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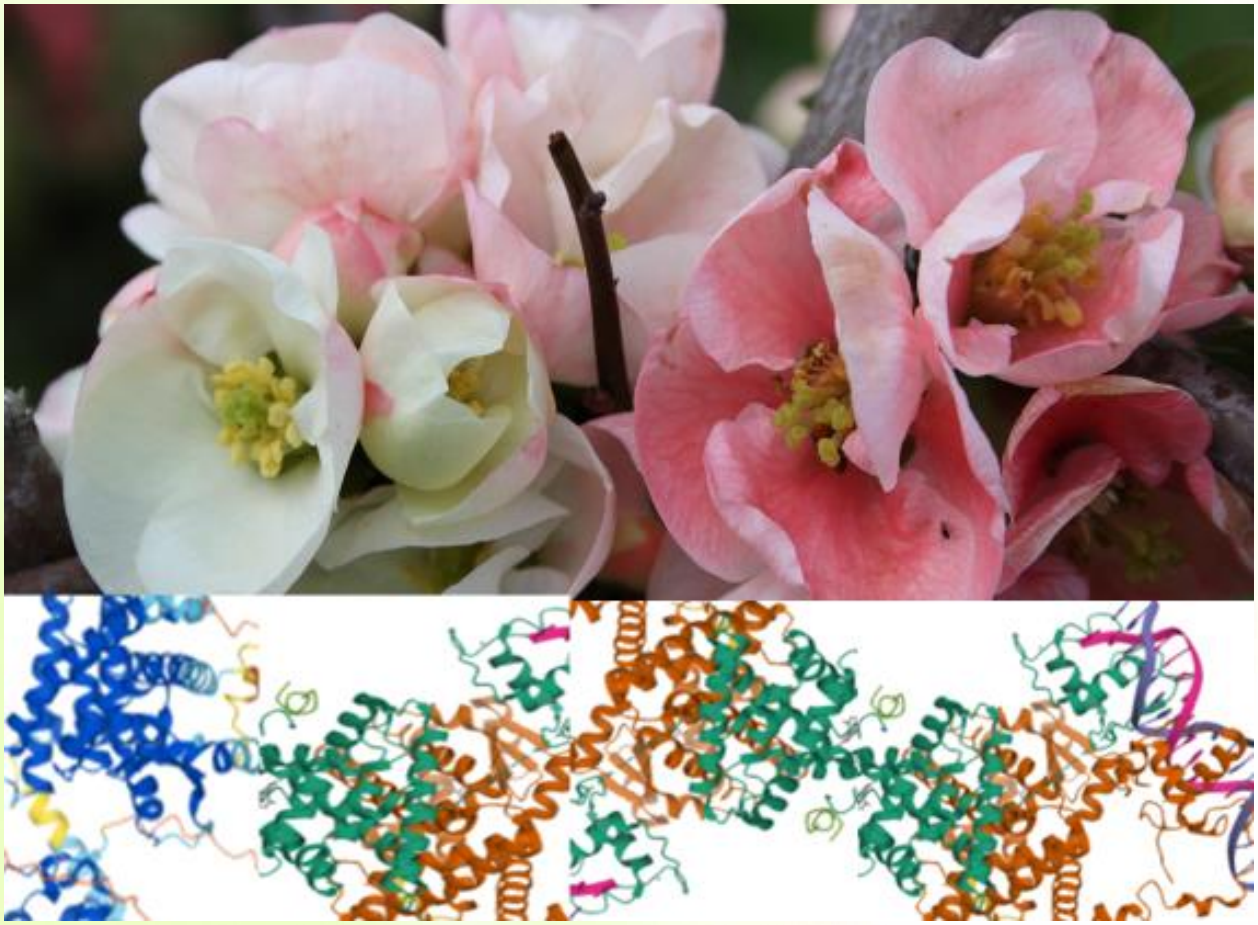

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# Combinational synergistic role of thymoquinone and celastrol in colon carcinoma cell line

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**Abstract**

Colon carcinoma (HCT-116) cells are highly aggressive cell line and cell proliferation of colon carcinoma cells are rapid and uncontrolled. Treatment of colorectal cancer cells can be achieved through the use of chemotherapeutic agents. However, the treatment with a single type of chemical may require high dosages, which leads to toxicity. To resolve this issue, synergistic combinational treatment of Thymoquinone (TQ) and Celastrol (CLS) can be promising strategy to reduce proliferation and cell viability of the colorectal cancer cells. Evaluation of cell viability and cell growth were determined for the combinational and alone treatments of TQ and CLS using MTT assay. The IC50 values of TQ and CLS were determined as 102 μM and 7 μM, respectively. Four different combinations of these two chemical agents were tested and the results revealed strong synergistic effect against HCT-116 colon cancer cells. Reactive oxygen species (ROS) production was also evaluated by monitoring the production of highly fluorescent DCF from DCFH-DA. Compared to the alone treatments of the both drugs, overproduction of ROS in combinational treatments supported the results obtained from cell viability. Our findings demonstrated that combinational strategy of TQ and CLS has strong synergistic activity against the HCT-116 cancer cells and it can be a promising strategy to increase the effect of the drugs.

**Keywords:** Colon cancer, thymoquinone, celastrol, synergistic combination, ROS production

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## Kolon karsinoma hücre hattında timokinon ve celastrolün kombinasyonel sinerjistik rolü

**Özet**

Kolon karsinoma (HCT-116) hücreleri oldukça agresif hücre dizisidir ve bu hücrelerinin proliferasyonu hızlı ve kontrolsüzdür. Kolorektal kanser hücrelerinin tedavisi kemoterapötik ajanların kullanılmasıyla sağlanabilir. Ancak tek tip kimyasalla tedavi yüksek dozlar gerektirebilir ve bu da toksisiteye yol açabilmektedir. Bu sorunu çözmek için, Timokinon (TQ) ve Celastrol'ün (CLS) sinerjistik kombinasyon tedavisi, kolorektal kanser hücrelerinin çoğalmasını ve hücre canlılığını azaltmak için umut verici bir strateji olabilir. Hücre canlılığı ve hücre büyümesinin değerlendirilmesi, TQ ve CLS'nin kombine grupları ve tek başına tedavileri için MTT methodu kullanılarak belirlendi. Bu iki ajanın kombinasyon konsantrasyonları ve kombinasyon indeksleri CompuSYN yazılım programı ile belirlendi. TQ ve CLS'nin yarı maksimum inhibitör konsantrasyonları (IC50) sırasıyla 102 μM ve 7 μM olarak belirlendi. Bu iki kimyasal ajanın kombine grupları, HCT-116 kolon kanseri hücrelerine karşı güçlü sinerjistik etkiyi ortaya çıkardı. Reaktif oksijen

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türlerinin üretimi ayrıca DCFH-DA'dan yüksek derecede floresan DCF üretiminin izlenmesiyle değerlendirildi. Her iki ilacın tek başına tedavileri ile karşılaştırıldığında kombinasyon tedavilerinde ROS'un aşırı üretimi hücre canlılığından elde edilen sonuçları destekledi. Bulgularımız, TQ ve CLS'nin kombinasyon stratejisinin, HCT-116 kanser hücrelerine karşı güçlü sinerjistik aktiviteye sahip olduğunu ve ilaçların etkisini arttırmak için umut verici bir strateji olabileceğini gösterdi.

**Anahtar kelimeler:** Kolon kanseri, timokinon, celastrol, sinerjistik kombinasyon, ROS üretimi.

## 1. Introduction

Colon carcinoma (HCT-116) cells are highly aggressive cell line and cell proliferation of colon carcinoma cells are rapid and uncontrolled. Researchers are trying to develop targeted therapies to inhibit proliferation of colon carcinoma cells. Chemotherapeutic agents have been long to be used to treat colorectal cancer, however, the use of these agents limited due to the severe side effects such as toxicity. To avoid potential adverse effects, synergistic combinations of anti-cancer medications with natural substances have been proposed as an alternative technique for treating a variety of cancer cell lines. In addition to toxic chemotherapeutic drugs, effect of natural agents may also improved via combination with another natural agent. Synergistic combination of natural flavonoids curcumin and quercetin have been shown to have better anti-cancer activity compared to single administration of these compounds. Similarly, synergistic avtivity of apigenin and curcumin against lung epithelium cancer [1]. Using natural anticancer agents minimize the side effects by lowering the dosage requirement for the cancer treatments.

Phytochemical agents such as thymoquinone have been reported to have anticancer [2], antioxidant [3], anti-inflammatory [4], and analgesic activities [5]. Chemical structure of thymoquinone is provided in Figure 1. Thymoquinone is a monoterpene and it can upregulates the caspase-3,8,9 and Bax expression. These genes are classified as apoptotic genes and they can induce apoptosis. It is also reported that thymoquinone can terminate JAK-2 and Src initiated phosphorylation of tyrosine kinase receptor. This process inactivates STAT3 genes, which induces apoptosis in colorectal canncer cells [6]. In addition, signalling of MEK-ERK can be distrupted due to TQ mediated alteration in the conformation of PAK1. This process also mediate apoptosis in colorectal cancer cells [7].

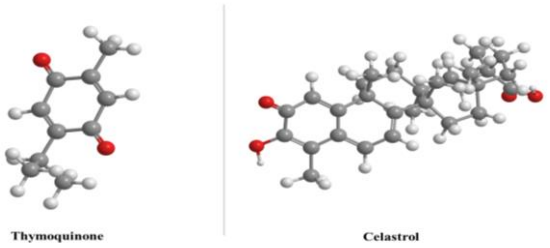


Figure 1. Chemical structures of thymoquinone and celastrol

Celastrol is a triterponoid and reported to have wide range of pharmacological activities. Possible toxicity of celastrol can be interlinked with the intramolecular hydrogen bonds to the carbonyl and hydroxyl groups [8]. Molecular mechanism behind the anticancer activity of celastrol shows variations depending on the type of cancer cell lines. It can reduce the function of NF-κB in colorectal cancer [9], suppress MMP3 and MMP7 via PI3K/AKT signalling [10], stimulates TGF-β1/Smad signalling [11], downregulates miR-21 and PI3K/AKT/GSK-3β pathway [12]. These mechanisms were detected upon celastrol treatments. In this study, synergistic combinations of thymoquinone and celastrol were determined to lower the dosage of administration while improving the anticancer activity against HCT-116 cancer cell lines by minimizing possible adverse effects.

## 2. Materials and methods

### *2.1 Materials*

MTT and dimethyl sulfoxide (DMSO), 2',7'-dichlorodihydrofluorescein diacetate (H2DCFDA), celastrol, PBS and thymoquinone were obtained from Merck. Fetal bovine serum (FBS), penicillin/streptomycin, L-glutamine and trypsin

were supplied from GIBCO Thermo Fisher Scientific (Waltham, Massachusetts, United States. Colorectal cancer cell lines (HCT-116) (ATCC-CCL-247) were obtained from the American Type Culture Collection in Virginia, USA.

*2.2 Cell Culture*

RPMI-1640 (10% fetal bovine serum (FBS), 1% L-glutamine, and 1% penicillin-streptomycin) was used as growth medium. Cells were detached from the surface of a cell culture flask using 0.25% trypsin/0.02% EDTA solution, and then resuspended in RPMI-1640 cell culture media. Following this process, cell suspension was transferred to samples in 96-well plates. Cells were cultured at 37 °C for 2 hours to allow for cell adherence and plates were incubated for another 48 hours at 37 °C [13].

*2.3 Cell Viability Assay*

HCT-116 cells ($5x10^3$ per well were seeded on a 96-well plate and cells were treated with various concentrations of thymoquinone (TQ) (10, 20, 60, 80, 100, 150, and 200 µM), celastrol (CLS) (1, 2, 4, 6, and 8 µM), and combinations of TQ and CLS at 37°C. After the incubation, the medium was removed and 10 µl of MTT solution (5 mg/ml in PBS) was added to each well and incubate for 3 hours at 37°C [14]. The produced purple formazan crystals were dissolved in 100 µl DMSO. The absorbance of each well was recorded at 540 nm. TQ and CLS IC50 values against HCT-116 cells were calculated using the Composyn software tool and evaluated with the GraphPad Prism 5 program.

*2.4 Combinational Analysis*

For the combination study analysis, the Chou-Talalay method was utilized to compute the combination index (CI). The combination index (CI) was determined with CompuSyn software.

*2.5 Determination of ROS Assay*

The determination of ROS levels is critical for understanding their relationship with apoptosis, aging, stress, and tumor growth. Reactive oxygen species oxidize the 2'7'-dichlorodihydrofluorescein diacetate (DCFH-DA) dye molecules to DCF. DCF molecules are fluorescent molecules and may be identified. HCT-116 cells are sown onto 96-well plates and incubated for 24 h. For 48 h, cells were treated with TQ, CLS, and their mixtures at their IC50 concentration. After incubation, DCFH-DA dye solution (20 µM) was applied to each well at 37 °C for 45 min. The fluorescence intensity of DCF was measured at 488 nm and 525 nm with a multiplate reader [13].

*2.6 Statistical Analysis*

To establish significant differences between treatments and controls, one-way ANOVA and Dunnett's multiple comparison test were utilized.

**3.Results**

*3.1 Cell viability*

The effect of different concentrations of TQ and CLS were tested on HCT-116 cell viability and results were presented in Figure 2a and 2b. After treatment with of TQ (10, 20, 60, 80, 100, 150 and 200 µM), CLS (1, 2, 4, 6 and 8 µM) alone, there was a significant reduction in the cell viability percentages for 48h. IC50 values of TQ and CLS were determined using Compusyn Software program. After 48 h treatment, IC50 values of TQ and CLS were found to be 102 µM and 7 µM, respectively.

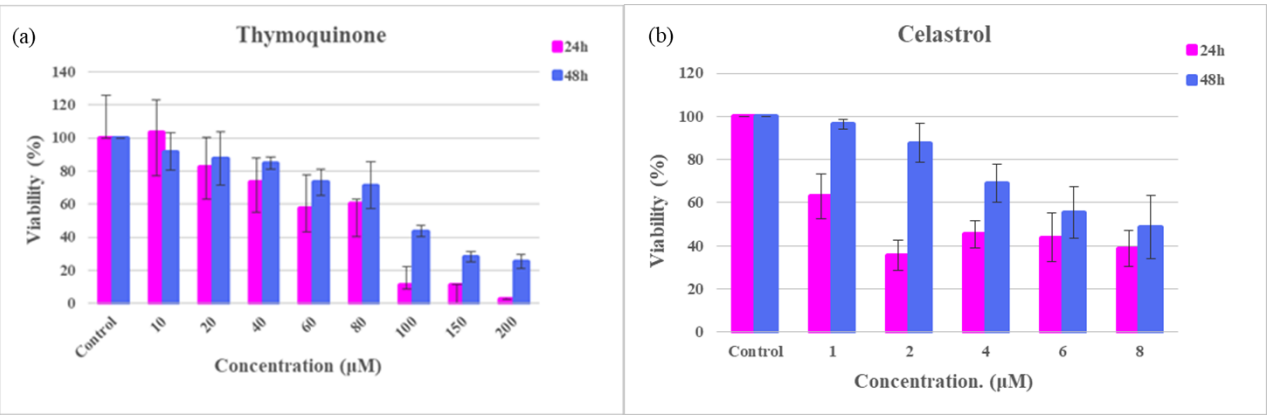


Figure 2. Cell viability % of various concentrations of **(a)** TQ, and **(b)** CLS toward the HCT-116 cancer cell line for 24 and 48 h

*3.2 Combined Effect of TQ and CLS on HCT-116 Cancer Cells*

Combinations of TQ and CLS enhanced their anticancer effects against HCT-116 cells. The dose-effect curves of TQ and CLS and their combinations are presented in Figure 3.

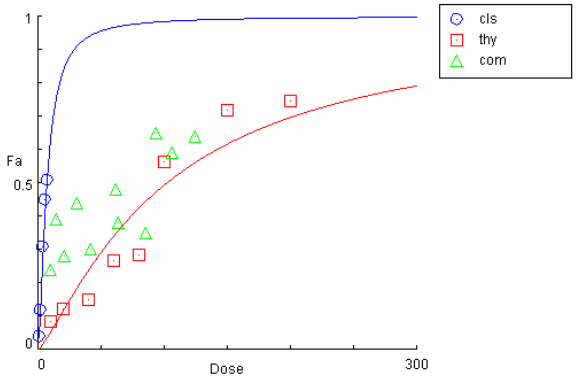


Figure 3. The dose-effect curves of thymoquinone, celastrol and their combinations

Combination index (CI) values were reported for the combination of TQ and CLS in Table 1. CI values are used to evaluate the synergistic activity between TQ and CLS. CI values expected to be smaller than 1 for synergistic activity. The strongest synergistic activity can be observed with smaller CI values. Based on CI values, 14 µM TQ with 1 µM CLS (CI: 0.38316, effect value: 0.39) and 10 µM TQ with 0.7 µM CLS (CI: 0.45194, effect value: 0.24) were determined as the best combination found due to smaller CI values compared to other combinations. It was observed that when TQ and CLS were used together, the same 50 % inhibitory concentration on HCT-116 cancer cells was achieved with a concentration approximately 3.5 times lower than when used alone. It has been observed that combination strategy of these two drugs significantly reduced the required dosage of each drug alone as shown in Figure 2 and this can minimize the possible toxic side effect of these chemicals.

*3.3 Effect on Reactive Oxygen Species of TQ, CLS and Their combinations*

Intracellular ROS production percentages for TQ, CLS and their combinations were presented in Figure 4. Based on experimental results, it was determined that TQ and CLS reduced intracellular ROS levels at their IC50 concentrations and alone treatments. Accumulation of ROS levels in cancer cells suppressed via the treatment of TQ and CLS. Combination-1, combination-2 and combination-3 treatments reduced the ROS production significantly at lower concentrations. Intracellular ROS production was effectively inhibited by combination-1 group among other combination groups.

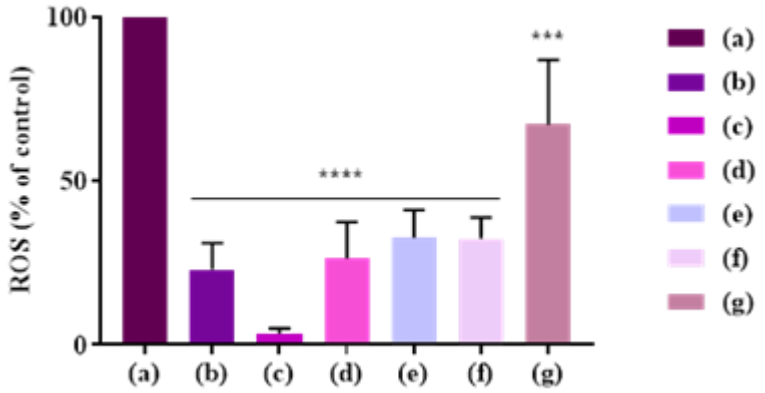


Figure 4. ROS production percentages of (a) control, (b) TQ, (c) CLS, (d) combination 1, (e) combination 2, (f) combination 3, and (g) combination 4. ROS production percentages were provided from three replicated measurements (n= 3). One Way ANOVA, Dunnett's multiple comparison assay used to evaluate the data by comparing with control as P<0.001, **** and p=0.0003, ***.

## 4. Conclusions and discussion

Synergisic activity is important as it often leads to enhanced therapeutic or beneficial effects compared to individual compounds. Synthetic compounds work together synergistically with natural substances, amplifying their overall impact on health. This synergism may improve absorption, increase antioxidant capacity, or target multiple pathways, making the combined effect more potent and valuable for various health applications.

In this study conducted in a similar manner, TQ alone inhibited HCT-116 cell growth at 48 and 72 hours, with a higher effect than 72 hours. Cell viability dropped to 27.8% at the highest TQ concentration (100 µg/mL), with an IC50 of 4.7 ± 0.21 µg/mL [15].

In different studies, HCT-116 cells treated with 50 µM TQ exhibited significant ($p<0.001$) reduction in cell proliferation. The IC50 of TQ against HCT-116 was calculated to be 64.15 ± 2.80 µM [16]. Fröhlich et al stated that the IC50 of TQ for HCT-116 cell line was 50.1 ± 6.1 µM [28].

In previous study, Kundu et al reported that apoptosis can be induce in HCT-116 cells upon STAT3 inactivation by inhibiting JAK2- and Src-initiated phosphorylation of tyrosine kinase [17]. Induction of apoptosis can also be mediated upon TQ treatment due to upregulation of apoptotic genes such as caspase-3, 8,9 and Bax [18]. Alterations in the conformation of PAK1 can block the signalling of MEK-ERK, which then eventually leads to the decrease in the proliferation of colorectal cancer cells [19]. Overall, oxidative stress mediated by TQ has been reported to downregulates JAK2/STAT3 pathway and this causes initiation of apoptosis in the cancer cells [20].

Several studies have demonstrated that CLS can effectively decrease tumor proliferation, migration, and angiogenesis in numerous tumor models, both in vitro and in vivo [21, 22]. In the present study, HCT-116 colon cancer cells were inhibited by 50 % at 7 µM of celastrol for 48 hours. Another study showed inhibitory effect to be more than 80 % after 48 h of treatment with 40 µM celastrol against HCT-116 colon cancer cells [23]. In different study, it was observed that CLS treatment caused a significant decrease in the proliferation of HCT-116 colon cancer cells in a dose-dependent manner [24]. All these molecular mechanisms inhibit the NF-κβ activity and blocks the nuclear translocation of NF-κβ [25]. These factors caused a reduction in the number of cancer cells.

Table 1. Dosages, and synergistic combination index values for combined groups of in thymoquinone (TQ) and celastrol (CLS) in HCT-116 cell line

| Combination | Thymoquinone (µM) | Celastrol (µM) | CI | Effect Value | Function of CI Values |
|---|---|---|---|---|---|
| **1** | 14.0 | 1.00 | **0.383** | 0.390 | **Strong Synergism** |
| **2** | 29.0 | 2.00 | **0.672** | 0.440 | **Synergism** |
| **3** | 10.0 | 0.700 | **0.451** | 0.240 | **Strong Synergism** |
| **4** | 20.0 | 1.40 | **0.776** | 0.280 | **Synergism** |

As previously determined by Moreira et al., CLS induces significant decrease in intracellular ROS production. This considerable reduction in intracellular ROS production can be observed with CLS at concentrations higher than 5 µM. At lower concentrations of CLS (0.1-1 µM), no significant difference or reduction can be observed as shown in our study [26]. It was determined that CLS decreased ROS production even better than TQ in parallel with cell viability results. Intracellular ROS assay further supported the synergistic effect of these combinational strategies against HCT-116 cells.

Recent studies on the combinational strategies of either TQ or CLS with various anticancer drugs are presented in Table 2. Strong synergistic activity of CLS (0.2 µmol/L) combination with 0.5 µg/mL vincristine on HCT-8 cell line determined with 0.18 CI value [27]. Combinations of TQ with 5-fluorouracil (5-FU) were reported to have low combination index values (CI< 0.5), which represent strong synergistic activity on glioblastoma cells. Reduction in the required concentrations of both drugs achieved by combinational treatments compared to alone treatments. This minimizes the possible side effects of the administered drugs [28]. In different study, synergistic activity of 60 µM TQ + 33 µg/mL Cisplatin was determined on MCF-7 cells with a CI value of 0.62 [29]. In this study, the concentration of TQ was further declined down to 14 µM upon combination with 1 µM CLS. This combination enabled the strong synergistic activity with 0.383 CI value.

In two separate studies, combination of TQ with silymarin and piperine showed synergistic effects on EMT6/P cells with CI values of 0.632 and 0.788, respectively [30, 31]. In another study, enhanced autophacy in MCF-7 cell line resulted upon 0.3 µM CLS + 10 µM tamoxifen combinational treatment [32].

Table 2. Recent studies on combinational strategies related to either TQ or CLS with function of CI values

| Combinational Strategies | Cancer Cell Line | CI Value | Function of CI Values | References |
|---|---|---|---|---|
| 0.2 µmol/L CLS + 0.5 µg/mL vincristine | HCT-8 cells | 0.18 | Strong Synergism | [27] |
| 33 µM TQ + 10 µM 5-Fluorouracil | U-251MG glioblastoma | 0.31 | Strong Synergism | [28] |
| 14 µM TQ + 1 µM CLS | HCT-116 cells | 0.383 | Strong Synergism | This study |
| 60 µM TQ + 33 µg/mL Cisplatin | MCF-7 cells | 0.62 | Synergism | [29] |
| 29.95 µM TQ + 25.23 µM Silymarin | EMT6/P Cells | 0.632 | Synergism | [30] |
| 425 µM piperine + 80 µM TQ | EMT6/P cells | 0.788 | Synergism | [31] |
| 0.3 µM CLS + 10 µM Tamoxifen | MCF-7 cells | Not available | Not available | [32] |

Overall, in this present study it was first time shown that the combination of TQ and CLS decreased the dose requirement compared to the alone administration of these drugs. Reducing the concentration of chemical agents required to treat colon cancer decrease the risk of any toxic, adverse effect of these drugs. Based on CompuSYN software, the combination of these two drugs showed strong synergistic activity against HCT-116 colon cancer cells. Cancer cells shows elavated levels of ROS compared to normal cells. This high level of ROS production can be reduced in dose dependent manner with TQ and CLS. Reduced ROS production in the combinational approaches decreased the HCT-116 cell growth and viability at low concentrations. This study showed that combination of two natural agents can be used to treat colon cancer cells by decreasing the required dose. Further studies required to be performed prior to clinical trails. These studies can be conducted to reveal the molecular basis of the action of combinational treatments on the specific cancer cell lines and also effect of combinational approaches required to be tested with in vivo models.

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# First faunistic data on soil mites (Acari: Oribatida: Punctoribatidae, Phenopelopidae) from the Acarlar Longoz Forest

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**Abstract**

In this study, soil mites found in the Acarlar Longoz Forest in Sakarya Province, Turkiye, which is the largest longoz of country, were investigated for the first time. The aim was to better understand the mite fauna of Türkiye by determining the species belonging to the families Punctoribatidae and Phenopelopidae (Acari: Oribatida). Knowledge of the oribatid mite fauna of Türkiye is very limited. The samples were collected in May, 2022. Three species belonging to the families Punctoribatidae and Phenopelopidae were recorded. The species *Punctoribates (Minguezetes) palustris* (Banks, 1895) and *Peloptulus (Sacculoptulus) sacculiferus* (Weigmann, 2008) are first records from Türkiye. SEM images of the species are presented.

**Keywords:** Acarlar Longoz Forest, First record, Phenopelopidae, Punctoribatidae, Türkiye

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## Acarlar Longoz Ormanı'ndan toprak akarları (Acari: Oribatida: Punctoribatidae, Phenopelopidae) hakkında ilk faunistik veriler

**Özet**

Bu çalışmada Türkiye'nin tek parça halindeki en büyük longoz (subasar) olan Acarlar Longoz ormanında bulunan toprak akarları faunistik açıdan ilk kez incelenmiştir. Punctoribatidae ve Phenopelopidae (Acari: Oribatida) familyalarına ait türlerinin belirlenerek Türkiye akar faunasına katkıda bulunmak amaçlanmıştır. Örnekler Acarlar Longoz Ormanı'ndan 2022 yılı Mayıs ayında toplanmıştır. Türkiye'nin oribatid akar faunası hakkında bilgi çok sınırlıdır. Bu araştırma sırasında Acarlar Longoz Ormanı'ndan Punctoribatidae ve Phenopelopidae familyalarına ait üç tür kaydedilmiştir. *Punctoribates (Minguezetes) palustris* (Banks, 1895) ve *Peloptulus (Sacculoptulus) sacculiferus* (Weigmann, 2008) türleri Türkiye'den ilk kez kaydedilmiştir. Türlerin SEM görüntüleri sunulmuştur.
**Anahtar kelimeler:** Acarlar Longoz Ormanı, yeni kayıt, Phenopelopidae, Punctoribatidae, Türkiye

## 1. Inroduction

Microhabitats in forest soils provide fairly stable and suitable conditions for their inhabitants, and therefore the species richness of the soil mesofauna (Mesostigmata, Oribatida, Collembola, Nematoda) in the old forest soils is quite high [1].

The oribatida is the most species rich suborder of Acari. There are 162 families and over 11,000 described species belonging to the suborder Oribatida, with nearly 4,000 of these species found in the Palearctic region [2,3].

The family Mycobatidae Grandjean 1954 was considered as a junior synonym of Punctoribatidae Thor, 1937 by different authors [4,5]. The family Punctoribatidae has 101 species in 12 genera [2]. The characteristic features of the family Punctoribatidae are the presence of movable or semimovable slender pteromorphs, tectum on the anterior margin of notogaster, shiny or variously ornamented notogaster, lamella with cuspides and translamella, and 10 pairs of notogastral setae. The family Punctoribatidae has 101 known species in 12 genera [6-8].

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The family Phenopelopidae Petrunkevitch, 1955 has 99 known species in 4 genera [2]. The characteristic features of the family are large body size (400-1000 micrometers), notogaster covered in a thick cerotegumental layer, prodorsal lamella flat and blade like, more or less pointed rostrum, movable or semimovable pteromorphs, presence of area porosae and 8-10 pairs of notogastral setae [9,10].

Five species belonging to family Punctoribatidae have been recorded from Türkiye, namely: *Minunthozetes (M.) pseudofusiger* (Schweizer, 1922), *Minunthozetes (M.) semirufus* (Koch, 1841), *Punctoribates (P.) punctum* (Koch, 1839), *Punctoribates (P.) angulatus* Bayartogtokh, Grobler and Cobanoglu, 2000, and *Punctoribates (Minguezetes) hexagonus* Berlese, 1908 [11-14]. At present, eight species belonging to family Phenopelopidae are known from Türkiye, namely: *Eupelops acromios* (Hermann, 1804), *Eupelops nepotulus* (Berlese, 1916), *Eupelops torulosus* (Koch, 1839), *Eupelops sulcatus* (Oudemans, 1914), *Eupelops occultus* (C.L. Koch, 1835), *Eupelops curtipilus* (Berlese, 1916), *Peloptulus phaeonotus* (Koch, 1844) and *Peloptulus montanus* (Hull, 1914) [14-18].

Acarlar Longoz Forest is bordered by Denizköy, Taşlıgeçit and Camitepe villages in Sakarya Province, northern Türkiye. Its width is 250–1250 m and it is approximately 7.5 km long in the east-west direction. The length of the coast is 12 km. It is a typical coastal set lake in terms of its formation. It is bordered by sand dunes with a height of 20-25 m between the Black Sea and the Longoz and low hills with an average height of 100 m in the south. It is located 6 km west of the Sakarya River, and its waters flow into the Sakarya River via the Okçu Stream. It has been reported that ash (*Fraxinus* sp.), alder (*Alnus* sp.), beech (*Fagus* sp.) and elm (*Ulmus* sp.) species are common in the forest area [19].

In this study, the aim was to contribute to a better understanding of the oribatid fauna of Türkiye by investigating the punctoribatid and phenopelopid mites of Acarlar Longoz Forest, which has unusual and distinctive wetland microhabitats, and has not been studied in terms of oribatid mite fauna before.

## 2. Materials and methods

The study area was Acarlar Longoz Forest located in the Marmara Region of Türkiye (Figure 1). Acarlar lake is one of the best models of the Longoz forest ecosystem in Türkiye. It is located about 6 km west of the point where the Sakarya River flows in to the Black Sea. The lowest altitude is 2 m and the highest altitude is 50 m. The Longoz forest area, which runs parallel to the coast, is approximately 1.5 km inland from the Black Sea coastline and is 7.5 km long. The area of the lake is about 2,517 hectares. It is covered with an dense vegetation and forest and it has Longoz features due to its wetland characteristics. The Longoz forest shelters rich flora and fauna due to its humid, wetland conditions [20]. The locations where the examined materials were collected are shown in Figure 2.

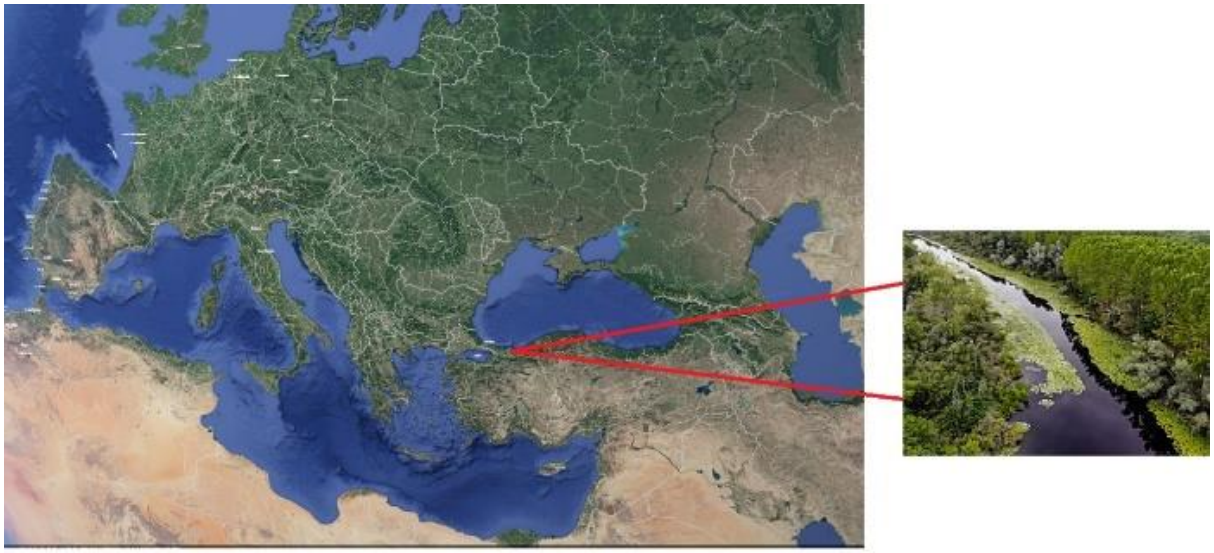

Figure 1. Map and view of the study area (Google Maps)


Figure 2. Localities A2, A3, A5.

Soil samples were collected from the Acarlar Longoz Forest located in Sakarya Province, Türkiye in May, 2022. The specimens were extracted using a Berlese funnel apparatus and sorted under an Olympus SZX51 stereo-microscope. The specimens were then examined under light (Leica DM 1000) and scanning electron (JEOL JSM 6060 LV, FEI Quanta FEG 450) microscopes. All measurements are given in micrometers (µm). Balogh and Balogh 1992, and Weigmann 2006 [6,8] were used for terminology and identification. The examined materials are stored at the Acarology Laboratory of the Science Faculty, Sakarya University, in Sakarya, Turkiye

## 3. Results

***Minunthozetes (M.) semirufus* (Koch, 1841) (Zetes) (Figure. 3)**

Family: Punctoribatidae

Genus: *Minunthozetes*

Subgenus: *Minunthozetes* (*Minunthozetes*)

Syn: *Punctoribates bicornis* Berlese, 1908, *Oribata fusigera* Michael, 1884, *Minunthozetes major* Mihelčič, 1957

Description.

Material Examined: A3, 41°11′86.8″ N 30°54′80.8″ E, 14 May 2022, soil where ivy was growing under an alder tree (*Alnus* sp.), Acarlar Longoz forest Turkey.

Measurements: Average body length 256 µm, average body width 163 µm (n=5).

Integument: Colour reddish brown. Prodorsum: Rostrum rounded. Sensillus fusiform with sharply pointed head, interlamellar and lamellar setae thin and smooth, rostral setae invisible. Lamellar cups and translamella present.

Notogaster: Notogaster oval with smooth surface. Notogastral setae short. Pteromorphae curled down. Areae porosae circular.

Ventral region: Apodems well developed, anal and genital plates medium not clear and distance between each other as long as the length of anal plate. Two pairs of anal, three pairs of adanal, one pair of aggenital and six pairs of genital setae present. Epimeral setation 3-1-3-3.

Distribution: Palearctic region and Canada [2]. Reports from Turkiye: Giresun and Samsun Provinces [11].

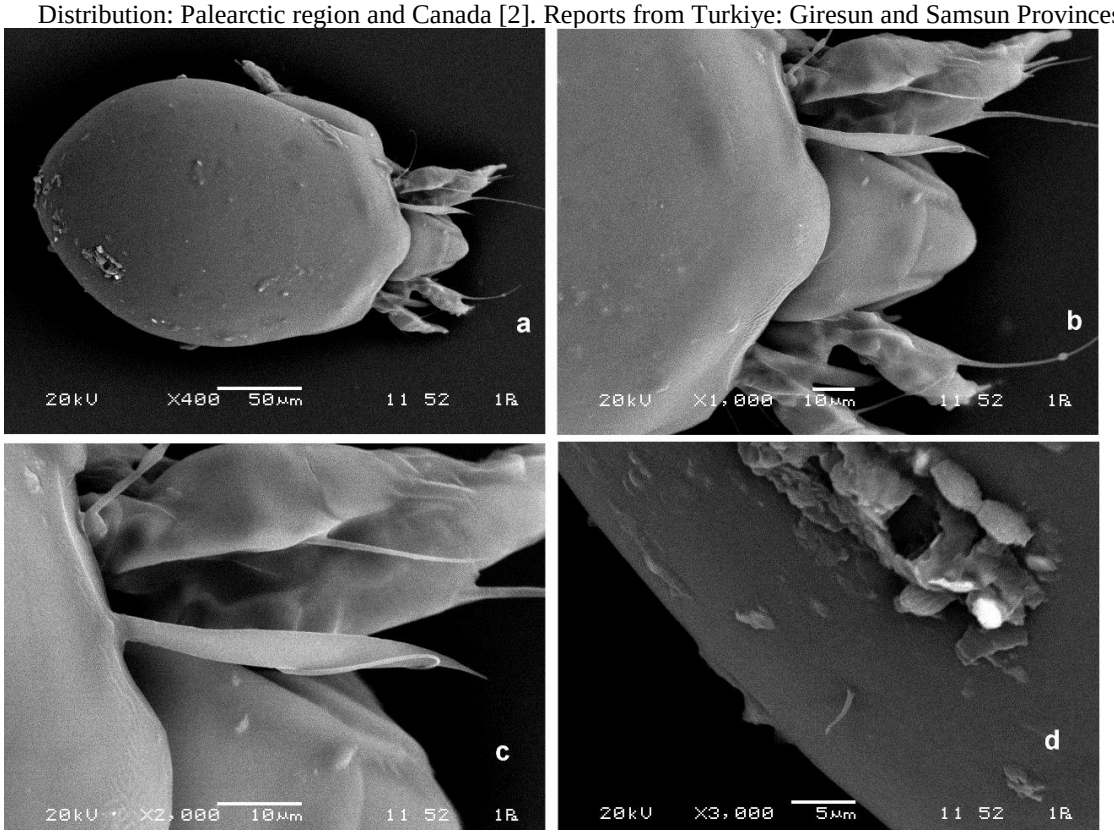


Figure 3. SEM image of *Minunthozetes* (*M.*) *semirufus*, a-Dorsal view, b-Prodorsum, c-Sensillus, d-Notogastral setae

***Punctoribates* (*Minguezetes*) *palustris* (Banks, 1895) (Figure. 4)**

***Punctoribates* (*Minguezetes*) *palustris* (Banks, 1895) (Figure. 4)**

Family: Punctoribatidae

Genus: *Punctoribates*

Subgenus: *Punctoribates* (*Minguezetes*)

Syn: *Galumna armipes*

Description.

Material Examined: A5, 41°11′87.5″ N 30°55′36.0″ E, 14 May 2022, soil from shrubland, Acarlar Longoz Forest, Turkiye.

Measurements: Average body length 433 µm, average body width 344 µm (n=3).

Integument: Colour dark brown.

Prodorsum: Rostrum rounded, rostral tetae thin. Anterior notogastral tectum relatively large, almost covering the prodorsum. Sensillus with short stalk and long fusiform head.

Notogaster: Anterior margin with strong u shaped tectum medially. Notogastral setae invisible, only represented by alveoli.

Ventral region: Anal plate bigger than genital plate and distance between each other shorter than the length of anal plate. Genital plates with longitudinal striations. Two pairs of anal, three pairs of adanal, one pair of aggenital and six or seven pairs of genital setae present. Epimeral setation 3-1-3-3.

Distribution: Holarctic region: Nearctic (frequent) and Western Palearctic (Italy and Caucasus) [2]. New record for the Turkish fauna.

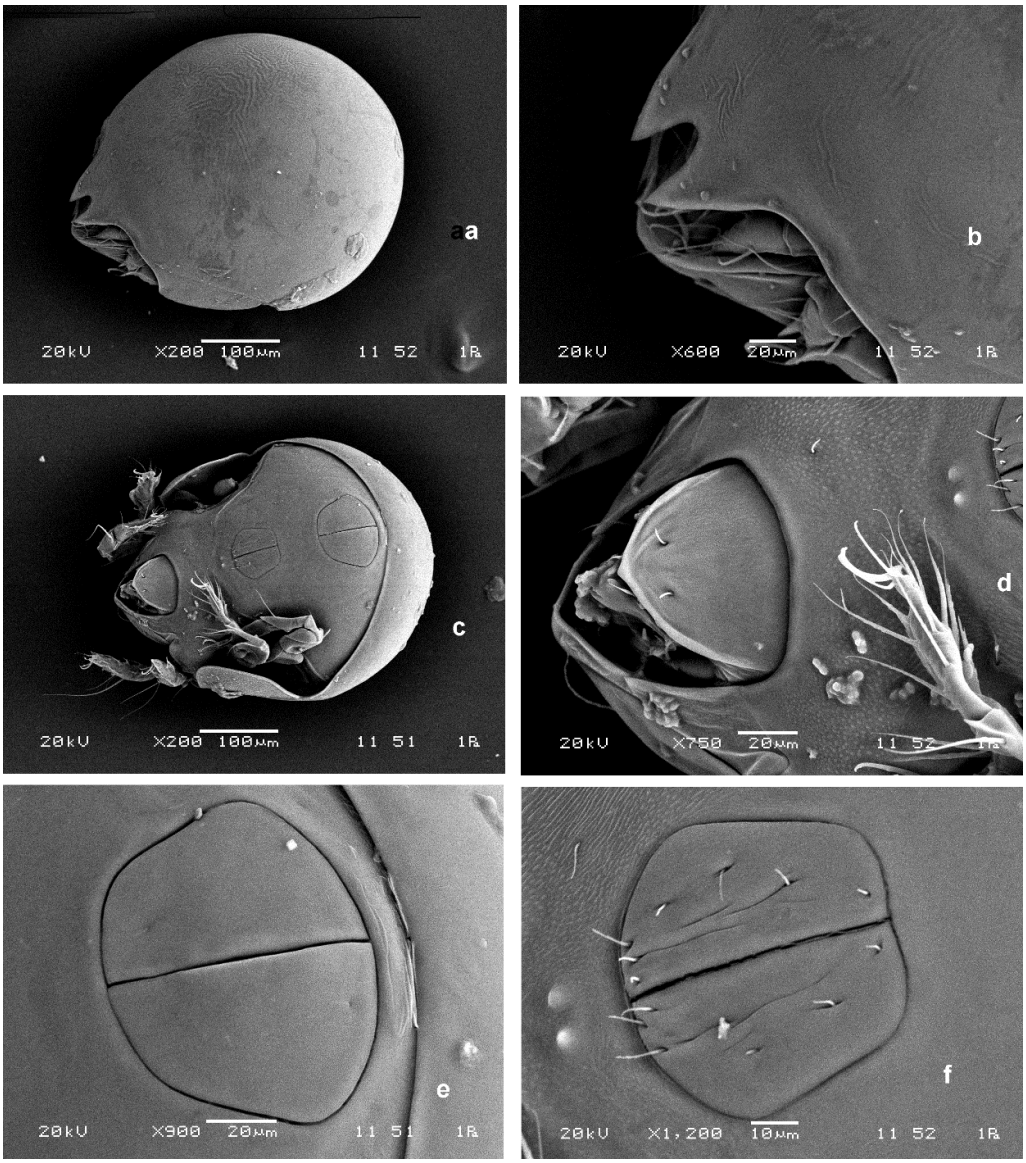


Figure 4. SEM image of *Punctoribates* (*Minguezetes*) *palustris*, a-Dorsal view, b-Prodorsum, c-Ventral view, d-Gnathosoma, e-Anal plate, f-Genital plate

***Peloptulus (Sacculoptulus) sacculiferus*** (Figure. 5)
Family: Phenopelopidae
Genus: *Peloptulus*
Subgenus: *Peloptulus* (*Sacculoptulus*)

Description.

Material Examined: A2, 41°11′87.7″ N 30°54′54.0″ E, 14 May 2022, Grassy soil, Acarlar Longoz Forest, Turkiye.

Measurements: Average body length 492 µm, average body width 330 µm (n=2).

Integument: Colour brown.

Prodorsum: Rostrum narrow and slightly rounded. Lamellae wide, lamellar setae barbed and originating on anterior edge of cusps; between cusps V-shaped narrow interspace present. Sensillus with long stalk and flattened claviform granulated head, interlamellar setae short.

Notogaster: with large movable pteromorphs connected to each other medially with broad bridge. Pteromorphs and bridge with granulated cerotegument on surface. Notogastral setae c2, lm, la, lp, h2 and h3 spicular and finely granulated. Notogastral setae h1 and p1 bacilliform.

Ventral region: Genital plate bigger than anal plate and distance between each other longer than the length of genital plate. Two pairs of anal, one pair of aggenital and six pairs of genital setae present, adanal setae invisible. Epimeral seta formula: 3-1-3-3.

Distribution:Portugal [2, 21]. New record for the Turkish fauna.

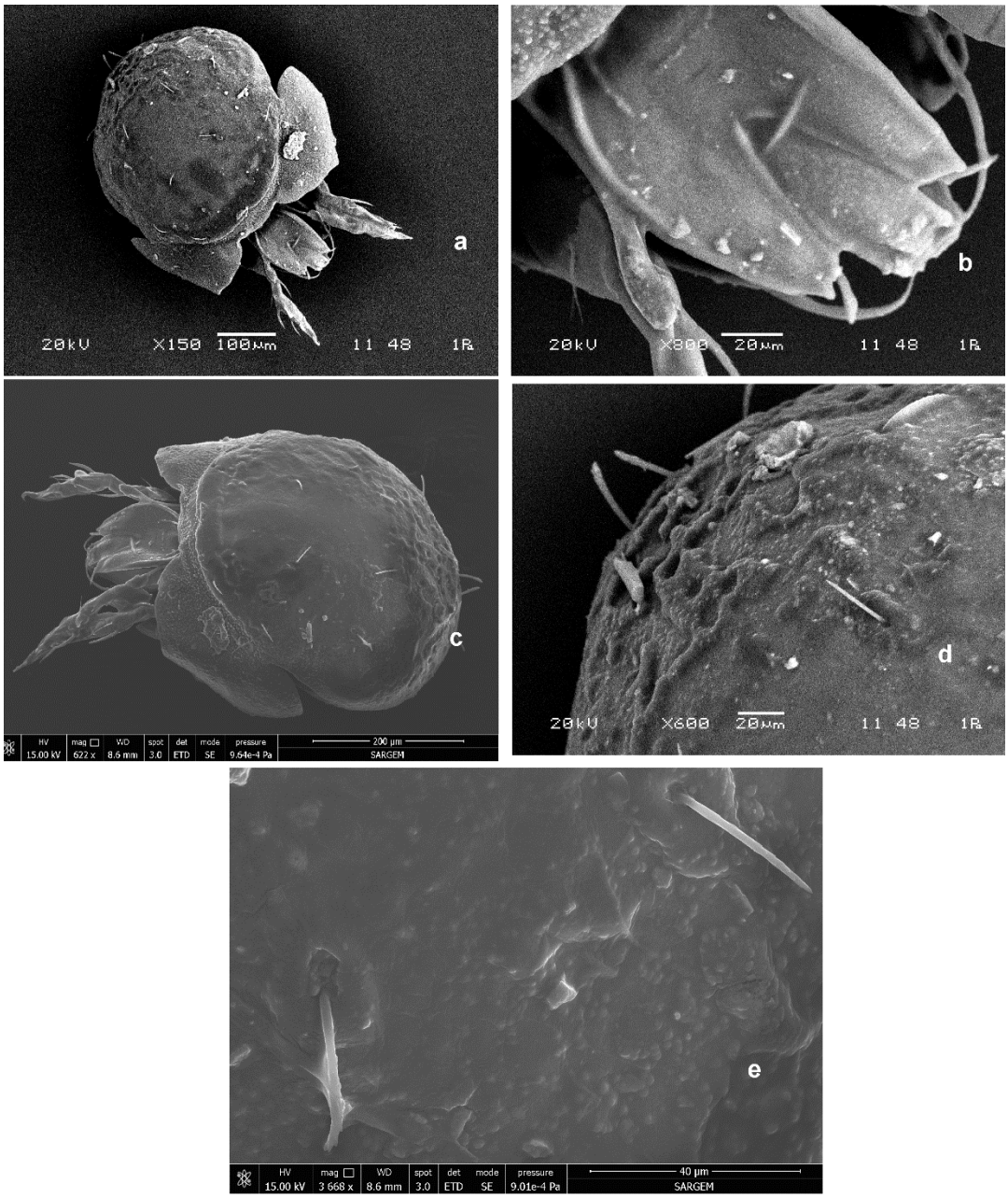


Figure 5. *Peloptulus* (*Sacculoptulus*) *sacculiferus* a- SEM image of dorsal view, b- Prodorsum, c- FESEM image of dorsal view, d- SEM image of setae *lp* and *h1*, e- FESEM image of setae *lp* and *h3*

## 4. Conclusions and discussion

## 5. Discussion and conclusions

In this study, soil mites of the Acarlar Longoz Forest, which is the largest longoz in Türkiye were investigated faunisticaly for the first time. Species belonging to the families Punctoribatidae and Phenopelopidae (Acari: Oribatida) were evaluated in the present study. *Minunthozetes (M.) semirufus* and *Punctoribates (Minguezetes) palustris* (Punctoribatidae) and *Peloptulus (Sacculoptulus) sacculiferus* (Phenopelopidae) were recorded.

*Minunthozetes (M.) semirufus* is known from the Palearctic Region and Canada, and has been reported from Giresun and Samsun Provinces, Türkiye [11]. This species is abundant in meadows, pastures, grasslands and soil surface moss [23]. We found it in grassy soil under analder tree. In this study, the average body size *of M. (M.) semirufus* specimens was 256/163µm. These measurements are in agreement with Bayartogtokh et al. (2002) but are smaller than the European specimens reported by Bayartogtokh et al. [11].

The second species, *Punctoribates (Minguezetes) palustris*, has frequently been reported from the Nearctic Region and also the Western Palearctic (Italy and Caucasus) [2]. This study reports it for the first time from Turkiye. *Punctoribates (Minguezetes) palustris* has been reported from bogs and flooded forests [24]. We also found it in flooded forests. The body length was 437-470 µm [25] and the body lengths of the our specimens were similar. This is the first record of the species from Turkey.

*Peloptulus* (*Sacculoptulus*) *sacculiferus* has previously only been recorded from the type locality; Portugal - coastal region, habitats adjacent to the sea, lagoon and river bank with meadow and shrub vegetation [2, 21]. Our specimens were also recorded from a longoz in a coastal area. Average body length and body width were 485/315 µm; the body dimensions of our specimens (492/330 µm) closely resemble provided earlier [21].

The sensillus in our specimens extends beyond the translamellar ridge, being longer than in the original description of the species in which it only comes up to the level of the translamella [21]. The other morphological features of our specimens are in agreement with the features given by Weigmann [21]. This is only the second record of *Peloptulus* (*Sacculoptulus*) *sacculiferus* worldwide, having previously only been recorded from Portugal [2,21]. Thus, it can be said that to date it is known to have a Mediterranean distribution.

The Acarlar Floodplain Forest in Sakarya Province, Turkiye is a vital protected wetland area that represents an ecosystem type that continues to be lost in many areas of the world. This area is likely to have many other unreported invertebrates, including mites, and possibly undescribed species.

**Acknowledgements**

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# Prediction of potential geographic distribution of *Capparis spinosa*

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**Abstract**

*Capparis spinosa* is a medicinal plant with economic (e.g., food, animal breeding, medicine) and ecological (e.g., erosion control, fighting wildfires) importance that is distributed in the western and southern coastal regions of Turkey. The MaxEnt model was used to simulate potential distribution areas of *C. spinosa* with the effect of environmental conditions. The results showed that the potential suitable area of *C. spinosa* is 6109 hectares, mainly distributed below 1000 meters in Babadağ Region. It was determined that the variables contributing to the model were bedrock, elevation, topographic position index and hillshade index, respectively. The acquired model presented excellent performance according to its AUC values (Training AUC: 0.909 and test AUC: 0.906). It is thought that the results revealed in the study will provide an insight for future investigations to be carried out for the species.

**Keywords:** Caper, habitat suitability, MaxEnt, species distribution modeling

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## *Capparis spinosa*'nın potansiyel coğrafi dağılımının tahmini

**Özet**

*Capparis spinosa*, Türkiye'nin batı ve güney kıyı bölgelerinde yayılış gösteren, ekonomik (örneğin gıda, hayvancılık, ilaç) ve ekolojik (örneğin erozyon kontrolü, orman yangınlarıyla mücadele) öneme sahip tıbbi bir bitkidir. Çevre koşullarının etkisiyle *C. spinosa*'nın potansiyel dağılım alanlarını simüle etmek için MaxEnt modeli kullanılmıştır. Sonuçlar, *C. spinosa* için potansiyel uygun alanın 6109 hektar olduğunu ve Babadağ Bölgesi'nde çoğunlukla 1000 metrenin altında dağıldığını göstermiştir. Modele katkı sağlayan değişkenlerin sırasıyla ana kaya, yükselti, topoğrafik pozisyon indeksi ve gölgelenme indeksi olduğu belirlendi. Elde edilen model, AUC değerlerine (Eğitim AUC: 0,909 ve test AUC: 0,906) göre mükemmel performans sergiledi. Çalışmada ortaya çıkan sonuçların türe yönelik ileride yapılacak araştırmalara ışık tutacağı düşünülmektedir.

**Anahtar kelimeler:** Kapari, habitat uygunluğu, MaxEnt, tür dağılım modellemesi

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## 1. Introduction

Non-wood forest products (NWFP) are among the biological elements whose sustainability has been under threat in recent years due to reasons such as population growth, deforestation, and climate crisis. Globally, the market share of NWFP has been reported as approximately 7.1 billion dollars. NWFP also has a large market share in Turkey. As of 2019 data, there is an 880 million dollars market share for 250 distinct taxa in an area of 1.7 million hectares in Turkey [1]. Among these species, taxa belonging to the genus Capparis attract attention due to their multifunctional benefits.

The genus Capparis is represented by about 250 species in a wide area, including Southern Europe, Northern and Eastern Africa, Madagascar, Western, central, and southeast Asia. Among these species, *Capparis spinosa* L. (C. spinosa) and *Capparis ovata* L. (*C. ovata*) are widely distributed in Turkey with a total of 5 varieties [2]. Varieties of *C. spinosa* are mostly distributed in the western and southern coastal regions, while varieties of *C. ovata* distribute in Central Anatolia, Eastern Anatolia, Southeastern Anatolia and locally in some parts of the Black Sea [3].

The name caper, which is based on Greek, is commonly used for the *C. spinosa*, which is also known by names such as kebere, gebreotu, cat's nail among the local people. Caper is a species having a number of benefits [4]. Archaeological findings give information for the aforementioned benefits. Previous research has showed that the species was used for food and medicine in ancient times and even cultivated. At present, capers are used by many people as an ornamental plant, cultivated for purposes such as erosion control and animal breeding, consumed as food and directly commercialized by generating income [5]. In addition, there are studies showing that it is used for disease treatment such as hypertension, atherosclerosis, and circulatory disorders [5]. Furthermore, Kart [6] found evidence that these species have analgesic, anti-inflammatory, antioxidant, antitumoral, diuretic, antidiarrheal, hypoglycemic, expectorant, antibacterial, antiallergic, antihypertensive, antifungal, and antihyperlipidemic properties

When the site factors of the *C. spinosa* are examined, it is seen that it is a species with production potential on the slopes outside the irrigation area or on the slopes where no species has been cultivated previously [5]. In this sense, determining the suitable ecological demands of the species and simulating its potential distribution areas form the basis of both sustainable and more effective utilization [7]. Species distribution modeling is pointed out as the most influential method that can serve this purpose [8]. Estimating the potential distribution areas of species using species distribution models has become an important component of conservation and planning studies on rare and endangered species [9;10], biodiversity studies [11], estimation of the effects of climate change on species distribution [12], and invasive species [13] issues in recent years. In this direction, various modeling techniques have been developed over time. These methods, which can be expressed as species distribution modeling, ecological niche modeling or habitat suitability modeling depending on the structure of dependent and independent variables. They provide practical and effective results by simulating the situations of species under various ecological and climatic conditions. For these reasons, the mentioned methods are becoming popular day by day. In the present study, the potential distribution areas of *C. spinosa* in Babadağ Region (Fethiye) were determined by using Maximum Entropy (MaxEnt), which is one of the most preferred modeling methods. Many species of non-wood forest products (NWFP), including *C. spinosa*, are distributed in Babadağ Region. It is important to determine the possible distribution areas of these species. In this direction, it is thought that the results acquired from present study will contribute to the determination of the NWFP potential of the region.

## 2. Material and method

### 2.1. *Study area and species occurrence data*

The study area is Babadağ Region, which is located within the borders of Fethiye district of Muğla province. The main tree species in the region are Red pine, Black pine, Crimean junipers, Taurus cedar, Mediterranean cypress [14]. The elevation range of the study area begins at 0 meters (m) above sea level and rises to 1938 m at the peak of Babadağ. The location map indicating the study area is given in Figure 1. In the present study, field studies were carried out in 200 sample areas and the presence data of *C. spinosa* were recorded at 18 locations.

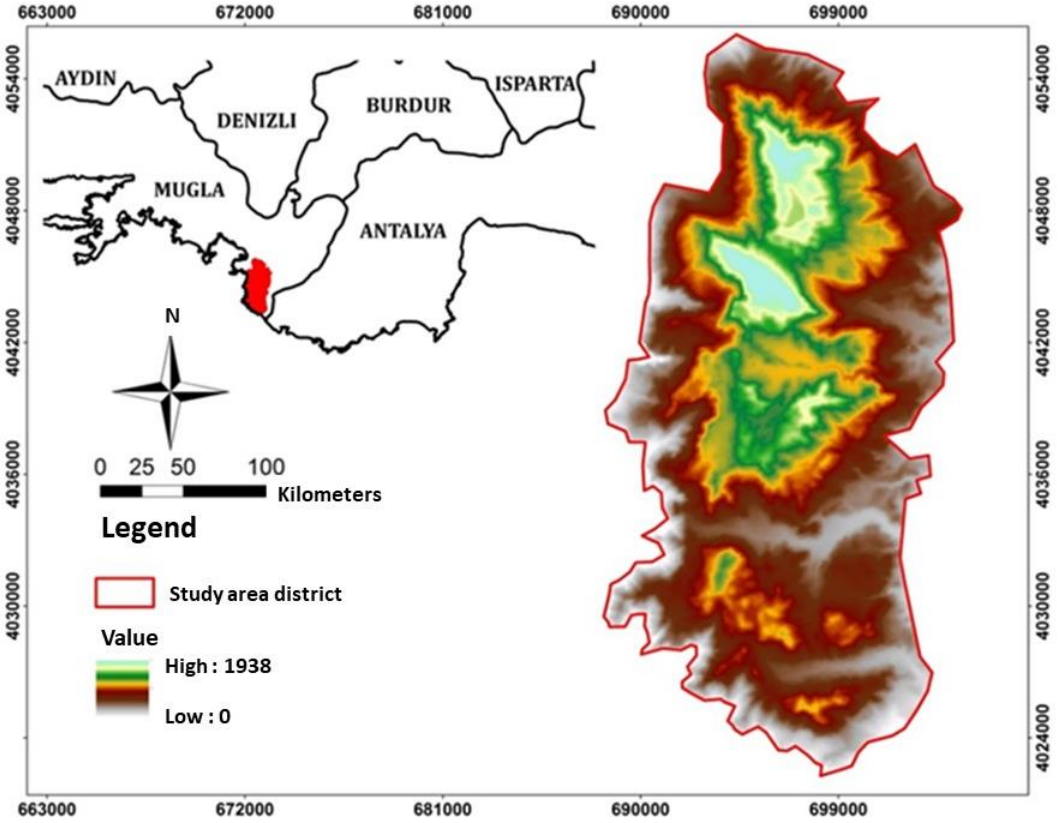


Figure 1. Location map of the Babadağ Region

*2.2. Environmental variables*

The bioclimatic variables were downloaded from WorldClim (www. worldclim.org/data/worldclim21.html) version 2.1 with 30 arc seconds spatial resolutions (~1 km). As a topographic variable, the elevation variable was first downloaded from the USGS database (www.earthexplorer.usgs.gov). Then, slope, aspect, roughness, roughness, shadowing, topographic position index bases were created by using the elevation pad in ArcMap software. Then, the layers for the aspect suitability index, heat index, and radiation index were created with the formulas in the literature. Finally, a total of 31 variables were determined by obtaining the bedrock map from the General Directorate of Mineral Research and Exploration (https://www.mta.gov.tr/en/) and the canopy shading map from the forest management data of Turkey General Directorate of Forestry.

*2.3. Variable selection and statistical analysis*

To reduce the multicollinearity issues, Pearson's correlation analysis was performed on environmental variables. Among the variables that were determined to be highly correlated (Pearson's $R \geq 0.80$) with each other, they were eliminated based on expertise. In other words, the variable known to be effective in the distribution of the species (according to the literature) was selected from two highly correlated variables [11].

Maximum Entropy (MaxEnt) method was employed to model the *C. spinosa* species. MaxEnt is one of the most preferred methods in modeling studies in recent years. The fact that the MaxEnt method, which only works with presence data, reveals more descriptive results with less data, is shown as one of the main reasons for the frequent use of the method. In the study, bootstrap option with 10 test percentages and 10 replicates were preferred as modeling parameters [15]. The bootstrap method was used because it gives better results with less data. As a result of the analyses, a potential distribution map and values ranging from 0-1 were obtained. Within the scope of the study, areas with values above 0.5 were accepted as potential areas. In this context, the final map was created by accepting the value of 0.5 as the threshold value.

Factor Analysis of Mixed Data (FAMD) method was used to reveal the potential effects of variables not included in the formation of the Maxent model. As a result of the analysis, the component with the highest explanation for *C. spinosa* was determined, and the variable with the highest correlation with that axis was associated with the distribution of the species.

## 3. Results

Previous literature has highlighted those environmental variables affect the distribution of species [16;17]. Therefore, the environmental variables to be employed in this study were determined based on the findings of these studies. Then, correlation analysis was performed between these variables to eliminate the multicollinearity problem. Pearson's Correlation analysis results are given in Figure 2.

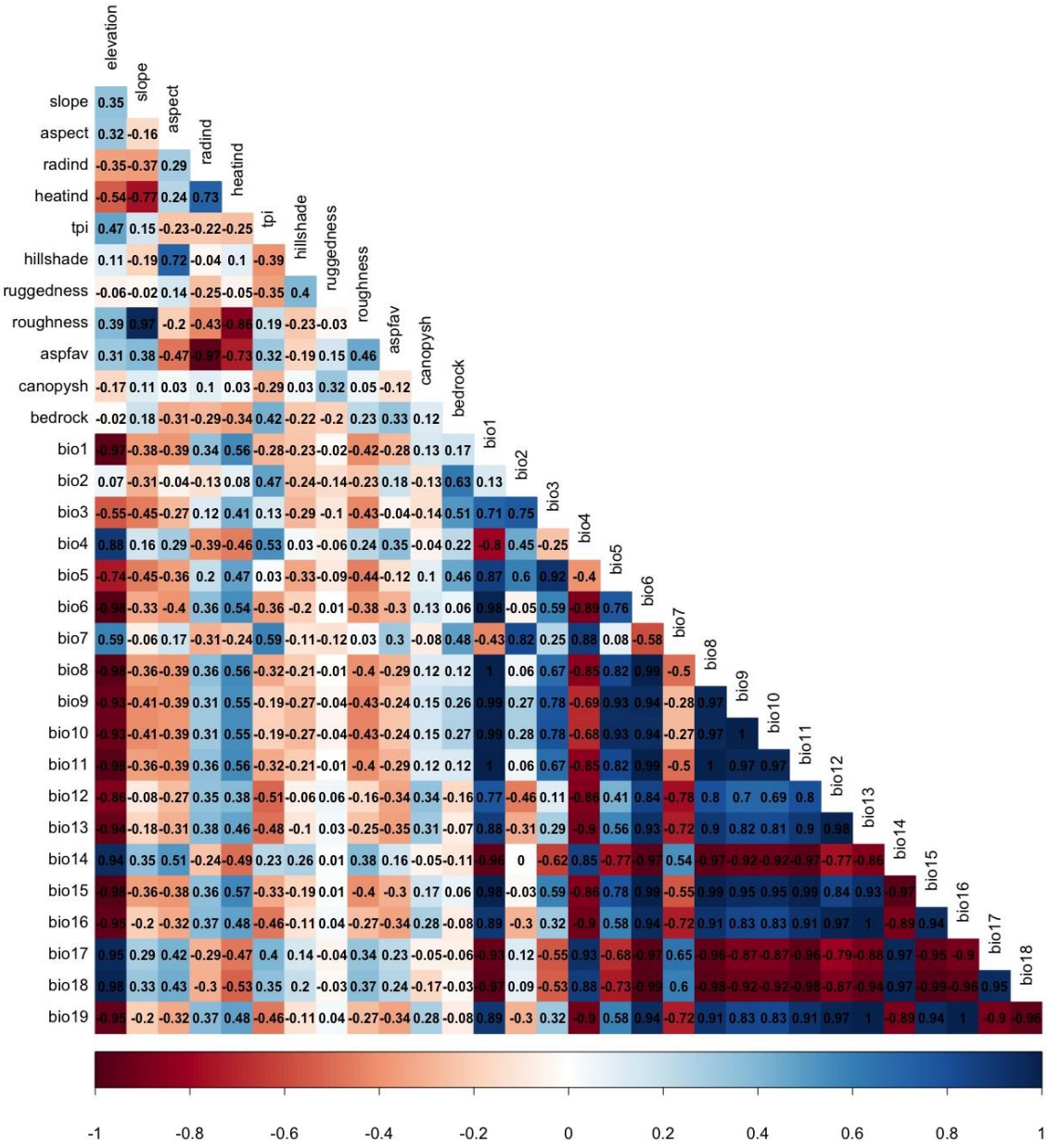


Figure 2. Results of the Pearson's Correlation analysis

As a result of this analysis, it was determined that 12 variables among the climatic and topographic variables could be used in the modeling. The variables that were included in the modeling are given in Table 1.

Table 1. Selected variables using Pearson's Correlation analysis

| **Variables** | **Variables** |
|---|---|
| Elevation | Roughness |
| Slope | Topographic position index (tpi) |
| Heat index | Canopy shading (canopysh) |
| Radiation index (radind) | Bedrock |
| Hillshade | Mean diurnal range (bio2) |
| Ruggedness | Maximum temperature of warmest month (bio5) |

In the present study, geographical distribution of *C. spinosa* that located in Babadağ Region, Fethiye, was assessed with Maximum Entropy method, considering environmental and climate variables. The Area Under Cover (AUC) value of the potential distribution model obtained as a result of the analysis was examined. The model performances were evaluated based on the AUC values (Training AUC: 0.909, Test AUC: 0.906).

The results showed that the most important variables affecting the distribution of the *C. spinosa* were bedrock 1 and 5 (alluvial and ophiolite), elevation, tpi, and hillshade, respectively. The marginal response curves of the variables indicated a negative relationship with elevation. The effects of the variables that create the model and the relationship between the target species and the response curves are examined. The responding curves of the variables contributing to the model are given in Figure 3.

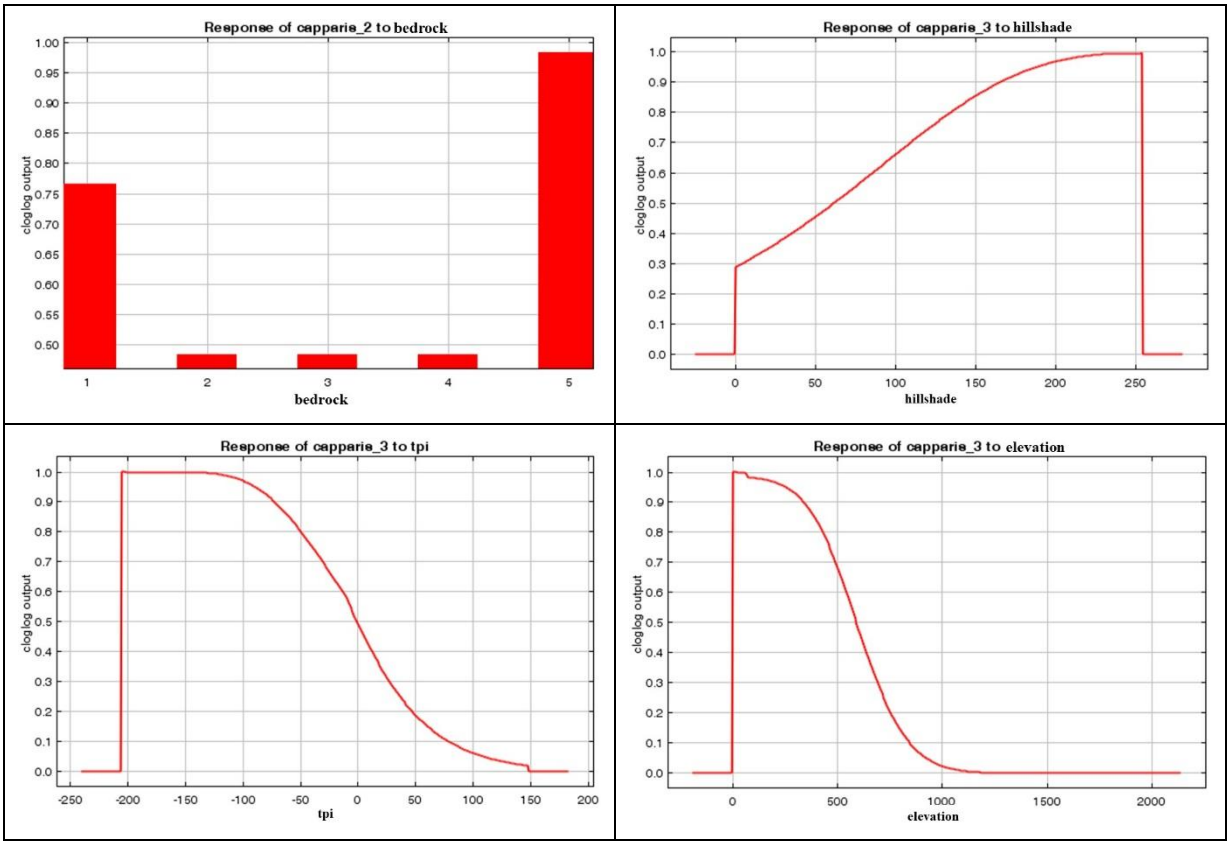


Figure 3. Marginal response curves of the variables contributing to the model

The prediction map provided by the MaxEnt program was arranged using ArcGIS software. The resulting map shows the potential distribution and suitable areas of the species *C. spinosa* (Figure 4a). In addition, the potential distribution map was classified according to 0.5 threshold value in terms of predictive values, and then a suitability map was created (Figure 4b).

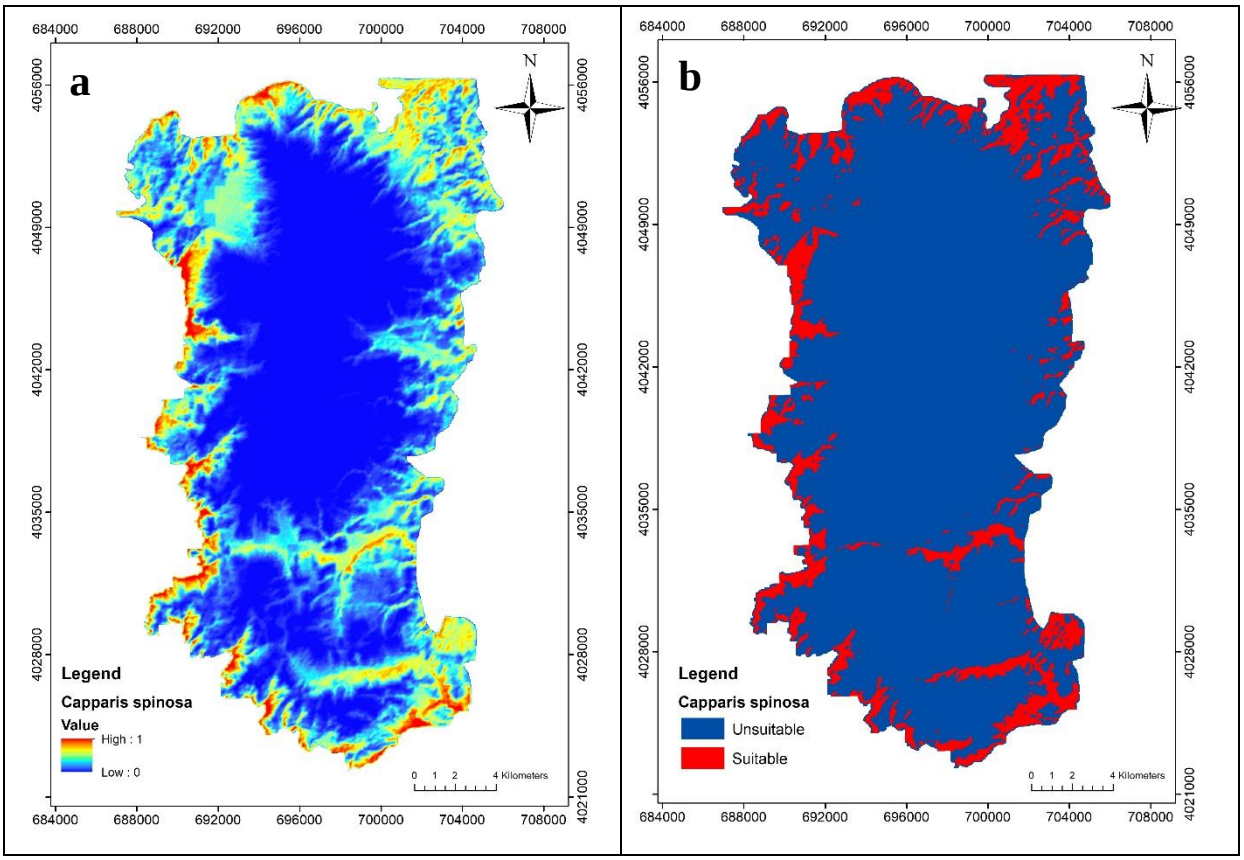


Figure 4. Potential distribution (a) and suitable areas (b) maps (0.5 threshold) of *C. spinosa*

On the figure 2b, the potential areas where the *C. spinosa* species is most likely to exist are displayed in red while the unsuitable areas for the species are blue.

Only 4 of the variables included in the modeling process (bedrock, hillshade, tpi and elevation) were included in the model obtained by the MaxEnt method. However, other variables are also likely to have an effect on the distribution of the species directly or indirectly. In modeling studies, evaluations are generally made on the variables that create the model. However, this does not mean that other variables have no effect. It is obvious that the predictive maps obtained with the variables that create the model offer practical application opportunities in practice. It is one of the best examples that can be given to the aforementioned applications, especially since it is an excellent base for breeding studies to be carried out in the natural areas of the species. However, this should not cause other variables to be ignored. Therefore, FAMD was applied in order to determine the effects of the variables not included in the MaxEnt model. The reason for using FAMD is that there are both continuous and categorical variables in the data. Principal component analysis (PCA) is preferred if all of the data are continuous, and multiple correspondence analysis (MCA) is preferred if all of the data are categorical. However, FAMD can work with both data types and is expressed as a version of PCA that can work with both categorical and continuous data [18].

The predictive values in the model map obtained by the MaxEnt method were also included in the MAFD as a variable (*C. spinosa*) to determine the effect of the variables on the species distribution. The eigenvalues and variance explanation rates obtained for the components as a result of MAFD are presented in Table 2.

Table 2. Eigenvalues and variance explanation ratios of components

| Components | Eigenvalue | Percentage of variance | Cumulative percentage of variance |
|---|---|---|---|
| **Component 1** | 3.932377 | 35.748886 | 35.74889 |
| **Component 2** | 2.035578 | 18.505253 | 54.25414 |
| **Component 3** | 1.438370 | 13.076094 | 67.33023 |
| **Component 4** | 1.062831 | 9.662096 | 76.99233 |
| **Component 5** | 0.774047 | 7.036791 | 84.02912 |

While commenting on the components, it is expected that the eigenvalue should be greater than 1% and the variance explanation rate greater than 10% [19]. Component 1 component 2 and component 3 meet these conditions.

That's why, evaluations were made on these three variables. Square Cosine values ($cos^2$) were used to evaluate the relations of the variables with the components (Table 3).

Square Cosine is a measure obtained as a result of PCA. When PCA transforms the dataset into a set of interconnected components, it obtains weight vectors that represent the relationship of each component with the original dataset. Sometimes, "cosine similarity" is used to measure the similarities between these weight vectors. Cosine similarity represents the angle between two vectors and expresses the similarity between vectors as an angle.

Square Cosine is calculated by taking the square of the cosine similarity value. It is a measure that assesses the similarity of each component with other components and is used in interpreting PCA results. If the square cosine value is close to 1, it indicates that two components are highly similar. If the value approaches 0, it means that two components are completely opposite to each other. These values are used to understand the relationships between variables in the dataset and to evaluate the accuracy of PCA results.

Table 3. Square Cosine values of the components

| | **Component 1** | **Component 2** | **Component 3** |
|---|---|---|---|
| **slope** | 0.795412 | 0.000000 | 0.000005 |
| **radind** | 0.000203 | 0.246500 | **0.148542** |
| **heatind** | 0.597991 | 0.003228 | 0.003429 |
| **ruggedness** | 0.161884 | 0.035264 | 0.002769 |
| **roughness** | 0.751617 | 0.000100 | 0.000927 |
| **bio2** | 0.003275 | 0.333213 | 0.002923 |
| **bio5** | 0.097201 | 0.067752 | 0.001705 |
| **canopysh** | 0.001135 | 0.068868 | 0.002406 |
| ***C. spinosa*** | 0.061751 | 0.001242 | **0.261998** |

As seen in Table 3, *C. spinosa*, the variable belonging to the predictive values, showed the highest $cos^2$ value with component 3. Therefore, the relationship of *C. spinosa* with other variables was interpreted only through component 3. In addition, it was seen that the variable with the highest $cos^2$ value with component 3 was radind. It was determined that the values of other variables were quite low. Therefore, only radind could be associated with *C. spinosa*.

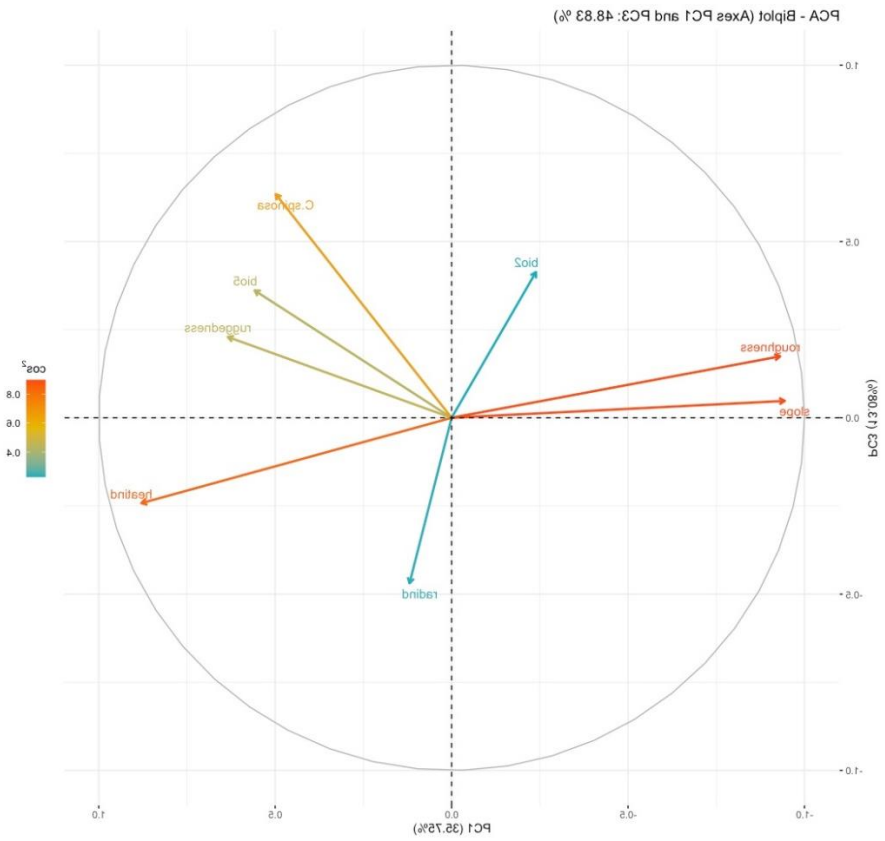


Figure 5. MAFD graph

According to component 3, there exists a negative relationship between C. spinosa and radial variables as illustrated in Figure 5. This relationship is determined through the utilization of aspect values that range from 0 to 1. As the value approaches 1, it signifies southern aspects characterized by greater insolation, whereas lower values correspond to northern aspects characterized by comparatively lower temperatures.

## 4. Conclusions and discussion

*C. spinosa* is a species that exhibits a widespread distribution across a considerable geographic range, while also holding economic significance [20;21]. Studies conducted on the species in different regions have revealed that factors influencing the species' distribution can vary depending on regional disparities [22]. Therefore, determining the habitat requirements of C. spinosa in different regions is of paramount importance for sustainable utilization. In this context, the MaxEnt method was employed in the study to identify the factors affecting the distribution of C. spinosa. Subsequently, the Factor Analysis of Mixed Data (FAMD) method was utilized to assess the extent to which non-included variables affect the species' distribution. According to MaxEnt results, it has been determined that the model performance and predictive power are excellent [23]. Swets [24] categorized AUC values as excellent (AUC>0,90), good (0.90>AUC>0.81), fair (0.80>AUC>0.71), poor (0.70>AUC>0.61) and fail (AUC<0.60). The marginal response curves of the variables indicated a negative relationship with elevation. The effects of the variables that create the model and the relationship between the target species and the response curves are examined. The responding curves of the variables contributing to the model are given in Figure 3. When the marginal response curves were examined, it was determined that the species preferred alluvial, and ophiolitic types as bedrock preference. The hillshade index also indicate that potential distribution areas of *C. spinosa* decreased in shading conditions with higher 200 values. In other words, the hillshade index showed that the species prefers shaded areas in all other aspects, except for the northern aspects. Topographic position index, which is another variable that contributes to the model, it is seen that the areas where *C. spinosa* species are widely distributed are plain and concave areas. A negative relationship was observed between elevation, which is the last variable contributing to the model, and potential suitable areas. In terms of studies, there are findings stating that the *C. spinosa* prefers low elevations, that it is a species that makes tap roots and can take its roots deep [5]. Therefore, the determination of elevation as an important variable for the present study might be due to the negative relationship between elevation and soil depth. On the other hand, Ashraf et al. [20] identified bio12 as one of the most important variables affecting the distribution of *C. spinosa*. In this study, it was determined that elevation showed a very high correlation with bio12 (Figure 2), and in this respect, it was aligned with the mentioned study. It has been concluded that there is a negative, but relatively more curvilinear relationship with the hillshade and tpi variables contributing to the model, similar to elevation. In terms of bedrock variable, it has been determined that there are 5 (alluvial, carbonate, limestone, detrial and ophiolitic) different bedrock types in the study area, of which alluvial, limestone and ophiolitic type rocks represent potential suitable areas. The areas with alluvial, limestone, and ophiolitic rock structures below 1000 meters in Babadağ Region are potential suitable areas for *C. spinosa*. In addition, it prefers plain and concave areas and shady areas on slope positions except for the northern. As can be understood from the evaluations of the model obtained from the MaxEnt method, the results coincide with the findings of the researchers regarding the *C. spinosa* [19;21].

The predictive map obtained for C. spinosa has been thresholded at a value of 0.5, categorizing areas into suitable and unsuitable categories. As a result of this classification, a predictive map consisting of two distinct colors has been generated. It is evident from this map that the species shows a preference for areas within the study area characterized by noticeably lower elevations. These areas also correspond to regions with relatively higher soil depth. These findings provide valuable insights into the distribution of the species within the study area.

The natural regeneration of *C. spinosa* primarily occurs through seed dispersal, although it is relatively weak. The species' seeds can remain dormant in the soil for extended periods and sprout after a few years. Therefore, it is crucial to investigate the seed germination biology, primary agricultural techniques for plant breeding, and the species' habitat requirements. The root system of *C. spinosa* reaches deep underground moisture sources, typically at depths of 10-18 meters or more [21]. Furthermore, its extensive root system and ground cover effectively prevent soil erosion. The plant stores moisture in its leaves, making it resilient against drought and summer heat, eliminating the need for irrigation. Additionally, *C. spinosa* is fire-resistant, making it suitable for planting in fire breaks to combat wildfires. The species also has significant economic value for food and medicinal purposes. Therefore, conserving and cultivating *C. spinosa* in appropriate natural areas is crucial for sustainable utilization. Modeling studies are valuable tools for understanding the ecological needs of the species. However, the study's findings suggest that relying solely on modeling methods may not sufficiently characterize the species' distribution. Consequently, incorporating different statistical approaches would be beneficial, and the results obtained using FAMD in this study serve as a notable example in addressing the aforementioned issue.

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# The effect of micro topographic features on the site selection of European ground squirrels (*Spermophilus citellus*): A case study from Tekirdağ province, Thrace Peninsula (Türkiye)

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**Abstract**

The scientific determination of the habitat selection of European ground squirrels (*Spermophilus citellus*), whose population has decreased significantly in recent years due to various human activities, is essential for the survival of this endangered species. Micro topography is one of the main factors affecting the habitat selection of this creature, which hibernates in its individual caves for a long period of time from early fall to early spring. Therefore, the aim of this study was to investigate the effect of micro topographic features on the nest selection of European ground squirrels. The study was shaped according to the results of a sample area in Tekirdağ province in the Thracian Peninsula of Türkiye (Turkish Thrace), one of the southernmost distribution areas of European ground squirrels in Europe. The main data of the study consisted of high-resolution digital elevation model (DEM) and ortho-photo images obtained using an unmanned aerial vehicle (UAV) during field studies. These data were processed with Geographic Information Systems (GIS) techniques using an ecological niche modeling (ENM) approach. As a result of the study, it was determined that among the micro topography features in the sample area, the aspect factor was more important (56.412%) in the nest preference of the species. The results of the study shed light on the plans to be made for the maintenance and protection of potential and existing habitats, as well as supporting specific measures to be taken to strengthen the population of the species, which is under threat of extinction, and to improve habitat features. It can also be used to expand areas important for the conservation of the species and improve the success of future reintroduction or introduction programs.

**Keywords:** Micro topography, aspect factor, GIS, DEM, Mediterranean climate.

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# Avrupa yer sincaplarının (*Spermophilus citellus*) yer seçiminde mikro topografya özelliklerinin etkisi: Tekirdağ ilinden örnek bir çalışma, Trakya Yarımadası (Türkiye)

**Özet**

Çeşitli insan aktivitelerine bağlı olarak son yıllarda popülasyonunda ciddi bir azalma görülen Avrupa yer sincaplarının (*Spermophilus citellus*) habitat seçiminin bilimsel bir şekilde belirlenmesi, nesli tükenme tehlikesi altında olan bu canlının hayatta kalması açısından çok önemlidir. Sonbaharın başlarından ilkbaharın başlarına kadar uzun bir süre boyunca bireysel yuvalarında kış uykusuna yatan bu canlının habitat seçiminde etkili temel faktörlerden birisi de mikro topografya özellikleridir. Dolayısıyla bu çalışmada Avrupa yer sincaplarının yuva seçiminde mikro topografya özelliklerin etkisinin incelenmesi amaçlanmıştır. Çalışma, Avrupa yer sincapların Avrupa'nın en güneyindeki dağılım alanlarından birisi olan Türkiye'nin Trakya Yarımadasındaki (Türkiye Trakyası) Tekirdağ ilinden örnek bir alan üzerinden yapılmış sonuçlara göre şekillendirilmiştir. Çalışmanın temel verilerini, arazi çalışmaları sırasında insansız hava aracı (İHA) kullanılarak elde edilmiş yüksek çözünürlüklü sayısal yükselti modeli (SYM) ve orto-foto görüntüleri oluşturmaktadır. Bu veriler Ekolojik Niş Modelleme (ENM) yaklaşımıyla Coğrafi Bilgi Sistemleri (CBS) teknikleriyle işlenmiştir. Çalışma sonucunda örneklem alanındaki mikro topografya özelliklerinden bakı faktörünün türün yuva tercihinde daha önemli (%56.412) olduğu belirlenmiştir. Çalışmanın sonuçları yok olma tehdidi altında bulunan türün

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popülasyonunun güçlendirilmesi ve habitat özelliklerinin iyileştirilmesine yönelik spesifik önlemlerin alınmasına destek olmasının yanında potansiyel ve mevcut habitatların bakımı ve korunması için yapılacak planlamalara ışık tutmaktadır. Ayrıca türün korunması için önemli alanların genişletilmesi ve gelecekteki yeniden yerleştirme veya tanıtım programlarının başarısının artırılması için kullanılabilir.

**Anahtar kelimeler:** Mikro topografya, bakı faktörü, CBS, DEM, Akdeniz iklimi.

## 1. Introduction

Old World ground squirrels (*Spermophilus* sensu stricto) are one of the most intensively studied mammalian groups regarding their ecology and behavior [1]. Only three species of this mammal group (Anatolian ground squirrel - *Spermophilus xanthoprymnus* [2], European ground squirrel - *Spermophilus citellus* [3] and Taurus ground squirrel - *Spermophilus taurensis* [4]) occur in Türkiye [5]. European ground squirrels are distributed in the Thracian Peninsula of Türkiye [6]. However, in recent years, especially the natural habitats of European ground squirrels have been dramatically decreasing due to human impacts [7]. In fact, the species is considered "Near Threatened" and "Vulnerable" due to the estimated population decline of 30% or more per decade [8]. Furthermore, according to the IUCN Red List, the European ground squirrel is listed as an endangered (EN) species [9].

To explain the existence and dynamics of animal colonies, it is ecologically important to determine the geographical distribution of species and the factors that control this distribution [10]. Thus, the habitat choices of animal species, which are their natural habitats, are more accurately understood scientifically [11]. Knowing the habitat selection of wild animal species, especially those under threat of extinction, is also essential for understanding why many have disappeared or for the planned conservation of the species against negative impacts [12]. For this purpose, many different innovative approaches have recently been used [13]. The most preferred of these is Geographic Information Systems (GIS) based Ecological Niche Modeling (ENM) [14]. Thus, inferences based on the biology and landscape features of the species can be easily made [11].

The scientific determination of habitat selection of European ground squirrels is very important for the survival of this endangered species [15]. In this respect, revealing the relationship between the density and distribution of European ground squirrels and natural environmental factors has become an important scientific goal [16]. In the related literature, it has been reported that climate, topography and vegetation/land use conditions are more effective in the habitat preference of the species [7]. However, it was also emphasized that more research should be carried out to reveal especially micro topography features in habitats with similar features in terms of other environmental conditions [15]. In this context, although there have been studies on both habitat features of European ground squirrels outside the borders of Türkiye [14, 7, 15] and nest selection using Unmanned Aerial Vehicle (UAV) based Remote Sensing (RS) technology [14], their habitats in Türkiye have not been sufficiently studied. Therefore, it is very important to examine the habitats of European ground squirrels in Türkiye regarding microconditions of some natural environmental features using UAV-based UA technology to fill the gap in the literature.

This study aimed to investigate the effect of micro topography features on European ground squirrels' nest selection. The study was shaped according to the results of a sample area in Tekirdağ province in the Thracian Peninsula, corresponding to one of the southern distribution areas of European ground squirrels in Europe. In this study, for the first time, the effect of micro topography features on nest selection of European ground squirrels in the Thracian Peninsula was determined using UAV-based UA technology. The study, which uses highly sensitive data with very high resolution, is very important in terms of guiding decision-makers in planning for the conservation and future of European ground squirrels.

## 2. Material and method

### *21. Study Area*

The study area corresponds to the habitat of European ground squirrels in the pasture of Köseilyas rural neighborhood of Süleymanpaşa district of Tekirdağ province, located in the Thracian Peninsula (Turkish Thrace) (Figure 1). The study area's surface area, suitable for the ecology of the European ground squirrel, is 3.06 ha.

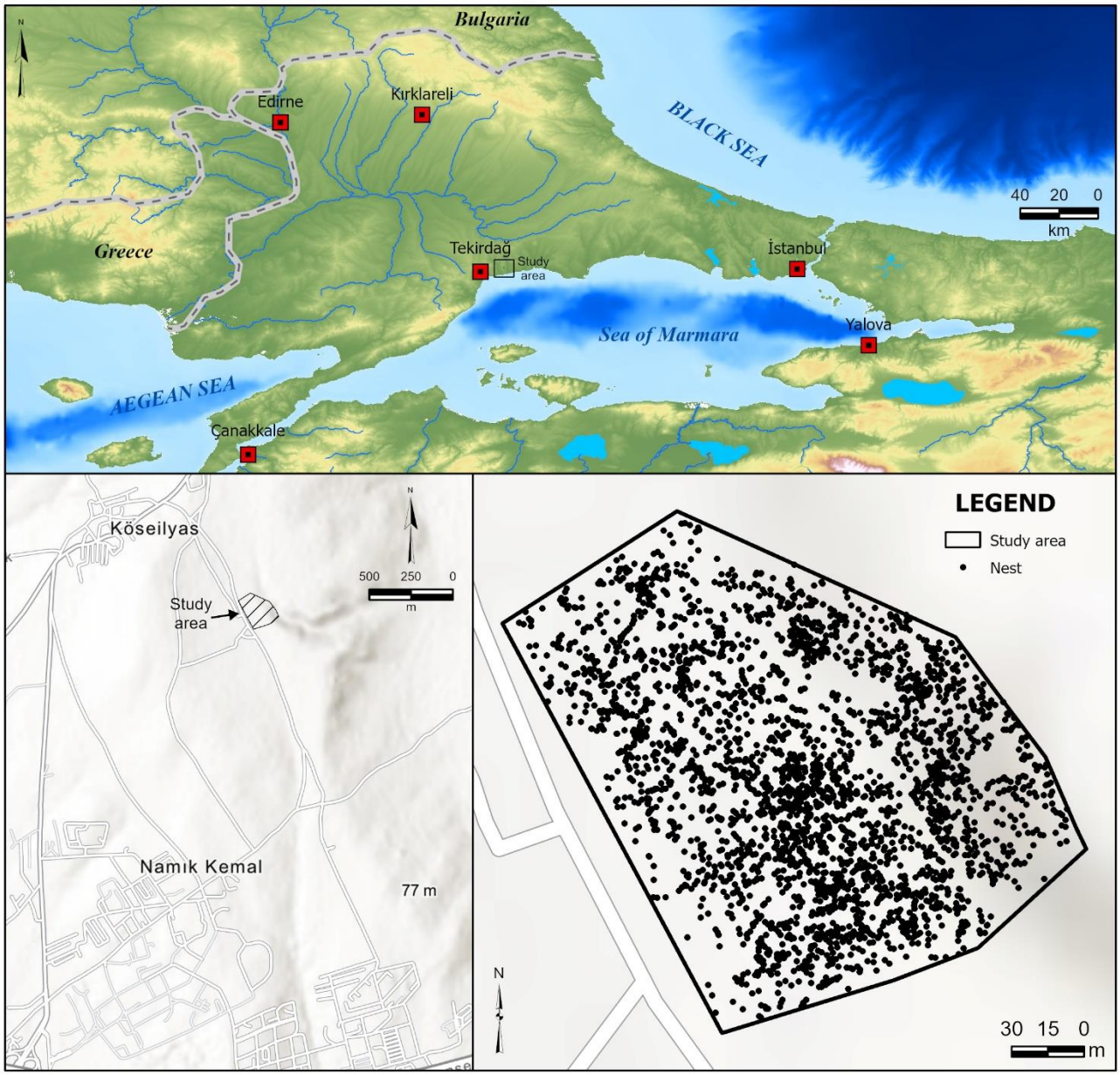


Figure 1. Location map of the study area (base map accessible using ArcGIS Pro provided by ESRI [17])

The study area has a homogeneous habitat features suitable for European ground squirrels in terms of factors other than micro topography (Figure 2; 3). In the study area, where the average annual temperature is 13.7 ˚C, and the total annual precipitation is 560 mm [18], pasture plants (wheatgrasses and legumes) characterized by local climatic features [19] are distributed [20]. The study area has a southeast-facing orientation, where the average altitude is 101.45 m, and the average slope is 7.24%. The soils in and around the study area are mostly heavily textured and rich in organic matter and nutrients (N, K, Fe and Zn). The soil reaction is acid since the study area is a grazed pasture [21]. The pastures in the region where the study area is located generally show a feature where animals are grazed lightly with free grazing from the end of May [20].

Figure 2. The study area provides a suitable habitat for European ground squirrels

*2.2. Method*

ENM method was used in this study. With this method, the distribution of a species in a geographical region where presence information is limited or incomplete can be estimated, as well as its relationship with ecosystem elements that are considered key to maintaining ecological functioning [14]. In this context, the habitat selection of European ground squirrels and its relationship with some environmental factors, especially micro topography features, were discussed. For this purpose, field studies determined a sample habitat that is homogeneous in terms of environmental factors other than micro topography features. Thus, it was tried to understand how much the micro topography features change and its effect on the nest selection of the species.

The study data were generated from a combination of field studies and UAV-based UA technology. First, digital aerial photographs of the study area were taken using an autonomous UAV system. The images were taken from a height of 20 m using 80% transverse and 75% longitudinal overlap ratios, respectively. After the photography, topsoil (0-20 cm) samples were taken randomly and systematically from some points of the site. The data obtained were processed in computer and soil analysis laboratories. Climate data were obtained from the long-term (1991-2022) average data of Tekirdağ Meteorological Station, which serves under the General Directorate of Meteorology [18]. Land use was created using data made available by ESRI [22]. Thus, basic data on the habitat features of European ground squirrels in the study area were obtained.

Using high-resolution (70 cm) ortho-photo images produced by computerized fusion of images taken using UAVs, nests of European ground squirrels were identified in the study area. These burrows are located in a geography where climate, soil and vegetation/land use factors are homogeneous, and micro topography features are heterogeneous. Therefore, this study analyzed the relationship between the nests in the study area, where topographic heterogeneity is high, and micro topography (altitude, slope and aspect) features. At this stage, the Average Nearest Neighbor Index was

based on GIS techniques, and the Forest-based Classification and Regression analysis of the Random Forest (RF) algorithm, one of the machine learning techniques, was used. Thus, a new perspective was gained on the effect of micro topography features on the nest site selection and spatial distribution of European ground squirrels [23]. The analyses and thematic maps in the study were carried out using ArcGIS Pro (Version 3.0.1), one of the GIS software packages.


Figure 3. Data in the study area was collected through field studies

## 3. Results

The sample habitat of this study was selected from the Thracian Peninsula, which corresponds to one of the distribution areas of European ground squirrels in the south of the European continent [24]. In this habitat, 3116 nests were identified. The spatial distribution of these nests is clustered according to the nearest neighbor index result (0.755260), which is based on the average distance. In addition, considering that the z value of these index results (-26.135698) is less than 1%, it can be said that this clustering pattern is not random (Figure 4).

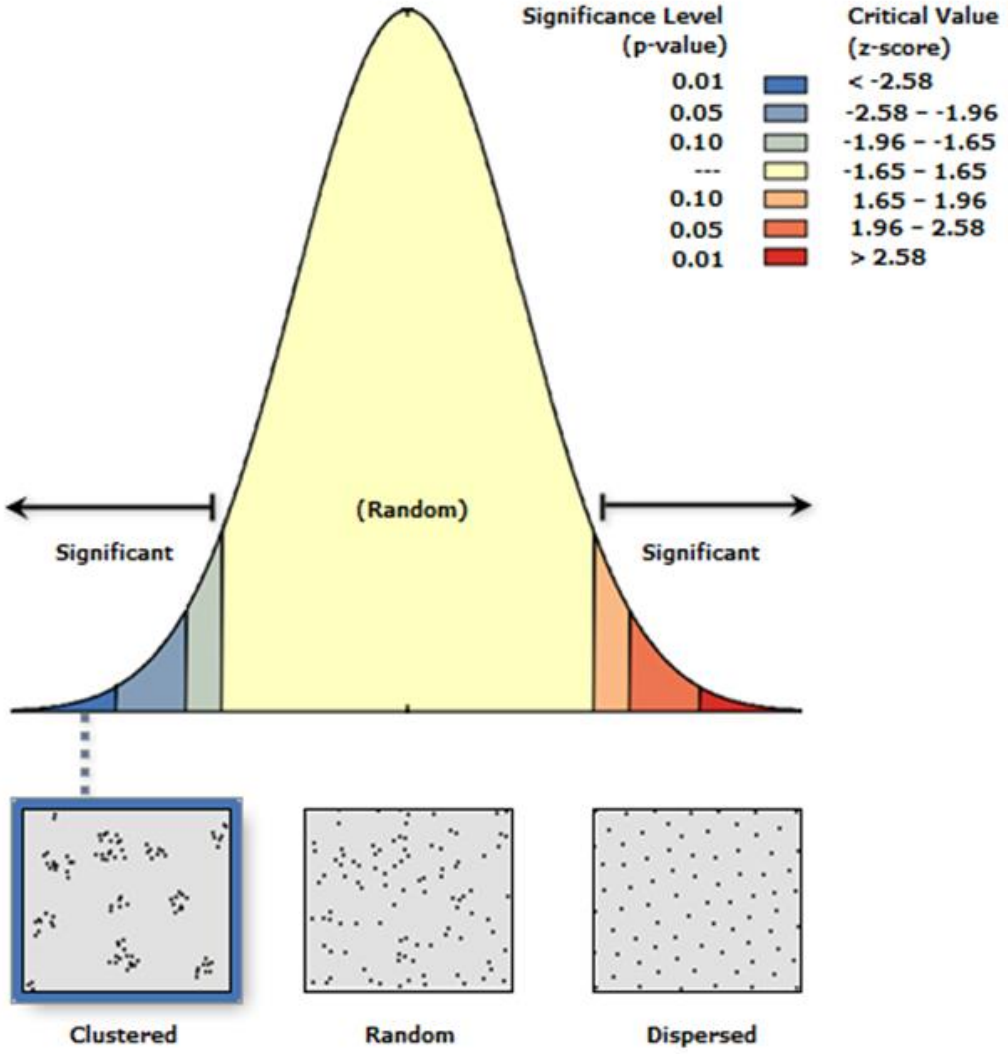


Figure 4. Representation of the Nearest Neighbor Index result of European ground squirrel nests in the study area using the standard distribution form [25]

In the study area, climate (precipitation and temperature), soil and land use factors that constitute the habitat features of European ground squirrels are homogeneous, while micro topography features are more heterogeneous. Gür [26] reported that the concept of heterogeneity, which is accepted as a measure of how much habitat features change, increases to the extent of the change in micro topography features in a given area. The study area, where the average annual temperature is 13.7 ˚C, and the average yearly total precipitation is 560 mm, corresponds to a roadside pasture area with clay loam soil characteristics (Figure 5; Table 1). Rammou et al. [14] stated that the species' nests are generally found in roadside pastures where the Mediterranean climate is effective, heavily textured soil characteristics are observed, and transportation is provided. In this respect, the nests of European ground squirrels in the study area are located in similar conditions that are effective regarding climate, soil, and land use factors.

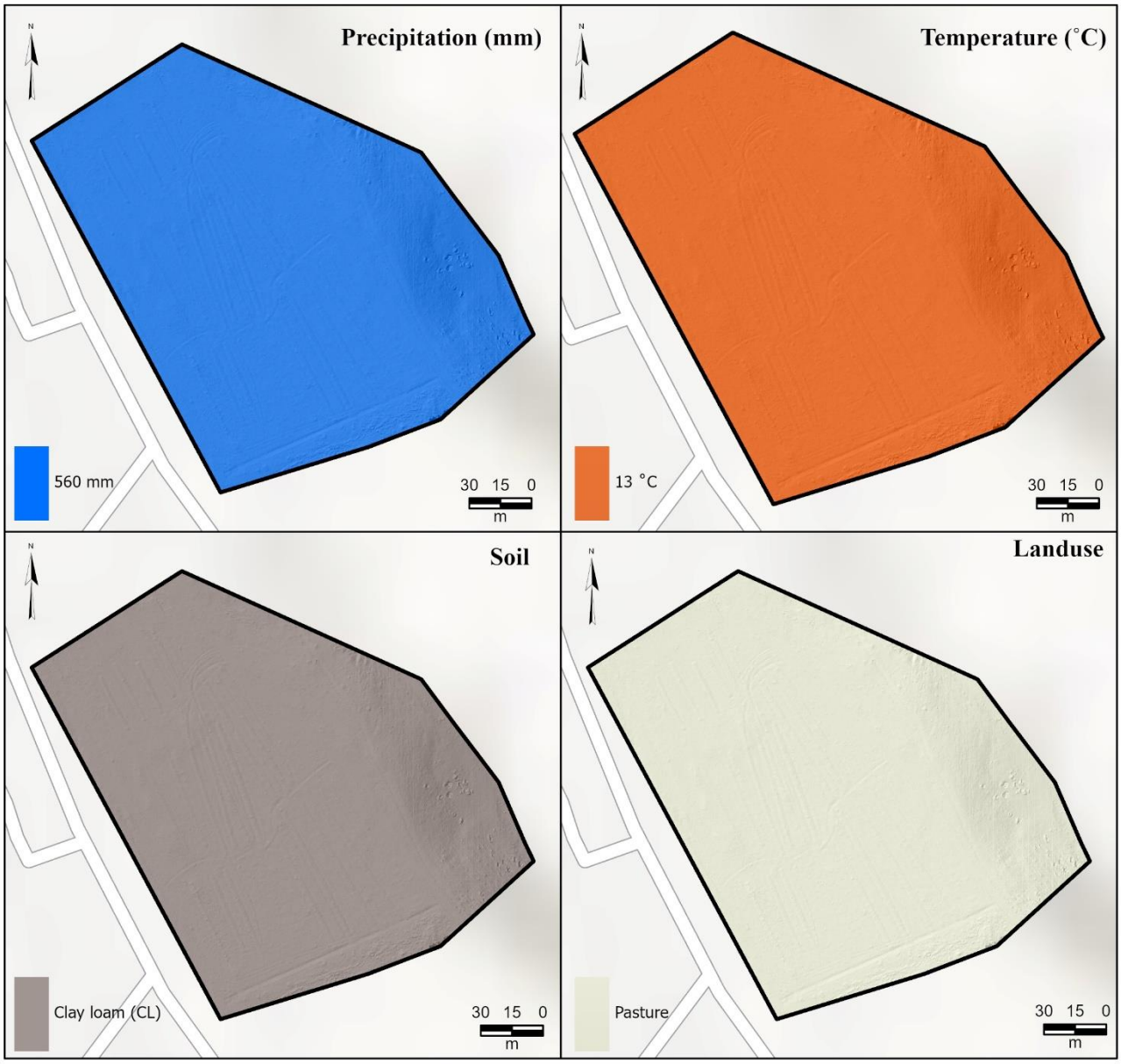


Figure 5. Map of homogeneous environmental factors affecting habitat features in the study area

Table 1. Distribution of some factors affecting habitat features at the study area and nest level

| Some factors affecting habitat features | | Study area | | Nest | |
|---|---|---|---|---|---|
| **Factor Name** | **Value** | **Area (ha)** | **Rate (%)** | **Number (units)** | **Rate (%)** |
| Precipitation | 560 mm | 3.062 | 100 | 3116 | 100 |
| Temperature | 13 ˚C | 3.062 | 100 | 3116 | 100 |
| Soil | Clay loam (CL) | 3.062 | 100 | 3116 | 100 |
| Landuse | Pasture | 3.062 | 100 | 3116 | 100 |

Micro topography features play a more decisive role in the spatial distribution of European ground squirrel nests in the study area. According to the micro topography features in the study area, 89% of the nests are located above 100 m, 77% are located below 10% slope and 50% are located in south-facing directions (Table 2; Figures 6; 7; 8; 9). This finding also indicates that European ground squirrel burrows in the study area are more related to aspect and slope features than altitude. Indeed, Zaharia et al. [7] noted that slope and aspect factors influence European ground squirrel density more than altitude (Table 2). Rammou et al. [14] emphasized that the nests of European ground squirrels are typically located in south-facing and gently sloping landscapes.

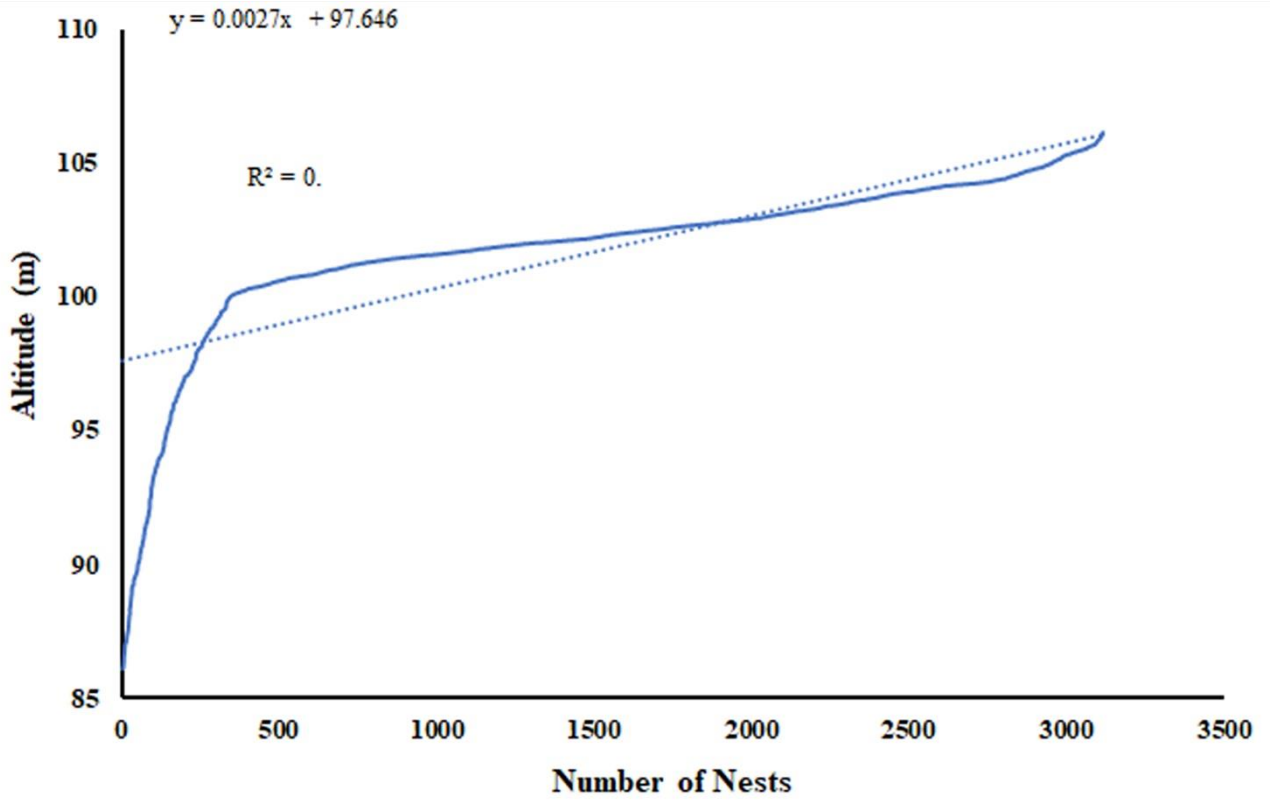


Figure 6. Graph of the relationship between European ground squirrel burrows and altitude factor in the study area

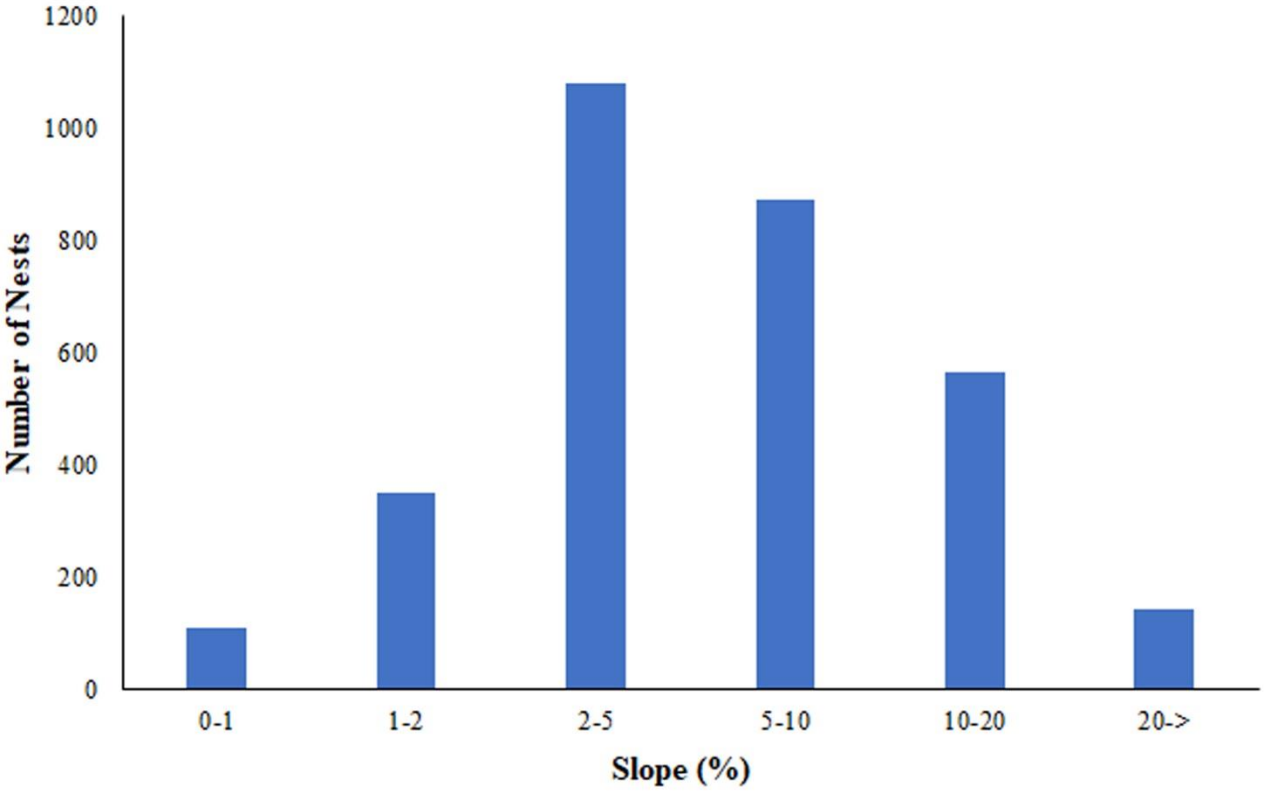


Figure 7. Histogram of the relationship between European ground squirrel burrows and slope factor in the study area

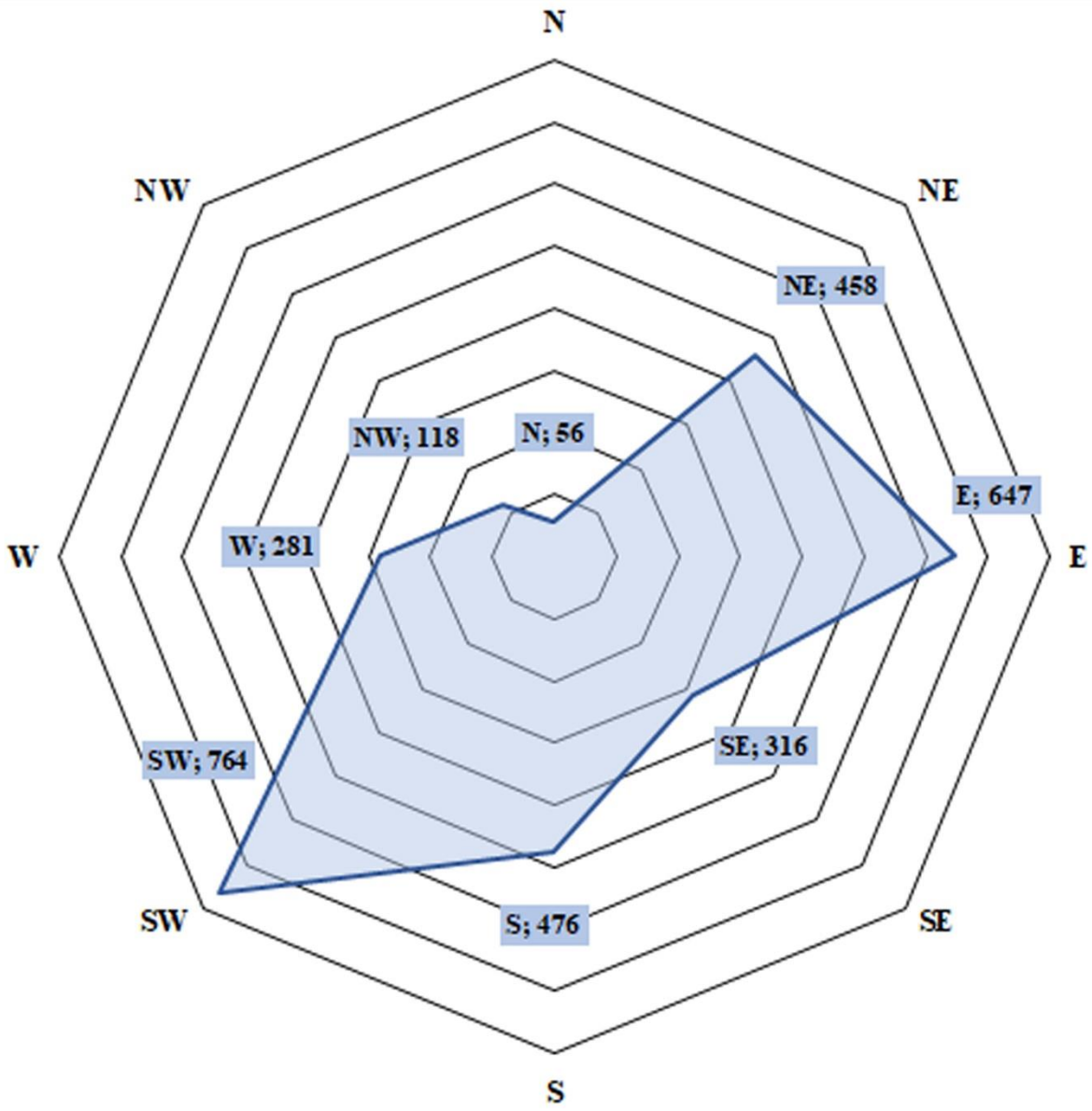


Figure 8. Rose diagram of the relationship between European ground squirrel burrows and aspect factor in the study area

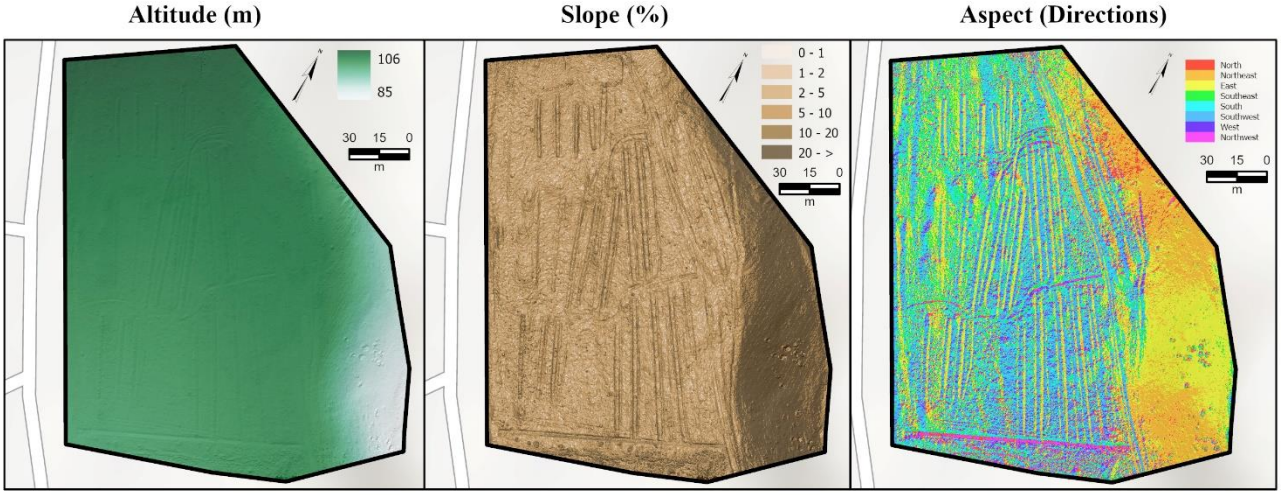


Figure 9. Distribution map of micro topography features that are effective in the spatial distribution of European ground squirrel nests in the study area

Table 2. Distribution of altitude, slope and aspect factors affecting habitat features according to the study area and nests

| **Altitude (m)** | **Study area** | | **Nest** | |
|---|---|---|---|---|
| | **Area (ha)** | **Rate (%)** | **Number (units)** | **Rate (%)** |
| <-100 | 0.415 | 13.6 | 342 | 11.0 |
| 100-> | 2.647 | 86.4 | 2774 | 89.0 |
| **Total** | **3.062** | **100** | **3116** | **100** |
| **Slope (%)** | **Study area** | | **Nest** | |
| | **Area (ha)** | **Rate (%)** | **Number (units)** | **Rate (%)** |
| 0-1 | 0.360 | 11.7 | 109 | 3.5 |
| 1-2 | 0.272 | 8.9 | 349 | 11.2 |
| 2-5 | 0.631 | 20.6 | 1081 | 34.7 |
| 5-10 | 0.865 | 28.3 | 873 | 28.0 |
| 10-20 | 0.606 | 19.8 | 563 | 18.1 |
| 20-> | 0.328 | 10.7 | 141 | 4.5 |
| **Total** | **3.062** | **100** | **3116** | **100** |
| **Aspect (Directions)** | **Study area** | | **Nest** | |
| | **Area (ha)** | **Rate (%)** | **Number (units)** | **Rate (%)** |
| N | 0.125 | 4.1 | 56 | 1.8 |
| NE | 0.545 | 17.8 | 458 | 14.7 |
| E | 0.698 | 22.8 | 647 | 20.8 |
| SE | 0.411 | 13.4. | 316 | 10.1 |
| S | 0.461 | 15.1 | 476 | 15.3 |
| SW | 0.526 | 17.2 | 764 | 24.5 |
| W | 0.191 | 6.2 | 281 | 9.0 |
| NW | 0.105 | 3.4 | 118 | 3.8 |
| **Total** | **3.062** | **100** | **3116** | **100** |

According to the Forest-Based Classification and Regression analysis, the degree of importance of micro topography features in the study area differs. Accordingly, the most important variable in the study area is aspect (56.412%). The other factors are slope (26.697%) and altitude (16.892%) from more important to less important (Table 3). This finding coincides with the study's findings in the nests of European ground squirrels recorded in Eastern Romania [7].

Table 3. Importance and percentage values of micro topography features in the study area

| **Micro Topography Features** | **Variable Importance** | |
|---|---|---|
| **Variable Name** | **Importance** | **Percentage** |
| Aspect (Directions) | 62052176 | 56.412 |
| Slope (%) | 29365969 | 26.697 |
| Altitude (m) | 18580981 | 16.892 |

## 4. Conclusions and Discussion

In recent years, the population of European ground squirrels has declined significantly due to various human activities. For this reason, it has become an important scientific objective to determine the relationship between the density and distribution of the species and natural environmental factors. Many studies have been conducted to determine the habitat features of various wild mammal species. However, studies on this subject in Türkiye are quite new [28]. In this study, the effect of micro topographic features was examined in a sample site located at the southernmost border of the habitat of European ground squirrels. Until today, no study has been carried out to determine the species' habitat selection in the Thracian Peninsula [29]. Moreover, the study area is even more important since it is one of the biogeographically designated "ancestral areas" [30] for the origin of the S. citellus lineage.

In this study, where the results supporting the literature were obtained, it was understood that the factors of aspect and slope were very effective in the nest preference of the species. Especially the aspect factor was found to be the most important variable. This is because the features of these aspects also shape the micro climate conditions. The fact that the slopes of the topography-oriented S-SE-SW-E are exposed to more solar radiation leads to the emergence of favorable features for the photosynthesis and productivity of the plants in the habitat area of the species. Thus, it controls the vital attributes of the species, such as body mass, reproductive success and survival rate [31]. In addition, the species' colony density is positively influenced by higher temperature and strong light on S-SE-SW-E oriented slopes [32] and negatively influenced by more humid conditions on N-NE-NW-W slopes that favor the occurrence of

mass movements of various species [7]. The slope factor also played a decisive role in the nest preference of the species. This must be related to the fact that the current habitat of the species typically corresponds to south-facing pastures with short-stature vegetation showing a lowland character with a slight slope (<3°) [14]. Because in such pastures, the low slope and short vegetation cover enables the species to easily detect predators. On the contrary, higher slope and vegetation conditions prevent the animal from seeing its surroundings [33]. Therefore, for the species, which is an important prey for a wide variety of carnivores, to easily detect predators and survive, it is important that the vegetation in the habitat area is short and the slope is low [7].

In this study, for the first time, the effect of micro topography features on the habitat selection of the species in a sample location selected from the Thracian Peninsula was determined using UAV-based UA technology. Because it has recently been reported that UAV-based image processing techniques have replaced traditional research methods for the evaluation of habitat features of the species [34], it has been suggested that more reliable results with better prediction accuracy and precision can be obtained [35]. In this study, the effect of micro topography features on the nest selection of the species was investigated and mapped using the GIS-based ENM method. There is a great need for studies on mapping the habitat features of wild animal species and their potential distribution in their habitats using GIS techniques [28]. In addition, the study and mapping of natural environmental features in ecological landscapes has become a standard and important tool for assessing an animal's habitat [27]. In this study, GIS techniques and very high-resolution data were utilized to determine the effect of micro topography features on both the nests of the species and habitat selection. Therefore, this study proves that the data obtained with new generation methods and techniques offer a different perspective for habitat assessment of endangered species.

The results of the study shed light on the planning to be made for the maintenance and protection of potential and existing habitats, as well as supporting specific measures to be taken to strengthen the population of the species, which is under threat of extinction, and to improve habitat features. For this reason, ecological plans should be made with a sustainable approach for the conservation of the species in the study area, taking into account the results of this study. These plans should be supported by biotope mapping and assessment method, which is considered one of the most important tools of nature conservation [36]. On the other hand, potential distribution areas in the immediate vicinity should also be determined for the conservation of the species. Thus, it is possible to get more practical and effective results in the planning process [37]. The results of the study will help researchers identify areas where action plans and conservation projects can be implemented to ensure the survival of the species [38]. In addition, these assessments can be used to expand the areas important for the conservation of the species and increase the success of future relocation or introduction programs. In future studies to be conducted with similar methods and techniques, it is recommended that the effect of micro topography features on the habitat selection of the species should be done comparatively according to different habitats.

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# Comparison of stem cell CD45/34 fluorescence intensity with stem cell mobilization in patients under and over 65 years of age

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**Abstract**

In our research, we attempted to compare CD45/34 MFI (Mean Fluorescent Intensity) in patients younger and over 65 years of age with hematopoietic stem cell mobilization (HSCM).The research involved a group of 76 individuals who had different types of cancer and were identified at the Bone Marrow Transplantation (BMT) Unit of Anadolu Medical Center Hospital from 2015 to 2016. To mobilize HSCs, participants were administered daily granulocyte colony-stimulating factor (G-CSF) (10 µg/kg/day) under the skin for 7-8 days. Calculating the appropriate level of peripheral blood (PB) CD34+ took into account the patients' WBC (White Blood Cell) counts. Our research revealed that HSCM patients above 65 had statistically greater CD45/34 MFI values than those under that age. Although the age factor for HSCM is important, according to our findings, age is not seen as a negative mobilization factor for HSCM in patients aged 65 and over, and should be supported by larger studies. Our research revealed that patients over 65 who underwent HSCM had statistically greater CD45/34 MFI values than younger patients. Given the decline in SC production observed in individuals aged 65 and above, along with the potential for other accompanying diseases, our results hold promise for elderly patients. However, it is important to validate these findings through multicenter studies with a larger patient population, while also taking into account the presence of other subsequent diseases in the patients under observation.

**Keywords:** Stem cell mobilization, mean fluorescent intensity, CD45/34, hematopoietic stem cell transplantation

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# Kök hücre CD45/34 floresan yoğunluğunun 65 yaş altı ve üstü hastalarda kök hücre mobilizasyonu ile karşılaştırılması

**Özet**

Araştırmamızda, hematopoietik kök hücre mobilizasyonu (HKHM) uygulanan 65 yaş altı ve üstü hastalarda CD45/34 MFI (Ortalama Floresan Yoğunluğu) değerlerini karşılaştırmayı amaçladık. 2015-2016 yılları arasında Anadolu Sağlık Merkezi Hastanesi Kemik İliği Transplantasyonu (KİT) Ünitesi'nde farklı kanser türlerine sahip 76 kişilik bir grupla çalıştık. HKH'leri harekete geçirmek için katılımcılara 7-8 gün boyunca cilt altından günlük granülosit koloni uyarıcı faktör (G-CSF) (10 µg/kg/gün) uygulandı. Uygun periferik kan (PK) CD34+ seviyesinin hesaplanmasında hastaların WBC (Beyaz Kan Hücresi) sayıları dikkate alınmıştır. Araştırmamız 65 yaş üstü HKHM hastalarının bu yaşın altındakilere kıyasla istatistiksel olarak daha yüksek CD45/34 MFI değerlerine sahip olduğunu ortaya koymuştur. HKHM için yaş faktörü önemli olmakla birlikte, bulgularımıza göre 65 yaş ve üzeri hastalarda yaş HKHM için olumsuz bir mobilizasyon faktörü olarak görülmemekte olup daha büyük çalışmalarla desteklenmelidir. Araştırmamız, HKHM uygulanan 65 yaş üstü hastaların genç hastalara kıyasla istatistiksel olarak daha yüksek CD45/34 MFI değerlerine sahip olduğunu ortaya koymuştur. KH üretiminde 65 yaş ve üzeri bireylerde gözlenen düşüş ve eşlik

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eden diğer hastalıkların potansiyeli göz önüne alındığında, sonuçlarımız yaşlı hastalar için umut vaat etmektedir. Bununla birlikte, bu bulguların çok merkezli çalışmalarla daha geniş bir hasta popülasyonuyla doğrulanması ve gözlem altındaki hastalarda diğer müteakip hastalıkların varlığının da dikkate alınması önemlidir.

**Anahtar kelimeler:** Kök hücre mobilizasyonu, ortalama floresan yoğunluğu, CD45/34, hematopoetik kök hücre transplantasyonu.

## 1. Introduction

Some hematological cancers can be cured with hematopoietic stem cell transplantation (HSCT). Peripheral SCs are mostly used as SC sources for autologous and allogeneic SC transplants. HSCM required for HSCT is provided by the G-CSF mobilization agent [1]. G-CSF blocks the CXCR4 receptor expressed by HSCs. HSCs are released into PB when the interaction of stromal-derived factor-1 (SDF-1) expressed by bone marrow (BM) stroma and CXCR4 receptor is inhibited [2]. The success of HSCM is determined by the number of CD34+ SCs that enter the peripheral blood. In our study, the MFI values of CD45/34+ cells were used. MFI is frequently used to measure the expression level of CD45/34 antigens across samples and cell types in flow cytometry. The CD45/CD34 antigen is a human leukocyte antigen belonging to the leucocyte common antigen (LCA) family, with a molecular weight ranging from 180 to 220 kilodaltons (kDa). It is expressed on all human leukocytes and is observed to have low levels of expression on hematopoietic progenitor cells. The CD45/CD34 marker is the main determinant of HSCs and is frequently used in the flow cytometer device in hematology laboratories to calculate the number of SCs

In one parameter histograms, data is expressed as either the % of a population's cells or the MFI. MFI value defined as the intensity of the fluorescent signals of the antigen-bound monoclonal test antibodies, is a surrogate marker for antigen density in the cell.

Several factors, including age, medical condition, chemotherapeutic regimen used for mobilization, previous sessions of chemotherapy or radiation treatment, and the time elapsed since the last chemotherapy cycle, can influence the process of HSCM [3]. Because various research has shown that elderly patients have lower mobilization capacity than younger patients, the results that are now available are inconsistent. In the study of Tempescul et al. (2010) with 359 patients, it was found that the HSCM success rates of patients below and above 65 years of age were similar (92% compared to 90.6%, respectively), However, a significant difference was observed in the number of CD34+ HSCs collected from the patients between the two age groups. Despite the significantly lower median number of CD34+ HSCs collected from the population aged 65 and over, this was still sufficient to perform one or more autologous SC transplants [4]. In light of all this information, we compared the CD45/34 MFI values in HSCM in individuals below and above the age of 65.

## 2. Materials and methods

From 2015 to 2016, a total of 76 patients were enrolled in a study conducted at the Bone Marrow Transplantation Unit of Anadolu Health Center Hospital. These patients had various diagnoses, with 37 (48.7%) cases of Multiple Myeloma (MM), 33 (43.4%) cases of Lymphoma, 2 (2.7%) cases of Testicular Cancer (Testicular CA), 3 (4%) cases of Solid Tumors, and 1 (1.3%) case of Acute Myeloid Leukemia (AML). All patients underwent HSCM procedures and were included in the study (Table 1).

Table 1. Diagnostic distributions

| Diagnosis | Frequency | Percent |
|---|---|---|
| MM | 37 | 48.68 |
| Lymphoma | 33 | 43.42 |
| Testicular CA | 2 | 2.63 |
| Solid Tumors | 1 | 1.32 |
| AML | 1 | 1.32 |
| MM | 2 | 2.63 |
| Total | 76 | 100 |

Multiple Myeloma (MM), Testicular Cancer (Testicular CA), Acute Myeloid Leukemia (AML)

The HSCM process usually begins with high-dose chemo and/or radiation therapy lasting about a week or two. This treatment aims to remove unwanted cells and make room for new cells to come in HSCT. Then, depending on the previous chemotherapy regimens received by the patients, mobilization chemotherapy is applied, which usually lasts for

1-2 days. One day after mobilization chemotherapy, G-CSF (10 µg/kg/day) is administered subcutaneously to patients daily for 7-8 days for HSCM. After 7-8 days, a PB sample was taken from the patient who came to the hospital to calculate the HSC and WBC count in the EDTA tube. The WBC count of the patients was considered to calculate the desired quantity of PB CD34+.

For CD45 FITC/34 PE staining with flow cytometer, 100 µl of the patient's mobilized PB sample was placed in the flow tube, and then 20 µl of CD45 FITC/CD34 PE (BD Bioscience, Cat No. 341071) antibody was added to it. Immediately after incubation in the dark for 20 min,2 mL of lysis solution was added to remove the erythrocytes in the sample. It was incubated for 10 min in the dark. After incubation, samples were washed twice with phosphate-buffered saline (PBS) for 5 minutes at 1800 rpm. Samples were resuspended with PBS and analyzed on a flow cytometer (BD Bioscience Facs Calibur).

The amount of CD34+ cells stained in the flow cytometer was multiplied by the number of total WBCs of the patient and the number of CD34 positive cells required for adequate mobilization is 10 per mL. In addition, the CD45/34 MFI given by the flow cytometer device was recorded in the analyzes performed for SC PB CD34 + on the day of HSCM. When the desired CD34+ SC count is obtained, the patient's SC is collected in the apheresis unit. The CD45/34 MFI number, which directly affects the number of SCs, was taken from the flow cytometer device, not the number of SCs calculated in the study. Flow cytometry analysis was performed on the first day of mobilization. The number of CD45/34 MFIs is associated with the number of SCs, and a high one indicates high mobilization (Figure 1)

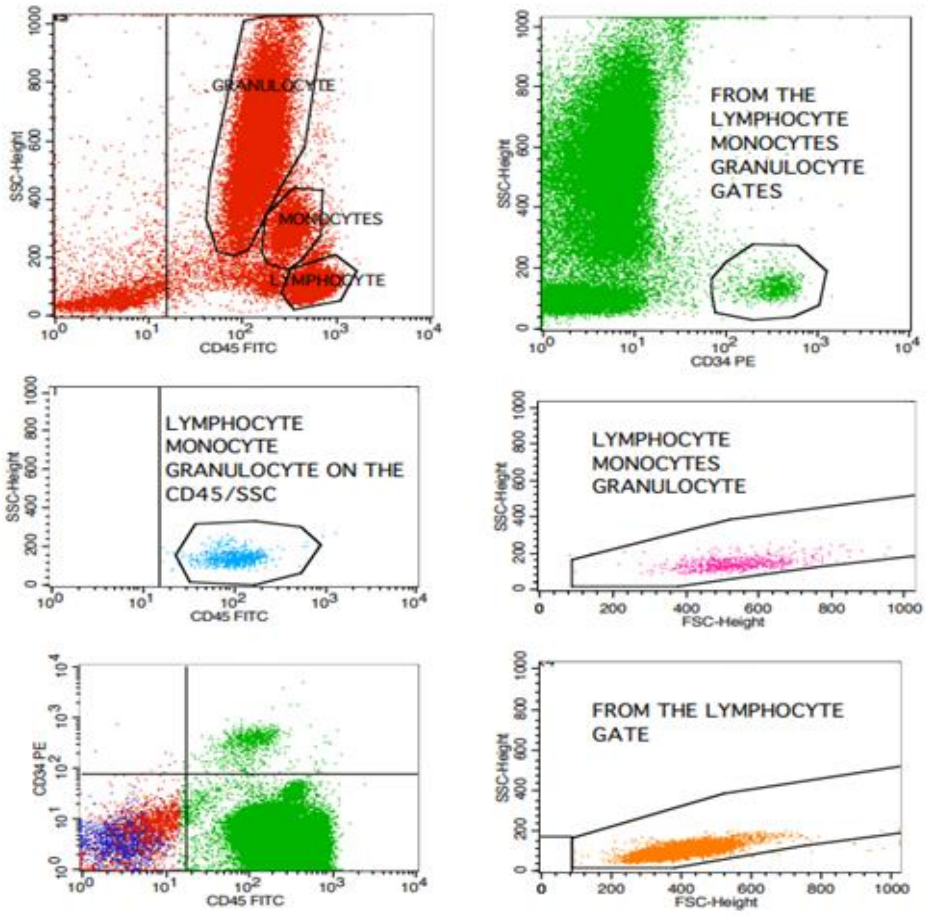


Figure 1. The representative flow cytometry plots (with gating strategy). The 2nd plot (green) gives the CD34 MFI percentage, and the 3rd plot (blue) the CD45 MFI percentage

Briefly, in our study, a technique called flow cytometry was used to calculate CD45/34 MFI (Mean Fluorescent Intensity) values. This process is specifically used to measure the intensity of antigens (such as CD45 and CD34) on the surface of cells. The process starts as described above by first preparing cell samples and staining them with special antibodies. The stained cells are then placed in a flow cytometer and exposed to a laser beam. The fluorescent light emitted by the antibodies was measured by the instrument and recorded as MFI values. These values reflect antigen levels on the surface of the cells, providing information on various biological and clinical conditions. For detailed methodology and flow cytometry techniques, the book [5] "Flow Cytometry: Principles and Applications (2007)" can be consulted. This book comprehensively covers the basic techniques used in flow cytometry, including

sample preparation, staining, flow cytometry analysis and data interpretation. It also provides detailed information on how MFI values are calculated.

The frequency and percentage were used to summarize the quantitative data, while the median (also represents the second quartile ($Q_2$), first quartile ($Q_1$), third quartile ($Q_3$), minimum (Min), and maximum (Max)) were used to summarize the quantitative variables. Independent group assessments were made using the Mann-Whitney U test and effect sizes were reported using rank-biserial correlation coefficients. JASP (Version 0.16.3) statistical software was used for statistical analysis. Statistical inferences were made at 5% significance level.

## 3. Results

Patients with HSCM were 44 (57.9%) men and 32 (42.1%) women (Table 4). While 52 of the patients were under the age of 65, 24 of the patients included in the study were over the age of 65. (Table 3). The 76 individuals who were a part of the research had an average age of 54.97. (17-77) (Table 2). There is a statistically significant difference between age groups in terms of CD45 and CD34 (respectively p=0.043 and 0.014). CD45 MFI distribution in the >=65 age group (Median=364.5, $Q_1$=279.25, and $Q_3$=394.25) is statistically significantly higher than in the <65 age group (Median=295.5, $Q_1$=221.5, and $Q_3$=361). CD34 MFI distribution in the >=65 age group (Median=424, $Q_1$=350.75, and $Q_3$=610.25) is statistically significantly higher than in the <65 age group (Median=342.5, $Q_1$=283, and $Q_3$=431.25). However, the effect sizes are low (respectively $r_{rb}$=-0.292 and -0.354) (Table 3). The distribution chart of CD45 MFI and CD34 MFI by age group is shown in Figure 2.

Table 2. Descriptive statistics for age

| | n | Mean | SD | Min | $Q_1$ | Median ($Q_2$) | $Q_3$ | Max |
|---|---|---|---|---|---|---|---|---|
| **Yaş** | 76 | 54.97 | 14.65 | 17 | 48.75 | 58.00 | 66.00 | 77 |

SD: Standard Deviation, Q: Quartile

Table 3. Comparison of age groups in terms of CD45 MFI and CD34 MFI

| | <65 age (n=52)<br>Median ($Q_1$-$Q_3$)<br>[Min-Max] | >=65 age (n=24)<br>Median ($Q_1$-$Q_3$)<br>[Min-Max] | W* | p | Rank-Biserial Correlation** |
|---|---|---|---|---|---|
| CD45 | 295.5 (221.5 - 361)<br>[137 - 766] | 364.5 (279.25 - 394.25)<br>[156 - 798] | 442.0 | 0.043 | -0.292 |
| CD45 | 342.5 (283 - 431.25)<br>[104 - 1038] | 424 (350.75 - 610.25)<br>[237 - 1313] | 403.0 | 0.014 | -0.354 |

*Mann-Whitney U test statistics. Q: Quartile

**For the Mann-Whitney test, effect size is given by the rank biserial correlation.

Table 4. Comparison of gender groups in terms of CD45 MFI and CD34 MFI

| | Male (n=44)<br>Median (Q1-Q3)<br>[Min-Max] | Female (n=32)<br>Median (Q1-Q3)<br>[Min-Max] | W | p | Rank-Biserial Correlation** |
|---|---|---|---|---|---|
| CD45 | 335.5 (276.75 - 407.25)<br>[151 - 798] | 278 (205 - 367.25)<br>[137 - 623] | 898.0 | 0.042 | 0.276 |
| CD34 | 363.5 (299.75 - 503.25)<br>[203 - 1038] | 359 (282.25 - 458.75)<br>[104 - 1313] | 789.5 | 0.371 | 0.121 |

*Mann-Whitney U test statistics. Q: Quartile

**For the Mann-Whitney test, effect size is given by the rank biserial correlation.

The statistical analysis shows that there is a statistically significant difference between gender groups in terms of CD45 MFI but there is no statistical significance in terms of CD34 MFI (respectively p=0.042 and 0.371). CD45 MFI distribution in the male group (Median=335.5, $Q_1$=276.75, and $Q_3$=407.25) is statistically significantly higher than in the female group (Median=278, $Q_1$=205 and $Q_3$=367.25). However, the effect size is low ($r_{rb}$=0.276) (Table 4). The distribution chart of CD45 MFI and CD34 MFI by gender groups is shown in Figure 3.

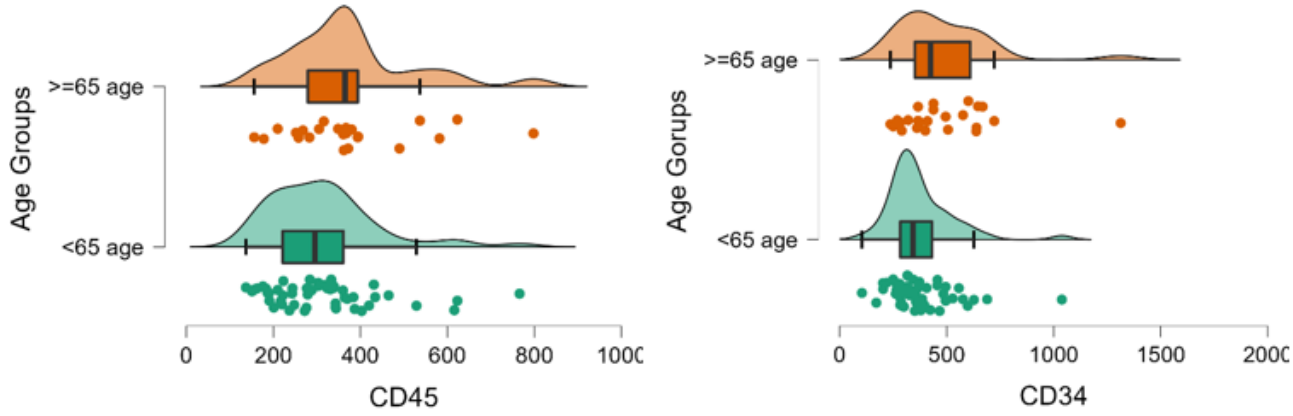


Figure 2. Distributions of CD45 MFI and CD34 MFI in the age groups

*Note: Points represent the observed (measured) values. Box-plot graphs represent the minimum, maximum, and first, second, and third quartiles. And one-sided violin graphs represent the estimated density of the distribution of the interested variable.*

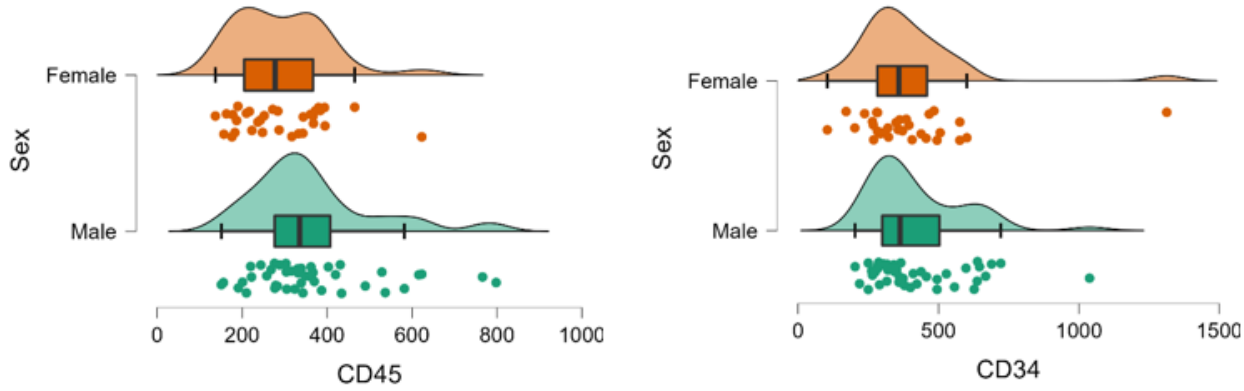


Figure 3. Distributions of CD45 MFI and CD34 MFI in the gender groups

*Note: Points represent the observed (measured) values. Blot graphs represent the minimum, maximum, and first, second, and third quartiles. And one-sided violin graphs represent the estimated density of the distribution of the interested variable.*

## 4. Conclusions and discussion

Patients with HSCM were 44 (57.9%) men and 32 (42.1%) women (Table 4). While 52 of the patients were under the age of 65, 24 of the patients included in the study were over the age of 65. (Table 3). The 76 individuals who were a part of the research had an average age of 54.97. (17-77) (Table 2). There is a statistically significant difference between age groups in terms of CD45 and CD34 (respectively p=0.043 and 0.014). CD45 MFI distribution in the >=65 age group (Median=364.5, $Q_1$=279.25, and $Q_3$=394.25) is statistically significantly higher than in the <65 age group (Median=295.5, $Q_1$=221.5, and $Q_3$=361). CD34 MFI distribution in the >=65 age group (Median=424, $Q_1$=350.75, and $Q_3$=610.25) is statistically significantly higher than in the <65 age group (Median=342.5, $Q_1$=283, and $Q_3$=431.25). However, the effect sizes are low (respectively $r_{rb}$=-0.292 and -0.354) (Table 3). The distribution chart of CD45 MFI and CD34 MFI by age group is shown in Figure 2.

The statistical analysis shows that there is a statistically significant difference between gender groups in terms of CD45 MFI but there is no statistical significance in terms of CD34 ]]9]MFI (respectively p=0.042 and 0.371). CD45 MFI distribution in the male group (Median=335.5, $Q_1$=276.75, and $Q_3$=407.25) is statistically significantly higher than in the female group (Median=278, $Q_1$=205 and $Q_3$=367.25). However, the effect size is low ($r_{rb}$=0.276) (Table 4). The distribution chart of CD45 MFI and CD34 MFI by gender groups is shown in Figure 3.

It is known that age-related phenotypic changes in cells are particularly important in elderly individuals. Such changes can affect cell function and response to therapies, making them crucial in studies involving elderly populations.

Age-related changes in cell biology and their impact on disease and treatment are discussed in "Brocklehurst's Textbook of Geriatric Medicine and Gerontology" [6] and how aging affects cellular function and response to therapies. Also according to Gabali, A. [7], it was explained that age-related phenotypic changes in haematolymphoid cells may be particularly important in the diagnosis of disease. According to the findings in this literature, it highlights the importance of detailed cellular analysis and the potential impact of age on cellular characteristics, which may affect the efficacy of treatments and diagnostic accuracy in elderly patients. This underlines the need for age-specific considerations in medical research, particularly studies focusing on haematopoietic stem cell mobilisation and related therapies.

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# Phylogenetic analysis of Kars' endemic plant species through amplification of the 26S rDNA gene region

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**Abstract**

The province of Kars, hosting 16% of Türkiye's plant cover, exhibits a high degree of floral diversity, including 71 endemic species. These species are located at the intersection of Türkiye's Caucasian lands, the Iran-Turan, Euro-Siberian, and Mediterranean flora regions. In this study, the 26S rDNA region of 14 endemic plant species sampled from Kars was amplified, and sequence data were obtained. NCBI GenBank database searches confirmed the first-time sequencing of the 26S rDNA gene region for these endemic plant species. The molecular characteristics of the 26S rDNA region of the 14 endemic plant species were examined, providing significant genetic data on the diversity and evolutionary relationships of endemic plants. Comparative analysis of the 26S rDNA sequences of the studied endemic species revealed notable genetic relationships within these plant groups, uncovering considerable variations among the species. The observed high polymorphism in the 26S rDNA region suggests its potential for accurate species identification. The genetic data obtained in this study have the potential to contribute to genetic research for the conservation of endemic species and biodiversity, emphasizing the importance of exploring and documenting the genetic uniqueness of endemic species. Furthermore, the genetic data obtained not only contribute to understanding the phylogenetic relationships among endemic species but also have implications for preserving and sustaining biological diversity by providing molecular identity to endemic plants in international databases.

**Keywords:** Kars, endemic, 26S rDNA, diversity, conservation

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## Kars'ın endemik bitki türlerinin 26S rDNA gen bölgesi amplifikasyonu ile filogenetik analizi

**Özet**

Türkiye'nin bitki örtüsünün %16'sine ev sahipliği yapan Kars ili, 71'i endemik olmak üzere yüksek oranda floral çeşitliliğe sahiptir. Bu türler, Türkiye'nin Kafkas toprakları, İran-Turan, Euro-Sibirya ve Akdeniz bitki coğrafyasının kesişim noktasında bulunmaktadır. Bu çalışmada, Kars ilinden örneklenen 14 endemik bitki türünün 26S rDNA bölgesi amplifiye edilerek sekans verileri elde edilmiştir. NCBI GenBank veri tabanı eşleştirmeleri bu endemik bitki türlerinin 26S rDNA gen bölgesinin ilk defa sekanslandığını göstermiştir. 14 endemik bitki türünün 26S rDNA bölgesinin moleküler özellikleri incelenerek, endemik bitkilerin genetik çeşitlilikleri ve evrimsel ilişkileriyle ilgili önemli genetik veriler elde edilmiştir. Çalışılan endemik türlerin 26S rDNA dizilerinin karşılaştırılması, bu bitki grupları arasında önemli genetik ilişkileri ortaya koyarak, türler arasında dikkate değer varyasyonları açığa çıkarmıştır. Yüksek polimorfizm 26S rDNA bölgesinin doğru tür tanımlamasındaki potansiyelini göstermiştir. Bu çalışmada elde edilen genetik veriler, endemik türlerin ve biyoçeşitliliğin korunmasına yönelik genetik araştırmalara katkıda bulunma potansiyeline sahiptir ve endemik türlerin genetik benzersizliğinin keşfedilmesinin ve belgelenmesinin önemini açığa çıkarmaktadır. Ayrıca elde edilen genetik veriler, endemik türler arasındaki filogenetik ilişkilerin anlaşılmasına katkı sağlamasının yanı sıra, uluslararası veri tabanlarında endemik bitkilere moleküler kimlik vermede kullanılabileceğinden biyolojik çeşitliliğin korunması ve sürdürülebilir olması açısından önem arz etmektedir.

**Anahtar kelimeler:** Kars, endemik, 26S rDNA, çeşitlilik, koruma

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## 1. Introduction

Endemic species, which are only found in specific geographic areas and not anywhere else in the world, are unique and often vulnerable components of biodiversity. These species make a significant contribution to overall biodiversity, and it is essential to conserve them in order to maintain ecosystem resilience and functionality [1]. Endemic plants have unique ecological roles and their presence contributes to the stability and functioning of ecosystems [2]. However, endemic plant species are often threatened by habitat loss, climate change, and other environmental pressures. Therefore, the molecular identification of endemic plant species is a crucial pursuit. Molecular identification techniques, such as DNA barcoding, are invaluable tools for studying and preserving these distinctive plants [3]. By using molecular identification, researchers can carefully monitor and identify these species, which empowers conservationists to develop targeted strategies for their preservation and sustainable management. Molecular methods also contribute to comprehensive biodiversity assessments by systematically identifying and cataloguing endemic plant species in specific regions [4]. Additionally, molecular identification allows researchers to study the biogeography of endemic plant species, providing insights into their historical distribution, migration patterns, and evolutionary relationships. This knowledge enhances our understanding of the complex processes that shape regional biodiversity [5, 6]. Molecular data are also crucial for reconstructing the evolutionary histories and phylogenetic relationships of endemic plant species. By identifying these species at the molecular level, we can effectively manage and utilize them as valuable genetic resources [7, 8].

The 26SrDNA gene region, which is part of the nuclear genome in plants, encodes a crucial segment of the 26S ribosomal RNA (rRNA), which is an essential component of the large ribosomal subunit. This gene region plays a fundamental role in protein synthesis and has become a focal point of scientific investigation [9]. Researchers use information from the 26SrDNA gene region, along with other molecular markers, to study plant systematics, taxonomy, and evolutionary relationships. The 26SrDNA gene region is highly conserved across a wide range of plant species, highlighting the fundamental role of ribosomal RNA in cellular translation. Phylogenetic studies, based on the 26S rDNA gene region, are key in unraveling the evolutionary history of plant species [10]. Due to its moderate evolutionary rate and versatility as a molecular marker, the 26S rDNA gene region is used to determine relationships at various taxonomic levels, from genera to families. Its universality and conservation make it an indispensable tool for comparing evolutionary relationships across diverse organisms. The gene region's evolutionary stability, combined with its ability to detect subtle variations, allows researchers to construct robust phylogenetic trees. These trees help us understand the relationships between different plant taxa and contribute to our knowledge of plant evolutionary history. The unique sequence variations in this region can be used as molecular fingerprints to distinguish between species. Phylogenetic studies using the 26S rDNA gene region also contribute to our understanding of biogeography and the historical distribution of species [11].

The Kars province in Türkiye has an exceptionally rich floral diversity, with 16% of Türkiye's flora consisting of 1615 species, 71 of which are endemic. This region represents Türkiye's Caucasian lands and serves as a convergence point for the Iran-Turan, Euro-Siberian, and Mediterranean flora regions [12]. Unfortunately, the region faces challenges such as excessive and uncontrolled grazing, as well as clearing activities, which pose a threat to the survival of various species, especially endemic ones [13]. To protect biodiversity, it is crucial to uncover and preserve the genetic diversity that allows species to adapt to changing environmental conditions. Among the 71 endemic species, 12 are specifically found around Lake Çıldır, Allahuekber Mountains, and Sarıkamış forests, which are designated as Important Plant Areas in Türkiye [12, 14]. By assigning molecular identities to endemic plants, we can make a significant contribution to the conservation and sustainable development of biological diversity in our country. In this study, our aim was to use the 26SrDNA gene region to perform phylogenetic identification of 14 endemic plant species in the Kars province. This will help us understand the phylogenetic relationships within and between species. The genetic data obtained in this study have the potential to contribute to genetic research for the conservation of endemic species and biodiversity. The use of molecular identification of endemic plant species could play an important role in understanding, conserving, and managing these species in the Kars province.

## 2. Materials and methods

### DNA extraction and PCR cycling

Leaf samples were systematically collected from diverse locations within Kars province, encompassing 14 distinct endemic plant species (Table 1). Nuclear DNA extraction from leaf tissues was conducted using the modified CTAB DNA isolation method devised by Kistler (2012) [15]. To assess DNA concentrations and quality, readings at 230 nm, 260 nm, and 280 nm were obtained using the Biodrop l Lite 7141 V.1.0.4 spectrophotometer. The 26S rDNA forward and reverse primer sequences, specifically 5’-TTCCCAAACAACCCGACTC-3’ and 5’-GCCGTCCGAATTGTAGTCTG-3’ [16], were employed for the PCR reaction. The reaction mixture, total volume 20 µl, comprised 4 µl HOT FIREPol Blend Master Mix (Solis BioDyne, Tartu, Estonia), 0.5 µl of 200 nM forward and reverse primers, 5 µl of template DNA (diluted to 10 ng), and 10 µl of water. The PCR protocol included an initial cycle

at 95 °C for 5 min, followed by 30 cycles of 30 s at 94 °C, 58 °C (Ta) for 30 s, and 72 °C for 45 s, with a final extension at 72 °C for 10 min. After PCR, electrophoresis was performed on 3% agarose gels at 90 V for 30 min.

Table 1. Information on 14 endemic plant species from Kars province

| **Endemic plant species** | **Distrubution** | **Endemism** | **Family** |
|---|---|---|---|
| *Onosma nigricaulis* Riedl | North East Anatolia, Kars | Local Endemic | Boraginacea e |
| *Onosma isaurica* Boiss. & Heldr. | Anatolia, Sarıkamış | Endemic | Boraginacea e |
| *Tragopogon aureus* Boiss. | North Anatolia, Kars | Endemic | Asteraceae |
| *Corydalis oppositifolia subsp. oppositifolia* DC. | North, South, East Anatolia, Sarıkamış | Endemic | Papaveracea e |
| *Rosa pisiformis (Christ) Sosn.* | North East Anatolia, Kars | Endemic | Rosaceae |
| *Lathyrus karsianus* P.H. Davis | North East Anatolia, Sarıkamış | Local Endemic | Fabaceaea |
| *Astragalus globosus* Vahl | North Anatolia, Kısır Mountain | Endemic | Fabaceae |
| *Lamium galactophyllum* Boiss. & Reuter | North East Anatolia, Seli | Endemic | Lamiaceae |
| *Salvia rosifolia* SM | Anatolia, Kağızman | Endemic | Lamiaceae |
| *Allium czelghauricum* Bordz | North East Anatolia, Göl | Local Endemic | Amaryllidac eae |
| *Papaver triniifolium* Boiss | North East and South Anatolia, Çıldır | Endemic | Papaveracea e |
| *Pastinaca armena subsp. dentata* (Freyn et Sint.) Chamberlain | North East Anatolia, Arpaça | Endemic | Apiaceae |
| *Vincetoxicum coskuncelebiana* S.Makbul& S.Güven | North East Anatolia, Çıldır, Tşbaşı village | Local Endemic | Apocynacea e |
| *Fritillaria michailovskyi* Fomin | North East Anatolia, Sarıkamış | Endemic | Liliaceae |

**Data analysis**

PCR products demonstrating the desired amplification were purified and sequenced at BM Labosis in Cankaya, Ankara. Chromatogram data visualization was performed using the TraceEditor tool included in MEGA 11 software. BLAST search [17] and CLUSTAL alignment [18] were conducted using MEGA 11 software [19]. MEGA 11 software was used to compute essential phylogenetic parameters and identify DNA polymorphism among the endemic species. BLAST analysis compared the 26SrDNA sequences of the 14 endemic species with sequences of closely related species from the NCBI database [20]. These analyses evaluated the correspondence between the acquired sequences and previously studied sequences of the same species or closely related species. To determine the evolutionary relationships among the endemic species, the Neighbor-Joining Method [21] was employed to construct a phylogenetic tree based on evolutionary distances [22]. The number of bootstrap replications was set at 500.

**3. Results**

A large amount of high-quality genomic DNA was obtained for many of the 14 endemic plant species studied using the modified CTAB method from Kistler and Shapiro (2011) [15] (Table 2). For the first time, the 26S rDNA region of the 14 endemic plant species from Kars province was successfully amplified and sequenced.

The length of the 26S rDNA region was found to be approximately 149 base pairs for all endemic species. However, due to the absence of previously published sequences of 26S rDNA for these species in the NCBI GenBank database [20], alignment with the same species could not be determined. Instead, the obtained sequences for these endemic plants were aligned with their relatives in the same genus or family (Table 3). Specifically, *Onosma nigricaulis* and *Onosma isaurica* showed 100% identity with *Echium plantagineum* (OL580770.1). Similarly, *Lamium galactophyllum* had the highest alignment with *Ballata nigra* (ON685391.1), while *Pastinaca armena* had the highest alignment with *Zizia*

*aurea* (MT610976.1), both with 100% identity. *Fritillaria michailovskyi* aligned with *Lilium michauxii* (AF205126.1) with a 99.31% identity value.

Table 2. DNA concentration of the studied endemic plants

| Species | DNA concentration (μg/ml) | 230/260 Ratio | OD | 260/280 Ratio | OD |
|---|---|---|---|---|---|
| *Onosma nigricaulis* | 837 | 0.65 | | 1.55 | |
| *Onosma isaurica* | 750 | 1.85 | | 2.02 | |
| *Tragopogon aureus* | 1057 | 1.25 | | 1.88 | |
| *Corydalis oppositifolia subsp. oppositifolia* | 1677 | 1.28 | | 2.04 | |
| *Rosa pisiformis* | 608 | 0.67 | | 1.19 | |
| *Lathyrus karsianus* | 1006 | 1.55 | | 1.99 | |
| *Astragalus globosus* | 2794 | 1.64 | | 1.68 | |
| *Lamium galactophyllum* | 655 | 0.40 | | 2.5 | |
| *Salvia rosifolia* | 316 | 0.99 | | 1.75 | |
| *Allium czelghauricum* | 27 | 2.83 | | 1.84 | |
| *Papaver triniifolium* | 65 | 2.81 | | 1.80 | |
| *Pastinaca armena* | 26 | 2.19 | | 1.84 | |
| *Vincetoxicum coskuncelebianus* | 178 | 2.53 | | 2.11 | |
| *Fritillaria michailovskyi* | 1171 | 0.98 | | 1.91 | |

The sequence of the 26S rDNA of *Tragopogon aureus, Corydalis oppositifolia, Rosa pisiformis, Lathyrus karsianus, Salvia rosifolia, Astragalus globosus, Allium czelghauricum*, and *Papaver triniifolium* corresponded with the sequence of members of the same genus. *Allium czelghauricum* exhibited the lowest sequence similarity with *Allium altaicum* (MK049255.1), with a 96.48% identity (Table 3).

Table 3. GenBank Alignment results of 26S rDNA gene region for the studied 14 endemic plant species

| Species | Amplified product length (bp) | Aligned species | Accession number | Query cover | e value | Percentage of identity |
|---|---|---|---|---|---|---|
| **26S rDNA** | | | | | | |
| *Onosma nigricaule* | 149 | *Echium plantagineum* | OL580770.1 | 98 | 1e-68 | 100 |
| *Onosma isaurica* | 149 | *Echium plantagineum* | OL580770.1 | 98 | 1e-68 | 100 |
| *Tragopogon aureus* | 149 | *Tragopogon dubius* | KT179725.1 | 97 | 9e-78 | 100 |
| *Corydalis oppositifolia subsp. oppositifolia* | 149 | *Corydalis wilsonii* | LN610850.1 | 100 | 2e-74 | 100 |
| *Rosa pisiformis* | 149 | *Rosa chinensis* | XR_002934681 | 98 | 6e-72 | 100 |
| *Lathyrus karsianus* | 149 | *Lathyrus decaphyllus* | KT459234.1 | 98 | 2e-72 | 99.32 |
| *Astragalus globosus* | 149 | *Astragalus canadensis* | MT610924.1 | 98 | 2e-70 | 99.32 |
| *Lamium galactophyllum* | 149 | *Ballata nigra* | ON685391.1 | 100 | 1e-69 | 100 |
| *Salvia rosifolia* | 149 | *Salvia carduaceae* | MK257800.1 | 100 | 3e-73 | 100 |
| *Allium czelghauricum* | 149 | *Allium altaicum* | MK049255.1 | 94 | 7e-57 | 96.48 |
| *Papaver triniifolium* | 149 | *Papaver somniferum* | XR_003342571.1 | 100 | 5e-68 | 99.33 |
| *Pastinaca armena* | 149 | *Zizia aurea* | MT610976.1 | 100 | 1e-69 | 100 |
| *Vincetoxicum coskuncelebianus* | 149 | *Asclepias tuberosa* | KY860923.1 | 100 | 2e-66 | 98.66 |
| *Fritillaria michailovskyi* | 149 | *Lilium michauxii* | AF205126.1 | 96 | 3e-65 | 99.31 |

When comparing the 26S rDNA sequences of 14 endemic plant species, we observed 38 variable sites (Figure 1). There was no variation between *Onosma isaurica* and *Onosma nigricaulis*. However, we did observe one variable site at the 52 bp location between *Lathyrus karsianus* and *Astragalus globosus*, which are both members of the Fabaceae family. In the Asteraceae family, there were four nucleotide substitutions between *Corydalis oppositifolia subsp. oppositifolia* and *Papaver triniifolium*, occurring at the 52, 77, 86, and 112 bp locations. Despite both belonging to the Lamiaceae family, *Lamium galactophyllum* and *Salvia rosifolia differed* at nine base positions in the 26S rDNA sequence (Figure 1). We have uploaded the sequence data for each endemic plant species to the GenBank database.

Figure 1. Multiple alignments of 26S rDNA gene region for the studied 14 endemic plant species

A phylogenetic tree was generated using the obtained sequences of 26S rDNA for 14 endemic plant species in order to detect the evolutionary relationships among them. The studied species were found to belong to different branches of the phylogenetic tree, with a low level of genetic differentiation among closely related taxa. Although members of each family were clustered together in separate genetic clusters, *Corydalis oppositifolia subsp. oppositifolia* and *Papaver triniifolium* were grouped with *Tragopogon aureus* and *Vincetoxicum coskuncelebianus*, respectively. When the 14 endemic species were analyzed, *Allium czelghauricum* and *Pastinaca armena* were found to be significantly different from the other endemic species (Figure 2).

## 4. Conclusions and discussion

The 26S rDNA region was successfully amplified and sequenced for the first time in 14 endemic plant species of Kars province, providing valuable insights into their genetic characteristics. The alignment results revealed intriguing relationships between the studied endemic species and their relatives in the same genus or family, emphasizing the genetic relationships within these plant groups. The comparison of 26S rDNA sequences among the 14 endemic plant species has revealed a notable level of genetic variation, as evidenced by the identification of 38 variable sites. These findings offer valuable insights into the molecular diversity within this group of plants and contribute to our understanding of their evolutionary relationships. The absence of variation between *Onosma isaurica* and *Onosma nigricaulis* suggests a high degree of sequence conservation within these two closely related species. Within the Fabaceae family, a single variable site at 52 bp distinguishes *Lathyrus karsianus* and *Astragalus. globosus*. This limited nucleotide substitution between members of the same family underscores the importance of exploring intraspecific variation, as even closely related species can exhibit subtle genomic differences that may have functional implications [25]. In contrast, a higher level of divergence within the Asteraceae family was observed in *Corydalis oppositifolia* subsp. *oppositifolia* and *Papaver triniifolium*, with four nucleotide substitutions at positions 52, 77, 86, and 112 bp, suggesting a greater evolutionary distance or potential ecological factors influencing genetic variation between these two species. Similarly, despite their membership in the same Lamiaceae family, *Lamium galactophyllum* and *Salvia rosifolia* exhibit variation at nine base positions in the 26S rDNA sequence. This highlights the complexity of genetic divergence even within taxonomically related groups, emphasizing the need for a nuanced understanding of evolutionary relationships at the species level [23].

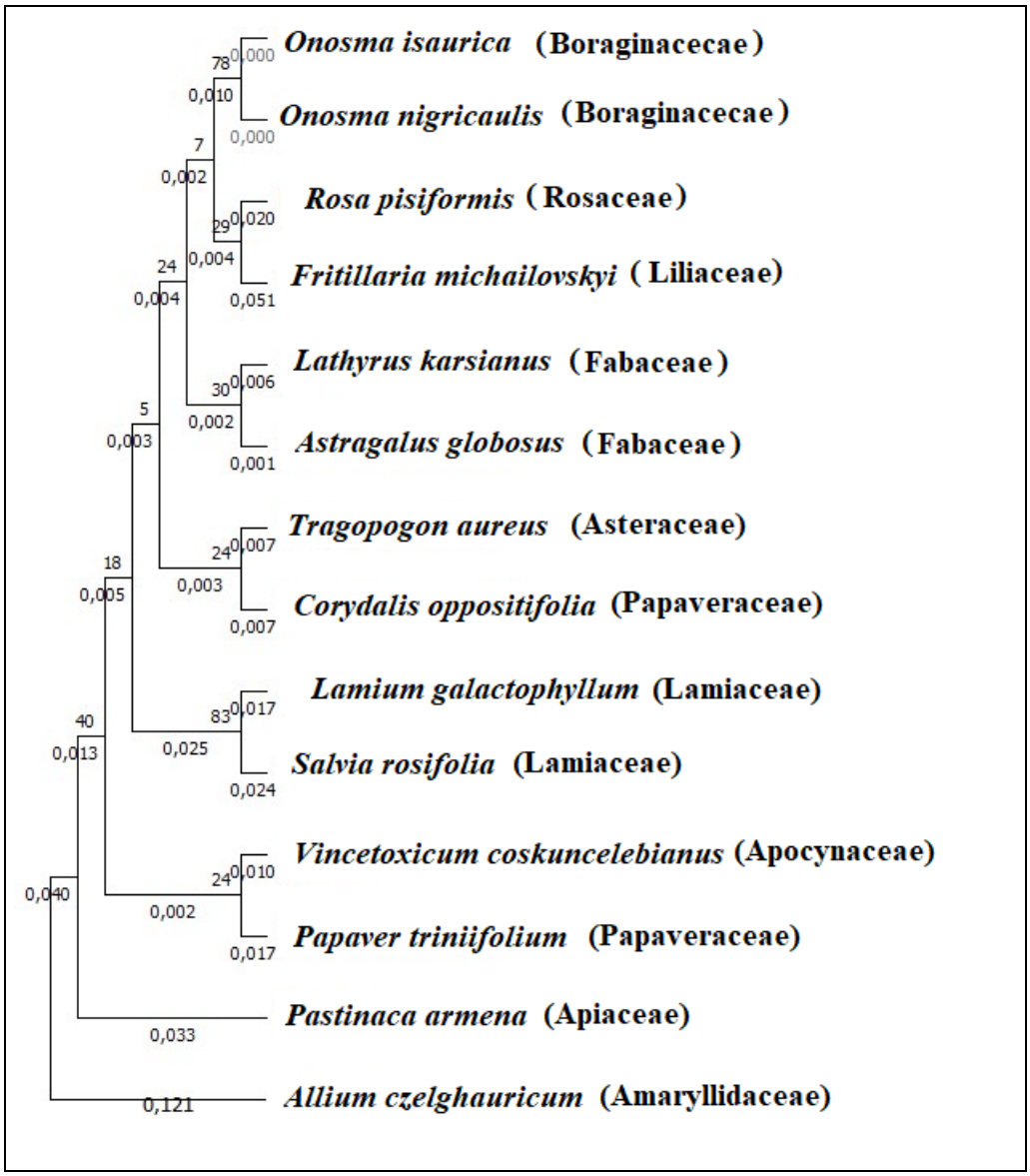


Figure 2. Phylogenetic tree based on the sequence of 26S rDNA gene region for 14 endemic plant species

The observed variable sites in the 26S rDNA sequences provide valuable markers for future studies aiming to explore the population genetics, phylogeography, and adaptive evolution of these endemic plant species [10, 11]. Investigating the functional significance of these variable sites could offer insights into their potential roles in adaptive processes or responses to environmental factors.

The construction of a phylogenetic tree using the 26S rDNA sequences of 14 endemic plant species has provided valuable insights into their evolutionary relationships. The phylogenetic analysis generally reveals a clustering of members within the same family. The observed clustering of members of each family into distinct genetic clusters aligns with expectations, suggesting a shared evolutionary history and genetic similarity among closely related species. However, the unexpected grouping of *Corydalis oppositifolia subsp. oppositifolia* and *Papaver triniifolium* with unrelated species challenge conventional taxonomic expectations. This discrepancy may be indicative of convergent evolution, or complex evolutionary processes that transcend traditional taxonomic boundaries [24]. A notable finding in the phylogenetic analysis is the significant genetic differentiation observed in *Allium czelghauricum* and *Pastinaca armena* compared to other endemic species. The distinct placement of these species in the phylogenetic tree suggests unique evolutionary trajectories or ecological adaptations that set them apart from their counterparts. The high polymorphism detected in the 26S rDNA region for these species indicates the dynamic nature of their genomes, reflecting ongoing evolutionary processes, potential gene flow, or adaptive responses to environmental factors [25]. It is seen that there is a paraphyletic relationship between the endemic species in terms of the 26S rDNA gene region. Although it is a very successful gene region in species identification, it is insufficient alone to explain the evolutionary relationship between these endemic species. The phylogenetic relationship between the studied endemic species can be revealed with different evolutionary approaches by studying different gene regions in addition to the 26S rDNA.

Genetic studies on endemic plant species have only recently begun to be carried out in Türkiye [26, 27]. It is clear that more detailed molecular identification and phylogenetic studies should be performed to protect the biodiversity of Türkiye.

Our study presents the first insights into the molecular characteristics of the 26S rDNA in 14 endemic plant species. The phylogenetic analysis of 26S rDNA sequences has revealed both expected and unexpected patterns in the evolutionary relationships among these 14 endemic plant species. This underscores the significance of our study in providing valuable genetic information for these endemic species. The establishment of genomic resources for these endemics may contribute to a broader understanding of plant diversity and evolution, emphasizing the ongoing need for efforts to explore and document the genetic uniqueness of endemic species. The preliminary results obtained from this study lay the groundwork for future genetic investigations aimed at conserving these endemic species and broader biodiversity. The addition of genetic data to national and international databases will contribute to a growing repository of information, facilitating collaborative research efforts and enhancing our understanding of plant evolution and diversity. The genetic data generated in this study serve as a valuable resource for future research initiatives focused on the conservation of Türkiye's rich biodiversity and contribute to the global discourse on biodiversity conservation.

**Acknowledgments**

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# The use of family planning methods by Somali women between the ages of 15-49 living in Turkey and their plans and attitudes towards these methods

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**Abstract**

Family planning (FP): individuals consciously plan the birth interval and regulate its timing. For FP, this effort requires resources such as drugs and devices and specific methods. This scientific research investigated the use of FP methods by Somali women aged 15-49 living in Turkey. Women were interviewed face to-face in 15 provinces with a survey consisting of 60 questions. According to the results of our scientific research, Somali women's cultures, traditions, social lifestyles, incomes, and religious beliefs have changed the use of FP methods at different levels. The pregnancy rate at a child age (<18) is 20%. 1/3 of women do not use any FP method. Belief, tradition, culture, tribe and family pressure are the biggest obstacles to FP. Income status changes the level of FP. As a result, proper planning of education and organized health delivery can reduce Somali women's FP approach and, therefore, obstetric problems.

**Keywords:** family planning, women's health, somali women living in Türkiye, birth control, women's obstetric knowledge level

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# Türkiye'de yaşayan 15-49 yaş arasındaki Somalili kadınların aile planlaması yöntemlerini kullanımı bu yöntemlere yönelik planları ve tutumları

**Özet**

Aile planlaması (FP); bireylerin doğum aralığını planlamak ve zamanlamasını düzenlemek üzere bilinçli çaba göstermeleridir. FP için bu çaba ilaç ve cihaz gibi kaynaklara ve belirli yöntemlere ihtiyaç duyar. Bu bilimsel araştırmada Türkiye'de yaşayan 15-49 yaş arası Somalili kadınların FP yöntemlerini kullanımları araştırıldı. 60 sorudan oluşan bir anket ile 15 vilayette kadınlarla yüz yüze görüşüldü. Bilimsel araştırmamızın sonuçlarına göre, Somalili kadınların kültürleri, gelenekleri, sosyal yaşam tarzları, gelirleri ve dini inançları değişik düzeylerde FP yöntemlerinin kullanımını değiştirmektedir. Çocuk yaşta (<18) gebelik oranı %20 düzeyindedir. Kadınların 1/3'ü hiçbir FP yöntemini kullanmamaktadır. İnanç, gelenek, kültür, kabile ve aile baskısı FP'nin önündeki en büyük engellerdir. Gelir durumu FP düzeyini değiştirmektedir. Sonuç olarak eğitim ve organize sağlık sunumunun doğru planlanması Somalili kadınların FP yaklaşımını dolayısıyla obstetrik sorunları azaltabilir.

**Anahtar kelimeler:** aile planlaması, kadın sağlığı, Türkiye'de yaşayan Somalili kadınlar, doğum kontrolü, kadınların obstetrik bilgi düzeyi

## 1. Introduction

According to the World Health Organization, FP is "the effort of individuals to plan and give birth to the desired number of children and to decide freely on the spacing and timing of birth." Approximately 75 million pregnancies worldwide are unplanned. This figure corresponds to 22% of total pregnancies. It is estimated that 61% of unplanned pregnancies are terminated because they are unwanted pregnancies. Since there is no complete data on the couples' relationships, these figures are only estimates. [1]. More than 200 million women in the world do not have access to modern birth control. FP practice can be more or less restricted due to socio-cultural realities, economic problems, psychological norms, lack of education, misinformation and beliefs. [2]. In

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underdeveloped societies, the pressure of patriarchal tradition, fear of social stigma, family management, myths about contraceptives, and traditional cultural interpretations are obstacles to FP. [3-5]. FP methods that can be used for a long time, such as long-acting family planning (LAFP) methods such as implants and extended-release drugs, can be a good solution for women's empowerment in reducing poverty, supporting economic growth, increasing female productivity and reducing fertility. [6, 7]. FP practices are subject to tradition, belief and tribal pressure as socio-economic factors reinforce the cultural loyalty of the demographic structure.[8]. Socio-economic factors, good health provision and education, can overcome this demographic blunting. [2]. Worldwide population growth has declined from its historic peak of 2.1% per year in the late 1960s to 1.7% today. However, Sub-Saharan Africa still faces the world's highest fertility and population growth rates.

While the population growth rate is becoming negative in some countries, the increase rate is high in African countries. As of 2021, the population growth rate is negative in Lithuania, Belarus, the Russian Federation, Romania, Estonia, Hungary, Armenia, Poland, Croatia, Germany, the United States, the Czech Republic and Japan. On the contrary, the population growth rate in countries such as Somalia, Djibouti, Ethiopia, Gambia, Mali and Chad is 5-8% [9, 10]. Somalia has the highest population growth rate (6.9-7.2%) [11].

## 2. Materials and methods

A critical questionnaire consisting of 60 questions was prepared for Somali women living in 15 provinces in Turkey. Ethics committee approval was obtained. Exclusions have been set. A power analysis of the research was done. A two-sample T-Test Power Analysis was used for power analysis. For statistical accuracy to be 99%, n=174 was determined as the ideal limit. For the group sample size, 99% accuracy was determined to detect a difference of 1.1 between the two groups, 87. Estimated group standard deviations of 1.9 and 1.6 to 2.2 were considered significant. A level of 0.05000 was accepted as a reference using a two-sided two-sample t-test. Statistically, 99.99% accuracy was determined for a total n ≥174(Machin, Campbell). The research was also done as n=196. The study was studied as n= 211. The prepared questionnaire was conducted face-to-face with the subjects in 15 provinces. In addition, he was sent to 209 universities (131 state, 11 technical, two fine arts, and 65 private and foundation universities) by obtaining permission through the Rectorate of Karabuk University. The answers are tabulated. Abstentions and non-answers were selected. Chi-square tests were applied to the continuous quantitative variables, and parametric tests were applied to the normally distributed data. Data with abnormal distribution were determined by non-parametric tests (Kolmogorov-Smirnov and Shapiro-Wilk).

## 3. Results

The average marriage duration of Somali individuals living in Turkey is 13.39 years. It generally refers to the young population. The current population average living in a household is 4.65 people, which is similar to the average family member of the Somali population living in developed countries [22]. The participants' marriage ages (mean: 20.63) and pregnancy ages (mean: 20.34) are very close to each other. The pressure of traditional culture sees the baby as the first product of marriage. For this reason, children should be requested as soon as possible without wasting any time. As a result, there is a very short period between the age of marriage and the age of first pregnancy ($p<0.05$).The study results related to the job and income status of families of Somali women living in Turkey are given in Figure 1.

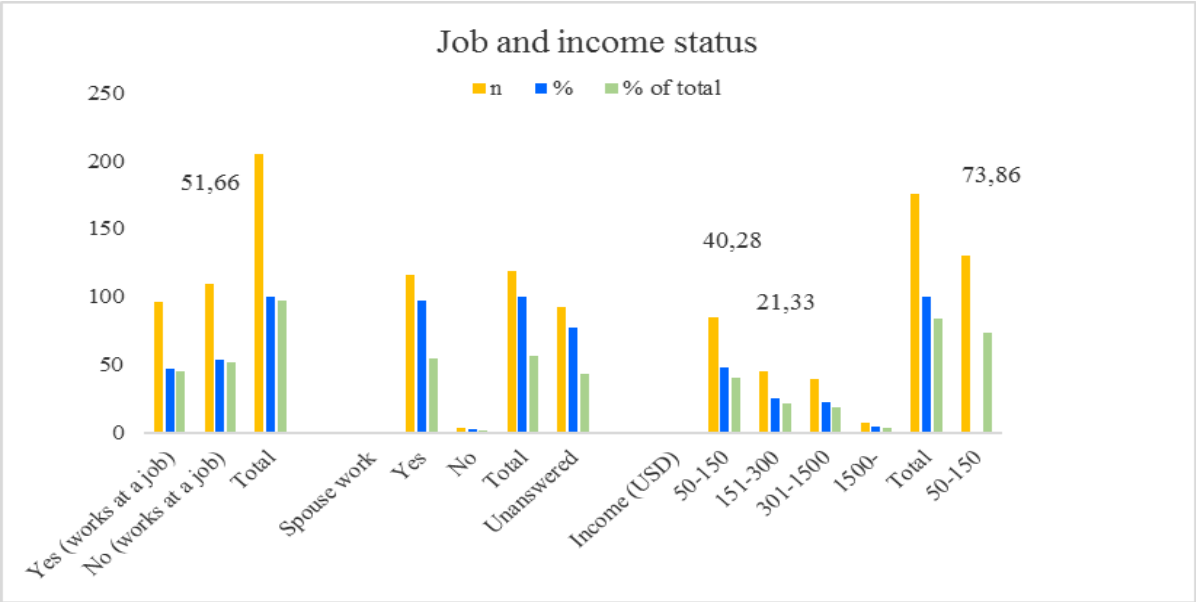


Figure 1. Job and income status of families. (*Working and income situations of Somali families living in Turkey. The income levels of families are insufficient and unstable. More than half of them do not have a job. Only the spouses of 54.98% of the participants have a job. 40.28% of the participants declared their total family income 50-150 dollars. The rate of those whose family income is between 150-300 dollars is 21.33%. Families with these two lower income levels correspond to 73.86% of the participants. Total family income is only around $50-$300. n: The number of participants, %: % of respondents among participants, % of total: Rate of respondents among all participants)*

Figure 2 indicates the women's attitudes towards pregnancy, birth interval, and abortion status of Somali women living in Turkey. The same figure indicate also the involuntary abortions and planned pregnancies. Based on the survey from this study the pregnancy age and status of the participants of Somali women living in Turkey has been shown in Figure 3. In addition, the study results related to participants about FP information resources, FP opposition reason, attitude about FP, the reason for abandoned FP as well as FP satisfaction, husband's FP claim status, and FP training with his wife were presented in Table 1.

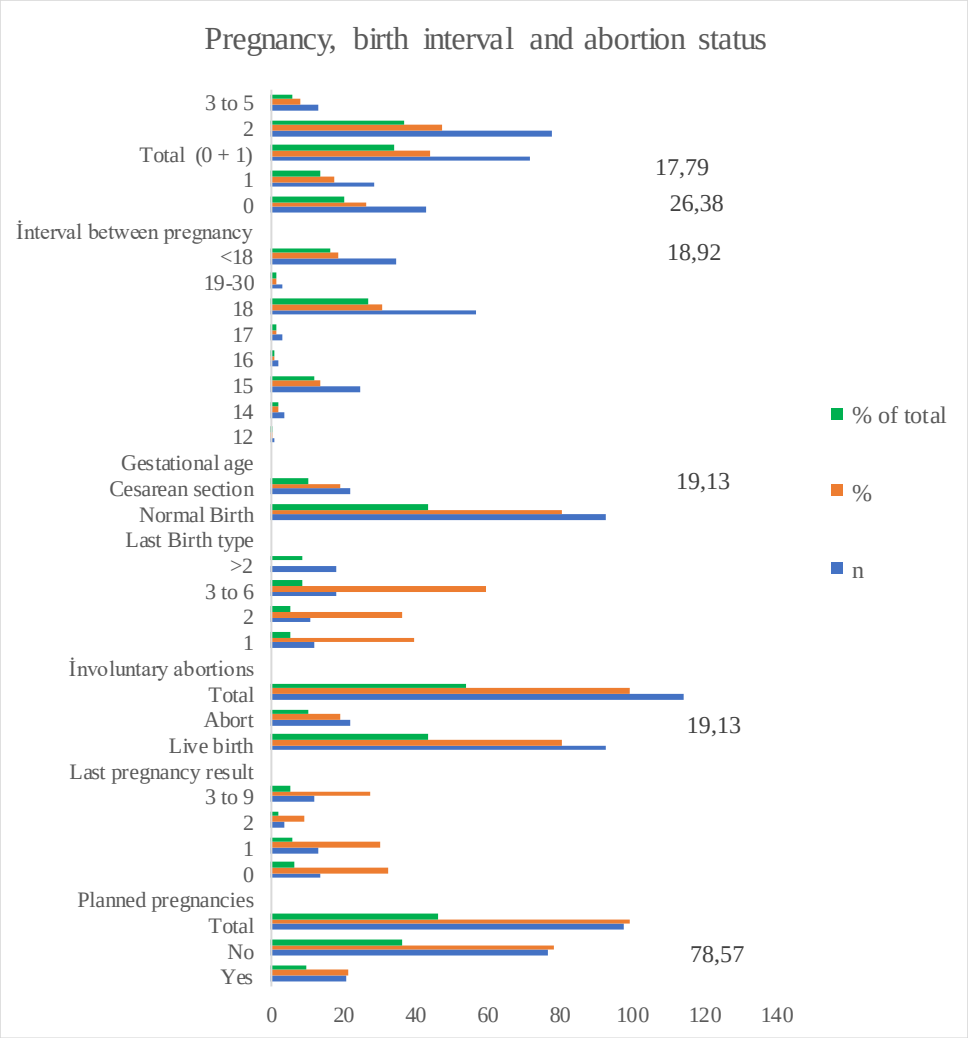


Figure 2. Women's attitudes towards pregnancy, birth interval, and abortion status (*All Somali women living in Turkey have had at least one pregnancy. Those whose interval between two pregnancies is less than 18 months is 20%. It is a partially accepted culture that the maternal age should be less than 18 for a healthy pregnancy (18.92%). One in every five women we met became pregnant as a child (<18). Gestational age is in childhood. Caesarean section is also available for birth at the same rate. The abortion and abortion rate is also quite high (19.13%). The majority of pregnancies (78.57%) occurred without FP. Pregnancy with FP is only 1/5. n: The number of participants, %: % of respondents among participants, % of total: Rate of respondents among all participants)*

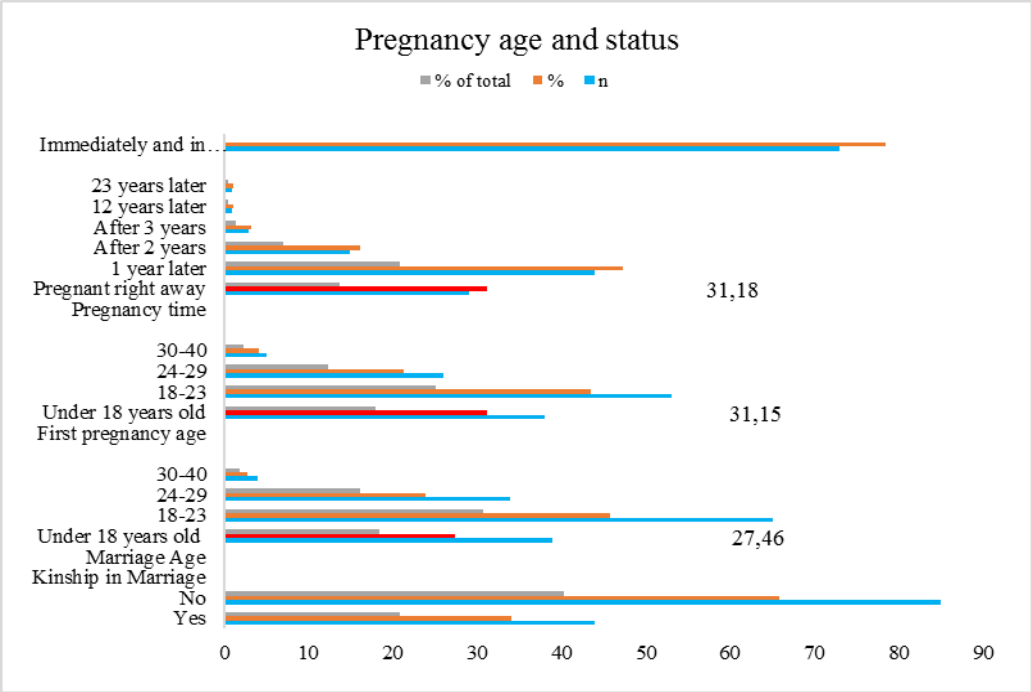


Figure 3. Pregnancy age and status of the participants (*The kinship level of Somali women living in Turkey with their spouses is 34.11%. Pregnancy during childhood is 1/3 of those who experience pregnancy at least once. The age of first pregnancy is usually between 18 and 29 years old. The rate of those who got pregnant again immediately after birth is 31.18%. The pregnancy rate within one year after birth is 47.31%. The rate of those with two years between births is 16.13%. Accordingly, the rate of those who got pregnant again until one year after birth (0-12 months) is 78.49%. n: The number of participants, %: % of respondents among participants, % of total: Rate of respondents among all participants)*

Table 1. Attitudes and behaviors about family planning

| **FP information resource** | **%** | | **Why FP opposition** | **%** |
|---|---|---|---|---|
| Other-Health personal-Internet-Relatives-neighbours | 5 | | Shame | 22,81 |
| Newspaper-Magazine-Book- Internet-Health personnel | 32 | | Spouse refuse | 8,77 |
| Social media | 2 | | Pregnancy wants | 14,91 |
| Internet-Other | 4 | | Haram (religiously forbidden) | 15,79 |
| Health personnel | 9 | | There is no reason | 35,09 |
| Radio-TV, Internet | 1 | | Disapprove pregnancy | 1,75 |
| Relative-Neighbor-Newspaper-Magazine-Book, | 47 | | Pregnant | 0,88 |
| **Attitude about FP** | **%** | | Shame + Haram + No reason +Wants pregnancy | 88,6 |
| Harms the unborn child | 3,16 | | **Why abandoned FP?** | **%** |
| No idea | 36,08 | | Menstrual irregularity | 16,67 |
| Sin | 10,76 | | Pain and ache | 11,11 |
| Not good | 12,66 | | Husband decision | 11,11 |
| Against | 20,89 | | Pregnancy claim | 16,67 |
| It is not healthy | 14,56 | | İncreased bleeding | 11,11 |
| Negative -Total | 62,03 | | No reason | 27,78 |
| Positive Total | 1,9 | | No idea | 5,56 |
| **FP satisfaction** | | | **Husband's FP claim status** | **%** |
| Menstrual problems | 7,69 | | Desire | 53,33 |
| Headache | 5,13 | | Not desire | 46,67 |
| No idea | 10,26 | | **FP training with his wife** | **%** |
| Positive | 76,92 | | Yes | 13,71 |
| | | | No | 86,79 |

**4. Conclusions and discussion**

This scientific research is the first detailed scientific research in Turkey. This study was done with an acceptable accuracy of 99.99%. The age difference between spouses is significant. The rate of those with a 10-16 year age gap is 10%. This situation is much lower in developed societies. The average family size, including siblings, is 7.42. There is no family smaller than five people. 20.21% of the participants are primary school graduates, 13.30% are secondary school graduates, and 515.43 are high school graduates. The rest are university graduates. There are many participants whose reason for living in Turkey is education. Therefore, the education level is quite low. The majority (60.20%) are married. Being divorced is hidden because it is considered shameful and a sin. Although the average age is expressed as 30.4 in a study conducted on refugees in Kampala the average age of individuals whose purpose of coming to Turkey is education and trade is 33.96 [12]. This difference can be interpreted as the difference between forced asylum and the choice of Somali refugees for commercial and educational purposes. Studies indicate that the average age of marriage is 18[13] or 18-19 [14]. Our research shows that marriages are most common between the ages of 18-23. Early marriage is routine in Somalia. According to the SHDS (The Somali Health and Demographic Survey), 36% of women aged 20-24 were married at 18. A woman who only marries at the age of 35 is almost absent. These data of SHDS exactly match our research [15]. Our research is consistent with this study.

Although previous studies found family size to be more than six or even 9 to 10 people, this number was found to be 5.13 in our study. Although the average family size is high in Somalia, the average number of family members going to developed countries for education and job opportunities is relatively low. Education awareness, the burden of population density, individuals' demand for a better life, and gaining digital and universal awareness through communication are the factors that cause the decrease in family size. [13].

In our study, unlike SHDS, the fertility rate was lower. Here, the number of children, the absence of social status, and the level of socio-cultural development are important. Although it is stated in the research that Somali women are mostly (62%)[16] or 73% (OCHA, 2023)[17] are housewives [16] or more than half of them do not practice any profession, the rate of homemakers in our research is 36%, and most of the rest are professionals. This situation; is the demand for integration into the socio-cultural structure of developed countries. Studies have found that 60.4% of women have knowledge of FP, and 38.6% of women have no knowledge of FP[3, 18]. Our study determined that 65.9% had knowledge of FP, while 28.4% had no knowledge of FP. Somali women consider their FP information confidential. However, as the level of education increases, this shyness ends. Reasons for not using any of the family planning methods in the study: Wanting to have a child is due to 6.6% pressure from her husband, 31.0% to avoid side effects of medications, and 8.9% to religious belief, culture and tradition. The rate of those who do not use FP for other reasons is low (36.3%).[18]. In our study, the spouse's rejection rate (6.6%) was lower than the others (12.8%). Socio-cultural integration in developed countries can also explain this situation. Reasons for not using FP in studies conducted on women in Norway and Somalia: For those living in Norway, 23.9% are religious, 42.3% do not want a spouse, and 30.6% want to give birth. It was determined that 76.1% of women in Somalia do not use it for religious reasons, 57.7% do not use it because their husbands do not want it, and 69.4% do not use it because they want to get pregnant. [19].

In Somalia, births determine self-confidence and status in society. Since the family type is patriarchal, it is considered shameful to discuss FP methods. Therefore, it is proportionally higher [20]. Although it was stated that any FP method was used in 38.0% and not used in 61.1% [21], in our study, the use was only 15.2%, and no FP method was used in the remaining 70.6%. We investigated whether the cost of the FP method poses a threat to method choice. It is clear that the cost of the FP method affects the choice (81.48%) ($p<0.05$) because they purchase the AP method they use (91.11%) by paying money ($p<0.05$). Although they did not specify the procurement method, the rate of those who stated that they obtained it somehow was 4.44%. The price rate not being determined by the FP method is 18.52%. Therefore, price is the determining factor when choosing a method. Some data indicate that 52% of those using modern methods are procuring from public institutions and organizations (TNSA, 2018). In our country, most participants benefit from free FP opportunities from health institutions.10.4% of the participants stated that they also received AP education consultancy. Again, 9.4% of the participants chose the method they used with their spouses. These rates are well below the averages of developed countries. Almost 8/10 of women believe that talking about private family matters, such as FP, is not well received in Somali culture and tradition.

The rate of participants abandoning any FP method is low (7.98%). There is a very high tendency to continue using the FP method they use (92.02%). The FP method may be abandoned due to menstrual irregularity (16.67%) and pain (11.11%). The main reasons for abandoning the FP method in different studies are menstrual irregularity (16.67%) and desire to get pregnant (16.67%). Abandonment of FP due to pain and ache complaints, partner's reluctance and

increased bleeding is 11.11%. They abandoned the current FP method (38%) because they wanted to get pregnant, (19%) because they could not use the method successfully, (9%) because it had side effects, and (7%) because they wanted more.

In our study, the average age of women and their spouses, family size, and number of households are parallel with Somali population data and Somali immigrants migrating to other developed countries.

Our country's average income level of Somali individuals is around 150 USD. Excluding seven families (3.32%) with monthly income above 1500 USD, the lower limit of the average household income is 150 USD. In this situation, the monthly earnings of Somali individuals living in Turkey are below the 2022 minimum wage. Therefore, although the lives of Somali families are well above Somali standards, they are below the minimum wage in Turkey. This income is below the hunger threshold of an average family of 4 in Turkey. Age at marriage is associated with the period from the first pregnancy to the second pregnancy (p=0.02). Because the biggest expectation from marriage is a baby. There is a tight statistical connection between the number of children conceived by planning (p=0.03) and the number of miscarriages (p=0.041). Because In unplanned pregnancies, the interval between two children is less than two years. Age at marriage is statistically significant and related to the number of people in the household (p=0.007). Because in the Somali family, Tradition and patriarchy are a measure of life.

First pregnancy after marriage: It is associated with the number of births (p=0.008), number of siblings (p=0.045) and number of children (p=0.041). Having the first pregnancy right after marriage shortens the time between two pregnancies (p=0.015). The reason for this is that women are a gender that is fertile from a young age (<18), and the more they give birth, the more prestigious they are. It was determined that there was a statistically strong relationship between the number of births a woman had and her attitude towards not getting pregnant after a certain age (p=0.001). An opposite relationship was found between the number of births and FP (p=0.875 >0.05). The number of births is higher because they do not internalize FP (p=0.57 >0.05). That means that the rate of unintended miscarriage is high in women who do not set a period between their first pregnancy and birth and do not plan to have children. The statistical significance between them (p = 0.036) confirms these conclusions. Using any FP method has nothing to do with the age at marriage, the time from the first pregnancy to marriage, how many pregnancies they had, how many children they had, the number of births, the age of their spouse, or their age. On the other hand, the family planning method used has a strong relationship with the age of the first pregnancy (p=0.004), the mother's awareness of how old she should be for a healthy pregnancy (p=0.001), and her attitude towards the period between two pregnancies (p=0.009). For this reason, Somali women who have been using the FP method for a certain period have an awareness about the age of first pregnancy and the age at which they should not become pregnant. There is a statistically significant relationship between the age of Somali women living in Turkey and involuntary abortion(s) (P=0.005). This situation can be interpreted as the fact that education cannot adequately solve traditional culture and traditions.

The time between the first pregnancy and the second pregnancy, the number of planned children, the duration of FP use and the number of abortions are independent of the age of the partner (p>0.005). There is a significant relationship between a healthy pregnancy and the partner's age (p=0.001) and the time between two pregnancies (p=0.009). This explains that the spouses' FP attitude is traditional and has stayed the same.

The time between marriage and two pregnancies, the woman's age (p=0.007), number of siblings, number of households, mother's healthy gestational age (p=0.003), partner's age (p=0.003), the time between two pregnancies (p=0.035) and the number of households (p=0.001) are closely related to the woman's family size. This situation reflects the socio-cultural structure from which the cultural and traditional family model originates. Clearly, women's education cannot change the family structure.

Marriage duration; regardless of the length of time they use any FP method and the number of children they plan to have. However, the relationship between income level (p=0.013), number of abortions (p=0.009), healthy gestational age of the mother (p=0.001) and the time between two pregnancies (p=0.006) is significant. This is because household income and awareness are more important than culture and traditions when using FP methods. It must be admitted that not every Somali woman has the opportunity to receive an education in a developed country like Türkiye.

Income level is directly related to the age of the spouse (p = 0.02), the number of planned children (p = 0.004), the duration of marriage (p = 0.013) and the attitude at which age one should get pregnant. This; It directly affects a healthy pregnancy (p=0.033). Household welfare level: It affects the obstetric awareness of families and the behaviours shaped by this awareness.

The number of households is closely related to the healthy gestational age limit (p=0.001). The period between two pregnancies (p=0.012), age at marriage (p=0.007), and age at first pregnancy (p=0.031) have a significant

correlation with the number of people in the household. Family size of Somali women living in Turkey changes FP education and perception.

**In conclusion**

There is a significant difference (p=0.041) between a woman's age at marriage and her unintentional abortions. Similarly, there is a close relationship between age at first pregnancy and unwanted miscarriage (p=0.015). The woman's age (p=0.05) and number of births (p=0.036) are associated with involuntary miscarriage. Likewise, there is a close statistical relationship between the duration of marriage, the number of children, the woman's age, the time between two pregnancies and the perception of the time between unwanted abortions (p=0.009).

Due to the prevalence of early and child marriages, the failure to obtain accurate information about birth from health personnel such as midwives and nurses after marriage seems to be the main reason for this situation.

The mother's age for a healthy pregnancy and the duration of any family planning method is directly related to the number of years the spouses have been married and how many people live in the house and have a very high statistical value (p = 0.001). Three important conclusions can be drawn from this:

1. It is essential that the spouse is of mature age for marriage and has sufficient health knowledge.
2. The experience gained during the years of marriage is of great importance for the continuation of the marriage and the health of children and parents.
3. It is vital that family planning methods can be received correctly and satisfactorily from health professionals and applied to the marriage process.

The rate of those who do not use FP because they do not want children is 8.1%. Those who do not use it due to peer pressure and embarrassment are 12.8%. 4.7% do not want to get pregnant. He does not use 1% as per his belief. 19% want to avoid specifying the reason. Due to the cultural differences between the countries and women's focus on education and working to improve themselves, the rate of those who do not use AP due to their desire to give birth is higher in our study. Since the first pregnancy occurs at the same time as marriage due to the culture, traditions and customs of Somali women, it does not seem possible to use family planning methods for the first child or to harmonize the knowledge, attitudes and behaviors of the mother and father. Spouses are together for a healthy pregnancy. The fact that one in every five married women is under the age of 18, the rate of pharaoh type (type-III) mutilation is over 97%, lack of nutrition, hygiene, sanitation, lack of access to clean water and sewer infrastructure make both birth and postpartum care difficult. This situation creates a vital problem not only for the mother but also for the newborn baby. The lack of health infrastructure and provision and the inadequacy of health personnel (midwives, nurses, doctors, etc.) should also be added to this. Therefore, although it is not impossible, it is extremely difficult for a young girl growing up in Somalia to know family planning, the methods used, to develop attitudes and to reflect the results of this in her behavior. The main reason we are in our country is to improve cultural knowledge, skills and attitudes, especially health. The average duration between two pregnancies is 1.42 years (17 months). The time between two pregnancies is inversely proportional to the level of education. The pregnancy period under one and a half years of age is generally considered to be an impossible period in terms of pregnancy health, family planning services, and adequate and effective health care. Although they are better than their spouses in terms of education at the undergraduate level, they are close to each other at primary, secondary and high school levels. It can be interpreted as an understanding of educational awareness for women. As the level of education increases, it is inevitable for FP presentation to be accurate and effective. Seeing women's education as a hope can be considered as a very important parameter. The number of unemployed men is quite low, and women are mostly housewives.

Lack of health insurance (73.9%) means that they cannot benefit from FP services, which include health services such as planning, monitoring, preventing pregnancy, and intervening in problems during pregnancy. Therefore, FP usage is directly affected. The rate of consanguineous marriages is lower. Since individuals studying in Turkey are above a certain economic level, their socio-cultural perceptions have changed. Despite this change, the FP methods known and used are classical and traditional, and 1/3 of the society is unaware of FP. Spouses' interest in FP is low (19.8%). Information; It was obtained from sources such as relatives, neighbors, friends, newspapers, radio, television and the internet. Therefore, the information is weak, insecure and insufficient.

It is traditionally considered rude to express an opinion about FP. FP is seen as unhealthy, harmful to pregnancy, has side effects, is not in line with traditional culture, is sinful, and is a practice.

The rate (<1%) of those who state that family planning is a feasible and good thing is very low (p<0.05). However, satisfaction with using any family planning method is quite high (96.7%). The rate of discontinuation of all

family planning methods used is quite low (5.7%). However; After the participants came to Turkey, their spouses' views on FP changed, their opposition to FP decreased, and their demands for FP increased. This contradiction can be described as follows: They are Somalis with their culture of life and imagination. However, they are like citizens of a developed country in terms of physical appearance, education and professional skills.

Anti-FP belief criteria are less influential than culture, tradition, tribal pressure, and money. This alone is an improvement.Even though they receive information about any FP method from healthcare professionals after coming to Turkey, the rate of those who are dissatisfied can be much higher. The situation of their wives is similar. If every developed country could provide free health education services to immigrant women, a butterfly effect could be created for citizens of underdeveloped countries. It is clear that 73.9% do not have health insurance, and they cannot benefit from the FP, which covers planning, monitoring or preventing pregnancy and intervening in problems during pregnancy. The rate of consanguineous marriages is lower among Somali women living in Turkey. Since individuals educated in Turkey are above a certain economic level, their socio-cultural perceptions have changed. Despite this change, the AP methods that are known and used are classical and traditional. Additionally, 1/3 of women are still unaware of FP.

Spouses' interest in FP is low (19.8%). FP information was obtained from relatives, neighbours, friends, newspapers, radio-television and the Internet. This information needs to be more robust, more secure and sufficient.
It is traditionally considered rude to express an opinion about FP. It is widely believed that FP is unhealthy and harmful to pregnancy. FP for them: It is a practice that has side effects, is incompatible with traditional culture, and is considered a sin.

The rate (<1%) of those who state that family planning is a feasible and good practice is shallow ($p<0.05$). On the other hand, satisfaction with using any family planning method is quite high (96.7%). The rate of abandoning all family planning methods is also meagre (5.7%). Participant spouses' opinions about FP changed after they came to Turkey. Their resistance to FP has decreased, and their demands have increased. This contradiction can be explained as follows: They are Somalis with their culture of life and imagination. However, they are like citizens of a developed country regarding physical appearance, education, and professional skills. Anti-FP belief criteria are less influential than culture, tradition, tribal pressure, and money. This alone is an improvement. The situation of their wives is similar. If every developed country could provide accessible health education services to immigrant women, a butterfly effect could be created for citizens of underdeveloped countries.

A significant ($p=0.048$) relationship exists between the age at first pregnancy and the number of children they plan. There is a high correlation ($p=0.004$) between age at first pregnancy, duration of family planning use and how many years they have used family planning. FP is directly proportional to awareness, culture and economic development.

This is because Somali women in Turkey receive complete FP services. A statistically significant ($p=0.016$) relationship exists between the duration of pregnancy after the first pregnancy and the number of pregnancies. On the other hand, there is a highly significant ($p=0.015$) relationship between the age at first pregnancy and the number of unintentional miscarriages. Child marriages and the short period between two pregnancies can be blamed for abortion. On the other hand, there is no significant difference between pregnancy and number of planned children, duration of family planning use and number of siblings. Receiving FP information and services free of charge in Turkey has changed perceptions.

The age at first pregnancy ($p=0.048$) and the time between two pregnancies ($p=0.05$) were statistically significant; this was the mother's last pregnancy. The age at which a woman can become pregnant is independent of the woman's age, length of marriage and the age of her husband. However, there is a high statistical significance ($p=0.004$) between the planned number of children and income status. There is a close relationship between the time taken for the first pregnancy after marriage and the number of births ($p = 0.008$). This situation arises from the identification of women with "birth", which comes from the culture and traditions of Somali women. There is a significant relationship between the number of children of Somali women in Turkey and the age at conception ($p=0.001$). It can be clearly understood how important the first pregnancy is. Therefore, the first birth after marriage, which is based on socio-cultural traditions, is for the woman's social status. The attitude is this: A woman is fertile with her feminine existence. Therefore, there is almost no age limit for a healthy pregnancy.

In terms of education and profession, developed countries, especially the United Nations, EU, FAO, World Bank and World Health Organization, should organize programs that encourage joint organizations, associations and cooperatives that will enable women to participate in working life.

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# A comparative haematological research on *Bufo bufo* (Linnaeus, 1758) and *B. verrucosissimus* (Pallas, 1814) in Türkiye

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**Abstract**

In this study, it is aimed to contribute to the systematic situation of *Bufo bufo* and *B. verrucosissimus* by comparing some blood parameters for the first time. For that, fieldwork was done in Lake Borçka Karagöl, Artvin for *B. verrucosissimus* and Lake Uzungöl, Trabzon for *B. bufo* in 2022. A total of 6 adult individuals (3 males, 3 females) were captured per species. For each individual, 1 ml blood sample was acquired from spontaneously pulsating heart ventricle and blood smears were prepared. Under a microscope, 40 randomly chosen erythrocytes from each smear were measured to acquire 9 distinct blood cell parameters. Additionally, erythrocyte and leukocyte counts were calculated using Neubauer hemacytometer. It was found that female individuals had larger values for measurement of blood cell parameters. Mean erythrocyte and leucocyte numbers were higher in the blood of *B. bufo* than *B. verrucosissimus* but there was not a significant difference between species for erythrocyte and leukocyte counts. In females, erythrocytes were larger and narrower in *B. verrucosissimus* than *B. bufo* species. Alike, nuclei were larger and narrower as observed in *B. verrucosissimus*, similar to erythrocytes. However, *B. bufo* had larger and wider erythrocytes but larger and narrower nuclei in males. Given the differences between sexes, these characters thought as not diagnostic. In addition, PCA analysis showed overlapped position of species in morphospace and supported the weak discrimination power of the characters. Our findings will contribute to the future studies as a reference source for basic blood parameters.

**Keywords:** blood cell, Common Toad, Caucasian Toad, erythrocyte, morphology

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## Türkiye'deki *Bufo bufo* (Linnaeus, 1758) ve *B. verrucosissimus* (Pallas, 1814) üzerine karşılaştırmalı bir hematolojik araştırma

**Özet**

Bu çalışmada, bazı kan parametrelerini ilk kez karşılaştırılarak *Bufo bufo* ve *B. verrucosissimus* türlerinin sistematik durumuna katkı sağlanması hedeflenmiştir. Bu amaçla, 2022 yılı içerisinde *B. verrucosissimus* için Artvin Borçka Karagöl ve *B. bufo* için Trabzon Uzungöl mevkisinde saha çalışması yapılmıştır. Tür başına toplam 6 yetişkin birey (3 erkek, 3 dişi) yakalanmıştır. Her birey için atmaya devam eden kalp ventrikülünden 1 ml kan örneği alınmış ve yayma preparatlar hazırlanmıştır. Mikroskop altında, 9 farklı kan hücresi parametresine ait veriyi elde etmek için her yaymadan rastgele seçilen 40 eritrosit ölçülmüştür. Ayrıca Neubauer hemasitometresi kullanılarak eritrosit ve lökosit sayıları hesaplanmıştır. Kan hücresi parametrelerinin ölçüm sonuçlarına göre dişilerin erkeklerden daha büyük değerlere sahip olduğu belirlenmiştir. *B. bufo* türünün kanındaki ortalama eritrosit ve lökosit sayıları *B. verrucosissimus* türüne göre daha fazla olsa da eritrosit ve lökosit sayıları açısından türler arasında anlamlı bir farklılık tespit edilmemiştir. Dişiler için eritrositler *B. verrucosissimus* türünde *B. bufo* türüne göre daha büyük ve daha dar şekle sahiptir. Benzer şekilde, *B. verrucosissimus* türünde çekirdekler, eritrositlerde gözlendiği gibi daha büyük ve daha dar şekillidir. Öte yandan, erkekler için *B. bufo* türüne ait eritrositler daha büyük ve daha geniş şekilli fakat çekirdekleri daha büyük ve daha dar şekillidir. Cinsiyetler arasındaki farklılıklar göz önüne alındığında, bu karakterlerin diyagnostik olmadığı

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düşünülmüştür. Ayrıca PCA analizi türlerin morfouzaydaki örtüşen konumlarını göstermiş ve karakterlerin zayıf ayırt etme gücünü desteklemiştir. Bulgularımız temel kan parametreleri için referans kaynağı olarak gelecek çalışmalara katkı sağlayacaktır.

**Anahtar kelimeler:** kan hücreleri, Siğilli Kurbağa, Kafkas Siğilli Kurbağası, eritrosit, morfoloji

## 1. Introduction

Haematological parameters allow the inference of physiological information specific to a species and offer valuable data for ecological studies on amphibian species [1]. In addition, the characteristics are evaluated as environmental indicators that react rapidly to changes in the surrounding environment [2, 3]. As a result, it is often used for assessing environmental stress. The systematic research on amphibian species benefits from the utilization of haematological markers besides their ability to detect changes in physiological, pathological, ecological, and environmental factors [4, 5]. It is feasible to draw conclusions regarding internal (gender, species-specific characteristics, age) and external (season, habitat, trophic) elements through the comparison of these metrics.

Amphibian blood cells are composed of leukocytes, platelets, and erythrocytes. Leukocytes are more resemble to those in human blood. Thrombocytes are nucleated spindle cells that fulfil the same function as mammalian platelets. Large erythrocytes of amphibians emerged through evolutionary processes distinguish them from other vertebrates. Compared to birds and mammals, their erythrocytes are more robust and persistent. The shape of erythrocyte cells is oval, nucleated, and biconcave [6, 7]. The number of erythrocytes varies with body size, age, gender, season, and environmental factors in addition to species and individuals within a population. Furthermore, the quantity of erythrocytes in amphibians differs greatly throughout species and presents useful data for systematic evaluations [8].

The *Bufo bufo* species group refers to a taxonomic group of toads within the genus *Bufo* distributing in Western Palearctic realm. The species group includes four closely related taxa namely Eichwald's toad *Bufo eichwaldi* Litvinchuk, Borkin, Skorinov and Rosanov, 2008, Spiny toad *Bufo spinosus* Daudin, 1803, Common toad *Bufo bufo* (Linnaues, 1758) and Caucasian toad *Bufo verrucosissimus* (Pallas, 1814). In the recent molecular studies, *Bufo bufo* and *B. verrucosissimus* have been assessed as sister species, and the broad distribution of both species have overlapped in Türkiye. Besides, the systematic situation of these taxa has become a subject in various studies based on molecular and morphological data [9, 10]. Lately, the presence of a narrow hybrid zone between these species has been reported in the northeastern Anatolia [11]. Unlike the presence of numerous comparative studies, there is no comparison in terms of serological characters and blood parameters between these species. Previous studies have focused only on the blood parameters of *B. bufo* species from Türkiye and described the characteristic of blood cells [12-15]. As a comparative study, Tosunoğlu and Taskavak [16] investigated 10 samples obtained from Manyas (Balıkesir) and Çamlıhemşin (Rize) districts in terms of blood-serum proteins. As a result of the study, they reported that there was no qualitative or quantitative difference in serum protein phenograms. Regarding their findings, the researchers reported that the samples from both localities should be considered as *Bufo bufo spinosus* subspecies and that the taxon called *B. bufo verrucosissimus* in Çamlıhemşin district could be synonymous. Given the current geographic distributions and presence of the hybrid zone between species, the comparison remained blur and did not adequately describe the haematological patterns.

The lack of a comparative study between *B. bufo* and *B. verrucosissimus* regarding blood parameters is an important gap in the literature that needs to be resolved. In addition, the absence of serological data on the *B. verrucosissimus* species is an essential taxonomic deficiency. In this study, it is aimed to contribute to the systematic situation of both species by comparing some blood parameters for the first time.

## 2. Material and method

Fieldwork was done in Lake Borçka Karagöl, Artvin (41.386124 N, 41.854107 E; 1450 m) for *B. verrucosissimus* and Lake Uzungöl, Trabzon (40.622108 N, 40.285267 E; 1100 m) for *B. bufo* in September 2022. For each species, a total of 6 adult individuals (3 males, 3 females) were captured. Adult samples were sexed following the external sexual characteristics: densely melanized fingers and presence of nuptial pad in males, and the opposite in females. Fieldwork and sampling were done with the permission of the Republic of Türkiye Ministry of Agriculture and Forestry General Directorate of Fisheries and Aquaculture Sampling (number: E-21264211-288.04-6387153), and the local ethics committee for animal experiments (Republic of Türkiye Recep Tayyip Erdogan University Local Ethics Committee for Animal Experiments, approval reference number: 2022/19).

The specimens were taken to the laboratory alive and snout-vent length (SVL) was measured using a digital calliper to the nearest 0.01 mm. Before sampling blood, individuals were anesthetized in 250 mg/L MS222 solution. For each individual, 1 ml blood sample was acquired from spontaneously pulsating heart ventricle using 21-gauge needle and 5 ml syringe. Afterwards, 4 different blood smears were fixed using methanol and was exposed to Wright’s stain

for 15 min. For each smear, a total of 40 randomly selected erythrocytes were measured using Olympus BX51 microscope at 200x and 400x magnifications for following characters: erythrocyte length (EL), erythrocyte width (EW), nucleus length (NL), nucleus width (NW), erythrocyte shape (ESh: EL/EW), nucleus shape (NSh: NL/NW), nucleus/cytoplasm shape (NCSh: NSh/Esh), erythrocyte size (ES: ELEW$\pi$/4) and nucleus size (NS: NLNW$\pi$/4). The erythrocyte (EN) and leukocyte (LeuN) counts were calculated by diluting Hayem and Turck solutions and using Neubauer hemacytometer.

Descriptive statistics were calculated using obtained measurements. Normality assumption was controlled using Kolmogorov-Smirnov test. The measurement differences between the sexes and species were compared using Student's t test and Mann-Whitney U test. Univariate analyses were run using the *stats* package. To reduce the dimension of dataset and to explain variable contribution, principal component analysis (PCA) was performed using log10-transformed data. For that, all specimens were used without grouping. The relationships between SVL and blood parameters were investigated using correlation analysis. All analyses were executed in R Programming Language v4.1.2 [17].

## 3. Results

Erythrocyte shape is oval and resemble to amphibian blood cell characteristic. Nuclei are generally elliptical and located at the centre of the erythrocytes. Cytoplasm is stained light purple whereas chromophilic nuclei are dark blue and purple. Descriptive statistics indicating measurements of blood cell characters were presented in Table 1.

For the variables showed non-parametric distribution ($p<0.05$), pairwise comparisons between sexes and species were carried out using Mann-Whitney U test. Regarding sexual comparison of whole data, significant differences were found in EL (Z= -4.426; p <0.01), EW (Z= -7.132; p <0.001), NL (Z= -3.420; p <0.01), NW (Z= -8.334; p <0.001), ESh (Z= -8.129; p <0.001), NSh (Z= -9.496; p <0.001), NS (Z= -3.715; p <0.001), NCSh (Z= -2.927; p <0.01) between sexes. When comparing species, significant differences were found in EL (Z= -2.880; p <0.05), EW (Z= -4.284; p <0.001), NW (Z= -6.149; p <0.001), ESh (Z= -5.145; p <0.001), NSh (Z= -5.514; p <0.001), NS (Z= -4.371; p <0.001), NCSh (Z= -2.838; p <0.05) for females and in EW (Z= -13.594; p <0.001), NL (Z= -3.750; p <0.001), NW (Z= -4.859; p <0.001), ESh (Z= -10.698; p <0.001), NSh (Z= -6.464; p <0.001), ES (Z= -8.426; p <0.001), NCSh (Z= -5.219; p <0.001) for males. In the variables showing normal distribution, there was only a significant difference between sexes in terms of SVL (t= 7.33; df=10; p<0.001). The distribution of data classified by species and sex was displayed using boxplots in Figure 1.

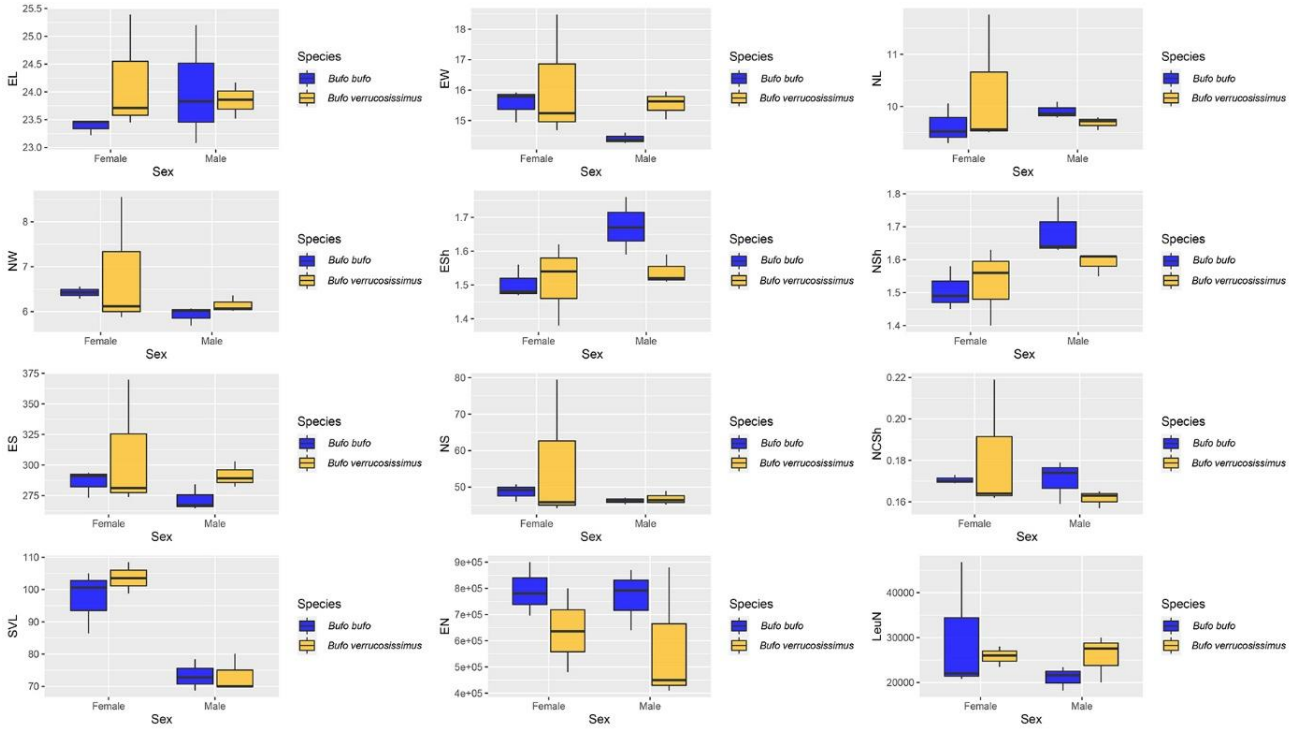


Figure 1. Boxplots representing differences in blood cell parameters between species based on sexes. The tick line in box is showing median. The lines positioning under and on the box are whiskers

Table 1. Descriptive statistics of SVL and blood parameters of *B. bufo* and *B. verrucosissimus*, respectively (mm: millimetre; µm:micrometre)

| | *Bufo bufo* (Uzungöl) / Females | | | | |
|---|---|---|---|---|---|
| Variable | N | Mean | St. Error | Min | Max |
| SVL (mm) | 3 | 97.33 | 5.61 | 86.40 | 105.00 |
| EN | 3 | 792000 | 59194.59 | 696000 | 900000 |
| LeuN | 3 | 29866.67 | 8473.75 | 20800 | 46800 |
| EL (µm) | 480 | 23.38 | 0.07 | 18.89 | 28.17 |
| EW (µm) | 480 | 15.55 | 0.05 | 11.22 | 19.43 |
| NL (µm) | 480 | 9.63 | 0.04 | 6.82 | 12.87 |
| NW (µm) | 480 | 6.43 | 0.02 | 4.66 | 8.62 |
| ESh (µm) | 480 | 1.51 | 0.00 | 1.14 | 2.20 |
| NSh (µm) | 480 | 1.51 | 0.00 | 1.00 | 2.56 |
| ES ($\mu m^2$) | 480 | 285.98 | 1.54 | 190.69 | 394.74 |
| NS ($\mu m^2$) | 480 | 48.64 | 0.29 | 29.02 | 80.19 |
| NCSh (µm) | 480 | 0.17 | 0.00 | 0.10 | 0.29 |
| | *Bufo bufo* (Uzungöl) / Males | | | | |
| Variable | N | Mean | St. Error | Min | Max |
| SVL (mm) | 3 | 73.30 | 2.81 | 68.70 | 78.40 |
| EN | 3 | 767333.33 | 67531.06 | 640000 | 870000 |
| LeuN | 3 | 21066.67 | 1524.613 | 18200 | 23400 |
| EL (µm) | 480 | 24.04 | 0.10 | 18.14 | 29.88 |
| EW (µm) | 480 | 14.41 | 0.04 | 11.47 | 20.58 |
| NL (µm) | 480 | 9.91 | 0.04 | 7.39 | 12.54 |
| NW (µm) | 480 | 5.92 | 0.03 | 4.36 | 7.96 |
| ESh (µm) | 480 | 1.67 | 0.00 | 1.17 | 2.50 |
| NSh (µm) | 480 | 1.6 | 0.01 | 1.06 | 2.46 |
| ES ($\mu m^2$) | 480 | 271.98 | 1.45 | 182.98 | 438.13 |
| NS ($\mu m^2$) | 480 | 46.25 | 0.35 | 26.05 | 67.88 |
| NCSh (µm) | 480 | 0.17 | 0.00 | 0.10 | 0.28 |
| | *Bufo verrucosissimus* (Karagöl) / Females | | | | |
| Variable | N | Mean | St. Error | Min | Max |
| SVL (mm) | 3 | 103.60 | 2.80 | 98.80 | 108.50 |
| EN | 3 | 638666.67 | 92385.66 | 480000 | 800000 |
| LeuN | 3 | 25820.00 | 1313.67 | 23460 | 28000 |
| EL (µm) | 360 | 23.78 | 0.10 | 16.24 | 30.18 |
| EW (µm) | 360 | 15.35 | 0.09 | 11.06 | 25.01 |
| NL (µm) | 360 | 9.78 | 0.06 | 6.75 | 13.79 |
| NW (µm) | 360 | 6.29 | 0.06 | 4.26 | 17.58 |
| ESh (µm) | 360 | 1.56 | 0.00 | 1.03 | 2.22 |
| NSh (µm) | 360 | 1.58 | 0.01 | 0.69 | 2.39 |
| ES ($\mu m^2$) | 360 | 287.68 | 2.58 | 180.01 | 562.87 |
| NS ($\mu m^2$) | 360 | 48.88 | 0.74 | 30.08 | 168.23 |
| NCSh (µm) | 360 | 0.16 | 0.00 | 0.10 | 0.43 |
| | *Bufo verrucosissimus* (Karagöl) / Males | | | | |
| Variable | N | Mean | St. Error | Min | Max |
| SVL (mm) | 3 | 73.30 | 3.40 | 69.80 | 80.10 |
| EN | 3 | 580050.00 | 150422.19 | 410000 | 880000 |
| LeuN | 3 | 25853.33 | 3010.23 | 20000 | 30000 |
| EL (µm) | 480 | 23.85 | 0.08 | 14.34 | 30.04 |
| EW (µm) | 480 | 15.54 | 0.05 | 10.97 | 19.32 |
| NL (µm) | 480 | 9.69 | 0.04 | 6.58 | 12.95 |
| NW (µm) | 480 | 6.15 | 0.03 | 3.74 | 8.37 |
| ESh (µm) | 480 | 1.54 | 0.00 | 1.00 | 2.11 |
| NSh (µm) | 480 | 1.59 | 0.01 | 0.79 | 2.41 |
| ES ($\mu m^2$) | 480 | 291.47 | 1.69 | 161.42 | 409.84 |
| NS ($\mu m^2$) | 480 | 46.83 | 0.31 | 25.31 | 74.41 |
| NCSh (µm) | 480 | 0.16 | 0.00 | 0.10 | 0.33 |

The first principal component explained 74.84 of total variance whereas the second component was loaded with 17.61 of total variance. In total, two principal components described 92.46% of total variance. Most of the variables showed positive loadings for PC1 more relevant to shape parameters of blood cells (Table 2).

Table 2. Principal component loadings, eigenvalues, and associated variances described by the first two components (PC1 and PC2) based on blood cell parameters

| **Variables** | **PC1** | **PC2** |
|---|---|---|
| EL | 0.206 | -0.628 |
| EW | 0.367 | 0.120 |
| NL | 0.332 | -0.362 |
| NW | 0.381 | 0.074 |
| ESh | -0.305 | -0.441 |
| NSh | -0.284 | -0.482 |
| ES | 0.366 | -0.116 |
| NS | 0.380 | -0.095 |
| NCSh | 0.337 | -0.045 |
| Eigenvalue | 6.73 | 1.58 |
| Variance (%) | 74.84 | 17.61 |
| Cumulative Variance (%) | 74.84 | 92.46 |

The highest loadings were in ES, NS, EW and NW variables. However, the highest load was observed for blood cell length (EL) in PC2 and most of variables were negatively loaded (Figure 2). The species were not clearly distinguished in the morphospace based on blood cell parameters (Figure 3).

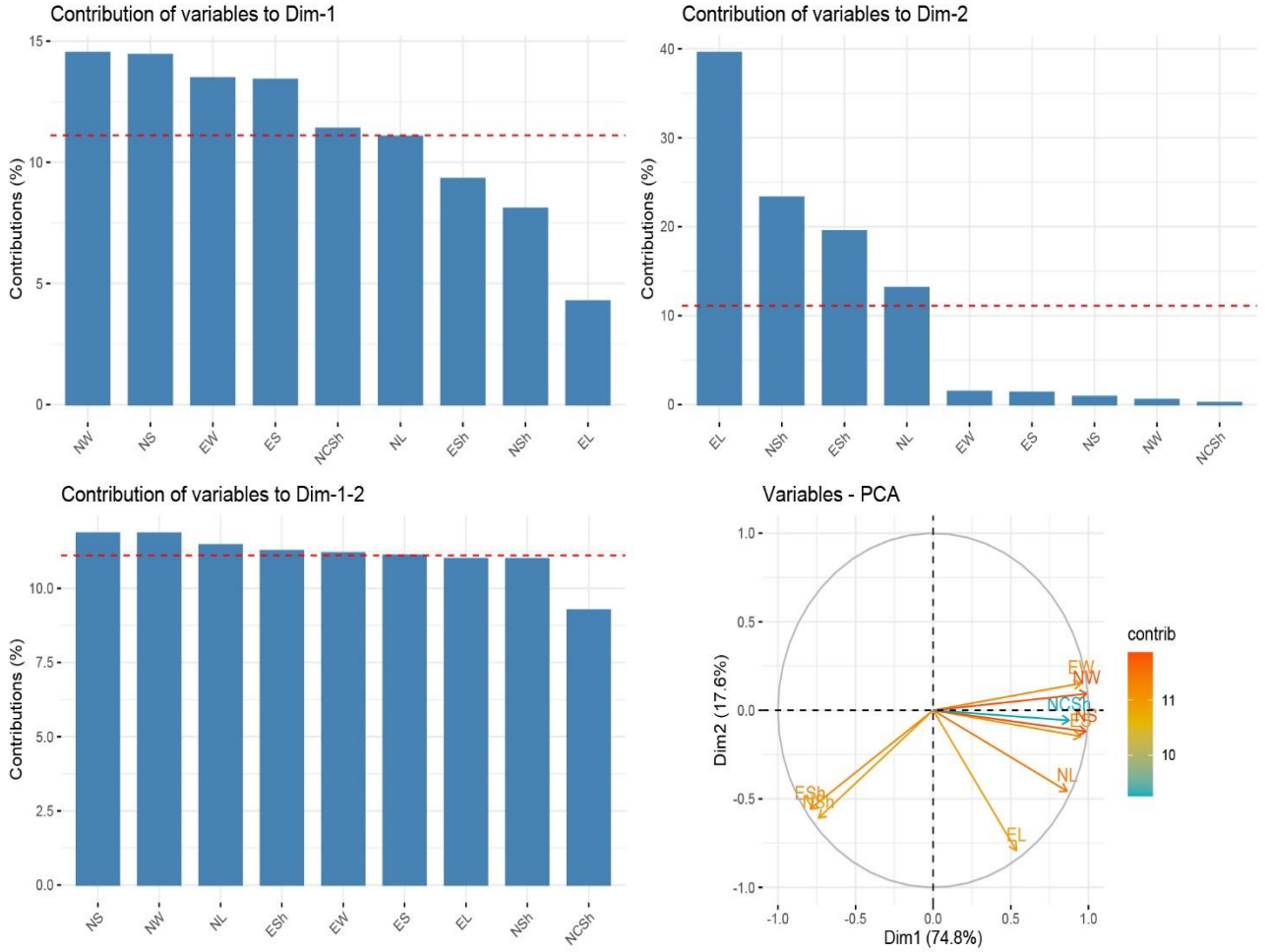


Figure 2. The contributions of each variable to the PC1, PC2 and cumulative PC1-PC2. Correlation circle demonstrates the relationship between blood cell parameters, and their relationship with the first two principal components

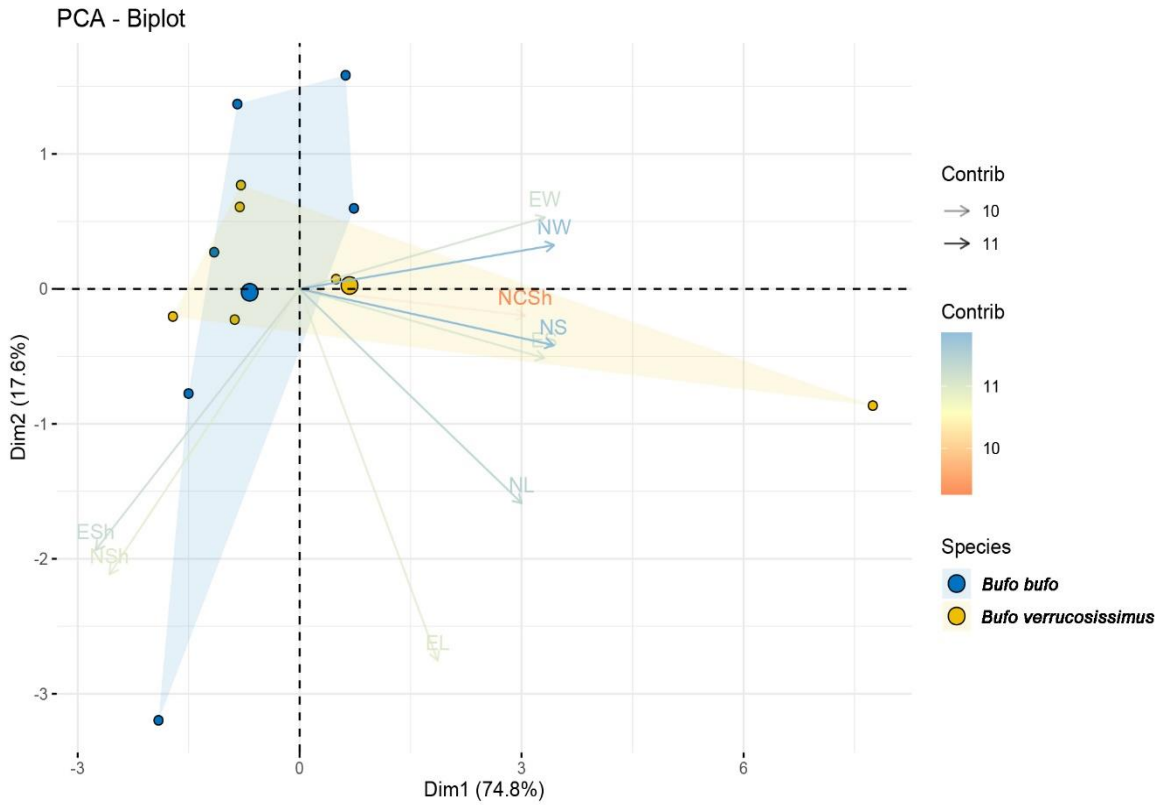


Figure 3. Principal component analysis of blood cell parameters based on species. Arrows indicate variable contributions

According to the correlation analysis of SVL and blood cell parameters, a significant negative correlation was found only between SVL and NSh (r = -0.61; p<0.05). Correlogram of all variables was demonstrated in Figure 4.


Figure 4. The matrix of scatter plots for visualizing the correlation between blood cell parameters and SVL (Bb: *Bufo bufo*; Bv: *Bufo verrucosissimus*)

## 4. Conclusions and discussion

In this study, the species were compared in terms of some blood parameters, and we contributed to the systematics of taxa regarding serological characters. Regardless of taxa, it was found that female individuals had larger values for measurement of blood cell parameters. The erythrocyte dimension was proportional to body size. Suljevic et al. [18] have investigated *Bufo bufo* in terms of general, seasonal, and sexual haematopoietic distribution and they reported that females had larger erythrocytes than males. Wei et al. [19] also handled erythrocyte morphology in some amphibians, and they indicated that the body size of organism is affecting erythrocyte dimension. From this aspect, we have found similar results in both taxa supported by literature data.

Our findings suggested mean erythrocyte and leucocyte numbers were higher in the blood of *B. bufo* than *B. verrucosissimus* but there was not a significant difference between species for EN and LeuN. The number of erythrocytes and leucocytes in amphibian blood demonstrates a wide range of interspecific variation regarding gender, age, season, and habitat conditions [20]. Considering the similarity of vegetation and altitude of Uzungöl and Karagöl [21, 22], sampling season as well as phylogenetic history of species [9, 10], we assume this observed situation is reasonable. On the other hand, it is asserted that terrestrial and aquatic anuran species have higher number of erythrocytes comparing to semi-aquatic species. For instance, Özgül et al. [23] indicated the mean erythrocyte number difference of *Pelophylax ridibundus* (mean: 119555.55) and *Bufotes variabilis* (mean: 186133.33) per 1 $mm^3$ blood samples. Similarly, Gül et al. [4] reported the number of erythrocytes in *Pseudepidalea viridis* (mean: 937666), *Pelobates syriacus* (mean: 765909) and *Hyla arborea* (mean: 733636) as terrestrial, *Pelophylax ridibundus* (mean: 886000) and *Rana dalmatina* (mean: 716660) as semi-aquatic. Although our results supported literature findings for *B. bufo*, the mean value of *B. verrucosissimus* was lower than referenced values. Therefore, this assertation is remained uncorrected with the recent data. However, Dönmez et al. [13] investigated haematological values in *B. bufo*, and they calculated number of erythrocyte 460000-920000 in females and 390000-900000 in males corresponding to range in our study. Moreover, Liu et al. [24] scored the number of erythrocytes and leucocytes between 443000-701000 and 23300-39400 for *B. gargarizans* in $1mm^3$ blood sample. In this study, number of erythrocytes and leucocytes were ranged between 640000-900000 and 18200-46800 for *B. bufo* (Uzungöl); between 410000-880000 and 20000-30000 for *B. verrucosissimus* (Karagöl). Regarding the comparison, it was observed that the average leukocyte and erythrocyte numbers of the three species were similar, but the number of erythrocytes of the *B. verrucosissimus* species was lower compared to the other two toads.

The measurement of erythrocyte cells became a subject of heamatological studies in *Bufo* taxa. Atatür et al. [13] handled erythrocyte sizes of some anurans from Türkiye, and they reported mean length, width, and size as 20,85 µm, 13,45 µm and 221,22 $µm^2$, respectively for *Bufo bufo* from Marmaris. In this study, we have found larger values in both taxa. This can be caused due to latitudinal and altitudinal differences because our sampling areas are located at the north and higher altitudes. Arıkan and Çiçek [15] also collected *B. bufo* samples from Marmaris (corresponding to *B. bufo*) and they recorded compatible values with Atatür et al. [12], but lower values than our study. As for other bufonid taxa, Xianguang et al. [25] studied on the blood cells of *Bufo gargarizans* in China, and they reported the mean erythrocyte length and width as 19.41 µm and 14.25 µm, respectively. Liu et al. [24] also assessed annual variation in peripheral blood cells in the same species and they reported erythrocyte length, width, and shape as 19.91-21.49 µm, 13.87- 15.47 µm and 1.38-1.46 µm; nucleus length, width, and shape as 8.29-10.69 µm, 5.43-6.5 µm, and 1.49-1.66 µm. These measurements were also lower than our samples. However, Wei et al. [19] handled evolution of erythrocyte morphology in amphibians, and they reported these mean length, width, and size as 28.17 µm, 20.18 µm and 447.56 $µm^2$. Accordingly, *B. gargarizans* species surpassing our measurements in both species.

In females, erythrocytes were larger and narrower in *B. verrucosissimus* than *B. bufo* species. Alike, nuclei were larger and narrower as observed in *B. verrucosissimus*, as observed in erythrocytes. However, *B. bufo* had larger and wider erythrocytes but larger and narrower nuclei in males. Given the differences between sexes, these characters thought as not diagnostic. Therefore, the other characters which are derived from these main measurements were also represented same situation. In addition, PCA analysis showed overlapped position of species in morphospace and supported the weak discrimination power of the characters.

To conclude, we presented serological comparison of two closely related taxa to literature for the first time. Our findings will contribute to the future studies as a reference source for basic blood parameters. New studies can deal with other peripheral blood cells such as granulocytes and their ratios between these species.

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# Determination of phylogenetic relationships of two species of *Phalangium* (Opiliones: Phalangiidae) by using 28S rRNA region

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**Abstract**

The species *Phalangium nalanae* Kurt, Erdek & Kurt 2023 and *Phalangium taylani* Kurt, Erdek & Kurt 2023 were previously described from Hakkari, Turkey based on morphological data and no molecular data are presented. In this study, the 28S rRNA gene region of these species belonging to the genus *Phalangium* was sequenced, and phylogenetic relationships of these species were revealed by performing maximum likelihood (ML) analysis and Bayesian inference (BI) analysis.

**Keywords:** 28S rRNA, Harvestmen, Phylogenetic analyses

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## 28S rRNA gen bölgesi kullanılarak iki *Phalangium* (Opiliones: Phalangiidae) türünün filogenetik ilişkilerinin belirlenmesi

**Özet**

*Phalangium nalanae* Kurt, Erdek & Kurt 2023 ve *Phalangium taylani* Kurt, Erdek & Kurt 2023 türleri morfolojik verilere dayanarak daha önceden, Türkiye (Hakkâri)'den tanımlanmış ve türlere ait moleküler veriler sunulmamıştır. Bu çalışmada; *Phalangium* cinsine ait bu türlerin 28S rRNA gen bölgesi dizilenmiş, maksimum olabilirlik (ML) analizi ve Bayesian çıkarım (BI) analizi yapılarak bu türlerin filogenetik ilişkileri ortaya konulmuştur.

**Anahtar kelimeler:** 28S rRNA, filogenetik analiz, otbiçen

## 1. Introduction

Opiliones, also known as harvestmen, are an order of arachnids that includes 6,740 known species worldwide. So far, more than 100 species have been reported from Türkiye [1, 2, 3].

*Phalangium* is a genus belonging to the order Opiliones (harvestmen) and approximately 42 species have been described worldwide [3]. Only 8 of these species are known from Türkiye [4, 5, 6, 7]. *Phalangium nalanae* and *P. taylani*, which belong to the genus *Phalangium*, were collected from Hakkari, Türkiye, and described based on their morphological characteristics. However, the study did not provide any information on the molecular data or phylogenetic relationships of the newly described species [6].

In recent years, morphological data as well as molecular techniques have been successfully used to identify and reclassify the species. The 28S rRNA gene region is commonly utilized to determine the phylogenetic relationships arachnids and other invertebrates [8]. At the same time, the 28S rRNA gene region is often used in conjunction with other gene regions for the determination of phylogenetic relationships among harvestmen [9, 10, 11, 12, 13, 14].

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This study aimed to analyze the phylogenetic positions and evolutionary relationships of *Phalangium nalanae* and *P. taylani* using 28S rRNA sequence data.

## 2. Materials and Methods

### *2.1.Sample collection and identification*

All specimens of *Phalangium nalanae* and *P. taylani* collected from Hakkari, Türkiye were identified by Dr. Kemal KURT (Gümüşhane University, Türkiye) and preserved in 70% ethyl alcohol in the GUSAL (The Arachnology Laboratory at Şiran Vocational School, Gümüşhane University, Türkiye) [6]. The specimens stored in GUSAL were used for molecular studies.

### *2.2. DNA extraction, PCR amplification and sequencing*

One adult individual from each species was washed with distilled water and the whole body and legs were smashed and genomic DNA was extracted using GeneAll & Exgene Tissue SV (Korea), following the manufacturer's instructions. The 28S rRNA gene was amplified with the primers ZX1: 5'-ACCCGCTGAATTTAAGCATAT-3' and ZR2: 5'-GCTATCCTGAGGGAAACTTCGG-3' [15].

PCR was performed using a total volume of 20 µl, which included 2 µl of DNA template, 10 µl of Mastermix (2x), 0.5 µl of each primer, and 7 µl of sterile distilled H2O. The amplification conditions consisted of an initial denaturation step at 95°C for 5 min, followed by denaturation at 95°C for 30 s, annealing at 52°C for 30 s, elongation at 72°C for 30 s, and a final elongation step at 72°C for 5 min (GeneAll, Seoul, Korea). The PCR products underwent evaluation for successful amplification through gel electrophoresis in 1% agarose. Subsequently, they were purified using a DNA gel extraction kit (WizPure™; Cat no: W1401).

### *2.3. Phylogenetic analyses*

28S rRNA sequences obtained were blasted with NCBI BLAST to confirm species identification and to find sequence data of similar species, and the results were downloaded from GenBank [16]. Phylogenetic analyses were performed based on 28S rRNA gene sequences obtained from *Phalangium nalanae* and *P. taylani* and additional sequences of other species were retrieved from GenBank. The accession numbers for all sequences used in the phylogenetic analysis are listed in Table 1. The 28S rRNA sequences were aligned using Bioedit 7.2.5 version Software program [17]. The best-fit substitution model was determined using JModelTest v.2.1.8 [18]. The model with the lowest AIC (Akaike's information criteria) degree was selected [19]. The phylogenetic tree was reconstructed using Bayesian Inference (BI) analysis (MrBayes v.3.2.6) [20] and Maximum Likelihood (ML) analysis (MEGA X) [21]. Bootstrap analyses with 1000 replicates were used to evaluate the ML trees. The statistical support of the resulting BI trees was determined based on Bayesian posterior probability (BPP). Nodes with a BPP of 95% or greater were considered significant [22]. Bayesian Posterior Probability (BPP) was used to determine the BI tree topology. Uncorrected pairwise sequence divergence among 28S rRNA gene were calculated using MEGA version X [21].

Table 1. GenBank accession numbers for the samples used in the phylogenetic analysis

| Species name | Sequence accession numbers | References |
|---|---|---|
| *Paroligolophus agrestis* | JQ437106 | [12] |
| *Rhampsinitus* sp. | GQ912757 | [10] |
| *Odiellus pictus* | JQ437107 | [12] |
| *Mitopus morio* | KP276371 | [23] |

## 3. Results

### Taxonomy

***Phalangium nalanae* Kurt, Erdek & Kurt 2023**

**Description:** Description of the species see Kurt et al., 2023[6].

**Specimens examined:** Türkiye, Hakkari Province, Ceyhanlı Village Road, 37°28'57.5"N; 43°33'49.8"E, 03.06.2020, leg. M. Erdek.

**Distribution:** Up to now only known from type locality in the Hakkari Province, Türkiye.

***Phalangium taylani* Kurt, Erdek & Kurt 2023**

**Description:** Description of the species see Kurt et al., 2023[6].

**Specimens examined:** Türkiye, Hakkari Province, Yüksekova District, Gürdere Village Road, 37°29'20.50"N 44°12'40.60"E, 03.06.2020, leg. M. Erdek.

**Distribution:** Up to now only known from type locality in the Hakkari Province, Türkiye.

**Phylogenetic analyses.**

A total of 506 base pairs of the 28S rRNA gene was obtained for two *Phalangium* species. According to model test results, the best-fit substitution model was chosen as HKY + G [24] for the 28S rRNA gene. Bayesian tree topology built on the basis of the 28S rRNA gene shows that the this two *Phalangium* species are quite different from *Phalangium opilio* species (Figure 1). The rooted tree is divided into two well-supported clades with 0.95 posterior probabilities. The two species are phylogenetically included in the genus *Phalangium* based on the 506-bp 28S rRNA. These two species diverged from each other with well supported 1.0 posterior probabilities. However, the genetic distance between *Phalangium* and other species was much lower (Table 2). This distance is about 0.04 % for the 28S rRNA gene of 506 bases between *Phalangium nalanae* and *P. taylani*.

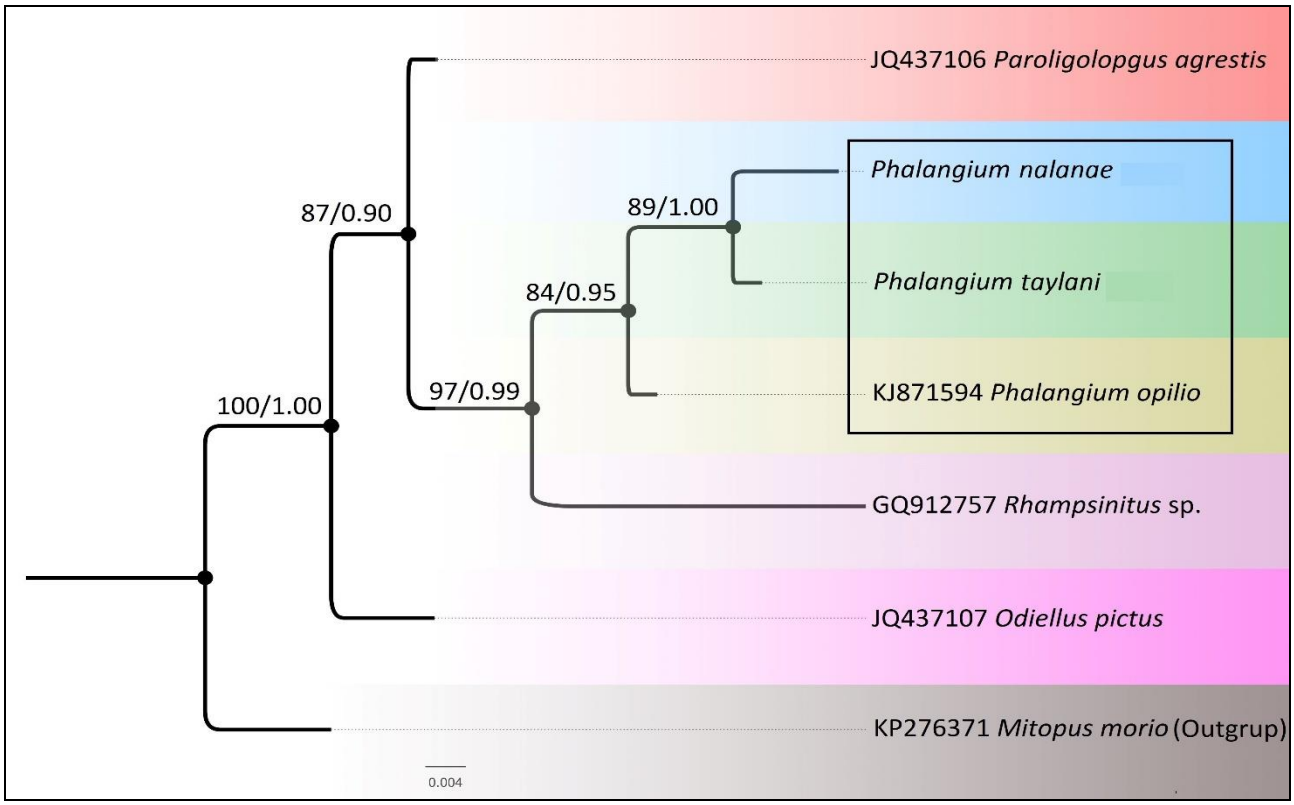


Figure 1. Phylogenetic tree based on combined 28S rRNA gene data. Bootstrap and posterior probability values are indicated above/below branches in order ML/BI

**4.Conclusions and discussion**

When the DNA sequences of the 28S rRNA gene obtained from GenBank and *Phalangium nalanae* and *P. taylani* species were analyzed, it was found that the genetic distances between the harvestmen species were very low. This did not lead to a situation where the species belonging to this group could not be separated from each other in the

phylogenetic tree. The phylogenetic analysis shows that the genus *Phalangium* is clearly separated from the other genera despite this low genetic distance, and the phylogenetic tree clearly shows that these two species are phylogenetically distinct from *Phalangium opilio*, a closely related species.

Since *Phalangium* species have been generally studied based on morphological data so far, this study is expected to make an important contribution to the literature. In addition, the lack of genetic data on *Phalangium* species in GenBank is very important in terms of adding new genetic data to the literature.

Table 2. Uncorrected genetic distances between some harvestmen species used in this study using a 506bp 28S rRNA gene fragment

| Species | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|
| *Phalangium nalanae* | - | | | | | | |
| *Phalangium taylani* | 0.004 | | | | | | |
| *Phalangium opilio* | 0.008 | 0.004 | | | | | |
| *Paroligolophus agrestis* | 0.016 | 0.012 | 0.008 | | | | |
| *Odiellus pictus* | 0.022 | 0.018 | 0.014 | 0.006 | | | |
| *Rhampsinitus* sp. | 0.024 | 0.020 | 0.018 | 0.020 | 0.026 | | |
| *Mitopus morio* | 0.030 | 0.026 | 0.022 | 0.014 | 0.014 | 0.034 | - |

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# Comparative research of findings of some medicinal plants in tradiotional medicine, folk medicine, and scientific studies

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**Abstract**

This article compares the significant 25 medical plants' (*Artemisia absinthium, Rosmarinus officinalis, Achillea millefolium, Nigella sativa, Laurus nobilis, Malva sylvestris., Althaea officinali*s., *Vitex agnus-castus, Cichorium intybus, Tilia platyphyllos, Urtica dioica, Valeriana officinalis, Rosa canina, Cistus creticus, Origanum majorana, Myrtus communis, Glycyrrhiza glabra, Sambucus nigra, Melissa officinalis, Viscum album, Pistacia lentiscus, Hypericum perforatum, Plantago lanceolata, Matricaria chamomilla, Arum maculatum*) medical usages in specified writings, folk medicine based on ethnobotanical researches and usage purposes in resent scientific studies.
The study reveals that the usages of specified 25 medical plants in 21 ancient books of medicine, in folk medicine, and in recent scientific studies are align with each other. The findings are detailed in this article by comparing three periods

**Keywords:** ethnobotany, ethnopharmacology, traditional medicine, folk medicine, medicinal plant

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## Bazı tıbbi bitkilerin kadim tıp konulu eserler, halk tıbbı ve bilimsel araştırmalardaki bulgularının karşılaştırılması

**Özet**

Bu makalede önemli 25 tıbbi bitkinin (*Artemisia absinthium, Rosmarinus officinalis, Achillea millefolium, Nigella sativa, Laurus nobilis, Malva sylvestris., Althaea officinalis, Vitex agnus-castus, Cichorium intybus, Tilia platyphyllos, Urtica dioica., Valeriana officinalis, Rosa canina, Cistus creticus, Origanum majorana, Myrtus communis, Glycyrrhiza glabra, Sambucus nigra, Melissa officinalis,Viscum album, Pistacia lentiscus, Hypericum perforatum, Plantago lanceolata, Matricaria chamomilla Arum maculatum*) belirlenen tıp yazma eserlerdeki tıbbi kullanımları ile etnobotanik derlemelere dayalı halk tıbbındaki kullanımı ve günümüz bilimsel çalışmalarındaki kullanım amaçları karşılaştırılmıştır. Çalışma sonucunda, belirlenen 25 tıbbi bitkinin, 21 geleneksel tıp kitabı ve halk tıbbındaki kullanımı ile günümüzde yapılan bilimsel araştırmalardaki kullanımı büyük oranda benzerlik göstermektedir. Elde edilen bulgular üç dönem karşılaştırması yapılarak bu makalede paylaşılmıştır

**Anahtar kelimeler:** etnobotanik, etnofarmakoloji, geleneksel tıp, halk tıbbı, tıbbi bitki

## 1. Giriş

Ülkemizin etnokültürel çeşitliliği, kültürlerin sürdürüldüğü coğrafyalardaki zengin bitki florasına ve kullanım çeşitliliği ile yansımıştır. Her etnik grup bitki kullanım bilgisini kendi kültürüyle nesilden nesile aktarılmıştır. Bitkilerin bu kullanım bilgisini derleme çalışmaları son yıllarda etnobotanik ve halk hekimliği araştırmalarıyla giderek yoğunlaşmıştır [1].

Etnobotanik araştırmalarda, bitkilerin gıdadan ilaca; yakacaktan süs eşyasına halkın bitkiyle temas kurduğu tüm yaşamsal alanları da içerdiğinden daha genel bir yaklaşımla derleme yapılmaktadır. Bitkilerin ilaç olarak kullanım amacı bu araştırmalarda daha çok ilaç araştırmacıları tarafından dikkat çekmekte; etnofarmakolojik çalışmalarda

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referans alınmaktadır. Halk ilaçları derlemeleri, biyoaktivite çalışmalarına ve klinik araştırmalara da temel oluşturmaktadır [1].

Gerek halk hekimliği çalışmaları gerekse de etnobotanik araştırmalar tez konusu olarak kayıtlara geçmektedir. Bilginin kaynağı genellikle kaynak kişilerin günümüze taşıdığı, nesilden nesile sözlü aktarımla gelen bilgilerden oluşmaktadır. Bu bilgilerin, tıp yazmalarında, günümüzde halk arasında ve modern tıptaki araştırmalarda kullanımının üçlü karşılaştırılması konusunda çalışma bulunmamaktadır. Halk ilacı araştırmalarında az da olsa derlenen bilgiler biyoaktivite çalışmaları ile test edilmektedir [1].

Bitkiler günümüzde fitoterapi alanında eğitimli hekimler tarafından reçete edilerek kullanılmakta ve bu konuya ilgi duyan hekim ve eczacı sayısı da giderek artmaktadır. Bu ilgi dolaylı olarak ilaç araştırmalarını da bitkilere, doğal olana daha fazla yönlendirmektedir. Araştırmalar da ilk olarak halk ilaçlarına yönlenmektedir. Fakat halk ilaçlarının yazılı kaynağı olan yazma tıp eserlerine yönlenen çalışmalar çok yetersizdir denilebilir [1].

Bu araştırma yaygın tıbbi kullanımı olan 25 tıbbı bitkinin geleneksel tıpta, günümüz halk tıbbında, modern tıpta kullanımını karşılaştırmalı olarak incelenmesini sağlayarak günümüz araştırmalarına kaynaklık teşkil etmeyi, Türkiye'nin bitkisel geleneksel ilaç potansiyeline yönelik araştırmalarda halk tıbbı ve geleneksel tıp eserlerinin önemine vurgu yapmayı amaçlamıştır. Araştırma, incelenen 21 geleneksel tıp ve Ege bölgesinde yetişen 25 tıbbı bitki ile sınırlandırılmıştır. Farklı geleneksel tıp kitap ve bitkilerle farklı sonuçlar elde edilebilir. Bununla birlikte halk ilaçları, etnobotanik çalışmalarla birlikte ilaç geliştirme süreçlerine kaynak olması amaçlanmaktadır. "Ege Yöresinde Yetişen Bazı Önemli Tıbbi Bitkilerin Geleneksel tıpta, Halk Tıbbında ve Bugünkü Araştırmalardaki Yerinin Karşılaştırmalı İncelenmesi" başlıklı yüksek lisans tezimizin bir özeti olan bu çalışmada farklı araştırmacılar tarafından günümüz diline çevrilerek yayımlanan yazma eserlerimizdeki bitki kullanım bilgisi, günümüzdeki halk ilaçları çalışmaları ve modern araştırmalardaki bulgularla karşılaştırılmaktadır.

## 1. Materyal ve yöntem

Bu makalede Türkiye florasında yetişen Tablo 1.'de listelenen 25 tıbbi bitki seçilmiş, bu bitkilerin geçmişten bugüne tıbbi kullanımları, kaynak taraması sonucu ulaştığımız verilerle karşılaştırmaya tabi tutulmuştur. Bitkilerin seçiminde şu ölçütler dikkate alınmıştır:

- Ülkemiz genelinde tıbbi maksatlı kullanılıyor olması
- Halk tıbbında kullanılıyor olması
- Geçmişten günümüze incelediğimiz geleneksel tıp metinlerinde yer alıyor olması

Çalışmamızda bitkiler şu konulara göre incelenmiştir:

### *2.1. Halk tıbbında kullanımı*

Bitkinin halk tıbbında kullanımını konu alan etnobotanik, etnofarmakoloji temelli araştırmalardan derlenen tıbbi kullanım bilgileri listelenmiştir. Çalışma kapsamına folklorik çalışmalardan derlenen, bitkilerin doğru tayinine dayalı Latince bitki adları bulunmayan kaynaklar alınmamış; farmasötik botanik, sistematik botanik gibi tıbbi bitki tanımlama konusunda uzmanlaşmış bilim dallarından bilim insanlarının bilimsel çalışmaları kapsama alınmıştır. Bitkilerin halk tıbbında kullanılışları, kaynaklarda verilen detaylar kadar çalışma kapsamına alınmıştır.

### *2.2. Yazma eserlerde kullanımı*

Bu bölümde yirmi bir (21) geleneksel tıp konulu eserin çoğunlukla kendisi ya da yazma eserin tıbbi bitkiler bölümünü konu alan bilimsel çalışmalar (tez, makale vb.) kaynak olarak kullanılmıştır. Yazma eserlerde bahsi geçen hastalıklar günümüz diline çevrilerek metinde yer verilmiş, böylece üç farklı başlık altında bilgileri karşılaştırma olanağı doğmuştur. Eserler seçilirken tüm dönemlerden eser seçilmesine özen gösterilmiştir. Araştırmamızda bitki kullanım bilgileri günümüzle karşılaştırılan eserler Tablo 2'de belirtilmiştir.

### *2.3. Modern araştırmalarda kullanım*

Bu bölümde araştırmaya konu olan 25 tıbbi bitkinin güncel araştırma sonuçları çeşitli bilimsel yayın veritabanlarından taranmıştır. Avrupa İlaç Ajansı (EMA), Dünya Sağlık Örgütü (WHO), Avrupa Fitoterapi Kooperatifi (ESCOP), Fitoterapi ve Farmakognozi Derneği (FFD) monografları ve Pubmed'de yayımlanan incelenen bitkiyle alakalı bilimsel araştırma bulguları temel bilimsel kaynakçaları oluşturmuştur.

Tablo 1. Kullanım bilgileri karşılaştırılan incelenen bitki türleri

| NO | BİTKİ | FAMİLYASI | DROG ADI / KULLANILAN KISMI |
|---|---|---|---|
| **1** | **ACIPELİN** - *Artemisia absinthium* L. | Asteraceae | *Artemisiae herba* – Acıpelin herbası |
| **2** | **BIBERIYE** - *Rosmarinus officinalis* L. | Lamiaceae | *Rosmarini folia* - Biberiye yaprağı |
| **3** | **CIVANPERÇEMI** - *Achillea millefolium* L. | Asteraceae | *Achilleae flos* - Beyaz civanperçemi çiçeği |
| **4** | **ÇÖREKOTU** - *Nigella sativa* L. | Ranunculaceae | *Nigellae semen* - Çörekotu tohumu |
| **5** | **DEFNE** - *Laurus nobilis* L. | Lauraceae | *Lauri folia* - Defne yaprağı |
| **6** | **EBEGÜMECİ** - *Malva sylvestris* L. | Malvaceae | *Malvae flos* cum *folia* - Ebegümeci çiçek ve yaprağı |
| **7** | **TIBBİ HATMİ** *Althae officinalis* L. | Malvaceae | *Althaeae radix* cum *folia* - *Hatmi kökü ve yaprakları* |
| **8** | **HAYIT** *Vitex agnus-castus* L. | Lamiaceae | *Vitex agni-casti fructus* - Hayıt meyveleri (hayıt tohumu) |
| **9** | **HİNDİBA** *Cichorium intybus* L. / | Asteraceae | *Cichorii intybi radix* - Hindiba kökü / Taraxaci radix – Karahindiba kökü |
| **10** | **IHLAMUR** - *Tilia plathyphyllos* Scop. | Tiliaceae | *Tiliae flos* - Ihlamur çiçeği |
| **11** | **ISIRGAN** - *Urtica dioica* L. | Urticaceae | *Urticae herba* cum *radix* - Isırgan herbası ve kökü |
| **12** | **KEDİOTU** - *Valeriana officinalis* L. | Valerianaceae | *Valerianae radix* - Kediotu kökü |
| **13** | **KUŞBURNU** - *Rosa canina* L. | Rosaceae | *Cynobasti fructus* - Kuşburnu meyvesi |
| **14** | **LADEN** - *Cistus creticus* L. | Cistaceae | *Cisti cretici folia* - Laden yaprağı |
| **15** | **MERCANKÖŞK** - *Origanum majorana* L. | Lamiaceae | *Origani majoranae herba* - Mercanköşk herbası |
| **16** | **MERSİN**- *Myrtus communis* L. | Myrtaceae | *Myrti folia – Mersin yaprağı* |
| **17** | **MEYANKÖKÜ** - *Glycyrrhiza glabra* L. | Fabaceae | *Liquiritiae radix* - Meyan kökü |
| **18** | **KARA MÜRVER** - *Sambucus nigra* L. | Adoxaceae | *Sambuci flos* cum *fructus* - Mürver çiçeği ve meyvesi |
| **19** | **OĞULOTU** - *Melissa officinalis* L. | Lamiaceae | *Melissae folia* - Oğulotu yaprağı |
| **20** | **ÖKSEOTU** - *Viscum album* L. | Santalaceae | *Visci albi herba* - Ökseotu herbası |
| **21** | **SAKIZ AĞACI** - *Pistacia lentiscus* L. | Anacardiaceae | *Gummi mastix* – Sakız |
| **22** | **SARI KANTARON** - *Hypericum perforatum* L. | Hypericaceae | *Hyperici herba* - Sarı kantaron herbası |
| **23** | **SİNİRLİOT** - *Plantago lanceolata* L. | Plantaginaceae | *Plantaginis lanceolatae folium* - Sinirliot yaprağı |
| **24** | **TIBBİ PAPATYA** - *Matricaria chamomilla* L. | Asteraceae | *Matricariae flos* – Papatya çiçeği |
| **25** | **YILAN YASTIĞI** - *Arum maculatum* L. | Araceae | *Ari dracunculi rhizome* - Yılanyastığı rizomu |

Tablo 2. Geleneksel tıp konulu incelenen bazı eserler

| No | Eserin Adı | Eserin Yazarı | Eserin Dönemi | İncelenen Kaynak ve Niteliği |
|---|---|---|---|---|
| 1 | De Materia Medica | Pedanius Dioscorides | 1.yy | Kitap (Türkiye Yazma Eserler Kurumu Başkanlığı, 2016) |
| 2 | El-Kanun Fi't- Tıb | İbn-i Sina | 10.yy | Kitap (Esin Kahya çevirisi, Atatürk Kültür Merkezi, 2020) |
| 3 | Tabibin Ahlakı ve Bir Saatte Şifa Risalesi | Ebu Bekr Muhammed bin Zekeriyya er-Razi | 10.yy | Kitap (Merkezefendi Geleneksel Tıp Derneği, 2013) |
| 4 | Tercüme-i Kâmilü's-Sınâ'a | Ali b. Abbas el Mecusi | 10.yy | Kitap (Tuncay Böler, Kesit Yayınları, 2014) |
| 5 | Physica | Hildegard von Bingen | 12.yy | Yüksek Lisans Tezi (Yurdagül Ertem, Fatih Sultan Mehmet Vakıf Üniversitesi Bilim Tarihi Anabilim Dalı, 2019) |
| 6 | Tercüme-i Müfredat-ı İbn-i Baytar | İbn-i Baytar | 13.yy | Kitap (Sağlık Bilimleri Üniversitesi, 2017) |
| 7 | Yadigar | İbn-i Şerif | 15.yy | Kitap (Merkezefendi Geleneksel Tıp Derneği, 2004) |
| 8 | Kitabu'l Müntehab fi't- Tıb | Abdülvehhab bin Yusuf bin Ahmed el-Mârdânî | 15.yy | Kitap (Merkezefendi Geleneksel Tıp Derneği, 2005) |
| 9 | Mücerrebname | Sabuncuoğlu Şerefeddin | 15.yy | Kitap (İlter Uzel, Atatürk Kültür Merkezi, 2000) |
| 10 | Hulasatu't- Tıb | Hekim Bereket | 15.yy | Yüksek Lisans Tezi ( Aydın Demir, Hacettepe Üniversitesi Sağlık Bilimleri Enstitüsü, 2010 – Gizem Doğru, Doğu Akdeniz Üniversitesi, 2015) |
| 11 | Müfid (Nazmü't Teshil) | Muhyiddin Mehi | 15.yy | Doktora Tezi (Emel Kaya Gözlü, Selçuk Üniversitesi Sosyal Bilimler Enstitüsü, 2008) |
| 12 | Kemaliyye | Şeyhülislam İbn-i Kemal | 15.yy | Kitap (Ali Haydar Bayat, Merkezefendi Geleneksel Tıp Derneği, 2007) |
| 13 | Musa bin Hamon ve Diş Tababetine Katkısı | Yayına Hazırlayan: Ahmet Zeki İzgör | 15.yy | Kitap (Ali Haydar Bayat, Merkezefendi Geleneksel Tıp Derneği, 2012) |
| 14 | Merkezefendi Terkipleri | Merkezefendi | 15.yy | Kitap (Hazırlayan: Efsun Sertoğlu, Merkezefendi Geleneksel Tıp Derneği) |
| 15 | Cerrah-name (Alaim-i Cerrahin) | İbrahim Bin Abdullah | 16.yy | Kitap (Türkiye Yazma Eserler Kurumu Başkanlığı, 2016) |
| 16 | Nüzhet-ül Ezhan fil Islah-il Ebdan | Hekim Davut bin Ömer El-Antaki | 16.yy | Doktora Tezi (Sadık Nazik, Çukurova Üniversitesi Sağlık Bilimleri Enstitüsü, 2015) |
| 17 | Neşati Yağı | Derviş Mehmed | 16.yy | Kitap (Nil Sarı, Ramazan Tuğ, Merkezefendi Geleneksel Tıp Derneği, 2009) |
| 18 | Terceme-i Cedide fi'l-Havasil-Müfrede | Mehmed bin Ali | 17.yy | Kitap (Merkezefendi Geleneksel Tıp Derneği, 2006) |
| 19 | Lehcetü'l-Lügat | Şeyhülislam Mehmed Efendi | 18.yy | Kitap (Ahmet Türkkılıç, Türk Dil Kurumu Yayınları, 1999) |
| 20 | Mâ-Hazara Fi't-Tıbbı'r- Rûhâni ve'l-Cismâni | Ebû Bekir Nusret Efendi | 18.yy | Yüksek Lisans Tezi (Şule Yüksel Özkaya, Marmara Üniversitesi Türkiyat Araştırmaları Enstitüsü, 2017) |
| 21 | Kolera Risalesi | İlyas Matar | 19.yy | Kitap (Kitapçı Karabet, 1983) |

## 2. Bulgular

İncelenen 25 tıbbi bitkiyle ilgili sırasıyla şu bulgulara ulaşılmıştır:

***Artemisia absinthium* (Acıpelin)** bitkisinin sindirim sistemi tedavisinde mide üzerindeki etkisi yazma eserlerde de verilmiştir. Acı pelinin antihelmintik etkisi günümüzde bilimsel anlamda etkileri EMA tarafından rapor edilmiştir [2]. Ayrıca modern veteriner hekimlik araştırmalarında da küçükbaş hayvanlar üzerinde etkisi üzerinde klinik çalışmalar bulunmaktadır [3]. İncelenen yazma eserlerde bitkinin sağırlık tedavisinde kullanım bilgisiyle ilgili klinik çalışmalar faydalı olacaktır kanaatindeyiz.

***Rosmarinus officinalis* (Biberiye)** bitkisinin yazma tıp eserleri ve halk tıbbında sindirim sisteminde mide ağrısı, mide hastalıkları, hazımsızlık, mide gazı ve iştahsızlık tedavisine yönelik kullanılmaktadır. Biberiye günümüzde hem baharat bitkisi hem de tıbbi bitki olarak yaygınlıkla kullanılmaktadır. Biberiye, hem geleneksel tıp eserlerde hem de günümüz halk tıbbı ve araştırmalarında saç dökülmesi ürünlerinde kullanılabileceği belirtilmiştir. 86 hastanın %15'in kontrol grubu olarak seçildiği çift körlü 7 ay süren bir klinik çalışmaya göre [3], biberiye uçucu yağının kafa derisine sürülmesi ile hastaların %44'ünde kellik tedavisine yardımcı olabildiği görülmüştür.

***Achillea millefolium* (Civanperçemi)** bitkisinin geleneksel tıp konulu kitaplarda yara tedavisinde kullanıldığı belirtilmektedir. Bu kullanımın bitkinin halk tıbbında ve günümüzdeki bilimsel kullanımı ile örtüştüğü görülürken, bitkinin karşılık bulunamayan araştırmalarına bakıldığında; göz bulanıklığı tedavisinde kullanıldığı Tercüme-i Müfredât ve Physica adlı eserlerde belirtilmesine rağmen aynı kullanımın halk tıbbı ve günümüzdeki bilimsel kullanımında yer almadığı görülmektedir. Civanperçeminin kan durdurucu etkisi Materia Medica'da ve halk tıbbında bahsedilmiştir. Fakat günümüzdeki incelediğimiz bilimsel çalışmalarda bu etkinlik üzerinde çalışılmadığı görülmüştür. Civanperçemi günümüz halk tıbbında yaygınlıkla kadın hastalıklarında da kullanıldığı görülmektedir. İncelediğimiz eserlerde civanperçemi bahsinde böyle bir kullanıma rastlanmamıştır. Bitkinin östrojenik aktivite gösterdiği yapılan bir invitro çalışma ile gösterilmiştir. Buna göre civanperçeminden elde edilen metanollü ekstrenin MCF-7 hücre testinde pozitif östrojenik etki gösterdiği bu etkinin de bitki bileşimindeki apigenin ve luteolin maddelerinden kaynaklandığı gösterilmiştir [4]. Civanperçemi hemoroit ve yara tedavisinde üç dönemde de literatüre bağlı olarak teyit edilmiştir. Hemoroit tedavisinde kullanımıyla ilgili 2017 yılında Fasa Üniversitesi'nden Mohammad Hashem Hashempur ve arkadaşları tarafından Geleneksel İran tıbbını baz alarak yaptıkları çalışmada civanperçemi dahil 105 bitkiyi incelemişler. Civanperçeminin etkinliği de bu çalışmada rapor edilmiştir [5].

***Nigella sativa* (Çörekotu)** bitkisi sindirim sistemi tedavisinde soğuk algınlığı, balgam söktürücü, ağrı kesici etkisi olduğunu belirten geleneksel tıp kitaplarımdaki kullanım bilgisiyle bu bitkinin kullanımı hem halk tıbbında hem de günümüzdeki bilimsel kullanımlarının örtüştüğü görülmektedir. Yine çörekotu bitkisinin süt arttırıcı, adet söktürücü olarak kullanıldığı yazma eserlerde belirtilmiştir. Bu kullanımın hem halk tıbbında hem de günümüzdeki bilimsel kullanımı ile örtüştüğü görülmektedir. Çörekotu günümüzde hem baharat hem tıbbi bitki olarak çok yaygın bir şekilde kullanılmaktadır. Halk tıbbında adet söktürücü, süt arttırıcı, soğuk algınlığı iyileştirici, mide ağrısı vb ağrılarda ağrı kesici etkileri için yaygınlıkla sağaltımda kullanılmaktadır. Bu kullanımların eskiden beri devam ettiğini geleneksel tıp eserler de bize teyit etmektedir. Çörekotu tohumlarından günümüzde soğuk pres yöntemiyle sabit yağ elde edilmektedir. Çörekotu yağı da tohumu da "timokinon" maddesi ihtiva etmektedir. Çörekotu bağışıklık sistemini güçlendirerek soğuk algınlığında iyileştirici etkisi bulunmaktadır. Timokinon maddesinin bağışıklık sistemini güçlendirici etkisi günümüz araştırmalarınca da gösterilmiştir [5].

***Laurus nobilis* (Defne)** bitkisinin mide ağrısı, hazımsızlık, iştahsızlık, sindirim zorluğu tedavisinde kullanıldığı bilgisi Materia Medica, Musa Bin Hamon'ın kitabında belirtilmiştir. Bu kullanımın hem halk tıbbında hem de günümüzdeki bilimsel kullanımı ile örtüştüğü görülmektedir. Aynı tıbbı bitkinin saç dökülmesi için kullanıldığı Alâim-i Cerrâhîn adlı eserde belirtilmiştir. Romatizma tedavisinde, ağrıların giderilmesinde ve soğuk algınlığında kullanımı Mücerrebname, Yadigar ve Alaim-i Cerrahin adlı tıp eserlerinde belirtilirken, bitkinin bu kullanımının halk tıbbı ile ve bilimsel kullanımı ile örtüştüğü görülmüştür. Hem uçucu yağ hem de sabit yağ günümüzde saç dökülmesi rahatsızlığı için yaygınlıkla kullanılmaktadır. Defne uçucu yağ bileşiminde 1-8 sineol maddesi içerdiği için antiviral aktivite gösterdiği için Loizzo ver arkadaşları tarafından HSV-1 ve SARS-CoV'e karşı etkinliği oldukça yüksek bulunmuştur [6]. Bu bilgiler bitkinin geleneksel tıp eserlerinde ve halk tıbbında kullanım bilgisini desteklemektedir.

***Malva sylvestris* (Ebegümeci)** bitkisinin, solunum yolu rahatsızlıklarında (öksürük, zatürre, bronşit, ses kısıklığı, nezle vb) etkili olduğunu belirten kitaplar; Yadigâr, Lehcetü'l-Lügat, Tercüme-i Müfredât-ı, Alâim-i Cerrâhîn, Müntahab-ı Fit't-Tıbb, Tahzibul Mathün El Kanun şeklindedir, bu bitkinin kullanımının hem halk tıbbında hem de günümüzdeki bilimsel kullanımı ile uyumludur [1]. Ebegümeci, ülkemizde hemen her bölgede, her dönemde görülebilmektedir. Bitki halk tıbbında, günümüz bilimsel araştırmalarında ve geleneksel tıp eserlerde kullanım amacı açısından benzerlik göstermektedir. Halk tıbbında hemen her bölgede ve her hastalıkta kullanımı olan bir bitki olduğu incelediğimiz halk tıbbı kaynaklarına dayalı olarak tespit edilmiştir. Bitkinin öksürük, bronşit gibi rahatsızlıklarda kullanımını günümüz araştırmaları da onaylamaktadır. Bileşimindeki yüksek oranda bulunun müsilaj maddesi bitkiye yumuşatıcı etkinlik kazandırmaktadır. Ebegümeci yapraklarında izole edilen bir polisakkarit antikompleman aktivite gösterdiğinden yangı yanıtını modülasyona uğrattığı bildirilmiştir [7]. Yine ebegümeci bileşiminde bulunan çinko sağlıklı bağışıklık sisteminin bitkisel kaynaklarından biridir. Bu sebeple de soğuk algınlığında iyileştirici olarak öteden beri kullanıldığına açıklık getirebilir. Günümüzde gerek ülkemizde gerekse de Avrupa ülkelerinde öksürük şurupları, bitki çayları vb takviye edici ürünler içerisinde kullanıldığı görülmektedir. Ülkemizdeki öksürük şurubu olan ürünler "gıda takviyesi" olarak sınıflandırılan ürünler arasındadır.

***Althaea officinalis* (Hatmi)** bitkisinin öksürük, grip, bronşit, solunum yolları tahrişi, ateş, baş ağrısı, göğüs yumuşatıcı etkileri için kullanıldığını belirten kitaplar Musa Bin Hamon, Physica, Materia Medica, Yadigâr, Hulāsatu't Tıbb, Müfîd (Nazmü't-Teshîl)'dir. Bu kullanımın bitkinin halk tıbbında ve günümüzdeki bilimsel kullanımı ile örtüştüğü görülmektedir [1].

ESCOP, WHO vb. otoritelerce monografları yayımlanan, farmakopelerde standartları belirlenmiş olan hatmi türü *Althaea officinalis* türüdür. Bu türün kökleri, çiçekleri ve yaprakları tedavide kullanılır. Ülkemizde genellikle hatmi olarak "*Alcea* sp." türleri kullanılmaktadır. Öksürük şuruplarında tıbbi hatmi ekstreleri de kullanılmakta; genellikle kekik, ebegümeci ile birlikte formüle edilmektedir. Ülkemizde de Geleneksel Bitkisel Tıbbi Ürün kapsamında hatmi içeren bir ürün öksürük ilacı olarak ruhsatlandırılmıştır.

Bitkinin öksürük karşıtı etkinliği için antitüssif aktivite çalışmaları da *in vivo* ve *in vitro* koşullarda yapılmıştır. Çalışmalarda hatmi kökünde bulunan ramnogalaktofuronan maddesinin bu etkiden sorumlu olduğu belirlenmiştir. Buna göre hatminin ekspektorasyonu arttırdığı, öksürme şiddeti ve atakları azalttığı rapor edilmiştir. Yine aynı çalışmaya göre narkotik etkili öksürük ilaçlarına göre daha zayıf etkili olduğu, narkotik olmayanlara göre de daha güçlü etkili olduğu tespit edilmiştir [8].

***Vitex agnus-castus* (Hayıt)** bitkisinin kadın hastalıklarının tedavisinde kullanıldığı bilgisi Materia Medica, Nüzhetü'l-Ezhân fi İslâhi'l-Ebdân, Terceme-i Cedide Fi'l-Havassi'l-Müfredede belirtilmektedir. Bu kullanımın bitkinin halk tıbbında ve günümüzdeki bilimsel kullanımı ile örtüştüğü görülmüştür [1].

Günümüzde yapılan bilimsel araştırmalar bitkinin kadın hastalıklarında etkin olduğunu rapor etmiştir. Jinekoloji uzmanlarının yürüttüğü çok merkezli gözlemsel bir çalışmada kontrol grubu olmadan, adet öncesi gerginliği (pms) olan 1634 hastaya, herbiri 1,6-3 mg ekstre içeren (20 mg drog eşdeğeri) hayıt kapsüllerinden günde 2 adet verilmiştir. Üç dönemlik uygulamadan sonra başlıca şikayetlerdeki (depresyon, anksiyete, yeme isteği, aşırı ödem vb.) değişiklikler konusunda anket düzenlenmiştir. Anket sonucuna göre %93 oranında azaltma/bitiş kaydedilmiştir. Doktorların %85'i genel değerlendirmede klinik etkisini iyi/çok iyi bulurken hastaların %81'i tedavi sonrası durumlarını çok iyi olarak değerlendirmiştir. Göğüslerdeki rahatsızlığın şiddeti 3 ay sonra oldukça azalmıştır [9].

Hayıt geçmişten günümüze tedavide kullanılan önemli tıbbi bitkilerden biridir. Geleneksel tıp metinlerinde bahsi geçen kullanım şekilleri ile halk arasındaki kullanım şekilleri ve amacı günümüzde de geçerliliğini korumaktadır.

Bilimsel çalışmalarda sadece hayıt meyvelerinin kullanımı aktarılmış olmasına rağmen gerek halk arasında gerekse de geleneksel tıp metinlerinde çiçek, yaprak ve meyveleri de kullanıldığı tespit edilmiştir. Bilimsel çalışmalar genellikle kadın hastalıkları konusunda etkilerine yoğunlaşmış, gelenekteki kullanım amaçları konusunda klinik olarak çalışma yapılmadığı görülmüştür. Hayıt bitkisi ile ilgili kadın hastalıklarındaki etkileri dışındaki etkinliklerin klinik çalışmalarla desteklenmesi faydalı olacaktır. Ülkemizde hayıt meyve ekstresi içeren, kadın hastalıklarında reçete edilen bir ilaç eczanelerde satışa sunulmaktadır.

***Cichorium intybus* (Karakavuk)** bitkisinin incelenen 21 geleneksel tıp kitapla, halk tıbbı ve günümüzdeki bilimsel kullanımına dayalı üç dönemi içeren ortak kullanım görülmüş, sindirim sistemine yönelik kullanımı da halk tıbbı ve günümüzdeki bilimsel kullanımı ile örtüşmektedir. Bitkinin kabızlık, karaciğer hastalıklarındaki kullanımı,

sarılıkta, böbrek taşlarının düşürülmesinde, kanserde kullanımı başlıkları da geleneksel tıp metinlerinde, halk tıbbında ve günümüz bilimsel literatüründeki bilgilerle uyumludur. *Cichorium intybus* bileşiminde bulunan polisakkaritlerin yapılan çalışmalara göre mideyi geçip kalın bağırsakta fermantasyona uğraması sebebiyle bağırsaktaki bifido bakteri popülasyonunu uyardığından kolon hastalıkları ve diyabet gelişiminde azalma gözlemlenmekte olduğu rapor edilmiştir. Yine hindiba yiyen yaşlı bireylerin karaciğer işlevlerinde düzelme görüldüğü bildirilmiştir [1].

***Tilia plathyphyllos* (Ihlamur)** bitkisinin soğuk algınlığı, nezle, öksürük tedavisinde etkili olduğu bilgisi Lehcetü'l-Lügatta belirtilmiştir. Bu kullanımın bitkinin halk tıbbında ve günümüzdeki bilimsel kullanımı ile örtüştüğü görülmüştür. Günümüzde bitkinin diüritik, ateş düşürücü, soğuk algınlığında iyileştirici etkileri için halk arasında yaygınlıkla kullanıldığı görülmektedir. Ihlamur, Türk, Alman, Avrupa, İngiliz, İsviçre, Macar Farmakopelerine ve Alman E Komisyonu monograflarına kayıtlı drog olarak bilimsel literatürde de yer almaktadır. Günümüzde Avrupa'da eczanelerde satışı yapılan ilaç formu da bulunmaktadır.
Yapılmış bir biyolojik aktivite bir çalışmasına göre ıhlamurun antimikrobiyal ve antioksidan aktivitesinin olduğu gözlemlenmiştir. Araştırmacılar, çalışma sonucuna ve etnobotanik bulgulara dayanarak, boğaz enfeksiyonlarında bitkinin ekstresinin yardımcı olacak bir ekstre olarak kullanılabileceğini belirtmiştir [11].

***Urtica dioica* (Isırgan)** bitkisinin böbrek taşı, romatizmal ağrılar, erkek üreme organları sağlığı tedavisinde etkili olduğunu belirten geleneksel tıp kitaplar; Lehcetü'l-Lügat, Nüzhetü'l-Ezhân fi İslâhi'l-Ebdân, Tercüme-i Müfredât-ı, Tahzibul Mathün El Kanun, El- Kanun Fi't Tıbb şeklindedir, bu bitkinin kullanımının hem halk tıbbında hem de günümüzdeki bilimsel kullanımı uyumludur. Bazı klinisyenler ısırgan yaprağı ekstresini klasik steroit olmayan yangı giderici ilaçlar (NSAEI'ler) ve diğer analjeziklerle kullanmaktadır. Eldeki kanıtlar, tedaviye ısırgan eklenmesinin, analjeziklerin dozunu azaltabilmekte olduğunu göstermektedir [12].
Isırgan hem geleneksel tıp metinlerinde hem de halk tıbbında romatizmal ağrılar, eklem ağrıları vb. durumlarda etkin olduğuna değinilmiştir. Günümüzde yapılan klinik çalışmalar da bu kullanımı doğrulamaktadır. Bir çalışmada 27 hasta üzerinde randomize çift körlü çalışmaya göre bilek ve baş parmaklarında orteoartriti olan hastalara bir hafta süreyle ağrı olan bölgeye ısırgan uygulanmış, plasebo olarak da *Lamium album* yaprakları kullanılmıştır. Sonuçlar anketle belirlenmiş, plaseboya göre belirgin farkta ısırgan etkin bulunmuştur. *Urtica dioica* bitkisinin idrar yollarına iyileştirici etkisi geleneksel tıp kitaplarda ve halk tıbbında geçmektedir. Bitki günümüzde de prostat rahatsızlıklarında kullanılmaktadır. İyi huylu prostathipertrofisinde etkinliğini incelemek amacıyla 6 ay süreli 620 hasta üzerinde yürütülen, randomize çift körlü ve plasebo kontrollü bir klinik çalışma yapılmıştır. 6 aylık süre sonunda ısırgan kullanan hastaların %81'inde idrar yolları problemlerinde azalma gerçekleşmiştir. Isırgan içeren prostat için hazırlanmış olan ürünler bugün eczanelerde satılmaktadır [13].

***Valeriana officinalis* (Kediotu)** bitkisinin sinir sistemi tedavisinde kullanıldığı bilgisi araştırılan 21 geleneksel tıp kitapta bahsedilmemektedir. Bitkinin halk tıbbı ve günümüzdeki bilimsel kullanımında olan sedatif etki, araştırılan geleneksel tıp kitaplarda rastlanmamıştır. *Valeriana officinalis* türü kediotu yerine Anadolu'da daha yaygın olarak yetişmekte olduğundan *Valeriana dioscoridis* türü kullanılmaktadır. GBTÜ kapsamında 2021 verilerine göre *Valeriana officinalis* ekstresi üç ürün bulunmaktadır. *Valeriana officinalis*'in sinirsel gerginlik ve uyku bozukluklarında kullanımı EMA, WHO, ESCOP ve Komisyon E monograflarında onaylanmıştır. Çift körlü bir çalışmada, *Valeriana officinalis* ekstresinin (450 mg-900 mg) plaseboya oranla uykuya geçiş süresini kısalttığı görülmüştür. Aynı çalışmada uyku şikayeti ve düzensiz uykusu olanlarda, uyku kalitesini arttırdığı görülmüştür [10].

***Rosa canina* (Kuşburnu)** bitkisinin sindirim tedavisinde etkili olduğu Tercüme-i Müfredât adlı eserde belirtilmiştir. Materia Medica'da dizanteri, sindirim bozukluğu ve boğaz tahrişi için kayıtlıdır. Bu kullanımın bitkinin halk tıbbında ve günümüzdeki bilimsel kullanımı ile örtüşmekte olup, ayrıca solunum sistemi tedavisinde kullanıldığı bilgisi araştırılan 21 geleneksel tıp kitapta geçmemesine rağmen fakat bitkinin solunum sistemine yönelik kullanımı halk tıbbı ve günümüzdeki bilimsel kullanımı uyumludur. Hemoroit, balgam söktürücü, nezle, grip, böbrek iltihabı, romatizma gibi rahatsızlıklar için halk tıbbında yaygınlıkla kullanılmaktadır. Kuşburnu halk tıbbında yaygınlıkla hemoroit tedavisinde kullanılmaktadır. Bu konuda içerisinde kuşburnu da olan bir preparatın etkinliği, *in vivo* olarak 96 hastada çift körlü ve plasebo kontrollü klinik çalışma yapılmıştır. Çalışma sonucuna göre semptomların giderilmesinde etkin bulunmuştur [14].
Yine bitkinin eklem ağrıları ve romatizmada kullanımı halk tıbbında kaydedilen kullanım şekilleridir. Bu yönde Warholm ve arkadaşları tarafından kalça ve diz eklemlerinde osteoartrit şikayeti olan 100 hasta üzerinde bir klinik

çalışma yapılmıştır. Araştırma sonucuna göre standardize kuşburnu tozu ile tedavi edilen hastaların %64,6'sında şikâyetlerde azalma görülmüştür**[15]**.

***Cistus creticus* (Laden)** bitkisinin solunum rahatsızlıkları ve soğuk algınlığı tedavisinde kullanıldığı Musa bin Hamon ve Müfid adlı eserlerde belirtilmiştir. Bu kullanım bitkinin halk tıbbında kullanımı ve günümüzdeki bilimsel kullanımı ile de örtüşmektedir. Yüksek oranda içerdiği fenolik bileşenler ve flavonoidler sebebiyle antibakteriyel, antioksidan ve DNA koruyucu etkileri ortaya konmuştur. İçerdiği yüksek orandaki epigallokateşin gallat maddesi sebebiyle antioksidan kapasitesi yeşilçaydan yüksek bulunmuştur. Bu polifenoller sayesinde antibakteriyel, antifungal ve antienflematuvar etkileri rapor edilmiştir. Bitkinin üst sonulum yollarında etkinliği ile ilgili de klinik çalışmalar bulunmaktadır. Ülkemizde yetişen *Cistus* türlerinin kurutulmuş örneklerinden hazırlanan ekstrelerin *Streptococcus faecalis, Staphylococcus aureus, Bacillus cereus, Escherichia coli* mikroorganizmalarına karşı etkinlikleri de saptanmıştır [16]. Günümüzde *Cistus* özleri içeren pastil, şurup vb ilaç formu eczanelerde soğuk algınlığı rahatsızlıklarına karşı satışa sunulmaktadır.

***Origanum majorana* (Mercanköşk)** bitkisinin sindirim sistemi (mide fesadı, mide ağrıları) tedavisinde kullanıldığı bilgisi Derviş Mehmed, Neşati Yağı risalesinde belirtilmiştir. Bu kullanımın hem halk tıbbında hem de günümüzdeki bilimsel kullanımı ile örtüştüğü görülürken, bitkinin halk tıbbı ve günümüzdeki bilimsel kullanımı ile ilgili karşılık bulunamayan bir sonucu ortaya çıkmadığı görülmektedir. Yeni yapılan bir çalışma mercanköşkün, ileri glikasyon ürünlerin (AGE) üretilmesini inhibe etmede etkili olduğu gösterilmiştir. Diyabetli hastalarda lipid peroksidasyonu inhibe ederek, oksidatif stresi hafifletip böbrek hasarını önlediği veya ertelediği belirlenmiştir. Ayrıca ileri glikasyon son ürünleri denilen AGE ile ilgili kronik tabloları önleyebileceği veya düzeltebileceğini ortaya koymuştur [1].

***Myrtus communis* (Mersin)** bitkisinin sindirim sistemi tedavisinde etkili olduğunu belirten geleneksel tıp eserleri Materia Medica, Yadigâr, Alâim-i Cerrâhîn, Derviş Mehmed'in risalesi, Terceme-i Cedide Fi'l-Havassi'l-Müfrede, Müfîd (Nazmü't-Teshî) şeklindedir, bu bitkinin kullanımının hem halk tıbbında hem de günümüzdeki bilimsel kullanımı ile örtüştüğü görülmekte, aynı tıbbı bitkinin integumenter sistem tedavisine yönelik kullanıldığı bilgisi ise Materia Medica, Kemâliyye, Tercüme-i Müfredât-ı, Derviş Mehmed'in risalesi, Terceme-i Cedide Fi'l-Havassi'l-Müfrede de belirtilmekte, bu kullanımın da hem halk tıbbında hem de günümüzdeki bilimsel kullanımı ile örtüştüğü görülmüştür. Bitkinin diş ağrısı üzerindeki etkisi olduğunu belirten geleneksel tıp kitapları; Alâim-i Cerrâhîn, Hulâsatu't Tıbb, Müfîd (Nazmü't-Teshîl) olup, bitkinin bu kullanımının halk tıbbında ve günümüzdeki bilimsel kullanımında karşılık bulduğu görülmektedir. Tüm dünyada yaygınlıkla kullanılan *Melaleuca alternifolia* (tea tree, çay ağacı) bitkisinin ait olduğu mersingiller familyasına adını veren bitki mersindir. Covid önlemleri sebebiyle çok sık tavsiye edilen çay ağacı gibi *Myrtus communis* bitkisinin de araştırılması gerekmektedir. Maryam Azimi, Fatemeh Sadat Hasheminasab isimli iki bilim insanı [17], geleneksel İran tıbbındaki kaynak eserlerindeki mersin bitkisinin kullanımından hareketle, mevcut covid-19 tedavi protokolüne "mersin" bitkisinin eklenmesiyle ilgili klinik çalışmaları devam ettirmektedir. Yine aynı ekip mersin şurubunu tedavi aracı olarak kullanma konusundaki çalışmalarını da sürdürmektedir. Günümüzde mersinin yapraklı herba aksamından distilasyonla uçucu yağ elde edilmekte, aromaterapi alanında değerlendirilmektedir. Mersin uçucu yağı jel kapsüllerin içersinde, standardize formda bronşit gibi üst solunum yolu enfeksiyonlarında Avrupa ülkelerinde kullanılmaktadır.

***Glycyrrhiza glabra* (Meyankökü)** bitkisi ülser, ishal vb. gibi sindirim sistemi hastalıklarında, Materia Medica, El- Kanun Fi't Tıbb, Physica, Nüzhetü'l-Ezhân fi İslâhi'l-Ebdân eserlerinde kullanımına değinilmiştir, Bitkinin bu kullanımının hem halk tıbbında hem de günümüzdeki bilimsel kullanımı ile örtüştüğü görülürken; bitkinin halk tıbbı ve günümüzdeki bilimsel kullanımı örtüşmektedir. Solunum sistemi üzerinde ses kısıklığı, balgam söktürücü, göğüs yumuşatıcı, öksürük, nefes darlığı vb. gibi hastalıklarda kullanımı Materia Medica, El- Kanun Fi't Tıbb, Physica, Merkez Efendi, Lehçetü'l Lügat, Alaim-i Cerrahin adlı eserlerde belirtilmiştir. Bitki halk tıbbında da günümüz araştırmalarında da ses kısıklığı, öksürük, bronşit, soğuk algınlığı şikâyetlerinde kullanılmaktadır. WHO tıbbi bitkiler monograflarının meyankökü bahsinde peptik ülserde kullanımı konusunda yapılan bir klinik çalışma sonucuna göre 15 hastada peptik ülser semptomlarının azaldığı görülmüş, iyileşmenin %75 oranında hızlandığı tespit edilmiştir. Yine WHO monograflarına göre antitüssif ve ekspoktoran etkinliği mukus sekresyonunu hızlandıran glisirizin maddesine bağlıdır diyerek öksürük tedavisinde kullanımını onaylamıştır [18]. Meyankökü günümüzde soğuk algınlığı ve üst

solunum yolları için çay, pastil vb. formlarda eczanelerde yer almaktadır. Kozmetik sektöründe de leke açıcı kremlerin içerisinde kullanılmaktadır.

***Sambucus nigra* (Karamürver)** bitkisinin solunum sistemi tedavisine yönelik olarak kullanıldığını Terceme-i Cedide Fi'l-Havassi'l-Müfrede adlı eserde belirtilmiştir. Bitkinin bu kullanımının hem halk tıbbında hem de günümüzdeki bilimsel kullanımı ile örtüştüğü görülürken; bitkinin halk tıbbı ve günümüzdeki bilimsel kullanımı ile ilgili karşılık bulunamayan bir sonucu yoktur. Kara mürver ülkemiz florasında doğal olarak yetişen, antosiyanince zengin, bağışıklık sistemi için çok kıymetli bir tıbbi bitkidir. Bitkinin gerek halk arasında, gerek günümüz tıbbında ve gerekse de geleneksel tıp eserlerde solunum rahatsızlıkları için kullanılıyor olması bitkinin öteden beri bu anlamdaki iyileştirici etkisini onaylar niteliktedir. Bitkiyle ilgili antiviral, antienflamatuvar, antikanser, antidiyabetik, bağışıklık sistemi, antibakteriyel, bronşiyal sekresyonu arttırıcı, antioksidan etki, diüritik etki çalışmaları yapılmıştır. Yapılan antiviral etki odaklı randomize, plasebo kontrollü, çift-körlü bir klinik çalışmada, meyvelerinden üretilen Sambucol meyve ekstresi, grip başlangıç semptomları yaşayan 60 hastaya, 5 gün süreyle günde 4 defa 15 ml verilmiştir. Semptomlar 8 gün süreyle izlenmiştir. Tedavi grubunda, ortalama 3-4 gün sonra iyileşme görülürken, plasebo grubununda iyileşme süresi 7-8 güne kadar uzamıştır [19]. Yine bitkinin bağışıklık sistemini güçlendirici etkisiyle ilgili bilinen immünstimülan ürünlerle karşılaştırılmış, kontrole göre stokinlerin üretimini daha yüksek oranda arttırdıkları, dolayısı ile immün sistemi aktive edici özelliği saptanmıştır [20]. 2020 covid-19 pandemisi sırasında salgını önlemek, bağışıklık sistemini güçlendirmek amacıyla bitkisel olarak en çok tavsiye edilen türlerden biri karamürver bitkisidir. Kara mürver'den "sambucol" gibi ilaçların yanısıra, farklı isimlerle şurupları, pastilleri eczanelerde günümüzde satışa sunulmaktadır.

***Melissa officinalis* (Oğulotu)** mide, bağırsak problemleri, ağız kokusu, nefes darlığı tedavisinde etkili olduğunu belirten geleneksel tıp eserleri El- Kanun fi't Tıbb, Materia Medica, Lehçetü'l Lügat, Tercüme-i Müfredât-ı'dır. Bitkinin bu kullanımı hem halk tıbbında hem de günümüzdeki bilimsel kullanımı ile örtüştüğü görülmektedir. Ayrıca kalp ağrısı, beyindeki tıkanıklık, uykusuzluk, depresyon, melankoli, huzursuzluk tedavisinde de kullanıldığı Materia Medica, El- Kanun fi't Tıbb, Lehcetü'l-Lügat, Mâ-Hazara Fiˈt-Tıbbıˈr-Rûhâni Veˈl-Cismâni adlı eserlerde belirtilmiştir. Bu kullanımın da hem halk tıbbında hem de günümüzdeki bilimsel kullanımı ile örtüşmektedir[1]. Oğulotunun günümüz modern literatürdeki sinirsel uyku problemleri, tedirginlik, huzursuzluk, asabiyet, kalp rahatsızlıkları, migren ağrılarının giderilmesinde kullanıldığı kayıtlıdır. Bitki haricen *Herpes labialis*'in sebep olduğu uçuk tedavisinde kullanılmaktadır Bitkinin günümüzdeki klinik araştırmalarına baktığımızda geleneksel tıp eserlerdeki zihinsel ve ruhsal marazlarda kullanımı konusunda klinik çalışmalarla doğrulanmış sonuçları görebiliriz. Bir çalışmaya [21] göre ciddi demans problemi olan 72 hasta üzerinde yapılan, çift-körlü, plasebo kontrollü bir çalışmada, *Melisa officinalis* uçucu yağı ile aromaterapi uygulaması yapılmış, 4 hafta sonrasında hastaların plaseboya göre daha az ajitasyon, olumlu aktivitelerde daha çok zaman geçirdikleri gözlenmiştir. Yine 65-80 yaşlarındaki hafif ve orta şiddette 6 aylık Alzheimer geçmişi olan hastalara, 4 ay süreyle günde 60 damla melisa ekstresi verilmiştir. Süre sonunda hastaların plaseboya göre bilişsel faaliyetleri anlamlı derece artmıştır. Ekstre alan kişilerde daha az ajitasyon görülmüş ve yan etki saptanmamıştır. Bitkinin geleneksel tıp metinlerinde ve günümüz halk tıbbında yatıştırıcı, huzursuzluk, uykusuzluk gibi durumlarda kullanıldığı kaydedilmiştir. Bu konuda da Cases ve arkadaşları [22] tarafından bir klinik çalışma yapılmıştır. Hafif, orta şiddetli anksiyete ve uyku bozukluğu tedavisi için yapılan tek merkezli çalışmada, *Melissa officinalis* standardize yaprak ekstresi, orta ve ileri şiddette anksiyete ve uyku bozukluğu olan 20 kişiye, 15 gün boyunca günde iki kez 600 mg uygulanmıştır. Araştırma sonucuna göre anksiyete oluşumu %18, uykusuzluğu %42 oranında azaltmıştır.

***Viscum album* (Ökseotu)** bitkisinin safra, ödem, tansiyon vb. hastalıkların tedavisinde kullanıldığı Müntahab-ı Fit't-Tıbb, Terceme-i Cedide Fi'l-Havassi'l-Müfrede adlı eserlerde belirtilmiştir. Bitkinin bu kullanımının hem halk tıbbında hem de günümüzdeki bilimsel kullanımı ile örtüştüğü görülmekte olup; aynı bitkinin karşılık bulunmayan araştırmalarına baktığımızda "Müntahab-ı Fit't-Tıbb" ve Hulāsatu't Tıbb'da bitkinin yara tedavisi ve kirpik batmasında etkisi olduğunun belirtilmesi halk tıbbında rastlanırken, günümüzdeki bilimsel kullanımında rastlanmamıştır ve ayrıca günümüzdeki bilimsel kullanımında ve halk tıbbında onkoloji tedavisinde kullanımına etkisi bulunduğu tespit edilirken, araştırmaya dahil olan geleneksel tıp kitaplarda bu bilgiye rastlanmamıştır. Ökseotu, Anadoluda halk tıbbında tanınan ve kullanılan bitkilerden biridir. Bitki halk arasında kabızlık tedavisinde, idrar söktürücü, kuvvet verici, tansiyon düzenleyici olarak kullanılır. Farklı ağaç türlerinde (ahlat, armut, elma, meşe, söğüt, çam, gürgen vb.) üzerinde parazit olarak yetişen ökseotları ile kanser arasında halk arasında bağlantı kurulmuştur. Ökseotu bu sebeple kanser tedavisinde

de geleneksel olarak kullanılagelmiştir. Araştırmamızda incelediğimiz halk tıbbı kaynakları ve modern araştırmalar bitkinin kanserde kullanımını doğrular niteliktedir. Sadece ökseotunun kanserde kullanımı konusunda çalışan ve farklı kanser türlerinden kemoterapide kullanılan ilaç geliştiren Hiscia Enstitüsü halen araştırmalarını sürdürmekte, Iscador adıyla ürettikleri ilaç kemoterapi ajanı olarak onkolojide kullanılmaktadır. Sekonder karaciğer kanseri üzerine yürütülen bir klinik çalışmada, primer odağı farklı 188 karaciğer kanserli hastaya Iscador tedavisi uygulanmış. Iscador kullanan hastaların yaşam süresi 9 aya çıkarken, tedavi almamış olan 122 hastada ortalama yaşam süresi 5 ay olarak tespit edilmiştir [23].

***Pistacia lentiscus* (Sakızağacı)** bitkisinin sindirim ülser, mide ve bağırsak hastalıkları tedavisinde etkili olduğu Nüzhetü'l-Ezhân fi İslâhi'l-Ebdân, Derviş Mehmed'in risalesi, Terceme-i Cedide Fi'l-Havassi'l-Müfrede, Mâ-Hazara Fiˈt-Tıbbıˈr-Rûhâni Veˈl-Cismâni, Mücerrebname, Müfîd (Nazmü't-Teshîl) eserlerinde belirtilmiştir, bitkinin bu kullanımı da hem halk tıbbında hem de günümüzdeki bilimsel kullanımı ile örtüştüğü görülürken; bitkinin halk tıbbı ve günümüzdeki bilimsel kullanımı ile uyumludur. Sakız ağacının gövdesinden elde edilen "reçine"ye damla sakızı denilmektedir. Bu şekilde bir ağacın verimli olarak sakız verebilmesi için 7-10 yaşlarına gelmesi gerekmektedir. Damla sakızı, gıdadan kozmetiğe çok farklı alanlarda kullanım alanına sahip önemli ekonomik bir üründür. Günümüzde halk arasında sıklıkla ülser, hazımsızlık vb mide rahatsızlıklarında ve yara tedavisinde kullanılmaktadır. Bitkinin bu amaçlarla kullanımı tarihten bugüne aktarıla gelmiştir. Günümüzde bitkinin midevi etkileri ile ilgili klinik çalışmalar da yapılmıştır. Bir çalışmaya göre, dispepsi şikayeti olan hastalarda çift körlü randomize plasebo kontrollü bir araştırmada 3 hafta boyunca 350 mg/gün dozda toz halde sakız verilmiştir. Tedavi sonrasında semptomların belirgin şekilde iyileştiği görülmüştür [24].

***Hypericum perforatum* (Sarıkantaron)** bitkisinin günümüzde yaygın şekilde depresyon tedavisinde kullanıldığı bilgisi araştırılan 21 geleneksel tıp kitapta bahsedilmemektedir, fakat bitkinin sinir sistemine yönelik kullanımı halk tıbbı ve günümüzdeki bilimsel kullanımı ile örtüşmektedir. Yara, romatizma, soğuk algınlığı, böbrek, mesane taşı, çeşitli ağrıların tedavisinde kullanımı Phsyica, Lehcetü'l-Lügat, Nüzhetü'l-Ezhân fi İslâhi'l-Ebdân, Terceme-i Cedide Fi'l-Havassi'l-Müfrede, Mücerrebname, Materia Medica eserlerinde belirtilmiş, günümüz halk tıbbı ve bilimsel araştırmalarla uyumlu bir kullanım olduğu tespit edilmiştir. Günümüz araştırmalarında antidepresan, antiromatizmal, antiviral, antibakteriyel, antikanser, hepatoprotektif etkileri monograflarda kayda geçmiştir. Bitkinin yara tedavisinde kullanımıyla ilgili WHO tıbbi bitkiler monograflarının sarı kantaron bahsinde yer verilen etkinlik testi şöyledir: Sarı kantaronun %20'lik sulu ekstresi kobay ve tavşanda oluşturulan yaralarda iyileşmeye sebep olduğu görülmüştür. Yaprakların %60 ekstresi sıçanlarda (0,1 ml/hayvan) oluşturulan yaralarda epitelizasyon oran ve kuvvetini arttırarak iyileşme sağlamıştır. [1]. Sarı kantarondan günümüzde önemli etken maddesi hyperisinin standardize edilmiş formları olan tabletler, zeytinyağında masere edilen sarı kantaron yağı ürünleri bulunmaktadır. Yine bitkiden reçete edilen Felis, Remotive gibi preparatlar eczanelerde satışa sunulmaktadır. Kantaron çayı da demet ve öğütülmüş formlarda aktar ve eczanelerden tedarik edilebilmektedir. Çayı mide problemleri için, yağı ise yara ve yanık tedavisinde halk arasında kullanılmaktadır.

***Plantago lanceolata* (Sinirliot)** bitkisi solunum sistemi hastalıkları (verem, balgam söktürücü, akciğer ağrısı öksürük vb) tedavisinde etkili olduğunu belirten geleneksel tıp eserleri Materia Medica, Physica ve Yadigâr'dır. Bitkinin bu kullanımı hem halk tıbbında hem de günümüzdeki bilimsel kullanımı, halk tıbbı ve günümüzdeki bilimsel kullanımı uyumlu olduğu çalışmamızda görülmüştür. Sinirliot türlerinden *Plantago major* de bu tür gibi halk arasında sıklıkla kullanılmaktadır. Halk tıbbında çıban olgunlaştırma, yara tedavisinin yanı sıra öksürük, soğuk algınlığı, verem, hemoroit, bronşit gibi rahatsızlıklarda kullanılmakta olduğu tespit edilmiştir. Bitki Komisyon E ve ESCOP monograflarında yer almaktadır [25]. Bitkinin geleneksel tıp metinleri, halk tıbbı ve günümüz bilimsel verilerine dayalı kullanımında solunum rahatsızlıklarında kullanıldığı görülmektedir. Bitkinin solunum yolları rahatsızlıklarında kullanımıyla ilgili klinik çalışmalar da bulunmaktadır. Bir çalışmada akut solunum rahatsızlığı ve öksürük olan 593 hastaya ortalama 10 gün boyunca bitkinin topraküstü kısmını (6,3 gr) içeren 31,3 ml şurup verilmiştir. Süreç sonunda hastalık belirtilerinde anlamlı bir azalma meydana gelmiş, belirtiler %65,5-79.9 oranında azalmıştır. Aynı çalışma bu kez 18 yaş ve altındaki 91 hasta ile yapılmış, 8 gün boyunca günlük ortalama 4,5 gr drog içeren 22,4 ml şurup verilmiş ve belirtilerin %58,3 oranında azaldığı bulunmuştur [26].

***Matricaria chamomilla* (Tıbbi papatya)** bitkisi nezle, yara, soğuk algınlığı tedavisine yönelik olarak da kullanıldığı Musa Bin Hamon'un eserinde ve El- Kanun Fi't Tıbb'da belirtilmektedir, bitkinin bu kullanımı da hem halk

tıbbında hem de günümüzdeki bilimsel kullanımı ile uyumludur. Tıbbi papatyanın antienflamatuvar, antigribal, antispazmotik, yara iyi edici etkileri klinik düzeyde çalışılmıştır. Rastgele seçilen çift körlü bir araştırmada, komplike olmayan diyaresi bulunan 79 çocuk (6 aylıktan 5 yaşına kadar) denek üzerinde çalışılmıştır. Bu çocuklara rehidrasyon ve diyete ek elma pektini ve papatya ekstresi içeren preparat ile plasebo verilmiştir. Tedavinin üçüncü gününün sonunda diyare, plaseboya kıyasla pektin/papatya grubunda anlamlı bir şekilde iyileşmiştir. Papatya çayı ishal süresini en az 5 saat kısaltmıştır [27]. Yine papatyanın soğuk algınlığı tedavisinde antigribal odaklı klinik etkinlik çalışması yapılmıştır. Soğuk algınlığı geçiren ve ayakta tedavi edilen 60 hastaya inhalasyon yoluyla papatya verilmiştir. Yapılan çalışma sonunda ekstrenin orta ve üst solunum yolu enfeksiyonuna karşı etkili olduğu, hiçbir yan etki taşımadığı gözlenmiştir. Etki, uygulamadan 15 dakika sonra başlamış ve 0,5-2 saat arasında maksimuma ulaşmıştır [28].

***Arum maculatum*** **(Yılanyastığı*)*** bitkileri yara kapatıcı, hemoroit iyileştirici, balgam söktürücü etkileri Yadigâr, Physica, Materia Medica eserlerinde belirtilmektedir. Bitkinin bu kullanımı hem halk tıbbında hem de günümüzdeki bilimsel kullanımı ile uyumlu olduğu görülmüştür. Bitkinin kanserde kullanımı da Alâim-i Cerrâhîn adlı eserde bahsedilmiştir. Bitki halk arasında, bölgelerine göre farklı türlerin kullanıldığı görülmektedir. Genellikle hemoroit ve kanser tedavisinde yaygınlıkla kullanıldığı anlaşılmaktadır. Hemoroit tedavisinde genellikle bitkinin yumrusu kullanılmaktadır. Kanserde kullanımı konusunda modern anlamda çalışmalar da bulunmaktadır.
Kozuharova ve arkadaşları (2020), tarafından *Arum* türlerinin hemoroit tedavisinde kullanılma potansiyelini araştırmak üzere *Arum* türlerinin geleneksel kullanımını, biyolojik olarak aktif bileşiklerini ve farmakolojik aktivitelerini incelemek üzere bir çalışma yürütülmüştür. *Arum maculatum, Arum italicum, Arum dioscoridis, Arum palaestinum* türlerinin yumru, yaprak kimyasal içerikleri araştırılmış ve biyoaktivite çalışması yapılmıştır. Arum türleri bileşiminde alkaloidler, polifenoller, glikozitler (favonoid, saponin ve syanojenik gruplar), monoterpenler, seskuiterpenler, lektinler tespit edilmiştir [29]. *Arum maculatum*'un semptomatik hemoroid tedavisindeki etkinliğini değerlendirmek için Yunanistan'da 53 hasta kullanılarak 2 haftalık bir test tedavi periyodu ile randomize kontrollü bir klinik çalışma yürütülmüştür Hastalar rastgele sıvı *Arum maculatum* karışımı veya standart bir antihemoroid özellikli bir krem verilmiştir. Tedavi başlangıcında ve tedaviden iki hafta sonra (SF-36, hasta sağlığı" anketi) sağlık araştırmasıyla yaşam kalitesi ölçülmüştür. Çalışma sonucuna göre *Arum maculatum*'un hemoroidli hastaların yaşam kalitesini artırdığını görülmüştür [30].

**4. Sonuçlar ve tartışma**

25 tıbbi bitkinin geleneksel tıp kitaplar, halk tıbbı ve günümüz araştırmalarıyla %84 uyumlu olduğu tespit edilmiştir. 25 bitkinin tıbbi kullanım amaçlarına bakıldığında, her türün birden fazla amaçla kullanıldığı araştırma sonucunda bulunmuş buna göre; türlerin %44'ü sindirim sistemi tedavisinde, %20'si solunum sistemi tedavisinde, %8'i boşaltım, sinir sistemi ve integumenter sistem tedavisinde kullanılırken; dolaşım sistemi, yara tedavisi, kadın hastalıkları %4 oranla en düşük kullanım amacını paylaşmıştır.

Araştırmamızda bitkilerin hemen hepsinin yılan, akrep vb zehirli hayvan sokmalarına karşı kullanıldığı yazma tıp kaynaklarında görülmüştür. Günümüz halk tıbbında da benzer uygulamalara rastlanmıştır. Fakat modern araştırmalarda bitkilerin bu yönüyle ilgili araştırmalar bulunmamaktadır. Bu yönüyle zehirli hayvan sokmalarına karşı bitkilerin kullanılması dönem insanının doğayla iç içe bir yaşam sürdüğünün bir karşılığı olarak geleneksel tıp dönemdeki kitaplarda yer bulmuştur. Yine halk tıbbında halen zehirli hayvan sokmalarına karşı reçetelerin kullanılmakta olduğu görülmektedir. Halk hekimliği, genellikle kırsal alanda, sağlık sistemlerine uzak köylerde yaygın olduğundan buralarda da önemli sorun olarak zehirli hayvan sokmalarına karşı uygulamalar devam etmektedir diyebiliriz.

Araştırmamızda bitkilerin halk tıbbında kullanım amacı ve şekli Türkiye genelinde hemen her bölgede benzerlik gösterdiği tespit edilmiştir. Halk tıbbında da, kaynak eserlerde de bitki kullanım bilgisi doz, hazırlama yöntemi ve kullanım süreleri gibi unsurların net olmadığı görülmüştür.

Çalışmamızı hazırlarken en çok sözlükler konusunda güçlük çekilmiş, kaynakların sonundaki sözlükler terkiplerde kullanılan bitkiler için yetersiz kalmıştır. Sadece tıp yazmalarındaki bitkileri ve diğer malzemeleri içeren disiplinler arası iş birliği ile hazırlanmış bir sözlük çalışması da araştırmacılara faydalı olacaktır.

Günümüzde kesin tedavisi ve nedeni anlaşılamayan bazı hastalıkların nedenleri ile devaları incelenen eserlerde detaylı olarak anlatılmıştır. Yazma eserlerdeki bitki kullanım bilgisinin günümüzde güncel olarak uyarlanması gerekmektedir. Bu da dil bilimci, eczacı, hekim, kimyager, tıp tarihçileri ve etnofarmakoloji, farmakognozi, farmasötik botanik gibi farklı disiplinlerdeki bilim insanları tarafından incelenmesi, klinik çalışmalarla etkinliklerinin teyit

edilmesi, devasız görülen marazların iyileşmesine vesile olabilecek ilaçların ortaya çıkmasını sağlayabileceği düşünülebilir. Çalışmamızda bahsi geçen 25 bitki ile sınırlandırılan kullanım bilgisi karşılaştırması bilgilerin kayıt altına alınmasının önemini de vurgulamaktadır. Halk hekimliği ilaçlarının kullanım bilgisi yaşlı kuşağın hafızasında saklı olduğu için bu bilgilerin kayıt altına alınması için çalışmalara katkı sunacaktır.

Üniversitelerimizin tıp, eczacılık, botanik, kimya, tıbbi ve aromatik bitkiler gibi alanları ile Sağlık Bakanlığı, TÜBİTAK, yerli ilaç sanayi gibi konunun ilgilisi paydaşlarının da katkı sunduğu, geleneksel tıp kitapları ve halk tıbbındaki bilgilerin etkinliğini araştıran milli bir kurum tarafından bu bilgiler veritabanında sistematik olarak incelenmeli, raflardan inip insanlığa faydaya dönüştürülmelidir.

Osmanlı dönemi tıp tarihi kaynaklarında ilaç yapımında kullanılan bitkiler incelenmek istendiğinde günümüzdeki karşılıklarını bulmak güçleşmektedir. Bu çalışmalar, araştırıcıların terkiplerdeki bitkilerin tam karşılıklarına ulaşabilmelerini sağlamaları açısından önemli görev üstlenmektedir.

İncelediğimiz yazma eserler genellikle dilbilimciler tarafından hazırlandığından bitkilerin sınıflandırılmasında ve doğru tanımlanmasında bazı hatalar olabilmektedir. Bu çalışmaların dibilbilimci, sistematik botanikçi, eczacı, tıp tarihçisi, hekim gibi meslek grupları iş birliği ile daha etraflı incelenip hazırlanması, bu eserlerdeki terkiplere dair çalışma yürüteceklere daha güvenilir kaynak sunacaktır diyebiliriz.

Etnobotanik araştırmalarında bitkinin ne amaçla, nasıl kullanılacağı genellikle kaynak kişilerin aktarımına göre kayıtlara geçmiş, fakat ne kadar süre kullanacağı, hangi dozda alınacağı genellikle belirtilmediğinden bu kullanım bilgilerinden hareketle yeni çalışma yapılması güçleşmektedir.

Çalışmamızın sonucuna göre bugünkü tecrübe edindiğimiz bilgilerin kaynağının, tarihin diğer dönemlerinde tecrübe edinilen deneyimlere dayalı olduğu görülmektedir. Tıp pratiğinde de günümüz insanının deneyimlemediği pandemi gibi tedavi pratiklerinin insanlık tarihi boyunca tecrübe edildiği görülmektedir. Bu konuda tıp büyüklerimiz İbn-i Sina ve Er-Razi'nin enfeksiyon hastalıklarındaki tecrübeleri, bugün de çözüm olarak sunulan karantina kavramının gelişimine katkı sağlamıştır. Yine günümüzde karantinanın yanısıra pandemi sırasında sağlık, diyet ve hijyen kurallarını ortaya koyan da yine bu hekimlerdir [1].

Çalışmamızda bitkilerin tıbbi tıbbi kullanım bilgileri üç dönemi içerecek şekilde karşılaştırılmıştır. Bitkilerden ilaç hazırlanma yöntemleri, kullanım süreleri, hastalıkların tedavisinin diğer yöntemlerinin incelenmesi günümüz tıbbına ve etnofarmakoloji bilimine katkı sunacaktır.

İncelediğimiz bitkilerin günümüzdeki kullanımlarını araştırırken rastladığımız bazı araştırmacılar günümüzde çaresiz görünen hastalıkların tedavilerini kendi ülkelerinde geleneksel tıp metinlerindeki bilgilerden yola çıkarak araştırmış, sonuçları bilim insanları ile paylaşmıştır. İbn-i Sina, Galenos, Dioscorides gibi Anadolu medeniyetine ilham olmuş tıp büyüklerimizin eserleri çaresiz görünen hastalıklar için bulunmaz birer hazine olarak sarrafını aramaktadır diyebiliriz. Bilim insanlarımız tarafından bu eserler incelenerek onlarca maraza deva bulunabilir diyerek çalışmamızı araştırmacıların dikkatine sunuyoruz.

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**Biological Diversity and Conservation**

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***Contents***



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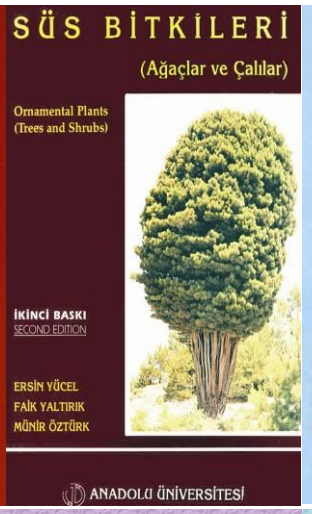


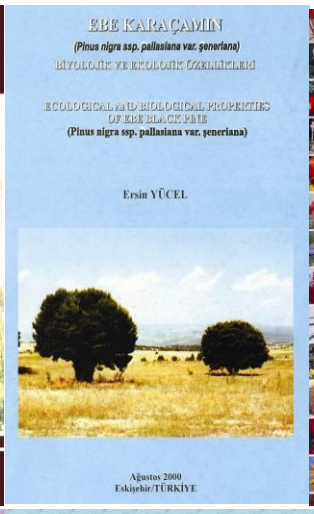


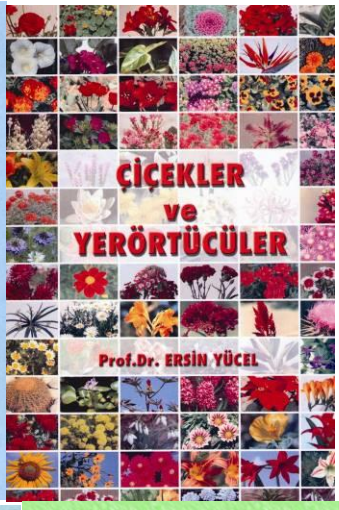


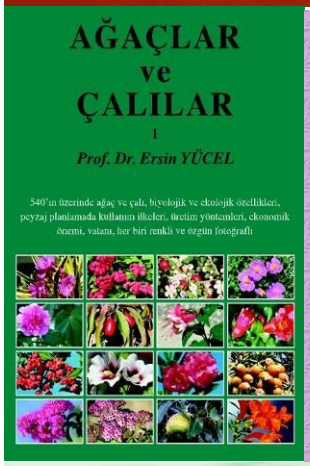


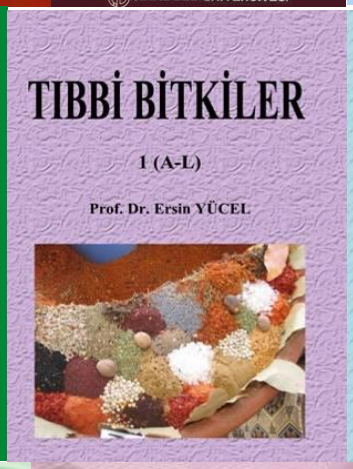



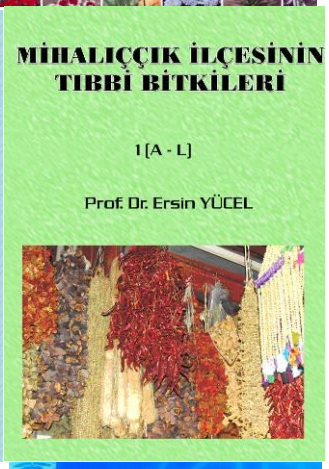


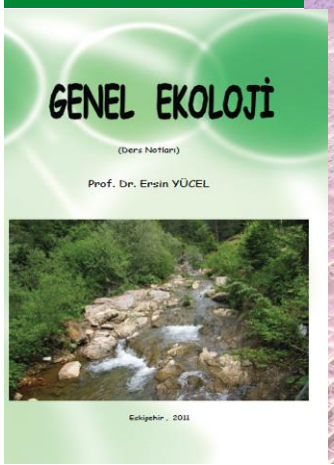


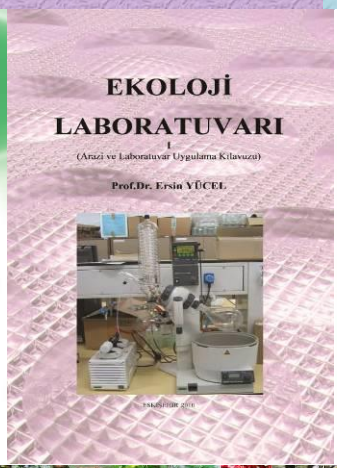


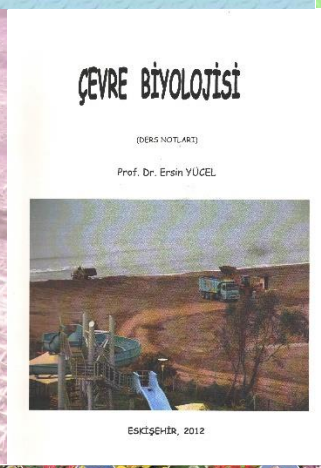


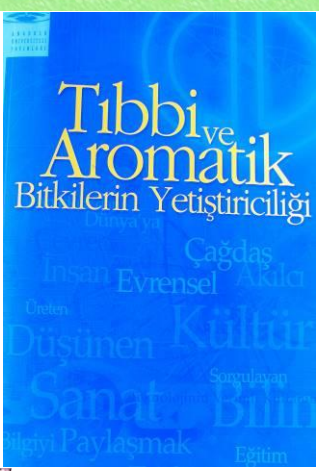



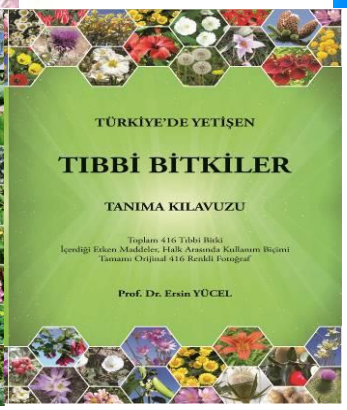


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