C O M M U N I C A T I O N S

FACULTY OF SCIENCES
UNIVERSITY OF ANKARA

DE LA FACULTE DES SCIENCES
DE L'UNIVERSITE D'ANKARA

Series C: Biology

VOLUME: 29 **Number: 1** **YEAR: 2020**

Faculy of Sciences, Ankara University
06100 Beşevler, Ankara-Turkey
ISSN: 1303-6025 E-ISSN: 2651-3749

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S e r i e s C : B i o l o g y

Volume 29 **Number : 1** **Year: 2020**

This Journal is published two issues in a year by the Faculty of Sciences, University of Ankara. Articles and any other material published in this journal represent the opinions of the author(s) and should not be construed to reflect the opinions of the Editor(s) and the Publisher(s).

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Print:
Ankara University Press
İncitaş Sokak No:10 06510 Beşevler
ANKARA – TURKEY

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Research Articles

Review Article

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Available online: January 13, 2020

Commun.Fac.Sci.Univ.Ank.Series C
Volume 29, Number 1, Pages 1-49 (2020)
ISSN 1303-6025 E-ISSN 2651-3749
https://dergipark.org.tr/en/pub/communc/issue/51836/619430

FLORA OF ÇALTEPE AND ÇELETEPE (BOLU)

AYDIN ÇELİK, İSMAİL EKER

ABSTRACT. This study was carried out to reveal the vascular plant diversity of Çaltepe and Çeletepe. During 2015−2018, 2340 plant specimens were collected from the research area and 363 genera and 767 taxa belonging to 81 families were determined. Of all the collected taxa, 66 are endemic and endemism rate is %8.60. The IUCN threat categories of endemic and rare plants at global level are as follows: 1 taxon in "CR" category, as well as 4 taxa "EN", 4 taxa "VU", 9 taxa "NT", and 45 taxa "LC". Also, 3 rare taxa are found in the "VU" category, as well as 1 rare taxon in the "DD" category at regional level. 56 taxa are new records for the province of Bolu. The largest families in the study area are as follows: Asteraceae 97 taxa (%12.65), Fabaceae 62 taxa (%8.08), Lamiaceae 51 taxa (%6.65), Rosaceae 44 taxa (%5.74), Poaceae 37 taxa (%4.82), Brassicaceae 35 taxa (%4.56), Caryophyllaceae 32 taxa (%4.17), Apiaceae 28 taxa (%3.65), Boraginaceae 27 taxa (%3.52) and Orchidaceae 24 taxa (%3.13). The distribution of taxa into phytogeographic regions are as follows: 234 taxa (%30.50) Euro-Siberian, 64 taxa (%8.34) Mediterranean, 46 taxa (%5.99) Irano-Turanian, and 423 taxa (%55.15) multiregional and/or unknown. *Geranium* and *Silene* (12 taxa) are the most common genera in the research area. These genera are followed by *Salvia* (11 taxa), *Trifolium, Veronica* and *Campanula* (each with 10 taxa), *Ranunculus*, *Euphorbia* and *Vicia* (each with 8 taxa), and *Poa* (7 taxa).

1. INTRODUCTION

Turkey has a rich variety of flora and vegetation due to its different climate types (continental, ocean and Mediterranean climates), geomorphological diversity, rich water resources (sea, lake and stream), great altitude differences (sea level−5000 m), and a wide variety of habitat types. In addition to this, as a result of the phytogeographical classification conducted on land owned by the whole of Europe and Asia, Turkey is divided into three different phytogeographic regions [1]. Our country is one of the important plant centers of the world in terms of its location at

Received by the editors: September 12, 2019; Accepted: December 12, 2019.
Key word and phrases: Flora, Bolu, Çaltepe, Çeletepe, Taxonomy, Turkey.

the intersection point of Iran-Turan, Euro-Siberian and Mediterranean phytogeographic regions, its connection of the continents of Europe and Asia which have different climatic and edaphic conditions [2].

The first registered floristic study in our country was carried out between 1701 and 1702 by the French botanist Joseph Pitton de Tournefort in Northern Anatolia [3]. The work entitled "*Flora Orientalis*" which was published in six volumes by the Swiss botanist Pierre Edmund Boissier between 1867 and 1888 covers the region extending from Greece to Afghanistan and mostly contains plant species of the Anatolian and Middle Eastern countries. The current study which included 6000 plants from Anatolia is the first work written about the flora of Turkey [4]. Then, the "*Flora of Turkey and the East Aegean Islands*" was written in 10 volumes under the editor-in-chief of English botanist Peter Hadland Davis between 1965 and 1988. It includes the floristic record of approximately 9000 plants from our country, and has become the main book of the researchers dealing with plant taxonomy [5-6]. Afterwards, Güner *et al*. [7] wrote the eleventh volume by adding 400 new taxa to Turkey's flora. In the years following the completion of flora-writing, problems were encountered while diagnosing a large number of materials collected, as a result, attention was drawn to problems of some genera. However, taxonomic problems persist in some species of many genera, especially large genera. Because of the limited of time and materials in the process of writing the "*Flora of Turkey*", the deficiencies regarding many species and sections has been highlighted in the flora but no adequate solutions have been proposed. In addition, it was emphasized that detailed floristic studies are needed in many regions of Turkey, especially in Eastern Anatolia. With the publication of the book entitled "*Türkiye Bitkileri Listesi-Damarlı Bitkiler*", the number of taxa in Turkey has reached 11707 while the endemism rate was 31.82% [8]. However, the extraordinary richness and diversity of the flora of Turkey has not yet been fully revealed. On the other hand, there is a need to carry out regional floristic and revision studies to better understand the distribution boundaries of taxa and determine the variation limits of taxa, in the process of writing the new volumes of the "*Resimli Türkiye Florası*" [9-10]. The first number of the current study, which was initiated by Turkish botanists, was published in 2014 and the second number was published in 2018. In the following processes, it is planned to be written the other volumes of the book to cover all plants of Turkey.

The revisional studies solve some taxonomic problems; however, they cannot sufficiently reveal the geographic distribution and transitions of taxa. Also, the researchers usually do not have time to do it. The serious consideration of regional

records based on floristic studies will reveal the true distribution of taxa in that region and ensure that the wrong records are extracted.

The studies on Bolu's floristic diversity in chronological order are as follows; Gerede and Aktaş Forests, and Köroğlu Mountain by Akman and Ketenoğlu [11-12], Bolu and Semen Mountains by Akman and Yurdakulol [13-14], Yedigöller Natural Park by Ekim and İlarslan [15], campus flora of Abant İzzet Baysal University by Turgut [16], Gökçeler Mountain by Uluğ [17], Lake Abant by Türker and Güner [18], Lake Yeniçağa by Sümer [19], Lake Gölcük by İkinci and Güner [20], Karakiriş Mountain by Aksoy [21], Lake Sünnet by İkinci [22], Kartalkaya by Sungurlu [23], Kale-Bolu Hazelnut Nature Reserve Area by Arslan *et al*. [24], aquatic plants of Gölköy and Yumrukaya by Bayındır [25], petaloid monocotyledonous flora of Bolu by Demir and Eker [26], the effect of intense construction and population pressure on flora changes in Gölköy Campus by Doğan *et al*. [27], Lake Sülüklü by Kanoğlu *et al*. [28], Taşlıyayla and Kızık surrounding by Tunçkol and Akkemik [29], Flora of Argözü Valley (Kıbrıscık-Bolu) by Güneş Özkan *et al.* [30], flora and e-flora of Gölköy Campus by Eker *et al*. [31-32], aquatic plants of Bolu by İkinci and Bayındır [33], endemic and rare plants of Bolu by Eker *et al*. [4] were studied. The vegetational studies are as follows: Köroğlu Mountains by Akman and Ketenoğlu [34], Bolu and Semen Mountains by Akman *et al*. [35-36], Gerede-Aktaş forest by Ketenoğlu [37], Mudurnu surroundings by Akman and İlarslan [38] and Plant communities and stand structure characteristics of Bolu-Ayıkaya region by Çoban [39] were studied.

Çaltepe and Çeletepe regions, which are selected as a research area, are located in the northeast of the province of Bolu and found in A3 square according to Davis' grid system in the "*Flora of Turkey and East Aegean Islands*" (Figure 1). In the southwest-northeastern direction of Bolu province, Bolu Mountains are located. The highest point of the Bolu Mountains is Çeletepe (Çeledoruğu) in 1987 m. It is the second highest mountain after Köroğlu Hill (2499 m) in Köroğlu Mountains in Bolu Province. Çaltepe has a height of 1890 m. Çeletepe with N 40° 51.940' – E 031° 42.123' coordinates and Çaltepe with N 40° 53.570' – E 031° 46.526' coordinates are integrated with each other. There are Banaz Plateau and Yedi Erenler on Çeletepe, and Merkeşler Plateau on Çaltepe, and Kadıköy Plateau are located between these two peaks. On the south of both hills, the villages of Tetemeçele, Mesciçele, Yeşilçele Çobankaya and Bağışlar, in the southwest the villages of Gölcük, Yakabayat, Hamzabey and Musluklar, in the southeast the villages of Merkeşler and Avşar, in the west the village of Çukuröre, in North towards the Yedigöller mountain ranges Sarımustan, Kapankayası, Gurbettaşı are located. The region has

various habitat layers such as damaged forest, steppe, coniferous and mixed forests, subalpine and alpine regions in the height range of 1000–2000 m. It is predominantly under the influence of the Euro-Siberian floristic region from the north and, the Mediterranean floristic region from the south-west. Although the region is one of the rare regions with alpin layer in Bolu, there has not been any floristic work related to Çaltepe and Çeltepe until this study.

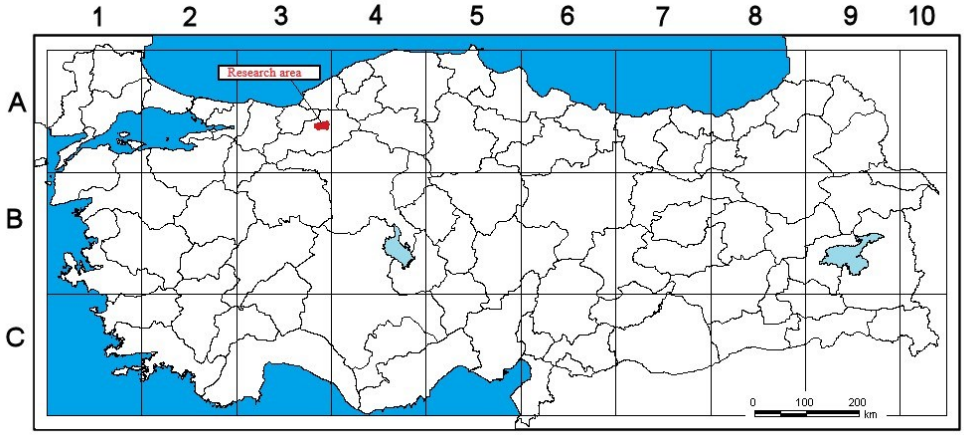

FIGURE 1. Map for Davis Grid System and the location of the research area in Turkey.

Considering temperature and precipitation data of Bolu, either semi-arid moist climate according to the De Martonne method or semi-arid Mediterranean climate according to Emberger drought index are observed (Akman, 2011). There is more rainfall in winter months and less rainfall in summer months in Bolu. Regarding the last 90 years rainfall data, annual mean temperature in Bolu city is 10.5 ºC. As the same data, mean maximum temperature is 27.9 ºC in August and mean minimum temperature is -3.6 ºC in January. Seasonal temperature avarages are 9.5 ºC in spring, 19.03 ºC in summer, 11.6 ºC in autumn and 1.7 ºC in winter. According to seasonal distribution of rainfalls, 160.8 mm in spring, 105.4 mm in summer, 115.5 mm in autumn, 164.1 mm in winter and annual mean rainfall is 546.8 mm. Depending on these data, the rainfall regime of Bolu city is classified as the 1st Lower Type of East Mediterranean rainfall regime [40] (Figure 2).

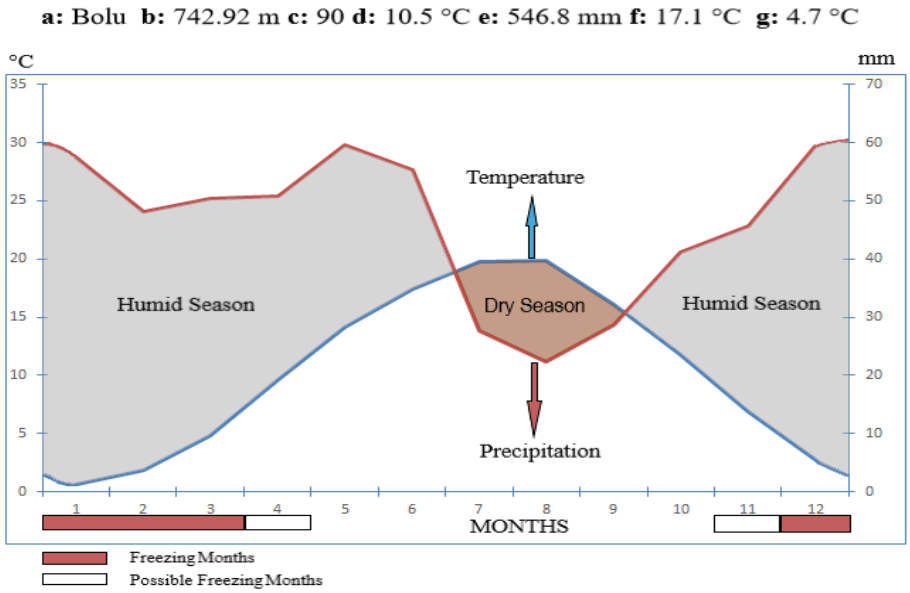

FIGURE 2. Climatic Diagram of Bolu (for the last 90 years). Explanations: a) Meteorological Station, b) Altitude of Meteorological Station, c) Data Collection Period (last ninety years), d) Mean Annual Temperature, e) Mean Annual Precipitation, f) Mean Maximum Temperature (ºC), g) Mean Minumum Temperature (ºC).

This study reveals the floristic diversity of Çaltepe and Çeletepe region in Bolu province. The species inventory lists of the research area were prepared, and observations were made regarding the distribution areas and population densities of endemic and rare species. Moreover, recommendations for the development of in situ conservation methods are presented.

The reasons for selecting Çaltepe and Çeletepe regions (Bolu) as the study area are as follows:

1. No studies have been conducted on the flora of Çaltepe and Çeletepe. In other words, this region is among the unknown or little-known regions of Turkey in terms of the floristic diversity.
2. The study area is an important region in terms of biodiversity due to the fact that it is under the influence of two different phytogeographic regions, and it has the second highest elevation after Köroğlu Hill in Bolu province.

The objectives of this study are formulated as follows in the order of priority:
1. To reveal the floristic inventory of the region.
2. To reveal the distribution areas and densities of endemic and rare species in the region by population observations.
3. To provide up-to-date regional reports about threat categories of endemic and rare species found in the IUCN Red Data List.
4. To determine the areas where biodiversity is high and to offer suggestions for in situ preservation.
5. To provide plant samples for the development of Bolu Abant İzzet Baysal University Herbarium (AIBU).

2. MATERIAL AND METHODS

In the research area, a total of 39 days of field studies were conducted from February 2015 to April 2018 and a total of 2340 flowering and fruiting plant specimens were collected from 19 main stations (Figure 3, Appendix 1) and substations. The photographs of taxa were taken in their natural environments, GPS coordinates, locality and habitat information were also noted. The collected samples were converted into herbarium material and the collector numbers were given, and then stored at the Bolu Abant İzzet Baysal University Herbarium (AIBU).

The specimens were diagnosed in the light of relevant literatures. Especially, for identification of the plants, 11 volumes of "*Flora of Turkey and East Aegean Islands* [5-7]" were used. To determine the correct and current scientific names of plant names "*Türkiye Bitkileri Listesi* [8]" were used as well as web sites "*World Checklist of Selected Plant Families* [41]", "*International Plant Name Index* [42]" and "*the Plant List* [43]". Author abbreviations were given according to IPNI [42].

Using "*International Union for Conservation of Nature* [44]" criteria, in determining of IUCN Red Data Book categories of endangered species of endemic and rare plants, the data in "*Türkiye Bitkileri Kırmızı Kitabı* [45]" were used. The abbreviations and meanings used in the identification of endangered species of plants are as follows: Extinct (EX), Extinct in the Wild (EW), Critically Endangered (CR), Endangered (EN), Vulnerable (VU), Near Threatened (NT), Least Concern (LC), Not Evaluated (NE), Data Deficient (DD). At the local level, population densities of endemic and rare taxa were categorized as "rare, low, medium-density, common" based on rough population observations.

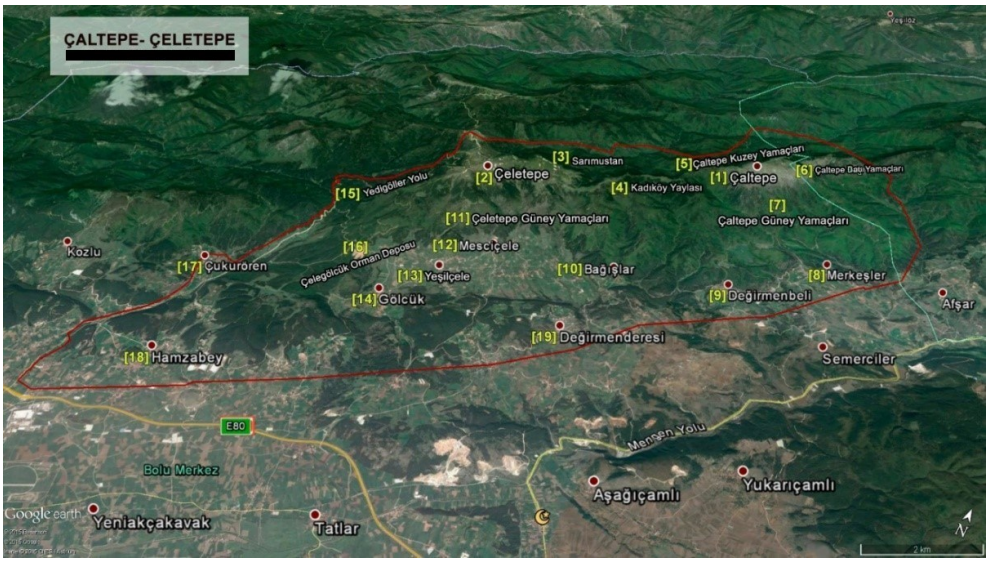

FIGURE 3. The main stations where fieldwork is carried out in the research area.

The complete floristic list was set out according to the the systematic order in "*Angiosperm Phylogeny Group III* [46]". Enumaretion of the taxa are given in the following order:

- Names of division, class, order and family in accordance with the taxonomic hierarchy
- Valid genus name, author(s)
- Valid species name, author(s); if it is present infraspecific category
- Colection information for specimen examined: location, altitude, habitat, GPS (Global Position System) coordinate, collection date, collector(s) number, identifier(s) and identification date
- If there is, endemism and rarity
- If known, phytogeographic region
- If it is a new record for Bolu, it is specified

In this study, climatic data of Bolu province were received from "Bolu Meterology Station" [47].

3. RESULTS

In the research area, 39 days of fieldwork was conducted between 2015–2018, and 2340 plant samples were collected from 19 different main locations and sublocations (Figure 3, Appendix 1). As a result of the diagnosis of these samples, 767 taxa (745 species) were identified (Appendix 2), of which 66 were endemic and 4 rare, belonging to 81 families and 363 genera. 15 of the taxa belong to the division Pteridophyta and the remaining 752 taxa belong to the division Spermatophyta. Five of the spermatophytic taxa belong to Gymnospermae and 747 belong to Angiospermae. Of the 747 taxa belonging to Angiospermae, 109 belong to Monocotylodonae and 638 belong to Dicotylodonae (Figure 4). 56 taxa are the new records for the province of Bolu (Appendix 2).

In the research area, characteristic vegetation types of Black Sea climate are seen. That is, forest, distorted forest, steppe, subalpine, alpine, rock, river and moist creek vegetation types are seen. Generally, below 1000 m, settlements, culture areas, degraded forests and steppe vegetation are observed. In these altitudes, the forest formation is characterized by the large-leaved *Quercus* spp., *Crataegus* spp., *Prunus* spp., *Corylus* spp., *Salix* spp. and coniferous *Abies nordmanniana* subsp. *equi-trojani* and *Pinus* spp. In the vicinity of the upper Kadıköy Plateau (1000–1500 m high) close to the north facing, the dominant species is Kazdağı fir (*Abies nordmanniana* subsp. *equi-trojani*). Also, beech (*Fagus orientalis*), hornbeam (*Carpinus betulus*), European bladdernut (*Staphylea pinnata*) and wych elm (*Ulmus glabra*) are intermingled in some places. On the south side, the black pine (*Pinus nigra* subsp. *pallasiana*) and yellow pine (*Pinus sylvestris* var. *hamata*) are dominant, but occasionally beech and hornbeam appear. In the southeastern region, up to 1200 m, mainly the oak species (*Quercus petraea* subsp. *iberica*, *Quercus pubescens* subsp. *pubescens, Quercus infectoria* subsp. *infectoria, Quercus macranthera* subsp. *syspirensis*) are included in the forest composition. This composition is accompanied by the cade juniper (*Juniperus oxycedrus* subsp. *oxycedrus* var. *oxycedrus*) in the southern and southeastern parts under 1200 m. At the 1700 m elevations where the subalpine zone is observed, dwarf juniper (*Juniperus communis* var. *saxatilis*) is dominant; geophytes, some alpine plants and moist plains dominate the alpine parts of both integrated mountain masses (Çeletepe and Çaltepe). At the creek edges, there are some trees such as poplar (*Populus nigra* subsp. *nigra, Populus tremula* subsp. *tremula*), willow (*Salix alba* subsp. *alba, Salix caprea*), nut (*Corylus avellana* var. *avellana*), common alder (*Alnus glutinosa* subsp. *glutinosa*) and some hygrophilous plant species such as horsetails (*Equisetum* spp.)

and saxifrages (*Saxifraga* spp.). *Salix caprea, Populus nigra* subsp. *nigra*, *Rosa canina, Colutea cilicica, Rubus* spp. are common tree and shrub species of dry creek bed in summer. On the other hand, common herbaceous species are *Chenopodium album, Mentha longifolia, Xanthium strumarium* subsp. *strumarium, Trifolium campestre* subsp. *campestre* var. *campestre, Lamium purpureum* var. *purpureum, Euphorbia pannonica*.

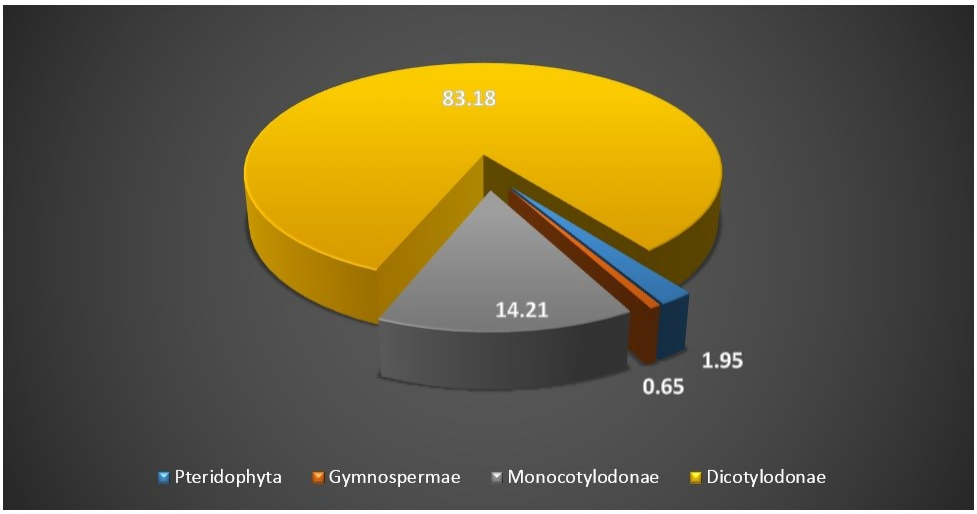

FIGURE 4. Spectrum of the distribution of species according to major taxa.

The distribution of taxa according to phytogeographical regions is as follows: 234 taxa (30.50%) Euro-Siberian, 64 taxa (8.34%) Mediterranean, 46 taxa (5.99%) Irano-Turanian and 423 taxa (55.15%) are the multizone and/or their phytogeographical regions are unknown (Figure 5). Of all the collected taxa, 66 are endemic and endemism rate is %8.60. The IUCN threat categories of endemic and rare plants are as follows: 1 taxon in “CR” category at global level, as well as 4 taxa “EN”, 4 taxa “VU”, 9 taxa “NT”, and 45 taxa “LC” (Table 1). Also there are 3 rare taxa which are found in the “VU” category and 1 rare taxon which is found in the “DD” category at regional level (Table 1).

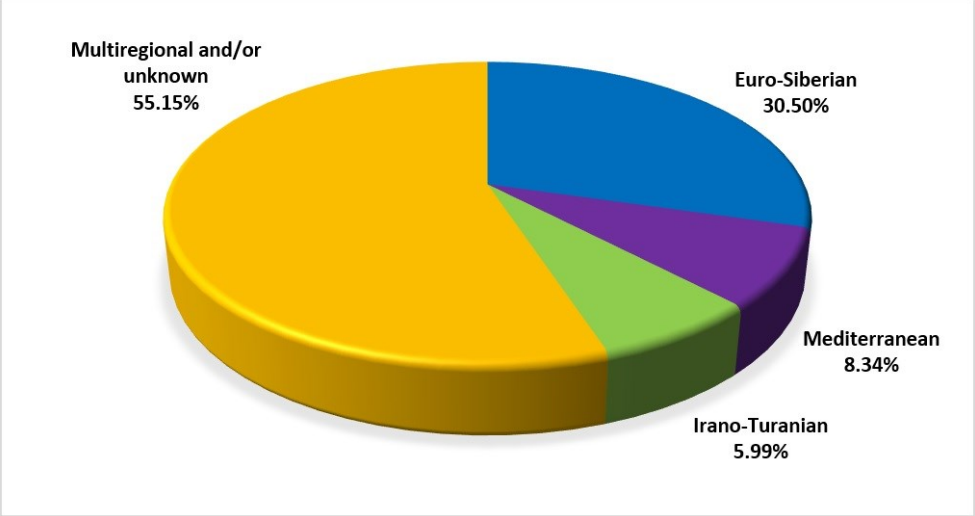

FIGURE 5. Distribution of species in research area according to phytogeographic regions.

Considering the population density of endemic and rare taxa detected in the research area, it can be said that especially *Asperula pestalozzae*, *Astragalus amoenus*, *Astragalus panduratus, Asyneuma rigidum* subsp. *sibtharpianum*, *Aubrieta olympica, Campanula grandis* subsp. *grandis, Cyanus pichleri* subsp. *extrarosularis, Delphinium fissum* subsp. *anatolicum, Hieracium paphlagonicum, Iris kerneriana, Lamium purpureum* var. *aznavourii*, *Lathyrus czeczottianus*, *Lathyrus tukhtensis, Melampyrum arvense* var. *elatius, Minuartia erythrosepala* var. *cappadocica*, *Papaver pilosum* subsp. *pilosum*, *Poa asiaeminoris*, *Salvia tobeyi, Silene olympica* var. *olympica, Trifolium aureum* subsp. *barbulatum, Turanecio hypochionaeus, Vicia freyniana, Hordeum murinum* subsp. *leporinum, Iris pumila* subsp. *attica* and *Lilium martagon* are rare taxa at regional level (Table 1).

TABLE 1. Endemic and rare plants, their IUCN Red Data Book categories and population frequency identified in the research area.

No	Endemic plants name	IUCN	Population frequency
1	*Abies nordmanniana* subsp. *equi-trojani*	NT (endemic)	Common
2	*Allium huber-morathii*	LC (endemic)	Medium
3	*Allium olympicum*	LC (endemic)	Low
4	*Arum hygrophilum* subsp. *euxinum*	LC (endemic)	Medium
5	*Asperula pestalozzae*	LC (endemic)	Rare
6	*Astragalus amoenus*	LC (endemic)	Rare
7	*Astragalus condensatus*	LC (endemic)	Low
8	*Astragalus mesogitanus*	LC (endemic)	Common
9	*Astragalus panduratus*	EN (endemic)	Rare
10	*Astrantia maxima* subsp. *haradjianii*	NT (endemic)	Common
11	*Asyneuma rigidum* subsp. *sibtharpianum*	LC (endemic)	Rare
12	*Aubrieta olympica*	EN (endemic)	Rare
13	*Campanula grandis* subsp. *grandis*	LC (endemic)	Rare
14	*Campanula lyrata* subsp. *lyrata*	LC (endemic)	Common
15	*Centaurea consanguinea*	LC (endemic)	Low
16	*Cirsium sintenisii*	NE (endemic)	Low
17	*Corydalis caucasica* subsp. *abantensis*	EN (endemic)	Medium
18	*Corydalis wendelboi* subsp. *congesta*	EN (endemic)	Common
19	*Crataegus tanacetifolia*	LC (endemic)	Medium
20	*Crataegus x bornmuelleri*	NE (endemic)	Low
21	*Crocus ancyrensis*	NT (endemic)	Common
22	*Cyanus pichleri* subsp. *extrarosularis*	LC (endemic)	Rare
23	*Dactylorhiza nieschalkiorum*	VU (endemic)	Medium
24	*Delphinium fissum* subsp. *anatolicum*	LC (endemic)	Rare
25	*Dianthus balansae*	LC (endemic)	Medium
26	*Dianthus carmelitarum*	LC (endemic)	Common
27	*Dianthus leucophaeus*	LC (endemic)	Low
28	*Digitalis lamarckii*	LC (endemic)	Common
29	*Eryngium bithynicum*	LC (endemic)	Common
30	*Euphorbia amygdaloides* var. *robbiae*	NT (endemic)	Medium
31	*Helichrysum arenarium* subsp. *aucheri*	LC (endemic)	Medium
32	*Hieracium paphlagonicum*	LC (endemic)	Rare
33	*Iris kerneriana*	LC (endemic)	Rare
34	*Jurinea alpigena*	LC (endemic)	Low
35	*Jurinea pontica*	LC (endemic)	Low
36	*Lamium purpureum* var. *aznavourii*	CR (endemic)	Rare
37	*Lathyrus czeczottianus*	LC (endemic)	Rare
38	*Lathyrus tukhtensis*	LC (endemic)	Rare
39	*Lathyrus undulatus*	VU (endemic)	Medium
40	*Linaria genistifolia* subsp. *confertiflora*	LC (endemic)	Low

41	*Linum hirsutum* subsp. *anatolicum* var. *anatolicum*	LC (endemic)	Common
42	*Lonicera orientalis*	LC (endemic)	Medium
43	*Melampyrum arvense* var. *elatius*	NT (endemic)	Rare
44	*Minuartia erythrosepala* var. *cappadocica*	LC (endemic)	Rare
45	*Muscari aucheri*	LC (endemic)	Low
46	*Noccaea iberidea*	NE (endemic)	Low
47	*Onosma bornmuelleri*	LC (endemic)	Common
48	*Onosma bracteosa*	LC (endemic)	Low
49	*Ornithogalum alpigenum*	NT (endemic)	Low
50	*Papaver pilosum* subsp. *pilosum*	LC (endemic)	Rare
51	*Paracaryum paphlagonicum*	NT (endemic)	Low
52	*Phlomis russeliana*	LC (endemic)	Common
53	*Poa asiaeminoris*	NT (endemic)	Rare
54	*Ptilostemon afer* subsp. *eburneus*	LC (endemic)	Low
55	*Quercus macranthera* subsp. *syspirensis*	LC (endemic)	Low
56	*Salvia tobeyi*	VU (endemic)	Rare
57	*Sempervivum gillianiae*	LC (endemic)	Medium
58	*Silene olympica* var. *olympica*	LC (endemic)	Rare
59	*Trifolium aureum* subsp. *barbulatum*	LC (endemic)	Rare
60	*Trifolium elongatum*	LC (endemic)	Common
61	*Tripleurospermum rosellum* var. *album*	VU (endemic)	Medium
62	*Turanecio hypochionaeus*	LC (endemic)	Rare
63	*Verbascum abieticola*	LC (endemic)	Low
64	*Verbascum bithynicum*	NT (endemic)	Common
65	*Verbascum caudatum*	LC (endemic)	Low
66	*Vicia freyniana*	LC (endemic)	Rare
67	*Hordeum murinum* subsp. *leporinum*	DD (rare)	Rare
68	*Iris pumila* subsp. *attica*	VU (rare)	Rare
69	*Koeleria pyramidata*	VU (rare)	Low
70	*Lilium martagon*	VU (rare)	Rare

The largest families in the study area are as follows: Asteraceae (97 taxa/%12.65), Fabaceae (62 taxa/%8.08), Lamiaceae (51 taxa/%6.65), Rosaceae (44 taxa/%5.74), Poaceae (37 taxa/%4.82), Brassicaceae (35 taxa/%4.56), Caryophyllaceae (32 taxa/%4.17), Apiaceae (28 taxa/%3.65), Boraginaceae (27 taxa/%3.52), Orchidaceae (24 taxa/%3.13). The number of plants of the 10 most common families constitutes 56.97% of the total number of plants (Figure 6).

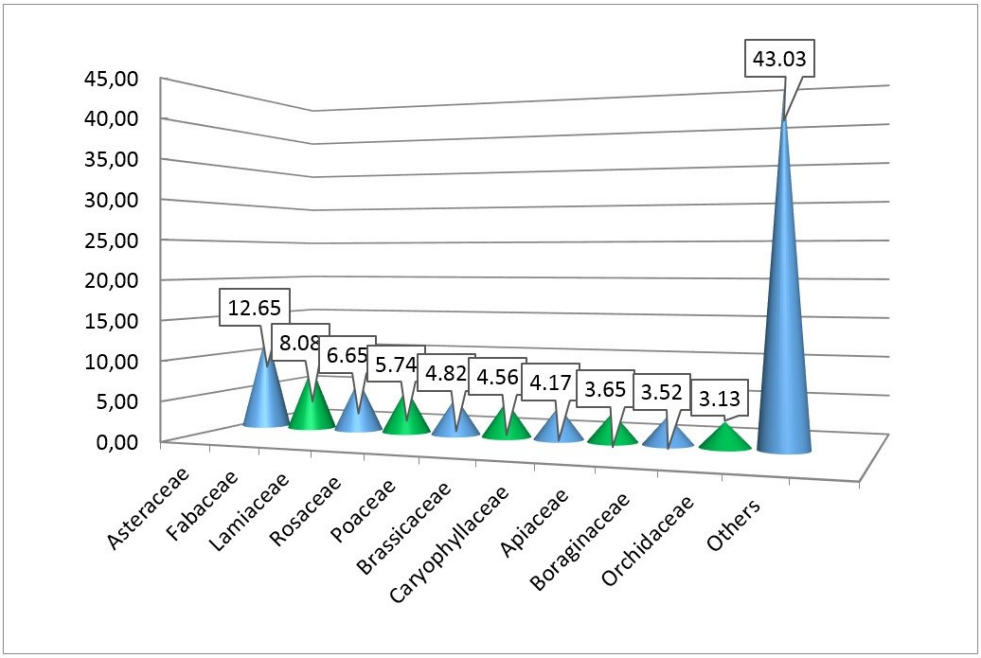

FIGURE 6. The largest families in the study area.

The largest genera in the study area are as follows: the most common two genera are *Geranium* and *Silene* (each 12 taxa). The following genera are *Salvia* (11 taxa), *Trifolium*, *Veronica* and *Campanula* (each with 10 taxa), *Ranunculus*, *Euphorbia* and *Vicia* (each with 8 taxa), and *Poa* (7 taxa) (Figure 7).

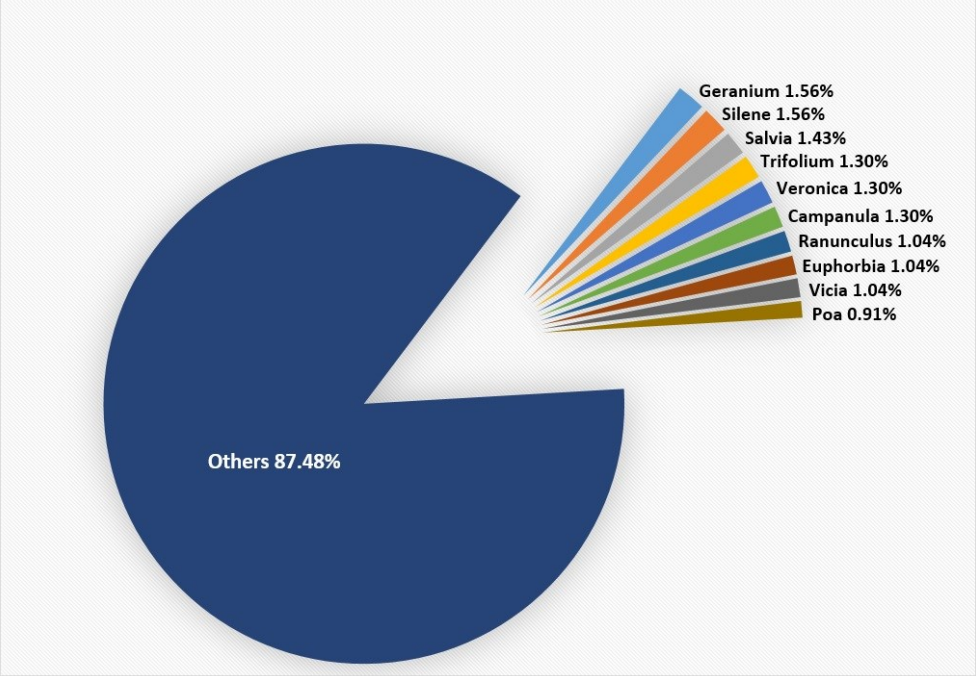

FIGURE 7. The largest 10 genera in the research area.

4. DISCUSSION

The research area is the place where the highest plant diversity is detected with 767 taxa which are compared with the previous floristic studies conducted in Bolu province. The number of taxa detected in the previous 13 floristic studies is between 174 and 660 (Table 2). The ratio of endemic plants identified in the current research area to the total number of taxa is 8.60%. In previous studies in Bolu, this rate is between 3.5−13.79%. It is observed that the four studies with a relatively higher rate of endemism [21-22, 29-30] were conducted in the southern parts of Bolu and the interactions of Iran-Turan and Mediterranean phytogeographic regions are more dominant in these areas. The range of endemism in these four studies is between 10.8% and 13.79%. In two studies in the north of Bolu which are closest to the current research area, the rate of endemism is 5.8−6.7%. In general, the endemism rate in province of Bolu is below the average of Turkey because Bolu is located in

the Euro-Siberian phytogeographic region having the least endemic taxa within the three phytogeographic regions of Turkey. However, with a rate of 8.60% endemism, the study area has a higher average of endemism compared to the average of endemism revealed in other studies carried out in the north of Bolu (Table 2).

TABLE 2. Comparison of floristic studies:

Abbreviations of studies: Studies: 1. Results of this study; 2. Güneş Özkan *et al.* (2016); 3. Akman & Ketenoğlu (1979b); 4. Akman & Yurdakulol (1981b); 5. Aksoy (2009); 6. Arslan *et al.* (2013); 7. Ekim & İlarslan (1982); 8. İkinci & Güner (2007); 9. İkinci (2011); 10. Kanoğlu *et al.* (2016); 11. Sungurlu (2011); 12. Tunçkol & Akkemik (2016); 13. Turker & Güner (2003); 14. Sümer (2002).

Compared Studies	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
Total Taxa	**767**	**554**	**315**	**286**	**511**	**240**	**245**	**453**	**324**	**406**	**174**	**573**	**660**	**345**
Endemic species (%)	%8.60	%11.73	%5	%4.1	%13.5	%5.8	%6.7	%3.5	%10.8	%9.36	%9.7	%13.79	%7.7	%3.5
Euro-Sib. (%)	%30.50	%19.68	%12.6	%19.5	%13.4	%37.5	%27.2	%30.8	%16.4	%19.46	%22.1	%22.5	%23.7	%24.7
Medit. (%)	%8.34	%14.08	%7.6	%7.6	%7.78	%5	%5.5	%5.2	%13.3	%5.91	%4.5	%9.07	%5.7	%3.2
Ir.-Tur. (%)	%5.99	%11.37	%7.6	%3.8	%26.1	%2.5	%2.3	%1.5	%10.2	%4.93	%4.5	%9.94	%4.8	%4.6
Multi-regional or phytogeographic region unknown (%)	%55.15	%54.87	%67.2	%65	%52.7	%55	%55.5	%58.4	%60.1	%69.7	%68.9	%58.46	%67.8	%65.5

According to phytogeographical regions, the Euro-Siberian elements are dominant with a ratio of 30.50% in the research area. In 12 of the 13 floristic studies previously conducted in Bolu province, the Euro-Siberian elements are in the first place and the ratio in all studies is found to be between 12.6−37.5%. In only one study [21], Irano-Turanian elements are in the first place. Similarly, with the exception of two of the remaining 13 studies [19, 29], in 11 studies, the Mediterranean elements are in the second place or have the same ratio with the Irano-Turanian elements. Although Bolu province is mainly under the influence of the Euro-Siberian phytogeographic region, it can be said that, as it moves from north to south, the Irano-Turanian phytogeographic region in the Seben and Kıbrıscık districts and the Mediterranean phytogeographic region in the Göynük district have a greater effect. Since the study area is in the north, the Euro-Siberian elements are denser and this result is in line with the range (27.2−37.5%) of the two studies [15, 24] conducted in the north of Bolu (Table 2).

The first three families with the most taxa in the research area are as follow respectively: Asteraceae (12.65%), Fabaceae (8.08%) and Lamiaceae (6.65%). The ordering of these three large families are compatible with the order in the "*Flora of Turkey* [5-7]". Considering the floristic studies conducted in Bolu, the result of this research is similar to the family ranking in the two studies, the same ranking is 14.1%, 11.8%, 7.5% in Aksoy [21] and 11.4%, 8.33%, 6.14% in Kanoğlu *et al.* [28]. In six of the remaining 11 floristic studies [18-19, 22-24, 29], Asteraceae ranked first in the 8.3−14.3% range while in other four studies [12, 14-15, 20], Fabaceae ranked first in the 8.48−11.1% range. In one study [30], Poaceae was the leading with a rate of 9.93%. On the other hand, Asteraceae, Fabaceae and Lamiaceae, which are ranking among the top three in the *Flora of Turkey*, were included in the first six ranking in all studies carried out in Bolu, and at least two of these three families were located in the first three. Poaceae or Rosaceae were sometimes included in the first three rankings. In addition to the three common families mentioned above, Brassicaceae was among the top ten families in all studies. The other families in the top 10 are mainly Caryophyllaceae, Boraginaceae, Apiaceae, Ranunculaceae and Plantaginaceae *s.l.*, and less frequently Asparagaceae, Campanulaceae, Cyperaceae, Ericaceae, Orchidaceae and Rubiaceae. In the present study, the ratio of the families in the top 10 in the total plant composition is 56.97%, which overlaps with the range (50.49−58.48%) of the other studies (Table 3).

TABLE 3. Comparison of our research with other surveys according to the 10 largest families.

Abbreviations of studies: 1. Result of this study; 2. Güneş Özkan *et al.* (2016); 3. Akman & Ketenoğlu (1979b); 4. Akman & Yurdakulol (1981b); 5. Aksoy (2009); 6. Arslan *et al.* (2013); 7. Ekim & İlarslan (1982); 8. İkinci & Güner (2007); 9. İkinci (2011); 10. Kanoğlu *et al*. (2016); 11. Sungurlu (2011); 12. Tunçkol & Akkemik (2016); 13. Turker & Güner (2003); 14. Sümer (2002).
Abbreviations of families: Api.: Apiaceae; Asp.: Asparagaceae; Ast.: Asteraceae; Bra.: Brassicaceae; Bor.: Boraginaceae; Cam.: Campanulaceae; Car.: Caryophyllaceae; Cyp.: Cyperaceae; Eri.: Ericaceae; Fab.: Fabaceae; Lam.: Lamiaceae; Orc.: Orchidaceae; Pla.: Plantaginaceae *s.l.*; Poa.: Poaceae; Ran.: Ranunculaceae; Ros.: Rosaceae; Rub.: Rubiaceae; Cyp.: Cyperaceae.

Compared Studies		**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
Total Numbers of Taxa		**767**	**554**	**315**	**286**	**511**	**240**	**245**	**453**	**324**	**406**	**174**	**573**	**660**	**345**
The largest 10 families and percentages	**1**	Ast. %12.65	Poa. %9.93	Fab. %8.48	Fab. %9.9	Ast. %14.1	Ast. %8.3	Fab. %11.1	Fab. %9.6	Ast. %9.9	Ast. %11.4	Ast. %9.2	Ast. %11.52	Ast. %10.4	Ast. %14.3
	2	Fab. %8.08	Ast. %9.21	Ast. %9.21	Ast. %7.5	Fab. %11.8	Ros. %7.9	Ast. %10.7	Ast. %9.3	Lam. %9.9	Fab. %8.33	Fab. %8.0	Lam. %7.11	Poa. %8.2	Poa. %9.6
	3	Lam. %6.65	Fab. %8.48	Pla. %6.0	Ros. %7.3	Lam. %7.5	Fab. %6.7	Ros. %5.5	Poa. %8.7	Fab. %9.3	Lam. %6.14	Poa. %8.0	Poa. %7.11	Fab. %7.1	Fab. %7.3
	4	Ros. %5.74	Ros. %5.96	Poa. %5.3	Lam. %6.6	Poa. %6.8	Lam. %6.3	Lam. %5.5	Lam. %5.3	Bor. %5.6	Bra. %5.70	Ros. %8.0	Bra. %6.77	Lam. %6.7	Lam. %5.8
	5	Poa. %4.82	Bra. %5.23	Lam. %5.0	Poa. %6.2	Ros. %5.2	Poa. %5.4	Poa. %4.7	Ros. %5.3	Ros. %4.0	Ros. %4.84	Lam. %6.3	Fab. %6.44	Ros. %5.3	Bra. %5
	6	Bra % 4.56	Lam. %4.87	Car. %5.0	Bra. %5.9	Bra. %3.9	Car. %4.2	Api. %4.7	Bra. %4.0	Bra. %4.0	Bor. %4.39	Car. %5.2	Ros. %5.08	Bra. %4.7	Ran. %4.7
	7	Car. %4.17	Car. %4.87	Bra. %4.7	Car. %4.8	Bor. %3.1	Bra. %3.8	Car. %4.3	Api. %3.8	Api. %3.7	Poa. %3.51	Pla. %3.0	Api. %3.72	Car. %3.4	Api. %4.4
	8	Api. %3.65	Lil. %3.61	Bor. %4.4	Ran. %3.6	Api. %3.0	Pla. %3.3	Bra. %2.6	Car. %3.8	Car. %2.8	Car. %3.70	Bra. %3.0	Bor. %3.38	Ran. %3.2	Cyp. %3.5
	9	Bor. %3.52	Bor. %3.43	Api. %3.1	Pla. %3.5	Pla. %1.95	Api. %3.3	Rub. %2.1	Bor. %3.2	Ran. %2.8	Api. %2.19	Rub. %3.0	Car. %3.38	Pla. %3.1	Ros. %2.9
	10	Orc. %3.13	Rub. %2.89	Ran. %2.8	Rub. %3.1	Cam. %1.56	Bor. %2.9	Eri. %2.1	Orc. %2.8	Pla. %2.4	Asp. %2.19	Asp. %3.0	Pla. %2.44	Api. %3.0	Car. %2.6
	Others	%43.03	%41.52	%46.01	%41.60	%41.09	%47.9	%46.7	%44.2	%45.60	%47.61	%43.3	%43.05	%44.9	%39.9

Geranium (12 taxa), *Silene* (12 taxa) and *Salvia* (11 taxa) are the first three genera that contain the most taxa in the research area. When we look at the floristic studies conducted in Bolu, *Geranium* (7 taxa) is ranked second in Kanoğlu *et al.* [28]. *Silene* with 6 taxa is ranked second in Arslan *et al.* [24] while similarly, in Ekim and İlarslan [15] it is ranked third with four taxa. However, as illustrated in Table 4, in five of the 13 studies [12, 14, 18, 24, 30] *Veronica* is ranked first with the range of 6−15 taxa. In two each studies [15, 23 and 20, 22] *Trifolium* and *Vicia* are the leading genera with the ranges of 4−9 and 7−9 taxa, respectively. In four separate studies [19, 21, 28-29], *Astragalus* (14 taxa), *Salvia* (12 taxa), *Ranunculus* (9 taxa) and *Centaurea* (7 taxa) are the most common genera. Other genera ranked in the first three apart from those mentioned above are *Allium*, *Ranunculus*, *Hypericum*, *Campanula*, *Lathyrus, Carex* and *Galium*. In this study, although the weight of the

first three ranks of genera differed significantly from the first three ranks of genera in the previous studies, it is seen that the similarity of the genera composition increased in the first 10 ranks. The ratio of the genera in the top 10 in the total plant composition is 12.48% and and overlaps with the intervals in other studies (12.47−21.91%).

TABLE 4. Comparison of our research with other surveys according to the 10 largest genera.

Abbreviations of studies: 1. Results of this study; 2. Güneş Özkan *et al.* (2016); 3. Akman & Ketenoğlu (1979b); 4. Akman & Yurdakulol (1981b); 5. Aksoy (2009); 6. Arslan *et al.* (2013); 7. Ekim & İlarslan (1982); 8. İkinci & Güner (2007); 9. İkinci (2011); 10. Kanoğlu *et al.* (2016); 11. Sungurlu (2011); 12. Tunçkol & Akkemik (2016); 13. Turker & Güner (2003); 14. Sümer (2002); O.= Others genera.

Compared Studies		1	2	3	4	5	6	7	8	9	10	11	12	13	14
Total Numbers of Taxa		767	554	315	286	511	240	245	453	324	406	174	573	660	345
The largest 10 genera, their numbers of taxa number and percentages	1	*Geranium* 12 (%1.56)	*Veronica* 10 (%1.81)	*Veronica* 12 (%3.81)	*Veronica* 7 (%2.45)	*Salvia* 12 (%2.35)	*Veronica* 6 (%2.50)	*Trifolium* 9 (%3.67)	*Vicia* 9 (%1.98)	*Vicia* 7 (%2.16)	*Centaurea* 7 (%1.72)	*Trifolium* 4 (%2.30)	*Astragalus* 14 (%2.44)	*Veronica* 15 (%2.27)	*Ranunculus* 9 (%2.6)
	2	*Silene* 12 (%1.56)	*Allium* 9 (%1.62)	*Trifolium* 9 (%2.86)	*Galium* 6 (%2.10)	*Astragalus* 10 (%1.96)	*Silene* 6 (%2.50)	*Campanula* 5 (%2.04)	*Trifolium* 8 (%1.76)	*Astragalus* 7 (%2.16)	*Geranium* 7 (%1.72)	*Allium* 4 (%2.30)	*Trifolium* 12 (%2.09)	*Ranunculus* 12 (%1.82)	*Veronica* 7 (%2.00)
	3	*Salvia* 11 (%1.43)	*Trifolium* 9 (%1.62)	*Lathyrus* 7 (%2.22)	*Ranunculus* 6 (%2.10)	*Centaurea* 8 (%1.57)	*Trifolium* 5 (%2.08)	*Silene* 4 (%1.63)	*Veronica* 7 (%1.54)	*Salvia* 6 (%1.85)	*Hypericum* 7 (%1.72)	*Hypericum* 3 (%1.72)	*Ranunculus* 10 (%1.75)	*Trifolium* 10 (%1.52)	*Carex* 7 (%2.00)
	4	*Trifolium* 10 (%1.30)	*Galium* 9 (%1.62)	*Sedum* 7 (%2.22)	*Poa* 6 (%2.10)	*Geranium* 8 (%1.57)	*Galium* 4 (%1.67)	*Lathyrus* 4 (%1.63)	*Poa* 6 (%1.32)	*Centaurea* 5 (%1.54)	*Ornithogalum* 6 (%1.48)	*Geranium* 3 (%1.72)	*Centaurea* 9 (%1.57)	*Carex* 10 (%1.52)	*Juncus* 6 (%1.80)
	5	*Veronica* 10 (%1.30)	*Ranunculus* 8 (%1.44)	*Myosotis* 6 (%1.90)	*Silene* 5 (%1.75)	*Euphorbia* 7 (%1.37)	*Allium* 4 (%1.67)	*Sedum* 3 (%1.22)	*Bromus* 6 (%1.32)	*Viola* 5 (%1.54)	*Salvia* 6 (%1.48)	*Epilobium* 3 (%1.72)	*Silene* 9 (%1.57)	*Galium* 8 (%1.21)	*Medicago* 5 (%1.50)
	6	*Campanula* 10 (%1.30)	*Lathyrus* 7 (%1.26)	*Campanula* 6 (%1.90)	*Cardamine* 5 (%1.75)	*Trifolium* 6 (%1.17)	*Lamium* 4 (%1.67)	*Geranium* 3 (%1.22)	*Carex* 5 (%1.10)	*Myosotis* 4 (%1.23)	*Trifolium* 6 (%1.48)	*Sedum* 3 (%1.72)	*Allium* 8 (%1.40)	*Allium* 8 (%1.21)	*Trifolium* 5 (%1.50)
	7	*Ranunculus* 8 (%1.04)	*Astragalus* 6 (%1.08)	*Ranunculus* 6 (%1.90)	*Carex* 5 (%1.75)	*Ornithogalum* 6 (%1.17)	*Campanula* 4 (%1.67)	*Epilobium* 3 (%1.22)	*Dianthus* 5 (%1.10)	*Carex* 4 (%1.23)	*Astragalus* 5 (%1.23)	*Galium* 3 (%1.72)	*Hypericum* 8 (%1.40)	*Poa* 7 (%1.06)	*Euphorbia* 5 (%1.50)
	8	*Euphorbia* 8 (%1.04)	*Sedum* 6 (%1.08)	*Astragalus* 6 (%1.90)	*Euphorbia* 5 (%1.75)	*Lathyrus* 5 (%0.98)	*Geranium* 4 (%1.67)	*Festuca* 3 (%1.22)	*Geranium* 5 (%1.10)	*Valerianella* 4 (%1.23)	*Campanula* 5 (%1.23)	*Myosotis* 3 (%1.72)	*Salvia* 8 (%1.40)	*Astragalus* 7 (%1.06)	*Vicia* 4 (%1.20)
	9	*Vicia* 8 (%1.04)	*Gagea* 6 (%1.08)	*Silene* 5 (%1.59)	*Crocus* 5 (%1.75)	*Galium* 5 (%0.98)	*Ranunculus* 4 (%1.67)	*Rubus* 3 (%1.22)	*Galium* 5 (%1.10)	*Dianthus* 3 (%0.93)	*Crataegus* 5 (%1.23)	*Euphorbia* 3 (%1.72)	*Quercus* 7 (%1.22)	*Salvia* 7 (%1.06)	*Lathyrus* 4 (%1.20)
	10	*Poa* 7 (0.91)	*Alyssum* 6 (%1.08)	*Galium* 5 (%1.59)	*Trifolium* 5 (%1.75)	*Carduus* 5 (%0.98)	*Cirsium* 3 (%1.25)	*Asperula* 3 (%1.22)	*Viola* 5 (%1.10)	*Silene* 3 (%0.93)	*Lathyrus* 5 (%1.23)	*Quercus* 3 (%1.72)	*Lathyrus* 7 (%1.22)	*Centaurea* 7 (%1.06)	*Centaurea* 4 (%1.20)
	O.	87.48	86.282	78.095	80.769	85.910	81.667	83.673	86.534	85.185	85.468	81.609	83.944	86.21	83.50

5. CONCLUSION

In the light of the information and comparisons given above, it would be fair to suggest that the research area is an important plant biodiversity center in Bolu. In more detail, the area reaching the summit of Çaltepe from the upper parts of Merkeşler Village, and the Gurbettaşı locality on the way of Sarımustan and Yedigöller has the highest variety of plants and these regions hosts many endemic and rare plants. Precautions should be taken to protect the *in-situ* (in place) of these

two regions. It will be crucial to consult with experts in order to prevent the destruction of nature in the case of activities such as construction and road building in the region.

Regional flora studies are very important for the determination of biological diversity of our country, the discovery of new species, and detection and protection of endangered species. In recent years, studies related to the conservation and rational use of plant genetic resources have been increasing in the world. First of all, the way of protection depends on the identification of the existing one. This study is hoped to contribute not only to the identification and preservation of our biological richness, but also to the establishment of information resources for the relevant institutions and organizations. The scientific data obtained in the present study will be beneficial for other disciplines, such as agriculture, biotechnology, biochemistry, pharmacy, medicine, food engineering, landscape sciences and pharmaceutical botany. Furthermore, it will provide a data for the development of economically important interdisciplinary projects. The data obtained from the floristic studies is of great importance in terms of pharmacy, particularly in terms of determining the potential of medicinal plants in the field of pharmaceutical botany, determination of plant gene resources related to agriculture and pasture breeding, recognition of important plants for beekeeping, and detection of natural forest areas.

Acknowledgement. This study was supported by Bolu Abant İzzet Baysal University (Project No: BAP–2015.03.01.817). We thank our institution for their financial contributions. We would like to thank Assoc. Prof. Dr. Barış ÖZÜDOĞRU, Assoc. Prof. Dr. Evren CABİ, Burçin ÇINGAY, Assoc. Prof. Dr. Mehmet SAĞIROĞLU, Assoc. Prof. Dr. Rıza BİNZET, Assoc. Prof. Dr. Mehtap TEKŞEN, Assoc. Prof. Dr. Ersin KARABACAK, Prof. Dr. M. Tekin BABAÇ and Assoc. Prof. Dr. Emel USLU for their help to diagnosis of some plants. We would like to thank Serdar ASLAN for his help in creating climate diagrams and Ayşe KAYA ÇELİK for supported us during our studies.

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APPENDICES

APPENDIX 1. Locations belonging to collected specimens.
(Abbreviations - Loc. no: Locality number)

Loc.no	Locality
1	Çaltepe summit and its environs
1.1	Merkeşler Upland
2	Çeletepe summit and its environs
2.1	Banaz Upland
3	Sarımustan and its environs
3.1	Gurbettaşı
4	Kadıköy Plateau and its environs
5	Northern slopes of Çaltepe
6	Eastern slopes of Çaltepe
7	Southern slopes of Çaltepe
8	Southern slopes of Çeletepe
9	Çele Gölcük forest warehouse
10	Çele Gölcük Village
10.1	Yakabayat Village
11	Değirmenderesi Village
11.1	Taşcılar Village
11.2	Banaz Village
12	Çukurören Village
13	Mesciçele Village
13.1	Tetemeçele Village
14	Değirmenbeli Village
15	Yedigöller road
16	Bağışlar Village
16.1	Semerciler Village
17	Merkeşler Village
17.1	Hayranlar Village
17.2	Afşar Village
18	Hamzabey Village
18.1	Bahceköy Village
19	Yeşilçele Village

APPENDIX 2. Enumeration of taxa.

(Abbreviations - Loc. no: Locality no; Col. no: Collecter no; Ph.R.: Phytogeographic region); EuSib: Euro-Siberian element; IrTu: Iran-Turan element; Med: Mediterranean element; * New record for Bolu Province.

No	Taxon name	Loc. no	Col. no	Ph.R.
	PTEROPHYTA			
	EQUISETOPSIDA			
	EQUISETALES			
	EQUISETACEAE			
1	***Equisetum arvense*** L.	15	143	
2	***Equisetum palustre*** L.	3;12;13.1;15	1631;513;820;1877	
3	***Equisetum hyemale*** L.	6;7;16	1430;322;993	
	POLYPODIOPSIDA			
	POLYPODIALES			
	ASPLENIACEAE			
4	***Asplenium adiantum-nigrum*** L.	4	1540	
5	***Asplenium ceterach*** L.	11.1	1663	
6	***Asplenium scolopendrium*** L	5	2273	
7	***Asplenium septentrionale*** (L.) Hoffm.	3	2155	
8	***Asplenium trichomanes*** L.	3	594;1235;1538	
	ATHYRIACEAE			
9	***Athyrium filix-femina*** (L.) Roth	3	2176	
	POLYPODIACEAE			
10	***Polypodium vulgare*** L. var. ***vulgare***	3;5;8	1353;2168;2272	
	PTERIDOPSIDA			
	ATHYRIALES			
	CYSTOPTERIDACEAE			
11	***Cystopteris fragilis*** (L.) Bernh.	6	1022	
	DENNSTAEDTIALES			
	DENNSTAEDTIACEAE			
12	***Pteridium aquilinum*** (L.) Kuhn	6;7;17	226;920;979	
	DRYOPTERIDALES			
	DRYOPTERIDACEAE			
13	***Dryopteris filix-mas*** (L.) Schott	1.1;3;7;15	1435;393;330;595	
14	***Polystichum aculeatum*** (L.) Roth ex Mert.	13.1	1514	
15	***Polystichum setiferum*** (Forssk.) Moore ex Woyn.	17	1244	
	GYMNOSPERMAE			
	PINOPSIDA			
	PINALES			
	CUPRESSACEAE			
16	***Juniperus communis*** L. var. ***saxatilis*** Pall.	1;1.1;8	648;73;175;388	

17	***Juniperus oxycedrus*** L. subsp. ***oxycedrus*** var. ***oxycedrus***	13;15;19	1334;756;53;267	
	PINACEAE			
18	***Abies nordmanniana*** (Steven) Spach subsp. ***equi-trojani*** (Asch. & Sint. ex Boiss.) Coode & Cullen	1;3;5;13.1	6;189;387;1874	EuSib
19	***Pinus nigra*** J.F.Arnold subsp. ***pallasiana*** (Lamb.) Holmboe	13.1	7;698;765;1028	EuSib
20	***Pinus sylvestris*** L. var. ***hamata*** Steven	7;9;13.1	5;100;432	EuSib
	ANGIOSPERMAE			
	EUDICOTYLEDONEAE			
	APIALES			
	APIACEAE			
21	***Angelica sylvestris*** L. var. ***sylvestris***	4;15	1290;1448	EuSib
22	***Anthriscus nemorosa*** (M.Bieb.) Spreng.	1;1.1;2.1	399;203;348;1848	
23	**** Anthriscus sylvestris*** (L.) Hoffm.	13.1	1124	
24	***Astrantia maxima*** Pall. subsp. ***haradjianii*** (Grintz) Rech.f.	1;1.1;4;6;17	1132;1239;2036	
25	***Bupleurum falcatum*** L. subsp. ***cernuum*** (Ten.) Arcang.	1;4	1127;2240	
26	***Bupleurum rotundifolium*** L.	15;18.1	1793;1937	
27	***Caucalis platycarpos*** L.	6;17	314;937	Med
28	**** Chaerophyllum aureum*** L.	3	1871	
29	**** Cnidium silaifolium*** (Jacq.) Simonk. subsp. ***orientale*** (Boiss.) Tutin	1.1;6	1015	
30	***Conium maculatum*** L.	18.1	1950	
31	***Daucus carota*** L.	17	944	
32	***Echinophora tenuifolia*** L. subsp. ***sibthorpiana*** (Guss.) Tutin	15	1315	IrTu
33	***Eryngium bithynicum*** Boiss.	11.2	1046	IrTu
34	***Eryngium campestre*** L. var. ***virens*** (Link) Weins	17.1	2066	
35	***Eryngium giganteum*** M.Bieb.	4;15	1204;1280	EuSib
36	***Gasparrinia peucedanoides*** Thell.	1;15	1195;1259	EuSib
37	***Heracleum platytaenium*** Boiss.	3;17	313;280;1869	EuSib
38	***Laser trilobum*** (L.) Borkh.	17	310	
39	***Laserpitium hispidum*** M.Bieb.	10.1;17	903;1381	EuSib
40	***Oenanthe fistulosa*** L.	1.1	2296	
41	***Orlaya grandiflora*** (L.) Hoffm.	17	305	
42	***Malabaila secacul*** (Mill.) Boiss. subsp. ***secacul***	11.2	2223	
43	**** Prangos ferulacea*** (L.) Lindl.	1.1;4;17	278;2131;2314	
44	***Sanicula europaea*** L.	1.1;2.1;15;17	246;377;596	EuSib
45	***Scandix iberica*** M.Bieb.	11.2;17	1551;1604	
46	***Seseli tortuosum*** L.	13.1	2336	

47	***Smyrnium perfoliatum*** L.	13.1;17	311;152;425	
48	***Torilis leptophylla*** (L.) Rchb.f.	17	936	
	ARALIACEAE			
49	***Hedera helix*** L.	7	1569	
	ASTERALES			
	ASTERACEAE			
50	***Achillea grandifolia*** Friv.	5	2282	
51	***Achillea millefolium*** L. subsp. ***millefolium*** var. ***millefolium***	1;1.1;17	1227;2125;2244	EuSib
52	***Achillea nobilis*** L. subsp. ***neilreichii*** (A.Kern.) Velen.	2;15	1221;2257;2258	EuSib
53	***Achillea setacea*** Waldst. & Kit.	15;17	663;1063;1779	EuSib
54	***Anthemis cotula*** L.	17;18.1	935;889;1951	
55	***Anthemis cretica*** L. subsp. ***anatolica*** (Boiss.) Grierson	11.2	2225	
56	***Anthemis cretica*** L. subsp. ***pontica*** (Willd.) Grierson	2;7;15	1453;362;324;651	
57	***Anthemis pseudocotula*** Boiss.	1.1;3;11	1010;846;2154	
58	***Arctium minus*** (Hill) Bernh.	16;17.2	1427;885;958	EuSib
59	***Bellis perennis*** L.	9;13;17;19	140;48;74;242	EuSib
60	***Carduus acanthoides*** L. subsp. ***acanthoides***	18.1	1927;1882	EuSib
61	***Carduus adpressus*** C.A.Mey.	1.1;17	1238;2115;2191	EuSib
62	***Carduus nutans*** L. subsp. ***nutans***	3;15	573;696	
63	***Carduus pycnocephalus*** L. subsp. ***albidus*** (M.Bieb.) Kazmi	18.1	1932	
64	***Carlina vulgaris*** L.	2.1;15	1155;2307	
65	***Carthamus lanatus*** L.	15	568	
66	***Centaurea consanguinea*** DC.	15;16;17.2	1411;2058;1304	IrTu
67	***Centaurea iberica*** Trevir. ex Spreng.	10.1;15;17	1366	
68	**** Centaurea kotschyi*** (Boiss. & Heldr.) Hayek var. ***persica*** (Boiss.) Wagenitz	1;7	2299	
69	***Centaurea phrygia*** L. subsp. ***stenolepis*** (A.Kern.) Gugler	10.1	1391;1386	EuSib
70	***Centaurea solstitialis*** L. subsp. ***solstitialis***	16;18.1	1409;1946	
71	***Chondrilla juncea*** L.	15;17.2	2079;1327	
72	***Cichorium intybus*** L.	11;18.1	1933;836;852;853	
73	***Cirsium arvense*** (L.) Scop.	15;17.2	1145;967	
74	***Cirsium hypoleucum*** DC.	12;15;17	768;249;462;882	EuSib
75	***Cirsium ligulare*** Boiss.	3;10.1;17.1	1154;1378;2075	EuSib
76	**** Cirsium sintenisii*** Freyn	1;2;6	2130;2096;2310	
77	***Cirsium vulgare*** (Savi) Ten.	10.1;15;17.1	1335;1376;2073	
78	***Conyza canadensis*** (L.) Cronquist	13.1;15	1316;2195	

79	***Cota tinctoria*** (L.) J.Gay var. ***pallida*** (DC.) U.Özbek & Vural	1.1;17;15	1240;1165;1255	
80	***Cota tinctoria*** (L.) J.Gay var. ***discoidea*** (All.) Özbek & Vural	2.1;12;15	1959;473	
81	***Crepis foetida*** L. subsp. ***foetida***	15	716	
82	**** Crepis reuteriana*** Boiss. & Heldr. subsp. ***reuteriana***	11.1;17	1552;1673;1326	Med
83	***Crepis sancta*** (L.) Bornm.	17;17.2	1557;2013;2077	
84	***Crupina crupinastrum*** (Moris) Vis.	14;17	2003	
85	***Cyanus depressus*** (M.Bieb.) Soják	17	1682	
86	***Cyanus pichleri*** (Boiss.) Holub subsp. ***extrarosularis*** (Hayek & Siehe) Wagenitz & Greuter	2.1	2179	
87	***Cyanus pichleri*** (Boiss.) Holub subsp. ***pichleri***	1;2.1;6;13.1	343;2122;418	
88	***Cyanus thirkei*** (Sch.Bip.) Holub	12	1814	Med
89	***Cyanus triumfettii*** (All.) Dostál ex Á.Löve & D.Löve subsp. ***triumfettii***	6;3.1;17	1246;2221;1190	
90	***Doronicum orientale*** Hoffm.	1.1;9;15	75;117;196;395	
91	***Echinops microcephalus*** Sm.	17.1;17.2	2065	Med
92	***Echinops sphaerocephalus*** L. subsp. ***sphaerocephalus***	12	1343;2135	EuSib
93	**** Echinops spinosissimus*** Turra subsp. ***bithynicus*** (Boiss.) Greuter	11.1;14	1300;2228	IrTu
94	***Filago arvensis*** L.	15	1139	
95	***Erigeron acris*** L. subsp. ***acris***	17	1242	EuSib
96	**** Erigeron acris*** subsp. ***pycnotrichus*** (Vierh.) Grierson	1.1;2.1	1215;2311	EuSib
97	***Eupatorium cannabinum*** L.	6;16	2085;1428	EuSib
98	***Helichrysum arenarium*** (L.) Moench subsp. ***aucheri*** (Boiss.) P.H.Davis & Kupicha	13	1078	IrTu
99	***Helichrysum plicatum*** DC. subsp. ***plicatum***	1.1;3.1;2;15	1189;2180;2110	
100	***Helminthotheca echioides*** (L.) Holub	1.1	2320	
101	***Hieracium oblongum*** Jord.	3;12;15;17	1811;223;470;527	EuSib
102	***Hieracium paphlagonicum*** Freyn & Sint.	1.1	2290	EuSib
103	***Hieracium vagum*** Jord.	3;3.1	2150;2166;2207	EuSib
104	***Inula britannica*** L.	6	2088	EuSib
105	***Inula oculus-christi*** L.	10.1	1374	EuSib
106	***Inula salicina*** L.	14	2235	EuSib
107	***Jurinea alpigena*** K.Koch	1;3.1;7;15	2129;1260;1261	EuSib
108	***Jurinea pontica*** Hausskn. & Freyn ex Hausskn.	10.1;11;15	850;1303;1380	IrTu
109	***Lactuca muralis*** (L.) Gaertn.	11;17.2	2078;841	EuSib

110	***Lactuca saligna*** L.	9;15	1473	
111	***Lactuca serriola*** L.	15	1301	EuSib
112	***Lactuca viminea*** (L.) J.Presl & C.Presl	13.1	1106	
113	***Lapsana communis*** L. subsp. ***intermedia*** (M.Bieb.) Hayek var. intermedia	1;6;12;17.2	1924;504;2052	EuSib
114	***Lapsana communis*** L. subsp. ***pisidica*** (Boiss. & Heldr.) Rech.f.	11.2;17.2	946;1056	
115	***Leontodon crispus*** Vill. subsp. ***asper*** (Waldst. & Kit.) Röhl. var. ***asper***	9;15;17	1795;779;1710	
116	***Leontodon hispidus*** L. subsp. ***hispidus***	1.1;11.2;12	1810;1059;2204	EuSib
117	***Pilosella hoppeana*** (Schult.) F.W.Schultz & Sch.Bip. subsp. ***testimonialis*** (Nägeli ex Nägeli & Peter)	13.1;17.2	806;962;1171	EuSib
118	***Pilosella piloselloides*** (Vill.) Soják subsp. ***magyarica*** (Peter) S.Bräut. & Greuter	11.2	1963	
119	***Onopordum tauricum*** Willd.	11;11.1;18.1	837;1921;1928	EuSib
120	***Petasites hybridus*** (L.) G.Gaertn.; B.Mey. & Scherb.	4;10.1;15	14;81;454;1545	EuSib
121	***Ptilostemon afer*** (Jacq.) Greuter subsp. ***eburneus*** Greuter	16	798	
122	***Scolymus hispanicus*** L. subsp. ***hispanicus***	17.2	2064	Med
123	***Scorzonera cana*** (C.A.Mey.) Griseb. var. ***jacquiniana*** (W.Koch) D.F.Chamb.	15;17	268;1628	
124	**** Senecio othannae*** M.Bieb.	3	2287	EuSib
125	***Senecio vernalis*** Waldst. & Kit.	13;14;19	1583;32	
126	***Senecio vulgaris*** L.	9	137	
127	**** Senecio viscosus*** L.	3.1	1156	
128	***Sonchus asper*** (L.) Hill subsp. ***glaucescens*** (Jord.) Ball ex Ball	18.1	1942	
129	***Sonchus oleraceus*** (L.) L.	6;11;12	842;1252;1947	
130	***Tanacetum corymbosum*** (L.) Sch.Bip. subsp. ***cinereum*** (Griseb.) Grierson	3;15	1205	EuSib
131	***Tanacetum parthenium*** (L.) Sch.Bip.	6;12;13.1	488;1110;2048	
132	***Tanacetum poteriifolium*** Grierson	12	1809	EuSib
133	***Taraxacum buttleri*** Soest	9;13;19	30;112;29	
134	***Taraxacum macrolepium*** Schischk.	9;15	142;748	
135	***Telekia speciosa*** (Schreb.) Baumg.	5	2263;2269	EuSib
136	***Tephroseris integrifolia*** (L.) Holub subsp. ***aucheri*** (DC.) B.Nord.	1;2.1;4;5	174;402;618	EuSib
137	***Tragopogon coloratus*** C.A. Mey.	3.1;11.1	541;287;1909	IrTu
138	***Tragopogon dubius*** Scop.	11.1;15;17.2	1775;1665;670	

139	***Tragopogon porrifolius*** L. subsp. ***longirostris*** (Sch.Bip.) Greuter	11.1;13.1;17	1909;1667;234	
140	***Tripleurospermum oreades*** (Boiss.) Rech.f. var. ***oreades***	1;4;9	1749;111;170	
141	***Tripleurospermum rosellum*** (Boiss. & Orph.) Hayek var. ***album*** E. Hossain	3;3.1;15	1635;1638;552	
142	***Tripleurospermum tenuifolium*** (Kit.) Freyn ex Freyn	15;17	2010;664	EuSib
143	***Turanecio hypochionaeus*** (Boiss.) Hamzaoglu	1;17	1136;1237	
144	***Tussilago farfara*** L.	4	1546	EuSib
145	***Xanthium strumarium*** L. subsp. ***strumarium***	11	2323	
146	***Xeranthemum annum*** L.	11;17.2	2056;1990;838	
	BRASSICALES			
	BRASSICACEAE			
147	***Alliaria petiolata*** (M.Bieb.) Cavara & Grande	1;9	82;202	
148	***Alyssum armenum*** Boiss.	3.1;9;17	144;180;288	
149	***Alyssum minutum*** Schltdl. ex DC.	14	1581	
150	***Alyssum murale*** Waldst. & Kit. subsp. ***murale*** var. ***murale***	13.1;17	281;436	
151	***Alyssum obtusifolium*** Steven ex DC.	16;17.2	956;1410	
152	***Alyssum sibiricum*** Willd.	1	624	
153	***Arabis alpina*** L. subsp. ***alpina***	1.1;2;2.1	370;1853;1867	
154	***Arabis hirsuta*** (L.) Scop.	9;15	516;553;1868	
155	***Arabis nova*** L.	3.1	555	
156	***Arabis sagittata*** (Bertol.) DC.	1;1.1;2;15	93;171;355;1256	
157	***Arabidopsis thaliana*** (L.) Heynh.	2.1	1838	
158	**** Aubrieta olympica*** Boiss.	3.1	2210	
159	***Capsella bursa-pastoris*** (L.) Medik.	9;13;19	129;31;133	
160	***Cardamine bulbifera*** (L.) Crantz	3;13	312;556	EuSib
161	***Cardamine hirsuta*** L.	9;13;19	46;76;83;116	
162	***Cardamine lazica*** Boiss. & Balansa ex Boiss.	3;2.1	558;1839	EuSib
163	***Conringia orientalis*** (L.) Dumort.	2	353	
164	***Draba heterocoma*** Fenzl	7	2302	
165	***Draba muralis*** L.	4	604	
166	***Draba verna*** L.	9;17;19	1564;45;49;130	
167	**** Eruca vesicaria*** (L.) Cav.	13.1	1620	
168	**** Erysimum uncinatifolium*** Boiss. & A.Huet	1;13.1	168;414;435	EuSib
169	***Lepidium draba*** L.	11	1599	
170	***Microthlaspi perfoliatum*** (L.) F.K.Mey.	2;9	125;364	

171	***Myagrum perfoliatum*** L.	15	747	
172	***Nasturtium officinale*** R.Br.	15	1803	
173	***Noccaea iberidea*** (Boiss.) Al-Shehbaz & Menke	2	1852	
174	***Noccaea ochroleuca*** (Boiss. & Heldr.) F.K.Mey.	9;1.1	104;191	
175	***Rapistrum rugosum*** (L.) All.	15	675	
176	***Rorippa amphibia*** (L.) Besser	15	659	
177	***Rorippa sylvestris*** (L.) Besser subsp. ***sylvestris***	15;18.1	743;1949	
178	***Sisymbrium loeselii*** L.	15	1773;1325	
179	***Sisymbrium officinale*** (L.) Scop.	13	1062	
180	***Turritis glabra*** L.	2.1;9;15	567;791	
181	***Turritis laxa*** (Sm.) Hayek	5;9;15	138;532;2270	
	RESEDACEAE			
182	***Reseda lutea*** L. var. ***lutea***	12;15;18	1323;1360;1813	
	BORAGINALES			
	BORAGINACEAE			
183	***Anchusa azurea*** Mill. var. ***azurea***	15	1786	
184	***Anchusa hybrida*** Ten.	13;15	714;1065	Med
185	***Anchusa leptophylla*** Roem. & Schult. subsp. ***leptophylla***	17	1677	
186	***Buglossoides arvensis*** (L.) I.M.Johnst. subsp. ***sibthorpiana*** (Griseb.) R.Fern.	14	1582	
187	***Buglossoides purpurocaerulea*** (L.) I.M.Johnst.	17	257	EuSib
188	***Cerinthe minor*** L. subsp. ***auriculata*** (Ten.) Domac	1;4;15;13.1	687;199;434;622a	
189	***Cynoglossum creticum*** Mill.	15	733;1767	
190	***Cynoglossum montanum*** L.	13.1;17	165;289;1064	EuSib
191	***Echium italicum*** L.	13.1;15;17	1185;803;1297	Med
192	***Echium vulgare*** L. subsp. ***vulgare***	13.1;17	291;456;1108	EuSib
193	***Heliotropium europaeum*** L.	10.1	1364	IrTu
194	***Myosotis arvensis*** (L.) Hill subsp. ***arvensis***	1;6;9	634;783;1253	EuSib
195	***Myosotis alpestris*** F.W.Schmidt subsp. ***alpestris***	1;2;9	77;376;357	
196	***Myosotis heteropoda*** Trautv.	3	2149	IrTu
197	***Myosotis lithospermifolia*** (Willd.) Hornem.	3.1;15	547;551	EuSib
198	***Myosotis ramosissima*** Rochel	13;19	43	
199	***Myosotis rivularis*** (Vestergr.) A.P. Khokhr.	15	466	
200	***Myosotis sylvatica*** Hoffm. subsp. ***cyanea*** (Hayek) Vestergr.	13.1;17	166;214	EuSib
201	**** Onosma aucherana*** DC.	4;13.1;17	1484;210;2220	Med

202	**** Onosma bourgaei*** Boiss.	16;17	293;800	IrTu
203	***Onosma bornmuelleri*** Hausskn. & Bornm.	2.1;13.1	419;823;1861	IrTu
204	**** Onosma bracteosa*** Hausskn. & Bornm.	4	338	IrTu
205	***Onosma heterophylla*** Griseb.	3.1	1757	EuSib
206	***Onosma taurica*** Willd. var. ***taurica***	11.1	1892	
207	***Paracaryum paphlagonicum*** (Bornm.) R.R.Mill	11.1;17;17.1	1699;1893;2071	IrTu
208	**** Phacelia tanacetifolia*** Benth.	17	1674	
209	***Trachystemon orientalis*** (L.) D.Don	4;917	22;85;286;197	EuSib
	CAMPANULACEAE			
210	***Asyneuma amplexicaule*** (Willd.) Hand.-Mazz. subsp. ***amplexicaule*** var. ***amplexicaule***	17	1243	
211	***Asyneuma limonifolium*** (L.) Janch. subsp. ***limonifolium***	1;11.1;17	1762;2229;2230	
212	***Asyneuma rigidum*** (Willd.) Grossh. subsp. ***sibtharpianum*** (Schult.) Damboldt	1;2	1220;2116	
213	***Asyneuma rigidum*** (Willd.) Grossh. subsp. ***rigidum***	1;15;13.1	673;2126;2251	IrTu
214	**** Asyneuma virgatum*** (Labill.) Bornm. subsp. ***virgatum*** (Labill.) Bornm.	1	1126	
215	***Campanula cymbalaria*** Sm.	1.1	2319	
216	***Campanula glomerata*** L. subsp. ***hispida*** (Witasek) Hayek	17;6;11.2	1974;2102;1216	
217	***Campanula grandis*** Fisch. & C.A.Mey. subsp. ***grandis***	3	2286	
218	***Campanula latifolia*** L. subsp. ***latifolia***	1;1.1	1265;2236	
219	***Campanula lyrata*** Lam. subsp. ***lyrata***	1.1;12;13.1	2027;303;325;253	
220	***Campanula olympica*** Boiss.	15	1441;581	EuSib
221	***Campanula persicifolia*** L. subsp. ***persicifolia***	17.2;15;17	972;910;2008	EuSib
222	***Campanula rapunculoides*** L.	2.1;17;17.2	878;980;1432;1983	EuSib
223	***Campanula rapunculus*** L. subsp. ***lambertiana*** (A.DC.) Rech.f.	4	2103	
224	***Legousia pentagonia*** (L.) Thell.	15;17	718;1716	Med
	CARYOPHYLLALES			
	AMARANTHACEAE			
225	***Chenopodium album*** L.	11	2324	
226	***Chenopodium foliosum*** Asch.	1.1;15	750;1254	
227	***Beta trigyna*** Waldst. & Kit.	4;15	571;612	
	CARYOPHYLLACEAE			
228	***Agrostemma githago*** L.	17	1715	

229	***Cerastium brachypetalum*** Desp. ex Pers. subsp. ***roeseri*** (Boiss. & Heldr.) Nyman	14	1574	
230	***Cerastium glomeratum*** Thuill.	14	1584	
231	***Dianthus balansae*** Boiss.	1;2;1.1;17	1231;1130;2243;2309	
232	***Dianthus carmelitarum*** Reut. ex Boiss.	15	1178;540	EuSib
233	***Dianthus carthusianorum*** L.	3.1;15;16	1466;1403;2067;2159	
234	***Dianthus calocephalus*** Boiss.	11;11.2	1980;851	
235	***Dianthus leucophaeus*** Sm.	2.1	1864	
236	***Minuartia erythrosepala*** Hand.-Mazz. var. ***cappadocica*** (Boiss.) McNeill	1	647	IrTu
237	***Minuartia hirsuta*** (M.Bieb.) Hand.-Mazz. subsp. ***falcata*** (Griseb.) Mattf.	13;19	41;1859	
238	**** Minuartia juressi*** (Willd.) Lacaita subsp. ***asiatica***	2	2260	Med
239	***Myosoton aquaticum*** (L.) Moench	12	2138	
240	***Moenchia mantica*** (L.) Bartl.	4;17	237;616	
241	***Petrorhagia prolifera*** (L.) P.W.Ball & Heywood	17	862	
242	***Saponaria glutinosa*** M.Bieb.	12;17.2	955;1342;2029	
243	***Scleranthus annuus*** L. subsp***. annuus***	2	345	
244	***Scleranthus perennis*** L. subsp. ***dichotomus*** (Schur) Nyman	2.1;2	1346;795	
245	**** Scleranthus perennis*** L. subsp. ***marginatus*** (Guss.) Nyman	2;15;17	115;259;562;2211	
246	**** Silene armeria*** L.	17	301	EuSib
247	***Silene argentea*** Ledeb.	11;11.1	857;858;1913	IrTu
248	***Silene compacta*** Fisch.	1;15;17	2024;579;829;1293	
249	***Silene coronaria*** (Desr.) Clairv. ex Rchb.	5	2279	EuSib
250	***Silene dichotoma*** Ehrh. subsp. ***dichotoma***	1;16	365;801	
251	***Silene gallica*** L.	17	869	
252	***Silene italica*** (L.) Pers. subsp. ***italica***	1.1;17;3.1	1029;282;366;1272	Med
253	***Silene latifolia*** Poir. subsp. ***alba*** (Mill.) Greuter & Burdet	18	1336	
254	***Silene noctiflora*** L.	15	1451	
255	***Silene olympica*** Boiss. var. ***olympica***	1;1.1	632;2246	IrTu
256	**** Silene otites*** (L.) Wibel	17.2	2060	
257	***Silene vulgaris*** (Moench) Garcke var. ***vulgaris***	2;15;17	787;514;1718;1941	
258	***Stellaria media*** (L.) Vill.	13;15;19	38;118;126	
259	***Vaccaria hispanica*** (Mill.) Rauschert	17	1712	
	POLYGONACEAE			
260	***Rumex acetosella*** L.	2;15	1345;507;1187;1277	
261	***Rumex crispus*** L.	15;17;18.1	1948;274;523;886	

262	***Rumex nepalensis*** Spreng.	4	1501	
263	***Rumex obtusifolius*** L. subsp. ***subalpinus*** (Schur) Celak	15	505	
264	**** Rumex pulcher*** L. subsp. ***pulcher***	17;13	1037;1073	
265	***Rumex tuberosus*** L. subsp. ***tuberosus***	1	1263	
	TAMARICACEAE			
266	***Tamarix parviflora*** DC.	11.1	2233	Med
	CELASTRALES			
	CELASTRACEAE			
267	***Euonymus europaeus*** L.	15;17.1	764;2053	EuSib
268	***Euonymus latifolius*** (L.) Mill. subsp. ***latifolius***	16	1419	EuSib
	CORNALES			
	CORNACEAE			
269	***Cornus mas*** L.	15;16	1418;700	EuSib
270	***Cornus sanguinea*** L. subsp. ***australis*** (C.A.Mey.) Jáv.	17.2	2055	EuSib
	CROSSOSOMATALES			
	STAPHYLEACEAE			
271	***Staphylea pinnata*** L.	3	2339	EuSib
	CUCURBITALES			
	DATISCACEAE			
272	***Datisca cannabina*** L.	6;15;17	489;884;1138;2084	
	DIOSCOREALES			
	DIOSCOREACEAE			
273	***Dioscorea communis*** (L.) Caddick & Wilkin	7;3;13.1	319;1397;2175;2330	
	DIPSACALES			
	ADOXACEAE			
274	***Sambucus ebulus*** L.	4;17.2	1500;947	EuSib
275	***Sambucus nigra*** L.	4;10.1;13.1	159;620;828;1390	EuSib
276	***Viburnum lantana*** L.	1;4;17	185;621;1229;2092	EuSib
	CAPRIFOLIACEAE			
277	***Centranthus longiflorus*** Steven subsp. ***longiflorus***	11	1992	IrTu
278	***Cephalaria gigantea*** (Ledeb.) Bobrov	1.1;2	1014;2152	EuSib
279	***Dipsacus laciniatus*** L.	4;17.1	1291;2076	
280	***Knautia involucrata*** Sommier & Levier	2;2.1;1.1	1276;1605;2120;2187	EuSib
281	***Lonicera orientalis*** Lam.	1.1;15;17	389;298;463;2104;2317	Med
282	***Morina persica*** L. var. ***persica***	11	1993	IrTu
283	***Scabiosa argentea*** L.	2.1	1047	
284	***Scabiosa atropurpurea*** L.	4;2.1;15	622b;866;1302;1929	
285	***Scabiosa columbaria*** L. subsp. ***ochroleuca*** (L.) Celák var. ***ochroleuca***	2;6	2097;2259	
286	***Scabiosa micrantha*** Desf.	11.1	1919	

287	***Scabiosa rotata*** M.Bieb.	11	1999	IrTu
288	***Valeriana alliariifolia*** Vahl.	6;15;17.2	2022;524;987;1872	
289	***Valerianella locusta*** (L.) Laterr.	17	1566	EuSib
290	***Valerianella turgida*** (Steven) Betcke	4	617	
	ERICALES			
	ERICACEAE			
291	***Erica arborea*** L.	6;17.2	2090;969	
292	***Monotropa hypopitys*** L.	15	525;1211	
293	***Orthilia secunda*** (L.) House	6	1000	
294	***Rhododendron ponticum*** L.	6;15	578;2051;996;1201	EuSib
	PRIMULACEAE			
295	***Anagallis arvensis*** L. var. ***arvensis***	11	848	
296	***Anagallis arvensis*** L. var. ***caerulea*** (L.) Gouan	17.1	2074	
297	***Anagallis foemina*** Mill.	15;18	1321;1361	Med
298	***Cyclamen coum*** Mill. subsp. ***coum***	13.1;17;19	1;57;284;229	
299	***Lysimachia punctata*** L.	6	2019	
300	**** Lysimachia verticiliaris*** Spreng.	6;15	2087;1206	EuSib
301	***Lysimachia vulgaris*** L.	6	974	
302	***Primula acaulis*** (L.) L. subsp. ***acaulis***	6;1.1;2.1	3;56;63;70;195	EuSib
	FABALES			
	FABACEAE			
303	***Argyrolobium biebersteinii*** P.W.Ball	2.1;15;17	520;1499;1984;2025	EuSib
304	***Anthyllis vulneraria*** L. subsp. ***boisseri*** (Sagorski) Bornm.	1;1.1;17	2005;655;1020	
305	***Astragalus amoenus*** Fenzl	2.1	1860	IrTu
306	***Astragalus angustifolius*** Lam. subsp. ***pungens*** (Willd.) Hayek	1	623	
307	***Astragalus condensatus*** Ledeb.	16	1400	IrTu
308	***Astragalus glycyphylloides*** DC.	1;2.1;17	1348;241;1289;1212;890	EuSib
309	***Astragalus mesogitanus*** Boiss.	11;11.1;17	2001;211;854;1657	IrTu
310	**** Astragalus panduratus*** Bunge	17	943	IrTu
311	***Astragalus ponticus*** Pall.	11;17.2	1996;1922	
312	***Bituminaria bituminosa*** (L.) C.H.Stirt	2.1;15;17	1957;682;933;2059	Med
313	***Colutea cilicica*** Boiss. & Balansa	11.1;17;17.2	1897;2002;881;950	
314	***Coronilla scorpioides*** (L.) W.D.J.Koch	15	686	
315	***Cytisus austriacus*** L. subsp. ***pygmaeus*** (Willd.) Briq.	1;2;15	178;360;539;1857	EuSib
316	***Cytisus hirsutus*** L.	6;15;17	2025;120;1151;1772	
317	***Dorycnium graecum*** (L.) Ser.	6;15;17	247;471;1144;2021	EuSib
318	***Dorycnium pentaphyllum*** Scop. subsp. ***anatolicum*** (Boiss.) Gams	15;17.2	680;953;1307	
319	***Dorycnium pentaphyllum*** Scop. subsp. ***herbaceum*** (Vill.) Rouy	11.2;15;17	1784;1960;922	
320	***Dorycnium rectum*** (L.) Ser.	6	996	Med

321	***Galega officinalis*** L.	4;15	1141;1122	EuSib
322	***Genista januensis*** Viv. subsp. ***lydia*** (Boiss.) Kit Tan &ZieI.	15;17	251;1181;472;1309	Med
323	***Genista tinctoria*** L.	17;17.2	963;905	EuSib
324	***Lathyrus aureus*** (Steven) D.Brandza	1;6;17	2047;139;220;465	EuSib
325	***Lathyrus czeczottianus*** Bässler	1	200;422	EuSib
326	**** Lathyrus corniculatus*** L. var. ***corniculatus***	12	2139	
327	***Lathyrus laxiflorus*** (Desf.) Kuntze subsp. ***laxiflorus***	15;17	121;225;464	
328	***Lathyrus nissolia*** L.	17	255	
329	***Lathyrus tukhtensis*** Czeczott	1.1	1019	EuSib
330	***Lathyrus undulatus*** Boiss.	4;13.1;17	816;1111;1292	EuSib
331	**** Lens ervoides*** (Brign.) Grande	15	482	Med
332	***Lotus corniculatus*** L. var. ***alpinus*** Ser.	10.1;13	1079;1373	
333	***Lotus corniculatus*** L. var. ***corniculatus***	1;15	417;665;1785	
334	***Medicago minima*** (L.) Bartal. var. ***minima***	11.1;17	870;1701;1899	
335	***Medicago orbicularis*** (L.) Bartal.	11.1;18.1	1900;1944	
336	**** Medicago rigidula*** (L.) All. var. ***rigidula***	13	1076	
337	***Medicago sativa*** L. subsp. ***sativa***	1;17	2294;860;2295	
338	***Medicago x varia*** Martyn	11.1;11.2;13	1956;1070;1888	
339	***Melilotus albus*** Medik.	17	932	
340	***Melilotus officinalis*** (L.) Pall.	1;15;18.1	653;695;1938	
341	***Melilotus spicatus*** (Sm.) Breistr.	17.2	1923	
342	***Onobrychis oxyodonta*** Boiss.	1;2;10.1	1093;644;1383;2313	
343	***Ononis spinosa*** L. subsp. ***leiosperma*** (Boiss.) Sirj.	13	1074	
344	***Scorpiurus subvillosus*** L. var. ***subvillosus***	11.1	1904	
345	***Securigera varia*** (L.) Lassen	13.1;15;17	296;909;804;2158	Med
346	***Trifolium arvense*** L. var. ***arvense***	4;17	1043;871;1268	
347	***Trifolium aureum*** Pollich subsp. ***barbulatum*** Freyn & Sint. ex Freyn	3	2171	EuSib
348	***Trifolium campestre*** Schreb. subsp. ***campestre*** var. ***campestre***	15	589;475;671;1140	
349	***Trifolium dubium*** Sibth.	17	2015	
350	***Trifolium elongatum*** Willd.	1;15;17	254;561;775;826;1207	
351	***Trifolium hybridum*** L. subsp. ***anatolicum*** (Boiss.) Hossain	15	1143;500	
352	***Trifolium patens*** Schreb.	15;17	145;272	
353	***Trifolium pratense*** L. var. ***pratense***	4;15;17	235;209;1798;2308	
354	***Trifolium repens*** L. var. ***repens***	15;17	258;705	
355	***Trifolium resupinatum*** L. var. ***resupinatum***	15	729	

356	***Vicia abbreviata*** Spreng.	1;13.1	201;437	EuSib
357	***Vicia cassubica*** L.	15	487	EuSib
358	***Vicia cracca*** L. subsp. ***cracca***	17	1714	EuSib
359	***Vicia freyniana*** Bornm.	1;15	449;534	EuSib
360	**** Vicia lathyroides*** L.	13;19	47	
361	***Vicia pannonica*** Crantz var. ***pannonica***	15;17.2	1805;2329	
362	**** Vicia sepium*** L.	6	2038	EuSib
363	**** Vicia villosa*** Roth subsp. ***villosa***	6;18.1	1934;2046	
	POLYGALACEAE			
364	***Polygala anatolica*** Boiss. & Heldr.	1;16;17	1241;1404;271;1671	
365	**** Polygala major*** Jacq.	11.1	1670	EuSib
366	**** Polygala pruinosa*** Boiss. subsp. ***pruinosa*** Boiss.	17	266;894	
	FAGALES			
	BETULACEAE			
367	***Alnus glutinosa*** (L.) Gaertn. subsp. ***glutinosa***	12	1344	EuSib
368	***Carpinus betulus*** L.	15;16;17	1420;1209;2099	EuSib
369	***Corylus avellana*** L. var. ***avellana***	8;1.1;13.1	1031;164;317;318	EuSib
370	***Corylus colurna*** L.	17.2	1036	EuSib
	FAGACEAE			
371	***Fagus orientalis*** Lipsky	6;15;17	2082;222;770;1431	EuSib
372	***Quercus infectoria*** G.Oliver subsp. ***infectoria***	16	1407	EuSib
373	***Quercus macranthera*** Fisch. & C.A.Mey. ex Hohen. subsp. ***syspirensis*** (K.Koch) Menitsky	14;17;17.2	224;960;2327	
374	***Quercus petraea*** (Matt.) Liebl. subsp. ***iberica*** (Steven ex M.Bieb.) Krassiln.	4;13;17	906;2326;2333	
375	***Quercus pubescens*** Willd. subsp. ***pubescens***	11;14;16	1401;2321;2322;2325	
	GENTIANALES			
	APOCYNACEAE			
376	***Vinca minor*** L.	13.1	2237	
377	***Vinca major*** L. subsp. ***major***	12	1629	Med
378	***Vincetoxicum fuscatum*** subsp. ***fuscatum*** (Hornem.) Endl.	15	1305	IrTu
	GENTIANACEAE			
379	***Blackstonia perfoliata*** (L.) Huds. subsp. ***perfoliata***	5	2264	
380	***Centaurium erythraea*** Rafn subsp. ***erythraea***	17.2	977	EuSib
381	***Centaurium erythraea*** Rafn subsp. ***rumelicum*** (Velen.) Melderis	13	1082	Med
382	***Gentiana asclepiadea*** L.	1;15	1439;1234;2113	EuSib
383	***Gentiana cruciata*** L.	6	1248	EuSib

	GERANIACEAE			
384	***Erodium cicutarium*** (L.) L'Hér. subsp. ***cicutarium***	4;15;18.1	1945;509;607;1071	
385	***Erodium acaule*** (L.) Bech. & Thell.	13;15;19	135;34	Med
386	***Geranium asphodeloides*** Burm.f. subsp. ***asphodeloides***	1.1;13.1;17	162;238;517;2203	Med
387	***Geranium bohemicum*** L.	7;15	331;479	
388	***Geranium columbinum*** L.	11.2	1975	
389	***Geranium dissectum*** L.	17	275	
390	***Geranium tuberosum*** L.	1;1.1	193;1753	IrTu
391	***Geranium purpureum*** Vill.	1.1;15;17.2	392;588;320;1033	
392	**** Geranium pusillum*** Burm J.	11.2	1607	
393	***Geranium pyrenaicum*** Burm.f.	4;13.1;17	153;335;508;2153	
394	***Geranium robertianum*** L.	1.1;13.1;17	157;216;421;2190	
395	**** Geranium rotundifolium*** L.	4	606	
396	***Geranium macrostylum*** Boiss.	1;1.1;2.1	176;386;1842	Med
397	***Geranium molle*** L.	14;17	276;1576	
	RUBIACEAE			
398	***Asperula involucrata*** Wahlenb.	12;15;17	217;460;1822	EuSib
399	***Asperula pestalozzae*** Boiss.	17	868	EuSib
400	***Asperula taurina*** L.	1;1.1	374;448	
401	***Cruciata laevipes*** Opiz	13.1	163	EuSib
402	***Cruciata taurica*** (Pall. ex Willd.) Ehrend.	1;2;15	91;359;638	IrTu
403	***Galium aparine*** L.	4;15;17	315;493;598	
404	***Galium odoratum*** (L.) Scop.	1.1;4;15	378;466;550	EuSib
405	***Galium palustre*** L.	12;15	1807;1168	EuSib
406	***Galium paschale*** Forssk.	6;16;17	883;915;1424;2030	Med
407	***Galium rotundifolium*** L.	2.1	1837	EuSib
408	***Galium tricornutum*** Dandy	14	1591	IrTu
409	***Galium verum*** L. subsp. ***verum***	2;4;17	611;861;984;1194	EuSib
	LAMIALES			
	LAMIACEAE			
410	***Ajuga reptans*** L.	12;13.1;17	263;153;154;1630	EuSib
411	***Ajuga chamaepitys*** (L.) Schreb. subsp. ***chia*** (Schreb.) Arcang.	15	1319;694	Med
412	***Ajuga orientalis*** L.	1.1;15	89;398	
413	***Ballota nigra*** L. subsp. ***anatolica*** P.H.Davis	11	849	IrTu
414	***Clinopodium grandiflorum*** (L.) Kuntze	6;3;1	1004;1511;2101;2148	EuSib
415	***Clinopodium graveolens*** (M.Bieb.) Kuntze subsp. ***graveolens***	17	1706	
416	***Clinopodium vulgare*** L. subsp. ***arundanum*** (Boiss.) Nyman	9;15;17	727;781;911	
417	***Clinopodium vulgare*** L. subsp. ***vulgare***	11.1;12	1917;2136	

418	**** Galeopsis bifida*** Boenn.	3	2172	EuSib
419	**** Glechoma hederacea*** L.	13.1	155;1621	EuSib
420	***Lamium album*** L. subsp. ***crinitum*** (Montbret & Aucher ex Benth.) Mennema	2;4	204;349	EuSib
421	***Lamium garganicum*** L. subsp. ***garganicum***	4;15	1542;1636	EuSib
422	***Lamium garganicum*** L. subsp. ***striatum*** (Sm.) Hayek var. ***striatum***	1	423	Med
423	***Lamium maculatum*** (L.) L.	1.1;15	369;554	
424	***Lamium purpureum*** L. var. ***purpureum***	1.1;2;13.1	1529;28;66;194	EuSib
425	***Lamium purpureum*** L. var. ***aznavourii*** Gand. ex Aznav.	17	299	EuSib
426	***Leonurus quinquelobatus*** Gilib.	1	1234;2109	EuSib
427	***Mentha longifolia*** (L.) L. subsp. ***longifolia***	15	735	
428	***Mentha longifolia*** (L.) L. subsp. ***typhoides*** (Briq.) Harley	15	1331	
429	***Nepeta italica*** L.	11.1	1916	Med
430	***Origanum vulgare L***. subsp. ***vulgare***	1.1;15;17.2	949;1016;2062	EuSib
431	***Prunella vulgaris*** L.	1;15;17	583;656;896	EuSib
432	***Prunella laciniata*** (L.) L.	13;15	1777;690;1080	EuSib
433	***Phlomis pungens*** Willd. var. ***pungens***	18.1	1931	
434	***Phlomis russeliana*** (Sims) Lag. ex Benth.	2.1;17	891;2007;300;1841	EuSib
435	***Salvia aethiopis*** L.	1;11.1;17	1902;2218;645	
436	***Salvia forskahlei*** L.	15;17.2	590;966;1161	EuSib
437	***Salvia glutinosa*** L.	5;17	2098;2277	EuSib
438	***Salvia sclarea*** L.	11;11.1;17	1903;2000;925;1032	
439	**** Salvia tobeyi*** Hedge	1	1752	EuSib
440	***Salvia tomentosa*** Mill.	2;11.2;17.2	952;1095;1954;2063	Med
441	**** Salvia verbenaca*** L.	10.1	1379	Med
442	***Salvia verticillata*** L. subsp. ***amasiaca*** (Freyn & Bornm.) Bornm.	11.1;15	667;1787;1901;1907	IrTu
443	***Salvia verticillata*** L. subsp. ***verticillata***	15;17	2011;587	EuSib
444	***Salvia virgata*** Jacq.	11;17.1	847;2061	IrTu
445	***Salvia viridis*** L.	17	1705	Med
446	***Scutellaria albida*** L. subsp. ***albida***	11.1	1911;2234	Med
447	***Scutellaria albida*** L. subsp. ***velenovskyi*** (Rech.f.) Greuter & Burdet	6	2026	Med
448	***Sideritis montana*** L. subsp. ***montana***	15	1328	Med
449	***Sideritis taurica*** Steph. ex Willd.	1;4;7	1264;2128;2239;2289	EuSib
450	***Stachys annua*** sl.	15;17;17.2	279;951;1688;2028	
451	***Stachys byzantina*** K.Koch	1;15	1770;443	EuSib
452	***Stachys iberica*** M.Bieb. subsp. ***iberica*** var. ***iberica***	2;15	2253;1173	IrTu

453	***Stachys sylvatica*** L.	6;5	2037;2278	EuSib
454	***Stachys thirkei*** K.Koch	11.1;15	703;1884;510;569	
455	***Teucrium chamaedrys*** L. subsp. ***chamaedrys***	1.1;11.1;11.2	1894;1027;1966;2189	
456	***Teucrium polium*** L. subsp. ***polium***	11.2;15;17	681;1955;864	
457	***Thymus longicaulis*** C.Presl subsp. ***chaubardii*** (Rchb.f.) Jalas	11.1;15	1180;1656	Med
458	***Thymus longicaulis*** C.Presl subsp. ***longicaulis***	2.1;17	1355;265	EuSib
459	***Thymus praecox*** Opiz subsp. ***jankae*** (Celak) Jalas	11.1;15	1883;543;570	EuSib
460	***Ziziphora capitata*** L.	11.1	1912	
	OLEACEAE			
461	***Ligustrum vulgare*** L.	17.1;15	713;1761;2057	EuSib
	RUTACEAE			
462	***Ruta thesioides*** Fisch. ex DC.	11.1;17.3	2224;1918	
	OROBANCHACEAE			
463	***Euphrasia pectinata*** Ten.	2;1.1;15	1090;1806;2193	EuSib
464	***Lathraea squamaria*** L.	1	149	EuSib
465	***Macrosyringion glutinosum*** (M.Bieb) Rothm.	1;2;15	1196;1257;2177	
466	***Melampyrum arvense*** L. var. ***arvense***	1.1;6	1018	EuSib
467	**** Melampyrum arvense*** L. var. ***elatius***	5;17	907;1273	EuSib
468	***Orobanche elatior*** Sutton	1.1	1017	
469	***Orobanche purpurea*** Jacq.	2.1	1350	
470	**** Orobanche gracilis*** Sm.	1;2;15	566;825;1103	
471	***Orobanche minor*** Sm.	15;17	273;661;691	
472	***Orobanche nana*** (Reutq.) Beck	17	1678	
473	***Parentucellia latifolia*** Caruel subsp. ***latifolia***	13;17;19	42;248	Med
474	***Pedicularis condensata*** M.Bieb.	1;15	444;536	EuSib
475	***Pedicularis comosa*** L. var. ***sibthorpii*** (Boiss.) Boiss.	1;2;15	1445;1135;1851	
476	***Rhinanthus angustifolius*** C.C.Gmel. subsp. ***grandiflorus*** (Wallr.) D.A.Webb)	2.1;4	1962;608	
477	***Rhynchocorys elephas*** (L.) Griseb. subsp. ***elephas***	1;3;7	316;2020;1875;2106	EuSib
	SCROPHULARIACEAE			
478	***Scrophularia canina*** L. subsp. ***bicolor*** (Sm.) Greuter	2;12;17	1713;1849;2068	Med
479	***Scrophularia scopolii*** Hoppe ex Pers. var. ***adenocalyx*** Sommier & Levier	4;15;17	240;334;492;2039	EuSib
480	***Scrophularia umbrosa*** Dumort.	3	2173	EuSib
481	***Verbascum abieticola*** Bornm.	1;4	438;1734	EuSib
482	***Verbascum bithynicum*** Boiss.	11.2;15;17	734;918;1964;2266	EuSib

483	***Verbascum caudatum*** Post	2	1866	IrTu
484	**** Verbascum cheiranthifolium*** Boiss. var. ***cheiranthifolium***	11.2;15	1885;2140	
485	***Verbascum gnaphalodes*** M.Bieb.	4;15	474;1735	EuSib
486	***Verbascum phlomoides*** L.	2;15;18.1	712;1864;1930	EuSib
	VERBENACEAE			
487	***Verbena officinalis*** L. var. ***officinalis***	17	895	
	MALPIGHIALES			
	EUPHORBIACEAE			
488	***Euphorbia aleppica*** L.	15	1320	
489	***Euphorbia amygdaloides*** L. var. ***amygdaloides***	4	1742	EuSib
490	***Euphorbia amygdaloides*** L. var. ***robbiae*** (Turrill) Stace	4;15;17	79;1639;21;232	EuSib
491	***Euphorbia falcata*** L. subsp. ***falcata*** var. ***falcata***	15	1322	
492	***Euphorbia glareosa*** Pall. ex M.Bieb.	10.1	1369	
493	***Euphorbia helioscopia*** L. subsp. ***helioscopia***	11,1;5	1598;746	
494	***Euphorbia pannonica*** Host	4,6,15	131;603;679;2040	EuSib
495	***Euphorbia seguieriana*** Neck. subsp. ***niciciana*** (Borbás ex Novák) Rech.f.	17	1039;2004	
496	***Euphorbia seguieriana*** Neck. subsp. ***seguieriana***	2;15;17	236;1091;476;1768	EuSib
497	***Euphorbia stricta*** L.	13;17;17.2	150;230;1034;1341	EuSib
498	***Mercurialis annua*** L.	17.3	1613	
499	***Mercurialis perennis*** L.	1	148	EuSib
	HYPERICACEAE			
500	***Hypericum bithynicum*** Boiss.	15	726;1192	EuSib
501	***Hypericum montbretii*** Spach	15;17	295;458;1152	
502	***Hypericum origanifolium*** Willd. var. ***origanifolium***	12;15	1700;1812	
503	***Hypericum perforatum*** L. subsp. ***perforatum***	2;10.1;15	1356;531;1377	
504	***Hypericum tetrapterum*** Fr. var. ***tetrapterum***	6;17	875;2089	
	LINACEAE			
505	***Linum bienne*** Mill.	1;11.2;17	867;1982;269;629;1075	
506	***Linum hirsutum*** L. subsp. ***anatolicum*** (Boiss.) Hayek var***. anatolicum***	11;11.2;15	844;1050;1914;1769	IrTu
507	***Linum tenuifolium*** L.	11.1;15	1766;685;1908	
508	***Linum trigynum*** L.	17	873	Med
	SALICACEAE			
509	***Populus nigra*** L. subsp. ***nigra***	8,15	329;741	EuSib
510	***Populus tremula*** L. subsp. ***tremula***	15	769	
511	***Salix alba*** L. subsp. ***alba***	15	1627;736	EuSib

512	***Salix caprea*** L.	1	433	EuSib
	VIOLACEAE			
513	***Viola arvensis*** Murray	4	605	
514	***Viola canina*** L.	13;19	58	
515	***Viola gracilis*** Sibth. et Sm.	2;13.1;15	1516;1162;88;351;354	
516	***Viola odorata*** L.	1;15;16.1	1553;86;186	
517	***Viola parvula*** Tineo	2	1648	
518	***Viola sieheana*** W.Becker	1.1;13;19	50;384;537	
519	***Viola suavis*** M.Bieb.	4;17	1560;20	
	MALVALES			
	CISTACEAE			
520	***Cistus creticus*** L.	9;10.1;17	778;1384;1828;1898	Med
521	***Cistus laurifolius*** L.	15	777	Med
522	***Fumana procumbens*** (Dunal) Gren. & Godr.	11.1	1886	
523	***Helianthemum nummularium*** (L.) Mill. subsp. ***nummularium***	1;15;17	264;658;693;1060	
524	**** Helianthemum ovatum*** Dun.	1.1	2202	
	MALVACEAE			
525	***Alcea apterocarpa*** Boiss.	5	2283	IrTu
526	***Alcea pallida*** (Willd.) Waldst. & Kit.	11.1;17	1906;923	
527	***Althaea hirsuta*** L.	15	1797	
528	***Malva neglecta*** Wallr.	17	2209	
529	***Malva sylvestris*** L.	17	2006;928	
530	***Tilia rubra*** DC. subsp. ***caucasica*** (Rupr.) V. Engl.	13	2338	EuSib
	PLANTAGINACEAE			
531	***Digitalis ferruginea*** L. subsp. ***ferruginea***	13;17;17.2	924;1083;1269;2284	EuSib
532	***Digitalis lamarckii*** Ivan.	2;11;11.1	1094;855;1895;1910	IrTu
533	***Kickxia elatine*** (L.) Dumort. subsp. ***crinita*** (Mabille) Greuter	15	1329a	Med
534	***Kickxia spuria*** (L.) Dumort. subsp. ***integrifolia*** (Brot.) R.Fern.	15	1329b	
535	***Linaria genistifolia*** (L.) Mill. Cf. subsp. ***confertiflora*** (Boiss.) P.H.Davis	1;2	1274;2256;2162	EuSib
536	***Globularia trichosantha*** Fisch. & C.A.Mey. subsp. ***trichosantha***	1	635;188	Med
537	***Plantago lanceolata*** L.	13;15	529;668;740;1069	IrTu
538	***Plantago major*** L. subsp. ***intermedia*** (Gilib.) Lange	17	1498	
539	***Plantago major*** L. subsp. ***major***	1;15;17	450;250;528	
540	***Veronica anagallis-aquatica*** L.	2;4;15	481;333;486;1878	
541	***Veronica bozakmanii*** M.A.Fisch.	14;15	559;1585	IrTu
542	***Veronica chamaedrys*** L.	1.1;15;17	51;218;381;557	EuSib

543	***Veronica gentianoides*** sl.	1;2	187;346;361;1855	EuSib
544	***Veronica hederifolia*** L.	11;15	127;1580	
545	***Veronica jacquinii*** Baumg.	11.1;15	1655;1184	EuSib
546	***Veronica officinalis*** L.	6;15	124;2050	EuSib
547	**** Veronica orientalis*** Mill. subsp. ***orientalis***	1;2;15	545;643;827;1854	
548	***Veronica persica*** Poir.	15	134;742	
549	***Veronica serpyllifolia*** L.	4	1743	
	THYMELAEACEAE			
550	***Daphne pontica*** L. subsp. ***pontica***	1.1;4;17	245;385;1744	EuSib
551	***Daphne oleoides*** Schreb. subsp. ***oleoides***	1.1	2245	
	MYRTALES			
	LYTHRACEAE			
552	***Lythrum salicaria*** L.	11.1	2231	EuSib
	ONAGRACEAE			
553	***Circaea lutetiana*** L.	2	2268	
554	***Epilobium angustifolium*** L.	11.2;13.1	1965;809	EuSib
555	***Epilobium hirsutum*** L.	11.2;15	1054;1436	
556	***Epilobium lanceolatum*** Seb. & Mauri	4;11.2;15	1952;459;1123	EuSib
557	***Epilobium montanum*** L.	15;17;17.2	549;;876;1142;1925	EuSib
558	***Epilobium parviflorum*** Schreb.	6;17.2	976;2086	
559	**** Oenothera biennis*** L.	13.1	1395	
	PIPERALES			
	ARISTOLOCHIACEAE			
560	***Aristolochia pallida*** Willd.	1;17;17.3	283;2226;442	EuSib
	RANUNCULALES			
	PAPAVERACEAE			
561	***Chelidonium majus*** L.	13.1;17.3	158;1612	EuSib
562	***Corydalis caucasica*** DC. subsp. ***abantensis*** Lidén	4;13.1;15	109;146;16;12;23	
563	***Corydalis caucasica*** DC. subsp. ***caucasica***	4	15;17;18	EuSib
564	***Corydalis wendelboi*** Lidén subsp. ***congesta*** Lidén & Zetterl.	2;13.1;15	13;10;62;71;108	
565	***Fumaria asepala*** Boiss.	11	1594	IrTu
566	***Fumaria officinalis*** L. subsp. ***officinalis***	1	657;1593	IrTu
567	***Glaucium grandiflorum*** Boiss. & A.Huet subsp. ***refractum*** (Nábělek) Mory	11	2227;1804	IrTu
568	***Papaver lacerum*** Popov	11.1;15	1668	
569	***Papaver pilosum*** Sibth. & Sm. subsp. ***pilosum***	2.1	2252	
570	***Papaver rhoeas*** L.	7;15;17.2	328;1104;1324;2229	

	RANUNCULACEAE			
571	***Adonis flammea*** Jacq.	11	1596	
572	***Clematis vitalba*** L.	2	2157	
573	***Clematis viticella*** L.	11.1	1915	
574	***Consolida regalis*** Gray subsp. ***regalis***	18	1359	
575	***Consolida orientalis*** (J.Gay) Schrödinger	15;18.1	1790;749;1943	
576	***Delphinium fissum*** Waldst. & Kit. subsp. ***anatolicum*** Chowdhuri & P.H.Davis	1;1.1	1234;2112;2292	
577	***Ficaria verna*** Huds. subsp. ***ficariiformis*** (Rouy & Foucaud) B.Walln.	2.1;17	65;1571	
578	***Helleborus orientalis*** Lam.	13.1;17	2;59;260	EuSib
579	***Nigella arvensis*** L. subsp. ***glauca*** (Boiss.) N.Terracc.	15	1318	
580	***Ranunculus arvensis*** L.	1;1.1	371;426	
581	***Ranunculus brutius*** Ten.	1.1;15	367;548;1759	EuSib
582	***Ranunculus constantinopolitanus*** (DC.) d'Urv**.**	15;17	141;239	
583	***Ranunculus dissectus*** M.Bieb. subsp. ***sibthorpii*** Davis	1.1;15;2	33;347;363;2205	
584	***Ranunculus gracilis*** E.D.Clarke	15	132	
585	***Ranunculus marginatus*** d'Urv.	15	480;518	
586	***Ranunculus neapolitanus*** Ten.	13	1086;1880	
587	***Ranunculus repens*** L.	15	744	
	ROSALES			
	MORACEAE			
588	***Ficus carica*** L. subsp. ***carica***	11.1	2232	Med
	ROSACEAE			
589	***Agrimonia eupatoria*** L. subsp. ***eupatoria***	17	2072;887	
590	***Agrimonia repens*** L.	10.1;17.2	945;2016;1392	
591	***Alchemilla erythropoda*** Juz.	13.1;15	818;1200	EuSib
592	***Alchemilla mollis*** (Buser) Rothm.	1;1.1	167;375	
593	***Alchemilla porrectidens*** Juz.	2;4	356;615	EuSib
594	***Alchemilla pseudocartalinica*** Juz.	1;1.1	427;382	
595	***Cotoneaster integerrimus*** Medik.	15	753;1193;1333	
596	***Cotoneaster nummularius*** Fisch. & C.A.Mey.	2;11.2	1052;2262	
597	***Crataegus microphylla*** K.Koch subsp. ***microphylla***	15;17	223;763	EuSib
598	***Crataegus monogyna*** Jacq. var. ***monogyna***	4;17	1733;899	
599	***Crataegus orientalis*** Pall. ex M.Bieb. subsp. ***orientalis***	15	1771	

600	***Crataegus tanacetifolia*** (Poir.) Pers.	12;15;17	2134;697;865	
601	***Crataegus x bornmuelleri*** Zabel ex K.I.Chr. & Ziel.	3;3.1;15	1164;1456;2161	
602	***Filipendula vulgaris*** Moench	1	2304	EuSib
603	***Fragaria vesca*** L.	13.1;15;17	122;261;898;1270	EuSib
604	***Geum urbanum*** L.	1;4;13.1	156;420;1844;2105	EuSib
605	***Malus sylvestris*** (L.) Mill. subsp. ***orientalis*** (Uglitzk.) Browicz var. ***orientalis***	2	1101	
606	***Mespilus germanica*** L.	15	731	EuSib
607	***Potentilla argentea*** L.	17	270;304	
608	***Potentilla astracanica*** Jacq. subsp. ***astracanica***	11.1	1662	EuSib
609	***Potentilla calabra*** Ten.	11.1	805	Med
610	***Potentilla crantzii (***Crantz) Beck ex Fritsch	17	874	EuSib
611	***Potentilla micrantha*** Ramond ex DC.	11.1;15	4;107;60	
612	***Potentilla recta*** L.	15;17	577;1796;2023	
613	***Potentilla reptans*** L.	1;6;10	428;1387;2033	
614	***Prunus avium*** (L.) L.	1.1;17;17.2	40;897;994;1061	EuSib
615	***Prunus divaricata*** Ledeb. var. ***divaricata***	9;15;17.2	797;959;2141	
616	***Prunus laurocerasus*** L.	1	2331	
617	***Prunus spinosa*** L.	16.1;12	1550;2133	EuSib
618	***Pyracantha coccinea*** M.Roem.	15	1512;724;755	EuSib
619	***Pyrus elaeagnifolia*** Pall. subsp. ***elaeagnifolia***	9;15	796;1467	
620	***Rosa canina*** L.	2;7;15	327;1472;2160	
621	***Rosa horrida*** Fisch.	1.1	2316	
622	***Rubus caesius*** L.	17.2	961	
623	***Rubus canescens*** DC. var. ***canescens***	6;15;17.2	986;754;2034	EuSib
624	***Rubus hirtus*** Waldst. & Kit.	15	576	EuSib
625	***Rubus idaeus*** L. subsp. ***idaeus***	2.1;3;15	1354;1186;2267	EuSib
626	***Rubus ulmifolius*** Schott.	11.1;13.1;17	863;1399;1920	
627	***Sanguisorba minor*** Scop. subsp. ***balearica*** (Bourg. ex Nyman) Muñoz Garm. & C.Navarro	15	692;1183	
628	***Sorbus aucuparia*** L.	1.1;2.1;4	396;1352;2216	EuSib
629	***Sorbus domestica*** L.	14	1299	EuSib
630	***Sorbus kusnetzovii*** Zinserl.	2	2332	
631	***Sorbus torminalis*** (L.) Crantz var. ***torminalis***	17	297	
632	***Sorbus umbellata*** (Desf.) Fritsch	1.1;6;15	1021;766;2121	
	ULMACEAE			
633	***Ulmus glabra*** Huds.	3	2340	EuSib

	URTICACEAE			
634	***Urtica dioica*** L. subsp. ***dioica***	2.1;4;15	119;1845;453	EuSib
	SANTALALES			
	SANTALACEAE			
635	***Thesium arvense*** Horv.	13;14;19	44;1592	EuSib
636	***Viscum album*** L. subsp. ***album***	3;10	1513;2174	
	SAPINDALES			
	ANACARDIACEAE			
637	***Rhus coriaria*** L.	17.2	1035	
	SAPINDACEAE			
638	***Acer campestre*** L. subsp. ***campestre***	16	1421	EuSib
639	***Acer heldreichii*** Orph. ex Boiss. subsp. ***trautvetteri*** (Medw.) A.E.Murray	1.1;15	758;2206	EuSib
640	***Acer platanoides*** L.	5	2285	EuSib
	SAXIFRAGALES			
	CRASSULACEAE			
641	**** Phedimus stoloniferus*** (S.G.Gmel.) 't Hart	3;5	2170;2275	EuSib
642	***Sedum acre*** L. subsp. ***acre***	11	856	
643	***Sedum album*** L.	1.1;11;15	1470;2117;845	
644	***Sedum pallidum*** M.Bieb.	5;11;11.2	2274;840;2094	EuSib
645	***Sedum urvillei*** DC.	11.1	1891	
646	**** Sempervivum gillianiae*** Muirhead	1;1.1;2	652;2124;2318	EuSib
	GROSSULARIACEAE			
647	**** Ribes uva-crispa*** L.	17	1684	
	SAXIFRAGACEAE			
648	***Saxifraga cymbalaria*** L.	3;9;15	586;784;1876	
649	***Saxifraga exarata*** Vill.	17	1236	
650	***Saxifraga rotundifolia*** L. subsp. ***rotundifolia***	15	535;1637	EuSib
	SOLANALES			
	CONVOLVULACEAE			
651	***Calystegia silvatica*** (Kit.) Griseb.	15;17.2	2031;572;771;954	
652	***Convolvulus arvensis*** L.	15;17;18	506;1698;1935;1936	
653	***Convolvulus cantabrica*** L.	13.1;14	1340;1765;2070;306	Med
654	***Cuscuta europaea*** L.	17.2	957	
	SOLANACEAE			
655	***Atropa belladonna*** L.	4;6;15	478;1266;2080	EuSib
656	***Datura stramonium*** L.	13.1	2194	
657	***Hyoscyamus niger*** L.	2.1;17	1214;1676	
658	***Solanum dulcamara*** L.	13.1;17	1396;1685	EuSib
	MONOCOTYLEDONEAE			
	ALISMATALES			
	ARACEAE			

659	***Arum hygrophilum*** Boiss. subsp. ***euxinum*** (R.R.Mill) Alpınar	11.2	1603	EuSib
660	***Lemna minor*** L.	1	2305	
	ASPARAGALES			
	AMARYLLIDACEAE			
661	***Allium guttatum*** Steven subsp. ***guttatum***	1;2;13.1	2301;1226;2248	
662	***Allium guttatum*** Steven subsp. ***sardoum*** (Moris) Stearn	15	1160;1182	Med
663	***Allium huber-morathii*** Kollmann; Özhatay & Koyuncu	6;15;17	1228;1191;2093	IrTu
664	***Allium jubatum*** J.F.Macbr.	15;17	717;1721	EuSib
665	***Allium olympicum*** Boiss.	15	1158	EuSib
666	***Allium rotundum*** L.	1;3.1;6	2123;1159;1230	
667	***Allium stamineum*** Boiss.	6;1;1.1;13.1	1006;2288;1030	Med
668	***Galanthus plicatus*** M.Bieb. subsp. ***plicatus***	1	147	
	ASPARAGACEAE			
669	***Muscari aucheri*** (Boiss.) Baker	11.2	2334	
670	***Muscari armeniacum*** Leichtlin ex Baker	15;4;1	90;19;26;27;179;231	
671	***Ornithogalum alpigenum*** Stapf	12	1808	Med
672	***Ornithogalum fimbriatum*** Willd.	1	182;1750	Med
673	***Ornithogalum narbonense*** L.	15	672	Med
674	***Ornithogalum oligophyllum*** E.D.Clarke	4;15;17	219;106;87;340	
675	***Ornithogalum sphaerocarpum*** A.Kern.	6	2041;2042	
676	***Ornithogalum sigmoideum*** Freyn & Sint.	1;13;19	52;637	EuSib
677	***Polygonatum orientale*** Desf.	1.1;17	397;285;1044	EuSib
678	***Prospero autumnale*** (L.) Speta	12;13;15	1434;1483;1480;2144	Med
679	***Ruscus hypoglossum*** L.	3	2335	EuSib
680	***Scilla bifolia*** L.	4;13.1;15	11;94;24;61;183	Med
	IRIDACEAE			
681	***Crocus ancyrensis*** (Herb.) Maw	2;2.1;13.1	8;68;72;97	IrTu
682	***Crocus olivieri*** J.Gay subsp. ***olivieri***	15	98	
683	***Crocus speciosus*** M.Bieb. subsp. ***speciosus***	13.1;15;17	114;1486;1495;1507	
684	***Gladiolus italicus*** Mill.	17	1717	
685	***Iris kerneriana*** Asch. & Sint. ex Baker	13.1	2213	EuSib
686	***Iris sintenisii*** Janka	6;15;17	302;721;2091	EuSib
687	***Iris pumila*** L. subsp. ***attica*** (Boiss. & Heldr.) K.Richt.	11.2	1611	EuSib
	LILIALES			
	COLCHICACEAE			

688	***Colchicum boissieri*** Orph.	13.1	2200	IrTu
689	***Colchicum speciosum*** Steven	13.1;15	1465;1487;2045;2111	EuSib
690	***Colchicum umbrosum*** Steven	15	1478	EuSib
	LILIACEAE			
691	***Gagea dubia*** Terracc.	13.1;15	9;113;101	
692	**** Gagea fragifera*** (Vill.) E.Bayer & G.López	4;15;2.1	25;96;64	
693	**** Gagea glacialis*** K.Koch	15	95	IrTu
694	***Gagea villosa*** (M.Bieb.) Sweet var. ***villosa***	2.1	67	Med
695	***Fritillaria pontica*** Wahlenb.	1;4;6	198;337;431;2044	EuSib
696	***Lilium martagon*** L.	5;15	2265;2276;2306	
	XANTHORRHOEACEAE			
697	***Asphodeline lutea*** (L.) Rchb.	6;17.2	989	Med
	ORCHIDALES			
	ORCHIDACEAE			
698	***Anacamptis coriophora*** (L.) R.M.Bateman	15	776	
699	***Anacamptis pyramidalis*** (L.) Rich.	11.2;15;17	704;833;1764	
700	***Cephalanthera damasonium*** (Mill.) Druce	1:17	309;654;1313	EuSib
701	***Cephalanthera epipactoides*** Fisch. & C.A.Mey.	11.3	1683	Med
702	***Cephalanthera rubra*** (L.) Rich.	1;13.1;15;17	511;683;1729;2018	
703	***Dactylorhiza nieschalkiorum*** H.Baumann & Künkele	13.1	817;813	
704	***Dactylorhiza romana*** (Sebast.) Soó subsp. ***romana***	1	822	
705	***Epipactis persica*** (Soó) Hausskn. ex Nannf.	4;15	1213;1284;1150	
706	***Epipactis helleborine*** (L.) Crantz subsp. ***helleborine***	4;5;13.1	1109;2280;1116;1285	
707	***Epipactis turcica*** Kreutz	1.1;13.1	1023;1024;1115	
708	***Epipogium aphyllum*** Sw.	4;15;17	1287;1202;2237	EuSib
709	***Himantoglossum caprinum*** (M.Bieb.) Spreng.	4;15;18	1338;1055;1728	EuSib
710	***Limodorum abortivum*** (L.) Sw. var. ***abortivum***	4;11.2;17	1267;1961;1727;1829	
711	***Neotinea tridentata*** (Scop.) R.M.Bateman; Pridgeon & M.W.Chase	11.1	1672	Med
712	***Neottia nidus-avis*** (L.) Rich.	1;3;9;13.1;15	430;468;1286;1502	EuSib
713	***Ophrys apifera*** Huds.	4;11.2;15	832;834;1337;1763	
714	***Ophrys oestrifera*** M.Bieb. subsp. ***oestrifera***	6;17	2017;1726	

715	***Ophrys transhyrcana*** Czerniak. ***subsp. paphlagonica*** Kreutz	15	1799	
716	***Orchis mascula*** (L.) L. subsp. ***pinetorum*** (Boiss. & Kotschy) E.G.Camus	7;9;17	128;262;522;1153	Med
717	***Orchis pallens*** L.	1.1;15	78;190;372;1131	EuSib
718	***Orchis purpurea*** Huds. subsp. ***purpurea***	17.2	970	EuSib
719	***Orchis simia*** Lam.	12;17	308;1823	Med
720	***Platanthera chlorantha*** (Custer) Rchb.	9;13.1;17	642;773;1117;1493	
721	***Spiranthes spiralis*** (L.) Chevall.	4;15	1433;1481;1488;2145	Med
	POALES			
	CYPERACEAE			
722	**** Carex caryophyllea*** Latourr.	16.1	1556	EuSib
723	***Carex flacca*** Schreb. subsp. ***erythrostachys*** (Hoppe) Holub	1.1;17	228;412	Med
724	***Carex pendula*** Huds.	3;6;17	321;2261;591;1873	EuSib
	JUNCACEAE			
725	***Juncus effusus*** L. subsp. ***effusus***	6;15	580;1879	
726	***Juncus inflexus*** L. subsp. ***inflexus***	16;17;17.2	277;1429;785;1926	
727	***Luzula campestris*** (L.) DC.	1;1.1	636;410	EuSib
728	***Luzula forsteri*** (Sm.) DC. subsp. ***caspica*** Novikov	17	1562	EuSib
729	***Luzula multiflora*** (Ehrh.) Lej. subsp. ***multiflora***	1.1;9	84;411	
	POACEAE			
730	***Aegilops geniculata*** Roth	15	702	Med
731	***Agrostis stolonifera*** L.	9;12;15	491;496;789	EuSib
732	***Aira elegantissima*** Schur subsp. ***elegantissima***	2	1223	Med
733	***Anthoxanthum odoratum*** L. subsp. ***alpinum*** (A.Löve & D.Löve) B.M.G.Jones & Melderis	1;4;15	92;177;208;341	EuSib
734	***Avena barbata*** Pott ex Link subsp. ***barbata***	1;4	172;206;336	Med
735	***Avena sterilis*** L. subsp. ***sterilis***	1.1;15;18.1	669;403;1939	
736	***Bothriochloa ischaemum*** (L.) Keng	1.1	413	
737	***Brachypodium sylvaticum*** (Huds.) P.Beauv.	12	498	EuSib
738	***Briza media*** L.	1.1;15;17	1174;904;2188;2297	
739	***Bromus hordeaceus*** L. subsp. ***hordeaceus***	13	1068	
740	***Bromus japonicus*** Thunb. subsp. ***japonicus***	1.1;6;17	934;2315	
741	***Bromus tectorum*** L.	12	497	
742	***Calamagrostis epigejos*** (L.) Roth	6;17.2	982;2081	EuSib

743	**** Chrysopogon gryllus*** (L.) Trin.	11.1	1887	
744	***Cynosurus cristatus*** L.	13	1081	EuSib
745	***Cynosurus echinatus*** L.	4,15;17	674;601;1066	Med
746	***Dactylis glomerata*** L. subsp***. glomerata***	1;4;17	415;326;447	EuSib
747	***Elymus hispidus*** (Opiz) Melderis subsp. ***barbulatus*** (Schur) Melderis	13	1067	
748	***Elymus repens*** (L.) Gould	15	678b	
749	***Festuca drymeja*** Mert. & W.D.J.Koch	15	494	EuSib
750	**** Helictotrichon versicolor*** (Vill.) Schult. & Schult.f.	2.1	1843;1856;609	EuSib
751	***Hordeum bulbosum*** L.	7;9;17;11.2	794;416;917;1057	
752	***Hordeum murinum*** L. subsp. ***leporinum*** (Link) Arcang.	18.1	1935;1940	IrTu
753	***Holcus lanatus*** L.	13.1	1105	EuSib
754	***Koeleria pyramidata*** (Lam.) P.Beauv.	17	938	EuSib
755	***Lolium perenne*** L.	15	1308;678a	EuSib
756	***Melica ciliata*** L. subsp. ***ciliata***	3;3.1;7	2151;2291	
757	**** Phleum aIpinum*** L.	1.1	406	EuSib
758	***Poa angustifolia*** L.	15	499	
759	***Poa annua*** L.	1;1.1;13.1	408;1617	
760	***Poa asiaeminoris*** H.Scholz & Byfield	1	446	
761	***Poa bulbosa*** L.	1.1;13;19	404;207;600;602	
762	***Poa pratensis*** L.	1	173	
763	**** Poa sterilis*** M.Bieb.	7;15	323;495;405	
764	***Poa trivialis*** L.	1.1;17	409;227	
765	***Sesleria alba*** Sm.	17	2219	
766	***Trisetum flavescens*** (L.) P.Beauv.	17	221	EuSib
	TYPHACEAE			
767	***Typha latifolia*** L.	3	1449	

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Available online: February 06, 2020

Commun.Fac.Sci.Univ.Ank.Series C
Volume 29, Number 1, Pages 50-60 (2020)
ISSN 1303-6025 E-ISSN 2651-3749
https://dergipark.org.tr/en/pub/communc/issue/51836/644632

ENNOMINAE, GEOMETRINAE AND LARENTIINAE (LEPIDOPTERA: GEOMETRIDAE) FAUNA IN GAZIANTEP PROVINCE, TURKEY

MÜRŞİT ÖMÜR KOYUNCU, MURAT KÜTÜK

ABSTRACT. This study was based on Geometridae species collected from Gaziantep province in spring and summer of 2009, 2010 and 2011. During the study, samples were collected from research area using by Robinson type light trap, portable light trap and insect net. In the study, 12 species belonging to 12 genera from 3 subfamilies have been determined. In addition, adult figures, material examined and distribution all species were given and.

1. INTRODUCTION

Geometridae is a family belonging to the Macrolepidoptera group of the order Lepidoptera, which includes all moths and butterflies. While the majority of geometrid moths display a nocturnal lifestyle, many fly by day [1]. Compared to other macrolepidopteran families, Geometridae species tend to be picky about living in certain habitats. Considering this characteristic of the family, the Geometridae species are regarded as useful environmental indicators by some researchers [2,3].

The larvae of species belonging to the family Geometridae are fed with annual plants, perennial forest plants and agricultural cultivated plants. Therefore, the diversity of plants to which larvae can be fed is quite more. More than 100 species belonging to the Larentiinae subfamily belong to the genus *Eupithecia* Curtis 1825 [4]. Generally, individuals are small. The forewingsare generally brown or whitish, and the rear wings are generally colorless. The wing pattern is transverse. There are M_1 vein and areole in the forewing [3]. The aim of the present study is to contribute to the of the Geometridae fauna of Gaziantep provinces and to summarize the geographic spread of the species.

Received by the editors: November 09, 2020; Accepted: February 06, 2020.
Key word and phrases: Fauna, Lepidoptera, Ennominae, Geometrinae, Larentiinae, Geometridae, Gaziantep, Turkey.

2. MATERIALS AND METHODS

Adult Geometridae specimens were collected with a Robinson Type Light Trap, insect net and stored in the laboratory. Then, these specimens were killed in ethyl acetate killing jars, they were brought to the Gaziantep University Entomology Laboratory. Then, they were prepared as standard museum materials.

Gaziantep is located in Turkey's southeast Anatolia. To the west of the region has been the Mediterranean and to the north has been the eastern Anatolia region (Figure 1).

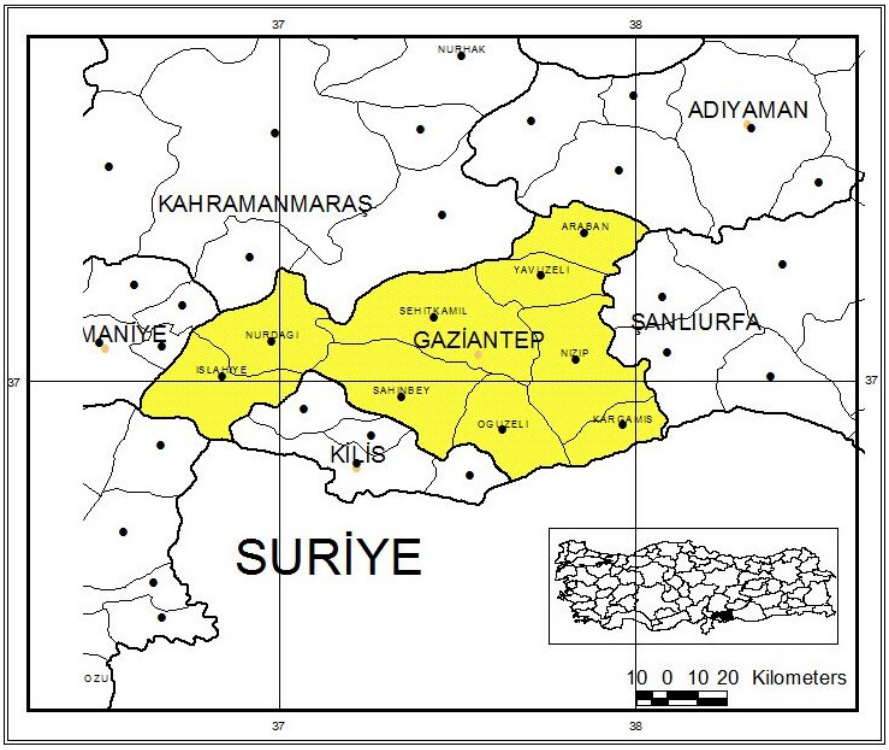

FIGURE 1. Study region (Gaziantep province in Turkey).

The genital structures were prepared according to Kornoşor [5]. Species were identifed by using the keys of Okyar and Aktaç [6], Hausmann [3], Doğanlar [7],

Mironov [8], Hausmann [9], Özdemir [10], Can [11], Redondo et al. [4] and Soku and Sihvonen, [12]. Adult specimens were deposited in the insect laboratory of Gaziantep University.

The elevation of is approximately the Gaziantep region varies between 450 and 1500 m. Summers are dry and hot, while winters are cold and rainy (Figure 2).

FIGURE 2. Some regions where adult Geometridae samples are collected.

3. RESULTS

In this study, 12 species from 12 genera belonging to 3 subfamily (Ennominae, Geometrinae and Larentiinae) were determined from Gaziantep province. Subfamilies and species listed alphabetical order (Table 1) and their photographs were given in Figure 3 and 4.

TABLE 1. Identified taxa

Subfamily	Genus	Specimens
Ennominae	*Aspitates*	*Aspitates ochrearia*
	Chiasmia	*Chiasmia clathrata*
	Neognopharmia	*Neognopharmia stevenaria*
Geometrinae	*Aplasta*	*Aplasta ononaria*
	Phaıogramma	*Phaiogramma etruscaria*
	Proteuchloris	*Proteuchloris neriaria*
Larentiinae	*Aplocera*	*Aplocera annexata*
	Camptogramma	*Camptogramma bilineata*
	Costaconvexa	*Costaconvexa polygrammata*
	Eupıthecıa	*Eupithecia centaureata*
	Protorhoe	*Protorhoe unicata*
	Xanthorhoe	*Xanthorhoe fluctuata*

All the collected material was preserved in the Entomological Laboratory of the Department of Biology, Gaziantep University. The altitudes, collecting dates and geographic coordinates of all collecting localities, are chronologically listed in Material examined.

Subfamily: Ennominae Duponchel, 1845
3 species belonging to 3 genera in Ennominae were determined.

Aspitates ochrearia (Rossi, 1794)
Material examined: Gaziantep, Burç, 36º 52ˈ N, 35º 52ˈ E, 640 m, 18.05.2011, 1 ♂.
World Distribution: Palearctic. Europe, North Africa, Turkey, Syria, England [13] and France [14].

Turkey Distribution: Çanakkale, Mersin, İstanbul, Amasya, Diyarbakır, Ankara, Edirne, Kırklareli, Kırıkkale, Kocaeli, Tekirdağ, Manisa, Kahramanmaraş, Kilis, Bursa, Uşak, Osmaniye [15].

Chiasmia clathrata (Linnaeus, 1758)

Material examined: Gaziantep, Huzurlu, 36º 58ˈ N, 36º 26ˈ E, 1030 m, 13.07.2010, 4 ♂♂.

World Distribution: Palearctic. North Africa, Europe and Turkey, Transcaucasia, Kazakhstan, Russia, Northwest China, Korea and Japan [4].

Turkey Distribution: Mersin, Van, Çankırı, İstanbul, Nevşehir, Amasya, Diyarbakır, Ankara, Edirne, Kırıkkale, Balıkesir, Konya, Ardahan, Bolu, Kahramanmaraş, Bursa, Osmaniye, Düzce [15].

Neognopharmia stevenaria (Boisduval, 1840)

Material examined: Gaziantep, Gökçük Village, 37º 27ˈ N, 37 º 35ˈ E, 575 m, 18.09.2009, 4 ♀♀; İbrahimli Village, 37º 05ˈ N, 37º 18ˈ E, 936 m, 11.04.2011, 5 ♂♂; İbrahimli Village, 37º 05ˈ N, 37º 19ˈ E, 944 m, 26.05.2011, 4 ♂♂.

World Distribution: Mediterranean-Turan, Mediterranean-Europe, Turkey, Cantabria Although there is a new record in the Iberian peninsula where there is the most widely peninsula [2].

Turkey Distribution: Çanakkale, Van, Diyarbakır, Ankara, Siirt, Konya, Malatya, Bitlis, Trabzon, Bolu, Kahramanmaraş, Hatay, Mardin, Bursa, Amasya [15].

Subfamily: Geometrinae Leaeh, 1815

3 species belonging to 3 genera in Geometrinae were determined.

Aplasta ononaria (Fuessly, 1783)

Material examined: Gaziantep, Yazıbağı Village, 37º 01ˈ N, 37º 07ˈ E, 768 m, 01.07.2010, 4 ♂♂.

World Distribution: Mediterranean "in southern Europe: Turkey, Transcaucasia, northern Iran, Israel, Lebanon and Syria. [2].

Turkey Distribution: Çanakkale, Mersin, Van, Amasya, Diyarbakır, Ankara, Elazığ, Kırklareli, Kırıkkale, Kırşehir, Aydın, Kocaeli, Konya, Tekirdağ, Bitlis, Bolu, Kahramanmaraş, Hatay, Bursa, Muğla, Osmaniye, Amasya [15].

Phaiogramma etruscaria (Zeller, 1849)

Material examined: Gaziantep, Oğuzeli, 37º 55ˈ N, 37º 32ˈ E, 641m, 17.06.2010, 4 ♂♂; Araban, 37º 31ˈ N, 37º 43ˈ E, 769 m, 29.06.2010, 3 ♂♂; Oğuzeli, 36º 55ˈ N, 37º 32ˈ E, 650 m, 03.07.2010, 3 ♂♂; İbrahimli Village, 37º 05ˈ N, 37º 18ˈ E, 936 m,

06.06.2011, 2 ♂♂; İbrahimli Village, 37º 05' N, 37º 19' E, 944 m, 06.06.2011 6 ♂♂; İbrahimli Village, 37º 05' N, 37º 18' E, 936 m, 05.07.2011, 7 ♂ ♂.
World Distribution: Turan Mediterranean, North Africa, Southern Europe, Turkey, Transcaucasia, northern Iran, Iraq, Kazakhstan, Turkmenistan, Uzbekistan, Tajikistan and Afghanistan [4].
Turkey Distribution: Çanakkale, Mersin, Van, Amasya, Kırklareli, Konya, Giresun, Trabzon, Bolu, Kahramanmaraş, Bursa, Osmaniye [15].

Proteuchloris neriaria (Herrich-Schâffer, 1852)
Material examined: Gaziantep, Oğuzeli, 37º 55' N, 37º 32' E, 641 m, 17.06.2010, 3 ♂♂.
World Distribution: It is a common species in Southern Europe. South Balkans, Turkey, Syria, Cyprus and Armenia [3] and Greece [2].
Turkey Distribution: Çanakkale, Mersin, Amasya, Bolu, Kahramanmaraş, Bursa, Osmaniye [15].

Subfamily: Larentiinae Duponchel, 1845
6 species belonging to 6 genera in Larentiinae were determined.

Aplocera annexata (Freyer, 1830)
Material examined: Gaziantep, Oğuzeli, 36º 55' N, 37º 32' E, 650m, 03.07.2010, 6 ♀♀.
World Distribution: It is a common species in Anatolia, Turkey, Azerbaijan, Caucasus, Armenia [13] and Ukraine [2].
Turkey Distribution: Çanakkale, Mersin, Van, Amasya, Diyarbakır, Ankara, Elazığ, Kırklareli, Kırıkkale, Kırşehir, Aydın, Kocaeli, Konya, Tekirdağ, Bitlis, Bolu, Kahramanmaraş, Hatay, Bursa, Muğla, Osmaniye [15].

Camptogramma bilineata (Linnaeus, 1758)
Material examined: Gaziantep, Gökçük Village, 37º 27' N, 37º 35', E, 575 m, 18.09.2009, 7 ♂♂; Oğuzeli, 37º 55' N, 37° 32' E, 641 m, 17.05.2011, 5 ♂♂; Oğuzeli, 37º 55' N, 37° 32' E, 641 m, 30.05.2011, 6 ♂♂.
World Distribution: Paleoarctic. It is widely headquartered in North Africa, Europe and Asia [4].
Turkey Distribution: Çanakkale, Mersin, Van, Çankırı, İstanbul, İzmir, Kars, Ordu, Amasya, Ankara, Edirne, Kayseri, Elazığ, Kırklareli, Siirt, Sinop, Şırnak, Balıkesir, Konya, Tekirdağ, Giresun, Malatya, Tokat, Bitlis, Manisa, Bolu, Kahramanmaraş, Bursa, Muğla, Osmaniye [15].

Costaconvexa polygrammata (Borkhausen, 1794)

Material examined: Gaziantep, Oğuzeli, 37º 55' N, 37º 32' E, 641 m, 17.06.2010, 1 ♀.

World Distribution: West palaearctik region, North Africa, Europe, Turkey and central Asia [4].

Turkey Distribution: Adana, Mersin, (Doğanlar, 2003), Çankırı, Amasya [15].

Eupithecia centaureata (Denis & Schittermüllerl, 1775)

Material examined: Gaziantep, Huzurlu, 36º 58' N, 36º 26' E, 1030 m, 13.07.2010, 5 ♂♂.

World Distribution: Palearctic region. North of Africa, Europe, Turkey, central Asia, Mongolia and China [4].

Turkey Distribution: Çanakkale-Gelibolu [16], Çanakkale, Edirne, Kırklareli, Tekirdağ [6].

Protorhoe unicata (Guenée, 1858)

Material examined: Gaziantep, Burç, 36º 52' N, 35º 52' E, 640 m, 17.05.2011, 5 ♂♂; Burç, 36º 52' N, 35º 52' E, 640 m, 24.05.2011, 7 ♂♂.

World Distribution: It is a species found in Anatolia and the Balkans [7].

Turkey Distribution: Mersin, Amasya, Kayseri, Kırıkkale, Konya, Manisa, Bolu, Osmaniye, Düzce [15].

Xanthorhoe fluctuata (Linnaeus,1758)

Material examined: Gaziantep, İbrahimli Village, 37º 05' N, 37º 18' E, 936 m, 26.05.2011, 3 ♂♂.

World Distribution: North Africa, Europe, Turkey, Asia, and is a common species in North America [4].

Turkey Distribution: Çanakkale, Mersin, Van, İstanbul, Niğde, Amasya, Ankara, Edirne, Kırklareli, Konya, Sivas, Bolu, Kahramanmaraş, Bursa, Muğla [15].

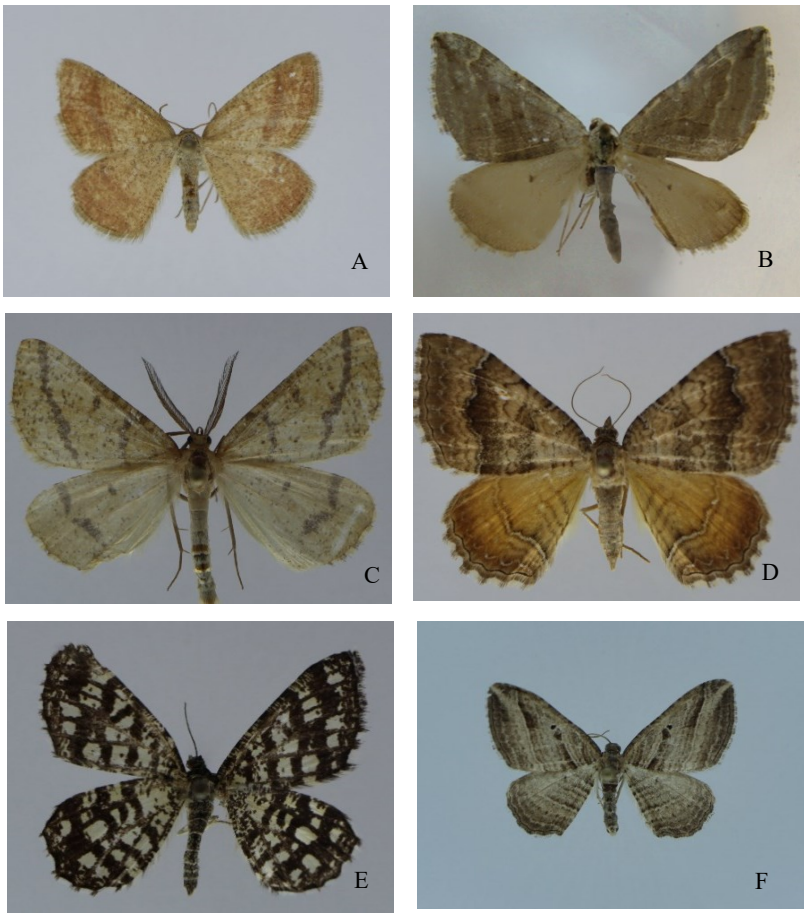

FIGURE 3. Geometridae adult: A. *Aplasta ononaria* B. *Aplocera annexata* C. *Aspitates ochrearia* D. *Camptogramma bilineata* E. *Chiasmia clathrate* F. *Costaconvexa polygrammata*.

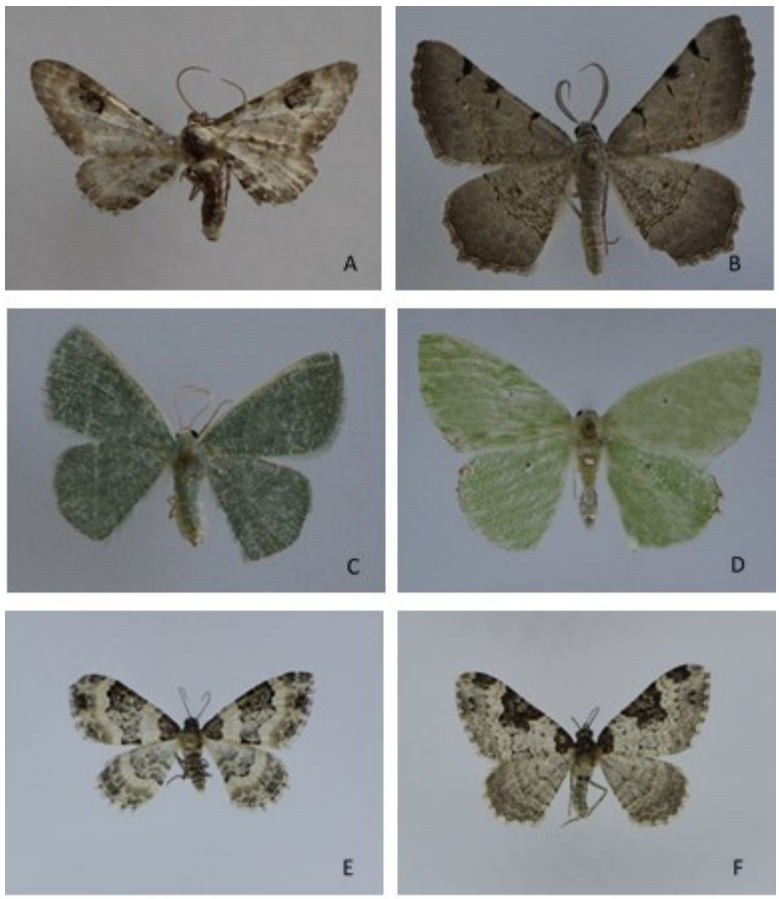

FIGURE 4. A. *Eupithecia centaureata* B. *Neognopharmia stevenaria* C. *Phaiogramma etruscaria* D. *Proteuchloris neriaria* E. *Protorhoe unicata* F. *Xanthorhoe fluctuate*.

4. DISCUSSION

Gaziantep is located in southeastern Turkey. Elevation is between 450 and 1500 m. There are significant temperature differences between summer and winter in Gaziantep. Winters are cold and rainy summers are hot and rainless. Therefore, it is similar to the desert climate in the region.

This study is based on the Geometridae specimens collected from Gaziantep province during 2009-2011 spring and summer period. In the study, we determined most common subfamily as Larentiinae with 6 species. Ennominae and Geometrinae distributed with respectively 3 species in the study region. There is no comprehensive study in Gaziantep province up to date. Together with this study, we contributed to distribution of Geometridae.

ACKNOWLEDGEMENT

We thank to Dr. Feza Can (Mustafa Kemal University, Faculty of Agriculture, Department of Plant Protection, Hatay, Turkey) for supporting of this study. This study was produced first author's Msc.

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Available online: February 24, 2020

Commun.Fac.Sci.Univ.Ank.Series C
Volume 29, Number 1, Pages 61-70 (2020)
ISSN 1303-6025 E-ISSN 2651-3749
https://dergipark.org.tr/en/pub/communc/issue/51836/688843

KARYOTYPE ANALYSIS OF *NANNOSPALAX XANTHODON* (SPALACIDAE, RODENTIA) AT THE EASTERNMOST PART OF ITS DISTRIBUTION RANGE

ALEXEY YANCHUKOV, ANDREI KANDAUROV,
MEHMET KÜRŞAT ŞAHİN and FERHAT MATUR

ABSTRACT. Chromosomal differentiation can play a crucial role in speciation. In order to reveal a complete picture of the speciation process within a taxon, it is important to determine the geographic distribution of all its chromosomal forms. The blind mole rats (subfamily Spalacinae) are one of the best models for studying chromosomal speciation due to extremely rich chromosomal variation across their large geographic distribution range. To fill the gap in our knowledge of the distribution of chromosomal races of small-bodied blind mole rats (*Nannospalax* sp.), we collected individuals from the region of Javakheti, Georgia – one of the easternmost localities known for *N. xanthodon*. We determined that this population has 9 meta- or submetacentric and 15 acrocentric chromosomes (2n=50, NFa=66, NF= 70). The same chromosomal formula is known for the blind mole rats from the nearby Erzurum-Kars plateau in Turkey. We compare our results with the other chromosomal races with the same diploid number 2n=50 found in Anatolia and Eastern Europe.

1. Introduction

The family of subterranean rodents Spalacinae originated from a Muroid-Cricetoid ancestor in Asia Minor or its vicinity in Oligocene or early Miocene and adaptively radiated in the Balkans, Ponto-Caspian steppes, and the Middle East, extending into North Africa [1]. The taxonomy within the group is uncertain, in part because of extremely high amount of cytogenetic variation in the genus *Nannospalax*, i.e. small bodied blind mole rats, with > 60 different, parapatric chromosomal forms described

Received by the editors: January 13, 2020; Accepted: February 24, 2020.
Key word and phrases: Chromosome, chromosomal evolution, blind mole rat, Georgia

to date [2-3], with some authors arguing for separate species status for the most distinct cytotypes [4]. Molecular DNA variation suggest the presence of several deep phylogenetic clades within Spalacinae, but with no obvious geographic barriers between their respective geographic ranges [5-7].

Despite multiple karyological studies elsewhere (see references with in Arslan et al. 2016), there are relatively few reports on the mole rat karyology in the Caucasus and Armenia, with the diploid chromosome numbers in north-western Armenian mole rats determined to be 2n= 50 [2,8]. At the same time, several distant mole rat populations with identical diploid chromosome numbers (2n= 50) are known, which however still differ by the number of chromosome arms (NFa= 66-68, NF 70-72 etc., reviewed in Arslan et al 2016). The different NF variation of 2n= 50 of *N.* xanthodon were recorded in the Norht-West, South and Eastern Turkey [2, 9-16]. The karyotype 2n= 50 is also known for the Balkan mole rat *N. leucodon* in Romania [17] and Hungary [1, 18-20], which is closely related to *N. xanthodon* [5,7].

Identification of all chromosomal forms and their distribution ranges is necessary to understand the evolutionary history of the entire group, as well as to construct a more realistic taxonomy. For the first time, we describe the karyotypic characteristics of the blind mole rat populations on the Javakheti plateau, Georgia, and compare their similarities with other chromosomal races that have the same number of diploid chromosomes 2n=50.

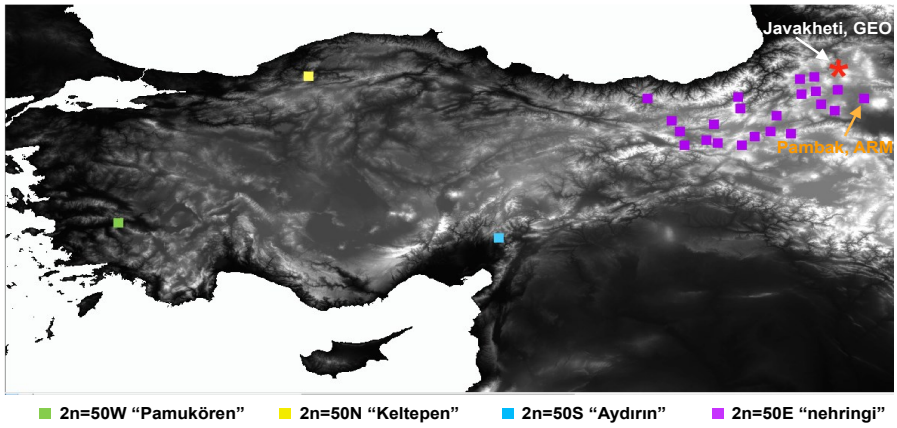
2. Materials and Methods

In this study, 10 (4 males, 6 females) blind mole rats were studied from two close localities on Javakheti plateau in Georgia: Agara (N41°22`55`, E43°12`36``) and Myasnikyani (N41°18`11``, E41°19`12``). Both these localities are of similar altitude (~1800 m a.s.l) and are characterised by similar alpine meadow / pasture habitat, but separated geographically by the deep Kura river canyon (Fig 1 a and b). The number of individuals analysed and karyological results are presented in Table 1.

Karyotypes were prepared from bone marrow according to Ford and Hamerton (1956) [21]. 25-30 metaphase cells, well-stained, and with chromosomes visibly separate and distinct, were examined from each animal. The diploid number of chromosomes (2n), the number of autosomal arms (NFa), the total number of chromosomal arms (NF) and the morphology of sex chromosomes were determined

from photographs of the metaphase plates according to the centromere position. Previously published karyotype data on *N. xanthodon* with diploid number 2n= 50 were compared with those of Georgian mole rats. We followed Matur et al. 2011 for classification of the corresponding cytopes (2n= 50S, 50N, 50E, 50W) [16].

a)



b)

FIGURE 1. a. Geographic distribution of 2n= 50 cytotypes of *N. xanthodon* and *N.leucodon*. Chromosomal races named according to Arslan et al. (2016). b. Locations of the two populations on the Javakheti plateau.

TABLE 1. The karyotype characteristics of known 2n= 50 cytotypes of *N. xanthodon* and *N. leucodon*. NF – fundamental number of chromosome arms, a – acrocentric, sm – small metacentric.

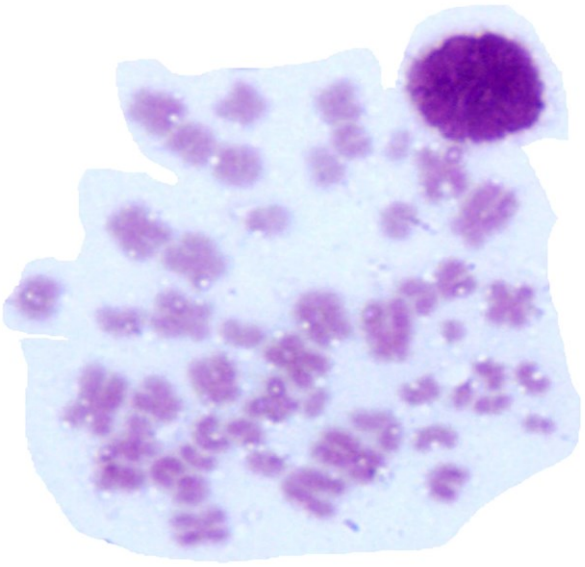
Species	**2n**	**NF**	**X**	**Y**	**Type locality**	**References**
N.xanthodon	**50Ge**	**70**	sm	a	**Javakheti, Georgia**	**This study**
N.xanthodon	50W	–	–	–	Aydın (Turkey)	Nevo et al. (1994, 1995)
N.xanthodon	50W	74			Alaşehir, Pamukören (Turkey)	Matur et al. (2011)
N.xanthodon	50	72			Maralik (Armenia)	Lyapunova et al. (1974)
N.xanthodon	50E	70	sm	–	Erzurum, Sarıkamış	Nevo et al. (1994), (1995)
N.xanthodon	50E	72	sm	a	Erzurum, Susuz, Ardahan	Sözen et al. (2000a)
N.xanthodon	50E	72			Eğribel Pass (Giresun), Ovid mount. (Rize), Demirözü (Bayburt), Yollarüstü (Erzincan)	Kankılıç et al. (2007b)
N.xanthodon	50E	70			Kandilli, Ilıca, Otlukbeli (Erzurum), Arpayazı (Erzincan)	Matur et al. (2011)
N.xanthodon	50E	70			Susuz, Selim (Kars)	Kankılıç et al. (2007b)
N.xanthodon	50N	70	sm	a	Karabük (Keltepe)	Sözen (2004), Matur et al. (2011)
N.xanthodon	50N	70	sm	a	Kahyalar	Sözen et al. (2006b)
N.xanthodon	50E	70	sm	a	Pasinler (Erzurum), Digor, Selim, Arpaçay (Kars), Hanak, Çıldır, Göle (Ardahan)	Coşkun (2003), Ulutürk et al. (2009)
N.xanthodon	50E	70			Göle (Erzurum)	Kankılıç et al. (2007)
N.xanthodon	50S	70			Andırın (Kahramanmaraş)	Matur et al. (2011)
N.xanthodon	50NE	72	sm	a	Başköy, Ovit pass	Sözen et al. (2006a)
N.leucodon	50	84			Transylvania, (Romania)	Raicu et al. (1968)
N.leucodon/ N.transsylvaticus	50	84			Hajduhadhaz, Hajdubagos (Hungary) Urziceni, Dabaca, Cluj-Napoca (Romania)	Raicu et al. (1968), Soldatovic (1977), Savic and Soldatovic (1977), Soldatovic and Savic (1983), Savic and Nevo (1990)

The specimens examined were deposited at Illia State University in Georgia, and the karyotype preparations were deposited in the Department of Biology, the Faculty of Sciences, Dokuz Eylül University in Turkey.

3. Results and Discussion

The chromosomal complement of the Javakheti mole rats included nine bi-armed and 15 acrocentric autosomes (NFa = 66: Fig. 2a,b, Fig. 3). NF = 70, X is a large sub-metacentric, and Y is a small acrocentric chromosome. Matur et al. (2011) [16] compared four 2n= 50 from Turkey, and named them according to their geographical distribution in Turkey; of these 2n= 50E is found in North-Eastern Anatolia [16]. Two NF types were described previously within 2n=50E: First is NFa = 68, NF = 72 from Pambak, Maralik (Armenia) and Erzurum, Erzincan, Susuz, Ardahan, Rize, Giresun, and Bayburt provinces in eastern Turkey [2, 11-12, 15, 22]. The second is NFa = 66, NF = 70 from Erzurum, Kars and Karabük provinces in Anatolia [4, 9-11, 13-16, 23-24]. It is apparent that the cytotype from Javakheti is more similar to the second described type of 2n=50E (NFa = 66, NF = 70), with the closest reported location less than 100 km away in the Eastern Turkey, but is different from the another nearby location in Armenia (Pambak, Fig. 1). Previously, the small-bodied blind mole rats were described in Georgia and Armenia as *Nannospalax nehringi* Satunin, 1898 - Nehring's Mole Rat [25-27], now synonymous with *N. xanthodon*.
Our results thus confirm that the cytotype 2n=50 is found at the north-eastern edge of *N. xanthodon* distribution range in Georgia. Both of its NF types (NF=70 and NF=72) were classified as a single chromosomal race "*nehringi*" by Arslan et al. (2016) [3], due to their continuous and distinct distribution ranges in the Eastern Turkey, Georgia and Armenia. At the same time, other *N. xanthodon* populations with the same diploid number 2n=50 are found in West, South and North of Anatolia (Matur et al. 2011) [16], and the cytotype 2n=50 of closely related *N. leucodon* is known from the Carpathian basin in Eastern Europe [18] (Raicu et al. 1968, Table 1). There is a substantial variation of NF types among these geographically distant populations (Fig. 2, Fig. 3), thus providing a support to the hypothesis that the same diploid number must have evolved independently in different populations. The cytogenetic variation in the blind mole rats corresponds only partly to their molecular DNA phylogeny, and sometimes even contradicts the accepted species taxonomy within the *Nannospalax* genus, as revealed, for example, by Matur et al. 2019 [7]. Further studies of phylogenetic relationship among the various chromosomal races of all species in the subfamily Spalacinae (both genera *Nannospalax* and *Spalax*), combining both molecular DNA and cytogenetic analyses, are needed to reveal the evolutionary history in this unique group.

a)



b)

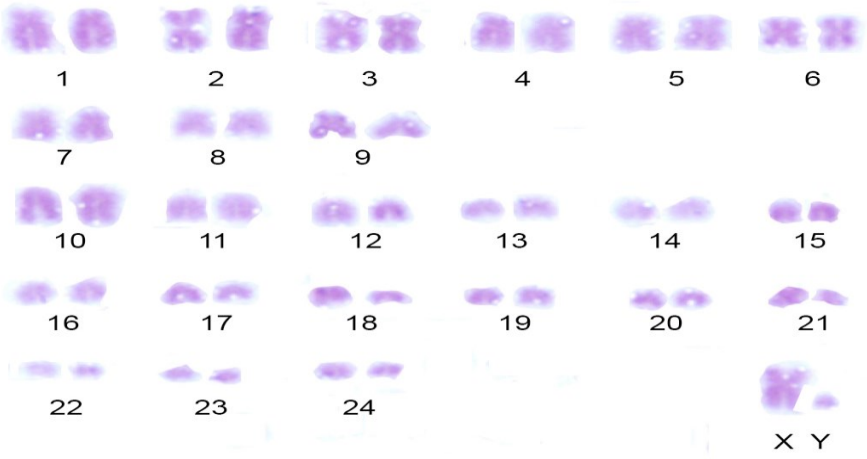


FIGURE 2. Karyotype analysis of Georgian Mole rats. (a) chromosome plate and (b) karyotypes of the samples.

FIGURE 3. Per chromosome comparison of 2n= 50 karyotypes in different populations of mole rats. The corresponding figures from the published sources shown in Table 1 were used. The comparison shows that 2n=50Ge from Javakheti is similar to the nearby 2n=50E in Turkey and 2n= 50leu (*N. leucodon*) has the largest number of bi-armed chromosomes among the other 2n=50.

Acknowledgement. We are grateful to David Tarkhnishvili and Georgi Jankoshvili for helping with logistics of the fieldwork in Javakheti and providing the karyotyping equipment used in our study. Andrei Kandaurov was supported by Georgia government program of scientific research (code 32 05 04).

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Available online: March 24, 2020

Commun.Fac.Sci.Univ.Ank.Series C
Volume 29, Number 1, Pages 71-94 (2020)
ISSN 1303-6025 E-ISSN 2651-3749
https://dergipark.org.tr/en/pub/communc/issue/51836/657262

COMPARATIVE BIOMETRIC AND MORPHOLOGICAL ANALYSIS OF SUBGENUS *TERRICOLA* (RODENTIA: *MICROTUS*) IN EASTERN BLACK SEA REGION FROM TURKEY

Perinçek Seçkinozan ŞEKER, Ercüment ÇOLAK, Teoman KANKILIÇ and Engin SELVİ

ABSTRACT. In the scope of current study, 41 specimens of the subgenus *Terricola* collected from nine different localities in Trabzon, Rize and Artvin provinces in Eastern Black Sea Region were subjected to detailed morphological and comparative biometric analyses. Based on those analyses; presence of two species, *Microtus* (*Terricola*) *subterraneus* and *Microtus* (*Terricola*) *majori* were determined in the region. Evaluation of the 12 specimens belonging to *M.* (*T.*) *subterraneus* showed that there is no intrapopulational variation within this species. Two different populations of *M.* (*T.*) *majori* were determined in the region studied, based on morphological differentiations in enamel cusp patterns of 29 specimens. Additionally, as a result of the evaluations made, it was determined that both species could be found in the similar habitats throughout study area and therefore that these two species can be coexist as sympatric within the same geographic area.

1. INTRODUCTION

The genus *Microtus* is distributed in Holarctic and contains a large number of species. About 65 species are present in this genus throughout its distribution range. Distribution map of the *Microtus* species includes various types of habitats such as meadows, pastures, forests and highlands. In this regard, by having quite different habitat types shaping by the influence of diverse ecological conditions, including mentioned habitats, Turkey is host to 13 *Microtus* species three of them are endemic. [1, 2].

Received by the editors: December 12, 2019; Accepted: March 10, 2020.
Key word and phrases: Terricola, Morphology, Biometry, Eastern Black Sea, Turkey.

The phylogenetic relationships within this genus are unclear, and there are difficulties in identifying species boundaries within the genus and in identifying subgenus [3]. At the same time rapid diversification and speciation processes which originated from mentioned complex relationships having determined by based on morphology, karyology and even mitochondrial DNA in the genus are known to be still continued [1]. This situation has recently led to the need for increase in the number of species described and even updates in the relationships at the subgenus or the genus level [2, 4]. As a natural consequence of this, with the support of the results of a subsequent molecular study too, the fact that *Chionomys* should be treated as a separate genus from *Microtus* was reinforced more. In addition to this, Palearctic species with characterized by the pitymoid condition of the first lower molar of *Microtus* were incorporated into *Terricola* subgenus, while, those with the similar molar teeth morphology in Nearctic were included in *Pitymys* subgenus as well [3].

The subgenus *Terricola* is one of the seven subgenus of *Microtus* and is represented by three species in Turkey [5-9]. *Microtus* (*Terricola*) *majori* and *Microtus* (*Terricola*) *subterraneus* along with the *Microtus* (*Terricola*) *daghestanicus* are closely related three species of *Terricola* and are known to be the members of pine voles group [10]. Distribution range of *M.* (*T.*) *subterraneus*, known as European pine vole, includes mostly Central and South Europe, northwestern Russia and the northern parts of Turkey. Its distribution in Turkey starts from Thrace in the west and lasts up to near Trabzon, which is type locality of *M.* (*T.*) *majori* in the east. Another species, mostly known as Caucasian pine vole, *M.* (*T.*) *majori*, is distributed in the northeastern parts of Turkey, Caucasus and northern parts of Iran. Although it has been previously suggested by some authors that this species has distributed in Europe including Thrace [6-9], Kryštufek et al. [11] has later showed that this species does not live in Europe. The moist forests on the southern coast of the Black Sea in northland of Turkey are the main habitats of these two small subterranean rodent species involved in *Terricola* subgenus. Previous studies has proposed that the distribution ranges of these two species in Turkey are not overlap and thus these species are not in sympatry event [1, 12, and 13]. Apart from these, although not enough data on the distribution of *M.* (*T.*) *daghestanicus*, Kryštufek and Vohralík [1] suggested that this species lives to a limited extent in the distribution range of *M.* (*T.*) *majori* in Turkey.

M. (*T.*) *subterraneus* and *M.* (*T.*) *majori* are morphologically quite similar species to each other and have been well adapted to the subterranean life with short toe and flat skull. Morphological distinction of these species is often not easy, and the distinction between these two species is mostly based on variations in molar tooth morphology

along with the external morphological characters' size. Except for slight differences, the karyotypes of both species are similar. The karyotype of *M.* (*T.*) *majori* consists of 54 chromosomes as it is in the Anatolian populations of *M.* (*T.*) *subterraneus*. Karyotype of European *M.* (*T.*) *subterraneus* populations is different from that of Anatolian populations and contains 52 chromosomes. These two species are found to be genetically very close and the low genetic distance based on allozyme data between them indicates that they have recently diverged [13]. It has later been suggested that this result is to be in conflict with the results of a subsequent DNA study including only one *M.* (*T.*) *majori* sample [3].

Among the representatives of the subgenus *Terricola* from Turkey, *M.* (*T.*) *subterraneus* and *M.* (*T.*) *majori* were more detailed examined. In the limited number of past studies, taxonomical assessments belonging to the subgenus *Terricola* were made by considering the variations in the fur coloration, molar tooth morphology, skull, baculum and karyology and biochemical [1, 12]. Although these studies provide valuable results, biochemical and karyotype studies among them were considered to have more satiable results for distinguishing both species [13], however, outcomes of those based on morphology were thought that they were not sufficient because they could not fully eradicate the complexities in their taxonomy. In addition, all those studies included either a limited number of samples or a limited number of localities from the area where the both species could be coexist. In this context, when the above-mentioned problems were considered, in particular on the representatives of this subgenus living in the localities from Eastern Black Sea Region, it was seen that there was no detailed study comprising morphological examination along with the comparative biometry employing multivariate statistics. Therefore, by present study, it was aimed to examine the morphological aspects of the populations of these two species living in the Eastern Black Sea Region by performing comparative biometric analysis, and thus to contribute to the taxonomic status and distribution of both species in this area.

2. Materials and Methods

A total of 41 samples of *M.* (*T.*) *subterraneus* and *M.* (*T.*) *majori* collected from nine different localities in the Eastern Black Sea Region were evaluated by morphological and biometric methods (Figure 1, Table 1). Samples were collected by the field studies performed between 2000 and 2008 years. In addition, a small number of museum samples were used as well. All samples used in the study were adult and there was no sexual dimorphism in individuals of the species of this subgenus. For detecting adult samples, the uterus and lactating status for female samples and the

testis status for male samples were considered and data recorded during preparation of the samples. All samples were firstly made into standard museum material. Then, in the morphological examinations, the skull, tooth and fur characteristics of the samples were taken into consideration. After treated at 70 °C for 15 minutes by 10% solution of ammonia (NH3) for removing the soft tissue remnants, the skulls were prepared for morphological examination. The skulls and teeth were detailed examined and photographed under the Scienscope SSZ Trinocular Stereo Zoom Microscope (Scienscope International Corporation, Chino, CA, USA). All skulls and skins were stored in the Ankara University Mammalian Research Collection (www.mammalia.ankara.edu.tr, AUMAC) for subsequent investigations.

Before starting the biometric analyses, the morphological criteria suggested by Ognev [14] and Osborn [15] were taken into consideration in the morphological diagnosis of both species. According to that, samples with an extra marked protrusion in the posterior of second upper molar tooth in the lingual side were grouped as *M.* (*T.*) *majori*, while, samples with no such protrusion were considered to be *M.* (*T.*) *subterraneus*. Also, *M.* (*T.*) *majori* samples were divided into two different groups as *M.* (*T.*) *majori* 1 and *M.* (*T.*) *majori* 2 based on the morphological variation determined in the third molar teeth of upper jaw (an extra protrusion in the labial and an additional recess). Ognev [14] has previously proposed that *M.* (*T.*) *majori* samples having such an extra protrusion in the labial and an additional recess from Sümela (Trabzon), where the type locality of this species is, were the nominate subspecies of this species. Therefore, *M.* (*T.*) *majori* samples used in the study were divided into two groups in the statistical analyses by taken into consideration this taxonomic rationale. Thus, all statistical analyses were performed on three different groups together with *M.* (*T.*) *subterraneus* samples.

The data set including four standard external, 22 cranial and eight dental measurements taken from the samples were used in the multivariate statistical analyses (TABLE 2). While preparing the data set, biometric characters frequently used in previous studies were taken into consideration [11, 12, 16 and 17]. For minimizing the measurement error, all the measurements were taken by the same person using the same digital caliper in the same laboratory conditions considering the applications in previous studies [18]. In the first place, the mean and standard error values of the internal and external character measurements of the populations belonging to the three groups were determined as descriptive statistics. Then, one-way analysis of variance (one-way ANOVA), minimizing the type I error in the multivariate data set, was used to determine whether there was significant difference between the group means. A multiple comparison test, Hochberg's GT2 that takes

into account the unequal sample size, were carried out to compare the means of the groups. With the similar purposes of one-way ANOVA, the multivariate analysis of variance (MANOVA) was used to determine if differences in biometric characters had significant effects on the mean vectors of the investigated groups, also whether there was any interaction among both groups and biometric characters. One another multivariate statistical method, the Discriminant Function Analysis (DFA), was carried out; (1) to estimate the relationships between groups and biometric characters, (2) to predict group membership of samples, (3) to test whether samples are classified as predicted and (4) to determine how much of the observed total variance among the groups can be explained by biometric characters.

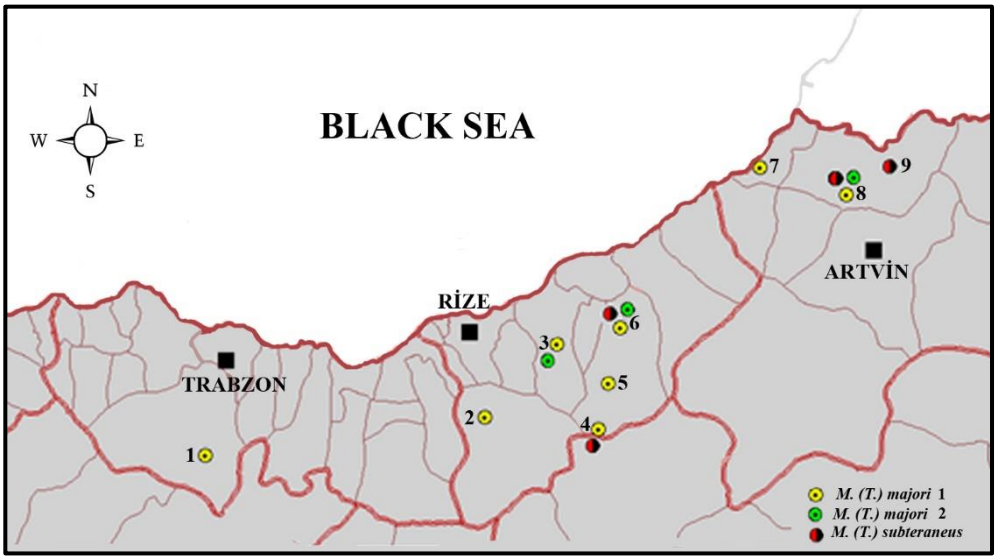

FIGURE 1. Collection sites of the samples; 1. Sümela (Trabzon), 2. İkizdere (Rize), 3. Kaptanpaşa (Rize), 4. Verçenik (Rize), 5. Çat (Rize), 6. Çamlıhemşin (Rize), 7. Hopa (Artvin), 8. Borçka (Artvin), 9. Karagöl-Borçka (Artvin).

In addition, the Principal Component Analysis (PCA), which is a size reduction method, was used to explain total variations among groups by fewer principal components that includes load of those biometric characters rather than a large number of correlated biometric characters. Before applying PCA, whether the data set was suitable for the analysis was checked by the Kaiser-Meyer-Olkin (KMO) measure of sampling adequacy and Bartlett test of sphericity tests. All those analyses

were performed implemented in SPSS 15.0 for Windows [19]. Lastly, a clustering analysis was performed based on the Manhattan distance produced from averages of biometric characters of three groups and the unweighted pair group mathematical averages dendrogram (UPGMA) was created by NTSYSpc 2.2 [20].

TABLE 1. Collection site and sample size of examined populations

Population	**Locality**	**n ♂**	**n ♀**
M. (*T.*) *majori* 1	Sümela	3	7
	Ikizdere	1	-
	Kaptanpaşa	2	-
	Verçenik	-	1
	Borçka	1	1
	Hopa	-	1
	Çat	-	1
	Çamlıhemşin	-	2
M. (*T.*) *majori* 2	Kaptanpaşa	4	-
	Çamlıhemşin	1	1
	Borçka	2	1
M. (*T.*) *subterraneus*	Verçenik	2	1
	Çamlıhemşin	1	-
	Borçka	3	3
	Karagöl	1	1

3. Results

3.1 Morphology

3.1.1 Cranial characteristics

Although the skull was smaller in *M.* (*T.*) *majori* 1 than others were, it was virtually in the same structure in each populations. All the skulls had the entirely delicate structure and were flat looking from the posterior of the nasal bone to the occipital bone. The rostrum region was short and curved downwardly in the anterior. The anterior of the nasal bone was not exceed the anterior of the incisors. The parietal bone was widen to the edges and, in its anterior, indented into the frontal bone in different forms. This indentation seemed like a spearhead in *M.* (*T.*) *subterraneus*, contrary to this, it was in the form of a slight arc in both populations of *M.* (*T.*) *majori*. The brain capsule was wide and flat. In addition, compared to the entire skull, the brain capsule was the most occupant part of the skull in the ventral view. Interorbital region was relatively narrow and long in *M.* (*T.*) *subterraneus*. The same region was relatively wider and shorter in the *M.* (*T.*) *majori* 1 population than in the

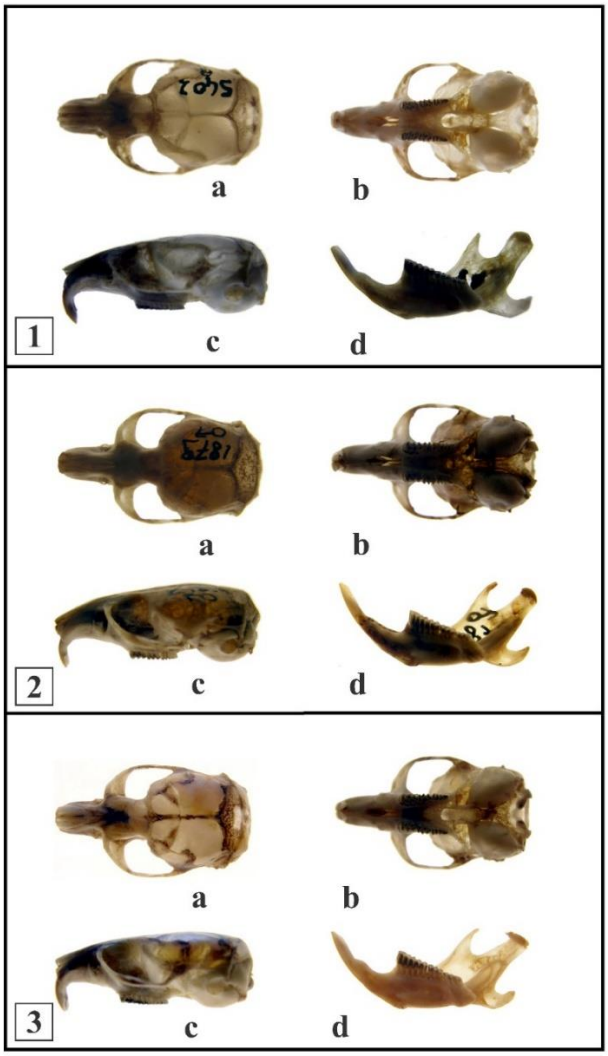

FIGURE 2. Dorsal (a), ventral (b), lateral (c) view of the skulls and right mandibles (d) of *M.* (*T.*) *subterraneus* (1), the first (2) and second (3) populations of *M.* (*T.*) *majori*.

M. (*T.*) *majori* 2 population. The zygomatic arch was fragile and relatively wide. The ventral part of the skull was narrow and triangular. In the ventral, the skull has a flat structure from the middle of the rostrum to the tympanic bullae. The posterior of the foramen incisive was in line with the anterior of the first upper molar teeth, and its

anterior was well behind the incisors. The pterygoid bones extended parallel to each other and ended by inclining towards the lateral from behind the anterior of the tympanic bullae. The tympanic bullae were of normal size compared to the skull. Mandibles were in a sensitive structure in all populations (FIGURE 2).

3.1.2 Dental characteristics

In all three populations, the incisors are orthodont (FIGURE 2, 1c, 2c and 3c). In all of the twelve specimens examined of *M.* (*T.*) *subterraneus* the crown of the first lower molar tooth (M_1) had a triangular like appearance, formed by 6 protrusions and 5 indentations in the lingual side, while, 5 protrusions and 4 indentations in the labial side (FIGURE 3, 1). A similar view was found in 19 of the 20 samples in the first population and in all of the samples of the second population of *M.* (*T.*) *majori* (Fig 3, 2 and 3). In one sample of the first population of *M.* (*T.*) *majori* (Sümela, Trabzon), the protrusion on the labial of the anterior lobe was unclear and thus four protrusions and three indentations were identified in M_1 (Fig 3, 2c). In addition, the same region of M_1 had a rather small and unclear additional protrusion and thus an indentation in three samples of the first population (Sümela: 1, Çamlıhemşin: 2) and in one sample of the second population of *M.* (*T.*) *majori* (FIGURE 3, 2a, 2b and 3a). On the other hand, this structure was not observed in the other samples of the same populations.

The appearance and number of the triangular like closed areas in the anterior lobe of the crown in M_1 was highly variable in all populations (FIGURE 3). This structure or appearance converged narrowly in seven samples of *M.* (*T.*) *subterraneus* (FIGURE 3, 1a and 1b), 13 samples of the first population (FIGURE 3, 2a, 2b and 2e) and seven samples of the second population of *M.* (*T.*) *majori* (FIGURE 3, 3b, 3c, 3d and 3e), whereas, in the remaining samples of each population, they were relatively broadly joined. The number of the triangular like closed areas in M_1 changed between four and six in all populations. A total of six triangular like closed areas in the crown of M_1 were detected in eight samples of *M.* (*T.*) *subterraneus*, twelve of the first population and six samples of the second population of *M.* (*T.*) *majori*. In the three samples of *M.* (*T.*) *subterraneus* (Borçka, Artvin), 12 samples of the first and three samples of the second population of *M.* (*T.*) *majori* (Kaptanpaşa: 2, Borçka: 1), five triangular like closed areas were determined in M_1 (FIGURE 3, 1a, 2c, 2d, 3a, 3b, 3c and 3d). This appearance was the result of the combination of the closed areas in the crown causing to form both the third protrusion in two sides and fourth labial, fifth lingual protrusions. In one sample of *M.* (*T.*) *subterraneus* (Borçka, Artvin), the closed area in the anterior lobe was unambiguously associated

with the closed area in the third lingual and labial protrusions and therefore the number of closed areas was determined as four (FIGURE 3, 1c).

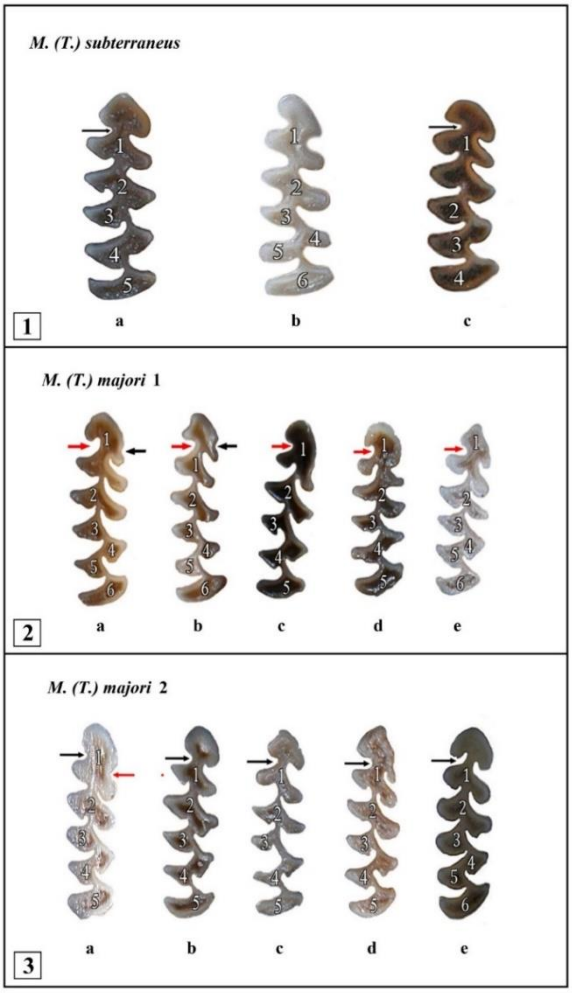

FIGURE 3. Variations of the first molar tooth (M_1) of the lower jaw in the subgenus *Terricola*. Numbers ranging from 1 to 6 show the number of closed areas in the crown of the teeth. In *M.* (*T.*) *subterraneus*, the black arrows show the narrow (1a) and wide (1c) junctions of the closed areas in the anterior lobe. In the samples of the first population of *M.* (*T.*) *majori*, the black arrows indicate an ambiguous indentation in the labial of anterior lobe (2a and 2b). The red arrows show two different types of convergence of narrow spaces (2a, 2b and 2e) and wide (2c and 2d). In the samples of the

second population of *M.* (*T.*) *majori*, black arrows show two different convergence of narrow (3b, 3c, 3d and 3e) and wide (3a) closed areas.

Despite the fact that there was an extra marked protrusion in the posterior of second upper molar tooth (M^2) in the lingual side in the samples from the two populations of *M.* (*T.*) *majori*, while, none of *M.* (*T.*) *subterraneus* samples had such a protrusion (FIGURE 4). This protrusion was unclear in eight samples of the first population and in three samples of the second population of *M.* (*T.*) *majori*. This was quite evident in the other samples of both populations. Triangular like closed areas in the crown of the M^2 formed by the second labial and lingual protrusions were discrete in two samples, completely unified in six samples and combined with a thin line in other samples of *M.* (*T.*) *subterraneus*. Similar morphological variations, in the same order, were detected in three, 13 and four samples of the first population, while, they were determined in three, five and one samples of the second population of *M.* (*T.*) *majori*.

In ten samples of *M.* (*T.*) *subterraneus*, the crown of the third upper molar tooth (M^3) had a triangular like appearance, formed by four protrusions and three indentations in the lingual side, while, three protrusions and two indentations in the labial side. In two samples (Borçka, Artvin), an unclear protrusion was observed in the labial near the posterior end of the M^3. The morphological structure of the M^3 varied considerably in the first population of *M.* (*T.*) *majori*. This structure was as in the *M.* (*T.*) *subterraneus* in 14 samples. The number of triangular like closed areas in the crown of 12 of these samples was three. In one of the remaining two samples, the number of triangular like closed areas was four (Sümela, Trabzon) and the other was two (Borçka, Artvin). The labial of the six samples had three protrusions and two indentations, while the lingual had a fifth an ambiguous protrusion and a fourth indentation in the posterior. In the second population, an extra protrusion and an indentation were observed in the posterior of the labial in the M^3, unlike the first population. Therefore, in all of the samples examined in this population, four protrusions and three indentations in the labial, a fifth ambiguous protrusion and a fourth indentation in the lingual near the posterior of the tooth were determined. In all of the samples of each populations, the number of closed areas in the crown of the M^3 was three (FIGURE 4).

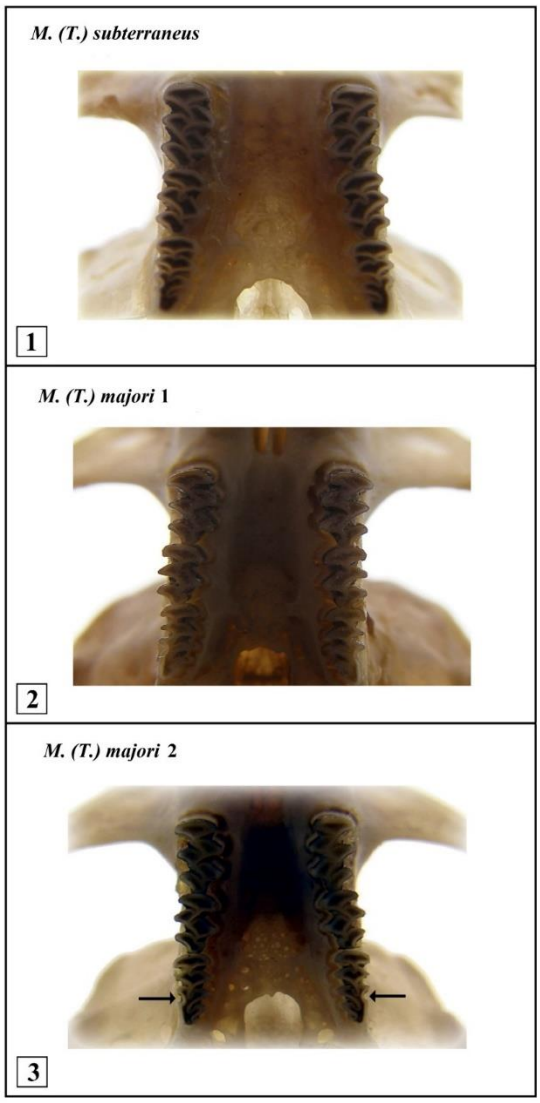

FIGURE 4. Variations in the second and third molar teeth (M^2 and M^3) of the upper jaw in the subgenus *Terricola*. In the samples of the second population of *M*. (*T*.) *majori* (3), the arrows in the right and left lower corners show the different structure formed by an extra protrusion-leading occurrence of a recess in the labial.

3.1.3 Fur coloration

In *M.* (*T.*) *subterraneus*, the fur color was in light brown tones in the dorsal part. The colour of the hairs in the dorsal fur was yellowish brown, gray and pale yellow. Mostly, the colorations on the lateral sides were lighter and grayer than the dorsal, while in some samples yellowish brown close to orange (Borçka: n = 3). There was a borderline separating the dorsal and ventral fur coloration in all samples. The ventral fur was dirty gray color including dominant white tones. In some samples, yellowish tones were observed in the ventral fur (Verçembek: n = 2, Borçka: n = 5, Karagöl: n = 1). The tail was two-color; the dorsal part was brownish gray and the ventral was whitish gray.

In the first population of *M.* (*T.*) *majori*, the dorsal fur colour of the samples changed from yellowish dark brown and gray to reddish. The dorsal fur of the samples obtained from the type locality was markedly reddish. In addition, the dark brown and dark greyish brown tones close to the cinnamon colour were seen in the samples with darker fur other than the type localities. The fur colour was lighter on the lateral sides than in the dorsal and varied from reddish to yellowish brown and gray. Dorsal and ventral fur separated by a distinct line. The ventral part was generally whitish-dirty gray. However, the white tones were seen intensely in the ventral fur of some samples, while, those of others included reddish yellow tones close to orange (Sümela: n = 3, İkizdere: n = 1, Çamlıhemşin: n = 1, Borçka: n = 2, Hopa: n = 1). The tail was in two colors, brown in dorsal part and white in ventral.

In the second population of *M.* (*T.*) *majori*, the dorsal fur colour was matte and varied. Both lighter and darker tones were observed than that of the first population of *M.* (*T.*) *majori*. It usually ranged from light and dark yellowish brown and blackish dark gray to dark brown close to cinnamon colour, even reddish. The fur on the lateral sides consisted of grey and reddish yellow hairs. The ventral fur was dirty greyish white and in some samples (Çamlıhemşin: n = 1, Borçka: n = 2) was markedly orange-yellow. The tail was two-color; greyish brown close to the black in dorsal and dirty white in ventral (FIGURE 5).

FIGURE 5. Dorsal and ventral colour variations of the fur in *M.* (*T.*) *subterraneus* (1a and 2a), the first population (1b and 2b) and the second population (1c and 2c) of *M.* (*T.*) *majori*.

3.1.4 Biometry

Mean and standard errors, as descriptive statistics, of the 34 biometric characters belonging to the three populations in the subgenus *Terricola* were indicated in TABLE 2. The one-way ANOVA results showed that statistically significant differences ($P<0.05$) were found between mean of the five (hind foot length, cranium length, upper molars alveoli length, M^2 length and M_1 length) of the total 34 biometric characters (TABLE 3). Hochberg's GT2 results demonstrated that mean of hind foot length was significantly differed between *M.* (*T.*) *subterraneus* and first population of *M.* (*T.*) *majori*. It was also determined that the mean of the other remaining four biometric characters were statistically different between *M.* (*T.*) *subterraneus* and second populations of *M.* (*T.*) *majori* by the same multiple comparison test. The MANOVA results showed no significant difference ($P> 0.05$) between group mean vectors of three groups (TABLE 4).

TABLE 2. Mean and standard error values of biometric characters in the subgenus *Terricola*

	M. (T.) subterraneus		***M. (T.) majori* 1**		***M. (T.) majori* 2**	
Biometric characters	**Mean**	**SE**	**Mean**	**SE**	**Mean**	**SE**
Total body length	135,2725	3,10691	141,2105	1,82321	143,4444	4,03840
Tail length	39,8183	1,57547	41,1055	0,72509	44,7778	2,38501
Hind foot length	16,3325	0,60302	18,1580	0,23241	16,8322	0,45389
Ear length	9,6675	0,53654	10,0525	0,43814	10,1678	0,42219
Zygomatic Breadth	13,5158	0,19176	13,7820	0,13058	14,0267	0,31796
Rostrum Breadth	3,8375	0,03289	3,8455	0,02805	3,9467	0,04272
Interorbital Breadth	4,1075	0,07145	4,0340	0,05807	4,2122	0,04542
Condylobasal Length	21,7408	0,23728	22,2275	0,15846	22,4267	0,38932
Condylonasal Length	23,2067	0,24986	23,7230	0,19287	24,0344	0,39311
Occipitonasal Length	22,8925	0,24912	23,3775	0,18100	23,6911	0,34280
Basal Length	20,5917	0,24820	21,0610	0,16132	21,0822	0,39444
Nasal Length	6,8025	0,11112	6,9270	0,08620	7,0378	0,13308
Nasal Breadth	2,9208	0,03331	2,8350	0,05206	2,9178	0,03789
Frontal Length	11,8808	0,17517	12,0530	0,10955	12,0611	0,18375
Parietal Length	3,5383	0,07248	3,6985	0,11406	3,4978	0,07940
Facial Region Length	14,4508	0,15946	14,8645	0,15327	14,9522	0,28418

Cranium Length	9,1550	0,15831	9,3760	0,09416	9,7144	0,19476
Mastoid Breadth	7,0025	0,08408	7,0225	0,05498	7,0789	0,08613
Cranium Depth	8,0675	0,10623	8,0580	0,05859	8,3144	0,11329
Cranium Breadth	11,3967	0,13270	11,4295	0,10550	11,7422	0,10460
Diastema Length	6,9383	0,12516	7,1575	0,07458	6,9511	0,18336
Incisive Foramen Length	3,8992	0,14170	3,9845	0,05220	3,8300	0,13995
Incisive Foramen Breadth	0,9733	0,03803	1,0375	0,02323	1,11267	0,10918
Tympanic Bulla Length	6,1042	0,09352	6,1480	0,04475	6,1956	0,13052
Mandible Length	13,2558	0,12697	13,4060	0,11239	12,0756	1,35466
Mandible Height	6,2742	0,10096	6,4650	0,11976	6,2244	0,12443
Upper Molars Alveoli Length	5,3892	0,07103	5,5615	0,03975	5,6667	0,08660
Lower Molars Alveoli Length	4,9400	0,06748	5,1215	0,04887	5,0856	0,05786
M^1 Length	1,8033	0,01920	1,8460	0,01177	1,8533	0,02682
M^2 Length	1,3700	0,03119	1,4145	0,01863	1,5200	0,05292
M^3 Length	1,7017	0,03128	1,7160	0,01210	1,7311	0,04373
M_1 Length	2,3675	0,03635	2,4540	0,03042	2,5289	0,05208
M_2 Length	1,2617	0,02081	1,2910	0,01591	1,2667	0,02261
M_3 Length	1,2050	0,02816	1,2775	0,01981	1,2833	0,03659

TABLE 3. One-way ANOVA results among three populations of the subgenus *Terricola*

Biometric characters	**S.S. (among groups)**	**S.S. (within groups)**	**F**	**d.f. (among groups)**	**d.f. (within groups)**	**P**
Total body length	404,219	3711,562	2,069	2	38	0,140
Tail length	133,693	936,981	2,711	2	38	0,079
Hind foot length	27,882	83,360	6,355	2	38	0,04*
Ear length	1,585	123,781	0,243	2	38	0,785
Zygomatic Breadth	1,365	18,612	1,393	2	38	0,261
Rostrum Breadth	0,077	0,573	2,543	2	38	0,092
Interorbital Breadth	0,200	2,104	1,806	2	38	0,178
Condylobasal Length	2,800	27,887	1,907	2	38	0,162
Condylonasal Length	3,791	33,503	2,150	2	38	0,130
Occipitonasal Length	3,489	29,101	2,278	2	38	0,116
Basal Length	1,925	29,222	1,252	2	38	0,298
Nasal Length	0,290	5,729	0,963	2	38	0,391
Nasal Breadth	0,073	1,280	1,087	2	38	0,347
Frontal Length	0,259	11,042	0,446	2	38	0,643

Parietal Length	0,331	6,091	1,034	2	38	0,365
Facial Region Length	1,698	18,098	1,782	2	38	0,182
Cranium Length	1,613	9,408	3,258	2	38	0,049*
Mastoid Breadth	0,032	2,616	0,230	2	38	0,796
Cranium Depth	0,450	3,718	2,299	2	38	0,114
Cranium Breadth	0,750	7,342	1,941	2	38	0,157
Diastema Length	0,469	6,602	1,349	2	38	0,272
Incisive Foramen Length	0,160	5,096	0,597	2	38	0,556
Incisive Foramen Breadth	718,467	7358,479	1,855	2	38	0,170
Tympanic Bulla Length	0,043	3,142	0,261	2	38	0,772
Mandible Length	11,573	139,055	1,581	2	38	0,219
Mandible Height	0,474	7,911	1,138	2	38	0,331
Upper Molars Alveoli Length	0,425	1,806	4,473	2	38	0,018*
Lower Molars Alveoli Length	0,254	1,750	2,762	2	38	0,076
M^1 Length	0,017	0,153	2,168	2	38	0,128
M^2 Length	0,120	0,462	4,925	2	38	0,013*
M^3 Length	0,004	0,323	0,264	2	38	0,769
M_1 Length	0,137	0,721	3,609	2	38	0,037*
M_2 Length	0,008	0,190	0,770	2	38	0,470
M_3 Length	0,047	0,350	2,554	2	38	0,091

TABLE 4**.** MANOVA results

Effect		**Value**	**F**	**Hypothesis df**	**Error df**	**Sig.**
Groups	Pillai's Trace	1,818	1,758	68	12	0,140***
	Wilks' Lambda	0,007	1,640	68	10	0,200***
	Hotelling's Trace	24,953	1,468	68	8	0,294***
	Roy's Largest Root	18,297	3,229	34	6	0,072***

Eigenvalue statistics were found to be significant for two canonical discriminant functions determined by DFA (Wilk's Lambda = 0.457, P < 0.001). As a result of DFA, the first canonical discriminant function explained the 81.4% of the total observed variations among three groups, while, the second one clarified 16.8% of the total variations. In the classification matrix, it was determined that 75.6% original group cases correctly classified (TABLE 5).

TABLE 5. Classification matrix obtained by DFA

GROUPS	**ACCURACY (%)**	**Predicted Group Membership**		
		1	2	3
1. *M. (T.) majori* 1	80	16	3	1
2. *M. (T.) subterraneus*	75	3	9	0
3. *M. (T.) majori* 2	66,7	2	1	6

According to the obtained canonical scores, the relative positions of the groups to each other was shown in the scatter plot. Pursuant to the scatter plot, the two populations of *M.* (*T.*) *majori* were spread closer to each other, while they were relatively more distant than the *M.* (*T.*) *subterraneus* (FIGURE 6).

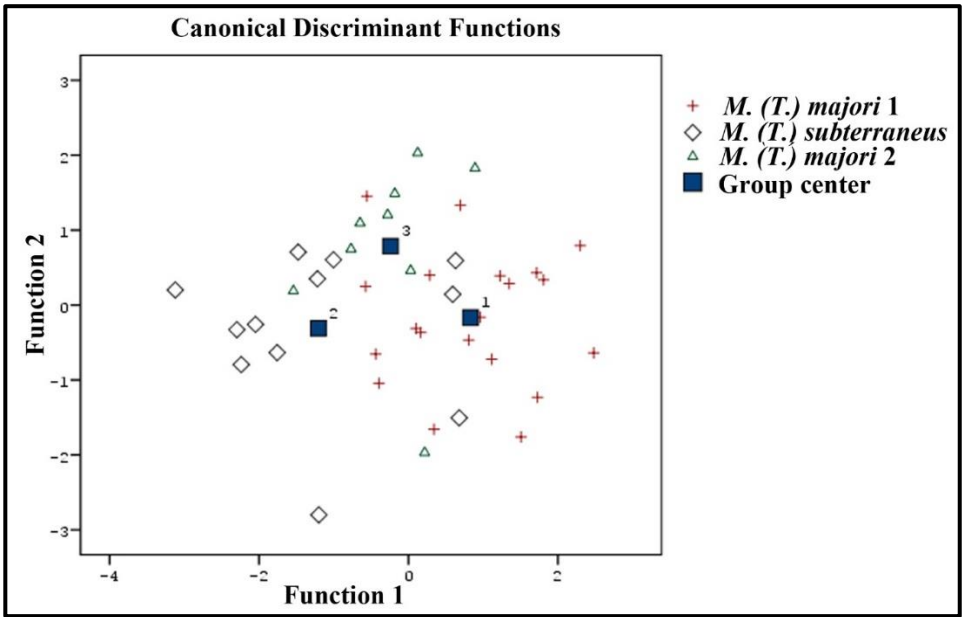

FIGURE 6. Scatter plot showing relative positions of three populations of *Terricola*

KMO measure of sampling adequacy was 0.645 and Bartlett's test of sphericity was significant at P<0.001 level, which indicated the data set was highly suitable for PCA. The first seven principal components, having eigenvalues greater than 1, explained a cumulative 74.487% of the total variation of the full data in the PCA. The loads of all biometric characters in the principal components were shown in the rotated component matrix (TABLE 6). According to the matrix, 13 cranial characters having loads whose absolute value greater than 0.5 were determined under the first principal component. Similarly, the total body length and the two dental characters (M^3 and M_3 Length) also contributed to the first principal component (explained variance: 41.956%). The second principal component included four cranial and one dental character (explained variance: 7.717%). As for the third principal component, the loads of dental characters, which were considered discriminative characters, predominantly contributed that (explained variance: 7.268%). While the fourth

principal component composed of the loads of external characters (explained variance: 5.877%), three cranial characters had the load on the fifth and sixth principal components (explained variance: 4.811% and 3.614%, respectively). Only hind foot length had the load on the last principal component (explained variance: 3.244%).

TABLE 6. Rotated component matrix produced by PCA

	Principal components						
Biometric characters	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Facial Region Length	0,924	0,029	0,172	0,123	0,043	-0,034	0,023
Basal Length	0,918	0,147	0,260	-0,057	0,079	0,017	0,061
Condylonasal Length	0,917	0,233	0,184	0,021	0,017	0,029	-0,040
Condylobasal Length	0,912	0,196	0,223	0,030	0,054	0,110	0,018
Occipitonasal Length	0,902	0,272	0,178	0,061	0,047	0,093	0,015
Diastema Length	0,854	-0,161	0,019	0,018	0,169	0,173	0,048
Nasal Length	0,797	0,211	0,153	0,045	-0,010	-0,120	0,200
Cranium Length	0,765	0,404	0,081	-0,193	-0,049	0,010	-0,075
Zygomatic Breadth	0,741	0,399	0,172	-0,026	-0,085	0,299	0,081
Upper Molars Alveoli Length	0,734	0,200	0,424	0,064	0,014	-0,041	-0,193
Tympanic Bulla Length	0,706	0,203	0,060	-0,168	0,056	-0,038	-0,095
Total body length	0,652	0,065	0,243	0,355	-0,071	0,366	0,218
Frontal Length	0,637	0,153	0,067	-0,010	0,159	-0,044	-0,229
Incisive Foramen Length	0,521	-0,081	-0,180	-0,131	0,346	0,450	-0,130
M_1 Length	0,498	0,375	0,424	0,066	-0,107	0,320	0,072
Interorbital Breadth	-0,072	0,840	-0,119	-0,062	0,138	-0,253	-0,086
Cranium Breadth	0,382	0,795	0,115	-0,041	-0,069	0,125	0,004
Cranium Depth	0,477	0,759	0,104	-0,083	-0,096	-0,065	-0,062
Rostrum Breadth	0,368	0,579	0,322	0,259	-0,188	-0,094	0,107
M^1 Length	0,422	0,506	0,415	0,038	0,237	0,077	0,194
M_2 Length	0,026	-0,099	0,727	-0,174	-0,248	-0,092	0,192
M^2 Length	0,379	0,125	0,680	0,206	-0,160	0,177	-0,280
M^3 Length	0,524	0,280	0,608	-0,057	0,148	0,035	-0,253
M_3 Length	0,588	-0,016	0,597	-0,036	0,058	-0,060	0,047
Lower Molars Alveoli Length	0,407	0,233	0,587	-0,006	0,106	0,014	0,252
Ear length	0,093	-0,022	-0,047	0,650	0,258	0,254	-0,006
Tail length	0,450	0,280	-0,019	0,626	-0,292	0,308	0,163
Mastoid Breadth	0,373	0,204	0,143	-0,526	0,136	0,109	-0,103
Mandible Length	-0,131	-0,043	0,373	0,421	0,244	-0,208	0,288
Parietal Length	0,201	0,281	-0,066	-0,036	0,717	0,114	0,100
Nasal Breadth	-0,025	0,278	0,022	-0,130	-0,652	0,091	0,088
Incisive Foramen Breadth	0,086	0,135	-0,013	-0,161	-0,005	-0,811	0,046
Hind foot length	-0,081	-0,036	0,059	0,185	-0,057	-0,064	0,855
Mandible Height	0,275	0,127	0,428	-0,295	0,319	0,157	0,465

The UPGMA dendrogram based on a pairwise matrix of Manhattan distances calculated by biometric differentiation among populations showed similar results to the results of DFA shown by the scatter plot. According to this, the first and second populations of *M.* (*T.*) *majori* were clustered together, and *M.* (*T.*) *subterraneus* was created a separate branch from them (Figure 7).

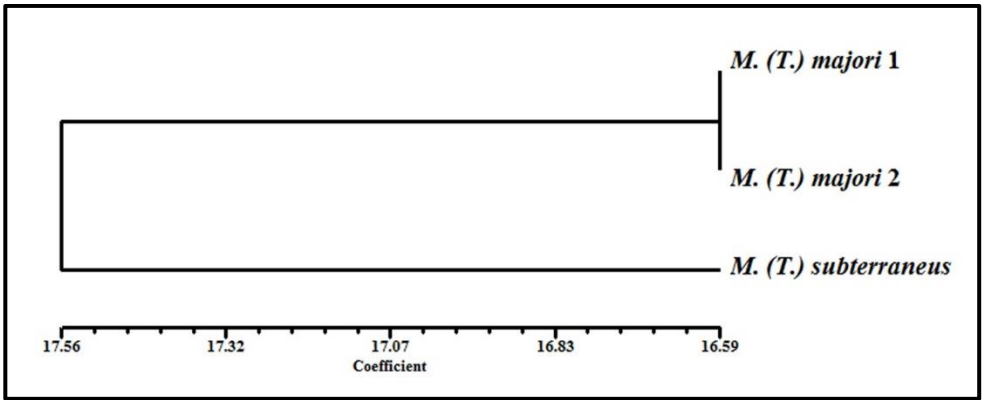

FIGURE 7. The UPGMA dendrogram based on a pairwise matrix of Manhattan distances

4. DISCUSSION

In the scope of this study, morphological and biometric aspects of populations of the subgenus *Terricola* from Eastern Black Sea Region in Turkey were investigated. According to the findings, it was thought that morphological and biometric differences observed in the molar tooth structure of the populations were more useful to some extent, rather than external morphological characters and skull characters to distinguish the populations. Additionally, unlike known distribution of *M.* (*T.*) *subterraneus* in Turkey, it was determined for the first time that this species could be occurred in the localities of Rize and Artvin, which are further east of Trabzon within distribution range of *M.* (*T.*) *majori*. Thus, that *M.* (*T.*) *subterraneus* and *M.* (*T.*) *majori* can be coexist as sympatric within the same geographic area was also detected.

Kryštufek and Vohralík [1] consider the east Anatolian populations of *M.* (*T.*) *subterraneus* to be easily separable from *M.* (*T.*) *majori* in terms of interorbital construction and the dorsal profile of the skull. In addition to this, they have stated that *M.* (*T.*) *subterraneus* has deeper skull than *M.* (*T.*) *majori* has. The skulls of the

examined populations of both species in the scope of current study were nearly in the same structure except for the some slight differences statistically insignificant. A narrower and longer interorbital construction in the skull of *M.* (*T.*) *subterraneus* than that of *M.* (*T.*) *majori* determined by this study could be perhaps considered to be a separator morphological differentiation between the skulls of two species as it was suggested by Kryštufek and Vohralík [1]. It was observed that the dorsal profile of all skulls of both species had no marked differences from each other unlike the findings (concavity) of Kryštufek and Vohralík [1], while, it was compatible with the determinations of Çolak et al. [12]. Additionally, no significant difference were detected too among cranium depth of both species unlike the statement of Kryštufek and Vohralík [1]. Except those, as a slight morphological distinction between the skulls of both species, it was detected by the study that the anterior of the parietal bone was in a spearhead form in *M.* (*T.*) *subterraneus* or slight arc form in *M.* (*T.*) *majori*. However, it is worth mentioning that a sample series is needed to be able to generalize this as a strong diagnostic character.

The dorsal and ventral fur color is highly variable and importantly differ between the two species. Alterations and dissimilarities in the fur color of examined samples is as partly in stated by Çolak et al. [12] and partly in stated by Kryštufek and Vohralík [1]. Accordingly, dorsal fur color can be regarded partly to be a separator character and respectively generalized as light brown in *M.* (*T.*) *subterraneus*, dark brown in the first population of *M.* (*T.*) *majori* and dull including lighter and darker brown tones in the second populations of *M.* (*T.*) *majori*. The color of the ventral fur in the distinct populations of both species includes the diverse intensity of each color tone, but is generally whitish-dirty gray. This coloration observed within all populations create more complexity rather than a distinction between them. Therefore, the color of the ventral fur does not exactly represent a distinctive character feature. Contrary to the observations of Kryštufek and Vohralík [1], there is a clear boundary line on the lateral of the specimens of both species that distinguishes the color of dorsal and ventral fur as previously determined by Çolak et al. [12]. The tail fur is bicolor and mostly incorporates brown above side and grey plume below side. They are also in highly variable tones of brown and grey, creating more complexity rather than a distinction.

All observed variations of the first lower molar, such as number of protrusion and indentation in labial and lingual, shape of the triangular like closed area in the anterior lobe and total number of closed area, were highly variable and shared within both species. Therefore, it is thought that first lower molar were not discriminative in respect to the morphological structure. This is a case determined before by

Kryštufek and Vohralík [1], and individuals with different tooth structures were evaluated as morphotype within each species according to the mentioned variations. In contrast to this complex case observed in morphology of the first lower molar, it was detected by the one-way ANOVA and Hochberg's GT2 that statistically significant differences was found between mean of the first lower molar length of *M*. (*T*.) *subterraneus* and second populations of *M*. (*T*.) *majori* by biometric evaluations. In a way that makes this statistics insignificant, it was seen that the first lower molar tooth length did not contribute to any principal component in PCA. In contradistinction to the complexity arising from the variable structure of the first lower molar, it is believed that the second upper molar is more powerful separator in real terms for distinguishing of both species because of the extra marked protrusion in its posterior. As a matter of fact, this morphological differentiation found in *M*. (*T*.) *majori* populations had formerly been proposed as a distinctive character [14, 15]. In addition to this apparent morphological differentiation in the second upper molar, biometric difference in the mean of the second upper molar length between *M*. (*T*.) *subterraneus* and *M*. (*T*.) *majori* were also found to be statistically significant. Supporting to this, it was detected that the load of mentioned biometric character was contributed to the third principal component that explains 7.26 % of total variations in PCA. In addition, unlike the morphological structure of the third upper molar in the first population of *M*. (*T*.) *majori*, that of the second *M*. (*T*.) *majori* population had a protrusion that leads to an extra indentation in the labial. However, it is useful to state that this morphological structure was found to be statistically insignificant. All specimens with such a tooth morphology were treated as nominate subspecies of *M*. (*T*.) *majori* by Ognev [14]. Since it is clear that additional studies are needed to define a new subspecies, no attempt was made in this sense and mentioned populations of *M*. (*T*.) *majori* was evaluated as two separate populations as first and second.

It has been suggested that the tail length is relatively longer in *M*. (*T*.) *majori* than that of *M*. (*T*.) *subterraneus* by Kryštufek et al. [11] and Çolak et al. [12]. The one-way ANOVA and Hochberg's GT2 results showed that there was no statistically significant difference between group means in terms of this character. Even more the load of this character contributed to the fourth principal component that explains a small percentage (5.87 %) of total variations observed in rotated component matrix produced by PCA. Therefore, it can be said that the relative differences in the tail length, which have been preciously used to be a discriminative character between the populations, was not statistically significant.

According to the results of Kryštufek et al. [11], 12 cranial characters employing in DFA was useful to distinguish the *M.* (*T.*) *subterraneus* and *M.* (*T.*) *majori* by explaining 91.8 % of total variations. By this study, more variables were utilized in DFA (34 morphological characters) and similarly with the findings of Kryštufek et al. [11], the high percentage of variation (81.4%) was detected among three groups. As it was stated by Kryštufek et al. [11], the suggestion that morphological characters used in DFA could be beneficial in separating the two species was well projected by the scatter plot showing relative positions of three populations of *Terricola*. Besides, the samples were grouped with a high percentage of accuracy in the classification matrix. Similar clustering of three populations in the UPGMA dendrogram to the scatter plot is another important result supporting this condition. Moreover, the fact that 12 of the total 21 characters contributing to the first and second principal component yielded by PCA are the same as those in DFA performed by Kryštufek et al. [11] was another finding that coherently supports the results of DFA performed by current study. However, approximate 46% of the total variance in the discriminant scores could not be explained by morphological differences between the groups, according to the Wilk's Lambda statistics revealed by DFA (Wilk's Lambda = 0.457, $P < 0.001$). The results of MANOVA also support this situation. This also could be thought of as a situation that shows that biometric characters used in the study have a not very strong discriminatory power, even though high the percentage of variation was detected.

Morphological evaluations and the results of multivariate statistical analyzes using biometric characters showed that there was a certain degree of morphological and biometric differentiation between the populations of the *Terricola* subgenus living in the Eastern Black Sea Region and that no definitive distinction could be made between these subspecies. Additional research using molecular techniques should be conducted to make a more definitive judgment on the taxonomic status of this subgenus in the study area.

Acknowledgement: This study was produced from Master's Thesis of Perinçek Seçkinozan ŞEKER, Ankara University, Graduate School of Natural and Applied Sciences, Department of Biology, 2009.

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Available online: March 24, 2020

Commun.Fac.Sci.Univ.Ank.Series C
Volume 29, Number 1, Pages 95-104 (2020)
ISSN 1303-6025 E-ISSN 2651-3749
https://dergipark.org.tr/en/pub/communc/issue/51836/ 644574

ANTIMUTAGENIC AND ANTIOXIDANT ACTIVITIES OF TEUCRIUM MULTICAULE AND ITS CYTOTOXIC EFFECT ON MURINE LR7 CELL

Mustafa PEHLIVAN, Önder YUMRUTAŞ, and İbrahim BOZGEYIK

ABSTRACT. In present study, antimutagenic, antioxidant and cytotoxic effects of *Teucrium multicaule* Montbret Et Aucher Ex Bentham methanol extract (TME) was investigated. Cytotoxic effects and antimutagenic and antioxidant activities of TME were determined by MTT, DNA protecting, DPPH scavenging methods, respectively. As a result, high dose of TME exhibited a cytotoxic effect on murine LR7 cells. TME also showed a strong antimutagenic activity in DNA protection test system, it exhibited modest activity in DPPH test system. Consequently, it may be used as natural agent for antioxidant and antimutagenic properties.

1. Introduction

Traditional medicine practices are common in many parts of the world [1-3]. Being used as medicinal plants family, the genus *Teucrium* contain about 300 species worldwide [4], 27 of which is spread in Turkey flora [5]. The main distribution area of the genus *Teucrium* is not only the Mediterranean, but also it has a high spread in other continents [6-8]. *Teucrium* species have many biological activities including antioxidant, antimicrobial [9], antitumor [10], and DNA protecting [11].

Teucrium multicaule Montbret & Aucher ex Benth. is perennial and suffruticose form. Stems 12-40 cm, many, erect or ascending, pubescent. Inflorescence laxly racemose. *T. multicaule* spreads between 500-1600 m altitude, and blooms between April and July [5].
The information on the biological activities of *T. multicaule* is very limited. According to our literature review, there is no study about the antioxidant and antimutagenic activities and cytotoxic effects of *T. multicaule*. Therefore, in this

Received by the editors: November 08, 2019; Accepted: March 10, 2020.
Key word and phrases: Cytotoxicity, DNA protection, Antioxidant, Antimutagenic, *Teucrium multicaule*

study, antioxidant activity in DPPH system, DNA protecting activity against to hydroxyl radical and cytotoxicity effect on murine LR7 cells of *T. multicaule* were determined. Besides, some of the phenolic compounds of *T. multicaule* used were identified by LC-MS-MS.

2. Materials and Methods

2.1. Collection and extraction of T. multicaule

Collection of T. multicaule and extraction were performed as previously described [12]. Briefly, leaf and flower materials were air-dried and standard Soxhlet isolation procedure was followed.

2.2. Determination of DPPH scavenging activity

DPPH scavenging activity of TME was determined according to the method applied by [13].

2.3. DNA protecting activity of TME against hydroxyl radical

DNA protection activities of TME were detected using the pBR322 supercoiled DNA. Standard solutions were prepared at 25, 50, 100, and 200 µg/mL ratios of the extract. First, 0.5 µg of plasmid pBR322 supercoiled DNA was put into the Eppendorf tubes, then, 10 µL of the standard extracts solutions were added into tubes. Also, 10 µL of Fenton's agent (30 mM H2O2, 50 µM ascorbic acid, and 80 µM FeCl3) was added into the prepared solution. The tubes incubated for 10 minutes at the room temperature. The final volume of the mixture was prepared to be 20mL and incubated for 30 minutes at 37 °C. Then, the DNA was analyzed by electrophoresis on 1% agarose gel containing ethidium bromide [14].

2.4. Cytotoxic effect of the TME on Murine LR7 cells

The viability of LR7 was determined by MTT (3- [4,5- dimethylthiazol- 2- yl]- 2,5- diphenyl- tetrazolium bromide) method. Briefly, different doses of TME (25, 50, 100, and 200 µg/mL doses) was applied on LR7 cells (1 × 106 mL) and incubated for 24 hours. After, cells were incubated with 1 mg/mL MTT for 45 minutes at 37 °C. Later, MTT was removed, and dimethyl sulfoxide (DMSO) was used to dissolve the dyes absorbed. Then, the absorbance for each well was measured at 570 nm in

an EZ Read 400 Microplate Reader (Biochrom, Cambridge, UK). The experiments were repeated three times.

2.5. Photochemical screening by LC-MS/MS

The sample was prepared for phytochemical analysis of TME by dissolving in methanol and filtering through a 0.22-μM filter. The separation of phenolic was performed with a LC-MS-MS apparatus of Nexera UHPLC (Shimadzu) with two LC-20AD pumps, DGU-20A3R degasser, CTO-10ASVP column furnace, and SIL-20AC auto sampler. Besides, C18 Intersil ODS-4 analytical colon (3.0mm x 100mm, 2μm) was used. The injection volume was 2 μL and flow rate 0.3 mL/min. Mobile phase A (water and 0.1% formic acid) and mobile phase B (methanol and 0.1% formic acid) were used in a linear gradient flow and the initial column temperature was set to 40 °C.

2.6. Statistical Analysis

The obtained data were determined as the mean ± standard deviation. SPSS version 22.0 software was used for statistical analysis. Intergroup evaluations were performed by a one-way ANOVA and post-hoc Tukey test. In all the analyses value of $p<0.05$ was considered statistically significant.

3. Results

3.1. Determination of DPPH scavenging and DNA protecting activities

DPPH scavenging percentage of different concentration of TME is shown in Figure 1. DPPH scavenging activity was increased after extract application in a dose-dependent manner (Figure 1). The TME exhibited a moderate antioxidant activity. 2 mg/ml dose of TME showed the highest scavenging activity ($p<0.05$)(40.8%). Furthermore, DNA protection activity of TME against the hydroxyl radical on plasmid DNA pBR322 was shown figure 2. As can be seen figure 2, supercoiled pBR322 DNA was protected by the presence of the all extract concentrations when compared with the control group.

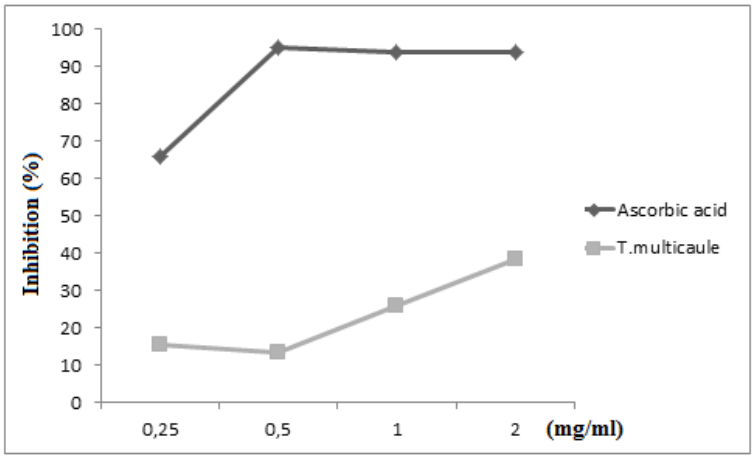

FIGURE 1. DPPH scavenging activity of TME.

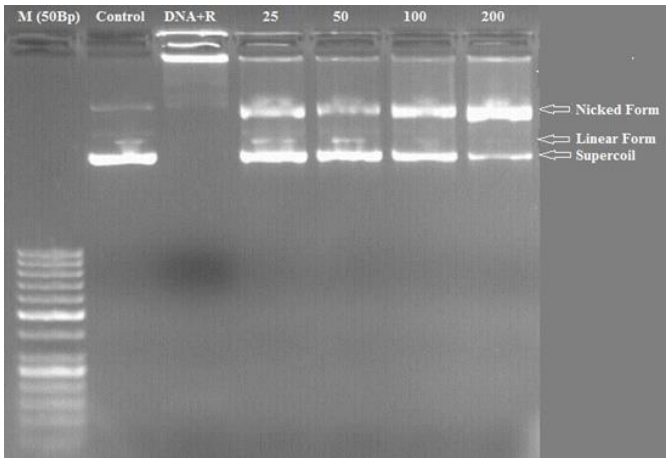

FIGURE 2. DNA protecting activity of TME. M: Marker, Negative control (DNA + R): pBR322 + OH radical, 25, 50, 100 and 200 µg / mL doses + OH radical.

3.2. Cytotoxic effects of TME on Murine LR7 cell

The murine LR7 cells were cultured in presence of the 25 to 200 µg/mL of TME at overnight (figure 3). It was observed that 25, 50, and 100 µg/mL of extracts did not

have an efficient cytotoxic effect on the cells, but the 200 µg/mL extract showed a significant cytotoxic effect ($p<0.05$).

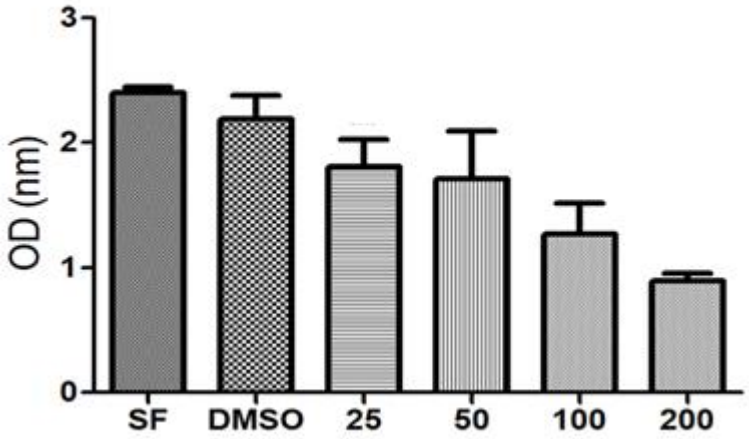

FIGURE 3. Effects of TME on cell viability of murine LR7 cells.

3.3. Determination of phenolic compounds of TME by LC-MS-MS

TME was screened to determine the phenolic compounds by LC-MS-MS. In total, 19 compounds were screened and only 13 were quantified. (Table 2). Among the identified phenolic compounds, it was found that TME contained the highest amount vanillic acid (50229.082 mg/kg) and Myrcetin (1005.257mg/kg). On the other hand, fumaric acid, gallic acid, ellagic acid, protocatechuic acid, kaempherol and thymokinone could not be detected in TME methanol extract.

TABLE 2. Compounds in TME by LC-MS-MS

	Compounds	**Amonts (mg/kg)**
1	Quercetine	12.78
2	Vanillic acid	50229.082
3	Resveratrol	212.608
4	Fumaric acid	0
5	Gallic acid	0
6	Caffeic acid	11.82
7	Phloridzindyhdrate	323.993
8	Oleuropein	77.631
9	Hidroxy cinnamic acid	418.114
10	Ellagic acid	0
11	Myrcetin	1005.257
12	Ptotocathecuic acid	0
13	Naringenin	0.857
14	Luteolin	11.858
15	Kaemperol	0
16	Thymoqunione	0
17	Alizarin	575.782
18	Hydroxybenzoic acid	5.486
19	Salicilic acid	3.972

4. DISCUSSION

In the present study, antimutagenic and antioxidant activities were determined by DNA protection and DPPH scavenging test systems, respectively. The DPPH method is one of the most practical methods used to determine the antioxidant activity of compounds. In the present study, different doses of TME showed a moderate antioxidant activity in a dose-dependent manner. The highest DPPH radical scavenging activity was observed at a dose of 2 mg/mL of extract. In previous studies, antioxidant activities of different species of *Teucrium* genus have been reported. *T. polium* exhibited strong antioxidant activity at low doses, while its petroleum ether and chloroform extracts exhibit low activity [15]. [16], reported that the methanol extracts of *T.* orientale collected during vegetative period showed strong DPPH scavenging activity.

DNA protecting activity was used as antimutagenic method. In this test, the hydroxyl radical formed as a result of the Fenton reactions cause fractures on DNA by

targeting the sugar-phosphate backbone [17]. If DNA is protected by an antioxidant molecule, the hydroxyl radical does not damage DNA. It was observed that in the DNA protecting activity test, the pBR322 was fragmented by the hydroxyl radical in the absence of TME, however, the harmful effect of hydroxyl radical on pBR322 was minimized at different concentrations of the TME. It can be said that our data is the first report to demonstrate in vitro DPPH scavenging and DNA protection activity of TME. In an *in vivo* study of ischemia-reperfusion conducted by [18], it was determined that *T. multicaule* reduce MDA level, as an indicator of tissue damage caused by radicals, and increased the level of GSH as an antioxidant compound.

Previous studies have also reported the effects of extracts of *Teucrium* species on different cells. For instance, [19] reported that *T. polium* water and ethanol extracts showed cytotoxic effects on human HepG2, A549, and HeLa cells. In addition to that, it reported that *T. polium* ethanol extract has cytotoxic effects on human A549, MCF7, BT20, and PC12 cells [20]. It was determined that neo-clerodane diterpenoid compounds obtained from *T. fruticans* did not show cytotoxic effects on U-2OS (human osteosarcoma cell line), NCI-H460 (human lung cancer cell line), and MCF-7 (human breast tumor cell line) cells. In our study, the cytotoxic effects of TME on murine LR7 cells were observed at a dose of 200 µg/mL.

Studies on the phytochemicals of TME are very limited. In a previous study, [21], identified germacrene D (13.2%), caryophyllene oxide, spathulenol, β-caryophyllene, and (6Z, 10Z)-pseudo phytol as major components among the 56 compounds in *T. multicaule* volatile oil. However, no studies on the phenolic compounds of TME have been found in the literature. In our study, TME was screened by LC-MS-MS for phenolic compounds. As shown in Table 2, 19 compounds were screened. It was found that vanillic acid and hydroxycinnamic acid among the phenolic acids, and myricetin, alizarin and resveratrol among the flavonoids, have the highest amounts among the determined compounds. On the other hand, fumaric, gallic, ellagic, and protocatechuic acid among phenolic acids and kaempferol and thymoquinone among flavonoids could not be detected.

In the light of the obtained data, it can be considered as an natural antioxidant and antimutagenic source due to it's the DNA protecting and DPPH radical scavenging activities and the determined phenolic compounds. Moreover, cytotoxic effect of TME on LR7 cells was found unimportantly.

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Available online: April 20, 2020

Commun.Fac.Sci.Univ.Ank.Ser.C
Volume 29, Number 1, Pages 105-118 (2020)
ISSN 1303-6025 E-ISSN 2651-3749
https://dergipark.org.tr/en/pub/communc/issue/51836/701003

CLASSIFICATION OF TURKISH HONEYS FROM AYDIN-KARACASU-DIKMEN VILLAGE BASED ON MELISSOPALYNOLOGICAL PARAMETERS

Ömür GENCAY ÇELEMLİ

ABSTRACT. The classification of Aydın -Karacasu-Dikmen honeys was practised based on melissopalynological parameters. A total of 65 honey samples from Aydın-Karacasu-Dikmen village located in Aegean Region of Turkey were collected during the 2018-2019 harvesting season. According to the melissopalynological results, 54 samples were determined as nectar (blossom), seven as honeydew honey and four as mix of nectar and honeydew honey (blend honey). In all the honey samples *Thymus spp*. pollens were observed. Also sensory analysis were done for the investigated honey samples. As a result, owing to *Thymus spp*. pollen contents in all the samples the aroma and the odour of Thymus were detected by sensory analyses. The honey types of the region were determined according to the botanical sources exhibited by the research.

1. INTRODUCTION

According to the Codex Alimentarius (Codex STAN 12-1981) and the European Union Legislation (2001/110/EC) “honey; is natural sweety substance produced by honeybees from the nectar or secretion of living parts of plants, or excretions of plant-sucking insects on the living parts of plants. Then the bees add their own specific substances, deposit, dehydrate, store and leave in the honeycomb to ripen and mature. Floral or nectar honey is made by honeybees from the nectar of blossoms, while honeydew honey is sourced from secretions of living parts of plants or exrections of plant-sucking insects on the living parts of plants [1].

Received by the editors: March 09, 2020; Accepted: April 01, 2020.
Key word and phrases: Terricola, Honey, melissopalynology, blossom, honeydew.

Melissopalynological analysis is a kind of method to determine the botanical source of the honey. Honey generally comprises so many pollen grains and honeydew elements (HDE; hyphae, fungal spores) that give an information about the source of honey. Quantitative and qualitative analysis of particules (pollen and honeydew elements) can be a step for characterization of honey group (as blossom or honeydew honey) and also type of blossom honey (monofloral, multifloral) [2]. Besides melissoplaynologcal analysis, physicochemical analysis are also necessary for certain results of botanical origin [3]. Knowing the botanical source of honey provides quality and economic value and also gives information to the consumer. Cause honey has beneficial properties depend on the floral sources, which improve human health [4].

The chemical composition of honey is variable, owing to the differences in plant types, climate, environmental conditions, and harvesting [5]. Its main components are carbohydrates, water, organic acids, enzymes, amino acids, pigments, pollen and wax; some are added by the bees and some of them are sourced from the plants [6]. Compare to nectar honeys, honeydew honeys are generally differentiated from nectar honey by higher values of pH, acidity, ash, electrical conductivity and lower monosaccharide content [7]. Moisture content is also an important criteria and determines the capability of honey to remain stable in storage without fermentation. Generally, a maximum moisture content of 21 g/100g honey is suggested [8]. Total phenolic acid content is another parameter to determine the quality of honey, owing to their antioxidant activities. These compounds have been used as chemotaxonomic markers in plant systematics; dark coloured honeys are reported to contain more phenolic acid derivates but less flavonoids than light colour ones [9].

Testing honey adulteration can be done by analyzing different physicochemical parameters like melissopalynological, sensory analysis, sugar and amino acid contents, enzyme activities. Owing to its geographical location, floral richness and climatical conditions, Turkey has a great potential for beekeeping. The production ratio of Turkish honey has been 114 471 tons in 2017. As well as, Aegean Region has an important role on the development of Turkish beekeeping. Cause it has the highest honey production compare to the other regions with a ratio 22.8% of the total [10]. Due to the floristic structure, in this region both honeydew and nectar honeys have been producing for many years now. Despite the high honey production potential of the Aegean region, the melissopalynological and physicochemical characteristics have not been researched together exhaustively. The researches about the region are mostly based on honeydew honeys.

The first aim of this study was to determine the honey types producing in Aydın-Karacasu-Dikmen village of Turkey. Secondly, characterize the identified honey types according to their botanical sources. In connection with, there is no any detailed data about the honey of research area, the results will be a data source for the region and will be useful for the characterization of different types honey.

2. Materials and Methods

2.1. Sampling

A total of 65 honey samples were collected from different beehives of the region (from Dikmen and Yeniköy villages), that has a rich plant cover for beekeeping. All the samples were collected during the year 2018 and 2019 period. Samples were stored at room temperature until the analysis.

2.2. Melissopalynological analysis

Microscopic analysis were done by qualitative and quantitative. Microscopic slides were prepared for melissopalynological analysis according to the method described by Louveaux et al. (1978) [11]. Besides the determination of botanical origin, the total pollen number in 10 g honey (TPN10) of all samples was calculated according to the method described by Moar et al. (1985) [12]. The honey samples were classified according to Maurizio's classification (1975) as Group I (<20.000) pollen grains per 10 g honey), Group II (20.000-100.000 pollen grains per 10 g honey), Group III (100.000–500.000 grains per 10 g honey), Group IV (500.000 –1000.000 grains per 10 g honey) and Group V (>1.000.000grains per 10 g honey) [13]. The honeydew elements (HDE) consist of fungal spores and hyphae were also recorded during the microscopic investigation for specifying honeydew honeys.

2.3. Physicochemical analysis

Moisture

Moustire analyses were done according to the Honey Product Inspection Manual of Canadian Food Inspection Agency (2012) by a non-digital refractometer and the results defined as % (w/v) ratio [14].

2.4. Sensory analysis

Sensory analysis were done according to the Marcazzan et al. (2018) [15]. The assessors evaluated the honey samples according to their colour intensity, odour intensity, sweetness, aroma and crystallisation rate.

3. Results

3.1.Melissopalynological characteristics

According to the melissopalynological results, in the 65 investigated honey samples, the pollen belong to the taxa of Asteraceae, Apiaceae, Betulaceae Brassicaceae, Boraginaceae, Campanulaceae, Caryophyllaceae, Chenopodiaceae, Cistaceae, Cyperaceae, Dipsecaceae, Euphorbiaceae, Fabaceae, Fagaceae, Geraniaceae, Lamiaceae, Liliaceae, Malvaceae, Myrtaceae, Oleaceae, Plantaginaceae, Poaceae, Polygonaceae, Portulaceae, Ranunculaceae, Rosaceae, Rubiaceae, Salicaceae and Scrophulariaceae families were identified. According to the melissopalynological results, honey samples divided into three groups; nectar honey (it is also divided as monofloral; Generally, a honey is considered as coming predominantly from a given botanical origin (unifloral –monofloral honey) if the relative frequency of the pollen of that taxon exceeds 45%. This ratio is; 13-68% for Thymus honey and >86% for chesnut honey, also from other plants in lower ratios and multifloral; sourced from various plant species, it has no any dominant species), honeydew honey (honeydew if the ratio of the number of honeydew elements (HDE) to that of pollen grains (PG) exceeds 3. [3]), compound honey (mix of honeydew and blossom honey). Main pollen identified in honey samples are given in the Table 1-5 and the classifying of the honey samples according to their TPN10 and HDE10 values are given in the Table 6. 54 of the samples were evaluated as nectar honey (multifloral;H3,5,6,8,15,21,23,24 and monofloral; H13: *Centaurea*, H25: Oleaceae, H4,7,20,22,26: *Thymus, H27-37 and H39-65: Astragalus sp., H38-2019: chesnut*), seven of them as honeydew honey (H9,10,11,12,16,18,19) and four as blend honey (H1,2,14,17). By this analysis a new type of honey; *Centaurea* honey was also identified. Also in all the investigated samples *Thymus* spp. pollen were observed in different ratios. Honeydew honey samples were probably sourced from *Pinus brutia* with contribution of Brassicaceae, Boraginaceae, Fabaceae, Lamiaceae, Plantaginaceae and Ranunculaceae.

TABLE 1. The ratios of the pollen of plant taxa identified in honey samples (%) (H1-15).

Plant family	Plant taxa	H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12	H13	H14	H15
Asteraceae		4	3,5	3,73	3,99	0,40	1,1	0	0,8	0	0	5,8	6,25	4,4	0,99	0,60
	Centaurea aphrodisea	0	0,96	26,86	23,65	16,39	0	31,75	19,65	6,73	20,45	9,80	6,25	62,68	10,89	31,70
	***Centaurea* sp.**	0	0	0	0	0	5,55	0,97	0	0	0	0	0	0	0	0
	Centaurea urvillei	0	0	0	0	0	0	0	1,74	0	0	0	0	4,47	0,99	0
	***Taraxacum* sp.**	4	0,96	0,74	7,01	0,40	0,55	6,35	3,49	1,03	25	7,84	3,12	2,98	0	0
Apiaceae		0	1,6	0	0,10	0,40	0,55	0,48	0	0,51	0	1,96	6,25	0	0	0
Brassicaceae		8	8,6	3,73	8,85	3,27	0,55	2,44	10,48	19,68	4,54	3,92	0	0	2,97	4,26
Boraginaceae		8	5,46	0,74	0,86	2,45	10	0	0,87	0	2,27	5,88	21,87	0	0,99	18,29
	***Alkanna* sp.**	0	0	0	0	0	0	0,16	0	0	0	0	0	0	0	0
	***Echium* sp.**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0,60
Campanulaceae		0	3,2	1,11	0,64	0,40	0,55	0	0,43	0	0	0	0	0	0	1,21
Caryophyllaceae		0	0	2,23	0,64	1,63	0	0	1,74	0	0	0	0	0	0	1,21
Chenopodiaceae		0	1,2	0,37	0,75	0	10,55	0	0	0	4,54	3,92	18,75	0	0,99	0,60
Cyperaceae	***Carex* sp.**	0	0	0,37	0	0	0	0	0	0	0	0	0	0	0	0
Dipsecaceae		0	0	0,37	0	0	0	0	0	0	0	0	0	0	0	0
	***Scabiosa* sp.**	0	0	0	0,97	0	0	0	0	0,51	0	0	0	0	0	0
Fabaceae		28	33,44	19,02	10,69	29,91	31,66	13,35	16,59	10,88	20,45	17,64	15,62	7,46	41,58	6,09
	***Astragalus* sp.**	0	2,25	1,11	0	3,27	4,44		1,3	1,55	0	0	0	0	0	0
	***Onobrychis* sp.**	0	0,32	0	0	0	0		0	0	0	0	0	0	0	0
	***Trifolium* sp.**	4	0	0	0	0	0	8,30	3,49	5,6	2,27	0	0	5,97	2,97	7,92
	Trifolium pratense	0	0,64	2,23	0	0	0	0	0	0	0	0	0	0	0	0
	Trifolium repens	0	8,36	11,19	7,12	15,57	9,44	0	0	0	0	0	0	0	0	0
	Vicia sp.	0	0	0	0	0,40	0	0,48	0	3,10	0	0	0	0	0	0
Lamiaceae		4	6,75	4,10	2,26	0,40	3,88	0	3,49	1,55	4,54	11,76	3,12	0	0,99	0,60
	Teucrium montanum	0	0	3,35	2,59	2,45	0	1,95	2,62	2,0	0	0	0	0	0	4,87
	Teucrium polium	0	0	0	0,86	0	0	2,44	7,42	8,29	4,54	0	3,12	0	0	0
	Thymus leucotrichum	8	10,61	8,95	18,35	7,37	6,66	18,07	5,67	12,4	4,54	5,88	3,12	8,95	0	6,09
	***Thymus* sp.**	0	0	0	0	0	0	0	0	0	0	0	0	0	32,67	10,97
Liliaceae		0	0	0	0,97	0	1,11	0,97	0	0	0	0	9,37	0	0	0
Malvaceae		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Oleaceae		0	0,32	0	0,21	0	0	0	0,43	0	2,27	0	0	0	0	0
Plantaginaceae	***Plantago* sp.**	8	0,64	2,61	3,34	0,40	0,55	0,97	0,43	3,10	0	19,60	3,12	1,49	0	0
Ranunculaceae		0	2,25	0	0	0	0	0	0	19,17	0	0	0	0	0	0
Rosaceae		4	3,5	1,49	2,05	1,63	2,7	2,2	0,43	0,5	0	1,96	0	0	1,98	0,60
Rubiaceae	***Galium* sp.**	0	0	0,74	0,32	0	0,5	0	0,436	0	2,27	1,96	0	0	0	0
Salicaceae	***Salix* sp.**	12	5,1	4,85	3,67	13,11	3,88	6,35	18,34	3,10	2,27	1,96	0	1,49	0,99	4,26

Dominant pollen (over 45%), secondary pollen (16-45%), minor pollen (1-15%); trace pollen (less than 1%) Dominant pollen for Thymus spp. (13–68%) for Castanea sativa (> 86%)

TABLE 2. The ratios of the pollen of plant taxa identified in honey samples (%) (H16-26).

Plant family	Plant taxa	H16	H17	H 18	H19	H 20	H21	H22	H23	H 24	H25	H26
Asteraceae		1,26	0	2,17	4,02	5,33	3,07	4,78	0,96	0,33	0	4,25
	***Carduus* sp.**	0	0	0	0	0	0	0	0	0	1,75	4,25
	Centaurea aphrodisea	19,19	11,90	6,52	1,72	3,91	21,53	9,57	31,25	7,87	1,22	0
	***Taraxacum* sp.**	20,20	2,38	2,17	5,74	7,82	1,53	0	0	0	0	0
Apiaceae		0,25	0	0	0,28	1,067	0	0	0	0	0	0
Brassicaceae		0,25	7,14	0	0	0,71	9,23	0	0	0	0	0
Boraginaceae		6,06	9,52	52,17	28,44	21,35	1,53	0	1,44	0	20,74	0
Campanulaceae		0	0	0	4,02	0,71	0	0	0	0	0	4,25
Caryophyllaceae		0	0	0	0	0	0,76	0	11,53	3,88	0	0
Chenopodiaceae		3,53	4,76	6,52	0	0,35	0	0	0	0	0	8,51
Cyperaceae	***Carex* sp.**	0	0	0	0	0	0	0	0	0	0	2,12
Dipsecaceae		0	0	0	0	0	0	0	0	0	0	4,25
	***Scabiosa* sp.**	0	0	0	0	0	0	0	0,48	0,34	0	0
Fabaceae		19,69	19,04	6,52	10,91	25,97	6,92	28,71	17,78	64,98	0,81	0
	***Astragalus* sp.**	0	0	0	0	0	3,076	4,57	2,40	0	0	6,38
	***Trifolium* sp.**	1,51	14,28	6,52	0,86	0	3,84	0	10,09	0	0	0
Lamiaceae		0,25	0	0	0	0	0	0	0	0	0,65	0
	Teucrium montanum	0,50	0	0	0	0	2,30	0	7,69	3,72	0	0
	Teucrium polium	1,01	2,38	4,34	0,57	1,42	4,61	0	0,48	0	0	0
	Thymus leucotrichum	2,77	7,14	13,04	12,64	**18,14**	6,15	4,72	6,73	7,37	0,39	8,51
	***Thymus* sp type I**							9,2		3,8		
	***Thymus sp*. type II**	11,61	2,38	0	6,03	1,06	0	**33,50**	3,84	12,32	0	**36,17**
Liliaceae		4,54	0	0	0,57	1,06	0	0	0	0	19,52	0
Malvaceae		0	0	0	0,28	0	0	0	0	0	0	0
Myrtaceae	***Eucalyptus* sp.**	0	0	0	0,28	0	0	0	0	0	0	0
Oleaceae		2,02	0	0	22,41	4,98	0	0	0	0	**54,48**	0
Plantaginaceae	***Plantago* sp.**	1,010	0	0	0	0,35	0,76	0	0,96	0	0	2,12
Portulacaceae	***Portulaca pilosa***	3,03	0	0	0,28	0	0	0	0	0	0	17,0
Ranunculaceae		0	0	0	0	0	2,30	0	0	0	0	0
Rosaceae		0,25	2,38	0	0,57	2,49	3,84	4,49	2,88	3,55	0	0
Rubiaceae	***Galium* sp.**	0,50	0	0	0	0,71	0	0	0,96	0	0	0
Salicaceae	***Salix* sp.**	0,50	16,66	0	0,28	2,49	28,46	0	0,48	0	0,40	2,12

TABLE 3. The ratios of the pollen of plant taxa identified in honey samples (%) (H27-41).

Plant family	Plant taxa	H27	H28	H29	H30	H31	H32	H33	H34	H35	H36	H37	H38	H39	H40	H41
Asteraceae	*Centaurea* sp.	0	0,5	0,5	0	0,5	1,5	0	0,5	0	0	0	0	0	0	2
	Cichorium sp.	0,5	0	0	1	0	0,5	0	1	0	0	1	0	0	0	0
Brassicaceae		0,5	0	0	0	0	0	0	0	0	0	0	0	0	0,5	0
Boraginaceae		0,5	0,5	0	0,5	0	0	0	0	0	0	0	0	0	0	0
	Echium sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0,5	0
Campanulaceae		0	0,5	0	0	0	0	0	0	0,5	0	0	0	0	0	0,5
Caryophyllaceae		0	1	0	0	0	0	1	0	0	0	1	0	0	0	1
Chenopodiaceae		0	0	0	0	0	0	0	0,5	0	0	0	0	0	0	0
Cistaceae		0	0,5	1	1	1	0	0	1	1,5	1	1	0	0,5	1	0
Cyperaceae	*Carex* sp.	0	0	0,5	0	0	0	0	0	0	0	0	0	0	0	0
Dipsecaceae	*Scabiosa* sp.	0	0	0	0,5	0	0	0	0,5	0	0,5	0	0	0	0	0
Fabaceae		0	0	0	0	0	0	0,5	0	0	0	0	0	0	0	0
	Astragalus sp.	90	83	93	85,5	92,5	87,5	86,5	81,5	75	81	87	13	93	83	82,5
	Onobrychis sp.	0	0	0	1	0	0	0	0	0	0	0	0	0	0,5	0
	Trifolium sp.	0	0	0	0,5	0	0	0	1,5	1	2	3,5	0	0,5	0	2
	Lotus sp.	0,5	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Medicago sp.	0	0	0	0	0	0	0	0	0	0	0	0	0,5	0,5	0
	Vicia sp.	0,5	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fagaceae	*Castanea sativa*	0	0	1	1	1	0	2	1	8,5	-	0,5	85	1	1	5
Lamiaceae																
	Teucrium sp.	1	0	1	0,5	0	2	0,5	1	0,5	2	0	0	1	4	0,5
	Thymus sp.	5	8	1	4,5	3,5	4	5	4,5	7,5	9	3	1	1,5	3,5	3,5
Liliaceae		0	3,5	0,5	1,5	0	2,5	0	3	1,5	2,5	1	0	0	4	0
Plantaginaceae	*Plantago* sp.	0	1,5	0,5	0	0	0,5	2	1,5	1,5	1,5	1,5	0,5	1	0,5	0
Polygonaceae	*Rumex sp.*	0	0	0	0,5	0	0	0	0	0	0	0	0	0	0	0
Rosaceae		1	2	1	2	1	1,5	2	1,5	2	1	0,5	0,5	0,5	2	2
Rubiaceae	*Galium* sp.	0	0	0	0	0,5	0	0	0	0	0	0	0	0	0	1
Salicaceae	*Salix* sp.	0	0	0	0	1,5	0	0,5	1	0,5	0	0	0	0,5	0	0

TABLE 4. The ratios of the pollen of plant taxa identified in honey samples (%) (H42-56).

Plant family	Plant taxa	H42	H43	H44	H45	H46	H47	H48	H49	H50	H51	H52	H53	H54	H55	H56
Asteraceae	*Artemissia* sp.						0,5									
	Centaurea sp.	0	1,5	0,5	0	1,5	0	0	0,5	0	0,5	0,5	0	0	0	0
	Cichorium sp.	0,5	0,5	1	0	0	0	0	0	0	0	0	0	0	0	0
	Taraxacum sp.	0	0	0	0	0,5	0	0	0	0,5	0,5	0	0	0,5	0	0
Betulaceae														1,5		
Brassicaceae									0,5	0	0	0,5	0,5	0,5	0	0
Boraginaceae	*Echium* sp.							0,5								
Campanulaceae			1													
Caryophyllaceae		0,5	1									0,5	0,5			
Cistaceae		0,5	0	1	1,5	0	1	0	0	0	0	1,5	0	2	1	0,5
Cyperaceae	*Carex* sp.			2									1,5	1,5		
Dipsecaceae	*Scabiosa* sp.			0,5								0,5	0	0,5		
Euphorbiaceae	*Euphorbia sp.*					0,5										
Fabaceae	*Astragalus* sp.	85,5	83	85	91	84,5	86	89	86,5	88,5	89,5	86,5	88	76	85	91,5
	Onobrychis sp.											0,5				
	Trifolium sp.	2,5	1	1												
	Lotus sp.	0	0	0	0,5	1,5	0	1,5	4	1,5	1	0,5	0	2,5	3,5	1
	Vicia sp.	0	0,5	0	0	2	1	0	0,5	0	0,5	0	0	0	0	0
Lamiaceae																
	Teucrium sp.	0	0,5	1												
	Thymus sp.	6	7	4	5	4,5	6,5	5,5	4	6	5	4	5,5	6,5	4,5	3,5
Liliaceae		1,5	1	2	0,5											
	Allium sp.						1	2	2	1,5	0,5	0,5	1	3	3,5	1,5
Plantaginaceae	*Plantago* sp.	1	0,5	0	0	0,5	0	0,5	0,5	0,5	0,5	0,5	0,5	4,5	1,5	0,5
Poaceae						1	1								1	
Polygonaceae	*Rumex sp.*										0,5					
Rosaceae		2	0,5	1,5	1	2,5	3	1	1,5	0,5	0	1,5	2	0,5	0	0,5
Rubiaceae	*Galium* sp.	0	0	0	0,5							0,5				
Salicaceae	*Salix* sp.			0,5							1	1	0	0,5	0	0,5
Scrophulariaceae	*Linaria sp.*					1	0	0	0	0,5	0,5	1	0,5	0	0	0,5

TABLE 5. The ratios of the pollen of plant taxa identified in honey samples (%) (H57-65).

Plant family	Plant taxa	H57	H58	H59	H60	H61	H62	H63	H64	H65
Asteraceae	*Centaurea* sp.						1	0	0,5	
	Taraxacum sp.	0,5	0,5						1	
Boraginaceae	*Heliotropium* sp.			0,5						
Caryophyllaceae			1,5	1						0,5
Cistaceae		1	0	2	0,5	1,5	1	0	0,5	0,5
Cyperaceae	*Carex* sp.			0,5	1	0,5				
Dipsecaceae	*Scabiosa* sp.	0,5						0,5	0	0,5
Fabaceae	*Astragalus* sp.	88,5	85,5	83,5	88,5	87	89,5	87,5	84	90
	Lotus sp.	2,5	1,5	1,5	1,5	0	1,5	1,5	1	0,5
Geraniaceae	*Geranium sp.*	0	0	1						
Lamiaceae	*Thymus* sp.	2,5	5	6,5	4	6	3	4	5,5	3,5
Liliaceae	*Allium* sp.	2,5	3	1,5	0,5	1,5	2,5	2,5	3,5	2,5
Myrtaceae	*Eucalyptus* sp.								0,5	
Plantaginaceae	*Plantago* sp.	0,5	1,5	0,5	2,5	1	0,5	0,5	0,5	1
Poaceae	*Zea mays*					0,5				
Polygonaceae	*Rumex sp.*	0	0,5							
Rosaceae		1	0	1	1,5	1,5	1	2,5	2	1
Salicaceae	*Salix* sp.	0,5	0	0	0	0,5	0	1		
Scrophulariaceae	*Linaria sp.*	0	1	0,5					0,5	

TABLE 6. TPN10, HDE10 values and sources of honeys.

Honey sample	TPN_{10}	Maurizio's classification	HDE_{10}	HDE10/TPN10	Source of honey	Type of honey	Moisture
H1	2416,5	Group I	6,867,947	2,842,105	Multiflower-,honeydew	Blend	17
H2	79 151,77	Group II	180796,8	2,284,178	Multiflower-honeydew	Blend	16.4
H3	40 354,98	Group II	30620,43	0,758777	Multiflower	Nectar	17.3
H4	63 387,39	Group II	4,621,195	0,00729	Monoflower	Nectar	18.4
H5	61 768,1	Group II	13485,25	0,218321	Multiflower	Nectar	17.4
H6	81 48,915	Group II	12570,13	1,542,553	Multiflower	Nectar	16.4
H7	19 237,7	Group I	-	-	Monoflower	Monofloral	17.5
H8	17 452,5	Group I	-	-	Multiflower	Nectar	17.6

Honey sample	TPN_{10}	Maurizio's classification	HDE_{10}	HDE10/TPN10	Source of honey	Type of honey	Moisture
H9	3 383,1	Group I	364891,5	1,078,571	Honeydew	Honeydew honey	18.4
H10	63 90,744	Group II	42338,68	6,625	Honeydew	Honeydew honey	15.7
H11	5 928,48	Group I	59284,8	10	Honeydew	Honeydew honey	17.4
H12	1 364,612	Group I	133049,6	97,5	Honeydew	Honeydew honey	17.3
H13	11 011,9	Group I	11827,59	1,074,074	Monoflower	Nectar	16.9
H14	11 288,76	Group I	27798,57	24,625	Honeydew, multifower	Blend	17
H15	30 902,98	Group II	6,349,927	0,205479	Multiflower	Nectar	17.7
H16	14 629,62	Group I	108154,7	7,392,857	Honeydew	Honeydew honey	16
H17	82 23,771	Group II	15895,2	1,932,836	Honeydew, multiflower	Blend	16.9
H18	44 81,509	Group II	55799,18	1,245,098	Honeydew	Honeydew honey	16.3
H19	27 064,8	Group II	111352,3	4,114,286	Honeydew	Honeydew honey	19.8
H20	18 160,36	Group I	18863,35	103,871	Monoflower	Nectar	14.4
H21	23 474,57	Group II	8,787,273	0,374332	Multiflower	Nectar	15.6
H22	208 297,9	Group III	-	-	Monoflower	Nectar	16.5
H23	28 664,69	Group II	-	-	Multiflower	Nectar	17.9
H24	79 0854,5	Group II	-	-	Multiflower	Nectar	17.3
H25	278 380,8	Group III	-	-	Monoflower	Nectar	18.5
H26	1 969,675	Group I	-	-	Monoflower	Nectar	14.7
H27	110 645	Group II	-	-	Monoflower	Nectar	16.1
H28	77 951	Group II	-	-	Monoflower	Nectar	16.2
H29	78 684	Group II	-	-	Monoflower	Nectar	16
H30	117 107	Group III	-	-	Monoflower	Nectar	16.7
H31	99 753	Group II	-	-	Monoflower	Nectar	16.8
H32	100 410	Group III	-	-	Monoflower	Nectar	16.6
H33	114 611	Group III	-	-	Monoflower	Nectar	16.9
H34	229 257	Group III	-	-	Monoflower	Nectar	16.7
H35	68 408	Group II	-	-	Monoflower	Nectar	16.4
H36	92 090	Group II	-	-	Monoflower	Nectar	16
H37	120 251	Group III	-	-	Monoflower	Nectar	16.6
H38	29 329,65	Group II	-	-	Monoflower	Nectar	16.4
H39	46 396,8	Group II	-	-	Monoflower	Nectar	16.5
H40	159 489	Group III	-	-	Monoflower	Nectar	17.3
H41	48 330	Group II	-	-	Monoflower	Nectar	16.4
H42	37 455,75	Group II	-	-	Monoflower	Nectar	16.2
H43	85 953	Group II	-	-	Monoflower	Nectar	16.4
H44	40 059	Group II	-	-	Monoflower	Nectar	16.5
H45	99 373	Group II	-	-	Monoflower	Nectar	16.5
H46	66 502	Group II	-	-	Monoflower	Nectar	16.9
H47	27 127	Group II	-	-	Monoflower	Nectar	16.6
H48	66 695	Group II	-	-	Monoflower	Nectar	16.7
H49	118 026	Group III	-	-	Monoflower	Nectar	16.5
H50	36 433,38	Group II	-	-	Monoflower	Nectar	16.7
H51	138 298	Group III	-	-	Monoflower	Nectar	16.3
H52	9 666	Group I	-	-	Monoflower	Nectar	16.2
H53	75 947	Group II	-	-	Monoflower	Nectar	16.5

Honey sample	TPN_{10}	Maurizio's classification	HDE_{10}	HDE10/TPN10	Source of honey	Type of honey	Moisture
H54	30 609	Group II	-	-	Monoflower	Nectar	16.3
H55	9 666	Group I	-	-	Monoflower	Nectar	16.5
H56	18 727	Group I	-	-	Monoflower	Nectar	16.4
H57	31 840	Group II	-	-	Monoflower	Nectar	16.6
H58	105 709	Group III	-	-	Monoflower	Nectar	16.5
H59	70 504	Group II	-	-	Monoflower	Nectar	16.5
H60	8 825	Group I	-	-	Monoflower	Nectar	16.5
H61	106 111	Group I	-	-	Monoflower	Nectar	16.5
H62	75 345	Group II	-	-	Monoflower	Nectar	15.8
H63	28 998	Group II	-	-	Monoflower	Nectar	16.5
H64	2 416,5	Group I	-	-	Monoflower	Nectar	16.1
H65	41 886	Group II	-	-	Monoflower	Nectar	16.4

3.2.Physicochemical analysis

The investigated honey samples are proper according to the moisture content. All the samples contained less than 20% moisture content which is safety against fermentation. It changes according to the climatic factors, harvesting season, the maturity degree of honey and environmental factors [16].

3.3.Sensory analysis

According to the sensory analysis colour intensity observed between 1-5. Mostly the colour of honeydew honeys were evaluated as degree 4 (Table 7). Intensity of odour were scored 1 to 3 and most of the samples evaluated as degree 2. Sweetness, intensity of aroma and crystallization rate were also scored. It is observed that crystallization ratios were low in honeydewhoneys as known.

By the assessors, it is mentioned that floral odour and aroma especially *Thymus* spp. odour was sensed in all the samples in different proportions.

TABLE 6. Sensory analysis results of the honey samples (H1-26)

Honey number		H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12	H13	H14	H15	H16	H17	H18	H19	H20	H21	H22	H23	H24	H25	H26
Colour intensity (from 1to5)		2	2	3	3	2	5	5	3	3	4	4	4	3	4	2	2	2	4	1	4	3	5	1	5	3	5
Intensity of odour (from 0 to 3)		2	2	2	3-Feb	2	2	2	2	2	2	2	2	1	2	2	2	2	2	2	2	3-Feb	2	2	2	2	2
	Floral	+	+	+	+	+		+	+						+	+		+			+	+	+	+	+	+	+
	Vegetal																										
	Woody	+	+	+						+	+	+	+		+		+		+	+							
Sweetness (from 1 to 3)		1	1	2	2	2	2	3-Feb	2	1	1	1	1	2	2	2	1	2	1	1	2	3-Feb	2	2	2	2	2
Intensity of aroma (from 0 to 3)		1	1	1	2	1	1	2	1	1	1	1	1	1	3	2	1	1	1	1	2	1	2	1	1	1	2
Crystallisation rate (from 1 to 3)		2	2	2	2	2	2	2	2	1	1	1	1	2	1	1	1	1	1	1	2	2	2	2	2	2	2

TABLE 6. (Continued) Sensory analysis results of the honey samples (27-49)

Honey number		H27	H28	H29	H30	H31	H32	H33	H34	H35	H36	H37	H38	H39	H40	H41	H42	H43	H44	H45	H46	H47	H48	H49
Colour intensity (from 1to5)		2	3	2	2	2	2	2	2	2	2	2	4	2	2	2	2	2	2	2	2	2	2	2
Intensity of odour (from 0 to 3)		2	2	2	2	2	2	2	2	2	2	2	3	2	2	2	2	1	2	2	1	2	2	2
	Floral	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Vegetal												+											
	Woody																							
Sweetness (from 1 to 3)		2	2	2	2	2	2	2	2	2	2	2	1	2	2	2	2	2	2	2	2	2	2	2
Intensity of aroma (from 0 to 3)		2	2	2	2	2	2	2	2	2	2	2	1	2	2	2	2	2	2	2	2	2	2	2
Crystallisation rate (from 1 to 3)		2	2	2	2	2	2	2	2	2	2	2	1	3-Feb	2	2	2	2	2	2	2	2	2	2

TABLE 6. (Continued) Sensory analysis results of the honey samples (27-49)

Honey number		H50	H51	H52	H53	H54	H55	H56	H57	H58	H59	H60	H61	H62	H63	H64	H65
Colour intensity (from 1to5)		2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
Intensity of odour (from 0 to 3)		2	2	2	1-2	2	1-2	2	1	2	2	2	2	2	2	2	2
	Floral	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Vegetal																
	Woody																
Sweetness (from 1 to 3)		2	2	2	1-2	2	1-2	2	2	1-2	2	2	2	2	2	2	2
Intensity of aroma (from 0 to 3)		2	2	2	1-2	2	2	2	2	1-2	2	2	2	2	1	2	2
Crystallisation rate (from 1 to 3)		2	2	2	2	1-2	2	1-2	2	2	2	2	2	2	2	2	2

4. CONCLUSION

As a part of the study, the honey type variety (multifloral, monofloral, blend, honeydew) was observed special to the Aydın-Karacasu-Dikmen village. As well as, by this research characterization of honey samples from Aegean region of Turkey has been done, which has not detailed with any other research before. This work comprises multifloral, monofloral (*Astragalus*, *Castanea sativa* Miller, *Centaurea, Thymus*, Oleaceae), honeydew honey and blend honey from this region. Also there is no any previous literature data about *Centaurea* honey characterized as monofloral honey by this research.

This results will highligt the rich variety of Aegean honeys and be a step for future researches.

Acknowledgement. This research is supported by the “Korda Energy”.

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Available online: April 24, 2020

Commun.Fac.Sci.Univ.Ank.Ser. C
Volume 29, Number 1, Pages 119-130 (2020)
ISSN 1303-6025 E-ISSN 2651-3749
https://dergipark.org.tr/en/pub/communc/issue/51836/650084

BIOFILM FORMATION AND CONTROL OF FACULTATIVE THERMOPHILE *BREVIBACILLUS AGRI* D505B

TUGBA KILIC AND ARZU COLERI CIHAN

ABSTRACT. *Brevibacillus agri* D505b is an aerobic, non-pathogenic, endospore-forming facultative thermophilic bacillus. Six abiotic surfaces (stainless steel, glass, polyvinyl chloride, polypropylene, polystyrene, and polycarbonate) were compared with viable cell enumerations. According to results, D505b cells could able to attach all these surfaces. Stainless steel (6.10 log CFU/cm^2) was found to be the most effective surface for biofilm formation. Polycarbonate (6.03 log CFU/cm^2) was found as the second best surface. Furthermore, the D505b biofilm was treated with 15 different sanitation agents and trichloroacetic acid (TCA) was determined to be the most effective one (80.3% removal). Our results showed that the strong biofilm producer *B. agri* D505b was very resistant to various sanitation agents. However, TCA significantly inhibited biofilm formation for the isolate.

1. INTRODUCTION

Biofilm coined by Bill Costerton in 1978, which is a heterogeneous structures comprising different populations of microorganisms surrounded by a matrix (mostly of exopolysaccharides) that allows their attachment to inert (e.g., glass, plastic) or organic (e.g., skin, mucosa) surfaces [1]. Furthermore, biofilms are formed over a surface, mostly industrial surfaces, including pipelines and membrane systems that come in direct contact with a flowing product [2,3]. Surfaces of food processing equipment, including closed systems such as pipes, valves and pumps or open systems such as conveyors, are regularly found to be contaminated by microorganisms [4]. Moreover, Bacilli can form biofilms on surfaces during dairy

Received by the editors: November 22, 2019; Accepted: March, 27, 2020.
Key word and phrases: Brevibacillus agri D505b, biofilm control, abiotic surfaces, enzymes.

processes [5]. Aerobic spore-forming bacteria, such as *Bacillus*, *Brevibacillus*, and *Geobacillus*, are able to survive industrial pasteurization and form biofilm within pipes and stainless steel equipment [6]. Stainless steel is widely used in industry and has an established record of biofilm formation responsible for deterioration and corrosion [7]. Thermophilic *Bacillus* species easily attach and grow on stainless steel surfaces. Furthermore, thermophilic bacilli grow and adhere to surfaces in the evaporators of milk powder manufacturing plants [8].

Biofilms cause many problems in food-processing industry, which are energy losses, blockage, cleaning, hygiene, corrosion, and material deterioration in equipment, sensors, detectors [9]. Furthermore, bacterial biofilms may both cross-contamination dairy products and may cause corrossion of the metal surfaces [3]. Bacterial contamination and product spoilage because of biofilm formation are recurring problems [10]. Thermophilic bacilli are potential contaminants in a variety of industries such as paper mills, canning, juice pasteurization, sugar refining, gelatin production, dehydrated vegetable manufacture and dairy product manufacture [11]. Bacterial biofilms are more difficult to eliminate from within a system than free-living cells. Adherent microorganisms become highly resistant to cleaning procedures and disinfection [3,4]. Cleaning and disinfection studies have focused on eliminating food-borne pathogens such as *Listeria* and have neglected other contaminating organisms such as thermophilic bacilli [10]. Enzymes are considered green countermeasures against biofilm formation in the food industry owing to their biodegradability and low toxicity [12]. Various sanitation agents for biofilm control are widely used. For example, the lytic action of lysozyme on bacteria can be ascribed simply to resolution of the rigid cell-wall structures [13]. Tsiaprazi-Stamou et al. (2019) determined that formulation A, containing amylase-protease-lipase was the most effective in biofilm cleaning [12]. Nisin is also useful for the inhibition of cell-wall synthesis [14], and it is effective against important Gram-positive foodborne pathogens and spoilage agents [15]. Takao et al. (2016) examined the effects of disinfectants such as Mazak P and benzalkonium chloride on a natural biofilm model of a dental unit waterline [16]. However, *B. agri* was found within the surviving bacteria. Biofilm control agents and regimes may not provide any effect for some bacteria. New strategies should be developed in this case. Similarly, Faille et al. (2002) found that the resistance of both *Bacillus cereus* and *Bacillus subtilis* spores to a cleaning procedure [4].

In our preliminary studies on biofilms, a facultative thermophilic isolate, *B. agri* D505b, was detected as a strong biofilm producer ($OD_{595\ nm}$: 3.365) [17]. Little is known about control of thermophilic bacilli biofilms. The aim of this study was to

detail the biofilm formation on abiotic surfaces of *B. agri* D505b isolate and its biofilm control. To the best of our knowledge, this is the first paper with regard to biofilm formation, and control of the facultative thermophile *B. agri*.

2. Materials and Methods

2.1. Bacterial Strain and Culture Conditions

The endospore-forming facultative thermophilic and aerobic bacillus *B. agri* D505b was isolated from sediment samples in the Dikili district of İzmir, Turkey. The 16S rRNA gene of the D505b isolate was registered with GenBank Accession Number FJ430048 [18]. The isolate was first cultured in tryptic soy agar (TSA) at 55 ^{o}C for 18 h and was subsequently incubated in tryptic soy broth (TSB) for 18 h at 55 ^{o}C in a shaking incubator. The culture was again incubated in TSB at 55 ^{o}C for 6 h under shaking. All biofilm assays were carried out with culture that was 6 h old in the mid-exponential growth phase.

2.2. Biofilm Formation on Surfaces

The bead vortexing method of Giaouris and Nychas (2006) with a few modifications was applied for cell viability assay on abiotic surfaces [19]. First of all, stainless steel (grade 316L), polypropylene, polystyrene, polyvinyl chloride, polycarbonate coupons (R: 14 mm), and glass slides (20 mm x 26 mm x 1 mm) were treated with isopropanol overnight and were washed for 30 min in a detergent solution. The coupons and glass slide were washed under running tap water and with deionized water, respectively. Afterwards, the surfaces were air-dried and autoclaved. The surfaces were placed into 6-well polystyrene microtiter plates containing TSB. Subsequently, bacterial culture was inoculated onto the plates and was incubated for 48 h. The surfaces were then removed with sterile forceps were rinsed with 4.5 mL of physiological saline to eliminate planktonic cells. Then, the surfaces were scratched. The surfaces and the suspensions were cited to tubes containing only glass beads and then were vortexed for 2 min. Viable cell numbers were calculated with the drop plate method [20]. Surfaces in TSB were used as negative controls. The results were converted to the logarithmic base (log CFU/cm^2). All assays were performed in duplicate.

TABLE 1. Sanitation agents for biofilm control and their effects.

Effect	Agents	Concentration	Temperature	Time	References
Protein	AP	0.16 U/g	37°C	60 min	[10]
	Protease	0.16 U/g	37°C	60 min	[10]
	Subtilisin	1%	37°C	30 min	[10]
	Trypsin	3%	37°C	3 h	[8]
	SDS	3%	100°C	10 min	[8]
Polysaccharide	α-Amylase	1%	37°C	30 min	[11]
	Cellulase	1.66%	37°C	30 min	[11]
	SM	100 mM	22°C	60 min	[8]
	Lysozyme	2%	37°C	60 min	[8]
	TCA	10%	100°C	15 min	[8]
Antimicrobial	Nisin	2 mg/mL	37°C	24 h	[8]
Pro-oxidant	PM	2 mg/mL	22°C	30 min	[8]
	ST	10 mg/mL	22°C	5 min	
Quorum sensing	Furanone	1 mg/mL	22°C	60 min	[22]
	Triclosan	2 mg/mL	22°C	60 min	[23]

2.3. Biofilm Control with Sanitation Agents

Protease, Alkaline protease (AP), lysozyme, α-amylase, cellulase, subtilisin, trypsin, nisin, furanone, triclosan, sodium metaperiodate (SM), potassium monopersulfate (PM) and sodium thiosulfate (ST) combination, sodium dodecyl sulfate (SDS), and trichloroacetic acid (TCA) were used in this assay. Bacteria culture (5 μL) and TSB (95 μL) were added to the polystyrene microtiter plates. The plates were incubated for 24 h at 45°C. Then the wells were emptied and rinsed with physiological saline. The plate wells were filled with 15 different sanitation agents under the suitable conditions with a few reference modifications (Table 1). Finally, the CV staining assay was applied to wells. The wells containing only the appropriate solvent without its cleaning agent were used as positive controls. The results were calculated using the formula of Pitts et al. (2003) [21].

2.4. Statistical Data Analyses

All statistical analyses were performed using SPSS 17.0 (SPSS Inc., Chicago, IL, USA). One-way ANOVA and Tukey and Dunnett tests were applied. Probability levels of < 0.05 were considered statistically significant.

3. Results

3.1. Biofilm Formation on Abiotic Surfaces

In this assay, scraping and bead vortexing were applied to cells. Six abiotic surfaces were compared with viable cell enumerations with the plate counting method such surfaces included stainless steel, polypropylene, glass, polyvinyl chloride, polystyrene, and polycarbonate. According to results, D505b cells could attach to all surfaces. The viable cell numbers were calculated based on all surface areas and varied from 4.47 to 6.10 log CFU/cm^2 for abiotic substrates. Grade 316 L stainless steel (6.10 log CFU/cm^2) was found to be the most effective surface for biofilm formation (Figure 1).

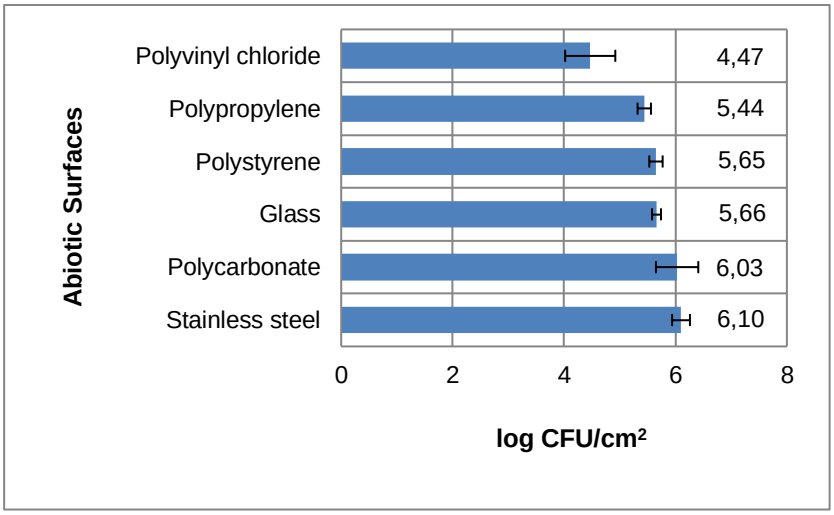

Figure 1. The biofilm formation of D505b cells on abiotic surfaces.

3.2. Biofilm Control with Various Agents

Fiftheen different sanitation agents were used for biofilm control. TCA (80.3%) provided the best biofilm removal among these agents. According to our results, other sanitation agents did not show sufficient effects for biofilm control (< 50%). It seems that TCA may have effect for *B. agri* biofilm control. Only TCA agent had an effect on the breakdown of surface polysaccharide in extracellular polymeric substance (EPS) matrix. Five different agents (AP, protease, subtilisin, trypsin, SDS) were used for degrade of surface proteins in EPS matrix. However, biofilm structure of the isolate was not affected by sanitation agents (Figure 2).

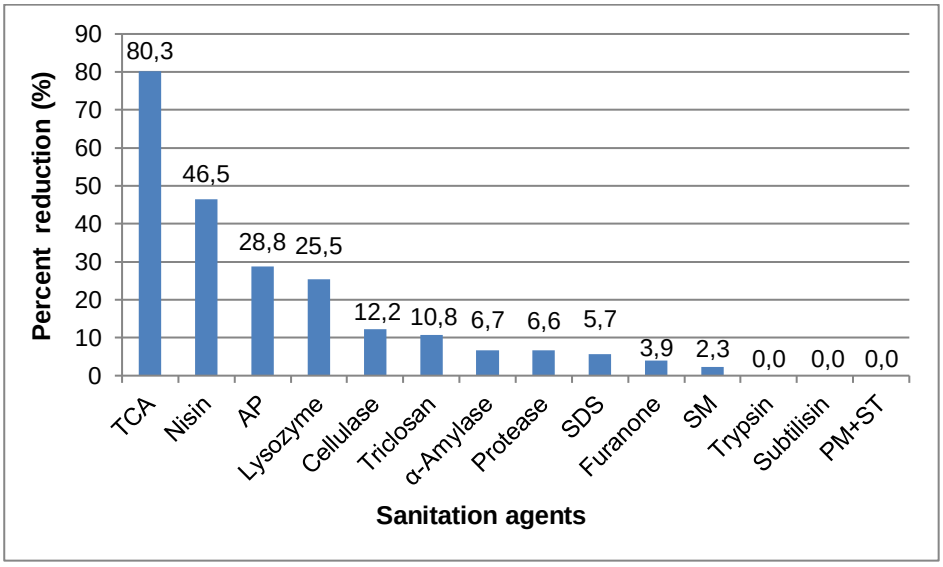

FIGURE 2. Biofilm control of the D505b isolate with different sanitation agents.

4. DISCUSSION

Bacterial spores are strongly hydrophobic. The spores have generally tended to adhere more to both hydrophobic and hydrophilic surfaces than to vegetative cells [24]. Food-related *Bacillus* species formed biofilms on polystyrene surfaces.

Polystyrene has been widely used in food packaging. Closed systems such as pipes, pumps, and valves or open systems such as conveyors are found to be contaminated by *Bacillus* spp., *Escherichia coli*, and *Listeria monocytogenes* [25]. In general, it is assumed that glass and stainless steel are hydrophilic materials, while plastics are hydrophobic materials [26]. Bacteria are able to attach to a wide variety of different materials, including glass, 304 and 316 stainless steel, plastics, rubber, polytetrafluoroethylene, and various organic polymers, which are used in modern processing equipment [21,28]. We tested both hydrophilic and hydrophobic materials for the biofilm formation of *B. agri* D505b. Our results indicated that D505b isolate formed biofilm on both hydrophobic and hydrophilic surfaces. The most suitable surface for biofilm production by D505b was grade 316L stainless steel (6.10 log CFU/cm^2). Its second choice was also detected as polycarbonate surface (6.03 log CFU/cm^2) (Figure 1). In a similar study, Mafu et al. (1990) showed that *L. monocytogenes* cells could attach to stainless steel, glass, and polypropylene surfaces [29]. In another study, Song et al. (2012) demonstrated that *E. coli* yielded differing amounts of biomass on stainless steel (1.7x10^7 CFU/coupon) and polycarbonate (4.1x10^7 CFU/coupon) [30]. A strain of *Yersinia ruckeri* form biofilms on solid supports such as fiberglass and polyvinyl chloride (PVC) [28]. The hydrophobic properties of endospores and their resistance to heat, disinfectants, and desiccation allow them to survive cleaning procedures [31]. Endospores of some bacterial species are known to have high thermal resistance and can survive disinfection and heat sterilization [24]. *L. monocytogenes* in biofilm was more resistant than single cells to sanitizers and heat [32]. Enzymes like protease and a-amylase, have gained attention as alternative agents that could demolish the EPS matrix and attack bacterial cells [12]. However, our study demonstrated that TCA was the most effective agent among 15 different sanitation agents for *B. agri* D505b biofilm control (80.3%). Similarly, Parkar et al. (2003) showed that TCA caused a 100% loss of viability of *Bacillus flavithermus* strain B12-C^m [8]. Nisin was determined as the second most effective agent (46.45%). The other agents were not able to be successful on biofilm removal. Our result showed that lysozyme (2%) reduced biofilm formation of *B. agri* D505b by 25.5% (Figure 2). Eladawy et al. (2020) reported that the highest reduction (19%) was seen in lysozyme concentration of 30 μg/mL for *Pseudomonas aeruginosa* [33].

5. Conclusion

Brevibacillus is one of the most widespread genera of Gram-positive bacteria, which recorded from the diverse environmental habitats [34]. Therefore, it is essential to

provide biofilm control of this bacterium. Our studies showed that *B. agri* could form biofilms on stainless steel, glass, polyvinyl chloride, polypropylene, polystyrene, and polycarbonate surfaces. To biofilm control may require investigation in a different time and temperature intervals on these surfaces. Current methods for controlling thermophilic bacilli and their biofilm growth in dairy manufacturing plants include increasing the cleaning frequency, the use of disinfectants, altering temperatures, reducing the surface area and the use of dual equipment [35]. Our data suggest that different new sanitation regimes should be tested for the biofilm control of *B. agri*. Besides, the synergistic effect of enzymatic detergents can be determined to biofilm control on different abiotic surfaces.

Acknowledgment. This research was supported by the Scientific Research Project Office of Ankara University (project number 11B4240003).

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Available online: May 03, 2020

Commun.Fac.Sci.Univ.Ank.Ser. C Bio.
Volume 29, Number 1, Pages 131-147 (2020)
ISSN 1303-6025 E-ISSN 2651-3749
https://dergipark.org.tr/en/pub/communc/issue/51836/xxxxx

DETERMINATION OF FUSARIUM SPECIES IN CARNATION GREENHOUSES IN ANTALYA, TURKEY

AYDIN ATAKAN AND HÜLYA ÖZGÖNEN ÖZKAYA

ABSTRACT. In this study, revealing of the identification and pathogenicity of *Fusarium* species isolated in carnation greenhouses of Antalya, Turkey were aimed. As a result of isolations, fungi included in *Fusarium* genus were identified using macroscopic and microscopic techniques. Pathogenicity of identified species were determined using Turbo carnation cutivar. As a result of the diagnostic studies, species belonging to the genus *Fusarium* were determined as *F. acutatum, F. avenaceum, F. chlamydosporum, F. equiseti, F. oxysporum, F. poae, F. proliferatum, F. sambucinum, F. solani, F. tricinctum* and *F. verticillioides*. *F. oxysporum* is the most commonly isolated species According to the pathogenicity test in Turbo carnation variety, the disease severity of *Fusarium* species were changed between 60-88 % and determined that *F. solani* had the highest pathogenicity rates among the others. Consequently, a total of 11 *Fusarium* species have been identified and has been demonstrated that have potential to cause problem in carnation cultivation in greenhouses in Antalya, Turkey.

1. INTRODUCTION

Turkey has a very wide variety of ecological vegetation and floristic features [1]. The economic importance of ornamental plants has been increasing in many countries, and international demand has quickly expanded. Carnation have an importance in basic ornamental crops, because it is the main export products particularly in the Antalya Province in Turkey. Carnation has a wide range of colors and patterns and is one of the rare flowers that decorate floral bouquets and it has

Received by the editors: March 10, 2020; Accepted: April 9, 2020.
Key word and phrases: Antalya, carnation, Fusarium spp., isolation, identification, pathogenicity.

been exported to many countries around the World. One of the most important factors limiting the carnation production and causes of losses are fungal diseases in Turkey.

Fungi spread in the ecosystem as parasites, pathogens and saprotrophic [2,3]. Among the important diseases of the upper parts are *Alternaria dianthi* F. Stevens & J.G. Hall and *Uromyces dianthi* (Pers.) Niessl. lead to significant losses in carnation greenhouses. However, it is reported that the most important losses are caused by soil-borne fungi, especially *Fusarium* species [4,5]. *Fusarium* genus is the most important group among soil-borne pathogens causing diseases in carnation and other ornamental plants. It is reported that *Fusarium* spp. lead to root and stem rot, wilt and plant deaths [6]. The most common species isolated from carnation greenhouses is *Fusarium* spp. in Antalya, Turkey [7]. The most important fungal pathogen causing root rot and wilt in carnation is *F. oxysporum* Schl. f. sp. *dianthi* W.C. Snyder & H.N. Hans [8,9]. In a study conducted in Iran, *F. proliferatum* (Matsush.) Nirenberg and *F. solani* (Mart.) Sacc. were reported as the first records of carnation, and the pathogenicity tests revealed that the virulence of *F. solani* was higher than *F. proliferatum* [10].

The aim of this study was to determine *Fusarium* spp. from infected plants in carnation greenhouses in Antalya Province. In addition, the pathogenicity tests were conducted using Turbo carnation cultivar and determined the effects on plant growth parameters. This is the first detailed study determining *Fusarium* species in this field where carnation production is made.

2. Materials and Methods

2.1 Collection of plant samples and pathogen isolation

Carnation samples showing disease symptoms (yellowing, wilting) were collected from a total of 29 carnation greenhouses in Antalya Province, Turkey. Collected plant samples were placed in plastic bags, taken to the laboratory, and subjected to isolation procedures.

For isolation procedure, the roots were cleaned and washed under running tap water and excess moisture was taken on the filter paper. For isolation, 4-5 mm tissue pieces were cut from the plant parts to containing diseased and healthy tissues and sterilized in 2% sodium hypochloride solution for 2 minutes. The surface sterilized plant parts

were rinsed twice with sterile distilled water and excess moisture were dried on sterile fitler papers. The sterilized tissues were placed in each petri dish containing the Potato Dextrose Agar (PDA) medium and incubated for 7 days at 24 °C. After the purification, *Fusarium* spp. were cultured on PDA, Carnation Leaf Agar (CLA) [11], Synthetic Nutrient Agar (SNA) [12].

2.2 Identification of *Fusarium* species

For the identification of *Fusarium* species, the isolates were cultured on PDA, SNA and CLA and than, incubated at 25 °C for 7-10 days. Colony diameters were measured at the end of the 4^{th} days development period and the daily growth rate was calculated. The lam culture technique was used to diagnose *Fusarium* cultures. The prepared lam cultures were incubated at 25 °C for 5-15 days [13]. Hyphal branching, fialid, microconidia, macroconidial shapes and sizes, chlamydospore and sporodochium formation of *Fusarium* species were observed using light microscope (Nicon/Eclipse E 100). During microscopic observations, for each species microconidia and macroconidia dimensions were measured with 10x and 40x objectives.

2.3 Determination of Pathogenicity of *Fusarium* species

The pathogenicity tests of *Fusarium* species were determined with pot trials in healthy carnation plants (*Dianthus caryophyllus* Linn. cv. Turbo). Rooted carnation plants were planted in pots with 10 cm diameters including peat and were grown in the climate room (16 hours of light, 8 hours of dark conditions, at 20 °C and 60-70% relative humidity. A selected isolate of each species was cultured on autoclaved wheat culture in 9 cm diameters petri dishes. Plants were inoculated by placing three grams of inoculum around roots. The control plants were inoculated three grams of autoclaved wheat cultures without pathogens in the experiments.

Pathogenicity of *Fusarium* species were evaluated according to scale 1-5 (1: Healthy plant; 2: Chlorosis in the bottom parts of the plant; 3: Bottom parts of the plant and 1/3 of the chlorosis or wiltness; 4: Wiltness on the upper part of the plant; 5; dead plant) [14]. At the end of the trial period, shoot length were measured with a ruler to evaluate the effects of pathogens on plant growth. At the same time, shoots and root weights are determined.

2.3 Statistical Analysis

The obtained data were analyzed by using variance analysis program of Minitab and the differences among the averages were determined by Tukey ($p<0.05$) multiple comparison test [15].

3. Results

In the present study, plant samples showing disease symptoms were collected and isolated from 29 carnation greenhouses in Antalya Province. As a result of surveys, a total of 11 *Fusarium* species have been identified and it has been proved that these species are potential problem in carnation production areas.

Species identified according to the morphological characteristics have been *F. acutatum* Nirenberg & O'Donnell, *F. avenaceum* (Fr.) Sacc., *F. chlamydosporum* Wollenw. & Reinking, *F. equiseti* (Corda) Sacc., *F. oxysporum* Schl., *F. poae* (Peck) Wollenw., *F. proliferatum* (Matsush.) Nirenberg, *F. sambucinum* Fuckel, *F. solani* (Mart.) Sacc., *F. tricinctum* (Corda) Sacc. and *F. verticillioides* (Sacc.) Nirenberg. Among the identified *Fusarium* species, *F. acutatum, F. avenaceum, F. chlamydosporum, F. poae, F. sambucinum* and *F. tricinctum* have been isolated for the first time in carnation, Turkey. The species having the highest and lowest prevalance were determined as *F. oxysporum* and *F. chlamydosporum*, respectively.

The pathogenicity of all *Fusarium* species in Turbo carnation cultivar were determined and the species had disease severity (%) at varying rates. The most virulent species were determined as *F. solani, F. sambucinum, F. oxysporum* and *F. acutatum.*

3.1 Cultural and Morphological Characteristics of *Fusarium* Species

Fusarium species isolated from carnation greenhouses of Antalya Province were identified by classical methods according to their cultural and morphological characteristics (Table 1).

TABLE 1. Distinctive features of *Fusarium* species

Fusarium species	Daily development rate on PDA(cm)	Pigmentation on PDA	Chlamydospor formation	Microconidia		Number of septa in macroconidia	Types of conidiogenous cells		Apical cell	Basal cell shape	Macroconidia sizes (µm)
				Shape	Number of septae		Monofialide	Polyfialide			
F. acutatum	1.1	Orange	+*	Oval Fusiform-Allantoid	0	3	+	+	Curved	Foot shape	30-54x2-3,5
F. avenaceum	1.2	Yellowish Brown	+	Fusiform	0-3	4-7	+	+	Smooth sickle	Foot shape	35-90x3,5-6
F. chlamydosporum	1.75	Burgundy	+	Smooth-Comma	0-2	3-5	+	+	Short curved-pointed	Foot shape Notched	30-37,5x3-5
F. equiseti	1.3	Brown	+	-	-	3-5-7	+	-	Alongated tapered	Foot shape	15-60x2,5-5,9
F. oxysporum	1,3	White-Violet	+	Oval Elipsoid Cylindrical	0-2	3-5	+	-	Curved	Foot shape	20-50x3-6
F. poae	1.5	Yellow- Red	-	Napiform Pyriform	0-1	2-3	+	-	Curved Tapered	Foot shape	18-38x3,8-7
F. proliferatum	1.1	Cream-Violet	-	Clavate Pyriform	0-1	3-5	+	+	Curved	Foot shape	30-58x3,3-4,4
F. sambucinum	1.1	Cream-Brown Red	+	-	-	3-5	-	+	Needle-tipped	Foot shape	22-50x4-5,6
F. solani	0.9	Brown - Orange	+	Clavate Ellipsoid	0-2	3-5	+	+	Alongated curved	Foot shape Notched	27-65x4,4-6,8
F. tritinctum	1.1	Red -Violet	+	Oval Pyriform Sitriform	0-1	3-5	+	+	Tapered Curved	Foot shape	24-50x3,2-4,6
F. verticilloides	1.2	Greyish cream-Violet	-	Oval-Clavate	0-2	3-7	+	-	Tapered Curved Needle-tipped	Foot shape Notched	30-58x2,7-3,6

* (+) available, (-) absent

F. avenaceum (Corda : Fr.) Sacc

The colony diameter was measured as 3-5.9 cm at 25 °C on the SNA medium at 4th days. *F. avenaceum* forms abundant aerial mycelium and its colour varies from yellow to whitish red. The pigmentation on the PDA is yellowish or brownish red. Conidiophores arising from aerial mycelium are simple, more or less branched. The microconidia in conidiogenous cells are fusiform shaped, 0-3 septate, and 6-30 x 2.5-4.5 (6) µm in size. Macroconidia are formed massively, it's fusiform-shaped, narrow in both sides, 4-7 septate, mostly 35-89 (90) x 3,5-4 (6) µm in size. Chlamydospores don't occur in mycelium, but they are rarely formed in the conidia. *F. avenaceum* causes root rot in wheat, rye, alfalfa and rough alfalfa. At the same time it leads to damage in vegetables, peach, apple, pear, oat, barley and wheat seeds [17,18] (Figure 2).

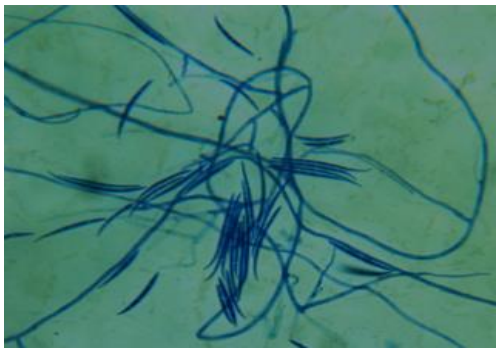

FIGURE 2. *F. avenaceum* macroconidia

F. chlamydosporum Wollenweber & Reinking

The colony diameter was measured as 7 cm at 25 °C on the PDA medium at 4th days. The aerial mycelium is white in young cultures. As the culture ages, its colour changes into greyish or burgundy. Microconidia are smooth or comma shaped, usually 0-2 septate, 10-26 x 2.5-4 µm in size. Microconidia are abundant and they are produced on both mono and poliphialides. Sporodochia formation is rare in PDA. In CLA, when sporodochia is produced, it is usually hidden on the bottom surface of carnation leaves. Macroconidia are thick walled and moderately curved. Macroconidia are usually 3-5 septate and have dimensions of 30-37.5 x 3-5 µm. Chlamydospore formation is abundant and very fast on CLA. Chlamydospores can be formed both singly and as a chain or bulk [19, 20]. (Figure 3)

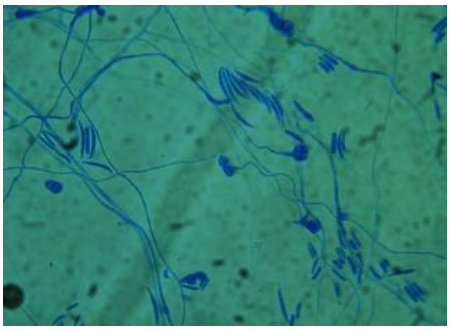

FIGURE 3. *F. chlamydosporum* macroconidia

F. equiseti (Corda) Sacc.

The colony diameter was measured as 4.5-6.9 cm in the 4th days at 25 °C on the PDA medium. The aerial mycelium varies from cream to yellowish brown in older cultures. In some isolates, sporodochia formation can be observed. Agar pigmentation of *F. equiseti* ranges from light brown to dark brown tones or it is formed peach colored pigments. Although microconidia are rare, it may be formed in some isolates fusoid or ovoid shaped, 0-2 septate and 6-24 x 2.5-4 µm in size. Macroconidia are sickle-shaped and they have 3-5 and rarely 7 septate. Dimensions of macroconidia are 15-60 x 2,5-5,9 µm. It produces abundant amounts of chlamydospores. Chlamydospores are formed in thin or thick-walled, intercalated, single, chain or clustered in hyphae and conidia [18]. (Figure 4)

FIGURE 4. *F. equiseti* macroconidia

F. oxysporum Schl.

The colony diameter was measured as 3-5.5 cm at 25 °C on the PDA medium at 4th days. The aerial mycelium has a cottony appearance and its whitish or peach colored. The pigmentation in the agar varies from cream to burgundy. In some races, orange colored sporodochia can be formed. Monophialides consist of branched or unbranched conidiophores. Microconidia are usually 0-2 septate, oval, ellipsoidal, cylindrical, smooth or slightly curved and 5-12 x 2.2-3.5 µm in size. They are formed in abundant amounts in short-branched phialides. Macroconidia are 3-5 septate, fusiform shaped, slightly curved, prominent in apical and basal cells and (20)27-46(50) x 3-4,5(6) µm in size. Chlamydospores are formed on hyphae or conidia, thin or thick-walled, semi-spherical shaped, terminal or intercalar of 5-15 µm in diameter. Chlamydospores can occur in both single and chain forms [18, 19]. (Figure 5)

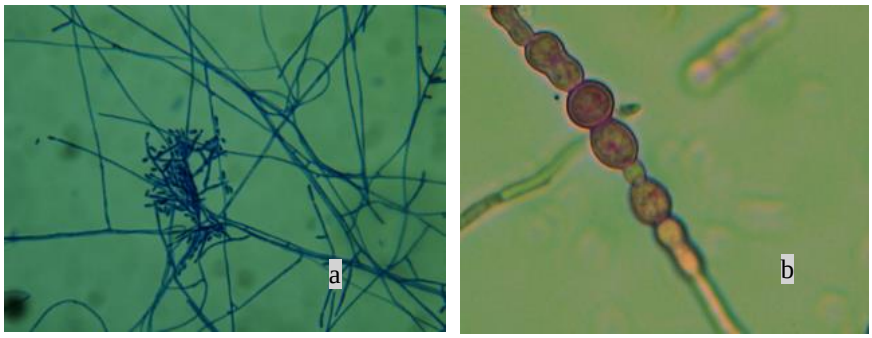

FIGURE 5. (a) *F. oxysporum* phialides, (b) chlamydospores

F. poae (Peck) Wollenw.

The colony diameter was measured as 5.5-8.8 cm at 25 °C on the PDA medium at 4th days. The aerial mycelium is cottony white or pale pink and may be close to the purple on the surface of the agar. Culture releases a scent that similar to peach smell. The pigmentation on the agar varies from yellow to shaded red. Sporodochia formation is not available. Microconidia formed in abundant amounts are napiform or pyriform shaped and their sizes are between 6-10 x 5.5-7.5 µm. Macroconidia are slightly curved, mostly 2-3 septate and 18-38 x 3,8-7 µm in size. Some macroconidia are 5 septate and bigger than 56 µm. Chlamydospore formation is absent, but hypha swellings are seen in some cultures. It has been reported to be isolated from cereal seeds, pepper and bean [18, 21]. (Figure 6)

FIGURE 6. *F.poae* macroconidia

F. proliferatum (Matsush.) Nirenberg

The colony diameter was measured as 3.5-5.5 cm at 25 °C on the PDA medium at 4th days. Color of aerial mycelium is white, pale pink or grayish violet. It can form a black sclerotium. Microconidia, produced in abundant amounts of aerial mycelium, are usually 0-1 septate. It is produced as a long chain or in conidiophores in bulk. The clavate-shaped ones are 7-9 x 2.2-3.2 μm and the pyriform-shaped ones are 7-11 x 4.7-7.7 μm. Macroconidia are rarely produced, usually 3-5 septate, smooth or sickle shaped and have dimensions of 30-46 x 3,3-4,1 μm, - 47-58 x 3,4-4,4 μm, respectively. There is no chlamydospore formation [18, 19]. (Figure 7)

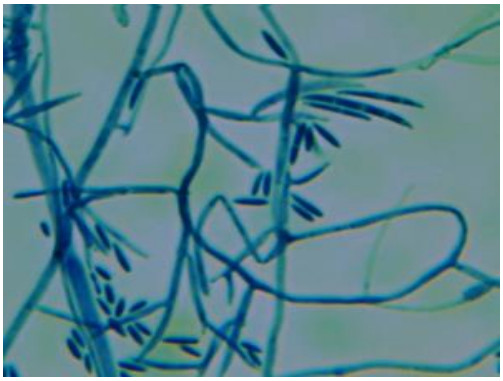

FIGURE 7. *F. proliferatum* macroconidia and microconidia

F. sambucinum Fuckel

The colony diameter was measured as 3.4-5.9 cm at 25 °C on the PDA medium at 4th days. The aerial mycelium is cottony and its color varies from white to dried rose. It forms white, yellowish, rosy or grayish red pigment on the agar surface. Dark red pigment is rarely seen in older cultures. It forms spore mass or sporodochia. There are no production of microconidia. Macroconidia are 3-5-7 septate, falcate, curved and thin-walled. Average dimensions of macroconidia are 22-50 x 4-5,6 μm. Chlamydospores occur singly, in the form of a chain, or as a mass [18, 19]. (Figure 8)

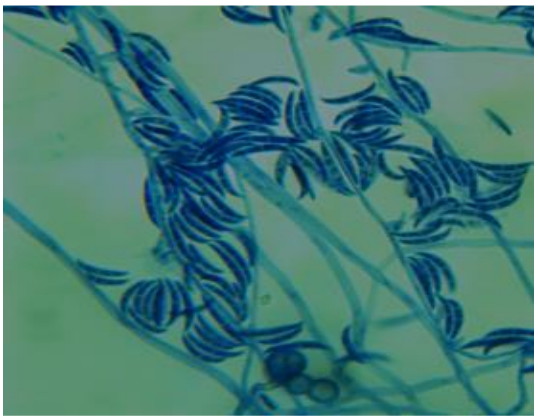

FIGURE 8. *F. sambucinum* macroconidia

*F. solani (*Mart.) Sacc.

The colony diameter was measured as 2.5-5.0 cm at 25 °C on the PDA medium at 4th days. The aerial mycelium is sparse or dense and its colour varies from greenish white to cream. Sometimes, on the surface of the agar forms a bluish-brown pigment from green. The conidial mass are also composed of sporodochia. Monophialides emerge from branched or unbranched conidiophores. Microconidia are generally ovoid, 0 to 1 septate and 8-16 (24) x 2-4 (5) µm in size and consist of long conidiophores with verticillate branching. Macroconidia are 3 to 5 septate (usually 3 septate) fusiform, cylindrical, slightly curved shaped and 27-52 (65) x 4.4-6.8 µm in size. The foot cell has a short apical cell that is not evident. Shape of chlamydospore varies from globose to ovoid and it forms hypha or conidia. Chlamydospores can form in terminal, intercalary, or chain form. Among the hosts were reported in carnations, avocados, beans, citrus, pea, peppers, potatoes and squash [18, 19, 21]. (Figure 9)

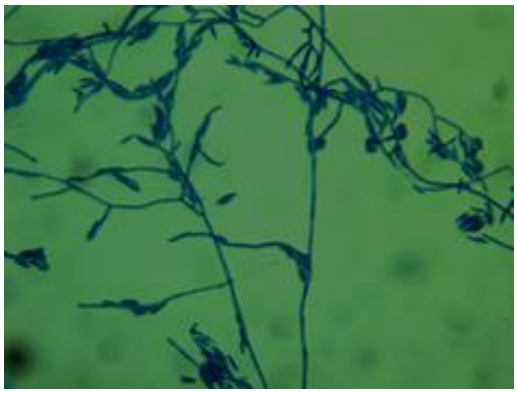

FIGURE 9. *F. solani* phialid and microconidia

F. tricinctum (Corda) Sacc.

The colony diameter was measured as 3.2-5.5 cm at 25 °C on the PDA medium at 4th days. The aerial mycelium is in a tight and fluffy form, and the color changes from red to purple, with the upper layer of these colors covered by a pale white mycelium. The agar color is generally red and purple tones, but in some cultures it produces yellowish pigment. Monophialides that are thin (10-30 x 2-3µm) and no simpodial branching are emerged from too many branched conidiophores. Microconidia are 0 to 2 septate, usually citriform, pyriform, napiform, ellipsoid or fusiform shaped, and 8-11 (14) x 4,5-7,5 µm in size. Sporodochium also produces a large number of macroconidia. Macroconidia are moderately curved and usually 3 to 5 septate and its dimension range from 24-46 x 3,2-4,1 µm to 33-50 x 3,6-4,6 µm. Chlamydospore formation is not common. It has been reported to be isolated from carnations, red alfalfa, cereals and soil [18, 19]. (Figure 10)

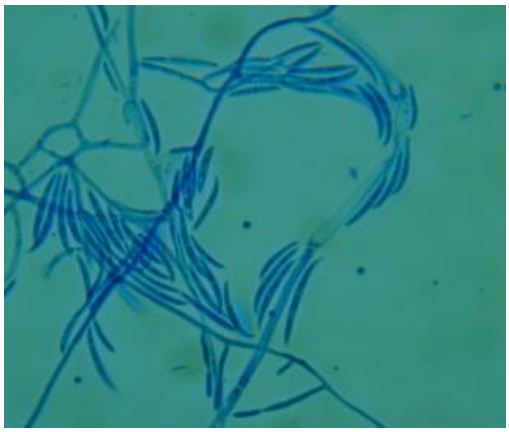

FIGURE 10. *F. tricinctum* macroconidia

F. verticillioides (Sacc.) Nirenb.

The colony diameter was measured as 3.5-5.5 cm at 25 ° C on the PDA medium at 4th days and 6.2 cm diameters in the 10 days on the SNA medium [9]. Microconidia are produced abundantly in aerial mycelium. It forms dark violet, lilac, wine red or shaded cream pigment on the agar surface. Microconidiophores are usually on aerial mycelium and don't show branching. The microconidia are in the form of long chains, rarely stacked, 0-2 septate, clavate shaped, with dimensions of 4.3-19 x 1.5-4.5 µm. Sporodochium and macroconidia are rarely seen in many races. Macroconidiophores show lateral and verticillate branching on hyphae. Macroconidia are thin, 3-7 septate smooth or slightly curved, fusiform, thin-walled, curved, apical cell elongated and basal cell stalked form. Sizes of macroconidia range from 30-46 x 2,7-3,6 µm to 47-58 x 3,1-3,6 µm. Chlamydospore formation is absent [18, 19]. (Figure 11)

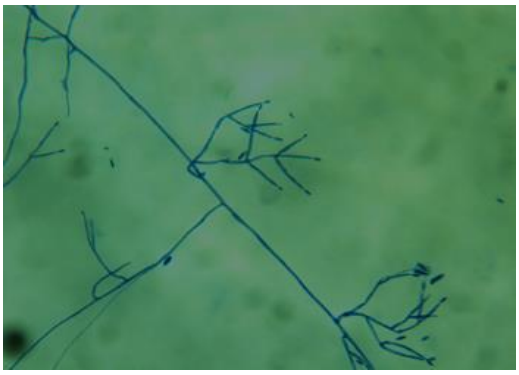

FIGURE 11. *F. verticillioides* phialid and microconidia

3.2 Pathogenicity Test

Pathogenicity tests of the identified *Fusarium* species were performed using Turbo carnation cultivar. According to the results, *Fusarium solani* had the highest disease severity, which was determined as 88%, followed by *F. acutatum* and *F. oxysporum* with 80% and 76% disease severity, respectively (Table 2).

TABLE 2. Disease index and disease severity after inoculation of *Fusarium* species (%)

***Fusarium* species**	**Disease Index**	**Disease Severity (%)**
F. acutatum	4.0 a*	80
F. avenaceum	3.2 b	64
F. chlamydosporum	3.2 b	64
F. equiseti	3.4 b	68
F. oxysporum	3.8 b	76
F. poae	3.0 c	60
F. proliferatum	3.4 b	68
F. sambucinum	4.2 a	84
F. solani	4.4 a	88
F. tricinctum	3.0 c	60
F. verticillioides	3.2 b	64

* The averages containing different letters in the same column are statistically different from each other according to the Tukey ($p<0,05$) test.

In this study, *F. solani, F. acutatum, F. sambucinum* and *F. oxysporum* significantly affected plant development in pathogenicity tests carried out at 25 ± 2 °C that they may cause serious problems in the production areas of carnation in our country. The inoculated *Fusarium* species were re-isolated from the infected carnation plants to prove the Koch's postulates.

According to the pathogenicity tests which conducted 28 days later, *Fusarium* species have reduced root weight, root length, shoot weight and shoot length compared to control (Table 3).

TABLE 3. Effect of *Fusarium* species on plant growth parameters

Pathogen	Root weight (g)	% Reduction	Root length (cm)	% Reduction	Shoot weight (g)	% Reduction	Shoot length (cm)	% Reduction
K**	3.01 a*	-	7.30 a	-	12.52 a	-	45.0 a	-
Fa	0.84 bc	71.9	1.62 b	77.8	2.93 b	76.6	7.6 c	83.1
Fav	0.54 bc	82.0	1.78 b	75.6	3.23 b	74.2	9.8 bc	78.2
Fc	0.61 bc	79.9	1.60 b	78.1	2.72 b	78.2	11.0 bc	75.6
Fe	0.98 bc	67.4	1.98 b	72.9	2.38 b	81.0	9.4 bc	79.1
Fo	0.81 bc	73.1	1.94 b	73.4	3.32 b	73.5	11.0 bc	75.6
Fp	1.02 b	66.3	1.54b	78.9	4.24b	66.1	12.0 bc	73.3
Fpr	0.94 bc	68.9	1.64 b	77.5	3.51 b	71.9	10.0 bc	77.8
Fs	0.92 bc	69.5	2.00 b	72.6	2.78 b	77.8	7.4 c	83.6
Fso	0.78 bc	74.0	1.88 b	74.2	2.31 b	81.5	8.6 bc	80.9
Ftr	0.69 bc	77.2	1.40 b	80.8	4.00 b	68.0	8.0 bc	82.2
Fv	0.79 bc	73.7	1.98 b	72.9	2.57 b	79.5	8.0 bc	82.2

* The averages containing different letters in the same column are statistically different from each other according to the Tukey ($p<0,05$) test.
** K: Kontrol, Fa: *F. acutatum*, Fav: *F. avenaceum*, Fc: *F. chlamydosporum*, Fe: *F. equiseti*, Fo: *F. oxysporum*, Fp: *F. poae*, Fpr: *F. proliferatum*, Fs: *F. sambucinum*, Fso: *F. solani*, Ftr: *F. tricinctum*, Fv: *F. vericillioides*

Fusarium species have reduced root and shoot lenghts by 72.6-80.8 % and 73.3-83.6 % respectively, compared to control. *F. verticillioides, F. solani, F. sambucinum, F. oxysporum*, and *F. equiseti* have brought about a reduction of 72.6-74.2% in root lenght. The other *Fusarium* species have caused more than 75 % reduction in root lengths. Pathogens have reduced root and shoot weight by 66.2-82 % and 66.1-81.5

%, respectively. While the greatest decline in root weight was made up by *F. avenaceum*, the greatest loss of shoot weight was caused by *F. solani.*

4. Discussion

From identified *Fusarium* species; *F. acutatum, F. avenaceum, F. chlamydosporum, F. poae, F. sambucinum* and *F. tricinctum* have been isolated for the first time in carnation, Turkey.

F. avenaceum has been reported to be pathogenic in watermelon, sainfoin, sugar beet and cotton. In a previous study, *F. chlamydosporum* was reported in spinach, watermelon, tomato and cucumber. *F. equiseti* has been reported to be pathogenic in carnation, gladiolus, tulip, melon, watermelon, cotton, tomato, onion, barley, wheat, chickpea, rice, bean, cabbage, carnivorous, spinach, radishes and celery, *F. oxysporum* was reported to be pathogenic in crops such as carnation, gladiolus, tulip, hyacinth, freesia, melon, watermelon, cucumber, chickpea, lentil, tomatoes, pepper, rice, cabbage, cauliflower, cowpea, cucumber, broom, corn, linen, groundnut, soybean, pea, bean, cotton, , banana, potato, onion, citrus fruits, apple and beet, *F. sambucinum* was reported to be pathogenic in crops such as tomato, pumpkin, cucumber, onion, cabbage, cauliflower, spinach, melon, watermelon, okra, lettuce, radish, carrot, cucumber, sunflower, sesame and tobacco, *F. proliferatum* was reported in carnation, wheat, bean, sainfoin, melon, watermelon and onion [21]. Among the hosts of *F. solani* were reported in carnation, avocado, bean, citrus, pea, pepper, potato and squash [18, 19, 21]. *F. tricinctum* was reported to be pathogenic in crops such as wheat, corn, cotton, pepper, eggplant, beet, onion and tomato, *F. verticillioides* was reported to be pathogenic in crops such as carnation, cereals, pomegranate and citrus [21].

In a conducted study in the Istanbul Province and its around, Özer and Soran [22] reported that *F. equiseti* was pathogenic by 70 % in carnation. In a conducted study in the Yalova Province, the pathogenicity of the Tempo carnation cultivar of *Fusarium* spp. isolated from carnation was determined. This resulted in virulence of *F. oxysporum* 76.70 %, *F. moniliforme* 100 %, *F. solani* 56.70 % and *F. culmorum* 66.70 % [23]. McCain [4] reported that *F. oxysporum* and *F. tricinctum* led to the disease in the carnation.

Acknowledgment. We thanks to Prof. Dr. Gürsel KARACA (Isparta University of Applied Science, Department of Plant Protection, Isparta, Turkey) for support in species identification. This study was produced first author's Msc Thesis.

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Available online: April 30, 2020

Commun.Fac.Sci.Univ.Ank.Series C
Volume 29, Number 1, Pages 148-160 (2020)
ISSN 1303-6025 E-ISSN 2651-3749
https://dergipark.org.tr/en/pub/communc/issue/51836/692077

REASSESSMENT OF IUCN THREAT CATEGORY FOR LOCAL ENDEMIC *CAMPANULA DAMBOLDTIANA* FROM ANKARA, TURKEY

GÜL AYYILDIZ, MERVE YILDIRIM, ALİ MURAT KESER, AHMET EMRE YAPRAK, AND GÜL NİLHAN TUĞ

ABSTRACT. In this study, threat category of local endemic plant species *Campanula damboldtiana* was reassessed according to IUCN Red List Categories and Criteria. *C. damboldtiana* prefers special habitat type with marly-gypsaceous soils. The assessment based on new field data such as current population size, distribution areas and the main threats to this taxon. Field studies were carried out during the vegetation periods between 2017 and 2018. In addition to those parameters, soil samples were taken from each location and physical and chemical analyses were performed including pH, EC, gypsum, texture, $CaCO_3$ parameters. Also, bioclimatic interpretations were made with the climatic data of the locations. With a recently discovered population, *C. damboldtiana* has three populations known from Ankara. The estimated total number of mature individuals was 8982. The area of occupancy and the extent of occurrence were calculated as 16 km^2 and 16 km^2, respectively. Considering our findings of *C. damboldtiana*, the IUCN threat category was reassessed as Critically Endangered (CR) as indicated in Red Data Book of Turkish Plants.

1. INTRODUCTION

In its geographical zone, Turkey is one of the richest countries in endemic plants. The narrowly distributed endemics live mainly in certain mountains and mountain chains and in certain habitats [1]. However, Turkey is very rich in endemic plants, some of these species that have very special habitat needs and restricted distribution areas, are faced with serious threats by anthropogenic drivers of global changes. According to recent studies, there are 11466 natural plant taxa, that 3649 of them are endemic (31.82%) throughout Turkey [2]. One of the major factors causing

Received by the editors: February 21, 2020; Accepted: April 18, 2020.
Key word and phrases: *Campanula damboldtiana,* IUCN, Endemic, Ankara, Conservation.

biodiversity erosion is habitat fragmentation, when the habitat is destroyed, patches of habitat remain. These habitat fragments are often isolated as differentiated or degraded areas [3]. *Campanula damboldtiana* P.H. Davis & Sorger is one of the local endemic taxon located in Ankara province. It belongs to Campanulaceae family and it is a perennial species with marly-gypsaceous soil preference. Flowering and fruiting periods occur between June and August. The species was first introduced to the scientific world by P.H. Davis and Sorger in 1979 and its type specimen was collected by Steppenhang at 16 km east of Ayaş, Aysantıbeli, which is located within the borders of Ankara province.

It was described as perennial herb with many stems. Roots cylindrical, thick, twisted. Flowering stems 5.5-22 cm. ascending, rigid. denselv retrorsely strigose with numerous leaves. Basal leaves absent. Cauline leaves oblong-linear, sessile, rigid, median ones 12-25 \ 2-4 mm. acute, patent or deflexed, densely antrorsely strigose, lower and upper decreasing in size. Inflorescence racemose or narrowly subpaniculate. 1.5-15 x 1-2 cm. upright. Corolla infundibular-campanulate. c. 12\ 12 mm, lilac-blue, adpressed pubescent outside, divided to 1/3, lobes ovate, acute, spreading (Figure 1). This species certainly belongs to Sect. *Dictyocalyx* (Fed.) Damboldt because of its inflated and reticulately-veined appendages in fruit; it is related to *Campanula stricta* L. [4,5].

The species was known from only two populations in Ankara: Ayaş, Aysantıbeli; Kahramankazan, Orhaniye locations [4,5], but a new location was discovered from Sincan district between Mülk and İncirlik villages during the fieldworks. Consequently, it has three populations known from Ankara province (Table 1). Additionaly, *C. damboldtiana* appears in the list of Bern Convention Appendix-I under title of "Strictly protected flora species" [6]. The threat category of *C. damboldtiana* is defined as Critically Endangered (CR) in Red Data Book of Turkish Plants [7].

FIGURE 1. *Campanula damboldtiana.*

TABLE 1. Locations of *C. damboldtiana.*

1	A4 Ankara: 16 km east of Ayaş, Ankara-Ayaş road 50th km, Aysantıbeli, 1180-1210 m
2	A4 Ankara: Sincan, between Mülk-İncirlik villages, 940-1000 m
3	A4 Ankara: North of Kahramankazan – Orhaniye village, Çaltepesi, 1070-1210 m

Because the species is rare, endemic and threatened, it needs to be protected. Therefore, the population size, distribution area, IUCN threat categories of this edaphic endemic species are determined. Physical and chemical analyses of soil

samples including pH, EC, gypsum, texture, $CaCO_3$are performed and bioclimatic interpretation is done using the climatic data of the locations.

2. Materials and Methods

2.1 Field studies and re-evaluation of IUCN Categories

The distribution areas of *C. damboldtiana* were determined by reviewing of literatures and visiting of the major herbaria of Ankara (ANK, GAZI, HUB). For the potential distribution areas, potential habitats around the known distribution areas were visited in the years between 2017 and 2018. By creating minimum convex polygon on Google Earth with GPS coordinates of locations, distribution areas were calculated. For determination of population sizes, small populations were counted one by one and for large populations it was estimated by extracting the mean of mature individuals in 25 m^2 of sampling areas with 10m gaps.

The threat category of each species re-evaluated in the light of the data obtained according to IUCN Red List Criteria [8] such as area of occupancy (AOO), extent of occurrence (EOO), number of mature individuals, number of locations and the main threats to the taxon. AOO and EOO values were calculated by using GeoCAT (Geospatial Conservation Assessment Tool program) [9] IUCN mapping program considering "Guidelines for Using the IUCN Red List Categories and Criteria" version 14 (Table 2) [10].

Table 2. Summary of some criteria in IUCN Red List threatened categories.

Threatened Categories	**Extent of occurrence (km^2)**	**Area of occupancy (km^2)**	**Number of mature individuals**	**Number of locations**
CR (Critically Endangered)	<100	<10	<250	=1
EN (Endangered)	<5.000	<500	<2.500	≤5
VU (Vulnerable)	<20.000	<2.000	<10.000	≤10

2.2 Bioclimatic Data

Climatic data of all locations obtained from General Directorate of Meteorology and their bioclimatic interpretations were made considering Emberger and Gaussen Methods [11]. There were two different meteorological observation stations covering the study areas (Table 3)

TABLE 3. Information of meteorological observation stations used in climate analysis of the study areas.

Station name	**Observation duration**	**Station altitude**	**Covered locations**
Etimesgut Airport	22 years	806 m	A4 Ankara: North of Kahramankazan – Orhaniye village, Çaltepesi, 1070-1210 m A4 Ankara: Sincan, between Mülk-Incirlik villages, 940-1000 m
Ayaş	15 years	910 m	A4 Ankara: 16 km east of Ayaş, Ankara-Ayaş road 50^{th} km, Aysantıbeli, 1180-1210 m

2.3 Soil Data

Within the scope of the study, soil samples were taken from each location of *C. dambodtiana* by considering 3 different heights as bottom, middle and top. Soil samples were dried and prepared as 2 kg each and sent to BIOTAR soil analysis laboratory for physical and chemical analyses. As physical parameter, texture analysis; as chemical parameters pH, EC (electrical conductivity), $CaCO_3$ and gypsum analyses were performed.

3. Results and Discussion

3.1 Field studies and re-evaluation of IUCN Categories

C. damboldtiana is known from three populations including Ayaş, Kahramankazan and Sincan in Ankara province (Figure 2). It has 8982 mature individuals in total. The AOO was calculated as 16 km^2 and the EOO was calculated as 16 km^2 (Figure 3). The main threats are urbanisation, agricultural and mining activities. The IUCN threat category was found as CR [8] (Table 4).

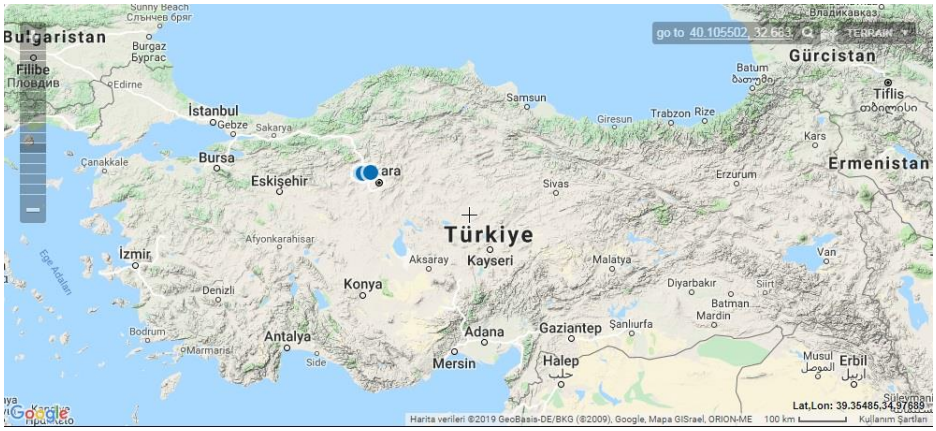

Figure 2. Location of three populations of *C. damboldtiana*.

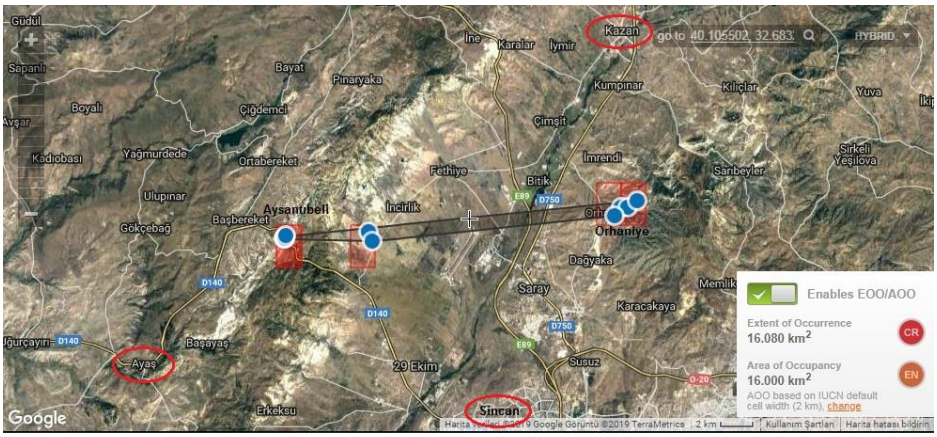

FIGURE 3. Area of occupancy and extent of occurrence of *C. damboldtiana.*

TABLE 4. Field data of *C. damboldtiana.*

Locations	**Number of mature individuals**	**Area**	**Threat factors**
A4 Ankara: North of Kahramankazan – Orhaniye village, Çaltepesi, 1070-1210 m	5610	≈28 ha	Mining activities (to obtain clay)
A4 Ankara: 16 km east of Ayaş, Ankara-Ayaş road 50th km, Aysantıbeli, 1180-1210 m	330	≈5 ha	Expansion of agricultural areas
A4 Ankara: Sincan, between Mülk-İncirlik villages, 940-1000 m	3042	≈18 ha	Expansion of agricultural areas, Proximity to urban areas, Soda Ash and Sodium Bicarbonate factory
Total	**8982**	**≈51ha**	

3.2 Bioclimatic Analysis

Bioclimatic analysis of the study areas was performed according to Emberger method (Table 5).

TABLE 5. Bioclimatic analysis of the study areas [12].

Stations / **Parameters**	**Etimesgut Airport** Kahramankazan and Sincan	**Ayaş** Aysantıbeli
P (mm)	381.9	427.1
M (°C)	31.7	29.4
M (°C)	-3.1	-2.9
Q	38.19	46.19
PE (mm)	65.5	58
S	3	1.97
Rainfall regime	Eastern Mediterranean Type 2	Eastern Mediterranean Type 2
Bioclimatic layers	Semi-arid "lower", very cold in winter, Mediterranean	Semi-arid "upper", cold in winter, Mediterranean

P: Mean total annual rainfall (mm),
M: Mean max.temperature of the warmest month (°C),
m: Mean min. temperature of the coldest month (°C)
Q: Rainfall-temperature coefficient
PE: Summer rainfal total (mm)
S: Drought index

Type of rainfall regime are characterized by Eastern Mediterranean Type 2 for all locationsof C.*damboldtiana*. In this regime type, spring is the rainy season and summer is the driest season [11].

Ombrothermic diagrams of the study areas were created according to Gaussen method [13] (Figure 4, 5).

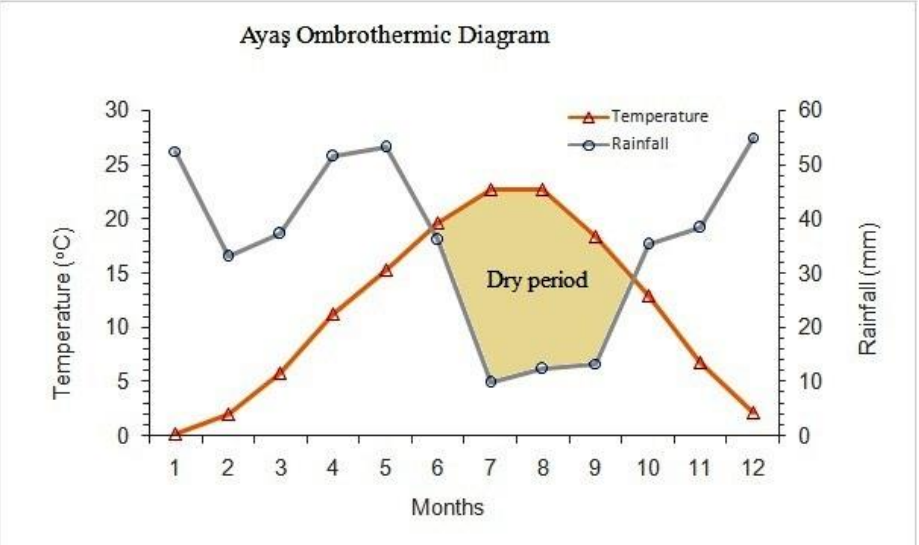

FIGURE 4. Ayaş (Aysantıbeli) ombrothermic diagram.

The dry periods, determined in the ombrothermic diagrams, starts with June and lasts at the beginning of October in Ayaş, Sincan and Kahramankazan.

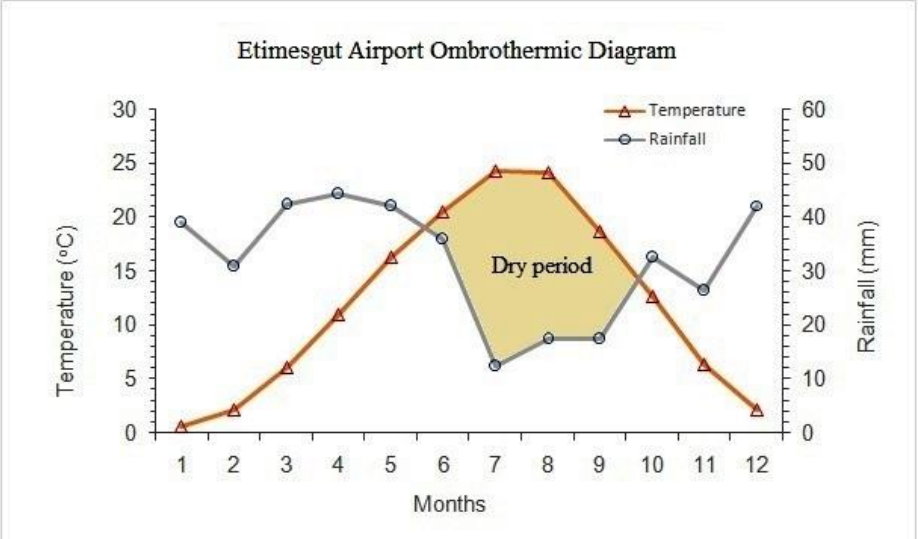

FIGURE 5. Etimesgut Airport (Kahramankazan and Sincan) ombrothermic diagram.

3.3 Soil Parameters Analysis

EC, pH, $CaCO_3$, gypsum and texture analyses were performed on soil samples taken from all locations (Table 6).

TABLE 6. Results of physical and chemical parameter analyses of soil samples.

Soil sample no	**pH (saturated soil paste)**	**EC (dS/m)**	**Salt (%)**	**$CaCO_3$ (%)**	**Gypsum (%)**	**Texture**
1 (Ayaş)	Slightly alkaline 7.89	0.540	Non-saline 0.0329	Strongly calcareous 43.85	0.135	Clay
2 (Sincan)	Slightly alkaline 7.85	0.440	Non-saline 0.0199	Strongly calcareous 45.28	0.101	Clay loam
3 (Kahramankazan)	Slightly alkaline 7.82	0.430	Non-saline 0.0113	Strongly calcareous 72.60	0.056	Loam

4. Conclusion

According to the results of climatic analysis, all of the study areas are under the influence of "semi-arid Mediterranean climate". In various type of "semi-arid Mediterranean climate", secondary steppe vegetation of anthropogenic origin is being dominant. In fact, all study areas have secondary steppe vegetation. This vegetation is sometimes covered with tree or shrub formations. However, the steppe vegetation without trees is the majority [11].

Considering the results of chemical analysis of soil samples, it can be inferred that all three locations have "non-saline, slightly alkaline and strongly calcareous" soils. Although there are slight differences between the locations in terms of texture, the clay content is dominant. Since all of them have predominantly calcareous-clay soil, they can be described as marly soil. In addition, according to the literature [14], since the gypsum contents are less than 2% in all samples, they cannot be classified as gypsum soils. However, all the soil samples contain gypsum in small quantities.

After the field studies and the examination of the data gathered from them, the EOO (Extent of occurrence), AOO (Area of occupancy), number of mature individuals (population size) and number of locations were determined. IUCN Red List Categories of each species were reassessed and all the results were summarized in Table 7.

Table 7. Results of IUCN Red List Criteria gathered from the field studies.

EOO (km^2)	**AOO (km^2)**	**Number of mature individuals**	**Number of locations**	**Categories in Red Data Book of Turkish Plants**	**Reassessed IUCN Red List Categories**
16	16	8982	3	CR	CR

For *C. damboldtiana* threat category did not change, it was reassessed as CR as before [CR B1ab (ii,iii)]. Even though the number of mature individuals seems relatively high, because of EOO size and the anthropogenic threat factors like urbanisation, agricultural and mining activities, it is better to keep the status in CR for this species.

To protect *C. damboldtiana,* there are some protection activities performed by Republic of Turkey Ministry of Agriculture and Forestry Ninth Regional Directorate of Nature Protection and Natural Parks, such as sending the seeds to gene banks and placing informative sign-boards to the locations.

Acknowledgements. This research was supported by "Ankara University Research Fund (Project no: 18L0430010)" and Republic of Turkey Ministry of Agriculture and Forestry Ninth Regional Directorate of Nature Protection and Natural Parks (Species Action Plan and Monitoring Project). Special thanks to Mecit Vural, Selçuk Tuğrul Körüklü, Ümit Subaşı, İsa Başköse, Batıkan Günal and Vildan Toprak for their supports in this study.

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Available online: June 02, 2020

Commun.Fac.Sci.Univ.Ank.Ser. C Bio.
Volume 29, Number 1, Pages 161-175 (2020)
ISSN 1303-6025 E-ISSN 2651-3749
https://dergipark.org.tr/en/pub/communc/issue/51836/663035

EFFECTS OF β-ESTRADIOL ON DNA METHYLATION CHANGES AND GENOMIC STABILITY IN *TRITICUM AESTIVUM* L. EXPOSED SALT

MURAT AYDIN, SEMRA YAĞCI, GÜLERAY AĞAR, AND
MAHMUT SINAN TAŞPINAR

ABSTRACT. Salt stress is considered one of the most important agricultural problems because of causing yield loss. Although it is well known that salinity damages to DNA and results in DNA methylation changes in plants, there is no report investigating the effect of mammalian hormones on plants under salinity stress. Therefore, the present study was aimed at investigating DNA damage levels (Genomic Template Stability) and DNA methylation changes in *Triticum aestivum* L. cv Kırik subjected to salinity stress and determine whether β-estradiol has any effect on these changes. RAPD (Randomly Amplified Polymorphic DNA) and CRED-RA (Coupled Restriction Enzyme Digestion-Random Amplification) were used to define the DNA damage levels and changes in the pattern of DNA methylation, respectively. The results showed that salinity stress led to an increase in RAPDs and CRED-RA profiles changes. Furthermore, salinity stress was a reduction in genomic template stability (GTS) and DNA methylation changes. The effects caused by salinity stress were decreased after treatment with different concentrations of β-estradiol. The results of this study have clearly shown that β-estradiol could be used effectively to protect wheat seedlings from the destructive effects of salinity stress in molecular levels.

1. INTRODUCTION

Abiotic stress causes economic losses because of reductions in productivity of agricultural crops. Salt stress is one of the major abiotic stresses for plants. The destructive effects of salinity on plants can be observed at the whole-plant level as the death of plants [1-3]. As a result of being exposed to salinity stress, plant growth

Received by the editors: December 22, 2019; Accepted: April 26, 2020.
Key word and phrases: Mammalian sex hormone, DNA damage, epigenetic change, wheat

and development are negatively affected. The effects of salinity plant devastation are related to osmotic stress, stress of soil solution, ionic equilibrium, specific ion effects, an increased amount of toxic ions, oxidative stress, the occurrence of genetic damage and epigenetic modification or a combination of all these factors [2-4].

Salt stress causes biochemical and physiological changes at the cellular and molecular levels, such as an increase in the plant stress hormone abscisic acid (ABA) and reactive oxygen species (ROS) levels, damaging cells and tissues by disturbing cellular structures, DNA methylation (e.g. cytosine methylation) and histone modification (e.g., acetylation, methylation and phosphorylation) changes, also plays a crucial role in regulation gene expression in plant [5,6]. In addition, several studies in recent years have demonstrated that environmental stresses such as water, cold, drought, salt, osmotic insults alter gene expression by DNA methylation and histone modification. Some studies have reported that the level of global DNA methylation decreases as the salt concentration is increased [7-9]. By contrast, salt stress has resulted in cytosine hypermethylation in rape, *Arabidopsis thailana*, and pea plants. It has been reported that different concentrations of salinity caused DNA methylation changes in *Jatropha curcas* L. Cytosine methylation plays an integral role in regulating gene expression at both transcriptional and posttranscriptional levels [10]. Surprisingly, methylation in the transcribed regions of endogenous genes is unexpectedly constitutes a common adaptation mechanism against stress in plants [11].

Plant growth regulators may help to improve the methods to increase the resistance of plants to adverse environmental conditions [12]. It has been reported that plant hormones modulate plant responses to oxidative stress generated by salinity [13,14]. On the other hand, a few studies have demonstrated that exogenous mammalian sex hormones (MSHs) such as progesterone, β-estradiol and androsterone have positive effects on plant growth and development. Moreover, they stimulate the activities of oxidative enzymes and synthesis reactions, reduce hydrogen peroxide (H_2O_2) content and lipid peroxidation (MDA) levels by inducing the activities of antioxidant enzymes, increase protein and nucleic acid contents and affect the inorganic constituents of plants under non-stress conditions [15,16]. Erdal [15], first recorded that MSH treatment stimulated superoxide dismutase (SOD), peroxidase (POX), catalase (CAT), ascorbate peroxidase (APX) and nitrate reductase (NR) activities and decreased in the lipid peroxidation (MDA) level, superoxide (O_2^-) production and H_2O_2 content following salt treatment. The same researcher demonstrated that MSH treatment generated a preventive effect against salt stress which promote the amount of dry weight, sugar, proline, protein, chlorophyll and glutathione (GSH)

[2]. Furthermore, according to the results of other studies in chickpea seedlings, the sodium, potassium and calcium content were increased by MSH treatment while the chloride content was reduced. Those results are critical for defusing salt stress because MSHs prevented the change in the K/Na and Ca/Na ratios [17, 18].

Previous results have suggested that MSH treatment modulates negative effects by salt stress in plants. However, the effect of β-estradiol on genetic and DNA methylation changes against salt stress has not been elucidated. The main aim of the present study is to determine whether β-estradiol has any protective effect against the adverse effects of salt stress in wheat

2. Materials and Methods

2.1. Plant material and treatment conditions

T. aestivum L. cv. Kırik seed samples known to be sensitive to salinity were obtained from the Department of Field Crops, Faculty of Agriculture, Ataturk University (Turkey). After sterilization with 1% w/v of sodium hypochlorite for 10 minute and washing with sterilize dH2O, sterilized seeds were soaked in solutions containing 0 (dH_2O), 10^{-8}, 10^{-9}, and 10^{-10} M β-estradiol at 25±1ºC for 24 hours. 20 pretreated seeds placed in each petri dishes with two layers of Whatman number 1 filter paper. NaCl (Sodium chloride) solutions (0, 100 mM and 200 mM) were added to each petri dishes. Petri dishes were kept in 22±1°C under 16-h light/8-h dark light conditions for 14 days for seed germination. Each treatment was replicated three times. Each petri dish was evaluated as a repeat. Bulk sample strategy was applied for molecular analysis. Three seedling were randomly taken from each repetition and a total of nine plants were used for each treatment. Samples were stored at -80ºC for DNA extraction.

2.2. Genomic DNA isolation

Genomic DNAs (gDNAs) was extracted from seedlings using the method described by Arslan et al. (2019) [19] and stored at -20ºC for further use. The quality and concentration of the gDNAs were measured using a Nano-Drop (ND-1000) spectrophotometer and electrophoresis in a 0.8% (w/v) agarose gel.

2.3. RAPD technique

13 primers (OPA-13, OPY-11, OPY-13, OPY-7, OPH-19, OPY-1, OPY-8, OPY-15, OPB-8, OPW-4, OPW-7, OPB-10 and OPW-5) were used in RAPD-PCR reactions (Table 1). PCR amplifications were carried out in thermocycler (SensoQuest GmbH, Göttingen, Germany) in a total volume of 25 µl, containing 50 ng gDNA, 10× PCR buffer (10 mM Tris–HCl, 50 mM KCl, pH=8.3), 400 µM dNTP, 10 pmol primer, 2.5 mM $MgCl_2$ and 1 U Taq DNA polymerase. The amplification profile composed of an initial denaturation at 95°C for 5 min, followed by 38 cycles at 94°C for 1 min, 36°C for 1 min 72°C for 2 min and a final extension of 15 min at 72°C.

2.4. CRED-RA technique

Genomic DNA sample from each treatment were separately digested with HpaII and MspI endonucleases. After checking digestion on agarose gel, 1µl of each digestion product were amplified with 8 random primers (OPY-11, OPY-7, OPY-13, OPH-9, OPW-6, OPB-8, OPW-4 and OPW-5). Amplification and visualization conditions for CRED-RA are the same as described for RAPD analysis.

2.5. Electrophoresis

The PCR products (7 µl) were mixed with 6x gel loading buffer (3 µl) and subjected to agarose (1.5% w/v) gel electrophoresis in 0.5x TBE (Tris-Borate- EDTA) buffer at 80 V for 120 min. Amplification products separated by gel electrophoresis were stained in ethidium bromide solution (2 µl Etbr/100ml of 1x TBE buffer) for 40 min. The amplified DNA products were detected using the Bio Doc Image Analysis System and analyzed using the UVI-soft analysis package (Cambridge Electronic Design Ltd, Cambridge, UK).

2.6. Molecular Patterns Analysis

RAPD patterns were evaluated using the Total Lab TL120 computer software. Genomic template stability (GTS, %) was calculated as follows: GTS = 100-(100x a/n), a in formula is the average number of polymorphic bands detected in each treated sample, and n is the number of total bands in the control. Polymorphisms in RAPD profiles included disappearance of a normal band and appearance of a new band compared with the control. The average was calculated for each experimental

group. To compare the sensitivity of each parameter, changes in these values were calculated as a percentage of their control (set to 100%) [19]. The average number of polymorphisms (%) was calculated for each dose to realize CRED–RA analysis. To calculate the number of polymorphisms (%), the following formula was used 100x a/n.

3. Results

In total, thirty-two oligonucleotide primers with 60-70% GC content were used for analyzing the PCR products of the *T. aestivum* L. cv Kırik genome, and only thirteen provided specific and stable results (Table 1). Compared with the PCR products obtained from the control DNA, β-estradiol and/or NaCl treatments resulted in apparent changes in RAPD patterns. These changes are characterized by variation in band intensity, loss of normal bands or appearance of new bands (Table 1). GTS was used for comparing the changes in RAPD profiles. GTS values decrease with increasing concentration of NaCl. This value was determined in 45.1% and 37.8% for 100 mM and 200 mM NaCl, respectively. Moreover, the application of β-estradiol did not cause any change in GTS. When the effect of β-estradiol and NaCl were considered together, it was determined that β-estradiol increased in GTS ratio in both NaCl concentrations. This increasing occurred in parallel with the β-estradiol dose increase. When the lowest dose of NaCl and highest dose of β-estradiol (H3+S1) were applied together, the GTS value was the highest (56.3%), and when the lowest dose of β-estradiol and highest dose of NaCl (H1+S2) were applied together, the GTS value was the lowest (46.7%) (Table 1).

CRED-RA analysis results were given in Table 2. Eight oligonucleotide primers which gave specific and stable results in RAPD analysis were used for CRED-RA analysis. Compared with the PCR products obtained from the control DNA, β-estradiol and/or NaCl treatments resulted in apparent changes in CRED-RA patterns (Figure 1). DNA methylation occurred at all doses of the combined treatments. Methylation value was 74.1% and 51.4% for 100 and 200 Mm NaCl applications, respectively. The highest methylation value was 42.2%, and the lowest was 27.9% in β-estradiol applications. Regarding the combined applications with the lowest dose of NaCl and highest dose of β-estradiol (H3+S1), the methylation value was the lowest (14.3%) with the lowest dose of β-estradiol and highest dose of NaCl (H1+S2) methylation value was the highest (38.7%).

TABLE 1. Molecular sizes (bp) of appeared (+)/disappeared bands (-) in RAPD profiles based on other treatments vs. control[1].

Primer	C	+/-	S1	S2	H1+S1	H2+S1	H3+S1	H1+S2	H2+S2	H3+S2
OPA_13	13	-	1438, 1284, 1184, 414, 322	1438, 1284, 1184, 934, 745,495, 414,322	1438, 1284, 1023, 745, 414,322	1438, 1284, 1023, 745, 414, 322	1438, 1284, 934,745, 414,322	1438, 1284, 1023, 745, 495,322	1184, 745, 495,322	1438, 1284, 1023, 745, 414,322
		+	1084,934	1084					849	
OPY_11	11	-	1387,715, 584	1687, 1387, 1062, 843, 438,352, 200	1687, 1062, 843,352	1062	1687, 1387, 1062	1387, 1062, 843,352, 200	1387, 1062, 438,352, 200	1687, 1387, 438,352, 200
		+				1237			922	
OPY_13	6	-	1121,800, 609,481	1121, 800, 481	928, 481	928, 481	800, 609	1121, 800	1121, 800, 481	1121, 800, 481
		+	692,322	322	652,288	556, 280	692	156	343	329,160
OPY_7	10	-	1300,1118, 388	1300, 1050, 689,388	1300,1 231, 1118, 1050, 541	1300, 1231, 970,541	1300, 1231, 970,541	1300, 1231, 1118, 1050, 754,388	1300, 1231, 388	1300, 1231, 388
		+	600	856				813		
OPH_19	10	-	693,	1220, 620, 491	1220, 693,	1220, 1073, 693	1220, 693,	1220, 1073, 947,776, 543	1220, 543, 387	1220, 620
		+	870		308		849,308			
OPY_1	9	-	671	1476, 952, 671	1476, 1269, 1138, 1076, 831	1476, 1269, 831	1476	1476, 831	1476, 1269, 952,459	1476, 1269, 1138, 831, 671,459
		+		568	533,353			788	1030	600
OPY_8	8	-	1353, 565	1353, 1233, 565	1353, 1233, 565	1353, 866	866, 565	1353, 565	1353, 565	1353, 565
		+	950,630	1166	805,484	1086,61	1093,48, 700		978, 653	
OPY_15	9	-	632,	764,632	764	1183, 764, 680	680	1183, 680, 632	1183, 764, 680,632	1183 680
		+	984,544	585,509	832534, 494,984	1458, 972, 867,529, 303	984,927, 504	849,566 240	914, 800, 240,572	800,372, 256

[1]: C (Control): 0 M β-estradiol + 0 mM NaCl, **S1:** 100 mM NaCl**, S2:** 200 mM NaCl**, H1:** 10^{-10} M β-estradiol**, H2:** 10^{-9} M β-estradiol**, H3:** 10^{-8} M β-estradiol, **H1+S1:** 10^{-10} M β-estradiol + 100 mM NaCl, **H2+S1:** 10^{-9} M β-estradiol + 100 mM NaCl, **H3+S1:** 10^{-8} M β-estradiol + 100 mM NaCl, **H1+S2:** 10^{-10} M β-estradiol + 200 mM NaCl**, H2+S2:** 10^{-9} M β-estradiol + 200 mM NaCl, **H3+S2:** 10^{-8} M β-estradiol + 200 mM NaCl

TABLE 1. (Continued).

Primer	C	+/-	S1	S2	H1+S1	H2+S1	H3+S1	H1+S2	H2+S2	H3+S2
OPB_8	11	-	1493,1313 1246,776	1493,1426, 1386,1313	1493,1426	1493,1426, 1386	1493,1426, 1386,1313,	1493,1426, 1386,1313, 1246,1153, 1053,984	1493,1426, 1386,1313, 1246	1493,1426, 1386,1313, 1246,1153, 1053,984
		+	861, 644, 541	876,845, 640,577, 510,443, 386	876,657, 615567, 472	876,657, 667,552, 447	858, 625, 577,472	867,833, 663,572, 500,424, 379,337	900,849, 707,611, 552,485, 421,389, 327	867,833, 663,572, 500,424, 379,337
OPW_4	12	-	1491, 1275, 1125, 886, 780, 674, 224	1491, 1275, 1125, 886, 674, 224	1275,575	1275,780	1491,1275, 780,481	1491,1275, 1125,886, 363	1491,1275, 1125,886	1491,1275, 1125,886
		+					982			
OPW_7	9	-	1376,1123, 983,762	1376,1123, 762	1123,983, 912,762	983,912, 762,	1376,983,	1376,1123, 983,762	1376,1195, 983,912, 762	1376,1195, 983,912,
		+	716	716,600, 574, 489, 400	834,708	826692, 581,489	658,356	617	850,634	640
OPB_10	11	-	1081,973, 800, 644, 516 300, 230	1081,973, 800,644, 516,300	644	644	800, 644	1081,973, 800,644 516,230	1081,973, 800,644, 516	1081,973, 800, 644, 516
		+	1300, 738, 590,178	1300,1172 738,590	1618,1445, 717	1618,1500, 1236,731	738	738,178	738,	909,724
OPW_5	10	-	1283,1058, 853,729, 600, 396, 296,	1283,1058, 853,729	1283,853, 396,296	729,296	1283,600, 396,296	1283,853	1283,1108, 853	1283,729
		+	917, 900, 574	917,574	992,523	1475,1183	671,567		548	
GTS%	100		45.1	37.8	48.6	51.9	56.3	46.7	47.2	49.5

TABLE 2. CRED-RA pattern analysis results[1].

Primer	Total bands				Total polymorphic bands						Polymorphism (%)			
	Control		HpaII		MspI		Hpa II		Msp I		Hpa II		Msp I	
	HpaII	MspI	S2	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2	S1
OPY-11	5	5	5	5	5	4	4	3	4	3	80	60	80	75
OPY-7	10	8	6	10	5	9	4	5	3	5	66.6	50	60	55.5
OPY-13	5	8	5	5	5	5	2	3	3	3	40	60	60	60
OPH-19	2	5	4	3	7	5	2	1	3	2	50	33.3	42.8	40
OPW-6	5	5	3	3	3	3	3	2	3	1	100	66.6	100	33.3
OPB-8	3	5	3	7	4	8	4	4	2	3	100	57.1	50	37.5
OPW-4	4	4	2	4	2	4	2	2	2	2	100	50	100	50
OPW-5	9	8	4	6	5	5	5	3	5	3	100	50	100	60
Total	43	48	34	43	36	43	26	23	25	22	-	-	-	-
Average	-	-	-	-	-	-	-	-	-	-	79.5	53.3	74.1	51.4

[1]: Abbreviations were listed in Table 1.

TABLE 2. (Continued).

Primer	Total bands								Total polymorphic bands						Polymorphism (%)					
	Control		HpaII			MspI			Hpa II			Msp I			Hpa II			Msp I		
	HpaII	MspI	H1	H2	H3	H1	H2	H3	H1	H2	H3	H1	H2	H3	H1	H2	H3	H1	H2	H3
OPY-11	5	5	5	4	5	7	3	5	2	1	0	2	0	0	40	20	0	28.5	0	0
OPY-7	10	8	9	9	11	11	7	10	3	2	1	3	4	2	33.3	22.2	9	27.2	57.1	20
OPY-13	5	8	4	4	9	1	6	7	2	3	4	7	2	1	50	75	44.4	100	33.3	14.2
OPH-19	2	5	2	3	3	4	4	5	2	1	1	1	1	0	100	33.3	33.3	25	25	0
OPW-6	5	5	5	4	4	5	5	4	0	1	1	0	2	1	0	25	25	0	40	25
OPB-8	3	5	6	7	5	2	7	8	3	4	2	5	2	3	50	57.1	40	100	28.5	37.5
OPW-4	4	4	5	3	4	5	3	2	1	1	2	1	1	2	20	33.3	50	20	33.3	100
OPW-5	9	8	10	10	6	8	9	11	3	2	3	3	5	3	30	20	50	37.5	55.5	27.2
Total	**43**	**48**	**46**	**44**	**47**	**43**	**44**	**52**	**16**	**15**	**14**	**22**	**17**	**12**	-	-	-	-	-	-
Average	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**40.4**	**35.7**	**31.4**	**42.2**	**34**	**27.9**

Primer	Total bands								Total polymorphic bands						Polymorphism (%)					
	Control		HpaII			MspI			Hpa II			Msp I			Hpa II			Msp I		
	HpaII	MspI	H1S1	H2S1	H3S1	H1S1	H2S1	H3S1	H1S1	H2S1	H3S1	H1S1	H2S1	H3S1	H1S1	H2S1	H3S1	H1S1	H2S1	H3S1
OPY-11	5	5	5	6	7	6	4	7	0	2	2	1	1	2	0	33.3	28.5	16.6	25	28.5
OPY-7	10	8	6	10	7	6	6	9	4	2	5	2	2	1	66.6	20	71.4	33.3	33.3	11.1
OPY-13	5	8	5	5	6	6	6	8	2	3	1	2	2	0	40	60	16.6	33.3	33.3	0
OPH-19	2	5	2	4	2	3	4	4	0	2	0	2	1	1	0	50	0	66.6	25	25
OPW-6	5	5	5	5	5	5	5	5	1	0	0	2	0	0	20	0	0	40	0	0
OPB-8	3	5	4	2	3	4	5	4	2	1	2	1	0	1	50	50	66.6	25	0	25
OPW-4	4	4	3	3	5	3	3	4	1	1	1	1	1	0	33.3	33.3	20	33.3	33.3	0
OPW-5	9	8	13	10	12	12	8	12	2	2	3	5	2	3	15.3	20	25	41.6	25	25
Total	**43**	**48**	**43**	**45**	**47**	**45**	**41**	**53**	**12**	**13**	**14**	**16**	**9**	**8**	-	-	-	-	-	-
Average	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**28.1**	**33.3**	**28.5**	**36.2**	**21.8**	**14.3**

Primer	Total bands								Total polymorphic bands						Polymorphism (%)					
	Control		HpaII			MspI			Hpa II			Msp I			Hpa II			Msp I		
	HpaII	MspI	H1S2	H2S2	H3S2	H1S2	H2S2	H3S2	H1S2	H2S2	H3S2	H1S2	H2S2	H3S2	H1S2	H2S2	H3S2	H1S2	H2S2	H3S2
OPY-11	5	5	3	5	7	3	6	7	2	0	0	2	1	1	66.6	0	0	66.6	16.6	14.2
OPY-7	10	8	5	13	7	7	10	8	4	4	0	3	2	1	80	30.7	0	42.8	20	14.2
OPY-13	5	8	7	8	5	4	8	5	0	3	2	0	0	1	0	37.5	40	0	0	20
OPH-19	2	5	5	5	5	6	5	6	0	3	3	1	0	2	0	60	60	16.6	0	33.3
OPW-6	5	5	4	5	4	5	5	6	2	0	2	2	0	1	50	0	50	40	0	16.6
OPB-8	3	5	7	2	4	5	8	3	2	2	2	2	3	1	28.5	100	50	40	37.5	33.3
OPW-4	4	4	3	3	3	3	3	3	2	1	0	2	1	0	66.6	33.3	0	66.6	33.3	0
OPW-5	9	8	7	9	7	8	8	6	2	3	0	3	6	1	28.5	33.3	0	37.5	75	16.6
Total	**43**	**48**	**41**	**50**	**42**	**41**	**53**	**44**	**14**	**16**	**9**	**15**	**13**	**8**	-	-	-	-	-	-
Average	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**40**	**36.8**	**25**	**38.7**	**22.8**	**18.5**

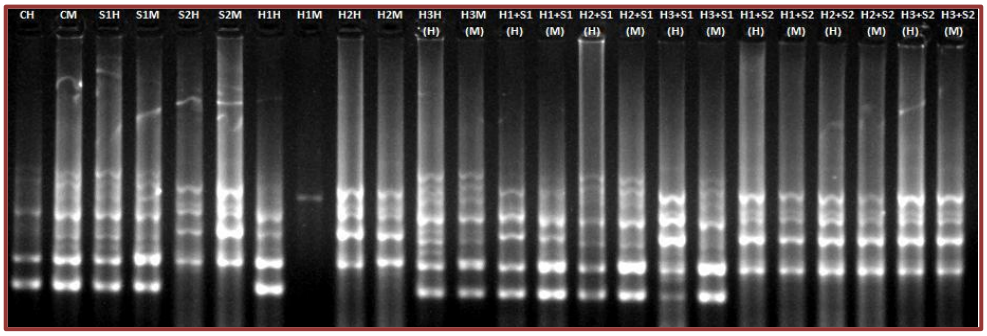

FIGURE 1. CRED-RA profiles based on treatments for primer OPY-13

4. DISCUSSION

Different abiotic stresses affect plant growth and productivity and cause economic losses. Salinity stress is one the most abiotic constraints for plant that also causes physiological drought indirectly. Recently, many studies showed that salt stress negatively affects plant growth and development, causing biochemical and physiological damages such as changes in dry weight, the levels of lipid peroxidation (MDA), ROS and chlorophyll and also the amount of proline, protein, sugar and enzyme activities in higher plants [2]. Several researchers reported that salt stress decreased the SOD, POX, CAT, APX, GSH and NR activities whereas it increased the O_2^-, H_2O_2 and MDA levels [2, 20].

ROS are permanently produced in cells even under optimal conditions in plants. Under non-stress conditions, plants maintain the balance between ROS production and activity of antioxidants. However, salt stress, like other abiotic stresses can upset the balance by increasing ROS production which caused to damage macro molecules such as DNA, protein and lipids. Previous studies have demonstrated that abiotic stresses such as drought, salt, water deficit and heavy metals cause DNA damage using different molecular techniques [21, 22]. In our study, we used RAPD to prove that salt stress caused to DNA damage in the wheat seedlings, a finding that was sign of GTS reduction. The differences in the DNA profiles observed in the present study were clearly dependent on extensive DNA damage (e.g. single and strand

breaks, modified bases, oxidized bases, DNA-protein cross-links, point mutations, complex chromosomal rearrangements, mutations in some oligonucleotide priming sites, large deletions and homologous recombination) induced by salt stress. The molecular mechanism responsible for the genotoxicity of salt stress suggested that salt stress could stimulate the release of free radicals and ROS such as O_2^-, hydroxyl radical (HO·), and H_2O_2 species [23-28]**.** Much of reactive oxygen does not appear to interact with DNA but they are precursors for hydroxyl radicals. The reaction of HO· with DNA generates a multitude of products because it attacks sugar, pyrimidines and purines, including guanine residues to form 8-hydroxydeoxyguanosine. In this instance, plants must develop a set of bio-defences to cope with these sources of damage by differential expression of several hundred genes and protein function in response to the different stresses. One of the molecular mechanisms by which plants could silence or super-activate the selected DNA templates is epigenetic modifications that change gene expression without changing DNA sequences [12,29]. Recently, several studies have demonstrated that salt stress alters gene expression through DNA methylation and histone modification [30-32]. Zhong et al. [33] reported that salt stress caused DNA methylation in *T. aestivum*. Similarly, Zhao et al. [34] suggested that demethylation positively contributed to salt tolerance and hypermethylation had a negative effect on salt tolerance in cotton. Lu et al. [21] suggested that both de novo methylation and demethylation events can help to plant adaptation under salt stress. The present study showed that different levels of salinity treatment caused DNA methylation changes in the whole genome. These changes can contribute to improve tolerance in plants under salt stress as well as its role in the control of plant development [33].

Exogenous applications in different stress conditions may help to increase tolerance in plants. Recently, some studies have emphasized that treatment with MSH may help increase to plant tolerance [2,17,18,35-37]. The studies have demonstrated that exogenous application of MSH (such as progesterone, β-estradiol and androsterone) substantially improved plant growth and development, augmented protein and nucleic acid contents, stimulated oxidative enzyme activities, and reduced H_2O_2 content and the MDA level under non-stress conditions [17].

Erdal and Dumlupinar [36] showed that MSH also affected the inorganic constituents of plants. The same researchers demonstrated that MSH treatment significantly decreased the Na content in chickpea seeds and barley leaves. In addition, other studies have shown that although MSH treatment increased the Na content in chickpea seedlings MSH treatment also increased K and Ca contents and decreased the Cl content [36,39]. Moreover, Erdal et al. [37] reported that MSH treatment together with salt stress increased the dry weight, sugar, proline, protein, chlorophyll, and GSH contents, as well as SOD, POX, CAT, APX and NR activities and reduced the MDA level, O_2^- production and H_2O_2 content compared with salinity alone.

Both earlier studies and our results suggest the protective role of MSH against stress related to osmo-protection, osmotic adjustment, carbon storage, radical scavenging and high antioxidant activities. According to the present findings, it is possible that the antigenotoxic effect of β-estradiol on salt stress might be related to its radical scavenging and high antioxidant activities. In the literature, no report is available the role of β-estradiol in salinity exposed wheat seedlings with regard to DNA methylation changes and genomic stability. To our knowledge, the present study represents the first report indicating the effects on DNA methylation changes of β-estradiol under non stress and salt stress conditions in wheat.

The contribution effect of β-estradiol against DNA methylation changes may be related to its effect on the transcription and translation processes of specific genes, improving the plant resistance under stress conditions. Many researchers have reported that the soluble protein content increases under salinity stress [2,39,40]. Therefore, β-estradiol could have adaptive significance for plants grown under salt stress. Based on the above findings, it is concluded that β-estradiol application to may be useful for large-scale agricultural benefit, and we intend to carry out such an investigation in the near future.

Acknowledgment. The authors thank the contributes of Atatürk University.

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Available online: May 25, 2020

Commun.Fac.Sci.Univ.Ank.Series C
Volume 29, Number 1, Pages 176-201 (2020)
ISSN 1303-6025 E-ISSN 2651-3749
https://dergipark.org.tr/en/pub/communc/issue/51836/672275

NEMATODE-DESTROYING FUNGI: INFECTION STRUCTURES, INTERACTION MECHANISMS AND BIOCONTROL

MEHMET KARAKAS

ABSTRACT. Fungi are pathogenic for different nematode groups, but their relationship with soil nematodes goes a grade beyond parasitism and into predation. Approximately, 200 species of taxonomically various fungi can attack active nematodes, which are effective animals nearly 0.1 to 1.0 mm long. Among these nematode-destroying fungi, only a few species are obligate parasites of nematodes; the majority are facultative saprotrophs. Nematode-destroying fungi have four general groups: (a) fungi with specialized structures (b) fungi with toxins; (c) fungi with spore germination; (d) fungi with colony-forming. Nematode-destroying fungi are natural enemies of nematodes in soil ecosystems and have potential as biocontrol agents against plant- and animal-parasitic nematodes. These predator fungi catches free-living nematodes in the soil ecosystem using traps produced by the fungal mycelium that cling to the worm, then, penetrate, kill, and digest the tissue of the nematode. Five kinds of trapping apparatus belonging to fungi are defined. These are adhesive or sticky column, adhesive or sticky knob, adhesive or sticky system, constricting and non-constricting rings.

1. INTRODUCTION

Nematode-destroying or hunting fungi are inherent enemies of nematodes called as roundworms. Nematode-destroying fungi can infect the eggs, larvae, or adult stages of the nematode. They reduce the population density by stopping the feeding activity of the nematode. These fungi contain more than 200 taxonomically distinct group types that can be classified as nematode-destroying or nematophagous fungi and endophytic fungi. The fungi that destroy the nematode are also divided into egg- and female-parasitic fungi invading nematode eggs or females with their hyphal ends, endo-parasitic fungi using their spores and toxin-producing fungi immobilizing nematodes before the invasion [1-4]. The taxonomy of nematophagous fungi, as well as their mode of action, is briefly shown in Table 1.

Received by the editors: January 8, 2020; Accepted: March 31, 2020.
Key word and phrases: Nematode-destroying fungi, pest management, biocontrol agents.

Nematode oppositional fungi have so far received a lot of attention, partly because of their high negative activity against both plant- and animal-parasitic nematodes, and their remarkable morphological adaptations in hunting and parasitizing nematodes [5, 6].

Studies on fungi that trap and hunt nematodes are considerably higher than studies with other organisms. Fungi forming traps are capable of catching nematodes by creating traps in various ways [7-10].

According to another classification, nematode-destroying fungi contain three main groups of fungi: nematode capture and endo-parasitic fungi that attack vermiform viable nematodes using special structures, and egg- and cyst-parasitic fungi that attack these stages with their hyphal ends [11-14]. The continuing interest in these fungi is partly due to their potential as biocontrol agents against plant- and animal-parasitic nematodes. Egg- and cyst-parasitic fungi have been thoroughly researched for their promise as biocontrol agents. Another reason for continued shooting in nematode-destroying fungi is remarkable morphological conformations and theatrical capture of nematodes by both nematode capture and endo-parasitic fungi [15-17]. In addition, both fungi and nematodes can be grown quite easily in the laboratory and provide a perfect model system for interaction studies [18-20].

In general, fungi that hunter fungi can be divided into two groups. They are good saprophytes, fast growing, sticky or adhesive hyphae network and more predators, catching nematodes by forming sticky knobs, constricting arms or sticky rings [21-22].

Nematode capture and endo-parasitic fungi are found in all major taxonomic fungal groups and are found mainly in any soil environment in which they survive as saprophytes [23, 24]. The ability to use nematodes as an additional source of nutrients gives them a dietary advantage. When fungi change their morphology, they enter parasitic stages and traps or mature spores are formed. The development of infectious structures is a pre-requisite for capturing nematodes. The mechanisms behind this development and the mechanisms behind the capture process, including the attraction, adhesion, penetration and digestion of nematodes, are the main themes of this article [25-27].

TABLE 1. Species of some nematode-destroying fungi and their trapping appatarus.

Nematode-destroying fungi	Classification	Trapping apparatus
Arthrobotrys brochopaga	Orbiliomycetes	Constricting rings
A.conoides	Orbiliomycetes	Adhesive networks
A.dactyloides	Orbiliomycetes	Constricting rings
A.haptotyla	Orbiliomycetes	Adhesive knobs
A.irregularis	Orbiliomycetes	Adhesive networks
A.microscaphoides	Orbiliomycetes	Adhesive networks
A.musiformis	Orbiliomycetes	Adhesive networks
A.oligospora	Orbiliomycetes	Adhesive networks
A.robusta	Orbiliomycetes	Adhesive networks
A.shizishanna	Orbiliomycetes	Adhesine networks
A.superba	Orbiliomycetes	Adhesive networks
A.thaumasia	Orbiliomycetes	Adhesive networks
Cystopage cladospora	Zygomycetes	Adhesive hyphae
Dactylaria candida	Orbiliomycetes	Adhesive knobs, non-constricting rings
D. eutermata	Orbiliomycetes	Adhesive networks
Dactylella bembicodes	Orbiliomycetes	Constricting rings
D. ellipsospora	Orbiliomycetes	Adhesive knobs
D. lobata	Orbiliomycetes	Adhesive hyphae
D. zhongdianensis	Orbiliomycetes	Adhesive networks
Dactylellina haptotyla	Orbiliomycetes	Adhesive knobs
D. sichuanensis	Orbiliomycetes	Adhesive knobs, non-constricting rings
D. varietas	Orbiliomycetes	Adhesive knobs, non-constricting rings
Drechslerella anchonia	Orbiliomycetes	Constricting rings
D. brochopaga	Orbiliomycetes	Constricting rings
D. dactyloides	Orbiliomycetes	Constricting rings
Duddingtonia flagrans	Orbiliomycetes	Adhesive networks
Geniculifera perpasta	Orbiliomycetes	Adhesive networks
Helicocephalum oligosporum	Zygomycetes	Adhesive hyphae
Monacrosporium bembicodes	Orbiliomycetes	Constricting rings
M. cionopagum	Orbiliomycetes	Adhesive networks
M. elegans	Orbiliomycetes	Adhesive networks
M. ellipsosporum	Orbiliomycetes	Adhesive knobs
M. eudermatum	Orbiliomycetes	Adhesive networks
M. gephyropagum	Orbiliomycetes	Adhesive branches
M. haptotylum	Orbiliomycetes	Adhesive knobs
M. megalosporum	Orbiliomycetes	Adhesive networks
M. psychrophilum	Orbiliomycetes	Adhesive networks
Peniophorella praetermissum	Basidiomycetes	Adhesive hyphae
Stropharia rugosoannulata	Basidiomycetes	Adhesive hyphae
Stylopage hadra	Zygomycetes	Adhesive hyphae
S. leiohypha	Zygomycetes	Adhesive hyphae

2. Characteristics of Nematode-Destroying Fungi

Nematode-destroying fungi infect the nematodes' eggs, juveniles, and adults and use them as foods. The fungi differ in their saprophytic-parasitic ability. While many of the trap-forming and egg-parasitic fungi can alive in soil ecosystem, the endo-parasites are mostly more dependent on nematodes as a nutrient that is called obligate parasites [28-32].

The ability to capture nematodes is linked to a certain developmental stage of the fungal mycelium. The trapping (predatory) fungi have developed advanced hyphal structures such as hyphal nets, rings, branches, or knobs, in which nematodes adhere or are mechanically captured (Figure 1). The different methods used by this type of nematode-destroying fungi to catch prey are also photographed in laboratory studies and presented in a guidebook [33]. Endo-parasites attack nematodes by their spores that adhere or assimilate to the surface of the nematodes. Regardless of the method of infection, the results are always the same: the death of the nematode. Examples of the first group are *Arthrobotrys* species, such as *A. oligospora, A. connoides, A. musiformis,* and *A. superba,* all of which form three-dimensional adhesive networks, and mechanical expansion of ring cells with nematodes, *A. dactyloides* [34, 35]. Sticky branches or arms and sticky buttons or knobs appear in the genus *Monacrosporium*. *M. haptotylum* (*Dactylaria candida*) produces both sticky knobs and non-shrinkable or constricting rings.

Among the endo-parasites, *Drechmeria coniospora, Hirsutella rhossoliensis, Haptoglossa dickii* and *Catenaria anguillulae* infect nematodes with their spores and engage their herbal lives in infected nematodes [36-37]. The *Nematoctonus* genus captures nematodes with both sticky traps and sticky spores, thereby forming a link between the two groups. Another mechanism for capturing nematodes is evident in wood-decomposed oyster mushroom *Pleurotus ostreatus*. Oyster mushrooms immobilize the nematode host with a toxin produced in special hyphal stems, and the hyphal ends grow chemo-tropically through the mouth of their victims and digest the content [38-39]. Egg parasite fungi, *Pochonia chlamydosporia* (*Verticillium chlamydosporium*) use appressoria to penetrate the nematode eggshells. Several stages of all these fungi have been described in a movie showing different strategies used by fungi [40-43].

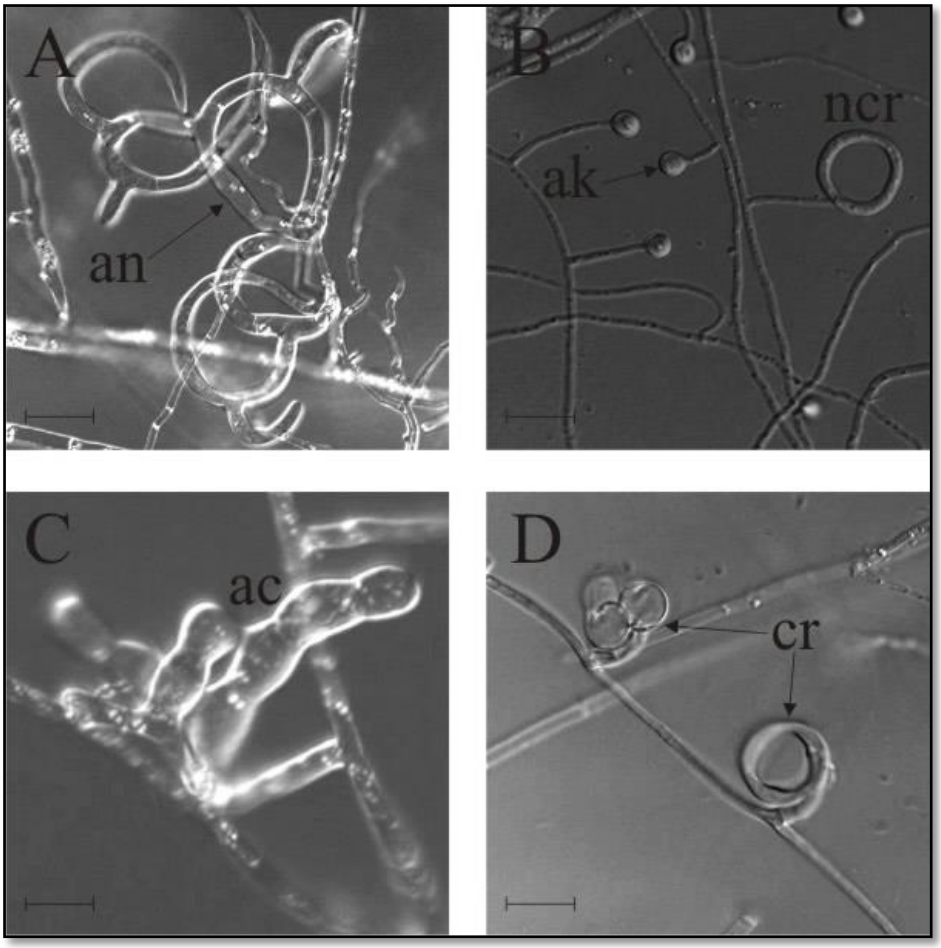

FIGURE 1. Natural nematode-trapping or capture apparatus. **A.** Adhesive network (**an**), **B**. Adhesive knob (**ak**) with non-constricting or non-compression rings (**ncr**), **C.** Adhesive column (**ac**), **D.** Constricting or compression ring (**cr**). Scale bars = 10 μm.

3. INFECTION STRUCTURES OF FUNGI

Nematode-destroying fungi show a large variety not only in terms of taxonomic distribution but also in the thrust structures formed (Table 2). The type of nematode capture structures formed depends on the species and even the strain of the species

as well as both biotic and abiotic environmental conditions. The most crucial biotic factor is living nematodes that not only stimulate the creation of trap structures by touching mycelium, but also act as a food source for the fungi after being invaded by fungi [44]. Thus, the relationship with the nematodes is two-fold: first, the nematodes can then induce the creation of the structures from which they were captured; and secondly, they serve as an extra nutrient source after the nematodes are invaded by the fungus [45-47].

For example, *Arthrobotrys* spp. it is generally more saprophytic than endo-parasites [48]. *Arthrobotrys* spp. They do not automatically create traps, but the fungi depend on environmental conditions, especially the presence of nematodes for the induction of traps. Trap structures of other fungi, such as branches, knobs, and collapsing rings, can be created automatically, indicating that these fungi need more nematodes as a food source [49].

Endo-parasites and spontaneous trap builders exhibit a large parasitic ability, while more saprophytic trap builders such as *Arthrobotrys* spp. has a unique ability to change their morphology to increase their parasitic abilities [23, 24]. As noted above, outer stimuli, such as nematodes, cause the formation of sticky traps in all trap-forming fungi. In *A. oligospora*, small peptides with highly non-polar and aromatic amino acids, or their low nutritional value, and amino acid components stimulate trap creation in both solid and liquid media. Based on this information, a growth technique has been developed in which the fungus can be studied both in its saprophytic and parasitic stages [9, 10, 38].

Most *Arthrobotrys* spp. are defined by an adhesive net trap. This trap can consist of a single ring or a fully developed three-dimensional network. Under some conditions, for example, *A. superba* may not develop full networks, but it can capture nematodes by sticky branches [7, 8]. Sticky branches form automatically in *Monacrosporium gephyropagum* regularly. Sometimes, such branches can merge to form simple rings. Sticky knobs are formed on the sensitive handle in the *M. haptotylum* mycelium. This species also procreates rings that do not contract on the sensitive stem. Both knobs and rings can be separated from the underlying mycelium and carried by nematodes [39].

TABLE 2. Species of some endoparasitic fungi and their mode of infection.

Endoparasitic fungi	Classification	Mode of infection
Catenaria anguillulae	Chytridiomycetes	Zoospores
C. vermiformis	Chytridiomycetes	Zoospores
Chlamydomyzium anomalum	Oomycetes	Zoospores
C. sphaericum	Oomycetes	Zoospores
Drechmeria coniospora	Deuteromycetes	Adhesive conidia
Haptocillium bactrosporum	Sordariomycetes	Adhesive conidia
H. balanoides	Sordariomycetes	Adhesive conidia
H. obovatum	Sordariomycetes	Adhesive conidia
Haptoglossa dickii	Oomycetes	'Gun cells', injection
H. erumpens	Oomycetes	'Gun cells', injection
H. heteromorpha	Oomycetes	'Gun cells', injection
H. mirabilis	Oomycetes	'Gun cells', injection
H. zoospora	Oomycetes	'Gun cells', injection
Harposporium anguillulae	Deuteromycetes	Ingested spores
H. bysmatosporum	Deuteromycetes	Ingested spores
H. leptospira	Deuteromycetes	Ingested spores
Hirsutella rhossiliensis	Deuteromycetes	Adhesive spores
Gonimochaete horridula	Oomycetes	Adhesive spores
G. latitubus	Oomycetes	Adhesive spores
G. lignicola	Oomycetes	Adhesive spores
G. pyriforme	Oomycetes	Adhesive spores
Meria coniospora	Deuteromycetes	Adhesive conidia
Meristacrum asterospermum	Zygomycetes	Adhesive conidia
Myzocytiopsis glutinospora	Oomycetes	Zoospores
M. humicola	Oomycetes	Zoospores
M. intermedia	Oomycetes	Zoospores
M. lenticularis	Oomycetes	Zoospores
M. papillata	Oomycetes	Zoospores
M. zoophthora	Oomycetes	Zoospores
Nematoctonus concurrens	Basidiomycetes	Adhesive hour-glass knobs, Adhesive spores
N. leiosporus	Basidiomycetes	Adhesive hour-glass knobs, Adhesive spores
Olpidium vermicola	Chytridiomycetes	Zoospores
Pythium (Lagenidium) caudatum	Oomycetes	Zoospores
Spirogyromyces vermicola	Unknown	Ingested spores
Verticillium balanoides	Deuteromycetes	Adhesive spores

Some groups of fungi form an adhesive network. These crotch-shaped loops are wound around the nematode body. The hyphae loops formed by the fungus hold the host and wrap the entire body of the nematode with a sticky substance. The body of the nematode is pierced through the parts where the hyphae loop is contacted (eg. *Arthrobotrys dactyloides* and *A. digospoa*). In addition, this group of fungi has the ability to enter the plant tissue. It is also known that they penetrate and kill *Ditylenchus dipsaci*, which develops in plant tissue.

Sticky knobs formed by fungi are small spheres or lobes and consist of 1-2 cells. *Stylopage harda*, *Doctylella lobata* and *D. cionopaga* are examples of fungi forming sticky knobs.

It may be less effective than fully developed traps in capturing nematodes. Some species (e.g. *A. superba*) may capture nematodes on initials or branches of adhesive nets, or even on adhesive hyphae, as in *Stylopage* and *Cystopage* spp. This growth pattern occurs in almost all trap-forming species when conidia are allowed to germinate in natural substrates such as cow manure or rhizosphere soil [50]. A mutant of *A. oligospora* does not only form conidial traps on the conidia when it is in upright conidiophores; it also produces large amounts of normal traps in mycelium. These examples may show an increased efficiency of these fungi to reduce the number of nematodes in the environment. Another morphological adaptation of the *A. oligospora* mycelium is the response to the presence of other fungi. *A. oligospora* can roam around hyphae and consume the contents of these cells called as mycoparasitism [27, 48, 49].

In addition, *A. oligospora* can create appressoria in response to plant roots. The winding of both the rhizosphere and the hyphae and appressoria are examples of the diversity of the ways nematode-catching fungi cope with changing environmental conditions. All these adaptations show the plasticity of the infection structures in nematode trapping fungi [27].

Endo-parasitic fungi are obligate parasites of nematodes that spend their entire vegetative life in the nematode they infect. Nematodes may encounter spores such as conidia or zoospores as they pass through soil pores. Spores infect the nematode in two ways: (a) orally, that is, when the spores are swallowed with food by the nematodes; or (b) percutaneous, i.e. spores adhere to the cuticle of the nematodes. In this case, zoospores float toward the nematode and are thrown around natural holes such as the mouth, anus, or vulva. There is a similar variety among endo-parasites.

D. coniospora creates a large number of conidia compared to hyphal material production. In a single contaminated nematode, *D. coniospora* can procreates as much as 10,000 conidia, while the single endo-parasite *H. rhossoliensis*, which does sports alone, procreates 100-1000 conidia per contaminated nematode. Both fungi develop a sticky bud in their conidia where they infect the nematode [51-53]. The genus *Harposporium* contains fungi that procreates spores of unusual forms that are ingested by nematodes. Due to their shape, spores get stuck in the oesophagus and from there they start a contamination of the nematodes. *C. anguillulae* contaminates nematodes with mobile zoospores that are thrown and moved over the nematode. Finally, spores in the genus *Haptoglossa* form a contamination "gun cell" that forcefully injects the infective principle into the nematode host [54, 55].

The fungi that parasitize the non-motile stages of nematodes, i.e. eggs use a different tactic. Hyphae of *P. chlamydospora* and other fungi grow towards the eggs, and appressoria occurs on hyphae ends that penetrate the eggshell. Fungi then digest the egg content of both immature and mature (containing juveniles) eggs [56]. Egg-parasitic fungi are those that use appressoria or zoospores to infect the eggs of plant-parasitic nematodes [57-60]. This group of fungi can survive saprotrophically in the rhizosphere and is relatively easy for mass culture [4].

An additional advantage of their potential is that their hosts are often stalk-free in the form of eggs, developing juveniles, and sedentary females (Table 3).

TABLE 3. Species of some nematode egg-and female-parasitic fungi and their infection mechanisms.

Nematode-destroying Fungi	**Classification**	**Mode of infection**
Dactylella ovaparasitica	Orbiliomycetes	Appressoria
Helicocephalum oligosporum	Zygomycetes	Adhesive hyphae
Lecanicillium psalliotae	Deuteromycetes	Appressoria
Nematophthora gynophila	Oomycetes	Zoospores
Olpidium vermicola	Chytridiomycetes	Zoospores
Paecilomyces lilacinus	Deuteromycetes	Appressoria
Pochonia chlamydosporia	Deuteromycetes	Appressoria
P. rubescens	Deuteromycetes	Appressoria
Rhopalomyces elegans	Zygomycetes	Appressoria

4. Interaction mechanisms

Nematodes are attracted by mycelium compounds and nematode trapping fungal traps and spores of endo-parasites. Both morphology and consequently saprophytic parasitic ability strongly affects the fascination of fungi [70, 71]. More parasitic fungi appear to have a stronger charm than more saprophytic ones; that is, endo-parasitic species infecting conidia and nematodes are more effective in fascinating nematodes than more saprophytic species with different trapping apparatus [72, 73].

The contact and adhesion of nematodes to the traps and spores of fungi that destroyed the nematode can be seen in the electron microscope. In *A. oligospora*, three-dimensional networks are surrounded by an extracellular fibril sheet. After contact, these fibrils are directed perpendicular to the surface of the host, possibly to simplify anchorage of the nematode and further fungal infestation [74, 75]. Endo-parasite *D. coniospora* shows a completely different type of adhesive, as if it consisted of spreading fibrils, regardless of whether contact with the nematode was established. In addition, *D. coniospora* spores adhere properly to the sensory organs at the tip of the nematode head, thereby preventing nematode charm. The chemical combination of surface fibrils of nematode-destroying fungi is not known in detail, but they contain both proteins and carbohydrate-containing polymers [76-78].

The adhesion of the traps in the nematode causes the fungi to differentiate. In *A. oligospora*, a penetration tube forms and pierces the nematode cuticle. This step probably includes both the activity of the hydrolytic enzymes that dissolve the macromolecules of the cuticle, and the activity of a mechanical pressure produced by the penetrating growing fungus. The nematode cuticle mainly consists of proteins, including collagen, and several proteases are isolated from nematode-destroying fungi that can hydrolyse the proteins of the cuticle. In any case, these proteases belong to the serine protease family and have been shown to have high homology to subtilisin-type serine proteins after obtaining data from sequencing [79, 80]. In endo-parasite *D. coniospora*, it appears that a chymoptrypsin-like protease is involved in the penetration process.

More detailed studies of subtilisin PII produced by *A. oligospora* have shown that such proteases may have a number of different functions [81]. Therefore, PII appears to have a nematotoxic activity, as well as being involved in the penetration and digestion of the cuticle and tissues of infected nematodes.

After penetration, the nematode is digested by the infected fungus. After entering the nematode, the penetration tube of *A. oligospora* is disintegrated to form a large bulb of infection. The development of bulbs and trophic hyphae occurs in parallel with dramatic changes in the infrastructure and physiology of the fungus. Dense objects are reduced in trap cells and ampoules. The bulb and trophic hyphae typically contain typical cell organelles, the endoplasmatic reticulum is mainly well developed. In the later stages, lipid droplets accumulate in trophic hyphae, possibly involved in the assimilation and storage of nutrients from the infected nematode [58, 64, 65].

Unlike trap-forming fungi, endo-parasite *D. coniospora* does not form an infection bulb upon penetration and does not have dense stems typical for trap-forming fungi. With the formation of lipid droplets, another way for *A. oligospora* to store host-derived nutrients is to produce a large amount of lectin in the cytoplasm [82]. This protein is *Arthrobotrys oligospora* lectin, AOL. Until recently, it is a member of the low molecular weight lectin family that shares similar primary sequences and binding properties that have been identified in only a few filamentous fungi [83, 84]. During infection of nematodes, AOL is rapidly synthesized in *A. oligospora* after the nematodes penetrate and digestion begins. Large amounts of AOL accumulate in trophic hyphae growing in the nematode. Lectin is then transported from the infected nematode to other parts of the mycelium, where it can break down and promote the growth of the fungus. It has been suggested that AOL, like other lectins, is involved in a recognition event during interaction with nematodes. Binding of the AOL lectin family to sugar structures specific to animal glycoproteins, including nematodes, but not found in fungi, supports this hypothesis.

Although the nematode infection patterns of other predatory fungi that use adhesive layers to capture nematodes (nets, hyphae or knobs) have been less studied, they often seem to be similar to those described for *A. oligospora*. In contrast, the catch mechanism of contraction rings is completely different. When a nematode moves into the ring, the three cells that form the ring trigger a response so that it swells inward quickly and closes around the nematode. Other stimuli can also trigger trap closure, such as touching a needle or heat. The reaction is rapid (0.1 s), irreversible, and is consorted by a large increase in cell volume, leading to the almost complete closure of the trap's opening [85]. Following capture, the fungus produces a diffusion tube that pierces the nematode cuticle. A small bulb of infection is formed inside the nematode, in which trophic hyphae develop.

The traps created by these fungi can be either sticky traps or sticky arms, sticky network, sticky knobs. Sticky arms are short lateral arms that are several cells long. They form a loop and attack the nematode. But they are never in the form of a mixed network. During the random movement of the nematode, these sticky arms come into contact with the nematode and catch it.

Dactylella ellipsospora's sticky hyphae loops adhere to the nematode, making the nematode completely immobile within two hours. Then the fungus hyphae penetrates into the nematode cuticle and develops and spreads inside the nematode body. After all, it absorbs the body fluid of the nematode, killing the nematode.

Non-suffocating rings formed by some group of fungi are only responsible for capturing the nematode. After the nematode is caught, the fungal hyphae grow rapidly, penetrating the nematode cuticle and absorb body fluid. *Dactylella doedyeoides* can be given as an example to this group of fungi.

The cells of the *Nematoctonus haptocladus* first secrete, the nematode that enters this secretion is caught by the fungus with short and sticky hyphae arms, and then the nematode is penetrated and killed.

The mechanism by which the compression rings are closed is not known in elaboration. Electron microscopy showed that during the ring cell enlargement, the outer cell wall of the ring cells was torn along a defined line on the inner surface of the ring. It has been suggested that this release of wall compression will lead to rapid water uptake and subsequently the enlargement of the flexible inner wall of the ring cells. The signal transduction track involved in bloating ring cells has been studied in *A. dactyloides*. In this fungus, the pressure exerted by a nematode on the ring appears to activate the G-proteins in the ring cells. Activation leads to increased calmodulin in cytoplasmic Ca^{2+} activation and finally opening of water canals. Ring cells tighten to narrow the ring, thereby fixing the nematode [86].

5. BIOCONTROL

Biocontrol or biological control is considered an alternative to chemicals, as it is not only an environmentally friendly measure but can also support sustainability in agricultural production [87-89]. Demonstrating that selected biocontrol agents can provide adequate control levels for political non-chemical disease management programs, along with political pressures, contributed to a change in attitudes towards biological control research [90-92]. Many organisms have shown antagonistic effect

against phytonematodes [93-95], and fungi among them are considered the most important group [4, 96-98]. These organisms often did not provide consistent or adequate control. However, the best results for biocontrol of soil microorganisms can be achieved when short-term conservation will result in significant yield benefits and where natural application of target areas is possible [99].

Many fungal plants from different taxonomic categories can adversely affect plant-parasitic nematodes [4], but having aggressiveness is not the only feature required to become a qualified biocontrol agent.

An important feature of nematophagous fungi is the possibility of using them for biological or biological control of plant- and animal-parasitic nematodes. Plant-parasitic nematodes, e.g. root-knot (*Meloidogyne* spp.) and cyst (*Heterodera* spp., *Globodera* spp.) nematodes are global pests that cause serious yield losses in agriculture and horticulture [100-104]. Many nematicides, such as methyl bromide, are prohibited due to health and environmental concerns. Therefore, new alternates are needed for nematode control. Biocontrol can be such an alternating [105-108].
There are two general ways to implement the biocontrol of nematodes using fungi that destroy nematodes: adding large amounts of fungus to the soil; or stimulating the activity of existing fungi using various changes. Initial experiments for plant-parasitic nematodes include nematode-trapping fungi, e.g. *Arthrobotrys* or *Monacrosporium* species and later endo-parasitic fungi, e.g. *H. rhossoliensis* and *D. coniospora* and egg-parasitic fungi, e.g. *P. chlamydosporia*. The performances of these biocontrol agents have been varied and so far no commercial products are available [60, 67].

The hyphae arms of the fungi forming stifling rings form a ring by bending backwards on it. These rings are 3-4 cells and the middle of the ring is empty. When the nematode enters the ring, it stimulates the ring cells as a result of the contact effect. The cell wall permeability of the stimulated cells increases and the cells reach 3 times the size by taking a lot of water from the environment. As a result, the ring space is narrowed and the nematode in the space is choked and their bodies are divided into two. *Monacrosporium lysipagum* is a good example of this group of fungi [67].

The use of nematode trapping fungi is of particular interest due to the increased knowledge of the biology of these fungi and partly because of the better formulation and application of fungal biocontrol agents to the soil. One way to improve the

control potential of nematode-destroying fungi would be to use genetic engineering to increase the pathogenicity and survival of the introduced fungus. Using genetic transmutation, it was possible to produce nematode-trapping fungi *A. oligospora* mutants that overexpress a protease gene (P II). Mutants containing additional copies of the P II gene developed a higher number of infectious structures and increased rates of catching and killing nematodes [81]. Also, it has recently been reported that the formulation of fungal *A. dactyloides* capturing nematodes can reduce tomato infection with knot-root nematodes in field experiments. In the same experiment, a similar decrease was not shown with egg parasite *P. chlamydosporia*. An important problem of adding nematode destructors and other biocontrol fungi to the soil is their low ability to form in a complex soil environment. Bourne et al. [109] it is of great importance that rhizosphere colonization is necessary for an accomplished enterprise, and therefore scanning the rhizosphere-authorized strains of nematode-destructive fungi [110, 111].

The interaction between nematode-hunting fungi and plant parasitic nematodes is complex. The activity of these fungi can be affected by soil pH, humidity, temperature and nutrients in the soil. On the other hand, their uncertainty in the invasion, their slow development, and their need for enormous amounts of food, sometimes very specific, hamper their success in being candidates for commercial production [42, 92].

Animal-parasitic nematodes cause disease and serious weight decrease in animal husbandry all over the world. The chemicals currently used to control these nematodes, anthelmintics have been shown to develop resistance in the parasitic nematode fauna. A promising approach has been presented in the feeding of grazing animals with fungal mycelium containing chlamydospores of nematode-trapping fungi; *Duddingtonia flagrans*. By allowing spores to be transported through the animal intestines and producing and producing traps in faeces and surrounding grass, it captures newly hatched offspring of parasites and reduces the nematode burden in the fields [112, 113]. The population structure of fungi that destroy nematodes is mostly unknown. This information is important to assess the fate and risk of undesirable spread of an applied biocontrol agent. Recently, the genetic variation in a worldwide collection of nematode-trapping fungus *D. flagrans* has been shown to be very low using various genetic markers [114]. The data show that *D. flagrans* is essentially clonal and recombination cannot be detected even within the same country. Therefore, recombination of the mass-applied *D. flagrans* strain with local isolates is unlikely.

Although not considered to be conventional biocontrol, another promising approach that nematode-destroying fungi as well as other soil fungi can be used to develop new tools to control animal- and plant-parasitic nematodes is to use the antagonist as a source for insulating new combinations with nematicidal efficiency [115-118]. According to the information I got from a nematologist, *Arthrobotrys irregularis*, one of the nematode predators, was produced commercially in France and launched as a preparation under the name of Royal 350. However, the fact that this fungus cannot grow below pH 6.5 limits the use of large areas in order to be successful, such as the necessity to use high doses and storage difficulties. Against *Ditylenchus myceliophagus*, a breed of *A. robusta* was produced and a commercial preparation named Royal 300 was obtained [18, 92].

6. Conclusions

Given the environmental safety, human health hazards and management costs, the fungal biocontrol agent is the best option, much safer and highly applicable. However, biocontrol of phytonematodes or plant-parasitic nematodes through nematode-destroying fungi provides irregular results, especially in field conditions, especially since the soil ecosystem is very complex.

Extensive research of fungi that have destroyed nematodes in the past decade has been carried out in many countries worldwide. However, most of these fungi have not yet been discovered. In addition, much research is needed on the identification of discoveries and their exploitation against economically important phytonematodes [119]. In recent years, scientists have succeeded in commercially exploiting several biocontrol agents such as *P. lilacinus*, *P. chlamydosporia, T. harzianum, A. niger* and *A. oligospora* against phytonematodes, but in all respects it was not promising [42, 92]. If one fungal biocontrol agent is successful in controlling one group of nematodes, the problem of the other group remains unresolved. It has been widely observed that if two or more species of phytonematodes are fed on a plant host, the fungal effect can only limit or control the population of one species.

Therefore, the problem of other mobile nematodes will remain unchanged. In addition, isolates of fungal biocontrol agents differ greatly in virulence and ability when installed in the soil, and therefore their results under field conditions are very uneven. On the other hand, another disadvantage is the presence of antagonists of these fungi in the soil, which, when applied in the field, often fails fungal biocontrol agents.

As a result, the use of fungal biocontrol agents is environmentally safe and the correct approach in the management of phytonematodes, but it is difficult to say that they replace nematicides. Fungi that destroy nematodes may not control the nematodes when the latter's inoculum level is too high in the soil, but the population of the nematode can be reduced to ultimately reduce crop yield loss. According to the researchers, the fungal biocontrol agent, combined with herbal and pesticides, seems to be one of the best options, as the seed treatment can prove to be economical, much safer and highly viable in field conditions.

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