,01). Such a low fluoride level may adversely affect public health because it is necessary for the body. The mean value of fluoride of packaged natural mineral waters samples in Sivas was found to be remarkably lower than the proposed level by Dissanayake (0.5-1.5 ppm) the best for dental health. Since the mean value of fluoride anion is low in Sivas, the use of these packaged natural mineral waters as a continuous drinking water can lead to an increase in frequency of tooth decay for the population. This should be seen as a warning on the stickers, or the addition of fluoride to the water, providing the best standard of 0.5-1.5 ppm necessary for the inhibition of tooth decay.

According to the ECDWD (1998), TWRHC (2005) and EPA (2014) allows maximum allowable level of iron cation is 0.20 ppm for drinking water. The iron is between 0.5 and 50 ppm in natural fresh waters. Although turbidity and color can be present, there is usually no noticeable taste at iron levels below 0.3 ppm. A guide value for iron was not suggested (WHO, 2017). In this study, iron cation observed from 0.00 to 0.30 ppm in sticker and laboratory findings for packaged natural mineral water samples (Table 1). The results show that the iron cation in Sivas is below the standard limit. Table 1, indicates there was no significant difference in iron between the sticker and the laboratory mean values (p=0.099).

In this study, the potassium anion observed from 0.00 to 1.50 ppm in sticker and laboratory findings for packaged natural mineral water samples (Table 1). Potassium is important for health in humans. Potassium in drinking water is rarely found in alarming levels in drinking water. It was not necessary to create for potassium a health based guideline value in drinking water (WHO, 2017). Table 1, indicates there was no significant difference in potassium between the sticker and the laboratory mean values (p=0.175) in Sivas.

According to the ECDWD (1998), TWRHC (2005) and WHO (2017) standards have based 250 ppm as the highest acceptable level of sulphate anion. Sulfate may cause a significant taste in drinking water. High levels of sulfate can have a laxative effect on consumers. The taste disorder varies according to the associated cation. The taste disorder is minimal below 250 ppm. No value was recommended for the sulphate (WHO, 2017). In this study, the sulphate anion observed from 0.00 to 8.00 ppm in sticker and laboratory findings for packaged natural mineral water samples (Table 1). The results show that the sulphate anion in Sivas is below the standard limit for ECDWD (1998), TWRHC (2005) and WHO (2017). According to the results of the dependent sample t-test conducted to test whether the difference between the mean values of the sticker and laboratory values of the chemical parameters were statistically significant (Table 1), the mean difference between the sticker and the laboratory values of the sulphate value was determined statistically significant at the 99 percent confidence level (t=8.052; p<0.01). For sulphate in Sivas, the sticker mean value ($\bar{x}$=5,83) is higher than the mean of the laboratory value ($\bar{x}$=0,00).

Also, a narrow variability of price was determined in the all samples of Sivas that the 0.5 L price of packaged natural mineral water ranged from a minimum of 0.50 Turkish Lira to a maximum of 1.00 Turkish Lira, while the average with standart deviation was 0.71±0.24 Turkish Lira. Even though it brings a financial burden, packaged natural mineral waters are one of the appropriate ways to meet the water needs of consumers in Sivas.

Most of the mean sticker values were higher in packaged natural mineral water samples than the mean laboratory values (Table 1). The comparison of the analyzed and recorded data using the dependent sample paired t-test indicated that the sticker findings were significantly higher than the laboratory findings for Fluoride (t=2.712; p=0.042*) and sulfate (t=8.052; p=0.000**). Conversely, according to the results of the dependent t-test comparison, laboratory findings were significantly lower than the label findings for magnesium (t=-4.098; p=0.009**). However, the pH (t=1.217; p=0.278), calcium (t=0.955; p=0.384), chloride (t=1.680; p=0.154), iron (t=-2.022; p=0.099) and potassium (t=1.581; p=0.175) in the sticker findings were not determined statistically different from laboratory findings. The water quality properties in the stickers are normally for water obtained at the resource, but the resource can change according to the quality over time. These differences between sticker and laboratory concentrations of packaged natural mineral water components presumably indicate that they are affected by post-packaging conditions. Especially, considering the low and high temperature in Sivas province may be as low -36.4 ºC and high 38 ºC, it may affect the concentrations of water components in packaged natural mineral water. Therefore, this study shows that packaged natural mineral water needs to be more strictly controlled in determining and monitoring its quality. Relevant authorities should strictly monitor the post-packaging conditions and quality of packaged natural mineral water provided to consumers to protect public health. In addition, further studies on microbiological tests and analysis of heavy metals are recommended for packaged natural mineral water.

CONCLUSION

Natural mineral waters contain many minerals such as calcium, magnesium, chloride, fluoride, iron. In this way, many minerals needed by the body are taken with drinking water. Because of the mineral content, natural mineral waters are a positive contribution to human health. This study highlighted that all packaged natural mineral water samples sold in Sivas were appropriate for drinking. Results like pH, calcium, chloride, fluoride, iron, magnesium, potassium and sulphate were in the range proposed by the ECDWD (1998), TWRHC (2005), EPA (2014), CDWQ (2017) and WHO (2017). On the other hand, the fluoride content of packaged natural mineral waters in Sivas was found to be significantly lower than the recommended level by Dissanayake (<0.5 ppm), and causing dental caries. For this reason, it is beneficial to pay attention to this situation when buying packaged natural mineral water in the province of Sivas.

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ARAŞTIRMA MAKALESİ **RESEARCH PAPER**

Abundance and Stock Management of Whiting (*Merlangius merlangus*) in Southeastern Black Sea Fisheries

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Abstract: In this study, 1579 *Merlangius merlangus* were obtained from bottom trawling operations (12 mm cod mesh size) using the research vessel 'Karadeniz' at depths between 5 m and 60 m off the coasts of Rize, Southeastern Black Sea. The collected samples' growth, recruitment, and stock structure were assessed using the ELEFAN-I, Y/R, B/R, VPA, and Thompson and Bell models within the FISAT-II software package. The von Bertalanffy growth parameters, including asymptotic length (L_∞), growth constant (K), and t_0, were calculated as 40.89 cm, 0.10 cm, and -1.1411 cm, respectively. The first catch length (Lc_{50}) varied between 11.82 cm and 13.40 cm. Total, natural, and fishing mortality rates were estimated at 0.85, 0.29, and 0.56, respectively. Three distinct recruitment periods were identified, occurring in March, August, and November. Based on VPA data, the fixed biomass was estimated at approximately 569.620,7 tonnes. The analysis suggests that the maximum sustainable yield (MSY) for whiting could be reached with an exploitation rate of 0.66. At the current exploitation level, calculated at 449.70 tonnes using the Thompson and Bell method with an F-factor of 0.56, the biomass has decreased. An F-factor of 1.0, representing the MSY, corresponds to 498.180 tonnes. At the current fishing rate (F = 0.56), the biomass has been reduced by 25% (168,026 tonnes) from its unfished state of 666,206 metric tonnes.

Keywords: Whiting, maximum sustainable yield (MSY), relative yield per recruit (Y′/R), relative yield per biomass (B′/R), stock, southeastern black sea.

Güneydoğu Karadeniz Balıkçılığında Mezgit (*Merlangius merlangus*) Bolluğu ve Stok Yönetimi

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Öz: Bu çalışmada, 1579 *Merlangius merlangus*, Güneydoğu Karadeniz, Rize sahillerinde 5 m ile 60 m arasındaki derinliklerden araştırma gemisi 'Karadeniz' kullanılarak yapılan dip trolü operasyonlarından (12 mm morina ağı boyutu) elde edilmiştir. Toplanan örneklerin büyüme, yeni birey katılımı ve stok yapısı FISAT-II yazılım paketindeki ELEFAN-I, Y/R, B/R, VPA ve Thompson ve Bell modelleri kullanılarak değerlendirilmiştir. Toplanan örneklerin büyüme, stoka katılım ve stok yapısı FISAT-II yazılım paketindeki ELEFAN-I, Y/R, B/R, VPA ve Thompson ve Bell modelleri kullanılarak değerlendirilmiştir. Asimptotik boy (L_∞), büyüme sabiti (K) ve t_0 dahil olmak üzere von Bertalanffy büyüme parametreleri sırasıyla 40,89 cm, 0,10 cm ve -1,1411 cm olarak hesaplanmıştır. İlk yakalama boyu (L_{c50}) 11,82 cm ile 13,40 cm arasında değişmiştir. Toplam, doğal ve balıkçılık ölüm oranları sırasıyla 0,85, 0,29 ve 0,56 olarak tahmin edilmiştir. Mart, Ağustos ve Kasım aylarında meydana gelen üç farklı katılım dönemi tespit edilmiştir. VPA verilerine dayanarak, sabit biyokütlenin yaklaşık 569.620,7 ton olduğu tahmin edilmiştir. Analiz, mezgit için maksimum sürdürülebilir verime (MSY) 0,66'lık bir sömürü oranı ile ulaşılabileceğini göstermektedir. Thompson ve Bell yöntemi kullanılarak 0,56 F faktörü ile 449,70 ton olarak hesaplanan mevcut sömürü seviyesinde biyokütle azalmıştır. MSY'yi temsil eden 1,0'lık bir F faktörü 498,180 tona karşılık gelmektedir. Mevcut avlanma oranında (F = 0.56), biyokütle avlanmamış durumdaki 666,206 metrik tondan %25 (168,026 ton) azalmıştır. Balıkçılık çabaları mevcut seviyede devam eder veya artarsa, *M. merlangus* stoklarının önemli ölçüde azalması beklenmektedir. Balıkçılık verimliliğindeki bu düşüşün, *M. merlangus* popülasyonlarına zarar verebilecek aşırı avlanma veya sürdürülemez uygulamalara işaret etmektedir. *M. merlangus*'un uzun vadeli sürdürülebilirliğini sağlamak için balık stoklarının ve balıkçılık faaliyetlerinin etkin yönetimi gereklidir.

Anahtar kelimeler: Mezgit, maksimum sürdürülebilir verim (MYS), stoklara katılım başına nispi verim (Y′/R), biyokütle başına nispi verim (B′/R), stok, güneydoğu karadeniz.

INTRODUCTION

Whiting (*Merlangius merlangus*) is widely distributed throughout the eastern Atlantic, including the North, Baltic, Black, and Mediterranean Seas. Its stock status varies regionally, with this cold-water species adapting to the temperate waters of the Black Sea, where it is commonly found in sandy, muddy substrates at depths of 60–80 meters and temperatures between 5–16 °C (Karahanlı, 2024; Ross et al., 2018). Whiting's spawning peaks between January and August, though it can occur year-round (Murua & Sabarido, 2003; Çiloğlu et al., 2001). Its distribution is dynamic as the fish move in and out of spawning grounds (Heino et al., 2005). Numerous studies have focused on whiting's reproductive patterns, population parameters, and distribution within Türkiye and globally (Yıldız & Karakulak, 2018; Ross et al., 2018; Salihoğlu et al., 2017; Raykov et al., 2008; Prodanov & Bradova, 2003).

Small-scale fishing is crucial for Black Sea nations, and whiting is a significant catch. In the 1960s, 26 fish species were economically fished in the Black Sea, but this has since declined to 21 due to decreased production of valuable species along the coasts of Bulgaria, Romania, Ukraine, Georgia, and Türkiye, which share Black Sea fishery resources (Zengin, 2019; 2000). Black Sea fisheries have traditionally focused on pelagic stocks, but ecological changes and fishing pressure have caused stock declines. With pelagic stocks collapsing, fishing efforts have increasingly shifted to limited demersal stocks through trawl modification (Avşar, 1998). In Türkiye, whiting production was 30.488 tonnes in 1988 but dropped to 9.074 tonnes by 2023 (BSGM, 2023), reflecting increased fishing pressure and ecological shifts that have raised serious sustainability concerns (Bat et al., 2005; Gücü et al., 2017). Whiting, a semi-pelagic species, is caught with bottom trawls in Turkey's central and western Black Sea coasts. In contrast, bottom trawling is prohibited in the Eastern Black Sea, where whiting is fished using trammel nets, handline, and gill nets. In this region, whiting is a primary target species for small-scale fisheries (Bat et al., 2005; Erdem et al., 2006; Kalaycı & Yeşilçiçek, 2014; Aydın & Hacıoğlu, 2017).

Research by Gönener and Özdemir (2013) and Gönener and Bilgin (2010) indicated that whiting dominated total biomass in the Black Sea's coastal waters, crucial data for understanding the ecosystem's dynamics and guiding sustainable fishery management. Another study revealed that whiting accounted for 98.8% of the fish caught in the Black Sea, underscoring its dominance in the region's fishery (BSGM, 2023). Global studies on whiting biomass and stock sizes also indicate recent increases in Black Sea whiting stocks, which may benefit the fishing industry and ecosystem. However, further research is necessary to understand this trend's drivers and potential effects (Nedreas et al., 2014). This study evaluates the stock status, management practices, and population structure of whiting in the Eastern Black Sea, given its economic importance for Türkiye and neighbouring Black Sea countries.

MATERIAL AND METHOD

Sampling area and data source: This study was conducted along Türkiye's southeastern Black Sea from March 2017 to February 2018 (Figure 1). For stock assessment, 1579 *Merlangius merlangus* were obtained from depths between 5 m and 60 m along the Rize coast of the Southeastern Black Sea (Figure 1). The samples were obtained from bottom trawling operations (12 mm cod mesh size) using the 'Karadeniz Araştırma' vessel of Recep Tayyip Erdoğan University.

Figure 1. Study area

Bottom trawling is prohibited across extensive sections of Turkey's Black Sea coastline to safeguard the marine environment. Whiting is exclusively fished beyond the restricted trawling zones (BSGM, 2024).

Laboratory examinations and data analysis: The length of each sample was recorded in the frequency data set after being measured with a precision of 1.0 cm; electronic scales with a precision of 0.01 grams were used to measure body weights. The following non-linear equation was used to establish the relationship between total length and weight for both sexes: $W = aL^b$ (Ricker, 1973). The allometric equation was considered when calculating the length-weight relationship. W stands for body weight (g), L for total length (cm), "***a***" for the regression coefficient, and "***b***" for the allometry coefficient (Ricker, 1973).

Population dynamics model: The Virtual Population Analysis (VPA) software, integrated into the FISAT II package, was utilized to process the data. The FISAT II package facilitated data analysis (Gayanilo et al.,

1997). By using the ELEFAN-I software included with FISAT II, we determined the asymptotic length (L_∞) and growth coefficient (K) (Saeger & Gayanilo, 1986). This estimation was made with the ELEFAN-I tool, part of the FISAT II package (Saeger & Gayanilo, 1986).

The value of t_0 was calculated using Pauly's empirical equation (Pauly, 1979).

$$log_{10}(-to) = -0.3922 - 02752log_{10}L\infty - 1.038log_{10}K$$

The length-converted catch curve method was used to determine total mortality (Z) (Pauly, 1984). The natural mortality rate (M) was calculated using the empirical relationship below (Pauly, 1986).

$$log_{10}M = -0.0066 - 0.279log_{10}L\infty + .06543log_{10}K + 0.4634log_{10}T$$

Where L_∞ represents the asymptotic length (cm), K denotes the VBGF growth coefficient, and T indicates the average annual temperature (°C). The required input for the study months was an average seawater temperature of 16.3 °C for 2017 and 2018 (Anonymous, 2023).

Fishing mortality (F) was calculated using the following relationship:

$F = Z - M$ (Avşar, 2005).

F is fishing mortality, Z is total mortality, and M is natural mortality.

The exploitation rate (E) was determined using Gulland's formula:

$E = {}^{F}/_{Z}$ (Gulland, 1971).

The length at first capture (L_{c50}) was calculated from the length frequency by applying the length-converted catch curve approach in the FISAT-II software. The ascending left arm of the length-converted catch curve was used to determine the capture probability of each length class using the FISAT-II package. By plotting the cumulative probability of capture against the mid-length of the generated curve, the length at the first capture (L_{c50}) was determined. Additionally, the lengths of the 25% and 75% catches were determined to represent the 25% and 75% cumulative possibilities, respectively. The Thompson and Bell method used the first capture lengths (L_{c50}) and (L_{c75}) as inputs.

The NORMSEP (Normsep component separation of normally distributed length-frequency samples) module attached to the FISAT-II package was used to determine the recruitment pattern of the *M. merlangus* population using the maximum probability method.

Y'/R and B'/R were determined using the "Beverton and Holt Y′/R Analysis" method integrated into the Fisat-II software. The capture probabilities ${Lc}/{L\infty}$ and ${M}/{K}$ values for the various length classes were used to determine the relative yield per recruit (Y'/R) and the relative biomass per recruit (B'/R).

The Beverton and Holt method provides the framework for the relative yield per recruit for the stock model given below (Beverton & Holt, 1966).

Relative yield-per recruit $({Y'}/{R}) = EU^{{M}/{K}}[{1 - 3U}/{(1+m)} - 3U^3(1+m)]$

Where $U = 1 - ({Lc}/{L\infty})$; $m = \frac{1-E}{M/K} = {K}/{Z}$; $E = {F}/{Z}$

Relative biomass per-recruit $= ({Y'}/{R})/F$

The first derivative of this function was used to determine $E_{0.1}$, $E_{0.5}$, and E_{max}. The linearised capture curve used to calculate Z was then estimated backward using the natural mortality rate (M) as an input in the FISAT-II package. Pauly's catch-curve analysis estimates the capture probability by projecting parameters and using moving averages (Jones & Van Zalinge, 1981).

Moving average $P_{L,i(new)} = P_{L,i-1} + P_{L,i(old)} + P_{L,(i+1)}/3$

Length-based virtual population analysis (VPA) is an appropriate approach for population reconstruction using fishing data. The VPA was computed using Whiting's length-based data.

The length-based virtual population analysis (VPA) provided fishing estimates of population densities by length class. The FISAT-II package used M, F, L, and K parameters as inputs (Pauly, 1984; Jones & van Zalinge, 1981). Thompson and Bell Yield-Stock estimation software is connected to the FISAT II package.

RESULTS AND DISCUSSION

Growth analysis: As this study aimed to estimate the whiting stock, no distinction was made between male and female samples. A total of 1579 whiting were sampled. Total length and weight ranged from 7.5 to 32.6 cm (mean 14.08±2.88) and 2.71 to 281.25 g (mean 25.19±20.79 S), respectively. When examining the sex of all specimens, 668 were identified as male (42.30%), 877 as female (55.54%), 28 as immature (1.77%), and 6 as of undetermined sex (0.37%). The female-to-male ratio was 1.31:1, and a Chi-square test indicated this ratio significantly deviated from an even 1:1 distribution (χ^2 df=1 = 28.72; $p<0.05$). Additionally, the Kolmogorov-Smirnov (K-S) test revealed a significant difference in the size distribution between males and females ($p<0.05$). The regression coefficient (a) and coefficient of allometry (b) in whiting growth were calculated as 0.0047 and 3.171, respectively (Table 1). Applying Pauly's empirical formula, t_0 was -2.14111 (Pauly, 1979).

Probability of capture : The length at first capture (L_{c50}) for whiting was 13.04 cm. In this sequence, the probabilities of L_{c25} and L_{c75} were estimated at 11.82 and 14.26 cm, respectively (Figure 2).

Table 1. Growth and population parameters of *Merlangius merlangus* in the southeastern Black Sea.

N	a	b	L_∞	W_∞	K	φ	t_0	Lc	Z	F	M	E	M/K	L_{c50}/L_∞	$E_{0.1}$	$E_{0.5}$	E_{max}	$Y_{(t)}$	$Y/U_{(t)}$	$Y/F_{(t)}$	$MSY_{(t)}$
3394	0.0047	3.1709	40.89	605.85	0.10	2.22	-2.1411	13.04	0.85	0.56	0.29	0.66	2.9	0.32	0.507	0.311	0.61	252.55	669.89	450.98	498.180

Y: Yield; Y/U: Total annual stock; Y/F: Standing stock; MSY: Maximum sustainable yield t: Thousand tons

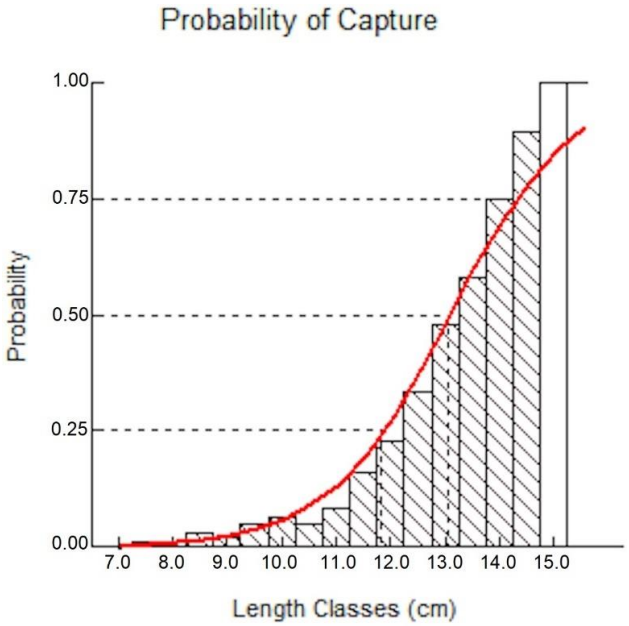

Figure 2. Logistic selection curve for the probability of capture, displaying 25, 50, and 75% selection lengths for *Merlangius merlangus.*

Mortality: Using the length-converted catch curve method, the total mortality rate (T), the natural mortality rate (N), and the fishing mortality rate (F) of the whiting in the southeastern Turkish waters of the Black Sea were estimated to be 0.85, 0.29, and 0.56, respectively. The exploitation rate (E) was estimated to be 0.66 using the same method (Figure 3, Table 1).

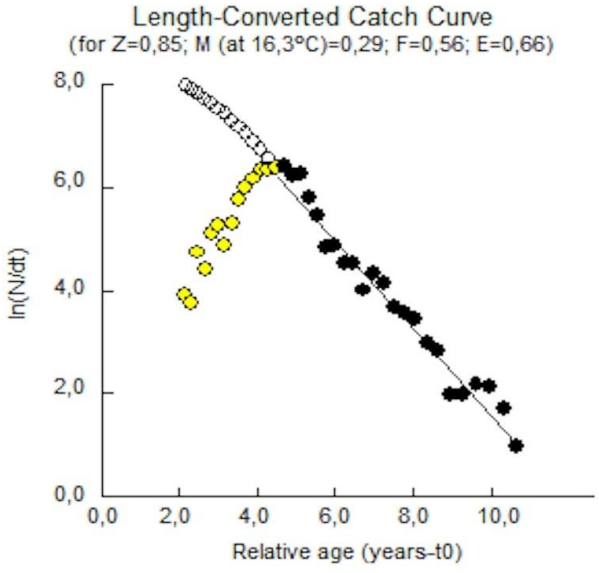

Figure 3. Length-converted catch curve for *Merlangius merlangus.*

Recruitment: Whiting recruitment peaked on the southeastern coast of the Black Sea, Turkish waters in March (16.28%), August (13.13%), and November (14.74%), respectively. However, recruitment fluctuates continuously throughout the year (Figure 4).

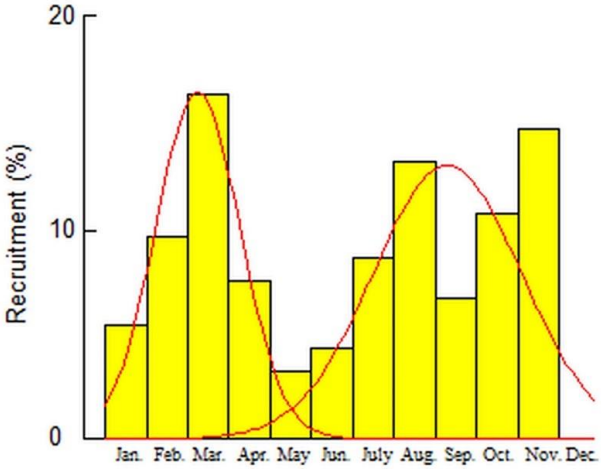

Figure 4. Recruitment pattern for *Merlangius merlangus.*

Length-based Virtual Population Analysis (VPA): Maximum natural mortality (M) and fishing mortality rates (F) in length classes were found to be between 7.5 cm and 11.5 cm and 12.0 cm and 14.5 cm, respectively (Figure 5). The largest length classes most affected by fishing were estimated to be between 14.0 cm and 16.5 cm, 20.0 cm and 25.5 cm, and 32.0 cm and 32.5 cm, respectively (Table 2). Based on the VPA data, the estimated steady-state biomass was 103,384.8 tons.

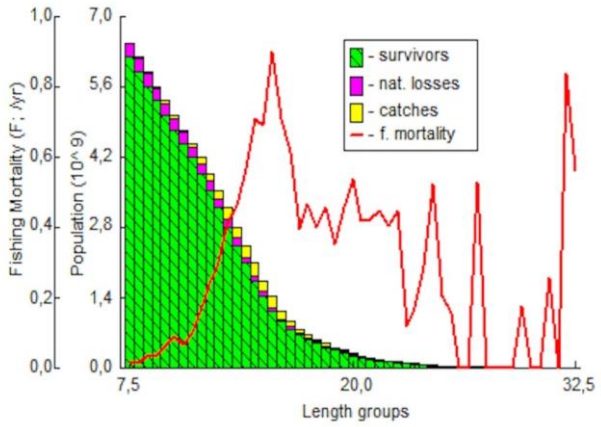

Figure 5. The length-structured VPA for *Merlangius Merlangus* in the southeastern Black Sea

Table 2. Length-based virtual population analysis (VPA).

Mid-Length (cm)	Catch (in numbers)	Population (N)	Fishing mortality (F)	Steady-state Biomass (tonnes)
7.5	16000000	6469807104	0.0169	1348.45
8.0	13000000	6179201024	0.0142	1604.75
8.5	36000000	5899979776	0.0405	1881.88
9.0	29000000	5606476288	0.0338	2178.28
9.5	51000000	5328914944	0.0618	2491.16
10.0	70000000	5038520832	0.0885	2809.96
10.5	50000000	4739102720	0.066	3141.18
11.0	71000000	4469475840	0.0981	3480.95
11.5	119000000	4188539136	0.1737	3794.78
12.0	151000000	3870826240	0.2358	4060.01
12.5	178000000	3534120192	0.3011	4267.09
13.0	223000000	3184689664	0.4158	4385.25
13.5	220000000	2806158592	0.4595	4413.73
14.0	238000000	2447311872	0.5657	4353.71
14.5	256000000	2087304704	0.7106	4167.95
15.0	210000000	1726830208	0.6912	3914.78
15.5	226000000	1428725504	0.9011	3586.73
16.0	146000000	1129993088	0.7099	3253.44
16.5	106000000	924352064	0.6127	3017.99
17.0	59000000	768182272	0.3938	2873.51
17.5	61000000	665738048	0.4641	2764.55
18.0	46000000	566620672	0.4004	2642.94
18.5	46000000	487301344	0.459	2514.94
19.0	31000000	412240192	0.354	2392.04
19.5	35000000	355845728	0.4586	2264.01
20.0	35000000	298715232	0.5397	2085.14
20.5	23000000	244909264	0.4171	1917.76
21.0	20000000	205918560	0.422	1779.76
21.5	18000000	172172880	0.445	1636.62
22.0	14000000	142443664	0.4066	1498.99
22.5	13000000	118457904	0.4453	1364.98
23.0	3000000	96991744	0.1171	1284.8
23.5	4000000	86560672	0.1716	1251.39
24.0	6000000	75801064	0.291	1183.5
24.5	9000000	63822060	0.5218	1057.13
25.0	3000000	49820300	0.2067	948.6
25.5	2000000	42611216	0.1552	897.22
26.0	0	36873016	0	873.55
26.5	0	33451282	0	869.77
27.0	5000000	30244536	0.5303	786.96
27.5	0	22510468	0	705.01
28.0	0	20199908	0	694.5
28.5	0	18050504	0	681.5
29.0	0	16056726	0	666.03
29.5	1000000	14213023	0.1772	624.05
30.0	0	11576899	0	580.79
30.5	0	10133417	0	559.84
31.0	1000000	8813233	0.2568	504.21
31.5	0	6684111	0	449.48
32.0	2000000	5727488.5	0.8384	341.72
32.5	2000000	3035714.25	0.56	537.47
Total	284.800.000.0	761.520.529,34		103.384,83

Yield per–recruit (Y′/R) and biomass per–recruit (B′/R): The study concludes that the Maximum Sustainable Yield (MSY) for whiting can be achieved with current fishing at an exploitation rate of 0.66. E0.1, the exploitation level, led to a marginal increase of 10% in relative yield per recruit, reaching 0.507. The study confidently estimates that the exploitation level at E0.5, equivalent to 50% of the relative biomass per recruit of the unexploited stock, is 0.311. Implementing proper management measures, such as adjusting fishing gear selectivity for larger fish, is essential to minimize the catches of small fish. Relative yield per recruit (Y'/R) analysis results for whiting in the Turkish seas off the eastern Black Sea indicate that increased fishing efforts will yield minimal additional harvest, suggesting a slight economic gain. Moreover, the study findings on biomass per recruit (B'/R) demonstrated a dramatic decrease as the exploitation rate increased (Figure 6).

Stock structure: The VPA analysis based on fish length was utilized for Thompson and Bell's long-term prediction (Thompson & Bell, 1934). This analysis produced the F-factor array, which provides yield, biomass, and values. The current level of fishing (approximately 449.70 tonnes) was predicted by an F-factor of 0.56. An F-factor of 1.0 corresponds to the maximum sustainable yield (MSY) of 498,180 tonnes (Table 3). As fishing pressure increased, the amount of product decreased inversely. However, after reaching the fishing level with an F-factor of 1.0, the amount of product declines by an average of about 2.0–2.5 tonnes for each incremental increase in the fishing level (F-factor) (Table 3). At the current fishing level (F = 0.56), the biomass is reduced by 25% (168.026 tonnes) of the unexploited biomass (666.206 tonnes). In terms of value, the maximum economic return is achieved at an F-factor of 1.0 and then declines (Figure 7).

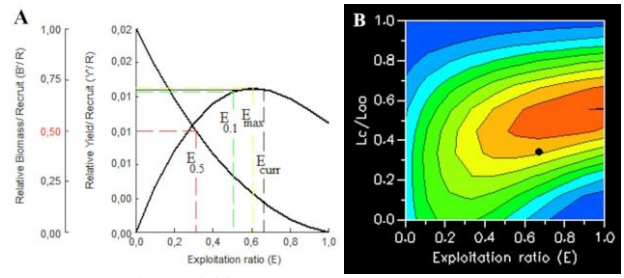

Figure 6. A) Relative yield per recruit, relative biomass per recruit (B′/R), and **B)** Yield isopleth per recruit (Y′/R) of *Merlangius merlangus* in the southeastern Black Sea.

Table 3. Predicted yield and biomass for different fishing mortality rates of *Merlangius merlangus* by the Thompson and Bell method.

F-factor	Yield (tonnes)	Mean biomass (tonnes)
0.0	000.000	666.206
0.2	279.567	431.246
0.4	411.701	298.624
0.5	447.349	254.901
0.6	470.860	221.129
0.7	484.853	194.763
0.9	497.101	157.308
1.0	498.180	143,852
1.2	495.037	123.695
1.4	488.295	109.522
1.6	480.219	99.096
1.9	467.806	87.785
2.0	463.814	84.798
2.2	456.186	79.656
2.3	452.563	77.423
2.4	449.071	75.375
2.6	442.469	71.744
2.8	436.348	68.617
3.0	430.669	65.889
3.2	425.388	63.485
3.4	420.466	61.345
3.6	415.866	59.426
3.8	411.554	57.692
4.0	407.502	56.115

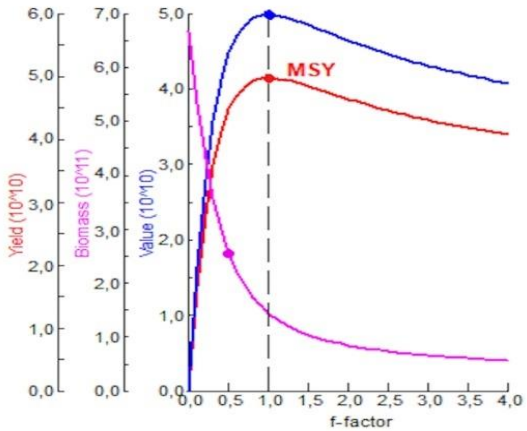

Figure 7. *Merlangius merlangus* yield-stock prediction in the southeastern Black Sea via the Thompson and Bell method.

Our study examined the growth and population parameters of *M. merlangus*, which are essential for practical stock assessment. The length distribution observed in our research ranged from 7.6 cm to 32.9 cm. In comparison, Samsun and Akyol (2017) reported a length range of 8.8 cm to 22.8 cm, while Sağlam and Sağlam (2012) found it to be between 10.0 cm and 26.0 cm. More recently, Şahin et al. (2021) documented a length range of 7.5 cm to 32.6 cm along Turkey's Eastern Black Sea coast, which closely matches our findings. In contrast, the results from Samsun and Akyol (2017) and Sağlam and Sağlam (2012) were narrower. Additionally, a study conducted in the Marmara Sea by Erdem et al. (2006) reported a length distribution for Whiting between 8.89 cm and 24.72 cm, which is more limited than our observed range. These variations may be due to differences in nutrient availability, temperature, and predator presence (Salihoğlu et al., 2017). Importantly, our growth estimates for the fish population provide valuable insights for fisheries management, enhancing understanding of population structure, size distribution in catches, and the size at maturity during fishing.

Table 4. Growth and population parameters of *Merlangius merlangus* in different habitats

Study area	L_∞	W_∞	K	t_0	Z	M	F	E	E_{max}	$Y_{(t)}$	$MSY_{(t)}$	Biomass (1000 t)	Literature
Marmara sea	35.74		0.124	–1.338	1.35	0.34	1.1	0.75					Karadurmuş, 2022
Black sea	31.87	271.01	0.130	–3.071									Şahin et al., 2021
Western Baltic sea	97.3 ♀		0.07										Ross et al., 2018
	58.0 ♂		0.16										
North sea	74.2 ♀		0.16										
	57.9 ♂		0.19										
Western Black sea	37.05		0.106	–1.63	1.19	0.37	0.82	0.69					Yıldız et al., 2018
SE Black sea	33.05		0.13	–2.93	0.98	0.22	0.76	0.77					Kasapoğlu, 2018
Northeast Atlantic Ocean	44.77 ♀		0.35	–0.95									Barrios et al.. 2017
	36.11 ♂		0.35	–1.06									
Black sea											4.941		Salihoğlu et al.. 2017
Mid. Black sea	28.69		0.21	–1.91	0.97	0.50	0.47	0.48					Samsun and Akyol, 2017
Mediterranean and Black sea							0.87-2.50 (F_{curr}) Fmsy/Fcurr=1.06-4.14 Fmsy/Fcurr=1.06 0.37 (F_{curr})				over exploited over exploited		Cardinale and Osio, 2013
SE Black sea	33.56		0.141		1.68	0.27	1.41	0.84					Sağlam and Sağlam, 2012
Southeast Black sea	20.29 ♀		0.805	–0.76									Bilgin et al.. 2012
	18.95 ♂		0.900	–0.24									
Southeast Black sea										40.421			Gönener and Bilgin, 2010
Western Black sea											595.6–606.966	51–68	Raykov et al.. 2008
Northeast Atlantic												8 (mill.t)	Heino et al.. 2005
Black sea												10-40	Ünlüata, 2005
Marmara sea	38.5	431.0	0.15	–1.47									Erdem, 2006
US and Canadian Pacific waters										190.400		1.851.400	Ishimura, 2003
Bulgarian waters	26.63	141.3	0.1981	–1.81									Bradova and Prodanov, 2003
Western Black sea												2.870,6	Prodanov and Bradova, 2003
Black sea	39.1		0.15	–1.53	1.63	0.39	1.24			30188			İşmen, 2002
Southeast Black sea	52.50 ♀		0.092	–2.39									Çiloğlu et al.. 2001
	37.19 ♂		0.114	–1.75									
Southeast Black sea	40.89	605.8	0.10	–2.14	0.85	0.29	0.56	0.66	0.61	252.55	450.98	666.206	This study

mill.t: million tonnes, mt: Metric tonnes, MYS: maximum sustainable yield, *SSB: Spawning stock biomass (mt).

A study conducted along Türkiye's Black Sea coast determined the von Bertalanffy seasonal oscillatory growth parameters as L_∞ = 39.1 cm, K = 0.15, t_0 = -1.05, C = 0.23, and t_s = 0.48, revealing differences in growth rates between males and females. Females grew faster and achieved a greater maximum length than males (Işmen, 2002). Similarly, research by Çiloğlu et al. (2001) on the southeastern Black Sea reported asymptotic length (L_∞) and growth constant (K) values of 52.50 cm and 37.19 cm for males and females, respectively, with t_0 calculated as -1.75 for males and -2.39 for females.

Aksu (2020) provided further insights into whiting growth parameters and length-weight relationships in the Central Black Sea region. For males, the length-weight relationship was $W = 0.0047L^{3}.1694$ ($r^2 = 0.97$), while for females, it was $W = 0.0058L^{3}.077$ ($r^2 = 0.96$). The study estimated von Bertalanffy growth parameters for all sexes as L_∞ = 30.57 cm, K = 0.937 y^{-1}, t_0 = -2.0407, and a growth performance index (φ) of 2.9423. Another study by Mazlum and Bilgin (2014) on the southeastern Black Sea highlighted that the Gompertz model was the most effective method for estimating whiting growth. Female growth parameters were determined as L_∞ = 32.3 cm, K = 0.1414 y^{-1}, t_0 = -4.69, and φ = 2.258, while combined-sex estimates were L_∞ = 22.3 cm, K = 0.2668 y^{-1}, t_0 = 3.87, and φ = 2.123. Sağlam and Sağlam (2012) found that male whiting can live up to 5 years, while females can reach 4 years of age. They calculated the weight-length relationship as $W = 0.0064L^{3}.0441$ and described the von Bertalanffy growth equation for both sexes as $L_t = 33.56(1 - e^{-0.141}(t+2.654))$. The study also determined mortality rates, with total mortality (Z) at 1.68, natural mortality (M) at 0.27, and fishing mortality (F) at 1.41. In another study, Yıldız and Karakulak (2018) found that most of the whiting population consisted of individuals aged 1 to 2 years. They calculated growth parameters for all individuals as L_∞ = 7.05 cm, K = 0.10 y^{-1}, and t_0 = -2, with mortality rates of Z = 1.19 y^{-1}, M = 0.37 y^{-1}, F = 0.82 y^{-1}, and an exploitation rate (E) of 0.69 y^{-1}.

Bilgin et al. (2012) investigated whiting growth and reproductive biology on the southeastern Black Sea, calculating von Bertalanffy growth parameters as L_∞ = 18.95 cm and K = 0.900 for males and L_∞ = 20.29 cm and K = 0.805 for females. These studies collectively emphasize the significance of understanding the growth dynamics of whiting to support effective fishery management and conservation efforts in the region.

Research on fish growth parameters along Türkiye′s Black Sea coast has highlighted consistent trends across multiple studies. For example, İşmen (2002) observed that females generally grow faster and reach larger sizes than males, with von Bertalanffy seasonal growth parameters recorded as L_∞ = 39.1 cm, K = 0.15, t_0 = -1.05, C = 0.23, and t_s = 0.48. Similarly, Çiloğlu et al. (2001) studied the southeastern Black Sea and reported asymptotic lengths (L_∞) of 52.50 cm for males and 37.19

cm for females, with growth rates (K) of 0.0092 and 0.114 and t_0 values of -1.75 for males and -2.39 for females. On the southeastern Black Sea, Erdem et al. (2006) estimated an L_∞ of 31.87 cm, an asymptotic weight (W_∞) of 271.01 g, and a growth rate (K) of 0.130. These findings are broadly consistent with studies along Türkiye′s Black Sea and the eastern Black Sea region (Radulescu, 2023; Şahin et al., 2021; İşmen, 2002; Çiloğlu et al., 2001). In contrast, studies conducted in the Bulgarian and Turkish waters of the Western Black Sea revealed lower growth parameter values (Bilgin et al., 2012; Bradova & Prodanov, 2003). This discrepancy underscores the need for further research. For instance, Barrios et al. (2017) studied the Northeast Atlantic region and reported asymptotic lengths (L_∞) of 44.77 cm for males and 36.11 cm for females, with identical growth rates (K) of 0.35. The t_0 values were calculated as -0.95 and -1.06 for males and females, respectively. Meanwhile, a study in the Western Baltic reported higher L_∞ values for females, attributed to environmental factors such as water temperature, nutrient availability, and predation pressure (Ross et al., 2018).

Numerous studies have also highlighted the negative impact of intensive fishing on *M. merlangus* populations in the Black Sea, the Sea of Marmara, and the Mediterranean Sea (Yıldız et al., 2018; Kasapoğlu, 2018; Cardinale & Osio, 2013; Erdem et al., 2006). Samsun and Akyol (2017) reported mortality rates for fish in the Central Black Sea, with total mortality (Z) at 0.97, natural mortality (M) at 0.50, and fishing mortality (F) at 0.47. In the eastern Black Sea, Sağlam and Sağlam (2012) reported higher rates: total mortality (Z) at 1.68, natural mortality (M) at 0.27, and fishing mortality (F) at 1.41, resulting in exploitation rates (E) of 0.48 and 0.84, respectively. Similarly, Kasapoğlu (2018) found mortality rates in the southeastern Black Sea of 0.98 for total mortality (Z), 0.22 for natural mortality (M), and 0.76 for fishing mortality (F).

In the Marmara Sea, Karadurmuş (2022) observed mortality rates of 1.35 for total mortality (Z), 0.34 for natural mortality (M), and 1.10 for fishing mortality (F), with an exploitation rate of 0.75. İşmen (2002) reported similar trends in the Black Sea, with total mortality (Z) at 1.63, natural mortality (M) at 0.39, and fishing mortality (F) at 1.24. A separate study in the Sea of Marmara recorded a natural mortality rate (M) of 1.35 and fishing mortality (F) of 1.10, resulting in an exploitation rate (E) of 0.75 (Karadurmuş, 2022). Our study found lower total mortality (M) and fishing mortality (F) rates than previous research, aligning closely with other studies on *M. merlangus* populations. This consistency highlights the significance of our findings in advancing the understanding of fish population dynamics and informing fisheries management.

The alarming trend in the Black Sea is driven by increasing pollution, the negative impacts of climate change on fish populations, the global decline in fish stocks, and the simultaneous reduction in fishery products. This study also assessed the relative yield and biomass per fish based on initial catch length. The maximum exploitation rate (Emax), representing the highest relative yield per juvenile fish (Y′/R), was calculated as 0.61, which fell short of the expected value of 0.66. Additionally, the current exploitation rate (E_{curr}) for *M. merlangus* exceeded Gulland's optimum exploitation rate of 0.50 (Gulland, 1971). Although *M. merlangius* catches were high in the eastern Black Sea until 2017, the western Black Sea was reported to have provided more catches after 2017 (Samsun, 2024).

These findings indicate that the *M. merlangus* stock in the study area is being exploited beyond sustainable levels, emphasizing the urgent need for effective fisheries management measures. Modifying fishing gear is recommended to enhance selectivity for larger fish and raise the minimum catch size to reduce the bycatch of smaller fish. The study also identified seasonal peaks in fish recruitment occurring in March, November, and August, underscoring the importance of accounting for seasonal fluctuations when designing fisheries management strategies to protect fish populations during critical life stages.

A study conducted in Bulgarian coastal waters revealed that the biomass and food web dynamics of whiting, which share similar breeding periods and depths with sprat, significantly impact sprat consumption along the coast. During the breeding season, whiting was observed to feed on giant ten-legged crabs and sprat, highlighting substantial competition between these species for shared food resources. Since 1985, reduced replenishment rates for both species have contributed to declining stocks of whiting and sprat (Prodanov & Bradova, 2003).

The survival of *M. merlangus* sperm is heavily influenced by environmental conditions, as it cannot persist in low-salinity, low-oxygen waters. Consequently, the recruitment of eastern Baltic whiting stocks was reported to be poor during periods of water stagnation. Strong year classes, supported by favorable environmental conditions in the late 1970s, were linked to a peak in spawning stock biomass (SSB) of nearly 700,000 metric tons in the early 1980s. However, this was followed by a steady decline, reaching approximately 100,000 metric tons in 1992-the lowest level recorded since 1966 (FAO, 2005).

Continuous recruitment monitoring is vital for evaluating the health and sustainability of fish populations. It offers critical insights for fisheries managers to implement effective harvest regulations and conservation strategies. A study by Henderson (2019) in Bridgwater Bay, UK, identified an intriguing pattern: haddock recruitment peaked five times annually between June and October, with the most pronounced peak occurring in June. This unique finding raises questions and warrants further investigation. Whiting recruitment, influenced by nutrient availability, habitat conditions, predators, and water temperature factors, adds another layer of complexity to marine ecosystems (Henderson, 2019).

In the Pacific waters off the USA and Canada, whiting biomass was estimated at 1.851.400 metric tons (Mt) (Ishimura, 2003). By comparison, our study recorded a whiting stock biomass of 666,203 Mt. The discrepancy between these findings could be attributed to differences in habitat size, water temperature, nutrient levels, fishing pressure, predator presence, and other ecological factors.

The biomass of *Merlangius merlangus* along the Bulgarian coast was recorded as 27,273 tonnes in 1976, 10,893 tonnes in 1988, and 16,072 tonnes in 1991 (Prodanov & Daskalov, 1995). These fluctuations were attributed to findings that the natural reproduction of whiting was severely impacted between 1987 and 1989 (Arkhipov & Rovnina, 1990), leading to a notable decline in juvenile fish after 1987. Similarly, research conducted in the northern Black Sea reported that whiting biomass in the coastal waters of Ukraine and Romania ranged from 595.6 to 606,966 tonnes (Raykov et al., 2008).

In contrast, studies on the west coast of North America reported a whiting yield of 264,000 tonnes, with a maximum sustainable yield (MSY) estimated between 127,000 and 193,000 tonnes (Hollowed & Francis, 1987). The present study estimated whiting biomass of 666,206 tonnes and an MSY of 498,180 tonnes, significantly higher values than those observed along the Bulgarian coast (FAO, 2022; Arkhipov & Rovnina, 1990).

Variations in biomass across regions can be attributed to factors such as pollution, temperature fluctuations, nutrient availability, and predation pressures. Understanding these influences is essential for effective fisheries management and conservation. For example, a study in the Pacific Ocean involving the USA and Canada reported a whiting yield of 264,000 tonnes, with a stock surplus ranging from 127,000 to 193,000 tonnes (Hollowed & Francis, 1987). Furthermore, two studies conducted in the Black Sea estimated that whiting biomass and yield range between 10 and 40 tonnes (Gönener & Bilgin, 2010; Ünlüata, 2005). These differences highlight the importance of environmental factors such as prey availability, habitat temperature, predator dynamics, and fishing pressure, underscoring the relevance of this research in understanding regional and global variations in whiting populations.

CONCLUSION

The resources of *M. merlangus* are being exploited to their maximum capacity, and any increase in fishing efforts is unlikely to lead to higher production. If fishing efforts continue at the current level or increase, *M. merlangus* stocks are expected to decline significantly. This decline in fishing efficiency indicates overfishing or unsustainable practices that could harm *M. merlangus* populations. Effective management of fish stocks, and fishing activities is essential to ensure the long-term sustainability of *M. merlangus*. Balancing fishing and sustainability will require implementing fishing limits, establishing protected areas, promoting responsible fishing methods, and monitoring population health.

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ARAŞTIRMA MAKALESİ RESEARCH PAPER

Determination of Changes in Polycyclic Aromatic Hydrocarbon (PAH) Levels in Hazelnut Oils After Storage

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Abstract: The oil of hazelnuts collected from a hazelnut garden in Piraziz District of Giresun Province in two different years, 2022 (called as S1) and 2023 (called as S2), was obtained and the polycyclic aromatic hydrocarbon (PAH) levels, known to have carcinogenic effects, were analyzed depending on the storage time and condition. The optimum parameters were maintained and analyzed by GC-MS. The highest total concentration of polycyclic aromatic hydrocarbons (Σ16PAHs) was 8.483 ± 0.032 ng/kg in sample S1 and 6.075 ± 0.024 ng/kg in sample S2. The value of naphthalene, the most abundant PAH analyte, is higher than that of the other analytes. The total amount of PAHs indicates that 92.35% of sample S1 and 87.82% of sample S2 are naphthalene. Notably, benzo[a]pyrene (BaP), a known carcinogen, was not detected in either sample.

Keywords: GC-MS, Hazelnut oils, Polycyclic aromatic hydrocarbons (PAHs), SPE sample preparation.

Depolama Sonrası Fındık Yağlarındaki Polisiklik Aromatik Hidrokarbon (PAHs) Düzeylerindeki Değişimlerin Belirlenmesi

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Öz: Giresun İli Piraziz İlçesindeki bir fındık bahçesinden; 2022 (S1 olarak kodlanan) ve 2023 (S2 olarak kodlanan) iki farklı yılda toplanan fındıkların yağı elde edilmiş ve bekleme süresine ve koşuluna bağlı olarak, kansorejen etkisi bilinen polisiklik aromatik hidrokarbon (PAH) değişimleri ölçülmek istenmiştir. Optimum parametreler sağlanarak GC-MS ile analizler gerçekleşmiştir. Polisiklik aromatik hidrokarbonların (Σ16PAH) en yüksek toplam konsantrasyonu S1 örneğinde 8,483 ± 0,032 ng/kg ve S2 örneğinde 6,075 ± 0,024 ng/kg olarak bulunmuştur. PAH standart karışımı içerisinde, en fazla miktardaki analit olan naftalinin bulunma miktarı diğerlerinden daha yüksektir. Toplam PAH miktarı, S1 örneğinin %92,35'inin ve S2 örneğinin %87,82'sinin naftalin olduğunu göstermektedir. Bilinen bir kanserojen olan benzo[a]piren (BaP) her iki örnekte de tespit edilmemiştir.

Anahtar kelimeler: GC–MS, Fındık yağları, Polisiklik aromatik hidrokarbonlar (PAH'lar), SPE numune hazırlama.

INTRODUCTION

The quality and safety of food are significant global concerns, with data on foodborne clinical incidents reaching alarming levels and posing a serious threat to human health. To ensure the safety of the food supply, it is essential to obtain, process and protect food in environments that do not adversely affect human health or create environmental problems. Nutrient contamination can occur at any stage of the production and transportation chain. Similarly, foods can become contaminated by bacteria and other microorganisms, pesticides, food packaging materials, detergents, disinfectants and other cleaning agents. Among the chemical hazards that threaten human health are polycyclic aromatic hydrocarbons (PAHs) (Balcıoğlu and Ceylan, 2019).

Hazelnuts are among the most widely cultivated hard-shelled fruits in the world. Turkey, one of the few countries in the world with favorable weather conditions for

hazelnut production, is at the forefront of world hazelnut production (FAO, 2017). Approximately 2/3 of hazelnut production and exports, which are among the traditional export products, are provided by Turkey (Yıldız, 2020), hazelnut oil is also produced in Turkey. The fat content of hazelnuts, which are rich in macronutrients and micronutrients, varies between 50% and 73% depending on the region, soil and hazelnut variety (Kesen et al., 2016). Hazelnut, which is a nut with a high fat content (~60%), is very rich in oleic, linoleic and palmitic fatty acids. Considering the content of hazelnuts and their effects on human health, they play a key role in human nutrition (Topçu, 2022). Hazelnuts contain high amounts of single and multiple fatty acids known to be effective in preventing heart disease (Crews et al., 2005) and are also good sources of high levels of minerals and B vitamins (B1, B6), vitamin E and natural antioxidants (Alasalvar et al., 2003; Köksal et al., 2006). Hazelnut is a good source of protein containing essential amino acids, mainly arginine, leucine, phenylalanine and valine, in addition to its high-quality oil content. The Hazelnut protein ratio is reported to vary between 9.3% and 22.5% g/100 g (Amaral et al., 2006a; Çetin et al., 2020; Köksal et al., 2006; Şahin et al., 2022; Venkatachalam and Sathe, 2006). They are also characterized by a high fat content with triacylglycerols as the main component and a high proportion of monounsaturated fatty acids, giving hazelnuts high nutritional value. The production of hazelnut crude oil involves the use of physical processes and extraction techniques, which do not involve chemical processing. The high oleic acid content facilitates digestion within the body. The amino acids present in hazelnuts have been demonstrated to exert anticancer, antidiabetic and arteriosclerotic effects (Alasalvar et al., 2003). Hazelnut oil is distinguished by its high content of oleic acid, a monounsaturated fatty acid (Alasalvar et al., 2006; Amaral et al., 2006a; Çetin et al., 2020; Crews et al., 2005). Hazelnut oil, which contains high levels of unsaturated fatty acids and small amounts of saturated fatty acids, has also been demonstrated to exert beneficial effects on human health, including a reduction in the risk of developing cardiovascular disease (Alasalvar and Shahidi, 2009; Mağden, 2023; WHO, 2019).

Polycyclic aromatic hydrocarbons (PAHs) are recognized as hazardous organic pollutants with the potential to cause cancer, genetic mutations and teratogenesis (Sakshi and Haritash, 2020). PAHs are organic compounds comprising at least two benzene rings arranged in angular, linear or cluster chains (Akinpelu et al., 2019; Bianco et al., 2023). Soil quality is a critical factor for plant, animal and human life, and soil pollution has detrimental consequences for a wide range of living organisms, with serious implications for human health as humans are the last link in the food chain. The levels of PAHs present within soil are influenced by various factors, including proximity to the source of pollution, temperature, and prevailing climatic conditions (Ortiz et al., 2012; Bozlaker et al., 2008; Chen et al., 2011; and Tasdemir and Esen, 2007a). In industrial areas, anthropogenic burning activities and atmospheric deposition constitute an important source of PAH pollution in soils (Smith and Harrison 1996; Lee et al., 2001). In urban areas, the use of fossil fuels for heating purposes and traffic density cause the PAH concentrations in the soils of the region to increase day by day (Nadal et al., 2004; Bozlaker et al., 2008).

PAHs are complex mixtures of light and heavy compounds in various categories of foods and beverages (Purcaro et al., 2013). PAHs are derived from the incomplete combustion of organic matter during industrial processes and other human activities (Bansal and Kim, 2015). Food processing, such as smoking, grilling, roasting and frying, can lead to the formation of high amounts of PAHs (Kim et al., 2021; Zelinkova and Wenzl, 2015). Unfortunately, edible vegetable oils, in addition to many other nutrients, are highly susceptible to organic contaminants due to their hydrophobic nature, and PAHs with high lipophilicity are considered a type of food contaminant commonly found in edible oils (Galeotti et al., 2017; Jing et al., 2021). PAH compounds have the potential to contaminate crops through air, water and soil in areas with industrial production. Many vegetable oils are contaminated by the environment during seed growth and coexist with hazardous substances such as mycotoxins, heavy metals, PAHs and pesticide residues (Ji et al., 2023). PAHs enter the human food chain extremely easily through the consumption of vegetable oils, posing a serious threat to human health. In recent years, serious concerns have been raised by consumers and health professionals about the presence of various toxigenic PAHs in foods.

In recent years, there has been considerable interest in PAH studies in Turkey, as well as worldwide. A substantial body of research has demonstrated that edible oils are susceptible to contamination by polycyclic aromatic hydrocarbons (PAHs). This finding has been corroborated by studies conducted by Camargo et al., (2012), Tfouni et al., (2014), Molle et al., (2017) and Ji et al., (2022). Furthermore, numerous studies have been conducted to characterize the lipid fraction of hazelnuts and evaluate its stability during shelf storage (Cialiè Rosso et al., 2021; Ghirardello et al., 2014; Savage et al., 1997; Turan, 2018). This study focuses on hazelnut plants and hazelnut oil, which are among the most important plants in our country, with production and export shares of over 70% worldwide. For this purpose, hazelnut samples were obtained from a garden in the Piraziz district of Giresun Province, It was aimed to monitor the PAH changes that may occur in the oil of

hazelnuts, which are expected to depend on the storage time and condition, and analyzed by GC-MS in this line. In addition to the analysis of the fatty acid methyl ester content of the hazelnut oil (FAME analysis), an attempt was made to determine the PAH content and accumulation.

MATERIAL AND METHOD

Chemicals and standards: The solvents hexane, dichloromethane, and acetone, which were grade for liquid chromatography, and all other chemicals used were purchased from Merck (Darmstadt, Germany). A PAH standard mixture that contained 16 compounds (100 ng/mL; 96–99.9% purity) was obtained from Sigma-Aldrich (Missouri, USA). (St. Louis, MO, USA). These compounds included naphthalene (Nap), acenaphthene (Ace), fluorene (Fle), phenanthrene (Phe), anthracene (Ant), fluoranthene (Flu), pyrene (Pyr), benz(a)anthracene (BaA), chrysene (Chr), benzo(b)fluoranthene (BbF), benzo(k)fluoranthene (BkF), benzo(a)pyrene (BaP), dibenz(a,h)anthracene (DahA), indeno(1,2,3-cd)pyrene (IcdP) and benzo(g,h,i)perylene (BghiP). Deionized water (resistivity of 18.2 MU cm-1) was produced by a Milli-Q water purification system (Millipore Co., USA).

Standard Preparation and Calibration Curves: The standard PAH solution (100 ng/mL) was diluted and stored in the dark at -20 °C. The six-point calibration curves corresponding to the PAH compounds were prepared at different concentrations because the concentration of each PAH analyte in the standard mixture is different. Each point was the average of three injections, and the target analytes were quantified via the external calibration method.

Sample collection and preparation for PAHs: Hazelnut samples were collected from the same nut trees in the Piraziz district of Giresun, Eastern Black Sea Region, during the harvest periods of 2022 (S1) and 2023 (S2) (Fig. 1) and extracted with hexane for 8 hours by Soxhlet to obtain oil. S1 hazelnuts were kept in a sack in the warehouse for a year, as the people did. A 5 g oil sample was weighed into a 10 mL volumetric flask, diluted to volume with n-hekzan and sonicated in an ultrasonic bath. Then, a 1.0 mL sample was extracted in an SPE cartridge previously washed with 20 mL of dichloromethane, dried completely by means of vacuum, and conditioned with 20 mL of n-hexane. The mixture of n-hexane: dichloramethane was 70:30 (v/v). PAHs were eluted, and the first 8 mL of eluent was discharged. The following 8 mL fraction was collected and concentrated under N_2 to approximately 20-30 µL, and the last residue was dissolved in 100 µL ACN and run in a GC-MS analysis system (Ergönül and Sanchez, 2013; Teixeira et al., 2007).

Apparatus and GC-MS System: The samples were filtered with SPE cartridges packed with a silica phase (Chromabond C18, octadecyl-modified silica, Germany) PAH mixture, and the samples were analyzed by a GC–MS instrument system (Agilent-Model 7890A-5975C inert MSD) in selected ion monitoring (SIM) acquisition mode. The method conditions of the chromatographic system were as follows: carrier gas, helium; flow rate, 1.5 mL/min; and initial injection port temperature, 30 °C. One microliter of the sample was run in pulsed splitless mode. An Agilent 5% HP-5MS phenyl methyl Silox column (30 m × 250 µm × 0.25 µm) was used, and the pressure was 18.635 psi. The GC oven parameters were set to increase from 55 °C for 1 min, then 25 °C/min to 320 °C for 5 min, and finally increased to 320 °C at 16.6 min. The mean recovery of PAHs ranged from 85.23% to 101.36% in this study (Table 1). Each standard mixture at different concentrations was analyzed in triplicate, and the standard deviation was < 5%.

Fig 1. Locations of the hazelnut samples.
Şekil 1. Fındık örneklerinin yerleşim yerleri.

RESULTS AND DISCUSSION

PAH analysis: Table 1 shows the results for the RT, LOD and LOQ ranges. The detection limits ranged from 0.25 ng/g to 0.43 ng/g. The mean recovery of PAHs ranged from 85.23% to 102.64% in this study (Table 2).

Table 1. Methodical description of PAH standard.

PAH	RT (min)	Identification (ion)	LOD (ng/g)	LOQ (ng/g)
Nap	5.544	(128) 129.127.102	0.21	0.50
Acy	6.306	(152) 151.150.153	0.22	0.55
Ace	6.486	(153) 154.152.151	0.25	0.60
Flu	6.977	(166) 165.167.163	0.35	0.70
Phe	7.898	(178) 176.179.152	0.36	0.72
Ant	7.941	(178) 176.179.152	0.31	0.65
Flua	9.049	(202) 203.200.101	0.24	0.58
Pyr	9.266	(202) 203.200.101	0.33	0.68
BaA	10.427	(228) 226.229.113	0.35	0.70
Chr	10.468	(228) 226.229.113	0.41	0.80
BpF	11.408	(252) 253.250.126	0.43	0.82
BkF	11.430	(252) 253.250.126	0.40	0.78
BaP	11.683	(252) 253.250.126	0.25	0.60
Inp	12.730	(276) 138.274.277	0.32	0.68
BghiP	12.997	(278) 276.279.139	0.34	0.69
DahA	13.021	(276) 138.274.277	0.30	0.62

Generally people who own hazelnut gardens prefer to keep hazelnuts for 1 year and sell them to be more economically profitable. Hazelnut samples collected in 2022 and 2023. PAH changes were investigated and analyzed. The

effects of 1 year of storage on the PAH contents and the total PAH contents are given in Table 3.

Table 2. Certified and observed concentrations (µg/mL) and % recoveries of all PAH analytes.

PAH	Certified	Observed	% Recovery
Nap	998.6	992.4	99.38
Acy	2000.2	1830.2	91.50
Ace	995.4	1002.5	100.71
Flu	198.6	201.3	101.36
Phe	98.7	95.6	96.86
Ant	99	98.1	99.09
Flua	196.2	197.5	100.66
Pyr	98.5	101.1	102.64
BaA	100.3	100.6	100.30
Chr	99.7	100.2	100.50
BpF	199.4	196.5	98.55
BkF	99.9	99.4	99.50
BaP	99.6	99.4	99.80
Inp	99.9	85.14	85.23
DahA	199.8	200.6	100.40
BghiP	199.2	201.4	101.10

Table 3. PAH16 components of the samples (ng/kg).

ng/kg	S-1	S-2
Naphthalene	7.834±0.015	5.335±0.021
Acenaphthylene	0.152±0.010	0.144±0.013
Acenaphthene	0.015±0.001	0.036±0.012
Fluorene	0.022±0.012	0.018±0.015
Phenanthrene	0.057±0.000	0.042±0.016
Anthracene	ND	ND
Fluoranthene	0.024±0.001	0.027±0.014
Pyrene	0.024±0.001	0.025±0.045
Benzo(a)anthracene	0.008±0.001	0.052±0.051
Chrysene	0.008±0.001	0.062±0.014
Benzo(b)fluoranthene	0.116±0.014	0.120±0.027
Benzo(k)fluoranthene	0.059±0.001	0.061±0.014
Benzo(a)pyrene	ND	ND
Dibenzo(a.h)anthracene	0.076±0.001	0.104±0.029
Benzo(g.h.i)perylene	0.088±0.001	0.047±0.010
Indenol(1.2.3-CD)pyrene	ND	ND
Σ16PAHs	8.483±0.032	6.075±0.024

The samples were harvested during the 2022 (S1) and 2023 (S2) seasons.

The detection rate of naphthalene was the highest among the PAH concentrations (S-1: 7,834 and S-2:5,335). Naphthalene was the most abundant PAH analyte, with 92.35% in sample S1 and 87.82% in sample S2. The sensitivity of hydrocarbons to microbial adsorption varies according to differences in their structure. These sensitivities can be listed in the following order: n-alkanes > branched alkanes > small-molecular-weight aromatics > cycloalkanes (Perry, 1984). The highest degradation rates were observed for the soluble compounds. This is followed by light aromatic compounds and high-molecular-weight aromatic compounds (Jobson et al., 1972). Occurrence rates of high-molecular-weight polycyclic aromatic hydrocarbons including naphthalene and phenanthrene were found to be associated with their dissolution in water rather than the total substance concentration (Thomas et al., 1986). The high level of naphthene observed in our study can be attributed to the factors like these (Jobson et al., 1972; Perry, 1984; Thomas et al., 1986). The Σ16PAH concentration in S1 was higher than that in S2 (Table 3). This may be due to the fact that all samples were selected from the same orchard; a one-year holding period would include samples collected from different areas and with different storage conditions, giving results that can be assessed statistically. In a study fifteen vegetable oils and butters were analyzed to determine the PAH4 (benz[a]anthracene, chrysene, benzo[b]fluoranthene and benzo[a]pyrene) contents of butters and vegetable oils (olive oils, palm oil, sunflower oil, almond oil, hazelnut oil and coffee oil) offered for sale in the Turkish market. The highest amount of PAH4 (20.76 µg kg^{-1}) was detected in sunflower oil, followed by olive oil (9.51 µg kg^{-1}), palm oil (6.05 µg kg^{-1}), coffee oil (5.25 µg kg^{-1}) and hazelnut oil (1.95 µg kg^{-1}). In another study, HPLC analysis was performed for the detection of benzo(a)pyrene (BaP) in 40 oil samples, and the concentration of BaP was calculated as 4.80 ± 0.01 ppb for hazelnut oil (Gülten et al., 2007). The SCF recommended the use of benzo(a)pyrene (BaP) as a marker for the presence and effects of carcinogenic PAHs in food, based on the evaluation of PAH profiles in food (European Food Safety Authority, 2008). According to the law, the maximum BaP level is 2 µg kg^{-1}, but BaP was not detected in either oil sample.

Fatty acid composition: Fatty acid composition was determined according to the methods of Oliveira et al. (2008), and Thermo Scientific Application Notes with some modifications. A sample of 100 µL of oil was used for fatty acid methyl esterification with 1 mL of methyl esterification reagent (2 N KOH solution in CH_3OH). The fatty acid profile was analyzed by an Agilent GC-7890 instrument with a chromatographic column (Thermo, 10 m × 0.10 mm ID × 0.20 µm film, Agilent, USA). The results are expressed as a percentage of the fatty acid composition.

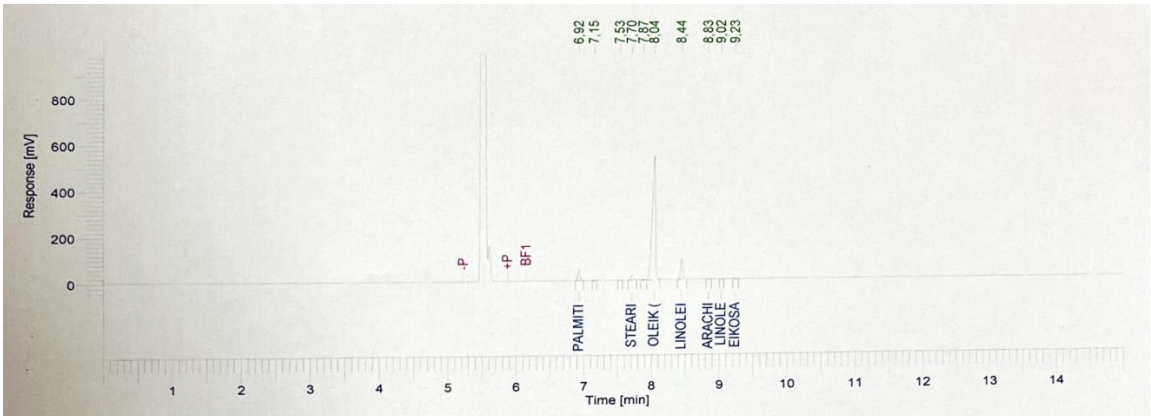

Fig. 3. Fatty acid composition of sample S2 (2023 harvest)

Table 4. Changes in fatty acid composition (%).
Tablo 4. Yağ asidi bileşimindeki değişimler (%).

% Fatty acids	S1	S2
Palmitic acid (C16:0)	5.95±0.01	4.61±0.02
Stearic acid (C18:0)	2.55±0.02	2.24±0.05
Oleic acid (C18:1)	84.2±0.12	82.81±0.13
Linoleic acid (C18:2)	7.3±0.02	10.05±0.06
Arachidik acid (C20:0)	-	0.09±0.01
Linolenic acid (C18:3. n-6)	-	0.07±0.01
Eicosanoic acid (C20:1)	-	0.13±0.01

The values are expressed as the means ± SDs (n = 3). (S1: 2022 harvest. S2: 2023 harvest).

Other researchers have reported similar results with respect to the fatty acid composition of other hazelnut oils (Amaral et al., 2006; Turan, 2018). The concentration of palmitic acid was found to be significantly greater in the oil of hazelnuts that had been stored for one year. A similar trend was observed for oleic acid, stearic acid and arachidic acid. Linolenic acid and eicosanoic acid were not detected in the oil of hazelnuts that had been stored for one year. Fresh hazelnut oil was used. The concentrations of these three fatty acids did not exceed 0.2% (Table 4). The extent of the degradation of linoleic and linolenic acids to monounsaturated acids was greater than that of oleic acid during the specified period. This result is consistent with those previously reported by Guillén et al., (2009) in which linoleic acid was oxidized throughout the oxidation process of sunflower oil, resulting in an increased oleic acid content.

CONCLUSION

A review of the literature revealed a limited scope of PAH analyses in hazelnut oil. In particular the 16-component PAH analysis was not identified as a topic of investigation within the literature. The objective of this study was to analyze the nature of the change in the PAH content of hazelnut oil as a result of storage with the aim of providing a reference point for hazelnut owners. Benzo[a]pyrene, a known carcinogen, was not detected in either sample, however naphthalene formation increased the amount of Σ16PAH. Furthermore, the variation in fatty acid composition was also examined. This condition was determined to be a consequence of the hazelnut being stored in a sack for one year in an environment with uncontrolled humidity. It is thought that storing hazelnut samples in temperature and moisture controlled stores will prevent the formation of naphthalene.

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ARAŞTIRMA MAKALESİ **RESEARCH PAPER**

Three New Records for Spider (Arachnida: Araneae) Fauna of Türkiye [*]

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Abstract: Bayburt Province has been insufficiently studied in terms of its spider fauna until now. As a result of this study conducted in Bayburt Province, three new records for Türkiye were identified. These species are *Agyneta fuscipalpus* (C. L. Koch, 1836), *Micaria lenzi* Bösenberg, 1899 and *Synaphris lehtineni* Marusik, Gnelitsa & Kovblyuk, 2005. The genus *Synaphris* Simon, 1894, belonging to the family Synaphridae, which has a very rare distribution, is represented by 11 species worldwide. Only 4 of these species are distributed in Europe. *Synaphris lehtineni* is extremely important as it represents the first recorded species of the family Synaphridae in Türkiye. The genus *Agyneta* Hull, 1911, belonging to the family Linyphiidae, one of the most diverse spider families in the world, comprises 201 species globally. Of these, 43 are also found in Europe. *Agyneta fuscipalpus* has been added as a new record to the genus, which previously had only three species in Türkiye. The genus *Micaria* Westring, 1851, of the family Gnaphosidae, which has a wide distribution, is represented by 124 species worldwide. Of these, 34 are also found in Europe. Prior to this study, 11 species of this genus had been identified from Türkiye. With this study, *Micaria lenzi* has been recorded for the first time in Türkiye. The specimens were collected from rocky habitats and beneath stones. The specimens were identified using a stereomicroscope and photographed using a digital camera. The morphological characteristics and photographs of the new records are presented with their detailed descriptions.

Keywords: Araneae, Bayburt, fauna, new records, Türkiye.

Türkiye'nin Örümcek (Arachnida: Araneae) Faunası İçin Üç Yeni Kayıt[*]

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Öz: Bayburt ili, örümcek faunası açısından bugüne kadar yeterince çalışılmamıştır. Bayburt ilinde gerçekleştirilen bu çalışma sonucunda Türkiye için üç yeni kayıt tespit edilmiştir. Bu türler; *Agyneta fuscipalpus* (C. L. Koch, 1836), *Micaria lenzi* Bösenberg, 1899 ve *Synaphris lehtineni* Marusik, Gnelitsa & Kovblyuk, 2005'dir. Oldukça nadir yayılış gösteren Synaphridae familyasına ait *Synaphris* Simon, 1894 cinsi dünya genelinde 11 tür ile temsil edilmektedir. Bu türlerden yalnızca 4'ü Avrupa'da dağılım göstermektedir. *Synaphris lehtineni*, Synaphridae familyasının Türkiye'de kaydedilen ilk türü olması açısından son derece önemlidir. Dünyadaki en çeşitli örümcek familyalarından biri olan Linyphiidae familyasına ait *Agyneta* Hull, 1911 cinsi dünya genelinde 201 tür içermektedir. Bu türlerin 43'ü Avrupa'da da bulunmaktadır. Türkiye'de yalnızca üç türü bulunan cinse, *Agyneta fuscipalpus* türü yeni kayıt olarak dahil edilmiştir. Geniş bir dağılıma sahip olan Gnaphosidae familyasının *Micaria* Westring, 1851 cinsi dünyada 124 tür ile temsil edilmektedir. Bu türlerden 34'ü Avrupa'da da bulunmaktadır. Bu çalışmadan önce Türkiye'den bu cinse ait 11 tür tespit edilmişti. Bu çalışma ile *Micaria lenzi* Türkiye'den ilk kez kaydedilmiştir. Örnekler kayalık habitatlardan ve taş altlarından toplanmıştır. Örnekler stereomikroskop kullanılarak teşhis edilmiş ve dijital kamera ile fotoğraflanmıştır. Yeni kayıtların morfolojik özellikleri ve fotoğrafları, detaylı tanımları ile birlikte sunulmuştur.

Anahtar kelimeler: Araneae, Bayburt, fauna, Türkiye, yeni kayıtlar.

INTRODUCTION

Based on the most recent data, there are 1282 spider species belonging to 57 families in Türkiye (Danışman et al., 2024).

The family Linyphiidae is represented by 4940 species belonging to 640 genera in the world (World Spider Catalog, 2025). In Türkiye, it contains 156 species belonging to 74 genera (Danışman et al., 2024). While the genus *Agyneta* belonging to this family is represented by 201 species in the world, there are only three species of the genus in Türkiye. These species are *Agyneta innotabilis* (O. Pickard-Cambridge, 1863), *Agyneta punctata* Wunderlich, 1995 and *Agyneta rurestris* (C. L. Koch, 1836) (Coşar et al., 2012; Danışman et al., 2024). Another species belonging to this genus was recorded from Türkiye by this study.

The family Gnaphosidae is represented by 2479 species belonging to 153 genera in the world. In Türkiye,

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it contains 163 species belonging to 34 genera. While the genus *Micaria* belonging to this family is represented by 124 species in the world, there are 11 species of this genus in Türkiye. These species are *Micaria albovittata* (Lucas, 1846), *Micaria bosmansi* Kovblyuk & Nadolny, 2008, *Micaria coarctata* (Lucas, 1846), *Micaria dives* (Lucas, 1846), *Micaria formicaria* (Sundevall, 1831), *Micaria fulgens* (Walckenaer, 1802), *Micaria pallipes* (Lucas, 1846), *Micaria pulicaria* (Sundevall, 1831), *Micaria rossica* Thorell, 1875, *Micaria silesiaca* L. Koch, 1875 and *Micaria sociabilis* Kulczyński, 1897 (Coşar & Danışman, 2021; Danışman et al., 2024; Demir et al., 2014; Demircan Aksan & Topçu, 2022; Demircan Aksan & Topçu, 2024; Efil et al., 2012; Topçu et al., 2007). With a new record from Bayburt Province, this number has increased to 12.

The family Synaphridae is represented by 13 species belonging to three genera in the world. The presence of the family Synaphridae in Türkiye is indicated by a genus record (*Synaphris* sp.) (Marusik & Kunt, 2011; Danışman et al., 2024). In this study, *Synaphris lehtineni* Marusik, Gnelitsa & Kovblyuk, 2005 was identified as both the first species record of the family and a new record for Türkiye.

MATERIAL AND METHOD

The specimens in this study were collected from Aydıntepe, Merkez and Demirözü Districts of Bayburt Province in 2024. *Agyneta fuscipalpus* and *Synaphris lehtineni* were collected from rocky areas, while *Micaria lenzi* was collected from under stones. The specimens were examined using an Olympus SZX-16 stereomicroscope. Photographs of the new records were taken with a Canon EOS 500D camera and edited with PhotoScape. Measurements are given in millimetres (mm). The new records were preserved in 70% ethanol. The locations where the species were collected from Bayburt Province are shown in Figure 1. The online databases Nentwig et al., (2025) and World Spider Catalog, (2025) were used for identification and zoogeographic distribution of spider species. Copulatory organs of *A. fuscipalpus* were mainly compared with Lee and Merrett, (2001), Thaler, (1983), Thaler and Noflatscher, (1990) and Wiehle, (1956); The epigyne and vulva of *M. lenzi* were primarily compared with Heimer and Nentwig, (1991), Marusik et al., (2014), Reimoser, (1937) and Roberts, (1998); The pedipalp of *S. lehtineni* was mainly compared with Lecigne, (2023) and Marusik et al., (2005).

RESULTS

Family: Gnaphosidae Banks, 1892
Genus: *Micaria* Westring, 1851
Micaria lenzi Bösenberg, 1899

Material Examined: (BUAM GNA-04/04), Türkiye, Bayburt Province, Demirözü District, surroundings of Demirözü Dam, 1♀, 40°07'46.19"N 39°53'53.76"E, 1720 m., 15.07.2024, collected from beneath stone, leg. N. Demircan Aksan.

Description: Female (Figure 2a-e). Total length 3.0. Carapace length 1.2, width 0.8. Abdomen length 1.8, width 1.1. Prosoma and chelicerae reddish brown. Legs yellowish brown with dark brown femur. Abdomen brown. Epigyne and vulva as in Figure 2d, e.

Distribution: Caucasus, Europe, Russia (Europe to South and north-eastern Siberia), China, Iran, Central Asia (World Spider Catalog, 2025).

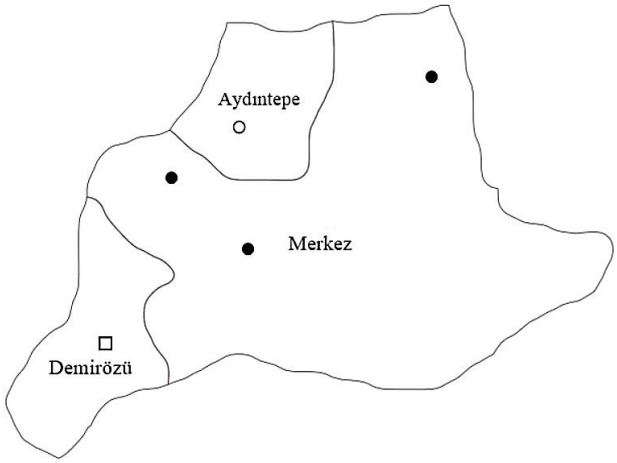

Figure 1. Collected locations of new records from Bayburt Province □ *Micaria lenzi* Bösenberg, 1899 ● *Agyneta fuscipalpus* (C. L. Koch, 1836) ○ *Synaphris lehtineni* Marusik, Gnelitsa & Kovblyuk, 2005.

Figure 2. *Micaria lenzi* Bösenberg, 1899 (♀) Habitus, a. dorsal view b. frontal view c. lateral view; Epigyne d. ventral view e. dorsal view (Scale: a-c. 0.5 mm, d, e. 0.1 mm).

Family: Linyphiidae Blackwall, 1859

Genus: *Agyneta* Hull, 1911

Agyneta fuscipalpus (C. L. Koch, 1836)

Material Examined: (BUAM LIN-01/03), Türkiye, Bayburt Province, Merkez, Akşar Village road, around Korgan Bridge, 1♂, 40°20'11.04"N 40°00'03.68"E, 1636 m., 29.08.2024; Bayburt Province, Merkez, Yoncalı Village road, 1♂, 40°29'38.47"N 40°33'42.26"E, 2300 m., 01.09.2024; Bayburt Province, Merkez, surroundings of Danişment Pond, 1♂,3♀, 40°14'48.91"N 40°09'59.77"E, 1650 m., 14.09.2024, collected from the rocky area, leg. N. Demircan Aksan.

Description: Male (Figure 3a, c-e) Total length 2.1. Carapace length 0.9, width 0.7. Abdomen length 1.2, width 0.6. Prosoma and chelicerae brown. Legs yellowish brown. Abdomen dark brown. Pedipalp with broad lamella characteristica as in Figure 3c-e.

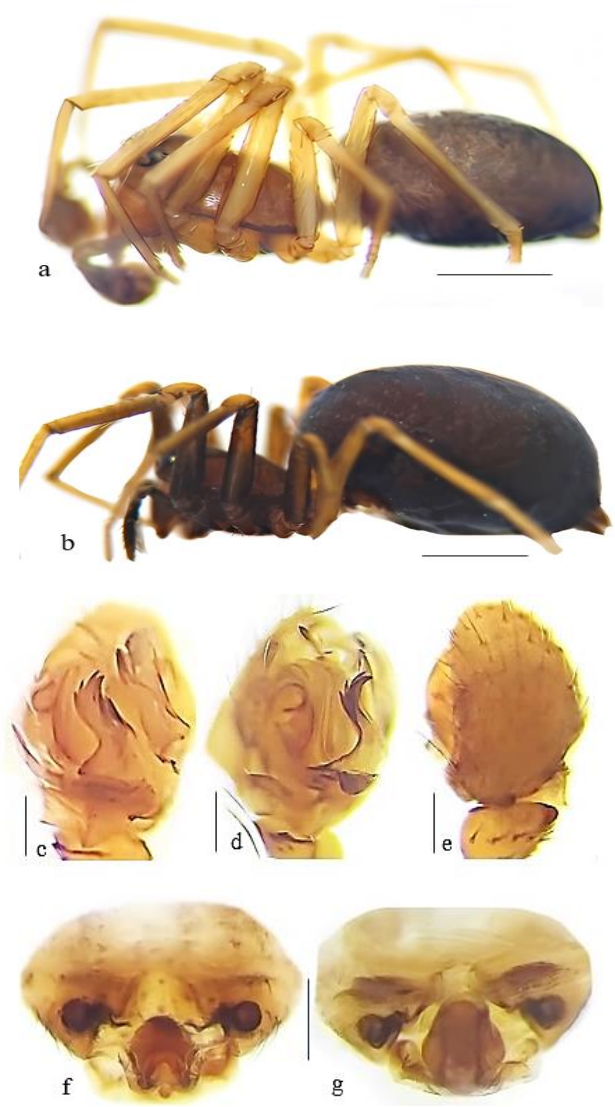

Figure 3. *Agyneta fuscipalpus* (C. L. Koch, 1836) (♂, ♀) Habitus, a. male, lateral view b. female, lateral view; Pedipalp c. ventral view d. retrolateral view e. dorsal view; Epigyne f. ventral view g. dorsal view (Scale: a, b. 0.5 mm, c-g. 0.1 mm).

Female (Figure 3b, f-g) Total length 2.5. Carapace length 0.8, width 0.9. Abdomen length 1.7, width 1.1. Prosoma and chelicerae brown. Legs yellowish brown. Abdomen dark brown. Epigyne and vulva as in Figure 3f-g.

Distribution: Azores, Cape Verde, Europe, North Africa, Caucasus, Russia (Europe to South Siberia), Central Asia, Iran (World Spider Catalog, 2025).

Family: Synaphridae Wunderlich, 1986

Genus: *Synaphris* Simon, 1894

Synaphris lehtineni Marusik, Gnelitsa & Kovblyuk, 2005

Material Examined: (BUAM SYN-01/01), Türkiye, Bayburt Province, Aydıntepe District, around Aydıntepe Plateau, 1♂, 40°25'47.62"N 40°07'47.77"E, 2240 m., 14.07.2024, collected from rocky and sparse vegetation, leg. N. Demircan Aksan.

Description: Male (Figure 4a-f) Total length 1.0. Carapace length 0.4, width 0.5. Abdomen length 0.6, width 0.5. Prosoma and chelicerae brown. Legs yellowish brown. Abdomen dark brown. Pedipalp with long embolus as in Figure 4d-f.

Distribution: Bulgaria, Romania, Ukraine, Greece (World Spider Catalog, 2025).

Figure 4. *Synaphris lehtineni* Marusik, Gnelitsa & Kovblyuk, 2005 (♂) Habitus, a. dorsal view b. frontal view c. lateral view; Pedipalp d. prolateral view e-f. retrolateral view (Scale: a-c. 0.2 mm, d-f. 0.1 mm).

CONCLUSION

As a result, this study has provided important contributions to the araneofauna of Türkiye. Three new records have been added to the spider fauna of Türkiye in the current study. It is expected that the microclimatic characteristics of Bayburt Province will significantly contribute to the spider diversity with the studies to be carried out.

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ARAŞTIRMA MAKALESİ **RESEARCH PAPER**

Unusual Winter Activity of the Ottoman Viper (*Montivipera xanthina*) in Türkiye

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Abstract: The seasonal dynamics of reptile species and their responses to fluctuations in environmental temperature play a crucial role in their overall ecological functions. Reptiles are ectothermic organisms, meaning their activity levels are largely influenced by external temperatures. While most reptile species in temperate regions enter a state of hibernation during colder months, some exceptions may occur as a result of environmental changes. In this study, an unusual instance of winter activity was documented in *M. xanthina* (Ottoman viper) in the Karalan-Eskişehir region of Türkiye. A single individual was observed on November 2, 2024, at an ambient temperature of 18.6°C. This observation provides valuable insights into the winter behavior of reptiles, which are generally considered inactive during this time of year. A review of existing literature reveals no prior records of *M.xanthina* exhibiting activity in November or during any other winter month. Therefore, this study presents the first known documentation of winter activity in this species, raising important questions about the potential effects of climate change on reptilian seasonal behavior. Rising temperatures and shifting seasonal patterns may be altering the hibernation strategies of reptiles, leading to extended activity periods. Such changes could have ecological consequences, affecting predator-prey interactions, reproductive cycles, and overall species distributions.

Keywords: Climate change, hibernation, reptiles, temperature,winter behaviors.

Şeritli Engereğin (*Montivipera xanthina*) Türkiye'deki Olağandışı Kış Aktivitesi

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Öz: Sürüngen türlerinin mevsimsel dinamikleri ve çevresel sıcaklık dalgalanmalarına verdikleri tepkiler, onların ekolojik işlevleri açısından önemli bir rol oynar. Sürüngenler ektotermik organizmalardır, yani aktiviteleri büyük ölçüde dış sıcaklık tarafından belirlenir. Ilıman bölgelerde yaşayan çoğu sürüngen türü soğuk aylarda kış uykusuna girerken, çevresel değişimlere bağlı olarak bazı istisnalar görülebilir. Bu çalışmada, Türkiye'nin Karalan-Eskişehir bölgesinde *M.xanthina* (Osmanlı engereği) türüne ait alışılmadık bir kış aktivitesi belgelendirilmiştir. Tek bir birey, 2 Kasım 2024 tarihinde, 18.6°C ortam sıcaklığında gözlemlenmiştir. Bu gözlem, genellikle bu dönemde hareketsiz olduğu düşünülen sürüngenlerin kış davranışlarına dair önemli bilgiler sunmaktadır. Mevcut literatür incelendiğinde, *M.xanthina'nın* Kasım ayında veya diğer kış aylarında aktivite gösterdiğine dair herhangi bir kayıt bulunmamaktadır. Dolayısıyla, bu çalışma, bu türde bilinen ilk kış aktivitesi kaydını sunarak, iklim değişikliğinin sürüngenlerin mevsimsel davranışları üzerindeki potansiyel etkilerine dair sorular ortaya çıkarmaktadır. Artan sıcaklıklar ve değişen mevsimsel düzenler, sürüngenlerin kış uykusu stratejilerini değiştirerek daha uzun aktivite dönemlerine yol açabilir. Bu tür değişiklikler, av-avcı etkileşimleri, üreme döngüleri ve türlerin genel dağılımı gibi ekolojik sonuçlar doğurabilir.

Anahtar kelimeler: İklim değişikliği, hibernasyon, sürüngenler, sıcaklık, kış davranışı.

INTRODUCTION

Ectothermic animals, such as reptiles, are highly dependent on seasonal temperature fluctuations to carry out their daily functions, as their body temperatures are determined by the surrounding environment (Kurnaz & Şahin, 2024). Consequently, snakes inhabiting temperate regions and higher latitudes enter hibernation during the winter and become active during warmer periods (Brito, 2003; Zuffi et al., 1999). During active periods, they engage in activities such as reproduction, feeding, biological functions, and escaping predators. However, in

colder seasons, physiological processes are impeded by low temperatures, rendering them incapable of performing these activities (Nordberg & Cobb, 2017). Hibernation enables them to survive the winter with minimal energy expenditure by sheltering underground or in suitable refuges until the next temperate season (Kurnaz & Şahin, 2024).

In recent years, rising temperatures due to global climate change have significantly impacted the behavior and biology of many reptiles (López-Alcaide and Macip-Ríos, 2011; Batum et al., 2025). High temperature fluctuations during winter months cause reptiles to awaken prematurely and exhibit unusual behaviors (Zuffi et al., 1999).

The Ottoman viper (*Montivipera xanthina* [Gray, 1849]) is a robust viper species, reaching lengths exceeding one meter. Its dorsal ground color is gray-brown, adorned with black zigzag-shaped bands. It has vertical pupils and venom that can be dangerous to humans (Baran et al., 2021). This species is found in Western, Central, and Southern Anatolia, as well as on some islands in the Aegean Sea within Greece, at elevations ranging from sea level up to 2000 meters (Yalcın et al., 2014). It prefers open slopes, valley sides, rocky and stony habitats (Gidiş & Başkale, 2020). It is primarily found in lowland areas or low-altitude open hills and can also be observed in agricultural lands (Cattaneo, 2022). *Montivipera xanthina* is listed as Least Concern (LC) on the IUCN Red List.

Numerous taxonomic, systematic, phylogenetic, and distributional studies have been conducted on *M. xanthina*. However, the majority of research has focused on its valuable venom (Nalbantsoy et al., 2013; Yalcın et al., 2014; Jablonski et al., 2015; Stümpel et al. 2016; Afsar et al., 2019). *Montivipera xanthina* has adapted to a narrow thermal range and mesophilic (temperate 20-35°C) environments. During the hot summer period, it exhibits more nocturnal behavior. The species is typically active from March to October (Cattaneo, 2022).

This study presents an observation of the winter activity of *Montivipera xanthina* in this region.

MATERIAL AND METHOD

A specimen of *M. xanthina* was observed near Karalan-Eskişehir by an individual who reported the sighting to us. GPS coordinates and a photograph of the observation were provided (39° 38.977'N, 30° 26.107'E, altitude: 884 m)(Figure 1).At the time of observation, the air temperature was recorded as 18.6°C. Temperature data were obtained from the website www.timeanddate.com (accessed on: 29 January 2025). The specimen was identified through direct observation and was determined to be an adult based on visual assessment (Figure 2).

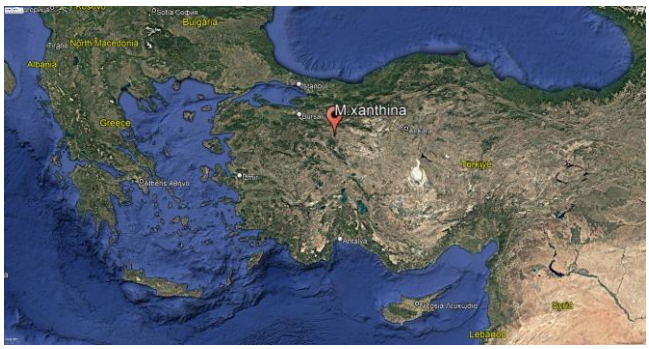

Figure 1. The location of the Ottoman viper (*Montivipera xanthina*) observation on the map (Google Earth).

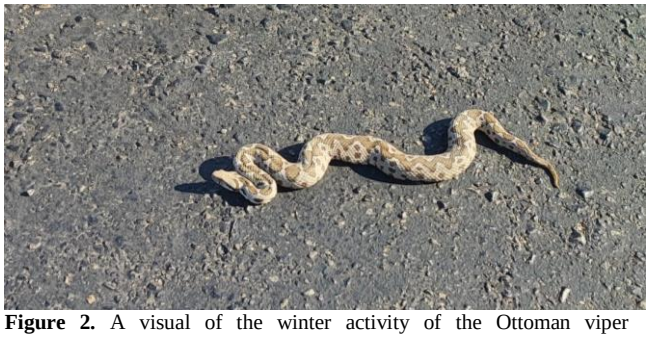

Figure 2. A visual of the winter activity of the Ottoman viper (*Montivipera xanthina*).

RESULTS

An adult *M.xanthina* was observed on 2 November 2024 at 14:22. The specimen was spotted on a road during a journey. The soil surface temperature at the observation site was measured at 20.3°C, while temperatures at depths of 6 cm and 10 cm were recorded as 19.2°C and 18.9°C, respectively. The humidity at the site was 35%, and the air pressure was recorded as 1019 mbar.

DISCUSSION

Ectothermic organisms, such as *M.xanthina* and other reptiles, are required to undergo hibernation during the winter months due to their limited activity levels. Their metabolism, which depends on ambient temperature, hinders their physiological functions, predation, and ability to evade from predators. This can lead to increased mortality and reduced body mass due to caloric deficiency in viper species (Zuffi et al., 1999; Brito, 2003; Kurnaz & Şahin, 2024).

To date, no studies on the winter activity of *M. xanthina* have been documented in Central Anatolia region. This study presents the first report of this species' activity in November. The observation of an air temperature of 18.6°C at the site is a significant factor contributing to this winter activity. *Montivipera xanthina* particularly favors temperate climates. The highest recorded daytime temperature of 19°C closely aligns with this viper's preferred temperature range (Cattaneo, 2022). Moreover, environmental activity temperatures for many

reptile species have been reported to range between 10C° and 25°C (Mori & Ota, 2002).

Global climate change has adverse effects on reptiles. According to the Turkish State Meteorological Service, the average winter temperature anomaly for 2024 was recorded at +3.3°C. The seasonal average temperature for the region normally 6°C. On the observation date, the average air temperature was 6°C, but just five days later, on 7 November 2024, the daily average temperature dropped to -6°C (www.timeanddate.com). These data suggest that fluctuations in daily air temperatures during winter months may causes reptiles to become active when conditions temperatures are sufficiently warm (Özgül et al., 2022; Kurnaz & Şahin, 2024; Batum et al., 2025).

A study by Ahmadi et al. (2019), projected significant habitat losses for *M. xanthina* by 2050 and 2070 due to climate change . These projections indicate 35% to 50% reduction in suitable habitat and a range contraction to ward higher altitudes. However, habitat fragmentation and anthropogenic barriers are expected to limit these shifts. Additionally, the species' low genetic diversity restricts its adaptive capacity, increasing its vulnerability to climate change. Climate change may also disrupt the reproductive cycles of this species, thereby affecting its demographic rates.

In conclusion, the disruption of hibernation cycles in reptile species poses a significant threat to their survival. These temperature fluctuations, combined with habitat loss may place the species at greater risk. Further field-based investigations are required to validate these findings and to elucidate the ecological mechanisms underlying winter activity in ectothermic reptiles.

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ARAŞTIRMA MAKALESİ **RESEARCH PAPER**

The Naturalness Level of Terrestrial Areas in Bartin Province (Northern Türkiye)

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Abstract: The level of naturalness of terrestrial ecosystems is the degree to which they are exposed to human influence. The high level of naturalness reflects low human impact and high biodiversity. Determining the level of naturalness of terrestrial ecosystems is extremely important for long-term land planning and conservation, sustainability, and resource management. This study examined the naturalness level of natural/anthropogenic areas within the administrative borders of Bartin province. Six spatial factors were used. These are settlement density, population density, road density, land cover/use, Normalized Difference Vegetation Index (NDVI), and Topographic Roughness Index (TRI). These six essential factors were inverted into the spatial fuzzy memberships using Geographic Information Systems (GIS). A final map was obtained by overlaying the fuzzy memberships in the fuzzy overlay model (FOM). According to this resulting map, the city center and its surroundings have a high anthropogenic impact and a low level of naturalness. The Küre Mountains National Park (KMNP) in the east of the province and the rugged mountain ranges in the south have a high level of naturalness, being remote from anthropogenic impact. Anthropogenic land degradation has expanded due to road construction, mining, and forestry activities. Uncontrolled economic activities constitute potential areas of human-induced degradation.

Keywords: Bartin, Degradation, Fuzzy overlay, Mapping, The level of naturalness.

Bartın İli (Türkiye'nin Kuzeyi) Karasal Alanlarının Doğallık Düzeyi

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Öz: Karasal ekosistemlerin doğallık düzeyini, insan etkisine maruz kalma derecesi belirler. Yüksek doğallık düzeyi, düşük insan etkisini ve yüksek biyolojik çeşitliliği yansıtır. Karasal ekosistemlerin doğallık düzeyinin belirlenmesi, uzun vadeli arazi planlaması ve korunması, sürdürülebilirlik ve kaynak yönetimi açısından son derece önemlidir. Bu çalışmada, Bartın ili idari sınırları içindeki doğal/antropojen alanların doğallık düzeyi incelenmiştir. Altı mekânsal faktör kullanılmıştır. Bunlar, yerleşim yeri yoğunluğu, nüfus yoğunluğu, yol yoğunluğu, arazi örtüsü/kullanımı, Normalize Edilmiş Fark Bitki Örtüsü İndeksi (NDVI) ve Topoğrafik Pürüzlülük İndeksidir (TRI). Bu altı temel faktör, Coğrafi Bilgi Sistemleri (CBS) kullanılarak mekânsal bulanık üyeliklere dönüştürülmüştür. Sonuç haritası, bulanık üyelerin fuzzy overlay modelinde (FOM) üst üste çakıştırılmasıyla elde edilmiştir. Ortaya çıkan bu haritaya göre, kent merkezi ve çevresi yüksek antropojenik etkiye ve düşük doğallık düzeyine sahiptir. İlin doğusundaki Küre Dağları Milli Parkı (KMNP) ve güneyindeki engebeli dağ sıraları, antropojenik etkiden uzak olmaları nedeniyle yüksek bir doğallığa sahiptir. Antropojenik arazi bozulması, yol yapımı, madencilik ve ormancılık faaliyetleri nedeniyle genişlemiştir. Kontrolsüz ekonomik faaliyetler, insan kaynaklı bozulmanın potansiyel alanlarını oluşturur.

Anahtar Kelimeler: Bartın, Bozulma, Bulanık Çakıştırma, Haritalama, Doğallık Düzeyi.

INTRODUCTION

Naturalness or natural area has been defined as being remote from human influence (Angermeier, 2000; Povilitis, 2002). The level of naturalness describes the degree of the influence of humankind on the natural environment (Potapov et al., 2017; Carver et al., 2013). Areas where naturalness has been disrupted or is in the process of being disrupted due to human intervention can be referred to as anthropogenic areas (under strong human influence). Naturalness, or the level of naturalness, estimates a gradual transition from less natural to more natural (Angermeier, 2000; Ekim et al., 2021). The boundaries among the different natural environments are fuzzy.

Human impact on natural ecosystems, based on balance and order, can be traced back at least 3000 years. During this period, the anthropogenic transformation of terrestrial ecosystems, particularly, has reached a global

scale (Ellis, 2015; Newbold et al., 2015; Ellis, 2021). In the history of human civilization, migrations, wars, agricultural and livestock systems, and the establishment and collapse of civilizations have all been significantly influenced by the natural environment. It is not possible to adequately explain the course of historical events and the establishment of archaeological sites and early settlements without considering the relationships between humans and the natural environment or without sufficient knowledge of the characteristics of the natural environment (Atalay, 2022).

Anthropogenic impacts on the natural environment have various effects that lead to a decrease in biodiversity and the degradation of natural resources. Human-caused degradation of water resources is particularly significant. This situation, combined with human-induced climate change, is expected to have much more significant impacts in the future (Findell et al., 2017; Morelli et al., 2020). The degradation of the natural environment humans use for their well-being and consumption inevitably has negative consequences for their future.

Land use/cover degradation is also directly related to population growth and industrialization (Duran & Günek, 2007; Findell et al., 2017). Approximately 41% of the Earth's surface has been subjected to human-induced land degradation. One-fifth of the affected land is in sub-Saharan Africa. South Asia is the most affected region, with 41% of its land area affected, and 70% of the affected area is severely degraded. This is followed by Southeast Asia, West Asia, and South America. These estimates do not include deserts. Human-induced degradation affects 34% of agricultural land. Expanding agricultural land into marginal areas leads to soil erosion and decreased soil-bound biodiversity. The intensive use of inorganic fertilizers in agricultural lands affects drainage systems and contributes to the degradation of water resources (FAO, 2024).

The Global Human Footprint Index (GHFI) is widely utilized in fields such as environmental research, natural resource management, the analysis of human impact, and the spatial assessment of habitat loss (Venter et al., 2016). However, the GHFI operates on a global scale. There are also different scientific studies on human-caused natural area degradation (Lambin et al., 2001; Reba et al., 2016; De Jong et al., 2021; Duran, 2024). Additionally, studies on natural areas need to be protected (Turoglu & Ozdemir, 2005; Şen & Erkan Bugday, 2015; Tunckol et al., 2020; Altunel et al., 2021). However, these studies do not include a study on spatial density reflecting human impact. A comprehensive geographical study within the administrative boundaries of Bartin province was not found.

This study investigates the spatial relationships between the natural environment and anthropogenic activities in Bartin province. The administrative border of the province was determined as the study area. Administrative boundaries are generally precisely defined units. It provides advantages in terms of conservation, resource management, and sustainability. Official (standardized) environmental data are typically collected by considering administrative boundaries, which allows for a comparative analysis of temporal and spatial changes. Multi-dimensional (integrated) plans and land-use policies can be implemented more effectively by decision-makers within these defined administrative units. The primary objective of this research is the gradual (fuzzy) determination of the naturalness level in Bartın province. It is also designed to serve as a basis for investigating future spatial changes. It also aims to identify spatial localities where human-induced degradation is intense and to develop sustainable management strategies. It will contribute to the literature by providing an example of studies that aim to reveal the level of naturalness in a local area.

MATERIAL AND METHOD

Study area: Bartin province is located north of Turkey and on the Black Sea coast. The geographic location of the province lies approximately between 41°17'00"-41°50'56" North latitude and 33°31'00"-32°06'20" East longitude. The administrative boundaries of Bartin cover an area of about 2120 km2. Its rough terrain is situated in parts of the Eastern and Southern. The Bartin River forms the western border of the Küre Mountains. The western part of the Küre Mountains National Park (KMNP) is located in this section. Bartin River flows north-south direction through the city center of Bartin. The valleys and lowlands formed by the Bartin River and its tributaries also include plains.

Bartin province comprises four district administrations (including the central district), four towns, and 260 village settlements. The total number of settlements established in the province is 268. According to the Turkish Statistical Institute data (TurkStat, 2023), the total population is 207238. Bartin province is one of the leading provinces in terms of natural areas. Bartin shares the Black Sea's climatic condition, which is found in the region of North Türkiye. The province has humid climate conditions. Bartin province shares administrative borders with Kastamonu, Zonguldak, and Karabük (Figure 1). Analyzing naturalness levels based on administrative boundaries provides a suitable foundation for sustainability by directly integrating it into management plans.

Method: This study aimed to assess the human impacts on the natural environment of Bartin province using a Geographic Information System (GIS). Various spatial data, including topographic maps, digital elevation models, land use/cover data, road data, and socioeconomic data, were integrated to create a comprehensive spatial database.

Six key indicators of human activity - settlement density, population density, road density, land use/cover,

Normalized Different Vegetation Index (NDVI), and Topographic Roughness Index (TRI)-were selected and inverted into fuzzy membership functions to account for the inherent uncertainties and gradual transitions in human-environment interactions. These fuzzy layers were combined using a fuzzy overlay method (FOM) to produce a spatial map delineating the extent and intensity of human impacts across the study area. Compared to traditional brutal classification methods, this approach provides a more nuanced understanding of the complex relationships between human activities and the natural environment (Figure 2). The study is similar to the "GHFI". The factors that better represent the local area were selected

Figure 1. Location map of the study area

This study utilized open-source ESA Sentinel-2 imagery (processed-licensed by Esri 2021) and land use/cover data. Open-source land use/cover data provided by Esri offers a publicly accessible spatial dataset for characterizing the Earth's surface. This resource facilitates various environmental and geographical analyses. This study classified settlements and agricultural areas as land use areas exposed to human impact. Forests were categorized as areas remote from human influence. Pastures, open areas, and sparse vegetation areas were classified as areas with a secondary level of human impact.

As human settlements increase, naturalness decreases. High settlement density exacerbates human-induced degradation of the natural environment. Areas with concentrated settlements in the spatial distribution of settlements also reflect human impact. The study area was mapped using kernel density interpolation in a GIS layer. The Kernel Density method calculates the density of settlement features (point features) in the study area. Areas with high population density indicate high human impact, while areas with decreasing population density are remote from human influence.

As the distance between rural settlements decreases/increases, the number of people per km² also decreases/increases. To determine population density, a specific area needs to be defined. Thiessen polygons are a simple and commonly used method for delineating the boundaries of rural settlements. This study also employed them to define the spatial density of rural population density. Boundaries were established at an equal distance between the village settlement center (point layer) and other neighboring settlements. All the sample points are connected to form a series of triangles. Then, the perpendicular bisectors of the triangle sides are drawn. Population data obtained from the address-based population registration system of TurkStat were divided into areas of Thiessen polygons (the population density per km²). Thus, a population density layer was created in the GIS. The obtained layer was converted into a fuzzy membership layer.

Another factor is road network density. Road networks, which provide human accessibility, were identified as artificial surfaces. In areas with low road density, human impact is typically lower. High road density, however, indicates fragmentation of natural areas and widespread human influence. Line density interpolation was used to define this factor in a GIS layer. The road data obtained from the Open Street Map was corrected by checking with platforms that provide open-access satellite imagery (with high-resolution images of Google Earth). The road network density using the line density tool was created by weighting the significant primary, secondary, and other roads.

NDVI is a standard index that measures the amount and health of vegetation using remote sensing data (usually satellite imagery). It is an index of dense vegetation and non-vegetation. High (positive) NDVI values indicate high levels of naturalness, while low (negative) NDVI values indicate low levels of naturalness. NDVI image is obtained by using 4, 5 bands of Landsat9 satellite imagery (=Bant 5 – Bant 4) / (Bant 5 + Bant 4).

The morphology of the land also limits human impact. Rough terrain limits human activity. As the roughness increases, naturalness increases. The decrease in roughness means a decrease in naturalness. TRI determined morphological roughness in the study area. TRI was calculated as the difference between a cell's maximum and minimum values and eight surrounding cells. TRI performed better than the Topographic Position Index (TPI). DEM data

"Produced using Copernicus data and information funded by the European Union - EU-DEM layers with 25 m resolution."(OpenDEM)

The naturalness level of the study area was determined using the fuzzy logic method. Fuzzy logic is preferred for identifying uncertain or intertwined situations without definite boundaries in the real world (Kainz, 2007). FOM is based on set theory, where a set generally corresponds to a class. The combining analysis step in FOM analysis quantifies each location's possibility of belonging to specified sets from various input rasters. The equation fuzzy Gaussian function is used. Spread defines the membership of the Gaussian function. It generally ranges from 0.01 to 1. Increasing the spread causes the fuzzy membership curve to become steeper. FOM quantifies the possibilities of each cell or location to a specified set based on membership value (Baidya et al., 2014; Çakıt & Karwowski, 2018; Tennakoon et al., 2023). It has advantages over set theory thanks to its membership process and flexible use. The analytical Hierarchy Process was not applied in the study. This assumes that the impact of each parameter on the output is equal.

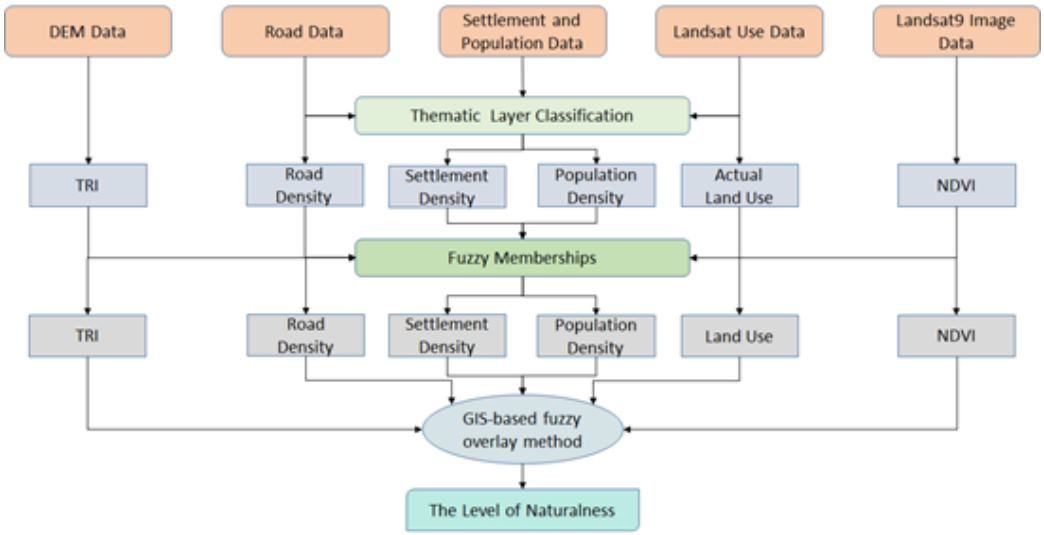

Figure 2. Flow chart of the FOM used to create the level of naturalness map.

RESULTS

Settlement density: Increased density in settlement areas results in the loss and fragmentation of natural habitats, consequently reducing biodiversity (McKinney, 2002; Seto et al., 2012). The settlements in the province are primarily located in mountainous, rugged terrain, coastal areas, and flatter lands around watercourses. The settlement pattern is generally dispersed throughout the province. The densest settlements are found along the courses of the Bartin Stream (central district settlement) and the Arit Stream, formed by the confluence of these two streams. Settlements established on the coast and nearby areas also constitute another high-population-density region. The construction of second homes for summer use in coastal areas has also contributed to the expansion of settlements (Figure 3).

Settlements are spread over larger areas on alluviums carried and deposited by rivers from highlands. Rural settlements have less infrastructure compared to urban settlements. In recent years, human-induced degradation of the natural environment has increased significantly. Natural risks such as landslides, floods, and earthquakes can be prevented with plans that will reduce the negative impact of settlements on nature.

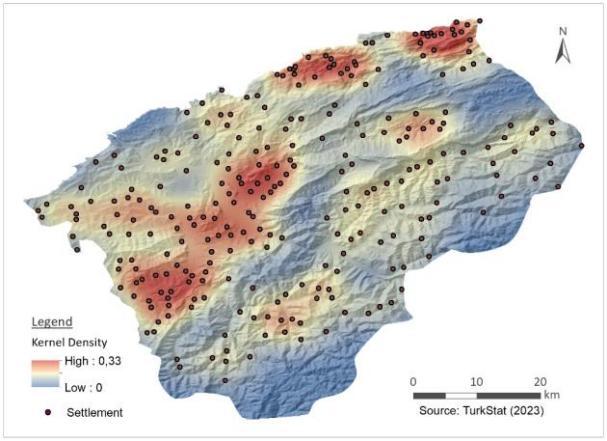

Figure 3. Kernel density estimation map of human settlements.

Population density: Population growth and density are central to research on the sustainability of natural resources (Crist et al., 2017). Natural ecosystems experience greater pressure in regions with high population density (McKee et al., 2003). Population density is typically calculated by dividing the total population of a settlement by its total area (or administrative boundary). However, in this study of Bartin province, geographic Thiessen polygons, which reference distance rather than administrative boundaries, were constructed to determine

population density. Boundaries between neighboring rural settlements were defined by bisecting the distance between them. The population density of the Bartın city center and its surrounding settlements is remarkably high. Amasra, Kurucaşile, Kumluca, Abdipaşa, and Ulus settlements also have high population density (Figure 4).

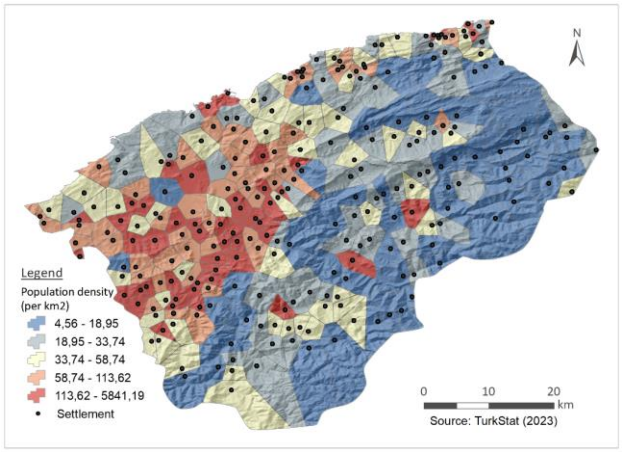

Figure 4. Population density map created with Thiessen polygons.

Road network density: The road network causes habitat fragmentation by breaking up natural landscapes. The differential effects of various road types and traffic volumes on habitat fragmentation and species movement have been identified (Fahrig & Rytwinski, 2009; Grilo et al., 2021). The road network in Bartin province is irregular due to the province's rugged topography. Road density was calculated using line density analysis. Road density generally includes significant roads from the city center to other district centers. Village roads connected to major roads have Secondary road density. The road density increases significantly along the corridor SW-NE and NW-SE directions within these routes. The road network and density decrease as distance from the coast and central settlements increases (Figure 5).

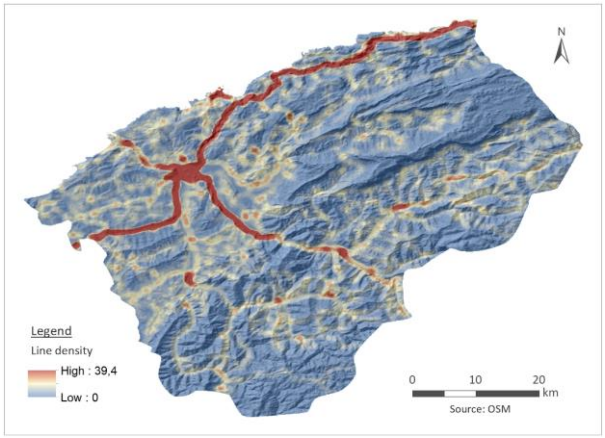

Figure 5. Line density estimation map of road network.

Land use/cover in Bartin province: The human-caused transformation of natural areas leads to habitat loss, biodiversity decline, and the degradation of ecosystem functions. Numerous studies have emphasized the importance of preserving natural land cover for sustainability (Foley et al., 2005; Sala et al., 2000). Global-scale land use projections have identified critical regions for future biodiversity losses (Rosa et al., 2023). It is the factor that directly affects how natural an area is. Land use in Bartin province is significantly influenced by its topography. Agricultural and settlement areas have been created on the foothills of mountainous terrain, on alluviums deposited by streams, and in irrigated areas. The slopes of the high mountain ranges are covered with forest cover. There are uses such as sparse/degraded vegetation, bare land, and pasture between forest and agricultural areas. Most artificial surfaces are around residential areas. Agricultural areas created around settlements are also areas where human activity is high. The areas with the lowest level of human impact are forest areas (Figure 6).

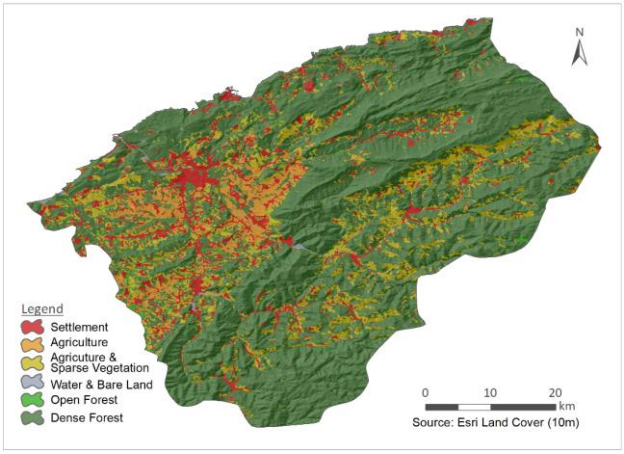

Figure 6. Actual land use in Bartin province.

Normalized Different Vegetation Index (NDVI): The NDVI measures vegetation density on the ground. The index is a value between -1 and 1. A positive (1) index value indicates dense vegetation, and a negative (-1) value indicates no vegetation. NDVI is high when green plants cover the entire pixel and low when there are no green plants. It also eliminates the effects of different solar angles. Decreased vegetation cover and degraded ecosystems due to human intervention have been observed to result in lower NDVI values (Goward et al., 1994; Lambin & Ehrlich, 1997). Human activities on natural environments, the impacts of climate change, and land degradation in arid and semi-arid regions are monitored using NDVI and trend analyses (Wang et al., 2021; De Keersmaecker et al., 2023). The NDVI in the study area ranges from -0.134 to 0.767. The negative index indicates artificial surfaces such as settlements, agriculture, and road networks without vegetation. A high positive index indicates natural areas with dense vegetation (Figure 7).

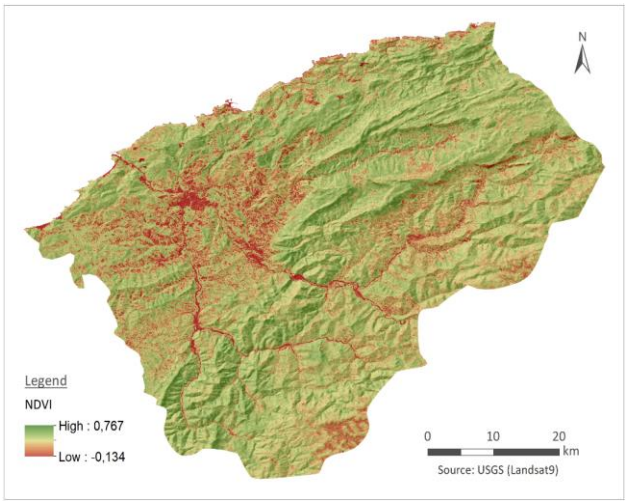

Figure 7. NDVI map in Bartin Province.

Topographic Roughness Index (TRI): The influence of landforms on terrestrial ecosystems is versatile, including ecosystem patterns, the spatial distribution of vegetation, and soil erosion. The TRI measures variability in the landscape surface. Topographic roughness is the land surface variability of a particular area and is a proxy for describing the potential of terrestrial propagation (Stambaugh & Guyette, 2008). The combination of difficult accessibility and rugged terrain keeps forests relatively undisturbed by human activities. High TRI (1) indicates a rugged terrain surface. A low TRI value (0) is a low, rugged terrain surface. TRI in the study area varies between 0.028 and 0.97. The low index is in the river network and its surroundings. The high index is a mountainous, hilly area (Figure 8).

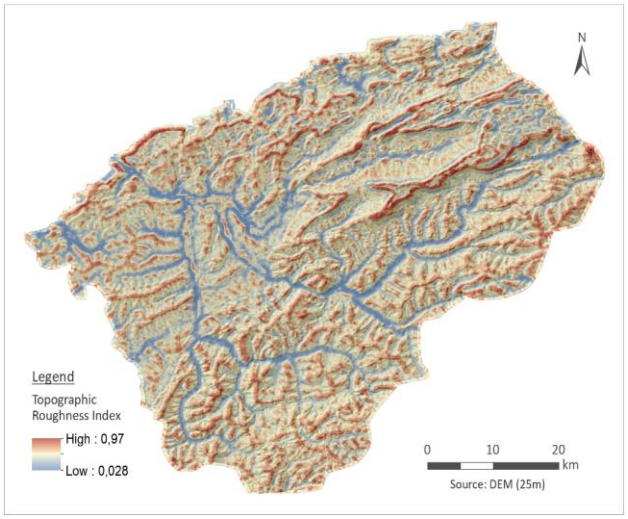

Figure 8. TRI map in Bartin Province

The Naturalness Levels of Bartın Province: Environmental degradation can be defined based on the presence of anthropogenic factors. Areas that overlap with various human-induced factors exhibit a high degree of degradation. Conversely, the absence of human-induced factors indicates a low level of spatial environmental degradation.

The naturalness level of any given land parcel can be determined based on the presence or absence of influential factors. Consequently, multi-criteria decision-making methods are widely employed for such assessments. Spatial clustering of these criteria aids in the classification of foreground regions/sub-regions. This study is similar to the GHFI, but it better describes the local characteristics of the province. This study identified six fundamental spatial criteria-land use/cover, population density, settlement density, road density, NDVI, and TRI-. Each criterion was converted into a fuzzy membership (Figure 9). The analytical hierarchy method (AHP) was not used. All factors were considered equally weighted. Overlaying these memberships resulted in a final map. The map range defines values from 0 to 1, where zero values are mainly formed from artificial surfaces. High values indicate naturalness (values approaching 1). According to this map, human-induced land change areas (anthropogenic areas) are concentrated in the central and coastal regions of the province. Remote from human influence, natural areas are found in rugged, high-elevation areas. Due to their functions in preserving naturalness, water, and soil, these rugged terrains are continuously covered by vegetation (absolute forest) and thus require protection. Semi-natural areas, with limited human impact, are potential degradation areas. Increasing human influence could transform these areas into anthropogenic ones. Fuzzy logic enables a more gradual visualization of spatial definitions. It is impossible to establish definite boundaries between natural and anthropogenic areas. Today, human accessibility and technological capabilities make conserving natural areas more challenging (Figure 10). Areas with a high level of naturalness are priority areas for sustainable land management and protection services.

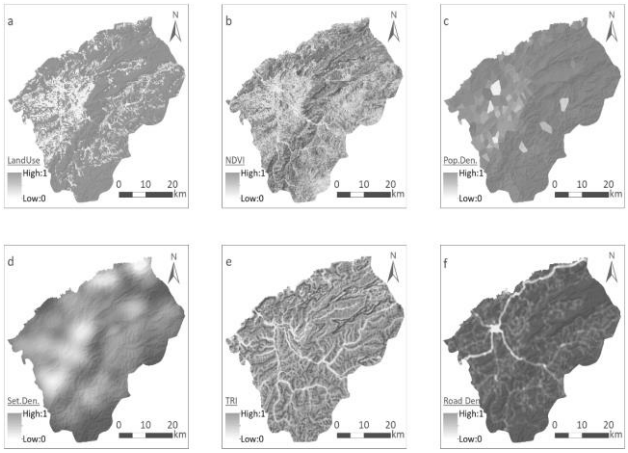

Figure 9. The six main factors are: a. Fuzzy member of the land use, b. Fuzzy member of the NDVI c. Fuzzy member of the population density d. Fuzzy member of the settlement density e. Fuzzy member of the TRI f. Fuzzy member of the road density

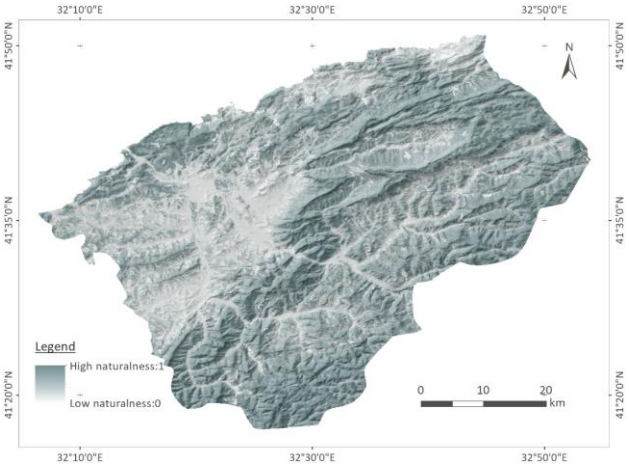

Figure 10. The naturalness level of Bartin province. (Combined pattern of six fuzzy members in the FOM).

CONCLUSION

Bartin province, renowned for its diverse physical geography, exhibits a high degree of naturalness due to limited human influence. However, the very attractiveness of its natural environment poses a risk of future degradation. To ensure sustainable land use and management, identifying priority areas for conservation and assessing the spatial sensitivity and direction of natural degradation processes are crucial. Furthermore, this study contributes to restoration efforts in human-induced degraded areas.

Topographic variations shape land use classes in Bartin province. Alluvial plains formed by rivers are intensively used for agriculture. The high mountainous regions in the east and south (West of the Küre Mountains) are covered with forests. Agricultural and settlement areas represent human-transformed landscapes, while forests are relatively undisturbed by human activities. Pasturelands, sparsely vegetated areas, and bare rocks constitute natural or human-induced semi-natural areas.

In conclusion, the naturalness level of a specific area can be correlated with human impact. Areas with high human influence exhibit low naturalness. Using the FOM, this study identifies Bartin's naturalness levels. Spatial results indicate that steep, rugged areas are priority conservation zones with high naturalness. Areas with moderate slopes, often covered by sparse vegetation, are semi-natural and moderate-level natural. A mix of settlements and agriculture areas characterizes flat and near-flat areas, typically found on alluvial deposits. These areas have non-natural and low-level naturalness. Steep and rugged terrains are less susceptible to socioeconomic pressures such as agriculture and grazing. Additionally, these areas offer pastoral and recreational opportunities.

The selected factors reflect the diverse impacts of human activities on natural environments. Therefore, their combined evaluation provides a scientifically justified approach to understanding an area's naturalness level and predicting future changes. Land use planning and policies should incorporate multi-faceted management models, especially in ecologically constrained areas, to ensure ecosystem sustainability, productivity, and rural development.

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ARAŞTIRMA MAKALESİ **RESEARCH PAPER**

Examination of Campus in Terms of Ecological Landscape Design Criteria: KTU Kanuni Campus

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Abstract: Today's cities face many problems, such as excessive population growth, environmental pollution, transportation, housing, economy, climate change, and natural disasters due to rapid urbanization. Due to these problems, the nature-human relationship in cities is also deteriorating. To regulate this relationship, it is necessary to create healthy and livable cities by integrating practical resources, energy, and water use with nature. At this point, ecological design criteria emerge in urban planning. Ecological design criteria create nature-friendly designs within the city. By providing energy and resource conservation in urban areas, it creates spaces that are close to nature and climate-friendly. At this point, many areas are suitable for ecological design in cities. University campuses are also suitable places for determining and implementing ecological design criteria. Campuses are areas where academic activities are carried out and also offer essential living spaces where environmental sustainability can be ensured. Therefore, campuses must be designed in line with ecological criteria to create a model for city centers. At this point, one of the campuses that is ecologically self-sufficient produces and uses its energy and offers sustainable solutions to increasing environmental problems, which is examined within the scope of the study. Thus, the KTÜ Kanuni campus, located within the borders of Trabzon City, was selected as the study area. The current situation of the study area was evaluated according to ecological design criteria. Thus, the importance of how much university campuses' current potential contributes to a city's sustainability and ecology was emphasized.

Keywords: Ecological design, ecological design criteria, university campuses, KTU kanuni campus.

Yerleşkenin Ekolojik Peyzaj Tasarım Kriterleri Açısından İncelenmesi: KTÜ Kanuni Yerleşkesi

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Öz: Günümüz kentleri, hızla kentleşme bağlı olarak aşırı nüfus artışı, çevre kirliliği, ulaşım, barınma, ekonomi, iklim değişikliği ve doğal afetler gibi birçok sorunla karşı karşı kalmaktadır. Bu sorunlara bağlı olarak kentlerde doğa-insan ilişkisini de bozulmaktadır. Bu ilişkinin düzenlenebilmesi için etkin kaynak, enerji ve su kullanımlarını doğa ile bütünleştirerek sağlıklı ve yaşanabilir kentler oluşturulması gerekmektedir. Bu noktada kentsel planlamada ekolojik tasarımlar kriterleri ortaya çıkmaktadır. Ekolojik tasarımlar kriterleri, kent içerisinde doğa dostu tasarımlar oluşturmaktadır. Kentsel alanlarda enerji ve kaynak korunumu sağlayarak, doğaya yakın ve iklim dostu mekânlar oluşturmaktadır. Bu noktada kentlerde ekolojik tasarım için uygun birçok alan bulunmaktadır. Üniversite kampüleri de, ekolojik tasarım kriterlerin belirlenip uygulanması açısından uygun mekanlardan bir tanesidir. Kampüsler, sadece akademik faaliyetlerin yürütüldüğü alanlar değil aynı zamanda çevresel sürdürülebilirliğin sağlanabileceği önemli yaşam alanları sunmaktadır. Bu nedenle kent merkezlerine model oluşturabilmek adına kampüslerin ekolojik kriterler doğrultusunda tasarlanmasın oldukça önemlidir. Bu noktada çalışma kapsamında ekolojik olarak kendi kendine yetebilen; kendi enerjisini üreten ve kullanan, artan çevre sorunlarına sürdürülebilir yaklaşımlarla çözümler sunan kampüslerden bir tanesi incelenmektedir. Böylece çalışma alanı olarak Trabzon kenti sınırları içerisinde bulunan KTÜ Kanuni kampüsü şeçilmiştir. Çalışma alanı mevcut durumunu ekolojik tasarım kriterlerine göre değerlendirilmiştir. Böylece üniversite kampüslerinin mevcut potansiyellerinin bir kentin sürdürülebilirliğine, ekolojisine ne kadar katkı sağladığının önemi vurgulanmıştır.

Anahtar kelimeler: Ekolojik tasarım, ekolojik tasarım kriterleri, üniversite kampüsleri, KTÜ kanuni yerleşkesi.

INTRODUCTION

Cities are a structuring that consists of small units where people perform functions such as accommodation, settlement, education and recreation (Keleş, 1998). Today, this structure of cities has become unsustainable cities where environmental quality has decreased, nat-ural resources have been depleted, and the natural environment

has deteriorated (Leitmann, 1999). However, a positive effort was made for the concept of sustainability, which first appeared in the Brutland report in 1987. In this respect, planning approaches that aim to transfer the sustainability of today's conditions to future generations and support the harmony between human and ecology should be kept at a high level (Yalçıner Ercoşkun, 2007).

The creation of sustainable cities is ensured by ecological urban designs compatible with nature. Protection of the environment and resources, rural-urban space planning, pedestrian-vehicle road organization, environmental impact assessments are among the subjects of sustainable ecological cities (Erdoğan & Öztürk, 2019; Yaşar & Düzgüneş, 2013). The ecological planning approach is provided by incorporating natural processes into small and large-scale planning for a sustainable planning. Since this process will be difficult in cities, especially due to the scarcity of natural areas, nature and ecological designs have gained importance in order to protect my existing resources (Korkut et al., 2017).

Ecological urban designs: It is to ensure the continuity of natural resources as a part of social, economic or urban ecological systems that provide continuity (Kartal, 2009). The basic principles of ecological designs McHarg (1969) developed the concept of ecology and is included in today's planning and design decisions, keeping its currency. According to McHarg (1969), the development processes of cities should be evaluated over time ac-cording to current conditions by including natural processes in planning and design studies. Especially since the places where the city users are will change over time according to their needs and environmental conditions, they should be designed with sus-tainable approaches by making plans according to the future climate and environmental conditions. Ecological designs are very effective in solving the current problems of the city and its region. The spaces designed in this context positively affect the environmental air conditioning and air quality, ensure water conservation, and make water reusable, protect and develop the biological diversity in the city by supporting the existence of natural plants, and provide natural resource conservation with the use of natural materials compatible with nature (McHarg, 1969; Selim,2021 Tregay, 1986).

Ecological landscape design: It aims to minimize energy consumption by protecting natural resources to make the city and its immediate surroundings more livable. In this way, it is an approach based on obtaining maximum benefit by mediating minimum re-source use in cities. However, with such design approaches, existing resource potentials are preserved, increasing the level of use and controlling the change over time (Alpay et al., 2013; Leitão & Ahern, 2002). The change of human use in cities with the understanding of design, the evaluation of current approaches to the solution of ecological problems in the city, and the establishment of human-nature relationship can be achieved with ecological landscape design (Cranz, 2000). Campuses are the most suitable places where ecological design criteria can be applied (Li et.al.,2018; Aksoy et.al.,2024). Campuses, like cities, are areas where social structure is created by creating a biological and physical network (Çorbacı et al.,2005; Ahern, 2007). Because campuses are compact city models by including all the factors that cities contain. In both areas, vital activities such as shelter, rest, transpor-tation, and work; as well as sustainable elements such as social, ecological and economic (Dober, 2000; Ertekin & Çorbacı, 2010; Şahin Körmeçli, 2022). While revealing the dynamic structure of cities and the human-nature relationship, campuses create an innovative and ecological lifestyle thanks to their users, mostly young people(Chen et.al., 2018; Cano et.al., 2023).

In this study, ecologically self-sufficient; It is aimed to create campuses that produce and use their own energy and offer solutions with sustainable approaches to increasing environmental problems. Campus areas, which can be considered as a small model of cities, are discussed. For this purpose, university campuses were examined in terms of ecological criteria. KTU Kanuni campus, which was determined as the study area, was evaluated according to the ecological design criteria within this scope.

MATERIAL AND METHOD

Materials: The study was carried out at the Kanuni Campus of Karadeniz Technical University in Trabzon. Trabzon city is in the Eastern Black Sea Region, between 40^0 33' and $41^0$07' northern latitudes and $39^0$07' and $40^0$30' east longitudes, the part not including the coastline is mountainous and hilly. The climate structure of the city is rainy in all seasons, hot in summers and warm in winters winters (Bayramoğlu, 2016). The study area, on the other hand, has the characteristics of the climate and topographic structure of the city due to its location. The campus has a sufficient level of open green space in terms of its natural and cultural structure and ecological and functional aspects (Bayramoğlu, 2016; Güneroğlu & Bekar, 2017).

Established in 1995 within the city of Trabzon, the campus includes 49 undergrad-uate and 36 associate degree programs in 12 faculties, 1 college, 8 vocational colleges; It has an area of 1,053,839 m² with 92 master's and 61 doctorate programs in 6 institutes (Karadeniz Technical University, 2023). With the vegetative presence of the open green areas on the campus, it not only meets the social needs of the campus user, but also provides an ecological environment on the campus. The campus is very rich in terms of natural plant existence and distribution

balance in the region. In this context, in order to evaluate the ecological planning criteria as a working environment, the KTU campus area was chosen and evaluated due to its location (Figure 1), density of use and the presence of open green areas.

Figure 1. Study area KTU Campus area.

Method: The methodological setup of the study is shown in the Figüre 2. In this direction, the methodologically used elements in the study are visualized.

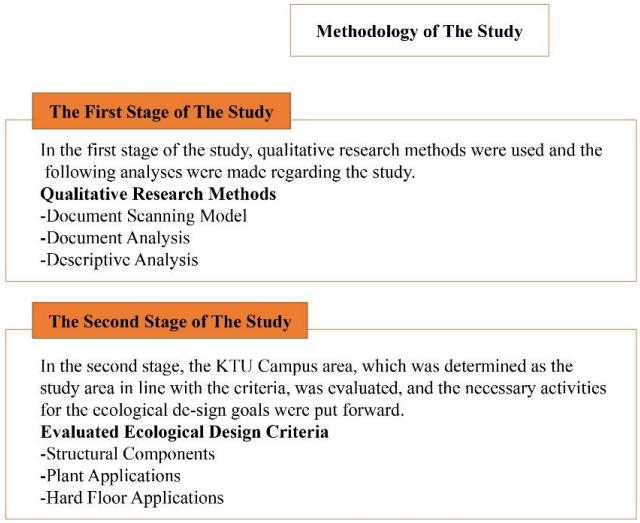

Figure 2. Methodology of The Study.

In the first stage of the study, document scanning model, one of the qualitative re-search methods, was used as a method. Qualitative research methods allow the researcher to access the data source directly. It provides detailed explanations necessary for a thorough understanding of the facts and context and generalizations in the light of the information obtained as a result of synthesis (Büyüköztürk, 2011). Document analysis was used in the process of obtaining and interpreting data within the qualitative research model. Document analysis is defined as the systematic handling of both printed and web-based in-formation and documents (Bowen, 2009). Descriptive analysis was performed to determine ecological design criteria. Structural components, vegetative application and hard-ground applications are discussed from ecological design criteria. According to Yıldırım and Şimşek (2008), In descriptive analysis, themes are predetermined, and data are summarized and interpreted according to these determined themes. In descriptive analysis, direct quotations are frequently used, and it is aimed to reflect the data requested to be researched in a striking way. descriptive analysis: It is important in terms of ensuring that the findings obtained are presented in an organized and interpreted way.

In the second stage, the KTU Campus area, which was determined as the study area in line with the criteria, was evaluated, and the necessary activities for the ecological de-sign goals were put forward. Suggestions were developed by determining the ecological design criteria. Evaluated ecological design criteria;

- **Structural components;** Bicycle path used for transportation, pedestrian and vehicle roads, all circulation routes, parking areas and roadside parking areas, permeability status of floor coverings.
- **Plant applications;** existence of natural plant species, wind curtain and shading ef-fect of plant tissue creating an ecological corridor, balance of plants' water needs in terms of water conservation, use of appropriate maintenance techniques.
- **Hard floor applications;** the use of ecological and recyclable materials in structural designs, a design approach suitable for the climatic conditions of the region, and ener-gy-efficient design approaches (water, wind and sun).

FİNDİNGS AND DİSCUSSİON

First, the current situation and analysis of the KTU Kanuni Campus area, the scope of which was evaluated, was made. For this purpose, the campus area was evaluated according to the vegetative and hard floor applications criteria determined in the method section. By analyzing the determined criteria, suggestions were developed according to the ecological campus design at the campus city scale.

Structural components: The entrance and exit points of the education and admin-istration buildings in the KTU Campus area operate according to their general functions and functions. Campus main entrance, A, B, C, D gates and vehicle and pedestrian en-trances are provided

by security controls. Vehicle roads provide the skeleton transporta-tion of the campus on the main arterial road. This road route serves public transportation vehicles and all other vehicle roads are connected to this main transportation network (Figure 3). The fact that public transportation vehicles are only on the main axis is positive in terms of reducing air and noise pollution. Vehicle roads are positioned to serve education and administration buildings. The vehicle road also provides opportunities for pedestrian transportation. There is a pedestrian access system on campus to building entrances, activity areas and dormitories (Kalayci Onac et.al., 2021). As Öner 1999 stated, pedestrian paths should carry pedestrians to assembly areas by providing uninterrupted circulation. Similarly, the pedestrian access in the campus area provides access to the assembly areas. However, although the festival area on the campus is sufficient in terms of use, it has problems in terms of transportation. The separation of pedestrian and vehicle roads is not clear. When the safety of pedestrian roads at night is examined, while night lighting is sufficient for areas with intensive use, the access roads on secondary roads, especially dormitories, are not sufficiently illuminated.

Figure 3. Structural components in the KTU Campus area (roadside parking lot).

There is no bike path on campus. This type of transportation vehicles come to the fore in sustainable planning approaches that provide great convenience in transportation and aim to reduce motorized transportation vehicles from the most important ecological trend to pedestrianization. The use of the BinBin Scooter, which is used as an alternative to cycling on campus and in the city, has also become widespread. The scooter, which provides pleasant, environmentally friendly and fast transportation to the campus, is also economical. However, the KTU Campus area does not have a suitable transportation network for scooter use. Using vehicle roads poses a security threat. It is aimed to reduce the use of vehicles, which is considered as an ecological planning approach, to increase the possibility of public transportation and to reduce carbon dioxide emissions with pe-destrian-oriented transportation. For this purpose, vehicle use should be reduced, espe-cially in campuses where pedestrianization would be most appropriate.

The other structural component is parking areas. Türeyen (2002) states that the parking areas within the campus should be in suitable numbers in suitable areas where vehicle roads and pedestrian roads meet (Figure 4). Similarly, the campus area is arranged near the educational buildings and in connection with the pedestrian paths. However, the parking areas on the campus do not meet the number of academic and student vehicles. Vehicles are parked along the main transportation axis of the road. This situation negatively affects the constantly flowing traffic, pedestrian transportation, bicycle and scooter use.

Figure 4. Structural components (pedestrian, bicycle and scooter transportation) in the KTU Campus area.

Within the scope of the study, completing the deficiencies of the structural components of university campuses in line with the ecological design principles would contribute to the spatial sustainability of the campuses (Telli & Aydın, 2024). Elements such as open and green areas, water elements, vehicle-pedestrian circulation systems, and building density stand out among the structural components on university campuses. Each of these elements has been seen to play a critical role in energy efficiency, conservation of natural resources, biodiversity, and user health, which are the basic principles of ecological design. Studies on this subject have been conducted in the literature. Interest in applying ecological planning principles to educational areas such as campuses has increased. Açıkay, 2025 and Ardıçoğlu et.al., 2024, especially in the studies carried out on the campus scaleemphasized the necessity of correlating spatial criteria such as ecological infrastructure, green area continuity, and building-green ratio. Yılmaz & Askan (2024) stated that green infrastructure on campuses is essential not only for aesthetics but also for increasing microclimatic comfort and supporting ecosystem services. As a result of the study conducted by Kawali (2021), it was seen that the widespread use of pedestrian-first transportation networks throughout the campus was effective in reducing the carbon footprint. Orenstein et.al., 2019 emphasized that many existing campuses are under urban growth pressure and that sustainable land use and transitional zoning strategies should be developed for their structural components. In this direction, this study revealed the necessity of conducting user experience-based evaluations

by considering the structural elements of campus areas quantitatively and qualitatively. It was argued that campuses should be evaluated not only as educational areas but also as an active part of the urban ecosystem. While this approach requires rethinking ecological design in the context of structural components, it also reveals the importance of multidisciplinary collaborations in campus planning.

Plant applications: In ecological landscape design criteria, the selection of species used in plant designs, healthy, well-grown, very little maintenance and application methods gain importance. Because the plant elements in the open green areas soften the climate of the city, provide permeable floors, and add an aesthetic dimension to the city. Vegetal designs should be planned sustainably, and the existing vegetation should be preserved and continue in accordance with the new designs. In herbal designs to benefit from the sun in winter; broad-leaved and dense-textured trees, evergreen, dense-textured tree-tree and shrub groups that aim to save energy by creating a shadow effect in summer should be used in plant designs. Climbing and climbing ivy species on the walls, gradual vegetation and creating a shielding effect for the winds provide heat gain (Birişçi et al., 2012). To cut the wind; Designs that are dense and sequential in the direction of the blowing wind, taking into account the seasonal prevailing wind directions, forming mounds and hillocks should be included (Alpay et al., 2013).

Figure 5. Plant applications in the KTU Campus area.

Kaya Şahin et al., (2020) determined that there are natural plants with high value in their study of plant taxa on campus. However, according to the study, the number of exotic plants was found to be close to the number of natural plants. In the study of Güneroğlu and Pektaş (2022) to determine the presence of edible fruit plants on campus (Figure 5), 46 different plant taxa with aesthetic and functional properties were identified. For this reason, nature-compatible plant groups should be preferred by giving place to more natural plant groups on campus. Because natural plant species do not require much maintenance, support sustainable design and are sensitive to current climatic conditions. At the same time, the water demands of natural plant groups are compatible with the climate of the region. Bayramoğlu (2016) examined the water demand levels of the plants in the main artery in accordance with xeric landscaping principles in KTU Kanuni Campus, and concluded that only 10 of the 53 plant species detected had low water de-mand, 13 had low/moderate water demand and 4 had high water demand.

Ecologically, one of the appropriate design criteria in plant applications is the selection of plant species that consume less water (Çorbacı & Özyavuz, 2024). Grass areas are the most consuming plant in landscaping applications. In the study conducted by Bayramoğlu (2016), she stated that grass mixtures are used extensively in herbal applications on campus. For this purpose, alternative species suitable for landscaping in terms of aesthetics and functionality should be preferred instead of these species. As an alternative to grass, instead of large grass surfaces, mulch, rubber, colored stones and glass shards can be used to cover the soil surface by keeping it moist. In terms of water management, applications that use and store water on campus and then use in open green areas should be preferred.

This study has revealed that plant applications should be evaluated in line with ecological design principles to contribute to spatial sustainability and ecosystem services on university campuses. Scientific studies, especially those on a campus scale, have also emphasized the importance of plant applications in campus system services.

Sarı & Karaşah, in their 2023 study, evaluated the ecosystem services provided by 147 woody plant taxa used in campus landscaping. The study revealed that campus plants provide cultural services such as air quality regulation, erosion control, pollination, aesthetic values , and education. Özdemir, in his 2024 study, showed that plant practices are essential in increasing spatial quality in an analysis conducted in the context of biophilic design principles on various university campuses in Türkiye. Tuna (2006) proposed a conceptual model for sustainable landscape planning on campuses. In the model developed within the framework of the green design approach, plant practices were evaluated in terms of their water management, energy saving, and habitat creation functions. These studies show that integrating plant practices with ecological design principles on university campuses can increase environmental sustainability, user satisfaction, and education quality. Considering factors such as local climate conditions, water consumption, maintenance requirements, and aesthetic values in planning plant arrangements will contribute to creating sustainable and functional campus areas.

Hard floor applications: Material selection and design features of floor coverings also gain importance in ecological planning approaches. Floor coverings cause the surface temperature to increase even more in hot regions.

For this reason, it also increases the urban heat island effect in spaces. For this reason, it is necessary to prefer to use materials that will reflect and absorb heat, especially in hot climatic regions. In humid and rainy areas, permeable floor coverings should be used to prevent surface water flow. Especially in these regions, the use of grass joint coating is appropriate. Since the study area is located in a rainy climate zone, permeable materials that prevent surface water flow and bring water together with underground water resources should be used. From this point of view, the campus area is insufficient in terms of permeable flooring materials. Vehicle roads are asphalt and walking paths consist of cobblestones. In certain periods when precipitation is high, water flows from sloping elevations and accumulates on vehicles and walking paths and threatens security (Büyükkurt, 2019).

In terms of environmental sustainability and user comfort on university campuses, hard floor applications should be integrated with ecological design principles. The widespread use of hard surfaces with impermeable materials causes problems in rainwater drainage, increases the heat island effect, and negatively affects biodiversity (Ardıçoğlu et al., 2024). At this point, applications such as sustainable material selection and permeable surface use are of critical importance in terms of the ecological performance of campuses. Hersek & Korkut, (2021) examined the xeriscape approach on the Tekirdağ Namık Kemal University campus and stated that microclimatic comfort decreased and green area continuity was disrupted due to the high impermeable rates of hard surfaces. In this study, it was suggested that permeable paving materials be spread by ecological design principles. Ak & Apaydın (2022) evaluated the hard floor covering materials used in landscape architecture according to visual impact and naturalness criteria and revealed the effects of user perception on material selection. This evaluation draws attention to the importance of a hard floor design that meets functional but also aesthetic and psychological needs. Açıkay emphasized the functional roles of hard floors in terms of user orientation, spatial definition, and pedestrian circulation in his study conducted in 2015, and stated that these components should be planned together with the plant texture and within ecological integrity. Finally, in the study of Ardıçoğlu et al. (2024), different campus typologies were compared regarding sustainability criteria, and the distribution of hard floors throughout the campus was evaluated regarding accessibility and water permeability. In this study, it is recommended that the rate of hard floors in campus design be reduced and natural materials used. These studies show that hard floor applications play critical roles not only in terms of spatial comfort and orientation functions, but also in terms of microclimate regulation, water cycle management and visual quality. Therefore, permeable, natural and user-friendly hard floor strategies that support ecological design on university campuses are indispensable for achieving sustainability goals.

CONCLUSION

Urban ecosystems are environments that construct the nature-human relationship in a heterogeneous and dynamic structure. Cities are spatial structures in which the foundations of sustainability are processed, and ecological functions are included in the design concept. Process and harmony are important in spatial planning for ecological functions to reach abiotic, biotic and cultural goals (Ahern, 2007).

Campuses are compact spaces of cities where natural and cultural elements come together. For this reason, it should be included in the planning with a holistic approach as a part of the cities, not separately from the cities. Campuses for educational purposes should be provided with opportunities where nature education can be practiced. For this purpose, campuses are also described as natural exhibition areas. When campuses are evaluated according to ecological criteria, they should be handled according to campus design components. Structural components, hard ground and vegetative applications should be planned and applied in this sense. In terms of architecture, energy use in buildings, plant preference suitable for the climate and floor coverings should be preferred.

Considering transportation from an ecological point of view, it is necessary to in-crease transportation with public transportation, reduce the use of private vehicles, and encourage users to use pedestrianization and bicycles. For transportation purposes, ed-ucation and management institutions should be designed in accordance with pedestrian transportation. For the ecological planning approach, facilities should be provided for non-motorized vehicles such as walking and bicycles. In order to save energy, the existing transportation route should be provided with ring services within the campus by in-creasing the public transportation opportunity, and the use of existing scooters should be made safe.

In addition to the presence of natural plants, natural plant species should be pre-ferred to reduce maintenance and cost costs in other planting designs. Water conservation approaches in cities should be adapted to campuses and supported for rainwater collection, storage and use in recreational activities.

Considering the intensity of use of campuses at all times of the year and every hour of the day, energy consumption is high. For this reason, energy use should be reduced, and renewable energy use should be supported. Wind, sun and rainwater should be used actively.

Reinforcement elements should be supported with solar panels, wind turbines and electricity generation should be provided in short-term use. Electrical energies to be used for lighting purposes should be evaluated within this scope. In addition, the use of water in the irrigation of open green areas should be provided from rainwater. Trans-portation is one of the most basic components on campuses. It is necessary for people to reach their desired destination safely and in a short time. For this reason, pedestrian, bi-cycle paths, wide structural grounds should be considered within the ecological im-provement model.

As a result; Measures should be taken to ensure energy efficiency in line with the principles of ecological campus planning.As the measures to be taken for this purpose provide energy savings during the operation process, they are capable of meeting the investment cost. While reducing energy consumption, which is one of the primary causes of global warming, ecological solutions will be obtained at the same time. As a result of the im-provement model developed on campuses, it will reduce the consumption of natural resources and preserve the qualities of existing resources. In the campuses, which are formed with ecological improvement activity targets, the targets of "energy efficient landscape design", "natural resource protection" and "bio comfort" are achieved.

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ARAŞTIRMA MAKALESİ **RESEARCH PAPER**

Soğuk Depolama Sürecinde Gökkuşağı Alabalığında (*Oncorhynchus Mykiss, L. 1758*) Meydana Gelen Fizikokimyasal Değişimlerin Gıda Güvenliği ve Besin Kalitesi Perspektifinden İncelenmesi [*]

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Öz: Bu çalışmada, Gökkuşağı Alabalığının (*Oncorhynchus mykiss, L. 1758*) 4±1°C'de depolama sürecinde meydana gelen fiziksel, kimyasal, biyolojik değişimler incelenerek besin kalitesi ve gıda güvenliği bakımından değerlendirilmesi amaçlanmıştır. Bu kapsamda ağırlık değişimi (g), % (nem , protein, yağ) değişimi, TBV-N (mg/100g), yağ asitleri ve aminoasit kompozisyonları incelenmiştir. Depolama süresinde ağırlık 110,95±0,55g –104,20±0,35g ve nem oranı %79,10±0,35–77,10±10 arasında değiştiği tespit edilmiştir. Serbest yağ asitliği (FFA % oleik asit olarak) %1,5±0,05-% 4,50±0,10; peroksit değeri 6,30±0,2–14,50±070 meq/kg; TVB-N değeri 15,30±0,02–32,23±0,15 mg/100g arasında değiştiği ve depolama boyunca yükseldiği belirlenmiştir. Yağ asitleri kompozisyonunda ΣSFA (toplam doymuş yağ asitleri) %24,34±0,18; ΣMUFA (toplam tekli doymamış) %31,45±1,05; ΣPUFA (toplam çoklu doymamış) %47, 42±1,13; dokosaheksaenoik asit (DHA) (C22:6n-3) 10,63±0,18 - 8,97±0,15, eikopentaenoik asit (EPA) (C20:5, n-3) %10,05±0,13- 3,38±0,15 olarak tespit edilmiştir. Esansiyel aminoasitlerin esansiyel olmayan aminoasitlere oranı (ΣEAA/ Σ NEAA) 0,66 olarak belirlenmiştir. Sonuç olarak, Gökkuşağı alabalığının 4±1°C'de soğuk depolamanın 4'üncü gün de fizikokimyasal reaksiyonların FFA (%), peroksit (meq/kg), üst sınırı aşan değerler bulunmuştur. Kimyasal bozulma ve tazeliğin değerlendirilmesinde önemli bir indikatör olan TVB-N(mg/100g) uygun olmayan sınıra yakın değer tespit edilmiştir. Kimyasal bozulma parametrelerinde artış depolama süresince devam etmiştir. Bütüncül bir değerlendirme yapıldığında Gökkuşağı alabalığının 4'üncü günden sonra besinsel kalite ve gıda güvenliği bakımından insan tüketimi için önerilemeyeceği belirlenmiştir. Bu veriler kapsamında 4±1°C'de soğuk depolamanın gökkuşağı alabalığının muhafazasında çok sınırlı bir etkiye sahip olduğu görülmüştür. Ancak besin kalitesi ve gıda güvenliği bakımından uzun süreli muhafaza için dondurarak muhafaza gibi daha etkin metotlarının kullanılması önerilmektedir.

Anahtar kelimeler: Aminoasit kompozisyonu, FFA, gökkuşağı alabalığı, peroksit, soğuk depolama, TBV-N.

Investigation of Physico Chemical Changes in Rainbow Trout (Oncorhynchus Mykiss, L. 1758) During Cold Storage from the Perspective of Food Safety And Nutritional Quality [*]

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Abstract: In this study, it was aimed to evaluate the physical, chemical and biological changes in rainbow trout (Oncorhynchus mykiss, L. 1758) during storage at 4±1°C in terms of food quality and food safety. In this context, weight change (g), % (moisture, protein, fat) change, TBV-N (mg/100g), fatty acids and amino acid compositions were analysed. It was determined that the weight ranged between 110,95±0,55g - 104,20±0,35g and moisture content ranged between 79,10±0,35-77,10±10% during the storage period. Free fatty acidity (FFA % oleic acid) ranged between 1,5±0,05%-4,50±0,10%; peroxide value ranged between 6,30±0,2-14,50±070 meq/kg; TVB-N value ranged between 15,30±0,02-32,23±0,15 mg/100g and increased during storage. In fatty acid composition, ΣSFA (total saturated fatty acids) were 24,34±0,18%; ΣMUFA (total monounsaturated) were 31,45±1,05%; ΣPUFA (total polyunsaturated) were 47, 42±1,13%; docosahexaenoic acid (DHA) (C22: 6n-3) was 10,63±0,18 - 8,97±0,15, eicopentaenoic acid and (EPA) (C20:5, n-3) was 10,05±0,13 - 3,38±0,15 %. The ratio of essential amino acids to non-essential amino acids (ΣEAA/ Σ NEAA) was 0.66. As a result, FFA (%), peroxide (meq/kg) and FFA (%) of physicochemical reactions of rainbow trout on the 4th day of cold storage at 4±1°C were found to exceed the upper limit. TVB-N (mg/100g), which is an important indicator in the evaluation of chemical spoilage and freshness, was found to be close to the limit. The increase in chemical spoilage parameters continued during storage. When a holistic evaluation was made, it was determined that rainbow trout could not be recommended for human consumption in terms of nutritional quality and food safety after the 4th day. Within the scope of these data, it was observed that cold storage at 4±1°C had a very limited effect on the preservation of rainbow trout. However, it is recommended to use more effective methods such as freezing for long-term preservation in terms of nutritional quality and food safety.

Key words: Amino acid composition, Fatty acid, Peroxide, Protein, Rainbow trout, TBV-N.

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GİRİŞ

Su ürünleri besin bileşimleri bakımından sağlıklı beslenmenin vazgeçilmez en önemli kaynaklarından biridir. Balıkta bulunan elzem besin ögeleri ve yüksek kalitedeki protein, balığı çok kıymetli bir gıda haline getirmektedir. Balık sadece bir hayvansal protein kaynağı olarak kalmayıp bir porsiyon balık (150g) ergin bir bireyin günlük protein ihtiyacının yarısından fazlasını karşılamaktadır (Öksüz vd., 2018). Özellikle beslenme bilincinin artması ve gelişen refah düzeyi balık ve balık ürünlerine olan talebi arttırmıştır. Bu nedenle su ürünlerinin gelecekte sağlıklı beslenmedeki öneminin giderek artacağı öngörülmektedir (Lymbery, 2000). Balık, içerik olarak su, protein, yağ, vitamin ve minerallerden oluşmuş bir gıdadır. İyot, vitamin D, kalsiyum, fosfor, potasyum, selenyum ve folik asit gibi vitamin ve mineraller ile vücut için esansiyel olan omega yağ asitleri ve amino asitlerce zenginbir kaynaktır. Protein dahil tüm besin bileşenlerinin biyoyararlılığı yüksektir. Bununla birlikte balık yağının doymamış yağ asitleri oranının yüksek olması, esansiyel yağ asitleri oranı n-6/n-3 dengeli içermesi balık yağını sağlıklı beslenme bakımından değerli yapmaktadır. Dengeli bir esansiyel yağ asitleri oranın (n-6/n-3), balık yağında yüksek oranda bulunan omega bazlı uzun zincirli eicosapentaenoic acid (EPA) ve docosahexaenoicacid (DHA) gibi yağ asitleri besinsel olarak önemli oldukları kadar kardiyovasküler ve kanser gibi hastalıkların önlenmesinde etkili oldukları belirtilmiştir (Foran, vd., 2005; Marchioli vd., 2009).

Protein, aminoasit, yağ ve yağ asitleri kompozisyonu bakımından önemli bir besin kaynağı olan balık fizyolojik olarak diğer gıda kaynaklardan farklı olmasından dolayı hızla bozulabilmektedir. Balık kalitesi; balığın türü, avlama bölgesi, avlama teknikleri, avlanmadan sonra uygulanan muhafaza teknikleri, ön işlemler, personel hijyeni, işleme ve depolamaya kadar geçen süre gibi faktörler tarafından etkilenmektedir (Özden & Gökoğlu 1997). Balıklarda bozulma avlama sonrası başlayan kimyasal, biyokimyasal (enzimatik) ve mikrobiyal olarak gelişen karmaşık reaksiyonlar zincirinden meydana gelmektedir (Hisar vd, 2004). Balık kas dokusundaki makro besin bileşenleri; protein, yağ, karbonhidrat ve suya bağlı olarak meydana gelen oksidatif reaksiyonlar da balığın bozulmasında etkili olmaktadır. Depolama şartlarına bağlı olarak yağda meydana gelen hidroliz ve peroksidasyon yanında, protein oksidasyonun da meydana gelmesi depolama sürecinde kalite kayıplarına neden olmaktadır (Çelik vd., 2008; Oğuzhan vd., 2006; Tunç, 1994; Şengör, 2020). Genellikle çiğ ve işlenmemiş yüksek protein içeren (%17-25) balık kas dokusunda protein oksidasyonu bozulma reaksiyonlarının gizli ve önemli nedenlerinden biri olduğu belirtilmiştir (Pearce vd., 2011). Protein oksidasyonunun genellikle lipid oksidasyonu ve biyokimyasal (enzimatik) reaksiyonlar gibi diğer oksidatif reaksiyonlarla ilişkili olduğu ifade edilmiştir (Soladoye, 2015). Su ürünlerinde fiziksel, mikrobiyolojik, biyokimyasal ve kimyasal bozulma reaksiyonlarının hızını azaltmak '*Su'dan – sofraya'* gıda güvenliğini sağlamak soğuk zincir içerisinde muhafaza edilmeleri ile sağlanabilmektedir (Ertaş, 1978; Gökoğlu & Varlık, 1992). Su ürünlerin muhafaza edilmesinde yaygın olarak soğukta muhafaza, dondurarak muhafaza ve buzda saklama gibi soğutma teknikleri uygulanmaktadır. Balığın taze olarak hızlı tüketim zincirinde uygulanan en yaygın muhafaza tekniklerinden biri soğuk muhafazadır. Etkili bir soğuk muhafaza işleminin yapılması için; balığın avlandığı andaki mikrobiyolojik yükü, yaşadığı suyun sıcaklığı, depolama sürecine kadar geçen süre ve yapılan ön işlemler, depolama şartları (sıcak, bağıl nem, depo atmosferinin bileşimi) gibi parametrelerin kontrol altında tutulması önem taşımaktadır (Varlık vd., 2004).

Bu çalışmada; kültür balıkçılığında yaygın olarak yetiştirilen su ürünlerinden biri olan Gökkuşağı Alabalığının hızlı tüketim zincirinde 4±1 °C soğukta depolama sürecinde meydana gelen fizikokimyasal ve biyolojik değişimlerin besin kalitesi ve gıda güvenliği açısından değerlendirilmesi amaçlanmıştır.

MATERYAL VE METOT

***Materyal*:** Çalışmada kullanılan Gökkuşağı Alabalığı (*Oncorhynchus mykiss, L. 1758)* İstanbul Üniversitesi Su Ürünleri Fakültesi Sabanca Balık üretme çiftliğinden temin edilmiştir. Çalışma kapsamına alınan balıkların aynı yaş grubuna ait olmaları, ortalama ağırlıklarının ve boylarının aynı olmasına özen gösterilmiştir. Balıkların önce iç organları temizlenmiş, yıkanıp kurulandıktan sonra eşit ağırlıkta olacak şekilde örnekler alüminyumla kaplanmış karton üzerine dörtlü gruplar halinde dizilerek streçle filmle kaplanmıştır. Başlangıç analizleri için (fiziksel, kimyasal) örnekler ayrılmış diğer örnekler 4 ±1 °C ve %95 nem ortamında soğuk depolamaya alınmıştır.

Metotlar

Ağırlık Değişimi: Depolama süresinde Gökkuşağı alabalığından iki gün ara ile numune alınarak hassasa terazide (Sartorius, 1615MP) tartılarak yapılmıştır.

NemTayini: AOAC, (1990 a), Memmert UM400 model etüv (Nürnberg,, Almanya) ile tayin edilmiştir. Kıyılmış homojenize edilmiş balıkdan hassas olarak sabit ağırlığa getirilmiş petri kapına 3,5 g örnek tartılmıştır. 105 °C'de ısıtılmış etüvde sabit tartıma ulaşılıncaya kadar

kurutulmuş ve ürünün nem miktarı ağırlık kaybından hesaplanmıştır**.**

Toplam Yağ Tayini: AOAC, (1990b), toplam yağ içeriği asit hidroliz yöntemi ile belirlendi. Bir santrifüj tüpü içerisine kıyılmış örnekler (1,5 g) tartılmıştır. Tüp içerisine seyreltik hidroklorik asit (Merck, Almanaya) (5 ml) ile ilave edilerek yaklaşık 45 dakika su banyosu üzerinde karıştırılarak ısıtılmıştır. Elde edilen karışım üzerine metanol (2,5 ml), dietil eter (7,5 ml) ve petrol eter (7,5 ml) ilave edilerek çözücü kombinasyona ile su banyosu üzerinde karıştırma ve ısıtma işlemi devam ettirilerek (30dakika) yağın eter fazına geçmesi sağlanmıştır. Daha sonra karışım santrifüj edilmiş, eter-yağ (üst faz) karışımı önceden darası alınmış bir yağ balonuna aktarılmıştır. Geri soğutucuda eter buharlaştırıldı ve örnekdeki yağ içeriği hesaplanmıştır.

Protein Tayini: Protein içeriğinin hesaplanmasında kıyılmış hohojenize edilmiş balık eti örneği kullanılmıştır. Protein içeriği, Gerhardt yarı mikro Kjeldahl yöntemi AOAC, (1990c), ve yanma yöntemine dayalı Leco CHNS 932 aparatı kullanılarak, AOAC, (1990d) distilasyon ve titrasyon yapılarak azot miktarı tayin edişlmiştir. (N × 6,25) kullanılarak protein oranı hesaplanmıştır.

Peroksit değerini (PV): Peroksit değerini belirlemek için, Gökkuşağı alabalığından elde edilmiş yağ ultra-turrax ile homojenize edilir, homojenize edilen yağdan bir erlene 5g tartılarak üzerine kloroform: asetik asit karışım çözeltisi (2:3) eklenirek 3 dakika çalkalanır. Üzerine 1,5 mL doymuş potasyum iyodür (KI) (Merck, Almanaya) çözeltisi eklenir ve 1 dakika çalkalanır, karanlıkda 5 dakika bekledilir. Daha sonra, üzerine 30 mL distile su eklenerek bir dakika daha çalkalanır. Daha sonra karışımın üzerine %1'lik nişasta çözeltisinden 1 mL ilave edilerek, 0,1 N sodyum tiyosülfat çözeltisi (NaS_2O_3) ile titre edilir. Bu işleme paralel olarak bir kör (örnek olmayan) titrasyon yapılır AOCS, (1994), Cd-5a-40). Titrasyonda harcanan sodyum tiyosülfat miktarı (mL) dikkate alınarak peroksit değeri meq/ kg olarak hesaplanır.

Serbest Yağ Asitliği (FFA% oleik asit): Serbest yağ asitliği AOCS, (1990), Ca-5a-40 standart titrasyon metoduna göre yapılmıştır. Erlen 2,5g balık yağı hassas olarak tartılır. Üzerine sıcak 50mL sıcak alkol ilav edilir, 2mL fenolfitalein(ff) indikatörü eklenerek 0,1N NaOH (Merck, Almanya) ile uçuk pembe renk verinceye kadar titre edilir. Harcanan NaOH miktarı (mL) dikkate alınarak serbest yağ asitliği hesaplanır.

Yağ Asitleri Kompozisyonu: IUPAC (1987), yağ asitleri kompozisyonu, International Union of Pure and Applied Chemistry Method 2. 301 talimatları izlenerek belirlenmiştir, 0.150- 0.200 g yağ örneği tartılarak üzerine 5 ml 0,5 N metanolik sodyum hidroksit (NaOH) (Merck - Almanya) çözeltisi eklenmiştir. Devamında 90 °C'de geri soğutucu altında su panyosu sabunlaştırma işlemi gerçekleştirilmiş, boron triflorür-¦metanol kompleksi (BF3) (Sigma Aldrich 8.01663, Darmstadt, Germany) ile metilasyon sağlanmış ve yağ asidi metil esterleri n-heptan fazına alınarak gaz kromatografisi (GC) (Shimadzu 2010, Japonya) cihazına enjekte edilmiştir.

GC cihazının çalışama şartları;

Enjektör sıcaklığı: 220 °C,

Kolon sıcaklığı: (75 °C, den 4°C /dak, artırılarak 200 °C çıkarılmıştır)

Dedektör Flame Ionization Detector (FID) sıcaklığı: 250 °C

Taşıyıcı gaz olarak helyum (He) (akış 30mL/dk), Split 1: 25.

Kolon (Supelco silica capillarry colon) (Uzunluk; 100m, film 0,20µm, yarı cap 0,25mm, Model SPTM-2380; Bellefonte, PA, USA).

Yağ asitlerinin tanımlanması ise, alıkonma zamanına göre yağ asidi metil esteri standartları kullanılarak yapılmıştır.

Toplam uçucu bazik azot (TVBN) tayini: Kimyasal analizlerden TVB-N (toplam uçucu bazik azot) analizi Antonocopoulos tarafından modifiye edilmiş, Lucke ve Giedel'e göre yapılmıştır (Schormuller, 1968).

Aminoasit Kompozisyonu: Örneğin hazırlanması; 0,1-1,0 g homojenize edilmiş örnek 50 ml'lik ağzı kapalı analiz şişesi içerisine alınır ve 6 N hidroklorik asit çözeltisinden 20 ml ilave edilir, şişe içine azot gazı verilerek ağzı sıkıca kapatılır ve 24 saat 110°C etüvde hidroliz edilir. Örnek, oda sıcaklığına getirilerek adi filtre kâğıdından süzülür. Süzüntüden 0,2 ml deney tüpüne alınarak azot gazı altında 50°C'de uçurulur ve üzerine 0,5 ml asetonitril konarak uçurma işlemi tekrarlanır. Tüp içindeki kalıntıya yaklaşık 0,5 ml asetonitril:metanol:trietilamin karışımı ve 0,1 ml türevlendirme çözeltisinden ilave edilerek 40°C etüvde 30 dakika süreyle türevlendirilir. Azot gazı altında 40°C'de uçurulduktan sonra üzerine 0,2 ml asetonitril ilave edilir ve azot gazı altında tekrar uçurulur. Üzerine 5 ml 0,02 M amonyum asetat çözeltisi ilave edilir. 0,2 µm filtreden süzülerek UFLC (Ultra hızlı likit kromatografi)'ye enjekte edilir (Dimova, (2003).

UFLC Çalışma koşulları:

Mobil Faz A: 1 L'lik balon jojeye 0,78 g sodyum dihidrojen fosfat dihidrat ve 0,88 g disodyum hidrojen fosfat dihidrat tartılır ve deiyonize su ile hacimine tamamlanır. Tampon çözeltisinin pH değeri 6,8-6,9 arasında olacak şekilde ayarlanır ve süzülür.

Mobil Faz B: Asetonitril

Kolon Sıcaklığı: 40°C

Dedektör: UV

Dalga Boyu: 254 nm

Enjeksiyon Hacmi: 10 µl

Akış Hızı:1 ml/dakika

Kolon: Agilent, Eclipse XDB-C18,5 µm, 4x6x150 mm

İstatistik Analiz: Bu çalışmada 4 depolanan Gökkuşağı alabalığının farklı günlerde ölçülen fiziksel, kimyasal parametrelerin ve kalite özelliklerinin zamana bağlı değişimini değerlendirmek için istatisitksel analiz yapılmıştır. Parametrelerin günler arasındaki farklılığını belirlemek için varyans analizi (ANOVA) uygulanmış ve anlamlılık düzeyi %95 güven aralığında ($p<0,05$) değerlendirilmiştir. Anlamlı farklılığın hangi gruplar arasında olduğunu belirlemek için LSD testi uygulanmış, sonuçlar ortalama ± standart sapma şeklinde verilmiştir. Tüm analizler, IBM SPSS 26 yazılım programı kullanılarak 3 tekerrürlü (n:3) yapılmıştır.

BULGULAR VE TARTIŞMA

Balıkların depolanma sürecini etkileyen önemli parametrelerin başında kas dokusunda bulıunan nem ve yağ, protein, karbonhidrat gibi makro besin bileşenleridir. Nem; kimyasal, enzimatik reaksiyonların ve mikrobiyolojik bozulmanın meydana gelmesinde önemli bir indikatördür. Balıkta'da nem üç farklı şekilde bulunmakatadır; makromolekül bileşikleri ile yakın ilişkili olarak bulunan bağlı su, organize protein yapıları içinde yer alan immobilize su ve ekstra-miyofibriler içinde bulunan serbest su şeklinde bulunur (Pearce, vd., 2011). Sun vd., (2018) tarafından yapılan çalışmada balığın 4±1 °C soğuk depolama sırasında protein ve su etkileşimi nedeniyle depolamanın erken aşamasında bağlı su ve serbest su arasında belirgin geçişler gözlenmiştir (Wang vd., 2018). Wang vd., (2019) yapılan çalışmada 4±1 °C'de somon balığında depolama süresinin uzamasıyla birlikte kas dokusundaki bağlı su oranın da azalma olurken serbest su oranında artış meydana geldiği bunun kas dokusunda yumuşamaya ve kas proteinlerinde kayıplara neden olduğu ifade edilmiştir. Nem kaybının ayrıca kas dokusunun fiziksel özelliklerini olumsuz etkilediği, proteinlerde kısmı denatürasyona ve kas dokusunun su tutma kapasitesinin azalmasına neden olduğu belirtilmiştir (Hultmann & Rustad, 2002).

Tablo 1. Gökkuşağı alabalığının soğukta depolama (+4 ±1 ºC) sürecinde (gün) meydana gelen, Ağırlık değişimi(g), % (nem, protein, yağ, serbest yağ asitliği), peroksit meq/kg, TVB-N mg/100g fizikokimyasal değişimler.
Table1. Weight change (g), % (moisture, protein, fat, free fatty acidity), peroxide meq/kg, TVB-N mg/100g physicochemical changes in rainbow trout during cold storage (+4 ± 1 ºC)

Değişkenler	0	2(gün)	4(gün)	7(gün)	10(gün)	12(gün)	14(gün)	17 (gün)	F	p
Ağırlık değişimi	110,95±0,55^{a}	109,80±0,60^{ab}	109,00±0,20^{ab}	107,35±0,75^{b}	105,80±0,20^{c}	105,10±0,30^{cd}	104,60±0,05^{d}	104,20±0,35^{d}	99,215	**0,000***
Nem (%)	79,10±0,35^{a}	78,70±0,10^{a}	78,40±0,05^{ab}	78,60±0,57^{a}	77,80±0,05^{b}	77,35±0,05^{c}	77,25±0,05^{d}	77,10±0,05^{d}	27,280	**0,000***
Protein(%)	21,13±0,21^{a}	20,15±0,05^{b}	19,15±0,90^{b}	19,33±0,49^{b}	18,95±0,05^{c}	18,92±0,03^{c}	18,60±0,20^{d}	18,60±0,10^{d}	14,731	**0,000***
Yağ(%)	3,80±0,10	3,75±0,15	3,75±0,05	3,70±0,20	3,75±0,05	3,80±0,10	3,72±0,10	3,70±0,10	0,346	0,919
Serbest yağ asitliği	1,15±0,05^{c}	1,60±0,10^{c}	4,10±0,10^{a}	3,30±0,20^{b}	3,60±0,10^{b}	4,25±0,25^{a}	4,15±0,15^{a}	4,50±0,10^{a}	439,178	**0,000***
Peroksit ((meq/kg	6,30±0,20^{f}	10,85±0,65^{e}	17,60±0,40^{a}	10,30±0,25^{e}	11,85±0,35^{d}	16,80±0,30^{b}	17,20±0,20^{a}	14,50±0,70^{c}	276,021	**0,000***
TVB-N (mg/kg)	15,30±0,02^{e}	14,85±0,15^{e}	20,10±0,30^{d}	12,30±6,32^{f}	21,75±0,25^{c}	24,98±0,03^{b}	25,10±0,10^{b}	32,23±0,15^{a}	25,282	**0,000***

P< 0,05; sonuçlar ortlama± standart sapma(n:3) olarak verilmiş olup; aynı satırda farklı harfleri (a, b, c, d, e, f) taşıyan ortalamalar arasındaki fark önemli dir.

Tablo. 1'de Gökkuşağı alabalığının makro besin bileşenlerinde meydana gelen fiziksel, kimyasal ve biyolojik değişimler verilmiştir. Ağırlık değişimi; 2,10 ve 12, nem; 2, 7, 10, 12, serbest yağ asitliği; 2, 4 ,7, 12, peroksit değeri; 2, 4,7, 10 ve12, protein; 2, 10,12 ve14, TVB-N değeri; 4, 7,10, 12 ve 17 günleri arasında meydana gelen değişimler istatistiksel olarak (P< 0,05) göre önemli olduğu belirlenmiştir.

Ağırlık değişimi: Örneklerde 17'nci gün sonunda en düşük ağırlık % 104,20±0,35 (g) olarak tespit edilmiştir (Tablo 1). Örneklerdeki ilk ağırlığa göre 110.95±0.55; F = 99.215, (p = 0.000), 17 günlük depolama sonunda toplam ağırlık kaybının 6,75 ± 0.20 g olduğu tespit edilmiştir.

Nem (su) kaybı: Balıkların kas dokusunun nem tutma kapasitesi etin sertliği, yumuşaklığı, çözünme sırasında damlama ve depolama ve taşıma sırasında ağırlık kaybını etkilemesinden dolayı önemli bir kalite parametresidir. Nem tutma kapasitesi ve nem kaybındaki değişkenlik balık dokusunu olumsuz etkilemekte ve kalite kaybına neden olmaktadır (Duun & Rustad, 2007; Davson, vd. 2018).

Çalışmamızda nem oranlarındaki değişim ise; depolama başlangıcında nem oranı %79,10±0,35 olduğu 17. Gün sonunda %77,10±0,05 düştüğü belirlenmiştir. Nem kaybı balığın tazeliği hakkında karar verilmesinde önemli bir parametre olması yanında kimyasal bozulmaların da tamamlayıcısı olarak da değerlendirilmektedir (Tunç, 1994). Özden (2005); Çelik & Küçükgülmez (2008) tarafından yapılan çalışmalarda taze gökkuşağı alabalığında nem oranları sırasıyla %76.23±2.02 ve %71.65±0.076 olarak belirlemiştir. Bulgularımız her iki çalışmada bulunan nem oranlarının bir miktar (%79.10 ± 0.35) üzerinde bulunmuştur.

Yağ Oranında değişim: Birçok çalışmada balıkların dondurarak depolanması sırasında yağ içeriğinin azaldığını belirtmiştir (Omotosho & Olu, 1995). Arannilewa, vd, (2005) tarafından yapılan çalışmada Tilapia balığının toplam yağ içeriğinin 60 günlük dondurulmuş depolama sırasında %9,72'den %7,20'ye düştüğünü tespit etmişlerdir**.** Gökkuşağı alabalığının soğuk depolama sürecinde yağ oranında anlamlı bir değişiklik olmamıştır (P<0,05). Ancak kısmı bir azalma meydana gelmiştir. (%3,80±0.10- 3.70±0.10). Ellahamy vd., (2023), tarafından yapılan çalışmada Gümüş ve Sazan balıklarında depolama sırasında toplam yağ oranlarında çok az bir azalma olduğunu bunun hidroliz ve oksidasyon reaksiyonları sonucu parçalanmadan ileri geldiğini belirtmişlerdir. Çalışmamızda gökkuşağı alabalığında

depolama sürecinde yağ oranında meydana gelen azalmanın aynı nedenlerden ileri geldiği düşünülmektedir.

Serbest yağ asitliğinde değişim (FFA%): Balık yağları yüksek oranda doymamış yağ asitleri içermeleri nedeniyle hidroliz ve oksidasyon reaksiyonlarına karşı aşırı hassastırlar. Yağda meydana gelen oksidasyon ve hidroliz reaksiyonları sonucu oluşan, aldehid ve ketonlar gibi bileşikler istenmeyen tat ve koku oluşmasına, besin değerinin düşmesine ve depolama süresinin kısalmasına neden olurlar (Boran vd., 2006). Secci ve Parisi, (2016) tarafından yapılan çalışmada taze yağlı balıkların buzda depolanması sırasında, yağlarda meydana gelen oksidasyon reaksiyonlarının ağırlıklı olarak kimyasal bozulmayı etkilediği, buzdolabı koşullarında depolanan balıklarda ise mikrobiyolojik (bakteriyel) bozulmanın hidrolitik reaksiyonları etkilediği belirtilmiştir. Özyurt, vd., (2007), tarafından kefal balığının +4 ºC buzdolabında depolanmasında serbest yağ asitliğinin 7'nci günde %5.26 yükseldiği belirtmişlerdir. Tablo. 1'de Gökkuşağı alabalığında depolama başlangıcında serbest yağ asitliğinin (FFA) %1,15 ±0,05 tespit edilmiş olup, 4. Gün sonunda %4,10±0,10' yükselmiştir. Depolamanın 17.gün sonunda FFA %4,50±0.10 yükseldiği belirlenmiştir.

Peroksit değerinde (meq/kg) değişim: Yağlarda meydana gelen oksidasyonun belirlenmesinde önemli parametrelerden biri de peroksit değeridir. Balık yağının yüksek oranda uzun zincirli yağ asitlerini içermesi okisdasyon eğilimini artırmaktadır. Okisadsyon çoklu doymamış yağ asitlerinde gelişerek balığın besin kalitesi ve raf ömrünü olumsuz etkilemektedir (Chakrabarty, 2003; Taheri, vd. 2012). Balıkta bulunan uzun zincirli aşırı doymamış eikosapentaenoik asit (EPA, 20:5ω-3), dokosaheksaenoik asit (DHA, 22:6ω3) ve PUFA gibi yağ asitlerinin oksidasyona karşı aşırı duyarlılık göstermeleri sonucu oluşan oksidasyon ürünleri (aldehitler, ketonlar , krbonil bileşikler) balık'da metalik ve acımsı tatların gelişmesine neden olmaları sonucu balığın besinsel kalitesinin bozulduğu ifade edilmiştir (Wen vd., 2023). Boran vd., (2006) tarafından farklı balık türlerinin yağ kalitesi üzerinde depolama sıcaklığının etkileri incelemiş sıcaklığın balık yağının debpolama süresi üzerinde önemli etkiye sahip olduğunu belirtmişlerdir. Özyurt vd., (2007) farklı yaş gruplarında kefal balığının (+4°C'de) depolanması sonucu balığın peroksit değerini 17.26 meq/kg – 19.73 meq/kg arasında değiştiği ve tazeliğini kaybettiğini belirtmişlertir. Çalışmamızda peroksit değeri başlangıçtan itibaren sürekli artış göstermiş olduğu 4'üncü günde (17,60 ± 0.40 meq/kg) düzeyine yükseltiği tespit edilmiştir.

Proteinlerde meydana gelen değişimler: Proteinler balık kas dokusunda bulunan önemli organik bileşiklerden biridir. Depolama sırasında bu bileşikler hem endojen hem de mikrobiyal enzimlerin etkisi ile parçalanması sonucu kas dokusunun su tutma kabiliyetinin bozulmasına ve kalite kaybına neden oldukları belirtilmiştir (Benjakul vd., 1997; Zhuang, vd, 2023). Su ürünlerinde soğuk depolama sırasında meydana gelen enzimatik reaksiyonlar ve bakteriyolojik faaliyetler sonucu protein içeriğinde ve kalitesinde düşümeye neden olduğu belirtilmektedir (Fallah *vd*., 2015; Wu & Bechtel, 2008). Çalışmamızda gökkuşağı alabalığının soğuk depolama sürecinde protein değerleri taze örnekte %21.13±0.21 ve 17 gün sonunda %18,60±0,10 olarak belirlenmiştir. ($F = 14.731$, $p = 0.000$) göre önemli bir azalma olduğu tespit edilmiştir. Depolamanın 2, 10, 12 ve14. günleri arasında protein değerlerinde istatistiki olarak $p<0$, 05 düzeyinde önemli bir değişiklik (azalma) tespit edilmiştir. Gökkuşağı alabalığında depolama sürecinde proteinlerde meydana gelen değişimlerin kısmen kas dokusundaki serbest su oranındaki artıştan ve enzimatik, bakteriyolojik faaliyetden kaynaklanabileceği düşünülmektedir.

Toplam uçucu bazik azot (TVBN) meydana gelen değişim: TVB-N, balık ve balık ürünlerinin kalitesini ve raf ömrünün belirlenmesinde yaygın olarak kullanılan bir parametredir (Fernández**,** vd. 1997; Fernandez-Segovia vd. 2012; Rizo vd. 2015). Trimetilamin, amonyak ve dimetilamin gibi uçucu bileşikler ve mikroorganizmaların faaliyetleri sonucu oluşan toplam uçucu bazik azot (TVB-N) olarak tanımlanır. Deniz ürünlerinin kalitesini ve gıda güvenliğini izlemek için kullanılan en önemli indikatörlerden biridir (Özoğul & Özoğul, 2000). Ayrıca TVB-N değerinin balık ürünlerinde tazeliğinin ve tüketim için uygunluğun belirlenmesinde kimyasal yöntemlerin en önemlilerinden biridir (Lang, 1979; Gökoğlu & Varlık, 1992; Fatih & Yeşim, 2000). Soğuk depolama sırasında TVB-N değerindeki artış, esas olarak enzimatik reaksiyonlar ve mikrobiyal aktivite ile proteinlerin parçalanması sonucu oluşan alkali özellikteki (amonyak, trimetilamin, dimetilamin ve diğer uçucu bazik azotlu) maddelerinden kaynaklandığı belirtilmiştir (Otero, 2019). TVB-N parametresi, balıkların tazeliğini etkileyen proteinlerin bozulma düzeyini değerlendirilmesinde kritik öneme sahiptir (Chu vd., 2023; Karsli vd., 2021). Farklı ambalaj tipleri ile ambalajlanan sazan balığı filatoları nın +4 ºC soğuk depolaması sırasında elde dilen TVB-N sonuçları, farklı ambalaj tipleri, depolama süresi ve protein oksidasyonu arasındaki ilişkiyi göstermesi bakımından önemli olduğunu belirtilmiştir (Ramadhan vd., 2025). Aynı çalışmada, acık atmosferik ambalajlarda mikroorganizma faaliyetlerinin yüksek olması nedeniyle proteinlerin amino asitler ve diğer polipeptitleri parçalanma oranının da yüksek olduğu buna bağlı olarak TVB-N değerininde yüksek olduğu belirtilmiştir.

TVB-N değeri taze balıklarda 5-20 mg /100 g arasında, buzda saklanan balıklar için TVB-N değerinin

30-35 mg /100 g arasında olması kabul edilebilir sınır değer olduğu belirtilmiştir (Connell, 1995). Özoğul & Özoğul (2000) tarafından gökkuşağı alabalığında TVB-N değeri 9 günde 40mg/100g, Özden vd, (2007), tarafından levrek balığının +4 ºC de depolanması sırasında TVB-N değerinin 7'nci günde 27,54 mg / 100g yükseltiğini tespit etmişlerdir. Çalışmamızda Gökkuşağı alabalığının başlangıç TVB-N değeri 15.30±0.02 mg/100 g olarak belirlenmiştir. Depolama sürecinde 4, 7, 10, 12, ve 17. günlerde değişim ($p<0.05$) önemli bulunmuştur. TVB-N değeri 17'nci gün'de 32.23±0.15 mg/100 g'a yükseldiği tespit edilmiştir. Metin & Varlık, (1997), tarafından yapılan çalışmada soğukta depolanan alabalıklar için TVB-N değeri 25 mg/100 g üst sınır olarak kabul edilebileceği belirtmişlerdir. Bu değerlendirmeye göre çalışmamızda gökkuşağı alabalığının soğuk depolama sürecinde TVB-N değerleri bakımından 12/14 günlerde ileri düzde bozulmuş oldukları ve TVB-N değeri bakımından üst sınırı aşmış olduğu tespit edilmiştir.

Bilgin vd., (2006) tarafından yapılan çalışmada pişmiş ve çiğ olarak +4±1 °C muhafaza edilen karideslerin duyusal, kimyasal ve mikrobiyolojik analiz sonuçlarına göre, çiğ karideslerin 2 gün, pişmiş karideslerin 4. gün bozulmadan saklanabildiğini, 5'inci gününden itibaren kimyasal, mikrobiyolojik ve duyusal olarak bozulmanın olduğunu belirtmişlerdir. Çalışmamızda 4 'üncü günde FFA, peroksit, TVB-N değerleri birlikte değerlendirildiğinde oksidasyonun başladığı ve krtik seviyeye geldiği belirlenmiştir. Depolama sürecinde bu değerlerde artış devam etmiş ve istatistiksel $p<0.05$ olarak bu artışlar önemli bulunmuştur.

Tablo 2. Gökkuşağı Alabalığının Soğuk Depolama Sürecinde (+ 4 °C) Yağ Asitleri Kompozisyonunda Meydana Gelen Değişimler (%).
Table 2. Changes in Fatty Acid Composition (%) of Rainbow Trout during Cold Storage (+ 4 °C).

Parametreler	**0**	**2**	**4**	**7**	**10**	**12**	**14**	**17**	**F**	**p**
Miristik **(C14:0)**	3,60^{c}±0,05	3,38^{c}±0,16	4,25^{b}±0,10	4,65^{a}±0,26	4,77^{a}±0,13	4,13^{b}±0,06	4,47^{a}±0,32	4,95^{a}±0,13	27,901	**0,000***
Palmitik **(C16:0)**	14,88^{b}±0,20	14,85^{b}±0,13	15,42^{ab}±0,20	15,65^{a}±0,13	15,50^{a}±0,30	15,43^{a}±0,31	16,00^{a}±0,26	16,93^{a}±0,15	26,606	**0,000***
Stearik (**C18:0**)	5,97^{c}±0,21	5,60^{c}±0,26	5,52^{c}±0,10	5,77^{c}±0,15	5,57^{c}±0,37	5,78^{c}±0,08	6,57^{b}±0,42	7,27^{a}±0,15	18,253	**0,000***
∑SFA	24,34^{d}±0,18	23,83^{d}±0,36	25,18^{d}±0,15	26,06^{c}±0,07	25,83^{c}±0,45	25,34^{cd}±0,30	27,03^{b}±0,49	29,15^{a}±0,23	110,178	**0,000***
Miristoleik **(C14:1, n-7)**	0,53^{c}±0,10	0,73^{b}±0,10	0,77^{b}±0,15	0,83^{a}±0,08	0,93^{a}±0,15	0,75^{b}±0,14	0,60^{c}±0,05	0,53^{c}±0,06	6,580	**0,001***
Palmitoleik **(16:1, n-7)**	5,95^{a}±0,23	5,33^{b}±0,10	4,72±0,08	5,43^{b}±0,15	5,23^{b}±0,08	5,20^{b}±0,10	4,78^{c}±0,13	4,78^{c}±0,18	25,672	**0,000***
Oleik (**C18:1, n-9**)	19,37^{c}±0,74	20,67^{b}±0,31	20,97^{ab}±0,21	21,18^{a}±0,23	21,35^{a}±0,40	21,27^{a}±0,32	21,40^{a}±0,36	21,15^{a}±0,59	7,979	**0,001***
Eikosanoikasit **(C20:1, n-9**)	3,68^{a}±0,13	3,07^{b}±0,15	2,60^{c}±0,26	2,47^{c}±0,15	2,35^{c}±0,15	2,35^{c}±0,22	3,25^{ab}±0,31	2,93^{b}±0,15	15,728	**0,000***
Dekasoneik asit **(C22:1, n-11)**	1,93±0,13	1,70±0,10	1,95±0,10	1,47±0,18	2,27±0,68	1,8±0,10	1,75±0,15	1,87±0,03	2,382	0,079
∑MUFA	31,45±1,05	31,50±0,09	31±0,22	31,53±0,63	31,97±0,83	31,37±0,46	31,65±0,71	31,27±0,55	0,682	0,686
Linoleik **(C18:2, n-6)**	18,38^{a}±0,71	18,18^{a}±0,10	18,25^{a}±0,05	17,68^{b}±0,23	18,72^{a}±0,13	17,65^{b}±0,05	17,57^{b}±0,28	16,90^{b}±0,20	10,904	**0,000***
Linolenik **(C18:3, n-6)**	5,35±0,23	5,40±0,05	5,18±0,10	5,32±0,08	5,20±0,10	5,25±0,15	5,22±0,13	5,10±0,17	1,364	0,293
Stearidonik asit **(C18:4, n-3)**	2,10^{c}±0,20	2,55^{a}±0,15	2,32^{b}±0,13	2,03^{c}±0,13	2,48^{a}±0,16	2,53^{a}±0,1	1,85^{d}±0,28	2,42^{b}±0,18	6,025	**0,002***
Eikopentaenoik **(C20:5, n-3)**	3,05^{c}±0,13	3,45^{b}±0,13	2,97^{c}±0,21	4,15^{a}±0,23	4,05^{a}±0,18	3,90^{a}±0,18	3,43^{b}±0,08	3,38^{b}±0,15	19,466	**0,000***
Dokosapentaenoik **(C22:5, n-3) (EPA)**	2,28^{a}±0,20	2,02^{a}±0,10	1,92^{ab}±0,08	1,82^{b}±0,10	1,77^{b}±0,10	1,68^{bc}±0,08	1,63^{c}±0,08	1,52^{c}±0,13	13,825	**0,000***
Dokosaheksaenoik **(C22:6, n-3**) (DHA)	10,63^{a}±0,18	10,43^{a}±0,13	10,13^{b}±0,08	9,78^{b}±0,16	9,60^{b}±0,23	9,32^{c}±0,18	9,20^{c}±0,26	8,97^{c}±0,15	52,198	**0,000***
ΣPUFA	47,42^{a}±1,13	46,80^{a}±0,23	45,32^{b}±0,56	44,72^{bc}±0,45	46,43^{a}±0,67	44,48^{c}±0,38	43,90^{c}±1,18	43,08^{c}±0,29	12,506	**0,000***
∑n-3	22,65^{b}±0,88	23,85^{a}±0,43	22,52^{b}±0,16	23,10^{b}±0,35	23,10^{b}±0,25	22,68^{b}±0,33	21,33^{c}±0,49	21,38^{c}±0,35	9,510	**0,000***
Σn-3/ Σn-6	1,27^{b}±0,03	1,31^{a}±0,03	1,23^{b}±0,01	1,30^{a}±0,04	1,23^{b}±0,02	1,28^{a}±0,03	1,21^{b}±0,02	1,26^{ab}±0,04	5,130	**0,005***
ΣPUFA/ ΣSFA	1,94^{a}±0,04	1,96^{a}±0,03	1,80^{b}±0,02	1,73^{c}±0,03	1,79^{b}±0,04	1,75^{b}±0,01	1,62^{d}±0,07	1,47^{d}±0,02	62,152	**0,000***

(P<0.0 5) güven aralığında sonuçlar; ortalama± standart sapma(n:3) olarak verilmiş olup; aynı satırda farklı harfleri (a, b, c, d,) taşıyan ortalamalar arasındaki farklar önemlidir. Not: SFA: doymuş yağ asitleri; MUFA: tekli doymamış yağ asitleri; PUFA: çoklu doymamış yağ asitleri; EPA: Eikosapentaenoik asit; DHA: dokosahekzaenoik asit

Yağ asitleri kompozisyonunda meydana gelen oksidasyon ve değişimler: Balık yağının yağ asitleri kompozisyonu ağırlıklı olarak uzun zincirli ve çoklu doymamış yağ asitlerinden oluşmaktadır. Yağ asitleri kompozisyonu beslenme, mevsimsel değişim, su sıcaklığı gibi birçok faktöre bağlı olarak değişiklik gösterebilmektedir (Williams & Hazel, 1992; Biderre, vd. 2000). Ayrıca tatlı su balıkları, deniz balıklarından daha fazla n-6 yağ asiti içermekte, buna karşılık daha az n-3 yağ asidi içermektedirler. Bununla birlikte su ürünleri yetiştiriciliğinde elde edilen balıkların n-6 yağ asiti içeriği balıkçılıkla elde edilenden daha yüksektir (Kocatepe & Turan, 2018). Balık yağı sağlıklı beslenme için vazgeçilmez önemli olan n-6, n -3 ve DHA (dokosahekzaenoik asit), EPA (Eikopentaenoik asit) gibi yağ asitlerinin doğal kaynağı durumundadır (Konar& Köprücü, 2002; Bayraklı vd., 2019). Özellikle çoklu doymamış yağ asitleri (PUFA) insanlar üzerindeki farmakolojik ve fizyolojik etkileri bilinmektedir (Singer & Calder, 2023; Duyar & Bayraklı, 2023).

Balık yağlarında doymuş yağ asitleri oranı oldukça düşüktür ve birçok çalışmada balık yağlarında doymuş yağ asitlerinin (SFA) palmitik (C16:0), stearik (C18:0), miristik (C14:0) asitlerden oluştuğu belirtilmiştir (Karslı, 2021; Bayraklı, 2023).

Tablo 2'de Gökkuşağı alabalığında soğuk depolama sürecinde yağ asitleri kompozisyonunda meydana gelen değişimler ($p<0.05$) güven aralığında değerlendirilmiştir. Taze durumda yapılan yağ asitleri kompozisyonu, ΣSFA (toplam doymuş yağ asitleri) %24,34±0,18, ΣMUFA (toplam tekli doymamış) %31,45±1,05, ΣPUFA (toplam çoklu doymamış) %47,42±1,13 olarak tespit edilmiştir. Depolama süresinde doymuş yağ asitleri %24,34±0,18 'den % 29,15±0,23 ($p<0.05$) göre anlamlı artış olduğu tespit edilmiştir. ΣMUFA değişim ise %31,45±1,05' den %31,27±0,55 $p<0.05$ göre önemli bir değişimin olmadığı belirlenmiştir. ΣPUFA %47,42±1,13'den % 43,08±0,29 azalma olduğu bulunmuştur. Kardiyovasküler hastalıklardan korunmak için ΣPUFA/ ΣSFA oranı minimum 0.45 olması

önerilmektedir (HMSO, 1994; Harper vd., 2005). Tablo 2'de gökkuşağı alabalığında ΣPUFA/ ΣSFA oranı depolama süresince %1,94±0,04-1,47±0,02 olduğu ve referans değerin çok üzerinde (3, 4 katı) olduğu belirlenmiştir. Bu verile ışığında gökkuşağı alabalığın kardiyovasküler hastalıkların önlenmesinde önemli etkiye sahip olduğunu söylenebilir. Çalışmamızda ΣPUFA yağ asitlerinde meydana gelen azalmanın oksidasyona hassas olmaları nedeniyle parçalanmaları ve kısmen doymuş yağ asitlerine dönüşmeleri sonucu olduğu öngörülmektedir. Balıkların depoılanması ile ilgili yapılan bir çalışmada balıklarda bulunan eikosanoik asit (EPA) ve dokosaheksaenoik (DHA) gibi yüksek doymamış yağ asitlerinin depolama süresine ve sıcaklığına bağlı olarak azalma görüldüğü belirtilmiştir (Chaijan vd., 2006; Tenyanga vd., 2017). Çalışmamızda Gökkuşağı alabalığın soğuk depolama sürecinde yağ asitleri kompozisyonundaki değişimlerin literatür bulguları ile uyumlu oldukları görülmüştür.

Aminoasitlerde meydana gelen değişimler: Gökkuşağı alabalığı birçok esansiyel aminoasitin kaynağı durumundadır (Wesselinova 2000; Li, vd.2009). Amino asitler geleneksel beslenme bakımından esansiyel aminoasitler (EAA) ve esansiyel olmayan (NEAA) aminoasitler olarak sınıflandırılmıştır (Wu, 2010). Arginin, sistin, histidin, lösin, lizin, metiyonin, treonin, triptofan, tirozin ve valin EAA' lerdir. Aspartik asit, serin ve alanin NEAA' lerdir. Esansiyel amino asitler diyetteki proteinlerden elde edilir ve diyet proteininin kalitesi bu proteinlerin varlığına ve bulunma oranına göre değerlendirilir. Bu nedenle, gıdaların besinsel kalitesinin belirlenmesinde yapılarındaki proteinin esansiyel amino asit (EAA) oranı önemli indikatördür (Young & Pellett, 1984). Gıdaların amino asit bileşiminin bilinmesi, potansiyel besin değerlerinin belirlenmesinde önemli bir parametredirler. Ayrıca, gıdaların hazırlanması, işlenmesi ve depolanması sırasında ortaya çıkabilecek besin değerindeki değişikliklerin ortaya konulmasına da imkân sağlayabilirler (Williams, 2005). Son yıllarda yapılan çalışmamalarda fonksiyonel amino asit (FAA) kavramı ortaya konulmuştur. Arginin, sistin, lösin, metiyonin, triptofan, tirozin, aspartat, glutamik asit, glisin, prolin ve taurin insan beslenmesi için FAA olarak sınıflandırılmışlardır. FAA su ürünlerinde besin kalitesi ve lezzetin yanı sıra depolama sırasında tazeliğin karakterize edilmesinde de önemli rol oynadıkları belirtilmiştir (Wu, 2013). Alvarez, vd (1999), tarafından dondurulmuş depolamanın balıkların amino asit üzerindeki etkisini belirlemek için yaptıkları çalışmada 4 ay boyunca -12°C'de depolanan Hake balığında (Merluccius sp.) lizin aminoasitinde azalma tespit emişlerdir.

Gökkuşağı alabalığı FAA olarak tanımlanan aminoasitlerin büyük bir bölümünü yapısında bulundurmaktadır. Iwasaki & Harada (1985) tarafından yapılan çalışmada alabalıkta aspartik asit, glutamik asit ve lizin aminoasitinin başlıca kaynağı olduğunu belirtmişlerdir. Ayrıca alabalıklarda avlanma zamanı ve yaşam bölgesi aminoasit kompozisyonu üzerinde etkili olduğu belirtmiştir (Wesselinova 2000).

Tablo 3. Gökkuşağı alabalığının soğuk depolama (+4 ±1 ºC) sürecinde aminoasit kompozisyonu değişim (mg/100 g)
Table 3. Changes of amino acid composition (mg/100 g) of rainbow trout during cold storage (+4 ± 1 ºC).

Değişkenler	0. gün	2. gün	4. gün	7. gün	10. gün	12. gün	14. gün	17. gün	F	p
Alanin	1115.00±5.00^{d}	1155.00.±5.00^{b}	1240.00±10.00^{a}	1130.00±5.00^{d}	1190.00± 5.00^{c}	1215.00±5.00^{b}	1190.00±10.00^{c}	1205.00±5.00^{b}	164.211	**0.000****
Arginin	1020.00±20.00^{b}	1020.00±10.00^{b}	1175.00±5.00^{a}	1030.00±30.00^{b}	1050.00±10.00^{b}	1040.00±10.0^{b}0	1040.00±10.00^{b}	993.33±15.28^{c}	32.882	**0.000***
Aspartik asid	1780.00±5.00^{c}	1880.00±5.00^{b}	2025.00±10.00^{a}	1865.00±5.00^{b}	1865.00±20.00^{b}	1860.00±10.00^{b}	1870.00±10.00^{b}	1855.00±15.00^{b}	106.521	**0.000***
Fenilalanin	705.00±5.00^{c}	703.33±7.64^{c}	780.00±10.00^{a}	730.00±20.00^{b}	720.00±10.00^{b}	700.00±5.00^{b}	715.00±5.00^{b}	715.00±15.00^{b}	14.845	**0.000***
Glisin	930.00±10.00^{d}	985.00±5.00^{c}	1165.00±5.00^{a}	895.00±5.00^{c}	1085.00±5.00^{b}	1075.00±5.00^{b}	990.00±5.00^{c}	905.00±5.00^{e}	719.862	**0.000***
Glutamik	3130.00±10.00^{e}	3265.00±5.00^{d}	3604.00±4.00^{a}	3320.00±10.00^{b}	3335.00±10.00^{b}	3320.00±5.00^{b}	3245.00±5.00^{d}	3295.00±5.00^{c}	1098.763	**0.000***
Histitin	535.00±5.00^{b}	545.00±5.00^{b}	615.00±15.00^{a}	590.00±10.00^{a}	605.00±5.00^{a}	550.00±10.00^{ab}	550.00±20.00^{ab}	520.00±20.00^{b}	19.729	**0.000***
Lizin	1725.00±5.00^{c}	1715.00±15.00^{c}	1915.00±15.00^{a}	1780.00±10.00^{b}	1815.00±15.00^{b}	1770.00±10.00^{b}	1785.00±5.00^{b}	1740.00±20.00^{c}	65.747	**0.000***
Losin	1305.00±5.00^{c}	1276.67±66.58^{abc}	1420.00±20.00^{a}	1345.00±5.00^{b}	1310.00±10.00^{c}	1325.00±5.00^{c}	1330.00±5.00^{c}	1365.00±5.00^{bc}	8.085	**0.001***
Metionin	440.00±10.00^{c}	455.00±5.00^{c}	520.00±10.00^{a}	520.00±20.00^{a}	510.00±10.00^{a}	490.00±10.00^{b}	540.00±10.00^{a}	515.00±5.00^{b}	27	**0.000***
Prolin	3150.00±10.00^{a}	1395.00±5.00^{d}	1390.00±5.00^{d}	1040.00±5.00^{e}	1445.00±5.00^{c}	1630.00±10.00^{b}	1590.00±10.00^{b}	1625.00±5.00^{b}	19917.13	**0.000***
Serin	413.33±297.38	630.00±10.00	700.00±10.00	600.00±15.00	650.00±50.00	650.00±50.00	650.00±25.00	595.00±5.00	1.902	0.145
Treonin	750.00±10.00^{c}	810.00±10.00^{b}	895.00±5.00^{a}	800.00±10.00^{b}	810.00±5.00^{b}	820.00±10.00^{b}	800.00±5.00^{b}	770.00±5.00^{c}	77.827	**0.000***
Triozin	420.00±10.00^{c}	425.00±5.00^{c}	470.00±10.00^{bb}	445.00±5.00^{b}	440.00±10.00^{b}	450.00±10.00^{bc}	515.00±5.00^{a}	440.00±20.00^{c}	22.91	**0.000***
Valin	990.00±5.00^{b}	1025.00±5.00^{a}	1133.33±44.81^{a}	1035.00±5.00^{a}	995.00±5.00^{b}	980.00±5.00^{b}	1038.33±62.12^{a}	985.00±5.00^{b}	9.558	**0.000***
İzolosin	740.00±10.00^{b}	730.00±5.00^{b}	790.00±10.00^{a}	740.00±10.00^{b}	710.00±10.00^{b}	760.00±10.00^{ab}	765.00±5.00^{b}	795.00±5.00^{a}	31.672	**0.000***

*Aynı satırda farklı harfleri (a,b,c) taşıyan ortalamalar arasındaki farklar istatiktiksel olarak önemlidir (P<0.05).
(P<0.0 5) güven aralığında sonuçlar; ortalama± standart sapma(n:3) olarak verilmiş olup; aynı satırda farklı harfleri (a, b, c, d,) taşıyan ortalamalar arasındaki farklar önemlidir.

Tablo 3'te Gökkuşağı alabalığında +4 ±1 ºC soğuk depolama sırasında aminoasit kompozisyonunda verilmiştir. Özellikle alanin, arginin, aspartik, glutamik ve fenilalanin gibi amino asitlerin depolama sürecinde değişkenlik gösterdiği belirlenmiştir. Gökkuşağı alabalığında taze olarak yapılan aminoasit analizlerinde esansiyel amino asitler; losin 1305.00±5.00 mg/100 g, tronin 750.00±10.00 mg/100g, metionin 440.00±10.00 mg/100 g, lizin 1725.00±5.00 mg/100 g, valin, 990.00±5.00 mg/100 g, histitin, 535.00±5.00 mg/100 g, fenilalanin 705.00±5.00mg/ 100 g olduğu tespit edilmiştir. Gökkuşağı alabalığında esansiyel olmayan amino asitlerin esansiyel amino asitlere oranı ΣEAA / ΣNEAA: 0.66 olarak tespit edilmiştir. Sabetian, vd. (2012) tarafından alabalığın aminoasitleri üzerinde yaptığı çalışmada bu oran vd. 0.73 olarak bulunmuştur. Depolama süresi boyunca arginin 1020.00±20.00 mg/100g - 993.33±15.28 mg/100 g, parolin 3150.00±10.00 mg/100 g - 1625.00±5.00 mg/100 g, histitin 535.00±5.00mg/100- 520.00±20.00mg/100g, glisin 930.00±10.00 mg/100 g-905.00±5.00 mg/100 g

arasın da değiştiği bu değişimlerin p<0.05 göre önemli olduğu belirlenmiştir. Bazı aminoasitlerde görülen azalmanın protein oksidasyonuna karşı hassasiyet göstermelerinden ileri geldiği öngörülmektedir. Castrillón vd., (1996) tarafından yapılan çalışmada Sardalya'nın (Clupea pilchardus) amino asit bileşiminin -20°C'de dondurulmuş depolama sırasında bile değiştiğini, özellikle; kükürt içeren amino asitlerinin (histidin, tirozin, lösin, lizin ve fenilalaninin) depolama sırasında azaldığını belirtmişlerdir. Aynı çalışmada donmuş depolama sırasında amino asitlerde gözlemlenen değişimlerin, oksidasyon, de aminasyon yoluyla bir amino asitten diğerine geçişinden kaynaklanıyor olabileceğini ifade edilmiştir. Çalışmamamızda +4°C soğuk depolama sürecinde aminoasit kompozisyonunda meydana gelen değişimlerin literatür verileri ile uyumlu oldukarı bulunmuştur.

SONUÇ

Sonuç olarak**,** verilerin birlikte değerlendirilmesi durumunda soğuk depolamanın (4±1°C'de) Gökkuşağı alabalığının muhafazasında çok sınırlı bir etkiye sahip olduğu görülmüştür. Soğuk depolama sürecinde 4'üncü günün sonunda fizikokimyasal parametrelerde % FFA%, peroksit (meq/kg), için üst sınırı aşan değerler bulunmuştur. Tazelik ve kimyasal bozulmanın önemli bir indikatörü olan TVB-N (mg/100g) üst sınır değerine çok yakı olduğu tespit edilmiştir. Depolama süresince fiziksel kimyasal bozulma parametrelerinde artışının devam etmiştir. Tüm veriler birlikte değerlendirildiğinde 4.'üncü günden sonra Gökkuşağı alabalığının besinsel kalite ve gıda güvenliği bakımından insan tüketim için önerilemeyeceği gözlemlenmiştir. Soğuk muhafaza (+ 4˚C±1) enzim aktivitesi ve mikroorganizma gelişimini kısmen yavaşlatması bakımından hızlı tüketim için ucuz ve kolay uygulanabilir bir yöntem olduğu için tercih edilebileceği, ancak uzun süreli gıda güvenliği ve besinsel kalitenin korunması bakımından uygun olmadığı, bunun yerine donmuş muhafaza gibi daha etkin yöntemlerin uygulanması önerilmektedir.

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ARAŞTIRMA MAKALESİ **RESEARCH PAPER**

Potential Use of *Beauveria bassiana* ET 10 in Biological Control of *Sitophilus zeamais* (Mots.) (Coleoptera: Curculionidae)

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Abstract: The use of entomopathogenic fungi as biological control agents against storage pests has become an effective method to reduce postharvest losses. This study aimed to determine the insecticidal effect of *Beauveria bassiana*, an important entomopathogenic fungus, on *Sitophilus zeamais* adults, known as the most important storage pest in maize. *B. bassiana* ET 10 isolate prepared in three different spore suspensions (10^6, 10^7 and 10^8 conidia/ml + Tween 80 (0.02%)) was applied to *S. zeamais* adults by the spraying method. The experiment was set up in three replicates according to the randomized plot design. For each suspension, one experimental group was set with corn grains and 10 adult *S. zeamais* adults, the number of dead insects was recorded every 24 hours and the data was recorded until the conclusion 264 hours. As a result of the application, mortality were observed in all suspensions from the first 24 hours, reaching 100% in the 10^8 conidia/ml suspension at the 144th hour. At the end of 264 hours, the mortality rates were recorded as 26.7% for 10^6 conidia/ml, 36.7% for 10^7 conidia/ml, 100% for 10^8 conidia/ml. The most effective dose among the application suspensions was determined to be 10^8 conidia/ml. It has been proven that *B. bassiana* ET 10 isolate can be used as a potential bioagent as an alternative in the biological control of *S. zeamais* and that biological control with entomopathogenic fungi can be a correct and positive strategy.

Keywords: *Beauveria bassiana*, biological control, maize weevil, mortality rate, *Zea mays* L.

Sitophilus zeamais (Mots.) (Coleoptera: Curculionidae)'in Biyolojik Mücadelesinde *Beauveria bassiana* ET 10'nun Kullanılma Potansiyeli

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Öz: Entomopatojen fungusların depo zararlılarına karşı biyolojik mücadele ajanı olarak kullanımı, hasat sonrası kayıpları azaltmak için etkili bir bir yöntem haline gelmiştir. Bu çalışmada mısırda en önemli depo zararlısı olarak bilinen *Sitophilus zeamais* (Mots.) (Coleoptera: Curculionidae)'in erginleri üzerinde önemli bir entomopatojen fungus olan *Beauveria bassiana*'nın insektisidal etkisinin belirlenmesi amaçlanmıştır. Üç farklı spor süspansiyonunda (10^6, 10^7 ve 10^8 konidi/ml+ Tween 80 (%0.02)) hazırlanan *B. bassiana* ET 10 izolatı spreyleme yöntemi ile *S. zeamais* erginlerine uygulanmıştır. Deneme tesadüf parselleri desenine göre üç tekerrürlü olarak yürütülmüştür. Her süspansiyon için mısır taneleri ile 10 adet ergin *S. zeamais* bireyi içeren deney grubu hazırlanmış, her 24 saatte bir ölü böcek sayıları kaydedilmiş ve 264 saatin sonuna kadar veriler kaydedilmiştir. Uygulama sonucu ilk 24 saatten itibaren tüm süspansiyonlarda ölümler gözlenmiş, 144. saatte 10^8 konidi/ml süspansiyonda %100'e ulaşmıştır. 264 saat sonunda ölüm oranları 10^6 konidi/ml için %26.7, 10^7 konidi/ml için %36.7, 10^8 konidi/ml için %100 olarak kaydedilmiştir. Uygulama süspansiyonları arasında en etkili dozun 10^8 konidi/ml olduğu belirlenmiştir. *B. bassiana* ET 10 izolatının *S. zeamais*'in biyolojik mücadelesinde alternatif olarak potansiyel bir biyoajan olarak kullanılabileceği ve entomopatojen funguslarla biyolojik mücadelenin doğru ve olumlu bir strateji olabileceği kanıtlamıştır.

Anahtar kelimeler: *Beauveria bassiana*, biyolojik mücadele, mısır biti, ölüm oranı, *Zea mays* L.

INTRODUCTION

Corn (*Zea mays* L.), is one of the important agricultural products in the family Gramineae (Poaceae) that can be cultivated almost everywhere in the world except Antarctica (Babaoğlu, 2019). The production of corn, which is the most cultivated product after wheat and rice, has been ~1.2 billion tons (annual grain) globally and ~8.5 million tons domestically (FAO, 2024).

Corn, which has a very important place in human nutrition, is used as a raw material in many different sectors other than agriculture (Şahin, 2001). The nutritional content of corn is 72% starch, 10% protein and 4% fat, providing 365 kcal of energy per 100 grams. Corn is used as the main

component of various products such as flour, sweeteners, oil, beverages, adhesives, industrial alcohol and fuel ethanol (Orhun, 2013; Ranum et al., 2014).

From the production of the corn plant to the public offering process, significant economic losses occur due to many diseases and pests. Approximately 15% of annual post-harvest yield losses are losses that occur during the storage process (Gwinner et al., 1996; Scheepens et al., 2011; Chen et al., 2018). Losses that occur during the storage process are caused by microorganisms, insects, mites and rodents (Casas, 1987). Insects are the most important of these pests (Ertugay & Certel 1991; Rajendran, 2002; Keskin & Özkaya, 2013; Chen et al., 2018). Species belonging to the orders Coleoptera, Lepidoptera and Psocoptera cause significant damage to stored products (Rees, 2004). Approximately 130 species of insects that cause postharvest economic losses have been recorded to the present day, and the majority of these are species belonging to the Coleoptera and Lepidoptera orders to the present day (Khare, 1994; Hagstrum & Subramanyam, 2009; Morales-Quiros et al., 2019). Among these species, the maize weevil *Sitophilus zeamais* (Motschulsky) (Coleoptera: Curculionidae) stands out as the species that causes the greatest damage to corn grains during storage in the world (Danho et al., 2002; Costa et al., 2006; Carneiro, 2019; Sebayang et al., 2023). In addition to corn, this pest also attacks many other stored agricultural products such as sorghum, rice, wheat, pasta and processed food products such as biscuits (Sebayang et al., 2023). The maize weevil, *S. zeamais*, is so harmful that it is reported to cause damage ranging from 20-90% in corn if precautions are not taken (Denning et al., 2009; Issa et al., 2011; Noosidum & Sangprajan, 2014). *S. zeamais* consumes the endosperm and/or germ of the corn grain during the larval stage and eats the grain from the outside during the adult stage, causing weight loss, decreased in nutritional value, and deterioration of quality (Keskin & Özkaya, 2013). Some studies report that this pest can cause 18-20% loss in corn, and in high water content conditions, the damage rate can reach up to 30-40%. This pest attack reduces product and nutritional quality, seed weight and germination percentage, which reduces the market value of the product (Sebayang et al., 2023).

Proper storage conditions and use of chemical pesticides have been shown to be control methods that can reduce postharvest insect reproduction in stored corn (Arthur & Subramanyam, 2012; Karim et al., 2017; Sikirou et al., 2018). Due to residues from chemical pesticide applications, the development of resistance of pests to pesticides, and harmful effects on non-target organisms, human health and the environment, many researchers have intensified the search for alternative environmentally friendly strategies (Udo, 2005; Salem et al., 2007; Mahdi & Rahman, 2008; Phillips & Throne, 2010; Wakil et al., 2021). Among alternative control methods, the use of entomopathogenic fungi in the control of stored product pests is a promising strategy that has the potential to eliminate the negative effects of pesticides, provides long-term protection, has a high ability to adapt to different environmental conditions, is reliable for human and environmental health, has a high reproductive capacity, and has a low risk of developing resistance (Nboyine et al., 2015; Sinha et al., 2016; Abd-Elgawad, 2019; Baker et al., 2020; Azhar et al., 2023; Khan & Khan, 2023). Entomopathogenic fungi penetrate the cuticle layer of the pest insect with their mycelium through both physical and enzymatic mechanisms, reach the internal organs and kill the pest (Altinok et al., 2019). The potential of entomopathogenic fungi to be used in different environments and against different pests has been demonstrated by many studies (Athanassiou et al., 2008; Kavallieratos et al., 2014; Abdel-Raheem et al., 2015; Mbata et al., 2018; Ak, 2019; Tozlu et al., 2019; Uçar et al., 2020; Wakil et al., 2021; Khan & Khan, 2023; Tekiner et al., 2023; Yaman & Güvendik, 2024). *Beauveria bassiana* (Bals.-Criv.) Vuill. (Hypocreales: Cordycipitaceae) is one of the most widely used and commercially produced entomopathogenic fungi with proven activity against a wide range of pests (Lord, 2001; Meyling et al., 2018; Karabörklü, 2022a; 2022b).

The aim of this study to determine the insecticidal potential of different spore concentrations of *B. bassiana* ET 10 against adult *S. zeamais* in vitro conditions.

MATERIAL AND METHOD

Maize weevil rearing: Insects obtained from the stock culture of *S. zeamais* (Atatürk University Faculty of Agriculture, Department of Plant Protection, Erzurum, Türkiye) were reared on cracked corn in the laboratory at 27±2°C and 65-75% humidity under 16:8 hours (h) light/dark photoperiod.

Production of conidia of Beauveria bassiana ET 10 isolate: The entomopathogenic fungus *B. bassiana* ET 10 isolate used in this study was isolated from *Sphenoptera antiqua* (Coleoptera, Buprestidae) adults that damage *Onobrychis sativa* L. (Fabacea), plant, was used as an entomopathogenic fungus (Tozlu et al., 2017). *B. bassiana* ET 10 isolate was obtained from the fungus culture collection of the Mycology Laboratory in the Plant Protection Department of Ataturk University Faculty of Agriculture. *B. bassiana* ET 10 isolate, which was preserved in Sabouraud Dextrose Agar (SDA, BD Difco), was transferred to petri (90 mm) dish containing Potato Dextrose Agar (PDA, BD Difco) and grown in an incubator for 2 weeks at 25±1°C for conidia production. Fungus spores were then collected in 10 ml of sterile water containing Tween 80 (0.02%). Three different spore dosses (10^6, 10^7 and 10^8

conidia/ml) were prepared using a hemocytometer (0.100 mm×0.0025 mm^2) under light microscope (Quesada-Moraga & Alain, 2004).

Insecticidal activity assay: For each application, 10 adult maize weevil *S. zeamais* were placed on corn seeds in a petri dish (90 mm) containing sterile filter paper (Whatman No. 1). Then, 10^6, 10^7 and 10^8 conidia/ml suspensions of the *B. bassiana* ET 10 isolate were sprayed onto 10 *S. zeamais* adults. As a control, sterile water containing 0.2 ml/l Tween-80 was applied. The treated petri dishes were covered with parafilm and incubated at 27±2°C with 65-75% humidity and 16:8 hour light/dark photoperiod. The laboratory experiment was designed in a randomized design with three replications. The counts were continued for 264 h at 24 h intervals from the application and during which the number of dead adult insects was recorded.

Percentage mortality rate (%) = 100 X Number of dead adults in the application / Total number of adults, in the application, calculated according to the formula (Abbott, 1925). According to Koch's Postulates, re-isolation was performed on infected insect adults with spores of the entomopathogenic fungus and the results were recorded.

Statistical analysis: The data obtained from the experiment conducted under controlled conditions in the laboratory were statistically analyzed using the JMP 5.0.1 program and the means were separated with LS Means Student's tests ($P \leq 0.01$). Differences between the applications were recorded after the analysis.

RESULTS AND DISCUSSION

In the study conducted to determine the insecticidal effect of *B. bassiana* on *S. zeamais* adults, data were recorded by counting dead and live insects between 24 and 264 hours (Table 1).

Table 1. Insecticidal effects of three different spore doses of *Beauveria bassiana* ET10 against *Sitophilus zeamais* adults under laboratory conditions.

	Percentage Mortality Rate (%)											
Spore doses/hour (h)	**24**	**48**	**72**	**96**	**120**	**144**	**168**	**192**	**216**	**240**	**264**	**Average**
10^6 Conidia/ml	6.7	6.7	10	13.3	13.3	13.3	13.3	13.3	23.3	26.7	26.7	15.15±6.87 B
10^7 Conidia/ml	6.7	6.7	6.7	10	10	13.3	13.3	13.3	26.7	36.7	36.7	16.37±10.9 B
10^8 Conidia/ml	13.3	60	80	83.3	83.3	100	100	100	100	100	100	82.12±25.4 A
Control	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.3	3.3	3.3	0.9±1.47 C
						CV:	0.49					
						LSD:	11.37					

According to the obtained data, the mortality rate of the treated insects was found to be significantly higher than that of the control group. It was determined that all different spore suspensions of *B. bassiana* ET 10 isolate had an insecticidal effect on *S. zeamais*. The insecticidal activities of the three different conidia suspensions tested were determined to be statistically different from the control at 11 different times and according to the results of the statistical variance analysis. The difference between the treatments was found to be statistically significant ($P \leq 0.01$). The differences between the groups were also determined using the LSMeans Differences Student's test (Percent mortality rate of *S. zeamais* F treatment: 48.8913; CV: 0.49; LSD treatment: 11.37; $P \leq 0.01$).

When the insecticidal effect of *B. bassiana* ET 10 isolate on *S. zeamais* is examined in terms of mortality rate, it was recorded that the effect started within the first 24 hours. At 144 h (6^{th} day) *B. bassiana* ET 10 isolate's mortality rate in 10^8 conidia/ml suspension was 100%. The lowest mortality rate was observed in the control group.

It was observed that the mortality rate of *S. zeamais* treated with *B. bassiana* ET 10 isolate increased with increasing concentrations. When three different spore suspensions were evaluated, it was determined that the most effective dose was 10^8 conidia/ml (82.12%) and the least effective dose was 10^6 conidia/ml (15.15%). It was also revealed that the insecticidal effects of different spore doses of the *B. bassiana* ET 10 isolate varied compared to the control (Table 1).

Entomopathogenic fungi are reliable alternative control agents that do not threaten the ecosystem, environment and human health in the control of stored product pests (Aminaee et al., 2010; Tozlu et al., 2017; Karabörklü, 2022b). *B. bassiana* is one of the most widespread and extensively studied species among entomopathogenic fungi and is the active agent of biopesticides that are in use and under development worldwide (Zimmermann, 2007; Mascarin & Jaronski, 2016; Javed et al., 2019; Tozlu et al., 2019; Tekiner et al., 2023; Erol & Erdoğan 2024).

Many researchers have applied *B. bassiana* against various storage pests and observed different mortality rates (Meikle et al., 2001; Kassa et al., 2002; da Paz Junior et al., 2012; Athanassiou et al., 2017; Batta, 2018; Uçar et al., 2020; Acheampong et al., 2023). Different researchers have reported that many factors (host specificity, physiological condition of the host, entomopathogenic fungus species, virulence of the entomopathogenic fungus species, application method and environmental factors) affect different levels of effectiveness (Wakefield, 2006; Padmini & Padmaja, 2010; Abdel-Raheem et al., 2015). Kaoud (2010) reported that after the death of insects treated with entomopathogenic fungi, the fungus can grow on the insect

and produce more spores, which can increase the mortality rate of other mobile insects in the same environment.

Adane et al. (1996) recorded the insecticidal effect of *B. bassiana* against *S. zeamais* with a mortality rate of approximately 88% on the eighth day. In this study, a mortality rate of 100% was recorded by the sixth day at a concentration of 10^8 conidia per ml of *B. bassiana* ET 10 isolate. Similar to our results, other researchers also confirmed that *B. bassiana* was effective against *S. zeamais* and reported that periodic counts were made for 24 h or longer (Meikle et al., 2001; Barra et al., 2013; Mbata et al., 2018). In Teshome and Tefera (2009), a single dose (10^8 conidia/ml) was used under laboratory conditions to determine the effectiveness of 11 local *M. anisopliae* and 6 *B. bassiana* isolates against *S. zeamais* and the most virulent isolates. They noted that the most virulent isolates (3 *M. anisopliae*, 2 *B. bassiana*) achieved a mortality rate between 84.4% to 98.3%.

Karabörklü (2022b) applied 10^6, 10^7, 10^8 conidia/ml doses to adults of *B. bassiana* YK23 and YK26 and *M. anisopliae* YK41 and YK45, *S. zeamais* and *Acanthoscelides obtectus* by spraying and counting the dead insects for 10 days. *B. bassiana* and *M. anisopliae* caused 100% mortality against *A. obtectus* at the lowest dose (10^5 conidia/ml), while it was found that it caused 100% mortality in *S. zeamais* at higher doses (10^6, 10^7 conidia/ml). The effectiveness of *B. bassiana* has also been proven useful in the biological control of *S. oryzea*, a rice storage pest belonging to the same genus as the corn pest (Kavallieratos et al., 2014; Er et al., 2018; Atmaca et al., 2022). Rehman et al., (2019) reported that the application of 10^8 conidia/ml of *B. bassiana* and *M. anisopliae* by the immersion method showed 100% and 96% mortality rate against *S. oryzae* under controlled conditions. In our study, it was determined that the most effective dose against *S. zeamais* was 10^8 conidia/ml and, this finding was similar to other studies in the literature.

In addition to being effective against storage pests of different *Sitophilus* genera, *B. bassiana* has been reported to effectively control important maize storage pests of different genera (*Prostephanus truncatus* and *Tribolium* spp.) with high mortality rates by different researchers (Meikle et al., 2001, 2002; Kassa et al., 2002; Acheampong et al., 2016; Akmal et al., 2020; Tekiner et al., 2023).

Isolates of the species *B. bassiana* are also used in the control of insects that damage different plants in addition to storage pests. Tozlu et al., (2020) tested fungal and bacterial biological control agents under controlled conditions on nymphs and adults of the pouched cochineal (*Icerya purchasi*), which is harmful to mimosa plants (*Acacia dealbata*). The fungal bioagent *B. bassiana* ET 10 was shown to cause mortality of up to 100% in nymphs and 80% in adults. Similarly, in the biological control of the boxwood moth, which damages boxwood trees, Tozlu et al., (2022) used bacterial (*Bacillus cereus* FD-63, *B. brevis* FD-1 and *Vibrio hollisae* FD-70) and fungal (*B. bassiana* ET 10) biological control agents, recording 100% mortality after 8 and 9 days in 10^8 conidia/ml suspension under laboratory conditions.

As a result, it has been demonstrated that entomopathogenic fungi have high potential if they are offered for commercial use in environmentally friendly pest management. In this way, dependency on pesticides can be reduced. In recent years, there has been a need to identify novel entomopathogenic fungi or to investigate more effective isolates in the biological control of stored product pests. According to the data obtained in this study, testing the storage applications of ET 10 isolate against *S. zeamais* in future studies by considering different temperature and humidity conditions in future studies (the effectiveness of entomopathogenic fungi may vary depending on the conditions) may be an important alternative method to control this pest. In addition, it has been concluded that ET 10 isolate is effective against many different pests and has a promising potential in this respect. It can be used as an alternative mycoinsecticide to chemical insecticides.

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