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Biological Diversity and Conservation

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Biyolojik çeşitlilik ve koruma üzerine yayın yapan hakemli uluslararası bir dergidir

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First photographic evidence of *Flos asoka* (de Nicéville, [1884]) (Lycaenidae: Theclinae: Arhopalini) in Uttar Pradesh, India: Recent confirmation and ecological insights

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Abstract

This study presents the first photographic evidence of *Flos asoka* (de Nicéville, [1884]) in Uttar Pradesh, India, marking a recent confirmation of its presence in this region. Additionally, ecological insights derived from the study provide a deeper understanding of the ecology and natural habitat of *Flos asoka* in the region. This finding represents a significant contribution to the knowledge of the distribution and ecology of *Flos asoka* and highlights the importance of photographic documentation in species presence and distribution research.

Keywords: Distribution, Dudhwa National Park, habitat, Rhopalocera, taxonomy

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Flos asoka'nın (de Nicéville, [1884]) (Lycaenidae: Theclinae: Arhopalini) Uttar Pradesh, Hindistan'da ilk fotoğraf kanıtı: Son doğrulama ve ekolojik içgörüler

Özet

Bu çalışma, Uttar Pradesh, Hindistan'da Flos asoka'nın (de Nicéville, [1884]) ilk fotografik kanıtlarını sunmaktadır, bu da bu bölgedeki varlığının son onayını işaretlemektedir. Ek olarak, çalışmadan elde edilen ekolojik içgörüler, bölgedeki *Flos asoka*'nın ekolojisi ve doğal yaşam alanı hakkında daha derin bir anlayış sağlamaktadır. Bu bulgu, *Flos asoka*'nın dağılımı ve ekolojisi hakkındaki bilgiye önemli bir katkı sağlamakta ve tür varlığı ve dağılım araştırmalarında fotografik belgelerin önemini vurgulamaktadır.

Anahtar kelimeler: Dağılım, Dudhwa Ulusal Parkı, Habitat, Rhopalocera, taksonomi

1. Introduction

The butterfly genus *Flos* (Doherty, 1889) belongs to the family lycaenidae with eight known species in India (Appendix I) [11]. The species belonging to this genus are commonly known as plushblues. The discovery of *Flos asoka* (de Nicéville, [1884]) in Uttar Pradesh, India, marks a significant milestone in butterfly research, as this species had eluded photographic documentation in the region until now. The paper also presents the first photographic evidence of *Flos asoka* within the Dudhwa National Park, situated in the Lakimpur-Kheri district of Uttar Pradesh. Through meticulous analysis of the newly captured photograph, the taxonomic identity of *Flos asoka* is verified, providing concrete confirmation of its presence in the region. The study not only fills a notable gap in previous literature but also offers valuable ecological insights into the habitat and natural environment of *Flos asoka* within Dudhwa National Park, the habitat was a dense forest and four individuals were seen in a week from the observed date. This finding contributes

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to our understanding of the distribution and ecology of *Flos asoka*, emphasizing the importance of photographic documentation in species presence and distribution research in India.

2. Materials and methods

On 17.vii.2023, researchers conducted a survey within the accessible areas of Dudhwa National Park (28°29'24.7"N 80°38'44.5"E), located in the Lakhimpur-Kheri district of Uttar Pradesh, India (Figure 2), at an elevation of 150 meters. During the survey focused on Rhopalocera species, they encountered and photographed a species identified as *Flos asoka* (de Nicéville, [1884]) based on available source of literature [14], [16]. The species is observed or sighted during the months of March through July exclusively, furthermore, *Flos asoka* may have specific host plants or breeding requirements that are available or optimal during the months of March to July. These host plants may provide essential resources for egg-laying, larval development, and adult feeding, influencing the timing of the species' presence in the area. Overall, the seasonal presence of *Flos asoka* from March to July likely reflects its adaptation to the environmental conditions and availability of resources during this period, highlighting the importance of understanding the species' life cycle and ecological requirements for conservation efforts.

This suggests a specific seasonal pattern in the species' appearance or activity, with sightings occurring within this particular time frame. Outside of this timeframe, sightings of the species are not reported yet. Following this initial discovery, the researchers revisited the area on 18.vii.2023 and 19.vii.2023, where they spotted and photographed three more specimens of the same species. To aid in the identification process, available literature sources such as [16], [10], [21], [19], [20] and [9] were consulted. The Rhopalocera specimens were photographed using either a DSLR Nikon D750. It's important to emphasize that no specimens were collected or harmed during the survey.

2.1 Study area

The study area encompassing a total of 490.29 square kilometers, which represents a significant portion of the once expansive Terai region, which stretches alongside the Himalayan foothills in Uttar Pradesh. This area is characterized by a diverse landscape comprising Sal forests, tall grasslands, and swamps that are sustained by periodic flooding. However, it faces substantial threats due to anthropogenic activities, with much of the Terai region having already been altered by agriculture and human settlements, leading to the depletion of its natural vegetation.

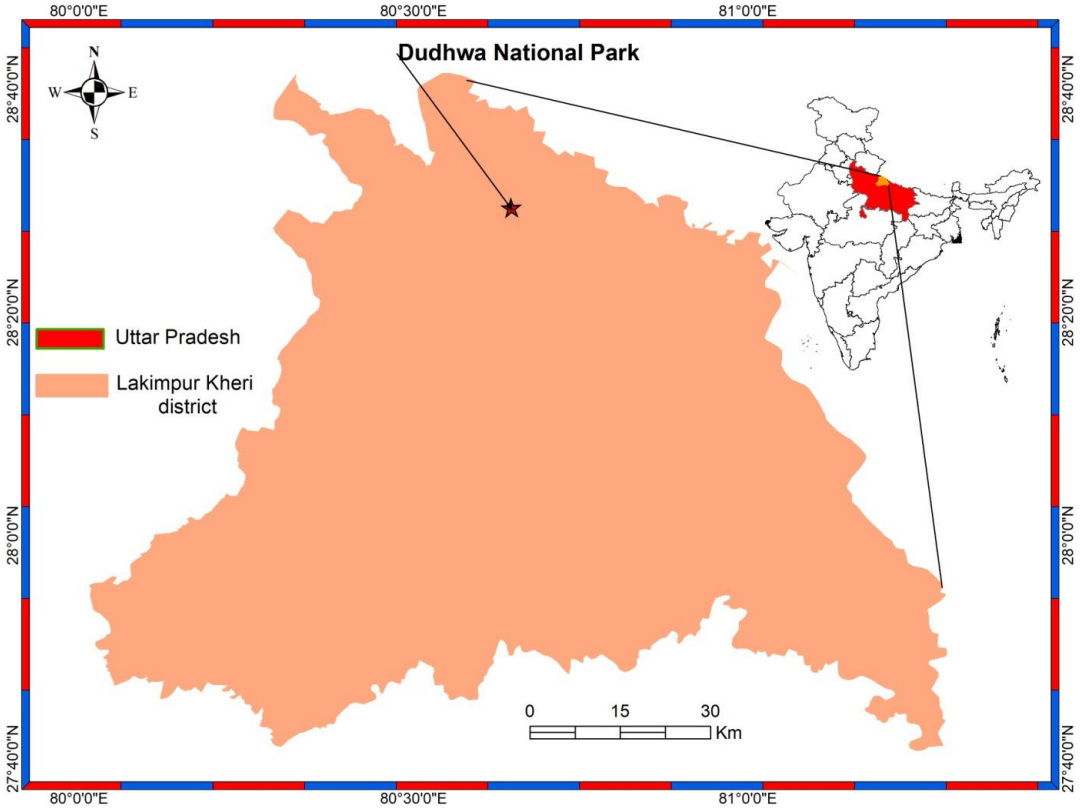

Figure 2. Map showing study area of Flos asoka (de Nicéville, [1884])

Dudhwa National Park is an integral part of the Dudhwa Tiger Reserve, which serves as the primary Protected Area Complex within the Terai region of India. As the sole National Park and Tiger Reserve representing the Terai-Bhabhar Biogeographic subdivision of the Upper Gangetic Plains Biogeographic province (7a), Dudhwa National Park holds significant ecological importance. The vegetation within the park falls under the category of North Indian Moist Deciduous type as classified by [5], boasting some of the most pristine Sal forests in the country. Ongoing documentation efforts have identified a wide variety of plant species and communities within the park, many of which hold conservation significance.

The Dudhwa Tiger Reserve harbors a unique and potentially viable population of the nominate sub-species of Swamp deer (*Rucervus duvaucelii duvaucelii* syn *Cervus duvaucelii duvaucelii*), making it the only location in the country where this species is found. Additionally, the reserve is home to five species of deer and supports a substantial tiger population. Several critically endangered species, including the Bengal Florican (*Hubaropsis bengalensis*) and Hispid Hare (*Caprolagus hispidus*), find sanctuary within Dudhwa National Park. Notably, the park has successfully reintroduced the Great Indian One Horned Rhinoceros. Furthermore, Dudhwa is home to numerous endangered species, with thirteen mammal species, nine bird species, and eleven reptile and amphibian species listed in Schedule–1 of the Wild Life (Protection) Act, 1972 [1].

3. Results

This study presents key findings regarding the confirmation of *Flos asoka* identity and its distribution in Uttar Pradesh, focusing on observations within Dudhwa National Park.

SYSTEMATIC POSTION

Class Insecta Linnaeus, 1758

Order Lepidoptera Linnaeus, 1758

Family Lyacenidae Leach, 1815

Subfamily Theclinae Swainson, 1831

Tribe Arhopalini

***Flos asoka* (de Nicéville, [1884]) (Figure 1)**

Nilasera asoka Nicéville, [1884]
Satadra chola Moore, 1884
Arhopala asoka vaya Fruhstorfer, 1914
Amblypodia asoka Evans, 1932

Identification features

The identification features include a short tail and a distinct lobe, along with metallic scales positioned at the lower tip on the underside of the hindwing. Additionally, this species exhibits a dark basal band extending up to the inner margin, a curved pale streak inside the wing, and a pale bar located at the end of the forewing cell on the underside. These specific traits are instrumental in accurately identifying and classifying this butterfly within its taxonomic group.

Confirmation of *Flos asoka* Identity

Taxonomic verification confirmed the identity of *Flos asoka* in Dudhwa National Park, establishing its presence in the region based on other available evidence such as field observations and literature references.

Distribution and Habitat Analysis

Flos asoka was observed in diverse habitats within Dudhwa National Park, characterized by the unique ecosystem of the Terai region. The park features a mosaic of tropical moist deciduous forests, grasslands (known as 'chaurs'), wetlands, and riverine ecosystems. Dominant tree species include sal (*Shorea robusta*), teak (*Tectona grandis*), and khair (*Acacia catechu*), providing dense canopies that support a rich diversity of wildlife. The park is traversed by several rivers, including the Suheli and Mohana, and is home to numerous oxbow lakes, marshes, and wetlands. These water bodies serve as vital habitats for aquatic species such as gharials, mugger crocodiles, and freshwater turtles, as well as breeding grounds for waterfowl and migratory bird species. Dudhwa National Park experiences distinct seasonal variations, with hot summers, cool winters, and a monsoon season. The park's climate, topography, and vegetation create a unique habitat mosaic that supports a diverse array of wildlife species, including *Flos asoka*. The findings provide conclusive evidence of *Flos asoka* presence within Dudhwa National Park, contributing to our understanding of its distribution and ecology in Uttar Pradesh.

Figure 1. *Flos asoka* (de Nicéville, [1884]) (Underwing)

Distribution in India

The species is rare in the Himalayas and is typically found at elevations of up to 900 meters. Its distribution ranges from Uttarakhand through northern Uttar Pradesh (Dudhwa National Park), Sikkim, northern West Bengal, Arunachal Pradesh, and northwestern Assam north of the Brahmaputra River (Chakrashila Wildlife Sanctuary), to northeastern India south of the Brahmaputra River (Meghalaya) [11]. Recorded from Manipur also [14].

Global distribution

India, Nepal, Bhutan, Bangladesh, Myanmar, Thailand, Laos, Cambodia, Vietnam, Hong Kong, South China [13].

4. Conclusions and discussion

Previous literature and checklists on butterflies of India [11] were thoroughly reviewed to verify the absence of published photographs of this species in Uttar Pradesh. The current research aligns with previous studies conducted in the state and neighboring regions, following similar methodologies and formats.

References to prior articles from Uttar Pradesh and adjacent states, such as those by [3], [4], [6], [6], [20], [8], [7], [21], [22], were consulted to contextualize the findings. Additionally, this study correlates with similar works on new butterfly records from adjacent areas, including studies by [12], [18], [23], [24] and [25].Interestingly, *Flos asoka* is not listed under the Wildlife (Protection) Act, 1972 (Anonymous, 2006), or its amendment in 2022 [2], highlighting the need for continued research and conservation efforts for this species and others in its genus. This research contributes to the understanding of butterfly distribution in Uttar Pradesh and underscores the importance of photographic documentation in biodiversity research. This research presents the first photographic evidence of Flos asoka in Uttar Pradesh, India, confirming its presence in the region. Through detailed analysis and ecological insights, the study enhances understanding of the species' distribution and ecology. The findings underscore the importance of photographic documentation in species research and highlight the significance of visual evidence in validating occurrences. This research sets a precedent for future studies and emphasizes the value of interdisciplinary approaches in biodiversity research.

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Appendix I

Checklist on Flos species in India as per updated checklist of (Gasse, 2018), (Kumar et.al., 2020), (Pandey et.al., 2024).

1. *Flos apidanus* (Cramer, 1773) - Identified as the Plain Plushblue
Subspecies: *F. a. ahamus* Doherty, 1891
Occurrence: Scarce at lower altitudes in northeastern India below the Brahmaputra River, with no records in Tripura or Mizoram [11].

2. *Flos adriana* (de Niceville, 1883) - Variegated Plushblue
Occurrence: Infrequent in the Himalayas, found up to 1100m altitude, spanning from Uttarakhand towards the east, Uttar Pradesh, Sikkim, North West Bengal, Arunachal Pradesh, and northeastern India below the Brahmaputra (Eastern Assam and Manipur) [11], [21].

3. *Flos areste* (Hewitson, 1862) - Tailless Plushblue
Subspecies: *F. a. areste* (Hewitson, 1862)
Occurrence: Uncommon in the Himalayas, between 600 to 1800m altitude, distributed across Sikkim, North West Bengal, and the hilly regions of northeastern India below the Brahmaputra (Meghalaya, Nagaland, and Manipur) [11].

4. *Flos asoka* (de Niceville, 1883) - Spangled Plushblue
Occurrence: Uncommon in the Himalayas, found up to 900m altitude, spanning from Uttarakhand towards the east through North Uttar Pradesh, Sikkim, North West Bengal, Arunachal Pradesh, and northwestern Assam above the Brahmaputra, as well as in northeastern India below the Brahmaputra (Meghalaya) [11].

5. *Flos chinensis* (C. & R. Felder, 1865) - Chinese Plushblue
Occurrence: Uncommon in the Himalayas, up to 1700m altitude, found in Uttarakhand, also seen in Sikkim, North West Bengal, and northeastern India below the Brahmaputra (not documented in Tripura or Mizoram) [11], [17].

6. *Flos diardi* (Hewitson, 1862) - Bifid Plushblue
Subspecies: *F. d. diardi* (Hewitson, 1862)
Occurrence: Rarely observed in the Himalayas, with limited sightings in Sikkim and Arunachal Pradesh at altitudes up to 1500m, and in northeastern India below the Brahmaputra (not documented in Tripura or Mizoram) [11].

7. *Flos fulgida* (Hewitson, 1863) - Shining Plushblue
Subspecies: *F. f. fulgida* (Hewitson, 1863)
Occurrence: Uncommon in the Himalayas up to 1700m altitude, observed from Central Nepal eastwards through Sikkim and North West Bengal to Bhutan, and in the hilly regions of northeastern India below the Brahmaputra (Meghalaya and Nagaland) [11].

8. *Flos anniella* (Hewitson, 1862) - Brilliant Plushblue
Subspecies: *F. a. artegal* Doherty, 1889
Occurrence: Extremely rare at lower elevations in northeastern India below the Brahmaputra (Meghalaya and Nagaland) [11].

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In vitro therapy investigation for breast cancer by B13-chloroquine application

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Abstract

Cancer disease still remains to be a strong treat to public health. New treatment approaches and agents with low side effects are needed for the treatment of breast cancer. Based on these, herein was aimed to investigate the cytotoxicity of a combination comprising a ceramidase inhibitor (B13) and an autophagy inhibitor (chloroquine) on a human breast cancer cell line. The antiproliferative activity was tested by Sulforhodamine B and ATP viability assays. For ultrastructural and morphological changes and apoptotic signs of MCF-7 cells were used TEM and confocal microscopy techniques. Results showed the high cytotoxic and antiproliferative activities of the combination along with the ultrastructural and morphological changes indicating apoptosis. B13+Chloroquine combinations were found to be effective in inducing cell death on MCF-7 cells and antiproliferative and cytotoxic effects on cells. Consequently, the new combination is suggested as a good candidate for further investigations to be an anti-cancer agent.

Keywords: MCF-7, combination therapy, cytotoxicity, B13, chloroquine

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Meme kanserinde B13-klorokin uygulamasıyla in vitro tedavi araştırması

Özet

Kanser hastalığı hala halk sağlığı için ciddi bir tehdit olmaya devam etmektedir. Meme kanseri tedavisi için yeni tedavi yaklaşımlarına ve yan etkisi düşük ajanlara ihtiyaç duyulmaktadır. Bunlara dayanarak, burada bir seramidaz inhibitörü (B13) ve bir otofaji inhibitörü (klorokin) içeren bir kombinasyonun insan meme kanseri hücre hattı üzerindeki sitotoksisitesinin araştırılması amaçlanmıştır. Antiproliferatif aktivite, Sulforhodamine B ve ATP canlılık deneyleri ile test edilmiştir. MCF-7 hücrelerinin ince yapısal ve morfolojik değişiklikleri ve apoptotik belirtileri için TEM ve konfokal mikroskopi teknikleri kullanılmıştır. Sonuçlar, kombinasyonun yüksek sitotoksik ve antiproliferatif aktivitelerinin yanı sıra apoptozu gösteren ince yapısal ve morfolojik değişikliklere neden olduğunu göstermiştir. B13+Klorokin kombinasyonunun MCF-7 hücrelerinde ölümü tetiklediği, antiproliferatif ve sitotoksik etkilere neden olduğu saptanmıştır. Sonuç olarak, yeni kombinasyonun antikanser bir ajan olarak ileri araştırmalar için iyi bir aday olduğu ortaya konulmuştur.

Anahtar kelimeler: MCF-7, kombine terapi, sitotoksisite, B13, klorokin

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1. Introduction

Breast cancer incidence and mortality rates remain unchanged despite the developments in its treatment [1, 2]. Due to the difficulties of traditional cures, it is important to develop effective treatment agents for cancer treatment [3, 4, 5]. Preclinical studies support the use of chloroquine as an anti-cancer agent with important antineoplastic effects and the opportunity for usage for the treatment of many aggressive and metastatic cancers [6, 7]. B13, has been indicated for its anti-cancer activity in different cells lines with the mechanism of inhibiting acid ceramidases [8, 9, 10]. Herein, we aimed to investigate the characteristics of changes in the morphology and ultrastructure of breast cancer MCF-7 cells applied with a B13+chloroquine combination.

2. Material and method

2.1. Materials

MCF-7 (ATCC® HTB-22™) cells were purchased from the American Type Culture Collection (Manassas, USA). B13 (D-NMAPPD), Chloroquine, fetal bovine serum, penicillin-streptomycin, dimethyl sulfoxide (DMSO), Sulforhodamine B (SRB), Trichloroacetic acid were obtained from Sigma-Aldrich (St. Louis, USA) and Roswell Park Memorial Institute medium (RPMI-1640) was from GIBCO (Grand Island, USA).

2.2. Cell culture

In the cell culture study, the MCF-7 cell line was grown in RPMI-1640 medium containing penicillin–streptomycin (100 units/mL-100 µg/mL) and fetal bovine serum (10%) at 37°C and 5% CO_2 in the humidified incubator until they become confluent. In all of the experiments cell culture flasks with at least 85% confluency.

2.3. Sulforhodamine B method (SRB)

When performing the Sulforhodamine B method (SRB), cells were planted in 96-well cell culture dishes at a density of 2500-7500, 100 µl per well. Cells were treated with B13 compound and chloroquine (between 1.5-100 µM doses) for 24 hours. Treated cells were fixed in situ with 50% (w/v) cold TCA and stained with 0.4% (w/v) SRB in 1% acetic acid for measurement. At the end of the treatment period, the SRB dye bound to the cells will be removed using unpuffed 10 mM Tris, and the optical density (absorbance) was measured at 530nm on a plate reader (Synergy HTX, Bio-Tek, USA). Viability percentages were calculated in comparison to the untreated cell.

2.4. ATP viability method

ATP level measurement is based on luminescence technology and is much more sensitive than other viability methods, however, even at low cell counts, it is more sensitive and reliable than colorimetric tests as there is an excellent correlation between the viable cell count and the RLU (relative light unit) values read on the device. For this purpose, B13 and chloroquine treatment (between 1.5-100 µM doses) were applied to MCF-7 cells in 96-well cell culture dishes with a density of $5x10^3$ cells/well for 24, 48 and 72 hours. ATP content in treated cells and control cells was measured using a luminometer (Synergy HTX, Bio-Tek, USA) with a measurement time of 1 second with the luciferin-luciferase bioluminescence reaction shown below. The results were analyzed as Relative Light Units (RLU).

$$ATP + Luciferin + O_2 \rightarrow\rightarrow\rightarrow\rightarrow\rightarrow AMP + 2Pi + CO_2 + Photon\ (RLU)$$

Viability percentages of the samples were calculated according to the RLU values that were obtained from the control cells. Viability calculation formula is as follows:

$$Viability\ (\%) = [100 \times (Sample\ RLU) / (Control\ RLU)]$$

2.5. Confocal microscopy

The morphological changes that B13 and chloroquine agents can cause in MCF-7 cells were examined by confocal microscopy method. In preparation for MCF-7 cells to be examined under a confocal microscope, $3x10^5$ cells were incubated with IC_{50} concentrations of B13 and Chloroquine on sterilized coverslips in 6-well plates. At the end of the incubation period, the medium was removed and the cells were fixed in glutaraldehyde by washing in phosphate buffer (PBS). After fixation, cells were washed again with PBS and double stained with acridine orange and phalloidin dyes [11]. The morphological changes of the cells were examined using a confocal microscope (Leica TCS-SP5 II) in Leica Confocal Software Version 2.00 and visuals were obtained.

2.6. Determination of ultrastructural changes by TEM

Ultrastructural changes of untreated MCF-7 cells with a density of $1x10^6$/mL and MCF-7 test cells exposed to IC_{50} concentrations of B13 and Chloroquine were examined under a transmission electron microscope (TEM). At the end of the incubation period, MCF-7 cells were fixed in glutaraldehyde overnight at +4°C, then subjected to secondary fixation in osmium tetraoxide by washing with buffer. Fixed cells were dehydrated in ethyl alcohol series (50%, 70%, 90%, 96% and absolute ethyl alcohol) and cells were exposed to propylene oxide followed by blocking in resin. The blocks were polymerized in an oven at 60°C for 48 hours. The resulting blocks were cut into sections (80-100 nm) and placed on copper grids. Samples were stained in uranyl acetate and lead citrate. Samples were visualized under a transmission electron microscope (TEM) at 120 kV (Biotwin FEI, USA) and subtle structural changes were detected.

2.7. Statistical analysis

Cytotoxicity test results were tested and determined by one-way analysis of variance (ANOVA). All tests were accepted at the α 0.05 significance level. Statistical analyzes were evaluated with GraphPad 8.0 computer package programs.

3. Results

3.1. Sulforhodamine B cytotoxicity results

Concentrated stock solutions of B13, Chloroquine and B13+Chloroquine compounds were prepared in DMSO. The MCF-7 cell line was incubated on a 96-well plate with different concentrations of B13, Chloroquine and B13+Chloroquine for 24 hours. According to the results of this experiment, IC_{50} concentrations were calculated and antiproliferative activity graphs were created. In these findings, it was observed that the viability of MCF-7 cells decreased dose-dependent after B13 administration. In MCF-7 cells, where the combination of B13+Chloroquine was applied for 24 hours. IC_{50} value for this combination could not be calculated. As a result of the Sulforhodamine B method (SRB), the concentrations (IC_{50} value) suppressing the growth of MCF-7 cells were determined as 19.3 µM for B13 substance, while this value was 61 µM for chloroquine. The viability of MCF-7 cells decreased after B13 administration depending on the concentration. It was found that cell viability decreased as the applied substance concentration increased. The inhibition graph formed by the viability values calculated from the absorbance obtained from the SRB test of MCF-7 cells where B13 and chloroquine are applied for 24 hours is given in Figure 1.

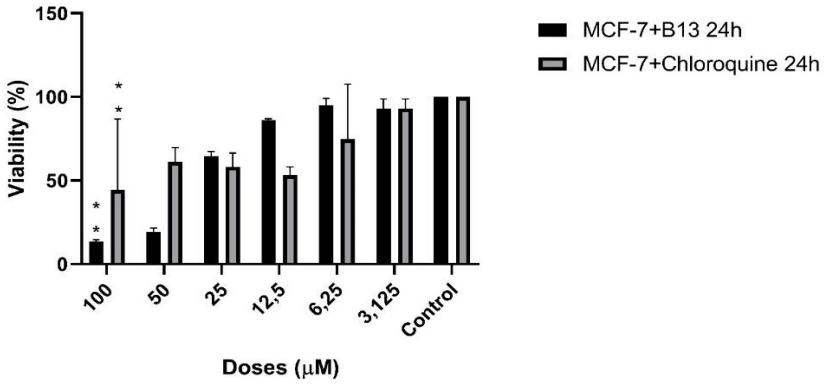

Figure 1. Viability inhibition in MCF-7 by B13 and chloroquine administration. (**; p: 0.0024) (**; p: 0.0046)

3.2. ATP bioluminescence cytotoxicity test results

ATP bioluminescence cytotoxicity test is defined as one of the fastest, most sensitive and easiest methods among viability tests performed in multi-well plates. In our study, 24 hours IC_{50} value for B13 was determined as 8,84 µM in this test results. This value was determined as 56.25 µM for Chloroquine and B13+Chloroquine combination 21.32 µM and the dose-dependent viability graph was shown in Figure 2A. B13, Chloroquine and B13 + Chloroquine combination concentrations applied to MCF-7 cells for 48 hours showed that the IC_{50} value of B13 could not be calculated. In the same application period, the IC_{50} value determined for chloroquine was determined as 18.99 µM, while this value of the B13+Chloroquine combination was determined as 4.96 µM. Concentration-dependent viability suppression graph is presented in Figure 2B. MCF-7 cells were treated with varied concentrations of B13, Chloroquine, and B13 + Chloroquine combined compound for 72 hours. As a result of this test, the determined IC_{50} values of B13, chloroquine, and B13 + Chloroquine combined compound are detected as 5.49 µM, 20µM and 12.75 µM respectively and the dose-dependent viability graph is shown in Figure 2C.

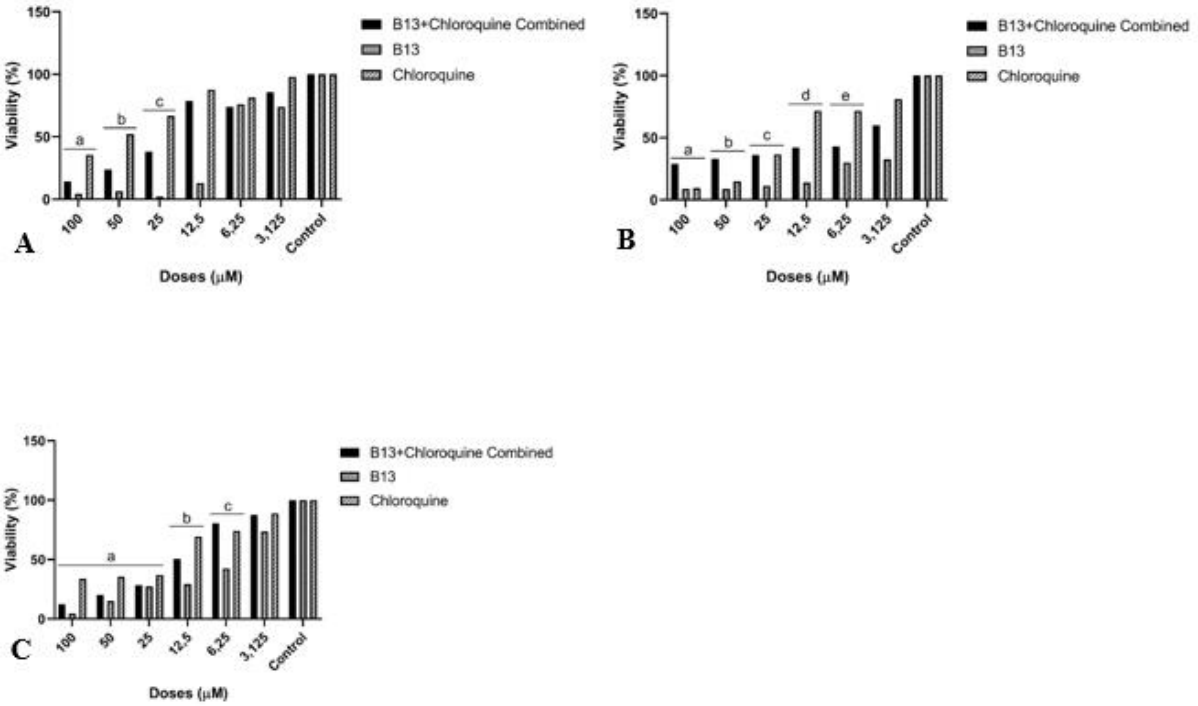

Figure 2**. (A)**Viability inhibition graph of MCF-7 cells exposed 24 hours to B13, Chloroquine and B13 + Chloroquine combination. (p values, a: **:0,0029; b: **:0.0082; c: *:0,0210) **(B)** Viability inhibition graph generated from ATP test findings with 48 hours of B13, Chloroquine and B13+Chloroquine combined compound on MCF-7 cells. (p values; a:***: 0.0006; b:***:0.0008; c: **:0.0025; d:*:0.0166; e:*:0.0358) **(C)** Viability percentages of MCF-7 cells exposed to B13, Chloroquine and B13 + Chloroquine combined compound for 72 hours. (p values: a=****:<0.0001; b=**:0.015; c=*0.0351)

3.3. *Confocal microscopy results*

When the obtained data is evaluated, morphological changes occurring on cells in the application period of 24 by B13+Chloroquine application are shown in Figure 3. The detected morphological changes were determined as nucleus fragmentation, chromatin condensation, cytoskeleton disruption and hole formation.

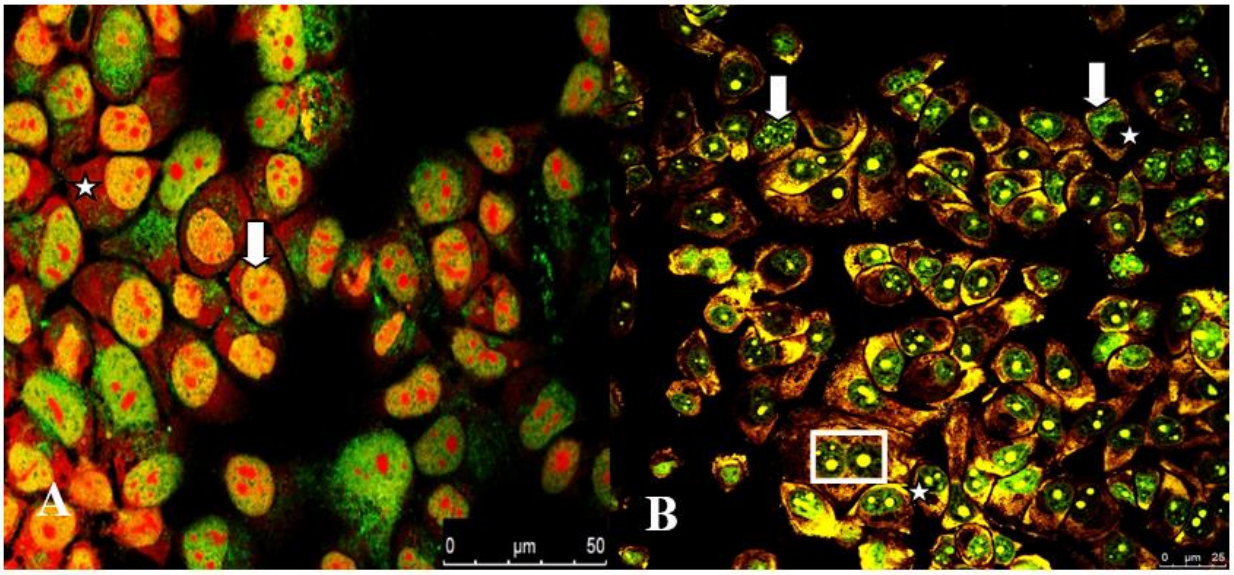

Figure 3**.** Confocal microscopic images of morphological changes of MCF-7 cells. (A) Control group MCF-7 cells: Arrow-free cell nucleus, Asterisk-undamaged cytoskeleton (B) MCF-7 cells in which IC_{50} concentrations of B13+Chloroquine are applied for 24 hours: Arrow-lysed cell nucleus, Asterisk-cytoskeleton disruption, Square-chromatin condensation.

3.4. Electron microscopic analysis of the ultrastructural changes

Electron microscopic analysis of the MCF-7 cell line exposed to B13+Chloroquine showed that the ultrastructure of the cell was changed. The detected ultrastructural changes of this cell group were chromatin condensation, blebbings on membranes, shrinkage on the nucleus membrane and loss of crystae of mitochondria in comparison with the control cells unchanged ultrastructure. These detected changes are shown in Figure 4.

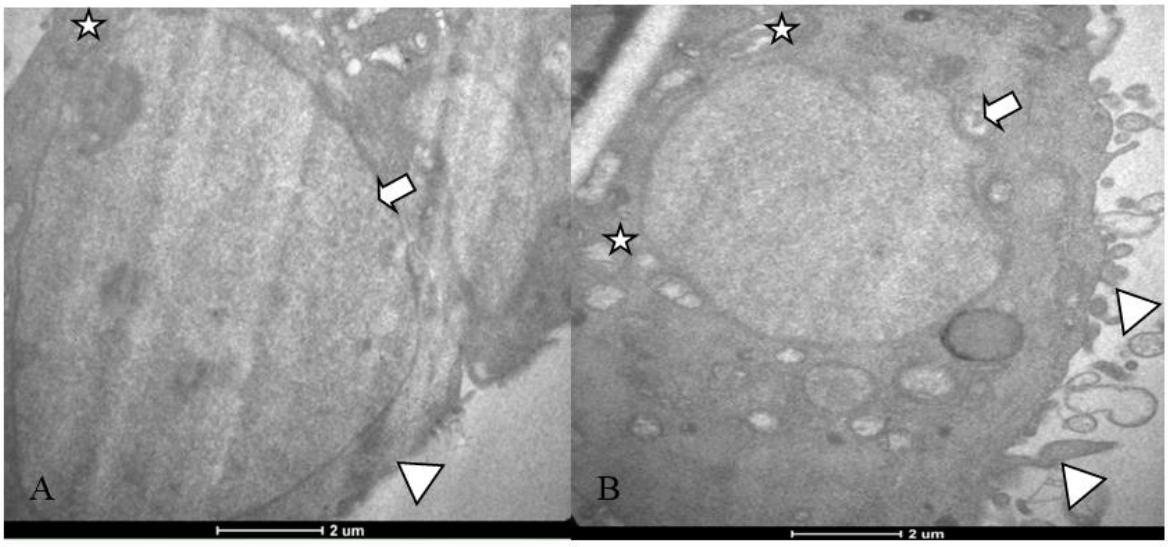

Figure 4. TEM images of ultrastructural changes of MCF-7 cells. (A) Control group MCF-7 cells: Arrow-free cell nucleus, Asterisk-intact mitochondria, Triangular-compact cell membrane. (B) MCF-7 cells in which IC_{50} concentrations of B13+Chloroquine are applied for 24 hours: Arrow-core membrane collapse and chromatin condensation, Asterisk-damaged crystae of mitochondria, Triangle-membrane blebbing.

4. Conclusions and discussion

Combination therapy is the focus of researchers due to its potential to reduce or completely eliminate multiple drug resistance in the treatment of many different types of cancer [12]. Today, combination therapy studies have become an important consideration in cancer treatments in order to strengthen therapeutic efficacy and overcome drug resistance and metastasis [13]. Autophagy is a self-digesting product that is evolutionarily protected and, however, is indicated as a process in which proteins and other cytoplasmic materials are recycled to back up cell survival under stressful terms (i.e. cancer treatment). It is recommended as a resistance mechanism to treatments such as radiotherapy and chemotherapy [14]. If we talk about another way of death such as autophagy, apoptosis is one of the main mechanisms of cell death in response to cancer treatments [15]. Changes in susceptibility to apoptosis not only contribute to neoplastic development. In addition to this contribution, it can also increase resistance to traditional anticancer treatments that are still being applied, such as radiation to the patient and treatments with cytotoxic agents [16]. In this study, we used chloroquine to inhibit autophagy and B13 to involve apoptosis in MCF-7 cells. ATP viability test results and SRB results imply to the enhanced cytotoxicity of the agent used in combination (Figures 1 and 2). Our ATP finding indicated to the dose and time dependency of the applied agent combination for an application time of 24 and 48 hours. The obtained IC_{50} value of the combination for 72 hours was found to be higher than that for 48 hours which may mean resistance development by the MCF-7 cells to the used combination (Figure 2). Our viability and cytotoxicity test findings were discussed as potent inhibition of authophagy and enhanced programmed cell death via potent arise in intracellular ceramide. Chloroquine is a lysosomal enzyme inhibitor and with it, it inhibits the late autophagy state by altering the pH of lysosomes and thus also affects the degradation of proteins enveloped in the autophagosome [17] and chloroquine, often used as an anti-malarial drug, it also has potential anti-cancer effects. It shows effects such as inhibition of cell growth in A549 (human lung cancer cells) cells and glioma cells. In addition, it has been shown to increase the inhibitory effects of other chemotherapeutic agents on tumors, thanks to its use in therapy [18]. Treatment methods in cancer are constantly improving, and recently there has been an increase in the work of classes of agents other than chemotherapy. Among these new agents being studied are targeted drugs, immunotherapies, and hormonal agents, but a clearer understanding of cancer biology and pathogenesis paves the way for molecular targeted therapies [4]. Through research, it has been shown that the biological effect of chloroquine is concentration-dependent. For example, at low concentrations, chloroquine has been found to inhibit the growth of the lung cancer cell line A549. At higher concentrations or longer durations, chloroquine has been reported to directly

induce apoptosis and necrosis [19]. Based on findings from different studies, ceramidase inhibitors are considered to be a potential new therapeutic class of antiproliferative and cytostatic drugs. D-NMAPPD, also known as B13, is a new member of the ceramidase inhibitor group. B13 has been shown to induce apoptosis on different cancer cells in in vitro and in vivo studies, and there is also evidence that the associated ceramidase inhibitor B13 can suppress acid ceramidase activity [9]. Our study describes a systematic time and dose-dependent approach to evaluate one of the drug combinations that are effective in killing cancer cells due to changes in drug exposure and duration. In a study with chloroquine alone, this agent has been found to inhibit the proliferation of breast cancer cells. Our study shows close results with our study only if the chloroquine inhibitor is examined. In a conducted study, different breast cancer cell lines (4T1, MCF-7, MDA-MB-231, MDA-MB-435S, T47D, and Bcap-37) were used to investigate the cell growth inhibitory properties of chloroquine. A comparison was made between the six cell lines examined in this study and showed that Bcap-37 cells were very susceptible to chloroquine treatment. IC_{50} values of MCF-7, MDAMB-231, MDA-MB-435S, T47D and Bcap-37 were shown as 63.98, 30.18, 35.84, 132.87, 21.68 μM after 48 hours of chloroquine treatment [20]. Paralelly with these findings, our results showed that the chloroquine+B13 combination resulted in lower IC_{50} values than that in the literature. Additionally, the antiproliferative and cytotoxic activities of the used combination were detected highly in the exposed MCF-7 cell ultrastructure and morphology (Figures 3 and 4). Confocal microscopic findings of morphological changes of MCF-7 cells exposed to the B13+Chloroquine combination were fragmentation of nuclei and cytoskeleton, chromatin condensation and hole formation. These findings were taken as morphological indicators of apoptotic cells death and interpreted to be caused by inhibition of ceramidase enzyme activity by B13 and autophagy by chloroquine (Figure 3). TEM findings of MCF-7 cells exposed to the used combination showed chromatin condensation, blebbing in membranes, collapse in the nucleus membrane, and loss of crystae of mitochondria as ultrastructural changes (Figure 4). Mitochondrial dysfunction was implied by the broken mitochondrial inner sides, especially its criste. This finding is taken as a clear apoptotic sign.

Herein, it has been determined that these compounds are potential anti-cancer agents but the combination was disscussed as the most relevant agent of the study to involve cell death with dual action, autophagy inhibition, and apoptosis induction. The potential of this combination as a treatment agent is thought to contribute to public health, and that is a good potent candidate for drug design and production after further and deeper studies. In line with the study we have conducted, it is recommended to carry out studies to produce the B13+Chloroquine combined compound as an anti-cancer drug. It is also anticipated that it can be used in the pharmaceutical field for synthesizing targeted therapeutic agent after drug design. In conclusion, the B13+Chloroquine combination is recommended for use in designing and synthesizing an effective pharmaceutical agent for cancer treatment, after investigating its further effects on other cancer cell lines and *in vivo*.

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Determination of model maps for the potential distribution of Anatolian black pine (*Pinus nigra* Arnold.) in natural forest areas in the Central Black Sea region

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Abstract

The purpose of this study was to determine potential distribution modeling and mapping of Anatolian black pine in the Vezirköprü district. Presence / absence data of the species was collected from 586 sample areas. Environmental variables (elevation, slope, bedrock, radiation index etc.) were obtained from the digital maps. In addition, climatic variables were downloaded from WorldClim database. Generalized Additive Model and Classification and Regression Tree were used for potential distribution modeling of the species. The validation value of Generalized Additive Model was 0.84 while the cross-validation test value was found to be 0.82. Also, the ROC values of the tree model were found to be 0.804 for the training data set and 0.750 for the testing data set. According to the Classification and Regression Tree method, the locations above 650 m and without meta-sandstone are suitable for the potential distribution of the species. Under the condition that there is meta-sandstone, the areas where the temperature is between 7.6-11.0 °C and the slope degree is more than 23% coincide with the potential distribution of the species. Also, Generalized Additive Model showed that places where gabbro, ophiolitic melange, serpentine, and mixed material were seen as main bedrock type, sloping sites where average elevation was from 600 to 1150 m and temperature is between approximately 8.5-11.3 °C were the most suitable conditions for the potential distribution of the species in the district. In both models, it was determined that especially rock formations, climatic variable, and altitude are effective in the potential distribution of the species. On the other hand, although there are some differences in the variables in the models, the potential distribution maps formed by these variables overlapped quite. As a result, the information obtained in the study is important for the forestry practices such as afforestation, regeneration and monitoring the species under future climate change conditions.

Keywords: generalized additive model, classification and regression tree, species distribution model, Vezirköprü

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Orta Karadeniz bölgesindeki doğal orman alanlarında Anadolu karaçamının (*Pinus nigra* Arnold.) potansiyel dağılım model ve haritalarının belirlenmesi

Özet

Bu çalışma Vezirköprü yöresinde Anadolu karaçamının potansiyel dağılımının modellemesi ve haritalaması amacıyla gerçekleştirilmiştir. 586 örnek alanda türün var / yok verisi toplanmıştır. Sayısal yükseklik modeli kullanılarak çevresel değişkenler (yükselti, eğim, anakaya, radyasyon indeksi vb.) elde edilmiştir. Ayrıca, WorldClim veri tabanında iklim değişkenleri indirilmiştir. Türün potansiyel dağılım modellemesinde Genelleştirilmiş Eklemeli Model ve Sınıflandırma (GAM) ve Regresyon Ağacı Tekniği (CART) kullanılmıştır. GAM modele ait geçerlilik oranı 0,84, çapraz geçerlilik katsayısı ise 0,82 bulunmuştur. Ayrıca, CART modelin geçerlilik (ROC) değerleri eğitim verisi için 0,804, test verisi için ise 0,750 bulunmuştur. CART yöntemine göre, 650 m'nin üzerindeki yükseltiler ve meta-

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kumtaşının olmadığı yerler türün potansiyel dağılımına uygundur. Metakumtaşının olduğu kısımlarda ise, yıllık ortalama sıcaklığın 7,6 – 11,0 ° C arasında ve eğimin %23'ten büyük olduğu yerler türün potansiyel dağılım alanlarını oluşturmaktadır. GAM yönteminde gabro, ofiyolitik melanj, serpantin ve karışık anakaya tiplerinin olduğu, eğimli arazi yapısına sahip 600 – 1150 m arasındaki yükseltiler ve yıllık ortalama sıcaklığının 8,5 – 11,3 °C olduğu yerlerde türün potansiyel dağılımı ile örtüşmüştür. Her iki yöntemde anakaya tipleri, iklim ve yükselti değişenlerinin türün yöredeki potansiyel dağılım alanlarında daha etkili olduğu tespit edilmiştir. Modellerde yer alan değişkenlerde bir takım farklılıklar olmasına rağmen, elde edilen potansiyel dağılım haritaları oldukça örtüşmüştür. Sonuç olarak, çalışmada elde edilen bilgiler türün ağaçlandırma ve gençleştirme gibi ormancılık uygulamaları ile iklim değişimi gibi süreçlerde izlenmesi için önem arz etmektedir.

Anahtar kelimeler: genelleştirilmiş eklemeli model, sınıflandırma ve regresyon ağacı, tür dağılım modellemesi, Vezirköprü

1. Introduction

Human activities above the forests, climate change, carbon emissions, and wildfires are all global issues that have a growing impact on all living organisms, including plant species distribution. In addition to these problems, there has been a significant contraction in forest areas worldwide due to possible climate change, severe drought, wildfires, rapid population growth, excessive and unsustainable illegal cutting, overgrazing, and other problems in recent years [1]. In response to this situation, the forest administrations of the countries have tried to manage the forests with a system that is more planned and sustainable way. At this stage, ecosystem-based multiple-use forest management (EBMFM) applications have been of great importance to ensure the sustainability of forest areas in a planned manner [2]. Especially, the modeling and mapping of the potential distribution areas of tree species, which are directly subject to forest management, constitute an important step for the EBMFM applications.

Potential distribution areas of a tree species can be determined as a result of modeling and mapping of ecological environmental conditions by utilizing the current status of species in forest areas. There are a number of variables that limit the distribution of tree species in forest ecosystems. Therefore, the species distribution modeling based on ecological data has recently been very popular for the conservation planning of the forest ecosystems [3]. In parallel with this situation, the modeling and mapping studies on the potential distribution areas of tree species, which are subject to direct forest management for any country, have begun to be carried out increasingly [4]. These models and maps can provide valuable information about the future of a tree species apart from the current situation. Therefore, it is evident that these models and model maps obtained in the forestry studies are able to operate and sustain tree species in a planned manner. In other words, the integration of these model studies into forest management plans is essential [5].

Just like in the world, pine species are very important forest trees for Turkish forests. One of them is Anatolian black pine (Pinus nigra Arnold.) which occupies a very large area of approximately 4.7 million ha in the forest ecosystems of Turkey [6]. It is an important tree species due to its ecological (e.g. carbon storage, biodiversity, and soil protection), economic and social (e.g. recreation, rural tourism) characteristics. It is also known as one of the most suitable species for afforestation of arid and rocky terrains [7]. In Turkey, it also covers a wide latitudinal distribution range from the southern and western parts to the inner and the northwestern parts.

One of the most important distribution areas of this species in the northwestern parts of Anatolia is Kunduz forests in Vezirköprü district. Following the information given above, identification and mapping of potential distribution areas in forested areas of this species, which is of such importance for the global scale and for Türkiye, is considered to be an appropriate application. As a result, the aim of this study was to determine the potential distribution areas of Anatolian black pine in the Vezirköprü district. Accordingly, two different nonparametric model techniques were applied in the study, it is aimed to reach the information that can contribute to the sustainability of the species in this region.

2. Material and Methods

2.1. Study area

The study area was located within the boundaries of Kunduz, Sarıçiçek, Gölköy and Narlısaray Forest Divisions under the Forest Directorate of Vezirköprü affiliated to the Regional Forest Directorate of Amasya and Osmancık Forest Division affiliated to the Forest Directorate of Çorum. The situated hinterland of the Central Black Sea Region, the study area is surrounded by Samsun province in the northeast, Amasya province in the south and Çorum province in the southwest (Figure 1). Kunduz (Vezirköprü) locality situates in the hinterland of the Central Black Sea Region covers an area of around 59,000 ha. Kızılırmak River flow into the Black Sea from Bafra cape in the shape of an arch starting from the west of this locality. The study area is surrounded by Ilgaz Mountain in the west, Küre Mountain in the northeast a Canik Mountain in the east. The summit of Kunduz Mountain after which the locality was named is Keltepe

that is 1791 m high. On the other hand, the coastal areas of Altınkaya Dam Lake located within the boundaries of Narlısaray Forest Division represent the areas of the locality with the highest elevation with around 190 m.

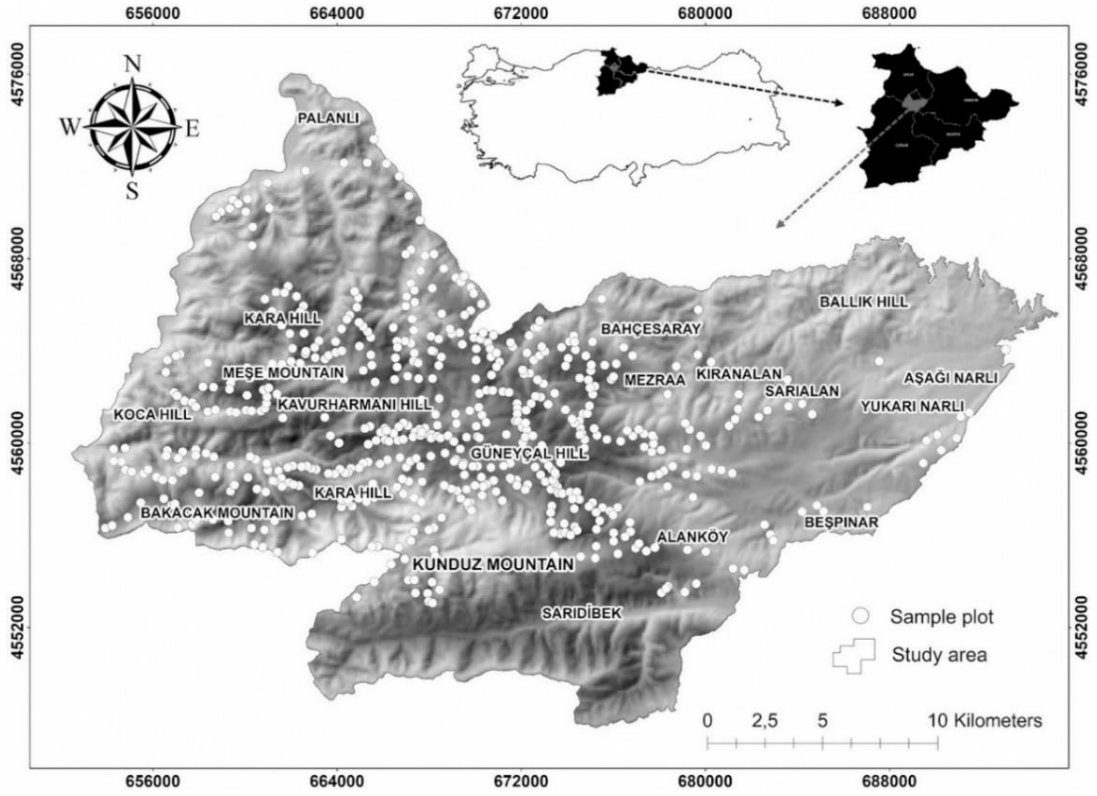

Figure 1. Location of the study area in the map of Turkey

It was found that the locality and its surrounding were located mainly on ophiolithic blocks containing sedimentary and magmatic rocks and belonging to Mesozoic Upper Cretaceous age [8]. Quartz minerals, gabbro, marble as well as different forms of magmatic basic rocks such as solid rock, tuff, and agglomerate in the Kunduz mountain mass. Ophiolithic rock blocks such as gabbro, amphibolite, pyroxenite, and spillite that are metamorphic rocks were found in Sarıdibek village and its surrounding [8].

Since the majority of the study area is located within the boundaries of Vezirköprü district, it represents the hinterland transition climate. According to the Köppen climate classification, the climate type in the vast majority of the study area is represented with warm winter and very hot summer while it is rainy in all seasons (Cfa); on the other hand, Çorum Osmancık section has cold semi-arid steppe climate (BSk) while the areas located within Amasya province have warm winter and very hot summer with an arid Mediterranean climate (Csa) [9]. The study area accommodates primary forest tree species such as Black pine, Brutian pine (*Pinus brutia*), Scotch pine (*Pinus sylvestris*), Nordmann fir (*Abies nordmanniana*), Crimean juniper (*Juniperus excelsa*), Beech (*Fagus orientalis*), Turkish oak (*Quercus cerris*) and Hornbeam (*Carpinus betulus*).

2.2. Data collection

Two sets of data (present: 1 – absent: 0) were collected from 586 sampling plots each with a size of 20x20 m. Stratified sampling method was used in the study [10]. Furthermore, the latitude and longitude of each sampling plot were recorded. On the other hand, environmental variables were created for potential distribution modeling and mapping (Table 1). Therefore, a Digital Elevation Model (DEM) with a cell size of 100×100 m was created taking into account the borders of the study area. DEM was used to generate topographic variables and the cell sizes of the variables obtained are 100×100 m. The elevation, slope and aspect maps were created based on the DEM in ArcGIS 10.2 software. A topographic position index map was created using the "Topographic Tool" extension in ArcGIS 10.2 software developed by Jennes [11]. Topographic position index is a variable that shows the structure of the landforms. Negative topographic position index values represent structures such as mainly canyons, valley floors, and values closer to zero represent flat areas, plains and lower slopes whereas positive values represent hills, mid-slopes, upper slopes, ridges, and mountain summits. The radiation index was obtained using the "TRASP" tool in the "Geomorphometric and Gradient Metrics Toolbox" extension in ArcGIS 10.2 software.

$$Radiation\ Index = [1 - \cos((\pi/180) \times (\theta - 30))] / 2 \quad (1)$$

Where, θ in the equation represents aspect values. The values calculated ranged from 0 to 1. "0" represented the shadowy aspects in the north-northeast direction while values closer to "1" represented lands that were hotter and dry slopes in the south-southwest direction [12].

The geology map of the study area was obtained from the General Directorate of Mineral Research and Exploration. Then, each bedrock type was drawn as polygons and recorded in the attribute table. Each bedrock type in the study area was exported to a raster format using the conversion tools.

Finally, the mean annual temperature and annual precipitation values used in the study area were downloaded from http://www.worldclim.org [13]. By using the cubic transformation option with the "*Resample*" tool in ArcGIS 10.2 software, the cell sizes of the downloaded climate variables (~ 1 km^2) were adjusted based on the cell sizes of the environmental variables (100×100 m).

Table 1. Definition of environmental variables

	Variable	**Codes**
Topographic Variables	Elevation (m)	ELVTN
	Slope (°)	SLOPE
	Aspect (°)	ASPECT
	Topographic Position Index	TPINDEX
	Radiation Index (0 – 1)	RADIN
Climate Variables	Annual Mean Temperature (mm)	BIO1
	Annual Precipitation (°C)	BIO12
Parent Materials	Alluvium	ALVM
	Gabbro	GABBRO
	Melange	MELANGE
	Metasandstone	METASAND
	Other types	OTHTYPE
	Sandstone, Mudstone	SANDMUD
	Sandstone, Mudstone, Limestone	SANDMUDLIM

2.3. Statistical assessment

In order to develop the potential black pine distribution model, the Generalized Additive Model (GAM) and Classification and Regression Trees (CART) technique were used. GAM is known to be the modified and non-parametric version of the generalized linear model. Non-linear distributions show higher associations in assessing the values [14, 15, 16]. It is often preferred in species distribution studies owing to the fact that it determines curvilinear relationships between dependent variable and independent variables. Different assessment methods are depending on the data type (such as presence-absence data, frequency, continuous between 0 and 1, counts, richness, positive integers, weights, size, biomass, etc.) of the dependent variable. In the study, GRASP (*Generalized Regression Analysis and Spatial Prediction*) plugin included in S - Plus 6.1 software was used [15]. Binomial distribution family was used to construct the model because of the fact that the data type of the target species is binary data. Different statistical methods are included in the GRASP extension and have model validations according to different tests. Among these statistical methods, the F test was selected and model was created.

On the other hand, CART is a rules-based non-parametric method. Independent variables were divided into homogenous sub-groups on the basis of the dependent variable and a tree model was created. The tree model obtained in a hierarchical order was connected through leaves and nodes. In the last node, the independent values are taken into consideration to write rules and prediction values are created [16]. The dependent variable in the study was categorical (present: 1 – absent: 0); thus, the method is named as classification tree. "*If then"* rules were written to calculating prediction value by using the value of the environmental variable in each terminal node [17]. On the other hand, using prediction values of the obtained CART model, the distribution map was visualized based on each cell value (100×100 m) of the study area (totally for 57603 grids).

The accuracy and performance of the models obtained from GAM and CART methods were checked by using Receiver Operating Characteristic (ROC) curve.

3. Results

The purpose of the study was to create the potential distribution model and map using the GAM and CART methods based on the present-absent data of black pine in 586 sampling plots. Elevation, slope, aspect, topographic

position index, radiation index and bedrock types from the environmental variables and the mean annual temperature and annual precipitation from the climate variables were used for the potential modeling mapping stage of the study.

The model obtained from the GAM technique was constituted by elevation, slope, bedrock types, and the mean annual temperature. The maximum contribution to the model came from bedrock types followed by elevation. The individual contribution of each variable was ranked as bedrock types (122.9403), mean annual temperature (121.9179), elevation (115.9843) and slope (11.2255).

The descriptive graphs relate to the GAM obtained after the analysis are presented in Figure 2 – 3. The graphs showed that black pine was potentially distributed at an elevation of approximately 700-1100 m, while its distribution decreased from about 1200 m to higher elevations. In particular, it preferred areas with a mean annual temperature of around 8.5 – 11.5 °C. On the other hand, slope is another variable that affected the potential distribution of black pine in the site, while areas with a slope of 10% - 30% were the most appropriate ones for the potential distribution of this species. Finally, the mixed formation of sandstone – pebble stone – limestone and melange were the most important rock formations that affected the potential distribution of black pine in the area while also gabbro and serpentine were the second most appropriate bedrock types for its potential distribution.

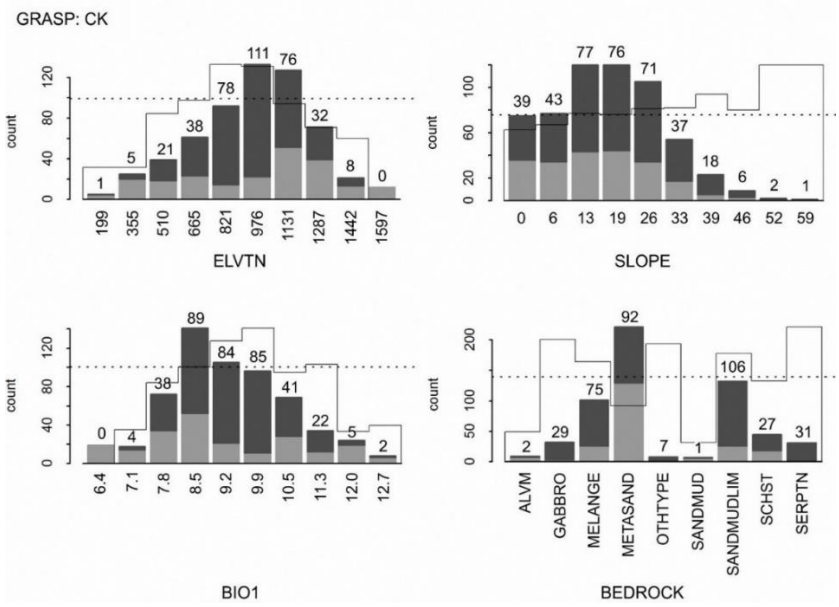

Figure 2. Histograms of the environmental variables to the modeling of Anatolian black pine predictive distribution.

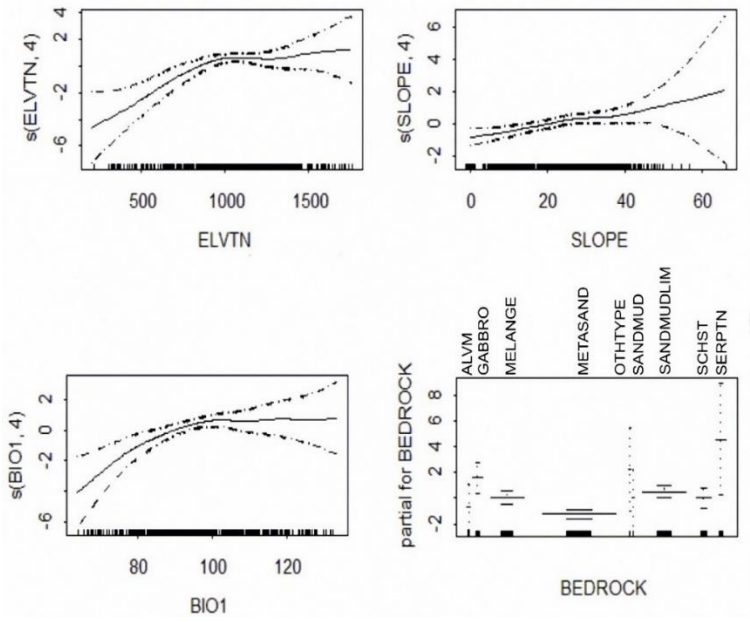

Figure 3. Partial response curves of the environmental variables to the modeling of Anatolian black pine predictive distribution

The validation value of the model for the main data set was 0.84 while the cross-validation test result was found to be 0.82. The potential distribution equation of black pine was obtained as Equation 2 from the model. In this formulation, 4 represents freedom.

$$CK \sim s(ELVTN, 4) + s(SLOPE, 4) + s(BIO1, 4) + BEDROCK \quad (2)$$

Where, CK is *Pinus nigra* Arnold., s represents spline smoother.

The tree model derived from the CART applied as the second method to model the potential distribution areas of the species under this study was structured with metasandstone, elevation, slope and mean annual temperature, respectively, as the variables according to their contribution (Figure 4).

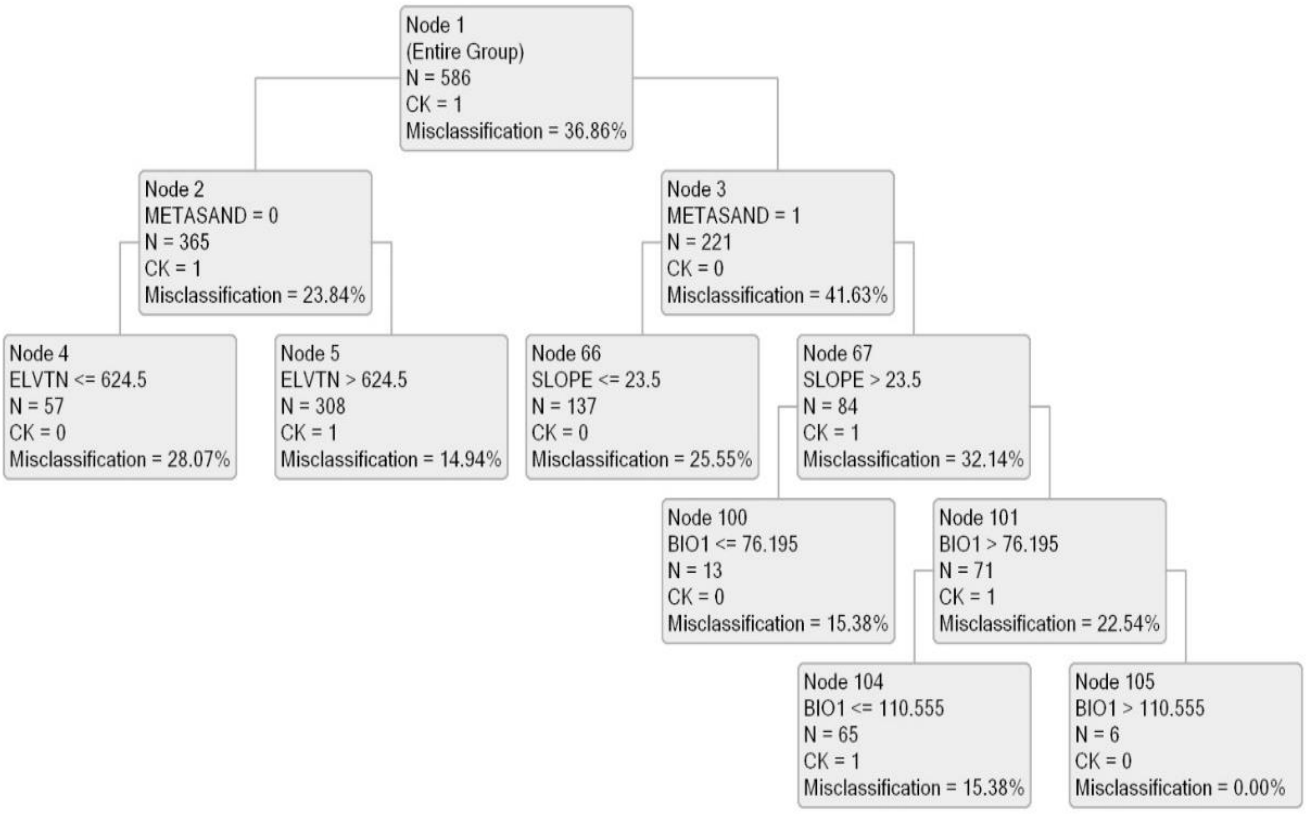

Figure 4. Classification tree of modeling black pine potential distribution

The decision tree of the CART model is shown in Figure 4. According to the tree model with 6 different terminal nodes, areas without metasandstone and areas with an elevation higher than 625 m were found to be the most appropriate potential distribution area of the species. On the other hand, in those sites with metasandstone, areas with a slope greater than 23.5% and mean annual temperature of 7.6 – 11 °C were also appropriate for the potential distribution of black pine. The ROC values of the tree model were found to be 0.804 for the training data set and 0.750 for the testing data set.

Finally, the potential distribution map of the GAM and CART created for black pine and intersection map of potential distribution of black pine using GAM and CART techniques was visualized in ArcGIS 10.2 software (Figure 5).

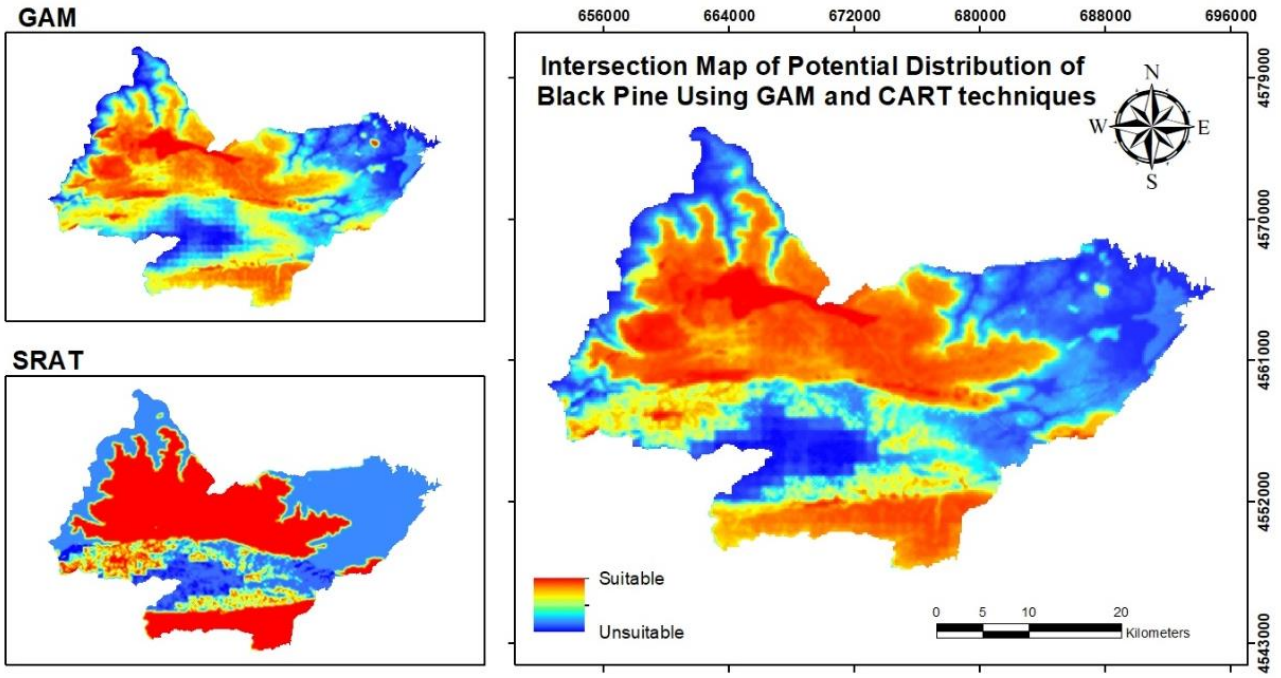

Figure 5. Intersection map of potential distribution of black pine

4. Conclusion and Discussion

The validity tests applied to the models developed through two analysis techniques used to determine the potential distribution areas of black pine revealed that these models had a high explanatory power. As a result of the model techniques applied, the descriptive variables that were statistically significant in the potential distribution areas of the species in that locality were bedrock, elevation, mean annual precipitation and slope according to the GAM technique and metasandstone, elevation, slope and mean annual precipitation according to the CART technique. In both models, it was determined that especially rock formations and climatic variables are effective in the potential distribution of the species. The results obtained from the models showed that in general, the best potential distribution areas for black pine were the ones located at an elevation of 650 – 1100 m with a mean annual temperature of 7.5 – 11.5 °C. Bedrock formation is another important variable that had an impact in that elevation a temperature range, while the mixed formation of sandstone– pebble stone– limestone as well as ophiolitic melange were the most appropriate geological formations for the potential distribution of black pine. On the other hand, gabbro and serpentine were the other appropriate formations for its potential distribution in the locality, whereas metasandstone was a limiting factor for its potential distribution. Moreover, the slope was another important variable found to be important in the models, while the potential distribution of the species increased especially at moderate and high slopes [16].

The association between black pine and elevation in the region can be explained with the fact that this species could meet appropriate climate requirements depending on that variable. The presence of the mean annual precipitation in the model supports this finding. As a matter of fact, in a study that determined the distribution areas of black pine in Turkey, they found that it was distributed on valley slopes and tectonic corridors in the Black Sea hinterlands starting from 600 m to 1400 m [17]. Moreover, they also showed that there was a significant variance in the distribution and productivity of black pine depending on elevation and temperature and its productivity decreased in general at elevations higher than 1400 m. Therefore, the findings regarding the associations with elevation and climate in the models developed for the potential distribution of black pine were also consistent with the results of the previous study. Furthermore, another study conducted in Aydınca (Amasya) to identify the potential distribution area of black pine in the hinterlands of the Central Black Sea showed that the elevations of 800 – 1100 m were the best distribution areas for this species. On the other hand, the main factor that had an actual impact was the climate conditions that altered depending on elevation. As in this study, it was stated that changing climate conditions with the increase in altitude affect the distribution of black pine [18].

In addition to the associations with climate requirements, as regards the rock formation which was another ecological condition that affected the distribution of black pine, the results of this study were consistent with the literature. It was reported that black pine trees could extend their roots deeper through the cracks and fractures of especially Mesozoic and Palaeozoic aged limestone rocks, and exploit the physiological depth and could establish pure and productive forests on this rock type in many areas [19]. It was also stated that very productive black pine forests were present on sandstone rock type at an elevation of around 1400 m in the west of Kastamonu tableland in the west Black Sea Region that also had similar habitat characteristics as that area [20]. Moreover, that study also showed that

very productive and widely distributed black pine stands were present in different parts of the Mediterranean Region (Pos-Karsantı area in the north of Çukurova, south slopes of Nur Mountain, Sütçüler and Eğirdir districts of Isparta province) and some locations of the Black Sea Region (for example, Dirgine and Camiyanı regions) as long as deep soil and good aeration conditions were available on serpentine bedrock. On the other hand, black pine trees could find cracks with their strong root systems on this rock formation, exploited the physiological depth and thus create an appropriate environment for their potential distribution. Therefore, it can be argued that all these rock types were suitable for the physiological depth for black pine and areas where it can be potentially present are formed when this condition exists. On the other hand, metasandstone was found to be a negative limiting factor for the potential distribution of black pine. This formation which is a metamorphic rock type is classified as semi-elastic very hard rock [21]. For that reason, as this is a very hard rock, insufficient soil formation is considered to be an important factor that limits the potential distribution of this species.

In our study, the slope was found to be another environmental factor that had an impact on the potential distribution of black pine. There aren't any literature findings that show the impact of this variable on the potential distribution of black pine. However, some studies found a positive association between slope and the productivity of black pine [22]. This can be explained as follows from a holistic perspective: the slope is an important factor that changes the environmental conditions such as erosion, soil depth, soil texture, the content of soil skeleton, surface runoff, soil temperature, water and nutrient economy [23]. Topographic factors such as elevation, different landforms, slope positions, slope degree play an important role in the distribution of black pine. Different landforms and degree of slope are important factors in the productivity of black pine. In general, while it is known that the productivity of black pine is low in areas with high slope degree, it is higher in places with low slope degree [24]. Güner et. al. [7] mentioned that the species was distributed in the degree of slope between 1-80% in the black pine afforestation in the study area. In addition, they determined about a positive relationship between the increase in the degree of slope and the height growth of black pine. Negiz et al. [25] found a negative relationship between the productivity of black pine and slope degree. In addition, as a result of obtained model, they were stated that the productivity of black pine on plain and lower slopes increased. As can be seen, the presence of black pine at many degree of slopes can be mentioned. Particularly, the distribution of black pine means that it can be possible even in areas with high slope degree. While the black pine potentially distributed in the slope values which were obtained to in the models, it was showed actual distribution in the other slope degrees of the species. That is to say, actual distribution of the black pine at high slope degrees in the district can be mentioned. Thus, it is more accurate to interpret the associations between the distribution and productivity of a species with the changing environmental conditions due to slope rather than directly based on slope. As a result, it can be suggested that the suitability of moderate and high slopes for black pine as found in this study should be explained with the other environmental conditions depending on the slope. In summary, black pine with a strong root system and the trunk has a better ecological tolerance to the abovementioned conditions compared to the other species present in the area, which increases its potential distribution at moderate and high slopes. For this reason, rather than claiming that these areas are the best places for its potential distribution, it is more accurate to suggest that these are the areas that are compatible with the ecological tolerance of the species.

Anatolian black pine is an economically and ecologically important species. The information obtained in this study can be used in certain forestry practices such as afforestation and regeneration a regional scale. The findings of the study are important in terms of ensuring the sustainability of the species in the region and observing the future changes about this species.

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The effect of different drying temperatures on the essential oil content and chemical composition of *Lavandula angustifolia* Mill.

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Abstract

Drying temperatures affect the content and composition of essential oils in plants containing essential oils due to the organs where essential oils are synthesized and stored. For this reason, many studies have been carried out to determine the appropriate drying temperature to obtain the highest amount and the best quality essential oil. In present study, it is aimed to determine the effects of different drying temperatures on essential oil content and components in lavender (*Lavandula angustufolia* Mill.). The samples dried at four different temperatures (35°C, 45°C, 55°C and 65°C) were isolated for 3 hours using Clevenger type apparatus and the obtained oils were analyzed by GC-MS. The essential oil contents obtained at 25°C, 35°C, 45°C, 55°C, and 65°C were 1.17%, 0.96%, 0.94%, 0.65%, and 0.18% respectively. It was determined that the major components of essential oils obtained at different drying temperatures were 1.8-cineole (17.88-50.15%), camphor (32.60-48.86) and borneol (3.46-9.45%). The highest 1,8-cineole ratio was found in samples dried at 55°C (50.15%) but the lowest in samples dried at 65°C (17.88%). The highest and lowest camphor ratios were determined in samples dried at 65°C (48.86%) and samples dried at 55°C (32.60%) respectively. The highest (9.45%) borneol ratio was obtained in samples dried at 65°C, while the lowest (3.46%) ratio was obtained in samples dried at 55°C. The results obtained in the present study showed that Lavender essential oil content and composition were affected by drying temperatures and the optimum drying temperature was 35 ºC.

Keywords: drying temperatures, essential oil, GC-MS, *Lamiaceae*, *Lavandula angustifolia* Mill.

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Farklı kurutma sıcaklıklarının *Lavandula angustifolia* Mill uçucu yağ içeriği ve kimyasal bileşimi üzerine etkisi

Özet

Kurutma sıcaklıkları, uçucu yağların sentezlendiği ve depolandığı organlar nedeniyle uçucu yağ içeren bitkilerdeki uçucu yağların içeriğini ve bileşimini etkiler. Bu nedenle en yüksek miktarda ve en kaliteli uçucu yağı elde etmek için uygun kurutma sıcaklığını belirlemek üzere birçok çalışma yapılmıştır. Bu çalışmada, farklı kurutma sıcaklıklarının lavantanın (*Lavandula angustufolia* Mill.) uçucu yağ içeriği ve bileşenleri üzerindeki etkilerinin belirlenmesi amaçlanmıştır. Dört farklı sıcaklıkta (35°C, 45°C, 55°C ve 65°C) kurutulan örnekler Clevenger tipi aparat kullanılarak 3 saat izole edilmiş ve elde edilen yağlar GC-MS ile analiz edilmiştir. 25°C, 35°C, 45°C, 55°C ve 65°C'de elde edilen uçucu yağ içerikleri sırasıyla %1,17, %0,96, %0,94, %0,65 ve %0,18'dir. Farklı kurutma sıcaklıklarında elde edilen uçucu yağların ana bileşenlerinin 1.8-cineole (%17.88-50.15), camphor (32.60-48.86) ve borneol (%3.46-9.45) olduğu belirlenmiştir. En yüksek 1,8-sineol oranı 55°C'de kurutulan örneklerde (%50.15), en düşük ise 65°C'de kurutulan örneklerde (%17.88) bulunmuştur. En yüksek ve en düşük kafur oranları sırasıyla 65°C'de (%48,86) ve 55°C'de (%32,60) kurutulan örneklerde tespit edilmiştir. En yüksek (%9,45) borneol oranı 65°C'de kurutulan örneklerde elde edilirken, en düşük (%3,46) oran 55°C'de kurutulan örneklerde elde edilmiştir. Bu çalışmada elde edilen sonuçlar, Lavanta uçucu yağ içeriği ve bileşiminin kurutma sıcaklıklarından etkilendiğini ve optimum kurutma sıcaklığının 35 ºC olduğunu göstermiştir.

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Anahtar kelimeler: kurutma sıcaklıkları, uçucu yağ, GC-MS, *Lamiaceae*, *Lavandula angustifolia* Mill.

1. Introduction

Lavender (*Lavandula angustifolia*), which is one of the characteristic plants of the Western Mediterranean Region, is a perennial and shrub form and is a valuable aromatic and medicinal plant that naturally spreads in Southern France, Central Italy, Spain and Greece. Although there are two different species of the genus (*Lavandula stoechas* subsp. *stoechas* and subsp. *cariensis*) in the flora of Türkiye, this species does not show a natural distribution. It is extensively cultivated in France, Bulgaria, Spain, Italy, Greece, England, Russia, USA, Austria and North African countries [1-3]. Three important and highly commercially valuable lavender species are cultivated: Lavander (*Lavandula angustifolia = L. officinalis = L. vera*), Lavandin (*Lavandula x intermedia = L. hybrida*) and Spike lavander (*Lavandula spica*). Best quality lavender oil from lavender, also called "English lavender" is obtained. The so-called "hybrid lavender" lavandin has a higher volatile content than lavender oil content, but lower essential oil quality has [4]. Lavender flower: It is a drug that has been used in folk medicine for a long time due to its effects such as relieving rheumatic pains, increasing urine, antiseptic, expectorant, healing eczema wounds, strengthening nerve and heart [1, 5, 6]. The essential oil obtained from the flowers of the lavender plant is important for the perfume, cosmetics, flavor and fragrance industries. In addition, it is used to give fragrance to some preparations in the pharmacy, and by leaving it in the wardrobe at home, it is ensured that the clothes smell good [5, 7]. Linanyl acetate and linalool, which are found in the essential oil of *Lavandula angustifolia* L., are important essential oil components and are desired to be found at high rates in the perfumery industry. These components, which determine the quality of the essential oil, are quality lavender oil with a camphor ratio of less than 0.5% in the essential oil [8].

Medicinal and aromatic plants have an important place in world markets. Medicinal and aromatic plants are used as raw materials in the food, pharmaceutical, cosmetic, cleaning and natural dye industries. Türkiye is the homeland of a significant part of these plants and has a flora where some of them can grow naturally. Also the parts of these plants can be used for herbal teas which is preferred to treat several illnesses especially in folk medicine [9, 10, 11]. Medicinal and aromatic plants should be processed immediately after harvesting in order to preserve the active substances contained in the process until they reach the consumer. Drying is the process of reducing the moisture content of medicinal and aromatic plants from the high moisture content (70-85%) after harvesting to a moisture level (10-15%) suitable for safe storage. The aim of the drying process is to reduce the product moisture to the final moisture value in the shortest time and with the least energy consumption without any deterioration in product quality. Selection of the appropriate drying method for medicinal and aromatic plants is the most important step for successful drying [12, 13].

Most of the harvested medicinal and aromatic plants are dried by natural drying methods. In shade and sun drying method, variations in temperature and air humidity make it difficult to obtain homogenous drying of the products. In some cases, natural drying methods can lead to the development of fungi in the products and the formation of harmful chemicals such as aflatoxin. Medicinal and aromatic plants with a high water content should be dried as soon as possible after harvest. The most important factor in drying these plants with heated air is the drying air temperature. Since high drying temperatures (> 40 °C) adversely affect the secondary metabolites and natural color of the plants, lower drying temperatures (~ 35 °C) are mostly used. Drying at low drying temperatures is completed in longer periods and energy consumption is high. Different studies investigating suitable drying conditions for drying medicinal and aromatic plants have reported that variable drying air temperature applications can better maintain product quality while shortening drying time [14]. Therefore, there is a need to determine this change in medicinal and aromatic plants by separate studies. In this study, it was aimed to determine the effect of drying at different temperatures on the essential oil yield and quality of *Lavandula angustifolia* and to determine the optimum drying temperature.

2. Materials and methods

In the research, plants harvested from Hatay Mustafa Kemal University campus were used as plant material. To determine the effect of different drying temperatures on essential oil content and components, the leaves of the harvested plants were dried in an oven at four different temperatures (35 °C, 45 °C, 55 °C and 65 °C) for 48 hours.

2.1. Isolation of the essential oils

Essential oil was obtained from dried leaves and flowers. A total of 50 g of each of the ground plant samples was used for the separate hydro distillation experiment. A weighed sample was individually and carefully placed into a 2 L flask. Distilled water was then added until it covered the sample completely. Essential oils were obtained by water distillation for 3 hours by using a Clevenger type apparatus according to the European Pharmacopoeia method. The trial was repeated three times. Essential oil yield was calculated according to dry weight of plant materials and amount of

essential oils obtained. The essential oils were dried over anhydrous sodium sulfate and stored in dark vial bottles at +4°C until analysis [15-16].

2.2. GC-MS Analysis

The components of the essential oils of the plants were determined by gas-chromatographic (GC-MS) method. Determination of essential oil components was carried out with Thermo Scientific ISQ Single Quadrupole model gas chromatographic device under the following conditions. TR-FAME MS model, 5% Phenyl Polysilphenylene-siloxane, 0.25 mm inner diameter x 60 m length, 0.25 µm film thickness column was used. Helium (99.9%) was used as the carrier gas for Peer Review only at a flow rate of 1 ml/min. Mass spectra were recorded at 70eV, the mass range was from 1.2-1200 m/z. Scan Mode was used for data collection. The MS transfer line temperature was 250°C, the MS ionization temperature was 220°C, the injection port temperature was 220°C, the column temperature was initially 50°C and the temperature was raised to 220°C with a rate of heat increase of 3°C/min. The structure of each compound was identified using mass spectra with the Xcalibur program (Wiley 9) [17].

3. Results

The essential oil ratio and components of medicinal and aromatic plants are largely determined by the genotype of the plant used in production. On the other hand, production location, production techniques, harvesting time, harvesting method, drying methods and temperatures, extraction/isolation methods used to obtain effective substances, product processing techniques and storage conditions are effective in determining the essential oil ratio and components of aromatic plants [8, 18-20]. The essential oil contents obtained by drying the leaves of the lavender plant at different temperatures are as given in Table 1. The highest essential oil content was obtained at 25 °C with 1.17%, followed by 0.96% and 0.94% at 35 °C and 45 °C, respectively. The least essential oil content was obtained from plants dried at 65 °C with 0.18% (Table 1).

When the main components of the plant, whose essential oils were obtained at different temperatures, were examined, it was determined that the main components were eucalyptol, and camphor (Table 1). Eucalyptol, which is an important oxygenated monoterpene, is one of the important components determining the utilization of medicinal plants, and a high ratio is desired for use as spice or herbal tea [21]. The eucalyptol ratio varied depending on different drying temperatures and the highest (50.15%) ratio was obtained in plants dried at 55 °C, followed by plants dried at 45 °C with 39.85% and 35 °C with 39.15%. The lowest eucalyptol ratio was found in plants dried at 65 °C with 17.88% (Table 1). While it was determined that the eucalyptol ratio increased with increasing temperature, it was determined that this ratio decreased after 55°C.

The camphor content in the oxygenated monoterpene structure, which is one of the most important main components of the essential oil, varied between 32.60-48.86 % depending on different drying temperatures. The highest camphor ratio (48.86 %) was obtained from samples dried at 65 °C, while the lowest ratio (32.60 %) was obtained at 55 °C (Table 1).

Since the organs of essential oil-containing plants where these oils are synthesized and stored are close to the outer surface of the plant, the drying methods and temperatures used have an effect on the ratio and composition of the essential oil [8, 18-20]. For this reason, many studies have been carried out to determine the appropriate drying temperature to obtain the highest quantity and quality of essential oil. In these studies, it has been reported that many ecological factors and cultivation practices, especially the genotypic characteristics of the plant material used in production, have an effect on the determination of yield and quality of medicinal aromatic plants. In addition, post-harvest practices (drying methods, drying time, distillation/extraction methods and time, storage conditions, etc.) are also effective in determining the yield and quality of the product [20, 22].

Table 1. Essential oil content and components of *L. angustifolia* plant dried at different temperatures.

RT	Compound Name	SI	RSI	Area %			
Temperatures (°C)				35 °C	45 °C	55 °C	65 °C
6.58	α-Pinene	717	730	0.58	0.61	0.81	nd
7.59	Camphene	991	994	0.73	0.72	1.23	nd
8.35	Cyclohexane	900	984	nd	nd	nd	0.97
8.4	β-Pinene	977	993	0.61	0.6	0.79	nd
8.72	β-Phellandrene	976	980	0.18	nd	nd	nd
10.1	Limonene	987	990	0.51	0.38	0.31	nd
10.8	Dehydrocineole	820	899	0.10	0.16	0.14	0.17
11.2	γ-Terpinene	868	923	0.09	0.22	0.14	0.45
12	Eucalyptol	988	992	39.15	39.85	50.15	17.88
12.7	o-Cymene	941	975	1.25	1.15	1.01	nd
17.3	1-Octen-3-ol	760	848	0.07	nd	nd	nd
18.2	Anon	925	961	0.08	nd	nd	0.41
18.9	n-Hexyl butanoate	961	973	0.13	0.1	0.07	nd
19.7	cis Sabinene hydrate	969	986	0.32	0.21	0.16	nd
20.7	Linalool	985	990	0.89	0.8	0.49	0.64
21.1	α-Santalene	904	957	0.11	nd	0.07	nd
22.3	Linalyl acetate	819	841	0.09	nd	nd	nd
22.9	trans Sabinene hydrate	955	972	0.19	0.12	0.08	nd
23.3	α-Campholene aldehyde	893	909	0.13	0.12	0.12	nd
24.2	Terpinen-4-ol	983	984	0.78	0.58	0.38	0.3
25	Bornyl formate	916	932	0.13	0.08	0.07	0.51
25.2	Isopinocarveol	967	970	0.43	0.47	0.33	0.61
25.4	Hexyl tiglate	954	974	0.12	nd	nd	nd
25.9	Camphor	988	991	38.78	36.38	32.6	48.86
26.2	Verbenol	888	898	0.23	0.33	0.28	0.39
27	α-Terpineol	889	957	1.33	1.22	0.93	0.32
27.5	Borneol	993	993	5.04	4.55	3.46	9.45
28.6	Myrtenal	974	983	0.37	0.54	0.39	0.56
28.8	Nopinone	849	914	0.15	0.36	0.16	nd
29.5	Myrtenol	964	974	0.17	0.22	0.17	0.37
30.7	trans-Carveol	952	971	0.18	0.19	0.16	0.17
31.5	Cumic alcohol	893	976	0.33	0.47	0.28	0.35
31.7	Carvacrol	797	809	0.76	0.87	0.53	0.67
31.9	Cryptone	949	973	0.89	0.8	0.54	0.54
32.5	p-Cumic aldehyde	943	967	0.84	0.72	0.56	1.28
32.7	Piperitone	862	938	0.09	0.08	nd	nd
33.1	Ethanone. 1-(methylphenyl)	883	915	0.08	0.12	nd	nd
33.5	Verbenone	936	963	0.14	3.31	0.17	0.28
36.1	Teresantalol	863	906	0.11	nd	0.09	0.44
37.8	Junipene	777	810	nd	nd	nd	0.18
38	3-Carene. 4-acetyl-	797	845	0.10	nd	nd	nd
39.7	Santalol	814	863	nd	0.22	0.12	0.61
40.1	Caryophyllene oxide	979	993	1.53	1.38	1.14	4.93
40.3	α-Cadinol	943	945	0.49	0.44	0.35	1.34
40.9	o-Allylguaiacol	771	823	0.13	0.15	0.1	0.36
41.3	α-Bisabolol	956	966	0.18	0.17	0.1	0.73
46.5	Methyl 6-octadecenoate	796	839	nd	nd	nd	0.19
49.5	Hexadecamethylheptasiloxane	774	841	nd	nd	nd	2.52
51	Vitamin A alcohol	766	766	0.67	0.13	0.1	0.47
53	Testosterone	793	798	0.21	0.08	0.07	nd
54.6	γ-Sitosterol	530	572	nd	nd	nd	0.23
56.2	Eremanthin	877	949	0.10	0.46	0.14	nd
Total identified (%)				46	39	39	32
Number of compounds				99.57	98.64	99.33	97.18
Essential Oil Content (%)				1.17	0.96	0.94	0.18

4. Conclusions and discussion

Katar et al., [23] in their study associated *Hyssopus officinalis*, reported that different drying times had a significant ($p<0.01$) effect on essential oil yield, and the optimum drying time should be 24 hours at 35 ºC. Aydın et al., [24] examined the effects of different drying temperatures on the chemical composition of *Salvia fruticosa* and determined that the optimum drying temperature was 35. Müller et al. [25] in the study in which they examined the effects of drying temperature on the essential oil of medicinal sage; They dried the products at drying temperatures ranging from 30-90 ºC until they dropped to 11% humidity. No loss of essential oil was observed at 60 ºC and they found that after this temperature the loss of essential oil increased.

Verma et al. [26] reported that the essential oil ratios obtained from the lavender plant varied between 0.80-1.30%. In the same study, they reported that they determined the main components as linalyl acetate (47.56%), linalool (28.06%), lavandulyl acetate (4.34%), α-terpineol (3.75%). Jianu et al., [27] reported the main components of essential oil obtained from *L. angustifolia* Miller as 24.12% caryophyllene, 16% beta-phelandrene and 15.69% eucalyptol (1,8-cineol). Maral et al., [28] reported the major components of essential oil obtained from *L. angustifolia* Miller as linalyl acetate (34.50%), linalool (33.68%), camphor (5.04%), and 1.8-cineole (4.3%). Arabaci and Bayram [29] reported in their study that the linalool ratio varied between 34.3–54.6%, and the linalyl acetate ratio varied between 24.0–29.0%. Kara and Baydar [30] reported that the ratio of linalool ranged between 28.5–43.9%, the ratio of linalyl acetate between 3.76–42.5%, and the ratio of camphor between 4.11-19.8%.

When the results obtained from the study were evaluated, it was determined that different drying temperatures were effective on both the content and components of the essential oil. To obtain the drug with the highest essential oil content, 35°C can be recommended as the most suitable drying temperature. Likewise, different drying temperatures were also effective on the components of the essential oil, and it was determined that it should be dried at 55 °C to obtain eucalyptol-rich essential oil in production. It can be determined that drying temperatures above 55°C should not be used as they reduce both the essential oil content and the ratio of main components.

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Fish fauna of lake Küçük Akgöl (Sakarya, Turkey)

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Abstract

This research was conducted to determine the ichthyofauna of lake Küçük Akgöl between March 2017 and March 2018. Sampling was conducted monthly and randomly using nets with different mesh sizes, fishing rods and fish scoops during field studies, resulting in a total of 99 fish samples collected. Through external morphology examinations based on traditional methods, it was determined that 9 fish species *Alburnus alburnus*, *Blicca bjoerkna*, *Carassius gibelio*, *Rutilus rutilus*, *Scardinius erythrophthalmus*, *Esox lucius*, *Gambusia sp.*, *Perca fluviatilis*, *Lepomis gibbosus*) belonging to 5 families (Cyprinidae, Esocidae, Poecilidae, Percidae, Centrarchidae) inhabit lake Küçük Akgöl.

Keywords: Sakarya, biodiversity, ichthyofauna, morphological species definition

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Küçük Akgöl (Sakarya, Türkiye) balık faunası

Özet

Bu araştırma, Küçük Akgöl ihtiyofaunasını belirlemek amacıyla, Mart 2017-Mart 2018 tarihleri arasında yürütülmüştür. Aylık, rastgele örnekleme yapılarak yürütülen arazi çalışmalarında, farklı göz açıklığına sahip ağlar, oltalar ve balık kepçeleri kullanılmış ve toplam 99 balık örneği yakalanmıştır. Geleneksel yöntemle dış morfoloji esas alınarak yapılan incelemeler sonucunda, Küçük Akgöl'de 5 familyaya (Cyprinidae, Esocidae, Poecilidae, Percidae, Centrarchidae) ait 9 balık türünün (*Alburnus alburnus*, *Blicca bjoerkna*, *Carassius gibelio*, *Rutilus rutilus*, *Scardinius erythrophthalmus*, *Esox lucius*, *Gambusia sp.*, *Perca fluviatilis*, *Lepomis gibbosus*) yaşadığı belirlenmiştir.

Anahtar kelimeler: Sakarya, biyoçeşitlilik, ihtiyofauna, morfolojik tür tanımı

1. Introduction

In Turkey, the lentic and lotic systems, separated from each other by natural geographical barriers, have presented a multitude of ecosystems with varying ecological conditions for living organisms. This separation has facilitated the development of various isolation mechanisms such as geographical and genetic isolation, greatly affecting the geographical distribution of species, and increasing diversity at the genetic, species, and ecosystem levels. The Sakarya river is the third-largest river in Turkey, and the longest river in northwest Anatolia in terms of its total length, surface area, and rainfall area (Figure 1). The basin of the Sakarya river is very rich in wetlands due to the abundance and diversity of underground and surface water sources [1]. Lake Küçük Akgöl, our research area, is located in the lower basin of the Sakarya river, very close to the river itself (Figure 2), and situated between 40°-41° northern latitudes and 30°'-31° eastern longitudes [1].

The research area is located in the northwest of Akgöl neighborhood, which borders the Söğütlü district in Sakarya province, situated 6 kilometers away from Söğütlü and 12 kilometers from Sakarya city center [2]. Lake Küçük Akgöl is fed by waters from below, and its average depth varies between 2 and 6 meters depending on the season [1].

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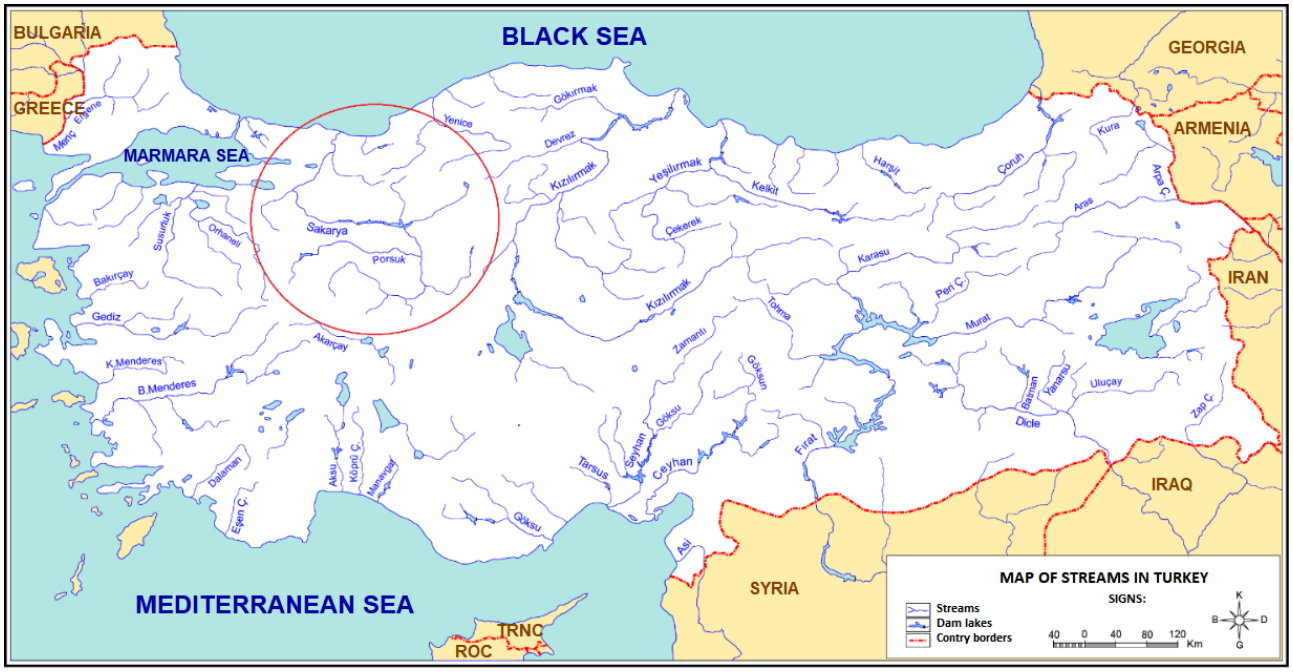


Figure 1. Map of rivers in Turkey [3]

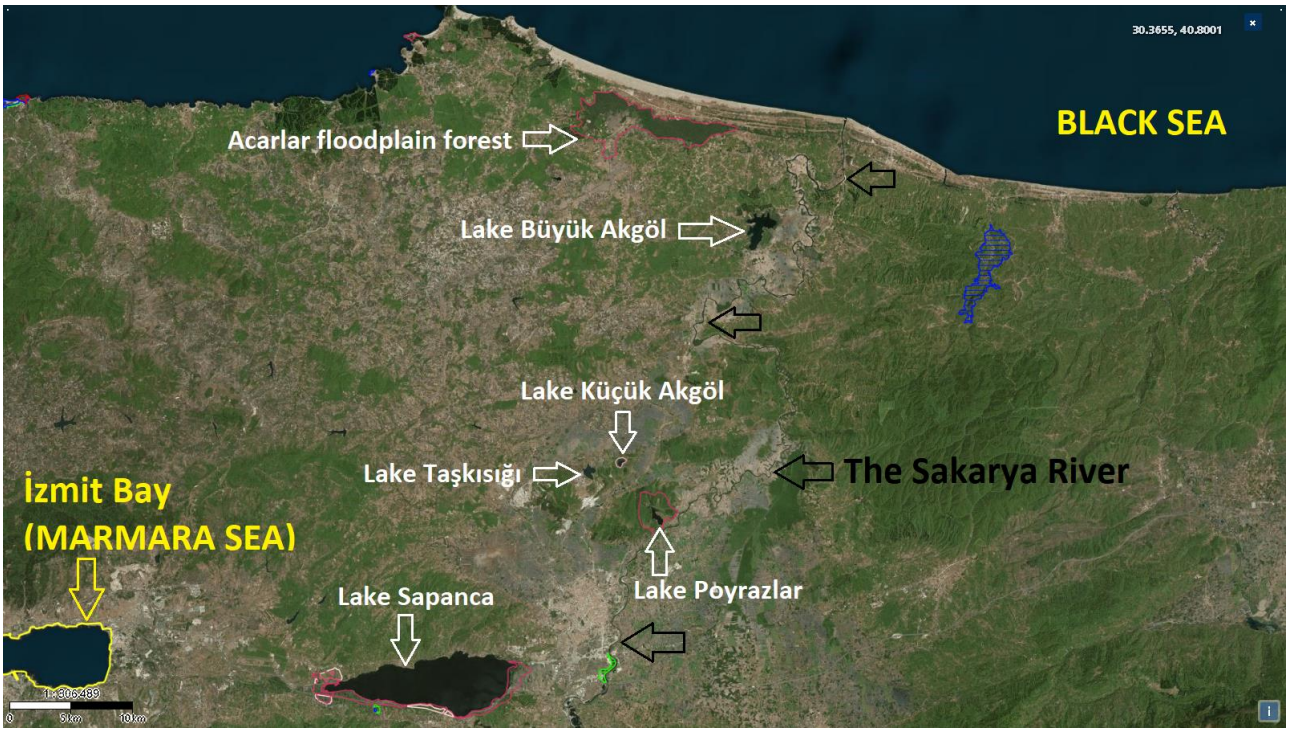


Figure 2. Map showing the lower basin of the Sakarya river [4]

According to the results obtained from geomorphological studies, lake Küçük Akgöl is a shallow alluvial embankment lake that is believed to have formed due to the Sakarya river and its tributaries, in response to past climate changes, sea level fluctuations, tectonic plate movements, and the high groundwater level in the Adapazari plain. This groundwater level is the primary factor in maintaining the existence of a lake assumed to have formed 5000 years ago. Additionally, there are many lakes assumed to have formed similarly in the lower basin of the Sakarya river [5].

Within the Sakarya river basin, there are special environmental protection zones such as national parks, nature parks, nature conservation areas, nature monuments, wildlife development areas, natural protected areas, nationally important wetlands, and wetlands of local importance [1].

Lake Küçük Akgöl and its nearby environment have been designated as two protected area at the national and regional management levels. Lake Küçük Akgöl has a surface area of approximately 20 hectares within a total region of 41 hectares. The lake has been declared a "Natural Site Area of Second Degree Importance" through decision number 8821, dated 2001, by Bursa Cultural and Natural Heritage Preservation Commission, which is affiliated with the Ministry of Culture and Tourism (Figures 3 and 4) [5]. In addition, a total region of 187 hectares, including lake Küçük Akgöl, has been designated as a "Wetland of Local Importance" by the General Directorate of Nature Conservation and National Parks affiliated with the Ministry of Agriculture and Forestry, on August 11th, 2019 [6].

Natural protected areas are classified into three categories: Sensitive areas for strict protection, qualified natural protection areas, and areas for sustainable protection and utilization under control [7]. The boundaries of these areas are represented respectively by pink, red, and blue colors in Figures 3 and 4.

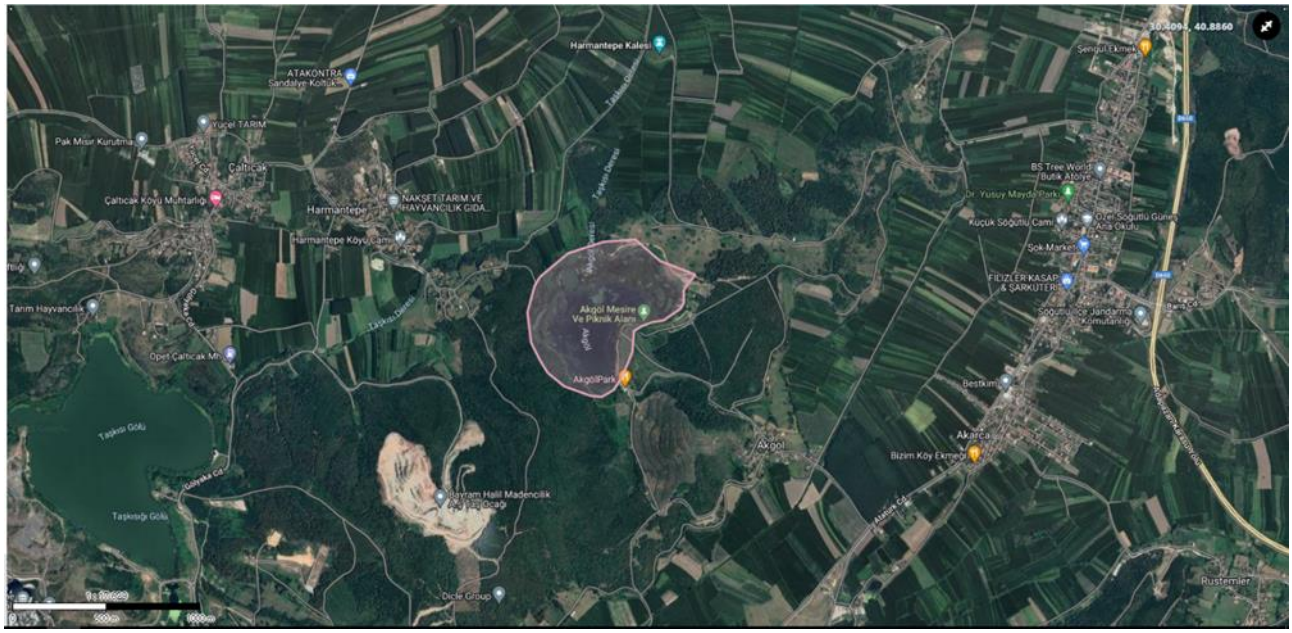
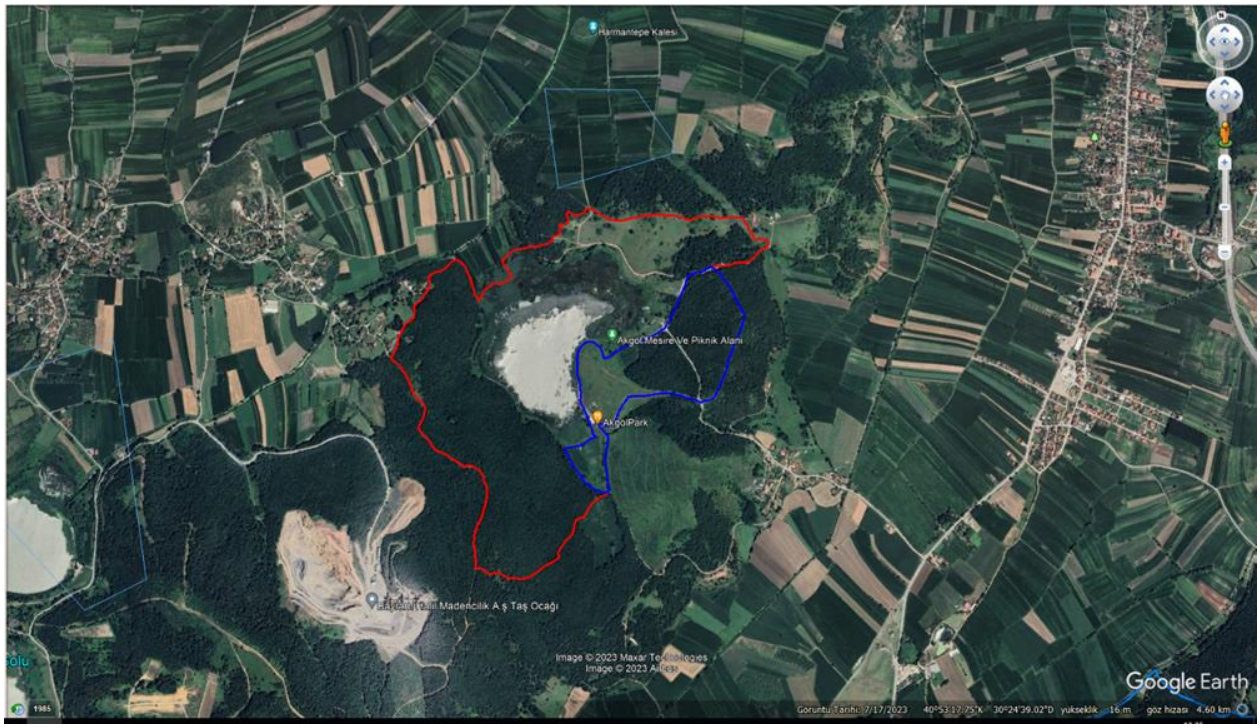


Figure 3. Map showing the natural protected area of lake Küçük Akgöl (Borders shown in pink) [4]

Figure 4. Map showing lake Küçük Akgöl and the surrounding areas suggested for protection through planning [4]



In addition to its current conservation status, lake Küçük Akgöl is not widely recognized as a wetland, thus its natural structure has remained largely intact without degradation. However, it is situated in close proximity to the Akgöl neighborhood, where human settlements, as well as agricultural and livestock activities, are still ongoing. Therefore, it is believed that the lake has been at least partially influenced by human activities (Figures 3 and 4) [5].

The total rainfall area of the Sakarya river basin constitutes approximately 1/8 of Turkey's landmass, spanning across parts or entireties of 13 provinces: Ankara, Eskişehir, Bolu, Kütahya, Bilecik, Afyonkarahisar, Bursa, Kocaeli, Düzce, Konya, Çankırı, Uşak, and Sakarya (Figure 1). Undoubtedly, the preservation or deterioration of wetland areas largely depends on the actions of the human inhabitants in these regions.

Lake Küçük Akgöl and its surrounding areas exhibit various features specified in the Regulation on Natural Protected Areas [7]. Some of these features are listed below:

a) It has species diversity, genetic diversity, and ecosystem diversity. For instance, the natural site area encompasses four different ecosystem types: Forest, lake, meadow, and swamp.

b) It possesses an adequate size that encompasses the primary elements of existing ecosystems and can sustain the functions of these ecosystems.

c) It holds social, cultural, and recreational values that contribute to the integrity of the landscape and its natural resources.

This study was conducted with the primary aim of determining the ichthyofauna of lake Küçük Akgöl. Subsequently, it sought to assess the damage to the fish community and natural structure of the study area caused by ongoing and increasing anthropogenic environmental degradation. Secondly, it aimed to understand how a degraded ecosystem responds to human intervention. Finally, it aimed to envision how the main ecological features and ecosystems in the area can contribute to achieving indicators of sustainable development targets through the status of the national protected area and the conservation efforts.

2. Materials and methods

This research was carried out to determine the fish species inhabiting lake Küçük Akgöl between March 2017 and March 2018. Due to the small size of the research area, fish sampling was conducted randomly on a monthly basis from the pelagic region of the lake during the research period. A total of 99 fish samples were caught using gillnets and trammel nets (with a wing length of 25-100 m, depth of 2-6 m, eye opening of 2-18 cm), fish scoops, and fishing rods.

The fresh fish samples collected during field studies were preserved as museum material, and identified based on external morphology using traditional methods at the family, genus, and species levels according to literature [8-20]. The current systematic position of each identified species' scientific name was referenced from Eschmeyer's Catalog of Fishes [21].

The following symbols and abbreviations are used in the text to denote the metric and meristic characters of the fish samples: SL (Standard Length), D (Dorsal fin), V (Pelvic fin), A (Anal fin), P (Pectoral fin), L.lat. (Lateral line scales), L.tran. (Linea transversal scales), Sq (Number of scales on a line between the back of the head and the beginning of the caudal fin in fish without a lateral line), PT (Pharyngeal teeth), GR_{Out} (Gill rakers on the outer margin of the first branchial arch), GR_{In} (Gill rakers on the inner margin of the first branchial arch), N (Specimen numbers).

3. Results

Following the evaluation of a total of 99 fish samples caught from the research area, it has been determined that 9 fish species (*Alburnus escherichii, Blicca bjoerkna, Carassius gibelio, Rutilus rutilus, Scardinius erythrophthalmus, Esox lucius, Gambusia holbrooki, Perca fluviatilis, Lepomis gibbosus*) belonging to 5 families (Cyprinidae, Esocidae, Poeciliidae, Percidae, Centrarchidae) inhabit lake Küçük Akgöl. The number of samples examined, along with qualitative and quantitative taxonomical characteristics, and original photographs for each fish species examined in this study, are provided below.

Family: Cyprinidae

Alburnus alburnus (Linnaeus, 1758)

SL: 91-113 mm. D: II-III 9-10 V: II 8 A: III 16 P: I 15-16 L.lat.: 47-48 L.tran.: 8-9/4 PT: 2.5-5.2 N: 4

The body shape is fusiform (Figure 5). The eyes are clearly large. The mouth is small and lacks barbels. The jaws are of equal length. In the middle of the upper and lower jaws, there is a recess and a round protrusion. The lateral line is complete and almost straight. Except for the head region and fins, the body is covered by cycloid scales, which are large and easily detached from the body. There is a prominent keel between the pelvic and anal fins. The body coloration is white with silver brilliance.

Figure 5. *Alburnus alburnus*

Blicca bjoerkna (Linnaeus, 1758)
SL: 93-171 mm. D: III 8-9 V: II 8 A: III 21-24 P: I 14-16 L.lat.: 46-49 L.tran.: 9-10/6 PT: 2.5-5.2 N: 6
The body shape is compressiform (Figure 6). The eyes are obviously large. The mouth is small, without barbels, and in a terminal position. The lateral line is complete and almost straight. Except for the head region and fins, the body is covered by cycloid scales, which are large and easily detached from the body. There is a prominent keel between the pelvic and anal fins, as well as in the predorsal region. The free edge of the dorsal and anal fins is concave. The body coloration is white with silver brilliance. Patterns such as spots or stripes are not seen throughout the body. Yellow-orange pigmentation in slightly lighter tones is especially visible in the ventral, anal, and caudal fins of mature specimens during the breeding season.

Figure 6. *Blicca bjoerkna*

Carassius gibelio (Bloch, 1782)
SL: 137-243 mm. D: IV 16-19 V: II 7-8 A: III 6 P: I 15-16 L.lat.: 30-33 L.tran.: 6-7/6-7 FD: 4-4 GR_{Out}: 52-53 GR_{In}: 58-64 N: 8
The body shape is short, blunt, and oval (Figure 7). The head is relatively small compared to the rest of the body. The eyes are quite prominent and large. The mouth is small, without barbels, and located terminally at the anterior part of the head. The lips have a slightly fleshy structure. The lateral line is complete and almost straight. Except for the head region

and fins, the body is covered by cycloid scales, which are large and easily detached from the body. The free edge of the last simple ray of the dorsal and anal fins has small serrations like sawtooth. The presence of a carina between the ventral fins and the anal fin is remarkable. The free edge of the dorsal and anal fins is straight. Although the body color varies depending on age, gender, and the ecosystem in which it lives, in general, the dorsal region and sides have an opaque silver color, and the ventral region has a bright white color. Patterns such as spots or stripes are not seen throughout the body.

Figure 7. *Carassius gibelio*

Rutilus rutilus (Linnaeus, 1758)
SL: 101-155 mm. D: (III) IV (9) 10-11 V: II (7) 8 A: III 10-12 P: I (14) 15-17 L.lat.: 41-43 L.tran.: 8/(3) 4 PT: 5-5, 6-5, 6-6 N: 14
The body shape is fusiform and slightly flattened from the sides (Figure 8). The head is relatively small compared to the rest of the body. The eyes are quite prominent and large, with orange-red pigmentation inside. The mouth is small, without barbels, protractile, and located terminally at the anterior part of the head. The lateral line is complete and slightly curved towards the ventral region, with the lateral line system continuing as pores on the head region. Except for the head region and fins, the body is covered by cycloid scales, which are large and easily detached from the body. There is a prominent keel between the pelvic and anal fins, as well as in the predorsal region. The free edge of the dorsal and anal fins is concave. The body coloration is white with silver brilliance. Patterns such as spots or stripes are not seen throughout the body. Yellow-orange pigmentation, neither dark nor light, is especially visible in the ventral, anal, and caudal fins of mature specimens during the breeding season.

Figure 8. *Rutilus rutilus*

Scardinius erythrophthalmus (Linnaeus, 1758)
SL: 128-165 mm. D: III 9-10 V: II 8 A: III (9) 11-13 P: I 14-16 L.lat.: 41-42 L.tran.: 7-8/3 PT: 2.5-5.2, 3.5-5.3 N: 8
The body shape is compressiform (Figure 9). The head is relatively small compared to the rest of the body. The eyes are quite prominent and large, with orange-red pigmentation inside. The mouth is small, without barbels, protractile, and located terminally at the anterior part of the head. The lateral line is complete and slightly curved towards the ventral region. Except for the head region and fins, the body is covered by cycloid scales, which are large and easily detached from the body. There is a prominent keel between the pelvic and anal fins, as well as in the predorsal region. The free edge of the dorsal and anal fins is concave. The body coloration is white with silver brilliance. Patterns such as spots or stripes are not seen throughout the body. Splendid dark red pigmentation is especially visible in the ventral, anal, and caudal fins of mature specimens during the breeding season.

Figure 9. *Scardinius erythrophthalmus*

Family: Esocidae
Esox lucius Linnaeus, 1758
SL: 443-515 mm. D: VIII, IX 14-15 V: II 9-10 A: VII 12-13 P: I 13-14 L.lat.: 120-130 L.tran.: 14/12-14 N: 3
The body shape is sagittiform (Figure 10). The head is relatively large compared to the rest of the body. The eyes are quite prominent and large. The mouth is large, elongated forward in the form of a duck's mouth, and the lower jaw is slightly longer than the upper jaw. Well-developed teeth are present on the jaws, tongue, and vomer bone. The lateral line is complete, but it is not clearly visible to the naked eye due to its spacing and the body's coloration. The lateral line system continues as pores all over the head region. A total of 10 submandibular pores were counted. The number of gill rays was counted at a total of 14-15. Except for the fins, the body is covered in small cycloid scales embedded in the skin. The dorsal and anal fins are located close to the caudal fin rather than the head. The vertical bands regularly found on the sides of the body in young and small immature individuals have turned into light-colored, nearly round-shaped, irregularly scattered spots in large and mature adult individuals.

Figure 10. *Esox lucius*

Family: Poeciliidae
Gambusia sp. Poey, 1854
SL: 10-40 mm. D: I-II 6-7 V: I 5 A: III (6-7) 8 P: (II-III) IV 8-10 Sq: 31-33 N: 34
The body shape exhibits a mosaic appearance; the head is depressiform, the trunk is fusiform, and the caudal is compressiform (Figure 11). The eyes are quite prominent and large. The dorsal part of the head is flat. The mouth is without barbels and in a superior position. The caudal fin is diphysercal shaped. Except for the head region and fins, the body is covered by cycloid scales, which are large and easily detached from the body. The lateral line is absent. The dorsal fin is located in the second half of the body. The body coloration is white with silver brilliance. There is black pigmentation like a large spot near the base of the anal fin on both sides of the body and black pigmentation like a thin stripe extending from the eyes to the lower chin. They are small-sized fish. In mature male individuals, the second and third simple rays and the first few branched rays in the anal fin are elongated by modification, forming a structure called a gonopodium, which serves as a copulation organ. Since all of our mosquitofish samples are female, we could not benefit from gonopodial features, and we did not identify at species level, so we decided to leave it genus level.

Figure 11. *Gambusia sp.* (Female)

Family: Percidae
Perca fluviatilis Linnaeus, 1758
SL: 102-204 mm. D_1: XIV-XVI D_2: II-III 13-15 V: I 5 A: II 9-10 P: II (10) 12-13 L.lat.: 62-66 L.tran.: 8-9/16-18 N: 10
The body shape is fusiform (Figure 12). The head is relatively large compared to the rest of the body. The eyes are quite prominent, large, and located closer to the top of the head. The mouth is large, protractile, in a terminal position, and without barbels. The free edge of the preoperculum is serrated like a sawtooth. The free edge of both operculum ends with a strong spine-like protrusion. The lateral line is complete and slightly curved towards the dorsal region, and the lateral line system continues as pores on the head region. The caudal peduncle can be thought of as thick compared to the height of the head and trunk. The space between the first and second dorsal fins is very small, almost none. Except for the fins, the body is covered by ctenoid scales embedded in the skin. There is a distinct black and large spot turning with a little orange pigmentation on the upper posterior margin of both operculum. There are a total of 8 dark-colored vertical bands positioned in parallel to each other on the sides of the body.

Figure 12. *Perca fluviatilis*

Family: Centrarchidae
Lepomis gibbosus (Linnaeus, 1758)
SL: 83-125 mm. D: X-XI 11-12 V: I 5 A: III (10) 11-12 P: II (10) 11 L.lat.: 40-44 L.tran.: 6-7/12-15 N: 12
The body shape is compressiform (Figure 13). The head is relatively small compared to the rest of the body. The eyes are quite prominent and large. The mouth is small, protractile, in a terminal position, and without barbels. The lateral line is complete and slightly curved towards the dorsal region, and the lateral line system continues as pores on the head region. Except for dorsal region of the head, throat and fins, the body is covered by cycloid scales, which are large and easily detached from the body. Cheeks, fins and the base of the fins are covered with scales. The free edge of both operculum are serrated like a sawtooth. They have wavy iridescent blue and brown lines on side of head. The lateral sides are covered densify spots colored of bright copper or gold. There is a distinct black and large spot on the upper-posterior margin of both operculum, as well as red-orange spot in a half-moon shape on the black ear flap in adults. They are small-sized fish.

Figure 13. *Lepomis gibbosus*

4. Conclusion and discussion

In this study, it has been identified that the fish community of lake Küçük Akgöl consists of 9 fish species (*Alburnus escherichii, Blicca bjoerkna, Carassius gibelio, Rutilus rutilus, Scardinius erythrophthalmus, Esox lucius, Gambusia holbrooki, Perca fluviatilis, Lepomis gibbosus*) belonging to 5 families (Cyprinidae, Esocidae, Poeciliidae, Percidae, Centrarchidae). Lake Küçük Akgöl is not a transparent lake; its water is turbid [1], and for this reason, although 9 taxa have been identified in this research, it is thought that there may be other fish species that cannot be captured. The best way to capture specimens belonging to the fish species forming the current ichthyofauna of a lotic ecosystem is fishing done using an electroshocker. This method is practical and not very time-consuming, and moreover, it makes it possible to collect fish samples in abundance and unspoiled. In seas, lagoons, and turbid lakes where the electrical conductivity is higher than freshwater sources with clear water, it is necessary to use a high-capacity generator to catch fish samples using an electroshocker device [16]. The electroshocker tool was not used in this study since the research area is a turbid lake.

The taxonomic characters of the fish species studied using the traditional method based on external morphology are similar to the data recorded in similar systematic studies published previously [8-20, 22-25]. In systematic studies where the external morphological characteristics of fish samples are examined, it is expected that all findings related to the taxonomic characters generally accord with the data recorded in similar studies conducted before. However, there may be small differences in at least a few taxonomic characters, and their importance can change depending on which character it is. Even within members of the same population, there may be similarities within species boundaries and endless variations on an individual basis because each individual is a partial representative of the gene pool to which it belongs [26]. Each ecosystem has its own unique elements and functions/dynamics. The ecological conditions in the aquatic environment have many different effects on the basic biological characteristics of living organisms, such as morphology, anatomy, physiology, metabolic rate, growth, development, ecological tolerance, geographical distribution, and migration [27], and these differences are reflected in biodiversity, with most of them considered as ecological variations within non-genetic individual variations [26]. Errors due to researchers can also cause differences. It is known that genetic and non-genetic variations create individual differences. If the differences are too remarkable to be neglected in terms of both qualitative and quantitative characteristics, the research area should be expanded, research methods should be diversified, and the number of samples should be increased, turning studies from the individual to the population level, and the possibility of a new species should be studied in more detail. For the reasons mentioned above, in similar systematic studies based on external morphology, it should be considered normal to find small differences in the taxonomic characters of the specimens belonging to the same species.

Lake Küçük Akgöl, with a surface area of 20 hectares, is an important food source for local people due to the qualities and quantities of its contained fish species. There is no doubt that the preservation of this wealth is as important as its determination. *Esox lucius* and *Perca fluviatilis*, found to be inhabiting lake Küçük Akgöl, have economic importance and are consumed by the regional people. *Carassius gibelio*, *Gambusia holbrooki* and *Lepomis gibbosus* are globally known invasive fish species inhabiting numerous wetlands, with the ability to rapidly breed, grow, and spread geographically [28, 29, 30]. It is known that invasive species can easily adapt to their environment, usually being euryoecious species, and can compete with native species for space and food. They can also damage eggs, larvae, juveniles as predators, spoil the genetic structure of native species by hybridizing with them, cause the extinction of native species through diseases and parasites brought from their original habitats, change interspecies relationships, damage certain abiotic features of the ecosystem, and enable changes in habitat features. Although there is not significant environmental degradation due to human activities in the natural structure of the study area, and it has had national protection status since 2001, the presence of invasive species is a concern.

It is possible to determine the current biodiversity and biogeographical regions in both aquatic and terrestrial environments through systematic research. Systematic studies have helped in correctly interpreting, protecting, and preserving the current biodiversity situation in the aquatic and terrestrial environment. They have also provided guidance for sustainable use and indicated directions for future studies.

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Three endemic *Verbascum* L. (Scrophulariaceae) species's stem and leaf anatomy

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Abstract

Three endemic species from the genus *Verbascum* L., whose gene center is Anatolia, distributed in Eskişehir and itas surroundings. nov., *V. eskisehirensis* Karavel., Ocak & Ekici sp. nov., *V. detersile* Boiss. & Heldr., *V. gypsicola* Vural & Aydoğdu leaf and stem anatomical structures and similarities and differences of the species were compared. The stem and leaf anatomy was illuminated by light microscopy, and the stem and leaf hairs were illuminated by scanning electron microscopy.

Keywords: Scrophulariaceae, *V. eskisehirensis*, *V. detersile*, *V. gypsicola*, endemic, anatomy

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Endemik üç *Verbascum* L. (Scrophulariaceae) türünün gövde ve yaprak anatomisi

Özet

Gen merkezi Anadolu olan *Verbascum* L. Cinsine ait Eskişehir ve çevresinde yayılış gösteren üç endemik türden *V. eskisehirensis* Karavel., Ocak & Ekici sp. nov., *V. detersile* Boiss. & Heldr., *V. gypsicola* Vural & Aydoğdu'ya ait yaprak ve gövde anatomik yapıları ile türlerin benzerlik ve farklılıkları karşılaştırılmıştır. Gövde ve yaprak anatomisi ışık mikroskobu, gövde ve yaprak tüyleri taramalı elektron mikroskobu ile aydınlatılmıştır.

Anahtar kelimeler: Scrophulariaceae, *V. eskisehirensis*, *V. detersile*, *V. gypsicola*, endemik, anatomi

1. Giriş

Dünyada 3000 kadar türe sahip büyük familyalardan Scrophulariceae familyasının en büyük cinsi olan *Verbascum* L., yaklaşık 366 taksona sahiptir ve ülkemizde yaklaşık 256 türü mevcuttur. Bu türlerden 131 türü hibrit ve 201 türü endemiktir [1]. *Verbascum* cinsi bazı türlerinin yaprak ve çiçekleri bronşit, astım, tüberküloz gibi solunum hastalıkları ile hemaroit, egzama gibi cilt hastalıklarını [2] ayrıca; hayvan yaralarını iyileştirmede, şeker hastalığı ve romatizma ağrılarını azaltmada kullanılmaktadır [3].

Yapılan çalışmalarda, *Verbascum* taksonlarının; monoterpen ve iridoit glikozitleri, saponinler, flavonoitler ve steroitler gibi farklı yapıda bileşiklerce zengin oldukları, sitotoksik, antioksidan ve antimikrobiyal etkilerinin olduğu görülmüştür [4].

Ancak, *Verbascum* cinsinin taksonomik açıdan teşhisinin zor olması, tür sayısının farklı bölgelere yayılmış çok sayıda olması ve hibritleşmenin yoğun görülmesi çalışmaların yeterli sayıda olmasını engellemektedir [3].

Bu nedenle, endemik üç *Verbascum* türünün gövde ve yaprak anatomik özelliklerinin incelenmesi ve aydınlatılmasının taksonomiye katkı sağlayacağı düşünülerek bu çalışma yapılmıştır.

Türlerimizden *V. eskisehirensis* ''Eski sığırkuyruğu'', sadece Eskişehir il sınırları içerisinde mevcut olup, 2009 yılında bilim dünyasına tanıtılarak yeni bir tür olarak kabul edilmiştir. *V. eskisehirensis*, kaya çatlaklarının içerisinde ve

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yükseklerde yetişmektedir. *V. gypsicola* ''Mermer sığırkuğruğu', 1993 yılında ismini üzerinde yaşadığı kayadan alarak bilim dünyasına tanıtılmış Ankara İli Nallıhan İlçesi'ne endemik bir türdür. Ülkemiz için endemik olan *V. detersile* 'Zinemit' ise, orman kenarlarında yaygın olarak yetişmektedir [5].

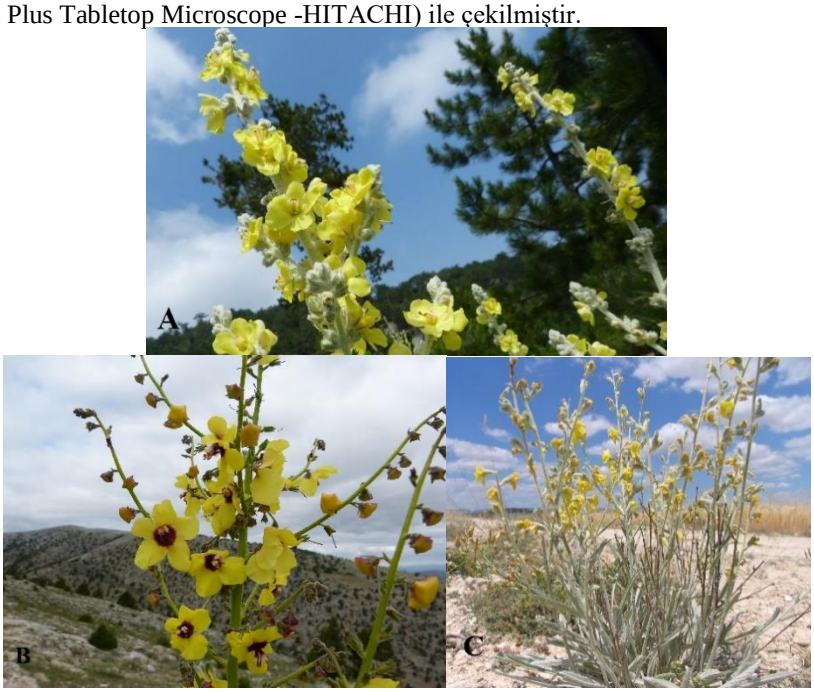
2. Materyal ve yöntem

2.1.Bitkisel Materyal

Araştırma materyallerinin lokalite bilgileri, toplanma tarihleri ve Anadolu Üniversitesi Eczacılık Fakültesi Herbaryum (ESSE) numaraları; *V. detersile* B3, Gülalan yolu, Eskişehir, 05.07.2019 (ESSE 15614), *V. eskisehirensis* B3, Kaymaz, Eskişehir, 19.06.2019 (ESSE 15616) ve *V. gypsicola* B4, Çayırhan, Ankara, 05.07.2019 (ESSE 15615) şeklindedir.

2.2.Anatomik

Bitkisel materyallerin anatomik çalışması için örneklerin bir kısmı %70'lik alkolde saklanmıştır. Bitki kısımlarından enine (gövde, yaprak) kesitler el yardımıyla alınmış, SARTUR reaktifi ile boyandıktan sonra daimi hale getirilmiştir. Kesitlerin anatomik fotoğrafları ışık mikroskobu Nikon ECLİPSE E200 ile, tüy yapıları taramalı elektron mikroskobu (TM3030 Plus Tabletop Microscope -HITACHI) ile çekilmiştir.



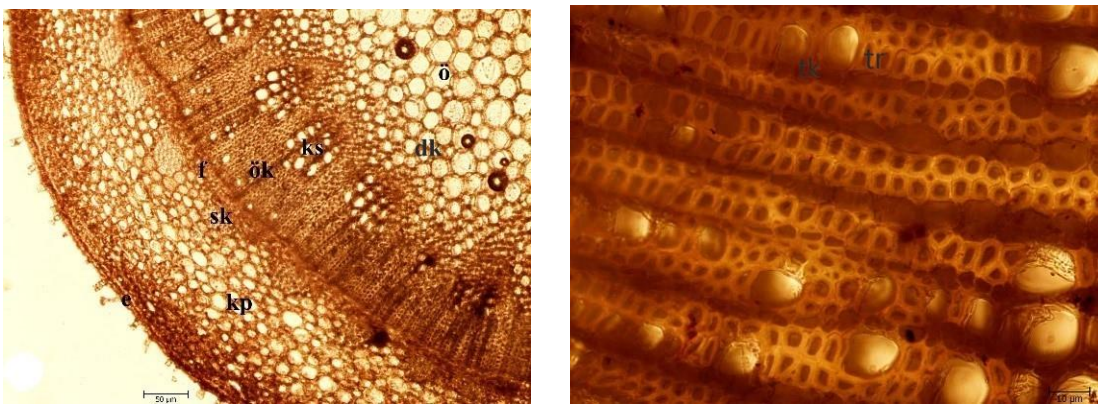
Şekil 1. Genel görünüm; (A) *Verbascum detersile,* (B) *Verbascum eskisehirensis,* (C) *Verbascum gypsicola*.

3. Bulgular

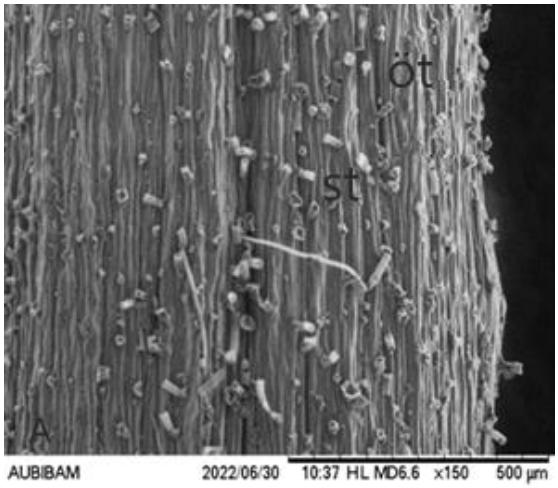
Verbascum detersile, *Verbascum eskisehirensis* ve *Verbascum gypsicola*'nın gövde enine kesitleri ve yapraklardan alınan enine ve yüzeysel kesitleri ile türlere ait anatomik özellikler belirlenmiştir.

3.1. Gövde

V. detersile; gövde enine kesitinde primer yapılar gözlenmiştir. En dışta üst ve alt çeperleri yan çeperlere oranla daha kalın olan bir sıralı geneli oval şekilli epidermis hücreleri yer almaktadır. Epidermis üzerinde ince çeperli kutikula tabakası ile örtü ve salgı tüyleri bulunmaktadır. Örtü tüyleri çok hücreli şamdan ve yıldız dallanmış tüylü, salgı tüyleri sap 1, baş 1, sap 2, baş 2,3 hücreli olmak üzere 3 tiptir. Epidermisin altında kloroplast içeren parenkimatik korteks tabakası 10-12 sıralı, hücreler arası boşlukları olmayan ince çeperli, oval hücrelerden oluşmaktadır. Endoderma belli değildir. Parenkimada druz kristalleri mevcuttur. Sklerankima demetleri kesintili 5-6 sıralı küçüklü büyüklü. Kambiyum belirlidir. Floem halka oluşturmuş 3-4 sıra basık ve oval hücreli, demetler primer ksileme doğru daralarak devam etmektedir. Trake ve trakeit sklerankima hücreleri düzenli sıralı radyal yöndedir. Öz parenkima hücrelerinde druz kristalleri bulunmaktadır. Öz kolları birkaç sıralıdır (Şekil 2, 3).



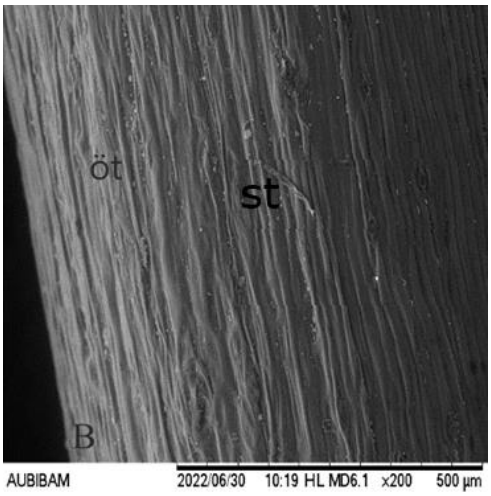
Şekil 2. *V. detersile* (ESSE15614). Gövde enine kesiti (e: epiderma, ku: kutikula, ko: kollenkima, kp: korteks parenkiması, dk: druz kristali, f: floem, ks: ksilem, sk: sklerankima ,tr: trake, tk: trakeid, ö: öz, ök: öz kolları)



Şekil 3. *V. detersile* (SEM). Gövde. (st: salgı tüyleri, öt: örtü tüyleri)

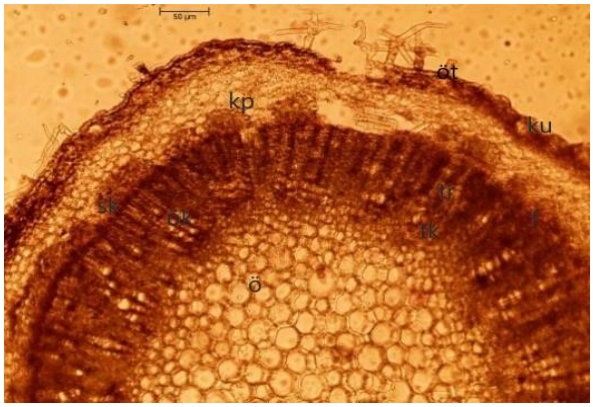
V. eskisehirensis; gövde enine kesitinde, en dışta tek sıralı büyüklükleri farklılık gösteren dikdörtgen ve oval şekilli epidermis hücreleri yer almaktadır. Epidermis üzerinde ince kutikula tabakası ve az sayıda örtü ve salgı tüyleri bulunmaktadır. Epidermisin altında korteks tabakası dar 5-6 sıra irili ufaklı kolroplast içeren parenkimatik hücrelerden oluşmaktadır, 2-3 sıralı irili ufaklı sklerankima demetleri yer almaktadır. Endoderma belirsizdir. Floemlerin geneli oval şeklinde ve 3-4 sıralıdır. Sekonder ksilemde demetler sıralı ve primere doğru küçülmektedir. Ksilem doku içerisinde trakeler belirgin, trake ve trakeid düzenli sıralı hücreli, sklerankima hücreleri düzenli radyal yönde sıralıdır. Öz kolları 1-2 sıralıdır. Öz ince çeperli, içerisinde druz kristalleri bulunan parenkimatik hücrelerdir (Şekil 4, 5).

Şekil 4. *V. eskisehirensis* (ESSE 15616). Gövde enine kesiti (e: epiderma, ku: kutikula, ko: kollenkima, kp: korteks parenkiması, f: floem, ks: ksilem, sk: sklerankima ,tr: trake, tk: trakeid, ö: öz, ök: öz kolları)

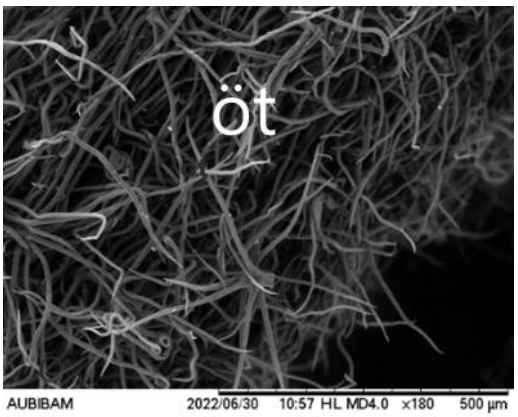


Şekil 5. *V. eskisehirensis* (SEM). Gövde. (st: salgı tüyleri, öt: örtü tüyleri)

V. gypsicola; gövde enine kesitinde, en dışta alt ve üst çeperleri kalın tek sıralı oldukça küçük oval ve yuvarlak epidermis hücreleri ve üzerinde şamdan ve salgı tüyler kaplı, kalın bir kutikula tabakası yer almaktadır. Örtü tüyler çok hücreli şamdan ve yıldız tüylü dallanmış, salgı tüyler sap 1 baş 1; sap1 baş 2; sap 2 baş 1 hücreli olmak üzere 3 tiptir. Epidermisin altında bulunan korteks tabakası 8-10 sıralı kalın çeperli parenkimatik hücrelerden oluşmaktadır. Kortekste epidermisin altına doğru bir iki sıralı kollenkima hücreleri, iç kısımlarda kloroplast taşıyan parenkimatik hücreler yer almaktadır. Endoderma belirsizdir. Kortekste 3-4 sıralı sklerankima demetleri bulunmaktadır. Kambiyum belirgindir. Floem 2-3 sıra basık hücrelerden oluşmuştur. Ksilem hücreleri primer ksileme doğru daralmaktadır. Trake ve trakeid düzenli sıralı hücrelerden oluşmaktadır. Öz kolları 1-2 sıralıdır. Öz druz kristalleri bulunduran parenkimatik çeperleri belirgin hücrelerden oluşmuştur (Şekil 6,7).



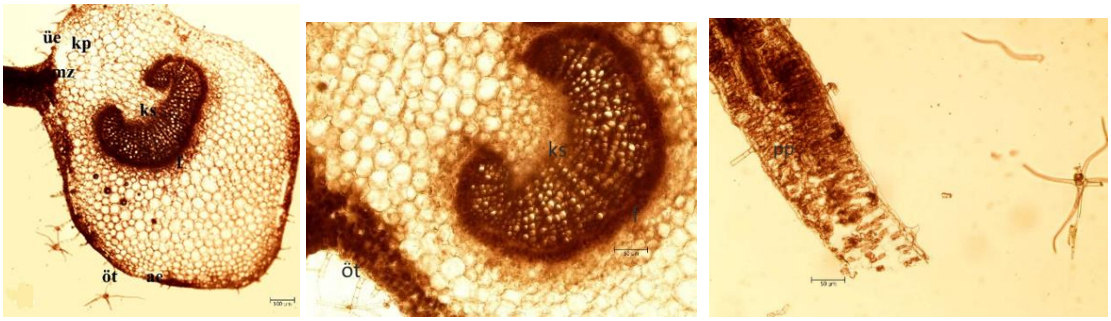
Şekil 6. *V. gypsicola* (ESSE 15615). Gövde enine kesiti. (e: epiderma, ku: kutikula, ko: kollenkima, kp: korteks parenkiması, f: floem, ks: ksilem, sk: sklerankima ,tr: trake, tk: trakeid, ö: öz, ök: öz kolları)



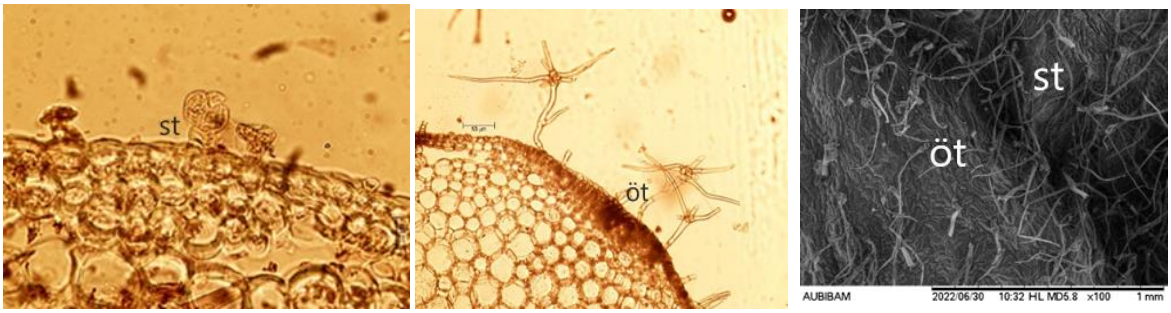
Şekil 7. *V. gypsicola* (SEM). Gövde. (öt: örtü tüyleri)

3.2. Yaprak

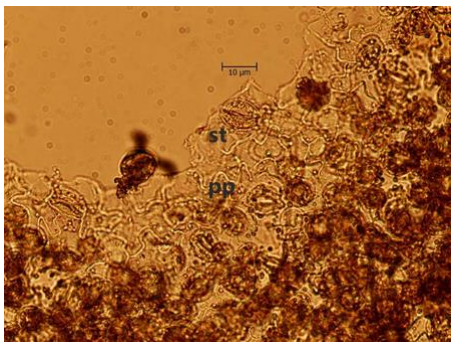
V. detersile; yaprak orta damar bölgesi kesitinde; bir sıralı kalın, küçük ve oval epidermis hücreleri, epidermisin üzerinde kutikula tabakası ile şamdan ve salgı tüyleri bulunmaktadır. Üst epidermis hücreleri alt epidermise göre büyüktür. Işınsal dizili trake ve tarkeid hücrelerinden oluşmuş ksilem üst epidermaya, floem alt epidermaya bakmaktadır. Koleteral iletim demetleri boynuz şeklinde iyi gelişmiştir. Mezofilde üst epiderma altında 10-14 sıra parenkimatik hücreler, alt epidermaya doğru büyüyen yuvarlak, altıgen şekilli 12-16 sıralı parenkimatik hücreler bulunmaktadır. Yaprak alt ve üst yüzeyi çıkıntılıdır. Yaprağın üst ve alt yüzeyinde stoma hücreleri (amfistomatik) bulunmakta ve epidermisin daha üstündedir (higromorf stoma) (Şekil 8,9,10).



Şekil 8. *V. detersile* (ESSE 15614). Yaprak orta damar ve mesofil enine kesiti (ku: kutikula, üe: üst epiderma, ko: kollenkima, ks: ksilem, f: floem, p: parenkima, ae: altepiderma, pp: palizat parenkiması, sp: sünger parenkiması)

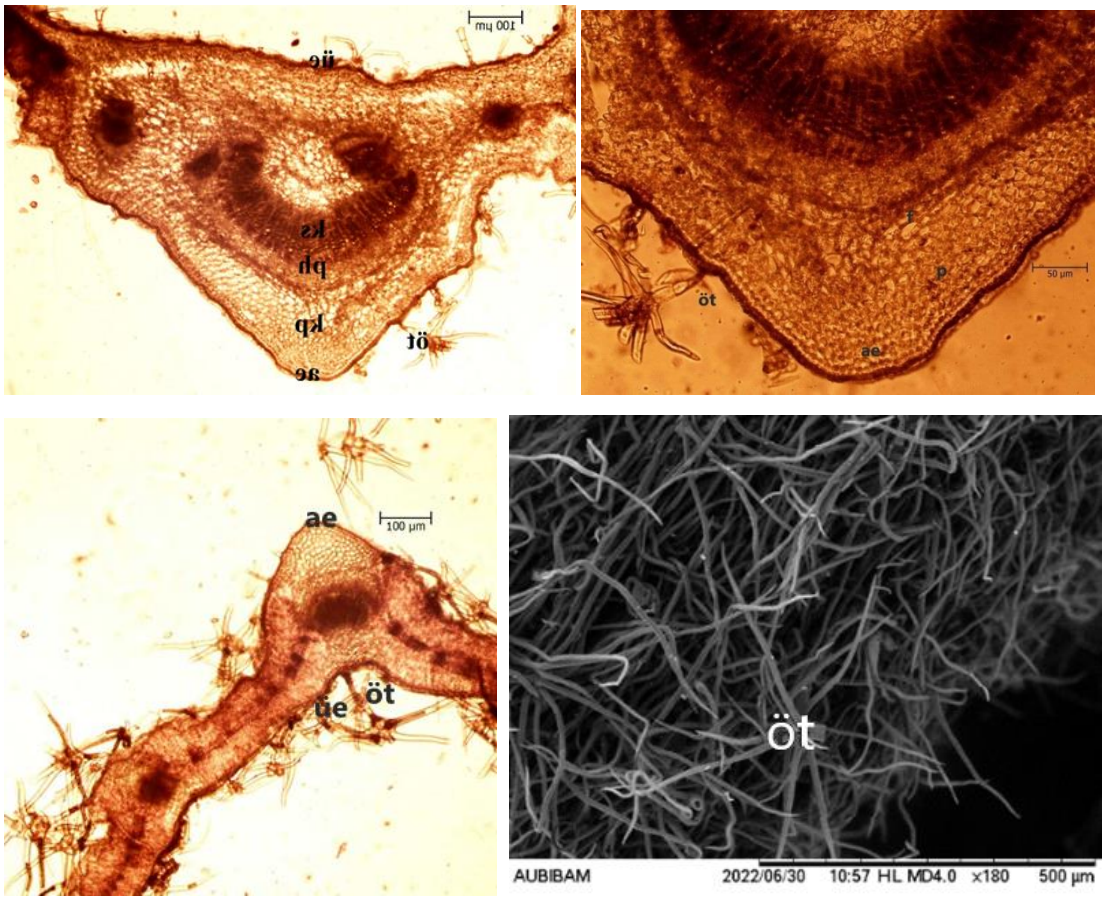


Şekil 9. *V. detersile* yaprak tüyleri (öt: örtü tüy, st: salgı tüy)

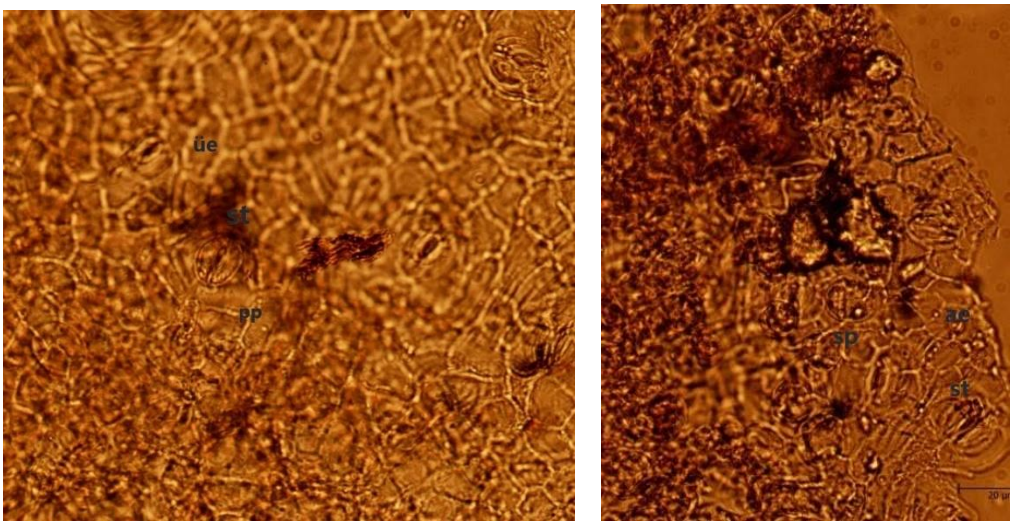


Şekil 10. *V. detersile*. Yaprak epiderma yüzeysel kesiti (st: stoma, pp: palizat parenkiması, sp: sünger parenkima)

V. eskisehirensis; yapraktan alınan enine kesitte, her iki yüzeyinde bir sıralı küçük oval epidermis hücreleri ve üzerinde kutikula tabakası ile yoğun şamdan ve salgı tipi örtü tüyleri bulunmaktadır. Glandular tüyler sap 1, baş 3, sap 1, baş1 hücrelidir. Korteks tabakası 9-10 sıralı parenkimatik hücrelerden meydana gelmektedir. Ksilem trakealleri ışınsal dizili aralarında parenkimatik oval, ince çeperli hücreler bulunmaktadır. Alt ve üst epiderma altında 1,2 sıra kollenkima hücreleri ardından, 10-12 sıra parenkimatik hücre bulunmaktadır. Orta damarın çift yanında yan ve kalın damarlar sıralı, alt yüzey dışa doğru belirgin çıkıntılıdır. Stoma hücreleri (amfistomatik) bulunmakta ve higromorf stoma tipidir (Şekil 11,12).



Şekil 11. *V. eskisehirensis* (ESSE 15616). Yaprak orta damar ve mezofil enine kesiti (üe: üst epiderma, ku: kutikula, ko: kollenkima, f: floem, ks: ksilem p: parenkima, ae: alt epiderma, pp: palizat parenkiması, sp: sünger parenkiması, öt: örtü tüy)

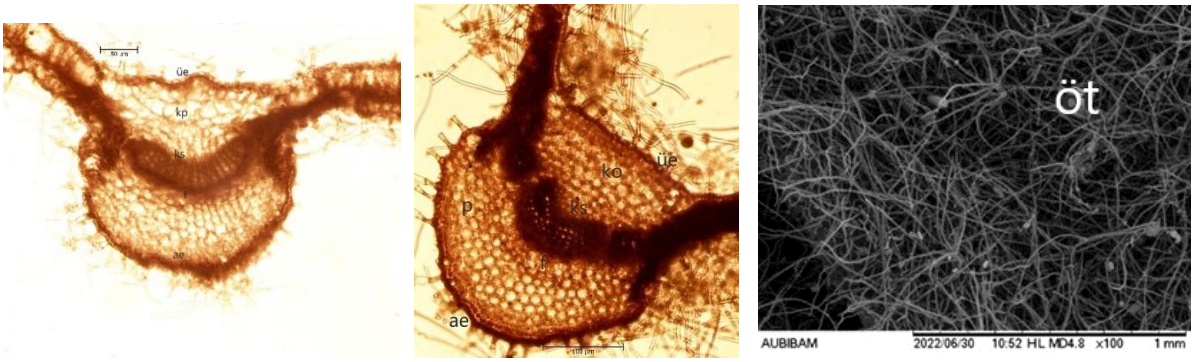


A B

Şekil 12. *V. eskisehirensis* (ESSE 15616). Yaprak üst (A) ve alt (B) epiderma yüzeysel kesiti (üe: üst epiderma, ae: alt epiderma, st: stoma, pp: palizat parenkiması, sp: sünger parenkima)

V. gypsicola; yaprak kesitinde, alt yüz çıkıntılı bir yapıdadır. Epiderma tek sıralı oval ve yuvarlaktır. İnce bir tabaka kutikula tabakası ile kaplıdır. Her iki yüzeyde şamdan tüyleri ve yıldız tüyler mevcuttur. Glandular tüyler sap 1,

baş 1, sap 1, baş 2, sap 2, baş1 hücrelidir. Üst ve alt epiderma hücreleri ve çeperlerinde belirgin bir büyüklük farkı gözlenmemiştir. Üst epiderma altında 2,3 sıra kollenkima, ardından 9,10 sıra parenkima elemanları bulunur. Floem altı parenkima hücreleride 12-15 sıradır. İletim demetleri gelişmiş ve hilal biçimlidir. Floem alt epidermaya, ksilem üst epidermaya yakındır. Ksilem trakeaları ışınsal dizilmiştir. Amfisitomatik yaprak ve higromorf stoma tipi mevcuttur (Şekil 13).



Şekil 13. *V. gypsicola* (ESSE 15615). Yaprak orta damar ve mesofil enine kesiti (üe: üst epiderma, ku: kutikula, ko: kollenkima, f: floem, ks: ksilem p: parenkima, ae: alt epiderma, pp: palizat parenkiması, sp: sünger parenkiması, öt: örtü tüy)

*V. eskisehirensis'*in, *V. gypsicola* ve *V. detersile* korteks parenkimasına göre daha dar, öz bölgesi daha geniş bir alan kaplamaktadır. Türlerin iletim dokuları silindir şeklinde sürekli dokulardır. Yüzeysel kesitlerde, yaprak stoma karakterleri çalışma türleri ile aynı özelliktedir. Türler, şamdan ve salgı tüylere sahiptir. Tablo 1' de bazı *Verbascum* türlerinin anatomik karşılaştırılması verilmiştir.

Tüm türlerin gövde kısmında ksilem ve floem devamlı doku şeklindedir. Türlerin iletim demetleri iyi gelişmiş olup, *V. gypsicola*, *V. basivelatum* ve *V. leptocladum* vasküler demetleri hilal şeklinde, diğer türlerde boynuz şeklindedir [6]. Yapraklar amfistomatik tiptir, stomalar higromorf ve yan damarlar, orta damar ile aynı yapıdadır, iletim demetleri daha da indirgenmiştir. Şamdan ve yıldız tüyler türlerde görülürken, salgı tüyler farklı tiplerde görülmektedir. Daha önce yapılan çalışmalarda *V. mucronatum* ve *V. davisianum*, bizim çalışmamızda *V. detersile* yaprak her iki yüzey çıkıntılı, diğer çalışma türlerinde yaprak alt yüzey çıkıntılıdır [7]. *V. stepporum* ve *V. tenue* türleri ile yapılan çalışmada yaprak enine kesitte yoğun örtü ve salgı tüyler belirlenmiş, gövde anatomisinde ayırt edici bir özellik bulunmaması dışında kollenkima hücre sayılarının farklılık gösterdiği belirtilmiştir [8]. Endemik olan bu türlerin anatomik yapılarının aydınlatılması tür taksonomisine ve tayin anahtarlarına katkı sağlamak adına önemlidir.

4. Sonuçlar ve tartışma

Literatürü incelediğimizde *Verbascum* cinsi ile ilgili yapılmış anatomi çalışmalarının sayıca az olması nedeni ile, türleri karşılaştırma imkanı azdır. Bu çalışmada üç endemik türün gövde ve yaprak anatomik özellikleri incelenmiş, farklı çalışmalar ile karşılaştırılmış, *V. eskisehirensis ve V. gypsicola* anatomisi ilk kez aydınlatılmıştır. Karşılaştırılan türlerin anatomik yapılarının benzer olduğu ancak, gövde sklerankima hücreleri ve floem ile yaprak parenkima hücre sayılarının farklılık gösterdiği görülmüştür. Türlerin gövde ve yapraklarında örtü tüylere ve salgı tüvlere sahip oldukları, yaprak stoma özelliklerinin amfistomatik olduğu gözlenmiştir (Tablo 1).

V. eskisehirensis tür koruma eylem planı dahilinde IUCN'nin kriterlerine göre CR (Çok tehlikeli) statüsündedir [9]. Eylem planında belirtilmiş olan faaliyetlerden biri de bu türe ait araştırmaların gerçekleşmesi olduğundan, çalışma türü incelenmek için toplanmış, türün yaşam alanlarının korunması ve neslinin devamının sağlamasına engel olunmamaya dikkat edilmiştir. *V. gypsicola*, nesli tükenme tehlikesi EN (Tehlikede) ile karşı kaşıya, *V. detersile*, VU (zarar görebilir) türler kategorisindedir [5]. Bulgularımızın Lersten'in [10] bulguları ile uyumlu olduğu gözlenmiştir.

Tablo 1. Verbascum türlerinin anatomik karşılaştırılması

		V. eskişehirensis	*V. detersile*	*V. gypsicoa*	*V. basivelatum*	*V. detersile*	*V. bellum*	*V. orgyale*	*V. leptocladum*	*V. mucronatum*	*V. davisianum*	*V. pestalozzae*	*V. pycnostachyum*	*V. oreophilum*
Gövde	Sklerankima h.	5-6 sıra	2-3 sıra	3-4 sıra	6-7 sıra	4-6 sıra	4-6 sıra	4-6 sıra	1-2 sıra	4-6 sıra	4-6 sıra	3-6 sıra	1-5 sıra	3-4 sıra
	Floem h.	3-4 sıra	3-4 sıra	2-3 sıra	3-7 sıra	3-8 sıra	3-6 sıra	3-8 sıra	3-4 sıra	3-8 sıra	3-8 sıra	3-8 sıra	8-15 sıra	Dar alanlı
Yaprak	Yaprak şekli	Alt yüz çıkıntılı	Her iki yüz çıkıntılı	Alt yüz çıkıntılı	Alt yüz çıkıntılı	Alt yüz çıkıntılı	Alt yüz çıkıntılı	Alt yüz çıkıntılı	Alt yüz çıkıntılı	Her iki yüz çıkıntılı	Her iki yüz çıkıntılı	Alt yüz çıkıntılı	Çıkıntılı	Alt yüz çıkıntılı
	Üst epidermis parenkimatik hücreler	10-12 sıra	12-16 sıra	12-15 sıra	2-3 sıra	10-20 sıra	10-20 sıra	10-20 sıra	3-5 sıra	10-12 sıra	10-12 sıra	5-10 sıra	25-30 sıra	Büyük ve az sayıda
	Alt epidermis parenkimatik hücreler	10-12 sıra	10-14 sıra	9-10 sıra	3-5 sıra	12-15 sıra	12-15 sıra	12-15 sıra	5-10 sıra	4-20 sıra	4-20 sıra	5-6 sıra	25-30 sıra	Küçük ve çok sayıda
	Vasküler demetler	Boynuz şeklinde	Boynuz şeklinde	Hilal şeklinde	Hilal şeklinde	Boynuz şeklinde	Boynuz şeklinde	Boynuz şeklinde	Hilal şeklinde	Boynuz şeklinde	Boynuz şeklinde	Boynuz şeklinde	Boynuz şeklinde	-
Indumentum	Gövde ve yaprakta Örtü tüyü	Gövdede seyrek basit örtü tüylü, yaprakta dallanmış çok hücreli şamdan ve yıldız tüylü	Dallanmış çok hücreli Şamdan ve yıldız tüylü	Dallanmış çok hücreli çok hücreli şamdan ve yıldız tüylü	Dallanmış çok hücreli şamdan tüylü	Çok hücreli şamdan tüylü	Dallanmış çok hücreli şamdan ve yıldız tüylü	Dallanmış çok hücreli şamdan ve yıldız tüylü	Çok hücreli yıldız tüylü	Çok hücreli Şamdan tüylü	Çok hücreli şamdan tüylü	Çok hücreli şamdan ve yıldız tüylü	Çok hücreli şamdan ve yıldız tüylü	Çok hücreli yıldız tüylü

Tablo 1. Devam ediyor

	Gövde ve yaprakta Salgı tüyü	sap 1 baş 3; sap1 baş 1 hücreli	sap 1 baş 1; sap 2 baş 2,3 hücreli	sap 1 baş 1; sap1 baş 2; sap2 baş 1 hücreli	1 gövde; 1 sap baş2;2 sap baş2 hücreli	sap 1 baş 1, sap 2 baş 1, sap 2 baş 2 hücreli	sap 1,baş 2; sap2 baş 1; sap 2 baş 2; sap 2 baş3;sap 3 baş 1 hücreli	sap 1, baş 1 hücreli, sapı 2 baş 1 hücreli, sapı 2 başı 2 hücreli	sap 1-2-3 baş 1; sap 1-2-3 baş 2; sap 2 baş 3 hücreli	sap 2 baş 1; sap 2baş 2; baş 3 sap2; baş 1 sap 3; baş 2 sap 3 hücreli	sap 2 baş 1; sap 2-3 baş 2 hücreli	sap 2 baş 1; sap 1 baş 2; sap 2 baş 3; sap 3 baş 1; sap 3 baş 2 hücreli	sap1 baş 1; sap1 baş 2; sap 2 baş 3 hücreli	Sap 1 baş 1, Sap 1 baş 2, sap2 baş 1 hücreli
	Stoma	Amfistomatik, higromorf stoma	Amfistomatik, igromorf stoma	Amfistomatik, higromorf stoma	Amfistomatik, higromorf stoma	Amfistomatik, higromorf stoma	Amfistomatik yaprak, amaryllis tip stoma	Amfistomatik yaprak, amaryllis tipi stoma	Amfistomatik yaprak	Amfistomatik yaprak	Amfistomatik yaprak	Amfistomatik yaprak	Amfistomatik yaprak	Amfistomatik yaprak
Kaynak					[6]	[7]	[11]	[12]	[13]	[13]	[13]	[14]	[14]	[15]

Teşekkür

Bitki gövde ve yaprak tüy anatomisinin SEM çekimleri ile aydınlatılmasında yardımcı olan AUBİBAM'a teşekkür **e**deriz.

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Effects of heavy metal pollution on population dynamics of another important pollinator insect group: Horseflies (Diptera: Tabanidae)

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Abstract

Horseflies represent important agents of pollination as well as other pollinator insects, like butterflies, bees and wasps. Horseflies belong to the genera *Atylotus*, *Dasyramphis*, *Glaucops*, *Pangonius*, *Philoliche*, *Chrysops*, *Hybomitra*, and *Tabanus* species are exclusive nectar feeders. Glucose, fructose and sucrose are major components of plant nectars which are used nourishing for developing eggs. Because of these features horseflies have a significant role in pollination of phanerogams. This study was conducted to investigate the effects of toxic heavy metals on the population dynamics of horsefly species. Monthly changes on the accumulation of heavy metals in the larval habitats of horsefly species were determined with Perkin Elmer Optical Spectrophotometer and also correlations between concentrations of heavy metals and population fluctuations of horse fly species were observed. The results demonstrated that lead, iron and cadmium have statistically important negative effects on the population dynamics of horsefly species.

Keywords: Diptera, heavy metal pollution, horseflies, population dynamics, Tabanidae

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Ağır metal kirliliğinin bir diğer önemli polinatör böcek grubu üzerindeki etkileri: Atsinekleri (Diptera: Tabanidae)

Özet

Atsinekleri, arılar ve kelebekler gibi diğer polinatör böcekler kadar önemli polenizasyon ajanları arasındadır. *Atylotus*, *Dasyramphis*, *Glaucops*, *Pangonius*, *Philoliche*, *Chrysops*, *Hybomitra* ve *Tabanus* cinslerine ait at sineği türleri özel olarak nektarla beslenirler. Glukoz, fruktoz ve sakkaroz, bitki nektarlarının başlıca bileşenleridir ve gelişmekte olan yumurtaların beslenmesinde kullanılırlar. Bu özellikleri nedeniyle at sinekleri fanerogamların tozlaşması için önemli bir role sahiptirler. Bu çalışma, toksik ağır metallerin at sineği türlerinin populasyon dinamiği üzerindeki etkilerini araştırmak amacıyla yürütülmüştür. At sineği türlerinin larval yaşam alanlarında aylık ağırlık artışındaki değişimler, Perkin Elmer Optik Spektrofotometre kullanılarak belirlenmiş ve aynı zamanda ağır metal konsantrasyonları ile at sineği türlerinin populasyon dinamikleri arasındaki ilişkiler gözlemlenmiştir. Sonuçlar, kurşunun, demirin ve kadmiyumun at sineklerinin populasyon dinamiği üzerinde istatistiksel olarak önemli negatif etkilere sahip olduğunu göstermiştir.

Anahtar kelimeler: Diptera, ağır metal kirliliği, At sinekleri, popülasyon dinamiği, Tabanidae

1. Introduction

Pollination is a significant issue for maintaining the ecological balance and ecosystem. Pollinator insects involve the majority of social and solitary bees, wasps, flies, beetles, butterflies, and moths. These pollinators are essential for the reproduction of fruits, vegetables, and grains for both animals and humans. Global changes, magnified

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land use, climate change, alien species, and the spread of pests, pathogens and intensive chemical use in agricultural lands have growing pressure on the insect pollinators [1, 2]. All these impacts not only change the status of pollinators but also effect their population dynamics, distribution, abundance, and dietary habits.

The horseflies belonging to the family Tabanidae are important regulators of the ecosystem. Although they are known as vector organisms, horseflies especially males need sucrose due to need large amounts of energy for flight, they obtain this energy from nectar and pollen. Despite bloodsucking behavior, long-proboscid Tabanidae species are significant nectar feedings and recognized as key species for pollination of plants of some regions [3]. Previous studies proved that long-proboscid Tabanidae co-evolved with flowering plants since the Late Jurassic and they are represented among the first pollinator insects of early angiosperms [4, 5]. Miller (1951) [6] confirmed that adult tabanids transport the pollens attached to their bodies. Horseflies belong to genus *Atylotus*, *Dasyramphis*, *Glaucops*, *Pangonius*, *Philoliche*, *Chrysops*, *Hybomitra*, and *Tabanus* are exclusive plant visitors for nectar, pollen, and plant exudates [7, 8, 9]. (Kniepert, 1980)[7] investigated the dietary preferences of adult male and female tabanids and reported that the 53% of female and 69% of male specimens carry nectar sugars. (Bosler & Hansens, 1974)[10] found that the greater than 80% of female *Tabanus nigrovittatus* had consumed nectars. Magnarelli & Anderson (1976) [11] reported that *T. nigrovittatus*, *Chrysops atlanticus* and *Chrysops fuliginosus* had used nectar sugars as normal diary diets. Furthermore, it is usual that most of the collected *C. celatus* had taken fructose, water-soluble sugar found in a variety of fruits for carbohydrates has been reported as a significant dietary elements for horseflies surely to renew decreasing energy reserves during flight [12, 13]. Magnarelli et al. (1979) [12] established that female *C. atlanticus* and *C. fuliginosus* visited yarrow and swamp rose many times, through the day and they also reported that 119 pollen grains, belonging to Compositae, Gramineae and Rosaceae, identified from digestive tracts of females. On the otherhand glucose, fructose and sucrose are the base components of plant nectars which are using for nourishing their developing eggs, were detected from adult digestive systems [11, 12, 13]. It can be easily argued that, horsefly species have important effect on the pollination of many phanerogam species of the Compositae, Gramineae, Rosaceae, Geraniaceae, lridaceae, Orchidaceae, Amaryllidaceae, Scrophulariaceae, Apiaceae, Amaryllidaceae, Convolvulaceae, Poaceae, Umbelliferae, as well as other pollinator insects [14, 15]. Consequently, changes of population dynamics of horsefly species effect the life cycle of phanerogams.

On the other hand, all stages of tabanids are absolute food source for other ecosystem components. Thereby, decreasing of population density of horsefly species affects many other species. Natural habitats sustain the pollinators, providing a strong and reciprocal pollination service that increases yield capacity [16, 17]. However, intensive chemical pesticide usage in the agricultural areas affect to the pollinator insects with target pests and decrease the crop quality which is consumed by pollinators. With the pesticides some heavy metals accumulate in the soil and water sources and affect all ecosystem agents. Horseflies prefer different habitat conditions like aquatic, semiaquatic and terrestrial zones in accordance with their life cycle. These habitats may be affected because of some pollution ingredients and population dynamics of horseflies may be damaged. This damage may cause decreasing pollination by tabanids and may be destroyed food chain. Some recent studies indicate that toxic heavy metals such as cadmium, ferric, aluminum, mercury and lead inhibit or restrict larval growth rates of fly larvae [18, 19].

This study therefore set out to assess the effect of the heavy metal accumulation in the larval habits and the effects on the population dynamics of horsefly species.

2. Materials and methods

The study was conducted in the marsh area, which is center of oak and pine forest, in Yarımca village in Eskişehir Province, Türkiye (39º 53’ 936” N, 30º 37’ 747” E, 1171m).

In order to determine the presence of heavy metal concentration in larval habits, eight different streams and four different soil samples were collected each month during three years in active periods of horseflies. Collected samples were frosted by dry ice and transferred to the laboratory with thermoflasks. Concentrations of the heavy metals in water and soil were examined with Perkin Elmer Optical Emission Spectrometer Optima 4300 DV. Larval habitats of horsefly species show great variability, larvae of many species have cannibalistic behavior and larval development periods vary as per to species. Therefore, population dynamics were investigated on adult samples. Adult horse flies were collected with water and malaise traps, in the active period durations of species (May to October 2017-2019).

Adult specimens were collected every 20 minutes from 09:00 to 20:00 and they were placed in ethyl acetate containing jars and carried to the laboratory. Species identification was made according to the Chvala et al. [20].

Population dynamics of the collected horsefly species were analyzed and given in (Table 1). The statistical models were modified from Karpakakunjaram et al. [21]. SPSS was used and datas were evaluated with the nanparametric Kendall's correlation coefficient test.

The weekly population data of species were converted to monthly means for analysis and were investigated for correlation with heavy metal concentrations. All data (populations and concentrations) were converted to normal varieties; $Z = (X - \mu) / \partial$; were Z is the standard normal deviation from the mean $(X - \mu)$ of the data, measured in units of standard deviations. The conversions were done to import the values of heavy metals and the population input to a comparable scale for plotting the histogram and also further analyses. Totally 50 correlation coefficient values were

obtained from Kendall's correlation coefficient tests. In order to eliminate significant correlations, nonparametric sequential Bonferroni tests were performed.

3. Results

Tabanidae family represented with 176 species and 15 subspecies in Türkiye [22]. In this study, totally 7234 adult samples belonging to 52 species were collected and identified. The list of species and seasonal distributions are given in Table 1.

Triticium vulgare, Brassica juncea and *Zea mays* are commonly harvested crops at study area (about 5 km^2). These wetland agricultural areas are well productive but insecticides are used concentratedly. According to the results of analysis, the accumulation of lead, iron, cadmium, mangan and aluminum were determined in the samples of soil and stream. Results of correlations between these heavy metals and population dynamics of selected specimens indicate that lead, iron and cadmium have significantly negative effects on the populations. Meanwhile the lead pollution is commonly originated from the presence of highway and dye; lead from processing fabrics; iron and cadmium pollution is generally based on insecticides, pesticides and other chemicals which are used for agricultural purpose.

Table 1. The list of the collected species and seasonal distributions

Species	**2017**					**2018**					**2019**				
	May	June	July	Aug	Sept	May	June	July	Aug	Sept	May	June	July	Aug	Sept
Tabanus bromius L.	4	141	528	75	19	2	47	86	43	5	2	133	367	51	12
Tabanus briani Lec.	-	-	2	-	-	-	-	-	1	-	-	-	-	-	-
Tabanus bifarius Loew.	-	47	26	9	-	-	11	5	1	-	-	268	26	2	-
Tabanus autumnalis L.	-	33	11	-	-	-	-	5	-	-	-	6	5	-	-
Tabanus armeniacus Kröb.	-	-	1	-	-	-	-	-	-	-	-	-	3	-	-
Tabanus cordiger Meig.	-	14	-	1	-	-	11	4	-	-	-	6	4	-	-
Tabanus cuculus Szi.	-	-	2	1	-	-	-	-	1	-	-	-	1	1	-
Tabanus eggeri Sch.	-	-	2	-	-	-	-	-	-	-	-	-	1	-	-
Tabanus exclusus Pand.	-	4	27	9	-	-	1	7	8	-	-	6	11	1	-
Tabanus glaucopis Meig.	-	-	10	17	1	-	1	12	11	2	-	-	7	13	3
Tabanus golovi Ols.	-	-	-	1	-	-	-	1	-	-	-	-	2	-	-
Tabanus indrae Haus.	-	-	1	1	-	-	-	2	3	-	-	-	3	-	-
Tabanus laetetinctus Bec.	-	-	2	-	-	-	1	-	-	-	-	-	1	-	-
Tabanus leleani Aust.	-	2	-	-	-	-	5	7	-	-	-	4	1	-	-
Tabanus lunatus Fabr.	-	4	37	3	-	-	1	22	14	2	-	10	138	11	1
Tabanus maculicornis Zet.	-	3	2	-	-	-	-	1	-	-	-	-	4	1	-
Tabanus martini Kröb.	-	-	1	8	1	-	-	-	3	2	-	-	-	7	1
Tabanus miki Brau.	-	-	18	4	-	-	1	5	2	-	-	8	11	8	-
Tabanus portschinskii Ols.	-	-	28	13	-	-	-	14	9	-	-	-	39	6	-
Tabanus prometheus Szi.	-	-	7	-	-	-	-	3	-	-	-	-	16	-	-
Tabanus quatuornotatus Meig.	9	379	9	-	-	7	118	3	-	-	3	77	5	-	-
Tabanus regularis Jaen.	-	-	-	1	-	-	-	-	4	-	-	-	-	7	-
Tabanus rupium Brau.	-	38	7	-	-	-	11	13	-	-	-	28	3	-	-
Tabanus spodopteroides O.M.C.	-	-	2	-	-	-	-	3	-	-	-	-	1	-	-
Tabanus spodopterus Meig.	-	-	27	3	-	-	-	4	3	-	-	-	11	2	-
Tabanus sudeticus Zel.	-	3	4	1	-	-	2	-	-	-	-	-	-	2	-
Tabanus spectabilis Loew	-	-	1	-	-	-	-	2	-	-	-	-	-	-	-
Tabanus tergestinus Egg.	-	-	7	-	-	-	-	2	-	-	-	-	4	-	-
Tabanus terterjani Dol. & And.	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-
Tabanus tinctus Wal.	-	-	4	-	-	-	-	1	-	-	-	-	2	-	-
Tabanus unifasciatus Loew.	4	141	37	7	3	3	14	29	13	4	3	67	-	4	11
Therioplectes tricolor Zel.	-	13	-	-	-	-	-	9	-	-	-	4	-	-	-
Philipomyia zizaniae Leg.	-	-	-	-	-	-	-	-	-	-	-	1	7	-	-
Philipomyia aprica Meig.	-	13	201	26	4	-	13	38	12	2	5	259	483	11	-
Atylotus fulvus Meig.	-	-	24	9	-	-	-		-	-	-	13	7	-	-
Chrysops caecutiens L.	-	2	13	2	-	-	-	4	1	-	-	17	15	2	-
Chrysops viduatus Fab.	-	2	3	1	-	-	-	1	-	-	-	3	4	1	-
Dasyrhamphis carbonarius Meig.	-	1	-	-	-	-	3	-	-	-	-	1	-	-	-
Dasyrhamphis umbrinus Meig.	11	155	21	-	-	8	21	-	-	-	8	119	-	-	-
Haematopota bigoti Gob.	-	3	1	-	-	-	-	-	-	-	-	-	2	-	-

Table 1. Continued

Haematopota crassicornis Wah	-	2	3	-	-	-	-	1	-	-	-	-	2	-	-
Haematopota grandis Meig.	-	-	1	1	-	-	-	1	-	-	-	-	1	-	-
Haematopota longantennata Ols.	-	-	1	-	-	-	-	1	-	-	-	-	1	-	-
Haematopota ocelligera Kröb.	-	6	-	-	-	-	5	-	-	-	-	1	-	-	-
Haematopota pluvialis L.	-	19	11	-	-	-	15	9	-	-	-	4	-	-	-
Haematopota scutellata O.M.C.	-	7	-	-	-	-	5	-	-	-	-	2	-	-	-
Haematopota subcylindrica Pand.	11	433	681	57	10	7	66	63	31	8	14	118	211	5	-
Hybomitra caucasica End.	6	4	-	-	-	7	3	-	-	-	9	2	-	-	-
Hybomitra ciureai Seg.	-	-	3	-	-	-	-	-	-	-	-	-	2	-	-
Hybomitra pilosa Loew.	-	4	-	-	-	2	-	-	-	-	-	3	-	-	-
Total	**3572**					**934**					**2771**				

3.1. Lead

Correlation coefficient data show that the lead concentration had significant effects on the populations of *T. bifarius* (T1= -0.482 and p = 0.012) (Fig. 1.a), *T. bromius* (T1= -0.310 p = 0.0008) (Fig. 1.b), *T. unifasciatus* (T1= -0.411 p = 0.0008) (Fig. 1.c), *T. quatuornotatus* (T1= -0.505 p = 0.012) (Fig. 1.d) and *T. rupium* (T1= -0.392 p = 0.012) (Fig. 1.e). These significance levels were lower than the α/k value (0.013) of the sequential Bonferroni test and hence the lead concentrations have negative effect on the population dynamics of these species. On the other hand, correlation coefficient data show that lead concentrations had no effects on the population dynamics of *T. portschinskii*, *D. umbrinus*, *P. aprica*, *H. pluvialis* and *H. subcylindrica*. These differences caused by the habitat preferences of species. Larvae of *T. bifarius*, *T. bromius*, *T. unifasciatus*, *T. quatuornotatus* and *T. rupium* are hemihydrobiont and concentration of lead in streams are higher than soil in the study area, hence lead pollution has important effect on the population dynamics of these species. But larvae of *T. portschinskii*, *D. umbrinus*, *P. aprica*, *H. pluvialis* and *H. subcylindrica* edaphebiont, and larval duration occurs terrestrial or semiaquatic places, hence lead pollution has negative effects on the population dynamics of these species, but the effects are not statistically important.

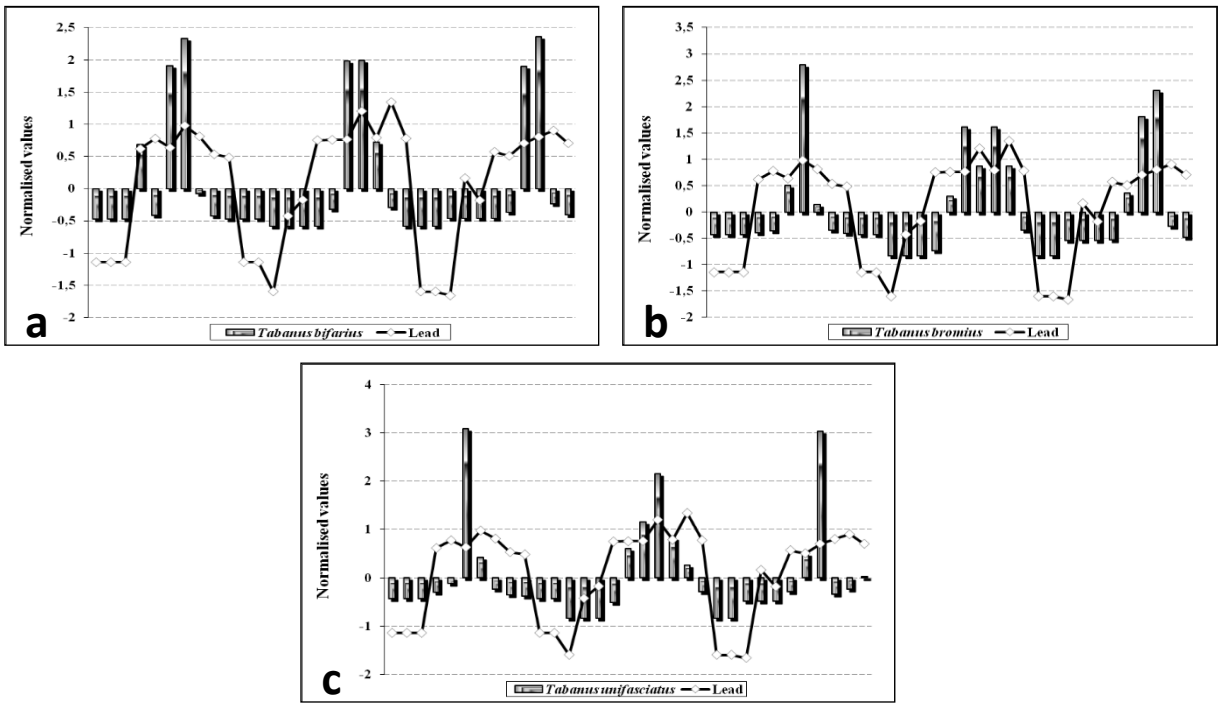

Figure 1. Effects of the lead on the population dynamics of *Tabanus bifarius* (a); *Tabanus bromius* (b); *Tabanus unifasciatus* (c)

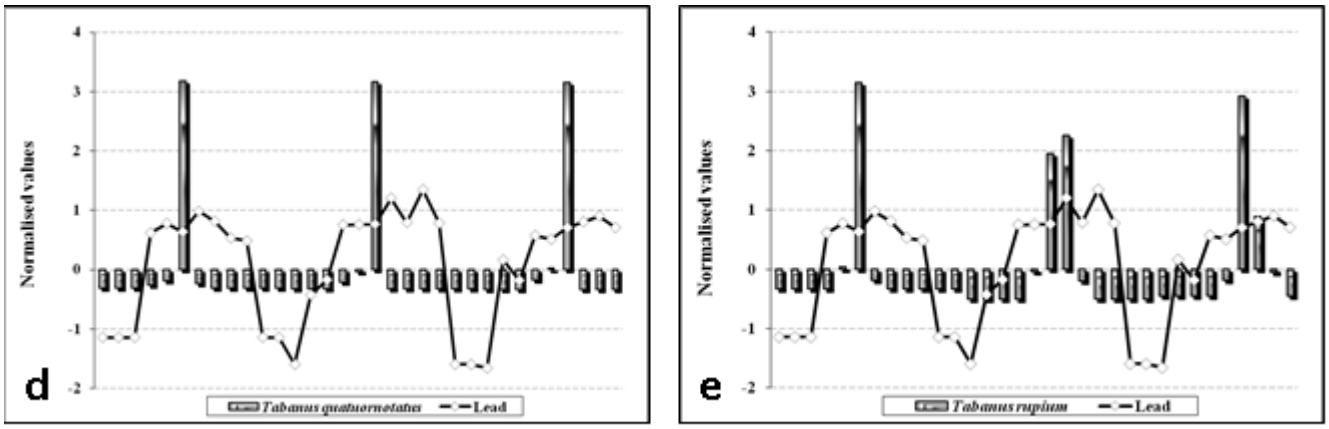

Figure 1. Effects of the lead on the population dynamics of *Tabanus quatuornotatus* (d); *Tabanus rupium* (e)

3.2. Iron (Ferric)

Iron has also negative effect on the population dynamics of species, and correlations between iron and *T. bifarius* (T1= -0.294 p = 0.012) (Fig. 2.a); *T. bromius* (T1= -0.412 p = 0.005) (Fig. 2.b); *T. quatuornotatus* (T1= -0.365 p = 0.0008) (Fig. 2.c); *T. rupium* (T1= -0.488 p = 0.005) (Fig. 2.d); *T. unifasciatus* (T1= -0.206 p = 0.0008) (Fig. 2.e), and *H. subcylindrica* (T1= -0.266 p = 0.005) (Fig. 2.f) are statistically important.

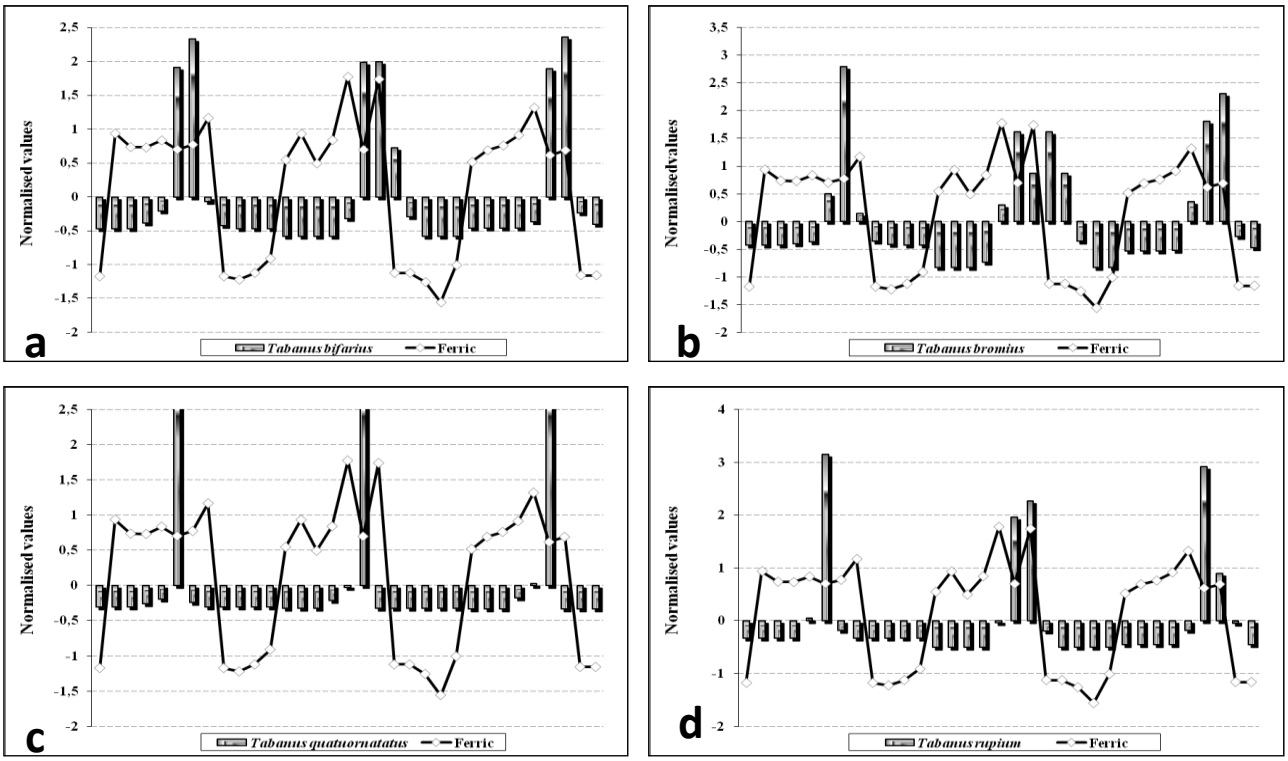

Figure 2. Effects of the iron on the population dynamics of *Tabanus bifarius* (a); *Tabanus bromius* (b); *Tabanus quatuornotatus* (c); *Tabanus rupium* (d)

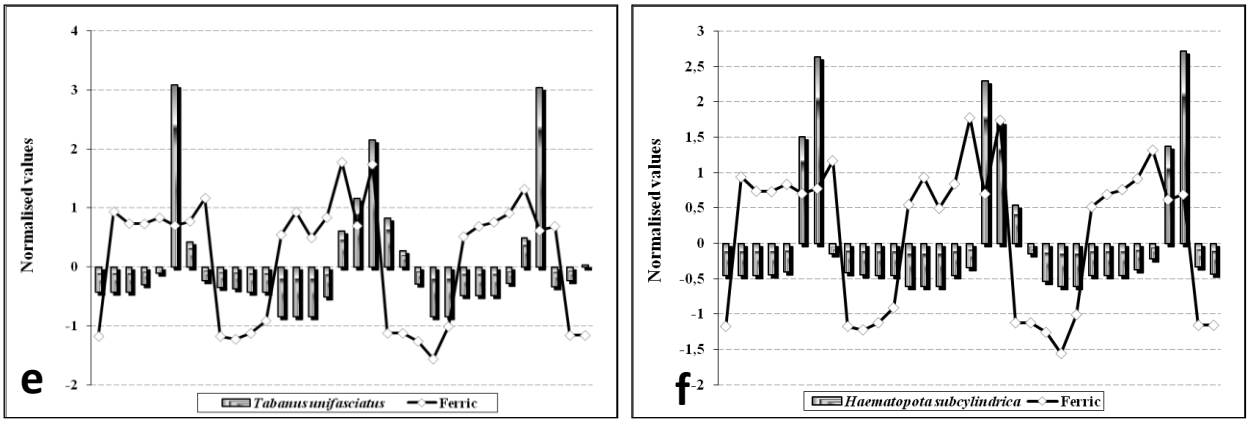

Figure 2. Effects of the iron on the population dynamics of *Tabanus unifasciatus* (e); *Haematopota subcylindrica* (f)

3.3. Cadmium

Comparing with iron and lead, less amounts of cadmium accumulation were detected. Due to the toxicity level of cadmium, it has further negative effects on the population dynamics of all species. However, correlations between cadmium and *T. bifarius* (T1= -0.311 p = 0.0012) (Fig. 3.a), *T. bromius* (T1= -0.304 p = 0.0012) (Fig. 3.b) and *T. unifasciatus* (T1= -0.403 p = 0.0005) (Fig. 3.c) were statistically important.

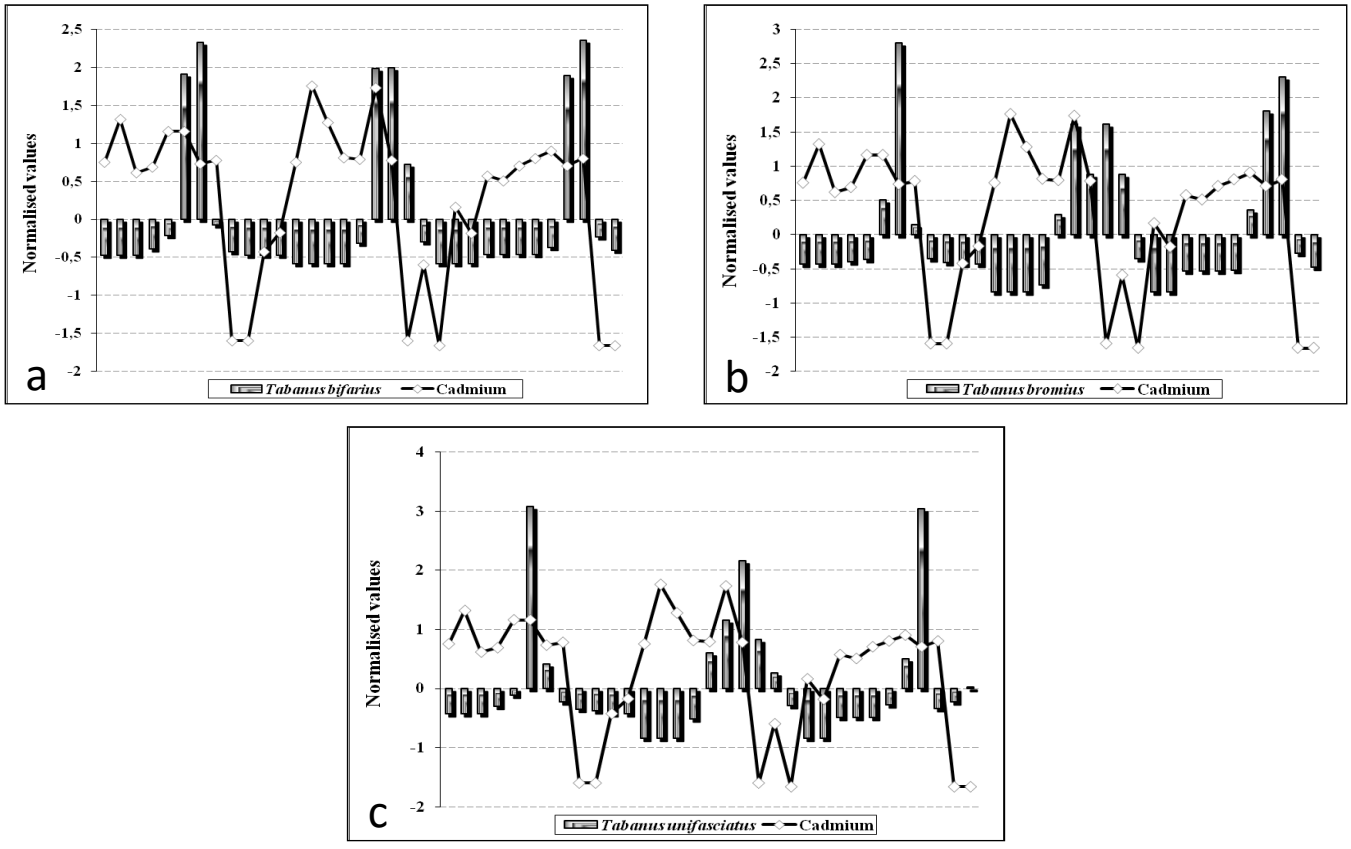

Figure 3.Effects of the cadmium on the population dynamics of *Tabanus bifarius* (a); *Tabanus bromius* (b); *Tabanus unifasciatus* (c)

4. Conclusions and discussion

Toxic heavy metal presence in water, air and soil is known as a serious problem that increasingly threatens the environment. There are many sources of heavy metal pollution, consisting of the chlor-alkali industries, natural gas, coal, paper, and dye industries [23]. Nevertheless, if heavy metal pollution exponentially grows over the years, the life quality of many species are affected irreversibly.

Heavy metal pollution can affect population dynamics of Tabanidae species as well as other groups. In this study, annual increase on accumulation of Lead (Pb), Iron (Fe), Cadmium (Cd), Mangan (Mn) and Aluminium (Al) were investigated in the samples of soil and stream. All toxicants have negative impacts on the population dynamics of Tabanidae species. Due to the accelerating concentrations and higher toxic effect, however, correlations between only lead, iron and cadmium were statistically important.

The many authors have investigated the effects of Zn, Pb, Cd and Cu on the population dynamics and diversity of different species and reported the negative impressions of stated heavy metals on the life cycles of studied populations [24, 25]. Hladun et al. [19] investigated the effect of soil-borne pollutants like selenium, methionine and elenomethionine on the honey bee and concluded that contaminated soil and plants have negative impact on the insects. Azam et al. [26] stated that Cr, Cu, Cd, Zn, and Ni in the water, soil and air can affect the species. They also determined that the various insect groups are potential indicators of heavy metal pollution and they may be used as bioindicator species. they conducted a study on heavy metal pollution in Salicornia europaea L. growing in wetlands and found that there was an increase in the accumulation of Pb and Zn. These heavy metals accumulated in water, soil and plants negatively affect both the horseflies, whose larvae are aquatic, and the ecosystem. Ay et al. [27] conducted a study on heavy metal pollution in *Salicornia europaea* L. growing in wetlands and found that increasing in the accumulation of Pb and Zn. These heavy metals accumulated in water, soil and plants and negatively affect both the horseflies, whose larvae are aquatic, and the ecosystem. Many stresses that are connected with all biological processes on the ecosystems and the living organisms are a hazard for pollinator welfare, diversity, and abundance. In addition to pollinators these pressures affect all species step by step, decreasing pollinators cause to decrease in plant species and decreasing the plant species also causes ecosystem degradation. It is urgent to restrict the negative compulsions of ongoing pollinator decreases for ecological services, human health and agricultural production. The present study which is the first attempt for Tabanidae in Türkiye, clearly emphasizes the requirement of adequate studies about the impacts of heavy metals on population dynamics in the field of observations on population ecology of Tabanidae species. Moreover, in the everchanging global world, the pollinator insects which are necessary for the ecosystem and their habitats should be protected from any human activities.

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First record of *Xerocomellus cisalpinus* for Turkish Mycobiota

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Abstract

Xerocomellus cisalpinus (Simonini, H. Ladurner & Peintner) Klofac is presented as new record for Turkish Mycobiota. This species is the sixth member of the genus *Xerocomellus* Šutara to be determined in Türkiye. A brief description of the species and the photographs, related to its macro and micromorphology are presented.

Key words: Biodiversity, Boletales, New record

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Xerocomellus cisalpinus'un Türkiye Mikobiyotası için ilk kaydı

Özet

Xerocomellus cisalpinus (Simonini, H. Ladurner & Peintner) Klofac, Türkiye Mikobiyotası için yeni kayıt olarak verilmiştir. Bu tür *Xerocomellus* Šutara cinsinin Türkiye'de belirlenen altıncı üyesidir. Türün kısa betimlemesi ile makro ve mikromorfolojisine ilişkin fotoğrafları verilmiştir.

Anahtar kelimeler: Biyoçeşitlilik, Boletales, Yeni kayıt

1. Introduction

Xerocomellus Šutara is a genus of the family Boletaceae. Members of the genus are characterized by small to medium-sized and often vividly coloured fruit bodies, velvety to often rimose-areolate pileus surface, minutely granulose, sometimes longitudinally striate but mostly non-reticulate stipe which is more slender compared to other boletes, initially palisadorem, typically encrusted pileipellis, and smooth to longitudinally striated, and not bacillate spore surface [1,2].

IndexFungorum currently lists 25 confirmed *Xerocomellus* species, five of which, *X. chrysenteron* (Bull.) Šutara, *X. porosporus* (Imler ex Watling) Šutara, *X. redeuilhii* A.F.S. Taylor, U. Eberh., Simonini, Gelardi & Vizzini, *X. truncatus* (Singer, Snell & E.A. Dick) Klofac, *X. zelleri* (Murrill) Klofac, were also reported to exist in Türkiye [3,4]. But the current checklists [4,5] and the latest contributions either as regional lists [6-8] or contributory records [9-13] indicated that *Xerocomellus cisalpinus* (Simonini, H. Ladurner & Peintner) Klofac has not been determined within the boundaries of Türkiye before.

The study aims to make a contribution to the macrofungal biodiversity of Türkiye.

2. Materials and methods

Fruit bodies were collected from Sarıyer (İstanbul) district during a field survey in 2023. Fruit bodies were photographed at their natural habitat, and necessary notes were taken about their ecological and morphological

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properties, and geographic position. Following collection, they were transferred to the fungarium in paper bags, and dried in an airconditioned room.

Investigations related to micromorphology were conducted under a Leica DM 2500 trinocular light microscope, equipped with a camera operating with a Relab Imaginary software. The specimens were mounted in potassium hydroxide, Congo-red, chrystal violet and safranin, and about 20 measurements were made for each micromorphological structure. The background of some photographs were cleaned with point net program without interfering with the cellular structure. With the help of the accumulated data from field and laboratory investigations, a description of the samples was prepared. Then, they were identified by comparing the determined characteristics with those given in literature [14-17]. The samples are kept at Karamanoğlu Mehmetbey University, Science Faculty of, Department of Biology.

3. Results

Boletaceae Chevall.
Xerocomellus cisalpinus (Simonini, H. Ladurner & Peintner) Klofac, Öst. Z. Pilzk. 20: 38 (2011) (Figure 1)

Syn: [*Boletus cisalpinus* (Simonini, H. Ladurner & Peintner) Watling & A.E. Hills, *Xerocomus cisalpinus* Simonini, H. Ladurner & Peintner].

Figure 1. Basidiocarps of *Xerocomellus cisalpinus*

Macroscopic and microscopic features

Pileus 30-50 mm in diameter, at first hemispherical, then convex to almost plane, surface dry, velvety, somewhat areolate in age, blackish to dark brown when young, pale brown to ochraceous at maturity with a reddish tint at marginal zone. Flesh whitish to creamy, soft, slightly blueing when injured. Tubes creamy yellow to pale yellow, slightly blueing when bruised. Pores almost concolorous with the tubes to olivaceous yellow, slightly blueing when injured. Taste and smell not distinctive. Stem 3−6.6(−7) × 5−9 mm, cylindrical without a ring, some slightly tapering towards the base, some slightly curved at the base or bulbose, yellow to olivaceous yellow in the upper half, gradually becoming dull red to ocher brown downwards, white fuzzy at the base, Context fibrillose and brittle, blueing when cut or bruised especially towards the base.

Basidia 32–43 × 10–11.5 µm, clavate, 2-4 spored (mainly 4) (Figure 2). Basidiospores (10.1−)10.4 − 12.5(−14.3) × (3.8−)3.9−4.8(−5) µm, subfusiform, thick walled, 1-3 guttulate (Figure 3).

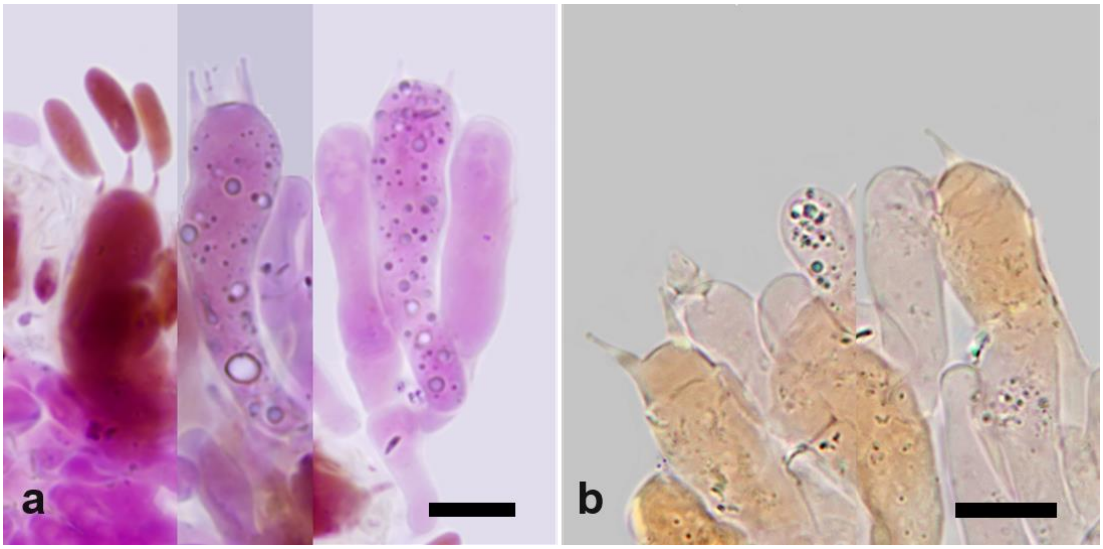

Figure 2. Basidia (a,b) of *Xerocomellus cisalpinus* (bars-10 µm) (a in crystal violet; b in Congo-red)

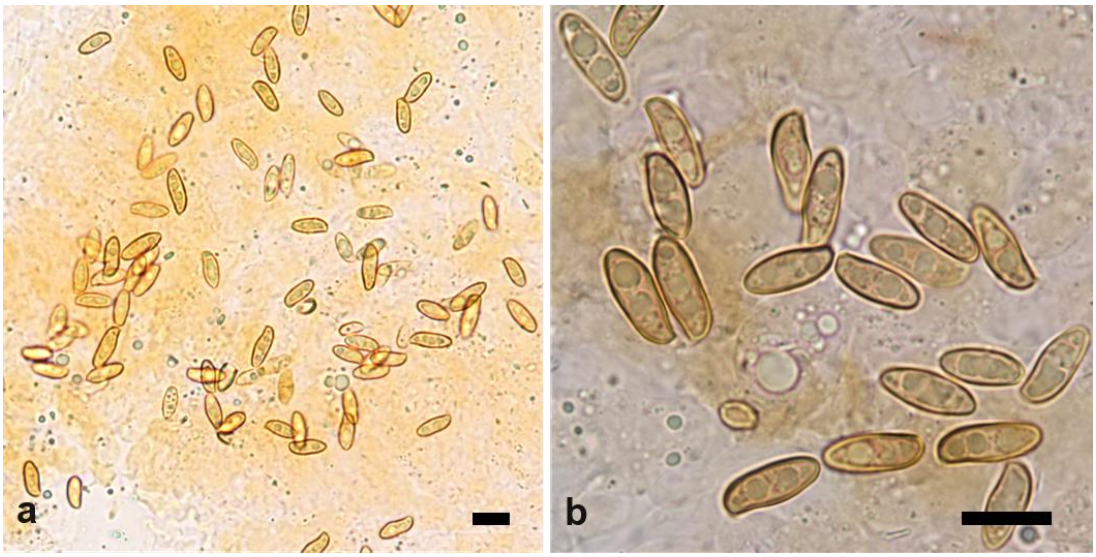

Figure 3. Basiospores (a,b) of *Xerocomellus cisalpinus* (bars-10 µm) (a,b in Congo-red)

Xerocomellus cisalpinus was reported to grow mainly under broad-leaved trees (*Quercus, Fagus, Populus, Betula*) as well as under conifers (*Pinus, Abies, Cedrus, Picea*) [14-18].

Specimen examined: İstanbul, Sarıyer, Belgrad Forest, Neşet Suyu Nature Park, under *Picea orientalis* and *Abies* sp., in mixed forest of *Picea*, *Abies*, *Carpinus* and *Fagus* spp., 41.190177N, 28.968502E, 150 m, 22.10.2023, YKaraduman 013.

Suggested Turkish name for the presented species is “Morcalı pöslen”.

4. Conclusions and discussion

Xerocomellus cisalpinus is added as a new record for Turkish Mycobiota. This species is the sixth member of the genus *Xerocomellus* in Türkiye. In general, the characteristics of the sample are in agreement with Peintner et al. [14], Læssøe and Petersen [15] and Assyov [16]. This species may have similar morphological appearance with *X. chrysenteron*, but the smooth spore surface of *X. chrysenteron* differs it from this species [15,16]. *Xerocomellus pruinatus* also have similar spore ornamentation with *X. cisalpinus*. But it lacks the ability of blueing [15].

Though *Xerocomellus cisalpinus* is mainly associated with broad-leaved trees such as Quercus, Fagus, Betula, Populus especially of *Quercus* spp. [14-18]. Association with some coniferous trees such as Pinus and Juniperus were also presented [14,18]. Turkish samples were collected under *Picea* and *Abies* spp., along with *Carpinus* and *Fagus* species.

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Planning and design principles of Artvin Çoruh University Ali Nihat Gökyiğit Botanical Garden in a sustainable approach

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Abstract

Botanic gardens are institutions holding documented collections of living plants for the purpose of scientific research, conservation, display, and education. Gardens and the cultivation of plants have been around for thousands of years with the first examples dating to around 3,000 years ago in ancient Egypt and Mesopotamia. In the last 50 years botanic gardens are increasingly recognised as being extremely important to conservation due to their existing collections and the scientific knowledge they possess in the propagation of plant species. There are 3765 botanical institutions, 1775 botanic gardens and arboreta in 148 countries around the world with many more under construction or being planned. 628 of them are being members of the Botanic Gardens Conservation International (BGCI) platform. The Artvin Çoruh University Ali Nihat Gökyiğit Botanical Garden (ANGBB) was established in 2018 and opened to the public in 2022. ANGBB is one of the 13 botanical gardens from Turkey that are members of this platform. There are around 30,000 plants belonging to approximately 2000 different species, including 400 rare, 200 medicinal-aromatic, and about 1400 decorative species, cultivated in the garden. A total of 594 seeds belonging to specialized species such as Medicinal Aromatic and endemic plants are preserved in the seed house of the botanical garden. Within the scope of this research, the purpose, functions, planning, and design of botanic gardens have been evaluated according to the economic, ecological, and social components of sustainability, and sustainable planning and design criteria have been established for the ANGBB. These criteria address structural landscape design, plant landscape design, administrative planning, spatial planning, educational, social, and cultural planning, as well as management and maintenance. The ANGBB has been evaluated in terms of sustainable planning and design criteria, and recommendations for enhancing the sustainability of the ANGBB have been provided based on the data obtained.

Keywords: ANGBB, Artvin, conservation, planning, sustainability.

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Sürdürülebilir bir yaklaşımda Artvin Çoruh Üniversitesi Ali Nihat Gökyiğit Botanik Bahçesi planlama ve tasarım ilkeleri

Özet

Botanik bahçeleri, bilimsel araştırma, koruma, sergileme ve eğitim amacıyla belgelenmiş canlı bitki koleksiyonlarını barındıran kurumlardır. Bahçeler ve bitki yetiştirme binlerce yıldır var olmuş olup, ilk örnekler Mısır ve Mezopotamya'da yaklaşık 3.000 yıl öncesine dayanmaktadır. Son yıllarda botanik bahçeleri, mevcut koleksiyonları ve bitki türlerinin üretiminde sahip oldukları bilimsel bilgi nedeniyle koruma açısından son derece önemli olarak kabul edilmektedir. Şu anda dünya çapında 148 ülkede 3765 botanik enstitüsü, 1775 botanik bahçesi ve arboretum bulunmakta olup, birçoğu inşa edilmekte veya planlanmaktadır. Artvin Çoruh Üniversitesi Ali Nihat Gökyiğit Botanik Bahçesi (ANGBB), 2018 yılında kurulmuş ve 2022 yılında ise halkın ziyaretine açılmıştır. ANGBB, bu platforma üye olan Türkiye'deki 13 botanik bahçesinden biridir. Bahçede, 400'ü nadir, 200'ü tıbbi-aromatik ve 1400'e yakın dekoratif tür olmak üzere 2000'e yakın farklı türe ait 30.000 civarında bitki yetiştirilmektedir. Botanik bahçesinin tohum evinde Tıbbi Aromatik ve endemik bitkiler gibi özel türlere ait toplam 594 tohum muhafaza edilmektedir. Bu araştırma kapsamında

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botanik bahçelerinin amacı, işlevleri, planlanması ve tasarlanması sürdürülebilirliğin ekonomik, ekolojik ve sosyal bileşenlerine göre değerlendirilmiş ve ANGBB için sürdürülebilir planlama ve tasarım kriterleri oluşturulmuştur. Bu kriterler yapısal peyzaj tasarımı, bitkisel peyzaj tasarımı, idari planlama, mekânsal planlama, eğitsel, sosyal ve kültürel planlama ile yönetim ve bakım ele alınmıştır. ANGBB sürdürülebilir planlama ve tasarım kriterleri açısından değerlendirilmiş, elde edilen veriler doğrultusunda ANGBB'nin sürdürülebilirliğinin geliştirilmesine yönelik önerilere yer verilmiştir.

Anahtar kelimeler: ANGBB, Artvin, koruma, planlama, sürdürülebilirlik

1. Introduction

People need to get away from their environment for a short or long period to regain their life integrity, which has been disrupted due to both the fast pace of life and the intensive working order, and for this purpose, they turn to natural or near-natural rural areas. However, the lack of conditions and opportunities often prevents the realization of this desire. In this case, urban outdoor spaces with sufficient opportunities to meet the recreation needs come to the fore. Botanical gardens, the first examples of which were found around 350 BC, although different from today's ones, have important scientific (research and conservation), educational (teaching, culture) and recreational functions [1, 2]. Botanical gardens constitute the center of the studies carried out in the world to identify plant species in danger of extinction, to protect plant species diversity, to ensure the continuity of living environments and biological diversity in the world, and to conduct research in the field of botany [2]. Botanical gardens, which have the mission of introducing their visitors to the natural vegetation of their regions as well as the plants growing in different parts of the world, contribute to the formation of environmental awareness and conservation awareness by providing information about plants to people of all age groups and introducing plant life. At the same time, it is noteworthy that many of the botanical gardens, which also serve purposes such as organizing various educational meetings, and creating institutional publications and documents, have recently included uses and organizations that will meet the active recreation needs of society in addition to their passive recreation opportunities [3].

British botanist Heywood defined botanical gardens as organizations that grow the world's natural and cultivated plants in a certain order under the purposes of the garden, educate children, students and the public by introducing them to them, and conduct scientific research on plants for various purposes [4]. Yaltırık defined botanical gardens as living plant museums where trees, shrubs and other herbaceous plants, each carefully labeled, are exhibited in a systematic arrangement and combined for scientific research [5]. According to Hohn [6], botanical gardens are open-air museums of living and non- living plants that provide plant material for branches of science such as medicine, chemistry, agriculture, forestry and botany, offer education, research and application opportunities, support them with units such as herbarium and museum, and help educate the public outside the school. In the horticultural dictionary of the Royal Historical Society, botanical gardens are referred to as gardens that form study and teach plant diversity, aim to cultivate plants, and organize them according to the classification system, not only for ornamental and utility purposes, but also for research and teaching about botanical science or both in an organized manner [7]. The definition of Botanical Garden accepted by the British Royal Horticultural Society in 1969 is as follows; It is a garden that includes plants separated according to botanical classification, arranged only for ornamentation and use, by a certain classification for research and cultivation. Today's botanical gardens, which have developed in the direction of raising public awareness, not only conduct horticultural research such as botany, pharmacology, selection and hybridization but also organize courses, seminars and exhibitions on a wide range of subjects that endear the plant world to people from all segments and age groups of the society [8]. Botanical gardens have a wide variety of functions together. They have recreational functions as well as being green areas for urban life. The most important functions are to identify, protect and contribute to the reproduction of various plants, especially those in danger of extinction. Botanical gardens also have functions as an institution. These include providing training, organizing meetings, creating documents, and organizing exhibitions [9, 10]. In addition to enabling various researches to be carried out, botanical gardens play an important role in providing information about plants to people of all ages and giving the love of nature to the society.

Ali Nihat Gökyiğit Botanical Garden, which was established in Artvin Province with the concern of keeping alive the plant species that disappeared especially with the construction of dams, is a life museum, an important educational institution, a scientific plant research facility and a conservation organization. The number of botanical gardens, which are very common and important abroad, is quite small in our country. ANGBB was established as the 13th Botanical Garden with international qualifications in Turkey. The establishment, design, renovation, transformation or

expansion of a botanical garden is a unique and invaluable activity with ecological, cultural, educational and economic benefits that will last for generations. Managing and guiding the development of botanical gardens presents challenges in terms of both economic and human resources.

1.1. Planning and design principles of botanical gardens

Considering the scientific functions of botanical gardens on the one hand and their educational and recreational functions on the other, it is seen that botanical gardens require a different planning and organization than parks and gardens. To create a sustainable botanical garden, first of all, the land must be suitable for the goals and objectives [11]. When the idea of developing a new botanical garden is put forward, the most suitable land is selected. A list of possible sites is considered. These lands are evaluated for their suitability to the vision and mission. Botanical gardens differ from other parks in terms of their size because they contain plant collections, greenhouses, administrative buildings, laboratories, technical equipment areas, and also because of their educational and research aspects. For this reason, the place to be allocated to the botanical garden should be carefully selected. For this, factors such as ecological conditions, transportation, irrigation facilities gain importance [10, 12].

It is also desirable to choose a moving terrain when selecting a location. Areas such as lakes, rivers, slopes and hills create enjoyable and non-monotonous areas for visitors as they move around the area. Slope is a desired principle in site selection. Sloping areas provide different perspectives. Location selection should be made in suitable areas outside the city center that can stay away from the physical pressure and development of the city with a long development projection [13].

In addition to the green building system that reveals the main space-defining structure of a botanical garden, its use for research, education, collection and other functions is based on scientific basic data. For scientific purposes, plants are organized in botanical gardens according to systematic classification, geographical regions, ecological requirements, dendrological characteristics, uses and flora regions. The first unit that requires the scientific use of plants in botanical gardens is the systematic section. A botanical garden usually includes an ecological section as well as a systematic section. Ecological grouping is the cultivation of plant species that show similar living environment in terms of soil and climate requirements of plants as well as their relations with each other. In order to carry out these studies, the desired ecological conditions must be provided in the garden in advance [6].

The planning and design principles of botanical gardens discussed in different sources can be listed as follows [1, 8, 14, 15, 16, 17]:

- Botanical gardens range in size from 5 hectares to 5,000 hectares.
- Since botanical gardens need to be in communication with universities or similar scientific institutions, it is appropriate to plan them close to these institutions.
- In terms of conservation, emphasis is placed on protecting species and gene resources used in agriculture.
- In the planning and materials of the units in the gardens, the local climate and materials are taken into account and care is taken to ensure harmony with the physiognomy of the environment.
- The educational role of botanical gardens should be taken into account. The use and design of space can be not only for scientific purposes, but also for practical knowledge and aesthetics-based activities and year-round interest.
- Research policies are established. Development areas are found for future needs.
- All parts of the botanical gardens that can be visited should be accessible for people with disabilities.
- Attention is paid to a multifunctional structure in functioning. It is necessary to divide the use of the area into basic sections such as management, education and research, production and recreation. Policies on Plant Collections are also established.
- An operating directive or management plan for the establishment and operation is prepared.
- Maintenance and protection operations must be continuous and regular,
- Botanical gardens include a planning that will realize environmental silence and an internal circulation texture that does not require auto traffic.
- Botanical gardens have cafe restaurants and seating areas for visitors to relax and meet their needs.
- Botanical gardens are usually open from 9:00 to 18:00 or 19:00. For this reason, lighting is not available in some botanical gardens. However, there is partial lighting for security purposes.
- Generally, the materials used for walkways in botanical gardens should be safe and in harmony with the environment (usually gravel, stabilized soil or grass). Benches and trash bins are provided on the walkways.

In its planning decisions, the Royal Botanic Garden at Kew has determined principles that emphasize the educational aspect of the garden. The planning criteria in a publication prepared by the Education Department are given below [7, 8]:

- Organizing the garden in line with the information it wants to give, especially determining what topics it wants to address and exhibit (permaculture practice, plant taxonomy, etc.),
- Identifying and promoting the resource values of the garden,
- Emphasizing some of the properties of plants that can be useful to society,
- Preparation of promotional signs about some plants in places,
- Creating special performance areas or surprise areas for small groups, learning spaces (surprise encounter spaces where walking paths intersect, small squares, etc.)
- Systematic arrangement of plants according to their root and leaf characters, or according to the purpose for which the plants are used, and also according to families,
- Exhibiting and explaining plants according to their local, cultural characteristics and history,
- Creating spaces for children to explore and investigate by playing in the garden.
- Arrangement of plants according to a theme or storytelling,
- First of all, the display of plants that visitors know a lot about,
- The garden interacts with adults, children and students,
- Providing practical work environments, especially for school groups, such as growing plants, harvesting crops and gardening,
- Displaying a product made from the plant source right next to the exhibited plant, thus satisfying visitors' desire to touch it.

1.2. Purpose and scope of the study

The aim of this study is to explain the design process and stages of the botanical garden that will ensure the sustainability of the plant wealth of Arvin Province, which is located in the east of the Eastern Black Sea, which has been destroyed by the dam constructions, as well as the protection and exhibition of the existing plant wealth, and at the same time to provide the city with a scientific recreation area. With the implementation of the botanical garden, it is aimed to reach a holistic structure that will serve users as a recreation, education, scientific study and research area with its structures such as greenhouse, education and management, cafe and restaurant, in addition to the important contributions to the urban landscape. This study aims to outline the proposed steps and strategic thinking for the planning, design and implementation of the ANGBB botanical garden project. It also evaluates the sustainable approach strategies adopted and implemented in the botanical garden.

2. Materials and methods

Artvin has an important position in terms of its geography. While the region has a mountainous topography and a continental climate, the valley of the Çoruh River, in which there are many dams, harbors Mediterranean climate characteristics. This increases the biodiversity of the region and allows plant species with different tolerances to grow in the region. The study area, which is the main material of the study, is administratively located within Artvin Çoruh University. It is located at the 13th km of the Şavşat-Artvin highway. The Botanical Garden is located within the borders of Salkımlı Village, Artvin province. It is on the Artvin-Ardahan road, an average of 525 m in elevation and is in the south slope. The area is situated between lat 41º11’12’’ – 41º11’21’’ N and long 41º51’13’’- 41º51’24’’ E and is approximately 140.000 m^2 in area (Figure 1).

The method of the study consisted of data collection, on-site identification, synthesis and evaluation stages. To determine the existing structure, natural and sociocultural characteristics of the study area, plans, maps, reports, analyzes and data were collected, sources were scanned, and determinations were made regarding the vegetative and structural assets of the Botanical Garden. In the synthesis and evaluation phase, the information obtained, the characteristics of the area and the factors that reveal the need for botanical gardens were evaluated together and long-term planning and design principles were put forward.

Figure 1. Location of the research area

3. Results

3.1. Establishment of the Artvin Çoruh University Ali Nihat Gökyiğit Botanical Garden

The Botanical Garden area was used as a prefabricated housing area for the employees of the Deriner Dam construction company, and at the end of the construction, the prefabricated houses were dismantled and the area remained idle as a pile of concrete (Figure 2). In 2015, with the initiatives of Prof. Dr. Özgür Eminağaoğlu, a faculty member at Artvin Çoruh University, work was started to establish a botanical garden in the area in question.

Feasibility studies were carried out for the area with the support of the Eastern Black Sea Development Agency (DOKA) and a Feasibility Final Report was prepared in 2017 [18]. In addition, with the support of TÜBİTAK in 2018, an international workshop was held with the participation of scientists from Turkey and abroad, and the workshop final declaration was published [19]. When the suitability of the area was revealed as a result of all these researches, the search for resources began. As a result of the meetings held within the framework of the Botanical Garden Project prepared under the coordination of Prof. Dr. Özgür Eminağaoğlu, Ali Nihat Gökyiğit, an investor from Artvin, the chairman of the board of ANG Foundation, supported the project and the process started. In this process, legal procedures were initiated for the transfer of the lands within the scope of treasury land to Artvin Çoruh University. As a result of the application for the allocation of the area to the University for the Establishment of the Botanical Garden, the General Directorate of National Real Estate allocated approximately 170 decares of land consisting of 11 parcels. Ali Nihat Gökyiğit Botanical Garden Directorate was established within Artvin Çoruh University and working groups were formed.

2. The construction site of the area before the botanical garden (May 2005- June 2018)

First of all, the borders of the area were enclosed with a fence in order to protect and reveal the existing plant presence in the botanical garden. Then, within the scope of the project, the infrastructure projects, structural landscaping and vegetative landscaping projects of the area were commissioned to an architectural office under the control of consultants appointed from the university. In 2019, the implementation of the infrastructure and structural landscaping projects of the Botanical Garden was awarded to a private company through a tender method. In 2019, the negativities experienced during the pandemic (covid-19), which was an unforeseen process, were overcome and the planned part of the construction stages was partially completed in 2021. However, it seems that construction activities in the area will continue for a while. 60% of the planned vegetative landscaping works have been completed. Planting activities are going and will continue at all times.

Since the garden has a sloping terrain, terracing was used. The planning of the botanical garden was organized on these pre-existing terraces. There are 16 terraces at different levels and of different sizes, 9 of which are open to visitors and 7 of which are closed to visitors. For these terraces, the term "Palya" was used within the scope of the garden. The names of the palyas are given according to their use and the presence of plants. There are various structures, plant arrangements and collections within the palyas. The collections and the Palyas in which they are located are listed as follows from the top level downwards: Entrance, administrative- educational-research units, greenhouse and nursery, rose garden, medicinal-aromatic plants, local houses and plants, honey and fodder plants, labyrinth and playground, theme area, production facilities, fruit species gene garden palyas (Figure 3).

Entrance Palya: The main entrance of the garden is at the top level. There is also an entrance at the lowest level. The palya at the top level, which is used as the main entrance, has a carriageway, security and a guesthouse. The carriageway is on this pavilion and ends at the parking lot. After this point, there is no vehicular circulation in the area. There are only pedestrian circulation areas and vehicles for the elderly and disabled. Only circulation areas suitable for the circulation of service vehicles and emergency vehicles have been organized within the area. There is also a section with the bust of Mr. Ali Nihat Gökyiğit at the entrance of the area. (Figure 3, 4).

Administrative - Education-Research Units Palya: It is the 2nd palya from the top of the garden. The management and administrative units of the garden are located here. It is an area where multifaceted and intensive activities such as education and research are carried out. In addition, the parking lot, observation terrace and pedestrian circulation area reaching the café are located in this pallet. There are also various plant collections in this area (Figure 3).

Greenhouse Palya: This palya houses the R&D and Exhibition Greenhouse. There are also shaded hard floors around the greenhouse where plants are exhibited and events are organized It is one of the most important structural areas of a botanical garden. Their purpose is to expand the range of plants in the botanical garden. Not only cultivated plants but also natural plant species can be propagated with all kinds of production techniques. The greenhouse needed for the production of plants to be used in ANGBB and for the display of visual plants was established in 2019 as 460 m^2. Later, a section of 200 m^2 was added to be used for production purposes (Figure 3).

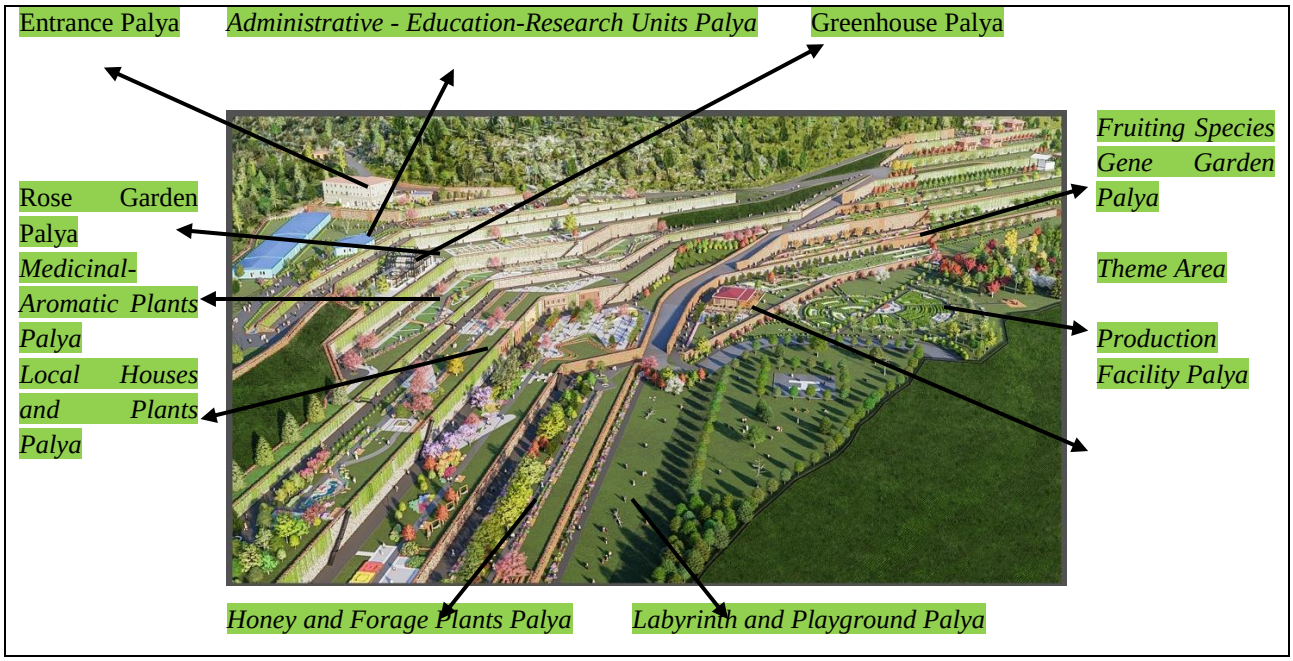

Figure 3. Botanical Garden renderings (Collections and Palyas)

Rose Garden Palya: A garden of dwarf and tall roses (*Rosa* spp.) with different characteristics. There are many varieties of roses in winding and dwarf forms. There are also examples of rose species (7 taxa) that grow naturally in Turkey (Figure 3).

Medicinal-Aromatic Plants Palya: The vascular plant flora of Artvin is represented by 2727 taxa, 2616 species, 397 subspecies, and 144 varieties belonging to 761 genera and 137 families [17, 20, 21]. The number of medicinal-aromatic plants with natural distribution in Turkey is around 1400 and 850 of these species are also found in Artvin [20]. There are nearly 1000 plants in the collection in this palya. There are 50 raised plant pads made for the cultivation of special medicinal-aromatic plants in this palya. There is an organic pond at the end of this palya. In addition, water surfaces were created on the ground. On the stagnant water surfaces, collections of local rare aquatic plants as well as aesthetic plants have been created (Figure 3).

Figure 4. General view of the entrance gate and its surroundings

Local Houses and Plants Palya: In this palya, there are rural architectural housing examples of Artvin, which are appliqued to the retaining wall surface. It is aimed to create a traditional street with the road passing in front of the houses and reaching the garden. In addition to the woody plant species of the region, there are many herbaceous plant species in the palya. The species are arranged alphabetically by genus and family. In addition, the garden has become an outdoor studio for photographers since its establishment. In order to increase the use of the garden by the public, heart-shaped iron profiles were created in certain areas to be wrapped with winding plants (Figure 3).

Honey and Forage Plants Palya: Approximately 800 honey plants and 250 forage plants are naturally distributed in Artvin province [20, 21]. In this palya, forage plants are included in addition to honey plants. The number of plant species is being increased day by day by transferring the plants removed from nature to the area; and it is also planned to build a honey house in this palya in the future. (Figure 3).

Labyrinth and Playground Palya: A playground for children arranged with thuja and viburnum plants. In the area organized as a labyrinth, children try to find their way out by wandering among the thuja. In addition, playgrounds are planned to be designed in natural environments where children will discover and experience adventure (Figure 3).

Production Facility Palya: The previously mentioned greenhouse and nursery palya is an important production center for the garden. It is also the palya where a production facility is planned to process the fruits produced in the garden and serve the local people (Figure 3).

Fruiting Species Gene Garden Palya: Turkey is an important country in terms of fruit diversity, where nearly 300 species have evolved and diversified. The number of protected fruits has reached 1534 grapes, 704 apple, 651 pear, and 627 citrus fruits [22]. ANGBB's fruit collection includes 21 different types of grape varieties. At the same time, a collection of other fruit varieties has also been created. This collection covers a total of five differently sized pallets. This palya, which is not open to visitors, is organized to increase interest in nature and botanical science as well as to exhibit the biodiversity of our country. In the following stages, it will be opened to visitors when suitable conditions arise (Figure 3).

Theme Area: It is organized as a reception and viewing area to present the developments in the field of floriculture. It is at the lowest level, visible from all parts of the botanical garden. Here, it is planned to exhibit new varieties obtained as a result of studies on periodically changing species. Experimental gardens have been created for annual and perennial and aesthetic natural plant species. Natural stones and gravel are used for the borders of the walking area, which is bordered by plants with high visual and aesthetic value (Figure 3).

3.2. Structural planning and design principles

When the land for the establishment of ANGBB was received, there were concrete floors of prefabricated structures that had been dismantled and taken away by the company that built the Deriner Dam. The general appearance of the site was that of a construction site with concrete floors in places (Figure 2). The company in question left its prefabricated administrative building, prefabricated meeting building and guesthouse building for the use of the botanical garden, while its villa at a point overlooking the landscape was demolished due to procedural reasons. In the structural planning of the site, the principle of preserving all existing buildings by functionalizing them, taking into account their economic value and needs, was adopted. Structures and structural sections within the Botanical Garden:

Guesthouse: The guesthouse building is located at the entrance of the site in its original form and is not yet in use. It is a three-storey reinforced concrete building with 10 rooms. Façade improvement and interior renovation are planned according to budget conditions.

Figure 5. Administrative - Education and Research Buildings

Administrative - Education and Research Buildings: The largest of the preserved prefabricated buildings. It is approximately one thousand square meters. It contains administration, offices, classrooms for training and courses, library, meeting room, laboratories, herbarium, sales office, exhibition hall, prayer room, kitchen and restroom. In order to make the external appearance of this building more harmonious with the natural environment, window openings and entrances were left empty and the facades were surrounded by a wire cage on all sides. This wire cage was wrapped with suitable vines to give it a green and flowery surface and a wooden surface in the form of veins in winter (Figure 5).

Meeting Building: It is a prefabricated building used in its original form without any renovation or modification. The structure has an amphitheater-type seating arrangement and seats one hundred people.

Cafe-Restaurant: This building was constructed on the site of the previously demolished villa. While the villa was located in the middle of the area dominating the view, the new building was set back and the front area, which has a panoramic view, was utilized as a large garden area. The facade of the building is clad in brick, a material in harmony with nature and used in the architectural tradition of the region. The façade also features large window surfaces for easy viewing of the view from all directions. The Café and Restaurant building consists of a food and beverage area, kitchen, administrative office, restroom and balcony units. In order not to interrupt the view and not to dominate the nature, care was taken not to exaggerate the scale of the building and to harmonize with the natural environment (Figure 6).

Figure 6. Botanical Garden cafe and view of the city

Greenhouse: The greenhouse is one of the largest structures built within the site, depending on the targeted production and exhibition capacity. Especially with its location that does not block the view, its transparent structure and its height that does not exceed the retaining wall, the scale concern was tried to be eliminated. Not only cultivated plants but also natural plant species can be propagated with all kinds of production techniques. The greenhouse needed for the production of plants to be used in ANGBB was established for the first time in 2019 at 460 m². The greenhouse, planned for R&D and Exhibition purposes, was built as polycarbonate on steel construction. The greenhouse consists of 3 sections with 3 different climates and 5 different irrigation and fertilization functions. A 200 m² production section was added to the greenhouse. The central part of the greenhouse has a gallery and the other sections are single storey. In the greenhouse; offices, department, main production section, Black Sea Plants section, tropical plants section, desert plants section, machine room, warehouse, germination room, and restrooms. Plants in different habitats that can live at different temperatures can be exhibited in the greenhouse (Figure 7).

Restrooms and Water Tank: There are three prefabricated restroom structures at different locations in the area. There is also a water tank with a capacity of 450 tons.

Parking Lot: There is a parking lot at the entrance of the Administrative-Education-Research Units Palya, where employees and visitors can park. This parking lot has a capacity of approximately 50 vehicles. It is also used as a proposed gathering area for botanical gardens.

Pathways and Stairs: Vertical circulation in the area is provided by stairs that follow each other vertically on the surface of the walls. Horizontally, a main road divides the area vertically in the middle and reaches from the top level to the bottom level of the area. This road is also used as a vehicular road, but vehicles are allowed to enter the parking lot. The section of the carriageway after the parking lot is planned to be used for service and emergencies, as well as for transfer vehicles provided by the garden. In principle, the garden is designed for pedestrian access. All palyas are connected horizontally and vertically by pedestrian circulation. The design of the pedestrian paths, is aimed to ensure that the plants in all palyas are easily seen from all directions and to ensure their continuity. Colored and uncolored concrete, keystones and natural stones are used on the ground of the paths.

Figure 7. General view of the R&D and Exhibition Greenhouse

Retaining Walls: ANGBB has a sloping terrain. For this reason, the land was used to form terraces. Depending on the terrace formations, there are high retaining walls on the land. One of the most important issues in the planning and design process of the site was to harmonize these retaining walls with the natural environment and to use the wall surfaces. For this purpose, pots were built at certain intervals at the bottom of the walls and covered with wire mesh in strips perpendicular to the wall surface. It is aimed that the vines in the pots will wrap these wires and create aesthetic vertical garden images on the surface (Figure 8).

Local Houses: Based on the idea of utilizing retaining walls as exhibition surfaces, a street of local houses was created. Portable applications were made on the retaining wall surface by selecting from the traditional-rural housing examples seen in the center and different districts of Artvin Province. These structures were scaled to the surface based on the height of the retaining wall. Five rural architectural housing examples are exhibited on the street. These Applications made with traditional materials such as wood, stone and brick were built as curtain walls on the steel skeleton built on the wall surface (Figure 8).

Figure 8. General view of local houses, organic pond and plant-covered walls

Biological Pond: Another structural element in the garden is the biological pond. It is located in the Medicinal-Aromatic Plants Palya. The pond is surrounded by a wooden railing and there are fixed metal frames in which interchangeable pots can be placed. The pond creates a surprising space with its location and its surroundings that provide a panoramic view.

As a structural element, there are fixed seating elements in different positions in the garden that follow the view. On these seating elements, there is a metal lattice-shaped cover that will be wrapped with plants to protect from the sun. There are also some decorative wooden elements in the area. There is an exhibition section where the products obtained from plants are exhibited in a glass case in the Medicinal-Aromatic Plants Palya. Here, plants and products can be viewed together.

3.3. Vegetation planning and design principles

Living materials used in landscape design are trees, shrubs and herbaceous plants that grow in different conditions. As with other living things, plants grow and spread in an ecology characterized by basic conditions such as soil, water, temperature and light. Differences in the structural structure of the elements that make up the ecology lead to the emergence of different ecological environments. The vegetation between the ANGBB buildings, the plants covering the high retaining walls, the fruit trees that refresh the memory of the past, the natural plant environments, the areas formed by the protected plants, and the structural differences of the plants exhibited create different ecological conditions.

One of the basic principles of plant design in the botanical garden is to support biodiversity and provide habitat for endangered species. First of all, the transportation and cultivation of plants that were under the waters of the Deriner dam has been one of the priority issues. In this context, locally distributed endemic species such as *Acer cappadocicum* subsp. *divergens, Alyssum artvinense, Alkanna cordifolia, Campanula troegerae, Centaurea pecho, Centaurea woronowii, Clypeola raddenea, Convolvulus pseudoscammonia, Micromeria elliptica, Lathyrus woronowii* were taken under protection in the botanical garden. In addition, the conservation and reproductive biology of endangered species, especially endemic plant species within the rich biodiversity of our country, are studied; ecological environments as suitable as possible for their habitats are created.

While designing the botanical garden, first of all, a scientific classification was made for the plants to be used in the garden. The "geographical distribution, ecological requirements, usage areas, habitats and dendrological characteristics" of the plants were investigated in detail. In addition to scientific classification, it also includes vegetation science (plant sociology). Therefore, plant species growing in similar habitats are exhibited together in terms of their ecological requirements and their relationships with each other. In plant design, emphasis is placed on the use of low maintenance plants, plant species compatible with the ecological structure of the region and natural plants.

Living plant collections in botanical gardens are living plant communities cultivated for specific purposes. Such collections can be considered on their own or as part of a more general garden collection. Botanic gardens also have a duty to preserve plant collections [11]. Plant collections in ANGBB are organized and arranged in a certain order and arrangement, and plant species with similar habitat characteristics are grown and exhibited together with the "ecological grouping" method. In this context, well-documented collections are kept and some plant species are also taken under protection. The existing plant collections are classified as bulbous plants collection, hedge plants collection, cactus and succulent plants collection, fern collection, climbing and climbing plants collection, medicinal-aromatic plants collection, local (Artvin) plants collection, honey and fodder plants collection, rock plants collection, woody plants collection, aquatic plants collection, fruiting species gene collection.

The Botanical Garden area was previously used as a construction site and lodging area for the construction of the Deriner Dam. The plants planted during this period and the woody plants living naturally on the site were preserved during the planning process of ANGBB and after its establishment. It is one of the first collections started to be created in the garden. During the establishment process, approximately 15.000 trees and shrubs were planted in the area between 2018 and 2023 [23]. There are many different genera of woody plants throughout the garden. The woody plant collection is one of the largest collections scattered throughout the garden. Broad-leaved and coniferous trees, shrubs and perennials with attractive flowers are native to the region. In the garden, there are woody plant species such as *Osmanthus decorus, Alnus glutinosa* subsp. *barbata, Castanea sativa, Fagus orientalis, Quercus petraea* subsp. *polycarpa, Quercus pontica, Quercus hartwissiana, Ostrya carpinifolia, Tilia dasystyla* subsp. *caucasica, Betula medwediewii, Pinus sylvestris, Abies nordmanniana, Picea orientalis, Vaccinium myrtillus, Epigaea gaultherioides, Cistus creticus, Cistus salvifolius, Acer trautwetterii, Acer campestre, Acer cappadocicum, Euonymus europaeus, Euonymus leiophloeus, Hypericum androsaemum, Hypericum xylosteifolium, Prunus laurocerasus, Rosa pimpinellifolia, Mespilus germanica, Jasminium fruticans, Carpinus orientalis, Crataegus monogyna, Sorbus aucuparia, Cotinus cogygria, Sambucus nigra, Buxus sempervirens, Juglans regia, Rhododendron ponticum, Rhododendron luteum, Punica granatum*. In addition, there are winding plants such as *Hedera colchica, Periploca gracea, Clematis vitalba.* There are many herbaceous plant species such as *Achillea millefolium, Galanthus krasnovii, Hypericum perforatum, Lilium kesselringianum, Primula vulgaris, Primula elatior, Sedum album, Saponaria prostrata, Salvia sclarea, Salvia verticillata, Origanum rotundifolium, Teucrium polium, Satureja hortensis, Trifolium pratense, Plantago major, Tussilago farfara* and *Cichorium intybus*.

Fruit trees were uprooted from different areas in Artvin and transferred to the fruiting species gene collection area. The planted fruit trees were mainly Walnut (*Juglans regia*), Mulberry (*Morus alba*), Apple (*Malus domestica*), Cherry (*Prunus avium*), Mandarin (*Citrus reticulata*), Pomegranate (*Punica granatum*), Persimmon (*Diospyros kaki*), Loquat (*Eriobotrya japonica*), Linden (*Tilia dasystyla* subsp. *caucasica*) and olive (*Olea europaea*) trees, as well as apple (*Malus* spp.) and pear (*Pyrus* spp.) rootstocks obtained from nurseries.

In the collection of honey and forage plants; there are a lot of honey plants such as *Acer campestre, Arbutus andrachne, Castanea sativa, Cistus salviifolius, Cornus sanguinea, Diospyros lotus, Diospyros kaki, Centaurea macrocephala, Echium vulgare, Echium italicum, Crataegus microphylla, Crataegus orientalis, Prunus laurocerasus, Laurus nobilis, Lythrum salicaria, Cotoneaster nummullaria, Origanum vulgare, Paliurus spina-christi, Satureja hortensis, Teucrium polium* as well as fodder plants such as *Onobrychis sativa, Medicago sativa, Vicia cracca, Lathyrus laxiflorus*. The collection is planned to be developed.

In the immediate surroundings of the buildings, grasses, ground covers and hugging plants that contribute to lowering the temperature felt in and around the building and on the retaining wall surfaces, creating microclimate environments were used. In addition to the aesthetic features of hedgerow plants, functional features such as wind and sound blocking, noise and image blocking were utilized in landscape planning. There are 15 different species of hedge plants in this collection. Hedge plants that can fulfill these functions are exhibited in front of the wire fence that defines the outer boundaries of ANGBB, in the labyrinth garden and many other areas. The hedge plants used include *Pyracantha coccinea, Platycladus orientalis, Viburnum tinus* and *Carpinus betulus*. ANGBB's "Fern Collection" was prepared to showcase Artvin's fern diversity. One of the remarkable plants of the collection is the King's Fern (*Osmunda regalis*).

Rock gardens are generally found in most botanical gardens around the world and their design is inspired by nature. The plants selected in this collection are generally selected from plants that can grow in very little soil and at high altitudes. In addition to rock gardens, there are also rock crack gardens in the garden, which contain plants that grow between rock cracks and contain less soil area. The collection of plants such as *Sedum album, Sedum gracile, Valeriana rubra, Valeriana erotica, Potentilla divina, Veronica liwanensis, Campanula betulifolia, Scrophularia chrysantha, Asphodeline lutea, Asplenium ceterach*, which are found in natural rocky areas in the province of Artvin, are transferred to different areas of the garden.

For the cactus and succulent plants collection, a special section has been created in the R&D and Exhibition greenhouse. For the bulbous plants collection, plants belonging to genera such as *Muscari, Galanthus, Fritillaria, Tulipa* transferred from nature are currently grown in the greenhouse.

In order to create vertical gardens on the retaining walls, a collection of climbing plants consisting of species such as *Wisteria floribunda, Wisteria sinensis, Campsis radicans, Clematis spp, Passiflora incarnata, Rhyncospermum jasminoides, Bougainvillea spectabilis, Bougainvillea glabra, Hedera helix, Parthenocissus tricuspidata, Parthenocissus quinquefolia* was created.

Aquatic plant collections were created with aesthetic plants such as *Lythrum salicaria, Juncus effusus, Nuphar lutea, Nymphaea alba, Typha latifolia, Epilobium hirsitum, Nasturtium officinale, Primula auriculata, Drosera rotundifolia, Nelumbo nucifera* in the biological pond and on the water surfaces created on the ground.

There are around 850 medicinal-aromatic plants with natural distribution in Artvin province [20]. 250 of these species are grown in the medicinal-aromatic plants collection area. In addition, while selecting the plant groups in the botanical garden, studies are carried out on the plants of the world in order to gain an international identity, as well as the plants in the local and Turkish flora, and it aims to show some special plants to its visitors. In this context, we cooperate with international botanical gardens and receive seed support. Thus, in the near future, it will become a garden with many international plants [24].

3.4. Strategies for sustainable approach in the botanical garden

The botanical garden differs from other parks because it contains plant collections, greenhouses, administrative structure, laboratories, technical equipment area and also because of its educational and researcher aspect. Ecological conditions, transportation and irrigation facilities are important for the sustainability of the garden. When the idea of developing a new botanical garden emerged, a feasibility study was conducted to assess the suitability and sustainable qualities of ANGBB's site [18], and an international workshop was organized with the participation of foreign scientists [19].

The main sustainable approach of ANGBB is to create a center where biological diversity is protected, preserved and recorded. Within the rich biological diversity of our country, it is to carry out studies for the protection and production of endangered species, especially endemic plant species, to study their biology, and to create suitable living environments as much as possible. For this purpose; water surfaces and water lines were created in the area.

Developing effective methods and techniques to ensure the continuity of plant collections in the botanical garden is important for sustainability. In addition to the ANGBB Herbarium, the DNA of plants is studied and recorded in the national gene bank. Scientific research activities are carried out to create and develop living plant and seed collections. In order to ensure the continuity of plant collections, plant materials are collected from nature, identified and systematically recorded by adding them to all collections. Research is carried out in the fields of taxonomy, phylogeny, biodiversity, ethnobotany and conservation biology to improve the collections.

In order to ensure the protection of entomological elements without harming the plant diversity in the botanical garden, biological control methods are used throughout the area instead of chemical spraying. In addition, there is a compost area in a section of the garden that is not open to visitors, which is created with green waste from the garden.

Animal manure purchased from livestock farmers in the region is stored in the storage area and is mixed with soil and sand when needed.

Low maintenance plants and plant species compatible with the ecological structure of the region are used in plant design. There is a goal of preserving and collecting plants belonging to the geography of Turkey and all geographies of the world in indoor or outdoor areas. In the parceling works carried out in line with this goal, it is aimed to carry out low-cost planting works in both maintenance and operation and installation of the remaining areas as well as areas where plants suitable for the ecological structure of the region can be modeled. For this purpose, natural plants of the region were used and their natural distribution was supported.

It is important to produce and cultivate plant species that are valuable in terms of economic sustainability and contribute to the national economy. In this direction, ANGBB provides the detection, identification, production, reproductive biology, chemical contents and ethnobotanical characteristics of plant species that can contribute to the national economy. It is thought that the research information obtained and the results of phytochemical analyzes will help the relevant parties and other researchers to work in this direction, not to cultivate plant species that can contribute to the national economy.

Recycling and renewable resources are also given importance in the garden. Solar panels are planned to be installed on the roofs to utilize solar energy and a storage area where rainwater is used for garden irrigation. One of the main factors in the sustainability of Botanical Gardens is that they appeal to all segments of society. In the design of the garden, the principle that solutions for disabled individuals are a part of the design has been adopted. In addition, accessibility has been ensured by considering that not only disabled individuals but also all disadvantaged groups can visit the area.

For the sustainability of botanical gardens, it is important to ensure their legibility and to take place in the memory of the society. For this, it is important to have informative signs and labels in addition to plant designs. In ANGBB, directional signs have been arranged to facilitate circulation within the area, but they have not yet been completed. Likewise, the labeling of plants in the area is currently done in the form of temporary labels. Design work is ongoing to design permanent plant labels so that the text on the signs and labels can be read and understood by everyone. In addition, it is planned to include barcode and QR code applications that will be associated with the ANGBB database and embossed text so that visually impaired individuals can recognize the plants.

A management scheme has been created for a sustainable administrative planning. This scheme consists of a directorate, central units and departments. The units reporting to the center are accounting, purchasing, business and marketing, administrative affairs, archives, library, security, internal and external relations, human resources and legal affairs. The departments under the center consist of seven divisions: administrative and institutional services division, plant sciences division, visitor services-education and courses division, property and facilities management division, plant health division, scientific agriculture division, seed bank and gene center division. While creating ANGBB, planning decisions were taken according to BGCI's accreditation checklist, especially to include structural and spatial units in the area. ANGBB received BGCI membership certificate in 2023. With this membership, it has gained international recognition by joining the largest global network of botanical gardens and conservation institutions worldwide. In addition, it is important in terms of sustainability that it provides advantages such as access to grants from the Global Botanic Garden Fund, training courses, and discounted participation in congresses.

Educational units such as the library and herbarium are planned to be easily accessible to researchers and students. Education is important for sustainability and ANGBB plans courses, trainings and events for this purpose. For example, courses such as medical aromatic plant cultivation and cosmetic product production training are offered; seed planting and sapling planting activities are organized for students in cooperation with the Directorate of National Education.

The number of people working in scientific, educational and horticultural activities should be in direct proportion to the size of the garden. In this context, the number of people working in ANGBB is quite insufficient. This naturally increases the number of responsibilities per person and causes some of the work in the garden to be unable to be done. The activities carried out in the garden are carried out with the support of the university, the ANG Foundation, donations and collaborations with other institutions. Although there is not yet a fee at the entrance to the garden, an entrance fee will soon be charged, creating an important source of income that will contribute to the sustainability of the garden.

4. Conclusions and discussion

ANGBB will fulfill its nature conservation mission with its 12 different collections. However, the small number of employees allows for the execution of mandatory works. However, there are volunteers working continuously in different parts of the garden. They help with events and general garden work. This also helps to spread the aims and objectives of the garden more quickly. Due to the large size of the area and its continuous development, more staff is needed and this need is met with the support of the staff working within the university.

In line with the planning and design principles of the Botanical Garden, it is important to define general and long-term goals in terms of sustainability. In this context, a sustainable strategic plan is needed. As part of creating a strategic plan, it is necessary to identify the major threats, opportunities, strengths and weaknesses that the garden may face while trying to achieve its goals. One of the best opportunities of ANGBB is that it is located within a university and is in constant collaboration with university academic staff for various scientific research. ANGBB actively cooperates with domestic and international organizations.

Despite the small number of personnel working in the botanical garden, the enthusiastic- excited working understanding and motivation should be maintained, trained and experienced gardeners should be included in the garden, and continuous professional training of garden staff should be provided. In addition, the diversity of activities in the botanical garden should be increased and improved. The development of infrastructure in education should continue. Scientific studies should be increased in the garden within the university. ANGBB should be promoted more in the media and digital platforms, and the contributions of the garden to the region should be explained and the public should be made aware of this issue.

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Characterization of chemical composition in the lipids from seeds bread of wheat (*T. aestivum* L.)

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Abstract

In this study, chemical components of whole seeds lipids, and extracted lipids in wheat genotypes were analysed and effective and important components in lysine, Zn, B_6 vitamin and the rate of linoleic acid/linolenic that is important for nutritional quality were determined by decision tree analysis. Bread wheat genotypes, Tosunbey, Alpu 01, ES26, Reis, Nacibey, Altay2000, Bayraktar 2000 and Rumeli, were used. The chemical compositions of whole seeds and seed lipids were investigated. The mean rate of components in lipids to total amount of while seed almost ranges between 53-68%. This means that rate in minerals, amino acids, fatty acids-enzymes-vitamins was about 53%, 63 and 68%, respectively. Besides, except for Ca and the rate of linoleic acid/linolenic acid, differences between whole seeds and lipids in genotypes for the other components were determined as significant at 1%. Differences for Ca and the rate of linoleic acid/linolenic acid were insignificant and significant at 5%, respectively. A substantial amount of components is present in wheat lipids. The ratio of these amounts to the whole seed is approximately one-third. Lipids amount and its content composition are important for bread quality. Therefore, consuming whole wheat flour containing embryo and bran provides a better quality nutrition. This rich content of wheat lipids makes it a valuable substance for the cosmetic industry. The results showed that Tosunbey-G1, Alpu 01-G2, ES26-G3 and Nacibey-G5 had the highest nutritional values and better activity. Mn, tryptophan, Na, N and Ca were found as effective components in the shaping and activity of lysine. SOD, Ca, Mg, N, Fe, Na and K were concluded as significant components in Zn activity. Significant components were found in whole seed and lipids such as linoleic, glutamine, N, Na and K for B_6. In linoleic/linolenic rate, linolenic, N, Na were important components.

Key Words: Bread wheat, whole seeds lipids, extracted lipids, biplot, decision tree

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Ekmeklik buğday (*T.aestivum* L.) genotiplerinde tam tohum lipidsleri ve ekstrakte lipids kompozisyonunun karakterizasyonu

Özet

Bu çalışmada, buğday genotiplerinde bulunan tam tohum lipitleri, ekstrakte edilmiş lipitlerin kimyasal bileşenleri analiz edilmiş ve lisin, Zn, B6 vitamini ve besin kalitesi açısından önemli olan linoleik asit/linolenik oranında etkili ve önemli bileşenler karar ağacı analizi ile belirlenmiştir. Ekmeklik buğday genotiplerinden Tosunbey, Alpu 01, ES26, Reis, Nacibey, Altay2000, Bayraktar 2000 ve Rumeli kullanılmıştır. Bütün tohumların ve tohum lipitlerinin kimyasal bileşimleri araştırılmıştır. Lipidslerdeki bileşenlerin toplam miktara oranı ortalama %53-68 arasında değişmektedir. Yani mineraller, amino asitler, yağ asitleri-enzimler-vitaminlerdeki oran sırasıyla %53, %63 ve %68 civarında bulunmuştur. Ayrıca Ca ve linoleik asit/linolenik asit oranı dışında diğer bileşenler açısından bütün tohumlar ve lipitler arasında genotiplerdeki farklılıklar %1 düzeyinde önemli bulunmuştur. Ca ve linoleik asit/linolenik

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asit oranı arasındaki farklar sırasıyla önemsiz ve %5 düzeyinde öenmli olmuştur. Buğday lipidsinde önemli miktarda bileşen mevcuttur. Bu miktarların tohumun tamamına oranı yaklaşık üçte birdir. Ekmeğin kalitesi açısından lipit miktarı ve içeriğinin bileşimi önemlidir. Bu nedenle embriyo ve kepek içeren tam buğday ununun tüketilmesi önerilmektedir. Buğday lipidsinin bu zengin içeriği, onu kozmetik endüstrisi için değerli bir madde haline getirmektedir. Sonuçlar Tosunbey-G1, Alpu 01-G2, ES26-G3 ve Nacibey-G5'in en yüksek besin değerlerine ve daha iyi aktiviteye sahip olduğunu göstermiştir. Lisinin şekillenmesinde ve aktivitesinde Mn, triptofan, Na, N ve Ca etkili bileşenler olarak tespit edilirken, SOD, Ca, Mg, N, Fe, Na ve K'nın Zn aktivitesinde önemli bileşenler olduğu sonucuna ulaşılmıştır. Bütün tohum ve lipidsde B_6 için linoleik, glutamin, N, Na ve K gibi önemli bileşenler bulunmuştur. Linoleik/linolenik oranında ise linolenik, N, Na önemli bileşenlerdir.

Anahtar Kelimeler: Ekmeklik buğday, tam tohum lipidsi, ekstrakte edilmiş lipids, biplot, karar ağacı,

1. Introduction

Wheat is one of the basic products that people use as flour, bread, biscuits and pasta.almost 50% of the calorie needs of the world's people are met from plant-based products (44% of which comes from bread alone) and 77% of the protein needs are met from plant-based products. Considering that, the approximate share of animal foods in providing daily calories is 20% and that animals are mostly fed with plant feeds; it is understood that humanity provides approximately 3/4 of its daily nutrition from grains. Wheat is showing itself as a plant that is becoming more and more important in terms of nutrition, nutrition and the future of humanity in the world. The gradual increase in terms of cultivation area and production amount is an indication of the increasing importance given to wheat. Among grains, wheat plays an important role in human nutrition and is a rich source not only of carbohydrates but also of proteins, amino acids, minerals, lipids, vitamins and dietary fibre [1]. In general, wheat flour contains 70% starch, 2% lipids, 2% pentosane, 12% protein and 12% moisture [2]. In this context, wheat lipids, minerals, amino acids and other components are mostly found in the embryo and bran. Wheat lipids, cell membranes, organelles and spherosomes are the source of wheat lipids. Depending on the degree of milling in wheat flour, the amount of lipids varies by 0.5% to 3% due to the mixing of other grain parts into the endosperm. The embryo has the highest lipids amount in wheat grain (11%). The bran layer also carries a significant amount of lipids [3]. The most abundant fatty acids in wheat are C16:0 (palmitic acid) and C18;0 (stearic acid), C18:1 (oleic acid), C18:2 (linoleic acid) and C18:3 (linolenic acid) [4]. Because of the binding of free glycolipids to gluten and gliadin in wheat flour, the gas retention property of gluten is improved. The complex structure of galacto lipids with starch is important in preserving the freshness of bread. The gas holding power of the dough is improved by closing the gas cells in the dough in the structure formed by the interaction of lipids with gluten and starch. Unsaturated lipids have very important functions in the oxidation of flours and thus improving the viscoelastic properties of dough. Unsaturated lipids, especially linoleic and linolenic acid, combine with atmospheric oxygen and undergo rapid oxidation through the lipoxidase enzyme. Hydro peroxides formed as a result of the oxidation of unsaturated lipids then oxidize sulfhydryl groups to disulphide groups [5]. Wheat lipids have significant potential in both plant growth, product quality and industrial fields (cosmetics). Wheat lipids is widely used in the pharmaceutical and cosmetic industries [6], as well as food, feed and biological insect control agent, and for the production of vitamin E [7]. Since, wheat lipids are located especially in the embryo layer; they contain significant amounts of vitamin E (tocopherol), antioxidants, carotenoids (vitamin A) [7]. Because it is dense in the embryo, it is rich and balanced in terms of B group vitamins (such as B1, B2, B6) [6], protein (mostly albumin and globulin) and therefore essential amino acids. Acid profile (especially lysine) [8], mineral substances such as potassium, magnesium, calcium, zinc and manganese [8], fibre, pentosane, sugars, antioxidant components [7]. As a result, wheat lipids, with the amino acids, unsaturated fatty acids, vitamins, mineral substances and antioxidant components they contain, are a very valuable product in terms of improving dough properties, nutrition, agriculture and the cosmetic industry. In this study, chemical components of whole seeds and lipids, extracted in wheat genotypes were analysed and effective and important components in lysine, Zn, B_6 vitamin and the rate of linoleic acid/linolenic that is important for nutritional quality were determined by decision tree analysis.

2. Material and method

Study was conducted in laboratory conditions of Eskisehir Osmangazi University, faculty of Agriculture in 2022. Bread wheat genotypes, Tosunbey, Alpu 01, ES26, Reis, Nacibey, Altay2000, Bayraktar 2000 and Rumeli, were used. Plant characteristics of bread wheat genotypes were given in Table 1.

Table 1. Plant characteristics of bread wheat genotypes

1 Tosunbey	**Spike characteristics;** brown awned, hard white grain; **agricultural features;** plant height, 100-110 cm, winter habit, mod-late, lodging resistant; **quality characteristics;** thousand seed weight, 38-42 g, test weight, 82-85 kg, protein, 13-14%, sedimentation 50-66 ml; **resistance to stress conditions;** resistant to yellow rust, brown rust, resistant to cold and drought.
2 Alpu 01	**Spike characteristics;** white awned, soft white grain; **agricultural features;** plant height, 90-100 cm, winter habit, mod-late, lodging resistant; **quality characteristics;** thousand seed weight, 40-44 g, test weight, 80-84 kg, protein, 10-11,5%, sedimentation 40-48 ml; **resistance to stress conditions;** resistant to yellow rust, brown rust, resistant to cold and drought.
3 ES26	**Spike characteristics;** brown awned, semi-hard white grain; **agricultural features;** plant height, 105-115 cm, winter habit, early, lodging resistant; **quality characteristics;** thousand seed weight, 37-38 g, test weight, 79-80 kg, protein, 10-12%, sedimentation 42-43 ml; **resistance to stress conditions;** resistant to yellow rust, brown rust, resistant to cold and drought.
4 Reis	**Spike characteristics;** brown awned, hard red grain; **agricultural features;** plant height, 105-110 cm, winter habit, early, lodging resistant; **quality characteristics;** thousand seed weight, 34-37 g, test weight, 77-79 kg, protein, 10-12%, sedimentation 43-46 ml; **resistance to stress conditions;** resistant to yellow rust, brown rust, resistant to cold and drought.
5 Nacibey	**Spike characteristics;** white awned, semi-hard red grain; **agricultural features;** plant height, 105-110 cm, winter habit, mod-late, lodging resistant; **quality characteristics;** thousand seed weight, 36-38 g, test weight, 76-78 kg, protein, 11-13%, sedimentation 46-48 ml; **resistance to stress conditions;** resistant to yellow rust, brown rust, resistant to cold and drought.
6 Altay2000	**Spike characteristics;** brown awned, semi-hard white grain; **agricultural features;** plant height, 100-110 cm, winter habit, mod-late, lodging resistant; **quality characteristics;** thousand seed weight, 32-34 g, test weight, 80-82 kg, protein, 11-12%, sedimentation 54-56 ml; **resistance to stress conditions;** resistant to yellow rust, brown rust, resistant to cold and drought.
7Bayraktar2000	**Spike characteristics;** white awned, semi-hard white grain; **agricultural features;** plant height, 90-100 cm, winter habit, early, lodging resistant; **quality characteristics;** thousand seed weight, 36-40 g, test weight, 78-80 kg, protein, 11-13%, sedimentation 48-52 ml; **resistance to stress conditions;** resistant to yellow rust, brown rust, resistant to cold and drought.
8 Rumeli	**Spike characteristics;** white awned, hard red grain; **agricultural features;** plant height, 100-110 cm, winter habit, mod-late, lodging resistant; **quality characteristics;** thousand seed weight, 44-46 g, test weight, 82-84 kg, protein, 13-15%, sedimentation 58-65 ml; **resistance to stress conditions;** resistant to yellow rust, brown rust, resistant to cold and drought.

The abbreviations of the genotypes used in the experiment based on the whole seed and seed lipids are given below; **Whole seed;** Tosunbey-**Seed G_1**, Alpu 01- **Seed G_2**, ES26 **Seed G_3**, Reis **Seed G_4**, Nacibey **Seed G_5**, Altay2000 **Seed G_6**, Bayraktar 2000 **Seed G_7** and Rumeli **Seed G_8**. **Seed Lipids;** Tosunbey-**Lipids G_1**, Alpu 01- **Lipids G_2**, ES26 **Lipids G_3**, Reis **Lipids G_4**, Nacibey **Lipids G_5**, Altay2000 **Lipids G_6**, Bayraktar 2000 **Lipids G_7** and Rumeli **Lipids G_8**

Chemical compositions of whole seed and seed lipids were investigated. Soxhlet Apparatus was used to extract seed lipids from wheat genotypes using a with petroleum ether for 5 h. The ether solvent was removed in a rotary vacuum evaporator at 50 °C, reckoned as % [9]. Methods of [10], for amino acid analysis, [11] for mineral analysis, [12] for A vitamin analysis, [13] for Vitamin C analysis, [14] for B_6 vitamin analysis, [23] for lipids acids analysis, [15, 24] for enzymes analysis were made. The changes on data of wheat genotypes were examined by t test and biplot [16], decision tree analyses [17] in Minitab 17, SPSS 25 programs.

3. Results

Wheat is one of the most grown cereals in the world due to its genetic value, carbohydrate content and wide adaptability; Many products are produced, especially bread, bulgur and pasta. Wheat, due to its widespread consumption feature; people meet their needs for many nutritional elements from wheat products in their daily diet. This feature increases the nutritional importance of bread wheat.

3. 1. Content analysis of bread wheat embryo and wheat lipids

When it comes to nutrition, which is an indicator of quality in wheat, the important factors that come to mind are protein ratio and sedimentation rate. In addition, minerals, amino and organic acids, saturated and unsaturated fatty acids, etc. Factors such as these are also factors that affect quality. These elements are mostly in the embryo and bran and are dispersed throughout the grain [18]. At the same time, wheat lipids contains minerals, amino and organic acids, saturated and unsaturated fatty acids, vitamins, etc. Elements such as are present in a certain amount [18]. Wheat lipids is mostly found in the embryo [19]. Wheat lipids, which has a rich content, has important functions in plant metabolism, in the formation and execution of biochemical events necessary for embryonisation, growth, maturation and seed formation, and in the formation of amino acids. Although the embryo and bran are actually the most functional parts of wheat; When it is ground with wheat, the unsaturated fatty acids it contains will darken in colour and taste bitter if it

comes into contact with air. This is an indication that wheat lipids has a rich structure in terms of saturated and unsaturated fatty acids, amino and organic acids and vitamins [19].

Table 2. Chemical contents of whole seeds and wheat lipids in bread wheat genotypes

					Whole Seed						
Gen.N.	**N (%)**	**Na (mg/kg)**	**K (mg/kg)**	**Ca (mg/kg)**	**Mg (mg/kg)**	**P (mg/kg)**	**Fe (mg/kg)**	**Cu (mg/kg)**	**Mn (mg/kg)**	**Zn (mg/kg)**	**Asparagine pmolµL^{-1}**
Seed G$_1$	1,97	180,79	7496,20	5103,91	2238,18	5942,97	151,64	23,53	28,47	**39,23**	10541,78
Seed G$_2$	2,00	177,98	**7747,86**	5024,52	2419,91	5730,01	**156,50**	21,37	29,43	36,36	11385,12
Seed G$_3$	1,90	208,13	7696,50	**5474,22**	2357,21	5636,99	149,75	27,96	**32,96**	33,82	**11726,67**
Seed G$_4$	1,79	**212,54**	7370,27	5395,33	2261,70	5821,24	131,83	**30,04**	29,91	32,81	10024,59
Seed G$_5$	1,86	209,25	7617,69	5311,46	2445,38	**6210,50**	136,19	27,14	30,91	37,74	10325,33
Seed G$_6$	1,92	200,54	7569,23	5123,65	2345,69	6152,36	149,65	29,65	28,96	39,12	10532,41
Seed G$_7$	1,88	199,89	7654,12	5214,89	**2445,65**	5896,74	150,63	27,45	29,65	36,54	10258,23
Seed G$_8$	**2,01**	204,48	7701,23	5301,26	2348,96	5963,41	148,74	25,62	27,98	37,25	10789,54
Mean	**1,92**	**199,20**	**7606,64**	**5243,66**	**2357,83**	**5919,28**	**146,87**	**26,60**	**29,78**	**36,61**	**10697.96**
б	**0,07**	**12,14**	**116,62**	**144,42**	**73,33**	**182,78**	**7,82**	**2,78**	**1,46**	**2,16**	**545.01**
					Whole Seed						
Lipids G$_1$	0,82	176,34	4336,62	5220,69	634,94	2701,41	20,57	7,51	**9,51**	17,58	9583,04
Lipids G$_2$	0,77	**178,83**	4074,74	**5536,02**	632,05	**2886,04**	18,90	**8,10**	8,30	18,06	**9711,28**
Lipids G$_3$	0,88	174,27	4371,56	4939,72	638,20	2590,63	20,87	6,99	9,46	20,06	9241,08
Lipids G$_4$	0,94	172,61	4270,11	5113,94	659,17	2836,42	22,13	7,00	8,97	21,23	8685,38
Lipids G$_5$	**1,92**	175,93	4253,21	4725,00	**666,04**	2708,33	**22,34**	6,16	8,96	**21,79**	9027,35
Lipids G$_6$	1,23	173,64	4251,89	5256,12	654,12	2754,89	19,54	6,98	8,99	18,95	9623,45
Lipids G$_7$	0,98	174,56	**4398,25**	5015,14	652,14	2756,89	20,01	7,89	9,12	19,54	9548,63
Lipids G$_8$	1,02	171,69	4184,51	5263,74	661,63	2841,12	21,05	8,01	8,56	18,96	9654,23
Mean	**1,07**	**174,73**	**4267,61**	**5133,80**	**649,79**	**2759,47**	**20,68**	**7,33**	**8,98**	**19,52**	**9384,31**
Б	**0,35**	**2,12**	**98,23**	**229,25**	**12,18**	**88,83**	**1,12**	**0,62**	**0,38**	**1,36**	**342,76**
(Lipids/Seed)*100	**55,73**	**87,72**	**56,10**	**97,90**	**27,56**	**46,62**	**14,08**	**27,56**	**30,15**	**53,32**	**87,72**
t test	**5,93****	**4,69****	**50,93****	**0,85ns**	**62,60****	**41,45****	**37,84****	**16,02****	**40,47****	**14,67****	**6,64****

					Whole Seed					
Gen.N.	**Glutamine pmolµL^{-1}**	**Glycine pmolµL^{-1}**	**Valine pmolµL^{-1}**	**Methionine pmolµL^{-1}**	**Tryptophan pmolµL^{-1}**	**Phenylalanine pmolµL^{-1}**	**Lysine pmolµL^{-1}**	**Hydroxyproline pmolµL^{-1}**	**Proline pmolµL^{-}1**	**Cystine pmolµL^{-1}**
Seed G$_1$	11796,02	3662,67	1069,59	2253,65	2022,76	1979,34	5014,47	2024,73	128,75	772,72
Seed G$_2$	11088,26	3711,73	**1083,92**	**2283,83**	**2049,85**	**2005,85**	5081,62	**2051,85**	**130,47**	741,31
Seed G$_3$	11642,67	3531,86	1031,39	2173,16	1950,52	1908,65	4835,38	1952,42	124,15	765,74
Seed G$_4$	12105,68	3319,30	969,32	2042,37	1833,12	1793,78	4544,36	1834,91	116,68	773,55
Seed G$_5$	**12710,96**	3450,11	1007,52	2122,86	1905,37	1864,47	4723,45	1907,22	121,27	**783,75**
Seed G$_6$	11568,23	**3789,12**	1012,31	2222,41	1958,12	1900,32	5021,47	2012,45	124,56	771,65
Seed G$_7$	12569,41	3698,10	1029,00	2021,45	1987,45	1887,45	**5096,21**	2013,69	122,56	772,58
Seed G$_8$	12111,56	3654,47	1022,36	2012,69	1899,32	1965,23	5045,77	1989,65	124,78	770,23
Mean	**11949,10**	**3602,17**	**1028,18**	**2141,55**	**1950,81**	**1913,14**	**4920,34**	**1973,37**	**124,15**	**768,94**
б	**502,44**	**146,05**	**33,56**	**100,97**	**66,09**	**64,37**	**186,78**	**67,27**	**4,00**	**11,46**
					Seed Lipids					
Lipids G$_1$	9020,87	511,44	710,87	1366,76	1833,65	1350,45	2397,09	1113,85	82,54	592,58
Lipids G$_2$	**9141,60**	543,26	767,24	**1407,57**	1724,01	1296,68	2290,46	**1211,65**	79,48	567,17
Lipids G$_3$	8698,92	**586,22**	**790,07**	1323,49	1809,89	1245,07	**2492,15**	1079,06	84,10	605,60
Lipids G$_4$	8175,75	473,03	694,24	1262,45	1735,04	**1376,76**	2254,09	1172,87	85,96	579,41
Lipids G$_5$	8497,70	510,37	714,88	1187,08	1821,48	1321,94	2452,57	1044,55	90,98	**618,61**
Lipids G$_6$	8563,45	489,56	741,23	1365,14	1789,63	1296,32	2356,41	1145,65	**91,65**	612,47
Lipids G$_7$	8674,13	500,11	699,26	1223,45	**1899,41**	1356,89	2357,14	1056,32	89,56	609,45
Lipids G$_8$	8769,49	517,82	715,63	1456,89	1756,32	1354,87	2436,65	1045,96	87,90	596,32
Mean	**8692,74**	**516,48**	**729,18**	**1324,10**	**1796,18**	**1324,87**	**2379,57**	**1108,74**	**86,52**	**597,70**
б	**282,28**	**32,65**	**31,90**	**87,19**	**54,16**	**40,71**	**76,07**	**58,93**	**4,01**	**16,40**
(Lipids/Seed)*100	**72,75**	**14,34**	**70,92**	**61,83**	**92,07**	**69,25**	**48,36**	**56,19**	**69,69**	**77,73**
t test	**12,35****	**55,95****	**22,83****	**19,83****	**5,54****	**18,37****	**34,56****	**26,70****	**14,01****	**40,71****

					Whole Seed					
Gen.N.	**Oleic mg/100g**	**Linolenic mg/100g**	**Linoleic mg/100g**	**CAT EU g/leave**	**POD IU/mg**	**SOD IU/mg**	**Avit mg/kg**	**B$_6$vit mg/kg**	**Cvit mg/kg**	**Linoleic/Linolenic**
Seed G$_1$	46,32	39,70	146,75	3,96	6,16	**6,87**	**29,99**	**40,52**	5,61	3,70
Seed G$_2$	44,99	34,37	150,09	4,01	5,82	6,61	27,19	39,16	5,45	4,38
Seed G$_3$	**54,17**	38,36	144,09	4,10	5,82	5,94	29,42	37,30	5,19	3,76
Seed G$_4$	43,87	**43,15**	150,22	3,38	5,72	6,09	26,59	38,90	5,53	3,48
Seed G$_5$	41,05	34,74	151,9	3,99	**6,22**	6,15	28,46	35,81	5,51	**4,37**
Seed G$_6$	45,69	39,13	**152,47**	3,89	5,96	6,59	29,65	36,00	5,21	3,90
Seed G$_7$	43,58	40,05	151,26	**4,02**	6,12	6,47	29,74	35,87	**5,71**	3,78
Seed G$_8$	52,63	43,06	151,74	3,95	6,14	6,52	28,96	35,96	5,62	3,52
Mean	**46,54**	**39,07**	**149,82**	**3,91**	**6,00**	**6,40**	**28,75**	**37,44**	**5,48**	**3,83**
б	**4,25**	**2,89**	**2,57**	**0,21**	**0,18**	**0,29**	**1,17**	**1,73**	**0,18**	**0,30**
					Seed Lipids					
Lipids G$_1$	31,23	**34,67**	**118,21**	2,22	**3,69**	3,86	16,45	25,65	3,07	3,41
Lipids G$_2$	33,90	22,23	117,14	**2,47**	3,65	3,90	16,65	**26,29**	3,13	5,27
Lipids G$_3$	**36,08**	21,72	106,9	2,26	3,60	3,93	16,88	25,93	3,18	4,92
Lipids G$_4$	29,78	18,66	111,33	2,04	3,53	3,78	17,05	22,89	3,19	**5,97**
Lipids G$_5$	31,96	21,02	112,17	2,20	3,65	3,84	17,42	23,24	3,42	5,34
Lipids G$_6$	35,32	19,54	114,25	2,20	3,64	3,89	17,02	23,65	3,12	5,85
Lipids G$_7$	34,56	20,94	115,13	2,21	3,62	3,78	16,98	24,56	3,48	5,50
Lipids G$_8$	33,36	33,79	117,07	2,19	3,67	**3,95**	**17,62**	26,12	**3,69**	3,46
Mean	**33,27**	**22,98**	**114,03**	**2,22**	**3,63**	**3,87**	**17,01**	**24,79**	**3,29**	**4,96**
б	**2,01**	**5,63**	**3,32**	**0,11**	**0,05**	**0,06**	**0,35**	**1,29**	**0,20**	**0,88**
(Lipids/Seed)*100	**71,68**	**86,49**	**78,14**	**56,01**	**61,17**	**61,72**	**61,29**	**69,76**	**67,34**	
t test	**9,38****	**6,80****	**27,48****	**28,31****	**42,41****	**22,95****	**24,71****	**17,99****	**30,84****	**-3,27***

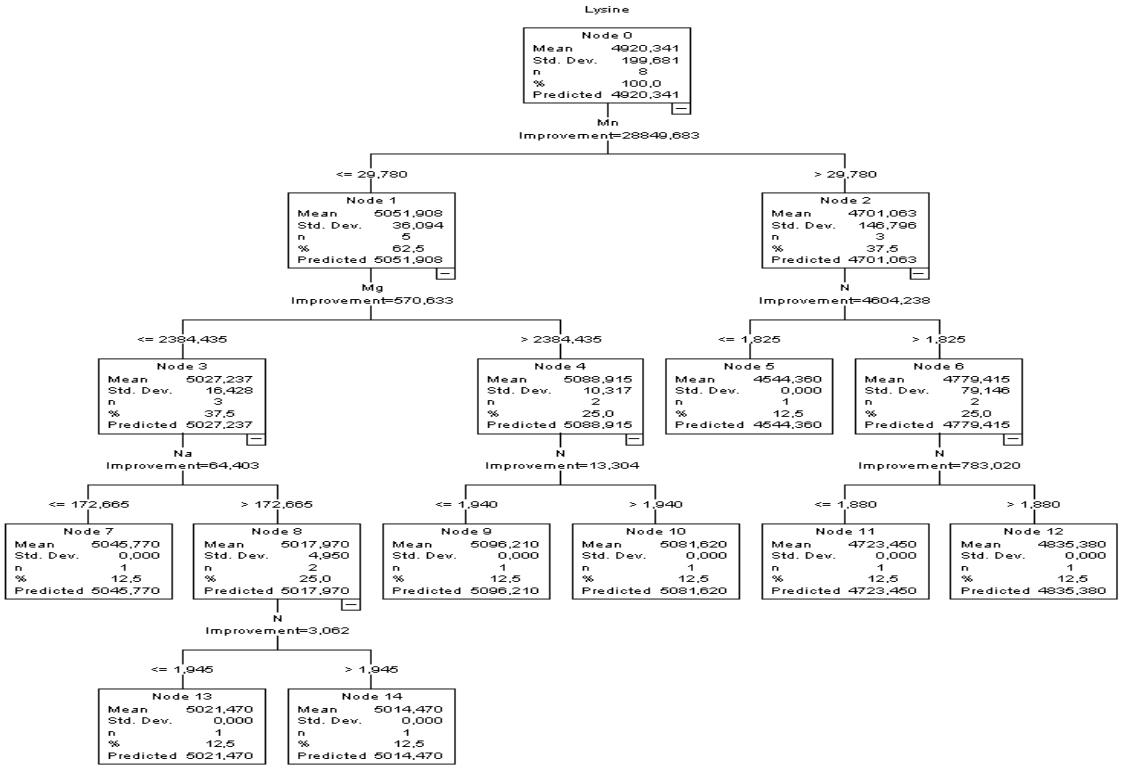
As seen in Table 2, whole seed and Lipids genotypes, having the highest values; **Seed G_1;** N, Zn, A vitamin, SOD and B_6 vitamin. **Seed G_2;** K, Fe, valine, methionine, tryptophan, phenylalanine, hydroxyl proline and proline. **Seed G_3;** Ca, Mn, asparagine and oleic acid. **Seed G_4;** Na, Cu, linolenic acid. **Seed G_5;** P, Mn, glutamine, cysteine, POD, linoleic acid/linolenic acid. **Seed G_6;** glycine, linoleic acid. **Seed G_7;** Mg, lysine, CAT and C vitamin.
Lipids G_1; Mn, linolenic acid, linoleic acid and POD. **Lipids G_2;** Na, Ca, P, Cu, asparagine, glutamine, methionine, hydroxyl proline, CAT, B_6 vitamin. **Lipids G_3;** glycine, valine, lysine and oleic acid. **Lipids G_4;** phenylalanine and linoleic acid/linolenic acid. **Lipids G_5;** N, Mg, Fe, Zn and cysteine. **Lipids G_6;** proline. **Lipids G_7;** K and tryptophan. **Lipids G_8;** A vitamin, SOD and C vitamin. **Seed G_1**, **Seed G_2**, **Seed G_3**, **Seed G_5**, **Seed G_7**, **Lipids G_1**, **Lipids G_2**, **Lipids G_3**, **Lipids G_4**, **Lipids G_5** and **Lipids G_8** showed better performance. Tosunbey-**G_1**, Alpu 01-**G_2**, ES26-**G_3** and Nacibey-**G_5** had the highest values and better activities. Table 2 showed that mean rate of components in lipids to total amount of while seed almost ranges between 53-68%. This mean that rate in minerals, amino acids, fatty acids-enzymes-vitamins was about 53%, 63 and 68%, respectively. Besides, except Ca and the rate of linoleic acid/linolenic acid, differences between whole seeds and lipids in genotypes for the other components were determined as significant at 1%. Differences for Ca and the rate of linoleic acid/linolenic acid were insignificant and significant at 5%, respectively. A substantial amount of components is present in wheat lipids. The ratio of these amounts to the whole seed is approximately one third. This result explains why wheat embryo lipids is widely used as a raw material in cosmetics, medicinal and aromatic fields and in other industrial fields. Wheat lipids, in addition to its cell regenerative feature, is very beneficial for burns, spots and wrinkles on the skin. Although it is effective in various hair problems; this lipids is also very effective for acne and skin dryness [19-20]. In this respect, it has an important place in the cosmetic industry. Besides, the fact that wheat lipids has a rich content in amino acids, minerals, enzymes and vitamins is an indication that this lipids contributes significantly to the biochemical processes in the plant, plant development, and seed quality. In fact, the amino acids, minerals, enzymes and vitamins contained in the seed have vital importance in the embryonisation and development of the plant in the early period, and in the accumulation of dry matter in the later periods. In particular, it has an important effect on the formation of protein and carbohydrates. This situation does not only provide high yield, but also plays an important role in seed quality and in resistance of stress conditions [20].

3. 2. Certain components, affecting nutritional quality

The substance composition of a seed and the amount of these components are important components that reveal the quality level and nutritional value of the seed. There are certain components in wheat seed that are considered in terms of nutritional quality. Lysine, Zn, B_6 vitamin and the rate of linoleic acid/linolenic are known as essential components, making nutritional quality better [20].

3. 3. Lysine

Lysine is an essential amino acid that cannot be synthesized in the human body and must be obtained from external sources. Lysine, whose chemical formula is C6H14N2O2, has two separate isomers [21] as L-Lysine and D-Lysine. The average daily amount of lysine required is between 800 mg and 3,000 mg. It especially helps the absorption of calcium and helps build muscle proteins. It helps protect bones and tissues thanks to calcium absorption. Lysine is an important amino acid that has many benefits such as reducing cold sores and anxiety, tissue repair and anti-aging. Without enough lysine, absorption of vitamins is reduced and collagen formation is inhibited. It also takes part in the synthesis of enzymes, hormones and antibodies [21]. Lysine, which has a direct effect on metabolic events that play a vital role in plant development, health and defence mechanism, takes part in protein synthesis [21], one of the basic building blocks in the plant. At the same time, the high/low amount of lysine in the flour obtained from the seeds helps determine the nutritional quality of that genotype. Decision tree analyses performed overall seed and extracted lipids in order to determine the factors affecting lysine are given in Figure 1 and Figure 2.



Main predictor is Mn;

if Mn≤29,780 Lysine is 5051,908
Main predictor is Mg
if Mg≤2384,435 Lysine is 5027,237
Main predictor is Na
if Na≤172,665 Lysine is 5045,770
if Na>172,665 Lysine is 5017,970
Main predictor is N
if N≤1,945 Lysine is 5021,470
if N>1,945 Lysine is 5014,470

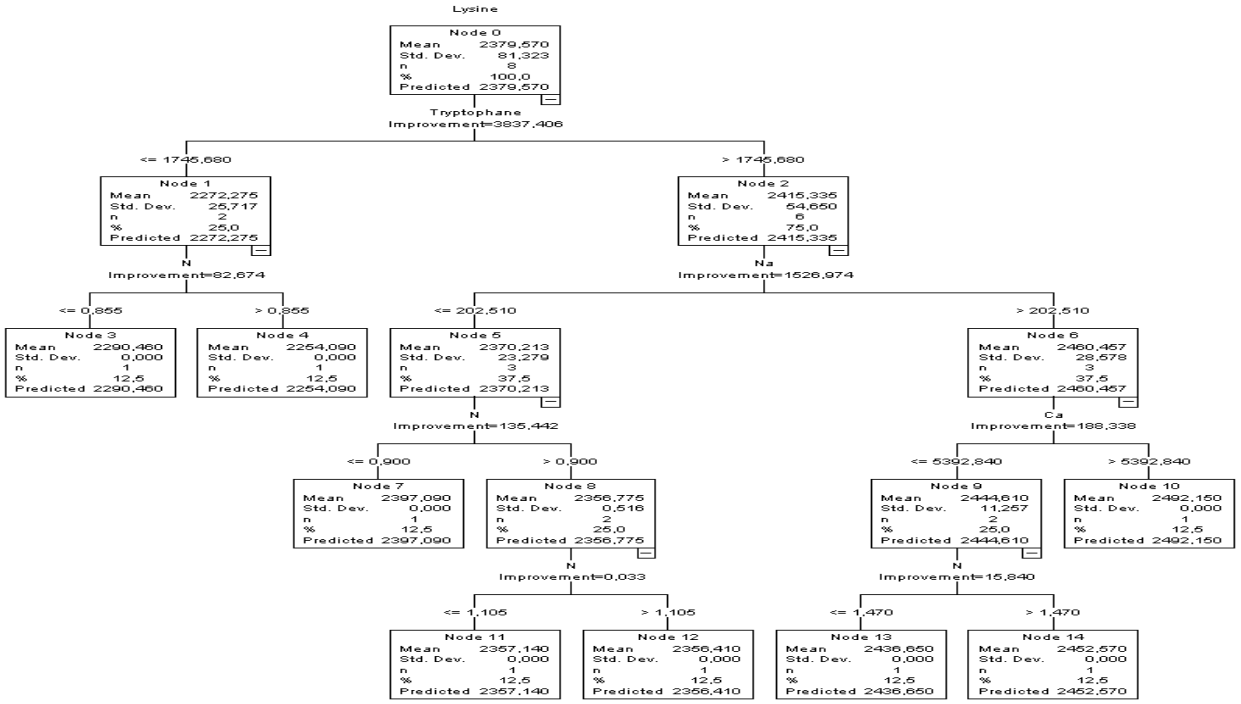
if Mg≤2384,435 Lysine is 5088,915
Main predictor is N
if N≤1,940 Lysine is 5096,210
if N>1,940 Lysine is 5081,620

if Mn≤29,780 Lysine is 4701,063
Main predictor is N
if N≤1,825 Lysine is 4544,360
if N>1,945 Lysine is 4779,415
Main predictor is N
if N≤1,880 Lysine is 4723,450
if N>1,880 Lysine is 4835,380

R^2: [1- $\frac{\textit{Risk Estimation}}{62}$ **]: [1-** $\frac{9835,45}{39872,51}$ **]: 1-0,25: 0,75**

Figure 1. Decision tree analysis to determine the factors affecting lysine on whole seed in wheat.

Decision tree analysis denoted that main predictor was Mn and sub predictors were Mg, Na and N in whole seed analysis. Increase in Mn made lysine level increase. Increasing Mg level caused decrease in lysine. Na was another main sub factor in low Mn levels. Higher Na levels decreased lysine levels. Besides, higher N contents had positive effect to lysine. Under higher Mn levels, increasing N contents raised lysine amount. Therefore, Mn, Na and N were determined as important components, effective in lysine (Figure 1). In lipids analysis, tryptophan as the main predictor; N, Ca and Na sub predictors, were found. Once tryptophan dropped off, Lysine amount raised. In lower tryptophan levels, higher Na and N levels caused decline in lysine content. On the contrary, at high tryptophan values, increasing amounts of nitrogen increased the lysine concentration. The major shaping of lysine concentration was possible in the presence of tryptophan, Ca, Na and N (Figure 2). So, Mn, tryptophan, Na, N and Ca were found as effective components in the formation and activity of lysine.



Main predictor is Tryptophan;

if Tryptophane≤1745,680 Lysine is 2272,275
Main predictor is N
if N≤0,855 Lysine is 2290,460
if N>0,855 Lysine is 2254,090

if Tryptophane≤1745,680 Lysine is 2415,335
Main predictor is Na
if Na≤202,510 Lysine is 2370,513
Main predictor is N
if N≤0,900 Lysine is 2397,090
if N>0,900 Lysine is 2356,775
Main predictor is N
if N≤1,105 Lysine is 2357,140
if N>1,105 Lysine is 2460,457

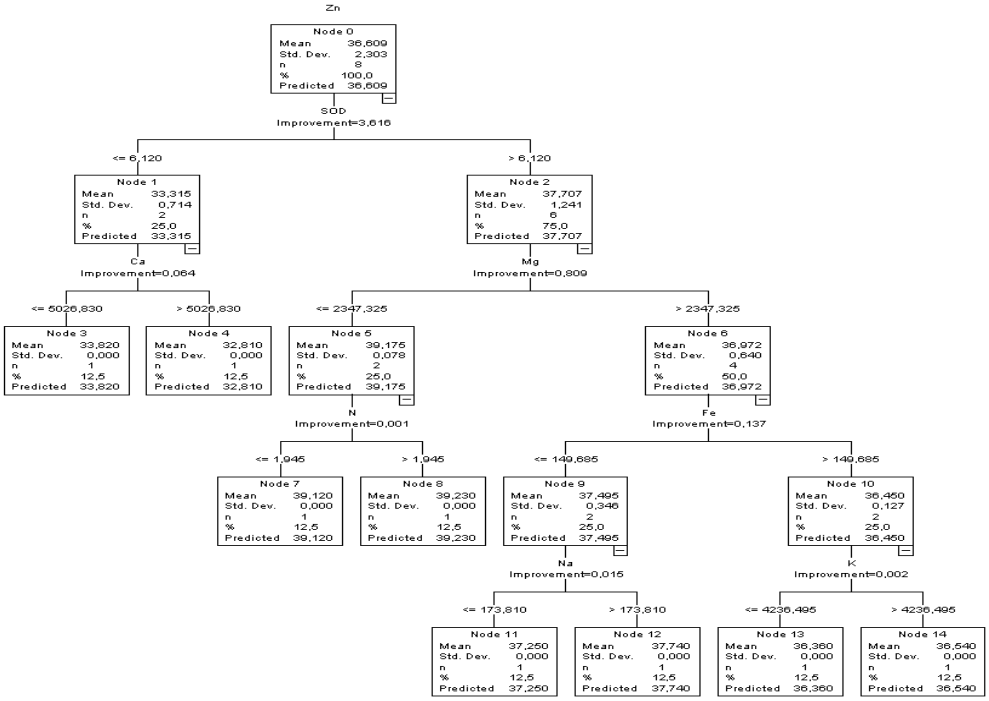
if Na>202,510 Lysine is 2370,513
Main predictor is Ca
if Ca≤5392,840 Lysine is 2444,610
if Ca>5392,840 Lysine is 2492,150
Main predictor is N
if N≤1,470 Lysine is 2436,650
if N>1,470 Lysine is 2452,570

R^2: [1- $\frac{\text{Risk Estimation}}{SS}$: [1- $\frac{1468,45}{6613,43}$]: 1-0,22: 0,78

Figure 2. Decision tree analysis performed to determine the factors affecting lysine in the lipids extracted from wheat embryo

3. 4. Zn

Zinc is one of the most important minerals necessary for both human and plant health. One of the most important minerals that benefit the healthy functioning of the human body is zinc. Zinc is a mineral that undertakes many important functions such as the immune system and metabolic activities. In addition, zinc is involved in many activities such as growth, development, protein synthesis, immune system function, reproductive health, tissue formation, and neurobehavioral development. It is predominantly found in muscle, skin, hair, and bone [22]. Zinc, which plays an important role in many biological and physiological processes, should be taken in sufficient amounts for a strong nervous system and immune system. The amount of zinc people need daily under normal conditions is; it is determined as 5 mg in infants, 10 mg to 15 mg in children, and 12 mg to 15 mg in adults [22]. On the other hand, although Zinc is involved in various metabolic events in plants, it plays a role in carbohydrate synthesis, the structure of enzymes, membrane stability, protein synthesis in photosynthesis and respiration [22]. Since, zinc is important in protein synthesis, the high protein content in the flour, combined with a high zinc level, indicates a high-quality variety. Decision tree analyses performed overall seed and extracted lipids in order to determine the factors affecting Zn are given in Figure 3 and Figure 4.



Main predictor is SOD;

if SOD≤6,120 Zn is 33,315
Main predictor is Ca
if Ca≤5026,830 Zn is 33,820
if Ca>5026,830 Zn is 32,810

if SOD>6,120 Zn is 37,707
Main predictor is Mg
if Mg≤2347,325 Zn is 39,176
Main predictor is N
if N≤1,945 Zn is 39,120
if N>1,945 Zn is 39,230

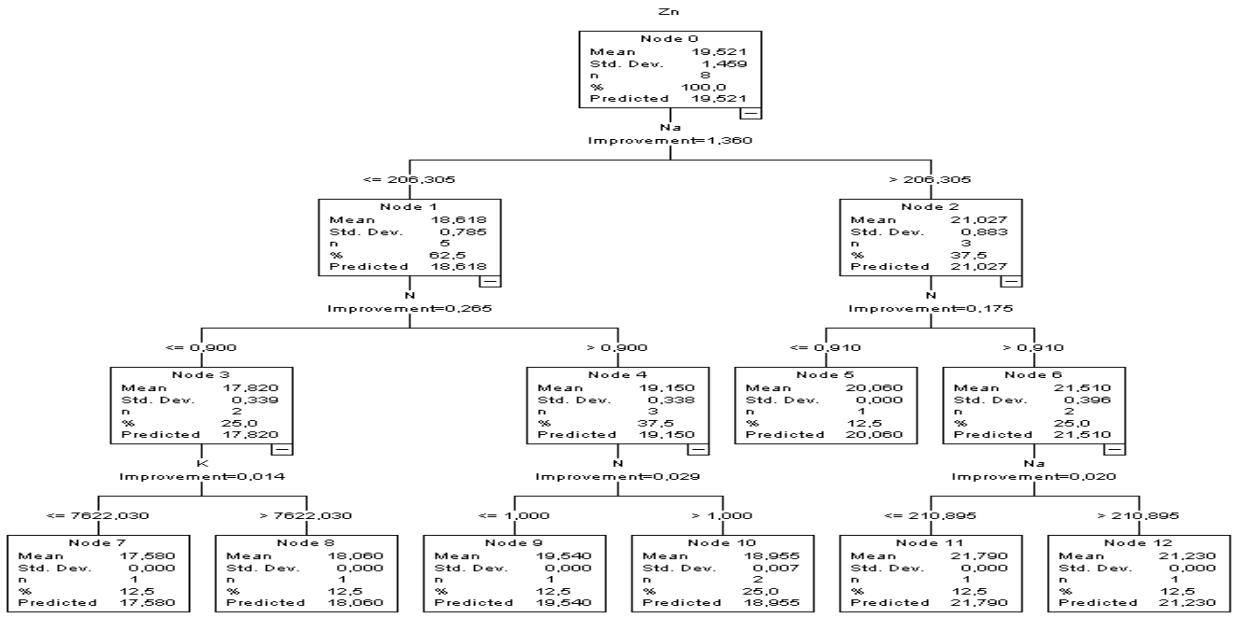
if Mg>2347,325 Zn is 36,972
Main predictor is Fe
if Fe≤149,685 Zn is 37,495
Main predictor is Na
if Na≤173,810 Zn is 37,250
if Na>173,810 Zn is 37,740

if Fe>149,685 Zn is 36,450
Main predictor is K
if K≤4236,495 Zn is 36,360
if K>4236,495 Zn is 36,540

R^2: [1- -[redacted]: [1- $\frac{1,59}{5,30}$]: 1-0,30: 0,70

Figure 3. Decision tree analysis to determine the factors affecting Zn on whole seed in wheat

Figure 3 exposed the effective components on Zn in whole seed. Main predictor was SOD, sub predictors were Ca, Mg, N, Fe and K. Na and N. Increase in SOD highly increased Zn amount. Second main determinants in lower and higher SOD levels were Ca and Mg, respectively. Zn increased with raising Ca in lower SOD level. Moreover, in higher SOD level, increasing Mg level caused decrease in Zn content. Main determinants in lower and higher Mg levels were N and Fe, respectively. In lower Mg level, raising N content made slightly increase in Zn. Rearing up Zn content occurred with raising Fe level in higher Mg level. In lower Fe level, increasing Na level increased in Zn content. In higher Fe level, Zn content increased with increasing K level. SOD, Ca, Mg, N, Fe, Na and K were determined as significant determinants in Zn activity.



Main predictor is Na;

if Na≤206,305 Zn is 18,610
Main predictor is N
if N≤0,900 Zn is 17,820
Main predictor is K
if K≤7622,030 Zn is 17,580
if K>7622,030 Zn is 18,060
if N>0,900 Zn is 19,150
Main predictor is N
if N≤1,000 Zn is 19,540
if N>1,000 Zn is 19,855

if Na>206,305 Zn is 21,027
Main predictor is N
if N≤0,910 Zn is 20,060
if N>0,910 Zn is 21,510
Main predictor is Na
if Na≤210,895 Zn is 21,790
if Na>210,895 Zn is 21,230

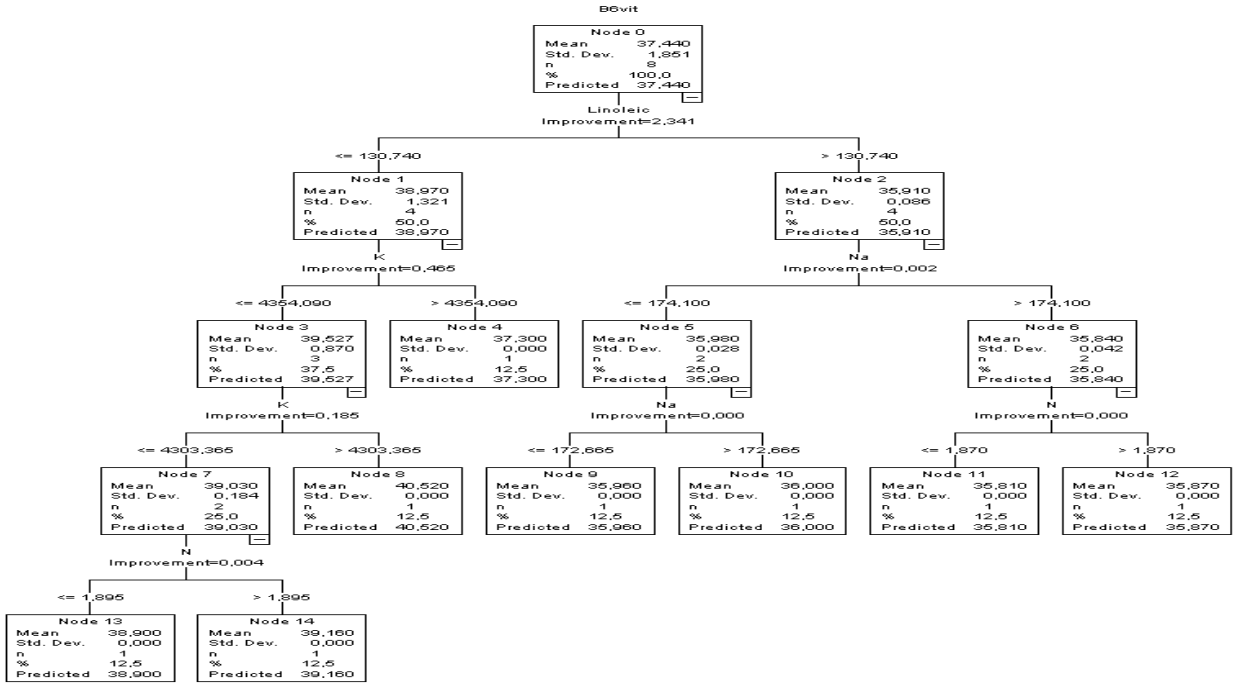
R^2: [1- -: [1- $\frac{0,66}{2,12}$]: 1-0,32: 0,68

Figure 4. Decision tree analysis performed to determine the factors affecting Zn in the lipids extracted from wheat embryo

Figure 4 assign the effective components on Zn on lipids. Main predictor and sub predictor were determined as Na and N, respectively. Increase in Na increased Zn amount. N was main predictor and N affects significantly increase Zn in lower and higher Na levels. In lower N level, K was predictor, and Zn raised with increasing K level. When N level was higher, increasing N content made slightly increase in Zn. Once Na was predictor in higher N, decrease was taken place in Zn, with increasing Na level. Na, N, K and Na were determined as important predictors. SOD, Ca, Mg, N, Fe, Na and K were concluded as significant components in Zn activity.

3. 5. B_6 vitamin

In order to meet and meet the body's basic needs, vitamin, mineral, protein, carbohydrate and mineral intakes are very important, and one of the important vitamins in meeting these needs is vitamin B_6 (pyridoxine). Vitamin B_6 supports many important functions such as metabolism of proteins, production of red blood cells, nervous system functions, immune system functions and skin health. Vitamin B_6 is found naturally in many foods, especially meat, fish, chicken, whole-grain bread and cereals, and vegetables such as potatoes, bananas, avocados, and kale. Vitamin B_6 has a regulatory function and plays a supporting role as a coenzyme and cofactor during the performance of many functions in the body. Vitamin B_6 has functions in many areas such as the hormonal system, nervous system, immune system and blood production. In order for the body to perform its daily functions, the need for macro and micronutrients must be fully met with a healthy and balanced diet. The daily need for vitamin B_6 is known to be 1.2-1.7 mg [23].



Main predictor is Linoleic;

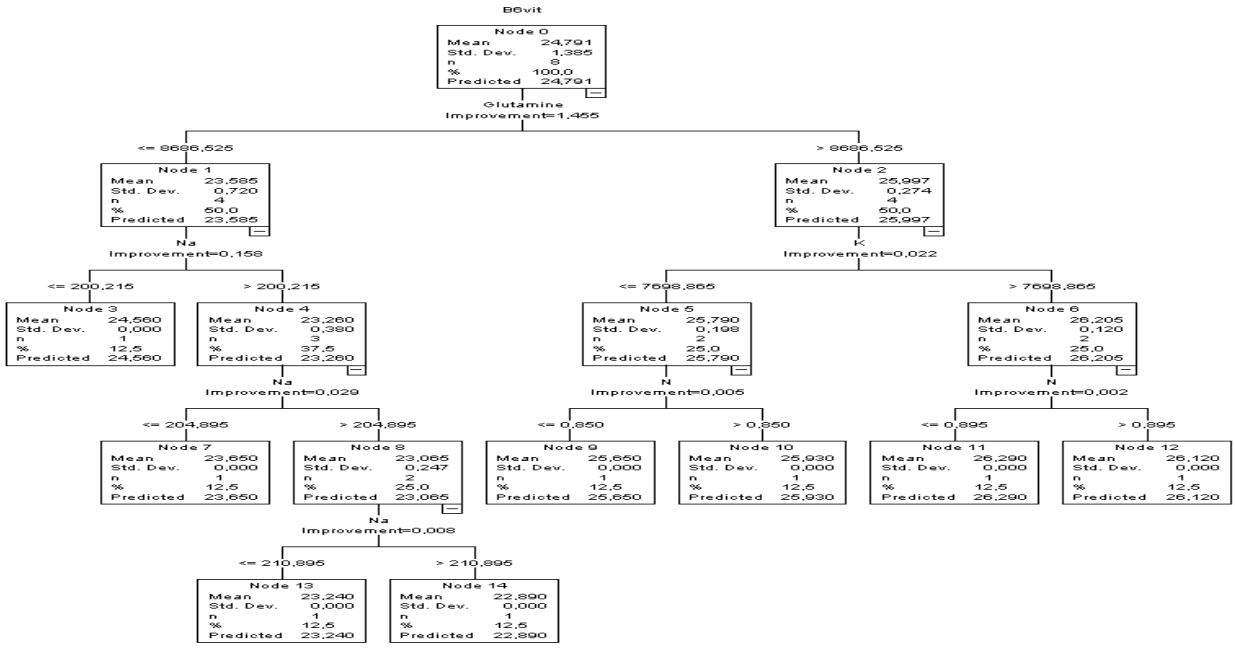
if Linoleic≤130,740 B_6 is 38,970
Main predictor is K
if K≤4364,090 B_6 is 39,527 if K>4364,090 B_6 is 37,300
Main predictor is K
if K≤4303,365 B_6 is 39,030 if K>4303,365 B_6 is 40,520
Main predictor is N
if N≤1,895 B_6 is 38,900
if K>1,895 B_6 is 39,160

if Linoleic>130,740 B_6 is 35,910
Main predictor is Na
if Na≤174,100 B_6 is 35,980 if Na>174,100 B_6 is 36,840
Main predictor is Na
if Na≤172,665 B_6 is 36,980 if N≤1,870 B_6 is 36,810
if Na>172,665 B_6 is 36,000 if N>1,870 B_6 is 36,870

R^2: [1- [illegible]: [1- 0,43 / 3,43]: 1-0,26: 0,74

Figure 5. Decision tree analysis to determine the factors affecting B_6 on whole seed in wheat

Vitamin B_6 is an important cofactor in many enzymatic reactions in plants and plays an important role in the plant's tolerance or defence system against abiotic stresses. Additionally, high levels of vitamin B_6 in the plant lipids and the whole seed lead to an increase in the nutritional quality of the variety [23]. Decision tree analyses performed overall seed and extracted lipids in order to determine the factors affecting B_6 are given in Figure 5 and Figure 6. Main important components for B_6 in whole seed were given in Figure 5. Main predictor was linoleic, sub predictors were K and Na. Increase in linoleic caused decline in B_6, and K decreased B_6 level in lower linoleic level. In lower K level, main predictor seemed K, with raising it, B_6 level increased. In higher linoleic level, raise in Na and N levels could not create certain changes in B_6 level (Figure 5). Linoleic, N, Na and K were determined as significant determinants in B_6 activity.



Main predictor is Glutamine;

if Glutamine≤8686,525 B_6 is 23,585
Main predictor is Na
if Na≤200,215 B_6 is 24,560
if Na>200,215 B_6 is 23,260
Main predictor is Na
if Na≤204,895 B_6 is 23,650
if Na>204,895 B_6 is 23,065
Main predictor is Na
if Na≤210,895 B_6 is 23,240
if Na>210,895 B_6 is 22,890

if Glutamine>8686,525 B_6 is 25,997
Main predictor is K
if K≤7698,865 B_6 is 25,790
Main predictor is
if N≤0,850 B_6 is 25,650
if N>0,850 B_6 is 25,930
if K>7698,865 B_6 is 26,205
Main predictor is N
if N≤0,895 B_6 is 26,290
if N>0,895 B_6 is 26,120

R^2: [1- [illegible]**: [1-** $\frac{0,49}{1,92}$ **]: 1-0,26: 0,74**

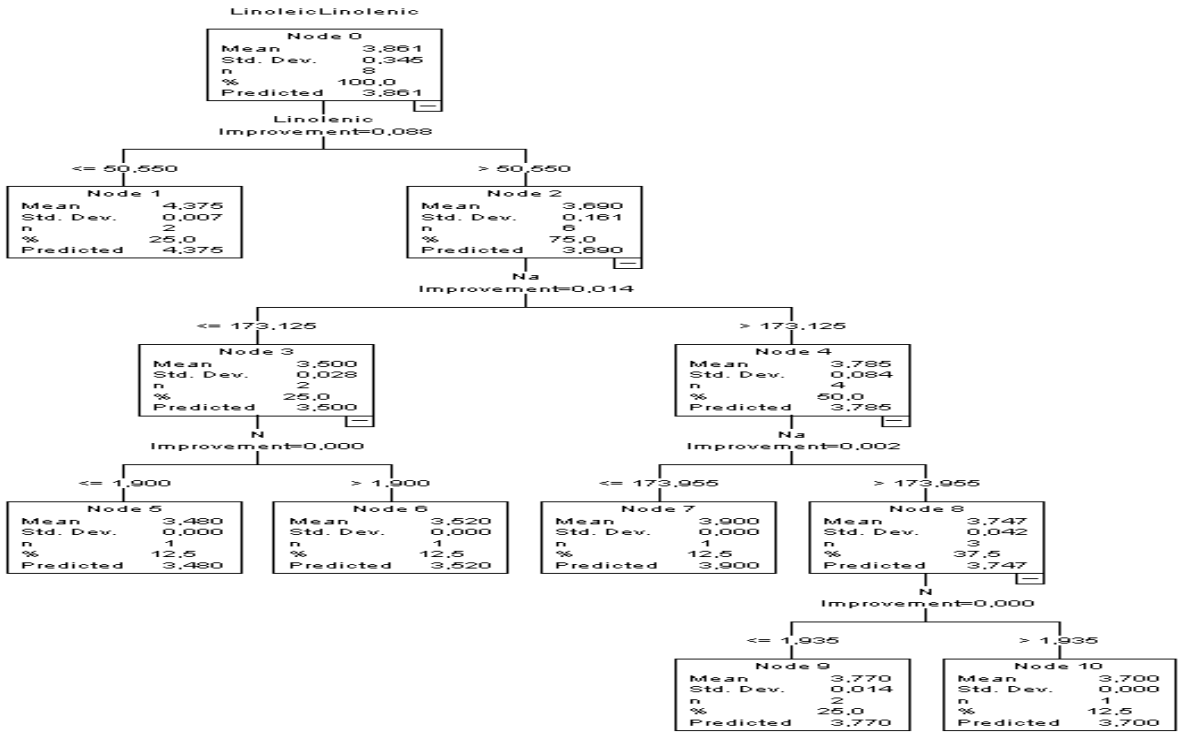
Figure 6. Decision tree analysis performed to determine the factors affecting B_6 in the lipids extracted from wheat embryo

Significant components on B_6 in lipids extracted were given in Figure 6. Main predictor was glutamine, sub predictors were K and Na. Increase in glutamine increased in B_6 in lower glutamine level, raising Na lowered B_6. In higher glutamine level, B_6 level increased with raising K amount. Though increasing N increased B_6 in lower K level, B_6 declined with increasing N level in higher K level. As in whole seed, glutamine, N, Na and K seemed significant components in B_6 action. Significant components were found in whole seed and lipids as linoleic, glutamine, N, Na and K for B_6.

3. 6. Rate of linoleic acid/linolenic acid

Essential fatty acids are fatty acids that cannot be synthesized in the body and that humans and animals must obtain from outside in order to survive. The most important fatty acids among essential fatty acids are linoleic acid and linolenic acid. Linoleic acid is a polyunsaturated fatty acid, a colourless or white lipids that is virtually insoluble in water but soluble in many organic solvents. It is one of the three essential fatty acids [24] that humans must obtain through diet. Since it is an essential fatty acid, linoleic acid consumption is vital for a healthy life. Another essential fatty acid is linolenic acid. Deficiency of both fatty acids causes serious problems, especially in brain and eye development. It also causes depression and behavioural disorders [24]. On the other hand, these two fatty acids have potential effects in the prevention and treatment of coronary heart diseases, hypertension, type 2 diabetes, ulcerative colitis, rheumatoid arthritis, depression, various cancers and chronic obstructive pulmonary diseases, with their properties such as anti-inflammatory, blood flow regulator and heart rhythm regulator. [24]. Linoleic acid and linolenic acid are large molecules and are formed by the combination of small molecules through dehydration reactions. Since there is a lot of hydrogen in their structure, because of their destruction by aerobic respiration in plants, abundant energy is produced and abundant metabolic water is formed. It participates in the structure of the cell membrane. It participates in the structure of some vitamins such as vitamin D. They are easy to destroy. Although it is richly found in many lipids such as olive lipids, cottonseed lipids, soybean lipids, corn lipids, hazelnut lipids, etc., it is present in a certain amount in wheat grain and lipids. It supports the plant defence mechanism against free radicals formed because of oxidative stress caused by free radicals formed by normal metabolism or environmental factors. It is involved in the cell membrane in cold resistance and ion uptake [24]. The World Health Organization states that a linolenic acid/linoleic

acid ratio between 5/1 and 10/1 is necessary for a healthy diet. It is reported that these ratios are the desired ratios in foods in an ideal diet. Therefore, varieties high in this ratio have high nutritional quality [24]. The decision tree analysis performed to determine the factors affecting the Rate of Linolenic acid is given in Figure 7 and Figure 8.



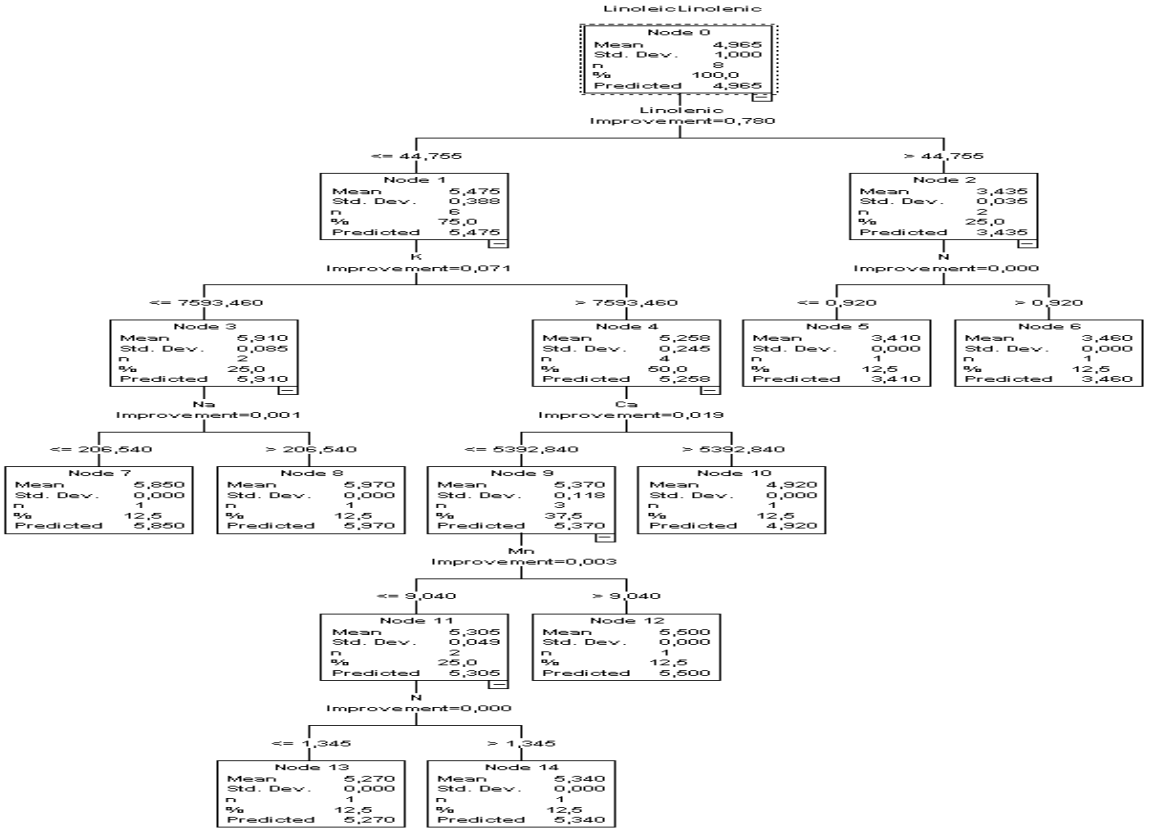
Main predictor is Linolenic;

if Linolenic≤50,550 Linoleic/Linolenic is 4,375 if Linolenic>50,550 Linoleic/Linolenic is 3,690
Main predictor is Na
if Na≤173,125 Linoleic/Linolenic 3,500 if Na>173,125 Linoleic/Linolenic 3,900
Main predictor is N Main predictor is Na
if N≤1,900 Linoleic/Linolenic 3,480 if N≤173,955 Linoleic/Linolenic 3,480 if N>173,955 Linoleic/Linolenic 3,747
if N>1,900 Linoleic/Linolenic 3,520 Main predictor is N
if N≤1,935 Linoleic/Linolenic 3,770
if N>1,935 Linoleic/Linolenic 3,700

R^2: [1- [illegible] **: [1-** $\frac{0,66}{0,12}$ **]: 1-0,34: 0,66**

Figure 7. Decision tree analysis to determine the factors affecting linoleic/linolenic on whole seed in wheat

Important effectual components for linoleic/linolenic in whole seed were given in Figure 7. Main predictor was linolenic, increase in linolenic caused decline in linoleic/linolenic rate. In higher linolenic level, sub predictor was Na, stepping up Na level increased linoleic/linolenic rate. In lower Na level, N was sub predictor and increment of N increased linoleic/linolenic rate. In higher Na level, Na and N were sub predictors. Once augmenting Na and N levels made decrease and increase in linoleic/linolenic rate, respectively (Figure 7). Significant components having effect on linoleic/linolenic rate were linolenic, N, Na.



Main predictor is Linolenic;

if Linolenic≤44,755 Linoleic/Linolenic is 5,475
Main predictor is K
if K≤7693,460 Linoleic/Linolenic is 5,910
Main predictor is Na
if Na≤206,640 Linoleic/Linolenic is 5,850
if Na>206,640 Linoleic/Linolenic is 5,970
if K>7693,460 Linoleic/Linolenic is 5,258
Main predictor is Ca
if Ca≤5392,840 Linoleic/Linolenic is 5,370
Main predictor is Mn
if Mn≤9,040 Linoleic/Linolenic is 5,305
Main predictor is N
if N≤1,345 Linoleic/Linolenic is 5,270
if N>1,345 Linoleic/Linolenic is 5,340
if Mn>9,040 Linoleic/Linolenic is 5,500
if Ca>5392,840 Linoleic/Linolenic is 4,920
if Linolenic >44,755 Linoleic/Linolenic is 3,435
Main predictor is N
if N≤0,920 Linoleic/Linolenic is 3,410
if N>0,920 Linoleic/Linolenic is 3,460

R^2: [1- [illegible] **: [1-** $\frac{0,29}{1,00}$ **]: 1-0,29: 0,71**

Figure 8. Decision tree analysis performed to determine the factors affecting linoleic/linolenic in the lipids extracted from wheat embryo

Only in Figure 8 was linolenic main predictor, rise in linolenic declined linoleic/linolenic rate. In higher linolenic level, sub predictor was N, increasing Na level increased linoleic/linolenic rate. In lower linolenic level, K was sub predictor and increment of K increased linoleic/linolenic rate. In lower K level, Na as a sub predictor, increased linoleic/linolenic rate. In higher K level, Ca was a sub predictor, decreased linoleic/linolenic rate. In lower Ca level, Mn was sub predictor and increasing Mn level increased linoleic/linolenic rate. In lower Mn level, N was sub predictor and made increase in linoleic/linolenic rate by increasing it. (Figure 8). Significant components were determined as linolenic, K, N, Na and Mn. Significant components in whole seed and lipids were found as linolenic, K, N, Na and Mn.

4. Conclusions and discussion

Wheat has a certain potential in terms of the components examined, but it is not sufficient in terms of nutrition alone. Especially the nutritional value of wheat, which we mentioned above, is far from meeting the daily needs of human beings. The solution to this need is through balanced nutrition. Instead of a one-way diet, it is necessary to have a balanced diet with plant foods such as vegetables and fruits and animal foods such as meat and chicken. In wheat embryo, especially in the embryo, lipids is used both as a building block and as an energy source in the structure of the cell membrane, in metabolic events that start from embryonisation to full maturity. As shown in this article, it contains approximately half of the minerals, amino acids, enzymes, sugars and other compounds present in the lipids. On the other hand, this lipids amount and its content composition are important for bread quality. Therefore, consuming whole wheat flour containing embryo and bran provides a better quality nutrition. This rich content of wheat lipids makes it a

valuable substance for the cosmetic industry. The results showed that **Tosunbey-G1, Alpu 01-G2, ES26-G3** and **Nacibey-G5** had the highest nutritional values and better activity. The ratio of minerals, amino acids, fatty acids, enzymes and vitamins in lipids to the whole seed was determined as 53%, 63% and 68%, respectively. Moreover, except for Ca and linoleic acid/linolenic acid ratio, the differences between whole seed and lipids in genotypes for the other components were found to be significant at the 1% level. The differences between Ca and linoleic acid/linolenic acid ratio were found to be insignificant and significant at the 5% level, respectively. In wheat, the ratio of lipids components to the whole seed is approximately one third. There are certain substances in wheat; lysine, Zn, B_6 vitamin and the rate of linoleic acid/linolenic are known as essential components, making nutritional quality better. The factors affecting the activity of these components were estimated by decision tree analysis. Mn, tryptophan, Na, N and Ca were found as effective components in the shaping and activity of lysine. SOD, Ca, Mg, N, Fe, Na and K were concluded as significant components in Zn activity. Significant components were found in whole seed and lipids as linoleic, glutamine, N, Na and K for B_6 vitamin. In linoleic/linolenic rate, linolenic, N, Na were important components. Studies that are more detailed are needed to better understand this issue.

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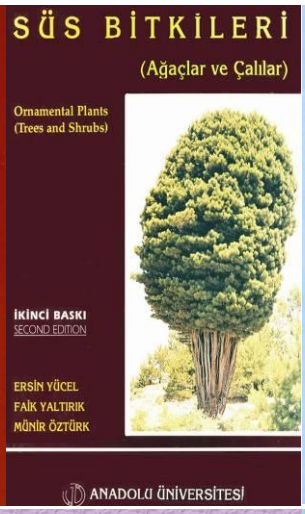

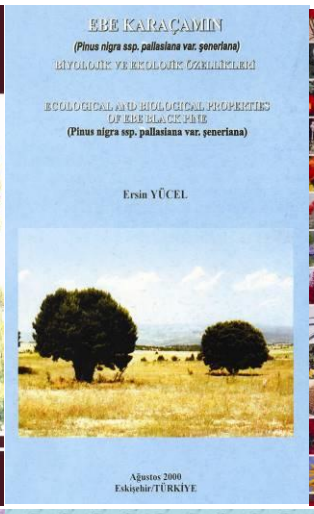

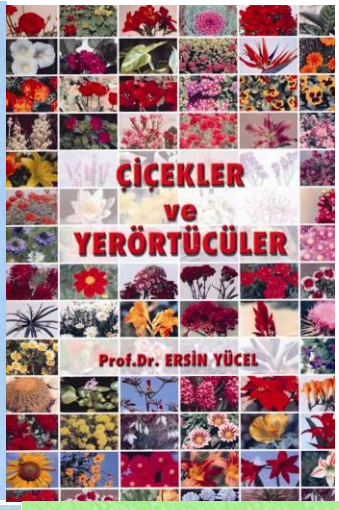

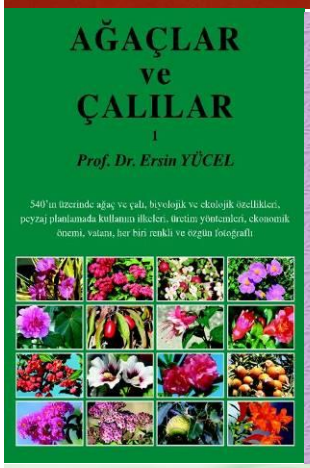

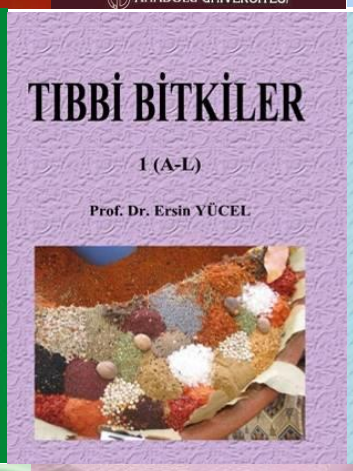

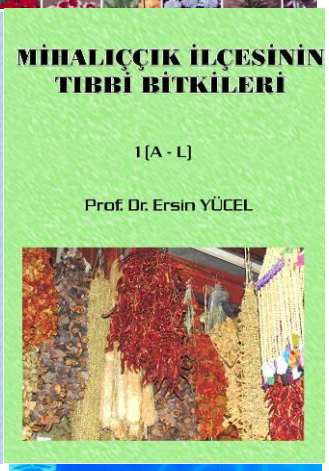

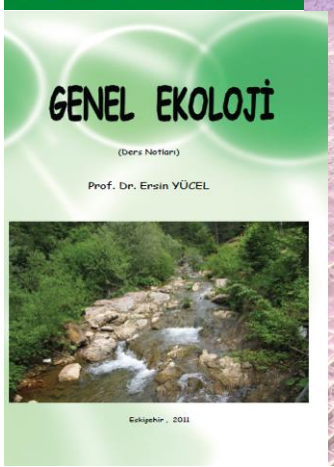

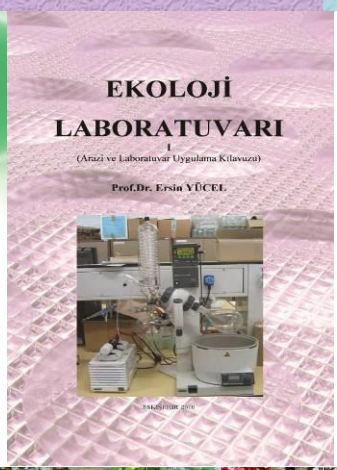

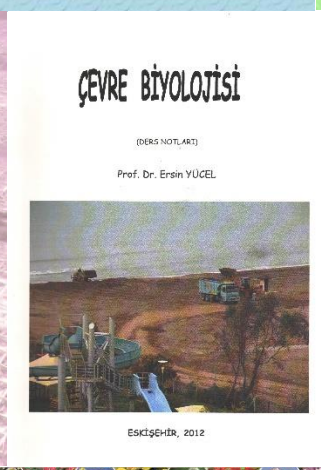

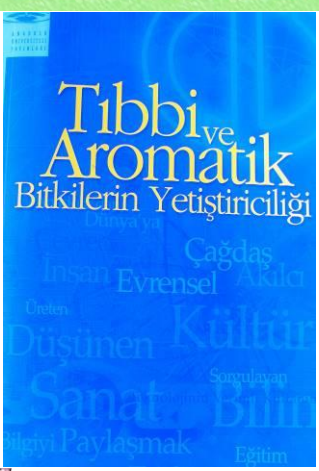

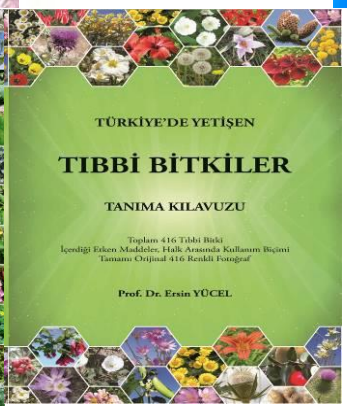

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