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Yönetim Yeri Adres / *Address*
Recep Tayyip Erdoğan Üniversitesi, Su Ürünleri Fakültesi,
Zihni Derin Yerleşkesi, Fener Mah., 53100, Rize / Turkey.
Tel: 90 464 223 33 85-1438 ve 1436
Fax: 90 464 223 41 18
İletişim: verepoglu@gmail.com, anatolianacademicsciense@hotmail.com

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ARAŞTIRMA MAKALESİ RESEARCH PAPER

Understanding the Possible Impact of Exotic Parrots on Human Health and Well Being

Esra PER
Department of Biology, Faculty of Science, Gazi University, Teknikokullar, Ankara, Turkey

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*: https://orcid.org/0000-0002-7764-1215

***Corresponding author's:**
Esra PER
Department of Biology, Faculty of Science, Gazi University, Teknikokullar, Ankara, Turkey esraper@gazi.edu.tr
✉: esraper@gazi.edu.tr

Abstract: Parrots are birds that live in the tropical and subtropical regions of the world. The international trade of parrots is carried out according to the CITES Convention. A total of 135 parrot species were imported and 40 parrot species were re-exported between 1982 and 2016 in Turkey. The introduction and establishment of alien species in any country is an undesirable situation. Alien species can have a negative impact on ecosystem, economic, human health and social factors in countries. Therefore, alien species should be monitored. In this study, the possible impact of exotic parrots on human health and well-being is assessed, which was given in three stages (literature search, bird watching database, ectoparasite investigation). Cage birds (pets) were the subject of 89% of theses, 88% of WoS articles, and 92% of Dergipark articles, according to the results. There have been several records of the sounds of the alien rose-ringed parakeet (Psittacula krameri) (RRP) and the Alexandrine parakeet (Psittacula eupatria) (AP) having negative impacts on human well-being. Both species have had no impact on human health to date in Turkey, but it is possible that in the future it could be determined that they carry various microorganisms and vectors. RRP and AP individuals live in the wild as a result of intentional or accidental introductions. Besides, pet trade on these species continues in Turkey. This situation poses a biosecurity concern. To overcome these issues, biosecurity measures should be increased in Turkey and incorrect scientific researches should not be considered in the management of these species.

Keywords: Alien species, biosecurity, incorrect assessment, public health, trade.

Egzotik Papağanların İnsan Sağlığı ve Refahı Üzerindeki Olası Etkisini Anlamak

***Sorumlu yazar:**
Esra PER
Biyoloji Bölümü, Fen Fakültesi, Gazi Üniversitesi, Teknikokullar Kampüsü, Ankara, Türkiye
✉: esraper@gazi.edu.tr

Öz: Papağanlar, dünyanın tropikal ve subtropikal bölgelerinde yayılış gösteren kuşlardır. Uluslararası papağan ticareti CITES Sözleşmesine bağlı olarak yapılmaktadır. Türkiye'de 1982-2016 yılları arasında toplam 135 papağan türü ithal edilmiş ve 40 papağan türü yeniden ihraç edilmiştir. Yabancı türlerin ülkeye girişi ve yerleşmesi istenmeyen bir durumdur. Yabancı türlerin ülkelerdeki ekosistem, ekonomi, insan sağlığı ve sosyal faktörler üzerinde olumsuz etkileri olabilmektedir. Bu nedenle yabancı türler izlenmelidir. Bu çalışmada Türkiye'de egzotik papağanların insan sağlığı ve refahı üzerindeki olası etkisi değerlendirilmiştir. Araştırma üç aşamalı olarak planlanmıştır (literatür araştırması, kuş gözlem veri tabanı ile ektoparazit araştırması). Tezlerin % 89'unun, WoS makalelerinin % 88'inin ve Dergipark makalelerinin % 92'sinin kafes kuşları (evcil hayvanlar) üzerine olduğu tespit edildi. Yeşil papağan (*Psittacula krameri*) (YP) ve İskender papağanı (*Psittacula eupatria*) (IP)'nın seslerinin insan refahı üzerinde olumsuz etkileri olduğuna dair birkaç kayıt vardır. Her iki tür de Türkiye'de şimdiye kadar insan sağlığı üzerinde herhangi bir etkiye sahip değildir, ancak gelecekte bu türlerin çeşitli mikroorganizmaları ve vektörleri taşıdığı tespit edilebilir. YP ve IP, kasıtlı veya kazara bırakılmalarının bir sonucu olarak vahşi doğada yaşıyor. Ayrıca Türkiye'de bu türlerin evcil hayvan ticareti devam etmektedir. Bu durum biyogüvenlik açısından endişe oluşturmaktadır. Bu konuların üstesinden gelmek için Türkiye'de biyogüvenlik önlemleri artırılmalı ve bu türlerin yönetiminde yanlış bilimsel araştırmalar dikkate alınmamalıdır.

Anahtar kelimeler: Biyogüvenlik, halk sağlığı, ticaret, yabancı türler, yanlış değerlendirme.

INTRODUCTION

People have been transporting parrots to various countries around the world since the 1400s. A total of 44 species have established new populations outside of their native ranges (Runde et al., 2007). The spread of non-native species was facilitated by the globalization of trade and travel. Some of these species have been established out of the native range and cause serious environmental, economic and human health effects (Keller et al., 2011).

Parrots are birds that locate in tropical and subtropical regions throughout the world. The international trade of parrots is carried out according to the CITES Convention. According to the CITES database, 135 parrot species were imported and 40 parrot species were re-exported in Turkey between 1982 and 2016 (Per, 2018). The Alexandrine parakeet (*Psittacula eupatria*) (AP) was first recorded in Ankara in 1990, and the rose-ringed parakeet (*Psittacula krameri*) (RRP) was first recorded in Ankara in 1975 (Per, 2019). These species have spread and established breeding populations in the wild in Turkey (Kirwan et al., 2008). The introduction and establishment of alien species in a country is an undesirable circumstance since alien species can have an adverse impact on a country's environment, culture, human health, and social factors. In order to make an accurate assessment, monitoring is required. Therefore, the Parakeet Census of Turkey study has been conducted since 2016.

The introduction of alien species by humans, for different reasons, has resulted in the introduction of more species into the local environment. The low level of quarantine measures and border controls are major issues in this field. For economic interests, many alien species have been deliberately introduced. Invasive alien species have different effects on people (Mcneely, 2001). Parrots can carry potential disease vectors, and threaten not only native bird species but also cage birds, poultry flocks, aviculture and human health (Runde et al., 2007). An impact evidence-mapping database on the ecological and social impacts of 11 non-native parrot species in Europe has been created. This database primarily focuses on two species: RRP (64%) and Monk parakeet (*Myiopsitta monachus*) (19%). Parrots have impacts on human health (5%) and human well-being (3%) based on this database (White et al., 2019). The issue of human health and human well-being in the management of introduced parrot species is included in the socio-economic assessment.

Through enhanced international cooperation, the Convention on Biological Diversity provides significant opportunities for addressing the complex global challenges of Invasive Alien Species (IAS) (Mcneely, 2001). Alien species are considered to be a threat to overall biodiversity loss worldwide (Bremner & Park, 2007). An important but often overlooked form of impact of native species is the parasite-mediated one. The alien species can carry parasites from their native distribution areas or receive parasites from native species, potentially raising the risk of parasite transmission and spread. The identification of parasites carried by alien species is crucial for determining and potentially avoiding negative effects (Mori et al., 2015). There is not any general assessment of invasive alien parakeet species and ectoparasites in the wild, Turkey.

As a result of improvements in Citizen Science, it is possible to effectively monitor IAS through community engagement (Roy et al., 2018). Invasion biology focuses on ecological effects by estimating the spread and developing control methods rather than documenting the economic and social impact on society. The next generation IAS science and policy should reflect the fact that IAS are becoming a major driver of global environmental change with implications for biodiversity and human welfare (Pejchar & Mooney, 2009). Public support can be critical to the success of management projects. Education activities can help raise public awareness and support (Bremner & Park, 2007). The Ministry of Agriculture and Forestry has begun to work for the management of IAS in marine and coastal ecosystems in Turkey. The new management approaches for terrestrial IAS will be developed by the ministry in the coming years.

The aim of this study is to understand the possible impact of exotic parrots on human health and well-being, as well as to evaluate current research and make recommendations for future studies.

MATERIALS AND METHODS

This research was carried out in three stages. A literature search was conducted in the first stage to determine the impact of parakeets and parrots on public health in Turkey, and a parrot research database was created by using Web of Science (WoS), the digital platform of academic journals published in Turkey (Dergipark), and theses. The database queries were used to make the numerical assessment.

For this database, the WoS and Dergipark systems were searched based on studies conducted in Turkey. The species in this database have been subjected to numerical assessments and comparisons. The following parameters were used to conduct database queries: “bird”, “aves”, “parrot”, “parakeet”, “parasite”, “bacteria”, “virus”, “impact”, and “human health” for the research method; “article”, “guide”, “book” and “project report” for the research type; “year” for the completion date.

The National Thesis Centre of the Turkish Council of Higher Education CoHE database was searched for master's theses and doctoral dissertations (Yöktez, 2019). The following parameters were used to conduct database queries: "bird", "aves", "parrot", "parakeet", "parasite", "bacteria", "virus", "impact", and "human health".

In the second stage, "The Turkey Parakeet Census Voluntary Network" was established in 2016. For RRP and AP observations, a Google Docs observation form was developed. Observers sent their different parrot observations by observation form. A database was established for exotic parrot monitoring in Turkey. This database includes information on parrots' impacts on the ecosystem, economy (as an agricultural pest), and social factors (disease and sound) (The Parakeet Census of Turkey, 2019). The observation results were used to make numerical assessment.

With the permission of the Ministry of National Defence, fieldwork was conducted in the Anıtkabir Campus (Memorial Tomb) (39°55'33"N 32°50'10"E), Ankara province, Central Anatolia. Anıtkabir is made up of the monumental blog containing the mausoleum and the Peace Park. Peace Park formed by 104 different species of plants, shrubs, and trees sent from different parts of Turkey and various countries of the world (Afghanistan, Austria, Belgium, Canada, China, Cyprus, Denmark, Egypt, Finland, France, Germany, Greece, India, Iraq, Israel, Italy, Japan, Norway, Portugal, Spain, Sweden, United Kingdom, United States and Yugoslavia) is a symbolic League of Nations (Culture and Tourism Directorate of Ankara, 2013). This campus is open to the public every day from 9:00 a.m. to 5:00 p.m. (17:00, till 4:00 p.m./16:00 in winter).

The RRP population was observed for a year and feathers were collected in Anıtkabir Campus for the third stage. Ectoparasite research was carried out in and around the mausoleum. The mausoleum building is home to RRP individuals (Figure 1).

Figure 1. RRP nest on the mausoleum building © Ömer Çetin.

The Featherbase template is used to identify bird feathers (Featherbase, 2019). Parakeets breeding on the mausoleum building had their contour feathers, flight, and tail feathers collected. In the mornings, parakeets are very active around their nests on the mausoleum building. Meanwhile, some of their feathers fell out when they entered and exited the nest. As soon as the feathers hit the ground, they were quickly gathered. Each bird feather was examined for ectoparasites. Each sample was first examined in a petri dish using an Olympus SZX7 stereo microscope at Gazi University, imaging procedures on the samples. In the current study, a total of 35 feathers (wing, tail, and body contour) (Figure 2; Figure 3) were deposited.

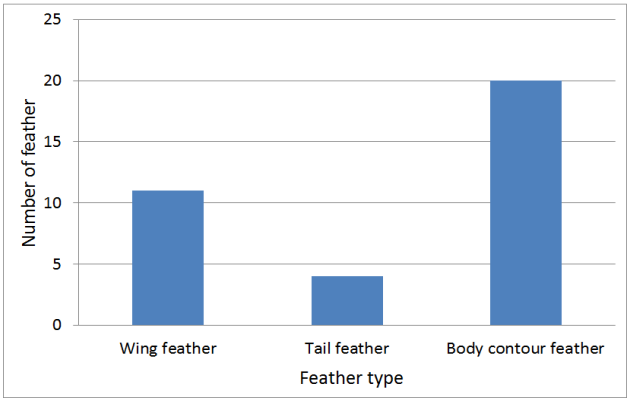

Figure 2. The ectoparasite examinations on feathers.

Figure 3. A-Flight feather, B-Body contour feather, C-Tail feather

RESULTS AND DISCUSSION

Alien parrots have ecological and social impacts in Europe and these effects are increasing (White et al., 2019). The number of exotic parrot species and their distribution areas are increasing in Turkey. The Citizen Science is used to monitor their ecological, economic, and cultural impacts (Per, 2019). The parrot research database was examined. The CoHE thesis database, WoS and Dergipark were assessed. Investigation of the publications on this subject revealed that 89% of theses, 88% of WoS articles and 92% of Dergipark articles are on cage birds (pets) (Table 1). There is no research on the impact of wild parakeets and parrots on human health and parasite interactions. All of the research on this subject has focused on cage birds.

Table1. The scientific database queries

Researches	CoHE thesis database	%	WoS	%	Dergipark	%
Breeding parakeet	1	11	2	12	2	8
Parrot as a pet	8	89	15	88	24	92
Total	9	100	17	100	26	100

According to the Parakeet Census of Turkey database, there are 247 AP observation records (in 19 provinces) and 2,265 RRP observation records (in 31 provinces) in and around urban areas. In Turkey, there are an estimated 2,000 RRP and 3,000 AP individuals. AP has established breeding populations in İstanbul. The breeding population of AP has increased dramatically in recent years in İstanbul. Provinces where RRP individuals have been observed; Adana, Adapazarı, Afyon, Ankara, Antalya, Balıkesir, Bolu, Burdur, Bursa, Çanakkale, Denizli, Gaziantep, Giresun, Eskişehir, Hatay, İstanbul, İzmir, Kastamonu, Kayseri, Kırşehir, Kocaeli, Manisa, Malatya, Mersin Muğla, Nevşehir, Osmaniye Samsun, Sinop, Şanlıurfa, Tekirdağ, Yalova. RRP has established breeding populations in ten provinces; İstanbul, İzmir, Ankara, Yalova, Antalya, Şanlıurfa, Eskişehir, Hatay, Adapazarı and Bursa.

There is no data on the ecological, economic, or social impact in 90% of the observation records. RRP individuals interact with Caucasian squirrel (*Sciurus anomalus*), common Buzzard (*Buteo buteo*), common myna (*Acridotheres tristis*), Eurasian magpie (*Pica pica*), hooded crow (*Corvus cornix*), jackdaw (*Coloeus monedula*), stray cat (*Felis catus*) and yellow-legged gull (*Larus michahellis*), while AP interacts with common myna, hooded crow and yellow-legged gull. The hybridization of AP and RRP individuals was not detected (impacts on the ecosystem). AP and RRP are widely spread in urban areas. So far, it hasn't been seen in rural areas, orchards and agricultural fields (no economic impact). There have been several observations of negative impact of both species due to their sounds (human well-being) in İstanbul's parks and gardens, where they roost in large flocks (social impact). Some observation records have been determined about AP and RRP individuals that have been illegally captured by humans.

The Anıtkabir campus is home to a wide variety of native and non-native tree species (Culture and Tourism Directorate of Ankara, 2013). This campus provides a shelter for parakeets, who feed on the fruits and seeds of the trees during the year.

The biggest breeding parakeet population of Ankara was established in Anıtkabir Campus (The Parakeet Census of Turkey, 2019). RRP individuals in Anıtkabir have been observed for a year. Their population reached 50 individuals in the winter season, while nine pairs were observed during the breeding season. Their nests are located on the top of the mausoleum building. The height of the Mausoleum building where they establish their nests are 17 meters (Figure 1). The feathers of the RRP individuals were collected from here every morning. Stereo microscopy was used to examine feather samples, but no ectoparasites were detected. Individuals of the Common swift (*Apus apus*) and RRP are both nesting in the same holes on the mausoleum building. However, feathers of common swift individuals were not found in the research field. As a result, It was impossible to determine whether common swifts nesting in this area has ectoparasites or not.

Ectoparasites can cause holes and structural problems on bird feathers (Per & Aktaş, 2019). There were no structural problems observed on any of the 35 feathers examined (flight (11), tail (4), and body contour (20)) (Figure 3 A - B). Only the wear and tear at the ends of the tail feathers of the parakeets were observed (Figure 3 C). They build their nests on the mausoleum building's narrow holes. It's possible that their feathers were broken when they entered and exited the nests. Three of the Mausoleum's facades are home to parakeets. The 35 feathers examined were collected from these three sites, but it's unclear how many parakeets they belonged to.

According to the first data on the macroparasite fauna of alien parrots in Italy, seven RRP individuals were infested by ectoparasites. Four ectoparasite species were identified by examining thirty-one arthropod individuals; *Neopsittaconirmus lybartota* is one of them, with a native range in India. The other three arthropod species (*Tarsopsylla o. octodecimdentata*, *Argas reflexus*, and *Laemobothrion cf. maximum*) are common and native to Italy (Mori et al., 2015). Some ectoparasites are carried by birds (feather mite, lice, tick, and bed bug). Feather mites live on the bird's skin, within the quill, down and contour feathers, flight feathers, and tail feathers, in four different microhabitats (Gaud & Atyeo, 1996). The feather collection is a good tool for studying feather mites, but parakeet individuals should be examined in detail for all ectoparasite groups. Ectoparasites carried by parakeets can be determined in the coming years by using the catching, marking, and releasing technique.

Parrot fever (*Psittacosis ornithosis*) disease is caused by bacteria called *Chlamydophila psittaci*. Many birds, especially parrots carry this bacteria species as a microparasite (Atay et al., 2012). RRP and AP individuals do not have any impact on human health until now in Turkey, but various microorganisms and vectors may be carried with alien parakeet species in the next years. There is a need for more research on this subject. However, for proper planning and interpretation of these studies, multidisciplinary studies in biology, veterinary, and public health are needed.

Psittacosis, salmonellosis, avian influenza, Newcastle's disease avian malaria, pasteurellosis, erysipelas, and tuberculosis are diseases that can be transmitted by parrots to humans or other birds (Runde et al., 2007). Introduced birds may act as a large reservoir for parasitic infections. They have the potential to spread

parasites to other native species (Eguchi & Amano, 2004). There is no data on this issue in Turkey. Parasitic examinations on parakeet individuals who are spreading in the wild and brought to veterinary faculties should be done and also blood samples should be taken. These samples can be used to detect internal parasites and ectoparasites of the parakeets which are distributed in the wild in Turkey.

There is no observation, evidence, or literature to indicate that two alien parakeet species (AP and RRP) have a negative impact on human health in Turkey. However, a presentation at the ESENIAS Conference was based on the incorrect assumption that these animals have a negative impact on human health (Oymak et al., 2017). These species carry risks with regard to human health in Turkey according to Oymak et al., 2017, but this is not correct since the wrong literature review and incorrect assessment were made with two articles. The species of parrot was not mentioned in the first article which was used as the literature (Tantaş et al., 1987). In the second article, a species of bacteria found on the grey parrot (*Psittacus erithacus*) was investigated (Tantaş et al., 1990). The word "parrot" is used in some articles about pet parrots in Turkey. The name of the parrot species is not written specifically, which is a lack of knowledge. In this research area, veterinarians should collaborate with ornithologists to identify species. According to scientific publications, AP and RRP species do not carry parasites as pets. Before this research, no preliminary study has been conducted in Turkey on ectoparasites carried by parakeets in the wild.

There are some observations about AP and RRP individuals that have been illegally captured by humans from breeding populations from time to time in Ankara, İstanbul and İzmir city centres (Per, 2019). Captured birds have the potential to get involved in the illicit trade cycle. Biosecurity measures should be increased regarding Invasive Alien Species in Turkey.

Nationwide controls and import bans are important measures to control the population growth of these alien species as well as limiting the spread of diseases (Stafford, 2003). To avoid the spread of diseases, the importation of wild birds from other countries into EU countries was banned in 2007 (Commission Decision, 2007). Prior to this ban, the deadly Newcastle disease entered Italy during shipment of parrots and finches imported from Pakistan in 2004. As a result of this incident, 4,000 birds were culled (World Parrot Trust, 2004). As a result of intentional or accidental introductions, RRP and AP now exist in the wild. The Republic of Turkey Ministry of Agriculture and Forestry banned rose-ringed parakeet imports in 2021, although the national trade of RRP individuals imported by Turkey in previous years will continue for some time. There is no import ban for AP in Turkey. In terms of biosecurity, this is a concern and some significant changes are required.

A new strategy should be developed for exotic parrots in Turkey. The trade of species that have established a breeding population should be banned in order to prevent their distribution from expanding further in the country. In the parrot trade, Turkey should implement quotas and restrictions based on some parrot species in the parrot trade. For illegally traded animals, a risk assessment should be done (Per, 2018). The risk analysis should be done for Invasive Alien Species based on their ecosystem, economic and social impact in Turkey.

This study provides the following insights for future research: firstly, Biosecurity measures should be increased in Turkey. Secondly, the management of these species should not be based on incorrect scientific studies. Thirdly, a risk analysis of the potential socio-economic impacts of parrots should be performed, and a monitoring report should be prepared based on the results of the risk analysis. Also, there is a need for non-profit citizen science research to increase public awareness on this issue. Finally, The development of new interdisciplinary studies in the fields of public health and ecology would help in the prevention of the introduction of invasive alien species to new areas and the providing of rapid management responses.

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ARAŞTIRMA MAKALESİ RESEARCH PAPER

LC–MS/MS and RP–HPLC–UV Analysis and Antioxidant Activities of *Arum italicum* Miller Edible and Nonedible Tuber Parts

Zeynep AKAR[1*] Çağlar DEMIR[1] Ozan ALKAN[1] Zehra CAN[2] Bülent AKAR[3]

[1] Department of Genetics and Bioengineering, Faculty of Engineering and Natural Sciences, Gümüşhane Universty, Gümüşhane, Turkey*
[2] Bayburt University, Faculty of Applied Sciences, Department of Emergency Aid and Disaster Management, Bayburt, Turkey
[3] Department of Food Engineering, Faculty of Engineering and Natural Sciences, Gümüşhane Universty, Gümüşhane, Turkey

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[*] : https://orcid.org/0000-0001-9262-8070
: https://orcid.org/0000-0001-8858-8475
: https://orcid.org/0000-0001-5419-4232
: https://orcid.org/0000-0002-7156-4941
: https://orcid.org/0000-0002-1421-374X

***Corresponding author's:**
Zeynep AKAR
Department of Genetics and Bioengineering, Faculty of Engineering and Natural Sciences, Gümüşhane University, Gümüşhane, Turkey
✉: zeynep_iskefiyeli@hotmail.com

Abstract: In the study, the phenolic components and antioxidant activities of edible and nonedible parts of tuber of *Arum italicum* plant were carried out to determine in methanolic and water extracts. In addition, the antioxidant potential of these parts of the plant was associated with their phenolic composition. Total phenolic content, FRAP, CUPRAC and DPPH tests as radical scavenging activity were performed to determine the antioxidant activity of the plant. The phenolic profiles in the two parts of the tuber were determined in the prepared methanolic extracts by RP–HPLC–UV and LC–MS/MS. The highest values of the total phenolic content, FRAP, CUPRAC and % DPPH were measured in methanolic extracts of nonedible parts of the tuber as 164 µg GAE/mL, 878 TEAC (µM), 0.064 TEAC (µM) and 19.41, respectively. Ferulic acid was determined as the main phenolic compound in methanolic extracts of both the tuber parts by LC/MS-MS. However, luteolin and rutin phenolics were measured as the major compounds in the edible and nonedible parts of the plant tuber with analysis based on RP-HPLC-UV, respectively.

Keywords: Antioxidant activity, *Arum italicum*, LC–MS/MS, Phenolic, RP–HPLC–UV.

Arum italicum Miller Bitkisinin Yenilebilir ve Yenmeyen Yumru Parçalarının LC–MS/MS ve RP–HPLC–UV Analizi ve Antioksidan Aktiviteleri

***Sorumlu yazar:**
Zeynep AKAR
Genetik ve Biyomühendislik Bölümü, Mühendislik ve Doğa Bilimleri Fakültesi, Gümüşhane Üniversitesi, Gümüşhane, Türkiye.
✉: zeynep_iskefiyeli@hotmail.com

Öz: Bu çalışmada, *Arum italicum* bitkisine ait yumrunun yenilebilir ve yenmeyen kısımlarından elde edilen metanolik ve sulu ekstraktların, fenolik içerikleri ve antioksidan aktiviteleri belirlenmiştir. Ek olarak, bitkinin bu kısımlarının antioksidan potansiyeli, fenolik kompoziyonu ile ilişkilendirilmiştir. Bitkinin antioksidan aktivitesini belirlemek için Toplam Fenolik Madde Miktarı, FRAP, CUPRAC ve radikal süpürme aktiviteyi belirleyen DPPH antioksidan aktivite testleri yapılmıştır. Bitki yumrusunun her iki kısmındaki fenolik profiller hazırlanan metanolik ekstraktlarında RP– HPLC–UV ve LC–MS/ MS cihazları kullanılarak belirlenmiştir. En yüksek toplam fenolik içerik, FRAP, CUPRAC ve % DPPH değerleri, yumrunun yenmeyen kısmının metanolik ekstraktında sırasıyla 164 µg GAE/mL, 878 TEAC(µM), 0.064 TEAC(µM) ve 19.41 olarak ölçülmüştür LC/MS-MS ölçümlerinde her iki yumru kısmının metanolik ekstraktlarında ferulik asit, ana fenolik bileşik olarak belirlendi. Bununla birlikte RP–HPLC–UV ölçümlerinde ise, bitki yumrusunun yenilebilir ve yenilmeyen kısımlarında sırasıyla luteolin ve rutin fenolikleri ana bileşikler olarak ölçülmüştür.

Anahtar kelimeler: Antioksidan aktivite, *Arum italicum*, Fenolik, LC–MS/MS, RP– HPLC–UV.

INTRODUCTION

Free radicals are formed in cells during usual metabolic activity and also various environmental factors such as air pollution, some chemicals, additives, and artificial nutrition are effective in their formation. They damage cells by breaking hydrogen atoms. However, molecules called antioxidant substances stop or minimize the effects of free radicals in the organism and prevent the formation of chain reactions which can cause the occurrence of various diseases. Thus, a balance between free radicals and antioxidants is obligation for cells and tissues to maintain their usual physiological activities. It is known that free radicals increase in the body due to the decline of the body antioxidant protective system. This causes many diseases, mainly cancer (Uttara et al., 2009; Valko et al., 2007). In order to maintain this system in a balanced way it is very important to consume natural compounds with antioxidant activity. Many plants showed high antioxidant, anti-inflammatory, antimicrobial activities due to their phytochemical compound content (Djeridane et al., 2006; Erez et al., 2019; Hirasa & Takemasa, 1998). Although these compounds called secondary metabolites do not have a direct relationship with the basic vital functions of the plant, they are chemical substances that are at least as important as primary metabolites such as protein, fat, carbohydrate. Plants include compounds having antioxidant activities such as carotenoids, lycopenes, coenzyme-Q, antioxidant vitamins, and phenolics (phenolic acids, flavonols, flavonoids, anthocyanins, lignins and tannins) (Cai et al., 2004; Mau et al., 2002; Ng et al., 2000; Xiao et al., 2000). Plant antioxidants have an important role in reducing of oxidative stress on organisms associated with industrialization and technology. Turkey, which has a rich flora, has great potential for natural antioxidant compounds. Consumed as food *Arum italicum* Miller in the family Araceae grows naturally in the northern regions of Turkey. Also, it is used as traditional herbal medicines to control some illness such as hemorrhoid, eczema, cancer, blepharitis, stye, abscess, muscle pain and antifebrile (Akbulut & Özkan, 2014; Bozyel et al., 2020). Generally, the tuber part of the plant is consumed as food boiled. However, the all of tuber part is not consumed as food by local people in Black Sea Region of Turkey. Edible part of the tuber is more delicious than the other part. It is the close to stem of plant and is separated from the nonedible part by a node. The nonedible part of the tuber is not consumed as food due to the unpleasant. Tubers of the plant are also used industrially due to the abundance of starch in the tubers of the plant (Ahmed et al., 2018). There are various antioxidant activity determination methods to detect the antioxidant activities of natural and synthetic components (Gidik et al., 2019; Tosun et al., 2015). These methods can be classified according to the type of measured antioxidants substance (hydrophilic or lipophilic, enzymatic or nonenzymatic), solvent character (organic or aqueous), reactive type (radical or non-radical) and reaction mechanism (electron transfer and hydrogen atom transfer) (Gülcin, 2012; Huang et al., 2005).

In the study, it was aimed to determine the antioxidant activity of methanolic and water extracts of both edible and nonedible parts of tuber of *Arum italicum* plant according to four antioxidant activity methods, which are widely used in the literature and have different reaction mechanisms. These methods are % DPPH Radical Scavenging Activity (Brand-Williams, 1995). Total Phenolic Method with Folin-Ciocalteu Separator (FCR), Iron (III) Reduction/Antioxidant Power (FRAP) Determination (Benzie & Strain, 1996). Copper (II) Reducing Antioxidant Activity (CUPRAC) (Apak et al., 2004). In addition, the phenolic contents of the methanolic extracts of both parts were determined with RP–HPLC–UV and LC–MS/MS devices comparatively.

MATERIALS AND METHODS

Preparation of Plant Extraction: The tuber parts of the *A. italicum* plant were collected in March 2017 from the Derecik district of Trabzon-Akçaabat province of Turkey. The edible and the nonedible of the tuber, were separated from each other (Table 1). After these parts were completely dried, they were and grounded into powder with blender. Then, edible and nonedible parts were extracted both water and methanol for two hours in a magnetic stirrer. Filtration of the extracts was performed with passing filter papers and through a 0.45 syringe filter respectively. Finally, the prepared solutions were separated for antioxidant analysis and RP-HPLC-UV phenolic analysis.

For LC/MS-MS, 1 g of each of the samples was added to 10 mL solvent (75% methanol + 25% dichloromethane) and the solutions were extracted on the shaker for 2 hours. Then, extracts were filtered and injected into the device with passing through a 0.45µm syringe filter.

Table 1. Abbreviations for solution extracts of tuber of *A. italicum.*

1S	Edible part of the tuber water extract
1M	Edible part of the tuber methanol extract
2S	Nonedible part of the tuber water extract
2M	Nonedible part of the tuber methanol extract

Determination of Antioxidant Activity

Total Phenolic Content (TPC): The total phenolic content of the edible part and the nonedible part of the *A. italicum* plant tuber was determined by using

Folin-Ciocalteu reagent, modified according to Slinkard and Singleton (1977) method. Firstly, 50 µL of sample solution was diluted with 2.5 mL of distilled water and 250 µl 0.2 N Folin–Ciocalteu reagent was added. Then 750 µl of Na_2CO_3 (7.5%) was put in the mixture and vortexed. The tubes were incubated at room temperature for 2 hours and the absorbance values at 765 nm were measured. The amounts of phenolic compounds in the samples were calculated in terms of gallic acid equivalent (GAE µg/mL) with using the function of the line obtained from the standard calibration graph of gallic acid prepared at six different concentrations (starting at 1000 µg/mL).

***Determination of Iron (III) Reduction/Antioxidant Power (FRAP)*:** Edible part and non-edible of the plant tuber were studied at a concentration of 100 mg/mL based on the FRAP method developed by Benzie and Strain (1996). As in determination of TPC, Trolox antioxidant standard was studied in six different concentrations (starting at 1000 µM). Samples were pipetted as triplicate together with a sample and reagent blank. After 20 minutes, the absorbance values were read at 595 nm. The results were calculated as µM TEAC comparing with the standard antioxidant substance Trolox.

***Copper (II) Reducing Antioxidant Activity (CUPRAC)*:** The method developed by Apak et al (2004) was modified and applied in this study. Cu (II) chloride and neocuproine solution, ammonium acetate buffer (pH = 7) and analysis solutions were added in equal volumes respectively. The volume of final solution was adjusted to 4.1 mL After 30 minutes, absorbance values were measured at 450 nm. As in FRAP, the antioxidant capacities of the samples in terms of Trolox equivalent were calculated as µM TEAC with using values obtained from the standard antioxidant Trolox graph studied at six different concentrations (starting from 1000 µM).

***DPPH Radical Scavenging Activity*:** In DPPH scavenging activity, 100 µM methanolic solution of DPPH radical was used to determine the activity of edible and non-edible parts of the tuber of the plant. The antioxidant standard and the extracts of the tuber parts of *A. italicum* were conducted in triplicate. In addition, a sample and a reagent blank were studied for each concentration of the samples. After the incubation period (50 min), the absorbance values of the solutions mixed with DPPH reagent were read at 517 nm and the % inhibition (DPPH• scavenging) values were calculated (Huang et al., 2005).
% inhibition (DPPH• cleaning) values were calculated using the following formula.

% Inhibition (radical cleaning power) = $[(A_{DPPH} - A_{Sample}) / A_{DPPH}] \times 100$

A_{DPPH}: Absorbance value of the DPPH solution

A_{Sample}: Absorbance value of the sample extract

Determination of Phenolic Components by HPLC–UV

RP–HPLC–UV Conditions: Analysis of phenolic compounds was carried out on HPLC (Elite LaChrom Hitachi Japan) device. The analysis were performed with using a reverse phase C18 (150 mm x4.6mm, 5µm; Fortis) column. In this process, the gradient program was applied containing 2% acetic acid (pure water) in a reservoir and 70% acetonitrile (pure water) in B reservoir (Can et al., 2015). In addition, it is optimized that the injection volumes of the standards and samples to 20 µL, the flow of mobile phase to 1.0 mL/min, and temperature of the column to 30 °C were fixed. In addition, for the gradient program the optimization was performed with passing through reservoir A as follows: 95% for 0-3 minutes, 85% for 5-8 minutes, 80% for 8-10 minutes, 75% for 10-12 minutes, 60% for 12-20 minutes, 20% for 20-30 minutes and 95% for 35-50 (Can et al., 2015).

LC–MS/MS Analysis: Phenolic component analysis was performed with LC–MS/MS Thermo Scientific/Dionex Ultimate 3000–TSQ Quantum device. LC–MS/MS analyzes were carried out by Hitit University Scientific Technical Application and Research Center. The analyses were performed using ODS Hypersil 4.6 * 250 mm, 5µm column and applying a gradient program including formic acid, water and methanol. Gradient program with 0.1% formic acid (pure water) in reservoir A and 100% methanol in reservoir B was applied. In addition, it is optimized that it is optimized that the injection volumes of the standards and samples to 20 µL, the flow of mobile phase to 0.7 mL/min, and temperature of the column to 30 °C were fixed. The gradient program was optimized by passing through the reservoir 100% A for 0-1 minutes, 5% A for 3 minutes in 22 minutes, and 100% B for 8 minutes in 26 minutes.

RESULTS AND DISCUSSION

Antioxidant Activity of A. italicum Extracts: There are many antioxidant activity determination methods. In the study, to determine the antioxidant activity of the plant tuber was used such as total phenolic content, FRAP, CUPRAC and % DPPH scavenging activity tests.

Total Phenolic Content: Polyphenols, flavonoids and phenolic compounds found in plants are natural antioxidants substances which prevent the harmful effects of oxidative stress induce by ROS. In the study, the total phenolic content of the edible and nonedible parts of the tuber of *Arum italicum* was determined using different solvents (water and methanol). Total phenolic content of methanolic extracts were determined higher than water extracts. While the 2M sample showed highest phenolic content it was followed by 1M, 2S and 1S, respectively (Figure 1).

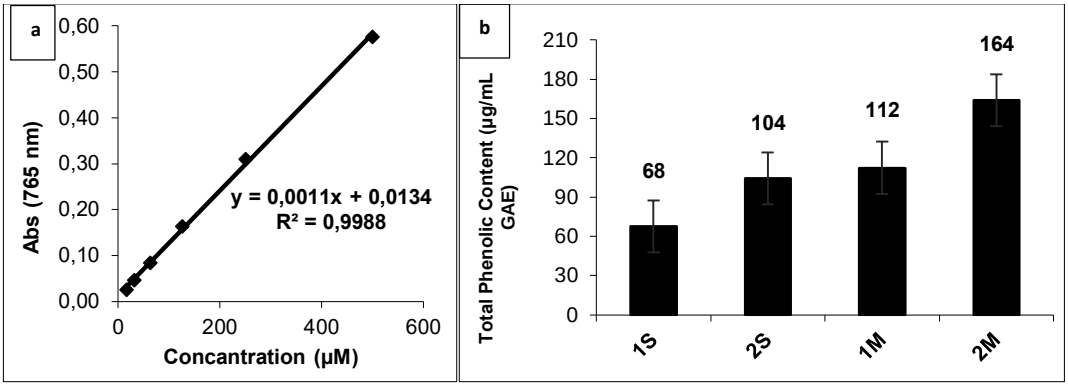

Figure 1. Calibration graphic of the gallic acid standard studied at different concentrations (a) and GAE (µg/mL) values of the Total Phenolic Content of the water and methanolic extracts of the two parts of the tuber (b).

Karahan et al. (2006) conducted experiments the TPC and TFC activity in the leaves of the *Arum dioscoridis* Sm. with using different organic solvents (acetone, ethanol, methanol and water). They report that the highest total phenolic and flavonoid contents were in the ethanol and methanol extracts respectively. In our study, also the total phenolic content was found to be higher in both methanolic extracts of edible and nonedible parts. The highest total phenolic content was determined as 164 µg GAE/mL in sample 2M, 112 µg GAE/mL in 1M sample, 104 µg GAE/mL in 2S sample, 68 µg GAE/mL in sample 1S.

***Iron (III) Reduction/Antioxidant Power and Copper (II) Reducing Antioxidant Activity Analysis*:** The reducing power of bioactive compounds is an indicator of the electron donating ability and this situation is related to the antioxidant activity of the compounds (Arabshahi-Delouee & Urooj, 2007). The antioxidant activity of the plant was determined with methods of FRAP and CUPRAC. In the FRAP test, antioxidant activity values were measured as higher in both water and methanolic extracts of the nonedible tuber part than the edible part (Figure 2).

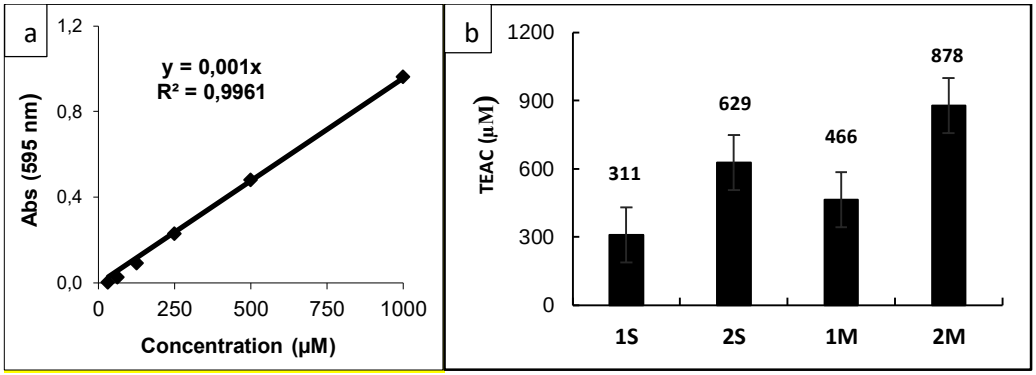

Figure 2. Calibration graphic of the Trolox standard studied at different concentrations (a) and FRAP values (µM TEAC) of water and methanolic extracts of the two parts of the tuber (b).

In CUPRAC test, Cu^{+2} reduction activity of methanolic extracts was measured higher than the water extracts. In addition, as with FRAP, the highest values of water and methanolic extracts were measured for the nonedible parts in CUPRAC test (Figure 3). FRAP results were determined as 878 TEAC (µM) in sample 2M, 629 TEAC (µM) in sample 2S, 466 TEAC (µM) in sample 1M, and 311 TEAC (µM) in sample 1S. The CUPRAC results were measured as 0.064 TEAC (µM) in sample 2M, 0.036 TEAC (µM) in sample 1M, 0.024 TEAC (µM) in sample 2S, 0.018 TEAC (µM) in sample 1S. The CUPRAC results are consistent with the results for the total phenolic content.

Antioxidant Activity Analysis with % DPPH Method: To determine the antioxidant activities, DPPH free radical scavenging test was applied to water and methanol extracts of both parts of the plant tuber. The DPPH radical scavenging method is commonly used in measuring the antioxidant activity of the phenolic compounds or plant extracts due to its easy to operate, rapid and sensitive nature (Uguzlar et al., 2012). Many plant extracts have antioxidant properties because they contain phytocomponents such as phenolic acids and flavonoids (Chu et al., 2000). When DPPH activity results were examined for the all the parts of the plant tuber , % DPPH scavenging activity the methanol extracts was higher than that of the water extracts. In addition, as in all the tests, the highest activity values of % DPPH in both methanolic and water extracts were found in the nonedible parts of the tuber. The % DPPH scavenging activity was determined as 19.41 in sample 2M, 19.02 in sample 1M, 18.23 in sample 2S, 14.78 in sample 1S, respectively (Figure 4).

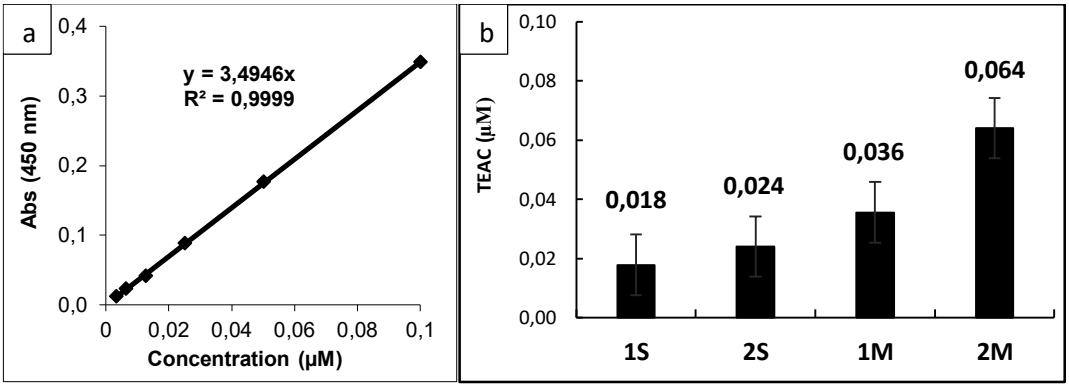

Figure 3. Calibration graphic of the Trolox standard studied at different concentrations (a) and CUPRAC values (µM TEAC) of water and methanolic extracts of the two parts of the tuber (b).

Antioxidant Activity Analysis with % DPPH Method: To determine the antioxidant activities, DPPH free radical scavenging test was applied to water and methanol extracts of both parts of the plant tuber. The DPPH radical scavenging method is commonly used in measuring the antioxidant activity of the phenolic compounds or plant extracts due to its easy to operate, rapid and sensitive nature (Uguzlar et al., 2012). Many plant extracts have antioxidant properties because they contain phytocomponents such as phenolic acids and flavonoids (Chu et al., 2000). When DPPH activity results were examined for the all the parts of the plant tuber , % DPPH scavenging activity the methanol extracts was higher than that of the water extracts. In addition, as in all the tests, the highest activity values of % DPPH in both methanolic and water extracts were found in the nonedible parts of the tuber. The % DPPH scavenging activity was determined as 19.41 in sample 2M, 19.02 in sample 1M, 18.23 in sample 2S, 14.78 in sample 1S, respectively (Figure 4).

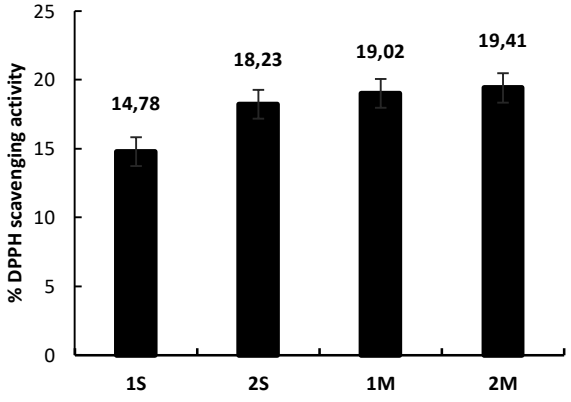

Figure 4. % DPPH radical scavenging activity of water and methanolic extracts of the two parts of the tuber.

***Phenolic Component Analysis by HPLC–UV and LC–MS/MS*:** The plants are rich source of secondary metabolite diversity. One of the most important groups of this metabolite diversity is phenolic compounds (Michalak, 2006). Phenolic compounds are present in plant parts such as fruit, leaves and stems and available in almost all plant parts. As their potential antioxidant properties and their possible role in preventing oxidative stress related diseases such as cancer, they have been attracted much attention (Odbayar et al., 2006).

Phenolic acids consist of two subgroups which are hydroxybenzoic acid and hydrocinnamic acid. In the study, the phenolic compositions of the methanolic extracts of the tuber were determined using both RP–HPLC–UV devices. In addition, methanol and dichloromethane extracts were prepared in LC–MS/MS devices.

The phenolic content in the samples was analyzed using 19 phenolic acid standards in RP–HPLC–UV device Table 2. More phenolic components were determined in the methanolic extracts of the nonedible parts than the edible part. In this case, the phenolic content is consistent with those of the antioxidant activity determination tests which are higher in nonedible part extract for all the tests.

Table 2. RP–HPLC–UV Phenolic Compounds.

Standards	1M(µg phenolic/g sample)	2M(µg phenolic/g sample)
Gallic acid	-	-
Protocateuic acid	6.02	-
p-OH Benzoic acid	4.539	-
Catechin	-	-
Caffeic acid	-	26.724
Syringic acid	-	13.179
Epicatechin	-	31.284
p-Coumaric acid	-	13.264
Ferulic acid	-	14.376
Rutin	-	247.754
Myricetin	-	-
Resveratrol	-	-
Daidzein	-	-
Luteolin	8.092	-
t-Cinnamic acid	4.970	2.696
Hesperidin	-	-
Chrysin	-	-
Pinocembrin	-	-
CAPE	-	-

*-: Not Detected

Rutin and epicatechin were major flavonoid components and caffeic acid was determined as hydroxycinnamic acid derivatives in the nonedible part. At the same time, the phenolic components of the two parts of the tuber of the plant were performed using LC–MS/MS device with 20 phenolic standards (Table 3). While ferulic acid, p-coumaric acid, epicatechin, vanillin, caffeic acid, p-hydroxy benzoic acid and salicylic acid are identified as

major components, rutin, catechin and taxipholine were measured at low levels at both parts of the tuber.

Table 3. LC/MS–MS Phenolic Compounds.

Standards	1M(µg phenolic/g sample)	2M(µg phenolic/g sample)
Gallic acid	-	-
Protocateuic acid	-	-
p-OH Benzoic acid	0.179	0.189
Benzoic acid	-	-
Catechin	0.014	0.090
Caffeic acid	0.579	0.716
Syringic acid	-	-
Epicatechin	1.165	0.711
Salicylic acid	0.159	0.108
Vanillic acid	-	
p-Coumaric acid	4.391	4.629
Ferulic acid	11.131	10.800
Rutin	0.014	0.021
Taxifolin	0.003	0.007
Protocatechuic aldehyde	-	-
Vanillin	1.111	1.263
Rosmarinic acid	-	-
Ellagic acid	-	-
Oleuropein	-	-
Resveratrol	-	-

*-: Not Detected

Phenolic components defined and measured in both RP–HPLC–UV and LC–MS/MS are compatible with each other except for minor differences. Ağalar et al (2017) reported that the whole tuber part of *Arum italicum* has hydroxycinnamic acid derivatives such as ferulic, caffeic and *p*-coumaric acid in analysis with LC–MS/MS. The results obtained in both RP–HPLC–UV and LC–MS /MS are similar to the results of study by Ağalar et al (2017). Ağalar et al (2017) stated that ferulic acid and caffeic acid were present in the leaves of plants belonging to the Araceae family, also p-coumaric acid in the seeds of some species of the same family. Hydroxycinnamic acids containing ferulic acid, caffeic acid, and p-coumaric acid prevent metastasis or invasion of cancer cells (Ağalar et al., 2017). It has been pointed out that rutin exhibits many pharmacological activities such as antitumor, antimutagenic, antibacterial, anti-inflammatory, antiulcer, antidiarrheal, vasodilator, hepatoprotective activities and immunomodulator (Janbaz et al., 2002; Kamalakkannan & Prince, 2006). In our study, rutin was detected in the nonedible part of the tuber with both RP–HPLC–UV and LC–MS/MS devices. It is known that this phenolic prevent ulcer in stomach where widespread in recent years. Ferulic acid was determined as 14.376 µg phenolic/g sample in 2M part with RP–HPLC–UV, 10.800 in 2M sample and 11.131 µg phenolic/g sample in 1M sample by LC–MS/MS. It has been reported that ferulic acid can be useful in the treatment of chronic diseases with its biological activity (de Oliveira Silva & Batista, 2017). In China, herbs rich in ferulic acid have been used in the repair of blood vessel damage and in the treatment of thrombosis diseases for many years (Ou & Kwok, 2004). In addition, it has been reported that ferulic acid has very high antimicrobial activity, showed particularly strong inhibitory effects on the growth of some human gastrointestinal pathogenic microbiota including *Helicobacteria pylori* and *Shigella sonnei* (Lo & Chung, 1999; Nilsson, 1999; Tsou et al., 2000).

CONCLUSION

It has been determined that the nonedible part of the tuber showed higher antioxidant activity than the edible part consumed as food in *Arum italicum* plant. Also, the phenolic profile determined by HPLC–UV and LC–MS/MS devices is richer in the nonedible part. According to these results, although nonedible tuber part has a bad taste it can be concluded that the part can be consumed as food and used for treatment in alternative medicine.

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ARAŞTIRMA MAKALESİ RESEARCH PAPER

Farklı Gökkuşağı Alabalığı (*Oncorhynchus mykiss*) Kuluçkahanelerindeki Damızlık Populasyonlarının Bazı Fenotipik Özellikleri ve Farklı Damızlıklardan Örneklenen Yumurtaların Aynı Çevresel Şartlarda Kalite Kriterleri

Boran KARATAŞ* **Muhammed ARABACI**
Van Yüzüncü Yıl Üniversitesi, Su Ürünleri Fakültesi, Van, Türkiye

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: https://orcid.org/0000-0003-4353-1293
: https://orcid.org/0000-0002-2462-6441

***Sorumlu yazarın:**
Boran KARATAŞ
Van Yüzüncü Yıl Üniversitesi, Su Ürünleri Fakültesi, VAN, Türkiye
✉: borankaratas@yyu.edu.tr

Öz: Bu çalışmada Van ilindeki altı adet gökkuşağı alabalığı kuluçkahanesinde bulunan damızlık populasyonlarının yumurta ve sağım ilgili bazı fenotipik özellikleri ile örneklenen yumurtaların aynı çevresel şartlarda kuluçkalanmasıyla yumurta ve larva kalite kriterlerinin belirlenmesi amaçlanmıştır. Bu amaçla kuluçkahanelerde bazı su kalite kriterleri, damızlıklarla ilgili kuluçkahane yönetim parametreleri, damızlıkların yumurta ve sağım ile ilgili fenotipik özellikleri ve farklı kuluçkahanelerden örneklenen yumurtaların aynı çevresel şartlarda (10,1±0,14°C) kuluçkalanarak yumurta ve larva kalite kriterleri belirlenmiştir. Kuluçkahane su kalite kriterlerinin literatürde verilen aralıklarla uyumlu olduğu ve damızlık refahını olumsuz etkileyecek düzeyde olmadığı görülmüştür. Kuluçkahanelerdeki toplam damızlık sayısı, yaşı, boyu ve ağırlığı ile toplam ve nispi fekonditeleri her bir kuluçkahane için farklı olup bakım ve beslemelerinde de farklılıklar görülmüştür. Damızlıkların sağım mevsimi ve sağım performansı da her bir kuluçkahane için farklı olup kuluçkahanelerden birinde Kasım başında sağılan damızlıklar gözlenmiştir. Aynı çevresel şartlar altında yapılan deneme düzeneğinde ise yumurtaların gözlenme oranı ve larva çıkış oranı istatistiksel olarak farklı ve önemli bulunmuştur($P<0,05$). Sonuç olarak tüm parametreler incelendiğinde kuluçkahaneler arasında fenotipik farklılıklar olduğu görülmüştür. Bu durumun nedeni kuluçkahanelerdeki su kalitesi ve damızlık yönetim parametrelerinin farklı olması olabilir. Ancak bu farklılıkların nedeni kuluçkahanelerin bünyelerinde farklı damızlık hatları bulundurmaları ve damızlıklar arasında olan genotipik farklılıklar da olabilir. Bu nedenle genotipik farklılıklarla ile ilgili detaylı çalışmalara ihtiyaç vardır. Yetiştiricilik açısından ise kuluçkahanelerden sadece birisinde bulunan ve erken sağıma gelen damızlıklardan, erken sağılan hat elde etmek mümkündür. Daha ekonomik bir yetiştiricilik için damızlıklarda yumurta sayısını ve kalitesini artırmaya yönelik besleme yöntemleri uygulanmalıdır. Ayrıca damızlık stok populasyonlarında homozigotluğu düşürmek için dişi erkek damızlık oranı kuluçkahanelerde eşitlenmeli ve erkek damızlık ihtiyacı porsiyonluk balıklardan karşılanmamalıdır.

Anahtar kelimeler: Damızlık stok, fenotip, gökkuşağı alabalığı, kuluçkahane, larva, yumurta.

Some Phenotypic Characteristics of Broodstock Populations Different in Rainbow Trout (*Oncorhynchus mykiss*) Hatcheries and Quality Criteria for Eggs Sampled from Different Broodstocks in the Same Environmental Conditions

Abstract: This study aims to determine some phenotypic characteristics of the broodstock populations in six rainbow trout hatcheries located in Van province and their egg and larval quality criteria by incubating the sampled eggs under the same environmental conditions. For this purpose, some water quality criteria, hatchery management parameters related to the broodstocks, phenotypic characteristics of the broodstocks were determined, and eggs sampled from different

hatcheries under the same environmental conditions (10.1±0.14°C) were incubated for determining egg and larva quality criteria. In the present study, it was observed that the water quality criteria were compatible with the ranges given in the literature and it was not at a level that would negatively affect the broodstock welfare. In addition to the differences observed in the feeding of broodstocks in these hatcheries, the total number of broodstocks, their age, height, weight, total and relative fecundities were also found different in each hatchery. The stripping season and performance of the broodstocks were also different in each hatchery, and the broodstocks stripping in early November was observed in one of the hatcheries. In the experimental setup conducted under the same environmental conditions, the eyeing rates of eggs and the hatching rates of larvae were found to be different and statistically significant ($P<0.05$). As a result, it was observed that when all parameters were examined, there were phenotypic differences between these hatcheries. These differences could be the result of the differences in water quality and broodstock management parameters in each hatchery. The reason for these differences, however, could be that the hatcheries different broodstock lines and genotypic differences between broodstocks. Therefore, detailed studies on genotypic differences are required. In terms of breeding, it is possible to obtain an early broodstock line from early stripping broodstocks in only one of the hatcheries. For a more affordable breeding, different broodstocks feeding methods should be applied for increasing the number and quality of eggs in broodstocks. Moreover, the ratio of female to male in broodstocks should be equalized in the hatcheries to reduce homozygosity in broodstock populations and the male table fish should not be used for male broodstock requirement.

***Corresponding author's:**
Boran KARATAŞ
Faculty of Fisheries, Van Yüzüncü Yıl University, VAN, Turkey
✉: borankaratas@yyu.edu.tr

Keywords: Broodstock stock, egg, hatchery, larva, phenotype, rainbow trout.

GİRİŞ

Gökkuşağı alabalığı (*Oncorhynchus mykiss*), Türkiye'de ve dünyanın birçok ülkesinde geniş çevresel koşullar altında yetiştirilmekte ve iç su balıkları yetiştiriciliğinin büyük bir kısmını oluşturmaktadır (Balta, 2020; Karataş vd., 2017). Geçtiğimiz son yirmi yılda, gökkuşağı alabalığı yetiştiriciliği dünyada büyük önem kazanmış ve 2017 yılında 811.590 tonu aşan yetiştiricilik miktarı ile dünyanın en önemli kültür balıklarından biri haline gelmiştir (FAO, 2019). Ancak gökkuşağı alabalığı kuluçkahanelerinin sürdürülebilirliği için düzenli olarak yüksek kaliteli yumurta ve larva temini gerekmektedir. Çünkü kaliteli yumurta ve larva üretimi başarılı bir su ürünleri yetiştiriciliği için öncelikli gerekliliktir. Dolayısıyla kuluçkahanelerin önemli bir hedefi, eldeki damızlıklardan yüksek miktarda ve kalitede yumurta ve larva elde etmektir. Bu durum ise her bir kuluçkahanede kullanılan damızlıkların fenotipik özelliklerinin ortaya çıkarılması ile mümkün olabilir. Bu nedenle kuluçkahanelerdeki damızlıkların fenotipik özelliklerini bilmek ve kuluçkahane yönetiminde bu özellikleri kullanmak, elde edilecek yumurta ve larva kalitesi açısından önemlidir (Bromage vd., 1992).

Damızlıkların üreme ile ilgili fenotipik özelliklerini ortaya koyabilmek için; damızlıkların fekonditesini, yaşlarını, boy ve ağırlıklarını, damızlıkların sağım mevsimini (sağımın başlangıç ve bitiş zamanı) ve sağım performansını (her sağımda sağıma katılan damızlık sayısı) belirlemek sağlıklı bir kuluçkahane ve damızlık yönetimi oluşturulmak için gereklidir. Damızlıkların sağım mevsimi, sağım performansını, yumurta ve larva kalitesi ile sayısı üzerine; su kalite kriterleri, stoklama yoğunluğu, yemleme oranı, yem miktarı ve kalitesi, damızlıkların yaşı ve büyüklüğü direk ya da dolaylı olarak etkilidir (Arabacı, 2007; Okumuş, 2002). Bu nedenle gökkuşağı alabalığı yetiştiriciliği yapan kuluçkahanelerde fenotipik verileri değerlendirebilmek için çevresel parametrelerin de bilinmesi ve izlenmesi önemlidir (Laird & Needham, 1988; Stevenson, 1987). Ayrıca damızlıkların fenotipik özelliklerinin bilinmesi; İlk damızlık stokların oluşturulması, damızlık stoklarının verim özelliklerinin arttırılması, seleksiyon ve ıslah gibi çalışmalar için gerekli ilk adımdır. Bu bağlamda ülkemizdeki gökkuşağı alabalığı kuluçkahanelerindeki damızlık stoklarının yumurta ve sağım ile ilgili fenotipik özelliklerinin bilinmesi, yumurta ve larva kalitesinin belirlenmesi açısından önemlidir. Geçmiş yıllarda bazı araştırıcılar tek bir kuluçkahaneyi ele alarak Türkiye'deki gökkuşağı alabalığı damızlıklarının yumurta ve sağım ile ilgili özelliklerini bildirmişlerdir (Güner & Tekinay, 2002; Kanyılmaz vd., 2016; Kurtoğlu vd., 1998; Özgür & Bayır, 2013). Ancak gen kaynağı ülkemizde olmayan gökkuşağı alabalığının sürdürülebilir üretimi için damızlıklarının yumurta ve sağım ile ilgili fenotipik özelliklerinin belirlenmesine yönelik daha çok çalışmaya ihtiyaç vardır.

Bu çalışmada, Van ilinde altı adet gökkuşağı alabalığı kuluçkahanesinde kullanılmakta olan damızlık stokların bazı fenotipik özelliklerinin belirlenmesi amaçlanmıştır. Damızlıkların bulundukları kuluçkahanelerdeki çevresel şartların birbirinden oldukça farklılık göstermeleri beklenmekle birlikte bu çalışmada; (i) Kuluçkahanelerde kullanılan suların bazı su kalite kriterleri, (ii) Kuluçkahanelerin damızlıklara yönelik bazı kuluçkahane yönetim parametreleri, (iii) Damızlıkların bulundukları çevresel şartlarda yumurta ve sağım ile ilgili bazı fenotipik özelliklerin belirlenmesi amaçlanmıştır. Ayrıca çalışmada (iv) Kuluçka performanslarının

kıyaslanabilmesi amacıyla, farklı kuluçkahanelerin, farklı damızlık stoklarından sağım yoluyla elde edilip döllenen yumurtalar, çevresel etkileri minimize etmek için aynı çevresel ortamda ve şartlarda kuluçkalanmış ve kuluçka performansları sayısallaştırılarak kaydedilmiştir.

MATERYAL VE METOT

Bu çalışma, Van il sınırları içinde bulunan Tarım ve Orman Bakanlığına kayıtlı 6 adet gökkuşağı alabalığı kuluçkahanesinde Ekim 2017–Mart 2018 tarihleri arasında yapılmıştır. Çalışmada kuluçkahaneler YSU (Yeşilsu), BSU (Beyazsu), KCM (Kırkçeşme), ELF (Elfa), OCK (Özçatak) ve SFA (Şifa) kısaltmaları kullanılarak isimlendirilmiştir.

Kuluçkahanelere ait bazı su kalite kriterlerinin belirlenmesi: Çalışmada kuluçkahanelere ait su kalite kriterleri kuluçkahanelere her hafta yapılan örnek toplama ve ölçüm ziyaretleri ile belirlenmiştir. Çalışmada damızlık refah parametresi olarak (MacIntyre vd., 2008) damızlık havuzlarının çıkış suyundaki çözünmüş oksijen (O2) konsantrasyonu, su sıcaklığı (oC) dijital oksijenmetre (YSI Pro 20) ile belirlenmiştir. Kuluçkahane suyunun pH'sı dijital pH metre (Thermo, Orion Star A211), kuluçkahanenin rakımı ise GPS cihazı (Garmin, eTrex Legend) ile belirlenmiştir. Ayrıca kuluçkahane suyunun total sertliği Egemen, (2006)'e göre ve debisi Arabacı, (2007)'e göre belirlenmiştir.

Damızlıklarla ilgili bazı kuluçkahane yönetim parametrelerin belirlenmesi: Hedef kuluçkahanelerde damızlık stok yoğunluğu, damızlık beslemesinde kullanılan ticari yem markası, damızlık besleme şekli, günlük besleme sayısı ve günlük verilen yem miktarı gibi parametreler kaydedilmiştir. Damızlık stok yoğunluğu kuluçkahanede bulunan toplam damızlık sayısına, damızlık büyüklüğüne ve havuz hacmine göre hesaplanmıştır (Yılmaz & Arabacı, 2010). Ayrıca her bir kuluçkahanenin toplam damızlık sayısı, kuluçkahanelerdeki dişi/erkek damızlık oranı ve kuluçkahanelerde döllemede kullanılan dişi/erkek damızlık oranı belirlenmiştir. Kuluçkahane yönetim parametreleri işletmecilerin beyanları ve elde edilen veriler doğrultusunda belirlenmiştir.

Hedef kuluçkahanelerdeki damızlıkların yumurta ve sağım ile ilgili bazı fenotipik özelliklerinin belirlenmesi: Çalışmada kuluçkahanelerdeki damızlıkların sağımı ile ilgili fenotipik özellikler sağım mevsimi (sağımın başlangıç ve bitiş zamanı) ve sağım performansı (her sağımda sağıma katılan damızlık sayısı) ile belirlenmiştir (Arabacı, 2000). Ayrıca her hafta sağım yapılan kuluçkahanelere gidilerek sağılan damızlıklarda yaş, boy, ağırlık ve döllemede kullanılan dişi/erkek damızlık oranı belirlenmiştir. Sağım dönemi sonunda ise bütün kuluçkahanelerden elde edilen veriler değerlendirilerek her bir kuluçkahanede sağılan damızlık sayısı, sağıma katılmayan damızlık sayısı ve kuluçkahanelerdeki dişi/erkek damızlık oranı belirlenerek damızlıkların sağım ile ilgili fenotipik özellikleri ortaya konulmuştur. Çalışmada kuluçkahanelerdeki damızlıkların yumurta ile ilgili bazı fenotipik özelliklerini belirlemek için her hafta sağım yapılan kuluçkahanelere gidilerek sağılan damızlıklarda yumurta çapı, toplam fekondite ve nispi fekondite belirlenmiştir. Gökkuşağı alabalığı damızlıklarından sağılan yumurtaların çapları Von Bayer sayım teknesi ile ortalama olarak hesaplanmıştır (Arabacı, 2007; Bromage vd., 1992; Huang & Gall, 1990).

Hedef kuluçkahanelerden örneklenen döllenmiş yumurtaların kuluçka performansları: İlde farklı kuluçkahanelerde, farklı damızlık stoklardan üretilen döllenmiş yumurtaların aynı ortamda kuluçkalanmasıyla, kuluçkahanelerin yumurta ve larva gelişim performanslarının karşılaştırılması amaçlanmıştır. Örneklenen yumurtalar aynı çevresel şartlar altında (10,1±0,14 °C) kuluçkalanmıştır. Örnekleme için her kuluçkahaneden 6 dişi damızlık rastgele örneklenmiş ve damızlıkların her biri yedişer adet erkek damızlıktan alınan sperm sıvısı karışımı ile döllenmiştir. Döllenmiş yumurtalardan her bir damızlığa ait rastgele 100 adet yumurta örneği alınmıştır. Alınan yumurtalar taşıma kabına yerleştirilerek aynı gün içinde sabit sıcaklıkta (10,0 °C), ortam şartları sabit olan, kaynak suyu kullanılan ve su sıcaklığı yıl boyunca 10,1±0,14 °C arasında değişen Kırkçeşme Alabalık Tesisindeki (Van) deneme düzeneğine getirilerek aynı inkübatörde kuluçkalanmıştır. Kuluçka tavaları örnekler getirilmeden önce altı eşit parçaya bölünmüş ve her bir tavaya tek bir kuluçkahanenin örnekleri konulmuştur. Yumurtalar burada gözlenme oranı, yumurtadan çıkan larva oranı ve larvaların yeme başlama oranı belirlenene kadar gözlemlenerek her bir kuluçkahanenin yumurta ve larva kalite kriterleri hesaplanmıştır. Yumurtaların döllenme oranı ise yumurtaları strese sokmamak için hesaplanmamıştır. Çalışmada hesaplamalar oransal olarak değerlendirilmiştir. Her bir damızlıktan örneklenen 100 adet yumurtadan gözlenenlerin oransal değeri belirlenerek yumurtaların gözlenme oranı hesaplanmıştır. Larva çıkış oranı ise gözlenen yumurtalardan çıkan larvaların oransal değeri belirlenerek hesaplanmıştır. Yeme başlama oranında ise yumurtadan çıkan larvalardan yem almaya başlayanların oranı hesaplanmıştır (Arabacı, 2000).

Verilerin değerlendirilmesi: Çalışmada farklı kuluçkahanelerdeki farklı damızlıkların yumurta ve larva kalite kriterleri ile ilgili sonuçların değerlendirilmesinde SAS 9.4 istatistik paket programından yararlanılmıştır. Kuluçkahaneler arasındaki farklılığı test etmek amacıyla öncelikle elde edilen verilerin normal dağılım gösterip

göstermediğine bakılmış ve verilerin normal dağılım göstermediği görülmüştür. Bu nedenle verileri karşılaştırmak için Non-Parametrik test olan Kruskal-Wallis çoklu karşılaştırma testi kullanılmış ve sonuçlar P<0,05 önem seviyesine göre anlamlı kabul edilmiştir. Kuluçkahaneler arasındaki farklılığın istatistiki olarak önemli çıkması durumunda bu farklılığın hangi kuluçkahanelerden kaynaklandığını tespit etmek için çoklu karşılaştırma testi Bonferroni düzeltmesi yapılmıştır. Bonferroni düzeltmesine göre kuluçkahaneler arası farklılıklar değerlendirilmiş ve farklar a, b, c olarak gösterilmiştir.

BULGULAR

Hedef kuluçkahanelerin su kalite parametreleri: Çalışmadaki gökkuşağı alabalığı kuluçkahanelerine ait su kalite kriterleri Tablo 1'de verilmiştir.

Tablo 1. Gökkuşağı alabalığı kuluçkahanelerine ait su kalite kriterleri*.
Table 1. Water quality criteria for rainbow trout hatcheries*.

Kuluçkahaneler/Parametreler	YSU	BSU	KCM	ELF	OCK	SFA
Havuz çıkış suyundaki çözünmüş oksijen konsantrasyonu (mg/L)	7,2±0,91	7,9±0,40	8,2±0,42	7,1±0,55	7,5±0,28	8,5±1,03
Su sıcaklığı (°C)	8,7±0,43	8,5±0,35	10,1±0,14	7,5±0,17	8,5±0,23	6,9±0,46
pH	8,45	8,3	7,9	7,7	7,78	7,7
Su sertliği (mg/L)	164	148	340	148	220	396
Rakım (m)	1626	1672	1664	1804	1628	1751
Su debisi (L/sn)	517	320	1201	1019	480	1000

* 2017-2018 yılı Ekim-Mart ayı ortalamaları (± standart sapma)

Damızlıklarla ilgili kuluçkahane yönetim parametreleri: Çalışmadaki gökkuşağı alabalığı kuluçkahanelerindeki damızlıklarla ilgili kuluçkahane yönetim parametreleri Tablo 2'de verilmiştir.

Kuluçkahanelerdeki damızlıkların yumurta ve sağım ile ilgili fenotipik özellikleri: Çalışmadaki gökkuşağı alabalığı kuluçkahanelerindeki damızlıkların sağım mevsimi ve sağım performansı Şekil 1'de verilmiştir.

Çalışmadaki gökkuşağı alabalığı kuluçkahanelerindeki damızlıkların sağım ile ilgili fenotipik özellikleri Tablo 3'de verilmiştir.

Çalışmadaki gökkuşağı alabalığı kuluçkahanelerindeki damızlıkların yumurta ile ilgili fenotipik özellikleri Tablo 4'de verilmiştir.

Kuluçkahanelerden örneklenen döllenmiş yumurtaların kuluçka performansları: İlde farklı kuluçkahanelerde, farklı damızlık stoklardan üretilen yumurtaların aynı ortamda kuluçkalanmasıyla kuluçkahanelerin yumurta ve larva gelişim performanslarının karşılaştırılması Tablo 5'de verilmiştir.

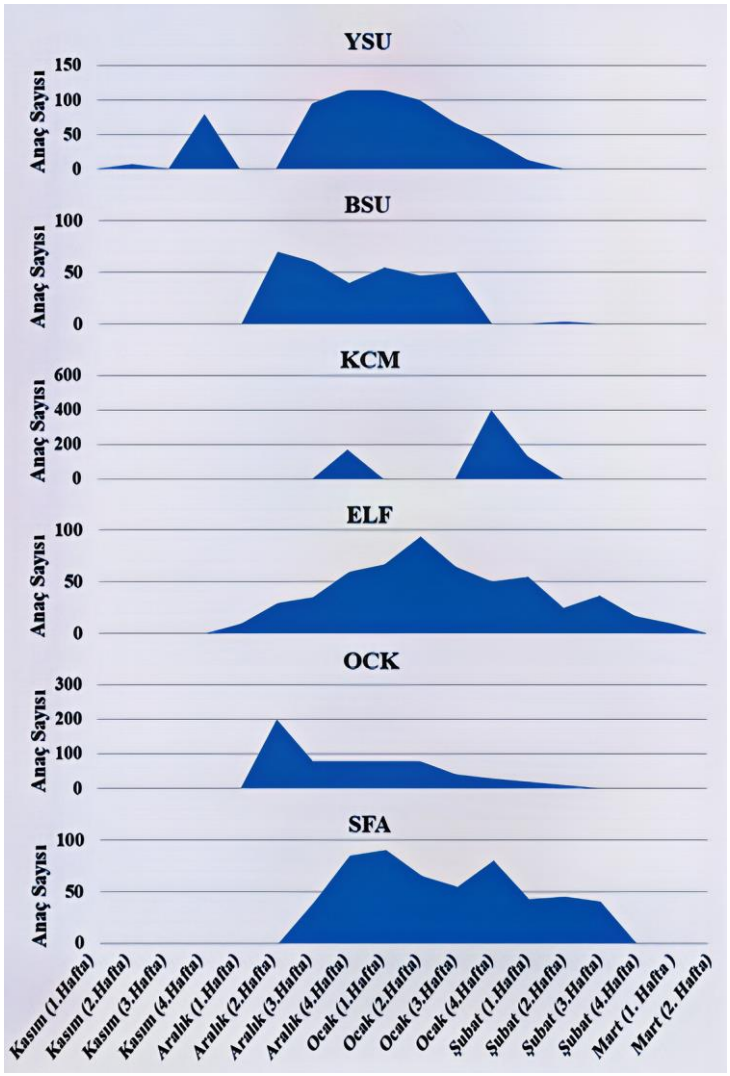
Tablo 2. Damızlıklarla ilgili kuluçkahane yönetim parametreleri.
Table 2. Hatchery management parameters related to broodstocks.

Kuluçkahaneler/Parametreler	YSU	BSU	KCM	ELF	OCK	SFA
Toplam damızlık sayısı	636	324	700	555	540	543
Dişi/Erkek damızlık oranı	2/1	1/2	3/1	9/1	1/1	2/1
Döllemede Dişi/Erkek oranı	2 /1	1 / 2	1 / 3	3 / 1	2 /3	1 /2
Damızlık stok yoğunluğu (adet/m^3)	2,33	5,4	5,18	2,02	2,72	2,94
Beslenmede kullanılan ticari yemler	A yemi	A ve B yemi	A yemi	A ve D yemi	C yemi	C ve D yemi
Damızlık besleme şekli	*Ad libitum*	*Ad libitum*	Besleme tablosu	Besleme tablosu	*Ad libitum*	*Ad libitum*
Günlük besleme sayısı (Yaz/Kış)	3/2	2/2	2/1	2/2	2/1	3/2
Günlük kilogram veya oransal olarak verilen yem miktarı (Yaz/ Kış)	25/15	50-75	%0,4	%0,2	25/15	35/35

Tablo 3. Damızlıkların sağım ile ilgili fenotipik özellikleri*.
Table 3. Phenotypic characteristics of brootstocks related to stripping.

Kuluçkahaneler/Parametreler	YSU	BSU	KCM	ELF	OCK	SFA
Damızlık yaşı (yıl)	4	3	2	3	4	2
Damızlık boyu (cm)	58,6±5,01	51±7,36	48±7,00	53±6,90	56±5,90	46±6,34
Damızlık ağırlığı (gr)	3391±845	2658±902	1889±859	2267±868	3436±904	1616±566
Sağılan damızlık sayısı	627	311	693	530	529	533
Sağıma katılmayan damızlık sayısı	9	13	7	25	11	10
Sağıma katılmayan damızlık oranı	%1,4	%4	%1	%4,5	%2	%1,8

*(ortalama ± standart sapma)



Şekil 1. Hedef kuluçkahanelerin zamansal ve damızlık sayısı itibarıyla sağım performansı.
Figure 1. The stripping performance of the target hatcheries in terms of time and the stripped number of broodstocks.

Tablo 4. Kuluçkahanelere göre damızlıkların yumurta ile ilgili fenotipik özellikleri*.
Table 4. Egg-related phenotypic characteristics of broodstock according to hatcheries.

Kuluçkahaneler/Parametreler	YSU	BSU	KCM	ELF	OCK	SFA
Yumurta çapı (mm)	5±0,35	4,7±0,41	4,9±0,17	4,9±0,30	5,1±0,34	4,5±0,47
Toplam fekondite (adet/damızlık)	4861±1003	4802±1248	3811±401	3859±833	4707±878	3232±1104
Nispi fekondite (yum/kg)	1433±0,29	1806±0,46	2017±0,21	1702±0,36	1370±0,25	2000±0,68

*(ortalama ± standart sapma)

Tablo 5. Kuluçkahanelere göre yumurta ve larva gelişim performansları*.
Table 5. Egg and larvae development performance according to hatcheries.

Kuluçkahaneler/Parametreler	YSU	BSU	KCM	ELF	OCK	SFA	**p.
Gözlenme oranı (%)	78 ± 13^{c}	94 ± 4^{ab}	96 ± 2^{a}	92 ± 3^{abc}	87 ± 7^{bc}	83 ± 27^{abc}	0,001
Yumurtadan çıkış oranı (%)	78 ± 5^{c}	91 ± 6^{a}	89 ± 9^{ab}	91 ± 8^{a}	74 ± 21^{bc}	84 ± 21^{ab}	0,021
Yeme başlama oranı (%)	99 ± 2^{a}	99 ± 1^{a}	99 ± 1^{a}	98 ± 1^{a}	99 ± 1^{a}	98 ± 2^{a}	0,735

*(ortalama ± standart sapma)
**Kruskal-Wallis Testine göre anlamlılık düzeyi; a,b,c: Çoklu karşılaştırma testi Bonferroni düzeltmesine göre gruplar arası farkı gösterir *(P<0,05)*

TARTIŞMA VE SONUÇ

Bu çalışmada gökkuşağı alabalığı kuluçkahanelerinin çözünmüş oksijen değerlerine bakıldığında kuluçkahanelerdeki damızlık havuzlarının su çıkışlarındaki çözünmüş oksijen değerlerinin 7,1 mg/L ile 8,5 mg/L arasında değiştiği görülmektedir. Gökkuşağı alabalığı yetiştiriciliğinde damızlık ve yumurtaların ihtiyacını karşılamak için havuz çıkış suyundaki çözünmüş oksijen miktarının 7 mg/L'den yüksek olması gerekmektedir (Arabacı, 2007; Okumuş, 2002). Kuluçkahanelerdeki damızlık havuz çıkışlarındaki

çözünmüş oksijen miktarı referans alınarak damızlıkların bazı su kalite kriterleri değerlendirildiğinde birçok noktada damızlıkların ortam koşullarının birbirlerine benzer ve iyi durumda olduğu ayrıca damızlık refahını olumsuz yönde etkileyecek düzeyde olmadığı görülmüştür (Tablo 1). Yapılan diğer çalışmalara bakıldığında da birçok araştırmacının gökkuşağı alabalığı damızlıkları için benzer su kalite kriterlerini bildirdikleri görülmüştür (Alabaster & Lloyd, 1980; Emre & Kürüm, 2004; Laird & Needham,1988; Stevenson, 1987).

Diğer taraftan gökkuşağı alabalığı yetiştiriciliğinde su kalite kriterleri kadar, damızlıklarla ilgili kuluçkahane yönetim parametreleri de önemli çevresel parametrelerden birisidir. Damızlık stok yoğunluğu, beslemede kullanılan yemler, damızlık besleme şekli, günlük besleme sayısı ve günlük verilen yem miktarı damızlıkların gelişimini etkileyebilir ve bu durum yumurta üretimi ve yumurta kalitesi üzerinde direk ve dolaylı etkilere sahiptir (Arabacı, 2007). Gökkuşağı alabalığı damızlıkları için stoklama yoğunluğu esas olarak su sıcaklığı ve çözünmüş oksijene bağlı olmakla beraber kuluçkahane rakımı ve suyun pH'sı da önemlidir. Damızlık havuz çıkışlarındaki çözünmüş oksijen miktarının 7 mg/L'nin üzerinde olması damızlık refahının iyi düzeyde olduğunu göstermektedir.

Çalışmada gökkuşağı alabalığı damızlıklarının beslenmesinde kullanılan yem markalarına bakıldığında, kuluçkahanelerde genellikle üç farklı yem markasının kullanıldığı görülmüştür. Her zaman kullanılmamakla beraber bazı dönemlerde kullanılan D yemi (ithal) ise fiyatının diğer yemlere oranla daha düşük olmasından dolayı bazı kuluçkahanelerde kullanıldığı belirlenmiştir. Damızlıkların beslenmesinde kullanılan yem marka sayısının az olmasının nedeni, bölgedeki bayilerin yaygın olarak tercih ettikleri yemlere yoğunlaşması ve yem bayilerinin sadece bu yemleri satmasından kaynaklanmaktadır. Ancak bölgeye gelen ve satılan yemlerin yol masrafları, kargo ücretleri gibi nedenlerden dolayı diğer bölgelere oranla daha yüksek fiyatlardan satılması kuluçkahanelerin daha ucuz olan ithal D yemini tercih etmelerine sebep olmaktadır.

Çalışmada damızlıkların besleme şekli karşılaştırıldığında ise kuluçkahanelerden dördünün damızlıkları *ad libutum* beslediği, ikisinin ise (KCM ve ELF) besleme tablosundan yararlanarak yemleme yaptığı görülmüştür (Tablo 2). Ancak kuluçkahanelerden birisinde (BSU) her ne kadar *ad libutum* yemleme yapılsa da kuluçkahanenin toplam damızlık sayısı ve damızlıkların ortalama ağırlıkları (Tablo 3) dikkate alındığında damızlıklara verilen yem miktarının normalden daha fazla olduğu görülmüştür. Ancak bu kuluçkahanenin damızlıkları fazla yem ile beslemesi yumurta ve larva kalitesini arttırmadığı görülmüştür *(P>0,05)*. Bu kuluçkahanenin (BSU) bu durumu göz önünde bulundurması ve gerekli önlemleri alması daha ekonomik bir üretim yapabilmesi açısından önemlidir. Bu nedenle bu kuluçkahanenin (BSU) damızlıklara verilen fazla yemi azaltarak yem maliyetini düşürmesi gerekmektedir. Çünkü kuluçkahanelerin en büyük girdilerinden olan yemin fazla kullanılması işletme maliyetini arttırmaktadır (Hoşsu vd., 2003). Ayrıca damızlıklara fazla yem verilmesi damızlıklarda yağlanmaya ve gamet performansının düşmesine neden olabilmektedir. Diğer taraftan tüm kuluçkahanelerde bir sonraki sağım döneminde yumurta sayısını ve kalitesini arttırmaya yönelik damızlık besleme yöntemlerinin uygulanması daha ekonomik bir yetiştiricilik açısından önemlidir. Bu nedenle kuluçkahanelerde *ad libutum* yemleme yerine damızlıklarda yumurta sayısını ve kalitesini arttırmaya yönelik besleme yapılması ekonomik ve sağlıklı bir kuluçkahane ve damızlık yönetimi oluşturmak için gerekli bir adımdır (Arabacı, 2007).

Çalışmada damızlıkların sağım ile ilgili fenotipik özelliklerine bakıldığında genel olarak sağım zamanının Kasım ayının başında başladığı ve Mart ayının ortasında bittiği görülmüştür. Ancak damızlıkların sağım mevsiminin her bir kuluçkahane için farklılık gösterdiği görülmüştür. Damızlıkların sağım performansı incelendiğinde ise gökkuşağı alabalığı kuluçkahanelerindeki damızlıkların sağım performansı kuluçkahaneler arasında farklılık göstermekle beraber Aralık ayının son haftası ve Ocak ayı boyunca kuluçkahanelerdeki damızlıkların büyük çoğunluğunun sağıldığı görülmüştür.

Şekil 1'de damızlıkların sağım performansına bakıldığında iki kuluçkahane (YSU ve KCM) hariç diğer kuluçkahanelerde her hafta sağım gerçekleştiği belirlenmiştir. Ancak YSU ve KCM kuluçkahanelerinde bazı haftalar hiç sağım yapılmadığı görülmüştür. Bu durum kuluçkahanelerdeki damızlıkların bir ihtimal farklı hatlara ait olmasından ve sağım zamanlarının farklı olmasından kaynaklanabilir. Karataş (2019), Van ilindeki kuluçkahanelerin farklı damızlık hatları içerdiğini yaptığı büyüme hormonu gen ekspresyonu ve genetik polimorfizm ile ilgili çalışmasında göstermiştir. Sağım zamanının en erken YSU kuluçkahanesinde başlaması, YSU kuluçkahanesinde erken sağıma gelen bir gökkuşağı alabalığı hattının olduğunun göstergesi olabilir. Bu durum erken yumurta almak isteyen kuluçkahaneler ve kış şartlarının ağır geçtiği bölgelerde olan kuluçkahaneler için önemlidir.

Türkiye'de yapılan çalışmalarda alabalıkların kültür şartlarında sonbahar sonu ve kış aylarında sağım sezonuna geldikleri bildirilmiştir (Güner & Tekinay, 2002; Kurtoğlu vd., 1998). Ayrıca Serezli vd., (2010), Türkiye'de gökkuşağı alabalıklarında sağım zamanının Kasım ayı

sonunda başladığını ve Nisan ayına kadar sürdüğünü, ancak sağımların büyük çoğunluğunun Ocak-Şubat aylarında gerçekleştiğini rapor etmişlerdir. Bu çalışmada ise Van ilindeki gökkuşağı alabalığı kuluçkahanelerinde sağım zamanının Kasım ayı başında başladığı ve Mart ayının ortasında bittiği görülmüştür. Yapılan diğer çalışmalar ile bu çalışma birlikte değerlendirildiğinde sağım mevsimi diğer bölgelerde yapılan çalışmalardan farklı olmasının nedeni kuluçkahanelerin kuluçkahane ve damızlık yönetim parametrelerinin farklı olması, kuluçkahanelerin bünyelerinde bir ihtimal farklı damızlık hatları bulundurmaları ve damızlık arasında olan genotipik farklılıklar olabilir.

Çalışmada kuluçkahanelerin damızlık stokları karşılaştırıldığında kuluçkahaneler arasında farklılıklar olduğu görülmüştür. Kuluçkahanelerdeki toplam damızlık sayısı, damızlık yaşı, damızlık boyu ve damızlık ağırlıkları her bir kuluçkahane için farklı bulunmuştur. Dişi/erkek damızlık oranlarına bakıldığında ise bir kuluçkahane hariç (OCK) diğer kuluçkahanelerin hepsinde dişi/erkek damızlık oranının eşit olmadığı belirlenmiştir (Tablo 2). Ancak damızlıklarda dişi/erkek oranı elde edilen döllerde akrabalığın ve homozigotluğun artmasını önlemek için eşit tutulması önemlidir (Arabacı, 2007; Okumuş, 2002; Salihoğlu vd., 2013). Dişi/erkek damızlık oranı eşit olmayan kuluçkahanelerin dişi/erkek damızlık oranlarını döllerde akrabalığın ve homozigotluğun artmasını önlemek için eşitlemeleri populasyonda heterozigotluğun korunması için önemlidir. Diğer taraftan kuluçkahanelerde homozigotluğa neden olabilecek başka bir faktör de porsiyonluk balıklardan erkek damızlık kullanılmasıdır (Arabacı, 2007). Çalışmada kuluçkahanelerin birisinde (ELF) erkek damızlık ihtiyacının çoğunluğunun porsiyonluk balıklardan karşılandığı görülmüştür. Bu uygulama akrabalığı arttıracağından homozigotluğu arttıran bir faktördür (Arabacı, 2007). Bu kuluçkahane homozigotluğu arttırmamak için özellikle erken cinsi olgunluğa erişen erkek balıkları kullanmamalı ve eşit erkek dişi damızlık oranını korumalıdır. Ayrıca kuluçkahanelerin tamamının etkin damızlık sayısını da dikkate alarak orta ve uzun vadede homozigotluğu arttırmamak için performans özelliği iyi bilinen, büyüme özellikleri iyi ve heterozigotluğu yüksek populasyonlardan kan tazelemesi sağlıklı bir yaklaşım olacaktır. Nitekim farklı populasyonlardan yapılan kan tazelemenin gelecek kuşaklarda heterozigotluğu arttırdığı bilinmektedir (Arabacı, 2007; Düzgüneş vd., 1996; Emsen, 2003).

Çalışmada damızlıkların yumurta ile ilgili diğer fenotipik özellikleri incelendiğinde ise damızlıklardan elde edilen yumurtaların, yumurta çapının 4,5±0,47 mm ile 5,1±0,34 mm arasında değiştiği görülmüştür. Konu ile ilgili Türkiye'de yapılan diğer çalışmalara bakıldığında bu çalışmada elde edilen yumurta çaplarının Kanyılmaz vd., (2016), Kurtoğlu vd., (1998) ve Serezli vd., (2010) tarafından yapılan çalışmalar da elde edilen yumurta çapları ile benzer aralıkta olduğu görülmüştür. Güner & Tekinay, (2002) ve Özgür & Bayır, (2013) tarafından yapılan çalışmalarda ise yumurta çapları bu çalışmada elde edilen yumurta çaplarından daha büyük olarak bildirilmiştir. Yapılan diğer çalışmalar ile bu çalışma birlikte değerlendirildiğinde damızlıkların yumurta çaplarındaki farklılık damızlık yaşları ile ilgili olabilir. Gökkuşağı alabalıklarında yumurta çapı ve damızlıkların yaşları doğru orantılı olarak artmaktadır (Bromage & Cumaranatunga, 1988).

Diğer yandan damızlıkların toplam fekonditelerine bakıldığında ise toplam fekonditesinin 3232±1104 adet/damızlık ile 4861±1003 adet/damızlık aralığında olduğu görülmüştür. Konu ile ilgili yapılan diğer çalışmalara bakıldığında bu çalışmada elde edilen toplam fekonditenin Güner & Tekinay, (2002), Kurtoğlu vd., (1998) ve Serezli vd., (2010) tarafından yapılan çalışmalardan daha yüksek olduğu, Kanyılmaz vd., (2016) ve Özgür & Bayır, (2013) tarafından yapılan çalışmalardan ise daha düşük olduğu görülmüştür. Yapılan diğer çalışmalar ile bu çalışma birlikte değerlendirildiğinde damızlıkların toplam fekoditelerindeki farklılık yumurta çaplarında olduğu gibi damızlık yaşları ile ilgili olabilir. Çünkü gökkuşağı alabalıklarında toplam fekondite de damızlıkların yaşları ile doğru orantılı olarak artmaktadır (Bromage & Cumaranatunga, 1988). Ancak nispi fekontide için bu durumu söylemek uygun değildir. Çünkü nispi fekondite 1 kg canlı ağırlığa düşen yumurta miktarını belirtiği için damızlık yaşı ile ters orantılıdır (Arabacı, 2007). Bu çalışmada damızlıkların nispi fekonditeleri incelendiğinde ise nispi fekonditesinin 1370±0,25 yumurta/kg ile 2017±0,21 yumurta/kg aralığında olduğu görülmüştür. Konu ile ilgili yapılan diğer çalışmalara bakıldığında bu çalışmada elde edilen nispi fekonditenin ise Serezli vd., (2010) tarafından yapılan çalışmadan daha düşük olduğu görülmüştür. Güner & Tekinay, (2002), Kanyılmaz vd., (2016), Kurtoğlu vd., (1998) ve Özgür & Bayır, (2013) tarafından yapılan çalışmalar da elde edilen ile nispi fekonditelerin ise bu çalışma ile benzer aralıkta olduğu görülmüştür.

Çalışmada farklı kuluçkahanelerdeki farklı damızlıklardan örneklenen döllenmiş yumurtaların aynı çevresel şartlarda yumurta ve larva gelişim performanslarına bakıldığında ise damızlıkların yumurta ve larva gelişim performansları arasında farklılıklar olduğu görülmüştür. Hem yumurtaların gözlenme oranı hem de yumurtadan larva çıkış oranı açısından damızlıklar arasındaki fark istatistiksel olarak önemli bulunmuştur. Damızlıklardan örneklenen yumurtaların gözlenme oranı incelendiğinde gözlenmenin %78±13 ile %96±2 arasında değiştiği görülmüştür. Yapılan diğer çalışmalara

bakıldığında bu çalışmada elde edilen gözlenme oranının Kanyılmaz vd., (2016) tarafından yapılan çalışmadan daha yüksek olduğu görülmüştür. Baki & Kalma (2011), Güner & Tekinay, (2002), Kurtoğlu vd., (1998) ve Özgür & Bayır, (2013) tarafından yapılan çalışmalar da elde edilen gözleme oranı ile ise benzer aralıkta olduğu görülmüştür.

Diğer taraftan larvaların yumurtadan çıkış oranı incelendiğinde çıkış oranının %74±21 ile %91±8 arasında değiştiği belirlenmiştir. Yapılan diğer çalışmalara bakıldığında bu çalışmada elde edilen larva çıkış oranının Güner & Tekinay, (2002), tarafından yapılan çalışmadan daha yüksek olduğu görülmüştür. Baki & Kalma (2011), Kanyılmaz vd., (2016), Kurtoğlu vd., (1998) ve Özgür & Bayır, (2013) tarafından yapılan çalışmalar da elde edilen larva çıkış oranı ile ise benzer aralıkta olduğu görülmüştür. Çalışmada larvaların yeme başlama oranına bakıldığında ise larvaların yeme başlama oranının %98±1 ile %99±2 arasında değiştiği görülmüştür. Yapılan diğer çalışmalara bakıldığında bu çalışmada elde edilen yeme başlama oranının Güner & Tekinay, (2002), tarafından yapılan çalışmadan daha yüksek olduğu tespit edilmiştir. Baki & Kalma (2011), Kurtoğlu vd., (1998) ve Özgür & Bayır, (2013) tarafından yapılan çalışmalar da elde edilen yeme başlama oranı ile ise benzer aralıkta olduğu görülmüştür.

Bu çalışmada ele alınan tüm parametreler incelendiğinde kuluçkahaneler arasında sağım mevsimi, sağım performansı, gözlenme oranı, yumurtadan çıkış oranı, serbest yüzmeye ve yem almaya başlama oranında farklılıklar olduğu görülmüştür. Bu durumun nedeni kuluçkahanelerdeki su kalitesi ve damızlık yönetim parametrelerinin farklı olması, kuluçkahanelerin bünyelerinde farklı damızlık hatları bulundurmaları ve damızlıklar arasında olan genotipik farklılıklardan kaynaklandığı düşünülmektedir.

Sonuç olarak elde edilen veriler yetiştiricilik açısından değerlendirildiğinde; (i) Daha ekonomik bir yetiştiricilik için damızlıklarda yumurta sayısını ve kalitesini arttırmaya yönelik besleme yöntemleri uygulanmalıdır, (ii) Kuluçkahanelerin birinde bulunan ve sağıma erken gelen damızlıklar, erken sağılan hat elde etmek için kullanılabilir, (iii) Damızlık stok populasyonlarında homozigotluğu düşürmek için dişi erkek damızlık oranı kuluçkahanelerde eşitlenmeli ve erkek damızlık ihtiyacı porsiyonluk balıklardan karşılanmamalıdır, (iv) Ayrıca damızlıklar arasındaki genotipik farklılıkları belirlemeye yönelik detaylı genotipik çalışmalar yapılmalıdır.

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ARAŞTIRMA MAKALESİ **RESEARCH PAPER**

Moda Tasarımı Öğrencilerinin Tekstil Ürünleri Tasarımında Çevreye Yönelik Tutumlarının Araştırılması

Onur TEKOĞLU
[1] Giresun Üniversitesi, Teknik Bilimler Meslek Yüksekokulu, Tasarım Bölümü, Giresun, Türkiye

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*: https://orcid.org/0000-0002-3608-7801

***Sorumlu yazarın:**
Onur TEKOĞLU
Giresun Üniversitesi, Teknik Bilimler Meslek Yüksekokulu, Tasarım Bölümü, Giresun, Türkiye
✉: onur.tekoglu@giresun.edu.tr

Öz: Moda tasarımı öğrencilerinin meslek hayatına atıldıklarında tekstil işletmelerinin doğa dostu üretim faaliyetlerine katkı sağlayabilmeleri için, çevre eğitimi konusunda kendilerini yeterli bilgi ile donatmaları gerekmektedir. Çevre kirliliklerinin oluşturduğu tehlikeler hakkında bilgisi olmayan bir tasarımcının, çalışacağı tekstil işletmesinde çevre dostu bir üretim sağlayamayacağı aşikârdır. Bu düşünceden hareketle yapılan çalışmada; son yıllarda tüm ülkeler için önemli bir sorun haline gelen çevresel kirliliklerin oluşturduğu tehlike boyutlarını; moda tasarımı öğrencilerinin anlama, farkında olma ve bilgi sahibi olma düzeyleri ile öğrencilerin tekstil ürünleri tasarımında çevreye yönelik tutumları araştırılmıştır. Araştırmada veriler, 2017–2018 eğitim-öğretim yılı Kasım ayında toplamda 124 moda tasarımı öğrencilerine uygulanan anket formu aracılığı ile toplanmıştır. Anketin birinci bölümünde; öğrencilerin sosyodemografik özellikleri, ikinci bölümünde çevre ve insan sağlığında risk olarak düşündükleri çevresel kirlilikleri ve risk düzeyleri ile tasarımda çevreye yönelik tutumları belirlenmiştir. Araştırmadan elde edilen sonuçlara göre; moda tasarımı öğrencilerinin çevre kirlilikleri hakkında yeterince bilgi sahibi olmadıkları, bu nedenle kirlilik yaratabilecek unsurların etkileri konusunda farkındalıklarının oluşmadığı ortaya çıkmıştır. Anketten elde edilen veriler değerlendirilerek, literatür bilgisi ile karşılaştırılmış ve öneriler geliştirilmiştir.

Anahtar kelimeler: Çevre farkındalığı, çevre kirlilikleri, moda tasarımı öğrencileri, tekstil endüstrisi.

Investigation of Environmental Attitudes of Fashion Design Students in the Textile Products Design

***Corresponding author's:**
Onur TEKOĞLU
Giresun University, Technical Sciences Vocational School, Department of Design, Giresun, Turkey
✉: onur.tekoglu@giresun.edu.tr

Abstract: When fashion design students start their professional life, they need to equip themselves with sufficient knowledge of environmental education in order to contribute to eco-friendly production activities of textile enterprises. It is clear that a designer who is not familiar with the hazards caused by environmental pollution cannot provide eco-friendly production in the textile enterprise. In this study based on this thought, fashion design students' attitudes towards environment in textile products design and their understanding, awareness and knowledge levels about the extent of the danger caused by environmental pollution, which has become an important problem for all countries in recent years, were investigated. The data were collected through a survey applied to 124 fashion design students in November 2017-2018 academic year. In the first part of the survey, the sociodemographic characteristics of the students and in the second part the environmental pollution that they think as a risk in the environment and human health, and these risk levels and their attitudes towards the environment in design were determined. According to the results obtained from the research; it has been revealed that fashion design students do not have enough information about environmental pollutions, so they are not aware of the effects of factors that may cause pollution. The data obtained from the survey were evaluated and they were compared with the literature and recommendations have been developed.

Keywords: Environmental awareness, environmental pollutions, fashion design students, textile industry.

GİRİŞ

Günümüzde çevre ve insan sağlığı bilincinin artması, tüm sanayi kolları ile beraber tekstil sektörünü de etkilemiştir. Tekstil mamullerinin çevre ve insan sağlığına zarar vermeden üretilmeleri önem kazanmıştır (Akdeniz Ar, 2009). Çevre koşullarındaki hızlı bozulma ve dünyanın sınırlarına yaklaşıldığı endişesi, işletme yöneticilerinin ekolojik çevreye bakış açılarını bir an önce değiştirmelerini ve işletme faaliyetleriyle ilgili kararlar alırken ekolojik çevreyi önemli bir faktör olarak değerlendirmelerini gerektirmektedir (Nemli, 2001). Çevrenin korunması konusunda tüketicilerden gelen talepler de işletmeleri çevreye karşı daha duyarlı olmaya yönlendirmektedir (Stead & Stead, 1996). Tüketiciler, daha az kirlilik ve atık, daha fazla geri dönüşüm istemekte, yenilenebilir kaynakların daha fazla kullanımını ve ürünlerin ekosistem için daha güvenli olmasını talep etmektedirler. İşletme yöneticileri de değişimin gerisinde kalmamak için bu yöndeki talepleri stratejik kararlarda dikkate almak ve çevreye karşı daha duyarlı bir yönetim anlayışı geliştirmek durumundadırlar (Nemli, 2001). Günümüz toplumlarında; iklim değişikliği, yeşil kimya, su kıtlığı ve insan hakları hususlarındaki konular tekstil ve giyim sektöründe de güçlü bir şekilde yerini almaktadır (Boström & Micheletti, 2016).

Moda ve tekstil endüstrisi bugün çevreye en fazla zarar veren sektörlerden birisi olarak kimya endüstrisi ile aynı derecede yargılanmaktadır. Sektör çok büyük miktarlarda kaynağı tüketmektedir ve hızla değişip tüketimi körükleyen trendlerin egemenliğindedir. Üretim için gerekli tüm kaynaklar ile üretim, kullanım ve ortadan kaldırılma aşamalarında ortaya çıkan atıklar, tekstil ürünlerinin yaşam döngüsünü oluşturmaktadırlar (Türkmen, 2009). Engellenebilir ve engellenemeyen atıklarla birlikte mevcut kaynakların da kullanılamaz hale gelmesinde olumsuz etkilere neden olan hazır giyim endüstrisinin konu ile ilgili farkındalığının artırılması için tasarım, üretim, kullanılan hammadde, tekstil yan malzemeleri ve ürün üretim proseslerinin incelenmesi gerekmektedir. Sektörü meydana getiren işletmelerdeki tasarımcıların, üretimde kullanılan kaynak, malzeme ve proseslerden sorumlu olduğu da dikkatten kaçmamalıdır (Koca vd., 2019). Tasarımcının dikkate alması gereken konulardan biri de moda döngüsünün üretim-tüketim sürecinde çevre ve insan sağlığına verdiği zarardır. Bu noktada modayı yönlendiren tasarımcılar bu hızlı üretim-tüketim ilişkisinin neden olduğu zararlar konusunda bilinç kazanmak durumundadır (Şahin & Odabaşı, 2018).

Tekstil endüstrisi ülke ekonomisine sağladığı fayda anlamında önemli bir yer teşkil etmektedir. Tekstil sektöründe önemli derecede su, boyarmadde ve kirlilik yaratacak diğer kimyasallar kullanılmakta olup bunun neticesinde kirlilik yükü çok yüksek olan atıksular ortaya çıkmaktadır. Tekstil endüstrisi kaynaklı atıksular, alıcı ortamda negatif etkiler meydana getirirken aynı zamanda üretim proseslerinde geri kullanım potansiyelini azaltmaktadır. Alıcı ortamda estetik sorunlar, sucul ekosisteme zararlı etkiler gibi pek çok istenmeyen etkiye de neden olmaktadır (Gergin & Cuci, 2017). Tekstil endüstrisinin oluşturduğu atıksu çözünmüş katı, yüksek KOİ, BOİ, güçlü renk ve tuz gibi karakteristik özellikleri nedeniyle yüksek miktarda kirlilik yükü ihtiva etmektedir (Kama, 2019). Tekstil atıksularındaki önemli kirleticiler temel olarak organikler, renk, toksik maddeler, inhibitör bileşikler, yüzey aktif maddeler, klorlu bileşikler (AOX), pH ve tuzlardır (Sandhya & Swaminathan, 2006). Tekstil sektöründe kullanılan 100.000'den fazla ticari olarak temin edilebilen boya mevcuttur ve yılda 7x105 tondan fazla üretilmektedir. Bu boyaların %10-15'inin atıksuya karıştığı tahmin edilmektedir. Arıtılmadan doğaya deşarj edilen atıksulardaki boyaların suda yaşayan organizmalar üzerinde toksik etkilerinin yanısıra insanlarda alerjiye, tahrişe, kansere ve hatta mutasyona neden olduğu bildirilmiştir (Kalıpcı vd., 2016; Namal & Kalıpcı, 2019; Namal & Kalıpcı, 2020).

Tekstil endüstrisinin kapsam ve tedarik zincirinin genişliği dolayısıyla üretim nedeniyle atmosferik kirleticilerin çıktısı da yüksektir (Coşkun & Doğan, 2021). Örnek verilecek olursa; kumaş üretim işlemlerinin pek çoğunda sera gazı emisyonu (CO_2 ve metan (CH_4) gazları vb.) ortaya çıkmaktadır (Keskin vd., 2017; Akhtar vd., 2017). Küresel karbon emisyonunun % 10'unu oluşturan tekstil ve moda endüstrisi, dünyayı ve çevreyi kirleten bununla birlikte suyu en fazla tüketen ikinci endüstri dalıdır (Lee, 2016). Yalnızca 1 adet pamuklu tişört üretebilmek için 2700 litre suya ihtiyaç olduğu göz önüne alındığında bu sonucun kaçınılmaz olduğu görülmektedir (Necef vd., 2020). Tekstil sanayi yüksek su tüketiminin yanı sıra aynı zamanda çok çeşitli kimyasal kullanımına ve çeşitli atıklara sahip bir sanayi dalıdır. Sıvı ve katı atıklar hava emisyonuna neden olmakta ve katı atıklar çevreyi çeşitli şekillerde etkilemektedir. Atılan bazı kimyasallar doğada toksik etkiler gösterebilmektedir. Bazı atıkların sulu ortama atılması, suda olabilecek toksisite nedeniyle çözünmüş oksijenin azalmasına neden olabilmekte ve suda yaşayan canlılar ve daha sonra bu suyu kullanan kişiler için tehdit unsuru olmaktadır (Özdoğan vd., 2007). Endüstriyel sektörler arasında yer alan tekstil endüstrisi; atıksu kompozisyonu ve deşarj hacmi dikkate alındığında yüksek kirleticiler olarak derecelendirilir. Özellikle tekstil sanayi, suyu büyük oranda tüketerek kayda değer oranlarda boyalı atıksular üretmektir (Dizge vd., 2008; Wang & Li, 2005). Tekstil sanayiinde kullanılan maddelerden atıksulara karışan ve endüstrisine özgü özelliklere sahip en tipik kirleticilerden biri renk parametresi olup bu parametre

canlılar üzerinde toksik ve kanserojen etkilere yol açarken aynı zamanda deşarj sahalarındaki sularda oluşturduğu renk değişimi fotosentezi yavaşlatmakta ve her yönden ekolojik dengeyi bozmaktadır (Karaçıray, 2019). Reaktif boyalar, insanların karaciğer, sindirim sistemi ve merkezi sinir sisteminde ciddi hasara yol açabilmekte ve yeraltı suları ile tarımsal yetiştiriciliği etkileyebilmektedir (Dhanapal & Subramanian, 2014).

Çevre sorunları, 19. yy'da başlayıp yirminci yüzyılda yaygınlık kazanmış olmakla birlikte, muhtemel olarak 21.yy toplumlarının çözmek zorunda kalacağı en önemli sorunu oluşturacaktır. Diğer bir tanımla modern dünyanın ürettiği sorunları, post-modern dünya çözmek zorunda kalacaktır (Ceritli, 2001). Çevre sorunları ile etkin şekilde mücadele etmek için sadece teknolojiyi ve kanunları kullanmak, sorunları çözüme kavuşturmada tek başına yeterli değildir. Çevresel sorunlarla mücadele edilebilmesi ve çözüm üretilebilmesi, ancak eğitimli, duyarlı ve bilinçli bireylerin yetiştirilmesi ile çözümlenebilir (Yener & Kalıpcı, 2007). Çevreye karşı olumsuz tutuma sahip bireylerin çevre sorunlarına duyarsız olacağı ve hatta çevreye sorun yaratmaya devam edeceği şüphesizdir (Uzun & Sağlam, 2006). Çevresel kirliliklerin oluşmasına neden olan bireylerin, bu tarz kirlenmelerin oluşmasının neden ve sonuçları ile nasıl giderileceği hakkında bilgi sahibi olmaları sağlanmalıdır. Çevreye yönelik bütün sorunlar; kayıtsız insan davranışından kaynaklanmakta, bu davranışların sonucunda ortaya çıkan sorunlar konusunda bilinçlendirilmeyen insanlar kendilerini doğrudan etkilemediğini düşündükleri olaylara karşı duyarsız kalmaktadırlar (Özmen vd., 2005).

Bu düşünceden hareketle yapılan çalışmada; eğitimlerini tamamladıklarında tekstil işletmelerinde çalışarak, işletmenin çevre ve insan sağlığına zarar vermeden üretim sağlamasında söz hakkı olacak moda tasarımcılarının ilköğretimden başlayarak şu anki eğitim süreci içerisine kadar geçen zaman diliminde çevre konularına ilgileri ve sahip oldukları bilgi seviyeleri ile öğrencilerin tekstil ürünleri tasarımında çevreye yönelik tutumlarının tespit edilmesi amaçlanmıştır.

MATERYAL VE METOT

Bu araştırma; özel durum çalışması olup, tarama modelinde yapılmıştır. Araştırmanın çalışma grubunu, 2017–2018 eğitim-öğretim yılında Giresun Üniversitesi Moda Tasarımı programında öğrenim gören toplamda 124 tane moda tasarımı öğrencileri oluşturmuştur.

Araştırmada veriler, rastgele seçilmiş olan toplamda 124 moda tasarımı öğrencilerine uygulanan anket formu aracılığı ile toplanmıştır. Anketin birinci bölümünde moda tasarımı öğrencilerinin sosyodemografik yapılarının tespitine yönelik 12 adet açık uçlu ve 2 adet de 5'li likert tipi olmak üzere toplamda 14 adet soruyu cevaplamaları istenmiştir. İkinci bölümünde ise öğrencilerin ürün tasarımında çevreye yönelik tutumlarını belirlemek için 5 adet açıkuçlu soru ile çevre ve insan sağlığı için risk olarak düşündükleri çevresel kirlilikleri ve risk düzeylerini belirlemek için ise 36 adet ifade verilmiş ve hiç risk oluşturmaz/düşük risk oluşturur/orta düzeyde risk oluşturur/ileri risk ve çok ileri düzeyde risk oluşturur şeklinde 5'li Likert skalasıyla değerlendirilmiştir. Araştırmada, düşük olarak belirtilen risk algılama düzeyi hiç ve düşük tanımlamaları birleştirilerek, ileri olarak belirtilen düzey ise ileri ve çok ileri tanımlamaları birleştirilerek elde edilmiştir. Anket formunun ilk bölümünde bulunan, moda tasarımı öğrencilerinin sosyodemografik özelliklerini belirlemeye yönelik sorular; Erten, (2005) Beyhun vd., (2007) ile Erten, (2002)'nin daha önceden kullanmış oldukları anket çalışmalarından alınarak kısmen değiştirilmiş ve modifiye edilerek hazırlanmıştır. İkinci bölümünde yer alan sorular ise, Öztaş ve Kalıpcı, (2009) ile Beyhun vd., (2007)'in yapmış olduğu çalışmalardan esinlenerek geliştirilmiş ve hazırlanmıştır. Bu anket, çalışmanın temel aracı olup moda tasarımı öğrencilerine 2017–2018 öğretim yılı Kasım ayında uygulanmıştır. Moda tasarımı öğrencileri anket formunu gözlem altında doldurmuşlardır ve soruları okumadan cevaplamalarını engellemek için uygulama yapılmadan önce konunun önemi anlatılmıştır. Ayrıca moda tasarımı öğrencilerine bunun bir sınav olmadığı belirtilmiş ve bu anketi tamamlamaları için yeterli süre verilmiştir. Bu test çevre eğitimi alanında uzmanlığı bulunan 3 öğretim üyesine kontrol ettirilmiştir. Yapılan anket çalışması sonucunda testin güvenilirliği hesaplanmıştır. Araştırma sonuçlarının değerlendirilmesi yüzde ifadeler ve hazır istatistik paket programı SPSS kullanılarak yapılmıştır. Sonuçlar literatür bilgisi ile karşılaştırılmış ve öneriler sunulmuştur.

BULGULAR

Araştırmaya katılan moda tasarımı öğrencilerinin sosyodemografik özellikleri Tablo 1'de verilmiş olup; anketin birinci bölümünde yer alan sorulara verdikleri cevaplara göre: katılımcıların 118'i (%95) kız, 6'sı (%5) erkek'tir. Moda tasarımı öğrencilerinin %55'i "Meslek Lisesi", %40'ı "Anadolu Lisesi", %5'i "Açık Lise mezunudur. Yaş ortalaması 21,0 ±1,0 (ortanca 21)'dir. Annelerin %73'ü (n=91), babaların %70'i (n=87) Lise ve üstü eğitim almıştır. Ailelerin %10'unun (n=12) gelir seviyesi 2000 TL ve altında, %74'ünün (n=92) 2000-5000 TL arasında ve %16'sının (n=20) ise 5000 TL ve üzerindedir.

Ankete katılan moda tasarımı öğrencilerinin ürün tasarımında çevreye yönelik tutumlarını belirlemek

amacıyla sorulan, 'Tekstil üretiminden kaynaklı atıkları yeni ürün tasarımında kullanmayı düşünür müsünüz? Neden?' sorusuna; öğrencilerin %79'u kullanabileceğini kullanma nedeninin ise tasarruf amaçlı olduğunu belirtmiştir. Öğrencilerin %21'i ise atık malzemeleri yeniden kullanmayacaklarını bildirmiştir. Atıkları yeniden kullanacağını belirten %79'unun ise sadece %23'lük kesimi geri dönüşüm bilinci ile kullanılması gerektiğini gerekçe olarak belirtmiştir.

Tablo 1. Moda tasarımı öğrencilerinin sosyodemografik özellikleri.
Table 1. Sociodemographic characteristics of fashion design students.

	Özellikler	**n**	**%**
Cinsiyet	Erkek	6	5
	Kız	118	95
Yaş	≤ 24	112	90
	> 24	12	10
Annenin eğitim seviyesi	Ortaokul ve altı	33	27
	Lise ve üstü	91	73
Babanın eğitim seviyesi	Ortaokul ve altı	37	30
	Lise ve üstü	87	70
Ailenin gelir seviyesi	2000 TL ve altı	12	10
	2000-5000 TL	92	74
	5000 TL ve üzeri	20	16
Çevre ile ilgili yapılan konferans, toplantı vb. katılım durumu	Hayır katılmadım	86	69
	Çok istedim ama katılamadım	29	23
	Evet katıldım	9	7
Üniversitede çevre koruma ile ilgili bir ders alma durumu	Evet	2	2
	Hayır	122	98

'Üreteceğiniz ürünlerde ekoetiket uygulamasının gerekli olduğunu düşünüyor musunuz? Neden?' sorusuna öğrencilerin %65'i gerekli olmadığını belirtmiştir. Gerekli olduğunu belirten öğrencilerin %72'si ise ürünün pazarlamasını kolaylaştıracağını düşündüğünü belirtmiştir.

'Tasarımlarınızda pazar kaygısı gütmeden çevre kirliliklerine farkındalık oluşturacak ürünleri tasarlamayı düşünür müsünüz?' sorusuna, öğrencilerin sadece %32'si 'tasarımlarında pazar kaygısı gütmeden çevre kirliliklerine farkındalık oluşturacak ürünleri tasarlamayı düşündüklerini' beyan etmiştir. %68'i ürünün satış rakamlarının daha önemli olduğunu vurgulamışlardır.

'Çevreye duyarlı bir üretim prosesinin ürün pazarlanmasını kolaylaştıracağını düşünür müsünüz?' Neden? sorusuna öğrencilerin %82'si ürünü iç piyasada tüketenlerin sadece fiyat ve kalite açısından değerlendireceğini, çevreye duyarlı bir proses ile üretilip üretilmediğinin tüketici açısından önemli olmadığını düşündüklerini beyan etmişlerdir.

'Tekstil işletmesinden kaynaklanan çevresel kirlilik yaratan unsurlar nelerdir?' sorusuna öğrencilerin sadece %38'i eksiksiz olarak cevap vermişlerdir.

'Ürünlerinizde kullanacağınız hammaddeyi alternatifinizin olması durumunda pahalı olan doğal hammaddelerden mi? yoksa uygun olan sentetik malzemelerden mi seçersiniz? Neden? sorusuna öğrencilerin %89'u ürünün maliyet fiyatı düşeceğinden daha kolay satılabileceği gerekçesiyle ucuz olan hammaddeyi tercih edeceğini bildirmiştir.

Moda tasarımı öğrencilerinin çevresel kirliliklerin oluşturduğu tehlike boyutlarını algılama düzeyleri Tablo 2'de verilmiştir. Yapılan araştırmada moda tasarımı öğrencileri tarafından ileri veya çok ileri derecede risk faktörü olarak algılanan çevresel faktörler sırasıyla; küresel ısınma (n=99, %80), iklim değişikliği (n=96, %77), ormanların azalması (n=93, %75), ozon tabakasının delinmesi (n=91, %73), suların kirlenmesi (n=89, %71), içme suyu miktarının azalması (n=86, %69), pestisitlerin kontrolsüz kullanılması (n=64, %52), toprağın kirlenmesi (n=55, %44), atık piller ve aküler (n=55, %44), erozyon ve çölleşme (n=54, %44), yeraltı kaynaklarının bilinçsiz tüketimi (n=54, %44), kimyasalların yaygın kullanımı (n=52, %42) olarak tespit edilmiştir. Çalışmada bulunan sonuçlar Kalıpcı vd., (2009) tarafından yapılan araştırma ile benzerlik göstermektedir.

Küresel ısınma, iklim değişikliği, ozon tabakasının delinmesi, ormanların azalması, gibi çevresel faktörlerin ankete katılan öğrenciler tarafından ileri veya çok ileri derecede risk faktörü olarak görülmesinin nedenlerinin, son yıllarda sıcaklıkların mevsim normallerinin üstünde olması, mevsim geçişlerinde yaşanan ani hava değişimleri ve yağış anormallikleridir. Bunun yanı sıra küresel ısınma, iklim değişikliği, orman yangınları vb. konular ile ilgili televizyon ve gazetelerde haberlerin sıklıkla yayınlanması da moda tasarımı öğrencilerinin bu konuların öncelikli çevre sorunu olduğu izlenimini uyandırdığı düşünülmektedir. Moda tasarımı öğrencilerinin, ormanların azalmasını ileri derecede tehlike olarak görmesinin bir diğer nedeni ise; ormanların azalmasıyla birlikte yağış miktarının da azalacağını düşünmeleridir. Kimyasalların yaygın kullanılmasının, ozon tabakasına zarar vereceğini düşünerek küresel ısınmanın artacağı kanaatinde oldukları görülmüştür. Yine bulunan bu sonuçlarda; Öztaş ve Kalıpcı, (2009) tarafından üniversite öğrencileri üzerinde yapılan bir araştırma ile benzerlik göstermektedir. Beyhun vd., (2007) tarafından 238 Tıp Fakültesi öğrencisi üzerinde yapılan bir diğer araştırmada; ankete katılanların %76,9'unun ozon tabakasının delinmesini, %64,8'inin küresel ısınmayı ileri veya çok ileri düzeyde risk faktörü olarak algıladıkları tespit edilmiştir. Küresel ısınma ve ozon tabakasının delinmesi ile ilgili bulunan sonuçlar da yine Beyhun vd., (2007) tarafından yapılan araştırma ile paralellik göstermektedir.

Moda tasarımı öğrencilerinin hiç risk oluşturmadığını veya düşük derecede risk oluşturduğunu düşündükleri çevresel faktörler ise sırasıyla; görüntü kirliliği (n=100, %81), kıyı ve sit alanlarının yok olması (n=85, %69), ürünlerin depozitolu ambalajlarda satılmaması (n=85 %69), ışık kirliliği (n=80, %65), metallerin ambalaj malzemesi olarak kullanılması (n=80, %64), gürültü kirliliği (n=80, %64), toplu taşıma

araçlarının yeterince kullanılmaması (n=80, %64), çöplerin ayrı toplama ve kompostlama yapılmadan bertaraf edilmesi (n=75, %60), tıbbi atıkların diğer çöplerle birlikte bertaraf edilmesi (n=70, %56), plastik ürünlerin kullanılması (n=67, %54), koku kirliliği (n=63, %51), atık yağların ayrı toplanmaması (n=62, %50), gecekondulaşma (n=60, %48) olarak tespit edilmiştir.

Tablo 2. Moda tasarımı öğrencilerinin çevresel kirliliklerin oluşturduğu tehlike boyutlarını algılama düzeyleri.
Table 2. Perception levels of fashion design students about hazard dimensions caused by environmental pollutions.

	Risk düzeyleri					
	Hiç risk olmaz veya düşük derece risk oluşturur		Orta derece risk oluşturur		İleri veya çok ileri derece risk oluşturur	
Kirlilik türleri ve diğer faktörler	**n**	**%**	**n**	**%**	**n**	**%**
Küresel ısınma	5	4	20	16	99	80
Elektromanyetik kirlilik (cep telefonları, bilgisayarlar, mikrodalga fırınlar vb.)	35	28	60	48	29	23
Suların kirlenmesi	10	8	25	20	89	71
Kentlerde yaşanan hava kirlilikleri	27	21	55	44	42	34
Görüntü kirliliği	100	81	14	11	10	8
Kıyı ve sit alanlarının yok olması	85	69	30	24	9	7
Sera etkisi	68	55	35	28	21	17
Nüfus artışı	22	18	58	47	44	35
Pestisitlerin kontrolsüz kullanılması	10	8	50	40	64	52
Toprağın kirlenmesi	20	16	49	40	55	44
Ozon tabakasının delinmesi	8	6	25	20	91	73
Asit yağmurları	15	12	67	54	42	34
Çarpık ve plansız kentleşme	25	20	55	44	44	35
Geri dönüşümü olmayan ürünlerin kullanılması	20	16	72	58	32	26
Erozyon ve çölleşme	22	18	48	39	54	44
Işık kirliliği	80	65	30	24	14	11
Katı atık (çöp) kirliliği	30	24	72	58	22	18
İklim değişiklikleri	6	5	22	18	96	77
Ormanların azalması	5	4	26	21	93	75
Atık piller ve aküler	29	23	40	32	55	44
Enerji tüketiminin bilinçsizce yapılması	32	26	71	57	21	17
Bitki ve hayvan türlerinin azalması	31	25	56	45	37	30
Gürültü kirliliği	80	64	30	24	14	11
Plastik ürünlerin kullanımı	67	54	37	30	20	16
Koku kirliliği	63	51	42	34	19	15
Gecekondulaşma	60	48	27	21	37	30
İçme suyu miktarının azalması	8	6	30	24	86	69
Kimyasalların yaygın kullanımı	20	16	52	42	52	42
Tıbbi atıkların diğer çöplerle birlikte bertaraf edilmesi	70	56	46	37	8	6
Metallerin ambalaj malzemesi olarak kullanılması (ör. metal kutular)	80	64	35	28	9	7
Suni gübrelerin kullanılması	30	24	58	47	36	29
Ürünlerin depozitolu ambalajlarda satılmaması	85	69	32	26	7	6
Atık yağların ayrı toplanmaması	62	50	40	32	22	18
Toplu taşıma araçlarının yeterince kullanılmaması	80	64	30	24	14	11
Yeraltı kaynaklarının bilinçsiz tüketimi	20	16	50	40	54	44
Çöplerin ayrı toplama ve kompostlama yapılmadan bertaraf edilmesi	75	60	45	36	4	3

Bu sonuçlara göre de; moda tasarımı öğrencilerinin bahsedilen bu kirlilik türlerinin çevresel etkileri hakkında bilgilerinin yeterli düzeyde olmadığı rahatlıkla söylenebilmektedir. Örneğin; moda tasarımı öğrencilerinin büyük çoğunluğu küresel ısınma ve iklim değişikliğini ileri veya çok ileri derecede risk faktörü olarak belirtirken, aynı zamanda da %55'i (n=68) tarafından sera etkisinin yaratacağı çevresel riskin, hiç risk oluşturmayacağını veya düşük risk oluşturacağını belirtmiştir. Plastik atıklar binlerce yıl doğada çürümeden, bozunmadan kalarak kirlilik oluşturmasına rağmen, moda tasarımı öğrencilerinin %54'ü plastik ürünlerin kullanılmasının hiç risk oluşturamayacağını veya düşük risk oluşturabileceklerini belirtmişlerdir. Aynı şekilde gürültü kirliliğinin insan vücudu üzerinde oluşturduğu psikolojik, fizyolojik ve sosyal yaşama olan olumsuz etkilerine rağmen moda tasarımı öğrencilerinin %64'ü tarafından hiç risk oluşturmayacağı veya düşük risk oluşturabileceği belirtilmiştir. Atık piller ve akülerin içerisinde bulunan kurşun, nikel, kadmiyum vb. ağır metallerden dolayı su kirliliği, toprak kirliliği gibi son derece önemli kirlilikler oluştuğu bilinirken, moda tasarımı öğrencilerinin yine %23'ü hiç riski olmadığını veya düşük risk oluşturduğunu belirtmişlerdir. Toplu taşıma araçlarının yeterince kullanılmaması kaynaklı hava kirliliği ve gürültü kirliliği oluşabileceği bilinirken, öğrencilerin yine %64'ü toplu taşıma araçlarının yeterince kullanılmamasının hiç riski olmadığını veya düşük risk oluşturduğunu belirtmişlerdir.

Moda tasarımı öğrencilerinin orta derecede risk oluşturduğunu düşündükleri çevresel faktörlerin ise sırasıyla; geri dönüşümü olmayan ürünlerin kullanılması (n=72, %58), katı atık (çöp) kirliliği (n=72, %58), enerji tüketiminin bilinçsizce yapılması (n=71, %57), asit yağmurları (n=67, %54), elektromanyetik kirlilik (n=60, %48), nüfus artışı (n=58, %47), suni gübrelerin kullanılması (n=58, %47), bitki ve hayvan türlerinin azalması (n=56, %45), çarpık ve plânsız kentleşme (n=55, %44), kentlerde yaşanan hava kirlilikleri (n=55, %44) olarak tespit edilmiştir.

TARTIŞMA VE SONUÇ

Yapılan bu çalışma sonucunda; ankete katılan moda tasarımı öğrencilerinin %98'inin Üniversitede çevre koruma ile ilgili herhangi bir dersi almadığı, %92'sinin ise çevre ile ilgili yapılan herhangi bir konferans veya benzeri bir toplantıya katılmadığını beyan etmesi öğrencilerin çevre farkındalıklarını oluşturacak bir eğitimi Üniversite yaşamlarında almadıklarını göstermektedir.

Öğrencilerin tekstil ürünleri tasarımında çevreye yönelik tutumlarına ilişkin sorulara verdikleri cevaplardan; tekstil endüstrisi atıklarını değerlendirme noktasında çevre bilinci ile hareket etmediği, geri dönüşüm ile yeniden kullanılması gerektiği bilincinde olanların sayısının çok az miktarda olduğu, ürün tasarımında artık malzemeleri tasarruf amaçlı kullanma bilincinde oldukları anlaşılmaktadır. Yine; öğrencilerin %65'inin ekoetiket uygulamasına gerek olmadığını düşünmeleri ve gerekli olduğunu belirten öğrencilerin %72'sinin ise ürünün pazarlamasını kolaylaştıracağını belirtmeleri, öğrencilerin zihinlerinde ürün tasarımı yaparlarken ağırlıklı olarak ekonomik maliyeti düşündükleri, çevreye yönelik kaygılarının öncelikli olmadıkları anlaşılmaktadır. Ayrıca; öğrencilerin sadece %32'sinin 'tasarımlarında pazar kaygısı gütmeden çevre kirliliklerine farkındalık oluşturacak ürünleri tasarlamayı düşündüklerini' ve %89'u ürünün maliyet fiyatı düşeceğinden daha kolay satılabileceği gerekçesiyle ucuz olan hammaddeyi tercih edeceğini belirtmesi bu durumu da desteklemektedir. Öğrencilerin; ürünün çevreye duyarlı bir proses ile üretilip üretilmediğinin tüketici açısından önemli olmadığını düşündüklerini beyan etmeleri de bunu açıklamaktadır.

Öğrencilerin 'Tekstil işletmesinden kaynaklanan çevresel kirlilik yaratan unsurlar nelerdir?' sorusuna sadece %38'inin eksiksiz olarak cevap vermesi çalışacağı sektördeki işletmenin ne gibi çevresel etkileri olacağının bilincinde olmadığını göstermektedir. Moda tasarımı öğrencilerinin çevresel kirliliklerin oluşturduğu tehlike boyutlarını algılama düzeylerine ait sorulara verdikleri cevaplara göre; öğrencilerin çevre kirlilikleri hakkında yeterince bilgi sahibi olmadıklarını, bilgi sahibi olmamaları nedeniyle de kirlilik yaratabilecek unsurların etkilerinin neler olacağı konusunda farkındalıklarının oluşmadığı söylenebilir.

Sonuç olarak; moda tasarımı öğrencilerinin meslek hayatına atıldıklarında tekstil işletmelerinin doğa dostu üretim faaliyetlerine katkı sağlayabilmeleri için; çevre bilinci ve farkındalığı konusunda yeterli bilgi ile donatılarak mezun edilmeleri gerekmektedir. Sanayiye dönük tüm endüstri kollarında çalışan bireylerde çevreye yönelik olumlu davranışların oluşturulabilmesi için, eğitimin ilk kademesi olan anaokullarından başlayarak üniversite eğitimine kadar her yıl bir adet zorunlu ve uygulamalı çevre eğitimi dersinin müfredata konulması gerekmektedir. Teorikte öğretilen bilgilerin uygulamalı olarak günlük yaşantıda alışkanlık haline dönüştürülmesinin çevre ile ilgili oluşabilecek problemleri anlama, farkında olma ve bilgi sahibi olma düzeylerine son derece yarar sağlayacağı düşünülmektedir.

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ARAŞTIRMA MAKALESİ **RESEARCH PAPER**

Gebe Sığırlarda İnaktif *Escherichia coli* Aşısının Kolostrum ve Buzağı Kan Serumlarındaki IgG Seviyelerine Etkileri [*]

Sinan SANCAK **Timur GÜLHAN***
Ondokuz Mayıs Üniversitesi, Veteriner Fakültesi, Mikrobiyoloji Anabilim Dalı, Samsun, Türkiye

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*: https://orcid.org/0000-0003-4798-1427
: https://orcid.org/0000-0001-6768-1048

***Sorumlu yazarın:**
Timur GÜLHAN
Ondokuz Mayıs Üniversitesi, Veteriner Fakültesi, Mikrobiyoloji Anabilim Dalı, 55220 Atakum, Samsun, Türkiye
✉: timur.gulhan@omu.edu.tr

Öz: Bu çalışmada, gebe sığırlara uygulanan inaktif *Escherichia* (*E.*) *coli* aşısının kolostrum ve buzağı kan serumlarındaki immunoglobulin (Ig) G seviyelerine olan etkisinin araştırılması amaçlandı. Bu amaçla, gebeliklerinin farklı dönemlerindeki sığırlara (son 60 ve 30 gün) ticari bir inaktif *E. coli* aşısı uygulandı. Aşılanan sığırların kolostrumu ve bu kolostrumlarla beslenen buzağıların kan serumlarındaki IgG seviyeleri ELISA ile incelendi.Aşılama grupları ve kontrol grubu arasında IgG seviyelerindeki farklılıklar ölçülerek, gruplar arasındaki IgG değerleri karşılaştırıldı. Kolostrum örneklerine ait veriler incelendiğinde, kontrol grubu ile tek doz ve iki doz aşılanan hayvanlara ait IgG seviyeleri arasındaki farkın önemli ($p<0,001$) olduğu belirlendi. Benzer şekilde buzağı serumlarına ait veriler değerlendirildiğinde; tek doz ve iki doz aşı yapılanan sığırların buzağılarında ölçülen kan serum IgG değerlerinde kontrol grubuna göre önemli oranda artış tespit edildi ($p<0,001$). Diğer yandan kontrol grubundaki 7 buzağının tamamında (%100), tek doz aşılanan sığırlara ait 7 buzağının 5'inde (%71,4) pasif transfer yetersizliği (PTY) görülürken, iki doz aşılanan 7 buzağının hiçbirinde PTY belirlenemedi. Böylece aşılanan hayvanlarda PTY oranlarında önemli oranda düşüş sağlanmış oldu. Sonuç olarak, gebe sığırlara uygulanan inaktif *E. coli* aşısı ile oluşturulan immun yanıtın pasif olarak buzağılara transfer edildiği,gebe sığırların gebeliklerinin son 60 ve 30. gününde iki doz aşılamanın, tek doz aşılamaya göre daha etkili olduğu kanaatine varıldı.

Anahtar kelimeler: ELISA,inaktif *E. coli* aşısı, IgG, kan serumu, kolostrum, sığır. .

Effects of Inactive *Escherichia coli* Vaccine on IgG Levels in Colostrum and Calf Blood Serum in Pregnant Cattle

***Corresponding author's:**
Timur GÜLHAN
Ondokuz Mayıs University, Faculty of Veterinary Medicine, Department of Microbiology, 55220 Atakum, Samsun, Turkey
✉: timur.gulhan@omu.edu.tr

Abstract: In this study, it was aimed to investigate the effect of inactive *E. coli* vaccine administered in pregnant cattle on immunoglobulin (Ig) G levels in colostrums and calf blood sera. For this purpose, a commercial inactive *E. coli* vaccine was administered in cattle at different stages of their pregnancy (last 60 and 30 days).The colostrums of vaccinated cattle and IgG levels in blood serum of calves fed with these colostrums were analyzed by ELISA. Differences in IgG levels between vaccination groups and control groups were measured and IgG values between groups were compared. When the data of the colostrum samples were evaluated the differencesamong control group, animals vaccinated with single dose, and animals vaccinated with two doses werefound to be significant ($p <0.001$). Similarly, when the data of calf sera were evaluated; significant increase was observed in blood serum IgG values measured in calves of cattle receiving single dose and two doses of vaccine compared with the control group ($p <0.001$). On the other hand, passive transfer failure (PTF) was observed in all 7 calves (100%) in the control group and in 5 (71.4%) of the 7 calves vaccinated with a single dose, while PTF could not be determined in none of the calves vaccinated with two doses. Thus, a significant decrease in PTF rates was achieved in vaccinated animals. As a result, it was concluded that the immune response generated by the inactive *E. coli* vaccine administered in pregnant cattle was transferred to calves passively, and two doses of vaccination on the last 60 and 30 days of pregnancy were determined to be more effective than single dose vaccination.

Keywords: Blood sera, bovine, colostrums, ELISA, inactive *E. coli* vaccine, IgG.

GİRİŞ

Neonatal (yenidoğan) buzağı hastalıkları ve bunlara bağlı buzağı kayıpları Veteriner Hekimlik açısından önemli bir konudur. Bir aylığa kadar olan buzağılar neonatal olarak sınıflandırılmaktadır. Sığırlarda, plasenta yapısı anne ile yavrusu arasında sınırlı geçirgenlik özelliği göstermesi nedeniyle (epitelyokordial), prenatal veya intrauterin dönemde immunoglobulinlerin buzağıya geçişi söz konusu değildir. Bu nedenle yenidoğan buzağılar hipo veya agammaglobulinemik olarak dünyaya gelirler. Hayatlarının en zayıf dönemlerinde buzağılar anneden aldıkları kolostrumla bağışıklık kazanırlar. Bağışıklığın istenilen düzeylerde olabilmesi için yüksek kaliteli ve koruyucu düzeyde immunoglobulin konsantrasyonu (>1500 mg/dl) içeren kolostrumla yeterince beslenme şarttır (da Silvavd., 2006).

Doğum sonrası 24 ve 48. saatlerde kan IgG seviyesi 10 mg/ml'den az olması buzağılar için pasif transfer yetmezliği (PTY) olarak kabul edilmektedir. PTY her ne kadar bir hastalık olmasa da, buzağıların hastalıklara olan direncini düşürerek, büyüme oranlarında azalma, üretkenlik kaybı, yemden yararlanmada azalma gibi ekonomik kayıplara neden olmaktadır. Doğal koşullar altında neonatal buzağılarda PTY oranının %10-35 olduğu bilinmektedir (McGee ve Early, 2019).

Enterotoksijenik *E. coli* (ETEC) suşları insan ve hayvanlarda ishallere neden olmaktadırlar. ETEC, çiftlik hayvanlarında *E. coli* kökenli ishal vakalarından en fazla izole edilen bakteridir. Özellikle ruminantlarda yaşamın ilk 12 saatinde bulunan ve 12. saatten sonra kaybolan fimbrialadhezinlerespesifik reseptörler, doğumdan sonra 1-2 saat ETEC suşlarının barsak yüzeyine tutunmasıyla buzağı septimesine sebep olmaktadır. ETEC, barsaktaki sıvı salgısında artıştan sorumlu enterotoksinler ve barsak kolonizasyonu için spesifikenterosit reseptörlerine bağlanmasını sağlayan adezinler/kolonizasyon faktörlerini üretebilmektedir. Adezinler içerisinde en iyi bilinenler F4 (K88), F5 (K99), F6 (987P), F17, F18 ve F41 fimbriya tipleridir. Bakterinin ince barsak epitel hücrelerine yapışmasını sağlayan F5 (K99) fimbrial antijeni, ishalli buzağılardan izole edilen klasik ETEC izolatlarında en yaygın saptanan antijenik yapıdır (Piccovd., 2015). ETEC, diğer enteropatojenlerle birlikte, ilk 4 gün ile 2 haftalık yaşa kadar olan buzağılarda yeni doğan ishallerine neden olmaktadır. ETEC'in hayvanlara bulaşması genellikle sindirim sistemi yoluyla olmaktadır(Mohammedvd., 2019).

K99 antijeninin özellikle ETEC suşlarınınjejunum ve /veya ileum epitelyum yüzeyindeki gilikoproteinlere tutunmada adherens antijeni olarak görev yaptığı ortaya konulmuştur. ETEC'in bağırsak epiteline sıkı bir şekilde yapışması, etkili toksin üretilmesine olanak sağlar. Barsak lümeninde su ve elektrolitlerin salgılanması toksin aktivitesinden kaynaklanır. İshal, % 10'dan az kuru madde içeren yumuşak dışkı olarak tanımlanmaktadır. Aşırı sıvı kaybına neden olur. Ölümcül ETEC infeksiyonları, özellikle ilk 24 saat içerisinde, ciddi dehidrasyon ve elektrolit dengesizliği sonucu ortaya çıkmaktadır (Dezfoulievd., 2019).

Veteriner aşılarının temel amacı, evcil hayvanların sağlığını ve refahını iyileştirmek, maliyeti düşürerek canlı hayvan üretimini etkin bir şekilde artırmak ve zoonoz hastalıkların hem evcil hayvanlardan hem de yabani hayvanlardan insana geçişini önlemektir (McGee veEarley, 2019).

ETEC fimbrialarına yönelik yapılan çalışmalar, bu organellerin biyolojisini ve patogenezdeki rolünü detaylandırmış, böylece yeni tanı teknikleri, profilaktik ve terapötik araçlar geliştirilmiştir. Fimbrialaların yüksek oranda immünojenik proteinler olduğu, maternalimmünizasyon ile indüklenen kolostral antikorların patojen *E. coli*suşlarınınenterositlere yapışmayı ve barsak kolonizasyonuna önleyerek neonatal yavruları koruduğu bilinmektedir. *E. coli* anti-adheziffimbria aşıları, veteriner aşı sektöründe oldukça başarılı bir şekilde kullanılmaktadır. Bu amaçla F4 (K88), F5 (K99), F6 (987P), F41 gibi antijenik yapılar tercih edilmektedir (Crouchvd., 2001; Dubreuilvd., 2016).

ETEC salgınlarının önlenmesinde fimbrialantijenlerin yanı sıra farklı toksijenik (ST, LT vs.) alt yapılar kullanılarak da aşılar elde edilmektedir. Farklı hayvan türleri için söz konusu antijenlerin purifikasyonu veya attenüasyonu ile geliştirilmiş çok sayıda ticari aşı bulunmaktadır. Ülkemizde, özellikle buzağıların *E. coli*infeksiyonlarından korunması amacıyla üretilen aşılar bakterinin K99+F41+STa virülensprofiline uygun olarak hazırlanmaktadır. Ancak, pek çok ülkede yaygın olarak kullanılan aşılama süreci ülkemizde sağlıklı bir şekilde sürdürülememektedir. Gebe sığırların neonatal septisemilerin önlenmesi amacıyla aşılanması ve takip edilmesi, saha uygulamalarındaki yetersizlik, hayvan yetiştiricilerinin yeterince bilinçli olmaması gibi faktörlere bağlı olarak tam kapsamlı bir şekilde yürütülememektedir.

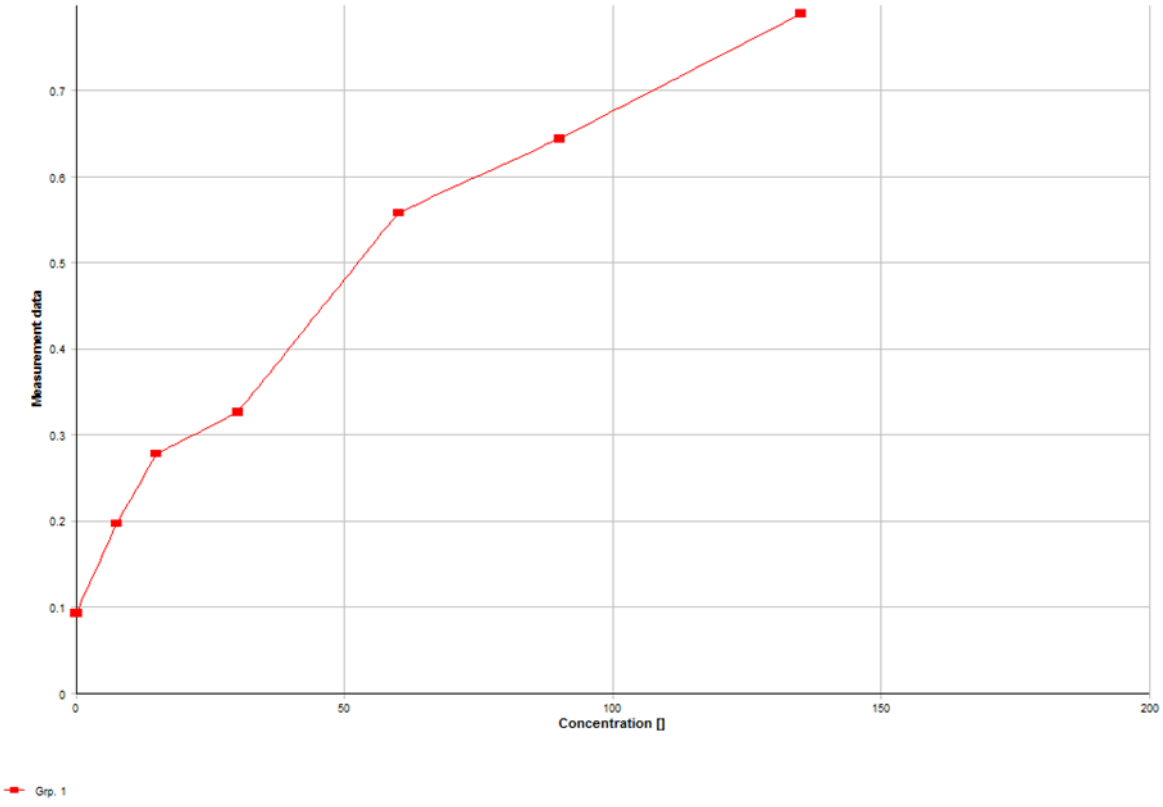
Bu çalışmada, gebeliklerinin son 30 (tek doz) ve 30. ve 60. günlerinde (iki doz) inaktif bir ticari *E. coli*aşısı ile aşılanan sığırların kolostrum ve bu hayvanlara ait kolostrumla yeterince beslenen buzağıların kan serumlarındaki IgG miktarlarındaki değişikliklerin izlenmesi amaçlandı. Aşılanan hayvan grupları ve kontrol grubundaki hayvanlar arasında kolostrum ve buzağı kan serumu IgG seviyelerindeki değişikliklerin izlenmesi ile sınırlı dahi olsa PTY miktarlarının ölçülmesi hedeflendi. Ayrıca sığır yetiştiricilerinin neonatal buzağı septisemilerinin önlenmesinde gebe sığırları aşılanması konusunda bilinçlendirilmesi ve bu konuda farkındalık oluşturulmaya çalışıldı.

MATERYAL VE METOT

Çalışma planı ve hayvan materyali: Çalışma kapsamında Sivas ili Suşehri ilçesindeki bir işletmede yarı intansif olarak yetiştirilen, klinik olarak sağlıklı, 21 Simental ırkı gebe sığır (3 yaş ve üzeri) ve bu hayvanlara ait 21 buzağıdan (1 günlük) oluşan 42 hayvan kullanıldı. Çalışma kapsamında aşılanacak hayvanlara yerel etik kurul (14.11.2019 tarih ve 68489742-604.01.03-E.25438nolu izin) ve hayvan sahibi onam belgesi (07.11.2019) doğrultusunda işlem yapıldı. Çalışmada kullanılan gruplardaki hayvanlar mümkün olduğunca benzer özellikte (yaş, ırk, klinik durum, önceki aşılama programı vb. açıdan) seçildi ve standart rasyon ile beslendi. Bu süreçte aşılanan hayvanların hiç birinde hayvanın sağlığını bozacak klinik semptom ve olumsuz değişiklik belirlenmedi. Araştırma kapsamında gebe sığırlara ticari bir inaktif *E. coli* aşısı (VBR K99 *E. coli* bakterin, Ata Fen) yapıldı. Bu amaçla, her grupta 7'şer gebe inek ve 7'şer buzağıdan oluşan 3 grup oluşturuldu. Birinci gruba gebeliğin son 60 gününde ilk aşı, izleyen 30 gün sonra ikinci aşı yapıldı. İkinci gruptaki sığırlara ise gebeliğin son ayında tek doz aşı uygulandı. Aşılama işlemi inaktif ticari aşının 2 ml'si gebe sığırlara deri altı (SC) yolla yapıldı. Kontrol grubundaki gebe sığırlara ise herhangi bir aşılama yapılmadı. Tüm gruplardaki hayvanlardan doğumu takiben kolostrum örnekleri (2'şer ml) alındı. Diğer yandan her guruptaki buzağıların yeterince (2,5 litre) kolostrum alması sağlandı. Kolostrumla beslenen buzağılardan 24 saat sonra, hayvanlara herhangi bir zarar vermeden klasik yöntemlerle yüksek lisans öğrencisi veteriner hekim tarafından vena jugularisten, kan örnekleri (5'er ml) alındı. Alınan kan örneklerinden çıkartılan kan serumları ve kolostrumlar -20°C'de stoklandı.

Ticari ELISA: Kolostrum ve kan serumu örneklerinde IgG seviyelerinin belirlenmesinde ticari sığır IgG ELISA kiti (Bovine Ig ELISA Kit, EB0001, FineTest, Wuhan Fine Biotech Co., Ltd., Çin) kullanıldı.

Test üretici firmanın önerileri doğrultusunda gerçekleştirildi. Bu amaçla tüm solüsyonlar oda ısısına getirilerek istenilen oranlarda sulandırıldı. Test içeriğinde sunulan standartların sulandırmaları yapıldı. Standartlar 135 µg/ml - 7,5 µg/ml olacak şekilde sulandırıldı ve elde edilen standart eğri Şekil 1'de verildi.



Şekil 1. ELISA standart eğri.
Figure 1. ELISA standard curve.

ELISA pleytinin ilk 6 kuyucuğunda50'şer µl standart sulandırmaları olacak şekilde, 7. kuyucuk blank amacıyla boş bırakıldı. 8. kuyucuktan başlanarak her kuyucuğa 40 µl örnek dilüsyon buffer eklendi. 8. kuyucuktan itibaren buzağı serum ve kolostrum örnekleri 10 µl eklenerek karıştırıldı. Pleyt 37°C'de 30 dk inkübe edildi. İnkübasyondan sonra pleyt, yıkama solusyonu ile 5 defa yıkandı. Blank haricinde tüm kuyucuklara 50 µl HRP-Konjugat eklendi. Benzer şekilde pleyt 37°C'de 30 dk inkübe edildi. İnkübasyon süresi sonunda pleyt, yıkama

solusyonu ile 5 kez yıkandı. Tüm kuyucuklara 50 µl Kromojen A solüsyonu ve 50 µl Kromojen B solüsyonu eklendi. Pleyt 37°C'de15 dk ışık almayacak şekilde inkübasyona bırakıldı. Bu sürenin sonunda tüm kuyucuklara50 µl stop solüsyonu eklendi. Böylece kuyucuklardaki renk maviden sarıya dönmesi sağlandı. Pleyt 450 nm'de ELISA reader cihazına yerleştirilerek sonuçlar okutuldu.ELISA ile elde edilen OD değerleri standart sulandırmalardaki IgG miktarları (135 µg/ml – 7,5 µg/ml) ile karşılaştırılarak her örnek için IgG miktarları (µg/ml) saptandı (Al-Alo, vd., 2018).

İstatistiksel Analiz: Araştırmadan elde edilen verilerin varyans analizi (Tek Yönlü Varyans Analizi) ve ortalamalar arasındaki farklılık (Duncan testi) SPSS 20.0 paket programı kullanılarak yapıldı (IBM Corp., 2011).

BULGULAR

ELISA Sonuçları: Kolostrumla beslenen buzağılardan 24 saat sonra alınan kan serumu ve kolostrum örneklerine ait ELISA sonuçları Tablo 1, Tablo2, Şekil 2 ve Şekil 3'te sunuldu.

Tablo 1. Buzağı kan serumlarına ait ELISA sonuçlarının gruplara göre dağılımı.
Table 1. Distribution of ELISA results of calf blood sera by groups.

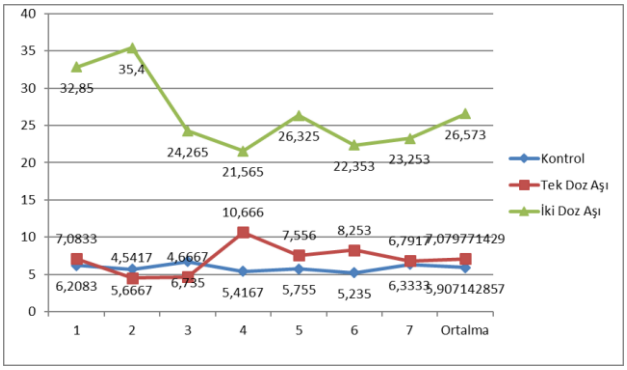
N	Kontrol	Tek Doz Aşı	İki Doz Aşı
1	6,2083	7,0833	32,85
2	5,6667	4,5417	35,4
3	6,735	4,6667	24,265
4	5,4167	10,666	21,565
5	5,755	7,556	26,325
6	5,235	8,253	22,353
7	6,3333	6,7917	23,253
Ortalama	5,907[b]	7,080[b]	26,573[a]
		Standart Hata	2,232
		P	0,000

a, b: Ig değerleri açısından gruplar arasındaki farklılıklar istatistiksel olarak önemlidir (P<0,001)

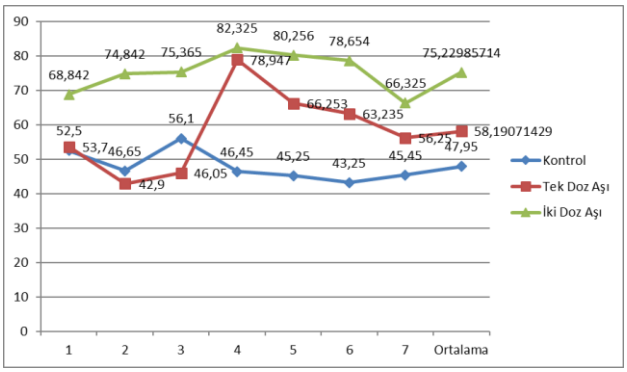
Tablo 2. Kolostrum örneklerine ait ELISA sonuçlarının gruplara göre dağılımı.
Table 2. Distribution of ELISA results of colostrum samples by groups.

N	Kontrol	Tek Doz Aşı	İki Doz Aşı
1	52,5	53,7	68,842
2	46,65	42,9	74,842
3	56,1	46,05	75,365
4	46,45	78,947	82,325
5	45,25	66,253	80,256
6	43,25	63,235	78,654
7	45,45	56,25	66,325
Ortalama	47,950[b]	58,191[b]	75,230[a]
		Standart Hata	3,055
		P	0,000

a, b: Ig değerleri açısından gruplar arasındaki farklılıklar istatistiksel olarak önemlidir (P<0,001)



Şekil 2. Gruplara göre buzağı kan serumu ELISA sonuçlarının dağılımı.
Figure 2. Distribution of calf blood serum ELISA results by groups.



Şekil 3. Gruplara göre kolostrum ELISA sonuçlarının dağılımı.
Figure 3. Distribution of colostrum ELISA results by groups.

Serum IgG seviyesine göre IgG seviyesi 8 µg/ml'den daha az olan buzağılar ile kolostrum örneklerinin IgG seviyelerine göre IgG konsantrasyonu 32 µg/ml ve altında olan örnekler PTY olarak değerlendirildi.

Buzağı serumlarına ait veriler değerlendirildiğinde, tek doz ve iki doz aşı yapılanan sığırların buzağılarında ölçülen kan serumu değerlerinde kontrol grubuna göre önemli oranda artış tespit edildi (p<0,001). Benzer şekilde kolostrum örneklerine ait veriler incelendiğinde, kontrol grubu ile tek doz ve iki doz aşılanan hayvanlardan elde edilen sonuçlar arasındaki farkın önemli (p<0,001) olduğu belirlendi. Diğer yandan iki doz aşılanan 7 buzağının hiçbirinde PTY belirlenemezken, tek doz aşılanan sığırlara ait 7 buzağının 5'inde (%71,4) ve kontrol grubundaki 7 buzağının tamamında (%100) pasif transfer yetersizliği (PTY) saptandı.

Diğer yandan çalışma populasyonundaki hayvanların uygulama sonrası klinik takiplerinde gruplar arasında önemli farklılıklar kaydedildi. Kontrol grubundaki 7 buzağının 6'sında, tek doz aşı yapılan hayvanlara ait 7 buzağının 3'ünde ishal belirtileri görülürken, iki doz aşılanan sığırlara ait 7 buzağının hiçbirinde ishal semptomları saptanmadı.

SONUÇ VE TARTIŞMA

Buzağılarda ETEC kökenli ishal ve ölüm vakaları genellikle doğumu takiben ilk birkaç gün içerisinde şekillenmektedir. Bu dönemde yapılacak antibiyotik tedavisi etkisiz kalabilmektedir. Çünkü antibiyotik tedavisinin etkili olabilmesi için en az 3-4 gün gerekmektedir. Bu nedenle buzağılar için kritik olan bu dönemde maternal antikorlar hayati öneme sahiptir. Kolostral antikorlar aracılığı (pasif transfer) ile buzağılarda bağışıklık oluşturmanın önemli olduğu ve düşük IgG seviyelerine sahip buzağılarda ölüm oranlarının daha yüksek olduğu bilinmektedir. *E. coli*'nin farklı antijenik yapıları kullanılarak gerçekleştirilen çalışmalarda; gebe sığırların aşılanması sonucu buzağılardaki neonatal ishallere bağlı ishal ve ölüm oranlarında önemli düşüşler kaydedilmiştir (Figueiredo vd., 2004).

Sağlıklı ve ishalli buzağıların kan serumlarındaki anti-*E. coli* antikor titreleri arasında önemli farklılıklar olduğu, ishalli buzağılardaki antikor titresinin sağlıklı olanlardan daha yüksek olduğu belirlenmiştir. Diğer yandan ishalli buzağılarda total IgG konsantrasyonunun sağlıklı buzağılara göre daha düşük seviyelerde olabilmektedir. Benzer şekilde, kolostral anti *E. coli* antikorları ile buzağı kan serumlarındaki anti *E. coli* antikorları arasında yüksek seviyelerde pozitif korelasyon saptanmıştır. Bu durum, ishallerin önlenmesinde maternal antikorlarla birlikte, anneden yavruya pasif (maternal) olarak aktarılan sitokinler gibi hücresel komponentlerin önemini göstermektedir (Al-Alo vd., 2018).

Gebelik dönemlerinde aşılanmamış sığırlardan doğan ve annelerinden maternal antikor almayan buzağıların neonatal dönemde aşılanmalarında daha düşük başarılar elde edilmiştir (Deluyker vd., 2004). Bu nedenle neonatal koliseptisemilerin önlenmesinde en etkili yol gebe sığırların farklı dönemlerde aşılanması ve annede oluşan maternal antikorların buzağılara kolostrum yoluyla aktarılmasıdır.

Bu amaçla *E. coli*'nin farklı antijenik yapılarından oluşan aşı kompozisyonları ile rota, corona ve parvavirüslerden oluşturulan kombine aşı seçenekleri kullanılarak hazırlanan ticari aşıların etkinliğini belirlemek için çok sayıda araştırma yapılmıştır. Araştırmaların bazılarında antikor titresinde artış sağlanmasına rağmen, buzağı ishallerinin ve ölümlerinin önlenmesine yönelik, koruyuculuk elde edilememiştir (Waltner-Toewsvd., 1985; Snodgrass, 1986). Diğer yandan çalışmaların çoğunda başarılı sonuçlar alınmış, aşılama ile oluşturulan anti K99 kolostral antikorların buzağılara yeterince aktarıldığı ve koruyucu seviyelerde olduğu (Wieda vd., 1987; Crouch vd., 2001), deneysel *E. coli* enfeksiyonlarına karşı tam korunma sağlandığı (Collinsvd., 1988), buzağı ölümlerinin önüne geçildiği (Mensikvd., 1989) aşının gebe sığırlarda hiçbir yan etkisinin olmadığı (Yano vd., 1995) rapor edilmiştir. İlave olarak tek doz aşı uygulanan gruptaki hayvanlardan elde edilen antikor seviyeleri, kontrol grubu ile karşılaştırıldığında önemli bir artış belirlenemezken, iki doz aşı yapılan gruptaki hayvanların kolostrum ve kan serumu örneklerinde kontrol grubundakilere göre istatistiksel anlamda artış saptandığı bildirilmiştir (Figueiredo vd., 2004).

Bu çalışmada, gebeliklerinin farklı dönemlerinde (doğuma 30 ve 60 gün kala) tek ve iki doz şeklinde inaktif bir ticari *E. coli* aşısı ile aşılanan sığırlara ait kolostrum örneklerinde ve bu kolostrumlarla beslenen buzağılardan doğumu takiben 24 saat sonra alınan kan serumlarında şekillenen IgG miktarları ELISA ile ölçüldü. ELISA verileri kontrol grubundaki verilerle karşılaştırıldığında, aşılanan sığırlarda oluşturulan immunoglobulinlerin buzağılara pasif transferinin gerçekleştiği, özelikle iki doz aşılama sonrası şekillenen antikor titresinde tek doz aşı ve kontrol grubuna göre önemli derecede artış belirlendi ve aradaki farkın istatistiksel olarak önemli ($p<0.001$) olduğu saptandı. Diğer yandan gruplar PTY açısından karşılaştırıldığında iki doz aşılanan hayvanlara ait buzağılarda PTY görülmezken, tek doz aşılanan hayvanlarda PTY %71.4, kontrol grubundakilerde ise %100 olarak kaydedildi.

Konuyla ilgili yapılan literatür taramasında geçmişten günümüze kadar en güncel çalışma verileri yorumlanmıştır. Ancak gebe sığırlarda yapılan aşı çalışmalarına ait çok güncel verilere ulaşılamamıştır. Bu nedenle tartışma mevcut literatür verilerine göre yapılmıştır. Önceki çalışmaların genellikle aşı etkinliğini tespit etmek ve bir kısmının oluşturulan antikorların deneysel enfeksiyonlara karşı koruyuculuk seviyelerini belirlemek amacıyla yapıldığı görülmektedir. Bu çalışmada inaktif *E. coli* aşısı ile oluşturulan IgG seviyelerinin kontrol grubu hayvanlardaki IgG seviyeleri ile karşılaştırılarak aşılamanın total IgG seviyelerine olan etkisi araştırıldı. Aşılama sonrası deneysel enfeksiyon oluşturularak, buzağılara pasif olarak aktarılan maternal anti-K99 antikorlarının koruyuculuk seviyeleri ölçülmedi. Aşı içeriğinde olan antijenlere (K99/F41/F17(Fy)) tek tek spesifik antikor artışı incelenmedi. Bu yönüyle çalışma aşıya ait spesifik bir IgG artışını ortaya koymakta sınırlı kalmaktadır. Ancak aşılanmamış hayvanlardan oluşan kontrol grubundaki hayvanlara ait verilerle aşılanmış hayvanlardan elde edilenler arasında istatistiksel olarak anlamlı farklılıklar belirlenmesi, klinik olarak aşılı gruptaki buzağılarda ishal sayılarında önemli düşüşlerin tespit edilmesi aşının koruyucu olduğuna işaret etmektedir. Böylece sınırlı hayvan üzerinde dahi olsa aşı etkinliği ve PTY oranlarında önemli seviyelerde azalma görüldüğü ortaya konulmuş oldu.

Bu araştırmada elde edilen verilerin sunulan aşı çalışmalarında belirtilen ve sığırların gebeliklerinin farklı dönemlerinde özellikle iki doz şeklinde gerçekleştirilen deneme sonuçları ile uyumlu olduğu görüldü. Her ne kadar bazı çalışma sonuçları ile farklılıklar olsa dahi, genellikle sonuçların paralellik arz ettiği görülmektedir. Diğer araştırmalarda da belirtildiği gibi sonuçlar arasındaki farklılıklar, çalışılan hayvan populasyonları arasındaki, ırk, yaş, bakım-besleme şartları vb. değişkenlere ve taramada kullanılan yöntem farklılıklarından kaynaklanabilmektedir.

Sonuç olarak bu çalışma kapsamında neonatal buzağı ishallerinin önlenmesine yönelik pilot bir aşılama çalışması gerçekleştirildi. Aşılanan hayvanlarda aşılama sonrası herhangi bir yan etki görülmedi. Tüm gruplardaki hayvanlar beklenen zamanda doğum yaptı ve buzağıların yeterince kolostrum alması sağlandı. Araştırma sonuçlarına göre aşılanan gruplar ile kontrol grubu arasında IgG seviyeleri açısından önemli farklılıklar saptandı. Özellikle gebeliklerinin son 60 ve 30. gününde iki doz şeklinde gerçekleştirilen inaktif *E. coli* aşılamasının hem kolostrum hem de buzağı kan serumlarındaki IgG seviyelerinde önemli derecede artış ve PTY'lerin önlenmesine yönelik başarı sağladığı kanaatine varıldı. Çalışma populasyonundaki hayvanların uygulama sonrası klinik takiplerinde gruplar arasında önemli farklılıklar kaydedildi. Kontrol grubundaki 7 buzağının 6'sında, tek doz aşı yapılan hayvanlara ait 7 buzağının 3'ünde ishal belirtileri görülürken, iki doz aşılanan sığırlara ait 7 buzağının hiçbirinde ishal semptomları saptanmadı. Ancak bu araştırma çok sınırlı bir hayvan üzerinde gerçekleştirildiği için, *E. coli* kökenli neonatal buzağı septisemilerinin engellenmesine yönelik daha fazla hayvan populasyonunda gerçekleştirilecek kapsamlı çalışmalara ihtiyaç duyulmaktadır. Böylece buzağı ishallerinden kaynaklanan ekonomik kayıpların önüne geçilmesi adına önemli adımlar atılabilecektir.

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ARAŞTIRMA MAKALESİ **RESEARCH PAPER**

Distribution and Fishery of the Blue Crab (*Callinectes sapidus* Rathbun, 1896) in Turkey Based on Local Ecological Knowledge of Fishers

Fikret ÖNDES[1*] **Gökhan GÖKÇE[2]**

[1*] *Faculty of Fisheries, Izmir Kâtip Çelebi University, Çiğli, Izmir, Turkey*
[2] *Faculty of Fisheries, Çukurova University, Balcalı, Adana, Turkey*

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*: https://orcid.org/0000-0002-9522-7050
: https://orcid.org/0000-0003-1830-0733

***Corresponding author's:**
Fikret ÖNDES
Faculty of Fisheries, Izmir Kâtip Çelebi University, Çiğli, Izmir, Turkey
✉: fikret.ondes@ikcu.edu.tr

Abstract: This study provides information on the distribution and ecology of *Callinectes sapidus* in Turkey, as well as its maximum daily catch in set nets (trammel nets and gillnets), and its commercial fishery in lagoons. Data were collected in 2020 by utilizing a telephone based questionnaire survey. Responses were gathered from fishermen (n = 6) who fish in the lagoons and the head or members of fishery cooperatives (n = 104) in 28 cities, including the coastal regions of the Mediterranean Sea (Levantine), Aegean Sea, Sea of Marmara and the Black Sea in Turkey. The results showed that *C. sapidus* is commonly distributed along the Levantine and the southern Aegean coasts of Turkey, whilst it is rarely observed in the Sea of Marmara and the Black Sea. Fishermen stated that *C. sapidus* has been seen in the Black Sea for the last decade. The maximum daily catch of blue crab in set nets showed a significant difference in the Levantine and Aegean coasts. The results indicated that the species was mainly produced in the lagoons, whereas many coastal fishermen returned it as discard and 79% of fishermen emphasized that *C. sapidus* shred the nets and caused an economic loss. Ovigerous females have been observed between March and October and we have discussed related management issues including current fishery season.

Keywords: *Callinectes sapidus*, fisheries management, invasive species, lagoon fishery, local ecological knowledge, marine invasion.

Balıkçıların Lokal Ekolojik Bilgilerine Göre Mavi Yengecin (*Callinectes sapidus* Rathbun, 1896) Türkiye'deki Dağılımı ve Balıkçılığı

***Sorumlu yazar:**
Fikret ÖNDES
İzmir Katip Çelebi Üniversitesi, Su Ürünleri Fakültesi, İzmir, Türkiye
✉: fikret.ondes@ikcu.edu.tr

Öz: Bu çalışma, *Callinectes sapidus*'un Türkiye'deki dağılımı, ekolojisi, uzatma ağlarındaki (fanyalı ve sade ağlar) günlük maksimum avı ve dalyanlardaki ticari balıkçılığı hakkında bilgi sağlamaktadır. Veriler, 2020 yılında yapılan telefon anketleri ile toplanmıştır. Dalyanlarda balıkçılık yapan 6 balıkçı ve Türkiye'nin Akdeniz, Ege, Marmara ve Karadeniz kıyılarını kapsayan toplam 28 ildeki su ürünleri kooperatifi başkanları ya da kooperatif üyeleri (örneklem sayısı = 104) anketleri cevaplamıştır. Bulgular, *C. sapidus* türünün Türkiye'nin Akdeniz ve Güney Ege kıyılarında yaygın olduğunu, Marmara ve Karadeniz kıyılarında ise nadiren gözlemlendiğini göstermiştir. Balıkçılar, *C. sapidus*'un Karadeniz'de son 10 yıldır görüldüğünü belirtmişlerdir. Uzatma ağlarındaki günlük maksimum yengeç avı Akdeniz ve Ege Denizi'nde anlamlı fark göstermiştir. Bulgular, bu türün çoğunlukla dalyanlarda üretilirken, çoğu kıyı balıkçısının ıskarta olarak suya geri bıraktıklarını göstermiş ve kıyı balıkçılarının %79'u *C. sapidus* türünün ağları parçaladığını ve ekonomik kayba neden olduğunu belirtmiştir. Yumurtalı dişiler Mart ve Ekim ayları arasında gözlemlenmiş olup, mevcut balıkçılık sezonu dâhil olmak üzere yönetim ile ilgili konular değerlendirilmiştir.

Anahtar kelimeler: Balıkçılık yönetimi, *Callinectes sapidus*, dalyan balıkçılığı, denizel istila, istilacı türler, lokal ekolojik bilgi.

INTRODUCTION

Callinectes sapidus Rathbun, 1896, is commonly known as blue crab or Atlantic blue crab and is regionally entitled as "Chesapeake blue crab" around the western Atlantic Ocean and the Gulf of Mexico (Taybi & Mabrouki, 2020). *C. sapidus* is one of the 100 worst invasive species and causes damage to nets and target fish species caught by nets (Streftaris & Zenetos, 2006). This species has been transported to Japanese and European waters via ballast waters of vessels and appeared in the Baltic, North, Mediterranean and Black Seas (Nehring, 2011). Like many other non-indigenous species, the distribution and adaptation of *C. sapidus* in Europe has been influenced by climate change (Nehring et al., 2008).

C. sapidus prefers the sandy and muddy habitats (Hill et al., 1989) and the depths between 0 to 90 m (Stasolla & Innocenti, 2014). This species exhibits a migration pattern; after mating females migrate to areas where salinity level is higher (Eggleston et al., 2015). *C. sapidus* mainly feeds on molluscs, arthropods, fishes and polychaetes, whereas algae species are rarely consumed (Belgrad & Griffen, 2016; Hines, 2003; Laughlin, 1982; Reichmuth et al., 2009). It was reported that *C. sapidus* can reach a maximum size (carapace width (CW)) of 20.9 cm in males and 20.4 cm in females (FAO, 2020).

Estuaries are major areas for *C. sapidus* populations (Türeli, 1999). Some environmental features including depth and salinity influence the population structure of *C. sapidus* in these areas (Jivoff et al., 2017). Adult individuals of *C. sapidus* generally mate in these areas then migrate to lower estuaries or offshore waters to spawn and hatch their eggs (Fitz & Wiegert, 1992). *C. sapidus* also can be a dominant species in lagoons within relatively short periods. For instance, Kampouris et al., (2019) stated that specimens of *C. sapidus* feed on economically important molluscs, fishes, and crustaceans in the Thermaikos Gulf and Papapouli Lagoon in Greece, and have negative impacts on the Greek national fisheries and aquaculture.

Despite negative effects cited above, it was noticed that blue crab meat has high nutritional quality and various blue crab products have been sold in markets in the US and Europe (Çelik et al., 2004). The global capture of blue crab was reported as 97 896 t in 2016 and it was mainly caught around the western Atlantic coast (FAO, 2020). The decrease of *C. sapidus* population in certain regions might be related to overfishing, reduced freshwater inflow into estuaries, and problems in larval recruitment (Weatherall et al., 2018). The main fishing gear used in the capture of blue crab is a crab trap, however they are also caught by trawls and set nets (Hammerschmidt et al., 1998). In spite of the fact that crab traps are known as selective fishing gears, due to storms, vandalism and vessel propellers, these gears can be lost at sea, which is commonly known as 'ghost fishing' (Anderson & Alford, 2014; Havens et al., 2008). The minimum landing size of *C. sapidus* is 127 mm CW for hard crabs and harvesting egg-carrying females is prohibited in the Chesapeake Bay (Carver, 2001). On the other hand, blue crab is commercially fished in Turkey and Greece (Gökçe et al., 2007; Mancinelli et al., 2017). Regarding the management of blue crab fishery in Turkey, there are some restrictions including, the minimum landing size (MLS) is 130 mm CW and the fishery closed season is between 1st May and 30th September. However, the Ministry of Agriculture and Forestry gives permission rights to its Provincial Directorates; hence the fishermen operating in respective provinces may also get special permissions during closed seasons to operate in lagoons (Anon., 2020a). Regarding recreational fisheries, the MLS is 130 mm CW and there is a bag limit which is 1 kg. In contrast to commercial coastal fishers, recreational fishers can fish blue crab throughout the year in Turkey (Anon., 2020b).

It was reported that *C. sapidus* introduced to the northern Aegean Sea between 1935 and 1945 (Artüz, 1990; Enzenroß et al., 1997). Then, the occurrence of *C. sapidus* was reported in different regions of Turkey; Aegean Sea (Kocatas, 1971; Tuncer & Bilgin, 2008), Mediterranean Sea (Levantine coasts) (Enzenroß et al., 1997), Sea of Marmara (Zaitsev & Öztürk, 2001) and the Black Sea (Aydın, 2017; Bilgin, 2019; Ceylan, 2020; Yağlıoğlu et al., 2014). However, previous studies reported this species from one or several localities. The distribution of *C. sapidus* in all Turkish coasts and its fishery have not been evaluated comprehensively yet. In addition, the negative impacts of *C. sapidus* on native species and ecosystem in the Mediterranean has not been sufficiently addressed (Czerniejewski et al., 2020). This study provides information on *C. sapidus* in Turkish waters including; geographical distribution, some bio-ecological characteristics (e.g. egg-carrying period, habitat and depth selectivity), and fishing techniques in lagoons as well as estimated maximum daily catch in the small-scale fisheries (trammel nets and gill nets) in Turkey. There is no doubt that an understanding of the ecological and socio-economic impacts of invasive species in their new ecosystems will help to manage aquatic resources more effectively.

MATERIALS AND METHODS

Data sampling: The objective of the questionnaire based study was to obtain information on spatial distribution, ecology and fisheries characteristics of *C. sapidus* in Turkey. The questionnaire mainly consisted

of the open-ended questions. Telephone based questionnaires were applied in 2020 to commercial fishers (president of fishery cooperatives) who is actively performing small-scale fisheries. If the president of the fishery cooperative did not fish actively, we could apply the questionnaire to other members of the fishery cooperatives. The fishers (n = 104) who completed the questionnaire surveys reported using fishing gears including trammel nets and gillnets to catch many different target species. The data were collected from 28 cities including 110 fishing grounds, in order to reliably represent the coastal regions of Turkey (Figure 1a). The questionnaire provided information on; i) some demographic characteristics of fishers (age, gender, fishing experience), ii) bio-ecological characteristics of *C. sapidus* (e.g. distribution, depth and habitat selectivity, temporal trends in abundance, estimation of spawning and molting periods), and iii) fishery characteristics (e.g. determination of the fishing gears types caught blue crabs, estimated maximum daily catch in 2019). If *C. sapidus* did not emerge in the fishers' fishing grounds, they were exempt from answering some questions. Additionally, another questionnaire was applied to fishers (n = 6) who produce blue crabs in lagoons with barrier traps, wire pots and fyke nets. This second questionnaire included identical questions to the first one, with the addition of questions related to the lagoon fishery.

Data analysis: The SPSS (version 20.0) was used for the statistical analyses in this study. A Mann Whitney U test was utilized to ascertain whether the maximum daily catch of blue crab shows a difference in Levantine and Aegean or not.

RESULTS

The age of fishers who participated in the questionnaire survey ranged between 25 and 70 with a mean value of 49 ± 9 years. All of them were male and their fishing experience ranged between 7 and 56 years, while the mean fishing experience was 32 ± 11 years.

***Distribution and some ecological characteristics of Callinectes sapidus*:** Concerning the occurrence of *C. sapidus*, 100 %, 72 % and 46 % of fishermen who fish around the Levantine coasts, Aegean coasts and Sea of Marmara (excluding Çanakkale Strait), respectively reported that they detected this species at least once in their nets. While only 17 % of fishermen have encountered with *C. sapidus* in the Black Sea to date. In other words, *C. sapidus* is commonly distributed around the southern and western coasts of Turkey, whereas it has rarely been observed in the Sea of Marmara and the Black Sea (Figure 1b).

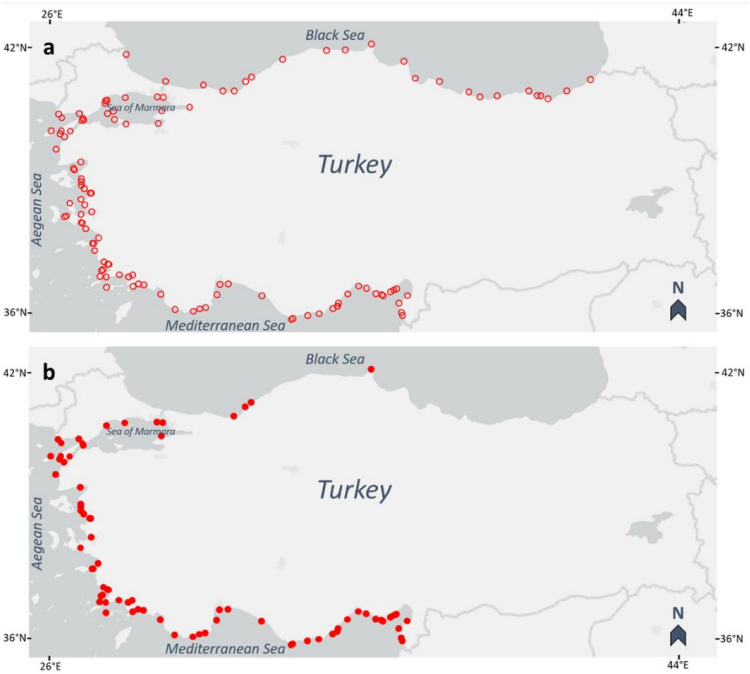

Figure 1. a) Spatial distribution of respondents, b) Spatial distribution of the recorded observations of *Callinectes sapidus* in Turkey.

In order to evaluate whether this distribution has changed over the years or not, fishermen with more than 20 years of experience were asked; "how long have you seen *C. sapidus* in your fishing grounds?" The answers related to this question proved that many fishermen around the Levantine coast of Turkey have seen this species for more than 20 years, whereas this species just started to be encountered in the Black Sea over the last several years (Table 1). Concerning the current status of *C. sapidus* population, 73 % of fishermen who fish around the Levantine coasts considered that its population has had stable characteristics for the last 10 years, whereas 44 % of fishermen who fish around the Aegean Sea reported an increased trend (Table 1). Meanwhile, a decreasing population was mainly detected by fishermen who fish around the northern Aegean Coast.

Table 1. Fishers' observations on the occurrence and population trend of *Callinectes sapidus* in Turkey.

	How long have you seen *C. sapidus*?			Population trend for the last 10 years		
Fishing ground	<10 years (%)	10-20 years (%)	>20 years (%)	Decrease (%)	Increase (%)	Stable (%)
Levantine	0	8	92	18	9	73
Aegean Sea	20	64	16	31	44	25
Sea of Marmara	90	10	0	NA	NA	NA
Black Sea	100	0	0	NA	NA	NA

The main habitats of *C. sapidus* were determined as muddy and sandy sediments by 94 % of fishermen. It was reported that this species was also rarely found in vegetated and rocky habitats. Based on commercial fishermen's responses who mainly fish at depths less than 200 m, *C. sapidus* was distributed in depths between 0.5 m to 40 m and was mainly caught in depths between 0.5 m to 10 m around Turkish coasts. In this study, ovigerous females were reported by trammel netters and gill netters from Levantine and Aegean coasts for the period between March and October, and March and September, respectively. Similarly, egg-carrying crabs were reported from lagoons between May and September. Even though we asked the question on molting periods of male and female crabs to fishermen, they could not clearly identify the molting periods. Furthermore, only a few fishermen who fished in the lagoons identified molting period as between May and August. However, they could not identify aforementioned period separately for male and female crabs.

Commercial fisheries

Coastal fishery: The results showed that *C. sapidus* was mainly caught by trammel nets and gillnets as a by-catch species. No fishermen identified the blue crab as the main target species. Fishermen who used these fishing gears noted that their main target species are red mullet, striped red mullet, shrimp, common sole, grey mullet, sea bass, blue fish and Atlantic bonito. Fishermen reported that *C. sapidus* was also rarely caught by encircling nets. In this study, a total of 9 fishermen indicated that they sold caught crabs, whereas others preferred to return them to the sea or, in rare cases, consume them. It was noticed that both male and female crabs were sold at the fishery cooperatives. The prices of *C. sapidus* ranged from 5 to 30 Turkish Lira (TL)/kg (0.56-3.36 €/kg) in 2020. There was a statistically significant difference in the reported maximum daily catch of *C. sapidus* between the Levantine and Aegean Sea (U = 48,000, P = 0.002). The higher daily catch values were found around the Levantine coasts (Figure 2).

In this study, 79 % of fishermen, used trammel and gill nets, expressed that *C. sapidus* causes damage to their fishing gears (e.g. shred the nets) and results in economic loss. Additionally, only 27 % of fishermen reported that *C. sapidus* has a negative impact on the aquatic ecosystems. Regarding blue crab fishery management in Turkey, all fishermen who sell blue crabs had information about the fishing season.

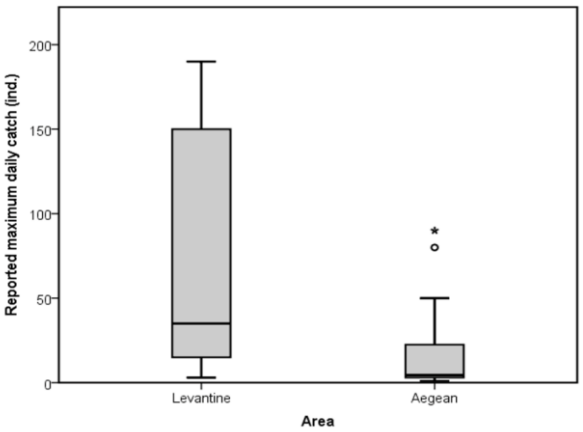

Figure 2. Reported maximum daily catch of *Callinectes sapidus* in set nets (combined data from trammel and gill nets) in Levantine coasts and Aegean coasts of Turkey in 2019.

Lagoon fishery: Blue crab fishery has been performed actively in several lagoons in Turkey. In addition to blue crab, gilthead seabream, European seabass, mullet, and European eel were stated by fishermen as the main target species in these lagoons. The blue crab has been fished at depths ranging from 0.5 to 2 m. Fishermen noticed that the main habitats of these lagoons were reported as muddy and sandy. In these lagoons, crabs have been caught mainly by barrier traps (Figure 3a). Scoop nets have been used to capture blue crabs in these barrier traps. In addition, other traps such as pots and fyke nets have been also used for catch. The pots used were mainly rectangular in shape with hexagonal wire mesh (Figure 3b) and the commonly used mesh bar size varied from 20 to 25 mm. Fish species including sardine, carp, mullet, smelt and also chicken were used as bait in traps. *C. sapidus* has been landed throughout the year in lagoons. In particular, the highest production period for blue crab was reported as between May and August. The maximum daily blue crab catch in lagoons was between 60 and 600 kg. Although the estimated annual production in these lagoons was between 1 and 15t due to limited demand, fishermen estimated the stock size of blue crabs can reach up to 150t in one lagoon in the eastern Mediterranean. Fishermen notified that the mean CW of sold crabs ranged from 13 to 16 cm in lagoons and all fishermen had information on the minimum landing size. Fishermen acknowledged that the ovigerous (sponge) was not sold from in the lagoon fishery. Both male and female crabs were exported live, however some fishermen noted that females were more resistant in transportation. Fishermen declared that these crabs were exported to China and the Netherlands and also some of them were sold at domestic markets and restaurants in Adana, Antalya,

Muğla and İstanbul. The prices of exported blue crabs ranged from 7 to 9 €/kg, while the prices of crabs sold to domestic markets ranged from 0.34 to 2.24 €/kg in 2020. All fishermen reported that blue crab causes damage to fishing nets and half of them believed blue crab also damaged other species in lagoons.

Figure 3. Fishing gears used in the lagoons; a) barrier traps, b) rectangular hexagonal mesh wire trap (photos by Gökhan Gökçe).

DISCUSSION

The occurrence of *C. sapidus* in Europe (Atlantic coast of France) has been detected since the earlier periods of the 1900's (Czerniejewski et al., 2020). The first record of *C. sapidus* in the Mediterranean was given by Giordani Soika, (1951). Due to the ballast waters, the species expanded rapidly (Mancinelli et al., 2017; Öztürk et al., 2020). *C. sapidus* was also reported from the Black Sea (Bulgurkov, 1968) and the northern Aegean Sea (Artüz, 1990). Then it spread to the Aegean and Mediterranean coasts of Turkey (Enzenroß et al., 1997). New records of *C. sapidus* found in different locations of Turkish waters were reported by Turkish Scientists (Aydın, 2017; Bilgin, 2019; Ceylan, 2020; Kocataş, 1971; Tuncer & Bilgin, 2008; Yağlıoğlu et al., 2014). The present study provided the most comprehensive information about the distribution of *C. sapidus* in Turkey. It should be noted that local ecological knowledge (LEK) is a valuable source to evaluate the distribution of invasive species in large geographical areas. Our results highlighted that the population size of *C. sapidus* was high throughout the Levantine coasts and some areas of the Aegean Sea, whereas this species was rarely observed by fishermen in the Sea of Marmara and the Black Sea (Figure 1b). This species was also recorded in some sites of the eastern Black Sea (Trabzon and Ordu-Fatsa) according to the previous studies (Bilgin, 2019; Ceylan, 2020).

The mating success and fecundity may be considered as important indicators for the adaptation of non-native species to new environments. *C. sapidus* exhibits high fecundity; ovigerous specimens can produce 8 million eggs per spawn in Chesapeake Bay (Prager et al., 1990). Previous studies showed that the Mediterranean population of *C. sapidus* also represented relatively high fecundity; which ranged from 244,000 to 7 million eggs in İskenderun, Turkey (Türeli, 1999) and from 742,652 to 7,359,642 eggs in Köyceğiz Lagoon, Turkey (Gülşahin, 2007). The success of reproduction and distribution of *C. sapidus* were dependent on water temperature (Nehring, 2011) and the optimal spawning conditions were noted as 19 – 22 ° C and 0 – 8 hr of darkness (Bembe et al., 2017). Thus, water temperature condition is proper for the reproduction and growth of *C. sapidus* in the Levantine coasts of Turkey, and this situation supports the highest catch that can be considered as relative abundance has been found in that region in the present study. However, due to the increasing trend of water temperature in the Sea of Marmara and the Black Sea, the recorded exotic species number has increased over the last years (Erdogan Saglam et al., 2010; Öztürk et al., 2020; Turan et al., 2018). Hence, the abundance of both the blue crab and other exotic species may show a dramatic increase in the Sea of Marmara and the Black Sea ecosystems in the future.

The present study revealed that, based on many fishermen's perspective, the population of *C. sapidus* was stable for the last 10 years around the Levantine coasts, whilst increase and decrease trends were reported from some fishing grounds of the Aegean Sea. Similarly, Cerri et al., (2020) collected data from recreational fishermen in Italy and reported that many fishers evaluated the population trend as "stable or fluctuating" (43.5%) or "increasing" (40.3%). On the other hand, the distribution area of *C. sapidus* widened; for instance, Mancinelli et al., (2017) noted that the number of blue crab records increased in the Mediterranean Sea and southern European waters in the last years.

Some invasive species have strong negative ecological and economic effects and cause damage to fishing gears (Galanidi et al., 2018). In the present study, many trammel netters and gillnetters (79%) reported that *C. sapidus* shredded the nets and caused significant economic loss in some fishing grounds, and 27% of coastal fishermen highlighted that this species has a negative influence on aquatic ecosystems. There is no doubt that the impact level of exotic species on fisheries depends on their abundance. For example, Cerri et al., (2020) evaluated the potential environmental consequences of *C. sapidus* in Italy, Croatia and Montenegro and many of the questionnaire survey participants did not have clear ideas about the environmental consequences, and few respondents believed *C. sapidus* has a negative effect on the environment and fishery. Besides, the economic impacts of *C. sapidus* in the small-scale fishery should be studied as a further study. However, it should be noted that not only blue crab but also other aquatic animals such as

pufferfishes, dolphins and sea turtles can cause damage to nets, such as shredding.

Our results showed that although some fishermen caught blue crab by trammel nets and gillnets around the Levantine and southern Aegean coast, due to its relatively low price compared with native species (e.g. red mullet, striped mullet, shrimp) and limited demand, this species has been mainly considered as a 'by-catch species' depending on catch rate in the coastal fishery. Hence, there is no standardized fishing gear and mesh size for the fishery of blue crab in Turkey. The size selectivity of traps and escape rates of *C. sapidus* in Turkey was evaluated in previous studies (Atar et al., 2002; Gökçe et al., 2007; Özdemir et al., 2015). For instance, Atar et al., (2002) compared the catch per unit effort (CPUE) of traps and hoop nets and noted that the mean CPUE of hoop nets was significantly higher than the mean CPUE of traps. Based on a laboratory study Gökçe et al., (2007) compared the escape success of *C. sapidus* in traps using three different square mesh barriers (35, 40, and 45 mm bar length). They reported that L50 for the 35, 40, and 45 mm bar length (mean ± s.e.) was 8.09 ± 0.12, 9.32 ±. 0.09, and 10.56 ± 0.11, respectively. Similarly, Özdemir et al., (2015) investigated the size selectivity in traps using four different mesh sizes (30, 35, 40, 45 and 50 mm) and they ascertained that using a 50 mm square mesh demonstrated high selectivity with a high escape rate of immature individuals.

The results of the present study demonstrated that the production of blue crab is mainly provided by lagoon fishery in Turkey. According to previous studies, the exploitation and management of *C. sapidus* in Turkey started in the early 1990's (Öztürk et al., 2020; Zaitsev & Öztürk, 2001). The present study also noted that the annual production of *C. sapidus* in one lagoon can reach up to 15 t. The abundance of blue crabs and its interactions with other species in lagoons should be studied in the future.

The MLS of *C. sapidus* increased from 8 to 13 cm CW in Turkey (Anon., 2020a; Anon., 2020b; Gökçe et al., 2007). The size onset of sexual maturity (SOM) of *C. sapidus* in the south coast of Turkey was determined by Türeli (1999) and the aforementioned study reported that females mature at 6.05 cm carapace length (CL), whereas males mature at 4.48 cm CL. It was reported that SOM of crabs show a difference depending on geographical areas (Gökçe et al., 2007; Türeli, 1999). Thus, to understand whether the current MLS is available or not more observations related to the SOM in the different sites of Turkey should be performed.

Another management measure related to the blue crab is fishery closed season for coastal fishery in Turkey. The current closed season of blue crab fishery (between 1st May and 30th September) spans across a relatively large period (Anon., 2020a) but according to our results ovigerous crabs can be captured from March to October. In addition, Türeli (1999) investigated the reproduction of *C. sapidus* in Yumurtalık (the southeastern coasts of Turkey) and noted that sponged females were observed from March to end of September (Table 2). Similarly, the ovigerous individuals were observed from May to October in Köyceğiz Lagoon, Turkey (Gülşahin & Erdem, 2009). On the other hand, the landing of ovigerous and soft crabs has not been prohibited in Turkey yet and the fishermen can fish blue crab with special permit throughout the year in lagoons. To develop the effective management tools, a priority should be given to investigate the ecological impacts of this invasive species on native species and the economic loss caused by *C. sapidus* in the small-scale fisheries.

Table 2. Temporal distribution of the ovigerous individuals of *Callinectes sapidus* and its current fishery closed season in Turkey.

Geographical area (Study)	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Yumurtalık (LE) (Türeli, 1999)												
Köyceğiz(AS) (Gülşahin & Erdem, 2009)												
Aegean Sea (This Study, 2021)												
Levantine (This Study, 2021)												
Current fishery closed season												

The abbreviations; LE: Levantine, AS: Aegean Sea

Consequently, the knowledge on spatial distribution of *C. sapidus* in Turkey has expanded over recent years. Due to the decline in stocks of native species in Mediterranean, some exotic species may be considered as an alternative food source for this region in the future. The blue crab is one example that started to be an economically important crustacean species of Turkey's lagoon fishery, whereas it causes monetary loss in gillnet and trammel net fishery and relatively few fishermen caught it as a secondary target species. More studies are required to reveal ecological and economic impacts of *C. sapidus* on the Mediterranean ecosystem.

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DERLEME MAKALESİ **REVİEWER PAPER**

Kedilerin Önemli Bakteriyel Zoonozu: *Helicobacter heilmannii*

Güvenç GÖKALP* **Ayşe Birsen GÖKALP**
Yozgat Bozok Üniversitesi Veteriner Fakültesi İç Hastalıkları Anabilim Dalı, Yozgat, Türkiye
Erciyes Üniversitesi Veteriner Fakültesi Mikrobiyoloji Anabilim Dalı, Kayseri, Türkiye

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*: https://orcid.org/0000-0001-9249-3567
: https://orcid.org/0000-0002-3405-6040

***Sorumlu yazarın:**
Güvenç GÖKALP
Yozgat Bozok Üniversitesi Veteriner Fakültesi İç Hastalıkları Anabilim Dalı, Yozgat, Türkiye
✉: guvenc.gokalp@yobu.edu.tr

Öz: Kedilerde zoonotik karaktere sahip olan *Helicobacter heilmannii* bakterisinin hem hayvanda hem de insanda görülme sıklığı sadece olgular dahilinde literatürlerce belirtilmiş bununla ilgili daha geniş bir hastalık ve etken taraması yapılmamıştır. Kedi sahipleri veya kedilerle aynı çevreyi paylaşan insanlarda bu bakterinin varlığı ve olası yaygınlığı gözardı edilmemelidir. Edinilen bulgularda göre *H. heilmannii*, insanlarda mukoza ilişkili lenfoid doku lenfoması (MALT)'ın olası nedenleri arasında gösterilmektedir. Şimdiye kadar yapılan çalışmalarda insanlardaki *H. heilmannii* insidansının kedilere nazaran daha az olduğu ve bulaşmanın genellikle kedilerden insanlara doğru seyir gösterebileceği görüşü yaygınlaşmaktadır. Zoonotik karaktere sahip olan *H. heilmannii* klinik belirtiler açısından diğer gastrik kökenli helikobakterlere benzerlik gösterebilmektedir. Enfekte kedilerde klinik olarak insanlardakine benzer tipik kronik aktif gastritis belirtileri görülebilmektedir. Helikobakter kökenli enfeksiyonların teşhisinde çeşitli yöntemler bulunmaktadır. Erken dönemdeki teşhiste Üre nefes testi, PCR, üreaz testi ve gastrik biyopsi yöntemlerinin yaygın olarak kullanıldığı bilinmektedir. *Helicobacter heilmannii*'nin ise etkene ait karakteristik morfolojisinin belirlenmesinde mikrobiyolojik tanı yöntemlerinden olan boyama yöntemleri kullanılmaktadır. *Helicobacter heilmannii*'den kaynaklı gastritislerin tedavi prosedürleri ise diğer helikobakter türlerinden kaynaklı gastritis tedavilerine benzerlik göstermektedir. Son olarak ise hastalığın takibi açısından uygulanan tedavi prosedürlerinin üç ay aralıklarla etken taraması yapılarak tekrarlanabileceği unutulmamalıdır. Bu derleme makalesi kedilerde bulunması muhtemel ve zoonoz olabilen *H. heilmannii*'nin halk sağlığı açısından önemine vurgu yapmayı amaçlamaktadır.

Anahtar kelimeler: *H. heilmannii*, kedi, malt, tedavi, teşhis, zoonoz.

Significant Zoonotic Bacteria of Cats: *Helicobacter heilmannii*

Abstract: The incidence of *Helicobacter heilmannii* bacteria, which has a zoonotic character in cats, in both animals and humans has only been reported in the literature within the scope of the cases, and a wider disease and agent screening has not been performed. The presence and possible prevalence of this bacterium in people who share the same environment with cat owners or cats should not be ignored. According to the findings obtained, *H. heilmannii* is shown as one of the possible causes of mucosa-associated lymphoid tissue lymphoma (MALT) in humans. In studies conducted so far, it is widely believed that the incidence of *H. heilmannii* in humans is lower than that of cats and that transmission can generally progress from cats to humans. *H. heilmannii*, which has a zoonotic character, may be similar to other gastric-derived helicobacteria in terms of clinical symptoms. The typical chronic active gastritis symptoms clinically similar to those in humans can be seen in infected cats. There are various methods for diagnosing Helicobacter infections. It is known that Urea breath test, PCR, urease test and gastric biopsy methods are widely used in early diagnosis. Staining methods, one of the microbiological diagnosis methods, are used to determine the characteristic morphology of *Helicobacter heilmannii*. The treatment procedures of gastritis caused by *Helicobacter heilmannii* are similar to the treatments for gastritis originating from other helicobacter species. Finally, it should be kept in mind that the treatment procedures applied in terms of the follow-up of the disease can be repeated by screening agents at intervals of three months. This review article aims to emphasize the importance of *H. heilmannii* in terms of public health, which can be found in cats and may be zoonotic.

***Corresponding author's:**
Güvenç GÖKALP
Yozgat Bozok University, Faculty of Veterinary Medicine, Department of Internal Medicine, Yozgat, Turkey
✉: guvenc.gokalp@yobu.edu.tr

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GİRİŞ

Helikobakter türlerinin kolonizasyonu ile gastritise ve mide ülserine sahip hasta köpek ve kediler arasında birebir bağlantı bulunmamaktadır. İnsanlarda ve evcil hayvanlarda görülen *Helicobacter pylori*, gastrit ve mide ülserinin önemli bir nedeni olarak görülse de *H. pylori* dışındaki Helikobakter türlerinin köpek ve kedilerde mide patojeni olarak rolü daha az bilinmesine karşın yaygın görülebilmektedir. Buna bağlı olarak, Helikobakter türlerinden *H. heilmannii, H. pylori, H. felis* ve *H. bizzozeronii* etkenleri ise farklı derecelerde gastritisli köpeklerden ve kedilerden izole edilebilmektedir (Tolbert & Gould, 2020). *Helicobacter heilmannii* sensu lato grubu, hayvanların midesini doğal olarak kolonize eden uzun, spiral şekilli bakteri bakterilerden oluşmaktadır (Liu vd., 2014). Hasebrouck vd., (2011) yaptıkları çalışmada, *H. heilmannii* sensu lato terimi tüm *H. pylori* olmayan helikobakterler (NHPH)'in ifade edilmesinde önerilmiştir. Bu etkenler insan ve hayvan midesinde histopatoloji, elektron mikroskobu ve ham taksonomik DNA tabanlı verilerle tespit edilmiştir. *Helicobacter heilmannii* sensu stricto terimi ise tür düzeyindeki bakteriyi ifade etmektedir (Hernandez vd., 2016). NHPH'lerin mide sağlığı açısından klinik önemi giderek artmaktadır. Bu bakteri grubunun, kediler, köpekler, domuzlar, primatlar, kemirgenler, çitalar ve tavşanlar dahil olmak üzere evcil ve vahşi hayvanların midesinde yaşayabilen, hayvanlar ve insanlar arasında geçiş yapabilen zoonotik özellikteki mikroorganizmalar olduğu bilinmektedir (Nakamura vd., 2016).

Spiral şekilli olmayan, *H. pylori* dışındaki Helikobakter türlerinin insan mide mukozasını da kolonize ettiği belirlenmiştir. İnsanlarda görülme sıklıkları ise %0,1 ile 6,2 arasında değişmektedir (Berlamont vd., 2020). *Helicobacter heilmannii* ise insan midelerinde nadirde olsa %0,1-2 oranlarda görülebilen bakteriyel bir ajandır (Ostahi vd., 2017). Van den Bulck vd., (2005) yaptıkları çalışmada bulunan en yaygın türün *H. suis* (%37) olmasının yanında bunu *H. salomonis* (%21), *H. felis* (%15), *H. heilmannii* (%8) ve *H. bizzozeronii* (%4) izlemektedir (Flahou vd., 2016). *Helicobacter heilmannii* enfeksiyonu insidansı, insanlar arasındaki tüm Helikobakter enfeksiyonlarının yaklaşık %1'ini oluştursa da sosyoekonomik durumu zayıf ülkelerde görülme sıklığının çok daha yüksek olduğu bilinmektedir (Majumber vd., 2020). Yapılan çalışmalarda ise *H. heilmannii* insanlarda semptomatik hastaların %5'inde bulunmasına karşın *H. heilmannii* tarafından oluşturulan kolonizasyonlardaki farklılıklar ile pylori negatif ve pylori pozitif hastalar arasındaki ilişkinin baz alındığı herhangi bir çalışma yapılmamıştır (Nakamura vd., 2016; Mladenova vd., 2017; Mohammadi vd., 2019).

Etiyoloji: Helikobakter türleri Gram (-), mikroaerobik, hareketli, fuziform, spiral şekilli veya spiral çubuk morfolojisine sahip olabilen bakterilerdir. Farklı türleri arasında sayısı ve bulunduğu yere göre değişkenlik gösteren flagellalarını kullanarak hareket edebilmektedir (Hong vd., 2015). Yüz elli omurgalı hayvanlardan alınan dışkı örneklerinde Polimeraz Zincir Reaksiyonu (PZR) ile 45 Helikobakter türü tespit edilmiştir (Elyasi vd., 2020). *Helicobacter heilmannii* ise %20-100 arasındaki prevalans oranları ile köpek ve kedilerin mide mukozalarını doğal olarak kolonize edebildiği bilinmektedir (Kubota vd., 2017; Matos vd., 2020). 16S rRNA gen dizilerinin analizi sonucu %96,6 benzerlik oranlarına rağmen *H. heilmannii* tip 1 ve tip 2 olarak sınıflandırılma yapılmış, morfolojik açıdan benzer organizmalar genellikle kedilerdeki *H. felis*, köpeklerdeki *H. bizzozeronii* ve *H. salomonis*, domuzlardaki ise *H. suis* olarak adlandırılmıştır (Ülgen vd., 2016).

Helicobacter heilmannii ve *H. felis*'in dahil olduğu *H. pylori* dışındaki Helikobakter türleri, *H. heilmannii* benzeri organizmalar (HHLO'lar) olarak da adlandırılmaktadır (Goji vd., 2015). *Gastrospirillum hominis*'in gastrik kökenli olan *H. heilmannnii*'nin ilk adı olduğu bilinmektedir (Mladenova vd., 2017). *Helicobacter heilmannii* ismi yapılan sekans analizleri sonucu bulunan 16S rRNA geni ile bu bakterinin mide mikroorganizmalarından Helikobakter cinsine ait olduğu belirlendikten sonra verilmiştir (Joosten, 2017). *Helicobacter heilmannii* mikroskobik tanım açısından *H. pylori*'nin sahip olduğu spiral morfolojiye benzemektedir. *Helicobacter pylori* 2,5-4 mm uzunluğunda iken, *H. heilmannii* ise 7-10 mm uzunluğundadır. *Helicobacter heilmannii* ise hücre başına dört ile altı dönüşlü spiral yapıya ve kutup başına 12 flagellaya kadar sahip olabilmektedir (Hernandez vd., 2016).

Kedilerde H. heilmannii: Kedilerdeki ana gastrik Helikobakter türleri öncelikle *H. heilmannii* ve *H. felis*'tir. Bu iki türün kedilerde prevalansının %57 ile 100 arasında olduğu bildirilmiştir (Hong vd., 2016). Helikobakter enfeksiyonlarının köpek veya kedi grupları arasındaki bulaşması kesinlik kazanmamasına bağlı olarak rezervuar konakçıları da tanımlanmamıştır. İnsanlarda Helikobakter enfeksiyonlarına bağlı elde edilebilen morbidite ve mortalite oranları zoonotik bulaşma riskinin artabileceğini göstermektedir (Blois, 2020).

Kedi ve köpeklerde yaygın olarak görülebilen ve *H. heilmannii* tip 2 grubunda bulunan *H. salomonis*'in insan midesinde de sıkça kolonize olabildiği bilinmektedir (Liu vd., 2014). *Helicobacter spp* prevalansının %41-100 oranlarında olmasına karşın baskın türler olan *H. heilmannii* ve *H. felis* , subglandüler ve gastrik mukozada hafif veya şiddetli diffuz lenfoplazmasitik infiltrasyona neden olabilmektedir (De sousa vd., 2019).

Helicobacter heilmannii'nin *Helicobakter spp.* pozitif vakalı olan kedilerle, gastrik hastalığı olan hasta insanlardan izole edilen Helikobakter türleri arasında % 99

oranında sekans benzerliği tespit edilmiştir (Kubota vd., 2017). *Helicobacter heilmannii* enfeksiyonu kronik aktif gastrit ile ilişkilendirilmiş olsa da köpeklerde ve kedilerde gastrit, peptik ülserasyon ve kronik kusma açısından değerlendirildiğinde bu hayvan türleri için patojenik önemi netlik kazanmamıştır ancak etkenin suşuna bağlı olarak enfeksiyonun patojenitesi değişkenlik gösterebilmektedir (Matos vd., 2020).

Helicobacter heilmannii enfeksiyonu ile birlikte MALT lenfoma: *H. heilmannii* sensu lato grubu bakterilerinin insan mide biyopsi örneklerinin %0,2-6'sında gözlendiği ve insanlarda kronik gastrit, peptik ülser ve gastrik düşük dereceli mukozayla ilişkili (MALT) ile ilişkilendirilmektedir (Liu vd., 2014). *Helicobacter heilmannii*'nin yüksek düzeydeki metabolizmasına bağlı olarak çok yönlü ve farklı çevresel etkilere tepki verme yeteneğine sahip olması insanlarda *H. heilmannii* enfeksiyonunun zoonotik doğasının temelini oluşturduğunu göstermektedir (Mladenova vd., 2017). *Helicobacter pylori* dışındaki Helikobakter türleri birçok hayvanın midesinde kolonize olsa bile ancak bazıları zoonotik potansiyel gösterebilmektedir. Özellikle kedi ve köpeklerle bulunabilen *H. suis* ve *H. heilmannii* etkenleri, gastritis, mide veya duodenum ülseri veya MALT lenfoması olan hasta insanlarda tespit edildiği bilinmektedir (Berlamont vd., 2020). *Helicobacter heilmannii* etkeni *H. pylori*'ye kıyasla gastrik MALT lenfomalarının oluşumunda daha fazla rol oynamaktadır. *Helicobacter pylori* esas olarak mukozal tabakayı kaplarken, *H. heilmannii* ise antral bezlerin derinliklerine kadar yayılım gösterebilmektedir (Shafaie vd., 2020). Nodüler yapılı gastritis vakalarının %40'ında, MALT lenfoma vakalarının %24'ünde, kronik gastiritislerin %17'sinde ve gastroduodenal ülser vakalarının %33'ünde Helikobakter türleri açısından pozitiflik saptandığı bildirilmektedir (Nakamura vd., 2020). İnsanlarda ise benzer semptoma sahip hastalara yapılan mide biyopsilerinin %8-19'unda *H. heilmannii*'ye rastlanılmaktadır (Bahadori vd., 2018; Matos vd., 2020).

Nakamura ve arkadaşlarının yaptıkları bir çalışma ise farelerde deneysel olarak oluşturulan *H. heilmannii* ilişkili gastritisin sadece midede değil aynı zamanda hepatik ve pulmoner kökenli MALT lenfomayı da tetikleyebildiğini göstermektedir (Sikiric & Brzozowski, 2020). Tedavi protokollerinde kullanılan antibiyotiklerden, özellikle klaritromisinin potansiyel immünomodülatör veya hatta doğrudan anti-neoplastik etkilerinin olabileceği ve bu yolla eradikasyon tedavisi açısından önemli katkıda bulunabileceği bildirilmektedir. Ayrıca kullanılan bu antibiyotiklerin, aynı zamanda MALT lenfoma gelişimi ile bağlantılı olan *H. heilmannii* gibi *H. pylori* dışındaki mikroorganizmaları da ortadan kaldırabileceği öngörülmektedir (Jung vd., 2021).

***Klinik Bulgular*:** NHPH enfeksiyonları ile ilişkili klinik semptomlar arasında akut veya kronik epigastrik ağrı, mide bulantısı, dispepsi, reflü özofajit, mide ekşimesi, kusma, hematemez, abdominal ağrı, düzensiz dışkılama, dışkı kıvamında ve iştahta azalmanın eşlik ettiği disfajinin bulunduğu bilinmektedir (Flahou vd., 2016). Bu semptomların yanısıra ot yeme, sarkık pozisyonda kalma, kaprofaji, allotriofaji, anokreksi, ruktus ve meteorizm gözlenmektedir (Ülgen vd., 2016). *Helicobacter heilmannii* enfeksiyonu sadece klinik bulgulara bakılarak gıdaya duyarlı enteropatiler, bakteriyel hipertrofi, IBD (Yangısal Barsak Hastalığı) veya parazitik enteropati gibi olgularla karıştırılabilmektedir (Rychlik & Kaczmar, 2020). *Helicobacter heilmannii* enfeksiyonu ile gözlenen gastrit, *H. pylori*'ye bağlı olandan daha az şiddetli olma eğilimindedir (Mladenova vd., 2017).

Mide kaynaklı helikobakterler, midede herhangi bir klinik semptom göstermeden bulunabilmekte, ancak safra kanalında fırsatçı patojen özellik kazanabilmektedir (Takemura vd., 2019). Hem sağlıklı hem de hasta köpek ve kedilerde Helikobakter'in yüksek prevalans göstermesi, bu hastalığın klinik önemi ve teşhisi açısından köpek ve kedilerde belirsizliğe neden olabilmektedir (Blois, 2020). Kronik kusma, Helikobakter enfeksiyonundan muzdarip kediler ve köpekler için açıklanan temel klinik bulgudur. İnsanlarda olduğu gibi kedi ve köpeklerde görülebilen Helikobakter türleri, kronik aktif gastritis ile gastrik kökenli lenfoma ise *H. heilmannii* enfeksiyonu ile ilişkilendirilmektedir (Joosten, 2017).

Tanı: Helikobakter enfeksiyonunu saptamak için farklı düzeylerde duyarlılık ve özgüllükte invaziv veya non-invaziv tekniklerin kullanıldığı çeşitli teşhis yöntemleri geliştirilmiştir. İnvaziv yöntemler, histoloji, kültür ve mide doku biyopsilerinin kullanıldığı hızlı üreaz testi ile endoskopik yöntemlerden oluşmaktadır. İnvaziv olmayan testler arasında üre nefes testi ve fekal antijen testleri biyopsi temelli tekniklerle karşılaştırılaştırıldığında doğru sonuca hızlı ulaşabilen teşhis prosedürleri olarak görülmektedir. Ayrıca, bu testlerin tedavi sürecinin takibi açısından tercih edilebilecek yöntemlerden olduğu bilinmektedir (Sabbagh vd., 2019).

Enfeksiyöz etiyolojilerin histopatolojik veya immünohistokimyasal açıdan tanımlanmasını gerekmektedir. Kedilerde ve köpeklerde Helikobakter ile ilişkili gastritis mide biyopsilerinden yapılan floresan in situ hibridizasyon (FISH) PZR uygulamaları kesin tanı açısından önemini korumaktadır (Tolbert & Gould, 2020). Dışkı antijen testi, Helikobakter eradikasyonunu doğrulamakta yararlı görülse bile poliklonal antikorların kullanıldığı test tipinin tanısal doğruluğu diğer tanı yöntemlerine göre düşük olabileceği düşünülmektedir. Dışkı antijen testi'nin, üre nefes testi kadar tanısal açıdan benzer doğruluğa sahip olabilmesi için test tipinde monoklonal antikorların

kullanımı önerilmektedir (Shiotani vd., 2016). Kubota vd., (2005) yaptıkları bir çalışmada *H. heilmannii* tanısına gitmek için yapılan ureAB genlerinin sekans analizleri sonucu *H. heilmannii* etkeninin neredeyse tüm Helikobakter-pozitif vakalarda büyük oranlarda tespit edildiği ve bu nedenle farklı NHPH ile enfekte olgular arasında gastrik histopatolojik tanıların bir karşılaştırmasının yapılamayacağı bildirilmektedir (Kubota vd., 2017). Köpeklerde ve kedilerde kronik enfeksiyon şeklinde seyreden gastrointestinal sistem hastalıklarında gastroduodenoskopi ve kolonoskopi ile kombinasyon halinde uygulanabilen endoskopik yöntemlerin teşhis açısından etkili olduğu bilinmektedir (Rychlik & Kaczmar, 2020). Endoskopik yöntemler, özellikle peptik ülser olgularına bağlı olarak Helikobakter varlığının belirlenmesinde kullanılan en etkili yöntemlerdir. Bununla birlikte, endoskopi, non-invaziv testlerden daha riskli olabilmekte ve uygulama esnasında birçok hasta için rahatsızlık verebilmektedir (Alzoubi vd., 2020). Hızlı üreaz testleri mide biyopsilerinde bakteriyel üreaz üretiminin saptanmasına bağlı olarak Helikobakter varlığının tespitinde kullanılan testlerdir. Ancak, hızlı üreaz testleri gibi biyopsi temelli olan histopatolojik testler ise tanısal açıdan hızlı üreaz testlere nazaran daha duyarlı ve spesifik testlerdir (Blois, 2020). Hızlı üreaz testlerinin prensibi bakteriyel üreazın üreyi amonyak ve karbondioksite ayrıştırması özelliğine dayanmaktadır. Bu yöntemde Helikobakter içeren bir mide biyopsisi üre içeren bir ortama yerleştirildikten sonra bakteriyel üreaz tarafından üretilen amonyağın pH'ı yükseltmesi ile birlikte pH indikatöründe renk değişikliğinin oluşmasına bağlı olarak bakteri varlığı tespit edilmektedir (Shiotani vd., 2016).

Üre Nefes Testi: Üre nefes testi (ÜNT), Helikobakter kökenli enfeksiyonların tanısında en çok önerilen, invaziv olmayan, yüksek duyarlılık ve özgüllüğe sahip bir yöntemdir (Hong vd., 2016). ÜNT, yaklaşık %95'lik duyarlılık ve özgüllük gösterebilen doğru teşhis yöntemi olarak kabul edilmektedir (Atkinson & Braden, 2016). ÜNT, patojenin yok edilip edilmediğinin belirlenmesinde güvenilir ve değerli bir yöntem olduğu bilinmektedir. Testin uygulanmasında öncelikle işaretlenmiş izotop içeren üre ağız yoluyla verilmektedir. Daha sonra, Helikobakter etkeni varlığında midede üretilen bakteriyel üreaz ölçümü yapılmaktadır. Bunun yanında, kanda ve solunum havasındaki karbondioksit miktarları ise kütle spektrofotometre yöntemiyle ölçülebilmektedir (Sabbagh vd., 2019). Kütle spektrometresinin yanısıra dağılmayan izotop seçici kızılötesi spektroskopi yöntemi de üre nefes analizinde kullanılabilmektedir (Atkinson & Braden, 2016). Bunun yanında, Helikobakterin üreyi hidroliz edebilme özelliğine dayanan işaretlenmiş karbon atomu uygulamasında radyoaktif izotop olan C^{14} veya radyoaktif olmayan C^{13} izotopları kullanılmaktadır. Bu izotoplarla işaretli üre etkenin ürettiği üreaz enzimi ile parçalanması sonucu açığa çıkan karbondioksitin solunum havasında saptanması esasına dayalı bir yöntem de bulunmaktadır (Gökalp, 2013).

Genellikle, *H. pylori* etkenli enfeksiyon teşhisi için kullanılan ÜNT, *H. heilmannii* ile enfekte hastalarda negatif çıkabilmektedir. Bu durum NHPH bakterilerinden kaynaklı enfeksiyonlarının, *H. pylori* enfeksiyonlarının aksine, daha sıklıkla fokal yolla bulaşmasından ve ağırlıklı olarak midenin antrumunda bulunmasından dolayı bu test türler arasındaki sınırlı tanısal değerleri ortaya koyabilmektedir (Joosten, 2017). NHPH kaynaklı olduğu bilinen enfeksiyonlarda ÜNT'inin hem negatif hem de zayıf pozitif sonuçlar verebilmesi üre nefes testinin NHPH kaynaklı enfeksiyonların tespitinde uygulanmasını zorlaştırmaktadır (Nakamura vd., 2020). Ağız boşluğu veya mideden izole edilen *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Proteus mirabilis*, *Enterobacter cloacae* ve *Citrobacter freundii* gibi çeşitli mikroorganizmalar da üreaz aktivitesi göstermekte ve yanlış pozitif sonuçlar vermektedir (Sabbagh vd., 2019). ÜNT'i, köpek ve kedilerde Helikobakter tedavi sürecinin takibinde kullanılmaktadır. Kombine uygulanan tedavi prosedürlerinden sonra kusma ve benzeri belirtilerin tekrar etmesi halinde tedavi sürecinin kontrol edilmesi açısından yeniden uygulanacak olan ÜNT'rinin yanında mide biyopsisi temelli uygulamalar da yapılmalıdır (Takemura vd., 2019). Enfeksiyon için uygulanan üçlü tedavi prosedürünü bitirdikten yaklaşık 6 hafta sonra eradikasyonun sağlanıp sağlanılmadığını öğrenebilmek için ÜNT'i ile dışkı antijen testlerine ihtiyaç duyulmaktadır (Alzoubi vd., 2020). Bazı çalışmalar ÜNT'inin gastritis, özofagus patolojileri ve ülserlerin varlığını doğrulayamadığını belirtmesine rağmen, bu test hastalığın erken teşhisi ve tedavi sürecinin etkin olabilmesi açısından dışkı antijen testi de dahil olmak üzere diğer birçok invazif olmayan testler arasından tercih edilmektedir (Sabbagh vd., 2019).

Mikrobiyolojik Tanı: Helikobakter türleri arasında ilk olarak kedilerin midesinden izole edilen ve köpeklerde de spiral şeklinde kolonileşmeyi yapan tür *H. felis*'tir (Nakamura vd., 2016). *Helicobacter heilmannii* ile benzerlik gösteren *H. felis*'in yanında *H. bizzozeronii* ve *H. salomonis* de kedi ve köpeklerden izole edilmektedir (Mladenova vd, 2017). 16S rRNA dizisinin analizi ile *H. heilmannii*'in tip 1 ve tip 2 alt sınıflandırmalarının yapılmasından sonra, *H. suis*'in *H. heilmannii* tip 1'i temsil eden tek tür olduğu ve *H.felis*, *H. bizzozeroniii*, *H. salomonis*, *H. cynogastricus*, *H. baculiformis* ve asıl *H. heilmannii* türlerinin ise *H. heilmannii* tip 2' yi temsil ettiği bildirilmektedir (Hernandez vd., 2016). *H. heilmannii*, *H. pylori* ile karşılaştırıldığında *H. heilmannii*'nin olduğu daha uzundur ve insandaki karakteristik morfolojisi ise hematoksilin-eosin, Giemsa veya gümüş boyama ile görülebilmektedir. *H.*

heilmannii'nin özellikle midenin antrumunda bulunduğu ve touch smear sitoloji ile daha iyi teşhis edilebildiği bilinmektedir (Ostahi vd., 2017).

PZR analizlerinde *H.pylori* ve NHPH (*H. suis, H. heilmannii, H. felis, H. bizzozeronii ve H. salomonis*) 'lere ait DNA ekstraksiyonları kullanılmaktadır. *H.pylori* için ureC geninin amplifikasyonu, NHPH türleri için de ureA geninin amplifikasyonu uygulanmakta ve DNA varlığının doğrulanması için de pozitif olan tüm örneklerde sekans analizi yapılabilmektedir (Bahadori vd., 2018). Helikobakter ile enfekte hayvanların mide dokusunda, sırasıyla CagA proteinini kodlayan cagA ve ureC sekansları ve üreazın alt birimi C, Polimeraz Zincir Reaksiyonu ile tespit edilebilmektedir (Gonciarz vd., 2021). Antibiyogramı yapılmış kedi izolatlarının çoğunda ampisilin, klaritromisin ve tetrasikline karşı direnç görülmesine rağmen *H. felis* pozitifliği olan örneklerde ise metronidazole karşı direnç görülmektedir (Goldstein & Abrahamian, 2015). Gastrik biyopsi muayenesinin morfolojik yönden teşhiste önemli olduğu bilinmektedir. *Helicobacter heilmannii*'nin karakteristik morfolojisi ise Hematoksilen-Eozin, Giemsa veya Warthin-Starry ile boyanmış biyopsi örneklerinde tanımlanabilmektedir (Goji vd., 2015)

Tablo 2. *Helicobacter heilmannii*'nin diğer gastrik Helikobakter türlerine kıyasla morfolojik ve biyokimyasal özellikleri (Joosten vd., 2016).

Helikobakter Türleri	Uzunluk(µm)	Periplasmik fibril	Flagella dağılımı	Üreaz aktivitesi	Nitrat üretimi	Alkalin Fosfataz aktivitesi
H. heilmannii	3–6,5	-	Bipolar	+	+	-
H. felis	5–7,5	+	Bipolar	+	+	+
H. bizzozeronii	5-10	-	Bipolar	+	+	+
H. salomonis	5-7	-	Bipolar	+	+	+
H. pylori	2,5-5,5	-	Monopolar	+	-	+

Bahadori vd., (2018) yaptığı çalışmada, histopatolojik analiz için alınan biyopsilerin %10 fosfat tamponlu formalin'de sabitlendikten sonra, rehidre edildiği ve 5 µm'de kesitli parafin içinde gömüldükten sonra parafinden arındırılarak hematoksilin ve eozin (HE) ile boyanarak ışık mikroskobik altında incelendiği ve elde edilen boyanmış gastrik biyopsilerde ise eğri şekilli veya spiral şekilli formlarda helikobakterlerin varlığının gözlemdiği bildirilmiştir (Bahadori vd., 2018). Dışkı antijen testinde ise dışkı örneklerindeki Helikobakter spesifik antijeni, bir enzim immünoassay veya immünokromatografi kullanılarak bakteri varlığı açısından saptanabilmektedir. İmmünokromatografik özellikteki dışkı antijen testlerinde laboratuvar ekipmanına ihtiyaç duyulmamaktadır. Monoklonal dışkı antijen test kitlerinin duyarlılığı poliklonal teknikten daha yüksek olduğu ise bilinmektedir (Atkinson & Braden, 2016).

Tedavi: Helikobakter etkenleri etkin antimikrobiyal tedavinin yokluğunda mideyi kolonize ettikten sonra bir ömür boyu konakçıda yaşayabilmektedir (Joosten vd., 2017). Helikobakter enfeksiyonlarında uygulanan gastrik biyopsi muayenesi enflamasyon derecesinin belirlenmesinde, enflamatuar ve neoplastik lezyonların (iyi huylu veya kötü huylu) ayırt edilmesinde ve uygulanan herhangi bir tedavi prosedürünün değerlendirilmesinde kullanılabilmektedir (Rychlik & Kaczmar, 2020). Helikobakter enfeksiyonlarının tedavisi için antibiyotikler ve asit baskılayıcılardan oluşan kombinasyon tedavi önerilmektedir. Köpek ve kedilerdeki Helikobakter kaynaklı enfeksiyonlar için uygulanacak tedavi şekilleri ise Helikobakter varlığında ortaya çıkabilen uygun klinik semptomlar ve mide lezyonlarının birlikte değerlendirilmesine göre değişkenlik gösterebilmektedir (Blois, 2020).

Tedavide kullanılan proton pompası inhibitörleri, antibiyotikler ve bizmut bileşikleri kombinasyonu yanıltıcı negatif sonuçlara neden olabilmektedir (Sabbagh vd., 2019). *Helicobacter heilmannii* tedavisi, ciddi klinik bulguları ve patolojisi olan insan hastalar için de endike olabileceği bilinmektedir. Günümüzde *H. heilmannii* tedavisi *H. pylori* eradikasyonu için kullanılan üçlü tedavi prosedürü ile aynıdır. Üçlü terapi prosedürü metronidazol veya amoksisilin ile birlikte iki antimikrobiyal ajan olan klaritromisin ve tetrasiklin ile omeprazol gibi bir proton pompası inhibitörünü içermektedir (Joosten, 2017). Köpek ve kedilerdeki gastrik *Helikobakter spp.* enfeksiyonlarının tedavisinde insanlardakinden farklı olarak asit supresyon takviyesine gerek duyulmadığı ancak bizmut türevlerinin tedaviye dahil edilmesinin gerekli olabileceği belirtilmektedir (Gökalp, 2013). Kedi izolatlarının çoğu ampisilin, klaritromisin ve tetrasikline duyarlıdır, oysa iki Helikobakter suşu metronidazole karşı direnç göstermektedir (Goldstein & Abrahamian, 2015). Bizmut veya bizmut içermeyen terapi modelinin (proton pompa inhibitörü, tetrasiklin, metronidazol ve bizmut tuzu) klaritromisine veya metronidazole karşı direncin olduğu durumlarda yararlı olabileceği bilinmesine rağmen bu modeldeki çoklu antibiyotik kullanımında tedavi sürecinin uzamasına bağlı olarak da antimikrobiyal direncin artabileceği bildirilmektedir (Garcia vd., 2021). Tedavinin değerlendirilmesinde hasta hayvandan alınan dışkı örneklerinden genomik DNA ekstraksiyonu uygulanmaktadır. Etkende bulunan üreaz B geninin çoğaltılmasında için de bir multipleks Helikobakter spesifik PZR testi yapılmaktadır. Hastalığın takibi açısından bu uygulama hasta hayvanlarda antibiyotik tedavisinden üç ay sonra tekrarlanabilmektedir (Hong vd., 2015).

Zoonotik Önemi: Kedilerden insanlara bulaşabilecek veya muhtemelen kediler ve insanların ortak yaşam alanlarından kaynaklardan edinebilecekleri çok sayıda hastalık bulunmaktadır. Bununla birlikte, evcil kedinin henüz tanımlanmamış birçok zoonoz için potansiyel rezervuar olabileceği bilinmektedir (Goldstein & Abrahamian, 2015). *Helicobacter heilmannii*'nin gelişmiş ülkelerde %0,5, Doğu Avrupa ve Asya ülkelerinde ise %1,2-6,2 arasında değişebilen düşük prevalanslara sahip olduğu bilinmektedir (Mladenova vd., 2017). Kedilerin ve köpeklerin, gastrik Helikobakter türleri için rezervuar konak olmasından dolayı zoonotik potansiyel göstermektedir. Köpek ve kedilerde görülen *H. pylori* dışındaki helikobakter türlerinin zoonotik potansiyeli bir dizi vaka çalışması ile belirlenmektedir. *Helicobacter heilmannii*'deki zoonotik potansiyel ise bu etkenin kedilerden sahiplerine doğrudan temas yoluyla bulaşabilmesinden kaynaklanmaktadır (Joosten, 2017).

Tablo 3. Kedilerde görülen Gastrik Helikobakter türleri ve bulunduğu konaklar (Joosten, 2017).

Takson	Doğal Konak	Zoonotik Potansiyeli
H. baculiformis	Kedi	Bilinmiyor
H. bizzozeronii	Kedi, Köpek	Var
H. felis	Kedi, Köpek,Tavşan	Var
H. heilmannii	Kedi, Köpek, Domuz	Var
H. salomonis	Kedi, Köpek,Tavşan	Var

Helicobacter heilmannii'nin görülme sıklığının değişkenliği bulaşma kaynağı açısından çeşitliliğin olduğunu göstermektedir. Öte yandan, *H. heilmannii*'nin insanlarda görülen gastrointestinal hastalıklar ile bağlantılı olduğu ve bunun histopatolojik bulgularla desteklenebildiği bilinmektedir (Shafaie vd., 2020). Evcil hayvanların midesinde, sütünde ve etinde insanlarda görülen helikobakter türlerindeki vacA genotiplerine benzer yüksek miktarda suşlarının bulunması hayvansal kaynaklı gıdalardan da Helikobakterlerin bulaşabileceğini göstermektedir (Park vd., 2020). Gastritise ait klinik belirtiler gösteren insanlarda yapılan endoskopik muayenelerde biyopsi sonucu Helikobakter türlerinin tespit edildiği ve bu türler arasında *H. heilmannii*'ye rastlanıldığı belirtilmektedir (Majumber vd., 2020). Ayrıca *H. heilmannii* tip 1 olarak adlandırılan *H. suis*, insana en çok bulaşan *H. pylori* dışındaki Helikobakter türlerinden olduğu bilinmektedir. Kedi ve köpeklerde baskın halde bulunan ve *H. heilmannii* tip 2 olarak adlandırılan *H. felis*, *H. bizzozeroniii*, *H. salomonis*, *H. cynogastricus*, *H. baculiformis* ve asıl *H. heilmannii* türlerinin ise *H. heilmannii* tip 1'e göre daha az oranda zoonotik etkisi olduğu belirtilmektedir (Nakamura vd., 2016).

Korunma: Helikobakter enfeksiyonunu veya bununla ilişkili patolojileri önlemeye yönelik bazı yeni yaklaşımların olduğu ve bu bağlamda Saccharomyces, Lactobacillus ve *Bifidobacterium spp.* gibi probiyotik kullanımlarının proflaktik etki yaratabileceği doğrulanmaktadır. Gelecekteki araştırmalarda, Helikobakter enfeksiyonu ile ilişkili hastalıkların önlenmesinde ve tedavisinde yeni stratejilerin potansiyel değerlerinin belirlenmesi ve antimikrobiyal direnç konusuna durumlara odaklanmanın önemi bilinmektedir (Pere Vedrenne vd., 2017). Immunoglobulin G antikorlarının tedaviden sonraki aylarda saptanabilmesi ve bu nedenle bakteriyel klirensten sonra bile pozitif sonuç verebilmesi dikkate alınmaktadır (Sabbagh vd., 2019). Tedavide kullanılan antimikrobiyal ajanların etken direncini arttırması eradikasyon tedavisinde yıllar boyunca daha az başarı elde edilmesine ve aşılama benzeri alternatif yaklaşımlara ihtiyaç duyulmasına neden olabilmektedir (Pan vd., 2018). Profilaktik ve terapötik aşılamanın, gastrik. Helikobakter enfeksiyonlarının önlenmesi ve tedavisi için en umut verici alternatif yaklaşım olduğu öngörülmektedir. (Joosten, 2017). Kanıtlar, aşılamada aşıların, farklı yollar, farklı antijenler ve adjuvantlardan oluştuğunu göstermektedir. Bu uygulamanın ise ülser ve mide kanseri riskinin azaltılmasında kullanılabileceği söylenmektedir. Tabii ki, aşılar söz konusu olduğunda, bazı aşılama stratejilerinin insanlarda test edildiği, ancak neredeyse hiçbir zaman eradike edici bağışıklığa ulaşamadığı bilinmektedir (Sabbagh vd., 2019).

Sonuç: Sonuç olarak, Helikobakter kökenli enfeksiyonların etken ve konak düzeyinde iyi sınıflandırılması gerekmektedir. Bunun için de sağlıklı anamnezin yanısıra teşhis metotlarının, insidans araştırmalarının, tedavi yaklaşımlarının ve zoonotik potansiyellerinin ciddi şekilde ele alınması önemlidir. Kedilerde görülen *H. heilmannii*'nin değerlendirilmesinde kedilerin yaşadığı çevre koşulları ve beslenme alışkanlıkları göz önünde bulundurulmalı hem kedi sahiplerinin hem de kedilerin sağlığını tehdit edebilen zoonoz karakter kazanmış olan *H. heilmannii* kökenli enfeksiyonların önüne geçebilecek etkin teşhis ve tedavi protokolleri oluşturulmalıdır.

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KISA MAKALE SHORT PAPER

A Preliminary Study on Zooplankton Fauna of Kızılca Pond (Konya-Seydişehir/Turkey)

Meral APAYDIN YAĞCI* **İsmail ERBATUR** **Abdullah DEMİR** **Abdulkadir YAĞCI**
[1] *Fisheries Research Insititute, Eğirdir, Isparta, Turkey*

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*: https://orcid.org/0000-0002-2108-1853
: https://orcid.org/0000-0003-1613-6650
: https://orcid.org/0000-0001-8881-2932
: https://orcid.org/0000-0002-7897-1734

***Corresponding author's:**
Meral APAYDIN YAĞCI
Fisheries Research Insititute, Eğirdir, Isparta, Turkey
✉: meralyagci@gmail.com

Abstract: Kızılca Pond has the most intensive population of Giant Spring Minnow fish (*Pseudophoxinus anatolicus*). To draw attention to the distribution area of the Anatolian endemic species, this pond was chosen as the study area and this preliminary study was carried out on one-time zooplankton species diversity in July 2018. Zooplankton samples were taken from the pelagic part of the lake at 8 m depth (sampling just once from this depth). Also, samples were collected with Hydro-Bios model plankton net with 55 µm mesh size. Physicochemical parameters of the lake (pH, conductivity, water temperature, dissolved oxygen concentration, saturation of dissolved oxygen) were measured. The zooplankton fauna of the pond consist of Rotifera (87%) and Cladocera groups (13%). The dominant species were recorded *as Asplanchna priodonta, Keratella cochlearis* and *Testudinella patina*.

Keywords: Cladocera, freshwater, kızılca pond, physicochemical parameters, rotifera.

Kızılca Göleti (Konya-Seydişehir/Türkiye) Zooplankton Faunası Üzerine Bir Ön Çalışma

***Sorumlu yazar:**
Meral APAYDIN YAĞCI
Su Ürünleri Araştırma Enstitüsü Müdürlüğü, Eğirdir, Isparta, Türkiye
✉: meralyagci@gmail.com

Öz: Kızılca Göleti en yoğun yağ balıkları (*Pseudophoxinus anatolicus*) popülasyonuna sahiptir. Anadolu endemik türünün yayılış alanına dikkat çekmek amacıyla çalışma alanı olarak bu gölet seçilmiş ve bu ön çalışma Temmuz 2018'de tek seferlik zooplankton tür çeşitliliği üzerinde gerçekleştirilmiştir. Zooplankton örnekleri 8 m derinlikte (bu derinlikten sadece bir kez örnekleme) gölün pelajik bölgesinden alınmıştır. Ayrıca, örnekler 55 µm göz açıklığındaki Hydro-Bios model plankton kepçesi ile toplanmıştır. Gölün fizikokimyasal parametreleri (pH, iletkenlik, su sıcaklığı, çözünmüş oksijen konsantrasyonu, çözünmüş oksijen doygunluğu) ölçülmüştür. Göletin zooplankton faunası Rotifera (%87) ve Cladocera gruplarından (%13) oluşmuştur. Baskın türler *Asplanchna priodonta, Keratella cochlearis* ve *Testudinella patina* olarak kaydedilmiştir.

Anahtar kelimeler: Cladocera, fizikokimyasal parametreler, kızılca göleti, rotifera, tatlısu.

INTRODUCTION

In the lake ecosystem, zooplanktonic organisms transfer carbon and energy from the primary producers to the living beings at higher trophic levels, such as invertebrates, fish and waterfowl, and limit the presence of phytoplankton. Rotifera is a large group of zooplankton (Apaydın Yağcı et al., 2015; Bulut & Saler, 2018; Tasevska et al., 2017). Some zooplankton types, especially Rotifers, are used as indicators to determine the water quality of freshwater ecosystems (Jeppesen et al., 2011; Saksena, 1987).

Turkey inland zooplankton checklist consists of a total of 661 taxa including 417 Rotifera, 103 Cladocera and 141 Copepoda (Ustaoğlu, 2015). In Turkey, there are studies on zooplankton fauna in some ponds such as Tadım, Beytepe, Topboğazı, TMİ 12, Alıç, Şeker-Reşadiye-Zincidere, Orduzu, Kaldırım and Halikan, Keçiborlu and Kapıaçmaz (Apaydın Yağcı et al., 2017; Bozkurt & Dural, 2005; Bulut, 2018; Bulut & Saler, 2016; Güher & Erdoğan, 2008; Gürel & Saler, 2015; Kaya et al., 2009; Korkmaz & Korkmaz, 2002; Saler & Arslan, 2007; Saler & Şen, 2002). The purpose of this study is to conduct zooplankton study for the first time in Kızılca pond and to contribute to the biodiversity of zooplankton in Turkey.

Figure 1. Anatolian endemic Giant Spring Minnow fish (*Pseudophoxinus anatolicus*).

MATERIAL AND METHOD

The body height of the Kızılca pond, which was built for irrigation purposes in Seydişehir District Kızılca Village by the Konya special provincial directorate of administration in 2010, is 22 m, its body length is 294 m, the maximum water elevation is 15 m and its area is 14.55 ha. The average depth is 6-8 m. Length of the pond: 943 m, width of the pond: 186 m (KOP, 2011). Classified as endangered in The IUCN Red List of Threatened Species, Giant Spring Minnow or Anatolian Minnow (*Pseudophoxinus anatolicus* Hanko, 1925) has a range that includes the Beyşehir-Seydişehir / Konya region (Figure 1).

Zooplankton samples were taken from the pelagic part of the lake at 8 m depth (from this depth, one time) in July 2018 (Figure 2, 3). The samples were collected horizontally with Hydro-Bios model plankton net with 55 µm mesh opening and fixed with 4% formaldehyde solution. Then they have been checked and diagnosed in invert, stereo and research microscope using the related resources for zooplankton species (Koste, 1978; Negrea, 1983; Nogrady & Segers, 2002; Segers, 1995; Smirnov, 1996; Ustaoğlu, 2004; Ustaoğlu et al., 2012; Ustaoğlu, 2015).

Figure 2. Study area (Coordinate; 37° 32' 44.05"N, 31° 42' 34.84"E).

Figure 3. Kızılca pond.

RESULTS

At the end of the study, a total of 15 species, 13 from Rotifera and 2 from Cladocera, were identified. The distribution of the identified species is provided in Table 1. In the pond, pH 8.58, conductivity 194 µmhos/cm, water temperature 27.1 °C, dissolved oxygen 7.9 mg/L, and dissolved oxygen saturation was measured as 94%. Zooplankton species show a distribution in two groups. Rotifera (87%), Cladocera (13%) (Figure 4).

Table 1. Kızılca pond zooplankton species.

Species	Density
Rotifera	
Ascomorpha sp.	▲
Asplanchna priodonta	▲▲▲
Colurella sp.	▲
Keratella cochlearis	▲▲
Keratella testudo	▲
Lecane bulla	▲
Lecane luna	▲
Lecane ludwigi	▲
Lecane lunaris	▲
Polyarthra dolichoptera	▲
Synchaeta pectinata	▲
Testudinella patina	▲▲
Trichocerca similis	▲
Cladocera	
Bosmina longirostris	▲
Daphnia cucullata	▲

▲▲▲: Most Abundant; ▲▲: Abundant; ▲: Few

The maximum number of species was identified in the Rotifera group (13 species). Dominant species were identified as *A. priodonta, K. cochlearis* and *T. patina* from Rotifera.

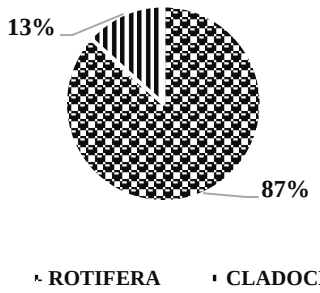

Figure 4. Distribution of zooplankton groups in Kızılca Pond.

DISCUSSION AND CONCLUSION

Biotic and abiotic factors have been reported to be effective in the density and distribution of zooplankton species. Of these factors, especially temperature, oxygen, nutrients, competition and predation affect zooplankton (Tasevska et al., 2017). Zooplankton is important in the change of ecological and trophic conditions of lakes related to nutrient elements and changes caused by climate in lakes (Jeppesen et al., 2011). Among the Zooplankton species, especially the Rotifera group organisms are used as indicators of lakes (Gannon & Stemberger, 1978). In the study on zooplankton fauna (Gürel & Saler, 2015) in the Orduzu (Malatya) pond, a total of 47 zooplankton species were reported, 7 of them (*A. priodonta, K. cochlearis, L. luna, L. lunaris, P. dolichoptera, S. pectinata, T. similis* and *B. longirostris*) are compatible with the zooplankton fauna of Kızılca pond. Additionally, a total of 24 species were identified in the seasonal change study of zooplankton in the Kapıaçmaz pond (Kovancılar, Elazığ), among which *A. priodonta, K. cochlearis, L. luna, P. dolichoptera, S. pectinata* and *B. longirostris* species were also identified in Kızılca pond (Bulut, 2018). In the study conducted on the Rotifera species of zooplanktonic organisms in some of the wetlands of Kayseri, Reşadiye-Şeker and Zincidere ponds, *K. cochlearis, L.luna, L. lunarıis, P. dolichoptera* and *S. pectinata* species were also detected in Kızılca pond (Kaya et al., 2009). *Polyarthra* sp, *Keratella* sp, *Lecane* sp, *Bosmina* sp and *Daphnia* sp species detected at the genus level in Beytepe (Ankara) pond are also similar to zooplanktonic organisms detected in Kızılca pond (Korkmaz & Korkmaz, 2002). In the study conducted on the Kaldırım and Halikan ponds, a total of 52 zooplankton species were identified in the Kaldırım pond, and 45 zooplankton species were identified in the Halikan pond, and 11 zooplankton species identified in the Kaldırım and Halikan ponds (*A. priodonta, K. cochlearis, L.luna, L. bulla, L. lunaris, P. dolichoptera, S. pectinata, T. similis, B. longirostris* and *D. cucullata*) have also been identified in the Kızılca pond (Bulut & Saler, 2016). In the research conducted in the Üçpınar (Uşak) pond, a total of 30 zoplankton species were identified, and *P.dolichoptera* and *T. similis* species were also found in the Kızılca pond (Ertosun et al., 2010). The zooplankton study conducted in Kızılca pond is the first one and all of the determined zooplankton species contributed to the region and zooplankton species diversity. It has become important that the biodiversity of the Kızılca pond ecosystem be studied thoroughly in the future in terms of ensuring the sustainability of the population of Anatolian endemic Giant Spring Minnow fish, which has a dense population in the region.

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ARAŞTIRMA MAKALESİ **RESEARCH PAPER**

Occurrence and Environmental Risks Assessment of DEET (N,N-diethyl-m-toluamide) Pesticide in Seyhan River, Turkey

Evşen YAVUZ GÜZEL
Cukurova University, Faculty of Fisheries, Department of Basic Sciences, Adana, Turkey

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*: https://orcid.org/0000-0002-8029-9254

***Corresponding author's:**
Evsen YAVUZ GÜZEL
Cukurova University, Faculty of Fisheries, Department of Basic Sciences, Adana, Turkey
✉: eguzel@cu.edu.tr

Abstract: The insect repellent DEET (*N,N*-diethyl-*m*-toluamide) is one of the most common organic chemical pollutants in water in a wide range of countries around the World. In this study, surface water samples were collected seasonally, from 7 stations in Seyhan River, following a period between December 2016 and November 2017. The DEET concentrations in Seyhan River were detected between 18.55-334.71 ng/L. DEET was detected in all seven sampling stations and detection frequency was 100%. Results obtained in autumn were higher than in summer. According to the environmental risk assessment, DEET in the Seyhan River, has no environmental risk (all measured RQ values were lower than 0.01).

Keywords: Environmental risk assessment, insect repellent, pesticide, seyhan river, water pollution.

Seyhan Nehri'nde DEET (N, N-dietil-m-toluamid) Pestisitinin Bulunurluğu ve Çevresel Risk Değerlendirmesi

***Sorumlu yazar:**
Evsen YAVUZ GÜZEL
Çukurova Üniversitesi, Su Ürünleri Fakültesi, Temel Bilimler Bölümü, Adana, Türkiye
✉: eguzel@cu.edu.tr

Öz: Böcek kovucu DEET, dünyadaki çok çeşitli ülkelerde suda bulunan en yaygın organik kimyasal kirleticilerden biridir. Bu çalışmada, Aralık 2016 ile Kasım 2017 tarihleri arasında, Seyhan Nehri'ndeki 7 istasyondan mevsimsel olarak yüzey suyu örnekleri toplanmıştır. Seyhan Nehri'ndeki DEET konsantrasyonları 18,55-334,71 ng/L arasında tespit edilmiştir. Yedi örnekleme istasyonunun tamamında DEET tespit edilmiş olup, tespit frekansı % 100'dür. Sonbaharda elde edilen sonuçlar yaz aylarına göre daha yüksek bulunmuştur. Çevresel risk değerlendirmesine göre Seyhan Nehri'ndeki DEET'in çevresel riski yoktur (ölçülen tüm RQ değerleri 0,01'den düşük bulunmuştur).

Anahtar kelimeler: Çevresel risk değerlendirmesi, böcek kovucu, pestisit, Seyhan Nehri, su kirliliği.

INTRODUCTION

Personal care products (PCPs) are compounds used in lotions, toothpastes, soaps, fragrances and sunscreen products. The classes of PCPs include insect repellents, preservatives, disinfectants, fragrances, and UV filters. PCPs are products for external use on the human body and are therefore not subject to metabolic changes. Consequently, large amounts of PCPs enter the environment through regular use (Brausch & Rand, 2011). PCPs are considered as environmental emerging pollutants due to their sustained release into the aquatic environment

and the persistence of ecotoxicological impacts (Calza, Medana, Raso, Giancotti, & Minero, 2011).

Insect repellents are organic synthetic chemicals that can be applied on garments, skin or other surfaces to prevent the effects of insects and insect bites. Mosquito, fly, fleas and spiders are the most common insect bites. Bites can produce many side effects such as skin irritation, allergic reactions, infections, fever or encephalitis. And even western Nile fever, malaria, dang can cause diseases such as spread (Molins-Delgado, García-Sillero, Díaz-Cruz, & Barceló, 2018). DEET (N,N-diethyl-*m*-toluamide or N,N-diethyl-3-methylbenzamide) is the most widely used insect repellent for the prevention of bites from insects (Calza et al., 2011; Guzel, Cevik, & Daglioglu, 2017).

DEET was developed in 1946 by the US military to protect against mosquito bites related diseases during World War II. In 1957, DEET was made available for public use. EPA has declared this product safe for normal use. However, there is still a debate on whether DEET constitutes a significant risk for people, animals and the environment (Campos et al., 2016; Merel, Nikiforov, & Snyder, 2015).

DEET is sold in a wide variety of products including lotions, sprays, and wristbands. The formulations used can vary from 4% to 95% of the active ingredient. Agricultural use of DEET has also been reported (Aronson, Weeks, Meylan, Guiney, & Howard, 2012). As of 2017, 27 companies in the United States reported that about 119 consumer products containing DEET were in use (Keith et al., 2017). However, such data could not be found for Turkey.

The presence of DEET in the aqueous environment contains many and potentially complex pathways. DEET can be released directly or indirectly to the environment by its presence in commercial products. There is no known natural source of DEET known to be environmentally important. Due to recreational activities such as swimming (via skin or clothing of swimmers or indirectly over spraying), DEET can directly enter surface waters. Although often neglected, domestic waters (through human activities such as showering and bathing) are now expected to be the major source of DEET in the aquatic environment (Keith et al., 2017; Merel & Snyder, 2016). These aquatic environments being often used as drinking or tap water sources. So DEET can be detected in water for human consumption (Merel et al., 2015; Merel & Snyder, 2016).

The insect repellent DEET is one of the most common organic chemical pollutants in water in a wide range of countries from all around the world (Merel & Snyder, 2016).

The first studies about the occurrence of DEET in the aquatic environment was in samples of Swedish municipal landfill leachate and in Mississippi river (USA) were published in 1993 (Öman & Hynning, 1993; Pereira & Hostettler, 1993). DEET has been identified and quantified in different aquatic environments, such as surface water (Bartelt-Hunt, Snow, Damon, Shockley, & Hoagland, 2009; Calza et al., 2011; Costanzo, Watkinson, Murby, Kolpin, & Sandstrom, 2007; Guzel, Cevik, & Daglioglu, 2018b; Ma et al., 2016; Yoon, Ryu, Oh, Choi, & Snyder, 2010), ground water (Sorensen et al., 2015; Stuart, Lapworth, Crane, & Hart, 2012), drinking water (Benotti et al., 2009), seawater (Dsikowitzky et al., 2014) and wastewater treatment plants (Bartelt-Hunt et al., 2009; Costanzo et al., 2007; Glassmeyer et al., 2005). Studies on the presence of DEET in surface waters have increased significantly since 2000. The reason for this is that the sensitivity of the analytical methods used to detect DEET has increased over the last two decades (Aronson et al., 2012). With the present methodology, DEET can detect even at concentrations of <0.1 ng/L. European studies about the occurrence of DEET are limited to Western countries. And Asian studies are mostly dominated by China but highest DEET concentrations were also reported for Indonesia (Dsikowitzky et al., 2014; Merel & Snyder, 2016).

Numerous reviews and researches have been published investigating toxicity (Legeay, Clere, Apaire-Marchais, Faure, & Lapied, 2018; Martinez, Vélez, Mayo, & Sastre, 2016; Swale, Sun, Tong, & Bloomquist, 2014; Weeks, Guiney, & Nikiforovz, 2012) and occurrence of DEET in aquatic environments (Bartelt-Hunt et al., 2009; Benotti et al., 2009; Guzel et al., 2018b; Sandstrom, Kolpin, Thurman, & Zaugg, 2005). But less attention has been paid to identify the environmental risk assessment of PCPs especially insect repellents release on aquatic environments (Aronson et al., 2012; Sun et al., 2016).

In the Seyhan River Basin, there are freshwater marshes, salt marshes and reed marshes suitable for proleferiation of mosquito species (TUBITAK MAM, n.d.). Although the municipalities in the basin apply pesticides to combat mosquitoes, mosquito populations can be quite intense in some areas close to wetlands.

The present study aims to investigate the occurrence of DEET pesticide in Seyhan River surface water samples for assessing seasonal and spatial variations (seasonal sampling over one year) in seven stations. And the other aim of this study is to calculate the risk quotient (RQ) with the measured concentrations of DEET obtained from Seyhan River and to determine the environmental risks. To our knowledge this is the first study about the occurrence and environmental risk assessment of DEET in Seyhan River, Turkey.

MATERIALS AND METHODS

Chemicals and materials: Methanol (MeOH), methyl tert-butyl ether (MTBE) and water were gradient grade for liquid chromatography and purchased from Merck Millipore, Germany. Solid-phase extraction (SPE) cartridges (Oasis HLB, 500 mg, 6 cm3), SPE extraction manifold (20-positions) which combined with vacuum pomp were purchased from Waters (Millford, MA, USA). DEET (<98%) was purchased from Sigma-Aldrich (Steinheim, Germany). Internal standard diazepam-d5 (99%) was obtained from Lipomed (Switzerland). Glass fibre filter disks (GF/C, pore size 1.2 µm) were purchased from Whatmann (VWR, Belgium). Allure® Pentafluorophenylpropyl (PFPP) column (50 mm x 2.1 mm, 5 µm) was purchased from Restek (Bellefonte, PA, ABD).

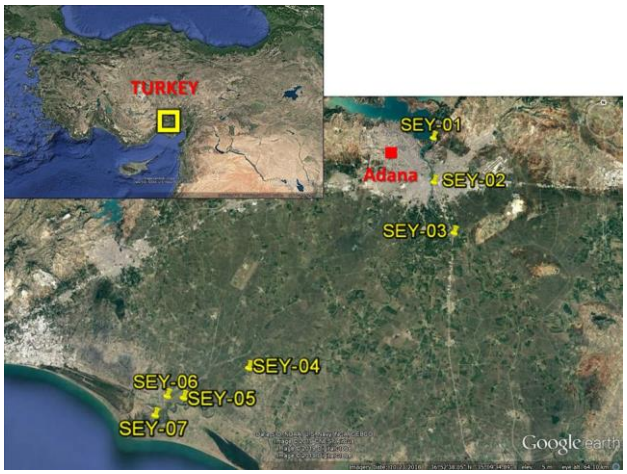

Figure 1. Geographic location of Seyhan River in Turkey and sampling stations.

***Sampling site and sample collection*:** The Seyhan River is the longest river in Turkey that flows into the Mediterranean Sea. It is located in the Seyhan Basin, south Turkey, and this basin has the total drainage area of 20.600 km^2. The total length of the Seyhan River is approximately 560 km. According to population and area information, population density is 83 person/km^2. Surface water samples were collected seasonally, from 7 stations in Seyhan River, following a period between December 2016 and November 2017. First two stations (SEY-01 (37° 1'59.94"N, 35°20'29.66"E) and SEY-02 (36°58'45.92"N, 35°20'5.90"E) are dominated by a large city centre (Adana) where 1.7 million inhabitants living. SEY-03 (36°55'6.59"N, 35°21'16.66"E), SEY-04 (36°47'4.66"N, 35° 3'47.58"E), and SEY-06 (36°45'39.10"N, 34°57'20.16"E) surrounded by a variety of land uses including agriculture and industry. SEY-05 (36°45'29.03"N, 34°58'36.87"E) is located on a drainage canal near Baharli Village that carries waste water. SEY-07 is located in the region where Seyhan River and drainage channel are mixed and poured into the Mediterranean Sea. Surface waters were sampled from a depth of approximately 25 cm. Water samples were transferred in a cooler bag to the laboratory. In the autumn season sampling, no sample was taken at SEY-03 station, as there was no water.

Sample preparation and extraction: Water samples were filtered through glass fibre filters (GF/C) and H_2SO_4 was added for pH adjustment (pH=2±0.1). Solid phase extraction (SPE) was used for sample preparation before instrumental analysis. 1 L filtered surface water samples were loaded through preconditioned (Oasis HLB cartridges) SPE cartridges at a flow rate of 15 mL/min using a vacuum extraction manifold (Waters 20-Position extraction manifold (Milford, MA, USA)).

Instrumental analysis: Chromatographic analyses were carried out with ultra-fast liquid chromatography (Shimadzu CBM-20A), automatic sampling system (Shimadzu SIL-20A/HT) and mass spectrometry (Shimadzu 8030, Kyoto, Japan). DEET was separated chromatographically on PFPP column (at 40 ºC, flow rate of 0.4 mL/min) using ultra-pure water with 10 mM ammonium formate (eluent A) and methanol (eluent B), with the gradient program started with 10% eluent B and was held for 10 min at a flow rate of 0.4 mL/min. Multiple reactions monitoring (MRM) mode was used for the determination of DEET (precursor: 192.2, products 91.1 and 119.1). Total method run time was 18 minutes. The retention time was 7.78 minute. The area ratio of the ions and retention time were compared by using the standard solutions under identical conditions. The limit of quantification (LOQ) was 0.3 and the limit of detection (LOD) was 0.2 ng/L for the DEET method. The correlation coefficients for calibration curve was higher than 0.99.

Environmental risk assessment calculations: Risk quotient (RQ) was calculated for the environmental risk assessment. The hazard and environmental risk level of DEET compound in the receiving environment can be determined by calculating the RQ value. PNEC value is calculated by using the smallest NOEC value calculated for many different species in ecotoxicology studies and the assessment factor (AF) suitable for the recipient environment (Aronson et al., 2012; Guzel, Cevik, & Daglioglu, 2018a). RQ formula is:

$$RQ=MEC/PNEC$$

In this formula, MEC is the measured environmental concentrations and PNEC is the no effect concentration in the surface water. If RQ values; < 0.1 means insignificant risk (no adverse effect expected), = 0.1-1 means low risk (potential adverse effects), = 1-10 means moderate risk (probable adverse effect) and > 10 means high risk (Ma et al., 2016). The PNEC value for DEET is 47000 (ECOSAR (Ecological Structure Activity

Relationship) class program Version 1.11 was used to obtain the pNEC value).

RESULTS

DEET was detected in all sampling stations, concentrations in Seyhan River were between 18.55 ng/L and 334.71 ng/L and detection frequency was 100%. Mean, median, maximum and minimum concentrations were listed in Table 1.

The lowest DEET concentration was found as 18.55 ng/L in winter and highest concentration was found as 334.71 ng/L in autumn. The mean concentrations of DEET were found as 32.58, 78.31, 81.46 and 171.66 ng/L in winter, spring, summer and autumn respectively (Fig.2).

Table 1. DEET concentrations in surface waters of Turkey and other countries.

Study Area	Mean (ng/L)	Median (ng/L)	Min. (ng/L)	Max. (ng/L)	References
Seyhan River, **Turkey**	88.01	75.89	18.55	334.71	*This study
Ceyhan River, **Turkey**	12.45	4.41	0.57	40.41	(Guzel et al., 2018b)
Konya Basin Lakes, **Turkey**	3.92	3.57	3.41	4.79	(Guzel et al., 2018b)
Antalya Basin Lakes, **Turkey**	3.43	2.73	1.54	7.77	(Guzel et al., 2018b)
Ceyhan Basin Lakes, **Turkey**	3.45	2.56	0.64	14.22	(Guzel et al., 2018b)
Seyhan Dam Lake, **Turkey**	5.50	1.78	1.01	17.43	(Guzel et al., 2018b)
USA	-	55	13	660	(Brausch & Rand, 2011)
The Northern River, **Germany**	-	-	0.11	1.09	(Weigel et al., 2002)
Po River, **Italy**	-	-	0.6	155.55	(Calza et al., 2011)
Schwarzbach, Modau, Winkelbach, and Weschnitz Rivers, **Germany**	124	-	<LOD	1292	(Quednow & Püttmann, 2009)
Zhujiang, Shijing Rivers, **China**	-	-	0.2	107	(Yang et al., 2013)
Jakarda River, **Indonesia**	-	-	30	24000	(Dsikowitzky et al., 2014)
Han River, **South Korea**	160	190	120	-	(Yoon et al., 2010)
5 stream sites, **USA**	-	-	30	180	(Mottaleb, Usenko, Brooks, & Chambliss, 2009)
Assunpink Creek, **USA**	-	-	45	340	(Alvarez et al., 2005)
139 stream sites, **USA**	-	60	-	1100	(Kolpin et al., 2002)

<LOD: lower than the limit of detection

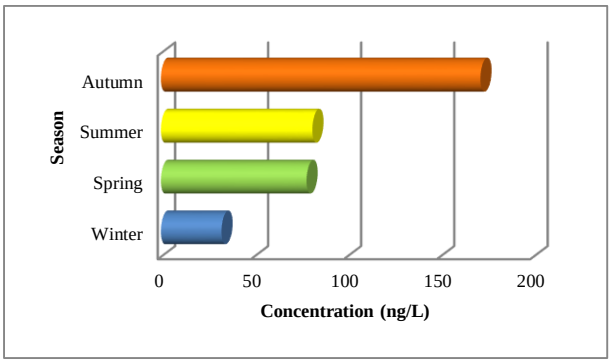

Figure. 2. Seasonal comparison of DEET concentrations (ng/L) in Seyhan River

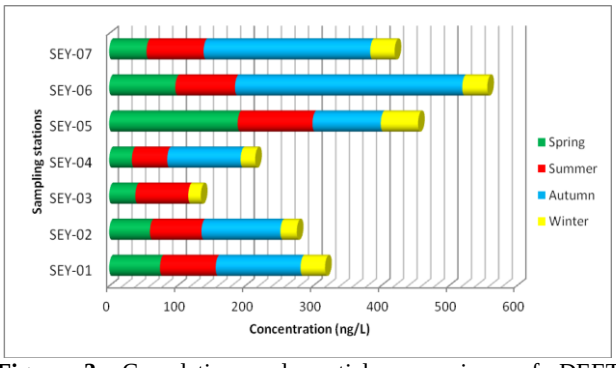

Figure 3. Cumulative and spatial comparison of DEET concentrations (ng/L) in Seyhan River

In Figure 3, the cumulative distribution of DEET concentrations measured in Seyhan River by stations was shown. DEET detected higher where there was the possibility of wastewater contamination, via human, industrial, and agricultural sources, entering the streams and near urban areas during summer and late winter (autumn). The station where the highest DEET concentration measured was SEY-06 was under the influence of the influent wastewaters of Seyhan wastewater treatment plant (WWTP), which is the largest WWTP in Adana Province, and during the field studies, it was observed that the mosquito population around the station was very high in autumn. Recent studies indicate that the highest DEET concentration in aquatic environments correlates with its increased application during the summer months and late winter (Keith et al., 2017; Merel et al., 2015; Sandstrom et al., 2005).

A study which was a summary of latest studies in surface water samples published in 2011 reported concentrations ranging from 13 to 660 ng/L for DEET throughout the USA (Brausch & Rand, 2011). In another review which reported worldwide DEET concentrations in water samples ranging from 40–3000 ng/L (Costanzo et al., 2007). Along the northern River, Germany DEET concentrations was between 0.11 to 1.09 ng/L (Weigel, Kuhlmann, & Huhnerfuss, 2002).DEET concentrations in Po River water samples were between 0.6 to 155.55 ng/L (Calza et al., 2011). Water samples taken from the Zhujiang and Shijing Rivers, concentrations of DEET were minimum 0.2 ng/L and maximum 107 ng/L. And in Assunpink Creek in Trenton, New Jersey DEET was detected at levels of 45–340 ng/L (Alvarez et al., 2005). DEET was detected in 74.1% of the water samples analyzed at a maximum concentration of 1100 ng/L (Kolpin et al., 2002). In Table 1, DEET concentrations in

Seyhan River compared with Ceyhan River (Turkey), Konya Basin Lakes (Turkey), Antalya Basin Lakes (Turkey), Ceyhan Basin Lakes (Turkey), Seyhan Dam Lake (Turkey), The Northern River (Germany), Po River (Italy), Schwarzbach, Modau, Winkelbach, and Weschnitz Rivers (Germany), Zhujiang and Shijing Rivers (China), Jakarda River (Indonesia), Han River (South Korea), 5 stream sites, Assunpink Creek and 139 stream sites (USA). Highest DEET concentrations in Turkey were detected for Seyhan River. Among the studies in the world, the highest DEET concentration was measured in Jakarda River, Indonesia (max: 24000 ng/L) (Dsikowitzky et al., 2014), followed by the results of Winkelbach, and Weschnitz Rivers (1292 ng/L) in Germany (Quednow & Püttmann, 2009), and the results of 139 stream waters (1100 ng/L) in the United States (Kolpin et al., 2002).

Risk assessment calculations were made for each station and season using DEET concentrations measured in the Seyhan River. According to the environmental risk assessment, DEET in the Seyhan River, pose no environmental risk (all measured RQ values were lower than 0.01, means no environmental risk) (Table 2). However, toxic effects may be higher when all pollutants behave together in natural environment.

As a result, DEET pesticide has been detected in all surface waters collected seasonally over a year from the Seyhan River, with concentrations varying between 18.55-334.71 ng/L. Aquatic organisms are thought to be affected as result of chronic exposure to DEET. The results obtained for Seyhan River were found lower than in USA (4700 ng L^{-1} in surface waters) and Europe. According to the risk assessment calculations, it was determined that the current concentrations of DEET pesticide do not pose an environmental risk.

Table 2. Measured environmental concentrations (MECs) and risk quotient values (RQs) of DEET detected in Seyhan River.

	MECs (ng/L)				RQs			
	Spring	**Summer**	**Autumn**	**Winter**	**Spring**	**Summer**	**Autumn**	**Winter**
SEY-01	74.749	82.096	124.964	36.125	0.002	0.002	0.003	0.001
SEY-02	59.99	75.886	116.257	24.185	0.001	0.002	0.002	0.001
SEY-03	38.491	77.952	-	18.547	0.001	0.002	-	0.000
SEY-04	33.388	52.065	108.089	21.457	0.001	0.001	0.002	0.000
SEY-05	189.121	110.308	100.505	54.985	0.004	0.002	0.002	0.001
SEY-06	97.414	87.849	334.713	36.898	0.002	0.002	0.007	0.001
SEY-07	54.985	84.047	245.442	35.894	0.001	0.002	0.005	0.001

CONCLUSION

This study was one of the first and most comprehensive studies in the region. The results obtained in autumn were found higher than in summer. It was unexpected, but it provides clue about the season that mosquitos are found more. The results obtained for Seyhan River were found lower than in USA and Europe. In future studies, bioaccumulation of DEET should be added to such research. Because DEET can accumulate in aquatic organisms and damage as a result of continuous exposure.

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DERLEME MAKALESİ **REVİEW PAPER**

Glukosinolatlardan Hidrolize Edilen Sülforafanın Potansiyel Etkileri ve Nrf2-Keap-1 Sinyal Yolağı ile İlişkisi

Deniz KARAKÇI
Tekirdağ Namık Kemal Üniversitesi Veteriner Fakültesi Biyokimya Anabilim Dalı Tekirdağ, Türkiye

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*: https://orcid.org/0000-0002-1884-1874

***Sorumlu yazarın:**
Deniz KARAKÇI
Tekirdağ Namık Kemal Üniversitesi, Veteriner Fakültesi, Biyokimya Anabilim Dalı Tekirdağ, Türkiye
✉: dkarakci@nku.edu.tr

Öz: Glukosinolatlar (Gls), brassica sebzelerinde bulunan ekonomik açıdan önemli olan ikincil bitki metabolitleridir. Glukosinolatlar ve bunların hidroliz ürünleri insanlar ve hayvanlar üzerinde birçok faydalı etkiye sahiptir. Gls, bitki içeriğinde ve bağırsak mikroflorasında bulunan mirosinaz enzimi ile hidrolize edilir ve bu şekilde biyolojik aktiviteleri ortaya çıkarabilmektedir. Glukosinolatların parçalanma ürünlerinden biri olan izotiyosiyanatlar bazı kanser türlerinin önlenmesinde önemli roller oynamaktadır. En çok incelenen izotiyosiyanat öncül maddesi sülforafan, memeli hücre koruyucu enzimlerinin güçlü bir uyarıcısı olarak brokoli özlerinden izole edilmektedir. Sülforafanın, bir sıçan memeli tümör modelinde, tümörlerin boyutunu indirekt bir antioksidan olarak çalışarak küçülttüğü bildirilmiştir. Nükleer faktör eritroid 2 ile ilişkili faktör 2 (Nrf2), antioksidan ve detoksifiye eden genlerin ekspresyonunu düzenleyen, spesifik bir gen tarafından kodlanan bir transkripsiyon faktörüdür. Nrf2, birçok Faz I ve Faz II ilaç metabolize edici enzimlerin ekspresyonunu kontrol etmektedir. Aynı zamanda, Kelch-benzeri ECH birleştirici protein 1 (Keap1) sistein aminoasitlerce zengindir ve oksidatif stresin oluşmasına bağlı olarak çalışabilen bir sensör görevi yapmaktadır. Hücre koruyucu proteinleri kodlayan genlerin çoğu, Keap1-Nrf2-ARE antioksidan yanıt elementi 'ARE' sinyal yolu boyunca ortak transkripsiyonel düzenlemeyi sağlamaktadır. Artan strese göre, Keap1 Nrf2'yi aktive eder ve ARE'yi uyarmaktadır. Özellikle glukosinolatlardan hidrolize edilen sülforafan, bu yolu kullanarak oksidatif hasara ve çeşitli kanser türlerine karşı vücudu koruduğu düşünülmektedir.

Anahtar kelimeler: Glukosinolatlar, kanser, oksidatif stres, sülforafan.

The potential effects of sulforaphane hydrolyzed from glucosinolates and relationship between Nrf-2 Keap-1 signal pathway

***Corresponding author's:**
Deniz KARAKÇI
Tekirdag Namık Kemal University Faculty of Veterinary Medicine Department of Biochemistry Tekirdağ, Turkey
✉: dkarakci@nku.edu.tr

Abstract: The glucosinolates (Gls) are economically important secondary plant metabolites which occur in all of brassica vegetables. Glucosinolates and their hydrolysis products have many beneficial effects on humans and animals. Gls are hydrolyzed by myrosinase enzyme found in plant or produced in intestinal microflora, in this way it can appear their biological activities. One of breakdown products of Gls called isothiocyanates and they have an essential role in prevention some cancer types. The most studies on isothiocyanate, sulforaphane (SFN) was isolated from extracts of broccoli as a potent inducer of mammalian cytoprotective enzymes. It is reported that sulforaphane reduced the size of tumors in a rat mammary tumor model by acting as an indirect antioxidant. NF-E2-related factor 2 (Nrf2) is a transcription factor encoded by a specific gene that regulates the expression of antioxidant and detoxifying genes. Nrf2 controls the expression of many Phase I and Phase II drug-metabolizing enzymes. Also, Kelch-like ECH associating protein 1 (Keap 1) is rich from amino acid cysteine and acts as a sensor that can work according to the existance of oxidative stress. Many of the genes encoding cytoprotective proteins share common transcriptional regulation through the Keap1-Nrf2-ARE pathway. According to increasing stress, Keap1 activates Nrf2 and induce the Antioxidant Response Element (ARE). Especially sulforaphane that is hydrolyzed from glucosinolates protects the body against oxidative damage and several cancer types by using this pathway.

Keywords: Cancer, glucosinolates, oxidative stress, sulforaphane.

GİRİŞ

Glukosinolatlar (Gls), Brassicaceae ailesinin ekonomik olarak öneme sahip, sülfür içeren ikincil bitki metabolitleridir. 120'den fazla farklı glukosinolat çeşidi yan-zincir yapısının değişikliğe uğramasıyla elde edilmektedir (Belenli vd., 2016; Chen vd., 2001). Glukosinolatların genel kimyasal yapısı, β-D-tiyoglukoz grubu, bir sülfonlanmış oksim parçası ve metiyonin, triptofan veya fenilalaninden türetilen değişken bir yan zincirden oluşur (Tripathi vd., 2007).

1970'lerden beri glukosinolatlar ve bunların hidroliz ürünlerinin, insan ve hayvan beslenmesi üzerine faydalı ve biyolojik etkileri üzerine çalışmalar yapılmaktadır. Ayrıca Brassica sebzelerinin karakteristik tat ve lezzetinden kısmen sorumlu oldukları bulunmuştur. Brassicaceae ailesi, brokoli, brüksel lahanası, lahana, yeşil lahana, karnabahar, şalgam ve tere gibi sebzeleri içermektedir. Bu sebzeler dünya çapında yaygın olarak yetiştirilmekte ve tüketilmektedir. Bu sebzeler içerisinde yer alan brokoli, ayrı bir öneme sahiptir ve brokolinin düzenli tüketimi ile bazı kanser türlerini önlemedeki etkileri birçok bilimsel çalışmada araştırılmıştır (Belenli & Polat, 2018; Cartea, 2008). Glukosinolatlar, bitkinin tüm kısımlarında bulunur ve fiziksel olarak mirosinaz (tiyoglukozit glukohidrolaz, EC 3.2.3.1) enzimi ile hidroliz ürünlerine ayrılır. Bitkiye ısıl işlem uygulanması veya vücuda alındıktan sonra sindirim sırasında bitki dokularının zarar görmesi ile mirosinaz enzimi aktive olur böylece glukosinolat ve biyolojik etkilere sahip hidroliz ürünlerinin açığa çıkması sağlanmış olur (Tripathi vd., 2007). Glukosinolatlar, hem bitkide bulunan hem de bağırsak mikroflorası tarafından üretilen mirosinaz enzimi tarafından hidrolize edilir ve parçalanma ürünleri serbest hale geçer (Bernardi vd., 2003; Cheng vd., 2004; Fenwick vd., 1983; Finiguerra vd., 2001; Mithen, 2001).

Glukosinolatların (Gls) hidroliz ürünleri, birçok hastalığa karşı koruma ve savunma sağlayan biyolojik aktivitelere sahiptir (Halkier vd., 1997). Hidroliz ürünleri şeker içermeyen bir aglikon bölümü, glikoz ve sülfattan oluşur. Aglikon kısmı kararsızdır ve Gls'in yapısına bağlı olarak izotiyosiyanatlar (ITC), tiyosiyanatlar, nitriller, oksazolidin tiyonlar ve epitionitriller gibi hidroliz ürünlerinden oluşmaktadır. Glukosinolatların biyolojik aktivitelerinin neredeyse tamamı, parçalanma ürünleri, özellikle izotiyosiyanatlar tarafından gerçekleştirildiği bildirilmiştir (Vig vd., 2009).

***İzotiyosiyanatlardan İzole Edilen Sülforafanın Biyolojik Etkileri*:** Paul Talalay vd., (2003) tarafından izotiyosiyanatlarla ilgili kabul gören ilk çalışmalar, faz I enzimlerinin inhibe edilmesi ve sitoprotektif (hücre koruyucu) faz II enzimlerinin transkripsiyonunu indükleyerek, DNA'yı mutasyona uğratacak olan mevcut kanserojenlerin seviyesinin azaltılması yönünde olmuştur. Yapılan ilk çalışmalarla elde edilen bu keşif, murin Hepa1c1c7 hücrelerindeki NAD(P)H:-kinon oksidoredüktaz-1'in (NQO1) artan transkripsiyonuna dayanan indüksiyon yeteneklerini taramak için kantitatif bir biyoanalizin geliştirilmesine işaret etmiştir. En çok çalışma yapılan izotiyosiyanatlardan biri olan sülforafan, brokoli ekstraktlarından, memelilerdeki sitoprotektif enzimlerin güçlü bir indükleyicisi olarak izole edilmiştir. Sülforafan, brokolinin ekstraksiyonu sonucu izole edilen, temel aktif bileşiğidir (Dinkova-Kostova vd., 2012).

İzotiyosiyanatlardan elde edilen sülforafanın çeşitli memeli patojenlerine karşı aktivitesi (örneğin *Escherichia coli, Salmonella typhimurium, Candida* sp.) yüzyıllardır yaraların tedavisinde kullanılmakta ve anti-tümör ajanlar olarak biyolojik özellikler göstermektedir (Fahey vd., 2001). Memeli dokuları mirosinaz enzimi içermemesine rağmen, memelilerde glukosinolatların izotiyosiyanatlara dönüşümü, gastrointestinal sistemin bakteriyel mikroflorasında bulunan mirosinaz ile gerçekleşmektedir (Dinkova- Kostova vd., 2012). Gönüllü insanlarda, sülforafan ve izotiyosiyanatın, bitkisel kaynağının sindirim sonrası metabolizması incelenmiş ve her iki çalışmada da, glukosinolatlardan izotiyosiyanatların hidrolizinde sindirim sistemindeki mikrofloranın önemli rolü olduğu görülmüştür (Shapiro vd., 2001).

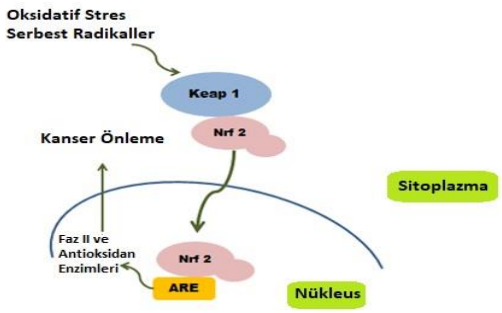
Bitki ekstraktlarından izotiyosiyanatların eldesi, genel olarak yüksek performanslı sıvı kromotografisi (HPLC) kullanılarak gerçekleşmektedir (Zhang vd., 1992; Kore vd., 1993). Günümüzde, HPLC için bu tekniğin modifikasyonu ile bitki ekstraktları gibi kompleks biyolojik sıvılarda 10 pmol'den az izotiyosiyanat ölçülebilmektedir (Zhang vd., 1996). Glukosinolatlardan ekstrakte edilen izotiyosiyanatlarda, saflaştırılmış mirosinaz enzimi ölçümü, belirlenen toplam glukosinolat titresi ve iyon çifti kromatografisi teknikleriyle doğrudan ölçülen glukosinolat seviyeleri arasında mükemmel bir korelasyon bulunmaktadır (Fahey vd., 1999).

Son yıllarda, birçok kanser türünün görülme sıklığını azaltmak için meyve ve sebzelerinden yararlanılmaktadır. Özellikle turpgil (Brassica) sebzelerinin tüketiminin artmasıyla bu konudaki çalışmalar kanıt niteliği kazanmaktadır (Doll, 1992; Fahey & Talalay 1999; Michaud vd., 1999; Steinmetz & Potter 1991; Verhoeven vd., 1996). Bu sebzelerin kanser kemoprotektif aktivitesinin en azından bir bölümünün glukosinolat içermelerinden kaynaklandığına inanılmaktadır (Fahey vd., 2001). Bu sebzelerin en önemli özelliği, Faz II enzimlerinin indükleyicileri olarak, apoptozu uyarır aynı zamanda redoks regülatörü olarak görev yapar ve Faz I enzimlerini inhibe ederek, metabolitlerin kanser önleyici olmasını sağlar. Sülforafan, sıçan meme tümörü modelinde (Fahey vd., 1997; Zhang vd., 1994), indirekt antioksidan

etki gösterir (Fahey & Talalay, 1999). Bunun yanında kolon kanseri hücrelerinde *in vitro* olarak seçici sitotoksik etki yapıp (Gamet-Payrastre vd., 1998), sitokrom p450'yi inhibe ederek memeli hücrelerindeki tümörün insidensinde ve büyüklüğünde azalmalar sağlamaktadır (Maheao vd., 1997; Morel vd., 1997). Ayrıca, *in vitro* olarak insan kolon kanseri hücrelerinde, hücre siklusunu durdurarak apoptozu indüklemektedir (Gamet-Payrastre vd., 2000). İzotiyosiyanatların, sıçan ve fare tümör modellerinde, akciğer ve yemek borusu kanserinin uyarılmasını da inhibe ettiği tespit edilmiştir. Bu etkiler, sitokrom p450 mekanizmasının da kuvvetli bir şekilde inhibe edildiğini göstermektedir (Fahey vd., 1997).

***Nrf2–Keap-1 Sinyal Yolağı ve Kanser Arasındaki İlişki*:** Nükleer faktör eritroid-2 ile ilişkili faktör 2 (Nrf2), oksidatif strese direnç gösteren ve çeşitli çevresel stres öncülerine karşı uyumlayıcı bir yanıtı gerçekleştirebilen bir transkripsiyon faktörüdür. Kelch-benzeri ECH ile ilişkili protein (Keap 1) ile Nrf2 ilişkisi, kansere neden olan somatik mutasyonların biyolojik önemini göstermektedir (Hayes & Dinkova-Kostova, 2014). Keap1, 624 amino asit içerisinde, 25 sistein kalıntısı bulunan, çinko metalloprotein grubuna ait uyarıcı bir sensördür. Keap1'in sistein açısından zengin olması, oksidatif stresin varlığına göre hareket edebilme özelliği kazanmasını sağlamaktadır. Hücresel strese karşı savunma mekanizmalarının ana düzenleyicisi olarak kabul edilen Nrf2/Keap1 sinyal yolağı, hasarlı makro moleküllerin onarımında veya ortadan kaldırılmasında görev alan çeşitli hücre koruyucu protein ağlarına ait gen ekspresyonlarını düzenleyerek, hücrenin stres koşullarında hayatta kalmasını sağlamaktadır. Hücre koruyucu proteinleri kodlayan genlerin çoğu, Keap1-Nrf2 ve antioksidan yanıt elementi 'ARE' yolağı aracılığıyla transkripsiyonel regülasyonda ortak rol oynamaktadırlar (Dinkova-Kostova & Kostov, 2012). Nrf2, sitoplazmada Keap1'e bağlıdır. Reaktif oksijen türleri (ROS), endojen antioksidan kapasitenin üstesinden geldiğinde Keap-1, Nrf2'yi serbest bırakır ve bu daha sonra, diğer transkripsiyon faktörleri ve ARE'nin de yardımıyla Nfr2 çekirdeğe geçer. Bu olay, glutatyon (GSH) sentezinden sorumlu olan başlıca anahtar antioksidanların ve sitoprotektif proteinlerin ve enzimlerin transkripsiyonel aktivasyonuna neden olur (Kavian vd., 2018).

İzotiyosiyanatlar, Keap1 spesifik sistein kalıntıları ile reaksiyona girer ve ubiqutinasyon ve degradasyon gerçekleştirmek için Nrf2'yi hedefleme kabiliyetini ortadan kaldırırlar. Sitozolde Nrf2'nin ubiqutinasyonunun ve proteozomal degredasyonunun önlenmesiyle Nrf2'nin nükleusa translokasyonu sağlamış olur (Mitsuishi vd., 2012). İnsan ve fare dokularında *in vivo* olarak Nrf2 transkripsiyon faktörü, ilaç metabolize eden enzimleri kontrol ederek, ekspresyona uğramaktadır. Nrf2-Keap1 sinyal yolunun, oksidatif strese hücresel yanıtta önemli rollere sahip olduğu tespit edilmiştir. Nrf2'nin aktivasyon mekanizması kapsamlı bir şekilde çalışmaları devam etmektedir (Dinkova-Kostova vd., 2005). Normal koşullar altında, Nrf2 sitoplazmaya yerleşir ve burada aktin bağlayıcı protein, Keap1, Nrf2 ile etkileşime girer ve onu aktive eder. Stresin arttığı durumlarda, Keap1 daha sonra hücre çekirdeğine göç eden ve ARE'yi azaltmak için DNA'ya bağlanan Nrf2'yi aktive eder ARE daha sonra çeşitli güçlü antioksidan enzimleri ve detoksifiye edici proteinlerin tekrar düzenlenmesini gerçekleştirir (Kobayashi & Yamamoto, 2005). Nrf2 hedef genlerinin promotor bölgesindeki ARE dizisine özgü bir şekilde bağlanır ve ilaç detoksifikasyonu, antioksidan cevap, NADPH rejenerasyonu ve metabolizmanın düzenlenmesinde rol oynayan genlerden protein kodlanmasını sağlar. Nrf2 hedef genleri, geniş bir antioksidan enzimler ağı, ksenobiyotik detoksifikasyonunda yer alan proteinler, hasarlı proteinlerin onarımı ve uzaklaştırılması, enflamasyonun inhibisyonu ve diğer transkripsiyon faktörlerinden oluşur (Yang vd., 2016). Son yıllarda, sitoprotektif gen ekspresyonunun düzenleyicisi rolünün bir parçası olarak, Nrf2'nin mitokondriyal işlevi etkilediği ortaya çıkmıştır. Artan Nrf2 aktivitesi, mitokondriyal toksinlere karşı koruma sağlar (Holmström vd., 2016). Paul Talalay vd. (2003) fare dokularında Nrf2'nin, Faz I ve Faz II ilaç metabolize eden enzimlerin ekspresyonunda rol oynadığını göstermiştir. Masayuki Yamamoto vd. ise Nrf2 negatif farelerde, GST (Glutatyon peroksidaz) ve NQO1'i uyardığını tespit etmiştir (Belenli & Polat, 2017; Dinkova-Kostova & Kostov, 2012).



Şekil 1. Nf2-Keap 1-ARE Sinyal Yolağı.
Figure 1. Nf2-Keap 1-ARE Signal Pathway.

Keap1, Nrf2'nin başlıca baskılayıcısıdır ve bu durum farelerde Keapl'in miktarının azalmasının gözlenmesi ile desteklenmektedir. Keap1'in, Nfr2 transkripsiyon faktörünün miktarını ve aktivitesini arttırmak için yeterlidir. Ayrıca, Keap1'deki somatik mutasyonlar, akciğer, meme ve kolon kanseri olan bazı hastalarda Keap1 promotörünün hipermetilasyonu şeklinde tümörlerdeki Nrf2'nin up-regülasyonuna neden olmaktadır (Hayes & Dinkova-Kostova, 2014).

SONUÇ

Özellikle glukosinolatların hidroliz ürünü olan sülforafanın kimyasal karsinojenlere karşı kemoprotektif özellikleri kanıtlanmıştır. Nrf2, sitoplazmada Keap1'e bağlıdır. Sülforafan tüketimi ile uyarılan Keap-1 harekete geçer ve Nrf2'yi serbest bırakır. Daha sonra, Nfr2 çekirdeğe geçer ve böylece Faz II enzimlerinin ekspresyonu sağlanmış olur. Faz II enzimlerinin ekspresyonu ile Nrf2-Keap-1-ARE sinyal yolunu indüklenerek antioksidan etkilerinin gösterilmesini sağlanır ve oksidatif hasara karşı koruma ve birçok kanser türünü önleme gerçekleşmiş olur. Bu derlemede, sülforafan dâhil olmak üzere düzenli olarak brassica sebzeleri ve özellikle brokoli tüketiminin bazı hastalıkların zararlı etkilerini azalttığı kanısına varılmıştır.

Glukosinolatların ve hidroliz ürünlerinin detaylı etkilerini incelemek için daha fazla araştırmaya ihtiyaç duyulmaktadır.

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ARAŞTIRMA MAKALESİ **RESEARCH PAPER**

Benfluorex, Friends or Foe? The Effects of Benfluorex on Oxidative Status in the Brain During Experimental Diabetes

Emine Gülçeri GÜLEÇ PEKER[1*] Barbaros BALABANLI[2] Çiğdem ÖZER[3] Şule COŞKUN CEVHER[2]
[1]*Department of Nursery, Faculty of Health Sciences, Giresun University, Giresun, Turkey*
[2]*Department of Biology, Faculty of Science, Gazi University, Ankara, Turkey*
[3]*Department of Physiology, Faculty of Medicine, Gazi University,, Ankara, Turkey*

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: https://orcid.org/0000-0001-7244-0281
: https://orcid.org/0000-0002-6670-8904
: https://orcid.org/0000-0002-2705-4522
: https://orcid.org/0000-0001-6204-2845

***Corresponding author's:**
Emine Gülçeri GÜLEÇ PEKER
Giresun University, Faculty of Health Science, Department of Nursery, Giresun, Turkey
✉: gulceri.peker@giresun.edu.tr

Abstract: Benfluorex is a pharmacological agent with antidiabetic and antihyperlipidemic properties. In this study, the brain's oxidative and non-enzymatic antioxidant status in diabetic and benfluorex administrated diabetic rats have been investigated. For the experimental procedures, three groups of 18 Wistar albino rats were used to control diabetes (induced by streptozotocin), and benfluorex treated diabetic rats (benfluorex administration intragastric 50 mg/kg daily for 21 days). Brain NOx, TBARS, GSH, AA levels, and MPO activity were determined spectrophotometrically. Benfluorex administration was caused that decreased lipid peroxidation and MPO activity while increased non-enzymatic antioxidant and NOx levels. These results showed that benfluorex treatment positively affects lipid peroxidation and the non-enzymatic antioxidant status of the brain during diabetes..

Keywords: Benfluorex, brain, diabetes, lipid peroxidation, non-enzymatic antioxidants, nitric oxide.

Benfloreks: Dost mu, Düşman mı? Deneysel Diyabette, Benfloreksin Beyindeki Oksidatif Olaylara Etkileri

***Sorumlu yazar:**
Emine Gülçeri GÜLEÇ PEKER
Giresun Üniversitesi, Sağlık Bilimleri Fakültesi, Hemşirelik Bölümü, Giresun, Türkiye.
✉: gulceri.peker@giresun.edu.tr

Öz: Benflureks, antidiyabetik ve antihiperlipidemik özelliklere sahip farmakolojik bir ajandır. Bu çalışmada, uygulanan diyabetik sıçanlarda benfloreks tedavisinin beynin oksidatif ve enzimatik olmayan antioksidan durumu üzerindeki etkileri araştırılmıştır. Deneysel prosedürler için, kontrol, diyabet (streptozotosin ile indüklenen) ve benfloreks ile tedavi edilmiş diyabetik sıçanlar (benfloreks 21 gün boyunca intragastrik 50 mg/kg dozda uygulanmıştır) olarak 18 Wistar albino sıçanından oluşan üç grup kullanıldı. Beyin NOx, TBARS, GSH, AA seviyeleri ve MPO aktivitesi spektrofotometrik olarak belirlendi. Benfloreks uygulaması, lipid peroksidasyonunu ve MPO aktivitesini azaltırken; enzimatik olmayan antioksidan seviyelerini ve NOx düzeylerini arttırmıştır. Bu sonuçlar, benfloreks tedavisinin, diyabet sırasında beyinde lipid peroksidasyonunu ve enzimatik olmayan antioksidan durumu olumlu etkilediğini göstermiştir.

Anahtar kelimeler: Benfloreks, beyin, diyabet, lipid peroksidasyonu, enzimatik olmayan antioksidanlar, nitrik oksit.

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disease which is one of the most important causes of morbidity and mortality worldwide (Ong et al., 2018). Therefore, the diabetes-related in vivo and in vitro studies remain its importance and popularity. DM becomes resulting from that either deficiency in insulin secretion from the beta (β) cells of the pancreatic islets of Langerhans or breakdown in the susceptibility of the insulin hormone or both of together exists. Streptozotocin (STZ, 2-deoxy-2-[3-methyl-3-nitrosoureido]-D glucopyranose) is one of the

most used pharmacological agents in order to create experimental diabetes. The diabetic effects of STZ are caused by the devastation of β pancreatic cells by natural killer (NK) cells (Szkudelski, 2001).

It is a known fact that increased Reactive Oxygen Species (ROS) production breaks down tissue homeostasis and it causes tissue damage. Particularly, lipids are vulnerable to ROS damage. Resulting of diabetic processes, hyperglycemia stimulates ROS generation from a variety of sources such as cytochrome P450 monooxygenases, nitric oxide synthase (NOS), oxidative phosphorylation, nicotinamide adenine dinucleotide phosphate oxidase, lipoxygenase, and glucose auto-oxidation (Pandey et al., 2010). ROS formation leads to devastation and injury on cell membranes by lipid peroxidation (Halliwell, 1994). Oxidative stress appears when ROS are produced in excess amounts or when antioxidant defense systems are impaired. Oxidative stress is the one of the causes in the pathogenesis and progression of complications from diabetes (Piero et al., 2014).

In every aerobic organism has antioxidant defense system existing of non-enzymatic antioxidant substances and antioxidant enzymes to protect the physiological activities of organisms against oxidative stress. Ascorbic acid (AA) and Glutathione (GSH) are a part of the non-enzymatic antioxidant that their cycle is known to act a fundamental role in the keeping of cellular redox homeostasis (Noctor et al., 2002).

The brain is one of the major organs of the body which responsible for maintaining of neuronal and hormonal processes and homeostasis. It is vulnerable to oxidative stress induced by diabetes consequence of its rich oxidizable polyunsaturated fatty acids content, high rate of oxygen consumption the existence of redox-active metals (Cu, Fe), and low defending of enzymatic antioxidant (Montilla et al., 2005; Uzar et al., 2012).

Benfluorex was an amphetamine derivative drug widely medicated by Type II diabetes patients through worldwide due to both lipid-lowering and antihyperglycemic effects until withdrawn in 2010 (Tribouilloy et al., 2012). In contrast to other antidiabetic drugs, the nonexistence of gastrointestinal side effects of benfluorex has increased the possibility of its preference (Moulin et al., 2009). Nevertheless, after the publication of several reports suggesting the link between the administration of benfluorex and serious cardiac valve regurgitation, it was withdrawn from the European market in 2010 (Rafel Ribara et al., 2003; Noize et al., 2006; Boutet et al., 2009; Frachon et al., 2010; Tribouilloy et al., 2010; Le Ven et al., 2011). The drug-induced cardiac side effects of benfluorex are caused by serotoninergic mechanisms via its metabolite norfenfluramine which has the ability to activate 5-HT_{2B} serotonin receptors in the heart valve, where it plays a role in the synthesis of glycosaminoglycans and collagen (Rothman et al., 2000; Roth, 2007; Tribouilloy et al., 2012). Besides known their cardiac and pulmonary adverse effects, amphetamines and their derivatives also could produce several neurological changes such as intracerebral vasculitis, ischemic stroke, and cerebral hemorrhage (Galvan-Arzate & Santamaria, 2002). On the other hand, there is no finding pointing to the effect of benfluorex on brain oxidant-antioxidant status during diabetes.

In this context, the present study was carried out to put forth the effects of benfluorex treatment on brain oxidative events throughout the diabetic process.

MATERIALS AND METHODS

Animals and Groups: Male adult Wistar albino rats were use in experiments (18, weighing 190–200 g). All animals were held in a temperature-controlled room with 12 hours of light and 12 hours of the dark cycle, in separate cages with access to water and food. All animal studies were performed in accordance with international ethical rules, and all animal procedure were approved by the Animal Experimentation Ethics Committee of Giresun University (Report no: 2019/06).

The animals have divided into three different groups as 1) Control (n=6) 2) Diabetes (n=6) and 3) Diabetes+benfluorex (n=6), randomly. Rats of the control group were given only an injection of 0.1 M, 1 ml citrate buffer at pH 4.5. On the Diabetes group, disease model was created by intraperitoneal injection of a single dose of STZ (Serva 35503) (45 mg/kg body weight) dissolved in 0.1 M, 1 ml citrate buffer at pH 4.5 (2). Rats were accepted diabetic if their fasting blood glucose (FBG) levels exceeded 200 mg/dl at 48 h after STZ injection. Rats of Diabetes+benfluorex group were treated with benfluorex (Sigma B-7522) intragastric (IG) 50 mg/kg daily for 21 days (Brindley et al., 1988; Serradas et al., 1993). The equal volume of tap water was IG delivered to control and diabetes groups for the same period.

After FBGs and body weights were measured, on day 22 of the experimental protocol, rats were sacrificed with taken blood from the heart under anesthesia. The brain tissues were removed rapidly and instantly freeze in liquid nitrogen and stuck at –80 °C until use.

Determination of TBARS: Lipid peroxidation in tissues was determined by the formation of TBARS (Casini et al., 1986). Brain tissues homogenized in cold trichloroacetic acid and centrifuged at 3000 rpm for 15 minutes. The supernatant then added to the tube containing an equal amount of thiobarbituric acid of 0.67% (w/v) and boiled at 100°C for 15 minutes. The absorbance of the samples determined at 535 nm.

Determination of GSH: The GSH levels of tissues were measured by the modified Elman method (Aykaç et al., 1985). Brain tissue samples homogenized in trichloroacetic acid solution and homogenate then centrifuged at 3000 rpm for 10 min. Supernatant added on the tube containing 0.3 mol/L $Na_2HPO_4 \cdot 2H_2O$ solution.

After, the dithiobisnitrobenzoate solution added in the tube and the absorbance was determined at 412 nm.

Determination of NOx: The Griess method was used to determine total nitric oxide (NOx) values in the brain tissues (Green et al., 1982). Tissue samples were homogenized in cold phosphate-buffered saline (pH = 7.5) then centrifuged at 3000 rpm for 5 min. Supernatant added to a tube containing 0.3 M NaOH. After 5% (w/v) ZnSO4 was added for deproteinization, mixture incubated for 5 min at room temperature, then centrifuged at 14,000 rpm for 5 min. The nitrate levels in brain tissues were measured spectrophotometrically by method of Miranda et. al. (Miranda et al., 2001). The Griess reaction was used to determine nitrite levels of tissues.

Determination of AA: Total ascorbate levels of brain tissues were measured by the modified Roe and Keuther method (Berger et al., 1989). Brain tissues homogenized on cold in 35% perchloric acid and centrifuged at 12,000 rpm for 3 min. Supernatants were combined with color reagent (0.6% copper sulfate, 5% thiourea, and 2,4-dinitrophenlyhydrazine at 1:1:20, v/v/v) and mixtures incubated 3 h at 37°C in a water bath. After they were cooled to 0°C, 65% (v/v) sulfuric acid was added and absorbances were measured at 515 nm.

Determination of MPO activity: MPO activity was measured by method of Glowick and Kaplan (1955). Tissue samples homogenized in cold phosphate buffer (pH 7.5). After centrifuged at 3000 g for 10 minutes at 4°C, supernatants were added to tubes containing 0.5 M phosphate buffer, 30% H_2O_2, 1% o-dianisidin, H_2O (10:1:2:3 v/v/ v/v). Mixtures incubated at 37°C for 30 minutes then HCl was added. One unit (U) of enzyme activity was defined as the amount of MPO present that caused a change in absorbance of 1.0/min at 410 nm and 37°C.

Statistical analysis: All data were submitted as the mean ± standard deviation. Evaluation of the values for all experimental groups was used by ANOVA variance analysis and nonparametric Mann–Whitney U test. The p-value less than 0.05 was well-considered significant.

RESULTS

The NOx levels of the brain in the control group were found to be 145.63 ± 14.88 μmol g^{-1}, while, they were 101.46 ± 7.61 μmol g^{-1} in the diabetes group and 115.06 ± 9.30 μmol g^{-1} in the benfluorex treatment group. In Figure 1, NOx levels are shown as diagrams. While the NOx levels were found to be significantly reduced in both groups than in the control group ($p<0.05$). In the benfluorex treatment group, NOx level was found to be significantly increased than the diabetes group ($p<0.05$).

The brain TBARS value in the control group was found to be 261.47±32.18 nmol g^{-1}, while they were 430.07±46.12 nmol g^{-1} in the diabetes group, and 262.65±44.74 nmol g^{-1} in the benfluorex treatment group. In Figure 2, TBARS values are shown as diagrams. TBARS level of the diabetes group was found to be elevated significantly compared to the control group ($p<0.05$). However, benfluorex treatment was significantly reduced TBARS levels compared to the diabetes group ($p<0.05$).

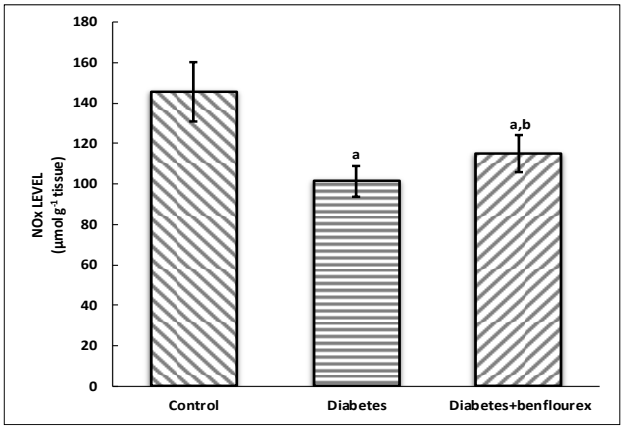

Figure 1. The effects of benfluorex administration during diabetes on the brain NOx levels.
[a] $p < 0.05$ compared to the control group; [b] $p < 0.05$ compared to the diabetes group.

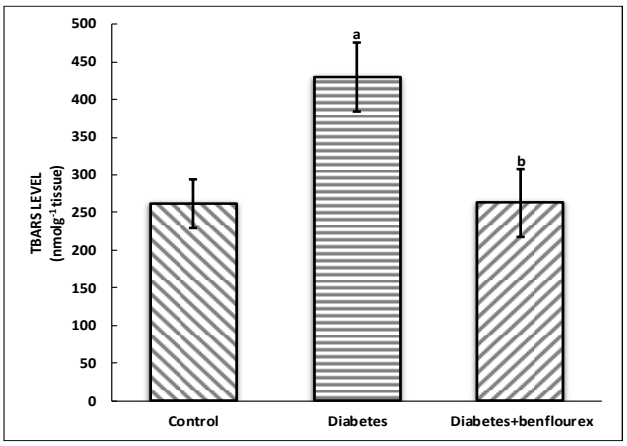

Figure 2. The effects of benfluorex administration during diabetes on the brain TBARS levels.
[a] $p < 0.05$ compared to the control group; [b] $p < 0.05$ compared to the diabetes group.

GSH levels of the brain were found to be respectively 3.86±0.36 μmol g^{-1}, 2.96 ± 0.49 μmol g^{-1} and, 4.48 ± 0.89 μmol g^{-1} in the control group, in the diabetes group and, in the benfluorex treatment group. In Figure 3, GSH levels are shown as diagrams. GSH level was found to be significantly decreased in the diabetes group compared to the control ($p<0.05$). Whereas with benfluorex administration, GSH level was shown to be significantly increased compared to the diabetes group ($p<0.05$).

The brain AA levels in the control group was 3.49±0.29 mg g^{-1}, while they were 2.98±0.21 mg g^{-1} in the diabetes group, and 3.48±0.16 mg g^{-1} in the benfluorex treatment group. In Figure 4, AA levels are shown as diagrams. AA level was found to be significantly reduced in the diabetes group compared to the control ($p<0.05$). Although in the benfluorex treatment group AA level was found to be significant enhancements than diabetes group ($p<0.05$).

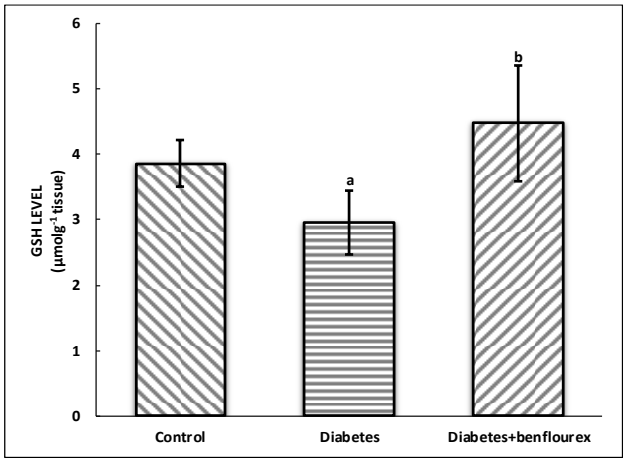

Figure 3. The effects of benfluorex administration during diabetes on the brain GSH levels.
[a] $p < 0.05$ compared to the control group; [b] $p < 0.05$ compared to the diabetes group.

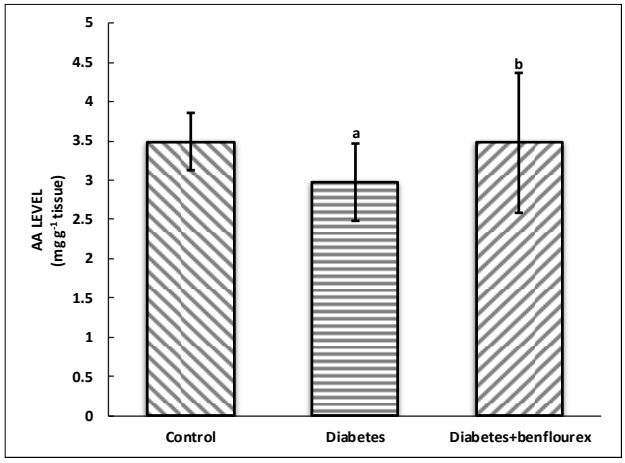

Figure 4. The effects of benfluorex administration during diabetes on the brain AA levels.
[a] $p < 0.05$ compared to the control group; [b] $p < 0.05$ compared to the diabetes group.

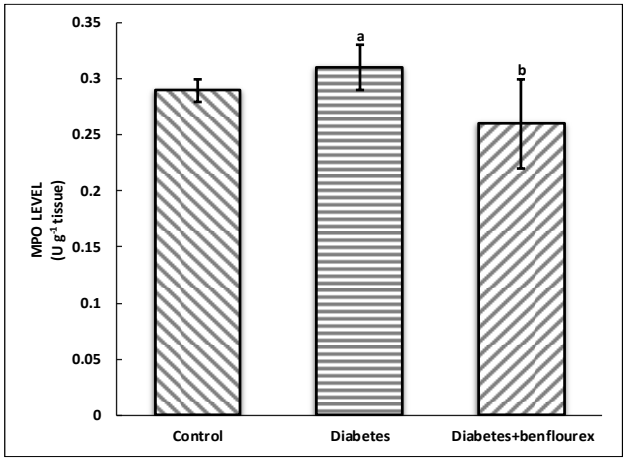

Figure 5. The effects of benfluorex administration during diabetes on the brain MPO activities.
[a] $p < 0.05$ compared to the control group; [b] $p < 0.05$ compared to the diabetes group.

MPO activity of the brain was found to be respectively 0.29±0.01 U g^{-1} tissue, 0.31 ± 0.02 U g^{-1} tissue and, 0.26 ± 0.04 U g^{-1} tissue in the control group, in the diabetes group and, in the benfluorex treatment group. In Figure 5, MPO activities are shown as diagrams. MPO activity of the diabetes group was found to be increased significantly compared to the control group ($p<0.05$). But, benfluorex treatment was significantly reduced MPO activity compared to the diabetes group ($p<0.05$).

DISCUSSION

This was the first study to show brain oxidative status, non-enzymatic antioxidants levels and MPO activity between diabetic rats who were treated with benfluorex compared with untreated diabetic rats.

Several studies have indicated that the altered oxidative state due to hyperglycemia may be induced to the diabetic nerve damage (Aragno et al., 2000; Ateş et al., 2006; Ateş et al., 2007; Zhang et al., 2008). One of the underlying causes of this damage has been that increased intracellular glucose concentration, therefore, the excessive formation of ROS originating from auto-oxidation. (Piero et al., 2014). Different investigators have been revealed that lipid peroxidation was elevated in the brain, arising from diabetes (El-Akabawy & El-Kholy, 2014; Muriach et al., 2014; Ogunyinka et al., 2016; Ibrahim, 2016; Fheem & Askary, 2017; Gürel-Gökmen et al., 2018). The results of this study showed that TBARS levels were increased in the brains of diabetic animals than in controls as demonstrated in other studies. Additionally, in the current results, non-enzymatic antioxidants GSH and AA levels, the inverse of rising lipid peroxidation, were found decreased in the diabetic group. Antioxidant defense system including antioxidant enzymes and non-enzymatic antioxidant compounds may be affected by diabetic processes (Kurutaş, 2016). Evaluation of TBARS and non-enzymatic antioxidant levels of the diabetic group has been suggested that excessive ROS production via both hyperglycemia and increased auto-oxidation of glucose during diabetes may cause lipid peroxidation. Also, it has been thought that of GSH and AA radical scavenging properties may be used to prevent from oxidative damage.

It has been demonstrated that several vascular complications related to diabetes may result from changes in the production and action of endothelially derived NO (Avogaro et al., 2006). In the literature, contradictory data about the amount of NO in diabetes have been reported. In the animal model of STZ-induced diabetes, some investigators have found to be increased NO levels in the cerebral cortex, hippocampus, cerebellum, brain stem, and spinal cord (Ateş et al., 2007; Xu et al., 2015), although in another study it was reported that NO levels were decreased in the hippocampus (Kino et al., 2004). However, Gurel-Gokmen et al. (2018) have observed that NO levels did not change in the brains of diabetic animals induced by STZ. According to the results of this study, the NOx levels of the brain during diabetes were found

decreased significantly compared to the controls. This situation may be elucidated by the raised catabolism of NO or the reduced production of NO by endothelial dysfunction due to diabetes.

Myeloperoxidase (MPO) is an important pro-oxidant enzyme that is physiologically released in circulating neutrophils, monocytes and some tissue macrophages including microglia as also described an inflammatory marker (39,42). When discharged to the extracellular environment as a component of inherent host defense, tissue damage can occur via MPO-derived oxidants (Lazarević-Pasti et al., 2015). MPO can also debilitate lipoprotein function, initiate endothelial dysfunction, and disrupt synthase of inducible NO (Avogaro et al., 2006). Consistent with other findings, the activity of MPO was found increased in diabetes group, and also at the same time, NO level was found the decrease in diabetic animals because of MPO's probably effect of triggering endothelial dysfunction.

Benfluorex was shown to have hypolipidemic and antihyperglycemic effects in diabetic animal models and in humans (Ravel & Laudignon, 1996). However, there has been never examined the relationship between benfluorex administration with oxidative events in the brain during diabetes. In the current study, the consequences of benfluorex treatment on the brain oxidative status were found to be interesting. According to the results of this study, benfluorex application was reduced TBARS level and MPO activity, but it was increased NO, GSH and AA levels. In the previous studies, benfluorex has been shown to reduce hepatic glucose production, ameliorate binding of insulin to its receptor, and increase aerobic glucose utilization in skeletal muscle (Bianchi et al., 1993; De Feo et al., 1993; Riccio et al., 1993; Kohl et al., 2002). Also, that has been showed that benfluorex affects the expression of genes encoding enzymes related to both glucose and fatty acid metabolism, resulting in inhibition of mitochondrial β-oxidation, that resulted reduces in gluconeogenesis (Kohl et al., 2002). On the other hand, it has been known that fenfluramine derivatives including benfluorex initiate serotonergic mechanisms via increasing synaptic levels of 5-HT. Because, norfenfluramine, a metabolite of benfluorex, has a strong agonistic effect for the 5-HT_{2B} receptor (Rothman et al., 2000). Furthermore, it has been established that 5-HT_2 receptor activation promotes glycogenolysis, which halted the process of gluconeogenesis. (Darvesh, & Gudelsky, 2003). The cumulative effect of all these metabolic paths constitutes both the antidiabetic and antihyperlipidemic effects of benfluorex. Based on our results, it may be suggested that hypolipidemic properties of benfluorex decrease lipid peroxidation, which as a result of changing lipid metabolism due to inhibition of beta-oxidation. In addition, its antidiabetic effects, in turn, may alleviate some metabolic and physiologic abnormalities associated with diabetes such as endothelial dysfunction, preternatural inflammation and, tissue damage via excessive ROS production. These facts about benfluorex affect mechanism may explain that the reason for decreased levels of TBARS and MPO activity, and also increased levels of NOx, GSH and AA in the brain during experimental diabetes.

CONCLUSION

Consequently, in this study, besides the known life-threatening cardiac side effects; from a different perspective, the effects of benfluorex on the brain during diabetes were examined. Taken together, the present study suggests that brain tissue with its inflammatory enzymatic process, ROS production, lipid peroxidation, and its non-enzymatic antioxidant capacity were affected by benfluorex treatment while experimental diabetes.

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ARAŞTIRMA MAKALESİ RESEARCH PAPER

Age, Growth, Length-Weight Relationship and Condition Factor of Beysehir Dace (*Squalius anatolicus* Bogutskaya, 1997) in Oymapınar Dam Lake (Antalya), Turkey

Elif SARI Zehra Arzu BECER*

Department of Basic Fisheries Sciences, Faculty of Fisheries, Akdeniz University,Konyaaltı, Antalya, Turkey

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: https://orcid.org/0000-0002-7277-0210
*: https://orcid.org/0000-0002-2793-4503

***Corresponding author's:**
Zehra Arzu BECER
Department of Basic Fisheries Sciences,
Faculty of Fisheries, Akdeniz University,
Konyaaltı, Antalya, Turkey
✉: abecer@akdeniz.edu.tr

Abstract: This study aimed to determine some biological parameters such as age, growth condition factor, length-weight relationship, and sex ratio of Beysehir daces (*Squalius anatolicus*) sampled from Oymapınar dam lake between March 2016 and February 2017. A total of 422 Beysehir daces were sampled using 100 m long and 2,5 m depth monofilament gillnets (20, 30, 40, 50 mm mesh size) from the Oymapınar dam lake. The ages of the Beysehir daces ranged between II and VIII. The oldest age for females was VIII and VII for males. The sex composition of the sampled fish showed that 47.39 % of the samples were females and 52.61% were males. It was determined that the fork lengths of the females varied between 20.0 cm and 55.1 cm and the weights varied between 93.37 g and 2307.88 g. Von Bertalanffy growth parameters for all individuals were determined as L_∞ = 60.57 cm, W_∞ = 3260.79 g, k = 0.160 and t_0 = -0.877 The b values of the length-weight relationship for females, males and all individuals were 3.202, 3.185 and 3.194, respectively. The average condition factor (C_F) for all individuals was calculated as 1.278 ± 0.006. The findings of this study will aid in the development and implementation of long-term management plans for the Beysehir dams in this lake, as well as serve as the foundation for future research on the biological parameters of fish living in this lake.

Keywords: Age, growth, oymapınar dam lake, *Squalius anatolicus.*

Oymapınar Baraj Gölü'ndeki (Antalya, Türkiye) Beyşehir Tatlısu Kefalinin *(Squalius anatolicus* Bogutskaya, 1997*)* Yaş, Büyüme, Boy-Ağırlık İlişkisi ve Kondisyon Faktörü

***Sorumlu yazar:**
Zehra Arzu BECER
Akdeniz Üniversitesi, Su Ürünleri Fakültesi,
Su Ürünleri Temel Bilimler Bölümü,
Konyaaltı, Antalya, Türkiye
✉: abecer@akdeniz.edu.tr

Öz: Bu çalışmada; Oymapınar Baraj Gölü'nden Mart 2016 - Şubat 2017 tarihleri arasında örneklenen Beyşehir tatlısu kefali (*S. anatolicus*)'nin yaş, büyüme, kondisyon faktörü, boy-ağırlık ilişkisi ve cinsiyet oranı gibi bazı biyolojik parametrelerin belirlenmesi amaçlanmıştır. Oymapınar Baraj Gölü'nden örneklenen 422 Beyşehir tatlısu kefalinin yaşları II-VIII arasında dağılım göstermiş, örneklerin % 47,39'nu dişiler, % 52,61'ini erkekler oluşturmuştur. Dişilerin çatal boylarının 20,0 cm ile 55,1 cm arasında, ağırlıklarının ise 93,37 g ile 2307,88 g arasında değiştiği saptanmıştır. Tüm bireyler için Von Bertalanffy büyüme parametreleri L_∞ = 60,57 cm, W_∞= 3260,79 g, k= 0,160 ve t_0= -0,877 olarak belirlenmiştir. Bu çalışmada balıklar için elde edilen b değerleri, izometrik b değerinden (3,0) istatistiki olarak önemli bulunmuştur. Yakalanan en büyük dişi VIII., erkek VII. yaşındadır. Tüm bireyler için ortalama kondisyon faktörü (KF) 1,278± 0,006 olarak hesaplanmıştır. Bu çalışmanın sonuçları, göldeki beyşehir tatlısu kefali için sürdürülebilir yönetim planlarının oluşturulması ve uygulanmasına yardımcı olacak ve gölde yaşayan balıkların biyolojik parametreleri ile ilgili gelecekte yapılacak çalışmalara temel oluşturacaktır.

Anahtar kelimeler: Büyüme, oymapınar baraj gölü, *Squalius anatolicus*, yaş.

INTRODUCTION

Squalius anatolicus (Bogutskaya, 1997) is a member of the Leuciscidae family and is commonly known as "Central Anatolian Pike Chub or Beysehir dace", which is endemic to Turkey (Freyhof, 2014; Çiçek, 2018, Bayçelebi et al., 2020). The population of the Beysehir dace is limited in distribution and is predominantly found in the Beyşehir lake, Saltlake, and Manavgat river basins (Özuluğ & Freyhof, 2011, Çiçek, et al., 2018). *S. anatolicus* is nutritious and appreciated by many people as a source of animal protein.

Studies on fish population are very important in resolving the management of fishing related issues (Polat et al., 1999). It forms the basis for calculations leading to knowledge of the growth, mortality, recruitment, and other fundamental parameters of fish populations. Growth is one of the most critical measurable characteristics of individuals, stocks, and species, and it is fundamental to our understanding of the life histories, demographics, ecosystem dynamics, and sustainability of fisheries (Pardo et al., 2013). Thus, knowledge of biological parameters such as the age, condition factor, growth parameters, length – weight relations and sex ratios of fish are important in understanding, the present and future status of the species due to environmental changes and the appropriate stock management measures to be taken (Wang et al., 2017).

There are some biological studies on *S. anatolicus* in Turkey (Aydogdu et al., 2015, Bayçelebi et al., 2020). But there is no previous study on the biological characteristics of *S. anatolicus* living in the Oymapınar dam lake.

In this study, the age, length and weight distribution, age-length, age-weight and length-weight relationships, sex distribution, growth parameters and condition factor of *S. anatolicus* were investigated to reveal the growth characteristics of the population. The results from this study will help in planning and implementing sustainable management plans for the Beysehir daces in this lake and will also act as the base for future studies on the biological parameters of fishes living in this lake.

MATERIALS AND METHODS

Study Area: This research was conducted at the Oymapnar Dam Lake in Antalya. Oymapınar Dam is located 76 km from the centre of Antalya province. It is a dam built on the Manavgat river from 1977 to 1984 for electric energy generation (Anonymous, 2021). The lake lies between latitude 36.908628°N and longitude 31.531694°E in the Antalya province of Turkey. Fish samples were collected from seven stations spread across different parts of the dam lake. Fish samples were collected from seven (7) sampling stations. The sampling stations were located at the points where the various sources of water enter the Oymapınar Dam Lake. The sampling point coordinates are presented in Table 1 and the sampling locations are shown in Figure 1.

Table 1. Sampling point coordinates.

Sampling Point	Latitude (°N)	Longitude (°E)
Power plant (1)	36.909	31.532
Kızlıca Stream (2)	36.917	31.534
Tepekli Stream (3)	36.923	31.537
Manavgat River (4)	36.921	31.549
Karpuz Stream (5)	36.908	31.57
Köprü Stream (6)	36.907	31.576
Aygir Stream (7)	36.903	31.576

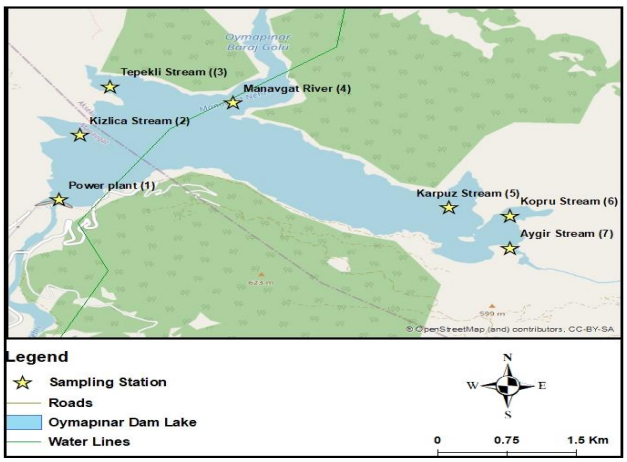

Figure 1. Oymapınar Dam Lake map showing sampling stations.

Fish Sampling and Measurements of Length and Weight: This study was conducted between March 2016 and February 2017. A total of 422 Beysehir daces were sampled using 100 m long and 2.5 m depth monofilament gillnets (20, 30, 40, 50 mm mesh size) from the Oymapınar dam lake. Beysehir dace was collected through random sampling from various points that could represent the lake. The nets were collected the next day after being thrown. The fork lengths (FL) of the samples were measured to the centimetre (0.1 cm) by placing them on the measuring board. Body weights were weighed with an electronic scale with an accuracy of 0.1 g.

Determination of Growth Parameters

***Age Determination*:** The scales of the Beysehir dace samples were used in age determinations according to the method specified in Lagler (1966) and Nikolsky (1963). Scales of fish were collected using dissecting scissors and forceps. The scale samples were cleaned and stored in small envelopes. The age of fish was determined from the scale using the profile projector tool at a 17.5X magnification.

Absolute and Proportional Growth: Growth was determined in terms of length and weight by sex, absolute and proportional growth. Fish samples were grouped

according to their age, average length, and weight (Çetinkaya et al., 1989). Absolute growth is determined as the length and weight attained at any age, and proportional growth is the growth in a certain period, the percentage of length or weight at the beginning of the period (Erkoyuncu, 1995).

The absolute length increase (AFL) and absolute weight increase (AW) were calculated as follows:

$AFL= (L_t - L_{t-1})$ and $AW = (W_t - W_{t-1})$

While proportional length increase (PFL) and proportional weight increase (PW) values were calculated using the equations suggested by Chugunova, (1963):

$PFL = [(L_t - L_{t-1}) / L_{t-1}] * 100$ and $PW = [(W_t - W_{t-1}) / W_{t-1}] * 100$.

In the equations, "L_t" is the average absolute length (cm) at any age, "L_{t-1}" is the average absolute length (cm) in the previous year, "W_t" is the average absolute weight (g) at any age, while "W_{t-1}" is the average absolute weight (g) in the previous year.

***Age-Length and Age-Weight Relationships*:** The age-length and age-weight relationships were determined by the von Bertalanffy Growth (VBBD) model (Beverton and Holt, 1957). For age-length and age-weight growth equations were used; $Lt = L_\infty^* (1-e^{-k(t-to)})$ and $Wt = W_\infty^* (1-e^{-k(t-to)})^b$, respectively (Lugart et al., 2016). In the equations, "Lt" is the expected length (cm) at a given time (t), "t" is the age, "Wt" (g) is the weight at a given time (t), "L_∞" is the asymptotic length (cm) of the fish, "W_∞" is the asymptotic weight (g) of the fish, "k" is the growth coefficient, "t_0" is the age at which the fish's length is theoretically zero, and "b" is the slope of the weight/length relationship.

***Length–Weight Relationship*:** The length-weight relationship was calculated according to Le Cren's (1951) equation, $W = aL^b$, by using the measured length and weight values of every individual. In this relationship, if the logarithms of both sides are taken to base 10, the relationship becomes a linear ($logW= loga + b.logL$) equation, where "W" is the fish weight (g), "L" is the fork length (cm), "a and b" are the regression coefficients.

***Condition Factor*:** Lagler's (1966) isometric growth equation was used to calculate the condition factor (C_F), known as the nutritional coefficient of individuals.

$C_F = (W/L^3) * 100$ where C_F = Condition factor, W = weight (g), L represents length (fork length, cm) (Nikolsky 1963; Çetinkaya 1989).

***Sex ratio*:** The sex ratio is defined as being the proportion of male or female individuals compared to the total number of individuals. It also gives an idea regarding the balance of the sexes within the population. The sex ratio is calculated as follows:

SR = F/ (M + F) x 100 where F= number of females, M = number of males.

Statistical Analysis: The data were subjected to statistical analysis using the computer package programs Microsoft Office Excel, and the IBM Statistical Package for Social Science (SPSS) version 21. All statistical analyses were considered significant at $P < 0.05$ and, all means are given with ± standard errors (SE).

The Chi-square (χ^2) test was used to compare sex ratios in age groups. The paired t-test was used to compare the mean lengths, weights, and condition factors of the female and male fish in age groups, and the paired t-test was used to compare the measured lengths and weights and the lengths and weights obtained by calculation (Kaptan, 1995). The student's t-test was used to test the statistical deviation of the obtained b values from the isometric range of 3.0 for fish.

RESULTS

Population Structure

Age, Length and Sex Distribution: The results of the age and sex distribution show that of the 422 Beysehir daces that were sampled from Oymapınar dam lake, 47.39% (200) were female and 52.61% (222) were male and the ages ranged from age groups II to VIII. The fork lengths of the 422 Beysehir daces sampled from the Oymapınar dam lake were measured and the lengths varied from 20 cm to 55.1 cm. Individuals in the 26 cm and 30 cm length groups were found to have the highest proportion with 47.16% of the population (Table 2). The fork lengths of the female fish varied between 20 cm and 55.1 cm, and the fork lengths of the male fish ranged from 20 cm to 51 cm. The female-to-male sex ratio was 0.90: 1.00. It was observed from the results that the most dominant age group in the population for both sexes was age group II. Sex distributions according to age groups are given in Table 2. The Chi-square (χ^2) test showed the differences between all age groups and, the sex ratio of Beysehir daces in total were not statistically significant ($P \geq 0.05$) Table 2).

Distributions of Weight Among Sampled Species: The weight of the 422 Beysehir daces examined varied between 93.37 g and 2307.88 g. Females weighed between 101.95 g and 2307.88 g while the weight of the males varied between 93.37 g and 2010.99 g. As shown in Table 3, the predominant weight class in the sampled population for female and male was 300 g with a rate of 15.88% for females and 21.33% for males. When females and males were mixed, the 300 g weight class had a rate of 37.20%, followed by the 100 g, 500 g and 900 g weight classes at 27.3%, 17.30%, and 5.69% respectively (Table 3).

Growth in Length: The average length, annual absolute length, and proportional length increase values of Beysehir daces in each age group sampled between March 2016 and February 2017 are presented in Table 4. The fastest growth rate in both sex groups occurred in age class

II. The proportional increase in length of male fish was greater than that of females (Table 5). The "t" test analysis showed that there was a significant difference between the average length of male and female fish in age class VI ($P \leq 0.05$).

Population growth: The growth of Beysehir dace was mathematically examined using the VBBD model and the growth parameters and growth equation obtained are presented in Table 5. The calculated Length values for both sex and age groups according to VBBD are shown in Table 4. In sex groups, it was observed that the difference between the measured length and the length obtained by calculation was not statistically significant according to the t-test analysis ($P \geq 0.05$).

Table 2. Sex distribution of Beysehir daces by age group

Age	Female		Male		χ^2 test	Female + Male	
	N	N (%)	N	N (%)		N	N (%)
II	71	16.82	85	20.14	0.891 (P>0.05)	156	36.97
III	44	10.43	60	14.22	0.371 (P>0.05)	104	24.64
IV	40	9.48	51	12.09	0.254 (P>0.05)	91	21.56
V	10	2.37	11	2.61	0.329 (P>0.05)	21	4.98
VI	7	1.66	13	3.08	0.130 (P>0.05)	20	4.74
VII	22	5.21	2	0.47	0.293 (P>0.05)	24	5.69
VIII	6	1.42	-	-	-	6	1.42
Total	200	47.39	222	52.61	0.700 (P>0.05)	422	100.00

Table 3. Dstribution of Beysehir daces by sex and weight classes

Weight Classes (g)	Female		Male		Female + Male	
	N	N%	N	N%	N	N%
100	55	13.03	62	14.69	117	27.73
300	67	15.88	90	21.33	157	37.20
500	36	8.53	37	8.77	73	17.30
700	8	1.90	12	2.84	20	4.74
900	13	3.08	11	2.61	24	5.69
1100	6	1.42	5	1.18	11	2.61
1300	7	1.66	3	0.71	10	2.37
1500	3	0.71	1	0.24	4	0.95
1700	1	0.24	0	0.00	1	0.24
1900	1	0.24	0	0.00	1	0.24
2100	2	0.47	1	0.24	3	0.71
2300	1	0.24	0	0.00	1	0.24
Total	200	47.39	222	52.61	422	100.00

Table 5. Growth Parameters and Equations of von Bertalanffy Calculated in terms of Length with Respect to Sexes of Beysehir daces.

Growth Parameters				
Sex	L_∞	K	t_0	Growth Equations
Female	66.18	0.140	-0.984	$Lt = 66.18\ [1- e^{-0.140\ (t+0.984)}]$
Male	57.15	0.170	-0.837	$Lt = 57.15\ [1- e^{-0.170\ (t+0.837)}]$
Female + Male	60.57	0.160	-0.877	$Lt = 60.57\ [1- e^{-0.160\ (t+0.877)}]$

L_∞ = asymptotic length, k = growth coefficient, t_0 = hypothetical age

Table 4. Average fork lengths (FL, cm), Standard Errors (SE), Minimum and Maximum Length Values, Annual Absolute (AFL) and Proportional Length Increase (PFL) Values in Beysehir daces by Sex and Age Groups

Age	Female				Male					Female + Male			
	N	FL±SE (min – max)	AFL	PFL %	N	FL±SE (min – max)	AFL	PFL %	T-test	N	FL±SE (min – max)	SFL	PFL %
II	71	23.67±0.18 (20-26.4)	-	-	85	23.68±0.17 (20-27.3)	-	-	0.395 P > 0.05	156	23.67±0.12 (20-27.3)	-	-
III	44	28.61±0.23 (26.1-31.8)	4.94	20.87	60	28.77±0.20 (26.2-32.4)	5.09	21.49	0.156 P > 0.05	104	28.70±0.15 (26.1-32.4)	5.03	21.25
IV	40	32.84±0.24 (29.8-35.5)	4.23	14.79	51	33.32±0.31 (29.5-37.8)	4.55	15.82	1.000 P > 0.05	91	33.11±0.20 (29.5-37.8)	4.41	15.37
V	10	36.36±0.24 (35.3-37.5)	3.52	10.72	11	38.37±0.27 (36.7-39.5)	5.05	15.16	0.344 P > 0.05	21	37.41±0.28 (35.3-39.5)	4.3	12.99
VI	7	39.05±0.19 (38.2-39.6)	2.69	7.40	13	42.49±0.48 (39.9-44.7)	4.12	10.74	0.037 P < 0.05	20	41.29±0.49 (38.2-44.7)	3.88	10.37
VII	22	42.99±0.50 (40-46.7)	3.94	10.09	2	49.55±1.44 (48.1-51)	7.06	16.62	0.142 P > 0.05	24	45.53±0.59 (40-51)	4.24	10.27
VIII	6	51.68±1.14 (48.5-55.1)	8.69	20.21	-	-	-	-	-	6	51.68±1.14 (48.5-55.1)	6.15	13.51

FL = Fork Length, SE = Standard Error, Min = Minimum, Max = Maximum, AFL = Absolute Fork Length and PFL = Proportional Fork Length

Growth in Weight: The average weight (g), absolute weight (AW) and proportional weight increase (PW) of the Beysehir daces, which were examined according to age and sex groups, are shown in Table 8. The "t" test analysis showed that the difference between the average weights of female and male fish in age class III and IV was statistically significant ($P \leq 0.05$).

The calculated von Bertalanffy weight growth parameters and equations are given in Table 6. The b values of 3.200, 3.186 and 3.195 obtained for female, male and female + male respectively was statistically different from the isometric b value range of 3 ($P \leq 0.05$). The calculated largest obtainable weight (W_∞) value for females (4294.08 g) is larger than that of males (2692.69 g), but the k value for the female is lower than that of the male. The measured weights (g) of the Beysehir dace and calculated weight using von the Bertalanffy growth equation are shown in Table 7. In the sex groups, it was observed that the differences between the measured weights and the calculated average weights statistically insignificant ($P \geq 0.05$).

Table 6. Calculated von Bertalanffy weight growth parameters and equations based on sex in Beysehir dace.

Growth Parameters						
Sex	W_∞	k	t_0	b	t-test	Growth Equations
Female	4294.08	0.140	-0.984	3.200	0.000, P < 0.05	$Wt = 4294.08\ [1- e^{0.140\ (t+\ 0.984\ )}]^{3.200}$
Male	2692.69	0.170	-0.837	3.186	0.001, P < 0.05	$Wt = 2816.17\ [1- e^{0.170\ (t+\ 0.837)}]^{3.186}$
Female + Male	3260.79	0.160	-0.877	3.195	0.000, P < 0.05	$Wt =5303.27\ [1- e^{0.160\ (t+\ 0.877\ )}]^{3.195}$

W_∞ = asymptotic weight, k = growth coefficient, t_0 = hypothetical age, b = is the slope of the weight/length relationship

Table 7. Measured weights (g) and calculated weights of the Beysehir dace using von Bertalanffy growth equation.

Age	Female			Male			Female + Male		
	N	Weighed (W)	Calculated (W)	N	Weighed (W)	Calculated (W)	N	Weighed (W)	Calculated (W)
II	71	166.12	137.91	85	167.70	126.25	156	166.93	134.94
III	44	297.11	283.05	60	301.77	258.44	104	299.80	277.25
IV	40	455.49	474.16	51	481.45	426.07	91	470.04	460.29
V	10	648.43	699.99	11	793.17	615.74	21	724.24	670.66
VI	7	843.39	948.63	13	1088.44	815.23	20	1002.67	895.46
VII	22	1130.20	1209.13	2	1725.96	1014.77	24	1179.84	1123.93
VIII	6	1951.63	1472.35	-	-	-	6	1951.63	-

Table 8. Average weight (W, g), standard errors (SE), minimum and maximum weight values of Beysehir dace based on age groups and sexes, annual absolute weight increase (AW) and proportional weight increase (PW) values.

Age	Female				Male				t-test	Female + Male			
	N	W±SE (min – max)	AW	PW (%)	N	W±SE (min – max)	AW	PW (%)		N	W±SE (min – max)	AW	PW (%)
II	71	166.12±4.64 101.95-248.76	-	-	85	167.70±4.37 93.37-247.45	-	-	0.058 (P > 0.05)	156	166.93±3.17 93.37-248.78	-	-
III	44	297.11±8.81 203.4- 435.74	130.99	78.85	60	301.77±7.36 219.51-465.37	134.07	79.95	0.034 (P < 0.05)	104	299.80±5.58 203.4-465.37	132.87	79.60
IV	40	455.49±12.47 302.3-647.56	158.38	53.31	51	481.45±15.22 284.94-836.17	179.68	59.54	0.001 (P < 0.05)	91	470.04±10.18 284.94-836.17	170.24	56.78
V	10	648.43±30.67 520.54-776.31	192.94	42.36	11	793.17±22.08 659.09-945.77	311.72	64.75	0.126 (P > 0.05)	21	724.24±24.30 520.54-945.77	254.2	54.08
VI	7	843.39±22.42 773.46-956.43	194.96	30.07	13	1088.44±35.20 956.38-1289.15	295.27	37.23	0.070 (P > 0.05)	20	1002.67±35.82 773.46-1289.15	278.43	38.44
VII	22	1130.20±41.00 873.9-1477.17	286.81	34.01	2	1725.96±285.02 1440.94-2010.99	637.52	58.57	0.194 (P > 0.05)	24	1179.84±53.67 873.9-2010.99	177.17	17.67
VIII	6	1951.63±123.70 1535.22-2307.88	821.43	72.68	-	-	-	-	-	6	1951.63±123.70 1535.22-2307.88	821.43	72.68

W = Weight, SE = Standard Error, Min = Minimum, Max = Maximum, AW = Absolute Weight, PW = Proportional Weight

Length – Weight Relationship: The length-weight relationship equation of Beysehir dace sampled from Oymapınar Dam Lake is given as follows; W= 0.0064 FL $^{3.202}$ (R^2= 0.982) for females (Figure 2), W= 0.0068 FL $^{3.185}$ (R^2= 0.981) (Figure 3) for males and males and W=0.0066 FL$^{3.194}$ (R^2= 0.982) for both females and males combined (Figure 4). The b value obtained from the regression equations used to determine the length-weight relationship was close to 3, indicating that there is positive allometric growth in the Beysehir population under study (Ricker 1968). The correlation coefficient in the relationships was found to be as high as 0.98. It was observed that in the first year, the growth in weight was faster than the growth in length in the sampled Beysehir dace population.

Condition Factor (C_F): The calculated average, maximum and minimum condition factors, and standard errors for each age class of the Beysehir dace samples are given in Table 9. In the Beysehir dace population sampled from Oymapınar dam lake, the average C_F values according to age classes and sex groups. The sum of female - male Beysehir daces varied between 1.237 and 1.410 in females. It was observed that the average C_F values generally increases with age. The difference between the average C_F values of Beysehir daces at the age class VII was statistically significant ($P \leq 0.05$). The average calculated C_F value of Beysehir dace for female, male and female-male combined was 1.284, 1.273 and 1.278, respectively.

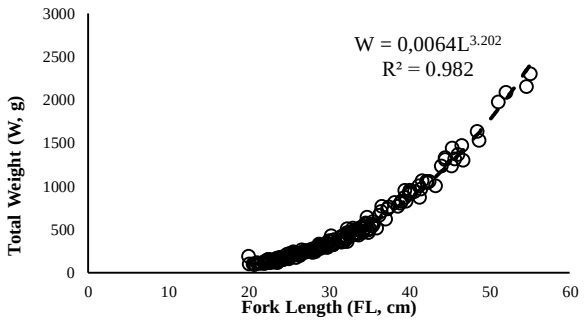

Figure 2. Length – weight relationship in female Beysehir daces.

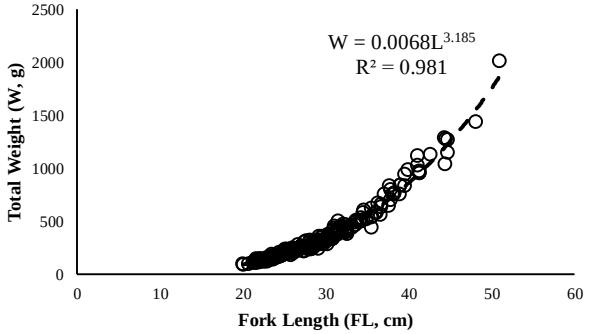

Figure 3. Length – weight relationship in male Beysehir daces.

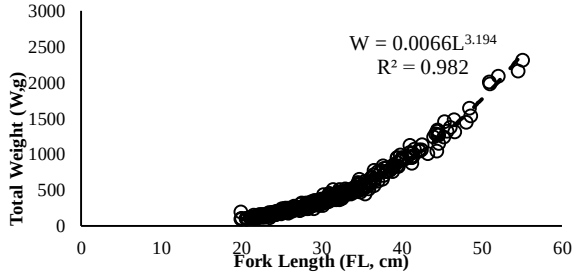

Figure 4. Length – weight relationship in both female and male Beysehir daces combined.

Table 9. The calculated average, minimum and maximum C_F values of Beysehir daces based on age classes and sex.

	Female		Male			Female + Male	
Age	N	C_F±SE (min - max)	N	C_F±SE (min – max)	t-test	N	C_F±SE (min – max)
II	71	1.237 ± 0.01 (0.902-2.469)	85	1.237 ± 0.01(1.017-1.503)	0.705 P > 0.05	156	1.237 ± 0.01 (0.902-2.469)
III	44	1.254 ± 0.02 (1.095-1.560)	60	1.256 ± 0.01(0.983-1.512)	0.004 P < 0.05	104	1.255 ± 0.01 (0.983-1.560)
IV	40	1.275 ± 0.02 (1.089-1.536)	51	1.284 ± 0.01(0.997-1.616)	0.620 P > 0.05	91	1.280±0.01 (0.997-1.616)
V	10	1.343 ± 0.04(1.115-1.583)	11	1.401 ± 0.04(1.286-1.534)	0.896 P > 0.05	21	1.373±0.03 (1.115-1.583)
VI	7	1.414 ± 0.05 (1.342-1.551)	13	1.417 ± 0.03(1.189-1.613)	0.547 P > 0.05	20	1.416±0.03 (1.189-1.613)
VII	22	1.411 ± 0.02 (1.240-1.550)	2	1.405 ± 0.10(1.294-1.516)	0.000 P < 0.05	24	1.410±0.02(1.240-1.550)
VIII	6	1.405 ± 0.05 (1.325-1.482)	-	-	-	6	1.405±0.05(1.325-1.482)
Total	200	1.284 ± 0.09	222	1.273 ± 0.09	0.228 P > 0.05	422	1.278±0.006

C_F = Condition Factor, SE = Standard Error, Min = Minimum, Max = Maximum

DISCUSSION

The understanding of variation in sex ratio is critical because it is a major factor in population fecundity. Unbalanced sex ratios can drive sexual selection, affect the mating system, and influence population persistence and conservation status, owing to the effect of the sex ratio on effective population size (Clutton-Brock 2007, Wang et al., 2017). The sex ratio of male to female in this study was 0.90:1, which is close to the 1:1 expected for most fish species (Bagenal & Tesch,1978). The ratio of females to males recorded in this study does not agree with the studies of Özcan & Serdar, (2019) that reported 1:0.9, female to male for the chubs living in Lake Yeniçağa, Bolu, Turkey Kılıç & Becer, (2016) also reported a higher female ratio in their studies. However, similar to the report, Benzer (2013) found a higher male to female ratio for chubs living in the Kirmir stream of the Sakarya River in Turkey. Carbonara et al., (2012) stated that differences in sex ratio are due to ethological behaviour responsible for the over-dispersion and segregated distribution of the sexes. Variation in sex ratio could be caused by several factors, such as offspring sex ratio, sex differences in mortality and migratory rates, and differ according to age at maturity (Donald 2007) and even due to environmental factors including temperature and toxicants (Wang et al., 2017).

The age classes of the sampled Beysehir daces varied from II to VIII. Out of the 422 sampled Beysehir daces, 156(36.97%) belong to age class II while age class III and IV followed closely with 104(24.64%) and 91(21.56%). In the studies of Balik et al., (2004), most of the samples were also in age class II for Çildir Lake and Isikli Lake. However, the oldest age class for females in this study was found to be age class VIII and age class VII for males. Kiliç & Becer (2016) reported a similar age range for chubs in Lake Yeniçaa, Bolu, Turkey, but Özcan and Serdar (2019) reported age class VI, the oldest age class for chubs in the Karasu River (East Anatolia, Turkey).

The average fork length of the individuals sampled varied between 23.67 cm and 51.68 cm. Fish lengths were grouped into a class interval of 4 cm, and the predominant length group was found to be 26 cm with 25.12% in the sample. The average length of Beysehir observed in this study is greater than the average length of chubs observed in Keban dam lake by Çolak in 1983 and those observed by Kara and Solak in (2004) from chubs sampled from Sır dam lake. The main reasons for the differences in the average length of Beysehir daces found in the study and studies conducted in other regions might be due to the different environmental conditions of the habitat and the differences in geographical conditions.

In the determination of the age – length relation of the Beysehir dace population in Oymapınar dam lake Beysehir dace, the calculated maximum theoretical length (L_∞) value was found to be 66.18 cm and 57.15 cm for females and males, respectively. When the females and males were combined, the calculated theoretical maximum length was found to be 60.57 cm. The values for the asymptotic length (L_∞) in this study are lower than the values obtained by Çolak (1983) for the same species in Keban dam lake and those obtained by Kara & Solak (2004) in Sır dam lake. While the L_∞ values obtained in this study is close to the L_∞ values reported by Tümgelir et al., (2007) for Beyşehir lake, they tend to be higher than the L_∞ values reported by Şaşı & Balık (2003), Balık et al., (2004), and Kılıç, (2011) for studies carried out for *Squalius anatolicus* in Topçam dam lake, Işıklı lake, and Yeniçağa lake, respectively. The higher L_∞ values obtained in this study may be due to better environmental conditions, water quality or diets available to the lake *Squalius anatolicus* population in the Oymapınar dam lake (Muchlisin et al., 2017).

The average weights recorded in this study for *S. anatolicus* population in Oymapınar dam lake are close to those reported by Tümgelir et al., (2007) for *Squalius anatolicus* population in Beyşehir Lake until age class V but lower than the average weights reported in Sır dam lake by Kara & Solak, (2004). From the reports of Kara & Solak, (2004), the chub population of Sır dam lake has a better growth performance than the Beysehir dace population in the Oymapınar Dam Lake. The average weight values reported on Topçam dam lake (Şaşı & Balık, 2003), Işıklı lake (Balık et al., 2004), Yeniçağal lake (Kılıç, 2011) and Keban dam lake (Aydın et al., 2015), are lower than the average weights recorded in this study. According to Kırankaya & Ekmekçi, (2007), besides climatic and geographical differences, the fact that the dam lakes are in different ontogenic stages, and the natural lakes and artificial lakes undergo different ontogenic developmental

processes might contribute to the different growth rates in the populations of different water bodies.

The asymptotic weight (W_∞) of the Beysehir dace population in Oymapınar dam lake from this study was 4294.08 g, 2692.69 g and 3260.79 g for females, males, and all individuals, respectively. The W_∞ values reported for female chubs in Sır Dam Lake by Kara and Solak, (2004) were higher than the values recorded in this study. The slope coefficient "b" of the regression in the length-weight relationship of Beysehir daces sampled from Oymapınar dam lake was found to be 3.195 for total individuals. The "b" values obtained in this study deviated statistically from the isometric b value range of 3.0 for fish. The "b" values obtained in this study are in agreement with the "b" values obtained by previous studies by other researchers from other lakes. It can be stated that the *S. anatolicus* population has a positive allometric growth between length and weight (Ricker 1968).

The condition factor is a useful index for the monitoring of feeding intensity and growth rates in fish (Oni et al. 1983). It is strongly influenced by both biotic and abiotic conditions and can therefore be used as an index to assess the status of the aquatic ecosystem in which fish live (Anene 2005). The average "C_F" value of female, male, and combined female and male Beysehir daces were calculated as 1.284, 1.273, and 1.278, respectively. The "C_F" values obtained in this study are lower than those reported by Benzer, (2013), and Kılıç & Becer, (2016). From the condition factors obtained in this study, it can be said that Oymapınar dam lake has a good nutrition level for the survival of fish. When the condition factor value is close to 1.00 and 1.00 above, it indicates that the nutritional level of the fish is good (Ünver & Tanyolaç 1999).

CONCLUSION

This study carried out on Oymapınar dam lake contains extremely important information about this species, which has been renamed within the framework of taxonomic regulations. It is seen that the information required to determine the protection and management strategies of Beysehir dace is very limited and no biological studies have been carried out for the Beysehir dace population in Oymapınar dam lake. This study is important in that it is the first research done on the growth characteristics of *S. anatolicus* in Oymapınar dam lake. A long-term study of the growth and reproductive biology of local fish species living in the reservoir environments is important in terms of monitoring the changes in the growth of these species and determining the best stock management system based on the results obtained. Also, it is believed that soon, pollutants will affect the life of aquatic creatures in this lake and contribute to future studies, as the water inputs into the dam lake are from streams close to settlements and agricultural areas.

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ARAŞTIRMA MAKALESİ **RESEARCH PAPER**

Determination of the Presence of *Klebsiella pneumoniae* and Phenotypic Antibiotic Resistance Profiles in Budgerigars and Parrots, Istanbul, Turkey

A. Ilgın KEKEÇ* **Barış HALAÇ** **Beren BAŞARAN KAHRAMAN** **Seyyal AK**
Istanbul University-Cerrahpaşa, Faculty of Veterinary Medicine, Department of Microbiology, Büyükçekmece, Istanbul, Turkey

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*: https://orcid.org/0000-0002-0821-8376
: https://orcid.org/0000-0002-3067-9937
: https://orcid.org/0000-0002-1736-093X
: https://orcid.org/0000-0002-6687-8401

***Corresponding author's:**
A. Ilgın KEKEÇ
Istanbul University-Cerrahpaşa, Faculty of Veterinary Medicine, Department of Microbiology, Büyükçekmece, Istanbul, Turkey
✉: ilginkekec@iuc.edu.tr

Abstract: In this study, it was aimed to investigate the presence of *Klebsiella pneumoniae* and phenotypically carbapenemase, extended-spectrum β-lactamase (ESBL), acquired-AmpC beta-lactamase, and multiple antibiotic resistance of the isolates in the faeces of budgerigars and parrots. A total of 96 faecal samples belonging to 54 budgerigars and 42 parrots were used in the study. Cultivation was performed on various media for the identification of *K. pneumoniae* from the collected stool samples. Biochemical properties of the presumptive isolates were determined by conventional methods. Besides, antibiotic susceptibility tests and ESBL, carbapenem, acquired AmpC screening and confirmation tests were applied to the identified isolates to phenotypically determine beta-lactam resistance. Beta-lactam, aminoglycoside, fluoroquinolone, tetracycline, chloramphenicol, and sulphonamide groups were used to determine the multidrug resistance. Isolates with resistance to 3 or more of the antibiotic and sulphonamide groups were accepted as multidrug-resistant isolates. *K. pneumoniae* was isolated from 2 (3.7%) of 54 budgerigars, and 1 (2.3%) of 42 parrot faecal samples from 3 (3.1%) of 96 faecal samples in total. No phenotypic resistance was detected in any of the isolates as a result of screening and confirming tests for ESBL, carbapenem, and acquired-AmpC resistance to determine phenotypic antibiotic resistance of the isolates. Multidrug resistance was detected in only one isolate. The presence of multi-resistant *K. pneumoniae* in cage birds that have close relationships with humans has been revealed.

Keywords: Budgerigar, faeces, *Klebsiella pnemoniae*, multidrug resistance, parrot.

Muhabbet Kuşu ve Papağanlarda *Klebsiella pneumoniae* Varlığının ve Fenotipik Direnç Profillerinin Saptanması, İstanbul, Türkiye

***Sorumlu yazar:**
A. Ilgın KEKEÇ
İstanbul Üniversitesi-Cerrahpaşa Veteriner Fakültesi Mikrobiyoloji Anabilim Dalı, Büyükçekmece, İstanbul, Türkiye
✉: ilginkekec@iuc.edu.tr

Öz: Bu çalışmada, muhabbet kuşu ve papağan dışkılarında *Klebsiella pneumoniae* varlığının ve fenotipik karbepenemaz, genişletilmiş spektrumlu β-laktamaz, kazanılmış- AmpC, beta-laktam ve çoklu antibiyotik direncinin belirlenmesi amaçlanmıştır. Çalışmada, 54'ü muhabbet kuşuna, 42'si papağana ait toplam 96 dışkı örneği kullanıldı. Toplanan dışkı örneklerinden *K. pneuomiae*, çeşitli besiyerleri kullanılarak izole edildi. Şüpheli izolatların biyokimyasal özellikleri konvansiyonel yöntemlerle belirlendi. Ayrıca beta-laktam direncini fenotipik olarak belirlemek için belirlenen izolatlara antibiyotik duyarlılık testleri ve ESBL, karbapenem, edinilmiş AmpC tarama ve doğrulama testleri uygulandı. Çoklu ilaç direncini belirlemek için beta-laktam, aminoglikozid, florokinolon, tetrasiklin, kloramfenikol ve sülfonamid grupları kullanıldı. 3 veya daha fazla antibiyotik ve sülfonamid grubuna dirençli izolatlar çoklu ilaç direncine sahip izolatlar olarak kabul edildi. *K. pneumoniae*, 54 muhabbet kuşu dışkı örneğinin 2'sinden (%3,7), 42 papağan dışkı örneğinin 1'inden (%2,3) olmak üzere toplamda 96 dışkı örneğinin 3'ünden (%3,1) izole edildi. İzolatların fenotipik antibiyotik direncini belirlemek için ESBL, karbapenem ve edinilmiş AmpC direncinin taranması ve doğrulanması sonucunda izolatların hiçbirinde fenotipik direnç saptanmadı. Yalnızca bir izolatta çoklu ilaç direnci tespit edildi. İnsanlarla yakın ilişkileri olan kafes kuşlarında çoklu ilaç direncine sahip *K. pneumoniae*'nin varlığı ortaya konuldu.

Anahtar kelimeler: Çoklu ilaç direnci, dışkı, *Klebsiella pneumoniae*, muhabbet kuşu, papağan.

INTRODUCTION

Companion birds like budgerigars and parrots are high in the ranking of popular pets worldwide. They have an important place for supporting children's love for animals, are preferred by families due to their close relationship with people and ease of feeding. Therewithal, the practice of keeping birds as pets has increased globally as these animals are used as companions, enjoyment, or psychological support (Ahmed et al., 2021). Resistance to antimicrobials frequently encountered among pathogen and commensal bacteria of animal origin is a growing concern in both veterinary and human medicine (Teuber, 2001). Bacterial pathogens carried by companion birds are considered a risk for birds, pet owners and also potential risks remain for human beings who come into close contact with companion birds in the home environment. The spread and contamination of commensal bacteria is thought to pose a risk to the release of caged birds, and also factors such as defecation in the home environment, frequent kisses by their owners, and oral feeding increase this risk. *Klebsiella pneumoniae* is considered one of the most important Gram-negative opportunistic pathogens and worrisome multidrug-resistant bacteria in nosocomial infections. The presence of *K. pneumoniae* in psittacines can create a potential risk to other birds and human beings (Davies et al., 2016). The increase in bacterial antimicrobial resistance is a natural phenomenon, an outcome of evolution (Fedorka-Cray et al., 2005). Unavoidable increase of antimicrobial resistance (AMR) in companion animals continues to be studied worldwide. However, a limited number of studies have been observed focusing on the resistance profiles of *Klebsiella pneumoniae* isolates in budgerigars and parrots belonging to the Psittaciformes family, which are considered among the most common and popular pet bird species in close contact with humans (Rueanghiran et al., 2019; Sigirci et al., 2020; Yame et al., 2017).

Considering these reasons, it was aimed to determine the presence of *K. pneumoniae* in the faeces of healthy and unhealthy budgerigars and parrots from home care centers, petshops, and veterinary clinics in Istanbul and to determine their antibiotic resistance profiles.

MATERIALS AND METHODS

Samples: Between April 2018 and January 2019, a total of 96 faecal samples from 54 budgerigars and 42 parrots were collected by visiting 9 sales focus, Faculty of Veterinary Medicine Animal Hospital and also 12 home care centers, in Istanbul. The samples were stored at +4 ° C until reaching the laboratory and were cultivated the same day.

Culture: The samples were cultured in Triptic Soy Broth (TSB; Merck, 1.05459)and incubated at 37 °C for 24 h. After incubation, a loopful of each culture was subcultured onto MacConkey agar (HiMedia, M081) with and without 1 mg/L cefotaxime (HiMedia), and incubated at 37 °C for 24 h. A presumptive colony was randomly selected and subcultured. Isolates were identified by routine conventional methods as *K. pneumoniae* (Krieg & Holt, 2005) and were confirmed with API 20E system (BioMérieux).

***Antibiotic Susceptibility Tests*:** The antibiotic susceptibility tests were performed according to the Kirby-Bauer method recommended by the Clinical Laboratory Standards Institute (CLSI) to select the optimal antimicrobial agent for treatment. Isolates were tested for antibiotic susceptibility to 15 different antimicrobials from 8 distinct classes: amikacin (30 µg), amoxicillin-clavulanic acid (30 µg), ampicillin-sulbactam (20 µg), aztreonam (30 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), gentamicin (10 µg), kanamycin (30 µg), levofloxacin (5 µg), nalidixic acid (30 µg), norfloxacin (10 µg), ofloxacin (10 µg), streptomycin (10 µg), sulfamethoxazole / trimethoprim (1.25/23.75 µg) and tetracycline (30 µg) (CLSI, 2014; EUCAST, 2015). Also, Extended-spectrum beta-lactamase (ESBL) production, carbapenem, acquired AmpC screening and confirmation tests were applied to the identified isolates to phenotypically determine beta-lactam resistance. The results were based on CLSI breakpoints (CLSI, 2018). For quality control, *E. coli* (ATCC 25922) and *K. pneumoniae* (ATCC 4352) were used. Beta-lactam, aminoglycoside, fluoroquinolone, tetracycline, chloramphenicol and sulphonamide groups were used to determine the multidrug resistance (MDR) defined as resistance at least three different antimicrobial classes (de Jong et al., 2018).

RESULTS

K. pneumoniae was isolated from 2 (3.7%) of 54 budgerigars and 1 (2.3%) of 42 parrots and in total from 3 (3.1%) of 96 faecal samples. All isolates were detected from different petshops and clinically healthy birds. Antibiotic susceptibility results for all isolates are shown in Table 1.

No resistance was found in any of the isolates as a result of screening and confirming tests for ESBL, carbapenem, and acquired-AmpC resistance to determine phenotypic antibiotic resistance of the isolates. Multidrug resistance was detected in only one isolate from budgerigar.

Table 1. Antibiotic susceptibility results for the isolates

Sources	OF	CIP	LEV	NOR	NA	AK	GE	ST	KA	TE	KL	SXT	AMC	SAM	AZ
Budgerigar	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	S
Parrot	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Budgerigar	S	S	S	S	R	S	S	S	S	S	**R**	S	S	S	S

Of: Ofloxacin; Cip: Ciprofloxacin; Lev: Levofloxacin; Nor: Norfloxacin; Na: Nalidixic acid; AK: Amikacin; Ge: Gentamicin; Strep: Streptomycin; Ka: Kanamycin; Tet: Tetracycline; Kl: Chloramphenicol; SXT: Sulfamethoxazole-Trimethoprim; AMC: Amoxicillin-Clavulanic acid; SAM: Ampicillin sulbactam; AZ: Aztreonam, S: Susceptible, R: Resistant

DISCUSSION

Although many articles are investigating the presence and antibiotic profiles of Enterobacteriaceae conducted on cage birds, the data about *K. pneumoniae* is scant. (Horn et al., 2015; Rueanghiran et al., 2019; Sigirci et al., 2019; Steger et al., 2020). Most studies focused on *K. pneumonia*, have been associated with companion animals, howbeit caged birds have been included in the category of companion animals in recent years.

A limited number of studies have paid attention to the presence of *K. pneumonia* from companion birds. Machado et al. (2015) reported that 6 of 79 samples obtained by cloacal swab method isolated *K. pneumoniae*. Yame (2017) reported 19 strains of *Klebsiella* spp. isolated from respiratory secretions of 46 diseased psittacines, 16 (16/19) were identified as *K. pneumoniae*, and three (3/19) were identified as *K. oxytoca*. It was reported that 3 strains of *K. pneumoniae* isolated from 100 parakeets, 23 parrots, 12 canaries, 12 Indian nightingales, 2 European goldfinches (Sigirci et al., 2019). Further, Rueanghiran et al. (2019) show that a total of 80/376 psittacine cases (21%) was diagnosed to have a respiratory problem and *K. pneumoniae* 7 isolates (8%) were obtained from 53 respiratory cases. Steger et al. (2020) was reported that 86 *K. pneumoniae* isolates from 811 birds of 20 zoological orders (mostly Psittaciformes 61.8 % and Passeriformes 14.5 % and from alive patients or pathological examinations) were found. Ahmed et al. (2021) emphasized that 17.6 % of the pet birds and 12.9 % of the human contacts were positive for *K. pneumonia*. Unlike previous studies, *K. pneumoniae* isolation rates were lower in the current study.

The authors pointed that *K. pneumonia* has become the most successful and modern pathogen by producing Extended Spectrum β-Lactamase (ESBL) and also shows high resistance to a broad spectrum of antibiotics including β-lactam antibiotics, fluoroquinolones, and aminoglycosides (Riwu et al., 2020). Davies et al. (2016) reported that the susceptibility profile of *K. pneumoniae* strains revealed a high resistance to ampicillin, nalidixic acid, sulphonamides and tetracycline. Further, Yame (2017) emphasized the antimicrobial susceptibility profile demonstrated high resistance to ampicillin (89.5%) and three strains of *K. pneumoniae* were positive for extended-spectrum beta-lactamase production. In another study conducted in our country, it was reported that the isolated *K. pneumoniae* species were all sensitive to the antibiotics tested and no resistance was observed (Sigirci et al., 2019).

In the current study, resistance was not detected in any of the isolates as a result of screening and confirming tests for ESBL, carbapenem and acquired-AmpC resistance to determine phenotypic antibiotic resistance. Besides, resistance to only chloramphenicol was detected in one of the two isolates taken from budgerigars, and it was observed that this isolate was susceptible to all other antimicrobials. However, the second isolate obtained from the budgerigar was resistant to all antimicrobials except aztreonam. Furthermore, it was observed that the only isolate detected from the parrot was susceptible to all the antimicrobials analysed. The reason for these differences in the findings between previous studies could be explained by the small sample sizes and the presence of geographical variants.

The complex hazard of AMR transmission from companion animals to humans has not been fully established. Consequently, studies on AMR and MDR, performed in various countries, unceasingly continue to provide more information. Unfortunately, data regarding MDR in companion birds are limited (Rueanghiran et al., 2019, Sigirci et al., 2020, Yame et al., 2017). According to the numerous reports, the MDR profiles of the *K. pneumoniae* strains reported between 25%- 57%, respectively (Ahmed et al., 2021, Ajayi & Egbebi, 2011, Davies et al., 2016, Rueanghiran et al., 2019). In the present study, MDR was noticed in only one isolate then this result is promising on its own.

As a result of this current study, the presence of antimicrobial resistance has been revealed in cage birds that have close relationships with humans. There are a limited number of studies on the antimicrobial resistance profiles of caged birds. Hence, monitoring pet birds as potential reservoirs of zoonotic bacterial pathogens is crucial to sustaining human health and it is necessary to develop routine analysis. Further studies are needed to evaluate risk factors, follow-up programs to prevent resistance. Because of the possibility that companion animals may act as reservoirs, the assessments must include commensal bacteria as well as pathogenic bacteria.

Especially the possibility that healthy animals contribute to the transmission of antibiotic resistance markers without being noticed makes the reports of antibiotic resistant isolates important.

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ARAŞTIRMA MAKALESİ **RESEARCH PAPER**

Investigation of *Aloe vera barbadensis* Miller Leaf Extract Effects On Glutamate and Glufosinate Induced Toxicity: In Vitro Study

Ayşegül YILMAZ[1] Ali TAGHIZADEHGHALEHJOUGHI[1,2*] Ahmet HACIMÜFTÜOĞLU[1] Aybike TÜRKMEN[3]

[1] *Ataturk University, Faculty of Medicine, Department of Medical Pharmacology, Erzurum, Turkey*
[2*] *Ataturk University, Faculty of Veterinary Medicine, Department of Pharmacology and Toxicology, Erzurum, Turkey*
[3] *Adnan Menderes University, Faculty of Veterinary Medicine, Department Pharmacology and Toxicology, Aydın, Turkey*

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: https://orcid.org/0000-0001-5843-1661
*: https://orcid.org/0000-0002-3506-0324
: https://orcid.org/0000-0002-9658-3313
: https://orcid.org/0000-0002-4119-7490

***Corresponding author's:**
Ali TAGHİZADEHGHALEHJOUGHİ
Ali Taghizadehghalehjoughi, Faculty of Veterinary Medicine, Department of Pharmacology and Toxicology, Ataturk University, Erzurum, Turkey.
✉: ali.tgzd@atauni.edu.tr

Abstract: *Aloe vera* is one of the medicinal plants whose importance has been better understood recently with its antibacterial, antimicrobial, antioxidant and anticancer properties. Although it is known that the aloe vera family has protective effects on neurons, the neuroprotective effect of the aloe barbadensis miller plant has not yet been fully explained. Glufosinate is structurally similar to glutamate and is an herbicide that blocks glutamine synthesis. Glutamate has been shown to induce cyclooxygenase-2 (COX-2), which causes toxicity. In the present study, neuron culture was exposed to *Aloe vera barbadensis* Miller plant extracts plant (25, 50, 100, 200, 400, 800, and 1600 µgr/ml doses) for 24 hours to protect against glufosinate (200 mM) and glutamate (10^{-5} mM) toxicity. After 24 hours, MTT, TAC, and TOS analyzes were performed and the results were revealed. In our study, it was seen that the aqueous extract of aloe barbadensis miller plant, glufosinate, and glutamate, could preserve the vitality of neurons (89% protection in AVB 400 µgr/ml group). At the same time, it was seen that while increasing the antioxidant level in neurons, it decreased the oxidant level. The group that increases the antioxidant value best is AVB 400 µgr/ml (the group that increases it 1.4 times). When the findings were evaluated, it was concluded that aloe vera and its components may have a neuroprotective effect.

Keywords: *Aloe vera barbadensis* Miller, glufosinate, glutamate, neuron.

Aloe vera barbadensis Miller *Yaprağı Ekstraktının Glutamat ve Glifosat Kaynaklı Toksisite Üzerindeki Etkilerinin Araştırılması: İn Vitro Çalışması*

***Sorumlu yazar:**
Ali TAGHİZADEHGHALEHJOUGHİ
Atatürk Üniversitesi, Veteriner Fakültesi, Farmakoloji ve Toksikoloji Bölümü, Erzurum, Türkiye.
✉: ali.tgzd@atauni.edu.tr

Öz: Antibakteriyel, antimikrobiyal, antioksidan ve antikanser gibi özellikleriyle son zamanlarda önemi daha da iyi anlaşılan *Aloe vera* şifalı bitkilerden biridir. Aloe vera ailesinin nöronlar üzerinde koruyucu etkileri olduğu bilinmesine rağmen aloe barbadensis miller bitkisinin nöroprotektif etkisi henüz tam olarak açıklanamamıştır. Glufosinat, yapısal olarak glutamata benzer ve glutamin sentetazı bloke eden bir herbisittir. Glutamatın toksisiteye neden olan siklooksijenaz-2'yi (COX-2) indüklediği gösterilmiştir. Ayrıca nöronlarda oksidatif strese neden olduğu bilinmektedir. Mevcut çalışmada, nöron kültürü, glufosinat (200 mM) ve glutamat (10^{-5} mM) toksisitesine karşı korunmak amacıyla 24 saat süreyle *Aloe vera barbadensis* Miller bitkisi ekstraktlarına (25, 50, 100, 200, 400, 800 ve 1600 µgr/ml) maruz bırakılmış olup, 24 saat sonunda MTT, TAC ve TOS analizleri yapılmıştır. Çalışmamızda aloe barbadensis miller bitkisinin sulu ekstraktının, glufosinat ve glutamat toksisitesine karşı nöron canlılığını koruyabildiği görülmüştür (AVB 400 µgr/ml grubunda %89 koruma). Aynı zamanda nöronlarda antioksidan seviyesini arttırırken oksidan seviyesini azalttığı gözlemlenmiştir. Antioksidan değerini en iyi artıran grup AVB 400 µgr/ml (1,4 kat artıran grup) olarak tespit edilmiştir. Elde edilen bulgular değerlendirildiğinde, aloe vera ve bileşenlerinin nöroprotektif etkisi olabileceği sonucuna varılmıştır.

Anahtar kelimeler: *Aloe vera barbadensis* Miller, glufosinat, glutamat, nöron.

INTRODUCTION

Aloe vera barbadensis Miller (AVB), one of the well-known medicinal plants recently, is preferred because of its multiple effects. This medicinal plant belongs to the Liliaceae family and is widely used in traditional medicine. The compounds found in aloe gel mainly contain polysaccharides that reduce inflammation and induce skin growth and regeneration. Recent studies have also shown that it has antibacterial, antimicrobial, antioxidant and anticancer properties (Khanal et al., 2021, Mahboubi, 2021). Although it is known that the aloe vera family has protective effects on neurons, the neuroprotective effect of the *Aloe vera barbadensis* Miller plant is not yet well known. Aloe vera and its components contain various properties. For example; salicylic acid, campesterol, β-sitosterol and C-glucosyl chromone are anti-inflammatory, vitamins A, C and E are anti-oxidant, anthraquinones and phorbol myristic acetate are anti-tumor, aloin and emodin are anti-microbial (Klaikeaw et al., 2020).

The use of pesticides in agriculture, industry, and domestic applications results in increased exposure to these chemicals (Sevim et al., 2019). As a result of acute exposure, it has been observed that human health is highly affected. Glufosinate is one of the most widely used herbicide-based pesticides worldwide (Comakli et al., 2019). Glufosinate, which is one of the main pollutants of rivers and various water resources, has a toxic effect not only on organisms, but also on food, feed and ecosystems (Singh et al., 2020). The European Chemicals Agency concludes that there are insufficient scientific data to classify glufosinate as a carcinogen, mutagen or reproductive toxicity for certain target organ toxicity (Levine et al., 2020, Matozzo et al., 2020, Pereira et al., 2021). Glufosinate toxicity was done by induction glutamate toxicity in inter synaptic area.

Glutamate is the principal excitatory neurotransmitter in the central nervous system. Elevated extracellular glutamate levels induce neuronal damage (Kumagai et al., 2019). In cerebral hypoxia/anoxia, and in the majority of nervous system diseases, glutamate transporter function is impaired, and extracellular glutamate levels increase and result in irreversible neuronal damage (Tehse & Taghibiglou, 2019). In addition, by attaching to N-methyl-d-aspartate (NMDA) and AMPA receptors for longer than physiological levels, glutamate causes Ca^{++} and Na^{+} influx. Strong evidence also exists that glutamate toxicity is significantly associated with NMDA receptors. These receptors are also significantly involved in the central sensitization processes associated with hyperalgesia (Zhao et al., 2019).

Previous studies of maternal exposure to Glufosinate have shown that pre- and postnatal exposures lead to calcium overload and glutamate excitotoxicity in the immature juvenile hippocampus (Cattani et al., 2017). The purpose of the present study was to evaluate different doses of *Aloe vera barbadensis* neuro protective effects against to Glufosinate and glutamate toxicity in vitro model.

MATERIALS AND METHODS

Chemicals: *Aloe vera barbadensis* (1000 mg film-coated tablets), was obtained from (Izmir, Turkey). Glufosinate, glutamate HCL, Dulbecco's modified Eagle's medium (DMEM), Fetal calf serum (FCS), Neurobasal medium (NBM), 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT), phosphate buffer solution (PBS), antibiotic antimitotic solution (100×), B27, L glutamine and trypsin–EDTA and dimethyl sulfoxide (DMSO) were obtained from Sigma-Aldrich (St. Louis, MO, USA).

In vitro studies

Cell cultures: Briefly, frozen cortex neuron cells were used for the study (Gibco™ Primary Rat Cortex Neurons, Catalog no: A1084001). The cryotube was open rapidly and centrifugation was done at 1200 rpm for 5 min. The collapsed cells were suspended with fresh medium (Neurobasal medium, FBS 10%, B27 2% and antibiotic 0.01%) and then the cells were seeded in 24-well plates (Corning, USA). The plate was stored in an incubator (5% CO_2; 37 °C) (Varmazyari et al., 2020).

***Glutamate and Glufosinate toxicity induction)*:** Adequate branches were observed to have formed in the cells by day 10. Glutamate 10^{-5} mM and Glufosinate 200 mM were used for toxicity induction. After 20 min, ABV different concentrations (50, 100, 200, 400, 800 and 1600 µgr/ml) were added to each well and incubated for 24 h (5% CO_2; 37 °C). These selected concentrations were added as a result of the literature search (Kang et al., 2014; Kaithwas et al., 2014; Klaikeaw et al., 2020). In addition, 150 µL of NBM only was added as a negative control, while the two positive controls each one contained separately 10^{-5} mM glutamate and 200 mM glufosinate (Singh et al., 2020).

3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) assay: MTT assay was according to the commercial kit protocol. Briefly, MTT reagent (10 µL) was added to the each well and incubated (5% CO_2; 37 °C) for 4 h. The medium was removed, then 100 µL of dimethyl sulfoxide was added to each well. The optical density was evaluated at 570 nm using a Multiskan™ GO Microplate Spectrophotometer reader (Thermo Scientific,

Canada, USA) (Taghizadehghalehjoughi et al., 2019). the cell viability (%) was calculated using the formula.
Viability % ratio: Sample Absorbance/control group absorbance×100.

Total oxidant status (TOS): TOS assay was done according to the commercial manufacture kit protocol. Briefly, 500 µl Reactive 1 solution was added to wells and the initial absorbance value at 530 nm. then 25 µl Reactive 2 solution was added to the same well, and the second absorbance was read at 530 nm. TOS levels were determined as H_2O_2 mmol equiv/mmol^{-1}.
The evaluation was done according the formula.
TOS =Δ example/ΔST2×20
Δ ST2 (Δ standard 2 = ST2 second reading - ST2 first reading), Δ Sample (Δ Sample= Sample second reading-Sample first reading)

Total Antioxidant Capacity (TAC): TAC assay was done according to the commercial manufacture kit protocol. Briefly, 500 µl Reactive 1 solution was added to wells and the first absorbance was read at 660 nm. Next, 75 µl Reactive 2 was added to the same wells and the second absorbance value was read at 660 nm. TAC levels were expressed as mmol equiv/mmol-1.
The evaluation was done according the formula;
TAC =(Δ ST1-Δ example)/(Δ ST1-Δ ST2)
Δ ST1 (Δ standard 1 = ST1 second reading - ST1 first reading), Δ ST2 (Δ standard 2 = ST2 second reading - ST2 first reading), Δ Sample (Δ Sample = Sample second reading- Sample first reading)

Statistical analysis: The analysis of the data of our study was evaluated with SPSS 21.0 program and One Way Annova method and P<0.05 was considered significant.

RESULTS AND DISCUSSION

In our study, the protective effects of *Aloe vera barbadensis* on neuronal cells against glutamate and glufosinate were investigated. For this purpose, MTT, TAC and TOS analyzes were performed 24 hours after the application and the results were shown in the figures.

When the MTT test in Figure 1 and 2 were examined, it was seen that AVB protected neuron viability against glutamate and glufosinate depending on the increasing dose. The best protection was seen in the AVB 400 µgr/mL (89%) group against glutamate, which caused a 31% decrease in viability. The highest protection against glufosinate toxicity was seen at the AVB 200 dose (85% protection). The neuroprotection rate of the same group against glufosinate toxicity (reduces viability by 36%) is 82%. While it was observed that the AVB 800 (86%) and 1600 (83%) µgr/mL groups also protected neuron cells against glutamate, the viability was decreased compared to the AVB 400 µgr/mL group. In glufosinate toxicity, viability was 8% (78%) lower in the AVB 800 µgr/mL group and 13% (70%) in the AVB 1600 µgr/mL group against glutamate toxicity.

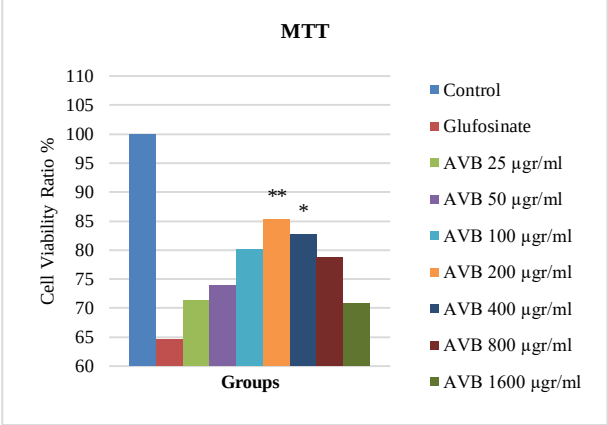

Figure 1. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay results for the Glufosinate induced toxicity in the neuron cell line after 24-h AVB treatment
* P<0.05, ** P<0.001 compared to control group.

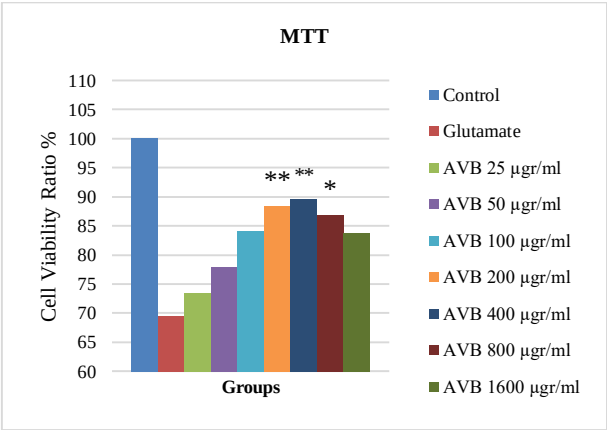

Figure 2. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay results for the Glutamate induced toxicity in the neuron cell line after 24-h AVB treatment
* P<0.05, ** P<0.001 compared to control group.

When the antioxidant results in Figure 3-6 were examined, it was seen that AVB increased the antioxidant level in a dose-dependent manner against glutamate. The highest antioxidant value was observed in the AVB 400 µgr/mL group (1.4-fold increase in antioxidant value against glutamate). When the antioxidant effects of AVB against glufosinate toxicity were examined, it was determined that the AVB 200 µgr/mL group increased antioxidant by 1.3. When the TOS results in Figure 3 were observed, it was determined that the oxidant level decreased depending on the increasing dose. It was determined that the best protection against glutamate toxicity was in the AVB 200 and AVB 400 µgr/mL groups, which reduced the oxidant level by 1.5 times. When the TOS results in glufosinate toxicity were examined, we found that the AB 200 µgr/mL group decreased the oxidant level 1.5 times.

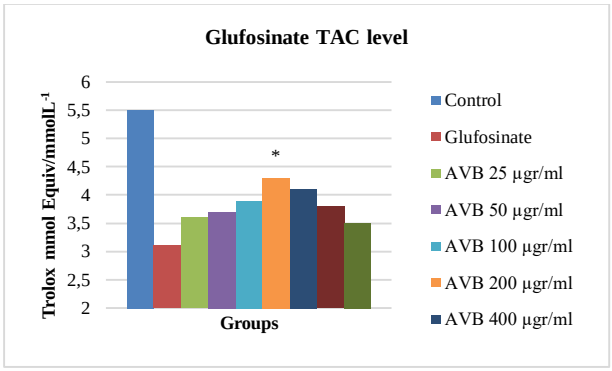

Figure 3. Total antioxidant capacity assay results for the Glufosinate induced toxicity in the neuron cell line after 24-h AVB treatment.
* P<0.05, ** P<0.001 compared to control group.

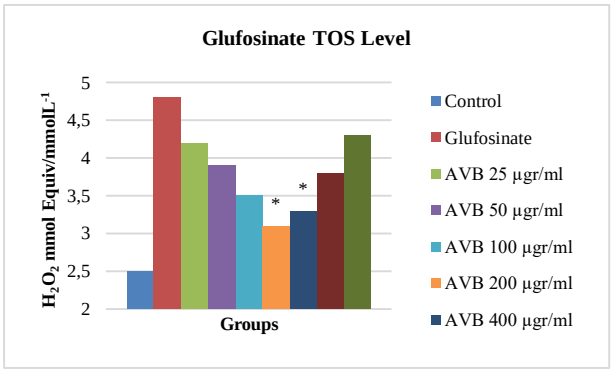

Figure 4. Total oxidant status assay results for the Glufosinate induced toxicity in the neuron cell line after 24-h AVB treatment.
* P<0.05, ** P<0.001 compared to control group.

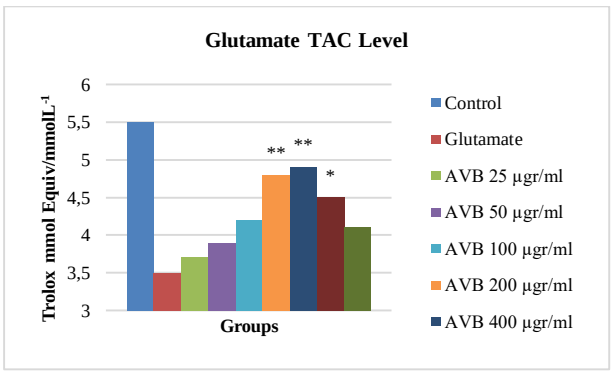

Figure 5. Total antioxidant capacity assay results for the Glutamate induced toxicity in the neuron cell line after 24-h AVB treatment
* P<0.05, ** P<0.001 compared to control group.

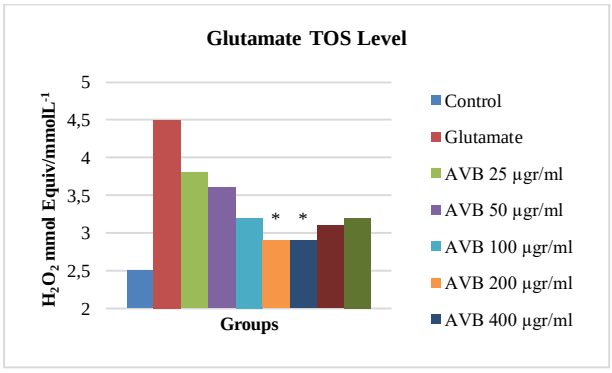

Figure 6. Total oxidant status assay results for the Glutamate induced toxicity in the neuron cell line after 24-h AVB treatment
* P<0.05, ** P<0.001 compared to control group.

DISCUSSION

In our study, the protective effects of *Aloe vera barbadensis* on neuronal cells against glutamate and glufosinate were investigated. Traditional medicine plays a critical role in the treatment of various types of diseases (Chinchilla et al., 2013). Nowadays, the use of complementary medicine and natural products has been increasing rapidly worldwide because they are effective and inexpensive and have fewer side effects. AVB contains several biologically active constituents, including vitamins, minerals, saccharides, amino acids, anthraquinones, enzymes, lignins, saponins, and salicylic acids (Mahboubi, 2021).

Medicinal plants contain various types of constituents, such as vitamins, amino acids, carbohydrates, and phenolic compounds. These compounds are active in controlling or neutralizing the reactive oxygen species (ROS). AVB also has function like an antioxidant through free radical- and superoxide radical-scavenging activities and anti-inflammatory activities (Pandhair et al., 2011; Parmar & Jasrai, 2009,).

Recent studies have shown that the application of glutamate to cortical and hippocampal axon terminals triggered exocytotic process, which appeared to be, at least partially, mediated by the efflux of Ca^{2+} ions from internal stores (Tarasenko et al., 2012). In light of the latest findings showing a close relationship between spontaneous transmitter release, Ca^{2+} efflux from internal stores and reactive oxygen species (ROS) generation (Martinez-Sanchez et al., 2020). The TAS and TOC tests were showed AVB 200 and 400 µgr/mL increased antioxidant activity while decreased oxidant status. Environmental stressors that are well known to induce oxidative stress and alterations to the cellular redox balance have been widely shown as apoptosis regulators (Peng et al., 2019). There are many evidence that glufosinate and glutamate induces cytotoxicity, oxidative damage, and apoptosis.

Examined, it was seen that AVB protected neuron viability against glutamate and glufosinate depending on the increasing dose. The best protection was seen in the AVB 400 µgr/mL (89%) group against glutamate, which caused a 31% decrease in viability. The neuroprotection rate of the same group against glufosinate toxicity (reduces viability by 36%) is 82%. While it was observed that the AVB 800 (86%) and 1600 (83%) µgr/mL groups also protected neuron cells against glutamate, the viability was decreased compared to the AVB 400 µgr/mL group. In glufosinate toxicity, viability was 8% (78%) lower in the AVB 800 µgr/mL group and 13% (70%) in the AVB 1600 µgr/mL group against glutamate toxicity.

When the antioxidant results in Figure 3-6 were examined, it was seen that AVB increased the antioxidant

level in a dose-dependent manner against glutamate. The highest antioxidant value was observed in the AVB 400 µgr/mL group (1.4-fold increase in antioxidant value against glutamate). Kaithwas et al. also stated that the antioxidant effects of aloe vera increased depending on the dose, and they observed the best antioxidant effect especially at the highest dose they used, 120 µgr/mL (Kaithwas et al., 2014). In our study, when the antioxidant effects of AB against glufosinate toxicity were examined, it was determined that the AVB 200 µgr/mL group increased the antioxidant by 1,3. When the TOS results in Figure 3 were observed, it was determined that the oxidant level decreased depending on the increasing dose. It was determined that the best protection against glutamate toxicity was in the AVB 200 and AVB 400 µgr/mL groups, which reduced the oxidant level by 1.5 times. When the TOS results in glufosinate toxicity were examined, we found that the AVB 200 µgr/mL group decreased the oxidant level 1.5 times. Kang et al., in their study with *Aloe vera barbadensis* miller component on Vero cells, seems to protect cell viability against AAPH-induced cell death (400 µgr/mL group). Again, protection was found in cells against AAPH-derived ROS products (Kang et al., 2014).

When high doses of AVB (400 and 800 µgr/mL) were examined, no effective protection was observed in neuronal cells against toxicity. In various studies, negative conditions such as decreased central nerve activity, slower growth, and diarrhea have been detected in rats as a result of high-dose aloe vera application (Herlihy et al., 1998; Herlihy et al., 1998; Shah et al., 1989).

CONCLUSION

Aloe vera, which has been used since ancient times, is very common, especially for skin, inflammation, and diabetes. With today's studies, its anti-cancer, anti-oxidant properties have made it an even more important compound. Although its effect on healthy and cancer cells is little known, its protective effect on neurons has not been clarified yet. Our study especially reveals its effect on neurons and hopes to be a pioneer for future studies. In our study, the protective effects of aloe barbadensis against toxicity (glutamate and glufosinate) especially on neurons were revealed. It has been demonstrated with its antioxidant effects that it can be used to reduce side effects against any component with known toxic properties. In summary, the protective effects of aloe barbadensis can be used both in practice and inspire future studies.

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ARAŞTIRMA MAKALESİ **RESEARCH PAPER**

The Global Problem of the Antibiotic and Heavy Metal Resistance in Aquatic Resources: An Examination of Gelevera Creek (Giresun), Turkey [*]

Hakan IŞIK[1]* **Tamer AKKAN[2]**

[1] *Giresun University, Natural Sciences Institutes, Biology Division, Giresun, Turkey*
[2] *Giresun University, Science and Art Faculty, Biology Department, Giresun, Turkey*

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: https://orcid.org/0000-0002-9907-9315
: https://orcid.org/0000-0002-9866-4475

***Corresponding author's:**
Hakan IŞIK
Giresun University, , Natural Sciences Institutes, Biology Division, Giresun, Turkey
✉: hknbiyoo@gmail.com

Abstract: Gelevera Creek was chosen as the sample area in this study, which aims to detect antibiotic and heavy metal resistance in water resources that threaten human, animal and ecosystem health on a global scale. For this purpose, surface water and sediment were collected monthly from four different stations starting from April 2017 and ending in March 2018. After the Gr (-) and Gr (+) bacteria isolation in selective media, the 325 isolated were tested for their resistance against 4 different heavy metals. The resistance levels of these bacteria against to cadmium, copper, lead and manganese resistance were detected respectively as 89%, 60.16%, 33%, 29.8% (Cd > Cu > Pb > Mn). The 82 isolates with high resistance against heavy metals in each station were also tested for their resistance against antibiotics. The levels of resistance against antibiotics of these isolated strains were found respectively as follows: cefazolin: 69.6%, cefuroxime: 59.4%, nalidixic acid: 51.7%, ampicillin: 46%, cefotaxime: 39.1%, meropenem: 31.4%, amikacin: 21.7%, erythromycin: 13%, and chloramphenicol: 3.3%. In our study, two isolates with high antibiotic and heavy metal, using the Vitek-II Compact System were identified as *Serratia marcenscens* (99%) and *Enterococcus avium* (91%). Furthermore, the multiple antibiotic resistance levels (MAR) of all isolates are 80,5%. The results indicate that the isolates taken from the Gelevera Creek (Giresun) were found to be extremely resistant against commercially used antibiotics and heavy metals, and this shows that there is antibiotic and heavy metal contamination in drinking water reserves.

Keywords: Antibiotic, Gelevera Creek, Giresun, Heavy Metal, Resistance.

Su Kaynaklarında Antibiyotik ve Ağır Metal Direncinin Küresel Sorunu: Gelevera Deresi'nin İncelemesi (Giresun, Türkiye)

***Sorumlu yazar:**
Hakan IŞIK
Giresun Üniversitesi, Fen Bilimleri Enstitüsü, Biyoloji Anabilim Dalı, Giresun, Türkiye
✉: hknbiyoo@gmail.com

Öz: Küresel boyutta mevcut olan, insan, hayvan ve ekosistem sağlığını tehdit eden antibiyotik ve ağır metal direncinin su kaynaklarında tespitinin amaç edinildiği bu çalışmada örnek alan olarak Gelevera Deresi seçilmiştir. Bu amaçla, Gelevera Deresi'ndeki dört istasyondan alınan yüzey suyu ve sediment örnekleri Nisan 2017-Mart 2018'a kadar aylık olarak toplanmıştır. Seçici bir şekilde Gr (-) ve Gr (+) bakteri izolasyonun ardından izole edilmiş olan 325 bakterinin 4 farklı ağır metale karşı direnç düzeyleri tespit edilmiştir. Bu izolatların kadminyum, bakır, kurşun ve manganese karşı direnç düzeyleri % ifadesiyle sırasıyla; %89; %60,16; %33; %29,8 (Cd >Cu >Pb >Mn) olarak tespit edilmiştir. Her bir istasyonda ağır metal direnç düzeyleri yüksek olan izolatların ilaveten antibiyotik direnç düzeyleri de saptanmıştır. İzole edilen bu suşların antibiyotik direnç düzeyleri sırasıyla; sefazolin: %69,6, sefuroksim: %59,4, nalidiksik asit: %51,7, amfisilin: %46, sefotaksim: %39,1, meropenem: %31,4, amikasin: %21,7, eritromisin: %13 ve kloramfenikol: %3,3 olarak saptanmıştır. Araştırmamızda antibiyotik ve ağır metal direnç düzeyi yüksek olan iki izolat Vitek-II Kompakt Sistem ile identifiye edilerek *Serratia marcencens* (%99) ve *Enterococcus avium* (%91) olarak tanımlanmıştır. Ayrıca yüzey suyu örneği izolatlarındaki çoklu antibiyotik direnç değeri (ÇAD) oranının %80,5' olduğu belirlenmiştir. Sonuç olarak bu çalışmada Gelevera Deresi (Giresun)'nden izole edilen izolatların ticari olarak kullanılan antibiyotiklere ve ağır metallere karşı yüksek düzeyde direnç gösterdiği ve bu durumun içme suyu kaynakları üzerinde antibiyotik ve ağır metal kirliliğinin olduğunu göstermektedir.

Anahtar kelimeler: Ağır Metal, Antibiyotik, Direnç, Gelevera Deresi, Giresun.

[*] *This study was produced from a master's thesis.* *Bu çalışma yüksek lisans tezinden üretilmiştir.*

INTRODUCTION

Water, an essential element for organisms to continue their lives, is one of our most important natural resources. Water is the most basic compound that is difficult to replace for life because it contains all the features related to life in its structure. Deterioration of the quality or balance of water in an environment for various reasons also leads to water pollution. It is assumed that 80% of all diseases in the developing world are caused by a lack of healthy water and cleaning conditions. More than 5 million people die each year from water pollution and more than half of them are children (Anonymous, 2007). Pathogenic bacteria, viruses, and parasites are among the harmful biological factors that can exist in water and endanger human health (Lawa, 1998). Although the first thought that awakens in our minds about the cause of microbiological pollution in the aquatic environments in question is fecal-based contamination, emergency action plans have been prepared for the 'Antibiotic Resistance', which seems to be the most important problem nowadays, and 'Antibiotic Resistance' has also been added among the reasons to make all people more sensitive about this issue. According to the report published by World Health Organization, medicine has regressed before the antibiotic age in the fight against bacteria and that many antibiotics, most of which are used for therapeutic purposes, do not work effectively, therefore a great disaster awaits in the rest of human life (Anonymous, 2014).

Nowadays, resistance is identified as a major problem in the path of new drug synthesis, reducing antibiotic resistance is a important public health issue in the world. (Hayder & Aljanaby, 2019). The four main forms of antibiotic resistance develop as; natural resistance (intrinsic, structural), the usage of antibiotics is not because of the resistance but is caused by the bacteria's structural particulars. (Kadhum & Hasan, 2019). This happen a result of intrinsic resistance, or microorganism which doesn't follow the goal antibiotic structure, or antibiotics which due to its characteristics do not encounter its goal (Waglechner & Wright, 2017).

Gained resistance; irrespective of resistance development due to change in the genetic features of bacteria, an gained because it is not affected by the antibiotics it was beforehand sensitive to it (Andersson et al., 2020). The source of this form of resistance generally comes from the main chromosome or extrachromosomal structures. (plasmids, transposons, etc.) (Aljanaby & Aljanaby, 2018).

The main reason for the quick increase in antibiotic resistance is the unconscious take of antibiotics. For this reason, a bacterium becomes resistant to the activity of an antimicrobial drug. As a result of the unconscious prescribing of antibiotics and their systematic application as growth accelerators in animal husbandry, this has worsened over the past decade (Acharya & Wilson, 2019). On the other hand, aquatic environments have contributed to the formation of antibiotic-resistant bacteria in humans and animals, particularly by the fact that drinking water can be obtained from surface water and antibiotic-resistant genes can enter non-pathogenic water bacteria (Akduman et al., 2020; Baquero et al., 2008; Şahin et al., 2021). The main reason for the growth of antibiotic-resistant genes in marine and freshwater ecosystems is the improvement of a long-term resistance reservoir (Di Cesare et al., 2015).

Heavy metal-resistant bacteria may develop in aquatic environments due to the presence of metal, mostly caused by anthropogenic activities and environmental factors (Altuğ & Balkis, 2009; Nadimpalli et al., 2020). Heavy metals can be described as metals with a density large than 5 g/cm^3. Heavy metals are not biodegradable and are considered an environmental pollutant associated with potential toxicity. A few important heavy metals such as iron, nickel, copper, and zinc are necessary for metabolic reaction and very important as trace elements for organisms. Other heavy metals, such as silver, cadmium and mercury, have no biological role in organisms and harmful effects on them even at very low concentrations (Alam et al., 2011).

Heavy metals are elements not dissolving biologically due to their high stability, and therefore they are environmental pollutants that have settled for a long time. They arise from the use of anthropogenic sources such as mines, power plants, smelters as heavy metal sources, as well as pesticides and fertilizers consisting of metal and mud (Zhu et al., 2013). İn the world heavy metal pollution is increasing day by day in many developed and growing countries because of rapid industrialization, mining activities, discharge of industrial wastes, long-term use of low-quality water for irrigation, and intensive agricultural practices (Rizvi et al., 2019). Heavy metal resistance frequently exists in bacteria exposed to metals in a variety of habitats and environments. (Pal et al., 2017). Water pollution caused by heavy metals results in the spread of integron-like Gene structures, which are described to play an important role in the development of multiple resistance to antibiotics (Marinescu et al., 2017).

The first step to being taken to protect the natural structure of aquatic environments and ensure that water is healthy and safe for the continuation of the vital activities of people is to determine the pollution standards and the factors that will harm the health of living things. For this reason, this study aims to determine the current antibiotic

and heavy metal resistance levels of isolates isolated from Gelevera Stream, one of the freshwater resources used for agricultural, drinking, and use purposes in Giresun province located in the Eastern Black Sea Basin, which is one of the most important basins of our country.

MATERIALS AND METHODS

Study Area: This study was conducted between April 2017 and March 2018 to determine the heavy metal and antibiotic resistance level in the bacterial flora of the Gelevera Stream, located within the borders of the Black Sea Region Giresun (Figure 1). The other name of Gelevera Creek is Özlüce Creek. It is fed by the Karadona, Karaovacık and Çukur creeks that originate from the Balaban Mountains in the province of Gümüşhane, as well as by streams and rivers with small flow rates. Gelevera Stream is 14 km away from the coastal part and has an estimated surface area of 351 km^2. The Gelevera Basin, which has a length of 80 km, is surrounded by Tirebolu, Güçü and Doğankent districts in the East, Yağlıdere Basin in the West, Black Sea in the North and Kurdün in the south. Gelevera Creek passes through the villages of Sapmaz, Ericek, and Direkbuku and flows into the sea from the east of Espiye district in Giresun province. It is known that the flow rate of the water is also fast due to the high slope of the stream bed. In addition, Gelevera Stream, one of the most important drinking water resources of Giresun, is under constant pressure from HEPP for energy production, domestic wastes, and quarries (Fig.1).

Figure 1. Sampling Area (atlas.gov.tr).

Collection of Surface Water Samples: To ensure the isolation of bacteria, previously sterilized dark glass sample bottles and water samples were taken in a sterile manner (250 mL) from 20 cm below the stream surface and brought to the laboratory within 2 hours after being stored with cold chain application (APHA, 1992).

***Bacteria Isolation*:** Gr (+) and Gr (-) bacteria isolation was performed from 1 mL water sample taken from surface water samples using the petri dish spreading technique on Nutrient Agar, PCA Agar, MacConkey Agar, and EMB Agar by serial dilution in sterile distilled water. 1 gr of the sediment samples was homogenized in 9 ml sterile water, and a serial dilution up to 10^7 was prepared in sterile water and plated. After confirming that the isolated bacteria are Gr (-) and Gr (+) by Gram staining and some biochemical tests, stock cultures were prepared in Plate Count Agar and Nutrient Agar.

Performing Bacterial Identification and Antibiotic Susceptibility Test: Isolates with high antibiotic and heavy metal resistance were determined. Microorganisms were identified by the Vitek-II (Biomerieux) automatic bacteria identification system. 9 different antimicrobial discs were used (KZ; cefazolin, CXM; cefuroxime, NA; nalidixic acid, AMP; ampicillin, CTX; cefotaxime, MEM; meropenem, AK; amikacin, E; erythromycin and C; chloramphenicol) through the disk diffusion method (Bauer et al., 1966) for determining antibiotic resistance profiles of isolated strains. To enrich the bacteria, it was incubated at 37ºC for 24 hours by sowing with the help of a loop into the LB broth medium. The incubated samples were placed in Nutrient Agar with the help of sterilized swabs and antibiotic-impregnated discs after the surface was dried with a sterile dispenser. The antibiotic resistance profiles of the bacteria were determined by measuring the zone diameters formed after the 24-hour incubation process.

***Multiple Antibiotic Resistance Index (MAR)*:** The multiple antibiotic resistance (MAR) index is calculated by the ratio of the number of antibiotics to which the test organisms are resistant to the total number of antibiotics tested (Ehindimu, 2003). If the isolate has been heavily exposed to antibiotics of human or animal origin, a MAR index value is greater than 0.2 results. However, if the antibiotic was used very rarely or not at all, the MAR index value is observed to be less than or equal to 0.2 (Krumperman, 1985).

***Determination of Heavy Metal Resistance*:** Minimal inhibitory concentrations (MIC) of 4 different heavy metals were calculated for each isolated strain. Heavy metal resistance of bacteria was determined in Müller Hinton Agar medium containing heavy metals Cu^{+2}, Cd^{+2}, $Pb^{+2,}$ and Mn^{+2} at concentrations of 100, 200, 400, 800, 1600, and 3200 µg/mL. If the bacterial strain studied can grow in an environment higher than the MIC of the control organism, it is considered resistant. *E. coli* K12 standard strain was used as a control organism according to (Table 1).

Table 1. Heavy Metal Resistance Levels of *E. coli* K12 (Akinbowale et al., 2007).

Heavy Metal Name	MIC Value
Cadmium	100 µg/mL
Copper	200 µg/mL
Lead	1600 µg/mL
Manganese	1600 µg/mL

RESULTS

Seasonal change values of heavy metal resistance levels of Gelevera Stream water and sediment samples according to stations; the heavy metal resistivity numbers of 77 isolates were isolated from the first station were determined as Cd: 60, Cu: 35, Mn: 7, and Pb: 7, respectively. Resistant isolates as a percentage were determined as Cd: 77.9, Cu: 45.5, Mn: 9.1, and Pb: 9.1, respectively. Also, 81 isolates isolated from the second station were determined as Cd: 71, Cu: 55, Mn: 17, and Pb: 12, respectively. As a percentage, the number of resistant isolates was determined as Cd: 87.7, Cu: 67.9, Mn: 21, and Pb: 14.8, respectively. Likely, the heavy metal resistance numbers of 84 isolates from the third station are as follows; Cd: 66, Cu: 56, Mn: 10, and Pb: 9. As a percentage, the number of resistant isolates was determined as Cd: 78.6, Cu: 66.7, Mn: 11.9, and Pb: 10.7, respectively. And, the heavy metal resistance levels of 83 isolates from the fourth station are as follows; Cd: 66, Cu: 60, Mn: 14, and Pb: 14. Resistant isolates as a percentage were determined as Cd: 79.5, Cu: 72.3, Mn: 16.9, and Pb: 16.9, respectively (Fig. 2).

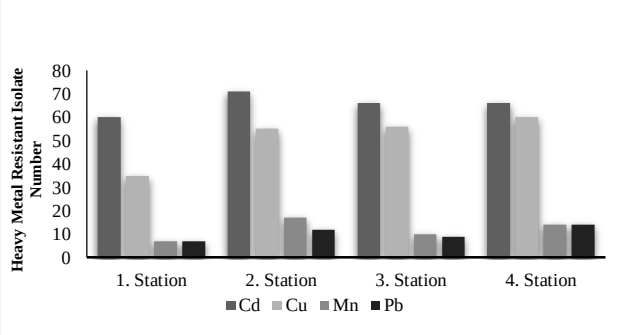

Figure 2. Stational changes value of heavy metal resistance levels.

Seasonal change values of the antibiotic resistance levels of Gelevera Creek water and sediment samples according to stations; antibiotic resistance levels of 11 isolates with high heavy metal resistance at the First Station were determined as follows: KZ: 81.8%, CXM: 63.3%, NA: 54.5%, AMP: 54.5%, MEM: 36.4%, CTX: 33.3%, AK: 18.2%, and E: 9.1%. Also, all isolates were determined of chloramphenicol sensitivity. Heavy metal resistance numbers of isolates with antibiotic resistance were determined as Cd: 11, Cu: 5, Mn: 3, and Pb: 2, respectively. Resistant isolates as a percentage were determined as Cd: 100 Cu: 45.5 Mn: 27.3 and Pb: 18.2, respectively. Also, antibiotic resistance levels of 33 isolates with high heavy metal resistance in the Second Station were determined as follows: KZ: 54.5%, NA: 51.5%, AMP: 45.5%, CXM: 42.4%, CTX: 24%, 2, M: 21.2%, AK: 21.2%, MEM: 15.2%, and C: 3%. Heavy metal resistance numbers of isolates with antibiotic resistance were determined as Cd: 29, Cu: 25, Mn: 15, and Pb: 10, respectively. Resistant isolates as a percentage were determined as Cd: 87.9, Cu: 75.8, Mn: 45.5, and Pb: 30.3, respectively. Likely, antibiotic resistance levels of 18 isolates with high heavy metal resistance at the Third Station were as follows: KZ: 72.2%, CXM: 66.7%, NA: 55.6%, AMP: 38.9%, CTX: 38.9%, MEM: 38.9%, AK: 22.2%, E: 16.7%, and C: 3% Heavy metal resistance numbers of isolates with antibiotic resistance were determined as Cd: 15, Cu: 8, Pb: 7 and Mn: 3, respectively. Resistant isolates as a percentage were determined as Cd: 83.3, Cu: 44.4, Pb: 38.9, and Mn: 16.7, respectively. And, antibiotic resistance levels of 20 isolates with high heavy metal resistance at Station Four are KZ: 70%, CXM: 65%, CTX: 60%, AMP: 45.9%, NA: 45%, MEM: 35%, AK: 25%. C: 10% and E: 5%. Heavy metal resistance numbers of isolates with antibiotic resistance were determined as Cd: 17, Cu: 15, Pb: 9, and Mn: 6, respectively. Resistant isolates as a percentage were determined as Cd: 85, Cu: 75, Pb: 45, and Mn: 30, respectively.

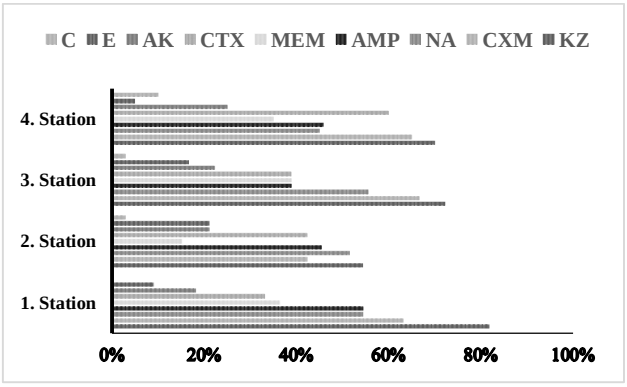

Figure 3. Stational changes value of antibiotic levels (%).

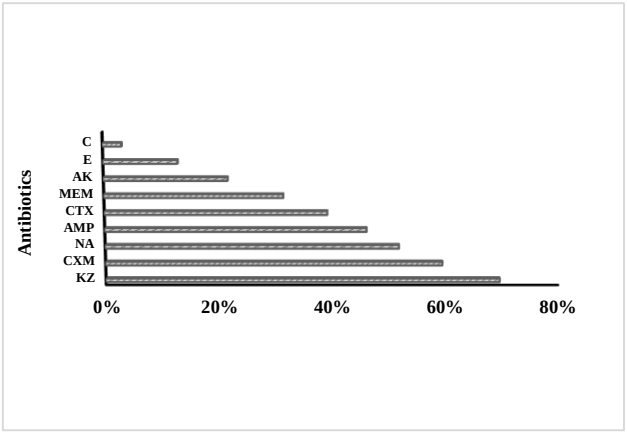

Figure 4. Antibiotic Resistance Levels (%).

During the study, resistance to at least 2 antibiotics was detected in 66 of the 82 isolates isolated and it was determined that 80.5% of the MAR Index reference range was exceeded (Table 2).

Table 2. MAR index value results of the isolates.

Number of antibiotics	**9**	**8**	**7**	**6**	**5**	**4**	**3**	**2***	**1**	**0**
Number of resistant isolates	1	0	0	6	16	15	16	12	10	6
MAR>0.2 N=82	66 (%80.5)									

DISCUSSION and CONCLUSION

When we look at the findings of the studies on antibiotic and heavy metal resistance levels of bacteria isolated from water resources worldwide, India, West Bengal, Kolkata, Dhapa, isolated from municipal waste *Bacillus* sp. heavy metal tolerant and antibiotic-resistant microorganisms were detected. In the presence of Cd^{+2}> Cr^{+6}> Ni^{+2}> Co^{+2} metals, respectively, the resistance ratios and antibiotic resistance of the isolates are determined by many factors such as kanamycin (30 µg / disk), ampicillin (25 µg / disk) and methicillin (5 µg / disk). It has also been observed to be resistant to antibiotics (Samanta et al., 2012). Resistant bacteria were investigated in sediment and seawater samples taken from the Aegean Sea between 2011 and 2013. All bacteria isolated from sediment samples showed 100% resistance to rifampicin, sulfonamide, tetracycline and ampicillin. It was noted that 98% of bacteria were resistant to nitrofurantoin and oxytetracycline, and bacteria isolated from sediment had higher antibiotic and heavy metal resistance than seawater samples. Among the isolates, the highest bacterial metal resistance was reported as 58.3% against copper, 33.8% against zinc, 32.1% against lead, 31% against chromium and 25.2% against iron (Altuğ et al., 2020).

Antibiotic and heavy metal resistance of bacteria isolated from different wastewater contaminated areas in Bucharest and its surroundings in Romania are 40% resistant to amoxicillin-clavulanic acid, 30% to cefazolin and tetracycline, 25% were resistant to cefoxitin and ceftazidime, 20% to ceftriaxone and sulfamethoxazole, that 15% were resistant to aztreonam, ciprofloxacin, piperacillin and imipenem, 10% to tobramycin and 5% to cefotaxime and amikacin respectively. It has been determined that 15% of the heavy metal resistance ratio of the isolates are resistant to mercury, 40% to copper, 75% to chromium and 80% to zinc, and 100% of all isolates are resistant to cadmium and aluminum (Marinescu et al., 2017). Antibiogram test of *P. shigelloides* (n = 182) isolated from 3 rivers of South Africa was performed. In this study, freshwater *P. shigelloides* isolates were tested against 24 antibiotics and it was determined that they have multiple resistance against cephalosporins, quinolones, and fluoroquinolones. 13 EUCAST (ERA) and 11 non-recommended antibiotics (NA), used as first-line agents in the treatment of gastroenteritis and extraintestinal infections, were tested. Resistance to ERAs cefoxitin 37.9%, cefuroxime 35.1%, cefepime 31.8%, ceftriaxone 29.6%, ciprofloxacin 18.1%, trimethoprim-sulfamethoxazole 10.4%, piperacillin 8.7%, ertapenem 4.9%, norfloxacin 4.4%, levofloxacin 2.7%, meropenem 1.1% and imipenem 0.5%. Although isolates have higher resistance (≥36.07) to NA's, it has been reported that they are susceptible to amikacin 67.5%, gentamicin 73%, and tetracycline 80.7% (Ekundayo & Okoh, 2020). In their study aiming to determine the antibiotic and heavy metal resistance of *S. aureus* associated with animal husbandry, which was first isolated from South African livestock production systems, heavy metal resistance is determined as cadmium 90%, zinc 88%, lead 86%, and copper 84%. When antibiotic resistance was examined, it was reported that 90.8% of the *S. aureus* isolates were resistant to at least three antibiotics and therefore were classified as multidrug-resistant and 98% of the tested isolates were resistant to penicillin G (Dweba et al., 2019). Compared to the findings of the studies carried out, the findings in our study have some similarities in terms of the isolates in the antibiotic and heavy metal resistance levels. Exposure to antibiotic-resistant bacteria poses a public health problem as it is directly linked to disease management. There can be several ways of dealing with resistant bacteria. Hospital and city wastewater appear to play an important role in spreading bacteria and antibiotic resistance genes to our environment (Aali et al., 2014).

In terms of determining sea fish 134 isolated *Enterobacteriaceae* antibiotics and heavy metal resistance levels, the findings indicated that antibiotic resistance levels of; erythromycin: 85%, cefazolin: 79.8%, cefotaxime: 78.3%, cefuroxime axetil: 71.6%, nalidixic acid: 60.4%, ampicillin: 58.9%, amikacin: 53.7%, tetracycline: 47.7% and streptomycin: 17.9%. In addition, it was determined that the rate of isolates exceeding the CAA reference value was 88% and the isolates had a high level of heavy metal resistance (Sipahi et al., 2013). Fecal bacterial density and resistance to antibiotics of a total of 232 Gram-negative bacteria isolated in Küçükçekmece Lagoon (Istanbul) are as follows: ampicillin 76.3%, amoxicillin-clavulanic acid 36.9%, streptomycin 20.7%, nalidixic acid, and tetracycline 16.8%, ceftazidime 16.4%, chloramphenicol 9.4%, imipenem 6.8% and amikacin 6.4% (Sivri et al., 2012). In another study, similar results were acquired by Mercimek Takci et al., (2021) who stated that MAR and MHMR index of 21 *E. coli* strains isolated from Seve Dam and Konak Pond, Kilis were recorded as 0.187 and 0.202, respectively. Resistance rates of Enterobacteriaceae isolates isolated from Batlama Creek (Giresun) are reported as follows: ampicillin: 75%, erythromycin: 64%, nalidixic acid: 48%, tetracycline:

39%, amikacin: 34%, cefazolin and chloramphenicol: 33%, cefuroxime: 32%, and cefotaxime: 23%. In the study, it was reported that 77% of the isolates exceeded the MAR reference limit and two isolates were resistant to all antibiotics tested (Akkan, 2017).

Antibiotic resistance profiles were determined as amoxicillin 77.5%, ampicillin 100%, cefazolin 65%, and cefoxitin 65% in isolates isolated from 6 different drinking water sources open to the public in Kilis (Şahin, 2018). Current antibiotic resistance development of *E. coli* isolated from different stations seasonally for 1 year from the central Batlama Creek of Giresun has been determined as follows: ampicillin 59%, tetracycline 50.8%, nalidixic acid 44.4%, erythromycin 42.9%, chloramphenicol 38.1%, cefazolin 36%, cefuroxime 35.9%, cefotaxime 28.4%. The ratio of multiple antibiotic resistance values (MAR) was reported as 73.28% (Topkaraoğlu, 2018). In the study conducted to determine the frequency of antibiotic and heavy metal resistance from water samples taken from Lake Sapanca between 2008 and 2010, the highest bacterial resistance among 84 isolates was found to be 90.4% to vancomycin, 88.1% to ampicillin, and 64,2% to amoxicillin-clavulanate. When the resistance was examined, rates varying between 10.7% and 59.5% were found against cefuroxime, kanamycin, aztreonam, ceftazidime, cefotaxime, and oxacillin. In terms of heavy metal ions, the highest frequency was recorded as 74.1% against nickel; and heavy metal resistance against copper, zinc, mercury, and cadmium were 52.3%, 46.4%, 33.3%, and 26.1%, respectively (Türetken et al., 2019).

The resistance levels of 325 bacteria against 4 different heavy metals were determined after the selective isolation of Gr (-) and Gr (+) bacteria. Resistance levels of 325 bacteria isolated in total to Cd, Cu, Pb and Mn heavy metals were determined as 89.0%, 60.16%, 33.0%, 29.8% (Cd> Cu> Pb> Mn), respectively. In addition, antibiotic resistance levels of the isolates with high heavy metal resistance levels were determined at each station. The 82 isolates with high resistance against heavy metals, antibiotic resistance levels of these isolated strains are as follows: cefazolin: 69.6%, cefuroxime: 59.4%, nalidixic acid: 51.7%, ampicillin: 46.0%, cefotaxime: 39.1%, meropenem: 31.4%, amikacin: 21.7%, erythromycin: 13.0%, and chloramphenicol: 3.3%. In our study, two isolates with high antibiotic and heavy metal isolate no 55 and 642 showing high resistance were identified by Vitek-II Compact System and defined as *Serratia marcescent* (99%) and *Enterococcus avium* (91%). In addition, the rate of multiple antibiotic resistance values (MAR) in surface water sample isolates is 80.5%. As a characteristic member of the *Enterobacteriaceae* family and supplementary to its competence for survival, *S. marcescens* characteristically exhibitions a tendency to express antimicrobial resistance. *S. marcescens* are uniformly resistant to a great range of antibiotics involving narrow-spectrum-penicillins and cephalosporins, cefuroxime, cephamycins, macrolides, tetracycline, nitrofurantoin, and colistin (Stock et al., 2003). The region around the Sangam region of Allahabad, Uttar Pradesh, India has shown a large variety of microorganisms out of which *S. marcescens* has shown significant antibiotic and heavy metal resistant patterns. To survive these metal stressed terms, microorganisms have developed to withstand the uptake of heavy metal ions. (Nies and Silver, 1995). *S. marcescens* is ubiquitous and can be found in water, soil, plants, insects, and animals (Abbott, 1999). Distinct strains of *Serratia* have shown different resistant patterns suggestive of plasmid-borne transfer of resistant genes or pump effluxing systems. But it has been proposed that increasing antibiotic resistance of Gram-negative bacteria is primarily because of mobile genes on plasmids that can easily travel through bacterial populations (Kumarasamy et al., 2010).

In conclusion, this study shows that isolates taken from Gelevera Creek (Giresun) have high resistance to commercially used antibiotics and heavy metals, and this suggests that there is antibiotic and heavy metal pollution on drinking water sources. When compared to the studies in the literature, it is concluded that the antibiotic and heavy metal resistance levels of the bacteria isolated from the Gelevera Course are at a considerable level and the current situation must be checked with a regular monitoring program.

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ARAŞTIRMA MAKALESİ **RESEARCH PAPER**

Japon Bıldırcını Diyetlerine Betain İlavesinin Büyüme Performansı, Karkas ve Duodenum Villus Uzunluğu Üzerine Etkisi

Mükremin ÖLMEZ
Kafkas Üniversitesi Veteriner Fakültesi Hayvan Besleme ve Beslenme Hastalıkları ABD, Kars, Türkiye

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: https://orcid.org/0000-0002-5003-3383

***Sorumlu yazarın:**
Mükremin ÖLMEZ
Kafkas Üniversitesi Veteriner Fakültesi Hayvan Besleme ve Beslenme Hastalıkları ABD, Kars, Türkiye
✉: mukremin.olmez@hotmail.com

Öz: Bu çalışma bıldırcın diyetlerine ilave edilen betainin performans, karkas randımanı ve bazı iç organ ağırlıkları ile duodenum villus uzunlukları üzerine etkilerini incelemek amacı ile yapılmıştır. Deneme 1 günlük yaştaki 150 adet japon bıldırcını ile yürütülmüştür. Araştırmada hayvanlar 1 kontrol ve 2 deneme grubu olmak üzere toplam 3 gruba ayrılmıştır. Gruplar, her birinde 10 civciv bulunan 5 alt gruba ayrılmıştır. Denemede kontrol grubu sadece bazal diyetle beslenirken deneme gruplarına sırasıyla; %0,3 ve %0,8 betain HCL eklenmiştir. Deneme rasyonları izokalorik ve izonitrojenik olarak hazırlanmıştır. Hayvanlara yem ve su ad-libitum verilmiştir. Çalışma 35 gün sürdürülmüştür. Elde edilen verilere göre betainin bıldırcın diyetlerine ilavesiyle canlı ağırlık, canlı ağırlık artışı, yem tüketimi, yemden yararlanma oranı ile duodenum villus uzunluğu üzerine istatistiksel olarak olumlu etkiler gösterdiği belirlenmiştir (P<0,05). Karkas randımanı ve bazı iç organ ağırlıkları üzerine etkisinin olmadığı tespit edilmiştir (P>0,05). Sonuç olarak; bıldırcın diyetlerine %0,8 düzeyinde betain ilavesinin büyüme performansı ve bağırsak sağlığını iyileştirdiği ve güvenle kullanılabileceği sonucuna varılmıştır.

Anahtar Kelimeler: Betain,bıldırcın, karkas, performans, villus.

Effects of Betain Using on Growth Performance, Carcass and Duodenum Villus Length in Japanese Quail Diets

***Corresponding author's:**
Mükremin ÖLMEZ
Division of Animal Nutrition and Nutritional Diseases, Faculty of Veterinary, Kafkas University, KARS, Turkey
✉: mukremin.olmez@hotmail.com

Abstract: This study was aimed to examine the effects of betaine supplemented to quail diets on performance, carcass yield and some visceral weights and duodenal villus lengths. The study was conducted with 150 one-day-old Japanese quails. In the study, animals were equally divided into 3 groups as 1 control and 2 experimental groups. Groups were divided into 5 subgroups of 10 chicks each. While the control group was fed only basal diet, the experimental groups were fed with; 0.3% and 0.8% betaine HCL were added in the trial. Diets were prepared as isocaloric and isonitrogenic. Feed and water were supplied ad-libitum. The study was lasted for 35 days. According to the data obtained, betaine was found to have statistically positive effects on body weight, body weight gain, feed consumption, feed conversion rate and duodenal villus length with the supplementation of quail diets (P <0.05). It was found that it had no effect on the carcass yield and some visceral weights (P> 0.05). It was concluded that 0.8% betaine supplementation to quail diets improved growth performance and intestinal health and could be used safely.

Keywords: Betaine, carcass, quail, performance, villus.

GİRİŞ

Betain, protein ve enerji metabolizmasında önemli rol oynayan üç metil grubundan oluşan amino asit türevidir (Ratriyanto vd., 2009). Kanatlı organizmasında betain metil donörü ve ozmoprotektan olarak iki önemli fonksiyona sahiptir (Metzler-Zebeli vd., 2009). Bunun yanında betainin sindirim kanalındaki besin maddelerinin sindirilebilirliğini arttırdığı bilinmektedir (Eklund vd., 2006). Betain, kolin ve metiyonin çiftlik hayvanlarının rasyonlarında 3-metil grubu sağlayıcılarıdır. Metiyonin protein sentezinde birincil kaynak olarak kullanılırken geri kalan kısmı metil grubu reaksiyonlarında değerlendirilmektedir (DasSarma vd., 2006). Kolin, asetilkolinin sentezinde kullanıldıktan sonra geri kalanı betain molekülüne dönüştürülmektedir (Niculescu & Zeisel, 2002). Betain, metiyonin metabolizmasının oluşturduğu homosisteine metil grubu vererek transsülfürasyon ile metiyonin veya sisteine dönüşmesini sağlar. Betain metiyonin dönüşümüyle protein sentezinin artmasını ve böylece kanatlılarda büyüme performansının daha yüksek olmasını sağlayabilmektedir (Rao vd., 2011). Hayvan beslemede karma yemlere betain ilavesinin kolin ve metiyonin ihtiyacını azalttığı, kolin ve metiyonin yararlanabilirliğini arttırdığı belirtilmiştir (Eklund vd., 2005). Kanatlı diyetlerine betain ilavesinin canlı ağırlığı ve yemden yararlanma oranını iyileştirdiği bildirilmekle birlikte Ayrıca mineral emilimini ve kasların su tutma kapasitesini arttırarak canlı ağırlık ve karkas ağırlığını arttırdığı da tespit edilmiştir (Esteve-Garcia & Mack, 2000).

Betainin hücrede birikmesi, ozmotik dengenin daha az enerji ile kurulmasını sağlamaktadır (Remus, 2001). Betain ozmotik etkisiyle bağırsak epitel hücrelerini koruyarak villus gelişimini desteklediği ve bağırsakta bulunan mikroorganizmaları destekleyerek besin maddelerinin sindirilebilirliğini arttırmaktadır (Dos Santos vd., 2019; Ratriyanto vd., 2017).

Bu çalışmada Japon bıldırcın diyetlerine farklı düzeylerde ilave edilen betainin büyüme performansı, karkas, iç organ ağırlıkları ve duodenum villus uzunlukları üzerine etkilerinin değerlendirilmesi amaçlanmıştır.

MATERYAL VE METOT

Hayvanlar, Deneme Dizaynı ve Yem: Bu çalışma Kafkas Üniversitesi Hayvan Deneyleri Yerel Etik Kurulu'nun KAÜ-HADYEK/2020-159 kodlu onayıyla yapıldı. Çalışma 35 gün sürdürüldü. Hayvan materyali olarak bir günlük yaşta 150 adet Japon bıldırcını (*Coturnix coturnix japonica*) kullanıldı. Çalışma 1 kontrol ve 2 deneme olmak üzere üç grupla yürütüldü. Her bir deneme grubu içerisinde on hayvan bulunan beş alt gruba ayrıldı. Araştırmada kontrol grubuna bazal diyet verilirken deneme grubu diyetlerine %0,3 ve %0,8 betain HCL ilave edildi. Her alt gruptaki bıldırcınlar 60×20×100 cm ölçülerine sahip türe özel olarak düzenlenmiş kafeslerde barındırıldı. Çalışmadaki tüm gruplar %24 HP ve 2950 kcal/kg ME içeren diyetle beslendi (Tablo 1). Diyet formülasyonu NRC'ye göre hazırlandı (Dale, 1994) ve AOAC'ye göre besin madde analizleri yapıldı (AOAC, 2019). Hayvanlara yem ve su *ad libitum* olarak sunuldu. Çalışma süresince tüm hayvanlar ilk üç gün boyunca 32-33 °C sıcaklıkta tutulduktan sonra sıcaklık her hafta 1-2 °C düşürülerek 25 °C'ye sabitlendi. Kafeslere 24 saat/gün aydınlatma sağlandı. Araştırmada kullanılan betain özel bir ticari firmadan (Betamar®, Vimar A.Ş.- İstanbul) tedarik edildi.

Tablo 1. Beslemede kullanılan rasyonun besin madde ve kimyasal analiz içeriği.
Table 1. Nutrient and chemical analysis content of the diet.

İçindekiler	%
Mısır	56,11
Soya küspesi (%48 HP)	40,20
Bitkisel yağ	1,00
Mermer tozu	1,33
Dikalsiyum fosfat	0,60
Tuz	0,25
L-lizin sülfat	0,15
L-treonin	0,11
Vitamin - Mineral karışımı[1]	0,25
Toplam	**100**
Besin madde analizi	
Ham protein (%)	23,80
*Metabolize enerji (Kcal/kg)	2950
*Kalsiyum (%)	1,19
*Fosfor (%)	0,31
*Lizin (%)	1,33
*Metiyonin (%)	0,80
*Treonin (%)	1,02
*Triptofan (%)	0,33

*Hesapla bulunmuştur.

Performans: Canlı ağırlık (CA) ve yem tüketimi (YT) tüm alt gruplarda haftalık olarak belirlendi. Yapılan tartımlar sonucu elde edilen farklardan canlı ağırlık artışı (CAA) ve yemden yararlanma oranı (YYO) hesaplandı (YT/CAA).

Karkas Parametreleri: Denemenin sonunda, karkas özelliklerini incelemek için her gruptan rastgele 10 adet bıldırcın seçilip aç bırakıldıktan sonra tek tek tartılıp kesim ağırlıkları belirlenerek kesildi. Kesilen hayvanların kanı boşaltıldıktan sonra tüyler yolundu. İç organlar (kalp, karaciğer ve taşlık) çıkarılarak karkas ve iç organları tartılıp ağırlıkları belirlendi. Sıcak karkas randımanı hesaplandı.

Histolojik Analizler: Çalışma sonunda bıldırcanlardan alınan duodenum doku örnekleri %10'luk formol solüsyonunda tespit edildikten sonra rutin histolojik işlemlerden geçirilerek parafinde bloklandı. Bu bloklardan alınan 5µ'luk kesitlere duodenum dokusunun genel yapısını incelemek amacı ile Crossman'ın üçlü boyama tekniği (Triple Boyama) uygulandı (Luna, 1968). Tüm grupların duodenum dokusunda villus uzunluk ölçümleri için image-j (vI. 50i) software programı kullanıldı. Villus uzunluk ölçümleri her bir grupta 4 farklı kesitteki toplam

40 alandan yapıldı (Akgül vd., 2015).

İstatistik Analiz: Elde edilen performans ve karkas parametreleri ile villus uzunlukları SPSS 20.0 (IBM-USA) istatistik programında değerlendirildi. Gruplara ait sonuçlar arasındaki farklılık tek yönlü varyans analizi (ANOVA) ile belirlendi. Gruplar arasındaki ikili karşılaştırmalarda Duncan çoklu karşılaştırma testi kullanıldı. Önemlilik P<0,05 derecesinde belirlendi.

BULGULAR

Bıldırcın diyetlerine betain ilavesinin performans üzerine etkileri Tablo 2' de verilmiştir. Deneme sonu CA, CAA, YT ve YYO betain içeren gruplarda istatistik olarak önemli derecede etkilenmiştir (P<0,05).

Tablo 2. Betainin performans parametreleri üzerine etkisi
Table 2. Effect of betaine on performance parameters

Parametreler	Kontrol	B1	B2	P
Çıkım, g	8,30±0,02	8,29±0,02	8,28±0,03	0,833
CA-35.gün, g	172,29±0,77^{b}	180,27±1,08^{a}	179,20±1,28^{a}	0,001
CAA, g/gün	4,69±0,02^{b}	4,91±0,03^{a}	4,88±0,04^{a}	0,001
YT, g/gün	16,45±0,11^{b}	17,07±0,11^{a}	16,79±0,15^{ab}	0,009
YYO, g/g	3,51±0,03^{b}	3,48±0,00^{ab}	3,44±0,00^{a}	0,016

CA: Canlı ağırlık, CAA: Canlı ağırlık artışı, YT: Yem tüketimi, YYO: Yemden yararlanma oranı.
^{a,b}: Aynı satırda farklı harflerle ifade edilen ortalamalar arasındaki farklılıklar önemlidir (P<0,05).

Çalışma sonuçlarına göre karkas randımanı, kalp, karaciğer ve taşlık ağırlıkları betain ilavesinden etkilenmemiştir (P>0,05) (Tablo 3).

Tablo 3. Betainin kesim parametreleri üzerine etkisi
Table 3. Effect of betaine on slaughter parameters

Parametreler	Kontrol	0,3% Betain	0,8% Betain	P
Karkas Randımanı, %	64,28±1,99	65,01±2,68	67,12±1,86	0,647
Kalp, g	1,66±0,07	1,52±0,04	1,51±0,08	0,716
Karaciğer, g	4,53±0,33	4,22±0,29	4,34±0,17	0,409
Taşlık, g	2,93±0,38	3,18±0,20	3,44±0,15	0,507

Histolojik parametreler incelendiğinde %0,8 düzeyinde betain HCL ilave edilen grupta duodenum villus uzunluğunun diğer gruplara göre daha yüksek olduğu tespit edilmiştir (P<0,05) (Tablo 4).

Tablo 4. Betainin duodenum villus uzunluğu üzerine etkisi
Table 4. Effect of betaine on duodenal villus length

Parametre	Kontrol	0,3% Betain	0,8% Betain	P
Villus uzunluğu (µm)	1309,60±53,25^{b}	1214,09±59,68^{b}	1640,58±76,98^{a}	0,001

^{a,b}: Aynı satırda farklı harflerle ifade edilen ortalamalar arasındaki farklılıklar önemlidir (P<0,05).

TARTIŞMA VE SONUÇ

Bu araştırma bıldırcın diyetlerine ilave edilen betain katkısının CA, CAA, YT, YYO, karkas randımanı ve iç organ ağırlıkları ile duodenum villus uzunlukları üzerine olan etkilerini tespit etmek amacıyla yürütülmüştür.

Betain ilavesinin çalışma sonunda performans parametrelerini istatistik olarak önemli derecede iyileştirdiği bulunmuştur. Deneme sonu CA ve ortalama günlük CAA incelendiğinde betain ilave edilen iki grupta da kontrol grubuna göre daha yüksek canlı ağırlık değerleri elde edilmiştir. Günlük ortalama en yüksek YT'nin %0,3 betain HCL içeren grupta olduğu belirlenirken en iyi YYO' nun ise %0,8 betain HCL içeren grupta olduğu tespit edilmiştir.

Sunulan çalışmanın sonuçları değerlendirildiğinde bazı çalışmalarla uyum içerisinde olduğu görülmüştür. Betainin etlik piliçlerde performans parametrelerinin değerlendirildiği bir çalışmada diyete %0.8 düzeyinde ilavesinin CA ve CAA'yı arttırdığı bildirilmiştir (Şahin vd., 2020). Chand vd., (2017) ise etlik piliç diyetlerine betainin 2g/kg düzeyinde ilavesi ile YT ve YYO'yu olumlu düzeyde etkilendiğini belirtmişlerdir. El-Husseiny, (2007) de broiler diyetlerine 0.75 g/kg düzeyinde betain ilavesinin YYO'yu iyileştirdiğini belirlemiştir. Mevcut çalışma sonuçları diğer bazı literatür bildirişleriyle de benzerlik göstermiştir (Liu vd., 2019; Sakomura vd., 2013b). Araştırmalardan elde edilen performans parametrelerinde görülen iyileşmenin betainin etkisiyle artan besin madde sindiriminden kaynaklanabileceği ifade edilmiştir (Sakomura vd., 2013b). Yine betainin enerji ve protein metabolizmasını etkileyerek büyüme performansını destekleyebileceği tespit edilmiştir (Eklund vd., 2005).

Waldroup vd., (2006)'nin betain ilavesiyle performans parametrelerinde herhangi bir değişiklik olmadığı yönündeki bildirişi ile Schutte vd., (1997)'nin betainin etlik piliçlerde performansı düşürdüğü yönündeki bildirişi mevcut çalışma sonuçları ile farklılık arz etmiştir. Betainin etkinliğinde görülen farklılıkların rasyonların protein ve enerji içeriğine bağlı olabileceği bildirilmiştir (Lawrence vd., 2002).

Yapılan araştırmalarda betain ilavesinin karkas randımanı ile kalp, karaciğer ve taşlık ağırlıkları üzerine etkisinin olmadığını bildiren sonuçlar mevcut çalışma sonuçları ile benzerlik göstermektedir (El-Shinnawy, 2015; Şahin vd., 2020). Benzer şekilde Uzunoğlu ve Yalcin, (2019) betain ilavesinin etlik piliçlerde karkas randımanını etkilemediğini bildirmişlerdir. Bu çalışmaların aksine betain ilavesinin su tutma kapasitesini artırarak, karkas randımanını yükselttiği yönünde araştırma sonuçları da bulunmaktadır (Chand vd., 2017; Waldroup & Fritts, 2005). Başka bir çalışmada ise etlik piliçlerde betain ilavesinin karkas randımanını arttırdığı, ancak karaciğer üzerine olumsuz etki gösterdiği belirlenmiştir (Jahanian & Rahmani, 2008). Etlik piliçlerde yapılan bir çalışmada ise betainin 100 mg/kg düzeyinde ilavesinin karkas randımanı üzerine iyileştirici etki gösterdiği tespit edilmiştir (Esteve-Garcia & Mack, 2000). Sonuçlarda görülen farklılıkların beslenme şartlarıyla birlikte betainin kullanım dozu ile ilişkili olduğu düşünülmektedir.

Bağırsaklarda görülebilecek bozukluklar veya

hasarlar besinlerin emiliminin azalmasına, epitel geçirgenliğinin artmasına, hastalıklara karşı direncin azalmasına ve bunun sonucunda da kanatlılarda performansın düşmesine yol açmaktadır (Wijtten vd., 2011). Bu açıdan bakıldığında bağırsak morfolojisi genel anlamda bağırsak sağlığı hakkında araştırmalara ışık tutabilmektedir. Bağırsak villus bütünlüğünün ve epitel hücrelerinin aktivitesinin korunması patojen mikroorganizmaların girişini önleyeceğinden bağırsak sağlığının iyileştirilmesinde önemli rol oynamaktadır (Wang vd., 2020). Nitekim mevcut çalışmada, betain ilavesinin duodenum villus uzunluğunu arttırdığı belirlenmiştir. Bağırsaklarda betain birikimi, enterositlerin su tutma kapasitesini geliştirdiğinden bağırsak epitel hücrelerinin yapısının korunmasında etkili olduğu ve kanatlılarda bağırsağın gerilme mukavemetini artırarak villus uzunluğunu olumlu etkilediği ortaya konmuştur (Kettunen vd., 2001a). Betaninin bu sayede kanatlılarda besin emilimini ve yemden yararlanma oranını arttırarak performansı iyileştirdiği düşünülmektedir. Dos Santos vd., (2019) etlik piliçlerde betain ilavesinin, duodenum villus genişliğini azaltıp, uzunluğunu artırdığını bunun da duodenumda emilim kapasitesini iyileştirdiğini bildirmişlerdir. Başka bir çalışmada ise betainin iyonofor antikoksidiyallerle birlikte kullanımının bağırsak sağlığını koruduğu belirtilmiştir (Kettunen vd., 2001b). Eklund vd., (2005) betainin etlik piliçlerde bağırsak villus bütünlüğünü koruyarak daha iyi besin emilimi ve sindirilebilirlik sağladığını tespit etmişlerdir. Betainin ısı stresi altındaki etlik piliçlerde villus uzunluğunu etkilemediğini bildiren çalışmalar da bulunmaktadır (Sakomura vd., 2013a). Ortaya çıkan bu farklılığın oluşturulan ısı stresinin boyutu ve süresi ile diyete katılan betain düzeyine bağlı olabileceğini düşündürmüştür.

Sonuç olarak; betain %0,8 düzeyinde canlı ağırlık, canlı ağırlık artışı, yemden yararlanma oranı ve duodenum villus uzunluğunu iyileştirmiştir. Kanatlı diyetlerine betain ilavesinin bağırsak sağlığını olumlu etkileyerek performansı artırabileceği ancak kesim parametrelerini artırıcı etkinliğinin araştırılması gerektiği kanaatine varılmıştır.

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ARAŞTIRMA MAKALESİ RESEARCH PAPER

Antibiotic Resistance and Virulence Gene Profiles in Staphylococci Isolated from Cattle with Mastitis

Mehmet Onur GÖKDAĞ **Alper ÇİFTCİ***
Ondokuz Mayıs University, Faculty of Veterinary Medicine, Department of Microbiology, Samsun, Turkey

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*: https://orcid.org/0000-0001-8370-8677
: https://orcid.org/0000-0003-4325-3772

***Corresponding author's:**
Alper ÇİFTCİ
Ondokuz Mayıs University, Faculty of Veterinary Medicine, Department of Microbiology, Samsun, Turkey
✉: aciftci@omu.edu.tr

Abstract: *Staphylococcus* spp. are the important bacterial agents of subclinical and clinical mastitis cases. This study was aimed to determine the vancomycin resistances, antibacterial resistance profiles, some virulence genes, and pheno- and genotyping of staphylococci from mastitis. For this aim, 121 staphylococcal isolates were analyzed. The identifications of isolates were confirmed with PCR for being *Staphylococcus* spp. and *Staphylococcus aureus*. The antibiotic resistance patterns were determined by Kirby-Bauer Disc Diffusion Tests and according to the resistance profiles, the isolates were antibiotyped. The vancomycin resistance genes were determined by PCR for investigating the *vanA*, *vanH*, *vanR*, *vanS*, *vanZ*, *vanY* and *vanX* genes. The vancomycin resistant isolates were genotyped with RAPD-PCR. The *nuc* gene was detected in 86 of 121 staphylococcal isolates examined and named as *Staphylococcus aureus*. The remaining 35 isolates were defined as *Staphylococcus* spp. *S. aureus* isolates were found to be resistant to penicillin G, amoxicillin/clavulanic acid, oxacillin, tetracycline, cefoperazone, teicoplanin, vancomycin and trimethoprim-sulfamethoxazole at 50% (43/86), 40.7% (35/86), 34.9% (30/86), 23.3% (20/86), 22.1% (19/86), 18.6% (16/86) 10.5% (9/86) and 8.1% (7/86) respectively. On the other hand, 4 (11.4%) of 35 *Staphylococcus* spp. isolates were resistant to penicillin G, 3 (8.6%) to amoxicillin/clavulanic acid, 2 (5.7%) to trimethoprim-sulfamethoxazole, 1 (2.9%) to oxacillin, cefoperazone, teicoplanin, while all isolates were sensitive to vancomycin and tetracycline. Of the 9 *S. aureus* isolates that were phenotypically resistant to vancomycin, *vanA* gene was detected in 2 and *vanR* gene in 3 isolates. Multiple resistances to three or more antibiotics were determined in 42 of 86 *S. aureus* isolates. In addition, *coa* gene was detected in 61 (70.9%) of 86 *S. aureus* isolates. 10 different gene polymorphisms were detected in *coa* gene positive isolates. While the *spa* gene was determined in all *S. aureus* isolates, it was revealed that they had 4 *spa* gene polymorphisms. Nine different genotypes with a similarity between 51-75% were detected in the genotyping of vancomycin resistant 9 isolates. In conclusion, multiple antibiotic resistance rates in *S. aureus* isolates investigated were found to be important for mastitis treatment. The results obtained from this study show that milk and dairy products containing these factors pose a public health risk due to the determination of vancomycin resistance in mastitis-derived *Staphylococcus* strains.

Keywords: Bovine, *coa*, mastitis, RAPD-PCR, *spa*, *Staphylococcus*, vancomycin resistance.

Mastitisli Sığırlardan İzole Edilen Stafilokoklarda Antibiyotik Direnci ve Virülens Gen Profilleri

Öz: *Staphylococcus* spp. subklinik ve klinik mastitis olgularının önemli bakteriyel etkenleridir. Bu çalışmanın amacı, mastitis kaynaklı stafilokokların vankomisin dirençlerini, antibakteriyel direnç profillerini, bazı virülans genlerini ve feno- ve genotiplendirmesini belirlemektir. Bu amaçla 121 stafilokok izolatının analizi yapılmıştır. İzolatların *Staphylococcus* spp. ve *Staphylococcus aureus* olmak üzere identifikasyonları PCR ile ve antibiyotik direnç paternleri de Kirby-Bauer Disk Difüzyon Testleri ile belirlendi. İzolatlar direnç profillerine göre antibiyotiplendirildi. *VanA*, *vanH*, *vanR*, *vanS*, *vanZ*, *vanY* ve *vanX* genlerinin PCR ile

[*] *This study was produced from the master thesis.*

araştırılması sonucunda vankomisin direnç genleri belirlendi. Vankomisine dirençli izolatlar, RAPD-PCR ile genotiplendirildi. İncelenen 121 stafilokok izolatının 86'sında *nuc* geni saptandı ve *Staphylococcus aureus* olarak identifiye edildi. *S. aureus* izolatlarının penisilin G, amoksisilin/klavulanik asit, okzasilin, tetrasiklin, sefaperazon, teikoplanin, vankomisin ve trimetoprim-sulfamethoxazole sırasıyla %50 (43/86), %40.7 (35/86), %34,9 (30/86), %23,3 (20/86), %22.1 (19/86), %18,6 (16/86) %10,5 (9/86) ve %8,1 (7/86) oranında dirençli bulundu. 35 adet *Staphylococcus* spp.'nin 4 (%11,4)'ü penisilin G'ye, 3 (%8,6)'ü amoksisilin/klavulanik aside, 2 (%5,7) 'si trimetoprim-sülfometaksazole, 1 (%2,9) 'i oksasilin, sefaperazon, teikoplanine direnç gösterirken, tüm izolatlar vankomisin ve tetrasikline duyarlıydı. Vankomisine fenotipik olarak dirençli olan 9 *S. aureus* izolatından 2'sinde *vanA*, 3'ünde *vanR* geni saptandı. 86 *S. aureus* izolatının 42'sinde üç veya daha fazla antibiyotiğe karşı çoklu direnç belirlendi. Ayrıca 86 *S. aureus* izolatının 61 (%70.9) 'inde *coa* geni tespit edildi. *coa* geni pozitif izolatlarda 10 farklı gen polimorfizmi tespit edildi. Tüm *S. aureus* izolatlarında *spa* geni belirlenirken, 4 *spa* gen polimorfizmi görüldü. Vankomisine dirençli 9 izolatın genotiplendirilmesinde %51-75 arasında benzerlik gösteren dokuz farklı genotip tespit edildi. Sonuç olarak, araştırılan *S. aureus* izolatlarında çoklu antibiyotik direnç oranları mastitis tedavisi için önemli bulunmuştur. Bu çalışmadan elde edilen sonuçlar, bu faktörleri içeren süt ve süt ürünlerinin mastitis kaynaklı *Staphylococcus* suşlarında vankomisin direncinin belirlenmesi nedeniyle halk sağlığı açısından risk oluşturduğunu göstermektedir.

***Sorumlu yazar:**
Alper ÇİFTCİ
Ondokuz Mayıs Üniversitesi Veteriner Fakültesi Mikrobiyoloji Anabilim Dalı, Samsun, Türkiye
✉: aciftci@omu.edu.tr

Anahtar kelimeler: *Coa*, inek, mastitis, RAPD-PCR, *spa*, *Staphylococcus*, vankomisin direnci.

INTRODUCTION

Bovine mastitis is a costly and multifactorial disease for businesses and producers due to reduced milk production, increased treatment costs, culling and mortality rates in dairy farms. More than 130 different types of microorganisms have been isolated in cow milk with mastitis. *Staphylococcus aureus* might be present in both subclinical and clinical mastitis cases as one of the most common chronic mastitis factors (Monistero et al., 2020). It has been reported that there are many factors involved in virulence of *S. aureus* strains that cause mastitis. Coagulase, DNAse, protein A, toxins (enterotoxins, leukotoxins, toxic shock syndrome toxin, exulsive toxin, etc.), hemolysins, fibrinolysins, and biofilm formation were responsible for the formation of *S. aureus* originated mastitis (Momtaz et al., 2010; Kot et al., 2016)

Although the discovery of effective agents used in the prevention and treatment of infections caused by bacteria and other pathogenic microorganisms is one of the most important developments in modern medicine, substances with anti-infective potential have actually been used for thousands of years. With the introduction of penicillin in the 1940s, the mortality rate due to staphylococcal infections decreased rapidly. However, shortly after, *S. aureus* strains started to produce penicillinase enzyme and developed penicillin resistance and these resistant strains spread rapidly. In the late 1950s, approximately 50% of strains became resistant to penicillin. At the same dates, strains of *S. aureus*, which showed multiple resistance to tetracycline, chloramphenicol and erythromycin, were reported (Schwarz et al., 2018). Methicillin, the first semisynthetic penicillinase resistant antimicrobial agent, entered clinical use in 1959. Two years later, in 1961, the first methicillin-resistant *S. aureus* (MRSA) isolates were reported from the UK (Jevons, 1961) and later became a problem in Europe in the 1960s and the USA in the 1970s (Hartstein & Mulligan, 1986). MRSA strains showing multiple resistance to antibiotics spread all over the world in the late 1980s and 1990s (Schmitz & Jones, 1997). MRSA is still among the most common nosocomial pathogens in hospitals of various sizes all over the world. Due to the increase in infections due to multiple resistant MRSA strains, vancomycin has been used for the treatment of staphylococcal nosocomial infections for the last 25 years. Vancomycin resistance was reported in clinical isolates of coagulase negative staphylococci in 1987 (Schwalbe et al., 1987). Clinical failures due to the selection of teicoplanin resistant isolates were reported after treatment of *S.aureus* infections with teicoplanin in 1990 (Kaatz et al., 1990). The emergence of MRSA isolates with reduced sensitivity to the vancomycin of Japan, USA and France in 1997 is very worrying (CDCP, 1997; Hiramatsu et al., 1997). These strains are resistant to most other antimicrobial agents and are isolated from patients who do not respond to vancomycin therapy. Vancomycin is a narrow-spectrum bactericidal antibiotic that was first isolated from *Streptomyces orientalis* on Borneo Island in 1956. Shortly after its isolation, it was purified in 1956 and entered clinical use. It lost importance after use of meticillin due to the impurity the preparations used in the first years and frequency of side effects, but gained importance with the first reporting of a methicillin-resistant *S. aureus* isolate in 1961 and the increasing MRSA infections since 1982.

In this study, it was aimed to investigate the vancomycin resistances, antibacterial resistance profiles,

some virulence genes, pheno- and genotyping of staphylococci from mastitis.

MATERIAL AND METHOD

Bacterial isolates: Within the scope of the study, 121 Staphylococcal isolates from bovine mastitis milks were examined. For the molecular identification, DNAs were extracted from fresh colonies by boiling method as described previously and their concentrations were equalized to 50 ng/microliter (Sezener et al., 2019). PCR was performed for the identification of isolates as *Staphylococcus* spp. and/or *S. aureus*. *S. aureus* specific *nuc* gene (279 bp) and *Staphylococcus* spp. specific 16S rRNA gene (756 bp) were investigated (Table 1) (Çiftci et al., 2009).

Table 1. Oligonucleotide primer sequences used for identification.

	Oligonucleotide sequence (5'-3')	Band size (bp)
Staph756F	AAC TCT GTT ATT AGG GAA GAA CA	756
Staph756R	CCA CCT TCC TCC GGT TTG TCA CC	
nuc 1	GCG ATT GAT GGT GAT ACG GTT	279
nuc 2	AGC CAA GCC TTG ACG AAC TAA AGC	

Antibiotic sensitivity tests: Antibiotic susceptibility tests of all isolates were performed under the conditions recommended by the Clinical and Laboratory Standards Institute (2013) by Kirby Bauer disc diffusion technique. For this purpose, standard antibiotic discs [oxacillin (10 µg), vancomycin (30 µg), teicoplanin (30 µg), trimethophrim-sulfamethoxazole (25 µg), tetracycline (30 µg), penicillin G (10 µg), cefoperazone (75 µg), amoxicillin-clavulonic acid (20/10 µg)] were used. The results were evaluated as sensitive (S), intermediate (I), and resistant (R) (CLSI, 2018).

Determination of vancomycin resistance level: Broth microdilution technique recommended by the Clinical and Laboratory Standards Institute (CLSI, 2018) was used to determine the minimal inhibition concentrations (MIC) of vancomycin resistance in staphylococcal strains. MIC values for staphylococci against vancomycin were evaluated as 4 µg/ml for susceptible, 8-16 µg/ml for intermediate susceptible (VISA), ≥32 µg/ml for resistant (VRSA).

Genotypic determination of vancomycin resistance: Vancomycin resistance genes of isolates which were determined to be resistant to vancomycin by diffusion method were determined by PCR. PCR was performed for these genes using specific oligonucleotides for the *vanA, vanH, vanR, vanS, vanZ, vanY,* and *vanX* genes (Table 2) in the *vanA* gene cluster of staphylococcal isolates (Dezfulian et al., 2012). Amplification products were visualized with UV transilluminator after 1.5% agarose gel electrophoresis.

Determination of virulence genes: Coagulase (*coa*) and protein A (*spa*) virulence genes of all isolates were determined by PCR. *coa* and *spa* genes presences and polymorphisms were detected by the method reported by Ciftci et al. (2009). The polymorphisms of the isolates were determined according to the band sizes displayed, and the isolates were typed with respect to their band sizes.

Table 2. Oligonucleotide primer sequences used to identify vancomycin resistance genes.

Primer		Oligonucleotide sequence (5'-3')	Band size (bp)	Annealing (°C)
vanR1	F	AGCGATAAAATACTTATTGTGGA'	645	53
vanR2	R	CGGATTATCAATGGTGTCGTT		
vanS1	F	TTGGTTATAAAATTGAAAAATAA	1155	47
vanS2	R	TTAGGACCTCCTTTTATC		
vanH1	F	ATCGGCATTACTGTTTATGGAT	943	55
vanH2	R	TCCTTTCAAAATCCAAACAGTTT		
vanA1	F	ATGAATAGAATAAAAGTTGCAATAC	1029	52
vanA2	R	CCCCTTTAACGCTAATACGAT		
vanX1	F	ATGGAAATAGGATTTACTTT	609	46
vanX2	R	TTATTTAACGGGGAAATC		
vanY1	F	ATGAAGAAGTTGTTTTTTTTA	912	47
vanY2	R	TTACCTCCTTGAATTAGTAT		
vanZ1	F	TTATCTAGAGGATTGCTAGC	454	51
vanZ2	R	AATGGGTACGGTAAACGAGC		

Genotyping and phylogenetic analysis: RAPD-PCR was performed using M13 (5'-AAG TAA GTG ACT GGG GTG AGC G-3') primer for genotyping of vancomycin resistant isolates (Fındık et al., 2011). The similarities and numbers of the bands among RAPD patterns were determined based on the Dice similarity coefficient. To create a dendrogram that graphed genetic relatedness among isolates, "Unweighted Pair Group Method with Arithmetic Averages (UPGMA)" was employed using CHEF-DR® III, Quantity One® Software (Bio-Rad Laboratories, Hercules, CA).

RESULTS

Genotypical identification of isolates: It was determined to contain *Staphylococcus* spp. specific 16S rRNA gene of all isolates and confirmed to be *Staphylococcus* spp. The *nuc* gene was detected in 86 of 121 staphylococcal isolates examined and named as *S. aureus*. The remaining 35 isolates were identified as *Staphylococcus* spp.

Antibiotic sensitivity test results: The resistance profiles of the isolates examined in the study are shown in Table 3-5.

Table 3. Antibiotic susceptibility profiles of *S. aureus* isolates.

	VA	TEC	OX	SXT	TE	PEN-G	CEP	AMC
R	9	16	30	7	20	43	19	35
I	0	10	5	14	16	6	18	6
S	77	60	51	65	50	37	49	45

S: sensitive; I: Intermediate; R: resistant. VA: vancomycin; TEC: teicoplanin; OX: oxacillin; SXT: trimethoprim-sulfamethoxazole; TE: tetracycline; PEN-G: penicillin G; CEP: cefoperazone; AMC: amoxicillin / clavulanic acid

Table 4. Antibiotic susceptibility profiles of *Staphylococcus* spp. isolates.

	VA	TEC	OX	SXT	TE	PEN-G	CEP	AMC
R	0	1	1	2	0	4	1	3
I	0	2	3	4	3	2	4	1
S	35	32	31	29	32	29	30	31

Vancomycin resistance level results: MIC values were determined for 9 isolates that were determined to be vancomycin resistant by the disc diffusion technique. As a result of the evaluation, 7 isolates were found to be resistant to 32 µg/ml and 2 isolates to 64 µg/ml vancomycin.

Table 5. Multiple antibiotic resistance profiles of isolates.

Number of antibiotics with resistance	*S. aureus* (n)	*Staphylococcus* spp. (n)
8	9	0
7	6	0
6	7	0
5	9	0
4	6	0
3	5	2
2	11	6
1	16	5
0	17	22

Genotypic determination of vancomycin resistance: Vancomycin resistance genes of 9 isolates determined to be resistant to vancomycin by disc diffusion method were determined by PCR method. As a result of PCR, it was determined that 2 isolates gave positive band for *vanA* (1029 bp) and 3 isolates for *vanR* (645 bp) gene. In 4 isolates resistant to vancomycin, the genes examined could not be determined.

Virulence gene results: *coa* and *spa* virulence genes of all *S. aureus* isolates (n = 86) included in the study were determined by PCR method. As a result of PCR performed to detect *coa* gene, 61 of isolates were found to be positive. The band profiles of the isolates for *coa* were presented Table 6.

Table 6. *Coa* polymorphism profiles of *S. aureus* isolates.

Coa group	Band size (bp)	Number of isolates
1	950	2
2	800, and 400	3
3	550	7
4	800	4
5	550, and 500	6
6	820, and 250	3
7	250	3
8	820, 620, and 580	2
9	120	31
10	negative	25

As a result of PCR with the presence of *spa* gene, 86 isolates were found to be positive. The band profiles of the isolates for *spa* were presented in Table 7.

Table 7. *Spa* polymorphism profiles of *S. aureus* isolates.

spa group	Band size (bp)	Number of isolates
1	130	26
2	200	31
3	290	16
4	310	13

Genotyping and phylogenetic analysis results: As a result of RAPD-PCR for genotyping of vancomycin resistant isolates, 9 different genotypes with a similarity between 51-75% were detected (Figure 1 and 2).

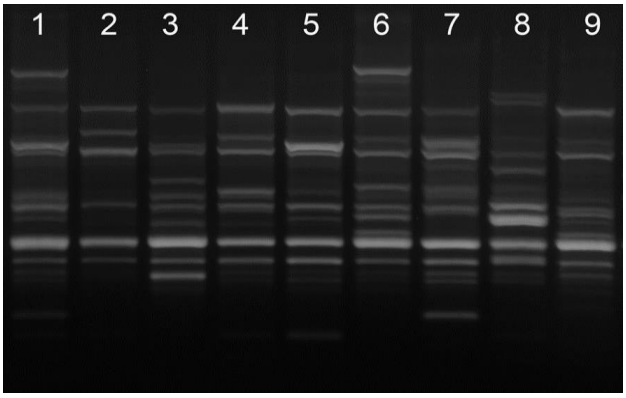

Figure 1. RAPD-PCR profiles of *S. aureus* isolates.

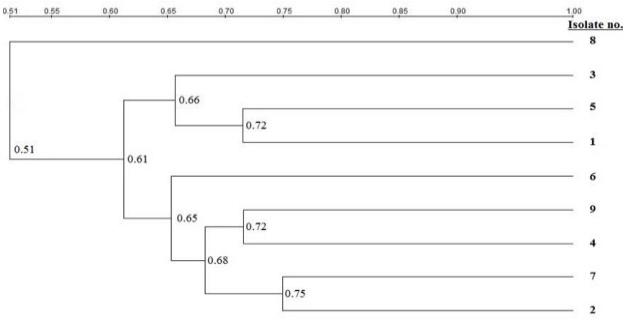

Fig. 2. Phylogenetic proximity analysis of *S. aureus* isolates.

DISCUSSION

Bovine mastitis is the most costly disease to the dairy industry worldwide as well as in Turkey. *S. aureus* is an important etiologic agent of mastitis in ruminants and also has an economical importance in cattle industry. There are many enzymes involved in the virulence of staphylococci. Coagulase is an extracellular proenzyme that coagulates the plasma by converting the fibrinogen to fibrin. Detection of this enzyme, which is accepted as a pathogenicity criterion for *S. aureus*, is routinely used to differentiate pathogenic staphylococci. Of the 121 isolates examined in the study, 73 were found to be coagulase positive and all of the positive isolates were *S. aureus*. It was observed that 13 *S. aureus* examined did not synthesize coagulase enzyme. The expression of the coagulase enzyme depends on the presence of the *coa* gene, and for this purpose, the presence of *coa* gene in the *S. aureus* isolates and the polymorphism in the gene were investigated for this purpose. As a result of PCR performed to detect *coa* gene presence and polymorphism, 61 isolates were found to be positive for *coa* gene and the remaining 25 isolates were found to be negative. The isolates found to be *coa* positive were grouped according to the band profiles they showed as a result of polymorphism occurring in the gene and 10 profiles were determined. While 25 of the isolates did not contain the *coa* gene, 2 isolates produced a band of 950 bp. On the other hand, we were determined that formed three isolates 800 and 400 bp 2

bands, 7 isolates 550 bp single band, 4 isolates 800 bp single band, 6 isolates 550 and 500 bp 2 bands, 3 isolates 820 and 250 bp 2 bands, 3 isolates 250 bp single band, 31 isolates 120 bp single band and 2 isolates formed 820, 620 and 580 bp size 3 bands. These results were found to be compatible with previous study (Karahan & Çetinkaya, 2007; Xu et al., 2015; Oliveira et al., 2016) data indicating that polysorphisms in terms of *coa* gene in mastitis isolate bacteria show that this shows diversity in mastitis isolate *S. aureus*.

The colonization process of *S. aureus* begins with its attachment to the host cell surface, and this takes place through the adhesins contained in the bacteria. Most of the adhesins contained in *S. aureus* are proteins found in cell peptidoglycans called Protein A (*spa*). Protein A is used as an important reagent in immunology and diagnostic laboratory technology for its properties such as binding to the Ig molecule and agglutinating bacteria against specific bacterial antigens. The gene responsible for the synthesis of protein A is the *spa* gene. As a result of the study, 86 gene isolates were found positive in terms of *spa* gene presence and PCR after polymorphism of isolates identified as *S. aureus*. It was determined that the isolates carrying the *spa* gene formed a band of different sizes and thus showed polymorphism in 4 different groups. It was seen that 26 of the isolates were 130 bp, 31 were 200 bp, 16 were 290 bp and 13 were 310 bp. As a result, it was determined that all mastitis isolates *S. aureus* carry the *spa* gene but there are polymorphisms in terms of gene. As this, *spa* genes are also common in several other studies on bovine mastitis, it can be assumed that these genotypes are prevalent among dairy cows (Schabauer et al., 2018; Pichette-Jolette et al., 2019)

The discovery of effective agents used in the treatment of infections caused by pathogenic bacteria is considered one of the most important developments in modern medicine. Like many other bacterial agents, there are antibiotics that staphylococci are resistant. *Staphylococcus* strains resistant to antibiotics cause problems in the treatment of bovine mastitis as in other diseases (Schwarz et al., 2018). There are many studies (Guimarães et al., 2017; Haubert et al., 2017; Sezener et al., 2019) showing that resistance to most antibiotics such as methicillin, vancomycin, used in the treatment of cattle mastitis of staphylococcal origin has developed over time.

In this study, *S. aureus* isolates were found to be resistant at penicillin G, amoxicillin/clavulanic acid, oxacillin, tetracycline, cefoperazone, teicoplanin, vancomycin and trimethoprim- sulfamethoxazole 50% (43/86), 40.7% (35/86), 34.9% (30/86), 23.3% (20/86), 22.1% (19/86), 18.6% (16/86) 10.5% (9/86) and 8.1% (7/86) respectively. On the other hand, 4 (11.4%) of 35 *Staphylococcus* spp. isolates were resistant to penicillin G, 3 (8.6%) to amoxicillin/clavulanic acid, 2 (5.7%) to trimethoprim- sulfamethoxazole, 1 (2.9%) to oxacillin, cefoperazone, teicoplanin, while all isolates were sensitive to vancomycin and tetracycline. Penicillin resistance is probably the best known antimicrobial resistance property of *S. aureus* and its frequency in the current study is in accordance with other studies that examined antibiotic susceptibility patterns of staphylococci isolated from cases of bovine mastitis. Resistance to penicillin among *S. aureus* from bovine mastitis has been encountered with increasing frequency throughout the world. However, the reported prevalence rates have varied extensively in different geographical regions. The percentage of penicilin resistant *S. aureus* isolates was found to be as high as 87% in China (Shi et al., 2010), 61% in Estonia (Kalmus et al., 2011), 50% in Turkey (Sezener et al., 2019), 45.3% in Kenya (Antok et al., 2020), 41% in Poland (Jagielski et al., 2014) 25% in 9 country cited by vetpath study group (de Jong et al., 2018).

Besides, MIC values of vancomycin-resistant *S. aureus* isolates were determined as 32 µg/ml for 7 isolates and 64 µg/ml vancomycin resistant (VRSA) for 2 isolates. Of the 9 *S. aureus* isolates that were phenotypically resistant to vancomycin, *vanA* gene (1029 bp) was detected in 2 and *vanR* gene (645 bp) in 3. In 4 isolates resistant to vancomycin, the genes examined could not be determined. Multiple resistance to three or more antibiotics was determined in many of *S. aureus* isolates (48.8%, 42/86). It was determined that *S.aureus* isolates were resistant to 8, 7, 6, 5, 4, 3, 2 and 1 antibiotics at 10.47%, 6.98%, 8.14%, 10.47%, 6.98%, 5.81%, 12.79% and 18.60% respectively. Seventeen isolates were also found to be sensitive to tested antibiotics. This shows the emergence of antimicrobial resistance in *S. aureus* isolated from bovine mastitis samples in Turkey. Recently, an increase in the number of antimicrobial resistant bacteria from bovine mastitis has been recognized, similar to the results obtained in this study (de Jong et al., 2018; Sezener et al., 2019). When evaluated in terms of multiple resistance, 5.71% of the *Staphylococcus* spp. isolates were found resistant to 3 antibiotics, 5.71% to 2 antibiotics and 17.14% to 1 antibiotic. Twenty-two isolates were found to be sensitive to all antibiotics tested. Multiple antibiotic resistance was found to be higher in *S. aureus* compared to other staphylococci. As a result, it was concluded that due to its multiple antibiotic resistance, mastitis originated *S. aureus* posed a risk both in the treatment of the animal and in terms of public health.

Molecular typing of bacterial isolates is done due to variations in chromosomal DNA structure (Goh et al., 1992; Carter et al., 2003). As with other bacterial species, *Staphylococcus* strains have many subtypes. Accurate identification of clones with high virulence or

epidemiologically spreading is considered important (Frenay et al., 1994; Frenay et al., 1996). Molecular typing systems have many advantages such as high performance and easy applicability compared to conventional methods. Although Pulsed-Field Gel Electrophoresis (PFGE), which is one of the molecular typing methods, is accepted as the "golden standard", PCR-based methods are frequently used for their quick results, ease and economy (Sabat et al., 2006). RAPD-PCR typing, which is one of the PCR based methods, is a typing method based on the principle of replicating DNA in short sections under the primer and variable reaction conditions that are randomly bound to DNA. This method is often used to determine genetic affinities among the isolates. It provides comparison of bacterial species depending on the regions to which the primer is attached (Qu et al., 2019).

Correct and fast typing of staphylococci is important in controlling the infection (Hookey et al., 1998). There are many subtypes of staphylococci, especially *S. aureus*, that vary depending on their virulence genes (DaSilva et al., 2006). Determining the genetic variation and heterogeneity in these bacteria is important for determining rational and effective strategies for staphylococci that cause mastitis (Kapur et al., 1995). In this study, as a result of RAPD-PCR performed using the M13 primer for genotyping of vancomycin resistant 9 isolates, 9 different genotypes with a similarity between 51-75% were detected. This detected diversity showed that there was diversity in mastitis-derived *S.aureus* strains and the clones causing infection were not closely related. Similar results were reported in other studies, with 2 predominant RAPD types of 6 representing 71% (Wang et al., 2016) and 60% (Qu et al., 2019) *S. aureus* isolates. Therefore, particular *S. aureus* strains might transmit more from cow to cow.

In conclusion, multiple antibiotic resistance rates in *S. aureus* isolates investigated were found to be important for mastitis treatment. The results obtained from this study show that milk and dairy products containing these factors pose a public health risk due to the determination of vancomycin resistance in mastitis-derived *Staphylococcus* strains. Very close genetic relationship could not be detected in *Staphylococcus* isolates isolated from bovine mastitis. This status indicates that there is a polymorphism genotypically among the isolates. Further studies are needed with Staphylococcal isolates from cattle with more mastitis.

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ARAŞTIRMA MAKALESİ RESEARCH PAPER

Condition, Length-Length and Length-Weight Relationships for Four Introduced Freshwater Fish Species from an Island Ecosystem (Gökçeada, Turkey)

Sevan AĞDAMAR[1]* Özcan GAYGUSUZ[2]

[1*]*Çanakkale Onsekiz Mart University, Gökçeada School of Applied Sciences, Gökçeada, Çanakkale, Turkey*
[2] *Istanbul University, Faculty of Aquatic Sciences, Department of Marine and Freshwater Resources Management, Laleli, Istanbul, Turkey*

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*: https://orcid.org/0000-0002-1268-0379
: https://orcid.org/0000-0001-6861-6221

***Corresponding author's:**
Sevan AĞDAMAR
Çanakkale Onsekiz Mart University, Gökçeada School of Applied Sciences, Gökçeada, Çanakkale, Turkey
✉: agdamars@gmail.com

Abstract: The present study aims to investigate the condition, length-weight (LWRs) and length-length relationships (LLRs) of four introduced freshwater fish species (*Carassius gibelio*, *Cyprinus carpio*, *Gambusia holbrooki*, and *Pseudorasbora parva*) inhabited in the inland waters of Gökçeada (Çanakkale, Turkey). Fish samples were collected using electroshocker from one lentic (Büyük Stream) and four lotic (Aydıncık, Dereköy, Gökçeada, and Uğurlu Reservoirs) ecosystems in September 2019, April 2020, May, 2020, and July 2020. The calculated values of parameter *b* in the LWRs were 3.275 and 3.230 for *G. holbrooki* (Uğurlu and Büyük populations, respectively), 3.031 and 2.937 for *C. carpio* (Aydıncık and Dereköy populations, respectively), 3.129 for *C. gibelio* and 3.047 for *P. parva*. Values of Fulton's condition factor (K) varied between 1.72 ± 0.23 (*P. parva*, Gökçeada population) and 3.60 ± 0.33 (*C. carpio*, Dereköy population). The coefficients of correlation (r) for all the LLR equations were greater than 0.95 and significantly linear. The present study provides the first knowledge about the selected biological parameters for four introduced freshwater fish species in Gökçeada.

Keywords: Biological traits, inland waters, insular ecosystem, non-native species.

Bir Ada Ekosistemindeki (Gökçeada, Türkiye) Dört Aşılanmış Tatlısu Balığı Türünün Kondisyon, Boy-Boy ve Boy-Ağırlık İlişkileri

***Sorumlu yazar:**
Sevan AĞDAMAR
Çanakkale Onsekiz Mart Üniversitesi, Gökçeada Uygulamalı Bilimler Yüksekokulu, Gökçeada, Çanakkale, Türkiye
✉: agdamars@gmail.com

Öz: Bu çalışma, Gökçeada (Çanakkale, Türkiye) içsularında yaşayan dört aşılanmış tatlısu balığı türünün (*Carassius gibelio*, *Cyprinus carpio*, *Gambusia holbrooki* ve *Pseudorasbora parva*) kondisyon, boy-ağırlık ilişkileri (BAİ) ve boy-boy ilişkilerini (BBİ) incelemeyi amaçlamaktadır. Balık örnekleri Eylül 2019, Nisan 2020, Mayıs 2020 ve Temmuz 2020 aylarında bir lentik (Büyük Dere) ve dört lotik (Aydıncık, Dereköy, Gökçeada, ve Uğurlu Baraj Gölleri) ekosistemden elektroşok cihazı kullanılarak toplanmıştır. BAİ kullanılarak hesaplanan *b* parametresi değerleri *G. holbrooki* için 3,275 ve 3,230 (sırasıyla Uğurlu ve Büyük populasyonları), *C. carpio* için 3,031 and 2,937 (sırasıyla Aydıncık ve Dereköy populasyonları), *C. gibelio* için 3,129 ve *P. parva* için 3,047'dir. Fulton'un kondisyon faktörü (K) değerleri 1,72 ± 0,23 (*P. parva*, Gökçeada populasyonu) ile 3,60 ± 0,33 (*C. carpio*, Dereköy populasyonu) arasında değişiklik göstermiştir. Tüm BBİ denklemleri için korelasyon katsayıları (r) 0,95'ten büyük ve önemli ölçüde doğrusal olarak belirlenmiştir. Bu çalışma, Gökçeada'da tespit edilen dört aşılanmış tatlısu balığı türü için seçilen biyolojik parametreler hakkındaki ilk bilgileri sağlamaktadır.

Anahtar kelimeler: Ada ekosistemi, biyolojik özellikler, doğal olmayan tür, içsular.

INTRODUCTION

Biological invasions are natural events and essentially point out range expansions of species into novel environments (Censky et al., 1998). One of the events for underlying biological invasions is an introduction of non-native species into new areas either intentionally or unintentionally (Copp et al., 2005). When these introductions are accomplished and end up with biological invasions, they have the possible to lead significant

economic and biodiversity losses, such as been sighted globally (Pimentel et al., 2005). Successful biological invasions include complicated relationships between the introduced species and the physical and/or biological traits of the novel environments (Hayes & Barry, 2008). It is important to determine some biological indices (e.g. condition factor, and length-weight relationships) and to evaluate these indices in order to track the invasion success of introduced species. The success of introduced fish species has been correlated with the spatial variability of life-history traits (Havel et al., 2005). Such variabilities support introduced species due to higher toleration to changes in biological indices and plasticity of life-history traits such as condition factor and length-weight relationships (Marchetti et al., 2004).

Condition factors (K), length-length relationships (LLRs), and length-weight relationships (LWRs) are significant instruments in fish biology, ecology, fisheries management, and conservation (Froese, 1998). Condition factors (K) are widely utilized for measuring the condition, fatness, or well-being of fish (Tesch, 1971). Fish condition is a cursor of recent physical and biological states (Le Cren, 1951), therefore, the condition factor ensures significant knowledge about present and future population success with its relationship via growth, reproduction, and survival. Length-weight relationships (LWRs) can be used to change the length to weight and the opposite. Length can be detected more readily than weight during the field surveys. Thus, weight can be determined after field surveys using LWR. Furthermore, LWR is helpful for comparing the life history of an organism in different environments and seasons (Gonçalves et al., 1997) which can be utilized for morphological comparison of species and populations (Petrakis & Stergiou, 1995). Length-length relationships (LLRs) are used for different varieties of fish length measurements in ichthyological explorations. Briefly stated, standard length (SL) is used in taxonomic research, whereas total length (TL) and fork (FL) length are generally utilized for the determination of fish growth (Moutopoulos & Stergiou, 2002). So, it is important to determine these LLRs to be helpful in comparing the results of different studies that use different measurement types in length.

Due to the absence of any native fish species except one species, reservoir ecosystems of Gökçeada are "empty" in terms of native fish species and it is indicated that introduced fish species have successfully filled all these empty niches. Hence, the aim of this study was to identify the K-values, LLRs, and LWRs for four introduced freshwater fish species gibel carp (*Carassius gibelio* Bloch, 1782), common carp (*Cyprinus carpio* Linnaeus, 1758), eastern mosquitofish (*Gambusia holbrooki*, Girard, 1859), and topmouth gudgeon (*Pseudorasbora parva* Temminck & Schlegel, 1846) in the lentic and lotic ecosystems of Gökçeada Island. Growth parameters of the introduced fish species currently living in the inland waters of the island were studied in order to discover the impact of the alterations in the fish composition in view of fish introductions in the inland waters. On the other hand, long-term survey is necessary to track the impacts of alterations in the fish composition on fish populations. The findings of this study would ensure a source for further assessment of the changes in fish growth following deliberately or accidentally introduction of non-native species into the insular ecosystems.

MATERIALS AND METHODS

Fish samples were collected using electrofishing (SAMUS-1000 portable electroshocker; frequency 50-55 Hz) from one lentic (Büyük Stream) and four lotic (Aydıncık, Dereköy, Gökçeada, and Uğurlu Reservoirs) ecosystems of Gökçeada between September 2019 and August 2020 (Figure 1). Data on the sampling sites and sampling dates were listed in Table 1. The map was created using QGIS ver. 3.4 software available from http://qgis.org.

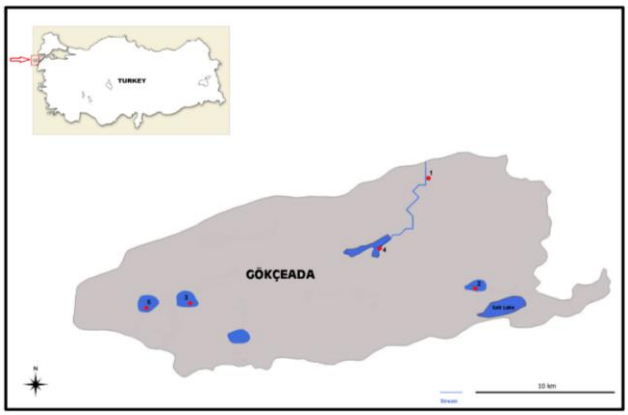

Figure 1. The study area and the sampling stations (1: Büyük Stream, 2: Aydıncık Reservoir, 3: Dereköy Reservoir, 4: Gökçeada Reservoir, 5: Uğurlu Reservoir).

Table 1. Data on the sampling sites and dates of four introduced fish species in Gökçeada.

Sampling Areas			Sampling Dates
Locality	**Locality No**	**Coordinate**	
Büyük Stream	1	40° 13' 24" N, 25° 53' 44" E	September 2019
Aydıncık Reservoir	2	40° 8' 53" N, 25° 55' 42" E	September 2019; May 2020; July 2020
Dereköy Reservoir	3	40° 8' 26" N, 25° 44' 9" E	September 2019; July 2020
Gökçeada Reservoir	4	40° 10' 45" N, 25° 51' 57" E	September 2019; April 2020; May 2020; July 2020
Uğurlu Reservoir	5	40° 8' 12" N, 25° 42' 49" E	September 2019; July 2020

***Statistical Analysis*:** The fish samples were measured for total (TL), fork (FL) and standard length (SL) to the nearest 0.1 cm and weighed for total body weight (W) on a digital balance with a 0.01 g accuracy. The length-weight relationship was calculated using the equation:

$W=aL^b$, where W is the total weight (g), L is the standard length (cm), *a* and *b* are regression parameters (Le Cren, 1951). The equation ($W=aL^b$) was converted into the natural logarithmic form ($lnW=lna+blnSL$)) and parameters *a* (regression intercept) and *b* (slope) were calculated by the regression analysis (King, 2007). 95% confidence intervals (CI) of parameters *a* and *b* were estimated by the equation: $95\%CI=x\pm(t_{0.05}\times SE)$ (x: *a* or *b*; t: table value of t (t-test at 95% confidence); SE: standard error value of *a* or *b*) (King, 2007).

The length-length relationship was calculated using linear regression analysis for being comparable to the results of different studies, which will use different length measures (Gaygusuz et al., 2006). The fish condition was assessed with Fulton's Condition Factor ($K=(W/L^3)\times100$) (Ricker, 1975).

RESULTS

In total, 288 specimens of four introduced fish species were collected from one lentic and four lotic ecosystems of Gökçeada Island (Table 1). The average standard length (SL ± SD) and body weight (W ± SD) of *G. holbrooki* were 1.9 ± 0.4 cm and 0.18 ± 0.12 g for Büyük population and 2.3 ± 0.7 cm and 0.37 ± 0.53 g for Uğurlu population, respectively. For *C. carpio*, the average SL ± SD and W ± SD were 4.5 ± 1.2 cm and 3.84 ± 5.40 g for Aydıncık population, and 4.8 ± 2.5 cm and 8.46 ± 24.20 g for Dereköy population. The average SL ± SD and W ± SD of *C. gibelio* were calculated as 11.8 ± 0.8 and 52.54 ± 12.36 g for Gökçeada population, and they were calculated as 4.7 ± 1.4 cm and 2.18 ± 1.71 g for *P. parva* in the same population. The estimated LWRs and condition factors of four species were listed in Table 2. The calculated values of parameter *b* in the LWRs were 3.230 and 3.275 for *G. holbrooki* (Büyük and Uğurlu populations, respectively), 3.031 and 2.937 for *C. carpio* (Aydıncık and Dereköy populations, respectively), 3.129 for *C. gibelio* and 3.047 for *P. parva* (Figure 2). Fulton's condition factor varied between 1.72 ± 0.23 (*P. parva*, Gökçeada Reservoir population) and 3.60 ± 0.33 (*C. carpio*, Dereköy population). The estimated LLRs of four species were summarized in Table 3. The coefficients of correlation (r) for all the LLR equations were greater than 0.95 and significantly linear.

Table 2. The descriptive statistics, estimated parameters of length-weight relationships and condition factor values of four introduced fish species living in Gökçeada (n: number of individuals, SL: standard length, W: body weight, Min: minimum, Max: maximum, *a:* intercept, *b*: slope, 95% CI: 95% confidence limit, r: coefficient correlation, K: condition factor, SD: standard deviation).

Species	Locality	n	SL, cm	W, g	Regression Parameters		95% CL of *a*	95% CL of *b*	r	K (±SD)
			Min.-Max.	Min.-Max.	*a*	*b*				
Gambusia holbrooki	Büyük Stream	38	1.1 – 3.0	0.02 – 0.56	0.018	3.230	0.016 – 0.021	3.057 – 3.403	0.99	2.14 ± 0.22
	Uğurlu Reservoir	89	1.3 – 5.0	0.03 – 3.40	0.016	3.275	0.015 – 0.018	3.168 – 3.381	0.99	2.02 ± 0.32
Cyprinus carpio	Aydıncık Reservoir	47	2.1 – 10.0	0.28 – 38.74	0.031	3.031	0.025 – 0.038	2.894 – 3.168	0.99	3.27 ± 0.32
	Dereköy Reservoir	51	2.6 – 16.5	0.63 – 162.45	0.039	2.937	0.035 – 0.044	2.863 – 3.010	0.99	3.60 ± 0.33
Carassius gibelio	Gökçeada Reservoir	33	10.4 – 13.9	35.12 – 86.68	0.023	3.129	0.006 – 0.079	2.623 – 3.635	0.92	3.13 ± 0.29
Pseudorasbora parva	Gökçeada Reservoir	30	2.0 – 7.0	0.12 – 6.57	0.016	3.047	0.012 – 0.020	2.889 – 3.204	0.99	1.72 ± 0.23

Table 3. Relationships between total, fork and standard length for four introduced fish species living in Gökçeada (n: sample size, TL: Total length, FL: Fork length, SL: Standard length, *a* and *b* are the parameters of linear regression analysis).

Species	Locality	n	SL = *a* + *b*TL	SL = *a* + *b*FL	FL = *a* + *b*TL
Gambusia holbrooki	Büyük Stream	38	SL = -0.022 + 0.771 TL (r = 0.97)	–	–
	Uğurlu Reservoir	89	SL = -0.094 + 0.815 TL (r = 0.99)	–	–
Cyprinus carpio	Aydıncık Reservoir	47	SL = -0.132 + 0.794 TL (r = 0.99)	SL = -0.130 + 0.887 FL (r = 0.97)	FL = -0.011 + 0.896 TL (r = 0.99)
	Dereköy Reservoir	51	SL = -0.149 + 0.806 TL (r = 0.99)	SL = -0.174 + 0.906 FL (r = 0.99)	FL = 0.026 + 0.890 TL (r = 0.99)
Carassius gibelio	Gökçeada Reservoir	33	SL = 0.755 + 0.732 TL (r = 0.97)	SL = 0.254 + 0.838 FL (r = 0.98)	FL = 0.616 + 0.872 TL (r = 0.98)
Pseudorasbora parva	Gökçeada Reservoir	30	SL = -0.066 + 0.812 TL (r = 0.99)	SL = -0.058 + 0.885 FL (r = 0.99)	FL = -0.008 + 0.918 TL (r = 0.99)

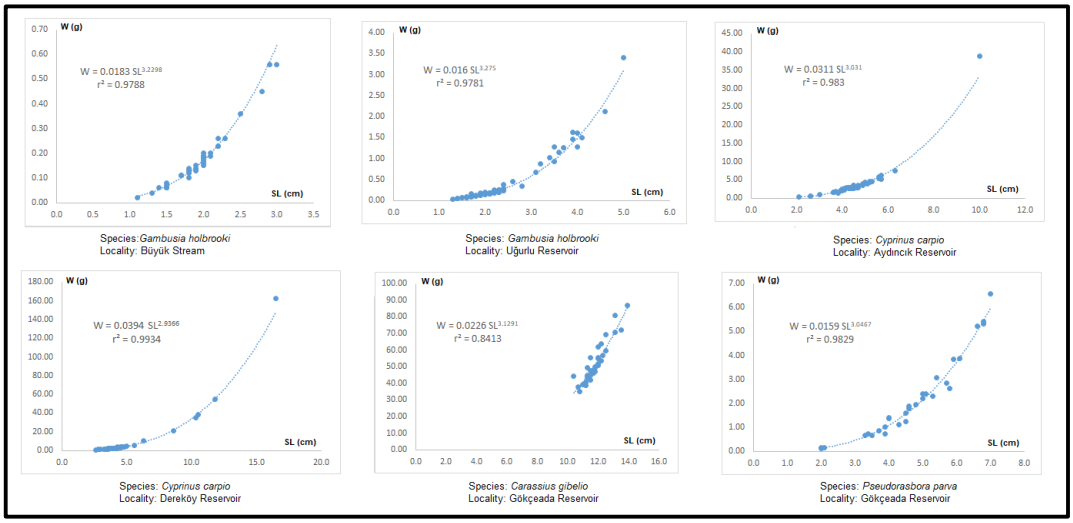

Figure 2. Diagram of the length-weight relationships of four introduced fish species living in Gökçeada inland waters.

DISCUSSION

Biological parameters have been used for ichthyological studies since the beginning of the last century (Froese, 2006) as a basic instrument to evaluate the well-being of species. The parameter of condition factor evaluates the condition of fish and can also be utilized to respectively distinguish and measure the impacts of length condition and other relevant factors (Le Cren, 1951). In the present study, values of K (see Table 2) varied between 1.72 for *P. parva* and 3.60 for *C. carpio* (Dereköy population). The condition factors of examined species found in this study were strikingly higher than the values reported in the previous studies (Table 4). Condition factors of ≥1 show a good status of feeding and suitable environmental factor (Ujjania et al., 2012). The K-values of all examined populations in the present study were ≥1 showing their proper environmental conditions of habitats.

Table 4. The comparison of K and *b* values of the current study and previous studies.

Species	K	References	Species	*b*	References
G. holbrooki	2.02	This study	*G. holbrooki*	3.230	This study
	2.14	This study		3.275	This study
	1.13	Eagderi & Radkhah (2015)		3.230	Öztürk & İkiz (2004)
	1.28	Ergüden & Ergüden (2008)		2.945	İlhan & Sarı (2015)
	1.38	Ergüden (2013)		2.960	Ergüden (2013)
				2.866	Kurtul & Sarı (2020)
C. carpio	3.27	This study	*C. carpio*	2.937	This study
	3.60	This study		3.031	This study
	1.87	Demirkalp (2007)		3.140	Tarkan et al. (2006)
	1.34	Karataş (2007)		3.319	Karataş et al. (2007)
	1.97	Yılmaz et al. (2010)		3.010	Çolakoğlu & Akyurt (2011)
C. gibelio	3.13	This study	*C. gibelio*	3.129	This study
	2.49	Bostancı (2007)		2.856	Birecikligil et al. (2016)
	2.68	Yazıcıoğlu (2013)		2.870	Reis et al. (2019)
				2.571	Ergüden (2015)
P. parva	1.72	This study	*P. parva*	3.047	This study
	0.96	Kırankaya et al. (2014)		3.324	Kırankaya et al. (2014)
	0.95	Arslan & Özeren (2019)		2.867	Benzer et al. (2016)
				3.121	Benzer & Benzer (2020)

In the present study, the coefficient of correlation (r) values for all the LLRs of four introduced fish species were greater than 0.95 (see Table 3) and these results would be useful for comparative growth studies of the same species. On the other hand, the *b* values in LWRs determine the growth type of the fish and when it is 3, the increase in weight is isometric (Bagenal & Tesch, 1978), otherwise it is allometric (positive allometric if $b>3$, negative allometric if $b<3$; see King, 1996). Additionally, this value usually estimated in fishes is within the range of 2.5–3.5 (Froese, 2006). In this study, the estimates of *b* values varied between 2.937 (*C. carpio*, Dereköy population) and 3.275 (*G. holbrooki*, Uğurlu population) for examined fish species (see Table 2) and these values are in the expected range. According to these findings, *C. gibelio, C. carpio,* and *P. parva* showed isometric growth ($b=3$), whereas *G. holbrooki* showed positive allometric growth ($b>3$). When these findings are compared with the results in the literature for the same ecosystems in Turkey, for the examined fish species in this study, the *b* values found in this study was both lower and higher than those reported by different authors (Table 4). Different environmental conditions in the habitat of fish have negative or positive impacts on growth (Nikolsky, 1963). This may clarify same fish species in different habitats can exhibit differences on growth. The other reasons for these differences could be the different TL ranges and different sample sizes (more small or large individuals) of the statistical populations examined in the different researches (Froese, 2006). Additionally, sex, health condition, gonad maturity, and stomach fullness could be thought the other factors that would be effective on these differences.

In conclusion, introduced species are able to survive a broad diversity of environments and adapt to high levels of habitat disruptions, being able to inhabit ecosystems that many other species are incapable to tolerate (Araújo et al., 2003). Thus, introduced species are in good status in terms of condition and other growth parameters. The results of this study stated that introduced species can be survived to different environmental factors by regulating their growth model. On the other hand, this study presented the first information about the selected biological indices (i.e. K and LWR) for four introduced fish species in Gökçeada that would be useful for researchers to better understanding its phenotypic plasticity in lotic and lentic ecosystems of the island.

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DERLEME MAKALESİ REVIEW PAPER

Diseases Transmission Via Semen in Cattle: Importance and Control Strategies

Cumali KAYA* **Melih AKAR** **Burcu ESİN**
Ondokuz Mayıs University, Faculty of Veterinary Medicine, Department of Reproduction and Artificial Insemination, Samsun, Turkey

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* : https://orcid.org/0000-0002-0666-5359
: https://orcid.org/0000-0002-9160-5026
: https://orcid.org/0000-0002-5728-1478

***Corresponding author's:**
Cumali KAYA
Ondokuz Mayıs University, Faculty of Veterinary Medicine, Department of Reproduction and Artificial Insemination, Samsun, Turkey.
✉: cumali.kaya@omu.edu.tr

Abstract: Disease transmission via semen is an extremely adversarial situation in terms of animal husbandry and reproductive productivity. Artificial insemination is the most widely used method in reproductive biotechnology. The main purpose of artificial insemination is to make genetic improvements. Millions of semen doses produce and distributed throughout the world. With this method, semen obtained both domestically and abroad affects animal husbandry throughout the country. Therefore, absolutely semen should be free from all kinds of disease factors. Control strategies should be determined and implemented in the entire process, from semen procurement to storage. Serious measures should be taken in semen production facilities; breeding animals should be checked regularly. Semen obtained from breeding animals in sperm stations should be investigated for various pathogens. Care should be taken to vaccinate the animals, and disease-free herds should be created. The purpose of this review is to review the importance of diseases via semen and the determination of control strategies.

Keywords: Cattle, control, disease, semen, transmission.

Hastalıkların Sperma Yoluyla Bulaşması: Önemi ve Kontrol Stratejileri, Türkiye

***Sorumlu yazar:**
Cumali KAYA
Ondokuz Mayıs Üniversitesi, Veteriner Fakültesi, Dölerme ve Suni Tohumlama Anabilim Dalı, Samsun, Turkey.
✉: cumali.kaya@omu.edu.tr

Öz: Sperma yoluyla hastalık bulaşması, hayvancılık ve üreme verimliliği açısından son derece olumsuz bir durumdur. Suni tohumlama, üreme biyoteknolojileri arasında yaygın olarak kullanılan yöntemdir. Suni tohumlamanın temel amacı genetik iyileştirme sağlamaktır. Milyonlarca doz sperma dünya çapında üretilmekte ve dağıtılmaktadır. Bu yöntemle hem yurt içinden hem de yurt dışından elde edilen sperma ile tüm ülke genelinde hayvancılık etkilenmektedir. Bu nedenle sperma her türlü hastalık faktöründen ari olmalıdır. Spermanın alınması, işlenmesi ve depolanmasına kadar tüm süreçte kontrol stratejileri belirlenmeli ve uygulanmalıdır. Sperma üretim tesislerinde ciddi önlemler alınmalı, damızlık hayvanlar düzenli olarak kontrol edilmelidir. Sperma üretim istasyonlarında damızlık hayvanlardan elde edilen sperma, çeşitli patojenler yönünden düzenli olarak taranmalıdır. Hayvanların aşılanmasına özen gösterilmeli ve hastalıktan ari sürüler oluşturulması sağlanmalıdır. Bu derlemenin amacı sperma yoluyla bulaşan hastalıkların ve kontrol stratejilerinin önemini değerlendirmektir.

Anahtar kelimeler: Bulaşma, hastalık, kontrol, sığır, sperma.

INTRODUCTION

Cattles, due to their high reproductive capacity, are essential for meat and milk production. Artificial insemination is used for most modern dairy cattle breeding (Givens & Marley, 2008). In cattle, artificial insemination's primary goal is to achieve continuous genetic improvement and prevent or eliminate venereal disease. As the most frequently used procedure today, artificial insemination (AI) has had and continues to have a tremendous impact on the various animal breeding industries. Today millions of semen doses produce and distributed for livestock improvement and easy application of artificial insemination throughout the world. Artificial insemination includes significant advantages such as genetic advancement, disease control, reproductive

management, and cost savings. Some of these advantages can also be considered disadvantages. This technology may cause the spread of genetic defects and some infections (Wentink et al., 2000). Diseases of a bull's testicle, epididymis, vas deferens, ampulla, seminal vesicle, prostate, urethra, penis, or prepuce or migration leakage infected blood cells into the male reproductive tract can readily contaminate semen. These conditions may detect the infectious pathogen in the semen, cattle via natural breeding or artificial insemination. Semen used for AI must therefore be free of infectious agents (Givens, 2018). Transmissible diseases that have the potential for severe and rapid spread, irrespective of national borders, are of serious socio-economic or public health consequence, which is of significant importance in the international trade of animals and animal products (Eaglesome & Garcia, 1997). At the international level, guidelines are published by the Office International des Epizooties (OIE) to enable the health of animals in AI centers to be maintained and facilitate the global distribution of semen free of specific pathogenic organisms. National standards for semen production and distribution are usually based on regulatory programs to ensure that diseases of importance are identified and appropriate tests are applied to all sires entering and residing in artificial insemination centers. Therefore necessitates daily testing of frozen semen under the most hygienic conditions (OIE, 2017). This review aims to determine potential semen-transmitted diseases and prevention strategies for these diseases, which play an important role in genetic progression.

***The Role of Sperm in Genetic Development and the Importance of Semen Production Facilities*:** Genetic development to satisfy global demand is put a greater focus on bull semen production facilities in order to increase the reproductive potential of bull. Testicular capability, daily sperm production (DSP) (Amann, 1970), daily sperm output (Amann et al., 1974), ejaculate ability in the laboratory, and timely bull management decisions all contribute to ample product production. Any sperm-producing company's goal is to optimize sperm yield while reducing the risk of injury to bulls and employees. To meet consumer demands for artificial insemination organizations to provide abundant products at a fair production cost and compete globally, it is necessary to taking advantage of bulls reproductive capability. A valid certification can be ensured by a periodic testing program of a representative number of animals in the region for the absence of diseases. When diseases are endemic, however, inquiries into the animals' be free from these diseases one month after semen collection seem to be inevitable. Fresh sperm is extremely dangerous and should be avoided at all costs (Lanyon et al., 2012).

The regular testing of semen donors under official veterinary supervision has been adopted by governments worldwide as a means of avoiding the spread of pathogens and reducing excessive contamination of semen by ubiquitous bacteria. National standards for semen production and distribution are usually based on regulatory programs to ensure that diseases of importance are identified, and appropriate tests are applied to all sires entering and residing in artificial insemination (AI) centers (Sanderson, 2009). These programs take into account the national health status as well as the health status of the herds and flocks of the semen donors. In interpreting health status, prime considerations include the sensitivity and specificity of tests, particularly when they are applied to individual animals, and the risk of latent infections. Testing programs need to be continually improved and updated as new information on the epidemiology, pathogenesis, and control of traditional diseases becomes available (Sibley, 2010). As hew infectious diseases emerge, these additional challenges and risks must also be met. The Office International des Epizooties (OIE) publishes guidelines at the international level to ensure the protection of animals in AI centers and to promote the global distribution of semen that is free of unique pathogenic species (Bayvel, 2004).

***Diseases Transmissible through Semen*:** Several pathogens can lead to various diseases in bulls or result in transmission through semen. The rate at which diseases are transmitted to other animals is determined by various factors, including the pathogen, the host, and the animal-to-animal contact structure. The number of animals infected by one typical infectious animal over the course of its infectious duration is a significant parameter in transmission. This number is highly dependent on the number of effective animal contacts. A contact is effective while the pathogen is transmitted when one of the animals is infectious (above the minimal effective dose excretion titer). These contacts may be direct (animal–animal) or indirect (by air, people, equipment, etc.) (Wentink et al., 2000). Viruses, bacteria, protozoa, and parasites can all be spread by bovine sperm. Infections of the testicles, epididymis, vas deferens, ampulla, seminal vesicle, prostate, urethra, penis, or prepuce, as well as the movement or leakage of infected blood cells into the male reproductive tract, can easily contaminate semen. Major viral pathogens have been researched in terms of transmission by sperm. Below is a summary of viral diseases through semen in Table 1 (Eaglesome & Garcia, 1997; Givens, 2018; Hornizek, 1990; Wentink et al., 2000) and bacterial and protozoon diseases through semen in Table 2 (Benchimol et al., 2008; BonDurant, 2005; Givens, 2018; Karlsson et al., 2010; Peter, 1997).

Table 1. A summary of viral diseases through semen.

Viral Diseases	Etiology	Incubation Period	Characteristic symptoms	Disease Risk via Semen	Diagnosis
BR/IPV	Bovine Herpes Virus type 1	2-5 days	Fever, nasal discharge, Balonopostitis	Transmission by semen is possible	Cornell semen test Virus isolation (SNT, ELISA or PCR)
Bovine viral Disease	Pestivirus	2-15 days	Fever, erosion of the oral and nasal mucosa	The primary source of infection is persistently infected animals present in a herd	Virus isolation (ELISA or PCR)
Leukosis	Oncovirus	Up to 35 days	Enlargement of superficial lymph nodes	Transmission by semen is very unlikely	Symptoms and lesions Virus identification (ELISA, AGID, PCR)
Blue Tongue	Orbivirus	3-6 days	Fever, cyanosis on tongue	Temporary infertility in bulls and shedding of BTV in semen may ocur	Symptoms and lesions Virus identification (ELISA or PCR)
Foot-Mouth Disease	Aphtovirus	2-8 days	Fever, viesicles in mucosa of mouth	Transmission by semen is very unlikely	Symptoms and lesions Virus identification (CFT or SNT) Electronmicroscopy
Rinderpest	Morbillivirus	4-15 days	Nasal and ocular discharge, diarrhoea	Transmission by semen is possible	Virus identification (ELISA or PCR)
Malignant Catharral fever	Herpes virus (BHV3)	From a few days to year	Fever, keratitis	Transmission by semen is very unlikely	Symptoms and lesions Virus identification (PCR)
Akabene virus	Akabanevirus	-	Congenital abnormalities	Transmission by semen is very unlikely	Serology, Culture techniques
Schmallenberg virus	Orthobunyavirus	1-5 days	Fever, diarrhoea	This virus can be detected in seminal plasma early in acute infections	Symptoms and lesions Virus identification (PCR)

Table 2. A summary of bacterial and protozoon diseases through semen.

Bacterial Diseases	Etiology	Characteristic symptoms	Disease Risk via Semen	Diagnosis
CBPP	*Mycoplasma mycoides* ssp.	Fever, dyspnoea, coughing, nasal discharges, and anorexia	Transmission by semen is possible	Culture techniques
Brucellosis	*Brucella abortus*, *Brucella melitensis*	Orchitis, epididymitis, seminal vesiculitis, ampullitis, decreased libido, and infertility	Transmission by semen is possible	Serology (CFT or BUA), Specific skin test
Bovine Tuberculosis	*Mycobacterium bovis*, *Mycobacterium tuberculosis*	Coughing, lymph nodes develop	Transmission by semen is possible	Serology (ELISA), Specific skin test
Johne's disease	*Mycobacterium paratuberculosis*	Diarrhea, emaciation, and submandibular edema	Transmission by contaminated semen or semen from contaminated bulls has never been demonstrated	Serology (ELISA or CFT), Specific skin test
Leptospirosis	*Leptospira hardjo*	Fever, icterus, hemorrhages, uremia, and blood-tinged urine	Transmission by semen is possible	Culture techniques, PCR
Bovine genital campylobacteriosis	*Campylobacter fetus* ssp. *venerealis*	Infertility	Mating and/or semen are the main causes of transmission	Culture techniques
Chlamydiaceae	*Cp. abortus*, *Cp. pecorum*, *Cp. psittaci*	Polyarthritis, encephalitis, intestinal infection, endometritis, vaginitis	Transmission by semen is possible	Serology (CFT or ELISA), PCR
Query fever	*Coxiella burnetti*	Infertility	Transmission by semen is limited or unlikely	Serology (CFT or ELISA)
Haemorrhagic septicaemia	*Pasteurella multocida*	Fever	Transmission by semen is very unlikely	Culture techniques
Protozoon Diseases	**Etiology**	**Characteristic symptoms**	**Disease Risk via Semen**	**Diagnosis**
Bovine genital trichomoniasis	*Trichomonas fetus*	Infertility	Transmission by semen is possible	Culture techniques

Matters to be Considered in Semen Production Facilities and Quarantine Procedure: First of all, preventive measures should be broad and applicable. A high transmission ratio in AI stations will be reached when the number of influential contacts is loud and the length of the incubation term is short. For protective measures, only disease-free animals should be admitted, have optimal sanitation for humans entering the premises, do 48-hour storage periods or disinfection of materials before entry into the station, and have to use piped water only and vermin eradication. Entries and exits of the personnel working in the center should be kept under strict control (Villarroel et al., 2007). Working personnel should wear special clothes at the breeding sperm production site, while workers should wear overalls and boots. However, infectious particles carried by air or birds remain a risk factor. In this situation, only long distances (greater than 1 km) between the AI station and other cattle in the region would suffice (Wentink et al., 2000). Care should be taken to ensure that technically competent persons who can control the spread of any disease, properly trained in disinfection procedures and hygiene techniques, are assigned at the production facilities (Collins & Stabel, 2011). Besides, the breed, date of birth, pedigree information of each animal, all health checks and vaccinations in the center will be kept in the health file, and this information should be checked during the inspections by the veterinarian (Mathevon et al., 1998).

The tests for the existence of infectious agents in the sperm are based solely on the sensitivity of the test methods since they are based on a single investigation. Consecutive investigations are used to continuously monitor semen donors for infectious diseases before and after semen collection, increasing the reliability by the application using several tests. Checking for the donor's freedom from infectious diseases 28 days after semen collection is without a doubt the best strategy for ensuring the health protection of the sperm (Sanderson, 2009). In terms of the lengths of incubation and infectious times, the amount of virus excreted, and the number of contacts made, all infectious animals are treated the same (Hage et al., 1996). The next move is to reduce the adverse effects of unintended agent introduction into AI stations. Disease agents that may be transmitted by sperm must be tested regularly , and animals that test positive must be isolated immediately (Wentink et al., 2000). In an AI station,

however, this is unlikely to happen because the animals are quarantined for four weeks and serologically checked before entering the stud. If the disease is spread through aerogenic transmissions, such as from a neighboring farm, several bulls can be infected right away (Sibley, 2010). In this situation, the chances of the initial infection spreading by chance are smaller, and more animals would have displayed seroconversion at the following sampling, raising the chances of detection (Colenbrander et al., 1993). The results are highly reliant on seroconversion. Medical symptoms will be apparent in many epidemics, the virus will be detected sooner, and semen will be withheld from trading. Furthermore, when assessing the protection of imported sperm, the endemic situation should be taken into account.

Moreover, diseases with a high transmission rate within the bull herd and an increased risk of transmission through semen should be regularly checked to ensure healthy goods. The best option for this group will be to test the animals before releasing the sperm 28 days after production (Sanderson, 2009). Clinical and serological tests of the health status may be done before release by using this protocol. AI stations in areas where specific diseases are declared to be eradicated should be able to deliver sperm without having to test the bulls (Hage et al., 1996).

CONCLUSION

Most pathogens can cause a variety of diseases and can potentially be transmitted by sperm. Understanding the specific infectious, bacterial, protozoal, and parasitic pathogens contaminating bovine sperm can help advance animal husbandry worldwide. In bulls, infection is not accompanied by either pathological lesions or modifications in the characteristics of the semen. Therefore quarantine of bulls before herd entry and adequate diagnostic testing during quarantine are wide musts used to avoid pathogen entry when specific pathogens are a concern. Efficient control protocols for entry of new bulls into herds for natural breeding and the importation of helpful novel genetics by artificial insemination can be established due to a thorough study of the characteristics of diseases that may trigger disease transmission semen. Adhering to disease control recommendations provided by CSS and the OIE can prevent pathogen transmission via semen.

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ARAŞTIRMA MAKALESİ **RESEARCH PAPER**

Harşit Çayı (Giresun, Türkiye) Epilitik Alglerinin Mevsimsel Değişimi Üzerine Bir Araştırma[*]

Bengü TEMİZEL[1*] Elif Neyran SOYLU[2]
[1*] *Giresun University, Natural Sciences Institutes, Biology Division, Giresun, Turkey*
[2] *Giresun University, Science and Art Faculty, Biology Department, Giresun, Turkey*

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: https://orcid.org/0000-0002-5217-3013
: https://orcid.org/0000-0002-7583-3416

***Sorumlu yazarın:**
Bengü TEMİZEL
Giresun Üniversitesi, Fen Bilimleri Enstitüsü, Biyoloji Anabilim Dalı, Giresun, Türkiye
✉: bengu.temizel@giresun.edu.tr

Öz: Harşit Çayı'nın epilitik alg kompozisyonu ve mevsimsel değişimleri, Eylül 2018 ile Ağustos 2020 tarihleri arasında beş istasyondan toplanan taş örnekleri kullanılarak incelenmiştir. Belirlenen beş istasyondan aylık periyotlarla toplanan taş örneklerinin sayımı ve teşhisleri yapılmıştır. Böylece alglerin mevsimsel değişimleri belirlenmiştir. Harşit Çayı epilitik alglerinde Bacillariophyta (54 takson), Chlorophyta (7 takson), Cyanobacteria (4 takson), Charophyta (1 takson) ve Cryptophyta (1 takson) divisiosuna ait toplam 67 takson tespit edilmiştir. Ayrıca bu beş istasyondan alınan su örneklerinden sıcaklık, pH, elektriksel iletkenlik ve TÇMM (toplam çözünmüş madde miktarı)ölçümleri de aylık olarak ölçülmüştür. Tüm bu verilere göre Harşit Çayı'nın kirlenme riski taşıdığı görülmüştür.

Anahtar kelimeler: Epilitik algler, harşit çayı, flora, mevsimsel değişim, kirlilik.

A Study on the Seasonal Variation of Epilithic Algae of Harşit Stream (Giresun, Turkey)

***Corresponding author's:**
Bengü TEMİZEL
Giresun University, Natural Sciences Institutes, Biology Division, Giresun, Turkey ✉: bengu.temizel@giresun.edu.tr

Abstract: The composition and seasonal variations of the epilithic diatoms of Harşit Stream were studied using samples collected from five stations between September 2018 and August 2020. The counting and identification of the stone samples taken periodically from the five determined stations were made. Thereby, seasonal variations of the algae were determined. A total of 67 taxa were identified belonging to divisio of Bacillariophyta (54 taxa), Chlorophyta (7 taxa), Cyanobacteria (4 taxa), Charophyta (1 taxa) and Cryptophyta (1 taxa) in the epilithic algae of Harşit Stream. In addition, the amount of temperature, pH, electrical conductivity and TDS (total dissolved solids) measurements from water samples taken from these five stations were calculated monthly too. As a result, it has been seen that Harşit Stream is at risk of pollution.

Keywords: Epilitic algae, harşit stream, flora, seasonal variation, pollution.

GİRİŞ

Ülkemizdeki içsuların hidrobiyolojik özelliklerinin bilinmesi, bu su kaynaklarından yararlanmada ekolojik dengenin korunması açısından büyük önem taşımaktadır (Ertan vd, 1997). Algler, akarsularda besin zincirinin birincil üreticileridir. Sucul ortamın oksijen ihtiyacını karşılarlar ve besin kaynaklarını oluştururlar. Bu nedenle iç sularımızdaki algler ve bunları etkileyen faktörlerin iyi bilinmesi büyük önem taşımaktadır (Palmer, 1980).

Algler, sucul ortamda en yaygın bulunan canlı grubudurlar ve su içerisinde bentik (bağımlı) veya fitoplankton (serbest) olarak yaşamaktadır. Bağımlı algler bitki üzerinde (epifitik), hayvanların üzerinde (epizoik), taş ve sert yüzeyler üzerinde (epilitik), sediment ve kum üzerinde (epipelik) yaşamaktadırlar. Bentik algler akarsu ve göl ekosistemlerinin oldukça zengin tür çeşitliliğine sahip üyeleridir. Bentik algler su kirlilik derecelerinin belirlenmesinde indikatör olarak kullanılmaktadırlar. Round (1993)'a göre epilitik diyatomlar suyun kalitesinin belirlenmesinde ve su kalitesindeki değişimleri izlemede

[*]*Bu çalışma, doktora tezinden üretilmiştir.*

[*]*This study was produced from the doctorathesis.*

uzun vadede kullanılan önemli organizmalardır.

Akarsudaki besin zincirinin primer üreticileri ve ekosistemdeki değişiklikleri yansıtmada biyomonitör organizmaları olan algler, yapılarında bulundurdukları pigmentler sayesinde su ortamındaki besin değerinin ve çözünmüş oksijen oranının artmasını sağlarlar (Round, 1973). Algler, organik karbon bileşiklerinin temel üreticileridirler ve yaşamın temelini oluşturmaktadırlar (Durgut, 2017). Dünyadaki toplam karbon fiksasyonunda büyük bir öneme sahip olmalarının yanı sıra organik kirliliğin ve ötrofikasyonun biyoindikatörü olarak çok iyi sonuç verirler. Kirli suların temizlenmesinde süzgeç görevi görerek bir çeşit doğal arıtma yapmaktadırlar. Yapılan çalışmalar neticesinde alglerin özellikle ağır metal gideriminde de kullanıldıkları ve oldukça olumlu sonuçlar alındığı tespit edilmiştir (Herreroet et al., 2006; Perales-Velaet et al., 2006;Satohet al., 2005; Vijayaraghavanet al., 2005).

Besin değeri yüksek olan algler, sucul organizmalar için besin, vitamin ve iz elementlerin en önemli kaynağı olarak kabul edilirler. Alg grupları arasında önemli bir yere sahip olan diatomeler çok çeşitli limnolojik ve çevresel değişkenler ile değişen fiziksel, kimyasal ve biyolojik çevre şartlarına karşı oldukça duyarlıdırlar (Moser et al., 1996). Silisli hücre duvarları sayesinde, kolayca örneklenebilir ve korunabilirler. Bu sayede kısa veya uzun vadeli değişimi değerlendirmek için kullanılabilecek kalıcı bir kayıt sağlarlar (Smol,1992).

Bu çalışmanın amacı, Giresun il sınırları içerisinden Karadeniz'e dökülen Harşit Çayı'ndaki epilitik alg florası ve mevsimsel değişimini belirlemek suretiyle mevcut akarsuyun su kirlilik potansiyelini belirlemektir. Bu çalışma ile Harşit Çayı'ndaki biyoçeşitlilik belirlenerek Türkiye alg florasına katkı sağlanması amaçlanmıştır.

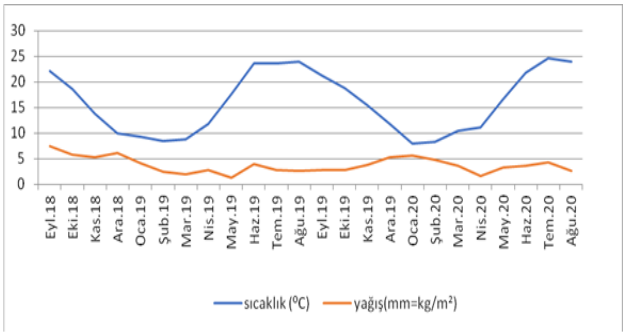
MATERYAL ve METOT

Çalışma Alanının Tanımı: Harşit Çayı, Gümüşhane il sınırlarındaki Vavuk Yaylası'ndan doğar, Günyüzü yakınlarında il topraklarına girer ve Tirebolu'nun doğusunda denize dökülür. İl sınırları içindeki uzunluğu 50 km olup toplamda 160 km ile ilin en uzun akarsuyu olma özelliğini taşımaktadır. Harşit Çayı üzerinde Doğankent I ve II hidroelektrik santralleri bulunmaktadır. Çayın debisi 232 m^3/sn'dir. Harşit Çayı 178 hm^3 yıllık tatlı su potansiyeline sahip olup il için en önemli tatlı su kaynaklarındandır. Bu çalışmada Harşit Çayı'nın Karadeniz'e deşarj olduğu noktadan, il sınırından çıkış yaptığı Günyüzü mevkiine kadar, 5 farklı noktadan taş ve su örnekleri toplama işlemi gerçekleştirilmiştir (Şekil 1).

Şekil1. Çalışma alanı
Figure 1. Study area

Çalışma Alanının İklimi: Giresun, ülkemizin en çok yağış alan bölgesi olan Doğu Karadeniz Bölgesi'nde yer almaktadır. Yıl genelinde ılık ve yağışlı iklim özellikleri gözlenir. Genel olarak en fazla yağış sonbaharda, en az yağış ilkbaharda görülür.

Giresun ili Meteoroloji Müdürlüğü'nden alınan verilere göre hazırlanan Eylül 2018- Ağustos 2020 tarihleri arasındaki Giresun ili sıcaklık-yağış grafiği Şekil 2' de görülmektedir.



Şekil 2. Giresun ili sıcaklık-yağış grafiği.
Figure 2. Temperature-precipitation graph of Giresun Province.

Örnek Toplama İstasyonları: Harşit Çayı'nın epilitik alg florası ile mevsimsel değişimi incelemek amacıyla 5 istasyon belirlenmiştir. İstasyonlara ait bilgiler Tablo 1'deki gibidir.

Suyun Fiziksel Değişkenlerin Tespiti: Harşit Çayı'nda belirlenen 5 istasyondan Eylül 2018- Ağustos 2020 tarihleri arasında alınan su örneklerinin bazı fiziksel özellikleri (sıcaklık, iletkenlik, çözünmüş oksijen, pH, TÇMM) yüzey suyu örneklerinde, örnek alma sırasında YSI 556 MPS cihazı kullanılarak yapılan analizlerle tespit edilmiştir (Tablo 2).

İstatistiksel Analiz: Harşit Çayı yüzey suyu örneklerinin toplandığı İstasyonlar arasındaki farklılıkların tespiti amacıyla Varyans analizi, One Way ANOVA yapılarak, Tukey çoklu karşılaştırma testi kullanılmıştır.

Tablo 1. Örnek toplama istasyonlarına ait özellikler.
Table 1. Features of sample collection stations.

İstasyon	Enlem-Boylam	Özellik
1	41°0'33.01"K 38°50'41.92"D	Karadeniz'e deşarj noktasını kapsamakta olup, dip yapısı çamurludur. Evsel, küçük çaplı sanayi ve tarımsal aktivite atıklarının yoğun etki ettiği lokasyonu içermektedir.
2	40°53'50.97"K 38°51'10.37"D	Dip yapısı çamur ve az miktarda küçük çakıldır. Dere yatağı yakınındaki yöre halkınca evsel çöp atıkları bırakılmakta, ayrıca istasyon yakınında kum ve çakıl işletmeleri bulunmaktadır.
3	40°49'34.89"K 38°54'24.40"D	Dip yapısı kum ve küçük çakıllardan oluşmaktadır.
4	40°46'47.41"K 38°56'5.53"D	Dip yapısı taşlıdır.
5	40°45'50.92"K 38°57'34.75"D	Dip yapısı taşlıdır. Harşit Çayı'nın Giresun il sınırları içerisine giriş yaptığı Günyüzü mevkii yakınlarını kapsamaktadır.

Tablo 2. YSI 556 MPS özellikleri (Akkan, 2013).
Table 2. Specification of YSI 556 MPS.

	Değişken	Sensör Tipi	Aralık	Hassasiyet
YSI 556 MPS	Çözünmüş Oksijen (ÇO)	Polarografik	0 ile 500 (%) hava doygunluğu 0 ile 50 mg/L	%0,1 hava doygunluğu 0,01 mg/L
	Sıcaklık	Termistör	-5 ile 45 °C	0,1 °C
	İletkenlik	4 Elektrot hücresi	0 ile 200 mS/cm	0,001 ile 0,1 mS/cm
	pH	Cam elektrot	0 ile 14	0,01
	Toplam Çözünmüş Madde Miktarı (TÇMM)	İletkenlik ve sıcaklığa göre hesaplama	0 ile 100 g/L	4 Dijit

Algolojik Özellikler

Epilitik Alglerin Toplanması ve İncelenmesi: Harşit Çayı'ndaki epilitik alglerin incelenmesi için taş örnekleri Eylül 2018- Ağustos 2020 tarihleri arasında belirlenen 5 istasyondan aylık periyotlarla alınmıştır. Örnek alınan tüm aylarda taşların eşit sayıda toplanmasına ve dış yüzeylerinin kaygan olmasına dikkat edilmiştir. Taşların üzerindeki algler arazi sırasında fırça yardımıyla kazınarak 100 ml'lik plastik kaplara toplanmıştır. Daha sonra toplanan bu örnekler lugol ve % 4'lük formaldehit ile fikse edilmiştir. Labarotuvara getirilen su örneklerin teşhisi için geçici ve kalıcı preparatlar hazırlanmıştır. Diyatome haricindeki alg türlerinin geçici preparatlarda ön sayımları yapılmıştır. Geçici preparat içindeki türün teşhisi için OLYMPUS BX51 marka mikroskop kullanılmıştır. Kalıcı preparatların hazırlanmasında Round (1973)'un metodu kullanılmıştır. Diyatomelerin teşhisinde kullanılan rafe ve stria gibi yapıların net olarak incelenebilmesi için asit ile kaynatma metodu kullanılmıştır. Bu yöntemde örnekler önce 1:1 oranında H_2SO_4 ve HNO_3 karışımında çekerocakta 120°C' de 15 dakika kaynatılmıştır. Kaynatılan örneklere 7 gün boyunca saf su ile seyreltme işlemi uygulanmıştır. Daha sonra örnekler entellan kullanılarak daimi preparat haline getirilmiştir. Sayımlarda, her istasyon için üç sayımın ortalaması alınmıştır (Sladeckova, 1962). Alglerin incelenmesi ve sayımı için x40, x100 büyütmeli OLYMPUS BX51 marka mikroskop kullanılmış ve mikrometrik oküler yardımıyla ebatları belirlenmiştir.

Harşit Çayı'nda bulunan alglerin teşhisi için; Krammer ve Lange-Bertalot, 1991a, Krammer ve Lange-Bertalot, 1991b, Krammer ve Lange-Bertalot, 1999a ve Krammer, Lange-Bertalot, 1999b ve John et. al. (2003) eserlerinden yararlanılmıştır**.** Ayrıca teşhis edilen türlerinsinonim durumları ve kategorileri, Algaebase veri tabanından (Guiry and Guiry, 2021) kontrol edilerek sınıflandırılmıştır.

BULGULAR

Harşit Çayı epilitik alg florasını belirlemek amacıyla belirlenen beş istasyondan Eylül 2018-Ağustos 2020 tarihleri arasında aylık periyotlarla taş örnekleri alınmıştır. Harşit Çayı epilitik alglerinin teşhis ve sayımları yapılmış, tespit edilen taksonların mevsimsel değişimleri incelenmiştir. Araştırma alanında epilitik alg florasına ait toplam 67 takson belirlenmiştir. Baskın divisio Bacillariophyta olarak belirlenmiş; *Asterionella formosa*, *Cymbella helvetica* ve *Ulnaria ulna* türleri sık bulunan taksonlar olmuşlardır. Tespit edilen taksonlar Tablo 3'de verilmiştir.

Harşit Çayı'nda alınan su örnekleri üzerinde de bazı fiziksel ölçümler yapılmıştır. Bu fiziksel analizlerin sonuçları Tablo 4'de gösterilmiştir.

Harşit Çayı'nda alınan su örneklerinde yapılan fiziksel ölçümler sonucunda; çalışmalar boyunca su sıcaklığı en düşük Ocak 2019 tarihinde 5. istasyonda 6,35 °C olarak ölçülmüştür. En yüksek sıcaklık ise Ağustos 2019 tarihinde 4. istasyonda 27,49 °C'dir. Suyun sıcaklığı arttıkça taşıdığı oksijen miktarı azalmakta ve bu durum o suda yaşayan canlıları olumsuz etkilemektedir (DSİ, 2014). Harşit Çayı'nda yapılan çalışmalar boyunca çözünmüş oksijen değeri en düşük Eylül 2019 tarihinde 4. istasyonda 7,06 mg/L, en yüksek çözünmüş oksijen değeri ise Aralık 2019 tarihinde 5. istasyonda 12,79 mg/L olarak ölçülmüştür.

Harşit Çayı'nda en düşük pH değeri Mart 2020 tarihinde 4. istasyonda 7,26 olarak ölçülmüştür. En yüksek pH değeri ise Temmuz 2020 tarihinde 4. istasyonda 8,24' dür. Harşit Çayı'nda elde edilen verilere göre en düşük elektriksel iletkenlik değeri Ekim 2019 tarihinde 3. İstasyonda 274µS/cm, en yüksek de Ağustos 2019 tarihinde 4. istasyonda 498µS/cm olarak ölçülmüştür. Harşit Çayı'nda çalışma süresince elde edilen verilere göre en düşük TÇMM değeri Aralık 2018 tarihinde 2. istasyonda 170mg/L, en yüksek değer de Ağustos 2019 tarihinde 4. istasyonda 631mg/L olarak ölçülmüştür. Ayrıca, Harşit Çayı yüzey suyu örneklerinin fiziksel değişkenlerinin istasyonlara göre değişiminde istatistiksel olarak anlamlı bir fark görülememiştir ($p>0,05$).

Tablo 3. Harşit Çayı'nda Tespit Edilen Epilitik Algler
Table 3. The list of epilitlic diatoms in Harşit Stream

Habitat	Epilitik				
İstasyon	1.	2.	3.	4.	5.
Alg Florası					
Divisio: Bacillariophyta					
Classis: Bacillariophyceae					
Ordo: Thalassiophysales					
Familya: Catenulaceae					
Amphora ovalis (Kützing) Kützing	+	+	+	+	+
Ordo: Tabellariales					
Familya: Tabellariaceae					
Asterionella formosa Hassall	+	+	+	+	+
Diatoma moniliformis (Kützing) D.M.Williams	+	+	+	+	
Diatoma tenuis C.Agardh			+	+	
Diatoma vulgaris Bory		+	+	+	
Meridion circulare (Greville) C.Agardh				+	+
Odontidium hyemale (Roth) Kützing		+	+		
Odontidium mesodon (Ehrenberg) Kützing		+			
Ordo: Cocconeidales					
Familya: Cocconeidaceae					
Cocconeis pediculus Ehrenberg	+	+	+	+	+
Cocconeis placentula Ehrenberg	+	+	+	+	
Ordo: Fragilariales					
Familya: Fragilariaceae					
Fragilaria capucina Desmazières		+	+	+	
Fragilaria crotonensis Kitton	+	+			
Fragilaria tenera var. *nanana* (Lange-Bertalot) Lange-Bertalot&S.Ulrich	+	+		+	
Ordo: Naviculales					
Familya: Naviculaceae					
Navicula cryptocephala Kützing		+	+	+	
Navicula gregaria Donkin	+	+			
Navicula radiosa Kützing		+	+		
Navicula rhynchocephala Kützing			+	+	
Navicula veneta Kützing		+	+	+	+
Familya: Diploneidaceae					
Diploneis ovalis (Hilse) Cleve		+	+		
Familya: Pinnulariaceae					
Pinnularia borealis Ehreneberg			+	+	
Pinnularia intermedia (Lagerstedt) Cleve		+	+		
Pinnularia viridis (Nitzsch) Ehrenberg			+	+	
Ordo: Cymbellales					
Familya: Cymbellaceae					
Cymbella affinis Kützing		+	+	+	+
Cymbella aspera (Ehrenberg) Cleve		+	+		
Cymbella cistula (Ehrenberg) O.Kirchner			+	+	
Cymbella cymbiformis C.Agardh	+	+	+	+	
Cymbella helvetica Kützing	+	+	+	+	+
Cymbella lanceolata C.Agardh		+	+		
Cymbella proxima Reimer			+	+	
Cymbella tumida (Brébisson) Van Heurck	+	+	+	+	
Didymosphenia geminata (Lyngbye) Mart.Schmidt in A.W.F.Schmidt	+	+	+	+	
Ordo: Rhopalodiales					
Familya: Rhopalodiaceae					
Epithemia adnata (Kützing) Brébisson	+	+	+		
Epithemia sorex Kützing		+		+	
Familya: Rhoicospheniaceae					
Rhoicosphenia abbreviata (C.Agardh) Lange-Bertalot			+	+	
Familya: Gomphonemataceae					
Encyonema silesiacum (Bleisch) D.G.Mann		+		+	
Encyonema ventricosum (C. Agardh) Grunow		+			
Gomphonella olivacea (Hornemann) Rabenhorst		+	+		
Gomphonema angustum C.Agardh	+	+	+	+	+
Gomphonema gracile Ehrenberg		+	+	+	
Gomphonema parvulum (Kützing) Kützing	+	+	+	+	+
Gomphenema truncatum Ehrenberg	+	+	+	+	+
Reimeria sinuata (W.Gregory) Kociolek&Stoermer	+	+		+	
Ordo: Melosirales					
Familya: Melosiaceae					
Melosira varians C. Agardh		+	+	+	+

Habitat	Epilitik				
İstasyon	1.	2.	3.	4.	5.
Alg Florası					
Ordo: Bacillariales					
Familya: Bacillariaceae					
Hantzschia amphioxys (Ehrenberg) Grunow	+		+	+	
Nitzschia acicularis (Kützing) W. Smith	+	+			
Nitzschia linearis W.Smith			+	+	
Nitzschia palea (Kützing) W. Smith	+	+	+	+	+
Ordo: Stephanodiscales					
Familya: Stephanodiscaceae					
Cyclotella meneghiniana Kützing		+	+		
Pantocsekiella ocellata(Pantocsek) K.T.Kiss&Ács	+	+	+	+	+
Ordo: Surirellales					
Familya: Surirellaceae					
Cymatopleura elliptica (Brébisson) W.Smith			+		
Surirella angusta Kützing		+			
Surirella librile (Ehrenberg) Ehrenberg	+	+	+	+	
Surirella minuta BrébissonexKützing		+		+	+
Ordo: Licmophorales					
Familya: Ulnariaceae					
Ulnaria ulna (Nitzsch) Compère	+	+	+	+	+
Divisio: Chlorophyta					
Classis: Trebouxiophyceae					
Ordo: Trebouxiales					
Familya: Botryococcaceae					
Botryococcus braunii Kützing	+	+	+	+	+
Ordo: Sphaeropleales					
Familya: Selenastraceae					
Ankistrodesmus falcatus (Corda) Ralfs		+	+		
Ankistrodesmus spiralis (W.B.Turner) Lemmermann		+	+	+	+
Familya: Scenedesmaceae					
Desmodesmus abundans (Kirchner) E.H. Hegewald			+	+	
Classis: Chlorophyceae					
Ordo: Chlamydomonadales					
Familya: Chlamydomonadaceae					
Chlamydomonas globosa J.W.Snow		+	+	+	
Classis: Chlorellales					
Ordo: Chlorellales					
Familya: Oocystaceae					
Oocystis pusilla Hansgirg		+		+	
Classis: Ulvophyceae					
Ordo: Ulotrichales					
Familya: Ulotrichaceae					
Ulothrix tenuissima Kützing	+	+			
Divisio: Cyanobacteria					
Classis: Cyanophyceae					
Ordo: Chroococcales					
Familya: Chroococcaceae					
Chroococcus minor (Kützing) Nägeli			+	+	
Chroococcus turgidus (Kützing) Nägeli		+	+		
Familya: Microcystaceae					
Microcystis aeruginosa (Kützing) Kützing	+	+	+		
Ordo: Oscillatoriales					
Familya: Oscillatoriaceae					
Oscillatoria limosa C. Agardhex Gomont	+	+	+		
Divisio: Charophyta					
Classis: Zygnematophyceae					
Ordo: Desmidiales					
Familya: Closteriaceae					
Closterium moniliferum Ehrenbergex Ralfs			+		+
Divisio: Cryptophyta					
Classis: Cryptophceae					
Ordo: Cryptomonadales					
Familya: Cryptomonadaceae					
Cryptomonas ovata Ehrenberg	+	+		+	+

Tablo.4.Yüzey suyu örneklerinin fiziksel değişimleri
Table.4. Physical properties of surface water samples

Parametreler	1.İstasyon	2. İstasyon	3. İstasyon	4. İstasyon	5. İstasyon	Total
			Ort. (±SH) Min.-Max.			
Su Sıcaklığı (ºC)	13,88 (±1,401) 6,41-26,03	14,14 (±1,369) 6,71-26,12	14,17 (±1,397) 6,75-26,72	14,15 (±1,480) 6,73-27,49	14,22 (±1,490) 6,35-27,38	14,11 (±0,628) 6,35-27,49
pH	7,61 (±0,044) 7,36-8,12	7,68 (±0,044) 7,49-8,14	7,73 (±0,048) 7,42-8,17	7,70 (±0,049) 7,26-8,24	7,66 (±0,052) 7,33-8,2	7,68 (±0,021) 7,26-8,24
Çözünmüş Oksijen (mg/L)	9,23 (±0,282) 7,16-77,98	9,19 (±0,284) 7,34-12,22	9,55 (±0,332) 7,11-12,36	9,39 (±0,364) 7,06-12,45	9,38 (±0,372) 7,12-12,79	9,34 (±0,145) 7,06-12,79
Elektriksel İletkenlik (µS/cm)	380,20 (±11,527) 275-489	392,04 (±9,976) 286-445	376,08 ±12,014) 274-487	394,75 (±11,033) 289-498	395,83 (±10,492) 289-479	387,78 (±4,906) 274-498
TÇMM(mg/L)	305,04 (±32,578) 171-614	306,43 (±33,876) 170-621	310,26 (±33,304) 171-609	309,56 (±33,218) 172-631	310,91 (±33,221) 173-623	304,34(±14,616) 170-631

TARTIŞMA VE SONUÇ

Harşit Çayı epilitik alg florasını belirlemek amacıyla Eylül 2018-Ağustos 2020 tarihleri arasında belirlenen beş istasyondan aylık periyotlarla taş örnekleri alınarak epilitik algler incelenmiştir. Epilitik alg florasında Bacillariophyta divizyosu üyeleri tür sayısı ve yoğunluğu bakımından baskındır. Harşit Çayı epilitik florasında Bacillariophyta (54 takson), Chlorophyta (7 takson), Cyanobacteria (4 takson), Charophyta (1 takson), Cryptophyta (1 takson) divizyolarına ait toplam 67 takson tespit edilmiştir. Bacillariophyta'ya ait *Asterionella formosa*, *Cocconeis pediculus*, *Cymbella helvetica* ve *Ulnaria ulna* türleri tüm istasyonlarda çalışma süresi boyunca devamlı mevcut olarak bulunmuşlardır.

Tüm istasyonlarda yüksek sayılarda görülen *Ulnaria ulna* sudaki kirliliğe karşı hem hassas hem de toleranslı bir türdür. 2019'da da 2020'de de özellikle 1. istasyonda ev atık sularından kirlendiği yaz aylarında daha yoğun olduğu gözlemlenmiştir. Bu da denize yakın konumdaki 1. istasyonun belirli zamanlarda kirlilik tehdidi altında olduğunu göstermektedir.

Asterionella formosa özellikle sonbahar ve kış aylarında daha yoğun olmakla birlikte, her mevsim tüm istasyonlarda yaygın olarak görülmüştür. Kozmopolit bir yayılış gösteren ve ötrofik göllerde yoğun olarak bulunan *Asterionella formosa* (Husted, 1985), ilkbahar ve sonbaharda düzenli artışlar göstermektedir (Lee, 1999). Yaz mevsimlerinde bolluğu azalan bu türün, 0,5-24 °C arasında en iyi gelişim gösterdiği bildirilmiştir (Goldman & Horne, 1983). Yaptığımız çalışmada da *Asterionella formosa* su sıcaklığının düşük olduğu kış aylarında ortaya çıktığı ancak aşırı yüksek sayılara ulaşamadığı gözlenmiştir. Reynolds vd. (2002) tarafından ötrofik sularda bulunduğu bildirilen *Asterionella formosa'*nın ayrıca sularda fosfor miktarından etkilendiği de belirtilmiştir (Akçaalan vd. 2007; Rott ve ark., 1999). Benzer şekilde Harşit Çayı'nın farklı noktalarında yıl boyunca toplanan su örneklerinde anyonik yüzey aktif madde kirliliğinin tespiti literatür ve araştırma bulgularımızı destekler niteliktedir (Akkan, 2017).

Çalışma boyunca devamlı görülen *Cymbella helvetica*'nın akarsuların oligotrofik-ötrofik bölgelerine uyumlu, yüksek elektriksel iletkenliğe sahip olan sularda sıkça görülen, sudaki kirliliğe hassas ve yaygın bir tür olduğu belirtilmiştir (Cox 1996).

Alkalinite diyatomeler için sınırlayıcı etki gösterebilmektedir. Harşit Çayı pH değerleri 7,26 ve 8,24 arasında değişmektedir. Ölçülen bu değerler derenin hafif alkali özellikte olduğunu göstermektedir. İstasyonlara göre pH değerleri ortalaması ilk istasyondan itibaren sırasıyla 7,61, 7,68, 7,73, 7,70 ve 7,66 olarak belirlenmiştir. Herhangi bir şekilde kirletilmemiş olan göl sularında pH değerinin 6-9 arasında değiştiği belirtilmiştir (Tanyolaç, 2000). Karadeniz Bölgesi'nde araştırılan göl ve akarsularda ölçülen pH değerleri, sularımızın genel olarak hafif alkali özellikte olduğunu göstermektedir (Yazıcı ve Gönülol,1994; Maraşlıoğlu vd., 2005; Soylu, 2011; Altürk, 2015; Memiş, 2019). Suyun elektriksel iletkenliği, sudaki iyonların derişimine ve sıcaklığa bağlı olarak değişmektedir. Sudaki iyonların derişimi ve sıcaklık arttıkça elektriksel iletkenlik de artar (MEB, 2011). Harşit Çayı'nda yapılan bu çalışmada elde edilen ölçüm verileri bu durumu desteklemektedir. Harşit Çayı'nda elde edilen verilere göre en düşük elektriksel iletkenlik değeri Ekim 2019 tarihinde 3. istasyonda 274µS/cm, en yüksek de Ağustos 2019 tarihinde 4. istasyonda 498µS/cm olarak ölçülmüştür. Dahası, akarsu hattı boyunca var olan çok sayıdaki kum ve çakıl tesisleri nedeniyle de suyun çoğunlukla bulanık aktığı gözlemlenmiştir. Ayrıca, Harşit Çayı üzerine kurulmuş HES'lerin su tutup bırakmaları, Harşit Çayı'na akan kanalizasyon suları ve evsel atıklar, 1. istasyon civarındaki hayvan kesim alanı, tarımsal gübre faaliyetleri gibi nedenlerle Harşit Çayı'nın kirlenme tehdidi ile karşı karşıya olduğu görülmüştür. Nitekim Harşit Çayı'ndan toplanan su numunelerinde ağır metal ve deterjan kirliliğinin tespit edildiği çalışmalar da bu durumu desteklemektedir (Akkan, 2017; Mutlu vd., 2018).

Sonuç olarak bu çalışma ile Harşit Çayı'nda bulunan epilitik alg taksonları ve bu taksonların deredeki dağılımları ile bu dağılımı etkileyen suyun bazı fiziksel özellikleri belirlenmiştir. Ölçülen pH değerlerine göre hafif alkali olan Harşit Çayı'nda sık görülen türlerin de kirliliğe toleranslı ve az da olsa kirli sularda yaşayan türler olduğu tespit edilmiştir. Bir bütün olarak ele alındığında Giresun ili ve ülkemiz için son derece önemli olan Harşit Çayı'nın algal olarak da kısmi düzeyde kirlenme tehlikesi altında olduğu görülmekle birlikte; ileriye yönelik koruma ve yönetme çalışmalarının en hızlı şekilde planlanıp hayata geçirilmesi gerekmektedir.

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ARAŞTIRMA MAKALESİ **RESEARCH PAPER**

Metagenomic Characterization of Planktonic Communities During a Mucilage Event in the Çanakkale Strait (Dardanelles), Turkey

Sevdan YILMAZ[1]* Mehmet Ali KUCUKER[2] Dilek KAHRAMAN YILMAZ[3]

[1*] *Department of Aquaculture, Faculty of Marine Sciences and Technology, Çanakkale Onsekiz Mart University, Çanakkale, Turkey*
[2] *İzmir Institute of Technology, Faculty of Engineering, Department of Environmental Engineering, Gülbahçe Campus, İzmir, Turkey*
[3] *Department of Marine Biology, Faculty of Marine Sciences and Technology, Çnakkale Onsekiz Mart University, Çanakkale, Turkey*

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*: https://orcid.org/0000-0002-4809-5809
: https://orcid.org/0000-0001-9648-8925
: https://orcid.org/0000-0002-9626-5446

***Corresponding author's:**
Sevdan YILMAZ
Çanakkale Onsekiz Mart University
Faculty of Marine Sciences and Technology
Çanakkale, Turkey
✉: sevdanyilmaz@comu.edu.tr

Abstract: The present study investigates the planktonic communities through metagenomics sequencing during a mucilage event in the Çanakkale Strait (Dardanelles), Turkey. Mucilage samples were collected in May 2021 during an intense period of mucilage formation in three different stations of Dardanelles (Station 1: 40°9'8.09"N, 26°24'16.19"E; Station 2: 40°6'21.62"N, 26°22'41.25"E; Station 3: 40°6'42.78"N, 26°23'57.00"E). The dominant planktonic eukaryotes, at the phylum level, were Dinoflagellata (38.57%), Protalveolata (15.03%), Diatomea (12.41%), Nematozoa (8.44%), Apicomplexa (6.79%) and Chlorophyta (5.43%), which constituted 86.68 % of the total number of sequences. The most dominant OTUs (>10%), were *Alexandrium* and Syndiniales Group II. other dominant OTUs (>2%) were *Viscosia* sp., *Lankesteria*, *Arcocellulus*, *Thalassiosira* and *Nannochloris*. This study has clarified the situation of planktonic communities during a mucilage event in the Çanakkale Strait (Dardanelles), Turkey. As a result, the most dominant genus was *Alexandrium*, which has been known to produce mucilage. Some *Alexandrium* species can produce toxins, cause severe impacts on human health, and lead to bivalve, shrimp, and fish mortality. Therefore, a more detailed study is needed to determine the *Alexandrium* toxins in the mucilage structure. In addition, the heavy metal content of the obtained mucilage was investigated, and the concentrations of the As and Cr are above the disposal limits in the landfill sites. Therefore, collected mucilage from the sea surface should be checked before sending it to landfill sites in terms of the heavy metal content.

Keywords: Çanakkale strait (dardanelles), eukaryotic biodiversity, heavy metal, metagenomic sequencing, mucilage.

Çanakkale Boğazı'nda (Dardanel) Müsilaj Oluşumu Sırasında Planktonik Toplulukların Metagenomik Karakterizasyonu, Türkiye

***Sorumlu yazar:**
Sevdan YILMAZ
Çanakkale Onsekiz Mart Üniversitesi
Deniz Bilimleri ve Teknolojisi Fakültesi
Çanakkale, Türkiye
✉: sevdanyilmaz@comu.edu.tr

Öz: Bu çalışmada Çanakkale Boğazı'ında (Dardanel) müsilaj oluşumu sırasında planktonik toplulukların kompozisyonları metagenomik yaklaşımla araştırılmıştır. Müsilaj örnekleri, Çanakkale Boğazı'nın üç farklı istasyonunda (İstasyon 1: 40°9'8.09"K, 26°24'16.19"D; İstasyon 2: 40°6'21.62"K, 26°22'41.25"D; İstasyon 3: 40°6'42.78"K, 26°23'57.00"D) yoğun müsilaj oluşumu gözlemlenen Mayıs 2021'de toplanmıştır. Filum düzeyinde toplam sekans sayısının 86.68% ini kapsayan baskın planktonik ökaryotlar, Dinoflagellata (%38,57), Protalveolata (%15,03), Diatomea (%12,41), Nematozoa (%8,44), Apicomplexa (%6,79) ve Chlorophyta (%5,43) olarak belirlenmiştir. En baskın OTU'ler (>%10), *Alexandrium* ve Syndiniales Group II olup, diğer baskın OTU'lar (>%2) ise *Viscosia* sp., *Lankesteria*, *Arcocellulus*, *Thalassiosira* ve *Nannochloris* olmuştur. Bu çalışma, Türkiye'de meydana gelen bir müsilaj olayı sırasında Çanakkale Boğazı'nda planktonik toplulukların durumunu netleştirmiştir. Sonuç olarak, en baskın cinsin müsilaj üretme kabiliyeti olduğu bilinen *Alexandrium* olduğu belirlenmiştir. Bazı *Alexandrium* türleri toksin üretebilir, insan sağlığı üzerinde ciddi etkilere neden olabilir ve çift kabuklu, karides ve balık ölümlerine yol açabilir. Bu nedenle müsilaj yapısındaki *Alexandrium* toksinlerinin belirlenmesi için daha detaylı bir çalışmaya ihtiyaç vardır. Ayrıca elde edilen müsilajın ağır metal içeriği araştırılmıştır. Müsilajın yapısındaki As ve Cr konsantrasyonları atıklar için belirlenen düzenli depolama sahalarına bertaraf etme sınırlarının üzerindedir. Bu nedenle deniz yüzeyinden toplanan müsilajın düzenli depolama sahalarına gönderilmeden önce ağır metal içeriği kontrol edilmelidir.

Anahtar kelimeler: Ağır metal, çanakkale boğazı (çanakkale), metagenomik dizileme, müsilaj, ökaryotik biyoçeşitlilik.

INTRODUCTION

The Dardanelles (Çanakkale Strait), a strait that separates Asian and European continents and connects the Aegean Sea and Sea of Marmara, is a significant watercourse for the international vessels. There are two major currents in the strait. The first one is a surface current flowing from the Black Sea to the Sea of Marmara through the Bosphorus and the Agean Sea through the Dardanelles. The second one is the undercurrent which flows from the Agean Sea to the Black Sea (Artüz et al., 2007; Yücel & Tarhan 2019). The pollution load of the Sea of Marmara increased incrementally, thus affecting the Dardanelles. The growth of population and industrialization on the seaside, insufficient treatment of domestic and industrial wastewater (Artüz et al., 2007; Okus et al., 2008), climate change, and global warming (Balkis et al., 2011) led to severe biologic problems in the marine environment. Consequently, intense mucilage has been found in Turkish seas in recent years for the first time. The mucilage problem, which was recorded in the Sea of Marmara (Aktan et al., 2008; Tüfekçi et al., 2010; Balkis et al., 2011) and the Dardanelles (Yentür et al., 2013) between 2007-2008 for the first time, reached a critical level to affect fishery, tourism, and social life in the first two quarters of 2021.

It has been reported that mucilage remarkably decreased the density of zooplankton groups, which are critical nutritional sources for larvae of marine species both in the Bosphorus (Okyar et al., 2015) and the Dardanelles (Yentür et al., 2013). Besides, research conducted between December 2020 and March 2021 reported the impacts of mucilage on endangered coral *Cladocora caespitosa*, which are essential for the Dardanelles and coral-rich habitats in Eceabat and Nara regions, which result in coral mortalities (Özalp, 2021).

It was reported that *Chrysophaeum taylorii*, which spreads through tropical and subtropical Atlantic and Western Pacific shores and produces mucilage in benthic areas, produced mucilage for the first time in the benthic area in the Aegean Sea in May 2011 (Aktan & Topaloğlu 2011). In another study conducted on the shores of Büyükada Island (the Marmara Sea) in 2008, it was detected that *Clindrotheca closterium, Pseudo-nitzschia sp., Skeletonema costatum, Thalassiosira rotula,* and *Gonyaulax fragilis* species were the dominant species in the mucilage structure (Balkis et al., 2011). In June 2007, when mucilage structure is observed in the north-eastern Sea of Marmara, it was observed that *Gonyaulax hyalina* and *Thalassiosira gravida* species, which are known to cause mucilage, were dominant (Tas et al., 2020). Moreover, the research conducted in the Sea of Marmara and the Gulf of İzmit between October 2007 and February 2008 reported that *Gonyaulax fragilis, Skeletonema costatum* and *Cylindrotheca closterium* were the dominant species of the mucilage structure (Tüfekçi et al., 2010). Yet information on the diversity of planktonic communities in the mucilage structure from the Sea of Marmara ecosystem is limited only to microscopic descriptions.

This study researches metagenome analysis of the mucilage structure reaching a critical level in the Dardanelles in the first two quarters of 2021, distribution of planktonic communities, and heavy metal content of the mucilage.

MATERIALS AND METHODS

Samples and Heavy Metal Analysis: Sampling was performed as part of three nearly simultaneous expeditions to different regions of the Dardanelles (Station 1: 40°9'8.09"N, 26°24'16.19"E; Station 2: 40°6'21.62"N, 26°22'41.25"E; Station 3: 40°6'42.78"N, 26°23'57.00"E) in May 2021. Sampling points in the Dardanelles is shown in Figure 1. Samples were collected using a 5L Niskin bottle at the maximum depth of 2 m according to the ISO 5667-9 method for the analyses by means of the Turkish Coast Guard ship in Çanakkale (ISO 5667-9, 1992). The mucilage samples were concentrated after centrifuging (Hettich, Rotofix 32A, Germany) at 6000 rpm for 10 min and then filtered through a pre-weighed glass fiber filter (Merck Millipore, AP40, Germany). The filter was heated to constant mass at 70 ± 1º C for 24 h and then weighed to determine the dry solid. The filters and a blank filter with 9 ml HNO_3 and 1 ml of H_2O_2 were digested in pressurized digestion vessels using a microwave oven (CEM, MARS-6, USA). The temperature was ramped to 210 °C within 15 minutes and held for 20 minutes at a constant microwave digestion temperature. Digested samples were diluted after cooling and centrifuging for soluble metal analysis. Soluble heavy metals in the diluted samples were analyzed by an inductively coupled plasma-optical emission spectrometry (ICP-OES / Agilent 5110 Dual View, USA).

DNA Extraction, PCR Amplification and Bioinformatics Analysis: Total DNA was extracted from the mucilage samples using GeneMATRIX Kit (EURx Poland) according to the manufacturer's instructions. The eukaryote nuclear 18S rRNA gene was amplified by PCR, using the eukaryotic-specific primers 18S-566F CAGCAGCCGCGGTAATTCC and 18S-1200R CCCGTGTTGAGTCAAATTAAGC (Hadziavdic et al., 2014).

Figure 1. Sampling points in the Dardanelles (Google Earth Map).

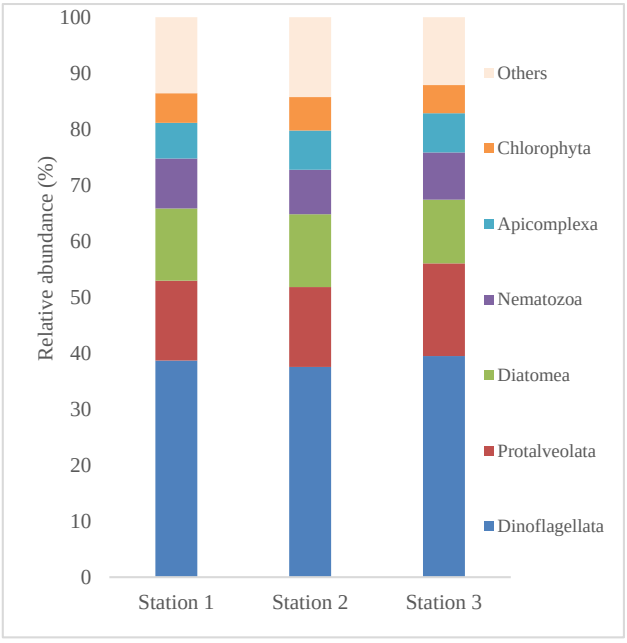

Figure 2. Planktonic community compositions during the mucilage event.

The first PCR amplification was performed in 25 µL volume, containing 10 µL of 2X KAPA HotStart ReadyMix (Roche, Switzerland), 5 µL of each primer (1 µM), and 2.5 µL of template DNA. Thermal cycling conditions were: 95 °C for 3 min followed by 25 cycles of 95 °C for the 30s, 55 °C for 30 s, 72 °C for 30 s, and a 5 min extension at 72 °C and a final hold at 4 °C. The second PCR amplification was performed in 50 µL volume, and reactions contained 25 µL of KAPA HiFi HotStart ReadyMix, 5 µL Nextera XT1 (N7xx), 5 µL Nextera XT2 (S5xx), 5 µL of cleaned PCR product and 10 µl PCR Grade water. The second thermocycling conditions were: 95°C for 3 min followed by 8 cycles of 95°C for 30 s, 55°C for 30 s, 72°C for 30 s, and a 5 min extension at 72°C and a final hold at 4°C. The sequencing (2×250 bp) was performed on the MiSeq platform. The processing and quality control was conducted using DADA2 (Callahan et al., 2016). Chimera check was conducted with DADA2. Amplicons with a quality score of more than 20 were retained, and amplicons were filtered and trimmed with DADA2. Taxonomic classification was performed against the SILVA 138 ribosomal RNA gene database (Quast et al., 2012).

RESULTS AND DISCUSSION

***Planktonic community compositions*:** The dominant planktonic eukaryotes, at the phylum level, were Dinoflagellata (38.57%), Protalveolata (15.03%), Diatomea (12.41%), Nematozoa (8.44%), Apicomplexa (6.79%) and Chlorophyta (5.43%), which constituted 86.68 % of the total number of sequences (Figure 2).

The most dominant OTUs (>10%) (Table 1) were *Alexandrium* with 25442 reads (35.36%), Syndiniales_Group_II with 7958 reads (11.06%). Other dominant OTUs (>2%) (Table 1) were *Viscosia* sp with 6305 reads (8.76%), Lankesteria with 4217 reads (5.86%), *Arcocellulus* with 3719 reads (5.17), *Thalassiosira* with 2133 (2.96) and *Nannochloris* with 1753 reads (2.44%).

Table 1. Top seven of planktonic eukaryotes. Results are given at species level or a higher taxonomic level if the sequences could not be assigned to a species.

	Dominant OTUs (%)
Alexandrium (**Dinophyceae**)	35.36
Syndiniales_Group_II (**Protalveolata**)	11.06
Viscosia sp (**Enoplea**)	8.76
Lankesteria (**Apicomplexa**)	5.86
Arcocellulus (**Diatomea**)	5.17
Thalassiosira (**Diatomea**)	2.96
Nannochloris (**Chlorophyta**)	2.44

A plethora of studies has been conducted on planktonic communities in water sources with metagenomic approaches. In the present study conducted in May 2021, when intense mucilage was observed, the dominant group was found to be the *Alexandrium* genus. Among many dinoflagellate species, the *Alexandrium* genus, in particular, produces toxins resulting in problems on human and environmental health as well as tourism economies (Vingiani et al., 2020). It was reported that *Alexandrium* species have the capability to produce mucilage and lead to shrimp and fish mortality in regions with algae bloom (Landsberg et al., 2002; Lewis et al., 2018). *Alexandrium minutum* is the most common toxic species in the western Mediterranean Sea (Vila et al., 2001). It caused larval mortality of some bivalve species and reduced valve gape and clearance rate of large-sized

class bivalves (May et al., 2010). The presence of *Alexandrium* species in the water column and sediment in the Gulf of Gemlik (the Sea of Marmara) between 2011-2012 was reported (Balkis et al., 2016; Balkis & Taş 2016). *A. minutum* cysts were determined in the sediments of Izmir Bay (Aydın et al., 2011; Aydın & Uzar, 2013). Moreover, vegetative forms of *A. minutum* were reported from the Marmara Sea (Balkis, 2004; Tüfekçi et al., 2010).

This study reports the second most dominant group as Syndiniales Group_II: endosymbionts of tintinnid ciliates, crustaceans, fish, protozoa, algae, and other dinoflagellates in particular (Luo et al., 2016). All the *Syndiniales* species identified have been reported to kill their hosts, including other protists and metazoans, and then release dinospores (Coats & Park., 2002; Guillou et al., 2008; Clarke et al., 2019). For this reason, *Syndiniales* species can potentially affect plankton population dynamics and biogeochemical cycling. The research in the literature has demonstrated that parasitic *Syndiniales* species assume the role of biological control agent on dinoflagellates that increase by algae bloom (Chambouvet et al., 2008; Mazzillo et al., 2011). As proof of this finding and parallel to the present study, 27.70% dinoflagellata and 12.81% parasitic Syndiniales Group I were dominant groups in polluted waters (Thessaloniki Bay, Greece) (Tsipas, 2020).

In this study, free-living marine nematode *Viscosia* sp. was detected in mucilage structure. In a similar vein, it was found among dominant nematode species in the Ligurian Sea, North-West Mediterranean (Moreno et al., 2009), Strait of Sicily, Central Mediterranean Sea (Sandulli et al., 2015), Aegean Sea, Eastern Mediterranean (Lampadariou & Eleftheriou, 2018) and the Black Sea (Ürkmez et al., 2016).

Gregarines, a large group of Apicomplexans, live in the body cavities of invertebrates, and some species attach to the epithelial tissues of the host, invading the intestinal lumen. In contrast, others invade the reproductive systems (Leander et al., 2016). As a member of this group, Lankesteria was found present in the mucilage structure at a rate of 5.86%. Lankesteria species were isolated in ascidians on the Central California Coast (Levine et al., 1981), and they were reported to cause large-scale infection in the ascidian *Ciona intestinalis* species (Mita et al., 2012). No report of Lankesteria species has been found in the literature for the Sea of Marmara and the Aegean Sea. However, the closest information to the present study area is *Lankesteria metandrocarpae* species in Varna Bay (the Black Sea) (Dzhembekova et al., 2017).

In this study, two Diatomea genus *Arcocellulus* and *Thalassiosira*, were detected in mucilage structure. Similarly, they were dominant groups during algae bloom periods in the Mediterranean Sea (Percopo et al., 2011). *Thalassiosira* species were reported among the most abundant diatom species forming resting stages (Montresor et al., 2013). Parallel to the present study, Tsipas (2020) reported that *Thalassiosira* was found among the dominant groups in polluted Thessaloniki Bay (Greece) at a rate of 2.41%.

It has been reported that the green algae *Nannochloris* genus belonging to the chlorellaceae family, identified among the dominant groups in the mucilage structure in the present study, contains species that lead to ecosystem disruptive algae blooms (Zhang et al., 2015; Mercado et al., 2021). Dzhembekova et al. (2017) detected the presence of *Nannochloris sp.* in Varna Bay (the Black Sea), though not intense, in their research on harmful algae. However, there has been no report of an algae bloom caused by *Nannochloris* species in Turkish waters in the literature.

This study determines planktonic communities in the mucilage structure, which started to be observed in Turkey's water column, surface, and bottom at the beginning of 2021 and reached severe levels as of May. The finding is that the *Alexandrium* genus, a dinoflagellate, and the Syndiniales Group_II, which is known to act as a biological control agent on the species belonging to this genus, are among the dominant groups is particularly significant. *Alexandrium* species have been known the capability to produce mucilage. Some *Alexandrium* species can cause severe impacts on human health and lead to bivalve, shrimp and fish mortality. Therefore, a more detailed study is needed to determine the *Alexandrium* toxins in the mucilage structure.

***The heavy metal content of the mucilage*:** There is limited information on the heavy metal concentration of mucilage in the literature. Therefore, the heavy metal content of the obtained mucilage from the Dardanelles was defined in this study. On the other hand, the collected mucilage from the surface of the Marmara Sea was sent to the landfill site for disposal. Thus, the concentration of the heavy metals in the obtained mucilage is crucial for environmental concerns. The microorganisms in the mucilage, such as microalgae and bacteria, have negative functional groups on their surface. These groups are more like to bind positive ions (i.e. heavy metals) in the sea. Due to this reason, the accumulation of heavy metals in the mucilage can occur. The heavy metal concentration of the mucilage and limitations for landfilling are given in the Table 2. The limit concentrations of heavy metals in non-hazardous waste in the Regulation on Landfilling of Wastes by the Republic of Turkey Ministry of Environment and Urbanization are 2, 100, 1, 10, 50, 0.2, 10, 10, 10, 0.5 and 50 mg/kg dry solid for As, Ba, Cd, Cr, Cu, Hg, Mo, Ni, Pb, Se and Zn, respectively (TMEU, 2019).

Table 2. The heavy metal concentration (mg/kg) of the mucilage and limitations for landfilling

Stations	As	Ba	Cd	Cr	Cu	Hg	Mo	Ni	Pb	Ba	Zn
Station 1	**3.10**	0.03	0.18	**10.80**	13.12	0.14	5.59	7.67	2.37	ND	0.77
Station 2	**4.45**	ND	0.12	**21.77**	1.08	0.18	7.96	7.22	2.06	ND	ND
Station 3	ND*	ND	ND	6.72	6.09	ND	4.73	3.71	ND	ND	ND
Limit Conc. **	**2.00**	100.00	1.00	**10.00**	50.00	0.20	10.00	10.00	10.00	0.50	50.00

*ND: under the detection limit

** Limit concentrations of heavy metals in landfilling of non-hazardous waste

The third sampling point (Station 3) is very close to the residential area. The water movement is very limited in that area as well; thus, the heavy metal content of the Station 3 could be lower than the other stations. According to the results, the total concentrations of all heavy metals in the mucilage complied with the regulation, except for As and Cr. Therefore, the collected mucilage from the sea surface should be checked before sending it to landfill sites in terms of the heavy metal content.

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ARAŞTIRMA MAKALESİ **RESEARCH PAPER**

Gundelia tournefortii Ekstraktlarının Antimikrobiyal Aktivitesi ve AMES/mikrozom Testi ile Antimutajenitesinin Belirlenmesi

Fatma Esen SARIGÜLLÜ ÖNALAN[1*] Hatice Aysun MERCİMEK TAKCI[2] Filiz UÇAN TÜRKMEN[2]
[1*] *Kilis 7 Aralık Üniversitesi Yusuf Şerefoğlu Sağlık Bilimleri Fakültesi Hemşirelik Bölümü, Kilis, Türkiye*
[2] *Kilis 7 Aralık Üniversitesi Fen Edebiyat Fakültesi Moleküler Biyoloji ve Genetik Bölümü, Kilis, Türkiye*

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: https://orcid.org/0000-0002-1374-4338
: https://orcid.org/0000-0002-5394-4959
: https://orcid.org/0000-0002-3653-9433

***Sorumlu yazarın:**
Fatma Esen SARIGÜLLÜ ÖNALAN
Kilis 7 Aralık Üniversitesi Yusuf Şerefoğlu Sağlık Bilimleri Fakültesi Hemşirelik Bölümü, Kilis, Türkiye
✉: esenonalan@kilis.edu.tr

Öz: Bu çalışmada ülkemizde özellikle Doğu Anadolu'da sıklıkla tüketilen *Gundelia tournefortii* (Kenger) bitkisinin genç saplarının AMES/mikrozom testi ile antimutajenik aktivitesinin ve antimikrobiyal aktivitesinin belirlenmesi amaçlanmıştır. Oda sıcaklığında ve nemsiz ortamda kurutma işlemi gerçekleştirilen kenger bitki ekstraktları su, metanol ve hekzan ile ekstrakte edilmiştir (1:10 (w/v)). Evaporatörde yoğunlaştırılan örnekler, son konsantrasyonları 1000 mg ml^{-1} olacak şekilde metanol ile ekstrakte edilmiştir. Ekstraktların *Bacillus cereus* ATCC 11778, *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella typhimurium* ATCC 14028 karşı *in vitro* antimikrobiyal özellikleri araştırılmıştır. 1000 mg ml^{-1} metanol ekstraktının metisilin dirençli *Staphylococcus aureus* ATCC 25923 suşuna karşı 15 mm çaplı antimikrobiyal etki gösterdiği saptanmıştır. Kenger bitkisinin su ekstraktlarının 21 mg ml^{-1}, 43 mg ml^{-1}, 87 mg ml^{-1} ve 175 mg ml^{-1} konsantrasyonlarının antimutajenik aktiviteleri Ames yöntemi ile tespit edilmiştir. 21 mg ml^{-1} konsantrasyondaki su ekstresinin S9 varlığında ve yokluğunda güçlü düzeyde antimutajenik aktivitesi belirlenmiştir.

Anahtar kelimeler: Ames/mikrozom testi, antimikrobiyal aktivite, antimutajenik aktivite, *Gundelia tournefortii* (Kenger).

Determinatin of Antimicrobial Activity and Antimutagenicity of *Gundelia tournefortii* Extracts with AMES/Microsome Test

***Corresponding author's:**
Fatma Esen SARIGÜLLÜ ÖNALAN
Yusuf Şerefoğlu Faculty of Health Sciences, Kilis 7 Aralık University, KİLİS, Turkey
✉: esenonalan@kilis.edu.tr

Abstract: In this study, it was aimed to determine the antimutagenic activity by using the AMES/microsome test and antimicrobial activity of the young stems of the *Gundelia tournefortii* (Kenger) plant, which is frequently consumed in our country, especially Eastern Anatolia. The plant extracts, dried at room temperature by without humidity, were extracted with distilled water, methanol and hexane (1:10 (w/v)). After evaporation, the samples were extracted with methanol at final concentration of 1000 mg ml^{-1}. *In vitro* antimicrobial properties of the extracts against *Bacillus cereus* ATCC 11778, *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella typhimurium* ATCC 14028 were investigated. It was determined that 1000 mg ml^{-1} methanol extract had an antimicrobial effect with a diameter of 15 mm against methicillin resistant *Staphylococcus aureus* ATCC 25923 strain. Antimutagenic activities of 21 mg ml^{-1}, 43 mg ml^{-1}, 87 mg ml^{-1} ve 175 mg ml^{-1} concentrations of distilled water extracts of Kenger plant were determined by Ames method. The strong antimutagenic activity for 21 mg ml^{-1} water extract was observed in the presence and absence of S9.

Keywords: Ames/microsome test, antimicrobial activity, antimutagenic activity, *Gundelia tournefortii* (Kenger)

GİRİŞ

3000'i endemik olmak üzere yaklaşık 12000 bitki taksonuna sahip ülkemiz bitki biyoçeşitliliği açısından oldukça zengindir. Özellikle besinsel ve fonksiyonel özelliklerinden dolayı yabani yenilebilir bitkiler geleneksel olarak halk arasında kullanılmaktadır (Konak vd., 2017). Yenilebilir kısımlarının vitamin, mineral ve besin elementleri açısından zengin olmasının yanı sıra yararlı fitokimyasal içerik miktarlarına göre antioksidan, antimikrobiyal, antiviral, antikarsinojenik, anti-inflamatuar, anti-parazit ve antimutajenik gibi biyolojik aktiviteleri de ortaya konulmuş yabani bitkiler insan sağlığı açısından önemli rol oynamaktadır (Konak vd., 2017; Saraç vd., 2019).

Asteraceae familyasına ait *Gundelia tournefortii* kenger otu, kenger sakızı, sakız otu, çadır dikeni ve kanak sakızı gibi çeşitli adlarla bilinen yenilebilir yabani bir bitki türüdür. Kenger, ülkemizde Anadolu'da Karaman, Ermenek, Toros dağları (Gülek civarı), Bayburt, Elazığ, Antalya (Yayladağı), Gaziantep, Silifke, Diyarbakır vb. bölgelerde farklı rakımlarda yetişebilen ve enginara benzeyen başçığı ve genç sapları nedeniyle sebze olarak yenilebilmekte, yem bitkisi olarak kullanılmakta ve gövdesinden sızan kıvamlı süt kenger sakızı olarak bilinmektedir (Karataş vd., 2014). Dondurma üretiminde iyi bir stabilizatör olarak kullanılmasına ek olarak, K, Ca, P, Na, Fe, Mg ve Zn gibi mineraller, yağ asitleri, tokoferol ve sterolleri de içerdiğinden yüksek besinsel içeriğe sahiptir (Al-Kadhi vd., 2020; Okcu ve Kaplan, 2018; Karaaslan vd., 2014).

20-30 cm boyunda, tek tohumlu, dikenli, çok yıllık otsu bir bitki olan kenger bitkisinin vücut kısmı hepatoprotektif ve kan temizleyici olarak, Doğu Anadolu'da ise kuru tohumları vitiligo tedavisinde, taze tohumları idrar söktürücü olarak, tohumlar ve diğer kısımlar yüksek antioksidan potansiyele sahip olup halk arasında tıbbi olarak kullanılmaktadır (Özaltun ve Daştan, 2019; Saraç vd., 2019; Ceylan vd., 2019). Ayrıca, karaciğer iltihabı, hipoglisemik, safra yolu iltihabı, siroz, kabakulak, ishal, bronşit ve kronik karaciğer hastalıklarına karşı etkisi de bildirilmiştir (Haghi vd., 2011; Rafii vd., 2017; Saraç vd., 2019).

İnsanoğlunun yaşamı boyunca çevresinde karşılaştığı pek çok mutajenik ve karsinojenik ajanlara karşı genomun korunması oldukça hayati önem taşımaktadır. Genomun kendi onarım sistemleri dışında, bitkilerden elde edilen antioksidan, antimutajenik özelliğe sahip biyoaktif bileşenlerle desteklenerek korunması da mümkündür. Bu açıdan günümüzde yabani yenilebilir bitki türlerinin çoğu antimutajenik potansiyelleri yönünden araştırılmaktadır. Bu nedenle çalışmamızda *Gundelia tournefortii* (kenger) bitkisinin genç saplarına ait kısımlarının Ames/mikrozom testi ile antimutajenik aktivitesi ve antibakteriyel aktivitesinin belirlenmesi amaçlanmıştır. Ülkemizde kenger bitkisi ile yapılmış antimutajenite aktivite çalışmasının bulunmaması sebebiyle bu projemiz ilk rapor olma niteliğini taşımaktadır.

MATERYAL VE METOT

Bitki Ekstraktlarının Hazırlanması: Kenger, *Gundelia tournefortii* bitkisi Mart ve Nisan ayları boyunca Urfa il pazarından toplanmıştır. Elde edilen bitkiler oda koşullarında gölgede kesilip kurutulmuş ve analizlerde kullanılana kadar karanlık şişelerde muhafaza edilmiştir. Bitki kısımları ev tipi öğütücüde (Arçelik K 3104) toz haline getirilmiştir. Ekstraksiyon için 30 gr toz haline getirilmiş kenger bitkisi kullanılmıştır. 72 saat oda sıcaklığında çalkalamalı koşullarda su, hekzan ve metanol çözücüleri (1:10 (w/v)) kullanılarak ekstraksiyon basamağı sürdürülmüştür. Ekstraksiyon işlemi tamamlanan örnekler filtre kağıdından (Whatman filter paper No.1) süzülmüş ve çözücülerin uzaklaştırılması için evaporatör kullanılmıştır. Evaporasyon sonrası örneklerin son konsantrasyonu 1000 mg ml^{-1} olacak şekilde metanol içerisinde süspanse edilmiştir. Antimutajenite çalışmaları için ise sadece su fazı ekstresi test edilmiştir. Antimikrobiyal analizler için 1000 mg ml^{-1} hekzan, saf su ve metanol ekstraktları kullanılmıştır. Ekstraktlar biyolojik aktivite analizlerine kadar renkli şişelerde -20°C'de stoklanmıştır.

Antibakteriyel Analizler: Kenger bitkisi ekstraktlarının bakterilere karşı biyolojik aktivitesini belirlemek amacıyla Kirby-Bauer disk difüzyon yöntemi kullanılmıştır (Bauer vd., 1966). Çalışmada, laboratuvarımız kültür koleksiyonunda bulunan standart bakteriler (*Bacillus cereus* ATCC 11778, *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella typhimurium* ATCC 14028) test edilmiştir. Bakterilere karşı belirlenecek aktivite için CLSI standartlarına uygun olarak Mueller Hinton Agar (MHA) besiyerleri kullanılmıştır. McFarland 0,5 standart bulanıklığında bakteri süspansiyonları hazırlanmış ve steril eküvyon çubuğu ile Mueller Hinton Agar yüzeyine inoküle edilmiştir. 6 mm çaplı steril blank disklere her bir kenger ekstrelerinden 10 µL emdirilip steril penset yardımı ile agar yüzeyine eşit aralıklarla yerleştirilmiştir. *Escherichia coli*, *Salmonella typhimurium* ve *Bacillus cereus* için Tetrasiklin (30 mcg/disk); *Staphylococcus aureus* için Metisilin (5 mcg/disk); *Pseudomonas aeruginosa* için Polimiksin B (300 unite/disk) antibiyotikleri standart olarak kullanılmıştır. Negatif kontrol olarak steril blank disklere 10 µL metanol emdirilmiştir. 37˚C'de 18-24 saat

inkübasyonu takiben disklerin etrafında bakterilerin üremediği şeffaf zonların varlığı incelenmiştir.

Antimutajenite Analizleri: Maron ve Ames (1983) tarafından geliştirilen Salmonella / mikrozom deneyi, kenger sulu ekstraktının antimutajenitesini saptamak için analiz edilmiştir. Analiz öncesi *Salmonella typhimurium* TA98 (HisD3052, pKm101, rfa, uvrB) ve TA100 (HisG46, pKm101, rfa, uvrB) suşlarının mutajen özellikleri test edilmiştir. Sitotoksisite analizi için 21 mg ml^{-1}, 43 mg ml^{-1}, 87 mg ml^{-1}, 175 mg ml^{-1}mg/plak kenger konsantrasyonları kullanılmıştır. Sitotoksik dozun belirlenmesi için, 100 µL kenger ektsraktı ve gece boyunca büyütülen 100 µl bakteri suşları (yaklaşık 1-2x10^9 cfu/mL), 2 mL üst agar içeren test tüpleriyle karıştırılmış ve Nutrient agar plağına dökülmüştür. 37ºC'de 24-72 saat inkübe edilen plaklarda test ve kontrol plağındaki koloni sayısı karşılaştırılarak toksik olmayan doz tespit edilmiştir. Mutajenite deneyi, seçilen dozlarla sürdürülmüştür.

Kenger bitkisinin antimutajenitesi karaciğer S9 fraksiyonun varlığında ve yokluğunda test edilmiştir. Karaciğer S9 karışımı, Ames tarafından açıklanan prosedüre göre hazırlanmıştır. (Maron ve Ames, 1983). Tüm testlerde, suşlara göre negatif kontroller (damıtılmış su) ve pozitif kontroller (S9 yokluğunda TA100 için sodyum azid (5µg/plak), TA 98 suşu için 4-nitro-o-fenilendiamin (5µg $plak^{-1}$); S9 varlığında TA100 için sodyum azid (5 µg $plak^{-1}$), TA98 suşu için 2-Aminofloren (7.5 µg $plak^{-1}$)) pozitif direkt mutajen olarak kullanılmıştır (Ekmekçi, 2010).

100 µl pozitif mutajen madde ve 100 µl gecelik bakteri kültürü (yaklaşık 1-2x10^9 cfu/mL) top agara ilave edilmiştir. Tüpler karıştırılarak MGA besiyerlerine dökülmüş ve 37ºC'de 48-72 saat inkübe edilmiştir. Inkübasyon sonrası koloni sayımı yapılmıştır. Negatif ve pozitif kontroller de deneye paralel olarak yapılmıştır. Pozitif kontrol için 500 µl sodyum-fosfat tamponu+100 µl pozitif mutajen madde+100 µl bakteri kültürü top agara ilave edilmiştir. Negatif kontrol için 500 µl sodyum-fosfat tamponu+100 µl su veya DMSO+100 µl bakteri kültürü top agara ilave edilmiştir. S9 yokluğundaki tüm işlemlerin aşamaları aynı şekilde yürütülmüştür. Sadece farklılık olarak S9 yokluğunda top agara eklenen 500 µl fosfat tamponu yerine 500 µl S9 karışımı eklenmiştir. Tüm analizler 3 tekrarlı yürütülmüştür.

BULGULAR VE TARTIŞMA

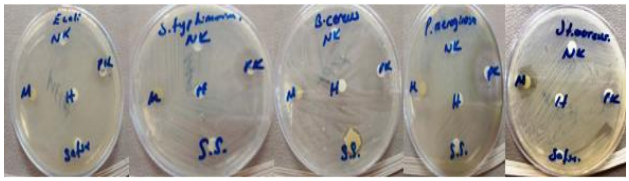
Kenger bitkisinin 1000 mg ml^{-1} hekzan, saf su ve metanol ekstraktlarının standart test izolatları (*Bacillus cereus* ATCC 11778, *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella typhimurium* ATCC 14028) üzerindeki antimikrobiyal aktivitesi Tablo 1'de verilmiştir.

Tablo 1. Kenger bitkisinin antibakteriyel aktivitesi (mm cinsinden).
Table 1. Antibacterial activity of the Kenger (terms of mm).

	Negatif Kontrol Negative Control	Pozitif Kontrol Positive Control	Su Water	Hekzan Hexan	Metanol Methanol
S. aureus ATCC 25923	–	–	–	–	15±0
P.aeruginosa ATCC 27853	–	15± 0	–	–	–
B. cereus ATCC 11778	–	28±2	–	–	–
S.typhimirum ATCC 14028	–	19±2	–	–	–
E. coli ATCC 25922	–	17,5±0,05	–	–	–

Tablodaki verilere göre Kenger bitkisinin sadece test mikroorganizmalarından *Staphylococcus aureus* ATCC 25923 suşu üzerinde metanol ekstraktının antibakteriyel aktivite gösterdiği belirlenmiştir. 2. dereceden bakteriyemi etkeni ve hastane enfeksiyonu açısından önemli bir enfeksiyöz bakteri olan metisiline karşı dirençli *S. aureus* üzerinde gözlenen antibakteriyel aktivite metanol ekstraktının biyolojik etkinliğine işaret etmektedir.

Kenger bitkisinin su ve hekzan ekstraktlarının denenen test organizmaları üzerinde herhangi bir antimikrobiyal aktivitesi saptanamamıştır (Şekil 2).



Şekil 2. Standart izolatlara karşı kenger bitki ekstratlarının antibakteriyel etkisi**.**
Figure 2. Antibacterial effect of kenger plant extracts against standard isolates.

Asadi-Samani vd., (2013) tarafından *Gundellia* sp. türü üzerinde yapılan çalışmada, metanol ekstraktının çeşitli mikroorganizmalara karşı antimikrobiyal etkinlikleri bildirilmiştir. Saraç vd., (2019) kenger tohumu su ekstraktının bazı bakteriler üzerindeki etkisini inceledikleri çalışmada, 8 farklı mikroorganizmaya karşı zayıf nitelikte aktivite saptanırken çalışmamıza benzer olarak sadece *S. aureus* ATCC 29213 bakterisi üzerinde 0,3125 mg/mL değeriyle orta düzeyde bir etkinliğin olduğu gözlenmiştir. 8 adet standart suş üzerinde test edilen diğer bir çalışmada ise kenger bitkisinin kök, yaprak ve uçucu yağ sulu ekstrelerinin antimikrobiyal aktivitesi incelenmiştir. Çalışmamızın sonuçlarını destekler nitelikte test mikroorganizmaları üzerinde genelde zayıf ve önemsiz aktivite belirlenirken, sadece *S. aureus* ATCC 29213 suşuna karşın ılımlı düzeyde (0,5 mg/mL) etki tespit edilmiştir (Özaltun ve Daştan, 2019). Kenger bitkisinin tüm kısımları ile yapılan diğer bir çalışmada ise, çoklu ilaç direnci gösteren *Escherichia coli* ve *Pseudomonas*

aeruginosa'ya karşı antibakteriyel aktivite gözlenmiştir. Ancak bu aktivitenin penisilin G ve eritomisin ile kenger bitkisinin kombinasyonu sonunda belirlendiği rapor edilmiştir (Darwish ve Aburjai, 2010). Diğer bir çalışmada, kenger bitkisinin metanolik ekstraktlarının farklı antibiyotiklerle kombinasyonu ile dirençli *S. aureus* suşu üzerinde antibakteriyel aktivite gözlenmiştir (Darwish vd., 2002).

Literatür çalışmaları ve projemizin sonuçları bazı bitki bileşenlerinin dirençli standart suşları üzerindeki antibakteriyel etkinliğinin yapısal farklılıklardan dolayı önemli düzeyde değişiklik gösterdiğini vurgulamaktadır.

Kenger sulu faz ekstresinin antimutajenite aktivitesinin belirlenmesi için öncelikle suşların mutant karakterleri ve kendiliğinden geri dönen koloni sayısı test edilmiştir. *S. typhimurium* TA98 ve TA100 suşlarının kendiliğinden geri dönen koloni sayısı aşağıdaki Tablo 2'de belirtildiği gibidir. Bu kendiliğinden geri dönen koloni sayısı her suş için olması gereken limitler aralığında bulunmuştur.

Tablo 2. *S. typhimurium* TA98 ve TA100 suşlarının kendiliğinden geri dönen koloni sayısı.
Table 2. Number of spontaneously returning colonies of *S. typhimurium* TA98 and TA 100 strains.

Suşlar	Referans değerleri	Kendiliğinden geriye dönen koloni sayısı
S. typhimurium TA98	20-50	30,5
S. typhimurium TA100	80-200	168,5

Antimutajenite deneylerinde kenger bitkisinin 21 mg ml^{-1}, 43 mg ml^{-1}, 87 mg ml^{-1} ve 175 mg ml^{-1} konsantrasyonlarındaki su ekstraksiyonları çalışıldı. Elde edilen verilere göre kontrol plaklarındaki koloni sayıları ve kenger bitkisinin bu konsantrasyonlarındaki koloni sayıları sayılamayacak kadar fazla olduğundan kenger bitkisinin bu konsantrasyonlarının sitotoksik etkilerinin olmadığı belirlendi.

Pozitif kontrollerin mutajenite oranları %100 (yani %0 antimutajenite) kabul edilerek, kenger bitki ekstraktlarının antimutajenite oranları [(A-B)/(A-C)]x100 formülüne göre hesaplanmıştır. (A= Bakteri+mutajen içeren besiyerinde geri dönen koloni sayısını; B= Bakteri+mutajen+ekstraktı içeren besiyerinde geri dönen koloni sayısını; C= Sadece bakteri içeren besiyerinde geri dönen koloni sayısını ifade etmektedir). İnhibisyonun aktivite yok veya zayıf antimutajenite olarak kabul edilmesi %0-25 aralığı baz alınarak, orta derece antimutajenite için %26-40 aralığı baz alınarak ve güçlü antimutajenite için ise %40 ve üzeri aralık baz alınmıştır (Uysal vd., 2016).

Kenger bitki ekstraktlarının antimutajenite test sonuçları Tablo 3 ve 4'de verilmiştir. Kenger bitki ekstraktlarının antimutajenik etkisi plak başına düşen revertant koloni sayılarının ortalaması ve bilinen mutajenlere karşı belirlenen % inhibisyon oranları değerlendirilerek belirlenmiştir. TA98 suşu üzerinde S9 yokluğunda sadece 21 mg ml^{-1} kenger ekstraktları orta dereceli (%38,41) antimutajenik aktivite gösterirken diğer konsantrasyonların zayıf antimutajenik etki gösterdiği gözlenmiştir. TA100 suşu üzerinde ise S9 yokluğunda 87 ve 43 kenger ekstraktları orta dereceli (%28-36) antimutajenik aktivite gösterirken 21 mg ml^{-1} kenger ekstraktında ise güçlü antimutajenik aktivite (%43,21) tespit edilmiştir.

S9 varlığında ise TA98 suşu üzerinde test edilen kenger konsatrasyonlarının tümü için % inhibisyonun arttığı saptanmıştır. 21 mg ml^{-1} kenger konsantrasyonunda S9 yokluğunda belirlenen orta dereceli (%38,41) inhibisyon, S9 varlığında ise güçlü antimutajenik aktivite (%44,87) ile sonuçlanmıştır. S9 varlığında TA100 suşuna karşın etkinliğin 21 mg ml^{-1} ve 43 mg ml^{-1} kenger konsantrasyonlarında artış gösterdiği tespit edilmiş olup, 87 mg ml^{-1} ve 175 mg ml^{-1} konsantrasyonlarda % inhibisyonda azalma gözlenmiştir. Buna göre S9 varlığında TA100 suşu için en yüksek mutajen inhibitör etki %63,35 güçlü antimuajenik aktvite ile 21 mg ml^{-1} kenger konsantrasyonunda rastlanmıştır.

Analizlerimizin sonuçları söz konusu antimutajenik aktivitenin madde konsantrasyonu artışına bağlı olarak % inhibisyonun artmadığını ortaya koymaktadır. Buna göre her iki suş için S9 varlığında ve yokluğunda en güçlü aktivite 21 mg ml^{-1} kenger konsantrasyonunda gözlenmiştir.

Kanser, günümüzde endüstriyel dünyada ölümlerin ana nedenlerinden biri olarak kabul edilmektedir. Bilim adamları, DNA dizisi ve sürekliliğindeki genetik materyalin hasar görmesi, genlerdeki mutasyon ve kromozomal yapılardaki diğer genetik değişikliklerin karsinogenezde önemli rol oynadığına inanmaktadır. Günlük yaşamda antimutajen ve antikarsinojen kullanımı, insan kanserini ve genetik hastalıkları önlemede en etkili prosedür olarak görülmektedir (Sarac, 2015).

Bitki türlerinin ortaya çıkardığı antimutajenik özellikler, insan sağlığında çok çeşitli ileriye dönük uygulamalara sahiptir. Aktif bişenler içeren bitkisel ilaçlar DNA'ya elektrofil (örneğin, serbest radikaller gibi) saldırısının yaşlanma ve kanser gibi yaygın sonuçlarına karşı koruma sağlamak için geliştirilmektedir. Kanserin ortaya çıkma oranı dünya çapında artmakta olup, kemopreventif veya kemoprofilaksi bileşiklerinin belirlenmesi kanser riskini azaltma çabasında önemlidir. Antimutajenite gösteren bir bitki özütü, bir antikarsinojen değildir; ancak, bu tür amaçlar için potansiyel adayların bir göstergesidir (Sarac, 2015).

Tablo 3. Kenger bitki ekstraktlarının S9 yokluğunda TA98 ve TA100 suşları üzerinde belirlenen antimutajenik etkileri.
Table 3. The antimutagenic effects of Kenger extracts on TA98 and TA100 strains in the absence of S9.

	Konsantrasyon ($mgml^{-1}$) *Concentration($mgml^{-1}$)*	TA98		TA100	
		S9 (-) His^+ revertant koloni sayısı *S9 (-) His + revertant colony count*	% İnhibisyon *Inhibition (%)*	S9 (-) His^+ revertant koloni sayısı *S9 (-) His + revertant colony count*	% Inhibisyon *Inhibition (%)*
Negatif Kontrol *Negative Control*	100µl/plak	24 ± 7^{f}		77 ± 8^{e}	
Pozitif Kontrol *Positive Control*	200µg/plak	$484,5\pm1,5^{b}$	%0	$369,5\pm0,5^{a}$	%0
	175mg/ml	439 ± 2^{c}	%9,88	$376,5\pm1,5^{a}$	%2,39
	87 mg/ml	$607,4\pm1,4^{a}$	-%0,26	$264,8\pm0,8^{c}$	%35,7
	43 mg/ml	$408,3\pm0,3^{d}$	%16,5	$283,3\pm1,3^{b}$	%29,47
	21 mg/ml	$307,6\pm2,7^{e}$	%38,41	$243,1\pm3,1^{d}$	%43,21

Negatif Kontrol: Su (100µlplak^{-1}) TA98 ve TA100 suşları için S9 yokluğunda negatif kontrol olarak kullanıldı.
Pozitif Kontroller: S9 yokluğunda TA 98 suşu için 4-nitro-o-fenilendiamin (5µgplak^{-1}), TA100 için sodyum azid (5µgplak^{-1}) pozitif direkt mutajen olarak kullanıldı.
^{abcde}Aynı sütundaki farklı harflere sahip gruplar arasındaki fark istatistiksel olarak önemlidir ($p< 0,05$).

Tablo 4. Kenger bitki ekstraktlarının S9 varlığında TA98 ve TA100 suşları üzerinde belirlenen antimutajenik etkileri.
Table 4. Antimutagenic effects of Kenger extracts on TA98 and TA100 strains in the presence of S9.

	Konsantrasyon ($mgmL^{-1}$) *Concentration($mgml^{-1}$)*	TA98		TA 100	
		S9 (+) His^+ revertant koloni sayısı *S9 (+) His + revertant colony count*	% İnhibisyon *Inhibition (%)*	S9 (+) His^+ revertant koloni sayısı *S9 (+) His + revertant colony count*	% İnhibisyon *Inhibition (%)*
Negatif Kontrol *Negative Control*	100µl/plak	43 ± 3^{f}	-	106 ± 25^{e}	-
Pozitif Kontrol *Positive Control*	200µg/plak	$632,5\pm17,5^{a}$	-	$347,5\pm5,5^{a}$	-
	175mg/mL	$593,5\pm19,5^{b}$	%6,61	343 ± 9^{a}	%1,86
	87 mg/mL	$538,5\pm1,5^{c}$	%15,95	$296,5\pm3,5^{b}$	%21,12
	43 mg/mL	$495,5\pm16,5^{d}$	%23,24	246 ± 3^{c}	%42,03
	21 mg/mL	368 ± 9^{e}	%44,87	$194,5\pm6,5^{d}$	%63,35

Negatif Kontrol: Su (100 µl plak^{-1}) TA98 ve TA100 suşları için S9 yokluğunda negatif kontrol olarak kullanıldı.
Pozitif Kontroller: S9 varlığında TA 98 suşu için 2-Aminofloren (7,5 µg plak^{-1}), TA100 için sodyum azid (5 µg plak^{-1}) pozitif direkt mutajen olarak kullanıldı.
^{abcde}Aynı sütundaki farklı harflere sahip gruplar arasındaki fark istatistiksel olarak önemlidir ($p<0,05$).

SONUÇ VE ÖNERİLER

AMES, maddelerin kanserojen ve mutajenik potansiyelleri arasındaki ilişkiyi araştıran en önemli *in vitro* test sistemidir. Ancak son yirmi yılda yapılan araştırmalar, mutajenik olmayan kanserojenleri belirlemeye yöneliktir. Sonuç olarak bir test maddesinin genotoksik etkileri, bakteriyel gen mutasyon analizini takiben mikronükleus, komet, kardeş kromatid değişimi, kromozomal anormallikler ve transgenik sıçan mutasyonu gibi *in vitro* ve *in vivo* test sistemleri ile araştırılmalı ve bu literatür verilerine dayanarak, ileriki çalışmalarımızda bu test sistemlerinde kenger bitkisi ile ilgili daha fazla çalışma yapılacaktır.

TEŞEKKÜR

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ARAŞTIRMA MAKALESİ **RESEARCH PAPER**

Determination of Some Heavy Metal Levels in Different Tissues of Common Carp (*Cyprinus carpio*, L., 1758) and Pike Barb (*Luciobarbus esocinus*, H., 1843) From Karasu River (Erzincan)

İsmail BAYIR[1*] **Cengiz MUTLU**[2]

[1*] *Giresun University, Institute of Science, Department of Biology, Giresun, Turkey*
[1] *Erzincan Binali Yıldırım University, Kemaliye HAA Vocational High School, Erzincan, Turkey*
[2] *Giresun University, Faculty of Engineering, Department of Environmental Engineering, Giresun, Turkey*

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: https://orcid.org/0000-0002-7273-3874
: https://orcid.org/0000-0002-9741-4167

***Corresponding author's:**
İsmail BAYIR
Giresun University, Institute of Science, Department of Biology, Giresun, Turkey
✉: ismmmail@gmail.com

Abstract: Heavy metals may be harmful to human health over the short and long term. Consumption of fish, one of the most important sources of protein in human life is one of the most common ways of heavy metal exposure. In this study for determine some heavy metals (Li, Na, Mg, Al, K, Ca, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Ag, Cd, Pb and U) concentration in different tissues as muscle, liver and gill of common carp (*Cyprinus carpio*, L., 1758) and pike barb (*Luciobarbus esocinus*, H., 1843) collected from Karasu River (Erzincan, Turkey) along the upper Euphrates Basin were collected between November 2019 and October 2020 on monthly. Heavy metal analysis was performed by ICP-MS -Bruker 820-MS. The results of heavy metals in tissues were compared to acceptable international limits. The studied metals were found at a considerable level in all seasons, indicating that the Karasu River biota is particularly vulnerable to heavy metal intake. Consequently, research has shown a strong connection between various elements and in addition, the human health risk assessment (HHRA) reveals are hazardous for consumption of fish.

Keywords: Karasu river, heavy metal, fish, public health.

Karasu Nehri'nden (Erzincan) Elde Edilen Sazan (*Cyprinus carpio*, L., 1758) ve Turna Balığı (*Luciobarbus esocinus*, H., 1843) Farklı Dokularındaki Bazı Ağır Metal Düzeylerinin Belirlenmesi

***Sorumlu yazar:**
İsmail BAYIR
Giresun Üniversitesi, Fen Bilimleri Enstitüsü, Biyoloji ABD, Giresun, Türkiye
✉: ismmmail@gmail.com

Öz: Ağır metaller kısa ve uzun vadede insan sağlığına zararlı olabilir. İnsan hayatındaki en önemli protein kaynaklarından biri olan balık tüketimi, ağır metallere maruz kalmanın en yaygın yollarından biridir. Bu çalışmada bazı ağır metallerin (Li, Na, Mg, Al, K, Ca, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Ag, Cd, Pb ve U) farklı dokulardaki konsantrasyonlarının belirlenmesi için Yukarı Fırat Havzası boyunca uzanan Karasu Nehri'nden (Erzincan, Türkiye) toplanan sazan (*Cyprinus carpio*, L., 1758) ve turna balığı (*Luciobarbus esocinus*, H., 1843) kas, karaciğer ve solungaç dokuları Kasım 2019 - Ekim 2020 tarihleri arasından aylık olarak toplanmıştır. Ağır metal analizi, ICP-MS -Bruker 820-MS ile gerçekleştirilmiştir. Dokulardaki ağır metallerin sonuçları kabul edilebilir uluslararası sınırlarla karşılaştırılmıştır. İncelenen metallerin her mevsimde önemli düzeyde bulunması, Karasu Nehri biyotasının özellikle ağır metal alımına karşı savunmasız olduğunu göstermektedir. Sonuç olarak, araştırmalar çeşitli unsurlar arasında güçlü bir bağlantı olduğunu göstermiştir ve ayrıca insan sağlığı risk değerlendirmesi (HHRA) balık tüketimi için tehlikeli olduğunu ortaya koymaktadır.

Anahtar kelimeler: Karasu nehri, ağır metal, balık, halk sağlığı.

INTRODUCTION

Fish is excellent and healthy source of protein, and its amino acid contents are richer in cysteine than other foods which has amino acid that humans consume (Attia et al., 2020; Kaushik & Seiliez, 2010; Mariotti & Gardner, 2019). Due to its acceptability, tastiness, and low cholesterol and low-fat, it is highly used for human

[*] *Bu çalışma, doktora tezinden üretilmiştir.*

[*] *This study was produced from the doctora thesis.*

consumption. It is also the cheapest protein source and other vital components for urban and semi-urban poverty regions. In recent years anthropogenic pollution sources emit heavy metals into aquatic environments, constantly (Akkan et al., 2018; Cornelis et al., 2005; Mutlu et al., 2020; Sipahi et al., 2013; Yilmaz, 2006). Metallic pollutants reach the aquatic environment through air deposition, by erosion of the geological matrix, or human sources, such as industrial effluents, mining wastes, and even food processing by-products (Borah et al., 2020; Bundschuh et al., 2020). These metals may be components of both food and pollution, such as the pesticide residue found in food. Field and laboratory research have investigated the bioaccumulation of trace metals in fish, combined with the underlying processes. Industrial operations and industrial waste, often from rubber and oil palm mills, are mostly responsible for the pollution (Ke et al., 2017; Newman & Watling, 2007; Tariq et al., 1996; Zhao et al., 2021).

The amount of heavy metal each individual fish has varies based on several factors such as how fast it grows, how much it consumes, and how it feeds (Al-Weher, 2008; Yılmaz, 2006; Yavuzcan Yildiz et al., 2017). An issue to consider is the variation in life cycle patterns across species and how it is related to their tropic and geographic distribution of life stages (Hunnam, 2021; Palomera, 2007; Petitgas et al., 2013). When a person exceeds the prescribed limit for trace metals, that person may become toxic (acute, chronic, or subchronic), which may cause neurotoxicity, carcinogenicity, mitogenicity, or teratogenicity (Abro & Gomez-Aguilar, 2019; Dżugan, 2010; Silbergeld, 2003; Tchounwou et al., 2019). According to a recently published studies, among of the most common symptoms reported for humans who have been exposed to the toxic metals include vomiting, convulsions, paralysis, ataxia, gastrointestinal disorders, diarrhea, stomatitis, depression, and pneumonia (Ayenimo et al., 2010; Ayua et al., 2020; Bashir, 2018; Flora, 2014; Guérin et al., 2011; Onakpa et al., 2018; Rweyemamu & Nkansah, 2021; Singh et al., 2017; Shelar et al., 2021).

In this study, it is aimed to determine the concentrations of some heavy metals in different tissues of Cyprinus carpio and Luciobarbus esocinus which are preferred as a nutrient by local people and affects to human health from different locations of Karasu River (Upper-Euphrates). It is expected that the results of this research will assist in acquiring information about the level of toxic metals in this area.

MATERIALS AND METHODS

The Euphrates, a river in Western Asia, is one of the longest in the region, on the eastern side of Anatolia (Kalender & Uçar, 2013). It joins the junction of the Karasu River and the Western Euphrates at 340 km point (211 miles) and the Murat River, which is an expansion of the Eastern Euphrates 650 km (400 miles of river). The Karasu River is located on Upper-Euphrates which is in Upper Euphrates Basin at Erzincan city in the North-East part of Anatolia (Bilen, 1994; Kalender & Uçar, 2013). It feeds Karakaya Dam Lake (KDL) which is the third largest reservoir in Turkey, located in the borders of Diyarbakır city in the east Anatolia region (Kalkan, 2008; Varol et al., 2017). For the study fish samples consisted of 30 individuals common carp (*Cyprinus carpio*, L., 1758) and 21 individuals pike barb (*Luciobarbus esocinus*, H., 1843) specimens collected from different parts of Karasu River between November 2019 and October 2020 on monthly basis (Özgür, 2016). The specimens were caught using cast nets and stored on ice until arrival at the laboratory.

All tissue samples (muscle, gill, and liver) were prepared with a preliminary digesting process (samples 0.5 g, 8 mL of HNO3 (65%) via a CEM MARS-5 model microwave instrument. The Heavy metal analysis was performed by ICP-MS-Bruker 820-MS. The reference materials were used to check the accuracy and reliability of the method. Metals concentrations are expressed in μg g-1. The estimated daily and weekly intakes (EDI-EWI) were calculated by Yilmaz et al. (2016), also The target hazard quotient (THQ) was finally calculated using formula: THQ: EDI/Reference Dose (mg/kg/day). One-way ANOVA and Tukey test were performed to test the differences of the metal levels among the specimens (significance level $p<0.05$). All statistical calculations were performed with SPSS 16.0 for Windows.

RESULTS AND DISCUSSION

The present study is about common carp and pike barb in Karasu River and supplies valuable information about metal contents in different tissues. The accumulation values of the respective metals in various tissues of common carp were as follows Li, gill > muscle > liver; Na, gill > liver > muscle; Mg, gill > muscle > liver; Al, muscle > gill > liver; K, muscle > liver > gill; Ca, gill >muscle > liver; Cr, gill > liver > muscle; Mn, gill > muscle > liver; Fe, gill > liver > muscle; Co, gill > liver > muscle; Ni, gill > muscle = liver; Cu, gill > liver > muscle; Zn, gill > liver > muscle; As, gill > muscle > liver; Se, gill > liver > muscle; Ag, muscle > liver > gill; Cd, muscle > gill > liver; Pb, muscle > liver > gill; U, liver > gill > muscle. The accumulation values of the respective metals in various tissues of pike barb were as follows Li, gill > liver > muscle; Na, gill > liver > muscle; Mg, gill > muscle > liver; Al, gill > muscle > liver; K, muscle > liver > gill; Ca, gill > muscle > liver; Cr, muscle > liver > gill; Mn, gill > liver

> muscle; Fe, gill > liver > muscle; Co, gill > liver > muscle; Ni, gill > muscle = liver; Cu, gill > liver > muscle; Zn, gill > liver > muscle; As, gill > muscle > liver; Se, gill > liver > muscle; Ag, gill > muscle = liver; Cd , liver > muscle = gill; Pb, liver > gill > muscle; U, liver > gill > muscle.

The mean concentration Li, Na, Mg, Al, K, Ca, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Ag, Cd, Pb and U in different tissues of common carp and pike barb were given in Table 1. 30 individuals common carp and 21 individuals pike barb were analyzed in this study. The highest metal concentrations were found in the gill on common carp samples. Especially Cu, Fe and Zn were the most abundant metals in the liver with the concentrations of 52250, 12060, 7.508, 48.160, 152.023 µg g-1, respectively. The second highest metal concentrations were found in the muscle. Al, Ag and Pb were the most abundant metals in the muscles with the concentrations of 518.9, 0.679, 2.80 µg g-1, respectively. Liver values showed dramatically lowest level of concentration throughout the study on common carp samples. Except U all metal concentrations in livers were lower than others.

There were significant differences among different tissues of pike barb. For the pike barb samples, the highest rates were found on gills, too. The differences were especially on Al, Cd, Mn, Fe, Co, Ni, Zn, As and Se values with the concentration of 674.71, 126290, 42.631, 30731.9, 18.052, 123.747, 511.457, 143.645, 258.389 µg g-1 for the liver samples. The second highest metal concentrations were found in the liver. Cd and Pb were the most abundant metals in the muscles with the concentrations of 0.524 and 2.532 µg g-1, respectively. With the concentration of 18497.25 µg g-1, heavy metal values in muscle exhibited lower rates than the amounts found in pike barb gills and liver, except K.

The total concentrations of Li, Na, Mg, Al, K, Ca, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Ag, Cd, Pb and U in common carp were 1.293 (±0.270), 3013.621 (±507.339), 874.574 (±131.135), 102.094 (±40.903), 10580.74 (±982.242), 8117.244 (±4496.401), 21.004 (±1.795), 6.781 (±1.267), 2186.789 (±1017.546), 1.453 (±0.615), 5.666 (±4.199), 25.638 (±2.770), 124.822 (±36.402), 9.997 (±5.369), 36.677 (±11.651), 0.066 (±0.056), 0.019 (±0.013), 1.026 (±0.207), 0.055 (±0.004) µg g-1, respectively. For the pike barb the total concentrations were found as Li: 1.049 (±0.193), Na: 2901.3 (±382.0), Mg: 1458.3 (±381.3), Al: 94.1 (±53.8), K: 10892.6 (±1083.6), Ca: 25261.5 (±11556.4), Cr: 23.565 (±1.246), Mn: 10.964 (±3.363), Fe: 6426.4 (±2819.0), Co: 3.904 (±1.703), Ni: 23.449 (±12.251), Cu: 37.077 (±6.565), Zn: 143.930 (±43.760), As: 28.379 (±12.995), Se: 65.585 (±23.014), Ag: 10.541 (±10.538), Cd: 0.124 (±0.065), Pb: 1.255 (±0.205) and U: 1.049 (±0.193) µg g-1 in this study.

Zafarzadeh et al (2018) reported that the accumulation of heavy metal concentrations in common carp muscle samples as Zn: 120.90 (477), Cd: 0.02 (0.14), Pb: 5.84 (21.86) and Cu: 7.92 (39.43) µg g-1. Bat & Öztekin (2018) were found that Zn was the highest in muscles and livers of C. carpio Altınkaya Dam Lake of Samsun Province as 9.85 and 25 µg g-1, respectively. Cu showed also high concentrations on the Common carp muscle and liver samples 0.90 and 7.6 µg g-1, respectively. Metal concentrations in carp from Euphrates river were found in as: 124.8(291), Cd: 1,29(2,02), Cu: 0,78 (0,97, Pb: 17.93(38.76), and Zn: 12.33(18.04) ppm (Varol & Sünbül, 2017). Kaymak et al, (2021) were reported the higher concentrations of Cu, Fe, Zn, Pb and Cd in common carp gill samples from Sapanca Lake as 4.67, 201.18, 1414.15, 1.09 and 1.02 µg g-1, respectively. The higher concentrations of Cu, Fe, Zn and Cd values were also reported 20.76, 53.69, 31.06 and 0.32 in muscles of common carp; 100.81, 631.97, 603.75 and 1.69 µg g-1, respectively (Kaymak et al, 2021). Güldiren & Tekin-Ozan (2018) reported that the heavy metals concentration from Carp Seyhan Dam Lake (Adana) were found in muscle; Cd: 0.38, Cr: 4.06, Cu: 48.89, Fe: 875.21, Mn: 36.52, Mo: 0.38, Ni: 178.93, Pb: 1.7, Se: 5.9, and Zn: 1129.36, liver; Cd: 45.86, Cr: 30.15, Cu: 210.51, Fe: 1048.89, Mn: 14.87, Mo: 2.41, Ni: 105.83, Pb: 1.84, Se: 12.72, and Zn: 2554.08, gill; Cd: 1.46, Cr: 3.43, Cu: 12.17, Fe: 745.61, Mn: 36.77, Mo: 0.51, Ni: 127.24, Pb: 1.72 Se: 7.7, and Zn: 1872.38 µg g-1. In this study the results of heavy metal total concentrations on common carp as mean (max.); Li: 1.293 (3.486), Na: 3013.6 (6267.6), Mg: 874.6 (1859.4), Al: 102.1 (518.9), K: 10659.9 (18219.9), Ca: 8117.2 (52249.9), Cr: 21.004 (36.119), Mn: 6.782 (17.901), Fe: 2186.8 (12059.7), Co: 1.454 (7.508), Ni: 5.666 (48.160), Cu: 25.638 (47.197), Zn: 124.822 (386.821), As: 9.997 (58.204), Se: 36.677 (152.023), Ag: 0.067 (0.680), Cd: 0.019 (0.136), Pb: 1.026 (2.800), U: 0.055 (0.087) µg g-1. According the mean concentrations of heavy metals on this study, K is the highest and Cd is the lowest values in all tissues of common carp samples. The highest mean concentration showed on K and Ca in gill samples. A total heavy metals concentration was found in this study K>Ca>Na>Fe>Mg>Zn>Al>Se>Cu>Cr>As>Mn>Ni>Co> Li>Pb>Ag>U>Cd. Moreover, there is a significant positive correlations between Al and Li; Ca and Mn; Mn and Al; Fe and Mg, Ca; Co and Mg, Fe, Ca, Ni and Mg, Ca, Fe; Cu and Mg, Ca, Fe, Co, Ni, Zn and Ca; Fe, Co and Ni, As and Mg, Ca, Fe, Co, Ni, Zn; Se and Ca, Fe, Co, Ni; Cd and Al, Mn found in carp tissues ($p<0.01$).

Varol & Sünbül (2018) were reported maximum values of As, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn in Pike barb muscle samples from Keban Dam Lake as 297.6, 2.52, 1.81, 1.58, 2.16, 17.0, 1.49, 1.55, 133.9 and 14.6 µg g-1,

respectively. In comparison to the other fish species, pike barb and common carp muscle samples had the highest amount of Co values (Varol & Sünbül, 2018). Heavy metal trainings obtained from L. esocinus samples taken from the Euphrates river showed values as Cu: 0.52, Fe: 16.08 and Zn: 9.01 µg g-1 (Düşükcan et al, 2017). In this study the results of heavy metal total concentrations on common carp as mean (max.); Li: 1.049 (2.932), Na: 2901.3 (5353.0), Mg: 1458.3 (4552.8), Al: 94.1 (674.7), K: 10892.6 (18497.3), Ca: 25261.5 (126290.0), Cr: 23.565 (31.762), Mn: 10.964 (42.631), Fe: 6426.4 (30731.9), Co: 3.904 (18.053), Ni: 523.449 (123.747), Cu: 37.077 (93.714), Zn: 143.930 (511.457), As: 28.379 (143.645), Se: 65.585 (258.390), Ag: 10.541 (126.464), Cd: 0.124 (0.524), Pb: 1.255 (2.532), U:1.049 (2.932) µg g-1. Analyses of the heavy metal levels in all tissues of fish samples showed that, on average, Ca is the highest and U is the lowest. The highest mean concentrations of Fe and Ca were found in the Pike barb gill samples. A total heavy metals concentration was found in this study Ca>K>Fe>Na>Mg>Zn>Al>Se>Cu>As>Cr>Ni>Mn>Ag> Co>Pb>Li>Cd>U. In addition, the pike barb tissues also showed significant positive correlations between Mg and Na; Al and Mg; Ca and Li, Na, Mg; Mn and Mg, Al; Fe and Li, Na, Mg, Ca; Co and Li, Na, Mg, Ca, Fe; Ni and Na, Mg, Ca, Fe, Co; Cu and Li, Na, Ca, Fe, Co, Ni; Zn and Na, Mg, Ca, Fe, Co, Ni, Cu; As with Li, Na, Mg, Ca, Fe, Co, Ni, Cu and Zn; Se with Li, Na, Mg, Ca, Fe, Cu, Ni, Cu, Zn and as; Ag with Li, Ca, Fe, Co, Ni, Cu, Zn, As and Se ($p<0.01$). As a result, heavy metal accumulation levels in various tissues of fish captured in the Karasu River are an extremely high level when compared to previous researches in the literature. In addition, that; its dramatically increase according to Human health risk assessment of heavy metals in muscle showed that the estimated daily and weekly intakes (EDI-EWI) for Ni, Zn, As, Cd, and U were above the reference doses for oral exposure (Table 4). The mean concentrations of heavy metals analyzed in the muscle of common carp were higher than the maximum permitted concentrations proposed by FAO (1983). Likely, the THQ showed that for health risk through ingestion, Mn (common carp and pike barb) had the lowest THQ value and Cr (common crap), Cr, Ni, and Ag (pike barb) had the highest potential for risk with a value >1.

Table 1. The heavy metal concentrations in different tissues of Common Carp (µg g^{-1}).

	Mean.±SE (Max.-Min.)			
	Common Carp			Total
	Muscle	Liver	Gill	
Li	1.435±0.732 (0.204-3.486)	1.262±0.380 (0.620-2.338)	1.181±0.330 (0.559-2.101)	1.293±0.270 (0.204-3.486)
Na	1380.2±122.4^{a} (1089-1680)	2697.7±252.6^{a} (2209-3402)	4962.9±750.5^{b} (3620.4-6267.6)	3013.6±507.3 (1089.0-6267.6)
Mg	841.6±76.9^{ab} (678.7-1020)	496.5±55.788^{a} (339.6-582.2)	1285.7±275.3^{b} (700.4-1859.4)	874.6±131.1 (339.6-1859.4)
Al	153.7±122.3 (9.505-518.9)	49.0±12.808 (24.7-83.7)	103.6±38.2 (51.8-216.8)	102.1±40.9 (9.505-518.9)
K	14395.7±1351.3^{b} (11917-18220)	9253.5±714.4^{a} (7576-11043)	8330.5±1075.3^{a} (6087.1-10581)	10659.9±982.2 (6087.1-18219.9)
Ca	1237±272.4^{a} (516.9-1808)	794.4±167.2^{a} (553.7-1264.7)	22320.2±11018.1^{b} (2377.2-52250)	8117.2±4496.4 (516.9-52249.9)
Cr	18.687±2.355 (13.857-24.306)	20.137±2.361 (15.717-26.793)	24.188±4.350 (17.187-36.119)	21.004±1.796 (13.858-36.119)
Mn	6.642±3.77 (1.937-17.9)	5.958±1.133 (2.845-8.270)	7.746±1.281 (4.729-10.116)	6.782±1.268 (1.937-17.901)
Fe	486.2±135.9^{a} (190-842.9)	618.2±116^{a} (408.8-896.1)	5456.0±2451.7^{b} (891.3-12059.7)	2186.8±1017.5 (190.0-12059.7)
Co	0.371±0.143^{a} (0.035-0.653)	0.496±0.066^{a} (0.310-0.606)	3.493±1.433^{b} (1.305-7.508)	1.454±0.615 (0.035-7.508)
Ni	0.000±0.000 (0.000-0.000)	0.000±0.000 (0.000-0.000)	16.999±11.391 (0.000-48.160)	5.666±4.199 (0.000-48.160)
Cu	21.658±2.930 (14.777-28.273)	26.182±2.57 (22.46-33.46)	29.074±7.742 (10.639-47.197)	25.638±2.771 (10.639-47.197)
Zn	25.675±0.558^{a} (24.34-27.068)	113±37.477^{ab} (68.78-225.18)	235.772±75.795^{b} (84.560-386.821)	124.822±36.402 (24.340-386.821)
As	1.589±1.274^{a} (0.000-5.343)	0.768±0.43^{a} (0.000-1.953)	27.635±12.636^{b} (2.776-58.204)	9.997±5.369 (0.000-58.204)
Se	18.401±1.847 (14.09-21.667)	23.023±13.574 (3.859-62.522)	68.607±28.145 (31.772-152.023)	36.677±11.651 (3.859-152.023)
Ag	0.17±0.170 (0.000-0.680)	0.029±0.029 (0.000-0.114)	0.002±0.002 (0.000-0.007)	0.067±0.056 (0.000-0.680)
Cd	0.034±0.034 (0.000-0.136)	0.000±0.000 (0.000-0.000)	0.024±0.024 (0.000-0.095)	0.019±0.013 (0.000-0.136)
Pb	1.357±0.618 (0.242-2.8)	0.898±0.106(0.689-1.191)	0.824±0.143 (0.567-1.116)	1.026±0.207 (0.242-2.800)
U	0.048±0.004 (0.037-0.057)	0.067±0.008(0.051-0.087)	0.049±0.006 (0.035-0.059)	0.055±0.004 (0.035-0.087)

Horizontally. letters a. b and c show statistically significant differences ($p < 0.05$). Vertically. letters x. y. z and w show statistically significant differences ($p < 0.05$). nd: not detected

Table 2. The heavy metal concentrations in different tissues of Pike barb (µg g^{-1}).

	Mean.±SE (Max.-Min.)			
	Pike barb			Total
	Muscle	Liver	Gill	
Li	0.735±0.067 (0.596-0.879)	0.900±0.169 (0.603-1.382)	1.513±0.513 (0.735-2.932)	1.049±0.193 (0.596-2.932)
Na	1589.3±96.1 (1384.7-1826.7)	2601.0±192.8 (2200.6-3121.5)	4513.7±297.3 (3978.6-5353.0)	2901.3±382.0 (1385-5353.0)
Mg	885.3±100.6 (697.7-1078.8)	453.1±47.3 (325.8-554.3)	3036.6±557.1 (2111.2-4552.8)	1458.3±381.3(325.8-4552.8)
Al	30.5±6.8 (18.5-49.7)	24.7±4.2 (16.9-36.7)	227.0±151.5 (31.0-674.7)	94.1±53.8 (16.91-674.7)
K	15442.7±1392.8 (12700.8-18497.3)	8840.6±706.0 (6777.5-9976.6)	8394.5±305.7 (7767.3-9231.6)	10892.6±1083.6 (6777-18497.3)
Ca	1881.2±428.0 (1320.3-3137.9)	858.9±165.1 (572.2-1218.7)	73044.4±18085.3 (45699.8-126290.0)	25261.5±11556.4 (572.2-126290.0)
Cr	24.033±2.859^{a} (18.004-31.762)	23.385±2.587^{ab} (16.178-27.909)	23.275±1.447^{ab} (20.262-26.193)	23.565±1.246 (16.178-31.762)
Mn	3.595±0.523 (2.460-4.887)	6.384±0.818 (5.208-8.764)	22.914±7.124 (8.629-42.631)	10.964±3.363 (2.460-42.631)
Fe	523.9±114.9 (351.1-860.1)	678.1±91.6 (488.7-927.2)	18077.1±4417.9 (10158.6-30731.9)	6426.4±2819.0 (351.1-30731.9)
Co	0.336±0.063 (0.188-0.487)	0.341±0.035 (0.258-0.420)	11.035±2.545 (5.944-18.053)	3.904±1.703 (0.188-18.053)
Ni	0.000±0.000 (0.000-0.000)	0.000±0.000 (0.000-0.000)	70.346±23.480 (21.610-123.747)	23.449±12.251 (0.000-123.747)
Cu	22.130±2.218 (19.401-28.734)	28.436±7.855 (17.343-51.229)	60.665±11.077 (47.504-93.714)	37.077±6.565 (17.343-93.714)
Zn	23.727±4.522 (13.274-34.246)	100.241±27.64 (31.580-157.411)	307.824±76.641 (148.345-511.457)	143.930±43.760 (13.274-511.457)
As	4.130±2.154 (0.000-9.220)	1.127±0.667 (0.000-2.910)	79.881±22.906 (35.015-143.645)	28.379±12.995 (0.000-143.645)
Se	9.191±2.536 (4.471-15.599)	29.113±9.652 (4.519-51.218)	158.450±36.720 (100.422-258.390)	65.585±23.014 (4.471-258.390)
Ag	0.000±0.000 (0.000-0.000)	0.000±0.000 (0.000-0.000)	31.622±31.614 (0.000-126.464)	10.541±10.538 (0.000-126.464)
Cd	0.000±0.000 (0.000-0.000)	0.373±0.125 (0.000-0.524)	0.000±0.000 (0.000-0.000)	0.124±0.065 (0.000-0.524)
Pb	0.672±0.306 (0.238-1.550)	1.513±0.411 (0.561-2.532)	1.578±0.176 (1.199-2.048)	1.255±0.205 (0238-2.532)
U	0.735±0.067 (0.596-0.879)	0.900±0.169 (0.603-1.382)	1.513±0.513 (0.735-2.932)	1.049±0.193 (0.596-2.932)

Horizontally. letters a. b and c show statistically significant differences ($p < 0.05$). Vertically. letters x. y. z and w show statistically significant differences ($p < 0.05$). nd: not detected

Table 3. Pearson correlation coefficients between heavy metal concentrations.

		Pike Barb																		
		Li	Na	Mg	Al	K	Ca	Cr	Mn	Fe	Co	Ni	Cu	Zn	As	Se	Ag	Cd	Pb	U	Mo
Common Carp	Li	1	0.692*	0.401	0.054	-0.401	**0.765****	0.038	-0.009	**0.741****	**0.710****	0.663*	**0.755****	0.678*	**0.736****	**0.773****	**0.888****	-0.284	0.163	0.226	
	Na	-0.280	1	**0.731****	0.381	-0.655*	**0.887****	-0.085	0.579*	**0.885****	**0.884****	**0.802****	**0.844****	**0.882****	**0.850****	**0.900****	0.584*	-0.147	0.418	0.168	
	Mg	0.016	0.397	1	**0.826****	-0.303	**0.808****	-0.028	**0.849****	**0.827****	**0.820****	**0.893****	0.678*	**0.782****	**0.789****	**0.847****	0.412	-0.438	0.220	-0.379	
	Al	**0.738****	-0.229	0.055	1	-0.268	0.419	-0.165	**0.852****	0.463	0.448	0.668*	0.310	0.514	0.421	0.557	0.095	-0.222	0.128	-0.619*	
	K	-0.293	-0.438	-0.114	-0.103	1	-0.419	0.180	-0.435	-0.428	-0.429	-0.401	-0.433	-0.506	-0.386	-0.512	-0.214	-0.221	-0.439	-0.104	
	Ca	0.127	0.405	**0.893****	-0.082	-0.421	1	0.095	0.485	**0.998****	**0.995****	**0.941****	**0.903****	**0.935****	**0.991****	**0.966****	**0.795****	-0.368	0.189	-0.003	
	Cr	-0.378	0.500	-0.166	-0.184	-0.012	-0.196	1	-0.113	0.091	0.099	0.059	0.068	0.016	0.100	0.042	0.192	-0.118	0.129	0.434	
	Mn	0.560	0.080	0.406	**0.758****	-0.226	0.292	-0.223	1	0.518	0.529	0.607*	0.365	0.526	0.460	0.566	-0.063	-0.217	0.360	-0.415	
	Fe	0.138	0.426	**0.891****	-0.056	-0.453	**0.998****	-0.195	0.330	1	**0.997****	**0.958****	**0.901****	**0.950****	**0.993****	**0.974****	**0.784****	-0.350	0.195	-0.030	
	Co	0.166	0.480	**0.867****	-0.068	-0.442	**0.987****	-0.112	0.317	**0.988****	1	**0.945****	**0.884****	**0.940****	**0.993****	**0.961****	**0.755****	-0.364	0.190	-0.031	
	Ni	0.163	0.348	**0.860****	-0.122	-0.363	**0.986****	-0.239	0.288	**0.981****	**0.980****	1	**0.835****	**0.943****	**0.947****	**0.968****	**0.744****	-0.332	0.152	-0.199	
	Cu	0.007	0.349	**0.620***	-0.330	-0.101	**0.719****	-0.227	0.199	**0.720****	**0.750****	**0.788****	1	**0.921****	**0.878****	**0.893****	**0.784****	-0.128	0.371	0.103	
	Zn	-0.056	0.677*	0.705*	-0.243	-0.489	**0.787****	0.036	0.271	**0.795****	**0.806****	**0.780****	0.650*	1	**0.938****	**0.951****	**0.764****	-0.085	0.285	0.003	
	As	0.057	0.471	**0.907****	-0.120	-0.421	**0.985****	-0.196	0.286	**0.984****	**0.966****	**0.966****	0.691*	**0.832****	1	**0.955****	**0.806****	-0.366	0.139	-0.022	
	Se	0.360	0.237	0.644*	-0.002	-0.547	**0.875****	-0.150	0.137	**0.867****	**0.868****	**0.864****	0.520	0.569	**0.822****	1	**0.762****	-0.333	0.286	-0.055	
	Ag	0.033	-0.272	-0.112	-0.111	0.293	-0.143	-0.377	-0.328	-0.160	-0.144	-0.134	0.004	-0.262	-0.166	-0.187	1	-0.174	-0.024	0.189	
	Cd	0.483	0.056	0.341	**0.725****	0.046	0.084	-0.303	**0.826****	0.108	0.089	0.080	-0.006	0.182	0.140	-0.094	-0.156	1	0.197	0.365	
	Pb	0.526	-0.264	0.060	0.672*	0.298	-0.117	0.052	0.627*	-0.106	-0.079	-0.082	0.025	-0.185	-0.190	-0.078	-0.279	0.546	1	0.236	
	U	-0.224	-0.025	-0.515	-0.062	-0.100	-0.419	0.281	0.029	-0.386	-0.383	-0.412	-0.068	-0.356	-0.460	-0.391	-0.279	-0.315	0.105	1	
	Mo	-0.227	-0.086	-0.203	-0.112	0.035	-0.139	-0.127	-0.019	-0.131	-0.141	-0.123	0.017	-0.109	-0.169	-0.256	-0.107	-0.132	-0.149	0.358	1

**Correlation is significant at the 0.01 level (2-tailed). *Correlation is significant at the 0.05 level (2-tailed).

Table 4. Summary of health risk assessment for metals in fishes from Karasu River.

Metal	THA*	THA[b]	TGA[c]	Total common carp HHA[d](HGA)[e] / THQ	Total pike barb HHA[d](HGA)[e] / THQ
Li				418 (60) / 0.5	411 (59) /0.04
Cr	–	–	–	5056 (722) / 3.4	4447 (635) / 3.03
Mn	980[g]	68.600	9800[h]	2506 (358) / 0.04	5968 (853) / 0.1
Co	–	–	–	1051 (150) / 0.1	2527 (361) / 0.3
Ni	35[g]	2450	350[f]	6742 (963) / 0.7	17325 (2475) / 1.8
Cu	3500[a]	245000	35000	6608 (944) / 0.3	13120 (1874) / 0.7
Zn	7000[a]	490000	70000	54155 (7736) / 0.4	71604 (10229) / 0.5
As				8149 (1164)	20110 (2873)
Se				21283 (3040)	36175 (5168)
Ag				95 (14) / 0.04	17705 (2529) / 7.2
Cd	7[a]	490	70	19 (3) / 0.04	73 (10) / 0.2
Pb	25[a]	1750	250	392 (56) / 0.2	355 (51) / 0.2
U				12 (2)	9 (1)

* Provisional Permissible Tolerable Weekly Intake (PTWI) in lg/week/kg body weight. Mean weekly fish consumption in Turkey is 0.14 kg per person (FAO. 2005).

[a] (FAO/WHO. 2004).

[b] PTWI for 70 kg adult person (lg/week/70 kg body weight).

[c] PTDI. permissible tolerable daily intake (lg/day/70 kg body weight).

[d] EWI. estimated weekly intake in lg/week/70 kg body weight.

[e] EDI. estimated daily intake in lg/day/70 kg body weight.

[f] WHO recommends a TDI (tolerable daily intake) of 5 lg/day/kg body weight. i.e. 350 lg/day for a 70-kg person (WHO. 1993).

[g] Calculated for a week (lg/week/kg body weight).

[h] EPA recommends a RfD (reference dose) of 0.14 mg/day/kg body weight. i.e. 9800 lg/day for a 70-kg person (EPA. 2008).

CONCLUSIONS

In this study, the accumulation of metals detected in fish samples obtained from the Karasu River was compared with the acceptable values determined by national and international standards. It was determined that some metals in fish tissues were above acceptable values. This negative situation originated by agricultural fields, mining and iron-steel factory along the Karasu River. Especially the wastes of the mines and iron-steel factory in the region, pesticides and fertilizers used in agricultural activities contain heavy metals. These metals reach The Karasu River especially through climate patterns (rains, snow melting etc.). In addition, the sewage and household wastes of the settlements around the river are mixed into the river. Especially, the local people living around the river, as well as the authorities in the region, should be made aware of these issues; the discharge of mine and iron-steel factory wastes into the aquatic ecosystem should be avoided; and they should be informed about the contents of fertilizers and the treatment of domestic waste.

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ARAŞTIRMA MAKALESİ **RESEARCH PAPER**

Effect of Salt Stress (Potassium Chloride) on the Ecological and Physiological Characteristics of Safflower (*Carthamus tinctorius* L.) Varieties

Sema LEBLEBİCİ[1,2*] Şifanur SÜLÜŞ[3] Gülçin ÇETİN KILIÇASLAN[1]
[1*]*Faculty of Arts and Sciences, Bilecik Şeyh Edebali University, Bilecik, Turkey*
[2]*Biotechnology Application and Research Center, Bilecik Şeyh Edebali University, Bilecik, Turkey*
[2]*Institue of Science, Bilecik Şeyh Edebali University, Bilecik, Turkey*

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: https://orcid.org/0000-0002-3762-6408
: https://orcid.org/0000-0002-4326-6568
: https://orcid.org/0000-0002-9625-224X

***Corresponding author's:**
Sema LEBLEBICI
Bilecik Şeyh Edebali University, Faculty of Arts and Sciences, Department of Molecular Biology and Genetics, Bilecik, Turkey
✉: leblebicisema@gmail.com

Abstract: Salinity, which is a significant abiotic stress factor, is among the most important factors that limit product quality and yield. In this study investigated phenological and physiological changes that occurred in three different safflower varieties that were exposed to KCl stress at concentrations of 0, 50, 100 and 150 mM. It was found that, based on increasing salt concentrations, the most significant decrease was in all phenological parameters at the concentrations of 100 and 150 mM KCl. The SOD and CAT activities showed a significant increase at high salt concentrations in the Balcı and Dinçer varieties. The APX and GR activities showed a parallelism in all safflower varieties, and they showed a significant increase based on increased concentration at the applications of 100 and 150 mM KCl. Among the three safflower varieties, the most resistant variety to salt application was Dinçer, while the most sensitive one was Remzibey. The Balcı variety was closer to the Dinçer variety in terms of its tolerance against salt stress.

Keywords: Antioxidant enzymes, phenological parameters, safflower, salt stress, seedling.

Tuz Stresinin (Potasyum Klorür) Aspir (*Carthamus tinctorius* L.) Çeşitlerinin Ekolojik ve Fizyolojik Özelliklerine Etkisi

***Sorumlu yazar:**
Sema LEBLEBICI
Bilecik Şeyh Edebali Üniversitesi, Fen-Edebiyat Fakültesi, Moleküler Biyoloji ve Genetik Bölümü, Bilecik, Türkiye
✉: leblebicisema@gmail.com

Öz: Önemli bir abiyotik stres faktörü olan tuzluluk, bitkilerde ürün kalitesini ve verimini ciddi şekilde sınırlandırmaktadır. Bu çalışmada, 0, 50, 100 ve 150 mM KCl uygulanan üç farklı aspir çeşidinde meydana gelen fenolojik ve fizyolojik değişiklikler incelenmiştir. Artan tuz konsantrasyonlarına bağlı olarak en önemli azalmanın tüm fenolojik parametrelerde 100 ve 150 mM KCl konsantrasyonlarında olduğu belirlenmiştir. Balcı ve Dinçer çeşidinde yüksek tuz konsantrasyonlarında SOD ve CAT aktiviteleri önemli bir artış göstermiştir. APX ve GR aktiviteleri tüm aspir çeşitlerinde paralellik göstermiş, 100 ve 150 mM KCl uygulamasında önemli bir artış göstermiştir. Üç Aspir çeşidi karşılşatırıldığında tuz stresine en dayanıklı çeşidin Dinçer, en hassas çeşidin Remzibey olduğu; Balcı çeşidinin ise tuz stresine tolerans bakımından Dinçer çeşidine daha yakın olduğu tespit edilmiştir.

Anahtar kelimeler: Antioksidan enzimler, aspir, bitki gelişimi, fenolojik parametreler, tuz stresi.

INTRODUCTION

Safflower, which is an important industrial plant, has an important potential in bringing barren and poor areas into production (Danicic et al., 2016). Safflower is one of the alternative products to be evaluated in dry and irrigated farming areas due to its better adaptation to arid regions, tolerance to high and low temperatures and salt stress, and high competition against weeds (Danicic et al., 2016; El-Shourbagy et al., 2017; Gengmao et al., 2015).

Soil salinity is an important abiotic stress factor that causes a decrease in agricultural production and is effective in the distribution of wild species. The salt tolerances of plant species and even varieties belonging to the same species show great variation (Elouaer and Hannachi, 2012). High amounts of salt negatively affect

seed germination, plant morphology, early development stages and later stages of growth (Acosta-Motos et al., 2017). Salinity reduces the amount of water in the seed by first removing the osmotic potential, and in the second stage, it causes a change in enzyme activity along with toxicity (Elouaer and Hannachi, 2012). It prevents plant development by affecting cell division and growth. It causes a decrease in mitotic activity and cell division rate in the root and stem in plants (Fahad et al., 2015). In the early development stage of the embryo, it directly affects the structural organization or synthesis of proteins. Besides, high KCl concentrations affect water and ion transport, disrupting plant nutrient status, ionic balance and physiological processes (Golldack et al., 2014). The growth and development of the plant is significantly affected, the growth slows down, and this process results in plant death (Elouaer and Hannachi, 2012).

Salinity causes oxidative stress through the increased formation of reactive oxygen species (ROS) like other abiotic stresses (Singh et al., 2015; Sourour et al., 2014). Excessive accumulation of ROS is highly cytotoxic due to their reactivity with various basic cellular components (Sharma et al., 2012; Sourour et al., 2014). High ROS concentrations adversely affect intracellular ionic homeostasis, activation of proteases and endonucleases, cell membrane integrity, protein synthesis, enzyme activities and photosynthesis efficiency (Gengmao et al., 2015; Golldack et al., 2014; Hussain and Al-Dakheel, 2018). This collective effect can lead to cell death (Kumari et al., 2015). Therefore, plants activate enzymatic and non-enzymatic antioxidant systems to prevent excessive ROS accumulation. The most common enzymatic antioxidant systems are superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX). These enzymes work in different parts of the cell and catalyse various reactions when cells are exposed to stress (Çulha Erdal & Çakirlar, 2014; El-Shourbagy et al., 2017; Golldack et al., 2014). SOD converts free oxygen radicals produced by xanthine oxidase into oxygen and hydrogen peroxide. SOD is recognized as a crucial intracellular antioxidant defence against free radicals (Stephenie et al., 2020). Catalase, an enzymatic antioxidant, plays a critical role in preventing cellular damage by efficiently reducing hydrogen peroxide (H_2O_2) to water and oxygen (Sharma et al., 2012). Ascorbate peroxidase is a central component of the AsA-GSH cycle, and it plays a crucial role in controlling intracellular ROS levels. APX uses two AsA molecules to reduce H_2O_2 to water and two monodehydroascorbate (MDHA) molecules (Sharma & Dubey, 2016). Glutathione reductase (GR), an enzyme linked to NAD(P)H, catalyses the reduction of GSSG to GSH, thereby maintaining a high cellular reduced/oxidized glutathione (GSH/GSSG) ratio. (Couto et al., 2016).

The purpose of this study is to i) evaluate the effect of salt (KCl) stress on three varieties of safflower plants at the germination and seedling stages, ii) identify salinity-tolerant safflower varieties and iii) determine some phenological and physiological characteristics of safflower varieties with different salt concentrations.

MATERIALS AND METHODS

***Phenological Parameters*:** This study used three different Safflower (*Carthamus tinctorius* L.) varieties as Balcı, Dinçer and Remzibey. The seeds to be used in the experiments were randomly selected. The selected seeds were sterilized by keeping them in 10% sodium hypochlorite (NaOCl) for five minutes (Jabeen & Ahmad, 2012). To conduct the germination experiments, a control group containing distilled water and groups containing 50, 100 and 150 mM KCl were prepared. The experiments were carried out with three replications. The seeds that were sown were kept in a 16-hour light/8-hour night photoperiod at 25°C and 60% humidity in a climatized room for 21 days. At the end of the 21 days, for the germinated individuals, the germination rate, root and shoot lengths, root and shoot fresh-dry weights, root and shoot biomasses and seed vigour indices were determined (Figure 1).

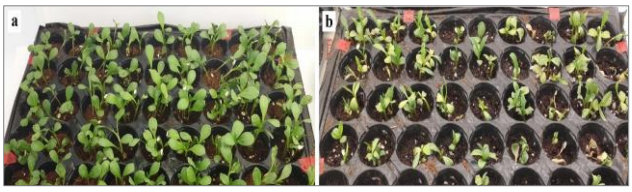

Figure 1. Plant samples **a)** Control groups, **b)** 150 mM KCl applied groups.

***The total protein concentration and determination of antioxidant enzyme activities*:** The total protein contents in the leaf samples were determined using the Bradford (1976) method. To determine the total protein contents, 20 µl of the enzyme solution was put onto 780 µl dH_2O. 200 µl of 5 X Bradford solutions was added to this mixture. The mixture was kept in the dark and at room temperature for 5 minutes before the absorbance values were measured in triplicates in the spectrophotometer at the wavelength of 595 nm. SOD activity was spectrophotometrically determined based on the method depending on photoreduction of Nitro blue tetrazolium (NBT) (Beyer & Fridovich, 1987). CAT activity in the leaf samples was determined according to the method reported by Tepe and Aydemir (2011), GR activity was determined according to the method reported by Rao et al. (1996), while APX activity was determined by using the method of Cervilla et al. (2007).

***Statistical analysis and evaluation of results*:** Experimental results with control groups and safflower cultivars exposed to three different concentrations of KCl (50, 100 and 150 mM) were statistically evaluated by using Tukey tests and one-way ANOVA (SPSS 21.0).

RESULTS

In the study, the results of three different KCl concentrations were compared with the control group of all three safflower cultivars. Experimental groups with statistically significant differences at the $p<0.05$ level were determined. Considering the germination rate of the safflower varieties used in the study, it was seen that this rate decreased based on increased KCl concentrations ($p<0.05$). When the varieties were compared, it was determined that the highest germination rate at the 100 mM and 150 mM salt concentrations was in the Dinçer variety (respectively 90%±4, 86%±3), while the lowest one was in the Balcı variety (respectively 88%±3, 82%±2) (Figure 2).

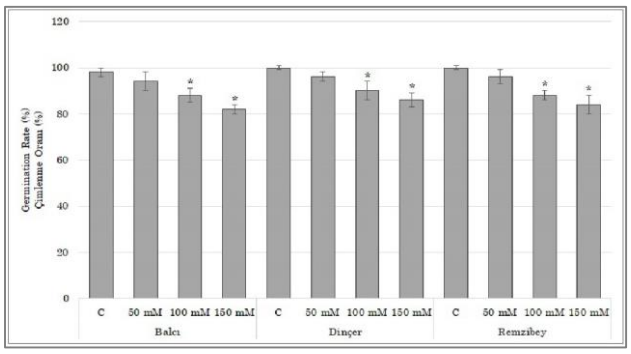

Figure 2. Seed germination rates for Safflower varieties (%)(*$p<0.05$).

In the comparison of the seed vigour index, the lowest seed vigour index was at the concentration of 150 mM KCl in Dinçer (75.8), while the highest was at 50 mM KCl in Remzibey (92). Among the varieties, the highest seed vigour index (92) was in Remzibey, while the lowest (75.8) was in Dinçer (Figure 3).

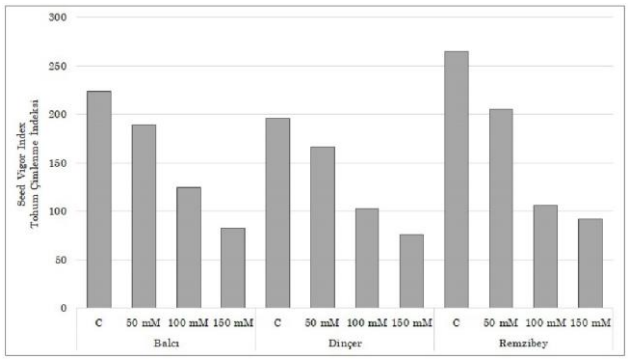

Figure 3. Seed vigor indices for Safflower varieties.

Based on increased salt concentrations, the root lengths of all varieties were shortened significantly ($p<0.05$). The Remzibey variety was the most affected by the KCl salt application, and it had the shortest root length at the 150 mM concentration (0.98±0.07 cm) (Figure 4). The shoot lengths of all cultivars decreased significantly, depending on the increasing salt concentrations ($p<0.05$). The Dinçer variety was the most affected by the KCl salt application, and it had the shortest shoot length at the 150 mM concentration (0.87±0.04 cm) (Figure 4). In all three safflower varieties, the reduction in the root and shoot lengths that occurred in the application of 100 mM and 150 mM KCl in comparison to the control group was found to be statistically significant ($p<0.05$) (Figure 4). It was determined that the root and shoot fresh-dry weight was negatively affected by the increased KCl concentration and decreased significantly ($p<0.05$). The decreases in the Balcı variety at the 100 and 150 mM salt applications in the root fresh weights (0.13±0.004 and 0.07±0.001 g), in the Dinçer variety at the 50, 100 and 150 mM salt applications in the root fresh and dry weights (respectively 0.23±0.004-0.04±0.001 g, 0.18±0.003-0.03±0.005 g, 0.05±0.002-0.01±0.003 g) and in the Remzibey variety at the 50 mM salt application in the root fresh weight (0.12±0.005 g) and at 100 and 150 mM in the root fresh and dry weight (respectively 0.05±0.004-0.02±0.001 g, 0.03±0.004-0.01±0.004 g) were found to be statistically significant ($p<0.05$) (Figure 5). The shoot fresh weight in the Balcı variety at 100 and 150 mM (0.67±0.01 and 0.52±0.03 g), the shoot fresh weight in the Dinçer variety at 50, 100 and 150 mM (1.47±0.04, 1.24±0.01 and 0.61±0.04 g) and the shoot fresh weight in the Remzibey variety at 100 and 150 mM (0.65±0.01 and 0.43±0.02 g) were significantly lower than those of the controls ($p<0.05$) (Figure 6).

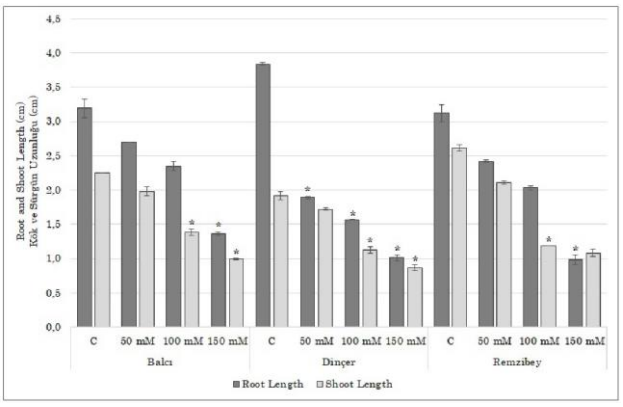

Figure 4. Root and shoot lengths for Safflower varieties (cm) (*$p<0.05$).

When the root and shoot biomasses of the control groups of the varieties were compared, the highest root (626.1±5.75 g ha^{-1}) and shoot (3521.7±42.26 g ha^{-1}) biomass was in the Dinçer variety, while the lowest root (321.7±5.50 g ha^{-1}) and shoot (2739.1±47.08 g ha^{-1}) biomass was in the Remzibey variety. The reduction in the

root and shoot biomass in the Balcı and Remzibey varieties at 100 and 150 mM (respectively 195.7±8.41, 132.8±6.41 g ha^{-1} and 126.8±4.87, 99.4±3.66 g ha^{-1}) and the reduction in the Dinçer variety in the root (respectively 300.3±7.51, 250.4±3.50 and 93.4±8.83 g ha^{-1}) and shoot biomass (2465.2±6.41, 2817.3±34.55 and 1408.7±26.48 g ha^{-1}) in all KCl concentrations were found to be statistically significant ($p<0.05$) (Figure 7).

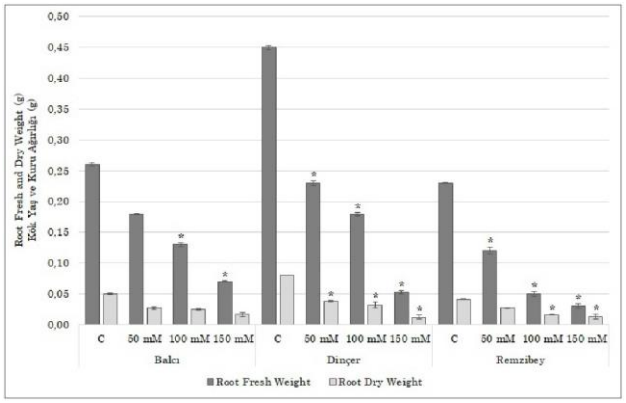

Figure 5. Root fresh and dry weights for Safflower varieties (g) (*$p<0.05$).

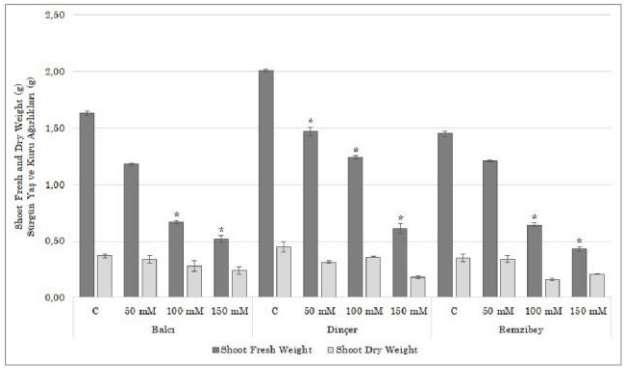

Figure 6. Shoot fresh and dry weights for Safflower varieties (g) (*$p<0.05$).

The SOD activity showed a significant increase at 100 and 150 mM KCl in the Balcı variety based on the increasing salt concentrations ($p<0.05$). As opposed to the Balcı variety, the Dinçer variety showed a decrease in SOD activity based on the increasing salt concentrations. The reduction in the Dinçer variety at 100 mM and 150 mM was statistically significant ($p<0.05$). The most significant increase in SOD in the Remzibey variety was determined at 150 mM (Figure 8a). In the Balcı variety, the CAT activity showed a decrease in comparison to the control as the salt concentration increased, and the reductions at 50 mM and 100 mM were statistically significant ($p<0.05$) (Figure 8b). In the Dinçer variety, there was a remarkable decrease in the CAT activity as the KCl concentration increased ($p<0.05$). While there was a significant decrease at 50 mM in the Remzibey variety in comparison to the control, a statistically significant increase was found at 100 mM and 150 mM KCl ($p<0.05$) (Figure 8b).

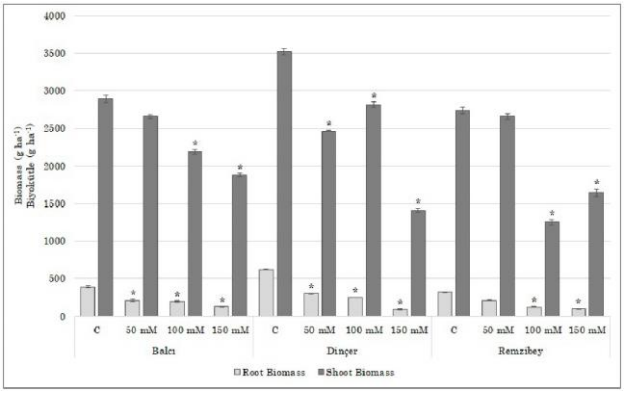

Figure 7. Root and shoot biomasses for Safflower varieties (g/ha) (*$p<0.05$).

In the comparison of the safflower varieties, it was determined that the APX and GR activities increased based on the increasing KCl concentrations (Figure 9a, 9b). The increase in the APX activity was significant at 100 and 150 mM in the Balcı variety and at all concentrations in the Dinçer variety. In the Remzibey variety, a significant increase was detected only at 150 mM KCl ($p<0.05$) (Figure 9a). In terms of the GR activity, the increases at 100 and 150 mM in the Balcı and Dinçer varieties and at 150 mM in the Remzibey variety were significant ($p<0.05$) (Figure 9b).

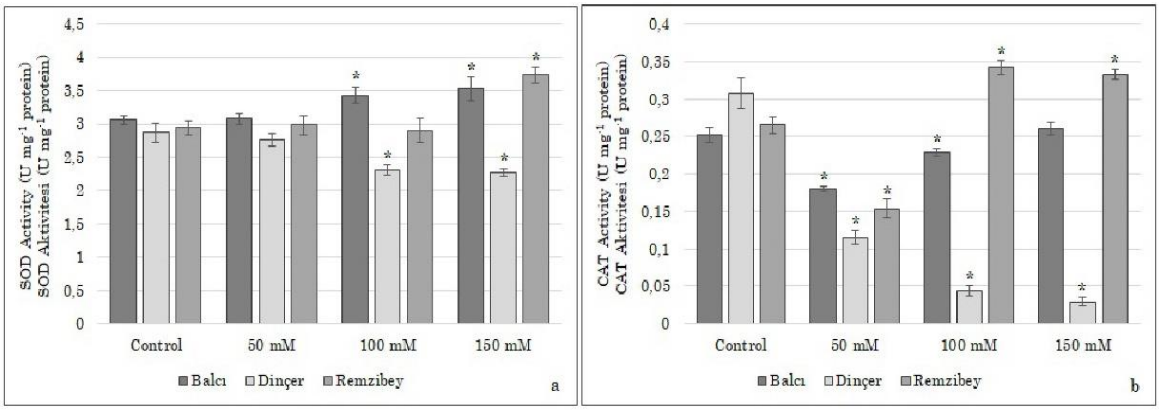

Figure 8. a) SOD and **b)** CAT activities for Safflower varieties (*$p<0.05$).

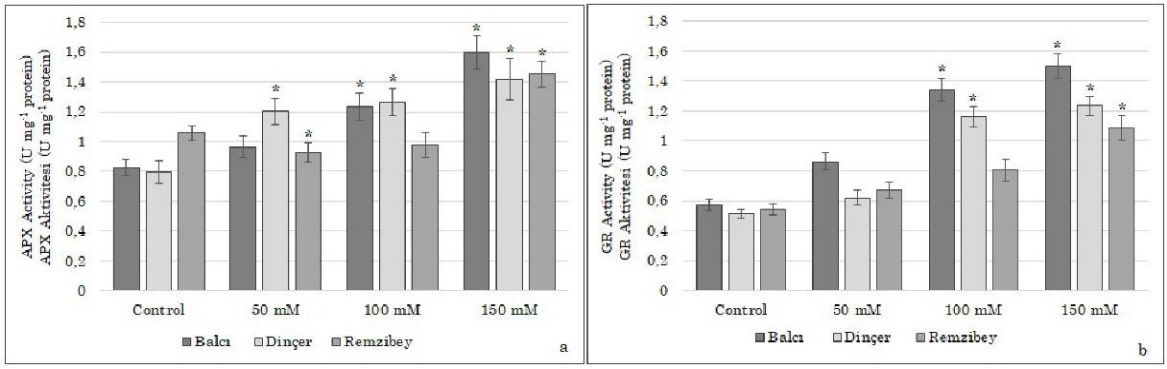

Figure 9. a) APX and **b)** GR activities for Safflower varieties (*p<0.05).

DISCUSSION AND CONCLUSIONS

Potassium chloride is the most widely used potassium source for agricultural crops, and Cl^- is considered an essential micronutrient for optimum growth (Zaman et al., 2012). However, KCl, which causes salinity, creates an osmotic potential outside the seed. This situation either inhibits water absorption or leads K and Cl to create a toxic effect. Thus, it causes a negative effect on seed germination. Salt tolerance in safflower is related to the ability to limit the uptake and transport of ions from the roots to the shoots. Safflower is a moderately salinity-tolerant species, but it has been reported to be sensitive to salinity at the germination stage (Elouaer and Hannachi, 2012). It has been reported that salinity-affecting germination causes insufficient imbibition, ion toxicity, inhibition of metabolic activity, imbalance in growth regulators or induction of oxidative stress and prevents germination of seeds (Ahanger et al., 2017; Liang et al., 2018). In our study, it was determined that the germination rate decreased based on the increase in the salt concentration in the Balcı, Dinçer and Remzibey varieties. In particular, a statistically significant decrease was detected in all three safflower varieties at 100 and 150 mM KCl ($p<0.05$). There are many studies in the literature about the effects of salt stress on the germination rate. Similar to our study, Chen et al. (2021) reported that 150 mM KCl and NaCl application reduced the seed germination rate in their study with sorghum (Chen et al., 2021). In the study conducted by Karimi et al. (2011), 0, 50, 100, 150 and 200 mM NaCl was applied on five different safflower genotypes, and a short-term toxicity experiment was performed. It was reported that the percentage of germination decreased due to the increase in concentration at all salt concentrations except the control (Karimi et al., 2011). In a study conducted with six safflower genotypes, five different NaCl concentrations were applied (0, -0.3, -0.5, -1 and -1.5 MPa), where the lowest germination rate was determined at -1.5 MPa (Khodadad, 2011).

In our study, the seed vigour index, which is another parameter related to germination, was compared among different varieties. In all three varieties, it was determined that the seed vigour index decreased at high salt concentrations, and the lowest seed vigour index was at the concentration of 150 mM KCl. Numerous studies in the literature have shown that salt stress significantly reduces seed vigour in many species, inhibiting germination and early seedling growth (Chen et al., 2021; Elouaer and Hannachi, 2012; Isik & Leblebici, 2016; Zaman et al., 2012).

Salt stress imposed during this period prevents plant growth by affecting cell division and elongation. It causes a decrease in mitotic activity and cell division rate in the root and stem. It inhibits the growth of the primary root system as a result of suppressing the cell cycle. Moreover, root hairs exposed to high salt concentrations become damaged and lose their activity (Sulus & Leblebici, 2020). In our study, Dinçer and Remzibey varieties were the most affected in the high KCl application. The decrease in the root and shoot length at 100 and 150 mM KCl in all three safflower varieties was found to be statistically significant in comparison to the control group ($p<0.05$). Similarly, it was determined that the root-shoot wet and dry weights decreased as a result of being negatively affected by the increasing KCl concentrations. As in other parameters, both root and stem biomass decreased based on the increasing KCl concentrations. The decrease in the root and shoot biomass of the three varieties at 100 and 150 mM KCl was found to be statistically significant ($p<0.05$). There are many studies in the literature on salt stress and phenological parameters. However, in many of these studies, NaCl has been used as a stress factor, and studies using KCl have been very rare. The results of studies in the literature have supported the data we obtained. In their study with sorghum, Chen et al. (2021) reported that 150 mM KCl application significantly reduced root length, shoot length, root fresh weight, shoot fresh weight, root dry weight and shoot dry weight by salt stress (Chen et al., 2021). In another study, 5 NaCl

concentrations of 0, -0.3, -0.5, -1 and -1.5 MPa were applied on six different safflower varieties. It was stated that root-shoot length and the root/shoot length ratio decreased significantly at -1.5 MPa (Khodadad, 2011). Five different safflower genotypes were used in the study of Karimi et al. (2011). 0, 50, 100 and 200 mM NaCl was applied on plant samples. It was reported that application of 100 and 200 mM KCl significantly reduced root-shoot length and root-shoot wet and dry weights (Karimi et al., 2011). In the study of Gengmao et al. (2015), 0, 50, 100 and 150 mM NaCl was applied on safflower seedlings for 30 days. The authors reported that the plant height, root length and plant dry weight of the samples were not affected at salt concentrations below 100 mM (Gengmao et al., 2015). Javed et al. (2014) used six safflower varieties in their study. They observed that salt stress significantly reduced the fresh and dry weights of the plant samples. They found that plant height and root length decreased depending on the increasing NaCl concentration (Javed et al., 2014). Studies with safflower have stated that root and stem biomass decreased at high salt concentrations, similar to the results of our study (Gengmao et al., 2015; Hussain & Al-Dakheel, 2018; Siddiqi et al., 2011; Zao et al., 2021).

Plants counteract stresses caused by changing ecological conditions by increasing their ROS production. Changes in ROS levels are controlled by the efficient work of antioxidant enzymes. Inadequate functioning of the antioxidant system leads to cellular degradation, occurrence of abnormalities in the metabolism, and ultimately, ROS-induced oxidative stress (Acosta-Motos et al., 2017; Ahanger et al., 2017; Golldack et al., 2014). In our study, depending on the increasing salt concentrations, the SOD activity increased in the Balcı and Remzibey varieties, but it decreased in the Dinçer variety. While the CAT activity decreased in the Balcı and Dinçer varieties, it increased in the Remzibey variety. The APX and GR activity increased in all varieties depending on the increasing salt concentrations. In particular, the increases at 100 and 150 mM KCl in the SOD, APX and GR activities and changes at all concentrations in the CAT activity were found to be statistically significant ($p<0.05$). In a study, 5 different NaCl solutions at concentrations of 0.06 mol L^{-1}, 0.12 mol L^{-1}, 0.18 mol L^{-1}, 0.24 mol L^{-1} and 0.30 mol L^{-1} were applied to two safflower varieties (TSF1 and SM). It was reported that SOD, CAT and APX activity increased in parallel with an increase in the NaCl concentrations. Additionally, it has been determined that the increase in the enzyme activities in the tolerant variety TSF1 was higher than that in the sensitive variety SM (Tian et al., 2019). Gengmao et al. (2015) applied 0, 50, 100 and 150 mM NaCl on safflower plants along 30 days. They reported that SOD and CAT activities increased significantly at all NaCl concentrations in comparison to the control. The authors stated that plant samples are resistant to medium-dose NaCl application (Gengmao et al., 2015). It was determined that SOD, CAT and POD activities were increased in safflower treated with 150 mmol L^{-1} NaCl (Siddiqi et al., 2011).

In a study which used the Arak, Isfahan, Horasan, C111, AC-Stirling and Saffire varieties of safflower, 5 different NaCl concentrations of 0, 50, 100, 150 and 200 mM were applied on the varieties. It was reported that SOD and APX activities differed between the genotypes. It was determined that the SOD and APX activity of the Isfahan variety increased at 200 mM, and the superior genotype was Isfahan (Golkar & Taghizadeh, 2018). In a study conducted with the Dinçer, Yenice and Remzibey varieties, it was stated that SOD activity increased significantly in Dinçer and Remzibey at 75 mM NaCl and higher salt concentrations. In the high-dose salt application, while the APX and GR activity of the Dinçer variety increased, the APX activities of the other varieties decreased. The lowest SOD, APX and GR activity was determined in the Remzibey variety (Çulha Erdal & Çakirlar, 2014). In the study by Karimi et al. (2011), five different NaCl concentrations (0, 50, 100, 150 and 200 mM) were applied on eight safflower varieties. It was reported that CAT activity decreased in most varieties grown under salt stress, and there were significant differences in the responses of the safflower varieties to salt stress (Karimi et al., 2011). In another study in which the Kairouan and Tazarka safflower varieties were used, 0 and 50 mM NaCl concentrations were applied on these varieties. It was reported that SOD, CAT and POD activity increased in Tazarka, and POD activity increased in Kairouan (Karray-Bouraoui et al., 2011). These examples in the literature have revealed that enzyme activities may differ in different varieties of the same species in their processes of coping with stress. In our study, in parallel with the literature, differences in SOD and CAT activities between varieties were determined.

As a result, the first visible effects of salt stress, which is naturally present in the soil or caused by the secondary effect of global warming, appear on morphological parameters. In this study, it was determined that the germination rate, seed vigour index, root-shoot length, root-shoot wet and dry weight and root-shoot biomass decreased depending on the increasing KCl concentrations in three different safflower varieties (Balcı, Dinçer and Remzibey). The change in the activities of antioxidant enzymes that are involved in the scavenging of ROS caused by osmotic stress is one of the most important factors for the plant to cope with stress. In our study, it was determined that the SOD, CAT, APX and GR activities increased in high salinity in all three safflower varieties. However, it was determined that there was a difference

between the Balcı and Dinçer varieties in terms of their SOD and CAT activities. Thus, it was demonstrated that different varieties of the same species may have different responses to stress. Considering the phenological and physiological results, it was determined that, among the three safflower varieties, the most resistant variety against the salt application was Dinçer, while the most sensitive one was Remzibey. The Balcı variety was closer to the Dinçer variety in terms of its tolerance against salt stress. In safflower cultivation in salty soils, the Dinçer variety may be recommended first, and the Balcı variety may be recommended as the second.

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ARAŞTIRMA MAKALESİ **RESEARCH PAPER**

The Effect of Different Cold Storage Period on Total Lipid Amount of *Tenebrio molitor* (Coleoptera: Tenebrionidae) Larvae

Evrim SÖNMEZ
Sinop University, Faculty of Education, Department of Mathematics and Science Education, Sinop, Turkey

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*: https://orcid.org/0000-0002-5412-5728

***Corresponding author's:**
Evrim SÖNMEZ
Sinop University, Faculty of Education, Department of Mathematics and Science Education, Sinop, Turkey
✉: esonmez@sinop.edu.tr

Abstract: The ever-increasing world population indicates that it is inevitable to consider insects such as *Tenebrio molitor*, which are used as live feed and even human food in some countries, as an alternative food source. Especially *T. molitor* larvae are a source of food with high nutritive value for including high lipid and protein. This study seeks answer to the question "will the insect continue to keep its lipid sources during the periods in which it is kept in the cold, or will it continue to use its energy sources since the physiological adaptations it develops are not enough?" The main material of this study was *T. molitor* cultures. Flour: wheat flour (250 g: 250 g) in a ratio of 1:1 was used as food. 25 g wheat germ and 5 g dry yeast was put in it. Larvae at stages 13-15th were grouped as control and trial groups and kept for 5, 10, 15 and 20 days at specified temperatures. The weights, total lipid content and percentages of the larvae whose storage period was over were determined. This study evaluates the total lipid amount and percentages of *T. molitor* larvae stored in refrigerator for different periods. Total lipid amount and percentages of the larvae stored in the cold for 5, 10 and 15 days were found to be higher when compared with the control group. A tendency to decrease was observed in larvae kept for 20 days. As a result, it is recommended for producers not to keep in the refrigerator for more than 15 days. Otherwise, it should be considered that there may be a decrease in important energy and food sources.

Keywords: Fat, low temperature, mealworm, rearing insects, temperature physiology.

Farklı Sürelerde Soğukta Depolamanın *Tenebrio molitor* (Coleoptera: Tenebrionidae) Larvalarının Toplam Lipid Miktarına Etkisi

***Sorumlu yazar:**
Evrim SÖNMEZ
Sinop Üniversitesi, Eğitim Fakültesi, Matematik ve Fen Bilimleri Eğitimi Bölümü, Sinop, Türkiye
✉: esonmez@sinop.edu.tr

Öz: Gittikçe artan dünya nüfusu *Tenebrio molitor* gibi canlı yem hatta bazı ülkelerde insan yiyeceği olarak kullanılan böcekleri alternatif besin kaynağı olarak değerlendirmemizin kaçınılmaz olduğunun habercisidir. Özellikle *T. molitor* larvaları fazlaca yağ ve protein içermesinden dolayı besleyici değeri yüksek bir besin kaynağıdır. Bu çalışma 'soğukta bekletildiği sürelerde böcek lipit kaynaklarını korumaya devam mı edecektir ya da geliştirdiği fizyolojik adaptasyonlar yeterli kalmayıp enerji kaynaklarını kullanmaya devam mı edecektir' sorularına cevap aramaktadır. Bu çalışmanın ana malzemesini *T. molitor* kültürleri oluşturdu. Besin olarak 1:1 oranında un:buğday unu (250 g:250 g) kullanıldı. İçerisine 25 gr rüşeym, 5 gr kuru maya konuldu. 13-15. larval aşamadaki larvalar kontrol ve deneme grupları oluşturularak belirtilen sıcaklıklarda 5, 10, 15 ve 20 gün süre ile bekletildi. Depolama süreleri biten larvaların ağırlıkları, toplam lipid miktarı ve yüzdeleri tespit edildi. Bu çalışmada farklı sürelerde buzdolabında depolanan *T. molitor* larvalarının toplam lipid miktarları ve yüzdeleri değerlendirildi. Soğukta 5, 10 ve 15 gün depolanan larvaların toplam lipid miktarları ve yüzdelerinin kontrol grubuna göre daha fazla olduğu belirlendi. 20 gün bekletilen larvalarda ise azalma eğilimi gözlendi. Sonuç olarak yetiştiricilere 15 günden fazla buzdolabında bekletmemeleri önerilmektedir. Aksi takdirde lipid gibi önemli enerji ve besin kaynaklarının azalabileceği göz önüne alınmalıdır.

Anahtar kelimeler: Yağ, düşük sıcaklık, un kurdu, böcek yetiştiriciliği, sıcaklık fizyolojisi

INTRODUCTION

It is estimated that the world population will exceed 9 billion by 2050 (Mirzaeva et al., 2020). This situation increases the demand for alternative food sources (Sogari et al., 2019). *Tenebrio molitor* (Coleoptera: Tenebrionidae) is used as both live feed for pets and also as human food for humans in some countries due to its high nutritional value and easy farming (Adamkova et al., 2017; 2020). It is the most farmed insect in Europe among edible insects (Costa et al., 2020; Kelemu et al., 2015; Ravzanaadii et al., 2012). Considering the rapidly increasing world population, it is no longer just a dream that in the future our food sources will be obtained mostly from insects rather than traditional meat sources (Belluco et al., 2013; Halloran et al., 2014).

Tenebrio molitor is actually an insect that damages stored products. It may take up to 18 months for larvae, which emerge 10-12 days after hatching, to mature depending on temperature and humidity. Adults may reach a weight of 300 mg and they live up to 2-3 months. Temperature and humidity are very important environmental factors especially in their life cycles (Finkel, 1948; Liu et al., 2009; 2020; Mirzaeva et al., 2020 Siemianowska et al., 2013). The insect needs 28 °C for its optimum growth, it can live only 48 hours in under 10°C (Mirzaeva et al., 2020). Low or high temperatures cause important physiological reactions in insects, as in other living beings (Dooremalen & Ellers, 2010; Helgadóttir et al., 2017). They can change the protein, carbohydrate or lipid amounts or compounds they contain (Rathee & Ram, 2018; Zou et al., 2010). This situation is one of the most important adaptations they gain to survive in changing climatic conditions (Duman et al., 1991; Lee et al., 1996). During the mass farming of this insect, producers keep larvae and their pupae with cold storage method when there is no demand from consumers during the periods when population is high (Levie et al., 2015; Tiencheu et al., 2013). Cold storage method extends the shelf life of insects. However, prolongation of cold storage period has negative effects on insects' life cycles (Errico et al., 2021). In addition to its negative effects such as larval and pupal development time, adult weight, deformation rate, it also affects important energy sources such as carbohydrate, protein and lipid. Cold storage can prolong the development time and adult weight of insects. It also can increase the rate of deformation. Substances such as carbohydrates, fats and proteins may also decrease or increase depending on the low temperatures and developmental stages to which they are exposed (Melis et al., 2018; Mirzaeva et al., 2020; Rathee & Ram, 2018).

Adipose tissue plays a very important physiological role in the life of insects. It regulates a large number of physiological and metabolic activities (Arrese & Soulages, 2010). Lipid metabolism is essential for growth and reproduction and it provides the energy needed during periods when feeding is stopped (Lee et al., 1996). Insects store their energy reserves in the form of glycogen and triglyceride in the main fat cells adipocytes (Arrese & Soulages, 2010). In addition, they synthesize most of the metabolites in the circulation with adipose tissue hemolymph proteins (Graham et al., 2000). The lipid content of insects can vary according to rearing conditions, temperature, feed content and developmental stages. Especially temperature is a factor that has a direct effect on insects' developmental stages and the energy sources they include (Aman et al., 2017; Azeez et al., 2014; Pant & Gupta, 1979; Patterson & Duman, 1978). They react to changes in temperature by providing different physiological adaptations specific to species. In low temperatures, they slow their metabolism and generally increase their energy stores such as lipid and protein (Sinclair & Marshall, 2018).

Tenebrio molitor larvae have been found to include more fat than pupae and adults (Jajić et al. 2019; 2020). Morales-Ramos et al., (2015) found 32.1% lipid in pupae and 35.9% lipid in larvae. The amount of lipid they contain may differ according to developmental stages, food, humidity and temperature (Stanley-Samuelson et al., 1988; Van Broekhoven et al., 2015). Van Broekhoven et al., (2015) found that *T. molitor* larvae fed with low quality food in terms of protein and starch content also reduced the lipid reserves required for energy. Jones et al., (1972) found total lipid percentage as 14.96% in larvae. Kröncke et al., (2019) found lipid amount as 27.13 g in 100 g when fresh, as 27.27 g when rock oven dried, as 29.57 g when vacuum dried and as 26.80 g when freeze dried. Mlček et al., (2019) found lipid rate by dry weight as 16.7 g, Siemianowska et al., (2013) found as 22%, Jajić et al., (2019) found as 25.19%, Danthine et al., (2013) found as 30% in those sold in the market and as 36% when bred in laboratory, Ravzanaadii et al., (2012) found as 32.7%, Finkel, (1948) found as 0.24 g, Adamkova et al., (2017) found as 24.56%. Selaledi & Mabelebele, (2021) found the content of sun-dried larvae higher than those oven-dried or freeze-dried. There are a large number of studies on the total lipid amount and the fatty acids contained in *T. molitor* in literature. However, the number of studies on how the cold storage period affects the total lipid content of this species during mass production is limited. The hypothesis in this study is that the weights, total lipid amount and percentages of larvae will not change during the period they are kept in the refrigerator. This is because temperature is one of the factors that affect insect

metabolism most. Therefore, it is expected that the metabolism of insect will slow and lipid amount will not change in low temperature. However, it is not known exactly what will become of these lipid sources with the prolongation of storage time. Will the insect continue to keep its lipid sources or will it continue to use its energy sources since the physiological adaptations it develops will not be enough? For this reason, the present study evaluated the effects of storage time on the weight, total lipid amount and percentages of *T. molitor* larvae and the points that should be considered by mass producers.

MATERIALS AND METHODS

This study was carried out in the Biology Laboratory of Sinop University, Faculty of Education, Sinop-Turkey in 2021. The main material of the trials consisted of *T. molitor* cultures which were available in the laboratory since 2017. Flour : wheat flour (250 g:250 g) in a ratio of 1:1 was given to the larvae and adults as food. 25 g wheat germ and 5 g dry yeast was put in it.

Trials were performed at 28 ± 2 and + 4 ºC, 60 ± 5% relative humidity and continues darkness. The insects were reared in plastic containers (size 30 × 20 × 5 cm). Wooddust was added in the containers to ease movement on the foodstuff. Small pieces (2 for each container, 4 × 4 × 6 cm) were cut from egg boxes for providing convenience for adults to mate and lay eggs. While the plastic containers were covered to prevent entrance of other living beings, small holes were opened on the top side to enable gas exchange. Potato was used for humidity (3 × 3 × 3 cm). They were wrapped into aluminium foil in order to prevent their contact and moisturing and decaying the food. The potatoes were changed every 3 d for the food layer not to get mouldy. The food layer was adjusted 4 - 5 cm thick. The food was renewed with intervals of 10 d. The larvae in the old food were separated by using a sifter and they were transferred on the new food. The containers were checked every day.

After 130-160 mg (in larval stage of 13-15th) larvae were obtained, they were weighed one by one. Control groups were also weighed. This was called the 'first weighing'. They were put in separate petri dishes one by one and given food. Control groups (0, 5, 10, 15 and 20 days) and trial groups (5, 10, 15 and 20 days) were formed. They were stored under specified laboratory conditions and in the refrigerator. The larvae, storage period of which was over, were taken out of the refrigerator and weighed. Control groups were also weighed. This value was called 'second weighing' and they were put in the freezer (Profilo 6600) until they were analyzed. The method applied by Folch et al., (1957) was used to find out the total lipid amount larvae included. Each larva was homogenized (Pro 2000) in 1:2 chloroform-methanol solution. The solution obtained was filtered with Whatman 41 paper. The volatile solution was evaporated under nitrogen gas. The sample obtained was weighed ('initial value') and placed in a desiccator containing silica gel. It was weighed until constant weigh ('final value'). At constant weigh, the total lipid amount was calculated in mg from the difference between the 'initial value' and the 'final value'. Total lipid percentage was found by dividing the mg value of the total lipid calculated to the 'first' and 'second' weight values of the larvae. Each trial group was replicated three times. 10 larvae were used for each trial. A total of 270 larvae were used for the whole study.

Statistical Analysis: SPSS 21.0 was used to evaluate the data. Data were tested for normality. They were found to be normally distributed according to Shapiro-Wilk test. It was found with One-way Anova whether there were differences between groups and with Tukey HSD test between which groups (control, cold storage for 5, 10, 15, 20 days). Paired comparisons between the 'first' and 'second' weighing of the control group and cold storage groups were determined with Independent samples t test ($P < 0.05$ was considered as significant).

RESULTS

In the control group, there was a statistical difference in the 'first weighing' (Control 1) from day 5 ($F_{8,260}$= 5.188, P<0.001, Tukey HSD), in the 'second weighing' (Control 2) from day 15 ($F_{8,260}$= 14.373, P<0.001, Tukey HSD) (Figure 1). Although there was no difference in the cold storage group (CS1 and CS2, Tukey HSD), a difference was found between 'first weighing' and 'second weighing' (F= 0.290, df=58, P= 0.009, Independent t test) (Figure 1).

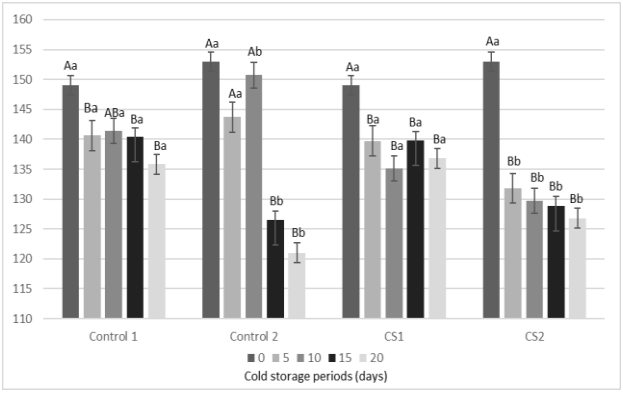

Figure 1. The effects of different periods of cold storage on larval weight before trial and after cold storage (mg). There is no statistical difference between the same uppercase and lowercase letters. Uppercase letters were used for comparison of days 0, 5, 10, 15 and 20 (Tukey HSD). Lowercase letters were used for 'first' and 'second' weighing groups (for exp. comparison of day 15 control group and day 15 cold storage, Independent t test). Control 1, Control 2, CS1: Before cold storage, CS2: After cold storage.

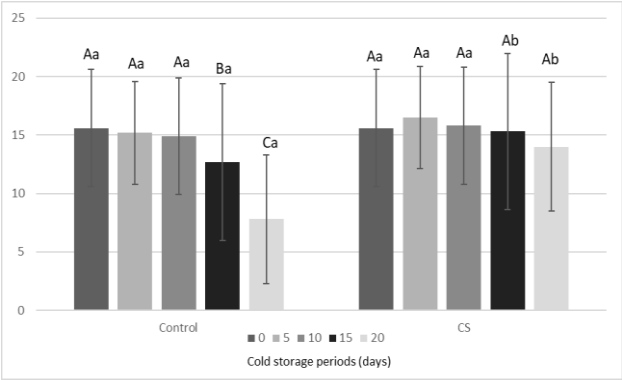

Figure 2. The effects of different periods of cold storage on total lipid amount of *Tenebrio molitor* larvae (mg). There is no statistical difference between the same uppercase and lowercase letters. Uppercase letters were used for comparison of days 0, 5, 10, 15 and 20 (Control and Cold Storage group, Tukey HSD). Lowercase letters were used for the comparison of control group day 15 and cold storage day 15 (Control/Cold Storage, Independent t test). C: Control, CS: Cold Storage.

Total lipid amounts of the control group were found to decrease depending on time (Figure 2). This shows that the control group uses lipids for their metabolic needs, perhaps making the required preparation for pupation. While total lipid amount was 15.6 ± 5.0 mg on day 0, it was found to be 7.8 ± 5.5 mg on day 20 ($F_{4,145}$= 35.688, P<0.001, Tukey HSD). Although there was no statistical difference in the cold storage group, there was a slight tendency to decrease on day 20 in total lipid amount ($F_{4,145}$=, P= 0.248, Tukey HSD). These values were found as 15.6 ± 5.0, 16.5 ± 4.4, 15.8 ± 6.4, 15.3 ± 4.8, 14.0 ± 4.1 on days 0, 5, 10 and 20, respectively. When the control group and the cold storage group were compared, a statistical difference was observed starting from day 15 (Figure 2) (F= 1.173, df=58, P<0.001, Independent t test).

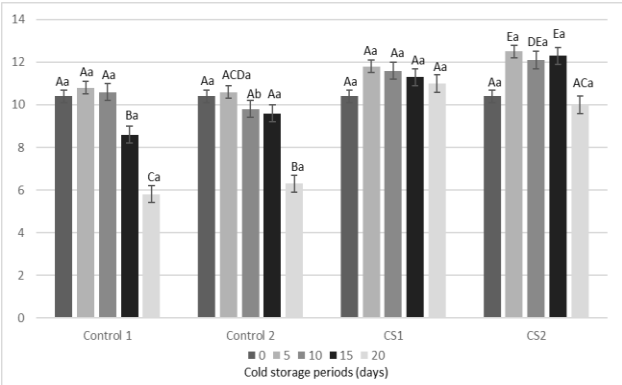

Figure 3. The effects of different periods of cold storage on total lipid percentage of *Tenebrio molitor* larvae (%). There is no statistical difference between the same uppercase and lowercase letters. Uppercase letters were used for comparison of days 0, 5, 10, 15 and 20 (Tukey HSD). Lowercase letters were used for first and second weighing groups (for exp. comparison of day 15 control group and day 15 cold storage, Independent t test).

In the 'first weighing', a statistical difference was found starting from day 15 ($F_{8,261}$= 24.887, P<0.001, Tukey HSD). No difference was found between the cold storage groups (CS1). Statistical difference was found in the 'second weighing' in control group. A decrease was found in total lipid percentage on day 20 in cold storage group ($F_{8,261}$= 31.009, P<0.001, Tukey HSD). No statistical difference was found when the first and second weighing were compared with each other (Figure 3) (F= 0.106, df= 58, P= 0.877, Independent t test). While lipid percentages decreased in parallel with the storage time in control groups (Control 1 and 2), a slight increase was found in the cold storage group on day 5, 10 and 15 (Figure 3).

DISCUSSION

The present study evaluated the effects of different periods of cold storage on the weights, total lipid amounts and percentages of *T. molitor* larvae. It was found that the weights of larvae in the control group decreased until day 20, while no difference was found in the cold storage group (Figure 1). In the control group, lipid use continued for metabolic needs. Resources required for survival and pup formation may have decreased depending on time. The differences between the 'first' and 'second' weighing in the cold storage group suggest that although the larvae slowed their metabolism in the cold, growth and development continued, even though partly. In addition, water loss, which is a method of adaptation to low temperature, might have caused a slight loss of weight. On the contrary, the fact that weights of *T. molitor* larvae stored in the cold for 5, 10, 15 and 20 days suggests that this group did not have enough time to use their reserves. In addition, it is known that insects slow their metabolism in the cold. This decreased metabolism is for protecting energy sources. In the control group, a time dependent loss of weight was seen although they were given food. This result shows that the control group used lipids for its metabolic needs and prepared for pupation, while on the other hand the cold storage group slowed their metabolism and tried to protect their existing sources. In a study conducted on *Cnephasia jactatana* (Lepidoptera: Tortricidae) by using three different temperatures, Ochieng-Odero, (1992) found that the females reared at 15 °C were heavier. The reason for this was explained with insects at 15 °C having a slower growth rate.

Edible insects have received increasing attention as a food product in recent years (Errico et al., 2021). They can be an alternative source of protein and lipid when compared with traditional sources of meat (Costa et al., 2020). The amount of lipid in insects varies depending on orders and species. In terms of dry matter, the highest total lipid amount was found in Lepidoptera and Coleoptera (up to 30%), while the lowest lipid amount was reported in Orthoptera and Odonata (less than 20%) (Dreassi et al., 2017). *T. molitor* larvae are generally rich in fat. Some insects store higher amount of lipid in the early stages of

life (Morales-Ramos et al., 2015; Paul et al., 2017). Jones et al., (1972) found total lipid percentage as 14.96% in their 98.66 mg *T. molitor* larvae. Mlček et al., (2019) found lipid ratio as 16.7% g by dry weight. These results are close to the results of the present study. In the present study, lipid amounts of day 0 control groups was found as 15.6 ± 5.0 mg and 10.4% (Figure 2 and 3). Interestingly, while the highest amount of lipid in the cold storage group was found as 16.5 ± 4.4 mg in day 5 group, the highest lipid percentage was found in the day 5 cold storage group as 12.5% ± 0.2. In the present study, while a decrease was found in the lipid amount of the larvae in the control group depending on time, no changes were found in the cold storage group (Figure 2). When we look at the lipid percentages, there is a slight tendency for increase in the cold storage group (Figure 3). This result shows that the lipids, which will facilitate survival in the cold and work as a source of energy, are protected and even increased (Azeez et al., 2014; Dooremalen & Ellers, 2010).

Panta & Gupta, (1979) found a significant increase in the protein, total lipid, phospholipid and triacylglycerol amounts of silkworm (*Philosamia ricini*) (Lepidoptera: Saturniidae) larvae at low temperature. In a study they exposed the larvae of Hepialus sp. (Lepidoptera: Hepialidae) insects to low temperature (0-3 °C), Zou et al., (2010) found that as unsaturated fatty acids increased in the total lipid content, saturated fatty acids decreased. They also reported that linoleic acid synthesis increased in larvae. In a study conducted with *Aedes aegypti* (Diptera: Culicidae) larvae, Sasmita et al., (2019) found that while the developmental period of larvae farmed at low temperatures decreased, energy reserves increased. The data of the present study also support the hypothesis that larvae increase their lipid reserves at low temperature, while they decrease energy consumption. However, it was found that with the prolongation of storage time, the larvae could not continue this resistance they developed.

Larval, pupal or adult developmental stages of the insect are also effective on this resistance (Irwin & Lee, 2003). They can store different amounts of lipid, protein or carbohydrates according to different developmental stages, or even if they are in the same developmental stage, large pupae, larvae or adult insects store more lipid (Scaccini et al., 2019). Therefore, the insect has more energy reserve and can easily overcome these extreme conditions (Irwin & Lee, 2003; Sinclair & Marshall, 2018). In a study conducted at very low temperatures in Mid-Western North America, it was found that *Eurosta solidaginis* (Diptera: Tephritidae) spent less energy when compared with those insulated under a layer of snow (Irwin & Lee, 2003). It has been found that Woolly Bear Caterpillar (*Pyrrharctia isabella*) (Lepidoptera: Noctuoidae), which is a freezing-resistant species, suppresses lipid consumption and metabolic speed in extremely low temperatures (Marshall & Sinclair, 2012). The lipids stored in larval period were found to continue their presence until adult (Aguila et al., 2007). These results show that protecting energy and lipids is one of the most important components of the success of surviving in low temperatures. In a study which examined the fatty acid compound of membrane and storage lipids of two different life stages (larvae and adult) of *Orchesella cincta* (Collembola: Entomobryidae) at low temperature, it was found that adult biomembranes had much more fatty acid compound than the larvae (Dooremalen & Ellers, 2010). The ability to adjust lipid compound to changes in ambient temperature is an improved adaptation to literature.

CONCLUSIONS

In this study, of the hypotheses, only the hypothesis that weight of larvae would not change with the prolongation of cold storage period was supported. With the prolongation of storage period, a slight decrease was found especially in the total lipid percentages of insects stored for 20 days. According to these data, the physiological adaptations developed by the insect did not show resistance to prolonged storage period. In the total lipid amount and percentages of cold storage groups, it was found that especially the insects stored on days 5, 10, and 15 slowed their metabolism, increased or kept their existing sources of energy. There was a slight tendency to decrease in total lipid amount and percentages only after day 15. It is an important result that the total lipid amount and percentages tended to decrease as of day 20 for mass producers. Considering that the amount of lipid started to decrease after day 15 and the quality will decrease in terms of the lipid amounts they included, it is recommended not to store these larvae for periods longer than 15 to 20 days. For this reason, future studies should store in longer periods of time and compare the total lipid amount in different developmental stages (for exp. pupa and adult). It will be useful for understanding how they react to low temperature at which stage and to understand the physiology of the insect. However, considering that they keep their lipid content until day 15, it can be thought that *T. molitor* larvae stored up to 15 days can be used as live feed and even become a source of lipid for humans in the future.

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ARAŞTIRMA MAKALESİ **RESEARCH PAPER**

Baraj Deformasyonları ve Al Kut Barajı (Irak) Örneği [*]

Mukhalad Sahib Hammoodi AL-JASSANİ[1] **Nükhet KONUK[1*]**
[1*]*Ondokuz Mayıs Üniversitesi, Mühendislik Fakültesi, Harita Mühendisliği Bölümü, 55270, Samsun, Türkiye*

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*: https://orcid.org/0000-0001-6099-4850
: https://orcid.org/0000-0002-9041-5570

***Sorumlu yazarın:**
Nükhet KONUK
Ondokuz Mayıs Üniversitesi, Mühendislik Fakültesi, Harita Mühendisliği Bölümü, 55270, Samsun, Türkiye
✉: nukhetg@omu.edu.tr

Öz: Deformasyon ölçümleri, yoğun nüfuslu yerleşim alanlarında en önemli mühendislik etüt faaliyetlerinden biridir. Mühendislik yapıları, tektonik hareketler, heyelanlar ve yeraltı suyu seviyesi gibi faktörler nedeniyle ya da ana kayadaki sıcaklık, yaşlanma ve dış etkenlerle deformasyona uğrarlar. Deformasyonların ölçümleri ve analizi, doğru araştırma ekipmanı ve doğru analiz yöntemlerinin kullanılmasını gerektirir. Hassas konumlandırmaya izin veren ve 24 saat yarı otomatik veri işleme sağlayan Küresel Navigasyon Uydu Sistemi (GNSS) araçları, deformasyon uygulamalarının izlenmesinde, yerkabuğunun yer değiştirmesinin hesaplanmasında yaygın olarak kullanılmaktadır. Bu çalışmada, baraj deformasyonlarının sebepleri, deformasyon türleri ve deformasyon izlemede GNSS araçlarının kullanımı araştırılmıştır. Ayrıca baraj derformasyonları Irak'ta yer alan Al Kut Barajı örneğinde incelenmiştir. 2017 yılında yapılan ilk gözlem ile 2019 yılında yapılan son gözlem arasındaki düşey fark 0.00683 m, mesafe farkı ise yatayda 0.0179 m olarak belirlenmiştir. Bu sonuçlara göre, hem yatay düzlemde hem de düşey düzlemde eşit olmayan farklılıklar gözlenmiştir.

Anahtar kelimeler: Baraj deformasyonu, deformasyon ölçümleri, küresel navigasyon uydu sistemi (GNSS), ölçme.

Dam Deformations and the Case of Al Kut Dam (Iraq)

***Corresponding author's:**
Nükhet KONUK
Ondokuz Mayıs University, Faculty of Engineering, Department of Geomatic Engineering, Samsun, Turkey
✉: nukhetg@omu.edu.tr

Abstract: Distortion measurements are one of the most important engineering survey activities in densely populated residential areas. The engineering structures are submitted to deformation due to the various factors such as tectonic movements, landslides and groundwater level, or due to the temperature, aging and external factors. As a results of the deformation measurements are directly related to the integrity of engineering structures and human life. avoiding misinterpretation of the displacements, a suitable distortion monitoring network must be established, and the data received from the distortion monitoring network should be carefully evaluated. Distortion measurements and analysis require the correct research equipment and correct analysis methods. GNSS instruments, which enable extremely accurate positioning and provide 24-hour semi-automatic data processing, are widely used in monitoring deformation applications and calculating the displacement of the Earth's crust. In this study, the factors efecting dam deformations, the types of deformations and the use of GNSS tools in deformation monitoring were investigated detailed. In addition, dam deformations were examined in case of Al Kut Dam in Iraq. The vertical difference between the first observation made in 2017 and the last observation made in 2019 was 0.00683 m, and the distance difference was 0.0179 m horizontally. According to these results, unequal differences were observed in both the horizontal and vertical planes..

Keywords: Dam deformation, deformation measurements, global navigate satellite system (GNSS), surveying.

[*]*Bu çalışma, yüksek lisans tezinden üretilmiştir.*

[*]*This study was produced from the master thesis.*

GİRİŞ

İnsanlığın ihtiyacı olan içme ve kullanma suyu temini, sulama suyu, su ürünleri üretimi, enerji üretimi için barajlara gereksinim duyulmuştur. Mühendislik yapıları olan barajlardaki değişimin izlenmesi, belirlenmesi ve yorumlanması oldukça önem arz etmektedir. Baraj, tünel gibi mühendislik yapılarında çeşitli etkilerle oluşan boyut değişimlerine deformasyon, bu deformasyonların ölçüm yöntemleri ile belirlenmesine de deformasyon ölçümleri adı verilir (Yıldırım, 2007). Baraj, ülke ekonomisindeki önemli altyapılardan biri olarak tanımlanabilir ve ulaşım ağı, su depolama ve elektrik üretiminde önemli bağlantılara sahiptir (Jaafar, 2017).

Barajlar iç ve dış yüklerden etkilenmekte ve deformasyona uğramaktadır. Yükler sabit değildir ve zamanla değişiklik gösterebilmektedir. Bir tankın hidrostatik basıncı, barajın merkez hattına dik olarak kalıcı yatay deformasyona neden olabilmektedir (Okiemute vd., 2018). Barajın ağırlığı ve tankın hidrostatik basıncı gibi su kuvvetleri, yapıların düşey hareketine yol açmaktadır. Kaya dolgu barajda elastik davranış daha küçüktür (Galán vd., 2013; Taşçi, 2008).

Deformasyon türlerinin ana nedenleri olarak değişen sıcaklıklar (yazın ısınması ve kışın soğuması), doğal deformasyonlar (yer kabuğunun deformasyonları), depremler ve volkanlar sayılabilmektedir (Abdulkahdum, 2015). Depremler genellikle yeraltındaki kayanın aniden bir fay boyunca kırılmasıyla olmaktadır. Meydana gelen ani enerji salınımı, yerin sarsılmasına neden olan sismik faaliyetlere yol açmaktadır (Barzaghi vd., 2018). Sismik faaliyetler, levha sınırları düzeyinde gerçekleşmektedir (Al-Husseinawi vd., 2018). Ardından, oluşan basınç nedeniyle kayalar kırılarak deprem meydana gelmektedir (Burgmann ve Dresen, 2008). Deprem sırasında veya sonrasında kaya plakaları veya blokları hareket etmeye başlamakta ve tekrar sıkışana kadar hareketine devam etmektedir (Jansen, 1983; Sêco e Pinto, 2010). Toprak dolgu ve kaya dolgu barajların depreme tepkisi, hareketin ivmesi ve hızına göre farklılık göstermekle birlikte, barajın jeolojik yapısına bağlıdır (Anastasiadis vd., 2004). İvme yeterince büyük olduğunda, sarsıntı geçici bir düşey harekete neden olmaktadır (Li vd., 2019). Baraj deformasyonlarında diğer bir kritik faktör de inşaat kalitesidir. Düşük derecede sıkışmaya sahip barajlar, depremlerde oldukça büyük zarar görebilmektedir.

Yeryüzü çeşitli hareketlere ve deformasyonlara maruz kaldığından, bu deformasyonları farklı ölçeklerde izlemek önem arz etmektedir (Abdullahi, 2016) . Jeodezik hesaplamalarla küresel levhaların hareketi ile temsil edilen jeodinamik mekanizmaları ölçmek mümkündür (Rodrigues vd., 2020).

Deformasyon iki şekilde belirlenebilmektedir. (i) bir, iki veya üç boyutlu yer değiştirme ya da coğrafi referanslı hareketleri ölçmek için Total Station, Nivo, Küresel Navigasyon Uydu Sistemi (GNSS) kullanımını içeren jeodezik ölçüm cihazları, (ii) yağ çubuğu, yüksek basınç ölçerler, yağmur ölçerler, termometre, barometre, eğim, ivme ölçer, deprem ölçer ve diğerleri gibi bazı araçların kullanımını içeren geoteknik ölçüm cihazları ile ölçülebilmektedir (Okiemute vd., 2018).

Barajlarda deformasyon, yapının boyutuna, türüne ve deformasyonun nedenine bağlıdır. Örneğin, yük altında veya yeni başlayan dengesizlik altında yapının temelinde deformasyona neden etkiler farklı büyüklük ve şekillerde olabilir (Moore, 1992). Yer değiştirme eğilimleri jeoteknik araçlardan tahmin edilerek inşaat projelerinde belgelenebilir (USACE, 2002).

Deformasyonların izlenmesinde farklı araçlar kullanılmaktadır. Literatürde bu araçlar ile ilgili farklı sınıflandırmalar mevcuttur. Örneğin Moore (1992), dikey hareketler, eğim (şakül dışı), yatay hareketler ve çatlaklar gibi ölçümlerin türüne göre izleme yaklaşımlarının ilkesini belirlemiştir. Düşey hareketler için hassas tesviye, eğim için lazer sistemi ve hassas optik şaküller, yatay hareketler ve GNSS sistemleri için teodolit ve Elektronik Mesafe Ölçüm Cihazları (EDM) önerilmiştir.

Hill ve Sippel, (2002), deformasyon izlemede kullanılan aletleri etüt, jeoteknik ve meteorolojik olarak üç gruba ayırmıştır. Hill ve Sippel (2002)'e göre tek boyutlu ölçümler jeoteknik ve meteorolojik aletlerle sağlanabilirken, üç boyutlu ölçümler, ölçme sensörleri kullanılarak yapılmaktadır. Ölçme yöntemi için olarak Total Station ve GNSS üzerine odaklanılmıştır. Ekstansometreler, eğimölçerler, piyezometreler, gerinim ölçerler, basınç hücreleri, eğim sensörleri ve çatlak ölçerler gibi jeoteknik sensörler izleme için yaygın olarak kullanılmaktadır. Meteorolojik aletler; sıcaklık, bağıl nem, barometrik basınç, rüzgâr hızı, rüzgâr yönü, küresel radyasyon (güneş enerjisi) ve yağış hakkında veri toplarken genellikle diğer sensörleri kalibre etmek için kullanılmaktadır.

USACE, (2002) ve Kalkan vd., (2010) ise izleme sensörlerini jeodezik sensörler ve jeodezik olmayan (jeoteknik) sensörler olmak üzere iki gruba ayırmıştır. Buna göre jeodezik yöntemlerde hassas teodolitler, lazer optik, Elektronik Mesafe Ölçüm Cihazları (EDM), Küresel Konumlandırma Sistemi (GPS), GLONASS ve GALILEO alıcıları, lazer tarayıcılar; jeodezik olmayan yöntemlerde ise eğimölçer, yerleşim sütunu, ekstansometre, piyezometre, ters sarkaç kullanılmaktadır.

Park vd., (2007) yaptıkları çalışmada; işlevsel olarak izlemede kullanılan sensörleri gruplandırmışlardır. Güvenliği kontrol etmek ve servis kolaylığı sağlamak için kullanılan sensörler, gerinim ölçerler gibi güvenlik sensörleri, ivmeölçerler ve GNSS gibi servis verilebilirlik

sensörleridir. Bununla birlikte, fotogrametri teknikleri gibi güvenlik ve servis kolaylığı için kullanılan görme tabanlı sensörler de bulunmaktadır.

Chanard vd., (2018) yaptıkları çalışmada; elastik dünya modeline dayalı olarak, yatay ve dikey yer değiştirmelerin, gözlemlenen yüzey kütle farklılıklarından türetildiğini ve bu yüzey kütle farklılıklarının da öncelikle kıtasal su depolaması, atmosferik basınç ve okyanus sirkülasyonu ile ilgili olduğunu belirtmişlerdir. Ayrıca, çalışmada diğer atipik fiziksel etkiler, GNSS gözlemleri ile yükleme modeli tahminleri arasındaki kalan farklılıkları açıklamaya katkıda bulunabileceği ortaya çıkmaktadır .

Xiao vd., (2019) yaptığı çalışmada; deformasyon sürecinin dolgu malzemesinin homojenliği, su seviyesi ve sızıntıdan etkilendiği açıklamışlardır. Ayrıca çalışmada, rezervuardaki su seviyesi ile baraj yüzeyinin deformasyonu arasında, su seviyesindeki değişikliklerin barajın deformasyonuna katkıda bulunan ana faktör olması gerektiğini gösteren önemli bir korelasyon ortaya çıkmaktadır.

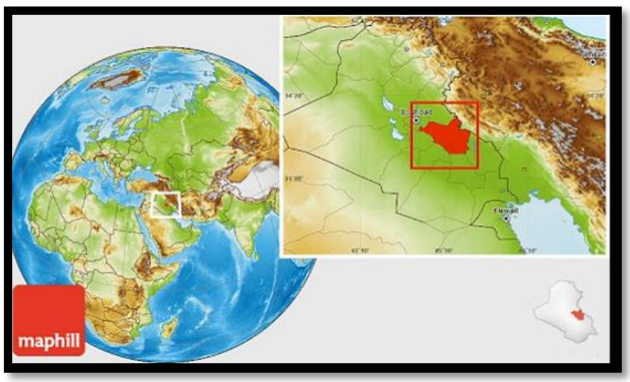
Li vd., (2019) yaptıkları çalışmada; Yarı dinamik veri doğruluğunu korumak için elektronik GNSS sisteminin yarı dinamik veri ile entegre edilmesini önermişlerdir. Yarı dinamik referans noktasındaki yüzey deformasyon modeli, kontrol noktalarının gerçek zamanlı güncellenmiş koordinatlarını sağlayabilir. e-GNSS'den gözlemlenen koordinatların zaman serileri ile yüzey deformasyon modelinden hesaplanan koordinatların zaman serileri arasındaki karşılaştırma, yarı dinamik referans noktasında yüzey deformasyon modelinin doğruluğunun izlenmesine yardımcı olmaktadır

Bu çalışmada, baraj deformasyonlarının sebepleri, nasıl gerçekleştiği ve deformasyon izlemede GNSS araçlarının kullanımı araştırılmıştır. Ayrıca, baraj derformasyonları Irak'ta yer alan Al Kut Barajı örneğinde incelenmiştir.

MATERYAL ve METOT

Çalışmada, deformasyonu izlemek ve ölçmek için Irak'ın Wasit ilindeki Al Kut Barajı seçilmiştir. Wasit ili, Irak-İran sınırında, depreme maruz kalan levhaların birleştiği noktada yer almaktadır. Şekil 1'de Wasit ilinin konumu gösterilmektedir.

Al Kut Barajı, Bağdat'ın 180 km güneyinde, Dicle Nehri kıyısındaki Al Kut şehrinde bulunmaktadır. Irak'taki en uzun barajlardan biridir. Baraj, 1939'da İngilizlerin gözetiminde inşa edilmiştir. Elektrik üretmek için kullanmanın yanı sıra su depolamaya da katkısı nedeniyle Irak'ın önemli barajlarından biri olarak kabul edilmektedir. Al Kut Barajı, Dicle Nehri üzerindeki en önemli sulama tesislerinden biridir, çünkü bu baraj, suyun Irak'ın güneyindeki illere dağıtımını sağlamakta ve illerdeki tarım projeleri için sulama imkânı sunmaktadır. 550 metre uzunluğunda olan Al Kut Barajı, her biri 6.00 x 6.50 m boyutlarında, 56 adet manuel ve elektrikle çalışan dikey kapılardan oluşmaktadır. Şekil 2'de Al Kut barajına ait bir görüntü sunulmuştur.



Şekil 1. Wasit ili konumu.
Figure 1. Wasit province location.

Şekil 2. Kut Barajı.
Figure 2. Kut Dam.

GPS ve Nivo cihazı ile ölçümler gerçekleştirilmeden önce, baraj dışındaki sabit temeller veya platformlar üzerindeki referans istasyonları için uygun noktaları seçmek ve baraj üzerindeki izleme noktalarını belirlemek için çalışma alanı ziyaret edilmiştir. Barajda, nivo kullanarak dikey izleme noktaları ve DGPS kullanarak yatay izleme noktaları olmak üzere iki takım gözlem noktası belirlenmiştir. Belirlenen noktalarda beton çivilerle tutturulmuş işaretleyiciler aracılığıyla ayırt edici gözetleme noktaları kalıcı hale getirilmiştir. İzleme noktalarının mekânsal kontrolleri Wasit Valiliği ve Su Kaynakları Bakanlığı ile koordineli olarak gerçekleştirilmiştir. Sabit referans için kalıcı bir izleme noktası tanımlanmıştır.

DGPS notları, TOPCON TOOL yazılımı kullanılarak bilgisayar sistemi klasörüne indirilmiş, indirilen veriler işlenip ve düzeltilip gerçek koordinatlara dönüştürülmüş ve çizilmek üzere AutoCAD programına aktarılmıştır. Şekil 3'te, GPS cihazının sabit noktasının (Best) konumunu ve bir Rover cihazı ile barajın merkezindeki deformasyonu izlemek için kullanılan diğer nokta gösterilmektedir.

Şekil 3. Sabit noktanın (Best) ve Rover cihazı ile barajın merkezindeki deformasyonu izlemek için kullanılan diğer noktanın konumu.(Google Earth, 2020)
Figure 3. The location of the fixed point (Best) and the other point used to track the deformation in the center of the dam with the Rover device.

Olası yer değiştirmeyi izlemek ve ölçmek için Al Kut Barajı jeodezik ağ izlenerek Su Kaynakları Bakanlığı / Etüt Genel Müdürlüğü tarafından periyodik olarak kontrol edilir. Bu ağ, baraj yüzeyine yerleştirilmiş kolonlardan oluşmaktadır. Bu çalışma, yüksek ölçüm doğruluğuna ulaştığı için GPS kullanılarak yatay deformasyonun düzenli izlenmesini ve nivo kullanılarak dikey seviyelendirmeyi sağlamaktadır. Çünkü baraj deformasyon süreci yavaştır ve kısa sürede ya da doğruluğu düşük cihazlarla gerçekleştirilememektedir.

Geleneksel araştırmalar bir "görüş hattı" gerektirmektedir ve sürekli gözetimsiz saha operasyonları (yüksek örnekleme oranları ve entegre ağ bağlantıları kullanarak) GPS kadar uygun değildir. Geleneksel ölçme sistemlerinde verilerin silinmesi GPS ile karşılaştırıldığında daha az karmaşık olsa da, yüksek hassasiyetli GPS analizinin güvenilir otomasyonu artık makul ölçüde rutin hale gelmiştir.

Çalışmada, detaylı yatay konumsal bilgileri sürekli doğrulukla elde etmek için GPS sistemi kullanılmıştır. Kullanılan Leica GPS sistemi, yatay: 2 mm + 0,5 ppm' ye kadar sürekli bir çözünürlükte ayrıntılı yatay konum bilgileri elde etmek için kullanılmaktadır. Yüksek doğrulukta sonuçları elde etmek için uzun süreli gözlemlerden yararlanılmış, üç yıllık bir dönem için (2017-2018-2019) yıllık gözlemler yapılmıştır. Tüm gözlemler, her nokta için iki saatlik bir süre boyunca her yılın Eylül ayında gerçekleştirilmiştir. Barajın su seviyesinin düşmesi ile barajın deformasyonu arasında doğrudan bir ilişki bulunmaktadır. Barajdaki su seviyesi azaldığında, barajın tepesindeki noktalar kaynağa doğru kayma eğilimindedir. Ters hareket durumunda, baraj eksenindeki noktalar aşağıya doğru kayma eğilimindedir. Bu durumda oluşacak hatanın üstesinden gelmek için noktalar ölçülmüş ve su seviyesi diğer gözlemlerden yaklaşık olarak alınmıştır. Barajda meydana gelen deformasyonu belirlemek için, çalışma yapılacak tesisten 900 metre uzakta, çalışma sahası dışında, biri sabit referans (Best) olmak üzere iki nokta seçilmiştir. Böylece, gelecekteki ölçümler yapılırken nokta, referans için güvenli bir şekilde sabitlenecektir.

Sabit referans noktası, Gerçek Zamanlı Kinematik (Real Time Kinematic (RTK)) yöntem kullanarak, izlemede kullanılan ikinci nokta ise, Rover kullanılarak belirlenmiştir. Mühendislik uygulamalarında aplikasyon ve detay alımı sırasında noktalara ait konumların gerçek zamanlı olarak belirlenmesi gerekmektedir. Bu nedenle nokta konumlarını gerçek zamanlı cm mertebesinde belirleyen RTK yöntemi geliştirilmiştir (Arslanoğlu, 2002). RTK yönteminde konumu yüksek doğrulukla bilinen bir referans istasyonu ve bu istasyona en fazla 15-20 km kadar uzaklıkta bulunan bir ya da daha fazla gezen alıcıya ihtiyaç duyulmaktadır. (İnal vd., 2014). Şekil 4'te uydu görüntüsü üzerinde sabit nokta ve hareket noktasının konumunu gösterilmektedir..

Şekil 4. 2020 yılına ait Google Earth görüntüsü üzerinde sabit nokta ve hareket noktasının konumu
Şekil 4. Position of fixed point and departure point on Google Earth image for 2020.

Her nokta GPS cihazı kullanılarak iki saat süreyle izlenmiştir. Aynı seansta RTK yöntemi ile araçların hareketinden kaynaklanan hatanın giderilmesini sağlamak için çok yüksek doğrulukta bir yatay izleme gerçekleştirilmiştir. Bu amaçla her 30 dakikada bir hareket etmeden aynı frekansta ve aynı noktada bağımsız 4 gözlem yapılmıştır. Düşey hareket ise, irtifanın doğruluğunu sağlamak, faz sistemi hatalarından ve dalgalardaki bozulmalardan etkilenmemek için dijital nivo kullanılarak belirlenmiştir.

Tablo 1'de ölçülen yatay ve dikey koordinatın üç yıllık gözleminde yıldan yıla bir fark olduğu görülmektedir.

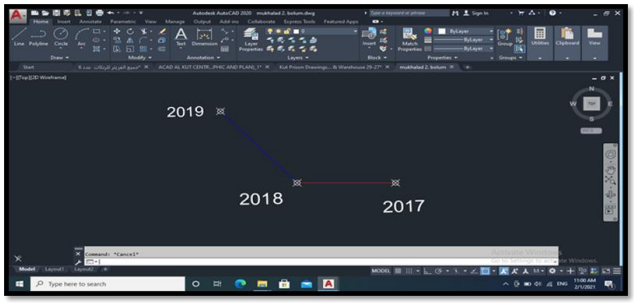
Tablo 1. 2017, 2018 ve 2019 yıllarında gözlemlenen noktanın koordinatları.
Table 1. Coordinates of the point observed in 2017, 2018 and 2019.

Gözlem Tarih	Dikey koordinat	Yatay koordinat	Yükseklik
2017	3596010.215	576806.222	22.41379
2018	3596010.216	576806.213	22.41723
2019	3596010.223	576806.206	22.42062

BULGULAR VE TARTIŞMA

Elde edilen sonuçlardan, hem yatay düzlemde hem de düşey düzlemde eşit olmayan farklılıklar gözlenmiştir. 2017 ve 2018 gözlemleri arasındaki fark, yatay düzlemde (0.009 m) ve düşey düzlemde (0.00344 m) olarak belirlenmiştir. 2018 ile 2019 yılları arasındaki fark ise yatay düzlemde (0.007 m) ve düşey düzlemde (0.00339 m) olmuştur. 2017 yılında yapılan ilk gözlem ile 2019 yılında yapılan son gözlem arasındaki fark ise yatayda (0,016 m) ve düşeyde (0,00683 m) olarak belirlenmiştir.

Yatay koordinatlardaki farkın (izleme ile sonraki arasındaki kayma ve bozulma), AutoCAD programındaki görünümü Şekil 5'te gösterilmiştir.



Şekil5. AutoCAD programına göre gözlemlenen noktaların konumlarındaki fark.
Figure 5. The difference in the positions of the observed points according to the AutoCAD program.

Kısmi taşıyıcı faz önyargısı ve uydu saat varyasyonunu içeren uydu sinyaline bağlı hata koşulları giderilmiştir. Ardından düşük gürültüleri sayesinde ham taşıyıcı çift fazlı sinyaller olarak L1 ve B1 kullanılmıştır. Gecikmenin dağılmamasını sağlamak için 15°'lik bir dar açı kullanılarak elimine edilen yayılma ortamı ile ilgili hata koşullarının yanı sıra çift farklılıkları gözlemleyerek hafifletilmiştir.

Noktanın gözlem tarihlerine göre yıllara göre yer değiştirmeleri 2017-1018 yılları arasında 0.0091 m, 2018-2019 yılları arasında, 0.0099 m ve 2017-2019 yıllarına bakıldığında ise 0.0179 m olduğu görülmektedir. Bu deformasyona barajın taban seviyesindeki sıcaklık farkı, deprem gibi doğa olaylarının yanı sıra, yapı malzemeleri, su akışının hızı ve yönü de neden olmaktadır. Tablo 2'de gözlem yapılan noktanın yıllar arasındaki mesafe farkı gösterilmiştir.

Tablo 2. Noktanın yıllar arasındaki mesafe farkı.
Table 2. Measurement difference between years.

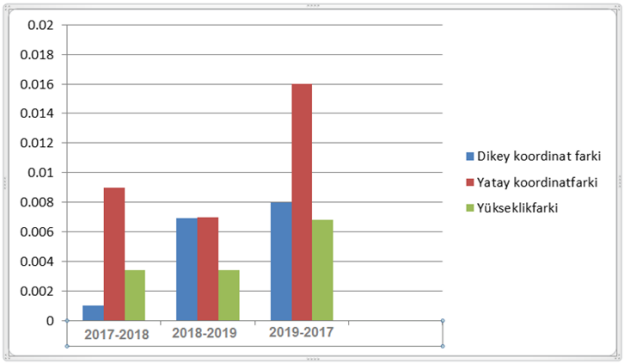
Gözlem Tarihi	Mesafe Farkı (m)
2017-2018	0.0091
2018-2019	0.0099
2017-2019	0.0179

Tablo 3.'de ölçüm yapılan noktadaki yıllar arasında gözlem farkı verilmiştir. Tablo 3'ten de görüleceği üzere, 2017-2019 yılları arasında dikey koordinat farkı 0.008 m, yatay koordinat farkı 0.016 m, yükseklik farkı ise 0.00683 m olarak belirlenmiştir.

Tablo 3. Ölçüm yapılan noktadaki yıllar arasındaki gözlem farkı.
Table 3. Observation difference between years at the measurement point.

Gözlem Tarihi	Dikey koordinat farkı (m)	Yatay koordinat farkı (m)	Yükseklik farkı (m)
2017-2018	0.001	0.009	0.00344
2018-2019	0.007	0.007	0.00339
2017-2019	0.008	0.016	0.00683

Yatay ve dikey eksenlerdeki koordinat farkları karşılaştırıldığında; dikey yöndeki koordinat farklarının, yatay yöndeki koordinat farklarının yaklaşık iki katı olduğu ve bunun sonucunda da su akış yönünün dikey yöne paralel olduğu söylenebilir. Şekil 6'da gözlem yapılan noktanın yıllar içinde ölçülen değerlerinin farkı grafik olarak gösterilmiştir.



Şekil 6. Gözlem yapılan noktanın yıllar içinde ölçülen değerlerinin fark grafiği.
Figure 6. The difference graph of the measured values of the observed point over the years.

SONUÇLAR VE ÖNERİLER

Sonuç olarak çalışmada Irak'ın Wasit ilindeki Al Kut Barajındaki deformasyon GNSS araçları kullanılarak incelenmiştir. İncelenen sonuçlara göre, hem yatay düzlemde hem de düşey düzlemde eşit olmayan farklılıklar gözlenmiştir. Deformasyondaki bu farklılıkların nedenlerinin aşağıdaki doğal ve fiziksel faktörlerden kaynaklandığı düşünülmektedir;

- Doğa olayları (2017 yılında İran'da meydana gelen (7,6) büyüklüğündeki deprem, yatay düzlemde ve düşey

düzlemde etkinin belirginleştiği büyük bir deformasyona neden olmuştur. Bu depremin, başta sınır bölgeleri olmak üzere Irak bölgelerinde de çok büyük etkisi olmuştur).

- Isı faktörü: (Barajdaki mevsimsel sıcaklık değişiklikleri)
- Yükler: (sabit ve ölü yükler)
- Su basıncı ve suyun hareket yönü. (Rezervuardaki su seviyesi ile baraj yüzeyinin deformasyonu arasında önemli bir korelasyon vardır. Bu da su seviyesindeki değişikliklerin barajın deformasyonuna neden olan ana faktör olduğunu göstermektedir).
- Barajın beton malzemelerinin oksidasyonu ve zayıflamasıdır.

Barajların güvenliğini sağlamak ve bunları büyük bir finansal maliyet veya mühendislik çabası ve çalışma tehlikesi olmadan izlemek için, baraj gövdesine sabit sensörlerin takılarak anlık değişimlerinin gözlenmesi tavsiye edilmektedir. Ayrıca hidrolik yapının farklı bölümlerindeki dolgu malzemeleri de dikkate alınması gerekmektedir.

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ERRATUM

In the original article titled "A New Trout Species from Southern Marmara Sea Drainages (Teleostei: Salmonidae)" published in the *Journal of Anatolian Environmental and Animal Sciences*, Vol: 6, Issue: 2, pp: 232-239, June 2021, a new fish species for the world was introduced. Zoobank numbers of the species and article have been corrected by adding them as follows. It is kindly submitted to your information.

Journal of Anatolian Environmental and Animal Sciences Editorial Board.

The zoobank code of the article: http://zoobank.org/urn:lsid:zoobank.org:pub:9832CB94-8F6A-4CE4-A9B3-25B448B3A389
The zoobank code of Salmo duhani: http://zoobank.org/urn:lsid:zoobank.org:act:C050C743-3FE3-40AD-9A65-DF97DB09FFD8

DÜZELTME

Anadolu Çevre ve Hayvancılık Bilimleri Dergisi, Cilt: 6, Sayı: 2, s: 232-239, June 2021 sayısında yayınlanan "A New Trout Species from Southern Marmara Sea Drainages (Teleostei: Salmonidae)" başlıklı özgün makalede dünya için yeni bir balık türü ileri sürüldüğünden türün ve makalenin Zoobank numaraları aşağıdaki gibi eklenerek düzeltilmiştir. Bilgilerinize arz olunur.

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